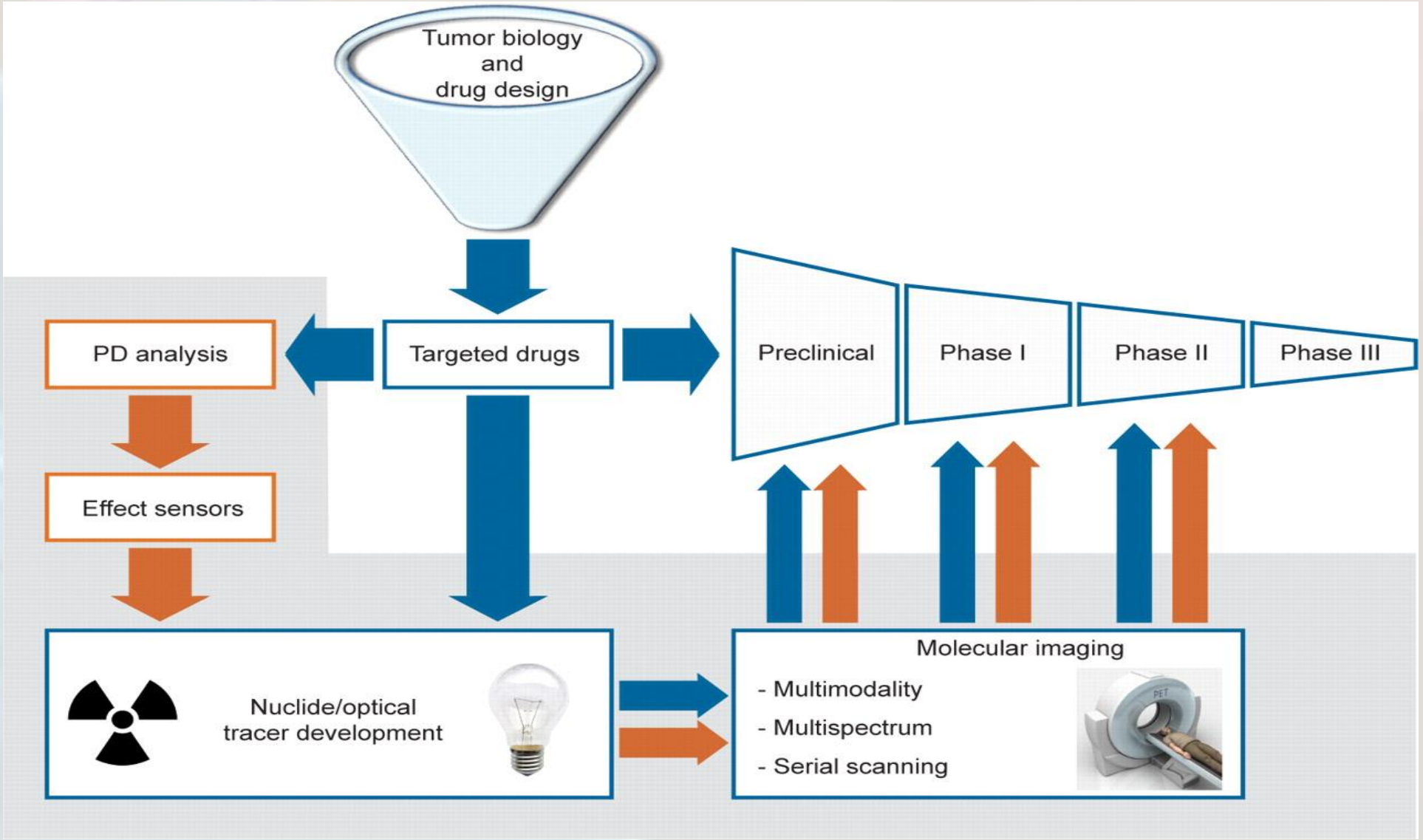
INTERNATIONAL SYMPOSIUM AT FACULTY OF MEDICAL SCIENCES
"Current achievements and future perspectives in medical and biomedical research"
November 24, 2015 - Stip

Drug development based on radiolabeled antibodies

- **Emilija Janevik**
- University Goce Delcev Stip
- Republic of Macedonia

Flow chart of molecular imaging in drug development.



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

First labelling …. For diagnosis

Schwarz, A. and Steinstrasser, A. (1987), A novel approach to Tc-99m-labeled monoclonal antibodies [abstract]. *J. Nucl. Med*., **28**, 721.

Figure 4 - Static images obtained with a gamma camera of the normal mice (up) and with tumor (down) after 24 hours post injection iv of the antibody ior egf/r3 labeled with 99mTc. Tumor (T), thyroid (Ty) and liver (L).

Radiopharmaceuticals in diagnostics and therapy

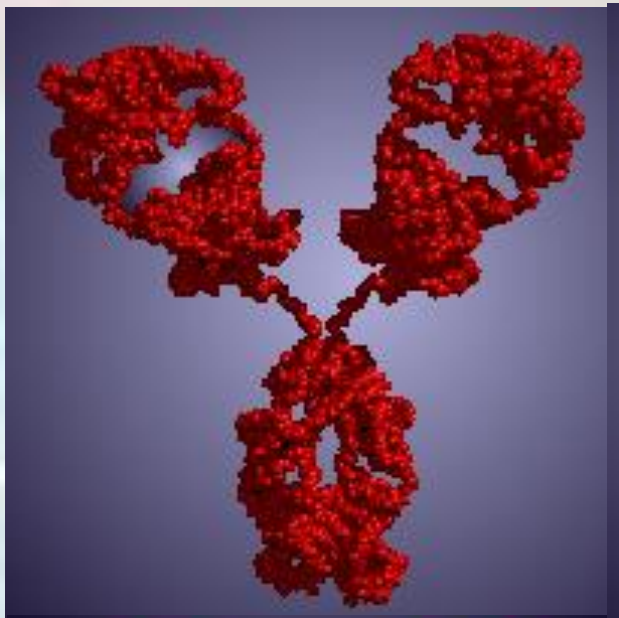
- **Radioimmunotherapy of cancer**
- **Radioimmunoscintigraphy**
- **Pretargeted imaging of cancer**
- Peptide receptor radionuclide imaging
- Peptide receptor radionuclide therapy

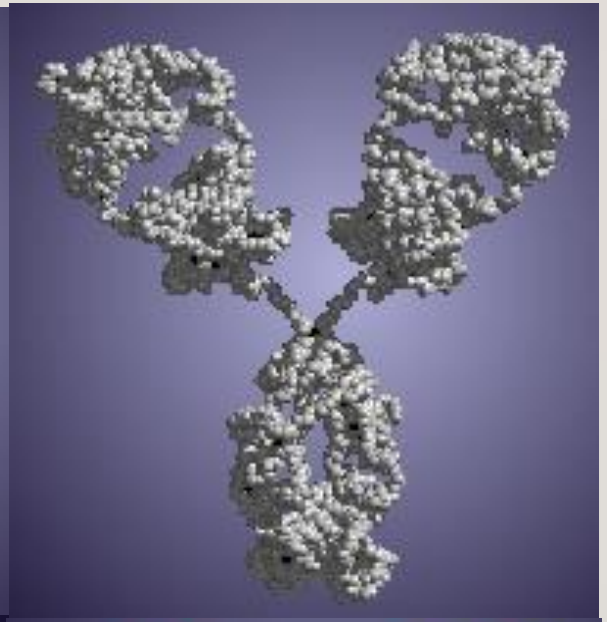
Established Therapeutic Radionuclides Pursued

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

Evolution of Monoclonal Antibodies

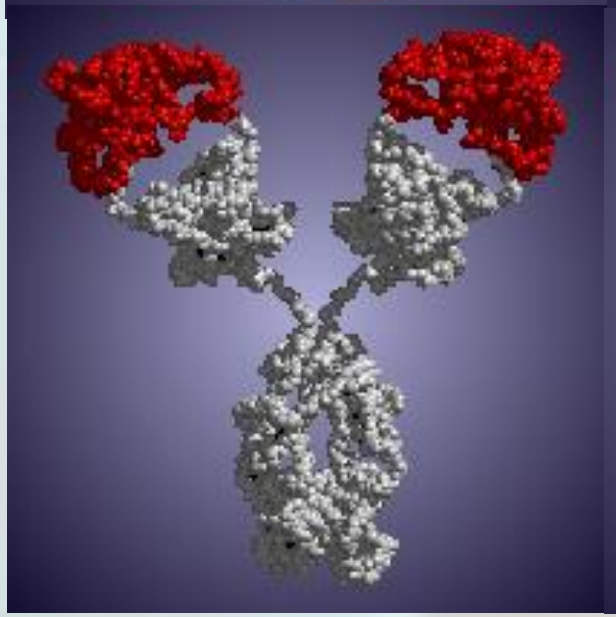
Mouse





Human

Chimeric

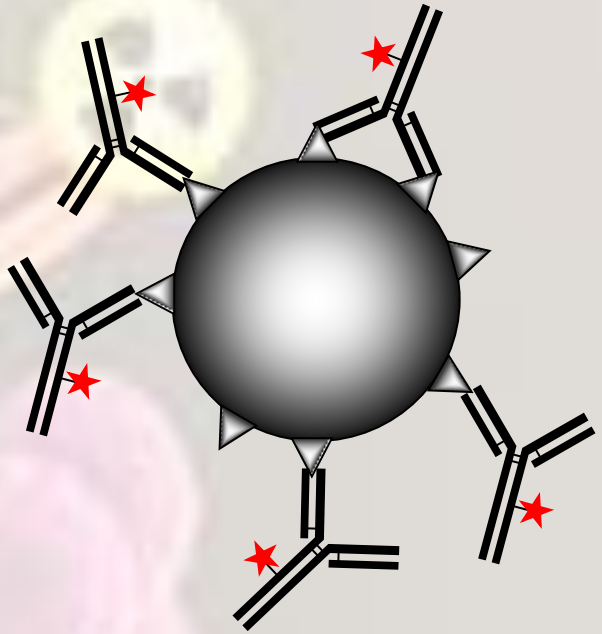


Humanized

Over houndred different types of antibodies – potential for therapy, potential for radioimmunotherapy

