SASNM Congress

18th Biennial Congress - Pretoria, 10 to 12 August, 2018

Therapeutic Radiopharmaceuticals - Importance of Preclinical Investigation for Effective Clinical Application

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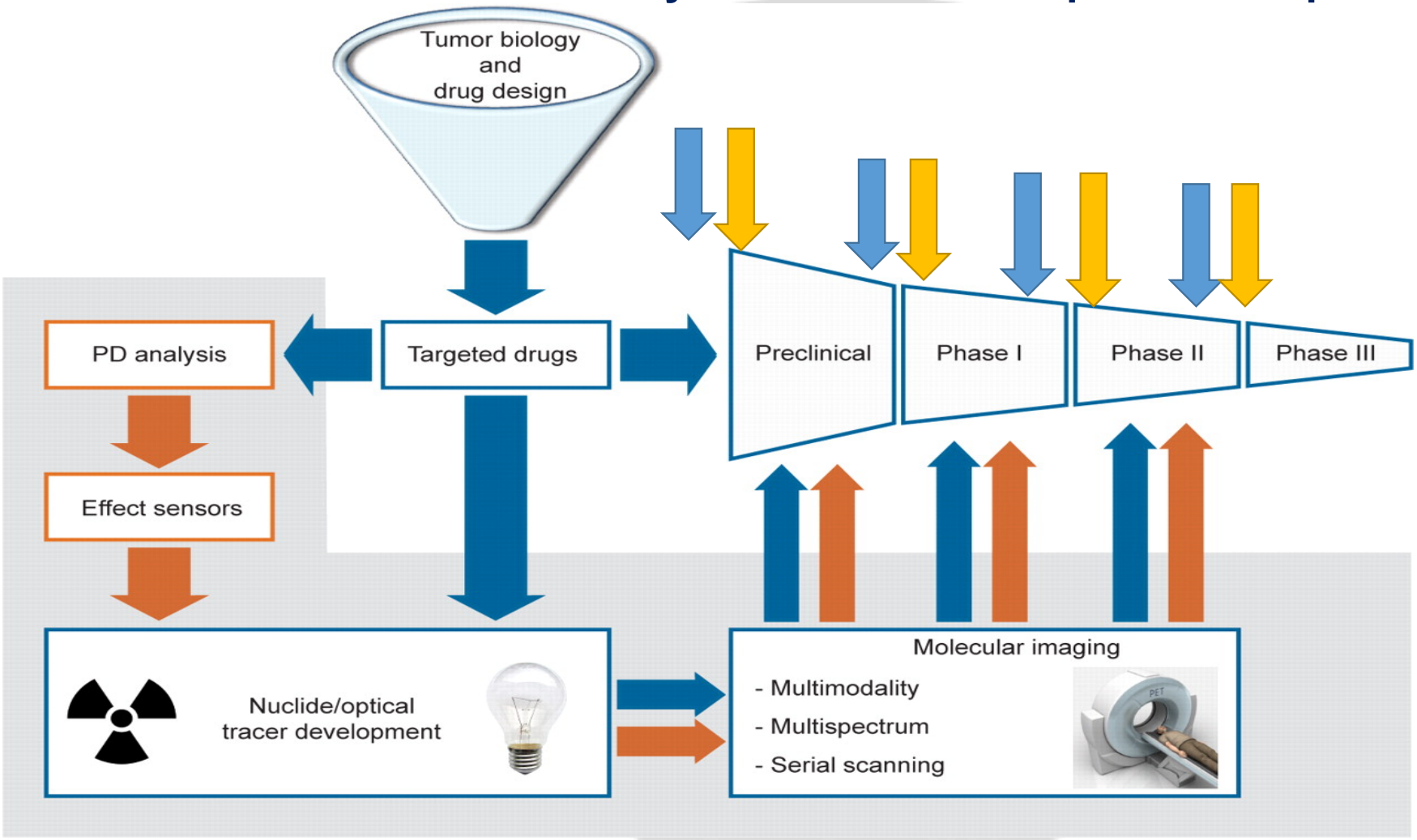
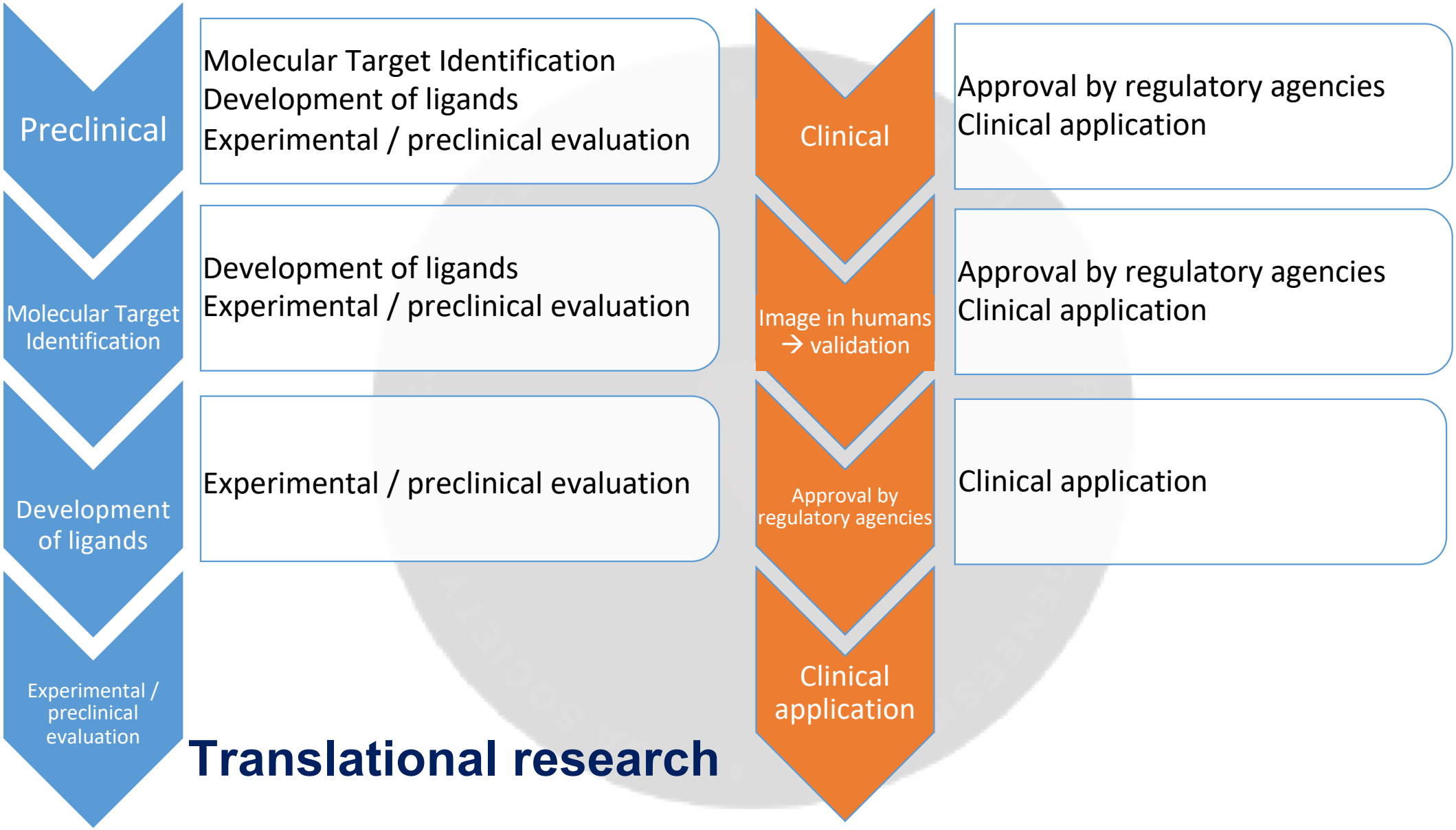
Can we say the same for therapeutic radiopharmaceuticals?



Flow chart of molecular imaging in drug development

Elisabeth G.E. de Vries et al. Cancer Discovery 2011;1:25-28


Preclinical
Molecular Target Identification
Development of ligands
Experimental / preclinical evaluation
Molecular Target Identification
Development of ligands
Experimental / preclinical evaluation
Development of ligands
Experimental / preclinical evaluation
Experimental / preclinical evaluation
Clinical
Approval by regulatory agencies
Clinical application
Image in humans → validation
Approval by regulatory agencies
Clinical application
Approval by regulatory agencies
Clinical application
Clinical application
Translational research

Development of *in vivo* probes / potential radiopfarmaceuticals for therapy

< 5% of *in vitro* targets allow development of an *in vivo probe?*

– *how many* potential radiopfarmaceuticals ? and how many for for therapy ?

- **<u>High TARGET activity / concentration</u>**
  - Affinity and specificity
  - Absence of biological barriers (i.e. endothelium, blood brain barrier, ...)
  - Stable labeling of compound
- **<u>Low BACKGROUND activity</u>**
  - Non-specific accumulation,
  - Circulating or interstitial activity
  - Renal or hepatic elimination
- **<u>Signal amplification</u>**
  - Cell trapping
  - Enzymatic conversion
  - "Reporter" molecules: fluorescence, radiation, magnetic

Preclinical safety testing of diagnostic and therapeutic radiopharmaceuticals - regulatory requirements

Toxicology

What are the toxicity target organs? Are toxic effects reversible?
Is the drug mutagenic, carcinogenic or toxic to reproduction?
Are there adverse effects on cardiovascular, neurological or respiratory function?
Are there any toxic metabolites?
Are there any toxic impurities in production batches for clinical use?

Toxicokinetics

How are toxic effects related to dose and systemic concentrations?
Which enzymes are involved in the drug's metabolism?
What are the metabolites and what is their activity on- and off-target?
Are there species differences in absorption, distribution, metabolism, and excretion?

Preclinical questions

What information is to be included in the Investigator's Brochure?
What (additional) safety endpoints need to be monitored in human trials?
What is the proposed human starting dose and its margin of safety?
What is the proposed human dose escalation step size?

ICH harmonisation for better health

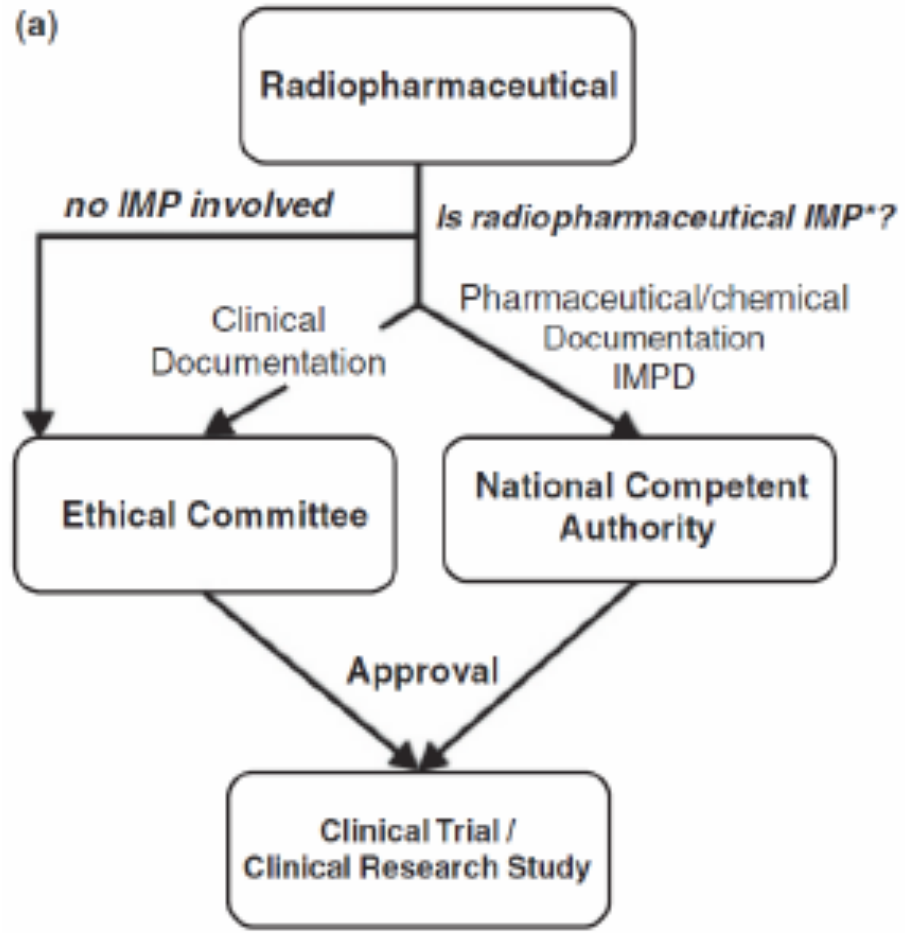
GUIDANCE ON NONCLINICAL SAFETY STUDIES FOR THE CONDUCT OF HUMAN CLINICAL TRIALS AND MARKETING|AUTHORIZATION FOR PHARMACEUTICALS
M3(R2)

