

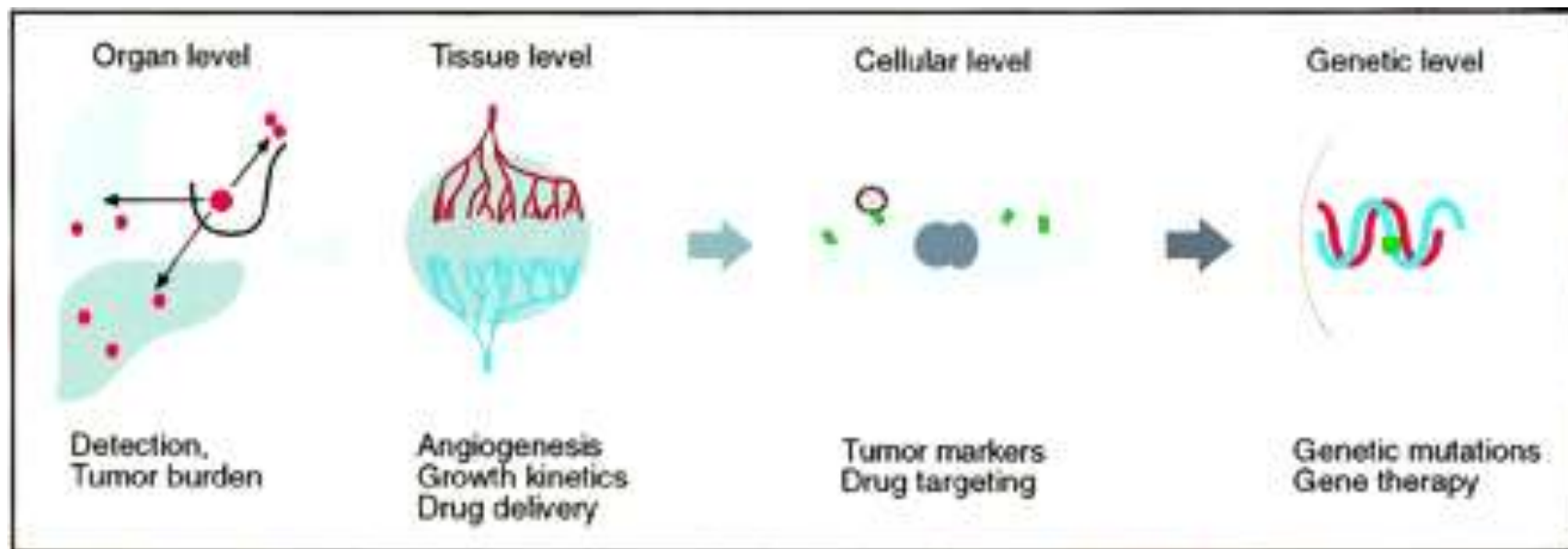


Biodistribution et Métabolisme par l'Imagerie

Claire Corot, JS Raynaud, JF Salazar, S Catoen RECHERCHE GUERBET

18 mai 2011 Journée Nationale Académie de Pharmacie

Imagerie et Biologie : Différents niveaux



Structure
Fonction
organe

Physio-
Pathologie
Tissue

Cellule
