In vivo Evaluation
Animal Models, Biodistribution, Metabolism Studies

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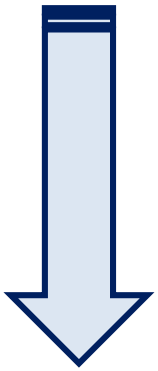
Medicis Promed

Summer School: Development and Pre-clinical Evaluation of Radiopharmaceuticals – 4-8 June 2018

Radiolabelled Biomolecules/Compounds with suitable Radiochemical and *In Vitro* Biological Profile



***In Vivo* Biological Evaluation**

In Vivo Biological Evaluation

- Biodistribution in Animal Model
- Pharmacokinetics in Animal Model
- *In Vivo* Stability / Metabolic Studies
- Molecular Imaging
- Assessment of Therapeutic Potential (*e.g.* Tumor regression)

Usefulness for clinical application as molecular imaging or radiotherapeutic agent

Molecular Imaging *is the visualization, characterization and measurement of biological processes at the molecular and cellular levels in humans and other living systems*

Measure physiological parameters:

- **Receptor, antigen, enzyme concentration**
- **Organ function**
- **Metabolic processes**

How these parameters change during disease?

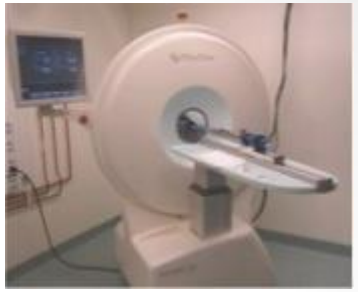
Screening of therapeutic responsiveness

Molecular imaging techniques

- Highly Specific
- Indispensable in Diagnostics
- Visualize specific molecular events
- Enable earlier diagnosis
- Monitor therapeutic responses (Radionuclide Therapy; Therapeutic Drug Development)

Molecular Imaging Modalities

Radionuclide Imaging



Imaging the in vivo biodistribution of radiotracers
Quantitative

SPECT

- Resolution 1 mm
- 80-250 keV energies
- Discriminate ≠ energies
- Higher doses of radioactivity
- Longer imaging times

PET

- Resolution 2 mm
- 511 keV energy
- More sensitive
- Lower levels of radioactivity
- Shorter imaging times

Molecular Imaging Modalities

Optical Imaging	Ultrasound Imaging	MRI	CT
1) Bioluminiscence 2) Fluorescence High Sensitivity; Rapid; Resolution - 5mm; High degree of scatter and absorption by tissues	Reflection of high-frequency sound waves in the body Low Sensitivity; Rapid; Resolution 50 µm; Operator dependent; Qualitative	Measure concentration and rates of relaxation of H atoms in magnetic field Very low Sensitivity; Rapid; Resolution 25 µm;	Anatomical information/ Absortion of X-rays Combined with PET and SPECT Very low Sensitivity; Resolution 50 µm

- **Design highly sensitive and specific imaging probes**

Various imaging modalities
(radionuclides, fluorophores, nanoparticles)
+
Targeting ligands
(Abs, Proteins, Peptides, Cells)

- **High affinity and specificity for target**
  (Nanomolar concentration)

- **Targeting selectivity**

Specific Interaction with biochemical and physiological processes

Radiolabelled Targeting Biomolecules

Molecular Imaging / Targeted Radionuclide Therapy / Theranostics

Selective Binding to target Antigens, cellular membrane or nuclear Receptors

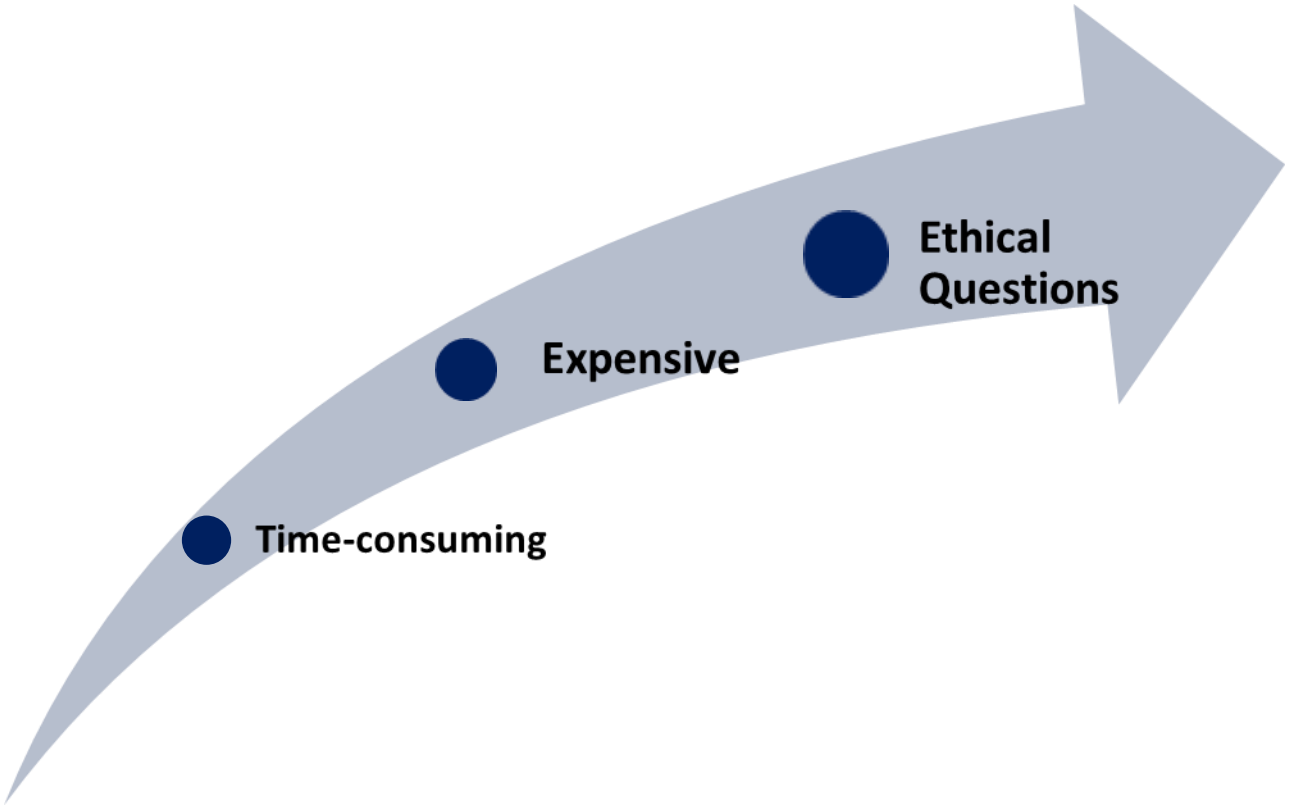
- **Increased Specificity** – essential to minimize the unnecessary radiation exposure
- Increased Accuracy
- Potential ability to eradicate primary tumor and disseminated metastasis

Targeting Biomolecules:

- Monoclonal Antibodies;
- Antibody Fragments;
- Small domain Antibodies
- Nanobodies;
- Peptides;
- Small molecules;
- ……

Determines the fate of radiopharmaceuticals after administration

Animal Models



Animal Models

Why use animal models in research?

- To try and model human diseases
- Understand molecular aspects of disease process
- Essential for the development of clinically useful (radio)pharmaceuticals
- Validation and quality control of (radio)pharmaceuticals
- Mechanisms of localisation of compounds
- Unique pharmacological and toxicological data
- Predict biosafety and clinical efficacy

Animal Models

3-Rs Principle

- **Replacement**

  In vitro techniques;
  microorganisms;

  computer modelling

- **Reduction**

  Study design – minimum animal number;
  Improve statistics;
  Use “lower” *vs* “higher” animals

- **Refinement**

  Reduce pain and stress

  Non-invasive techniques

  Improve conditions

Animal Models

Principles for animal experiments

- Essential for significant relevant information
- Obligation to treat animals with respect
- Investigator has ultimate responsability
- Balance between effects on animals and benefit for health

- Appropriate species
- Bred in captivity
- Scientifically valid using minimum number
- Well trained and competent staff
- Brief experiments
- No unnecessary repeats

Animal Models

Normal Animals

- **Small rodents**

  Rat; mice

- **Many physiological similarities**

  Preserved basic layout and function of most organs

- **Provide useful information**

  Biodistribution;

  ***In vivo* stability;**

  Interaction with molecular target in biological environment;

  Neuropeptide receptors widely expressed in mice (*e.g.* somatostatin analogues) usually interact as efficiently as human receptors;

  Most monoclonal antibodies towards human targets do not bind to their rodent equivalent.

Animal Models

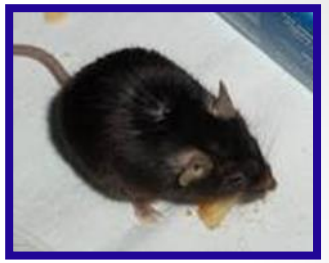
Disease Animal Models

- **Infection/ Inflamation Animal Models**
- **Tumour-Bearing Animals**
- **Transgenic Animals**

Biomolecules specifically bind *in vivo* to infection sites, antigens, overexpressed receptors,….