Antibody targeting of tumors

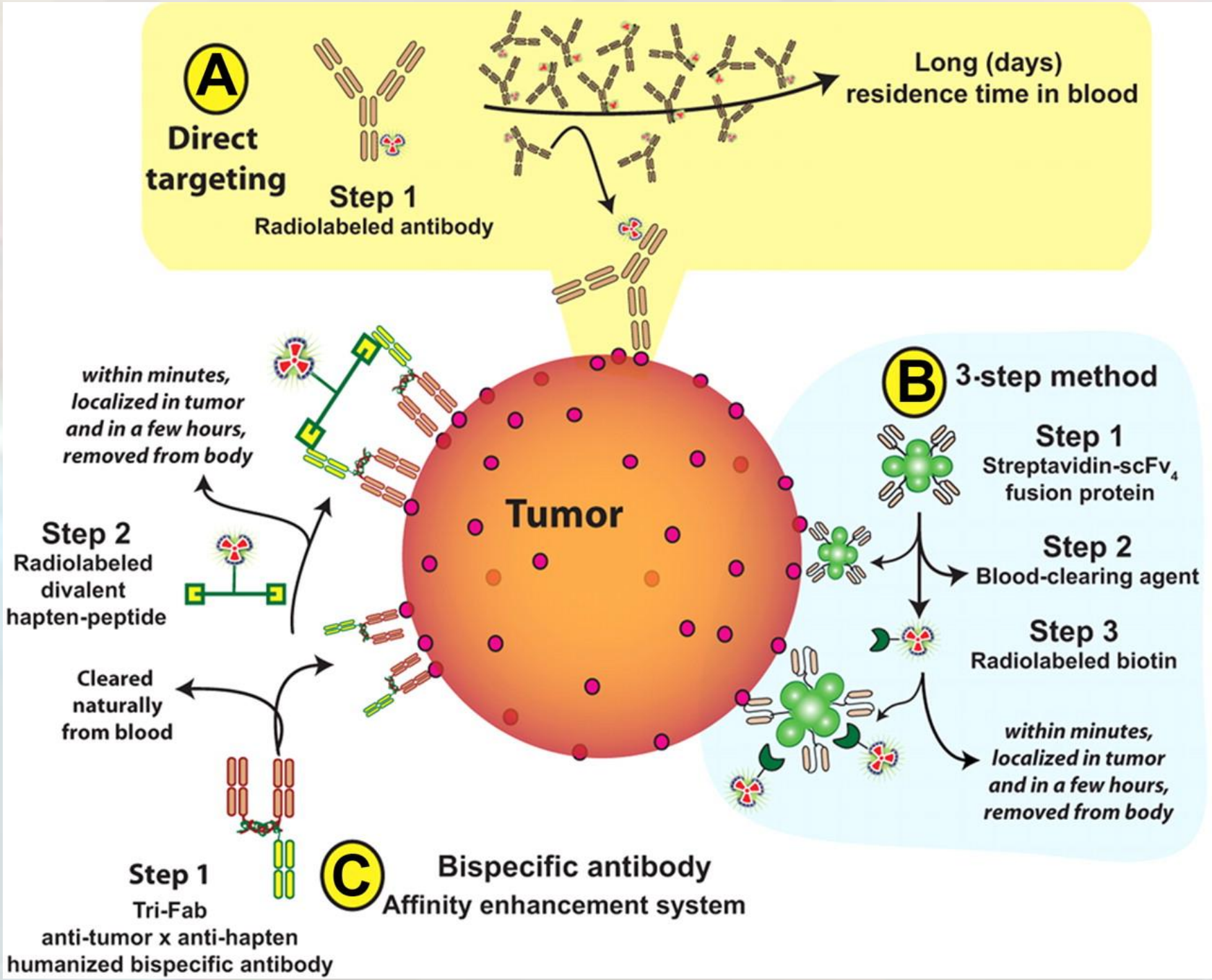
Radiolabeled antibody



Some like it hot: radioimmunotherapy

David M. Goldenberg

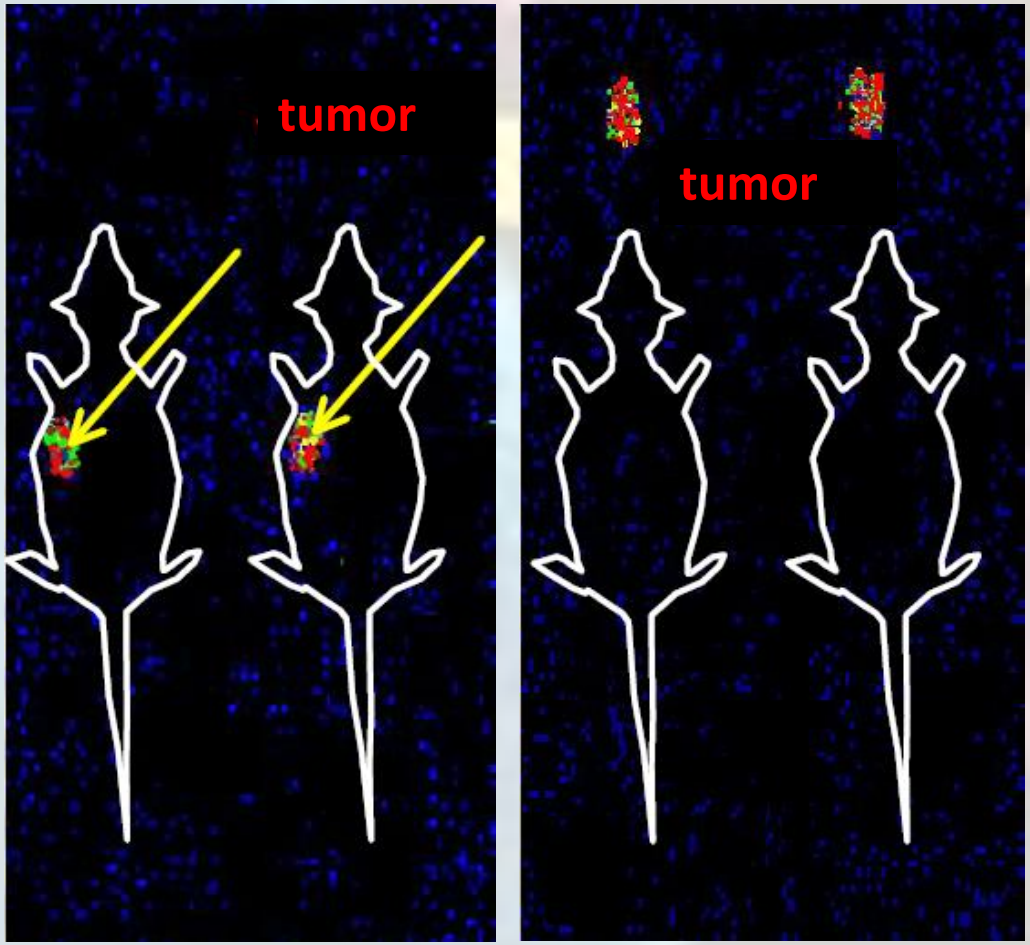
lood 14 MAY 2009 I VOLUME 113, NUMBER 20

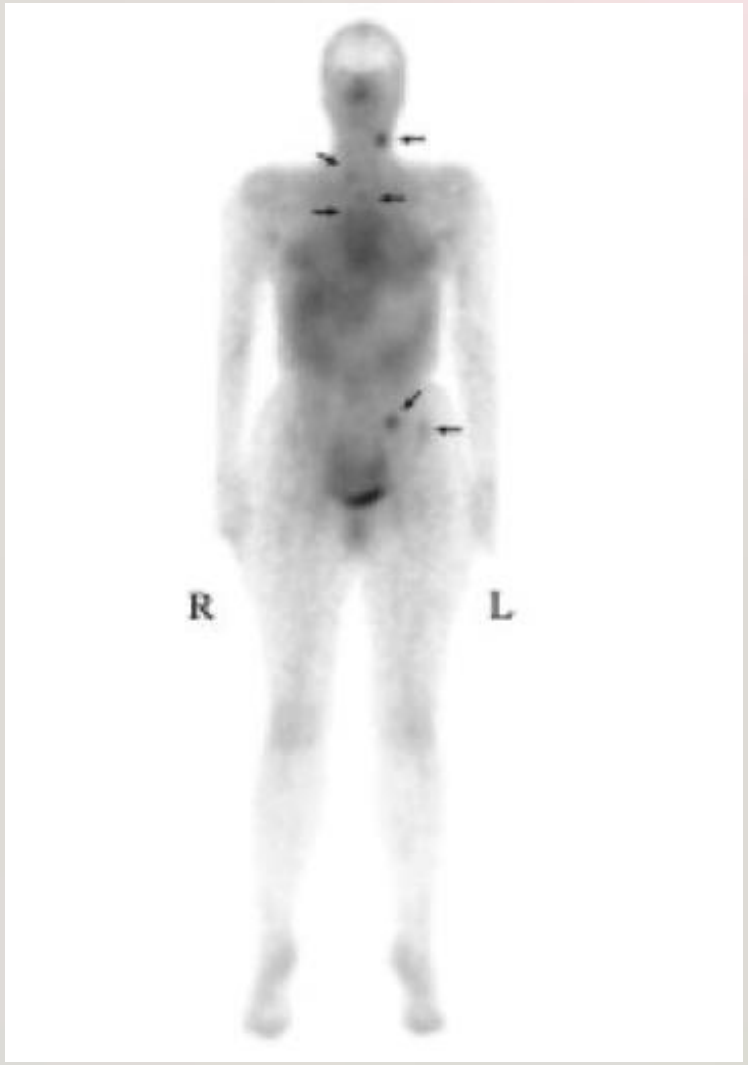


valent hapten-bearing peptides designed for
dine-131 pretargeted radioimmunotherapy
Janevik-Ivanovska, E Gautherot, M Hillairet de
oisferon, M Cohen, ...
oconjugate chemistry 8 (4), 526-533

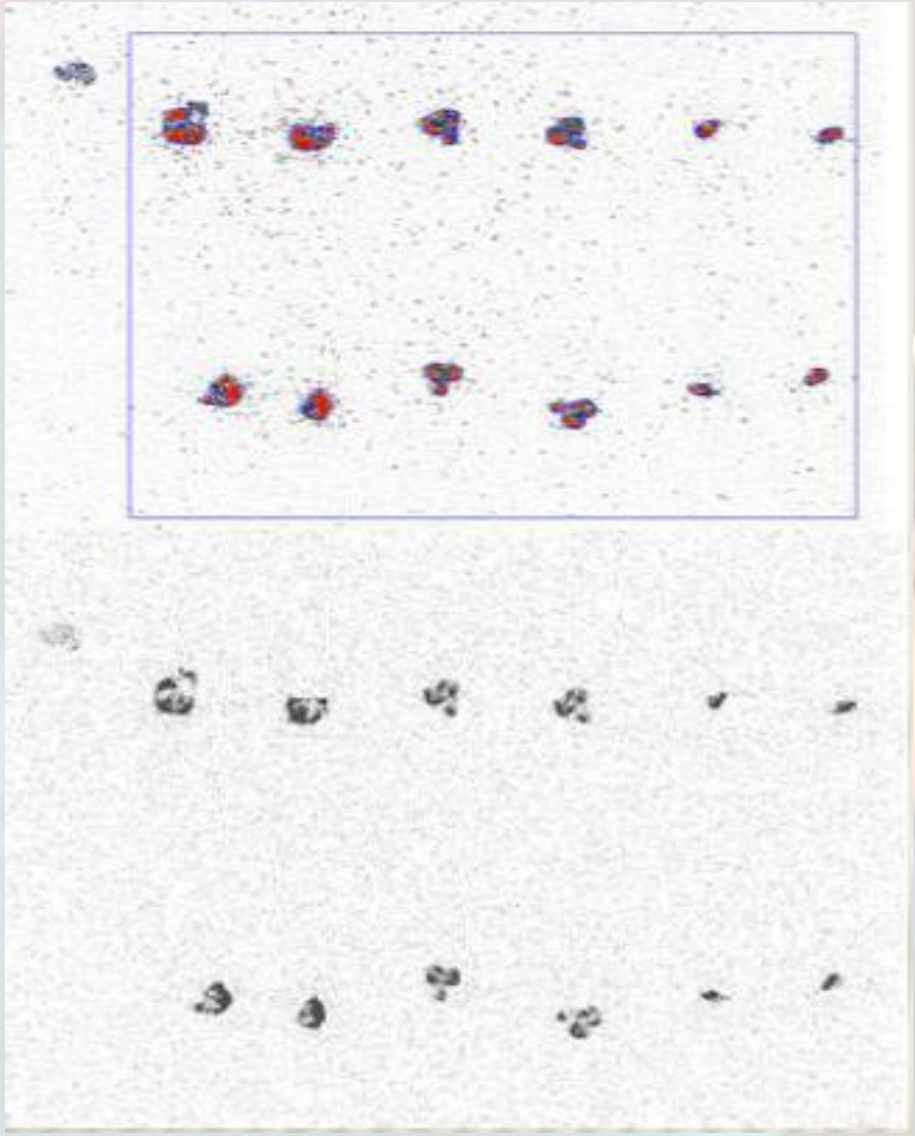
adiolabeled bivalent haptens for tumor
mmunodetection and radioimmunotherapy
Gruaz-Guyon, E Janevik-Ivanovska, O Raguin, C De
abriolle-Vaylet, ...
he Quarterly Journal of Nuclear Medicine and
olecular Imaging 45 (2), 201

Preclinical and clinical application of bispecific antibodies anti-CEA/anti histamine labeled with 99mTc/188 Re :