Composant
Matrice

Genome

Les facteurs clés de succès en imagerie



Résolution spatiale

Résolution temporelle

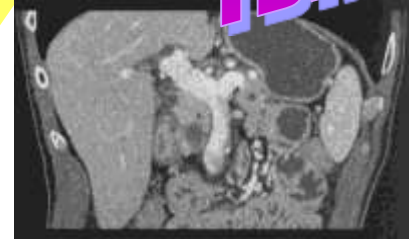
Imagerie Médicale :
Les fondamentaux

Contraste

IRM



TDM



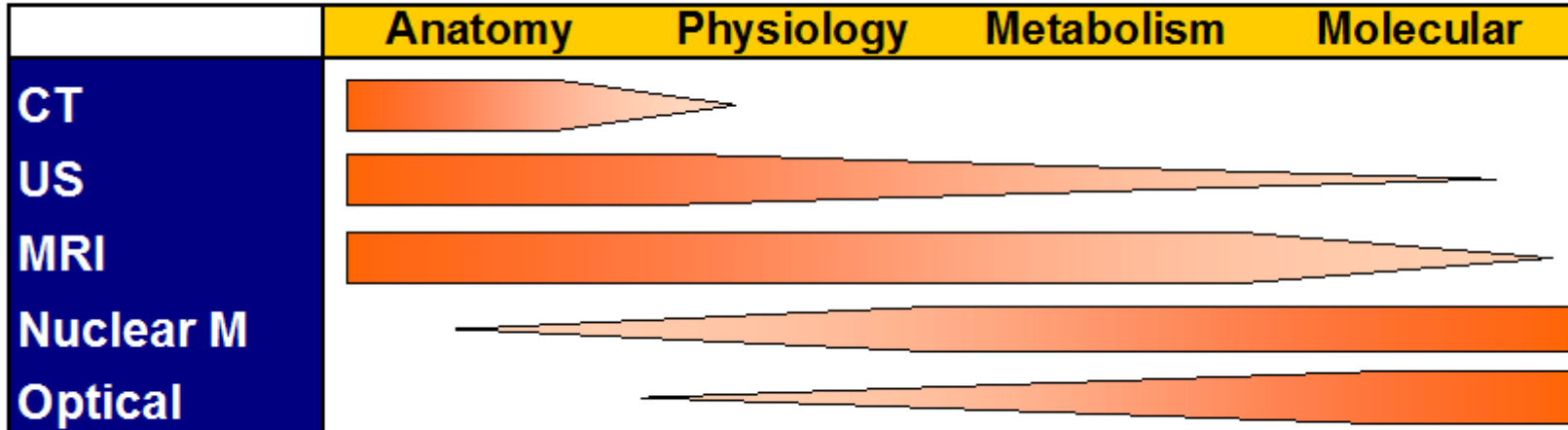
UltraSon



Med Nucl



Modalités d'imagerie : Positionnement