Animal Models

Tumour-Bearing Animals



To predict the likely behaviour of the radiolabelled biomolecule in a cancer patient

Depends on:

- **Tumour source;**
- **Imunocompetence of the animal;**
- **Genetic manipulation**

Syngeneic Model – animals bearing tumours of their own species

- **Spontaneous or carcinogen-induced**
- **Transplanted by administration of tumor cells**

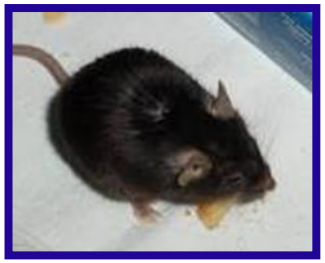
(unnatural location; changes in the intratumoral signaling)

Orthotopic Model - transplant of the tumor to the site as its origin

(*e.g.* mamary gland, eye, bone marrow)

Animal Models

Tumour-Bearing Animals



Syngeneic Model

- Well characterized cell lines
- Immunocompetent hosts
- Reproducible tumors
- Low cost
- Poor representation of human disease (diferent receptor subtypes, expression level,…)
- Lack of target molecule homology between species

Animal Models

Tumour-Bearing Animals

Xenogeneic Model – animals bearing tumours of human origin

Animals with imunodeficient system:

Genetically modified

1. **Nude strains of mice or rats – lack of thymus; do not generate mature T cells**
2. **SCID Mice (*severe combined immunodeficient*) - have a mutation, complete loss of humoral and cellular immune system**

Animal Models

Tumour-Bearing Animals

Xenogeneic Model

- Well characterized cells
- Simple to implement
- Expression of human homolog of the target
- Homogeneity in tumor
- Reproducible

Animal Models

Tumour-Bearing Animals

Xenogeneic Model

- Immunosupressed non human hosts
- Tumor cells of human origin
- Murine peritumoral milieu (blood vessels, stromal cells)
- Imune environment of tumor
- Different human tumor histology
- More expensive
- Require microbe-free animal housing

Animal Models

Tumour-Bearing Animals

Orthotopic Model

- Best mimicking human carcinogenesis and metastatic patterns
- Limited number of hosts
- Surgical skills
- Complex logistics
- Non-homogeneity/ non reproducibility in tumor growth

Animal Models

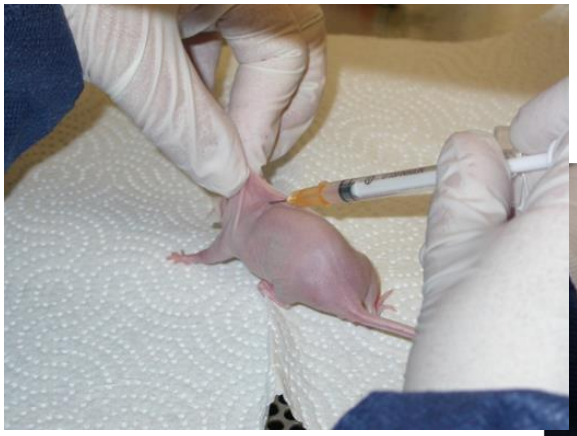
Induction of Xenotransplant

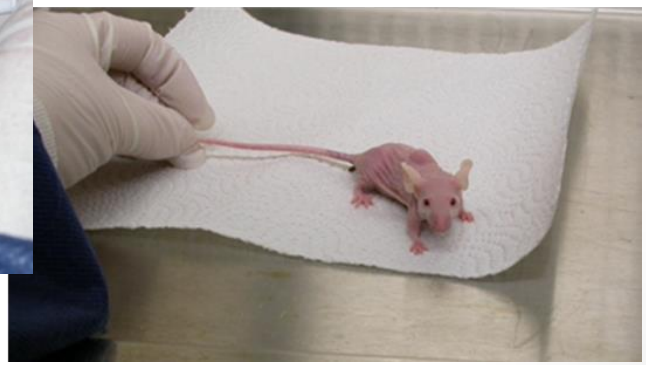
Administration routes – subcutaneous administration of tumor cell suspension

Tumor cells of human origin

Murine stromal cells and blood vessels

Abnormal imune environment of tumor





Animal Models

Transgenic model

Genetically modified animals to alter expression of target molecule

Models of human disease

1. **Administration of transfected cells** (1 receptor subtype; different levels of expression)
2. **Reporter-gene imaging** (expression of target molecule controled by a particular gene)
3. **Transgenic mice** (incorporation of human gene, random, transient, relatively inefficient)
4. **Gene targeting**
5. **“Knockout” mice** (disruption of function of a selected gene)

Animal Models

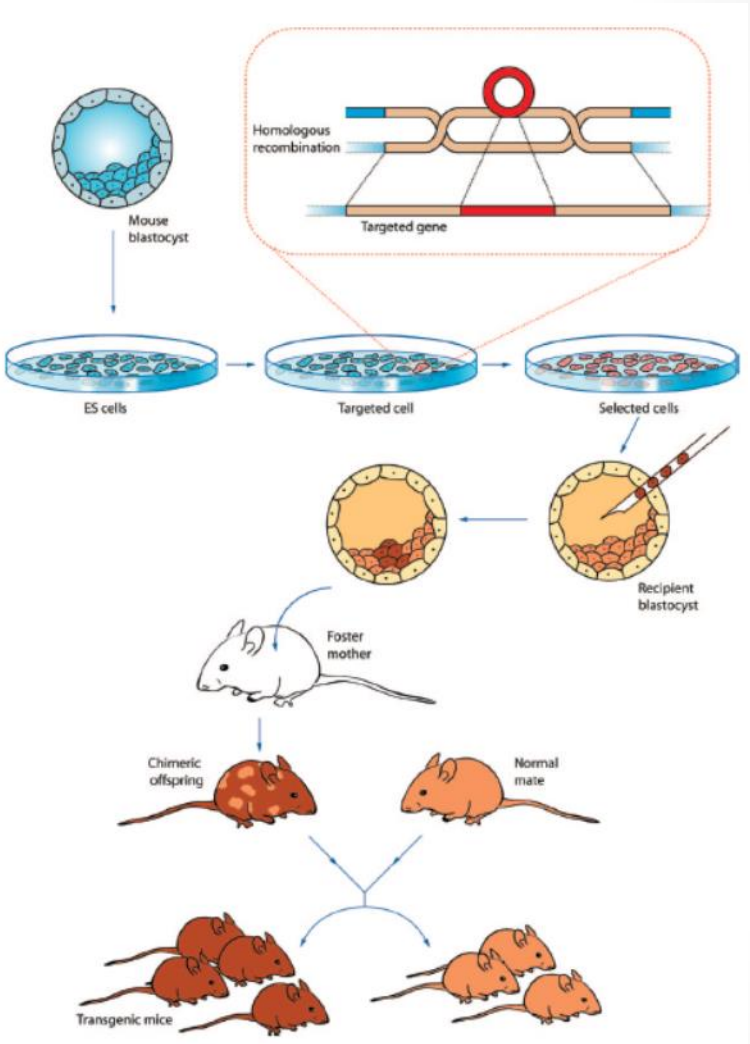
Gene targeting

Genetically modified animals

Introduction of human DNA homologous to the target mouse gene into embryonic stem cells

Selected cells implanted in foster mothers

Birth of 2 types of mice (only human gene or only mouse gene)



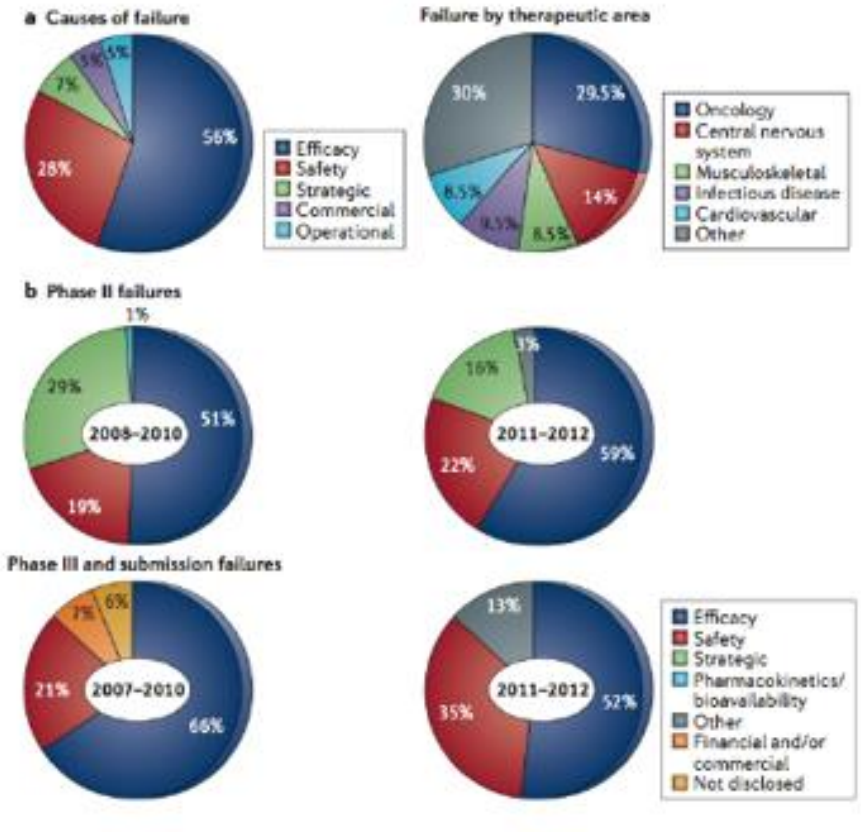
Animal Models

Transgenic model

- Controlled cancer progression in selected organs;
- Resemble human carcinogenesis;
- Immunocompetent host;
- Limited availability;
- Expensive;
- Restricted experience;
- Variations in tumor growth rates;
- Demanding statistics