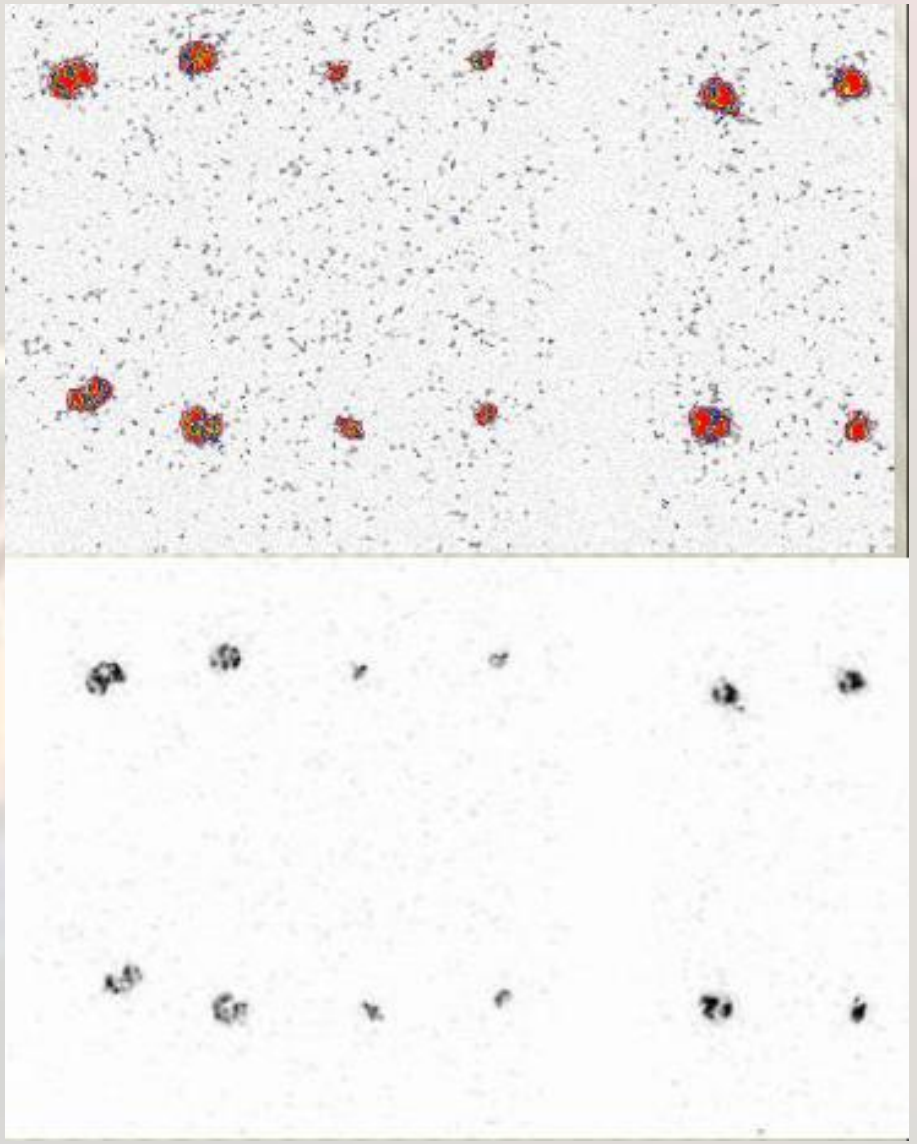




Fusion images - autoradiography / microscopic photo

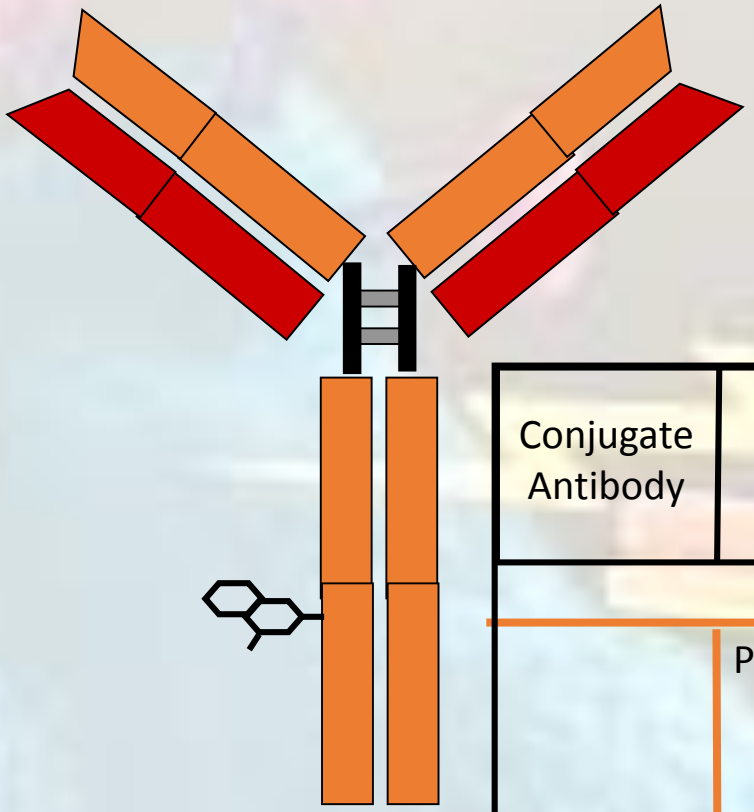


Tumor 3 hour after application



Tumor 3 hour after application

Antibody Conjugate



These changes are not necessarily independent

The vast majority of these occur at miniscule levels or not at all

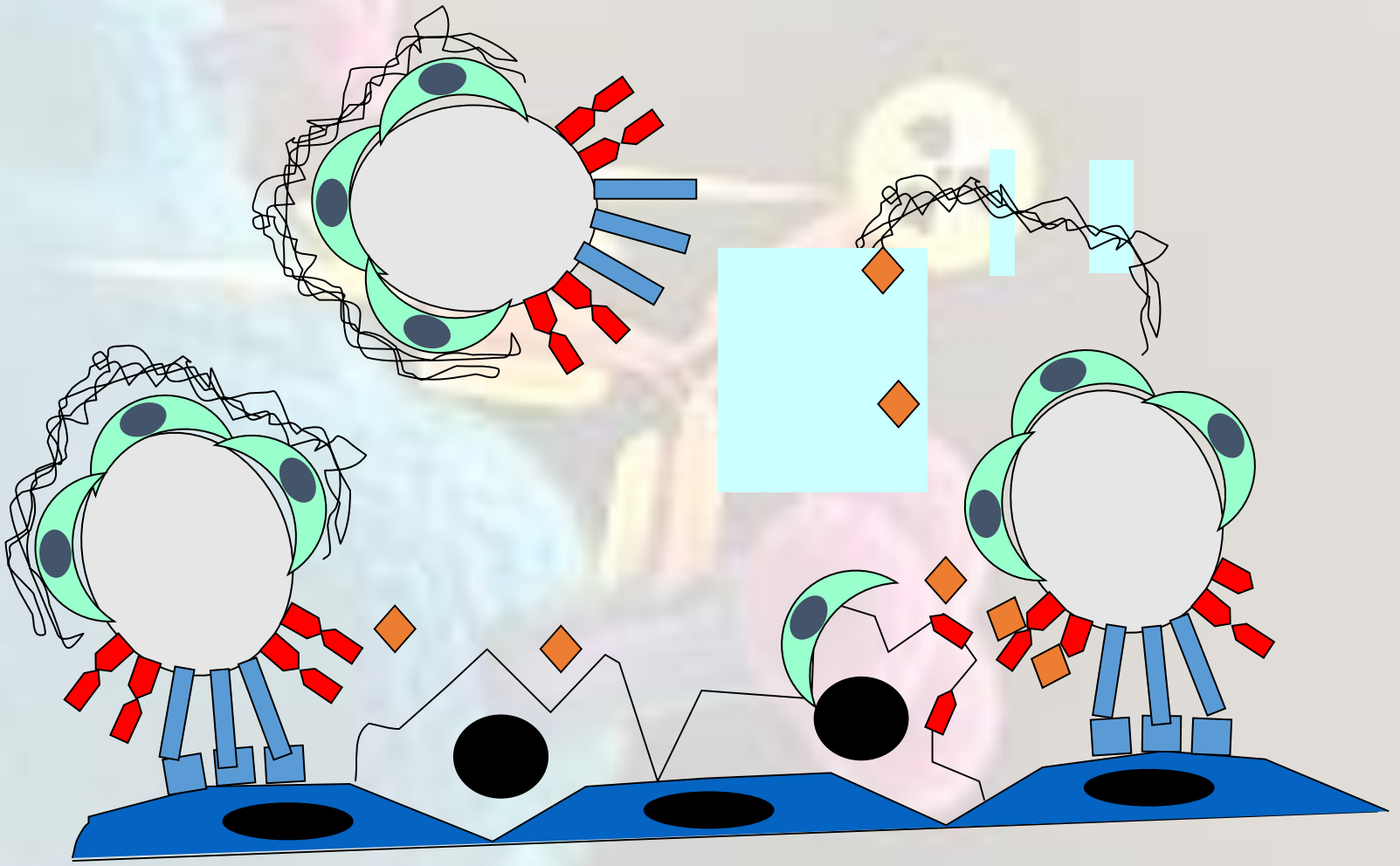
They are generally not characterized in many products

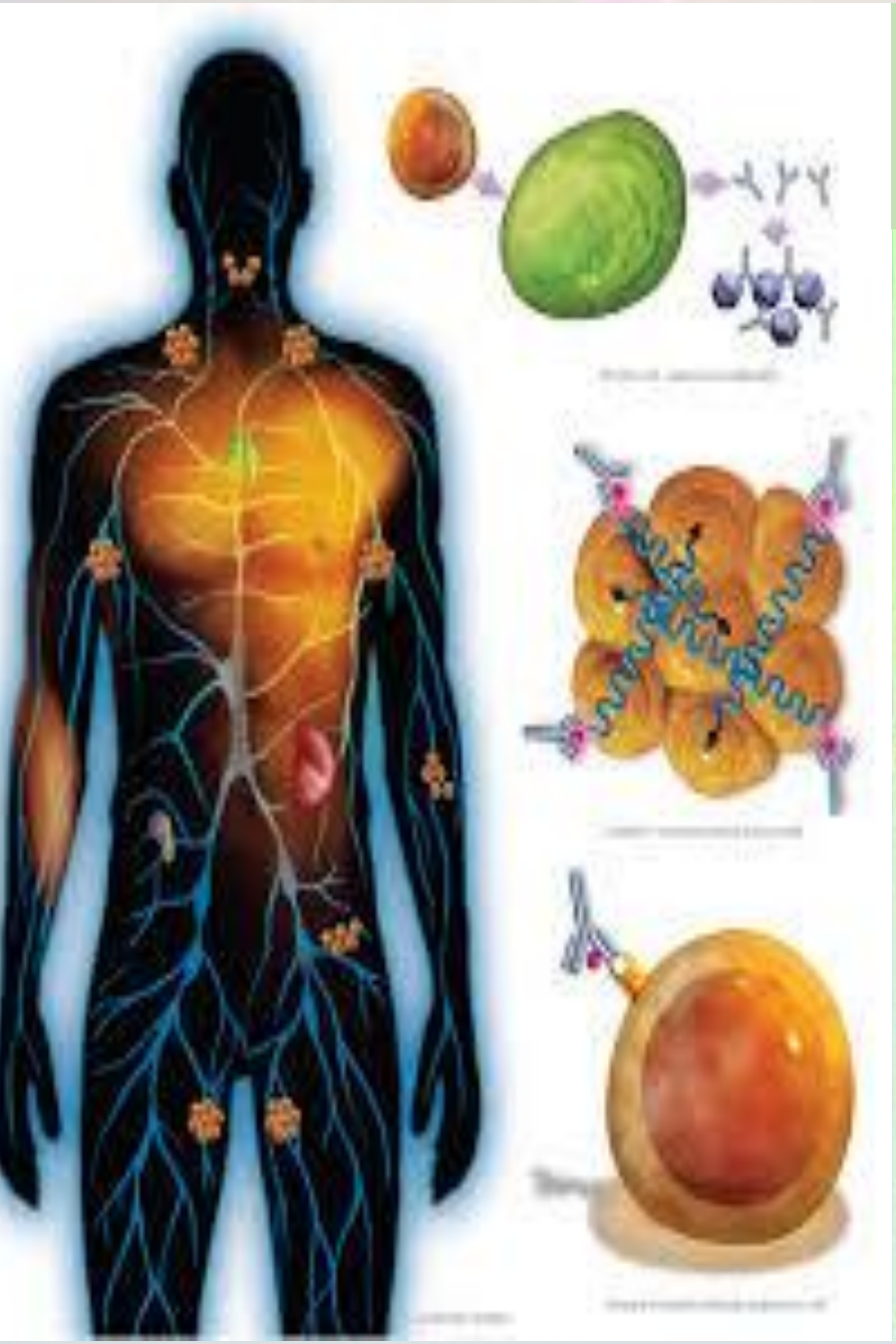
Characterization of structure and heterogeneity is not enough-→

Conjugate Antibody	Linkage OK	MAb OK	Toxin OK	Detect-able	Scen-ario #	End State
					1	Accept.
	P=?	P=?	P=?		2	Batch Fails
				P=?	3	No Efficacy
					4	Batch Fails
				P=?	5	Toxicity
					6	Batch Fails
				P=?	7	Toxicity