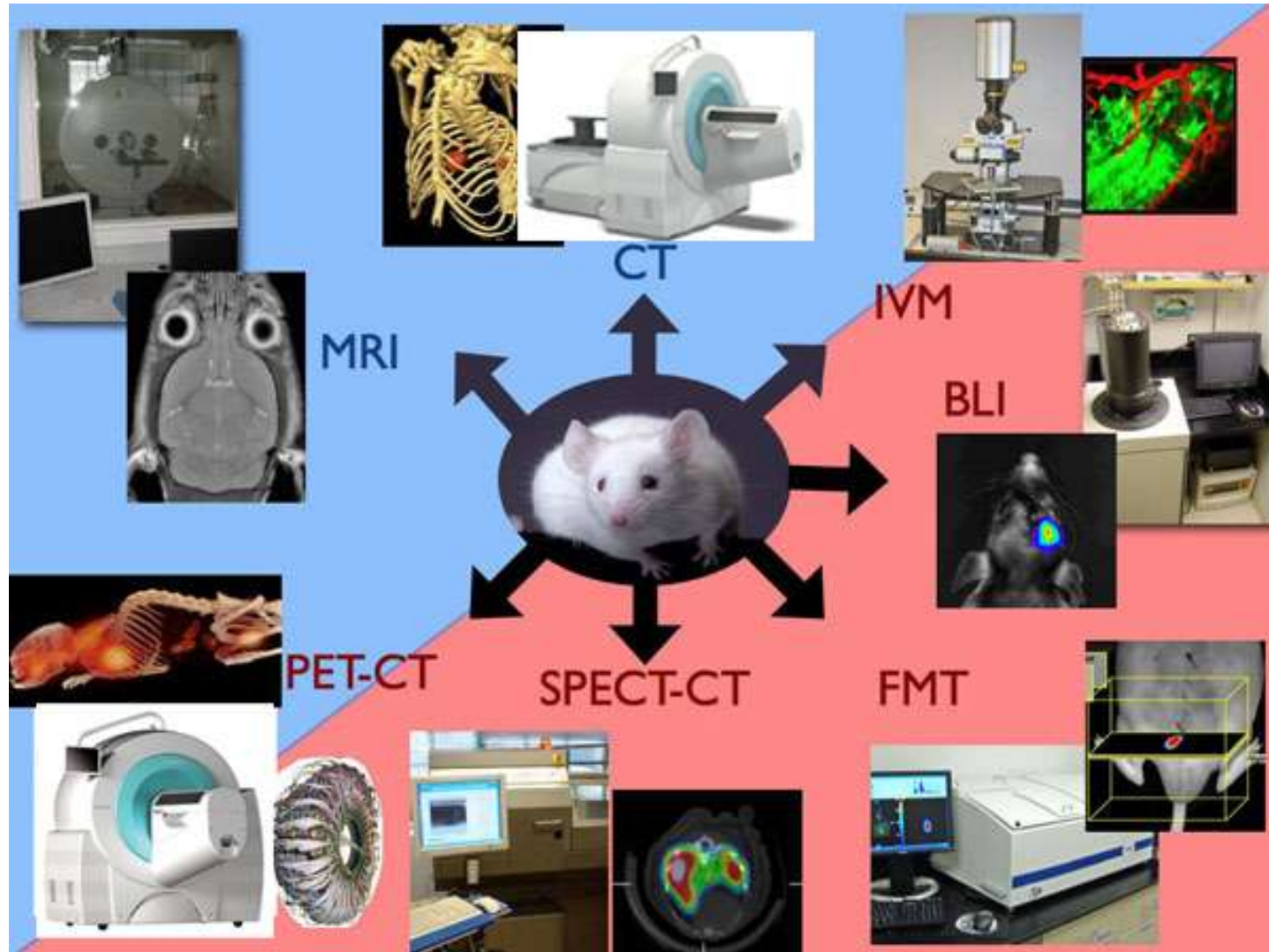


Relative Attributes of Molecular Imaging Modalities

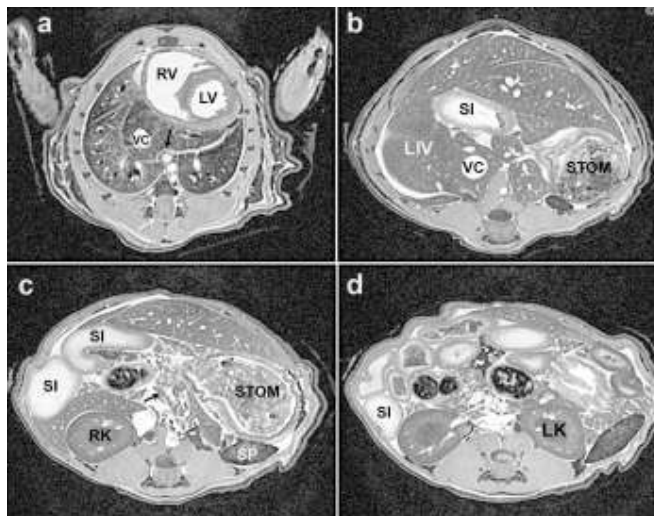
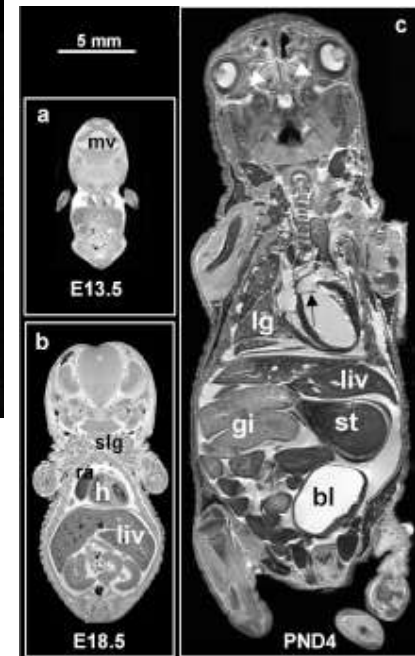
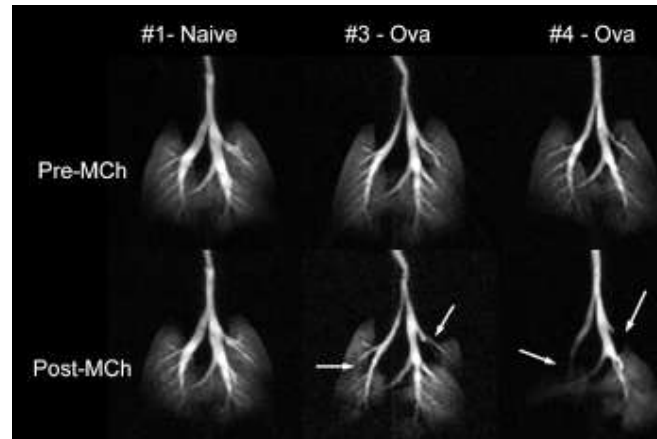
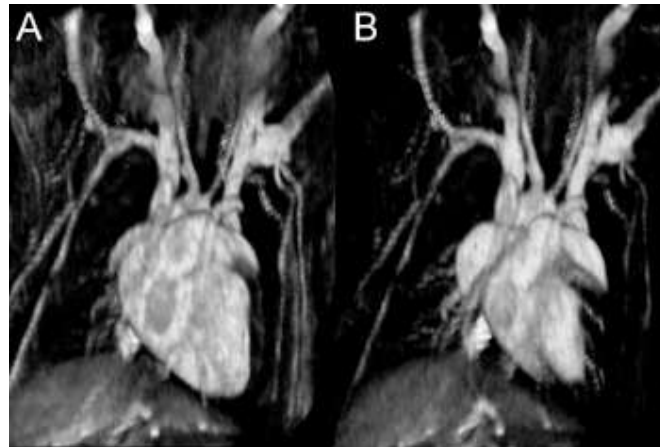
Modality	Sensitivity	Resolution		
		Spatial	Temporal	Contrast
CT	+	++	+	++
MR imaging	++	+++	++	+++
Nuclear medicine	+++	+	+	++
Optical imaging	+++	+	+++	+++
US	++	+++	+++	++

Note.—+ = low resolution, ++ = intermediate resolution, +++ = high resolution.

Développement de plateformes d'imagerie pour le petit animal Center For Molecular Imaging Research MGH - Boston