Animal Models

Patient Derived Xenograft (PDX) Model



Emerging platform for Translational Cancer Research;

Observation: High failure rate of new molecules in late clinical development in oncology;

Lack of efficacy

J.Arrowsmith, P.Miller, *Nat Rev Drug Discovery* **2013**

Animal Models

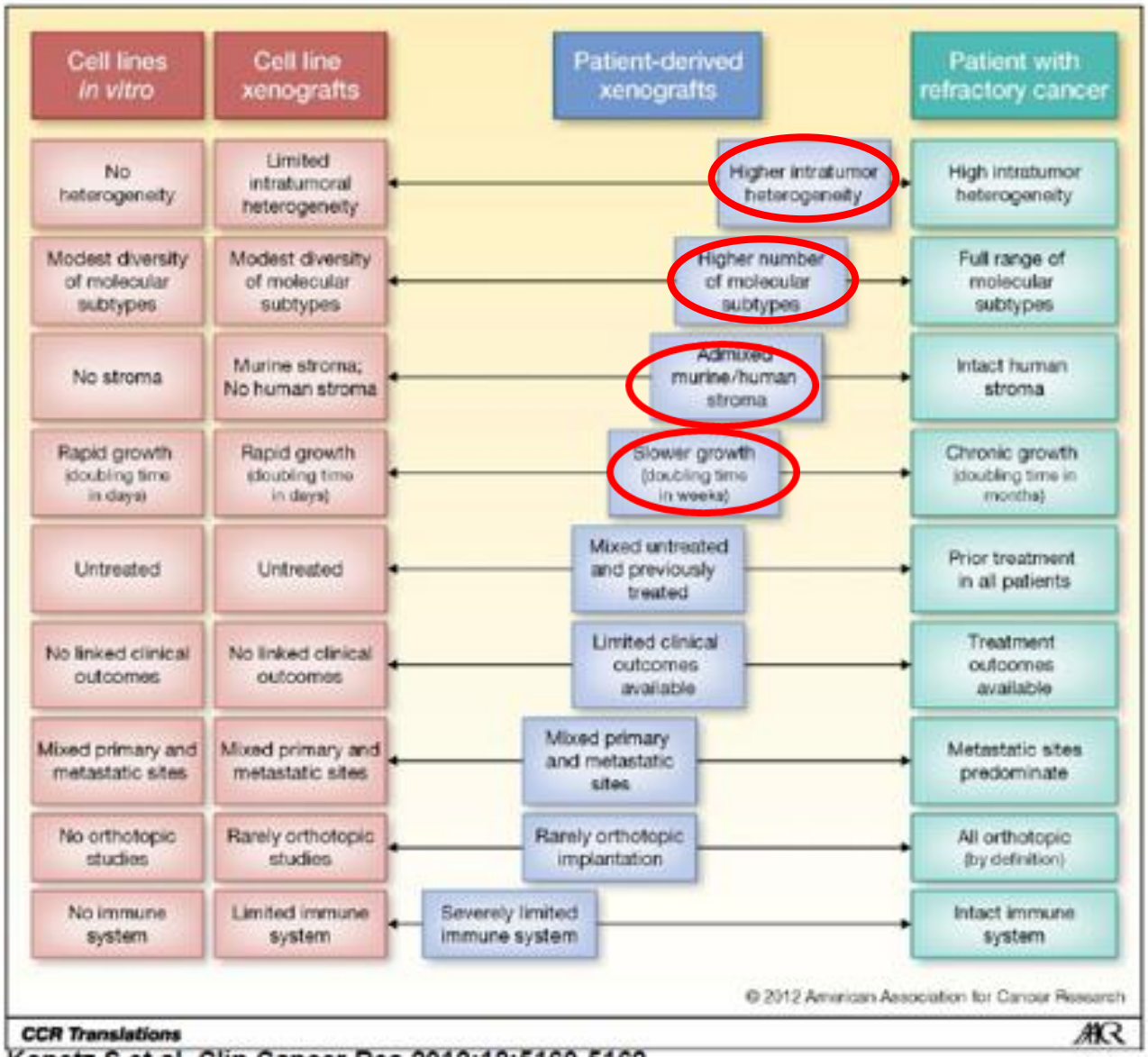
PDX Model

Way to increase the predictibility of preclinical studies

- **Use PDX animal model that more closely reproduce the heterogeneity of human cancers**
- **Perform studies for genotype/response correlation**
- **Maintain high correlation with the original tumor from patients**
- **Still complementary to other models**

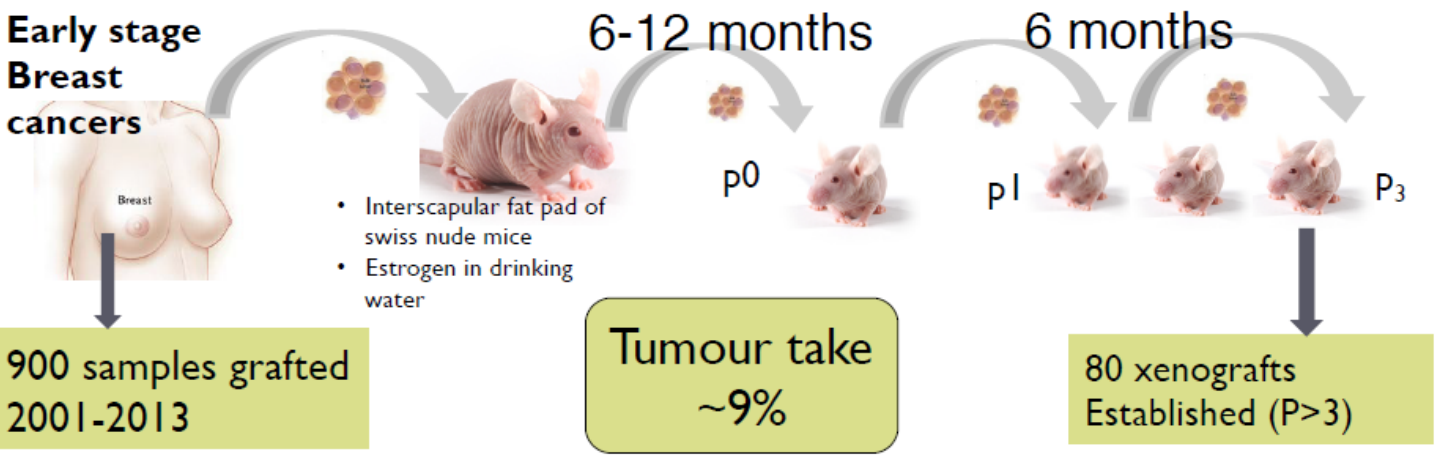
Animal Models

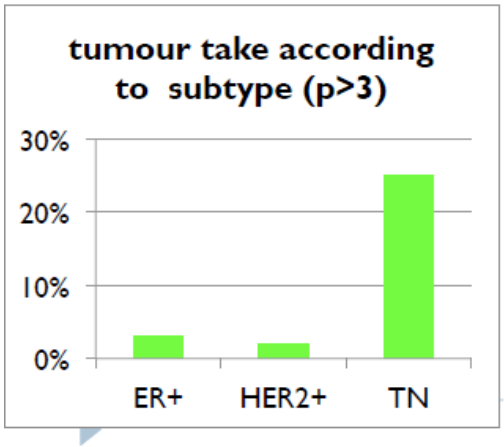
PDX Model

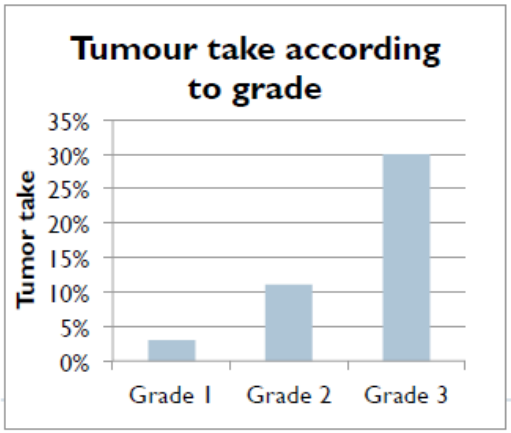


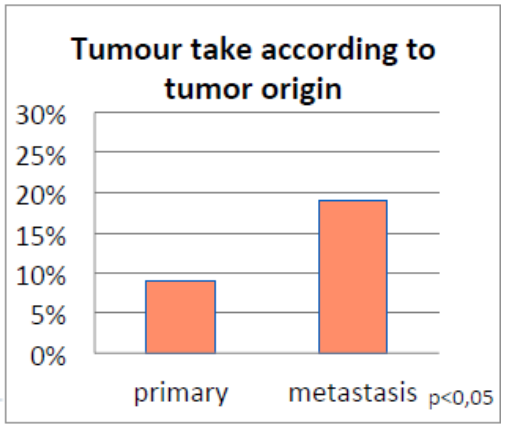
Kopetz S et al. Clin Cancer Res 2012;18:5160-5162