S1A/B/C	Carcinogenicity Studies
S2	Genotoxicity Studies
S3A/B	Toxicokinetics and Pharmacokinetics
S4	Toxicity Testing
S5	Reproductive Toxicology
S6	Biotechnological Products
S7A/B	Pharmacology Studies
S8	Immunotoxicology Studies
S9	Nonclinical Evaluation for Anticancer Pharmaceuticals
S10	Photosafety Evaluation

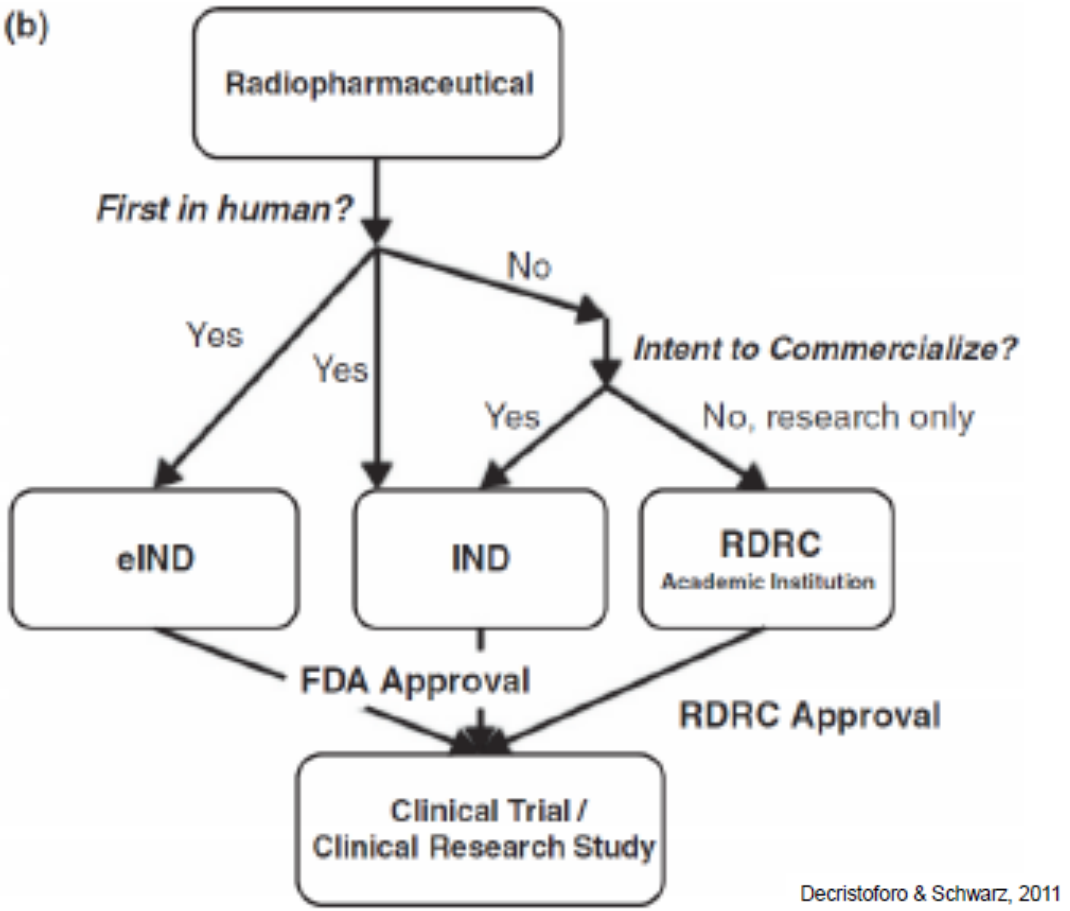
All toxicology/safety studies according to GLP

Radiopharmaceuticals - regulation and leglisation

EU



USA



Decristoforo & Schwarz, 2011

Investigational Medicinal Products (IMPs)

“Medicinal products” are defined by Directive 2001/83/EC as "…*prepared industrially or manufactured by a method involving an industrial process*…".

The **Clinical Trials Directive 2001/20/EC, Article 2** (d), provides the following definition for an Investigational Medicinal Product (IMP): "*a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including products already with a marketing authorization but used or assembled (formulated or packaged) in a way different from the authorised form, or when used for an unauthorised indication, or when used to gain further information about the authorised form*."

Radiopharmaceuticals which may be classified as IMPs include radiolabelling kits, radionuclide generators and radionuclide precursors.

EU **Investigational Medicinal Product (IMP) Safety documentation requirements for radiopharmaceuticals** [radionuclide generators, kits, radionuclide precursor radiopharmaceuticals and industrially prepared radiopharmaceuticals] :
Standard requirements for medicinal products [= ICH M3(R2) for preclinical safety] Radiation dosimetry - Organ/tissue exposure to radiation; - Absorbed radiation dose estimates for a given route of administration according to a specified, internationally recognised system.

(http://ec.europa.eu/health/files/pharmacos/docs/doc2006/07_2006/def_imp_2006_07_27_en.pdf)

Therapeutic Radiopharmaceuticals Single-dose toxicity: These studies may give some indication of the likely effects of acute overdosage in man and may be useful for the design of toxicity studies requiring repeated dosing in the relevant animal species

Reproductive function and foetal toxicity: Studies may be required in certain cases, especially if the radiopharmaceutical is intended for repeated use in women of child-bearing potential. Otherwise the study on reproductive function may justifiably be limited to ascertaining the effect on fertility.

Mutagenic potential: Characterization of the mutagenic potential of the non-radioactive equivalent of the product; may be limited to screening for gene and chromosome mutations.

Carcinogenic potential: An evaluation of any carcinogenic potential of the substances involved must be presented. If no carcinogenicity tests are performed, this must be clearly indicated.

Investigational New Drug (IND) Application - FDA

Oct 5, 2017

There are two IND categories:

- Commercial
- Research (non-commercial)

The IND application must contain information in three broad areas:

- **Animal Pharmacology and Toxicology Studies** - Preclinical data to permit an assessment as to whether the product is reasonably safe for initial testing in humans. Also included are any previous experience with the drug in humans (often foreign use).

- **Manufacturing Information** - Information pertaining to the composition, manufacturer, stability, and controls used for manufacturing the drug substance and the drug product. This information is assessed to ensure that the company can adequately produce and supply consistent batches of the drug.

- **Clinical Protocols and Investigator Information** - Detailed protocols for proposed clinical studies to assess whether the initial-phase trials will expose subjects to unnecessary risks. Also, information on the qualifications of clinical investigators--professionals (generally physicians) who oversee the administration of the experimental compound--to assess whether they are qualified to fulfill their clinical trial duties. Finally, commitments to obtain informed consent from the research subjects, to obtain review of the study by an institutional review board (IRB), and to adhere to the investigational new drug regulations.

https://www.fda.gov/drugs/developmentapprovalprocess/howdrugsaredevelopedandapproved/approvalapplications/investigationalnewdrugindapplication/default.htm

Emergency Investigational New Drug (EIND)

A physician may decide to request use of an investigational antiviral product through a single-patient

Emergency Investigational New Drug (EIND) application if:

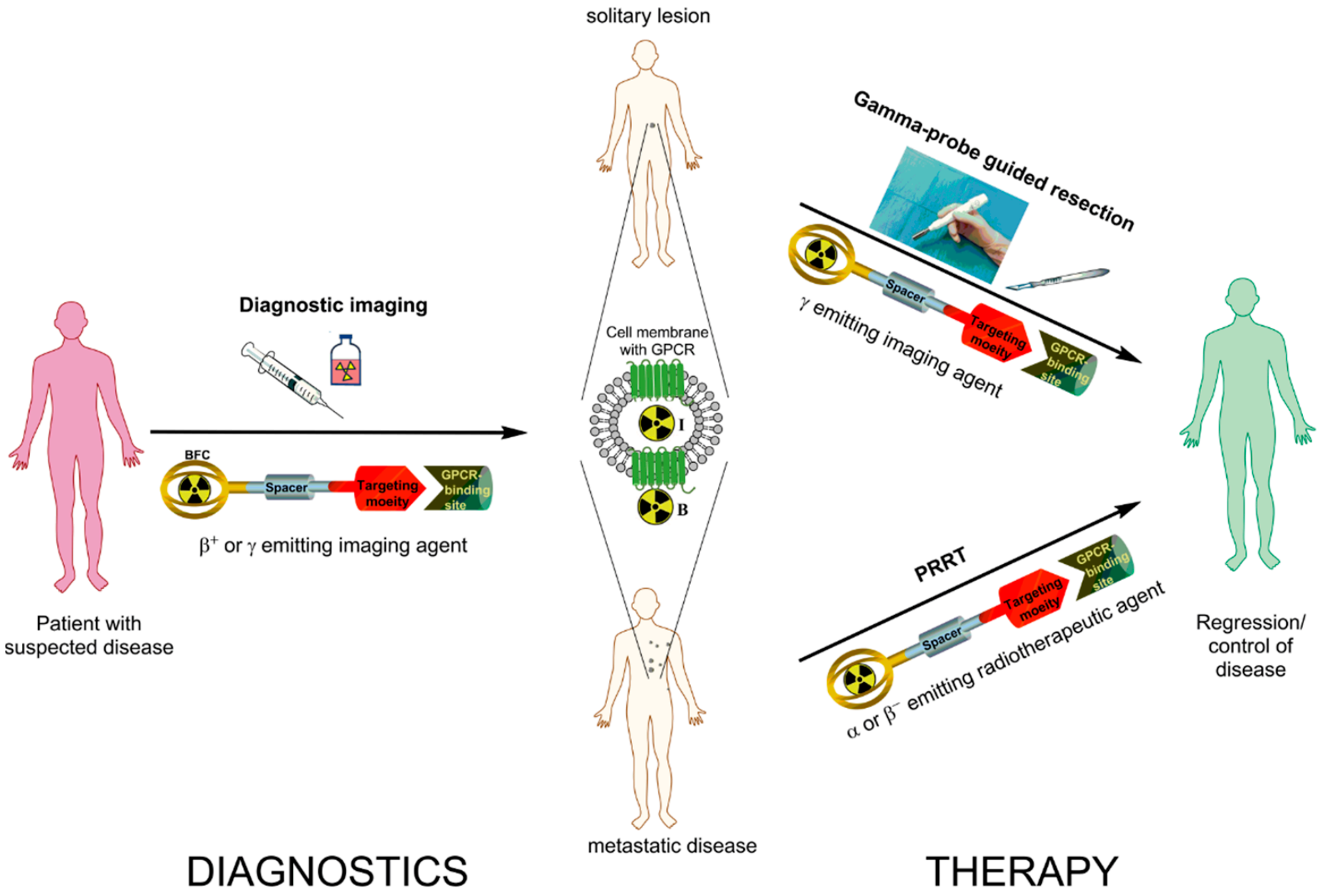
- the physician considers the product may be urgently needed for the patient's serious or life-threatening con
- no satisfactory alternative therapy is available; and
- the patient cannot receive the product through any existing clinical trials or expanded access protocols