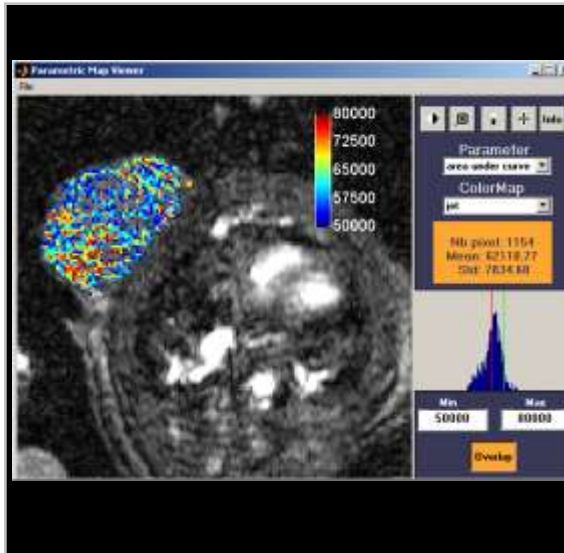
L'IRM à haute résolution





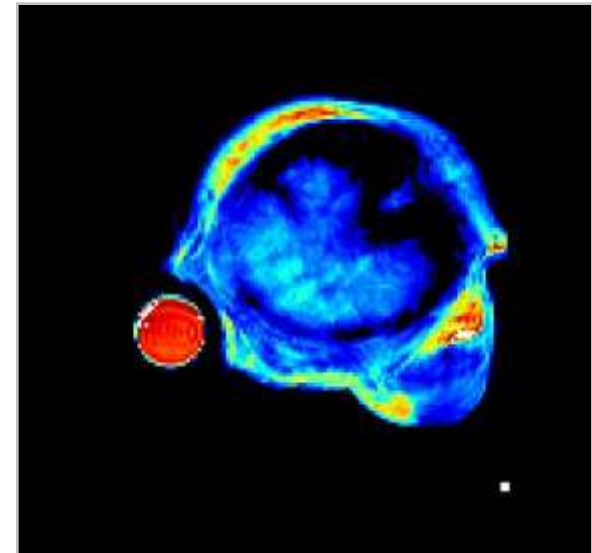
- Imagerie anatomique

1^{ère} génération de produits de contraste