Establishment of the xenografts: tumor take



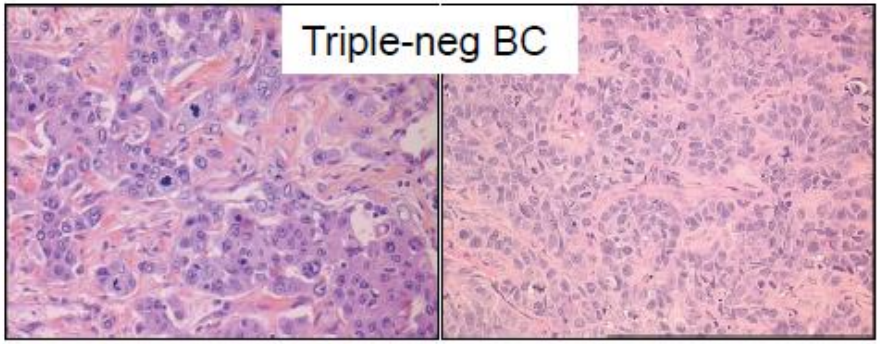




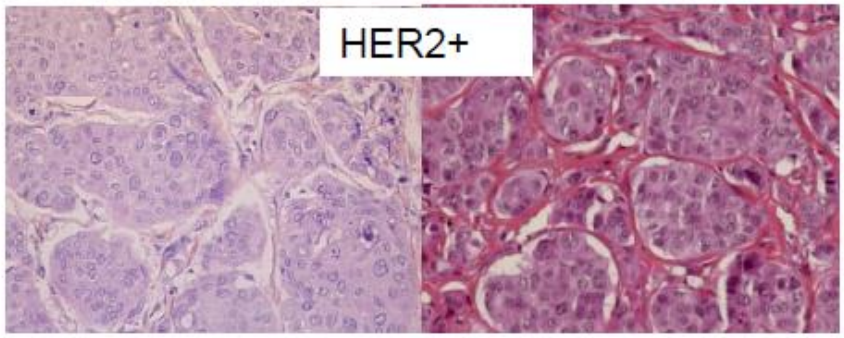


Marangoni et al 2007; Cottu et al 2012

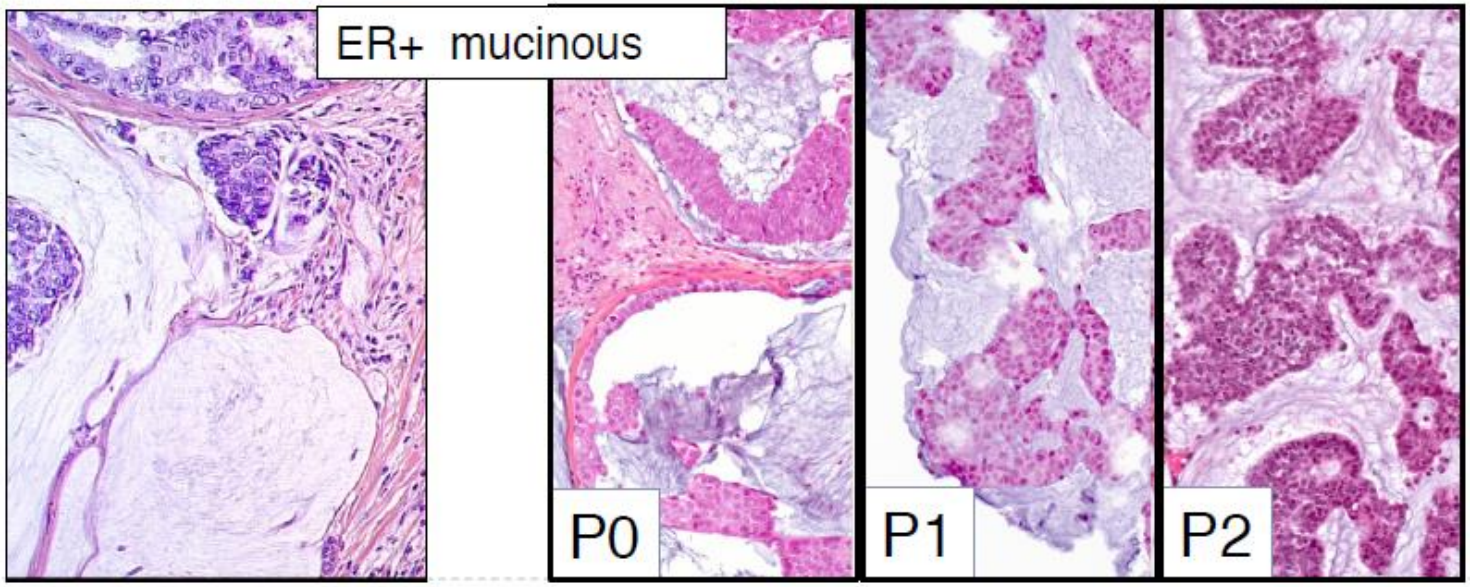
Tumor morphology is reproduced in xenografts



patient xenograft

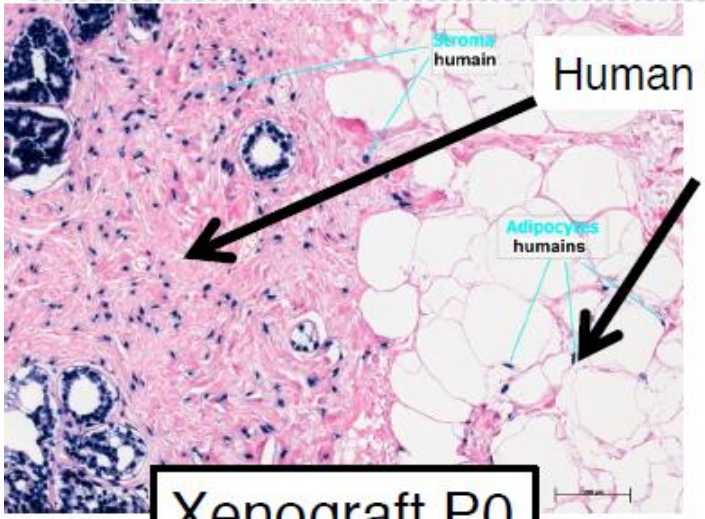


patient xenograft



patient xenografts

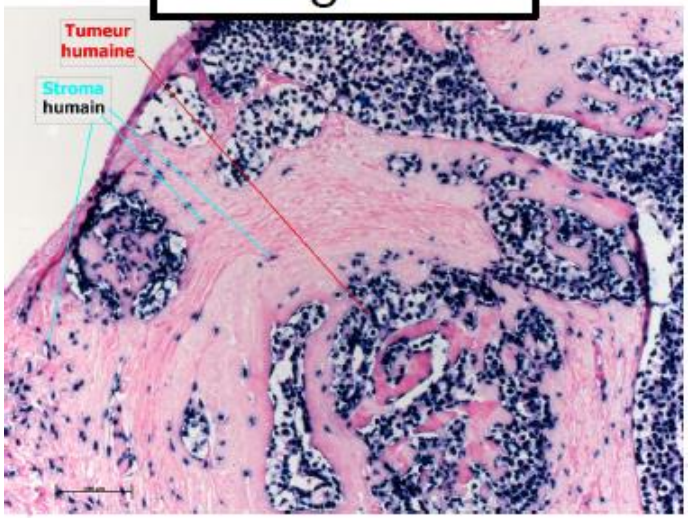
Human stroma is still detected at passage 0 but it is progressively lost and replaced by mouse stroma