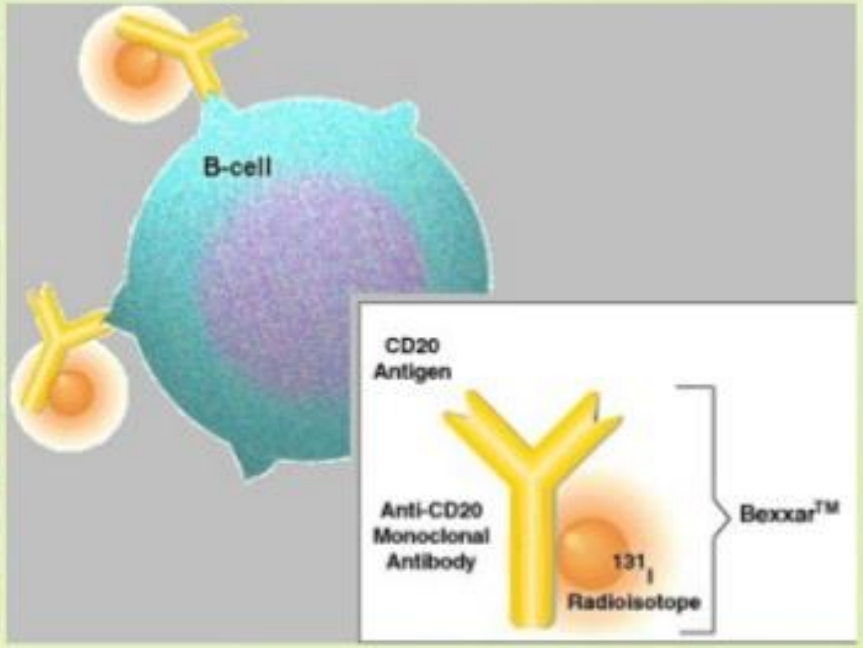
P=?
Probabilistic Risk Assessment

Very Complex Combinations





Commercially available – only TWO!! – TREATMENT OF NHL - hematology

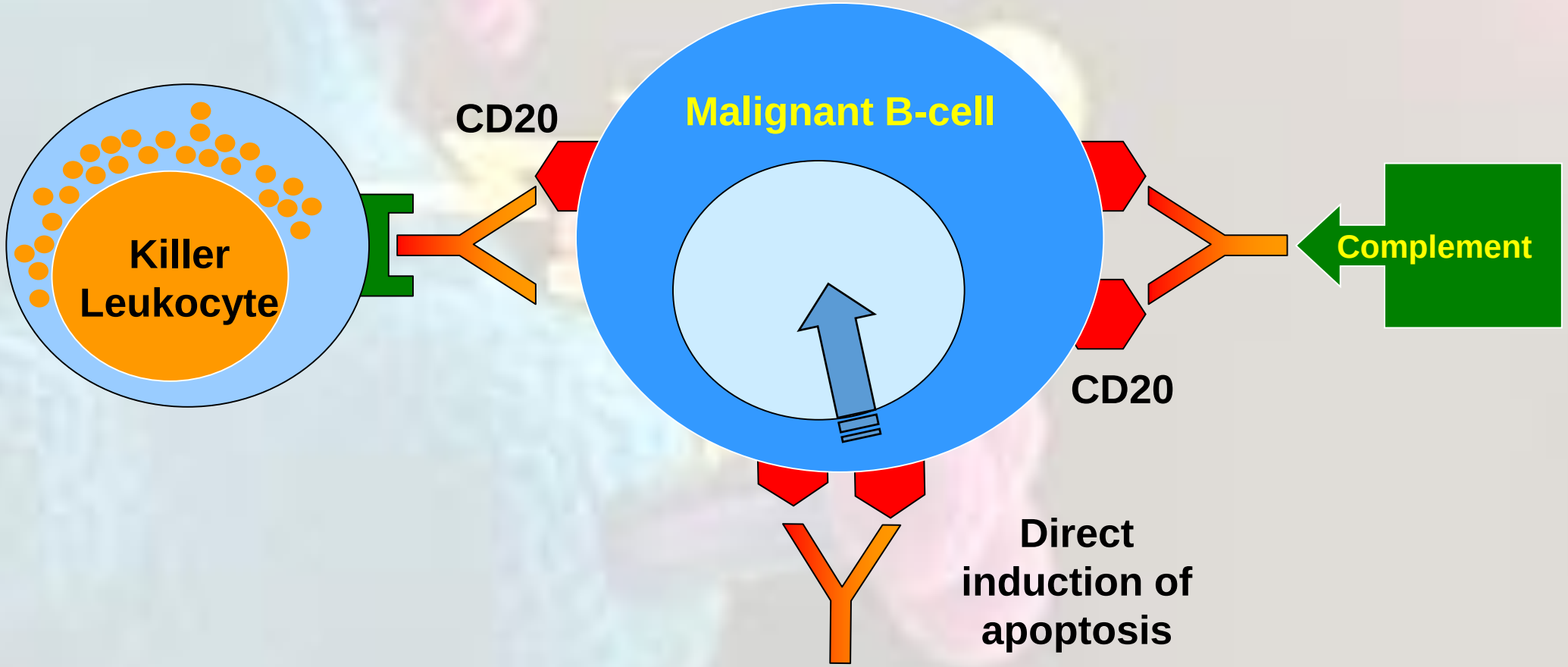


Bexxar – ^{131}I

Zevalin – ^{90}Y

Anti-CD20 (Rituximab= Mabthera®)

mechanism of action



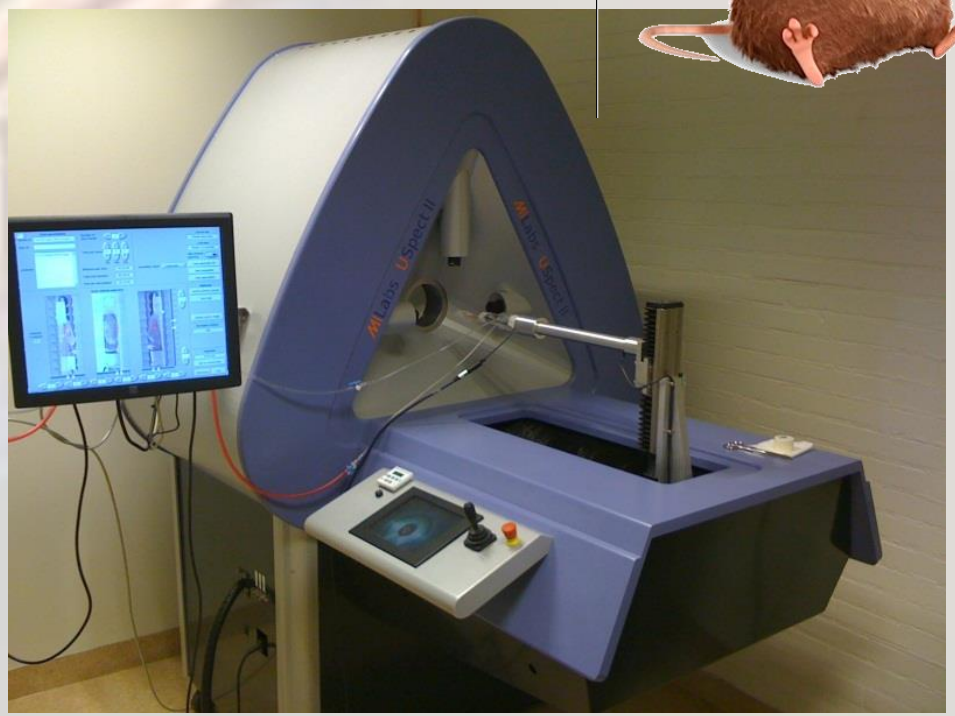
Adapted from Male D, *et al., Advanced Immunology* 1996: 1.1–1.16

Who is the best one…

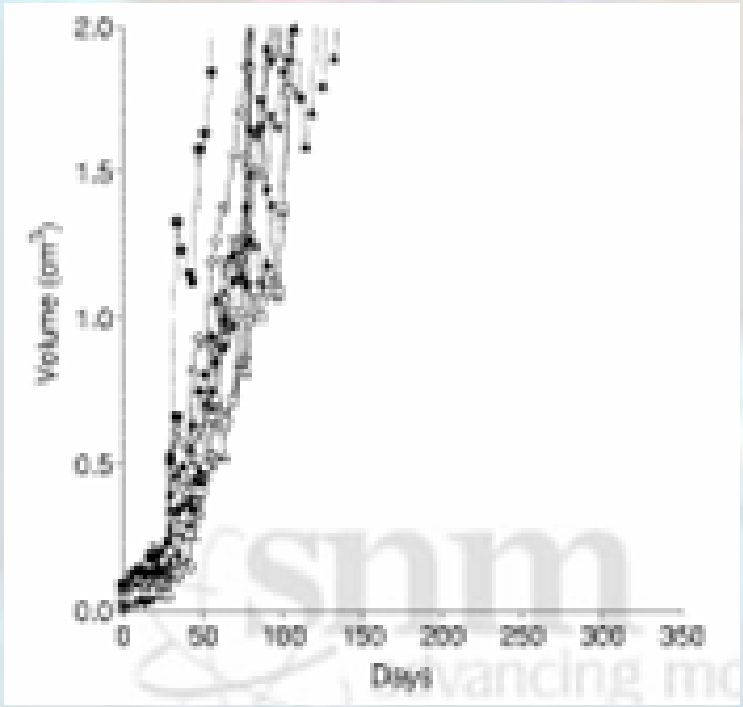
- Antibody…
- Isotope…
- Labelling methods…
- Animal model…
- Clinical trials …