- Imagerie fonctionnelle

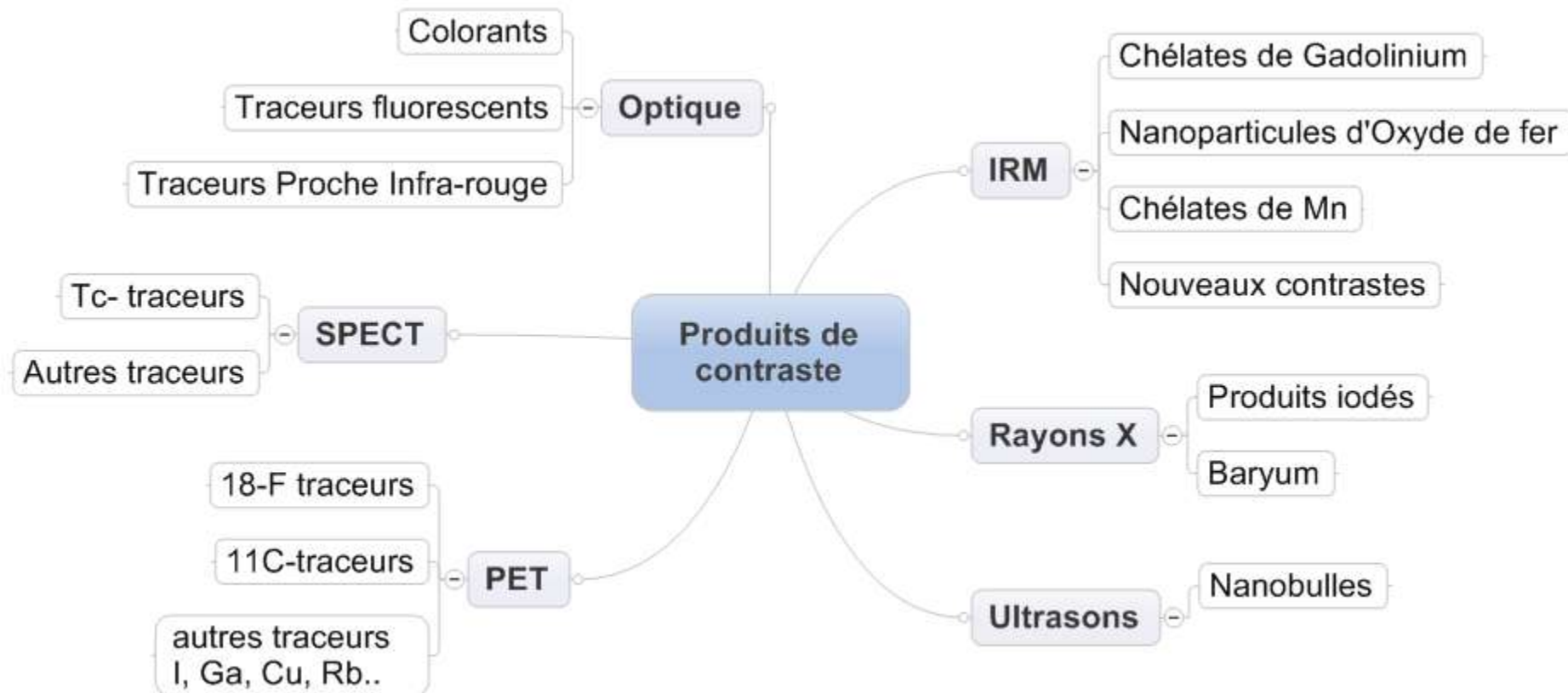
2^e génération de produits de contraste

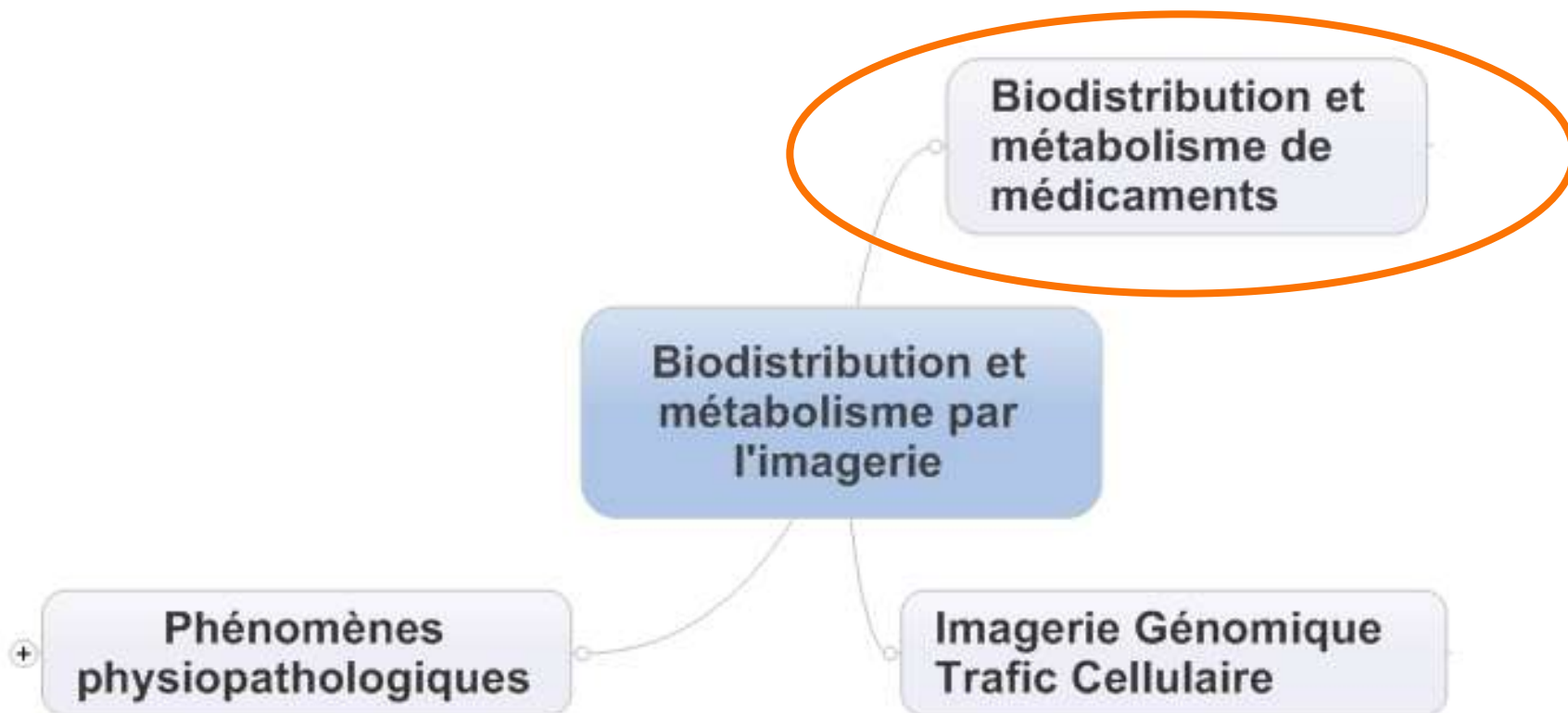


- Imagerie moléculaire