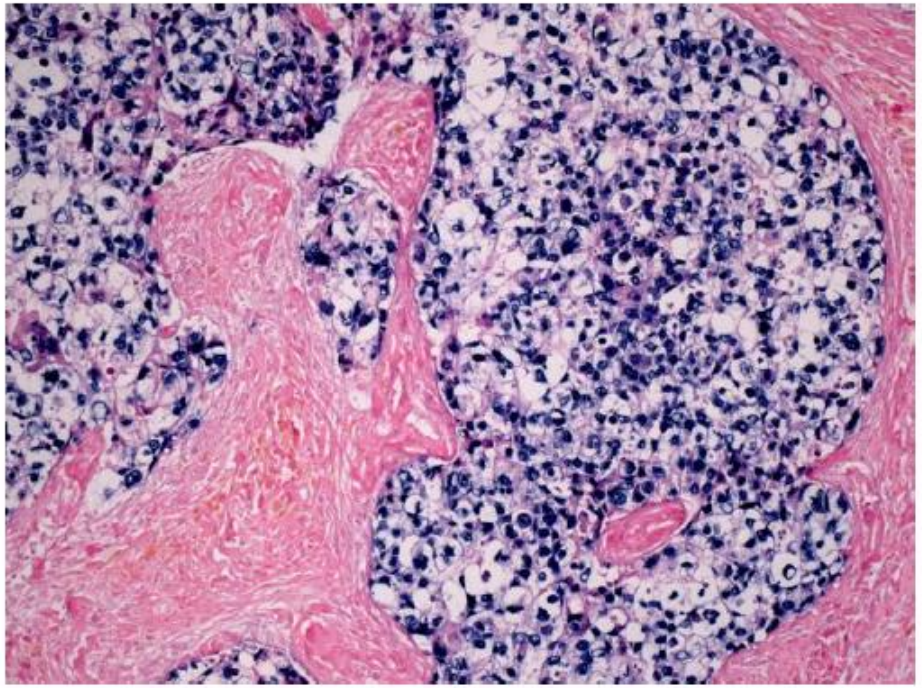


Human fibroblasts

Human adipocytes

Xenograft P0





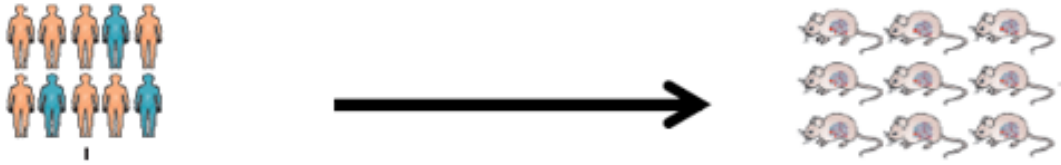
Xenograft P2

Alu staining

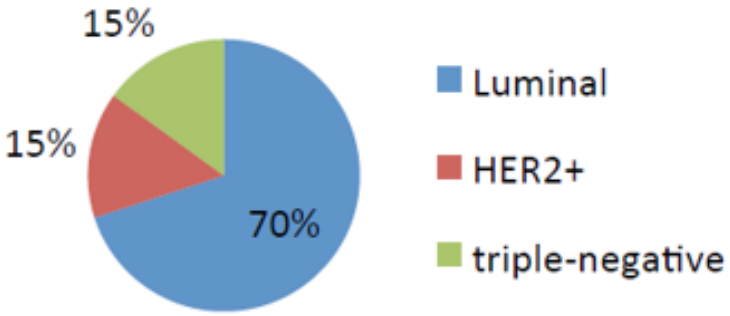
Animal Models

PDX Model

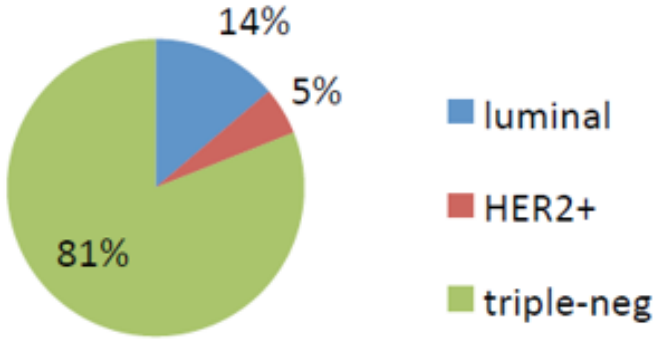
Enrichment in triple-negative breast xenografts, grade 3, invasive ductal carcinoma



Subtype of breast cancer in patients



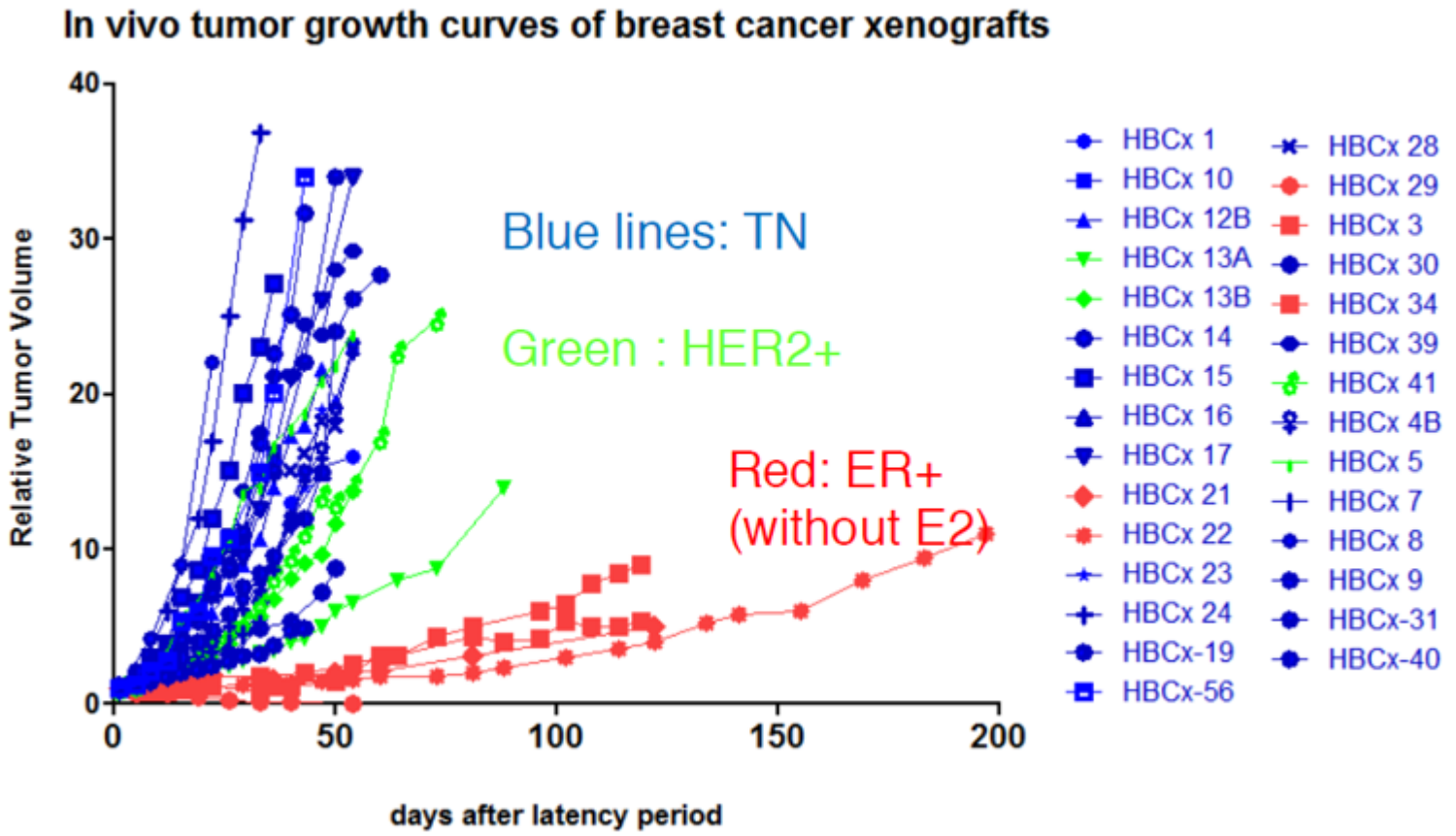
Established xenografts (p>3)



Histology

Animal Models

PDX Model



PDX of Triple Negative Breast Cancer (TNBC) can be obtained with relative high rates compared to ER+ or Her2+ since these TNBC xenografts are highly proliferating

Animal Models

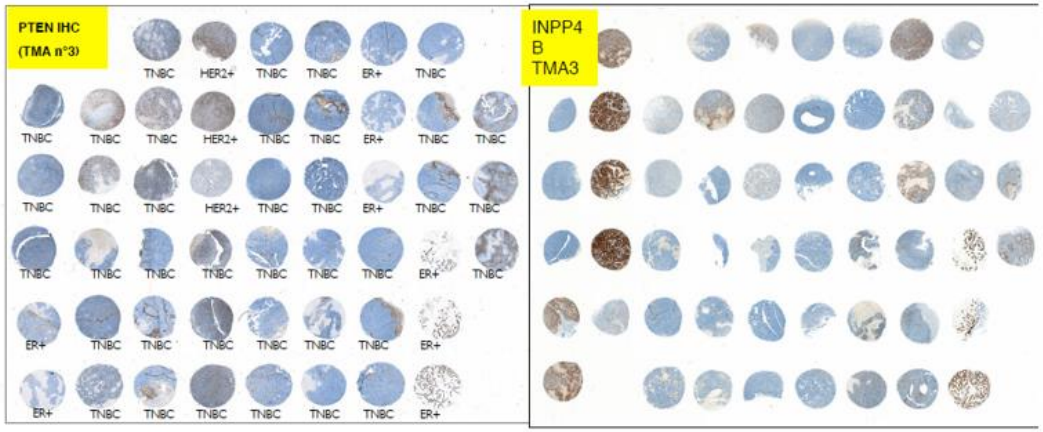
PDX Model

Gene expression is stable over time (tumor passage)

BUT

There are gene differences when comparing the xenograft with the patient

Expression of tumor supressors is lost in 60-70% xenografts



Radiopharmaceuticals Efficacy

- **Biodistribution and Pharmacokinetics**
  - Rapid uptake in target tissue
  - High affinity and selectivity
  - Rapid clearance from blood and non-target organs
  - Residence time in target tissue long enough
  - Predominant kidney excretion with no tubular reabsorption
- **Specific Activity**
- **in vitro/ in vivo stability**

Factors affecting performance of radiolabelled biomolecules

- Affinity for the target (receptor)
- The target density
- The target accessibility (membrane or nuclear receptor)
- Non-target expression of the receptor
- The *in vivo* stability of biomolecule
- The choice of radionuclide
- The stability of the radiolabelled biomolecule complex
- The physicochemical properties of the radiolabelled biomolecule (size, charge, lipophilicity)