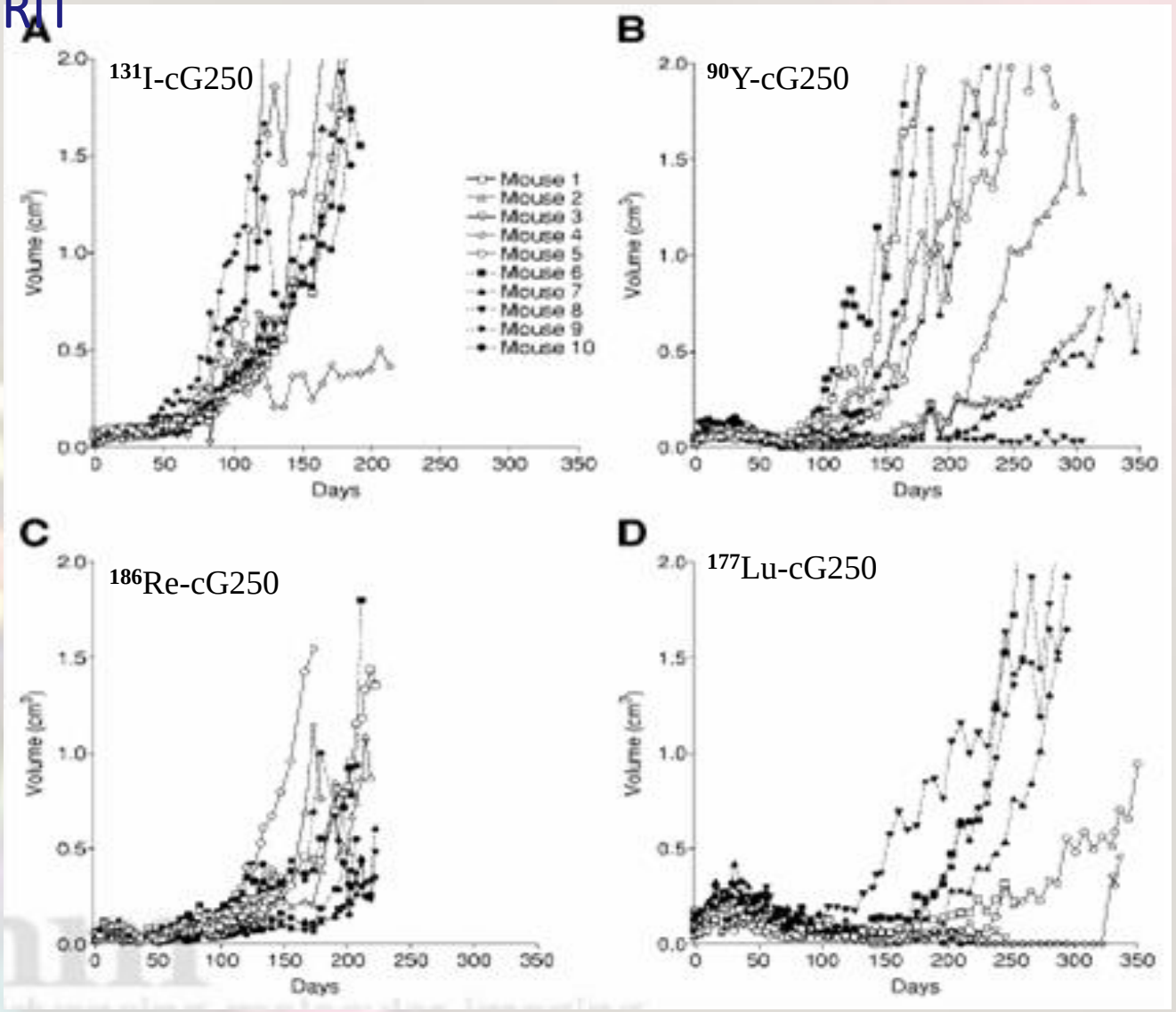
waiting on the door

Preclinical data …animal models



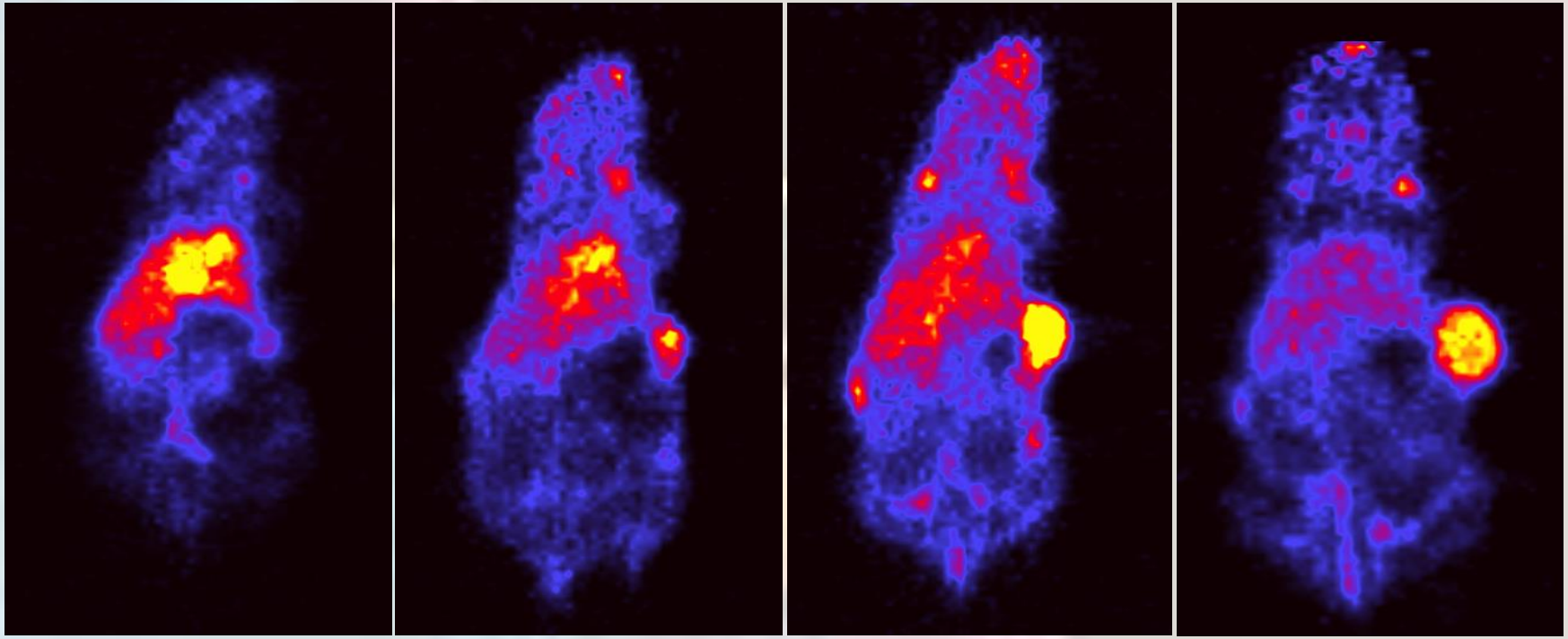
The Quest for the optimal radionuclide for RIT





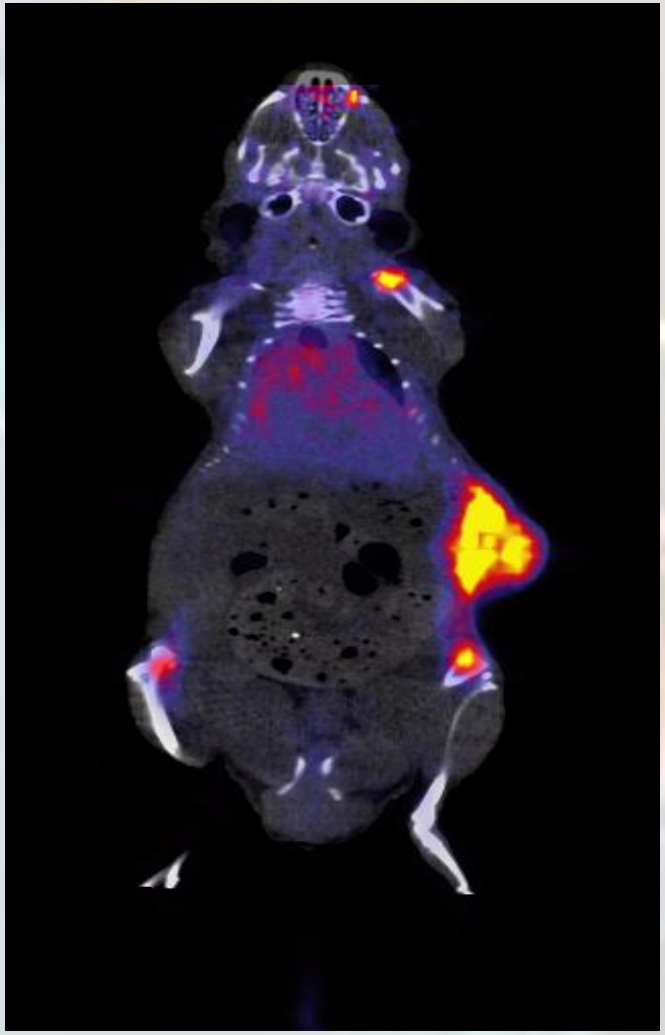
J Nucl Med. 2004;45:327-337

microPET imaging of VEGF-A expressionwith ^{89}Zr-bevacizumab

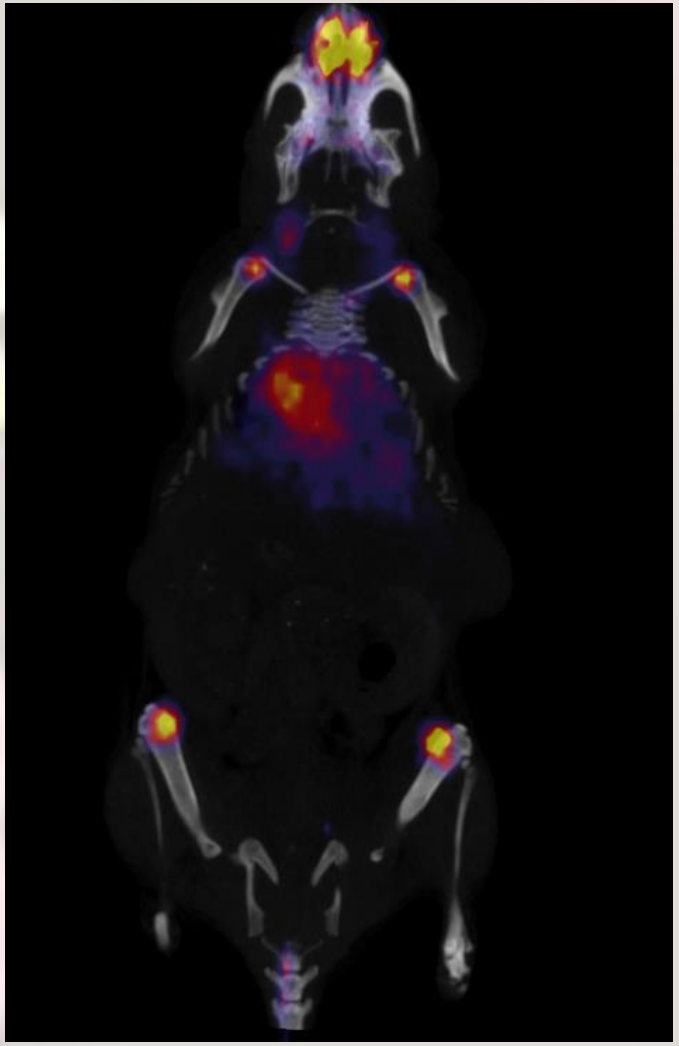


day 0 **day 1** **day 4** **day 7**

microPET imaging of VEGF-A expressionwith ^{89}Zr-bevacizumab



^{89}Zr-bevacizumab, 7 days p.i.