World Health Organization

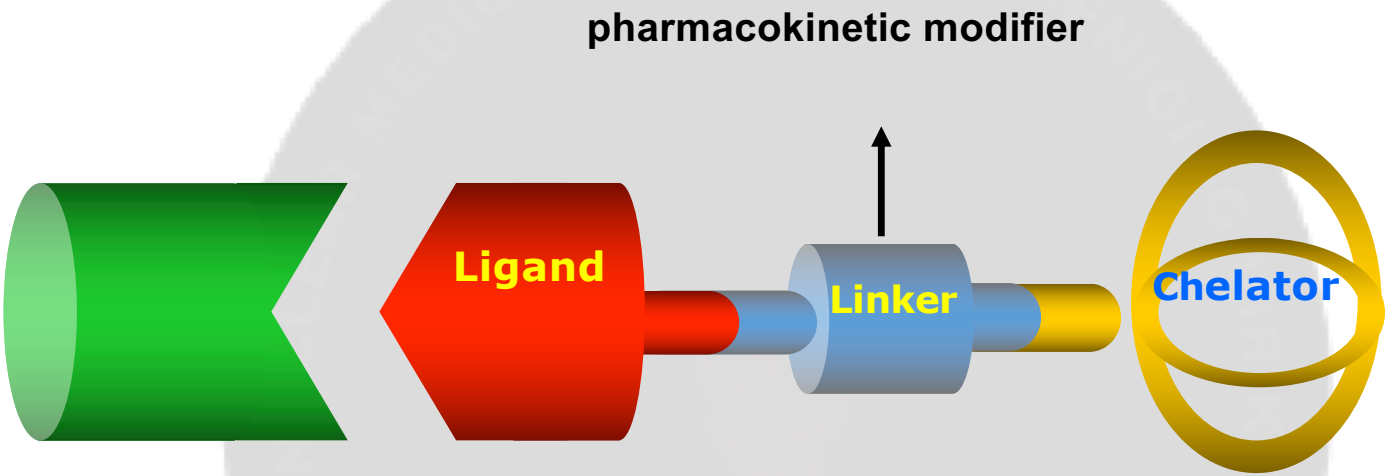
International Pharmacopoeia (Ph. Int.)

Biodistribution A physiological distribution test is prescribed, if necessary, for certain radiopharmaceutical preparations.
The distribution pattern of radioactivity observed in specified organs, tissues or other body compartments of an appropriate animal species (usually rats or mice) can be a reliable indication of the expected distribution in humans and thus of the suitability of the intended purpose.

The preparation meets the requirements of the test if the distribution of radioactivity in at least two of the three animals complies with the criteria specified in the monograph.


solitary lesion
Diagnostic imaging
Cell membrane with GPCR
I
B
BFC
Spacer
Targeting moeity
GPCR-binding site
β+ or γ emitting imaging agent
Patient with suspected disease
metastatic disease
Gamma-probe guided resection
γ emitting imaging agent
PRRT
α or β− emitting radiotherapeutic agent
Regression/ control of disease
DIAGNOSTICS
THERAPY

Schematic Representation of a Drug for Imaging and Targeted Therapy



Target

- Antigens
- GPCRs
- Transporters

Molecular Address

- Antibodies, their fragments and modifications
- Regulatory peptides and analogs thereof
- Amino Acids

Reporting Unit

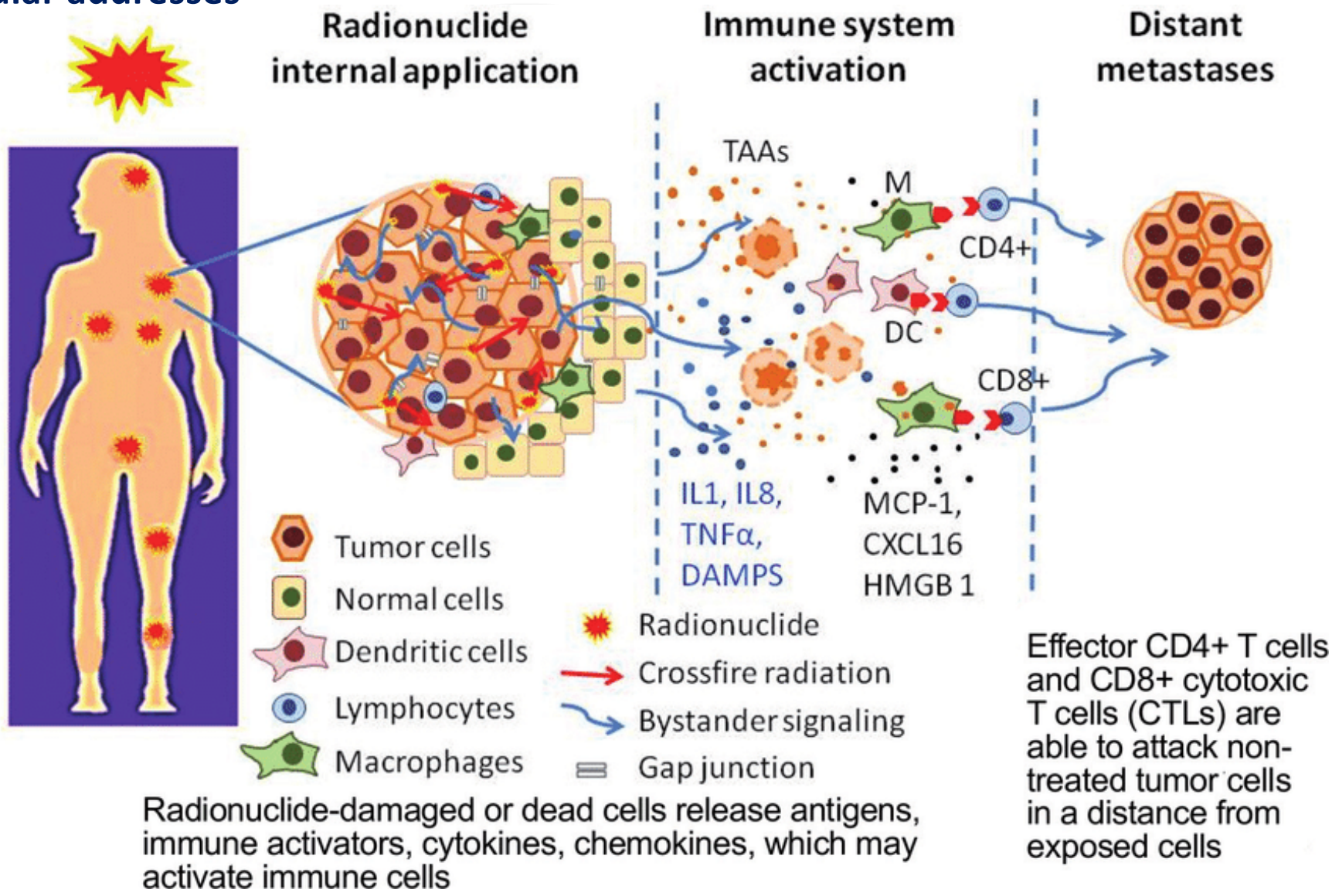
- **SPECT**
- **PET**
- Gd^{3+}

Cytotoxic Unit

- **THERAPY**

H.R. Maecke

Target and molecular addresses



RADIONUCLIDES FOR THERAPY

WHAT WE HAVE

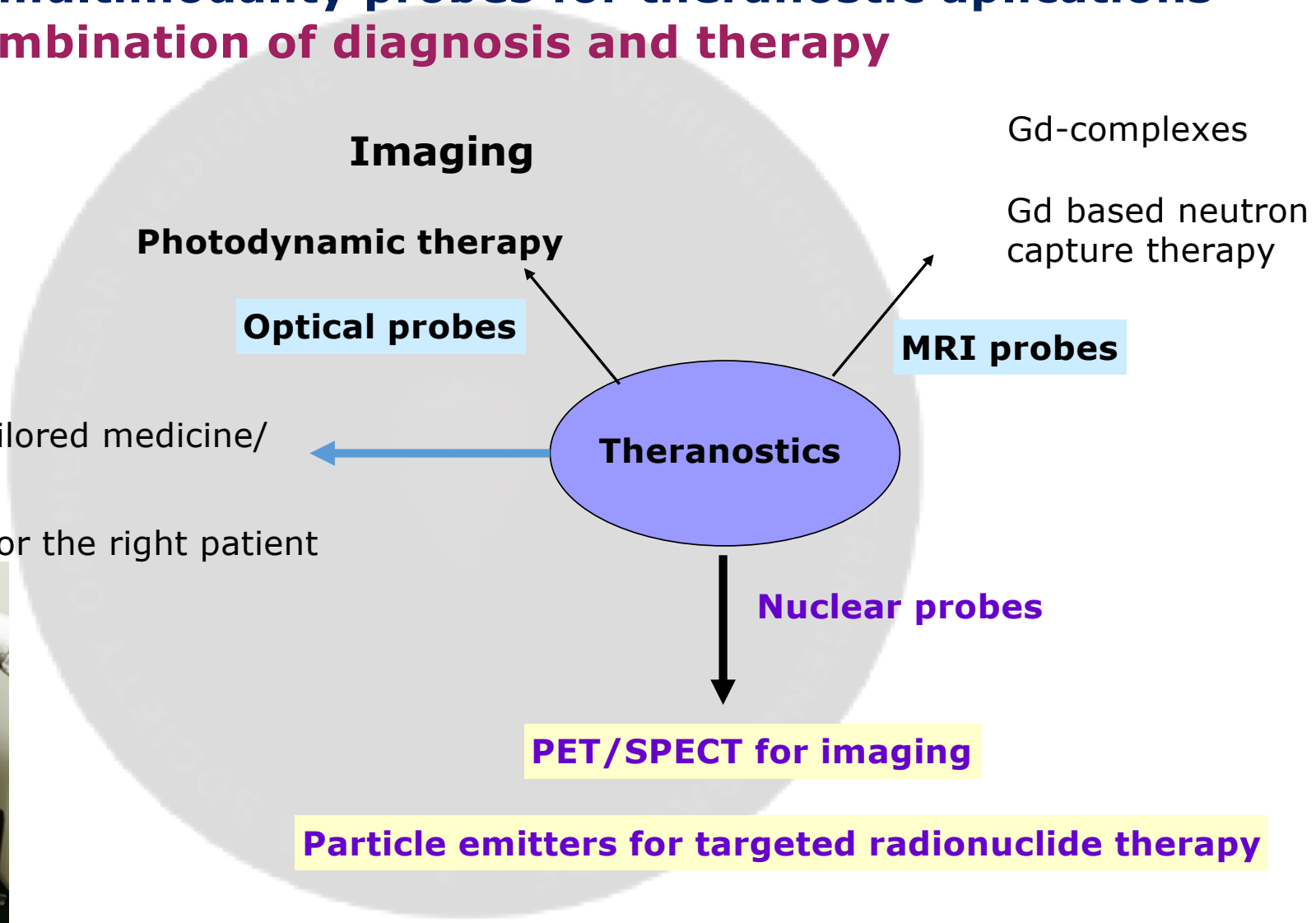
Radionuclide	Half-life	Mode of decay	Energy (keV)
THERAPY			
^{90}Y	64.1 h	β^-	2282.0
^{131}I	**8.0 d**	**β^-, γ**	**970.8**
^{153}Sm	46.3 h	β^-, γ	808.4
^{89}Sr	50.5 d	β^-	1496.6
^{177}Lu	6.7 d	β^-, γ	498.2
$^{188/186}Re$	16.9 h	β^-, γ	2120.4

WHAT IS COMING

Radionuclide	Half-life	Mode of decay	Energy (keV)
THERAPY			
^{211}At	7.2 h	α	6790
^{67}Cu	61.9 h	β^-, γ	577
$^{212/213}Bi$	60/46 min	α	8320
^{225}Ac	10.0 d	α	5750
^{223}Ra	11.43 d	α	5780

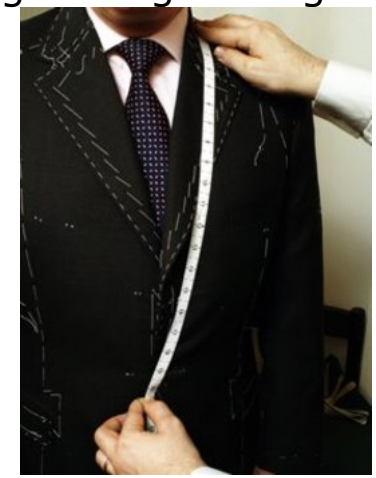
Development of multimodality probes for theranostic aplications

Theranostics: combination of diagnosis and therapy



Personalized medicine/tailored medicine/

Matching the right drug for the right patient



Matched β^+/β^- pairs

- $^{64}Cu/^{67}Cu$
- $^{86}Y/^{90}Y$
- $^{44}Sc/^{47}Sc$
- $^{124}I/^{123/131}I$

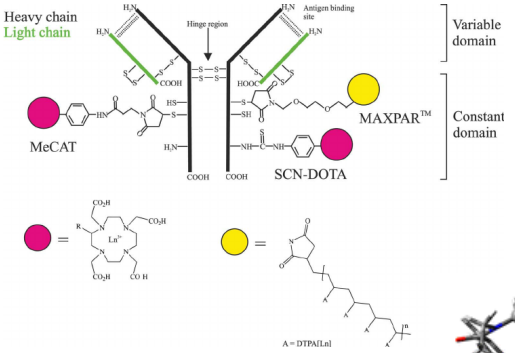
The „twin" isotope of the same element can be used for diagnostic imaging or therapy follow up, while the other is used for therapy using the same carrier molecules.