Prerequisite for effective *in vivo* tumor targeting

- ***In vivo*** **metabolic stability in the biological milieu**

  (metal chelate; enzymatic peptide chain)
- **Radiolabelled antibodies must retain imunoreactivity**
- **Radiolabelled peptides must retain receptor binding ability**
- **High radiochemical purity**
- **High specific activity** **(at least 1Ci/umol peptide)**

Unlabeled peptide bioconjugate would occupy saturable receptor sites

Prerequisite for effective *in vivo* tumor targeting

- **High target-to-background ratio**
- **Rapid clearance from non-target organs**

  (high contrast images – diagnosis

  Minimize radiotoxicity – therapy)
- **Rapid excretion into urine**
- **Minimal hepatobiliar excretion**
- **Rapid clearance of radioactivity from kidneys improve accuracy of diagnostic and minimize nefrotoxicity during therapy**

Pharmakokinetic Aspects of Radiolabelled Antibodies *vs* Peptides

Radiolabelled Antibodies

- **Slow blood clearance (MW; circulating antigens)**

Radiolabelled Peptides

- **Rapid blood clearance**
- **More favourable pharmacokinetics**

Preclinical screening of radiolabelled biomolecules

- Determine biodistribution overtime (depends on the application)
- Determine % Radioactivity Excretion;
- Determine % I.A. per organ; % I.A. per gram;

  Dissection and counting

  Quantification by PET or SPECT camera

  Autoradiography
- Target-to-non target ratio
- Clearance of radiolabelled biomolecule and its radioactive metabolites

Fate of Radiolabelled Biomolecules in the Body

- **Absorption** **X**
- **Distribution** Reversible pass Vascular Comp → Tissues/ Organs
- **Elimination (Excretion + Metabolism)**

Pharmacokinetics parameters

Clearance = Rate of elimination / Plasma concentration

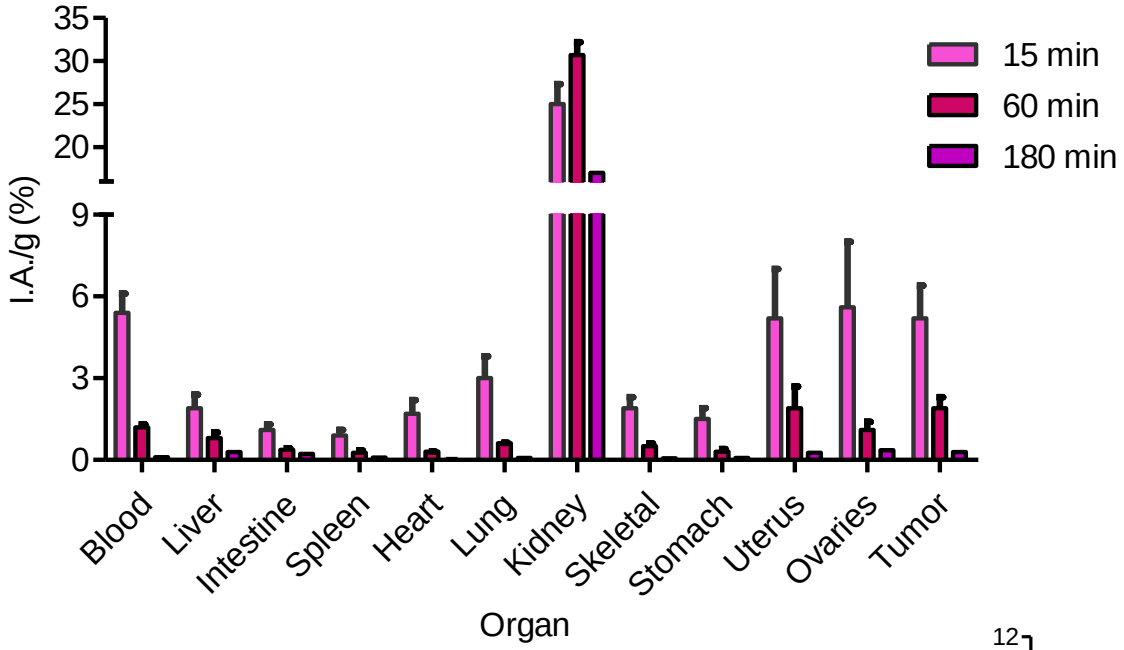
Mean residence time = 1 / *K* *k*= elimination rate constant

Biodistribution Studies

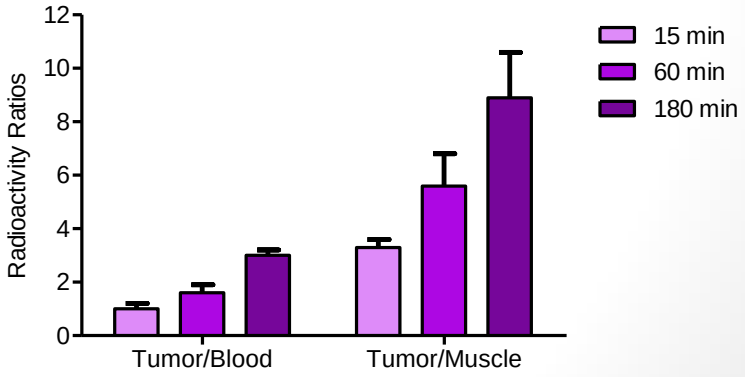
- Distribution of radiolabelled biomolecule in main organs
- Uptake and retention time in receptor-negative tissues *vs* receptor –positive tissues
- Blocking experiments by co-administration of unlabelled biomolecule
- Rate of blood clearance
- Rate and route of excretion
- In vivo stability of radiolabeled biomolecules

Biodistribution Studies

Female MCF-7 xenografts
Balb/C nude mice



- **Fast blood clearance**
- **High kidney uptake**
- **Uptake in ER rich organs (ovaries, uterus) and xenografts**



Biodistribution Studies

Species Variation

Major variations between species:

Uptake by specific organs

Clearance

Mouse heart beats much faster than the human;

Mice breath much faster

- **Shorter tissue perfusion times**
- **Shorter gastrointestinal transit time**
- **More rapid pharmacokinetics**

Biodistribution Studies

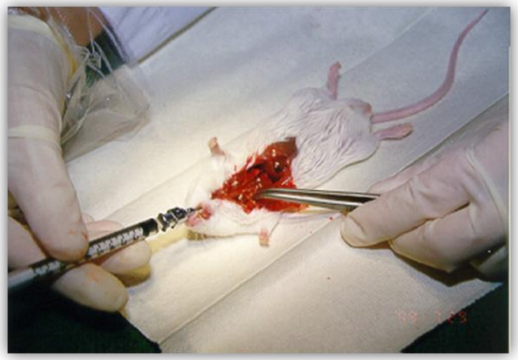
There are some limitations in extrapolating data from animal models due to:

- **Different genotypes between mice and men**
- **Size difference – specially dosimetric calculations**
- **Faster sequestring and metabolizing**

Biodistribution Studies

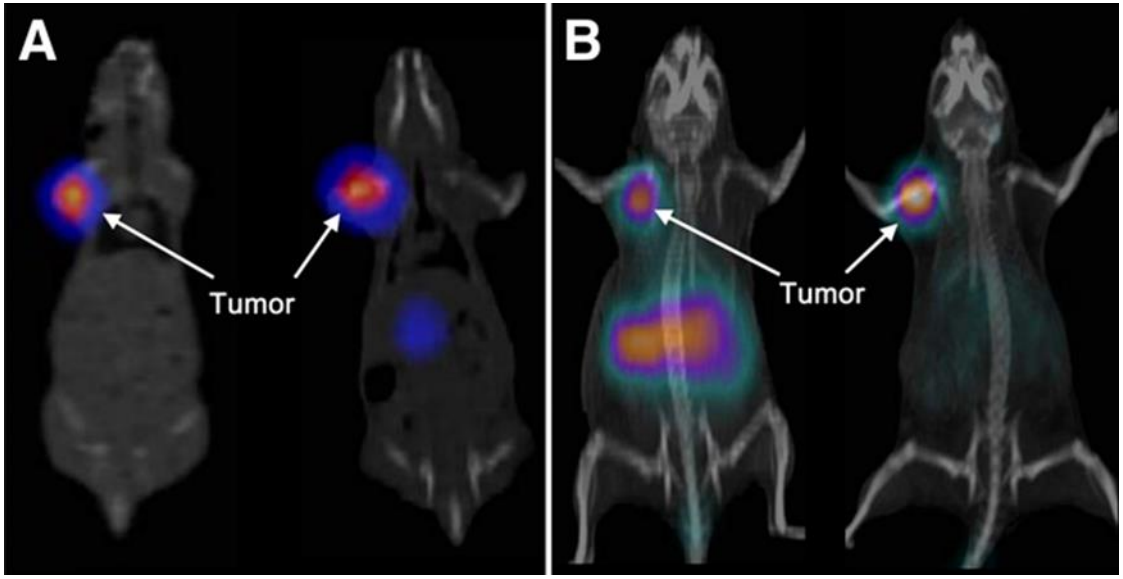
Experimental Procedure

- Administration of radiolabelled molecules (i.v.; i.p.);
- Measure I.A.;
- Sacrifice , weight, whole body radioactivity measrument;
- Organ dissection, weight and counting
- Determination- % Excreted activity; %I.A./g ; % I. A. /total organ