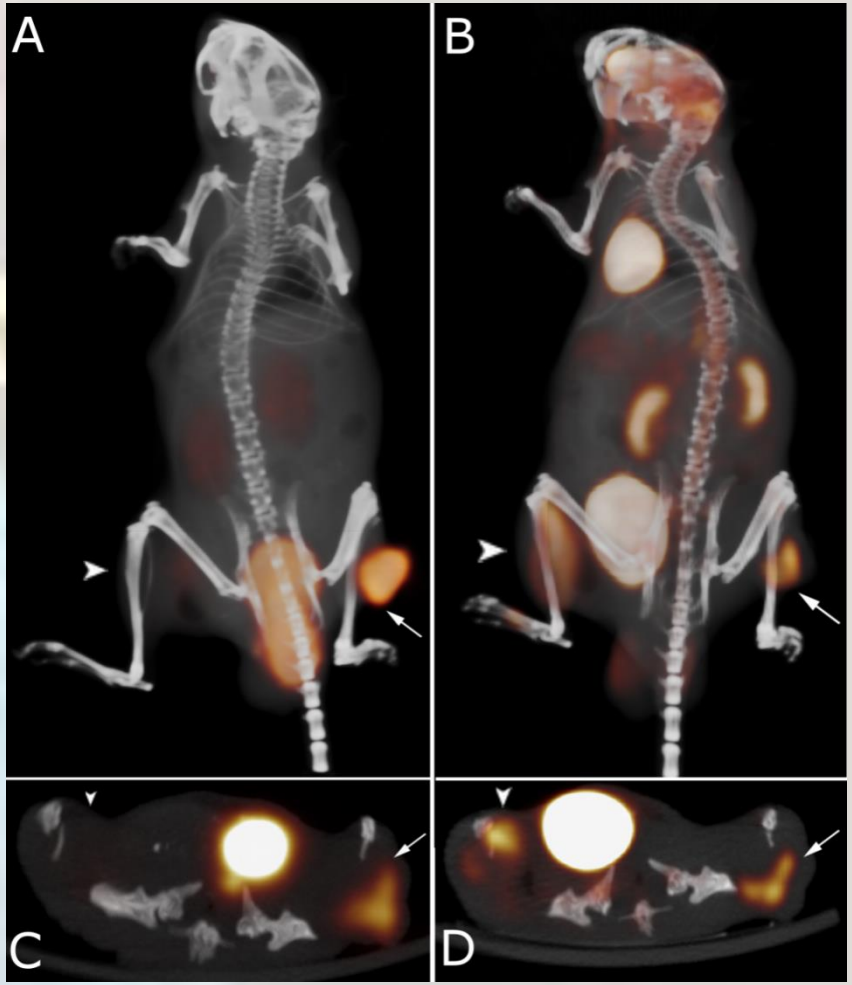


^{89}Zr-bevacizumab + 300 µg cold

Pretargeted immunoPET imaging of cancer

^{68}Ga-immunoPET

Inflammation

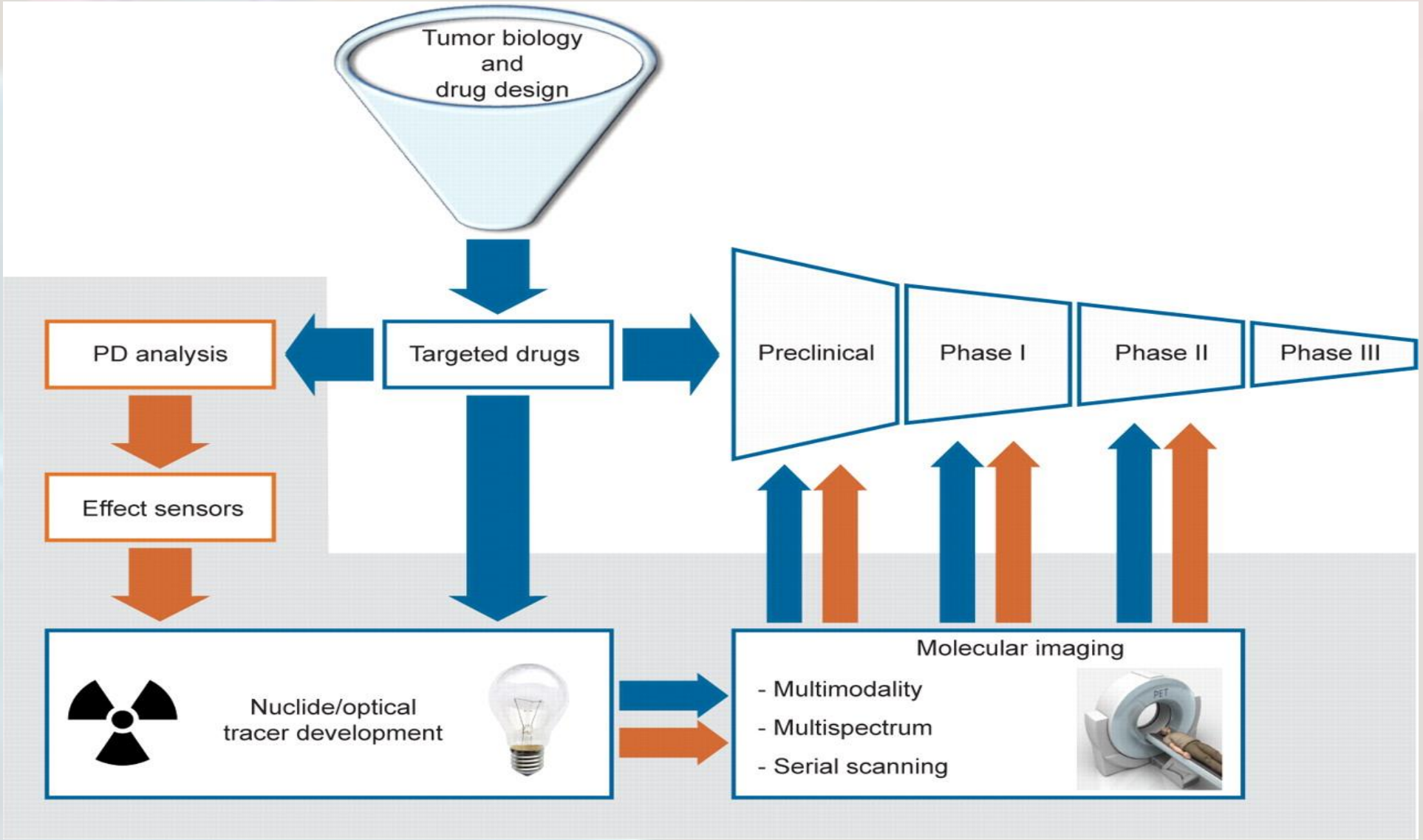


[^{18}F]FDG

Tumor

Schoffelen *et al.* Mol Cancer Ther 2009; in press

Flow chart of molecular imaging in drug development.

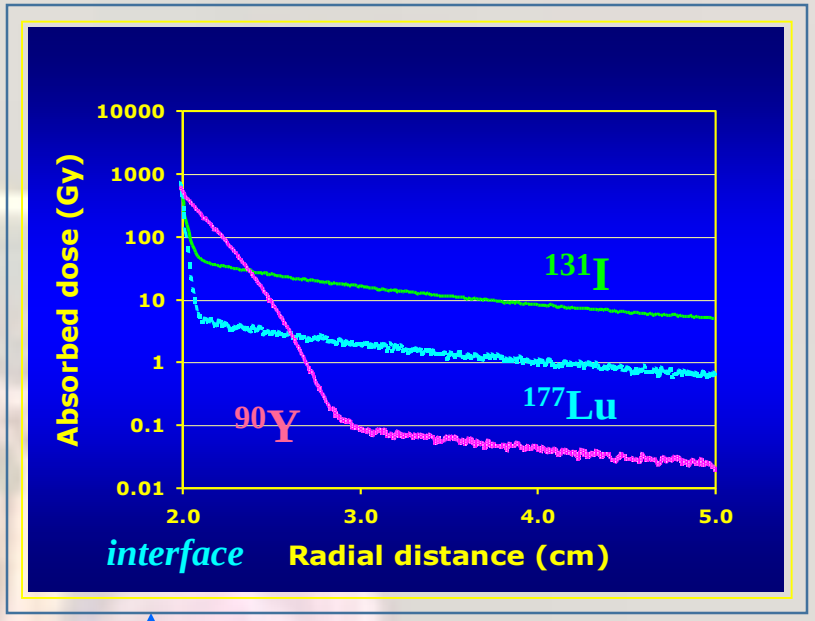


Elisabeth G.E. de Vries et al. Cancer Discovery 2011;1:25-28

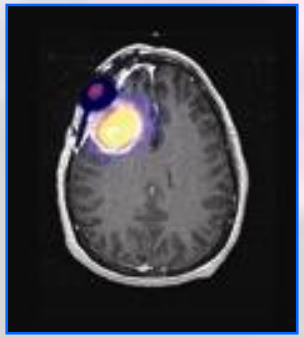
Clinical trials……

Potential Advantages of ^{177}Lu for Brain Tumor Treatment

Property	^{131}I	^{177}Lu
Half-life (days)	8.1	6.7
Ave. β-energy (keV)	182	133
Mean range (mm)	0.91	0.67
Max. range (mm)	2.3	1.8
γ-ray energy (keV)	364, 637	113, 208
γ-ray intensity (%)	81,7	7, 11

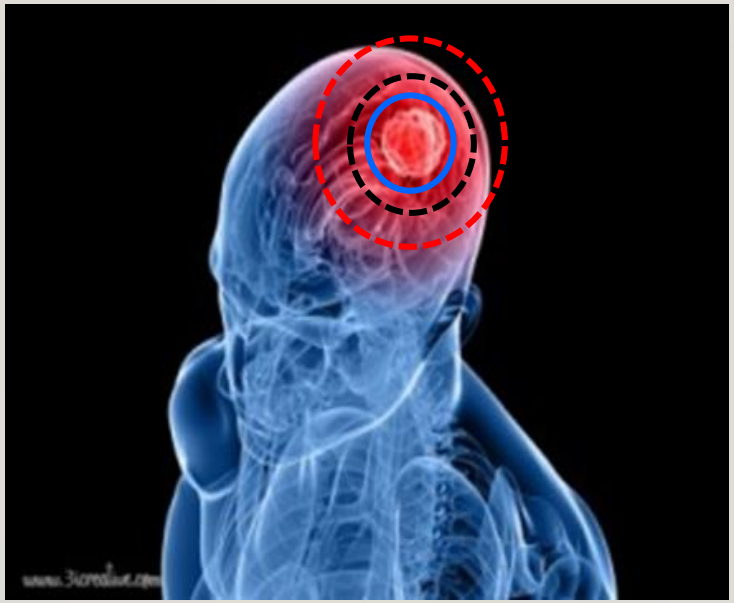


Assume 2-cm radius cavity (33.5 cm^3)

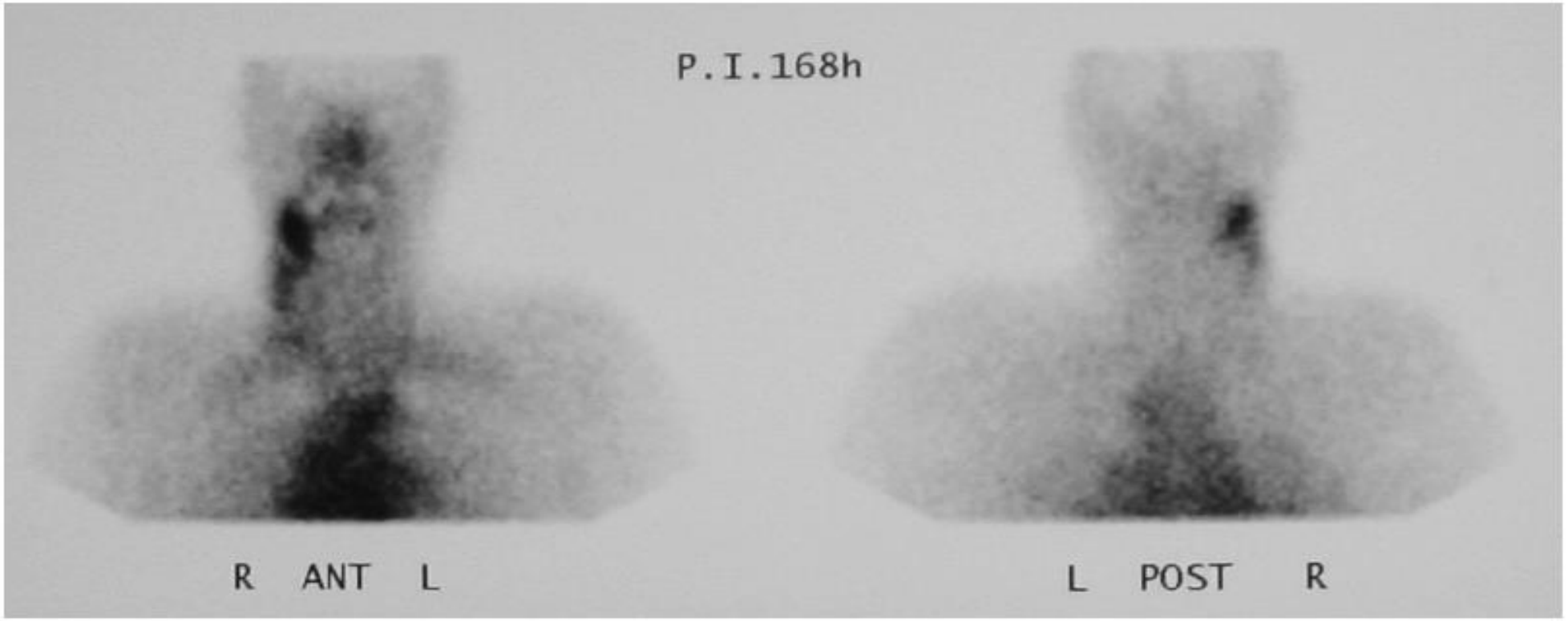


Dose to Brain beyond SCRC Interface

Threshold Dose (Gy)	Brain volume (cm^3) > threshold dose		
	^{131}I	^{177}Lu	^{90}Y
110	2.05	1.02	11.70
50	4.73	1.79	18.12
10	180.3	3.64	31.14



Preliminary results (H. R. Mäcke)