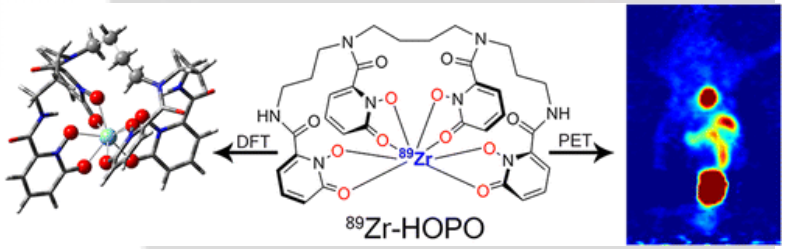
Matched Radionuclide Pairs for Imaging and Therapy (edited by A. Bockish)
Eur J Nucl Med Mol Imaging, Vol 38, Suppl 1, June 2011

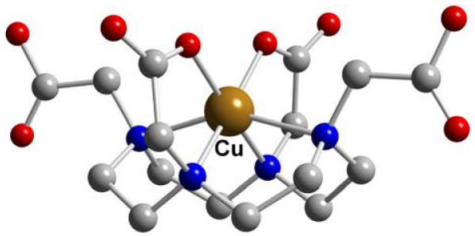
Chelators

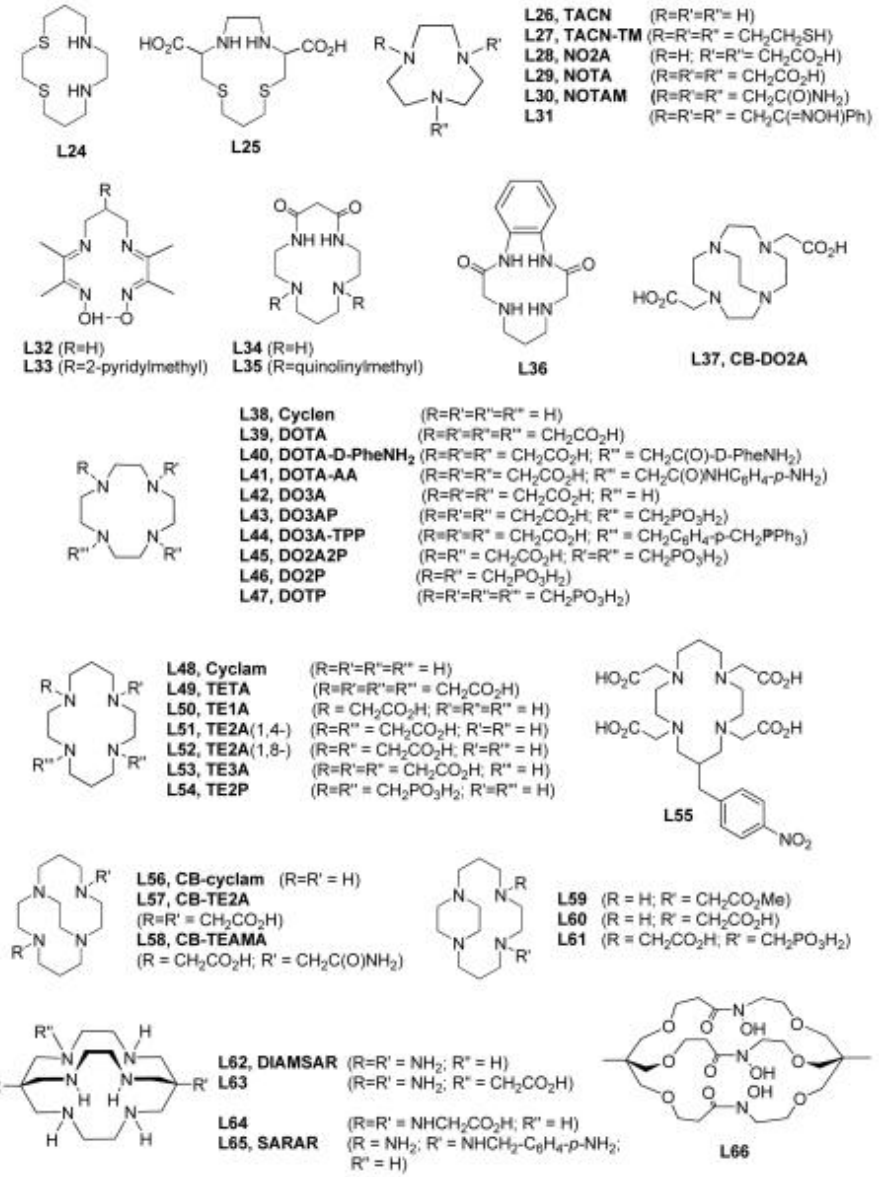
used to bind the a radioisotope molecule

so that when injected into a patient, the targeting molecule can be delivered without any radioisotope loss





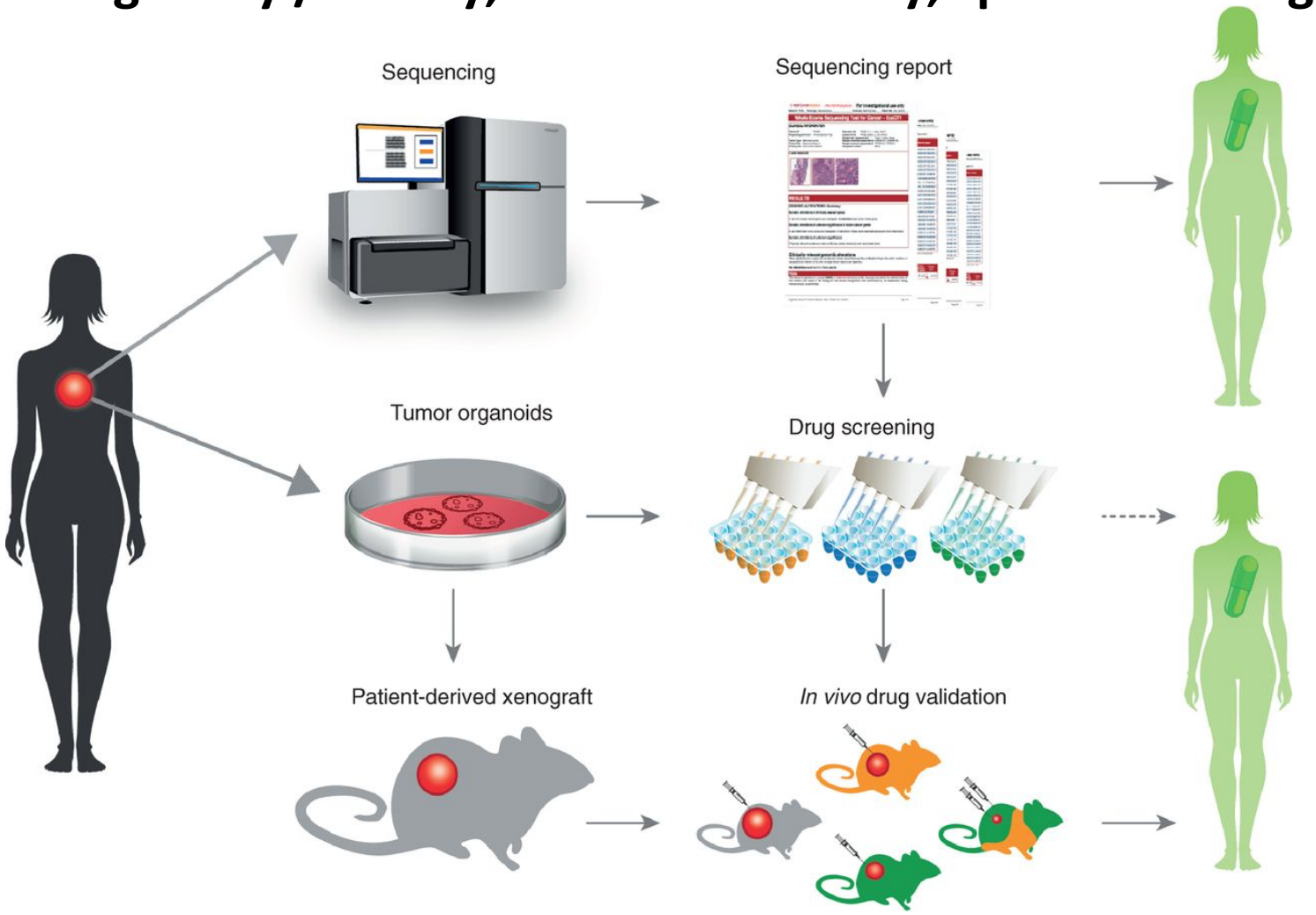




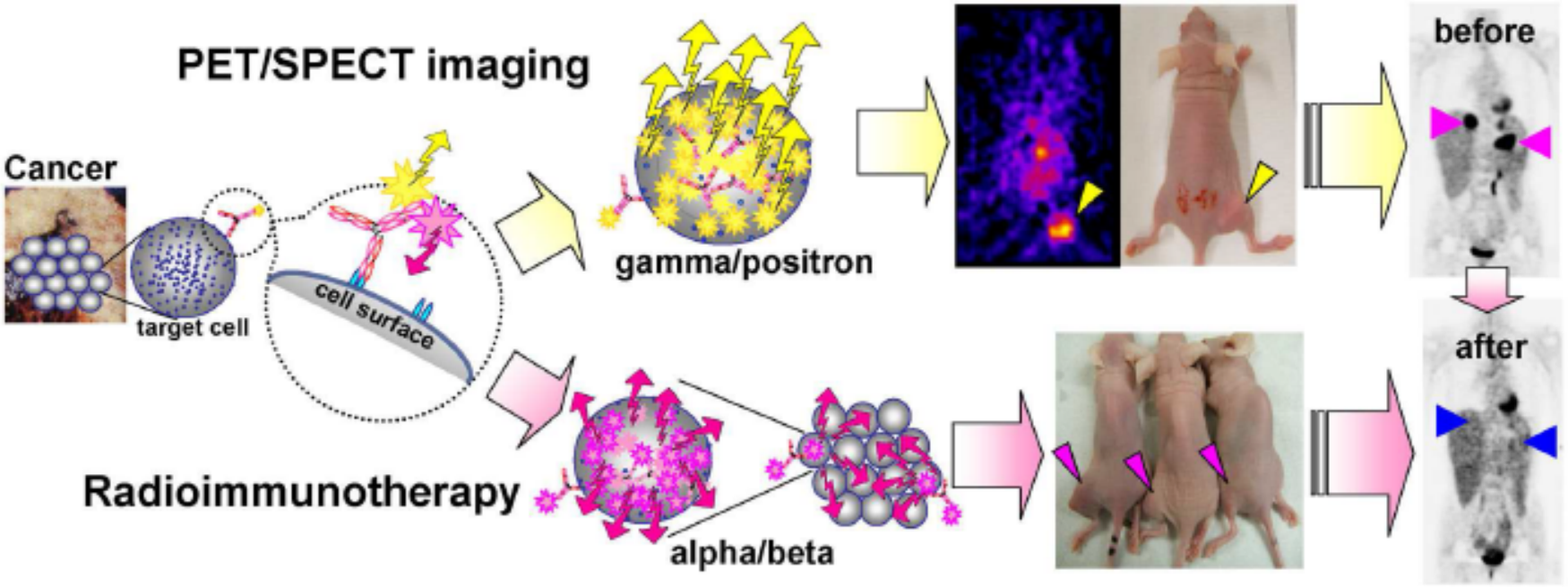
Wadas TJ et al. Chem Rev 2010;110(5):2858-2902