Bombesin antagonis radioligands

SPECT/CT PET



^{99m}Tc ^{111}In ^{68}Ga ^{64}Cu

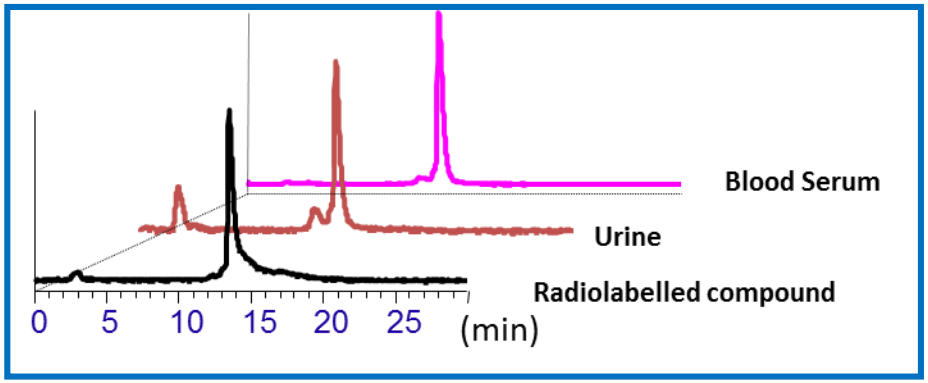
Agonist radioligands have been preferred since they internalize after receptor binding promoting intracellular accumulation of radionuclide

Antagonists can bind more receptors - High receptor occupancy

Biomolecules labelled with different radionuclides – Different biodistribution patterns

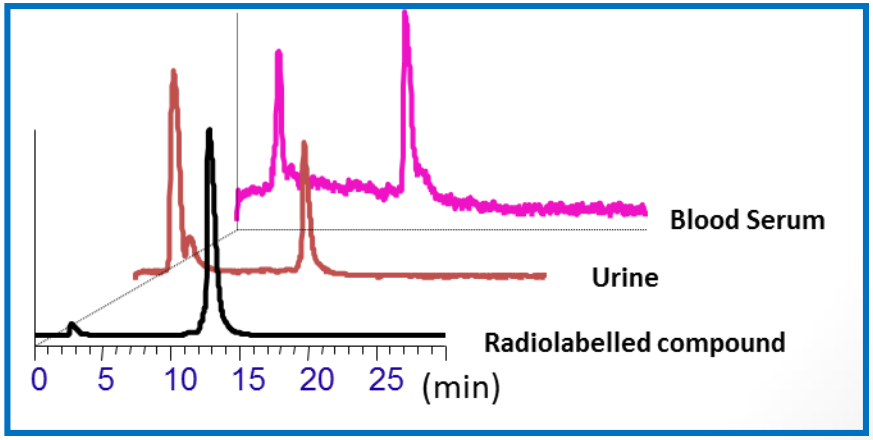
In vivo Stability Studies

HPLC analysis of samples of blood serum; collected urine and organ homogenates (liver, kidney, brain,…)



Treatment of biological samples (protein precipitation)

Chromatographic analysis (HPLC)



In vivo Stability Studies

HPLC analysis of samples of urine collected at sacrifice time

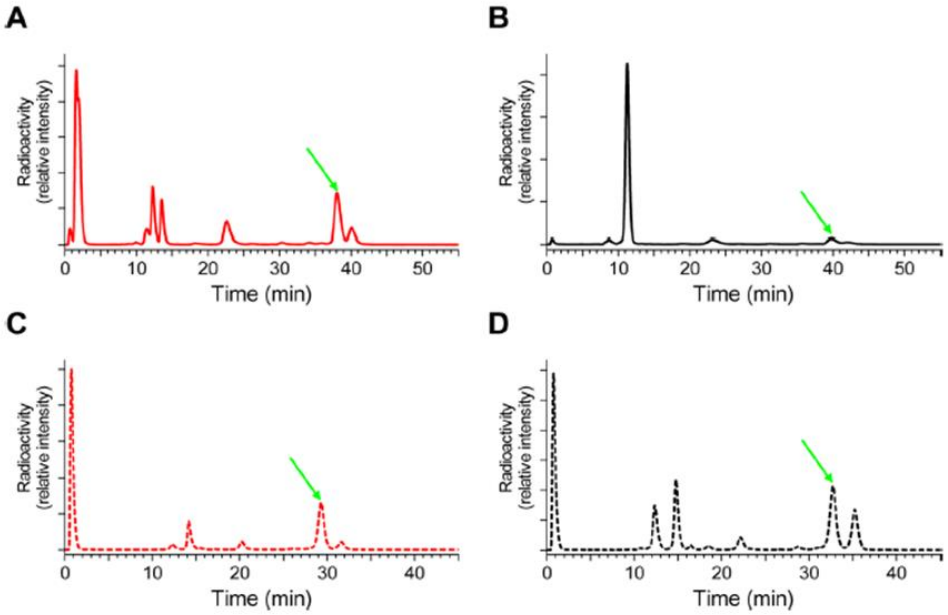


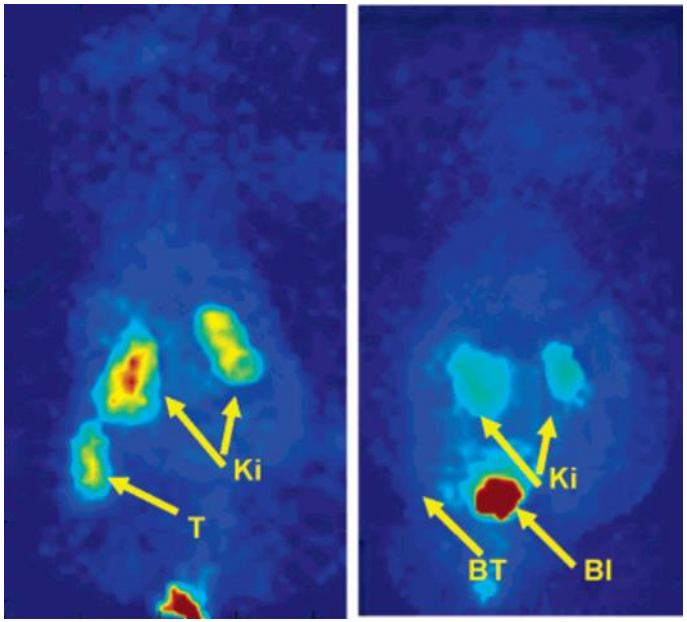
Figure 4. Representative radiochromatograms of blood samples collected 5 min postinjection in mice for (A) [^{111}In]**1** (t_R = 38.0 min), (B) [^{111}In]**2** (t_R = 39.8 min), (C) [^{111}In]**3** (t_R = 29.3 min), and (D) [^{111}In]**4** (t_R = 32.7 min); co-injection with labeling reaction samples indicated the position of parent radiopeptides (green arrow); chromatographic system 3 was applied in analyses.

J Nucl Med 2011 52:1970

Biodistribution Studies

Receptor blockade experiments

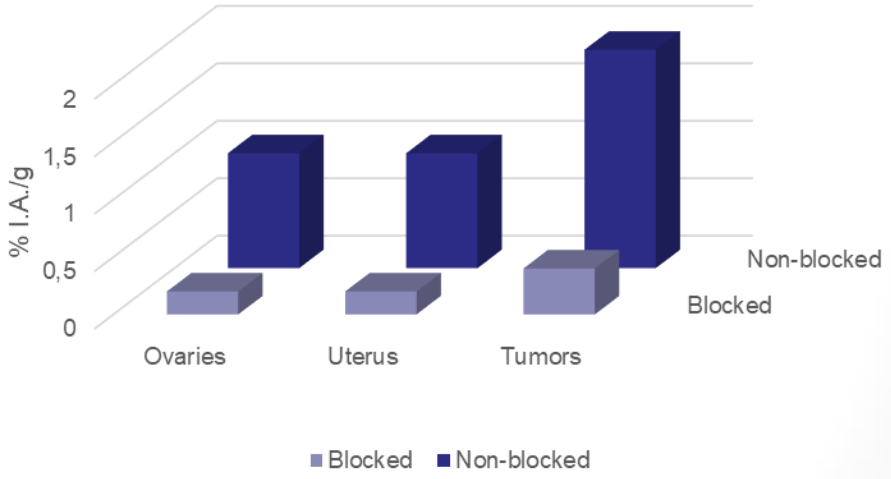
PC-3 xenograft bearing mice



100ug Tyr-BBN

J Med Chem 48:100

Blockade with co-injection of peptide excess



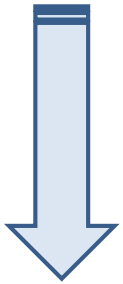
Uptake decrease in receptor rich organs

Peptide Radionuclide Receptor Therapy

- Specific
- Rapid tumor uptake
- Long residence time into tumor
- Rapid clearance from non target organs

- Improve patients quality of life
- Pain relief
- Tumor regression
- Decrease level of tumor markers

Clinical success of therapy



Nefrotoxicity

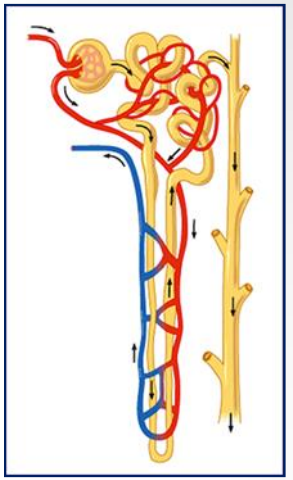
Mechanisms of Urinary Excretion

- Glomerular Filtration

- Tubular Resorption – Active process (proximal tubule)