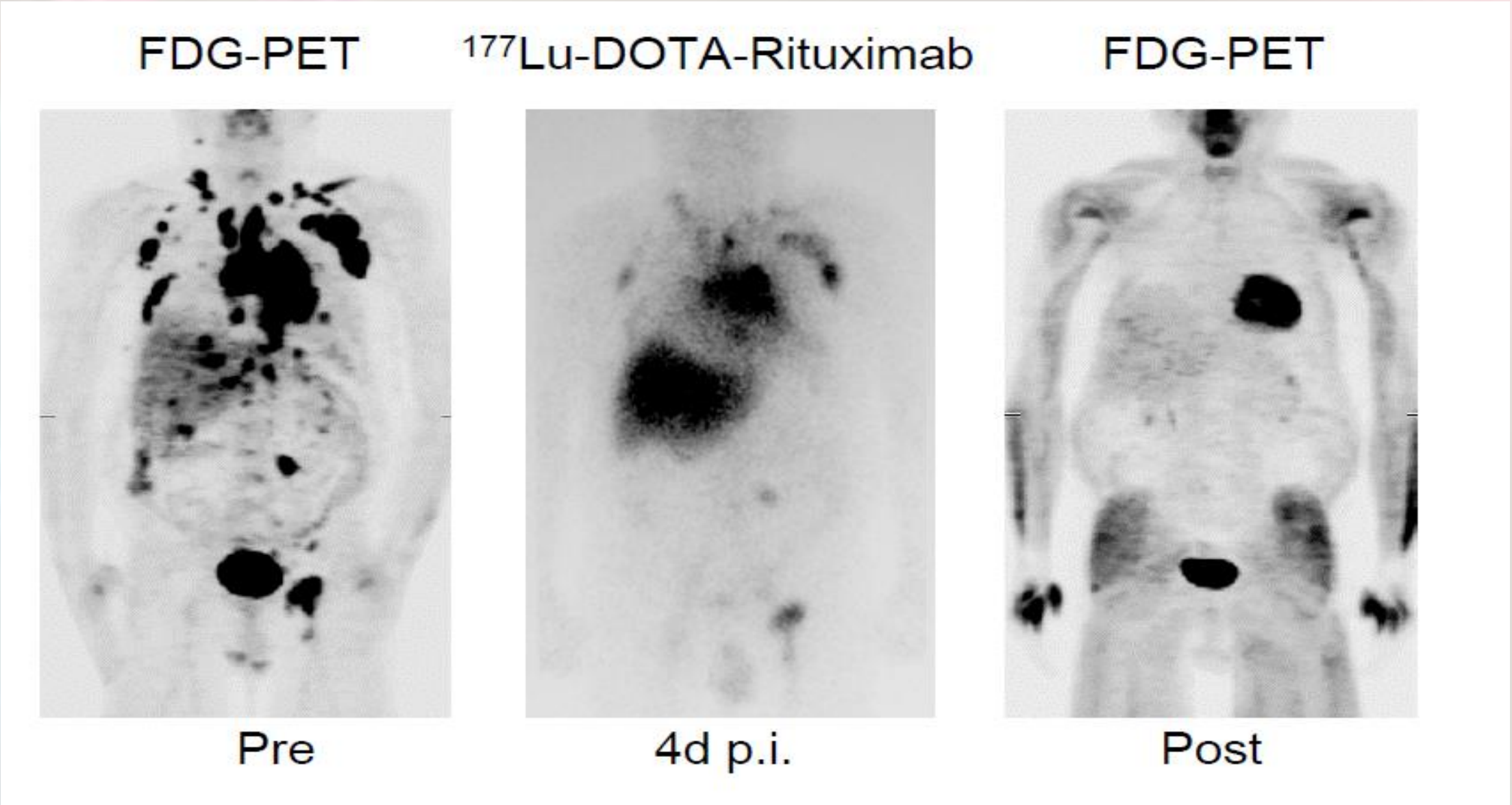


1480 MBq (40 mCi) ^{177}Lu-DOTA-Rituximab

1 week p.i.

Mantle cell lymphoma

Follicular lymphoma



Alpha Particle Emitter Radiolabeled Antibody for Metastatic Cancer: What Can We Learn from Heavy Ion Beam Radiobiology?

- ^{213}Bi labeled anti-CD33 monoclonal antibody HuM195 was investigated in patients with myeloid leukemia
- ^{211}At-anti-tenascin for glioblastoma
- ^{225}Ac-HuM195 for myeloid leukemia
- ^{212}Pb-Trastuzumab for ovarian cancer
- ^{211}At-MX35 F(ab')2 for ovarian cancer
- ^{213}Bi-substance P for glioblastoma

Theranostics: combining imaging and therapy

Radioiodine: the classic theranostic agent

β^+/β^- *isotopes* -actual

^{64}Cu and ^{67}Cu

^{67}Ga and ^{68}Ga

Work performed in Faculty of Medical Sciences – from 2011…

Establishment and standardization of a technology for ready to use production of cold kit formulation of DOTA-Rituximab and peptide based radiopharmaceuticals for labeling with Lu-177 and Y-90

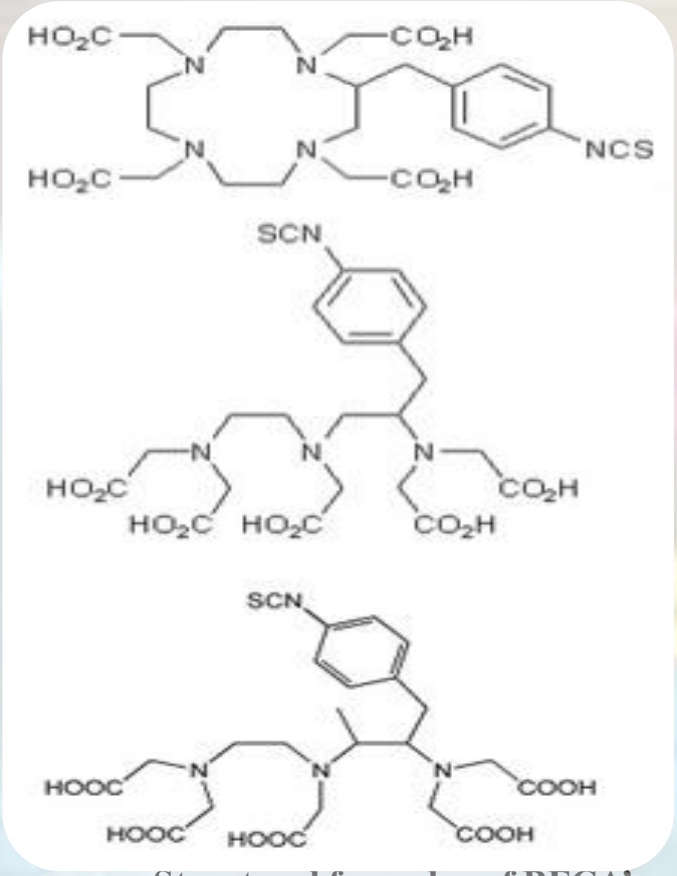
Part of the IAEA's Coordinated Research Project (CRP):

Development and preclinical evaluation of therapeutic radiopharmaceuticals based on Lu-177 and Y-90 labeled monoclonal antibodies

IAEA - Radioisotope Production and Radiation Technology Section, Doctoral Grant under CRP

Darinka Gjorgieva - Ackova
Subject: Chemical analysis of labeled product using not radioactive Lutetium / Ytrium and determination of the structure of obtained freeze dried products

Katarina Smilkov
Subject: Established protocol for freeze-draying of bifunctional ligand -Rituximab

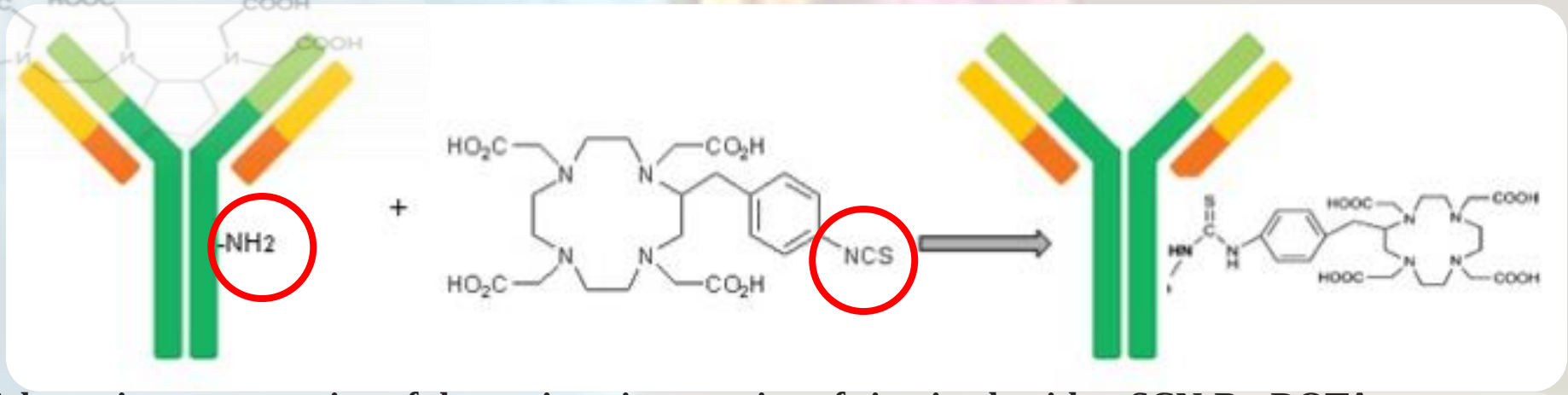


_p_-SCN-Bn-DOTA
(2-(4-izothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid)

_p_-SCN-Bn-DTPA
(2-(4-izothiocyanatobenzyl)-diethylenetriaminepentaacetic acid)

1B4M-DTPA
2-(4-izothiocyanatobenzyl)-6-methyl-diethylenetriaminepentaacetic acid

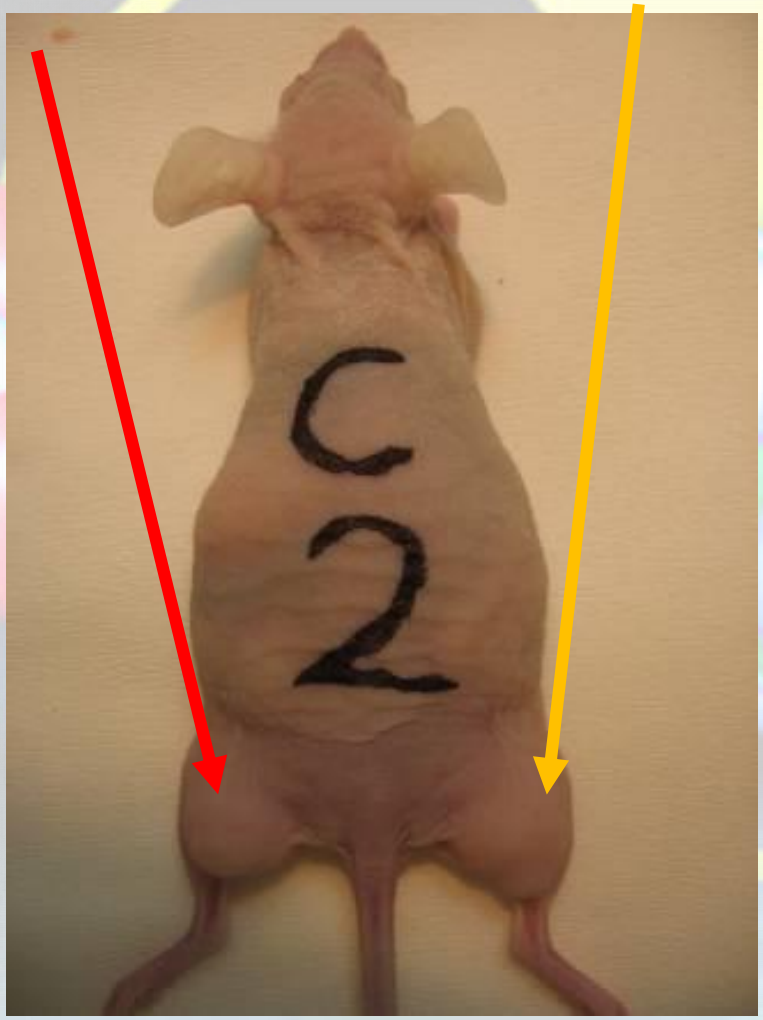
Structural formulae of BFCA's used

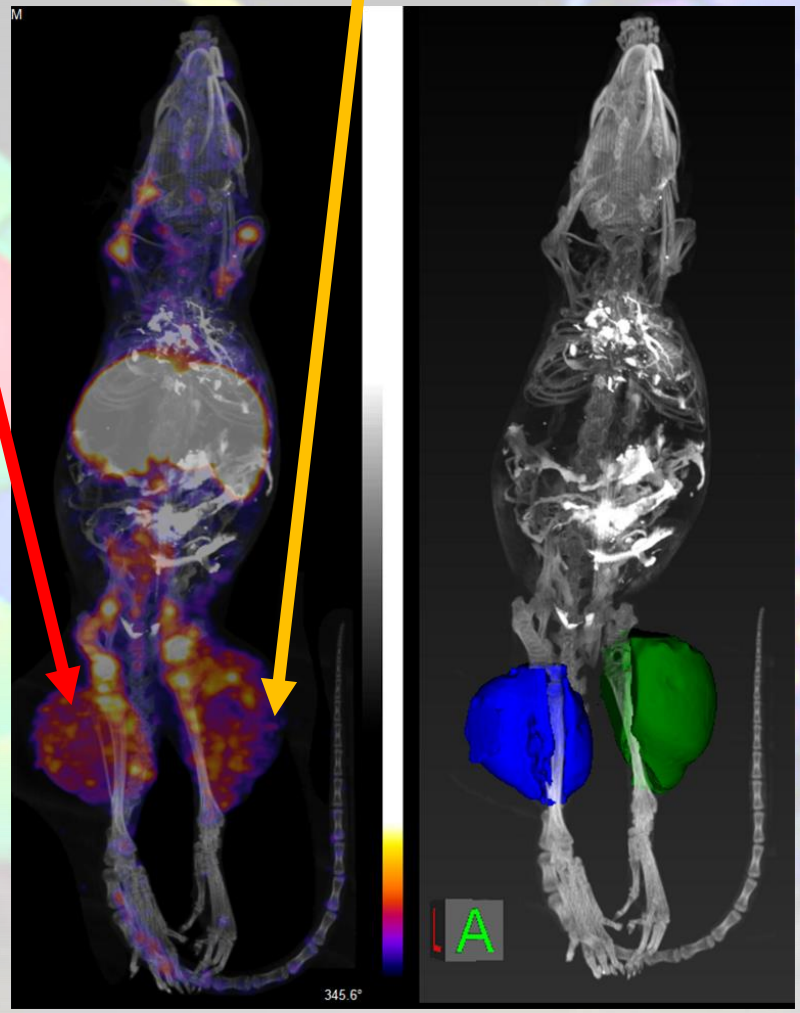


Schematic representation of the conjugation reaction of rituximab with p-SCN-Bn-DOTA