The “right” cells - the good model

In vitro binding study /affinity, immunoreactivity, specific binding



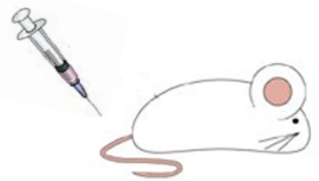
BASIC / PRECLINICAL RESEARCH



- Study of mechanisms of disease development and progression
- Detection and activity of receptors and pathways Pharmacokinetics / pharmacodynamics of target drugs

In vivo study in Balb/c mice or Wistar rat

Injection of
Radioisotope/ ligand



Biodistribution

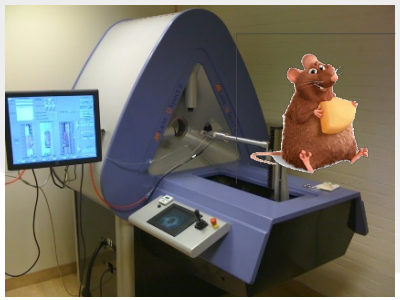
4 h
24h
48h
72h

In vivo studies in tumor bearing mice

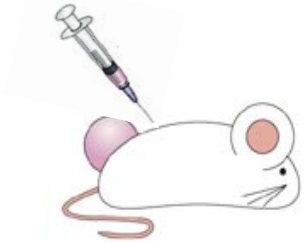
Tumor cell culture

Mice subcutaneously
xenografted with 2 mln cells

2–3 weeks



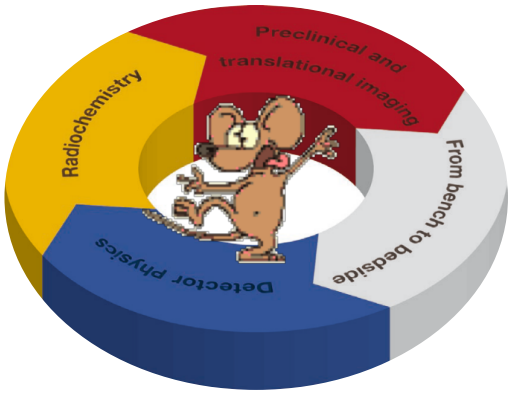
Injection of
Radiolabeled ligand



Animal scanner
or Optical Imaging

Biodistribution

4 h
24h
48h
72h



Animal model in preclinical studies

Animals have long been used as subject in laboratory experiments, as they were considered viable alternatives of the use of humans.

Of Mice and Humans: Are They the Same?

Challenge – to identify an animal model that is comparable to humans

- **The shift to applied research**
- **Medicalization of research**
- **Technical development**
- **Linkage**

Problems:

- **The design** (small/larger groups) of animal studies, the experimental execution, **the evaluation of the data** are under purview of one, no masked person – elucidated certain weakness, lack of masking and limit the translation value to human application.

- **Choice of the animal** – often young, rarely comorbidities, not exposed to the full range interventions that human often received.

- **To select positive animal data**, but to ignore equally valid but negative work when planning clinical trials

- **Reporting experimental design**
  - Masking and randomization
  - Comparison the treatment effects
  - Use systematic review of human and clinical studies

Animal handling and Anesthesia
Why ?

- decrease motion artifact
- decrease pain and stress?

Indications for awake imaging

- avoid influence of anesthesia on: blood flow, metabolism, neural-vascular coupling
- elucidate disease pathophysiology
- drug/radiopharmaceutical development
- mimic the human state

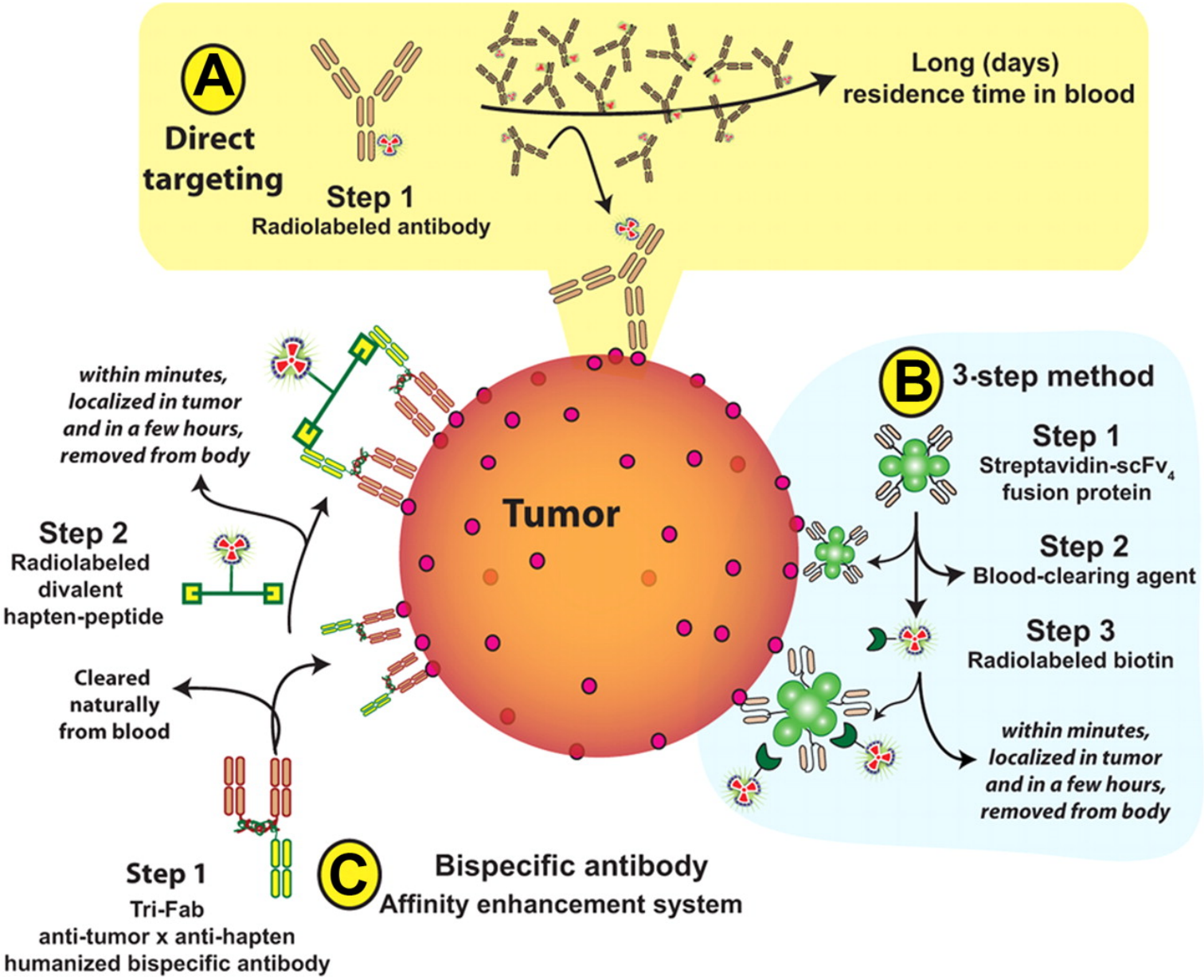
Some like it hot: Radioimmunotherapy

From our experience

David M. Goldenberg
blood 14 MAY 2009 I VOLUME 113, NUMBER 20

Bivalent hapten-bearing peptides designed for iodine-131 pretargeted radioimmunotherapy
E Janevik-Ivanovska, E Gautherot, M Hillairet de Boisferon, M Cohen, ...
Bioconjugate chemistry 8 (4), 526-533

Radiolabeled bivalent haptens for tumor immunodetection and radioimmunotherapy
A Gruaz-Guyon, E Janevik-Ivanovska, O Raguin, C De Labriolle-Vaylet, ...
The Quarterly Journal of Nuclear Medicine and Molecular Imaging 45 (2), 201

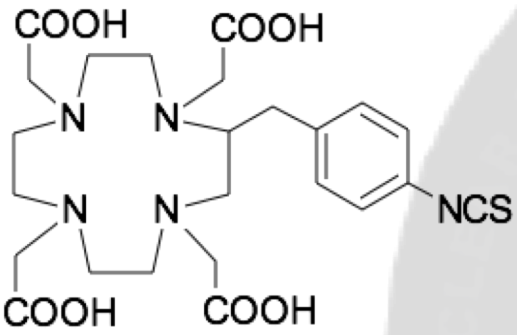


OUR EXPERIENCE:

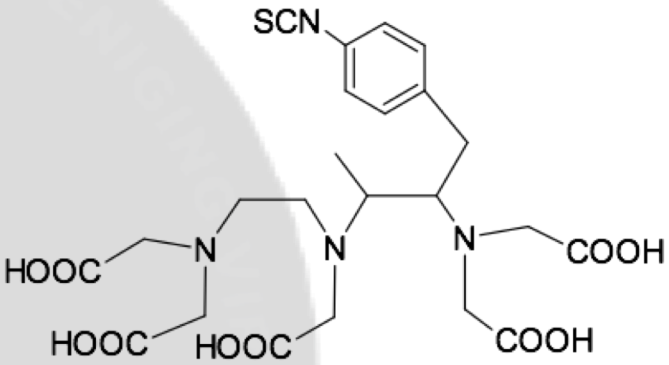
ESTABLISHMENT AND STANDARDIZATION OF A TECHNOLOGY FOR THE PRODUCTION OF READY-TO-USE COLD KIT FORMULATIONS FOR LABELLING OF CONJUGATED ANTIBODIES WITH Lu-177

Rituximab:BFCAs
1:10
1:20
1:50

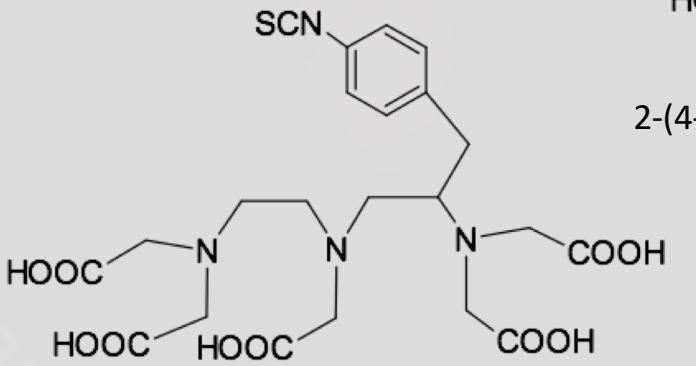
Rituximab:BFCAs
1:20



***p*-SCN-Bn-DOTA**
Para isothiocyanatobenzyl derivate - DOTA



1B4M-DTPA
2-(4-isothiocyanatobenzyl)-6-methyldiethylene - DTPA



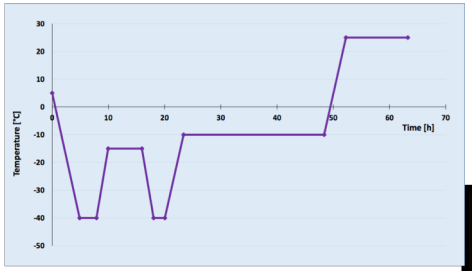
***p*-SCN-Bn-DTPA**
Para isothiocyanatobenzyl derivate of DTPA