  Passive process (distal tubule)

- Tubular Secretion – active process (proximal tubule)



Peptide Radionuclide Receptor Therapy

Nefrotoxicity – limits the administration dose

Neuropeptides

- Predominant renal excretion
- Glomerular filtration
- Resorption in proximal tubule
- Retention in lisossomes

High radioactivity concentration in the kidney

Peptide Radionuclide Receptor Therapy

Strategies to reduce nefrotoxicity → **Reduce kidney uptake**

- **Co-administration of positive charged aminoacids solution (lysine and arginine) - 33 a 40%**

- **Gelofusine**

  Increases excretion of megalin ligands

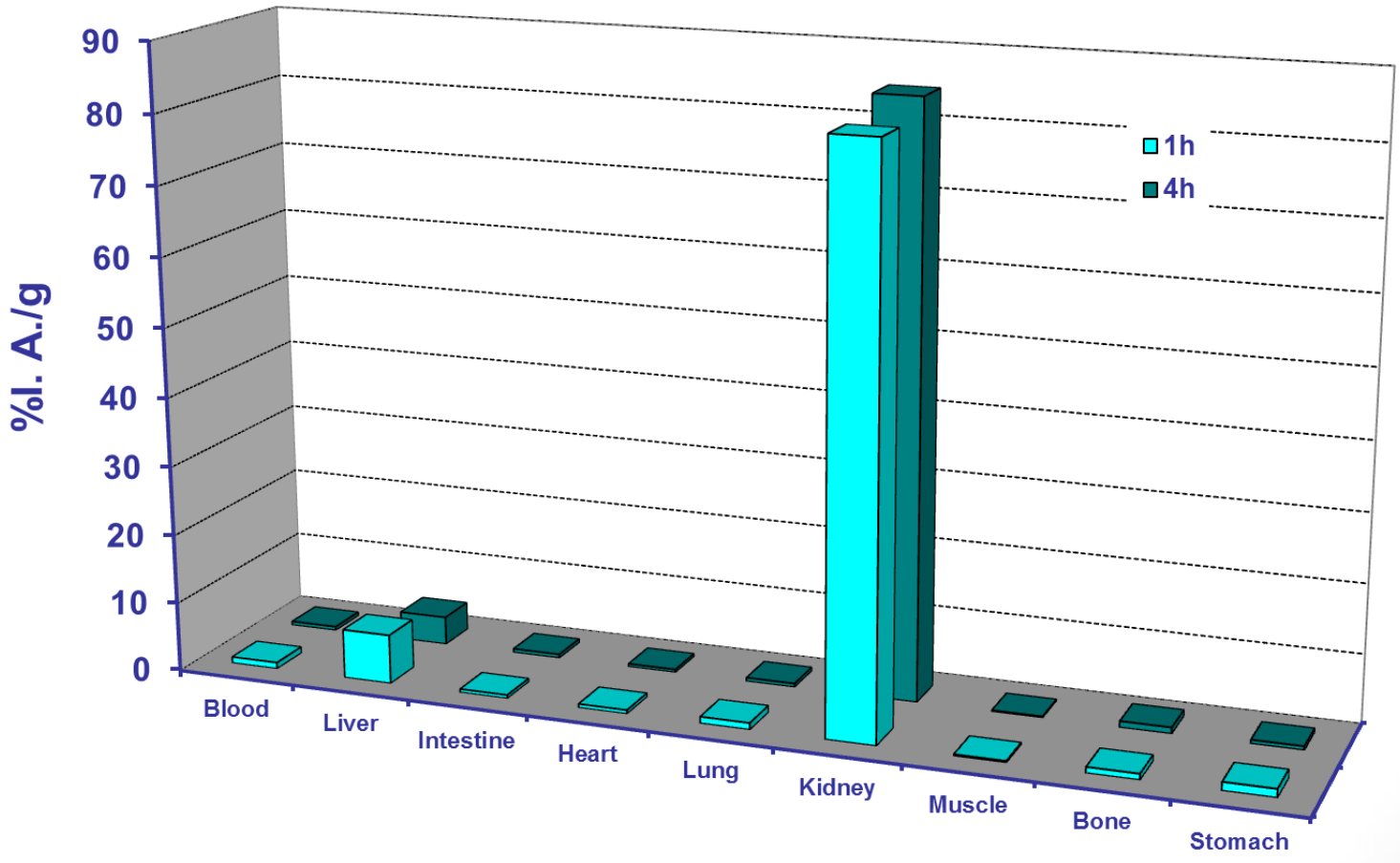
- **Colchicine**

  Blocks microtubules function – essential to endocitosis

- **Co-administration of albumin fragments (3-50 kDa)**

  Interfers megalin mediated resorption

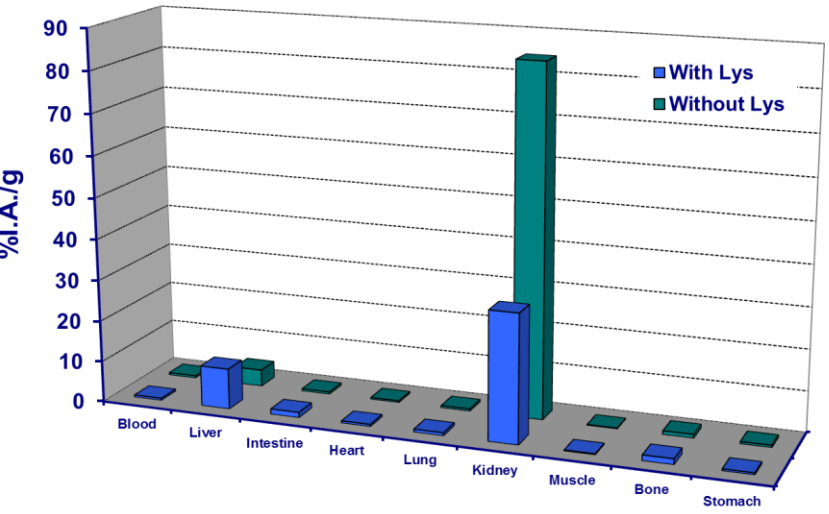
Biodistribution in mice



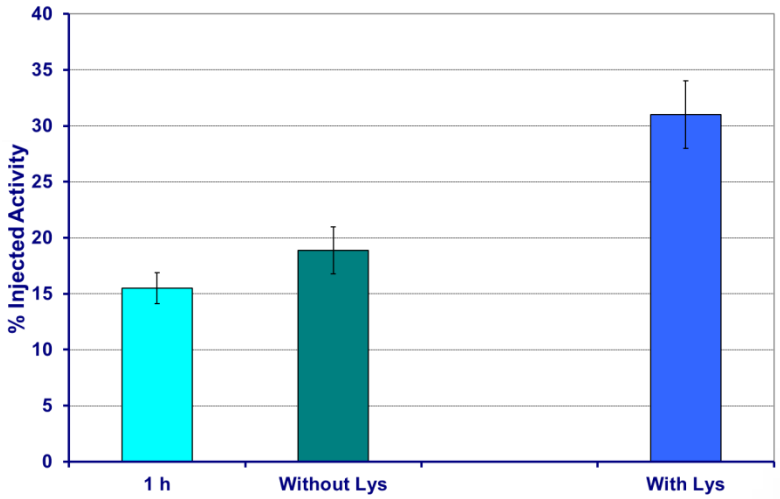
Strategies to reduce nefrotoxicity

Treatment with Lysine

Biodistribution profile



Excretion

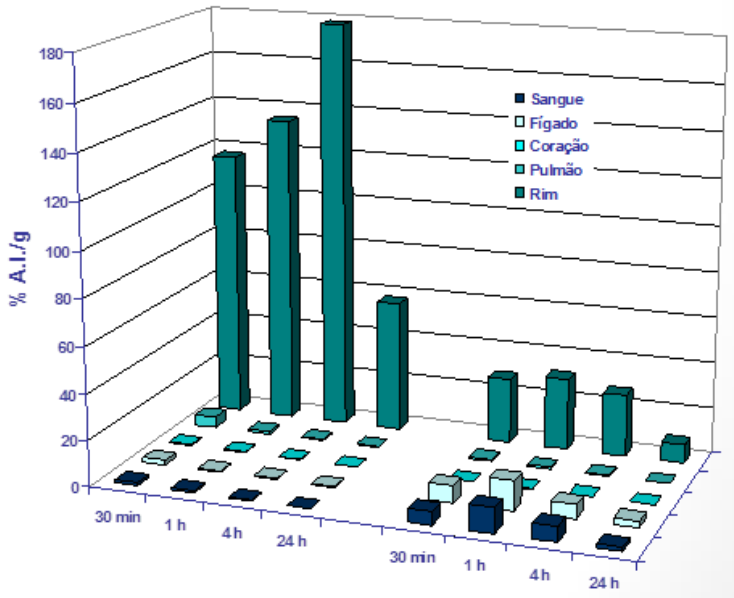


Peptide Radionuclide Receptor Therapy

Strategies to reduce nefrotoxicity

New peptide analogues with improved biological profile

- Higher receptor affinity
- Prolonged tumor retention time
- Faster renal clearance and rapid excretion




Thank you!