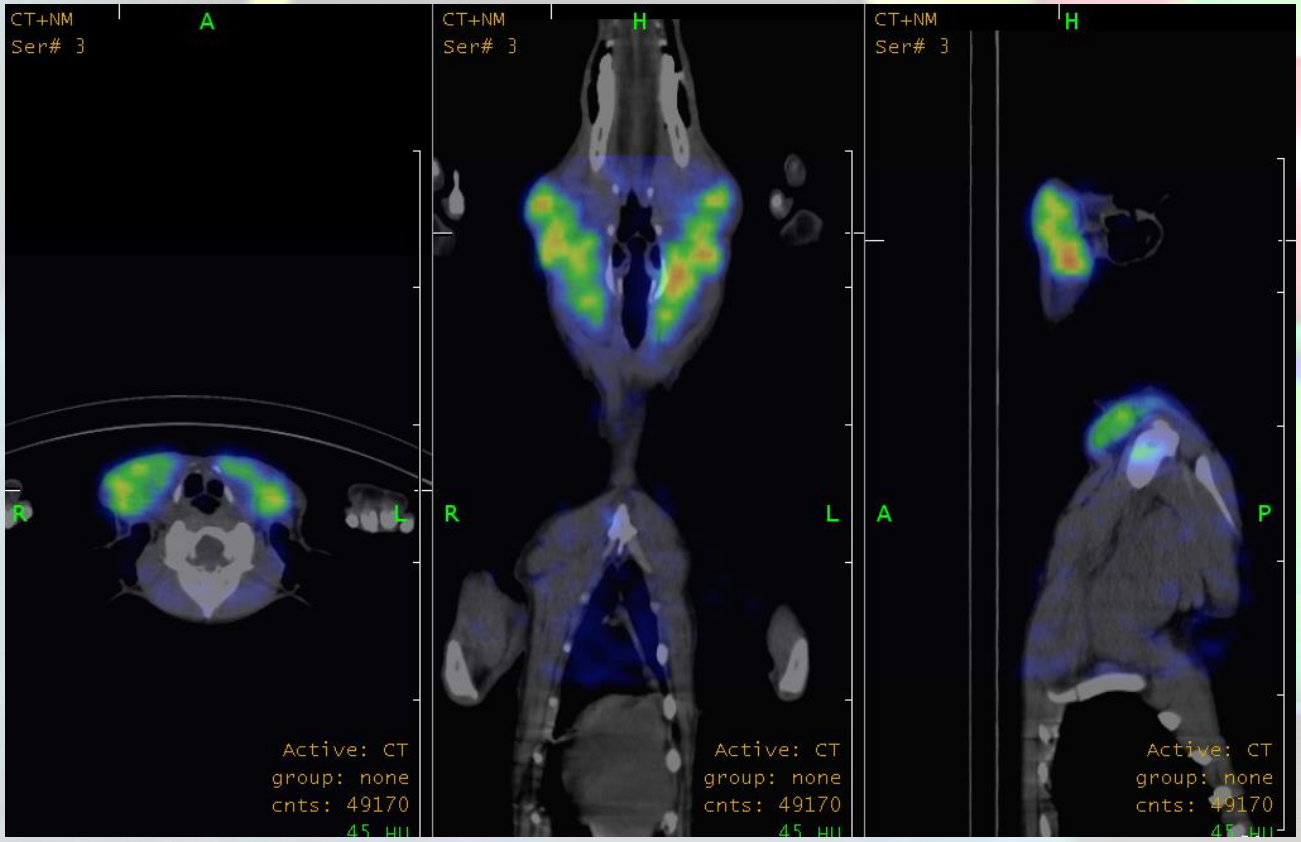
Animal studies - Double xenografts in Nude mice

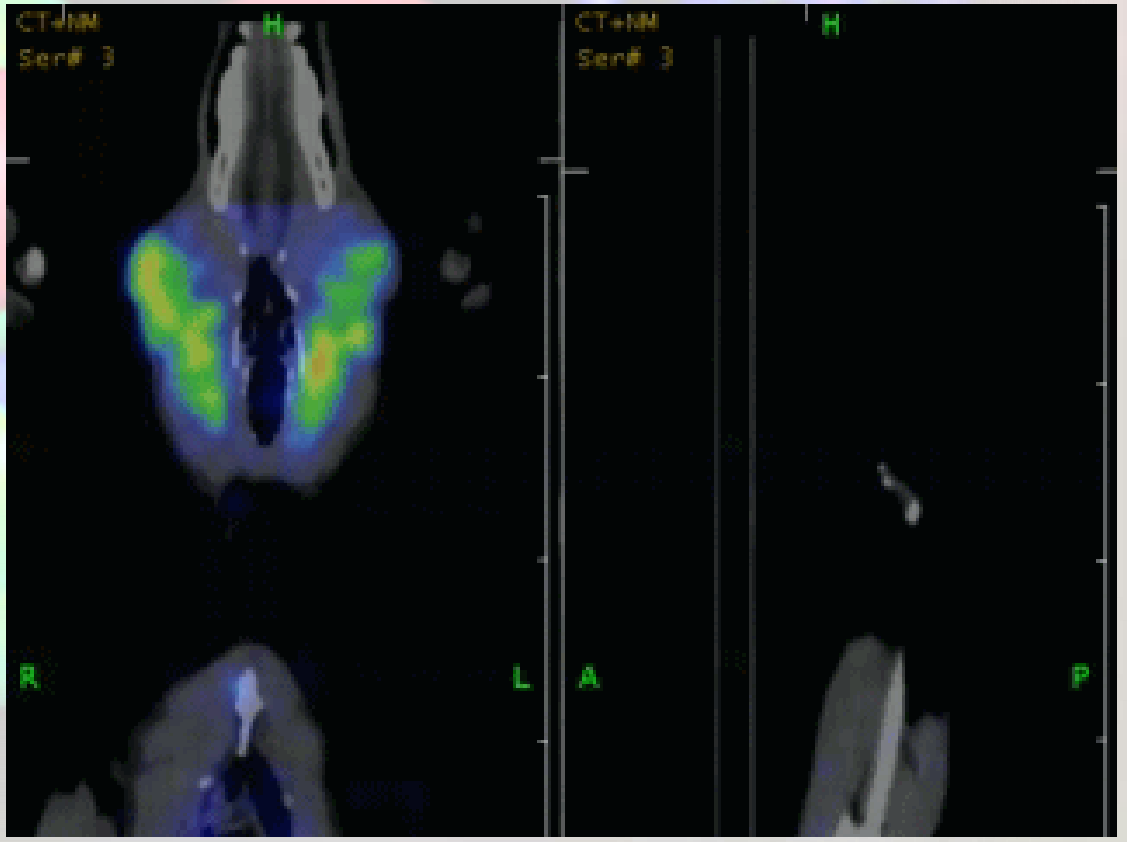
specific vs non-specific uptake





Patient – Dog with B limphoma

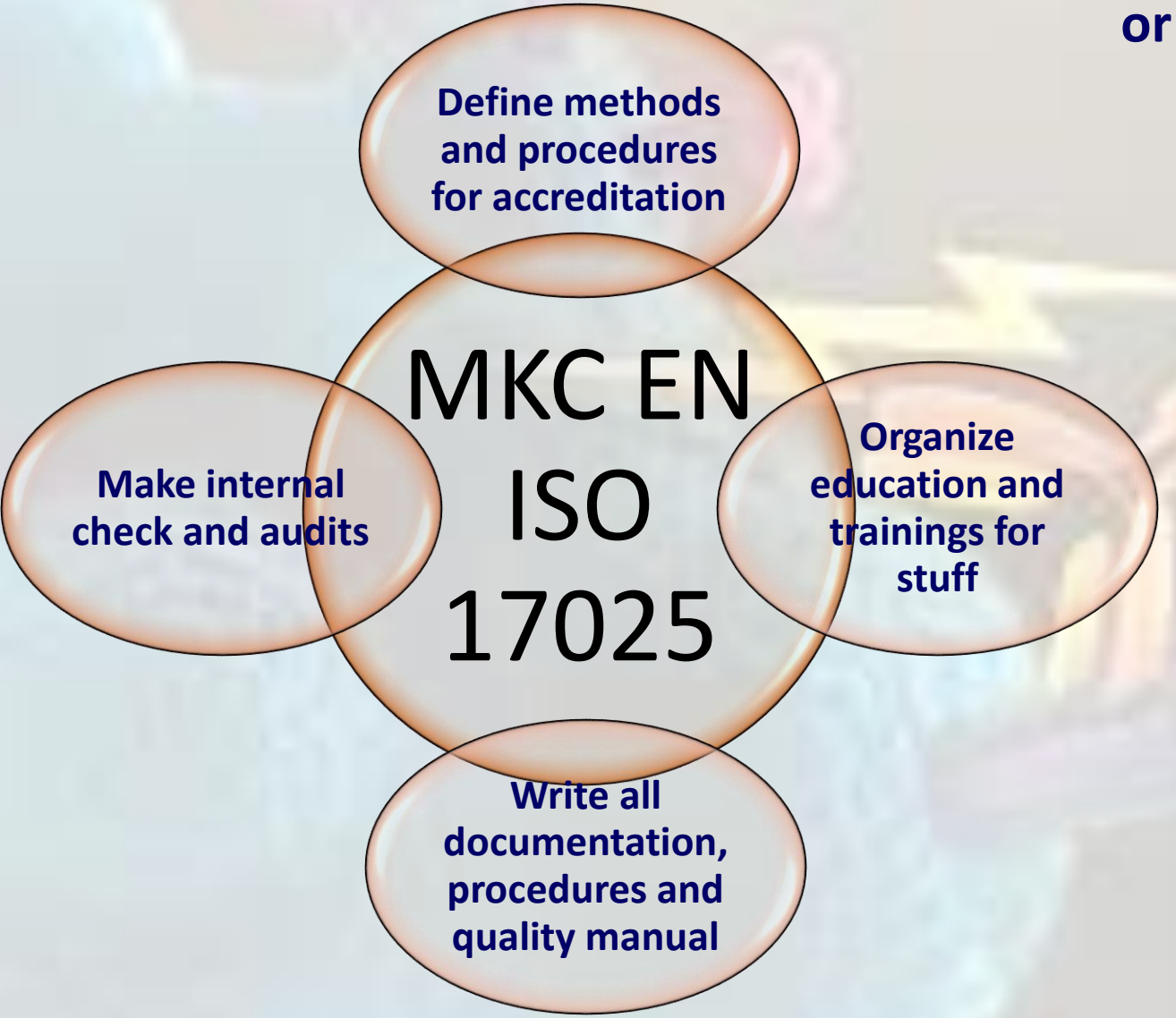





Next Step

- To use the same method of freeze drying for the other working antibodies, other anti CD-20 (biosimilary), - formulation of stable immunoconjugate of the HER2-targeting trastuzumab – potential for rapid labelling with Gallium-68
- Other isotopes with the same antibody
- To participate in clinical trial – existing PET facility with production laboratory dedicated for GPM small scale production of radiopharmaceuticals for therapy

Accreditation of the Laboratory of Radiopharmacy – requested requirements or need of challenge



Thank you