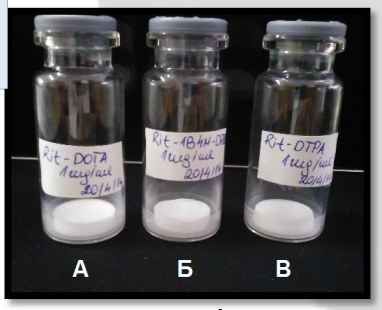
Bifunctional chelating agents

Part of the IAEA's Coordinated Research Project (CRP)

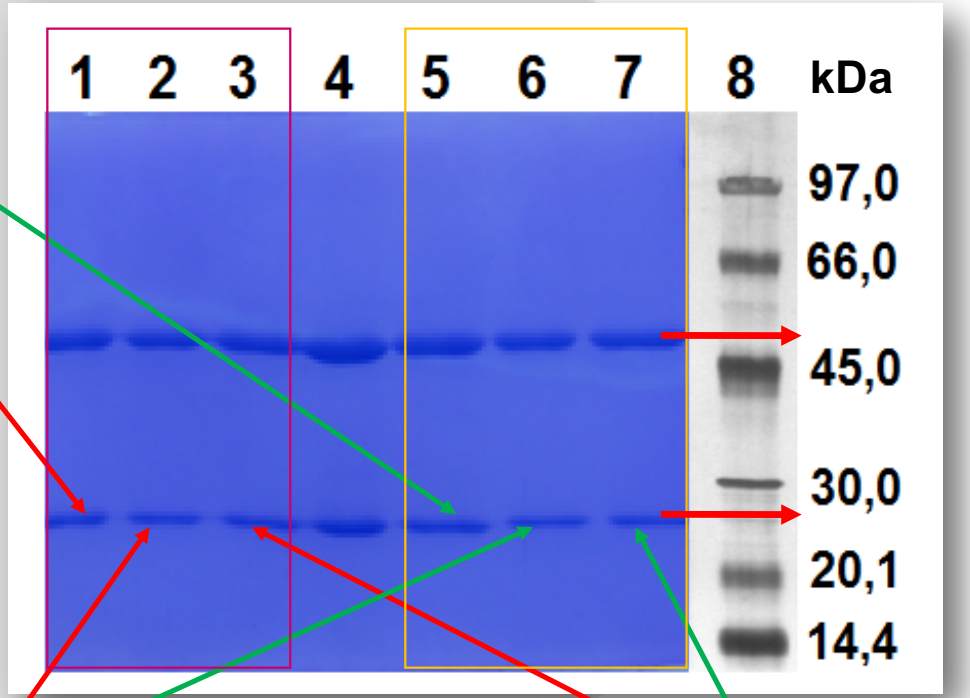
SDS-PAGE electrophoresis of immunoconjugate

Rituximab-DTPA-SCN





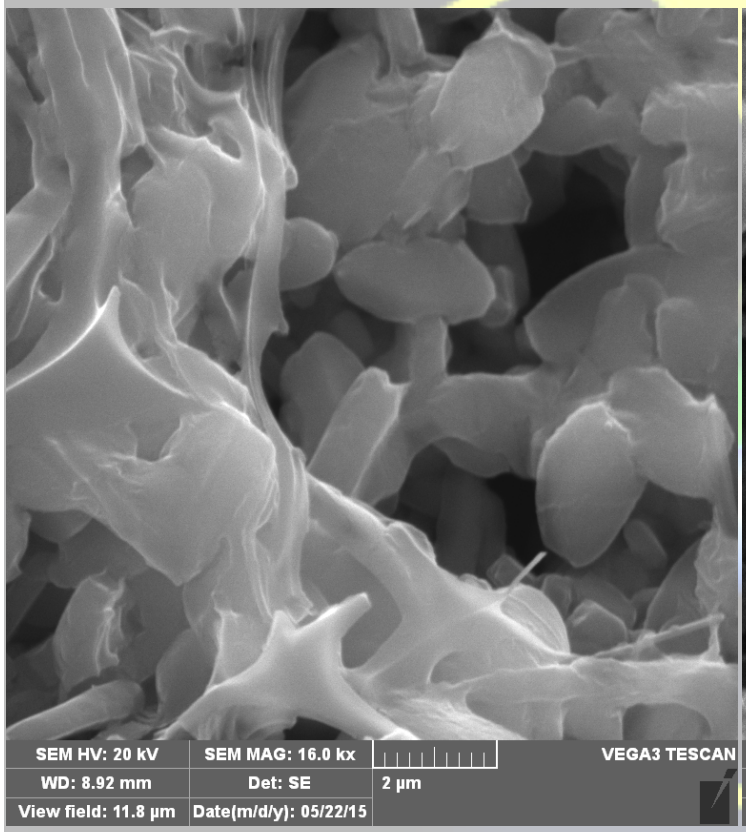
Rituximab -DOTA-SCN



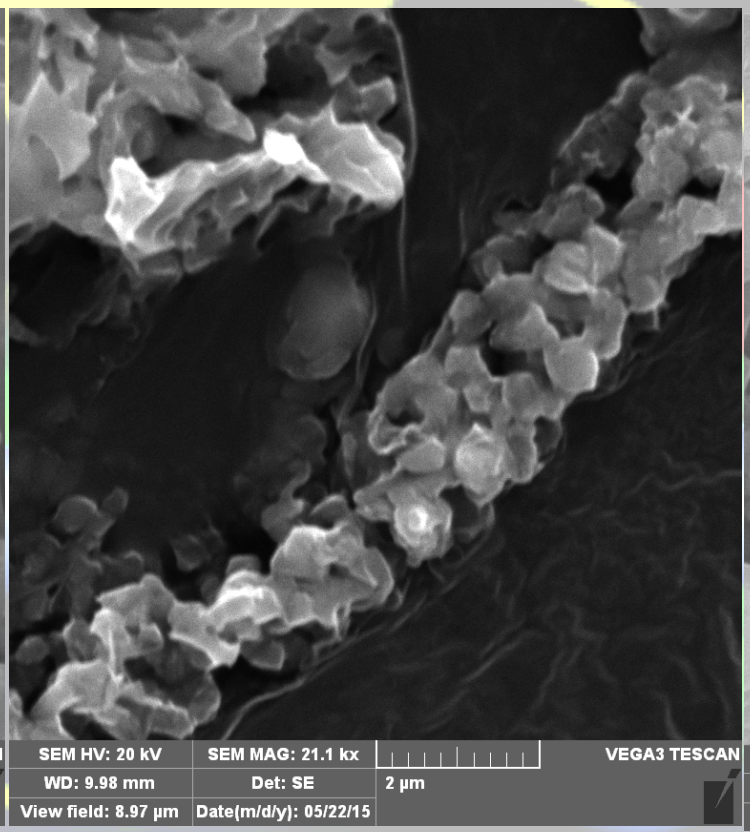
Rituximab -DTPA-1B4M

Scanning electronic microscopy of freeze drying Rituximab conjugate 2 μm

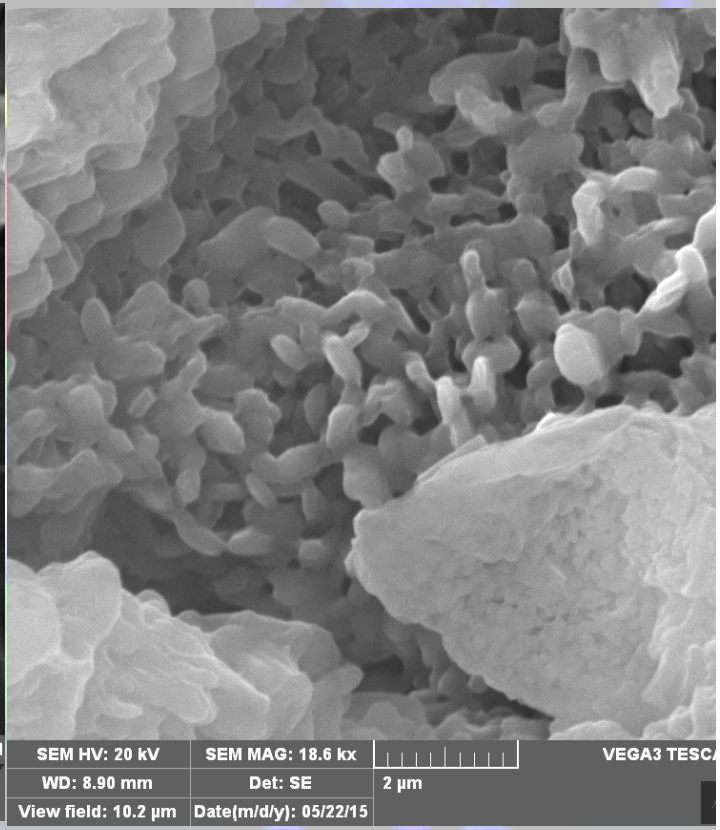
***p*-SCN-Bn-DOTA**



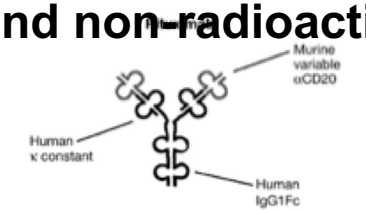
***p*-SCN-Bn-DTPA**



1B4M-DTPA



Schematic process of conjugation and non-radioactive labeling of three BFCA-rituximab



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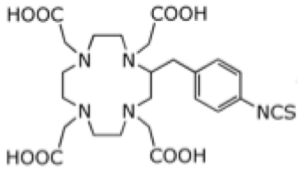
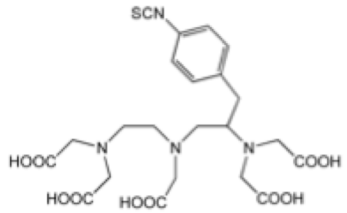
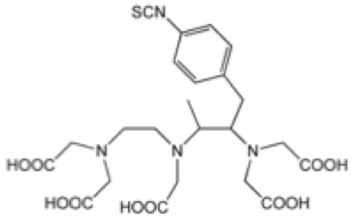


p-SCN-Bn-DOTA [2-(4-Isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-tetraacetic acid]



p-SCN-Bn-DTPA [2-(4-Isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid]



1B4M-DTPA [2-(4-isothiocyanatobenzyl)-6-methyl-diethylenetriaminepentaacetic acid]

freeze-dried preparation

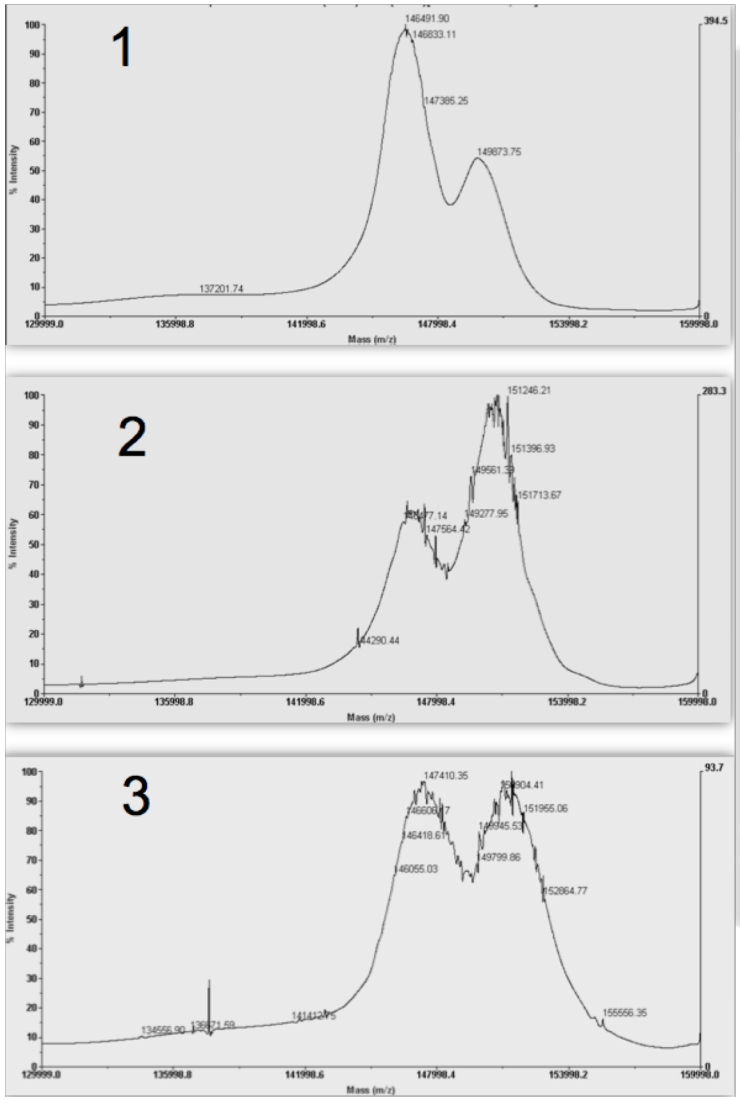
dissolved with sterile saline, and labelled with 1.0709 µg Lu chloride

Incubation

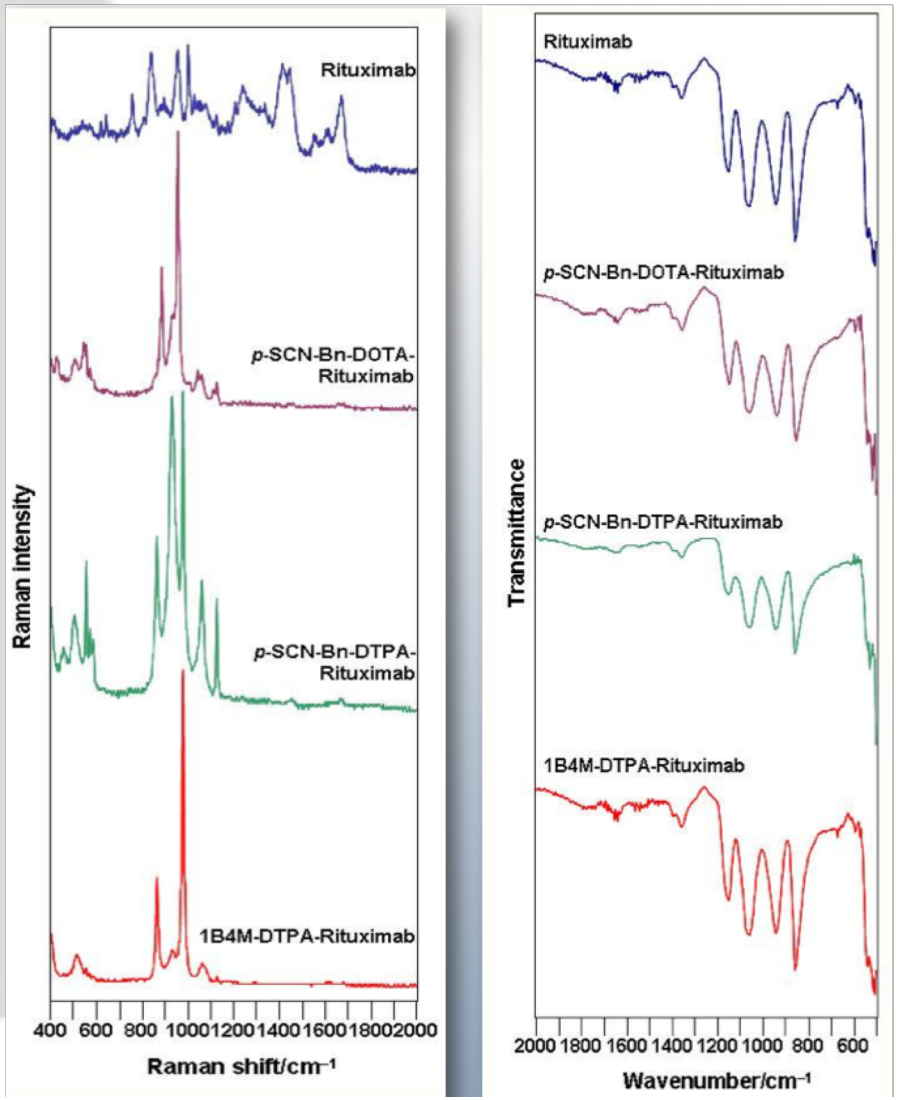
- 30 min at room temperature (p-SCN-Bn-DTPA-rituximab and 1B4M-DTPA-rituximab)
- 60 min at 40°C (p-SCN-Bn-DOTA-rituximab)

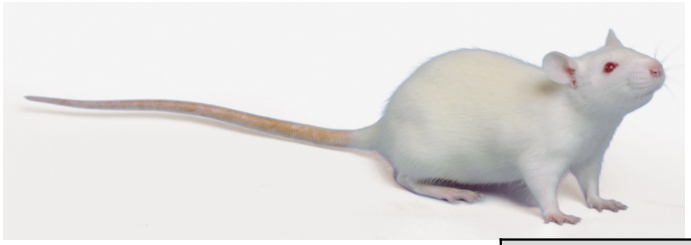
MALDI-TOF results for three BFCA-rituximab

(1: p-SCN-Bn-DOTA; 2: p- SCN-Bn-DTPA and 3: 1B4M-DTPA)



Raman and ATR-IR spectra of rituximab -conjugates

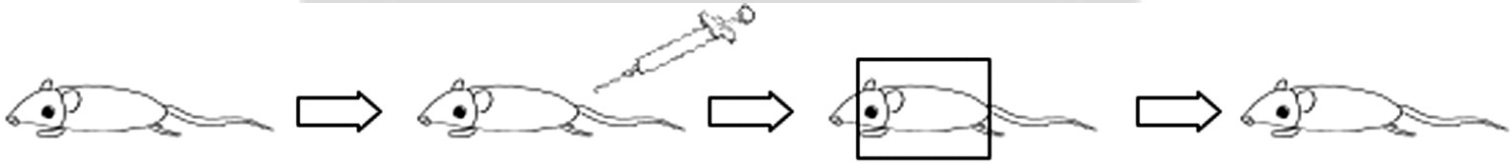




Animal studies with not radioactive Lu/Y-labeled imunoconjugate of Rituximab for determination of normal tissue toxicity

Group	RBC	WBC	PLT
	Normal values		
	7,21 – 8,45 x10^{12}/L	7,2 – 12,6 x10^{9}/L	250 – 1200 x10^{9}/L
Average value from group I (Lu-DOTA-Rituximab)	3,40	7,8	1496,5
Average value from group II (Lu-DTPA-Rituximab)	3,05	8,06	558
Average value from group III (Lu-1B4M-DTPA-Rituximab)	3,69	9,375	984
Average value from group IV (Y-DOTA-Rituximab)	2,83	10,74	770,66
Average value from group V (Y-DTPA-Rituximab)	3,288	9,66	1802
Average value from group VI (Y-1B4M-DTPA-Rituximab)	3,575	9,10	1332,5

*** Results are average from the values after 3 weeks aplication**

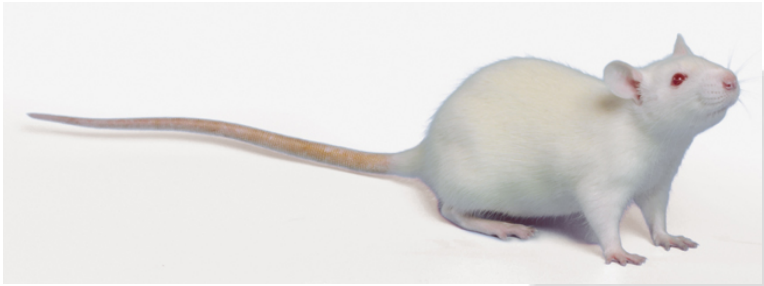


Blood sample
(6 groups x 5 animal)

Rituximab conjugatет
(3 weeks)

Blood sample
(every week)

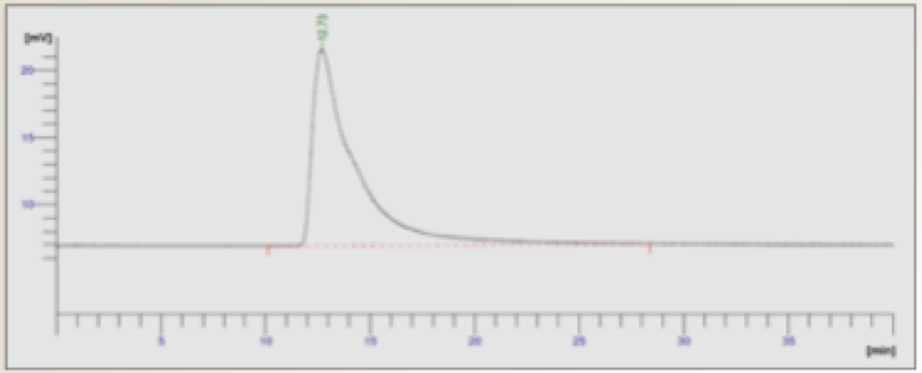
Blood sample + liver an spleen sample for immunochemistry (week 4)



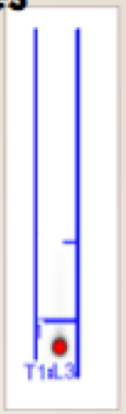
Animal studies with not radioactive Lu/Y-labeled imunoconjugate of Rituximab for determination of normal tissue toxicity after 4 weeks from the last treatment

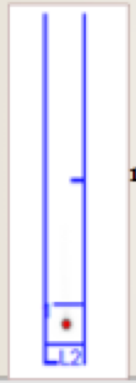
Group	RBC	WBC	PLT
	Нормални вредности		
	7,21 – 8,45 $x10^{12}/L$	7,2 – 12,6 $x10^{9}/L$	250 – 1200 $x10^{9}/L$
Average value from group I (Lu-DOTA-Rituximab)	**6,90**	**6,50**	652
Average value from group II (Lu-DTPA-Rituximab)	**5,39**	**1,80**	**71**
Average value from group III (Lu-1B4M-DTPA-Rituximab)	**7,05**	**3,80**	963
Average value from group IV (Y-DOTA-Rituximab)	**4,21**	**0,70**	**30**
Average value from group V (Y-DTPA-Rituximab)	**6,80**	**7,10**	824
Average value from group VI (Y-1B4M-DTPA-Rituximab)	**6,98**	**3,30**	1075

Radiolabeling with ^{177}Lu after reconstitution of lyophilisates



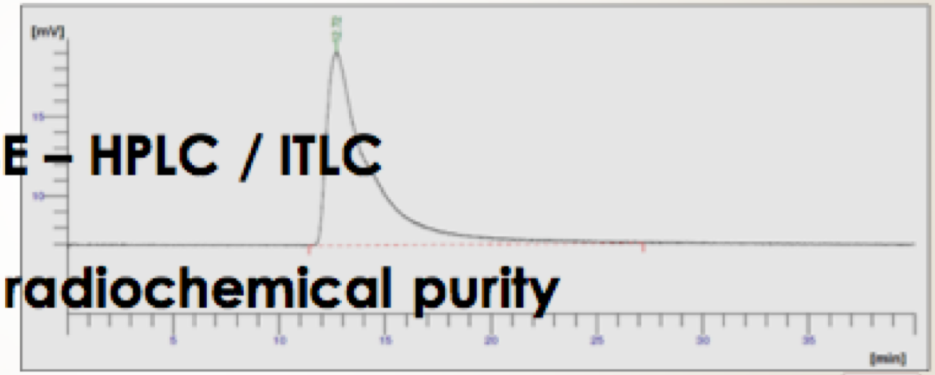
^{177}Lu-DOTA-rituximab



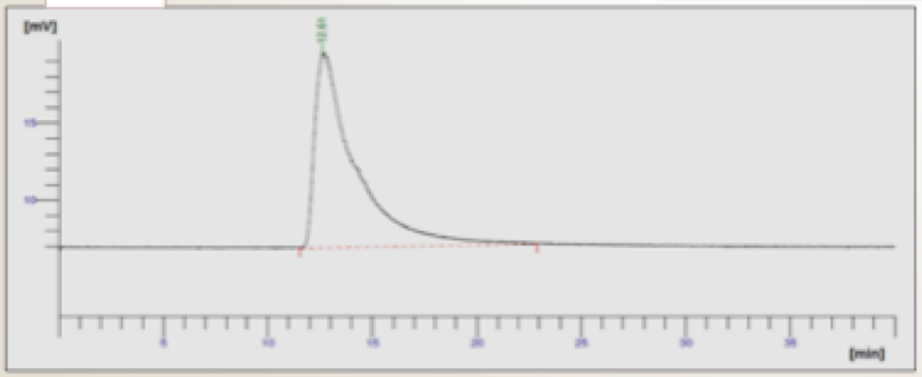


^{177}Lu-DTPA-rituximab

SE – HPLC / ITLC



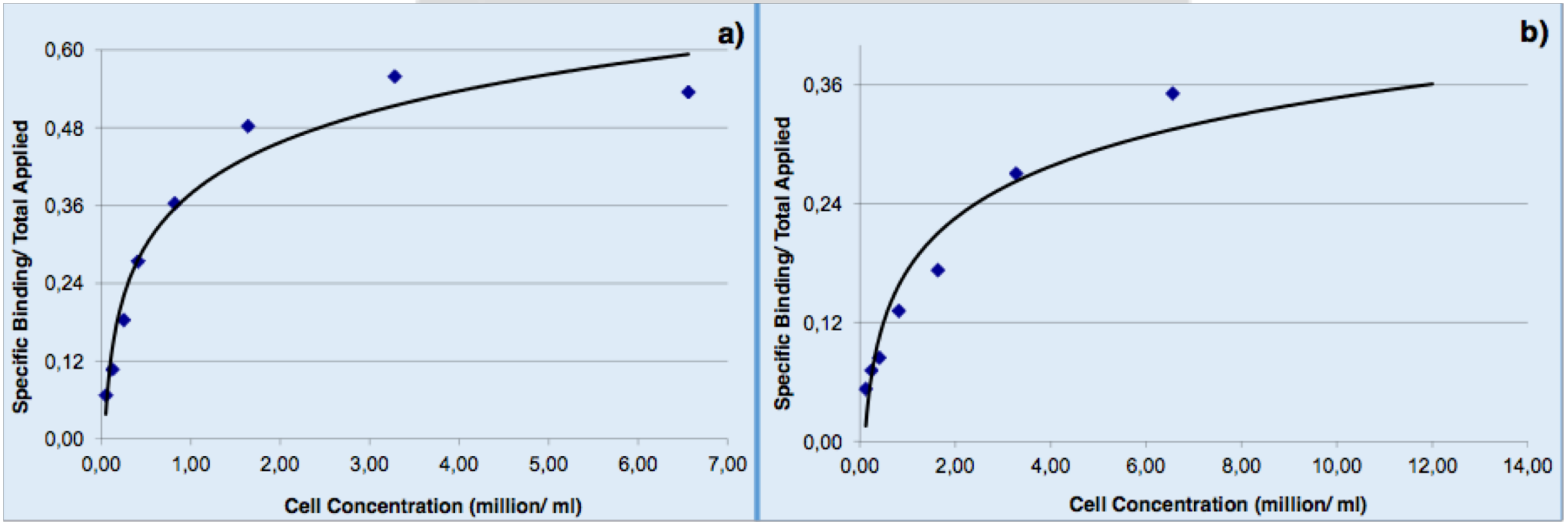
- **High radiochemical purity**



^{177}Lu-1B4M-DTPA-rituximab

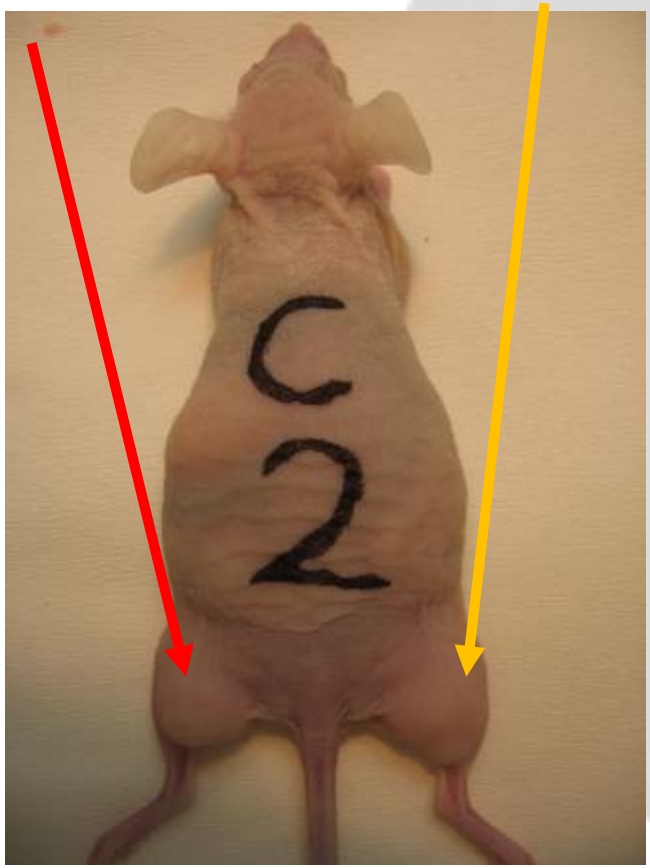
Specific binding over total applied radioactivity as a function of cell concentration in:

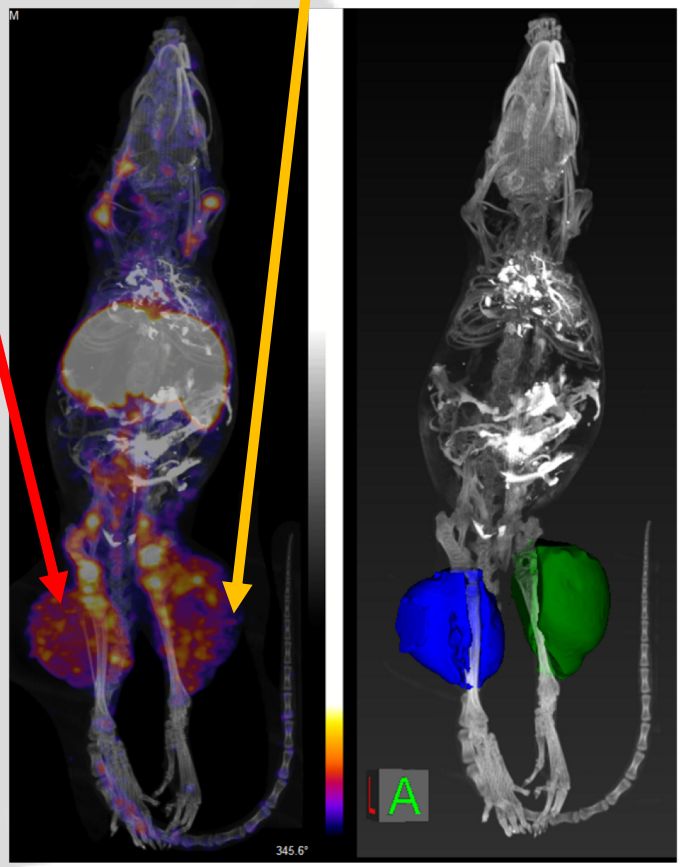
a) ^{177}Lu-DOTA-rituximab and **b) ^{177}Lu-DTPA-rituximab.**



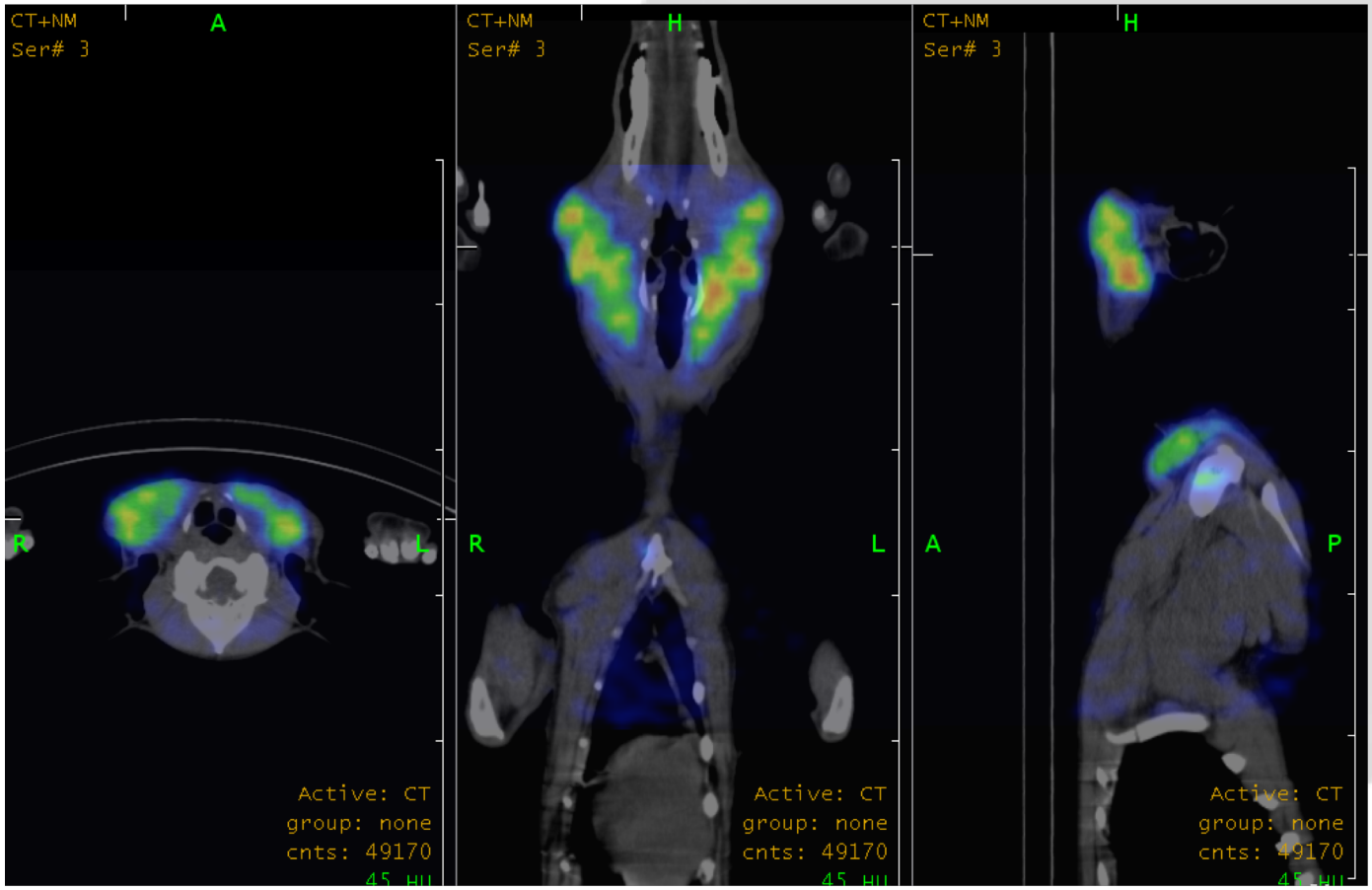
Animal studies - Double xenografts in Nude mice

specific vs non-specific uptake





Patient – Dog with B limphoma




УНИВЕРЗИТЕТ „ГОЦЕ ДЕЛЧЕВ"
ШТИП
THANK YOU