3^e génération de produits de contraste

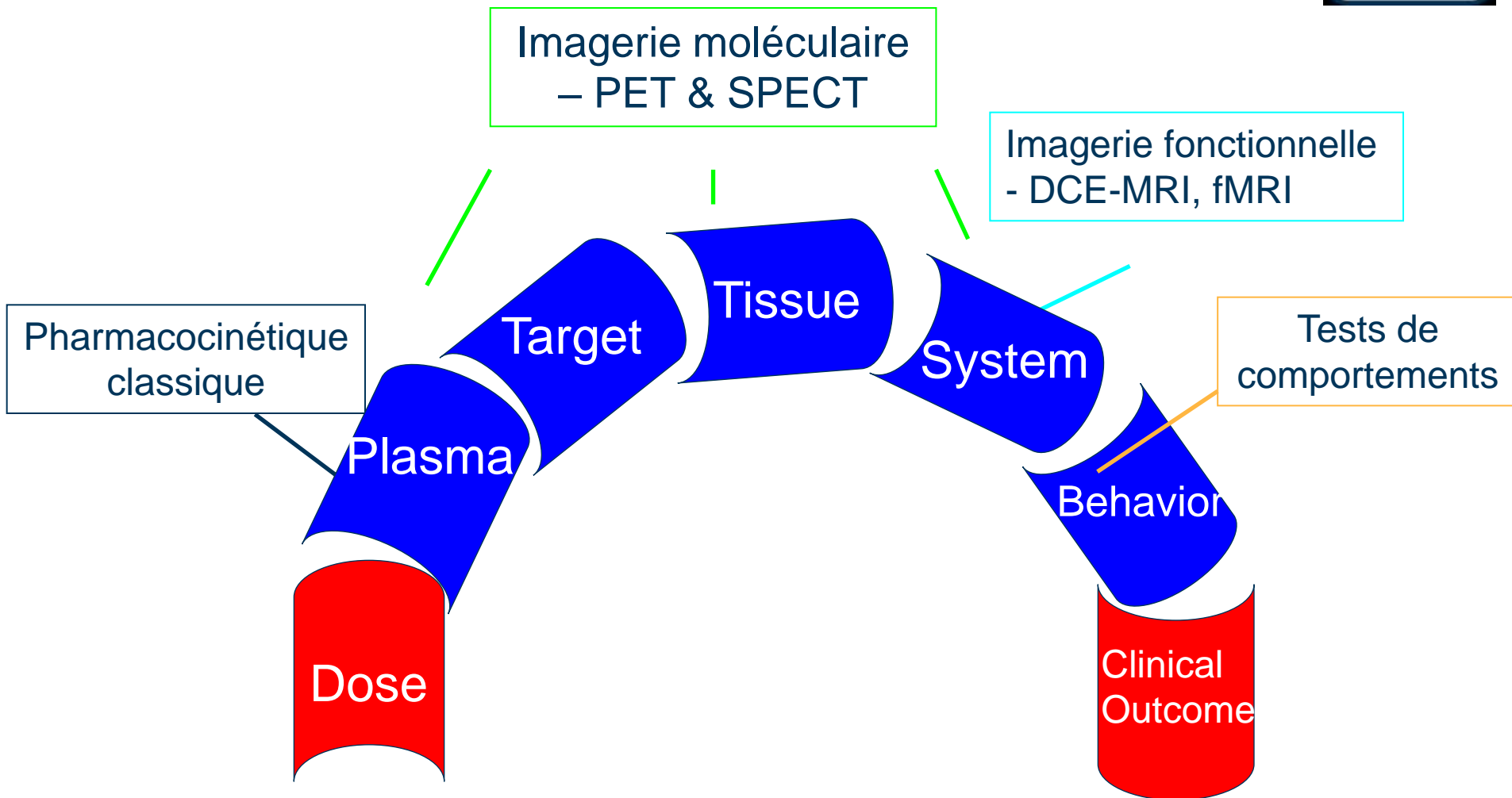
Les agents de contraste en imagerie



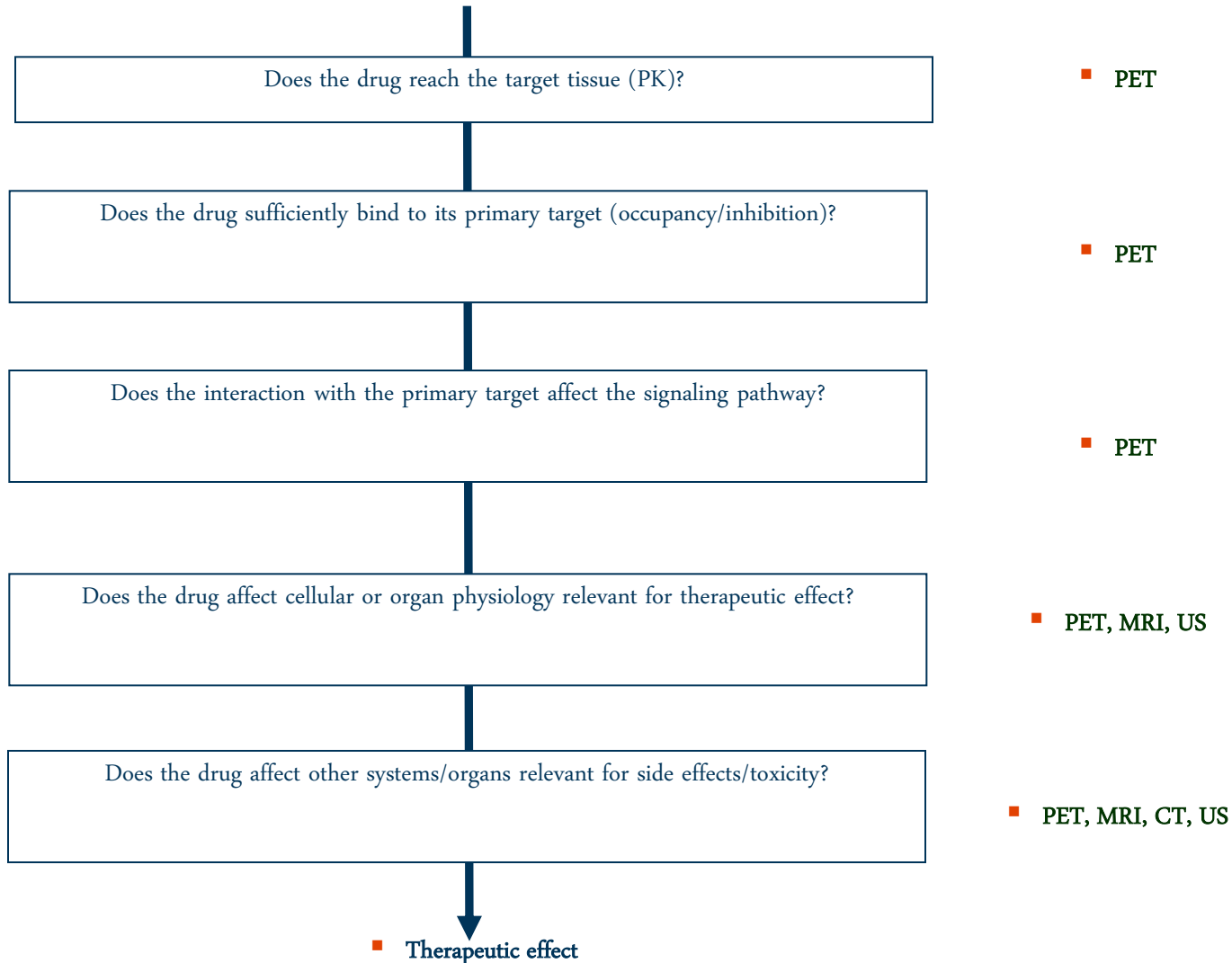


Imagerie translationnelle

Développement de médicaments en Neurologie



DÉveloppement de médicament : Objectifs en imagerie expérimentale



Le champ d'applications de l'imagerie médicale

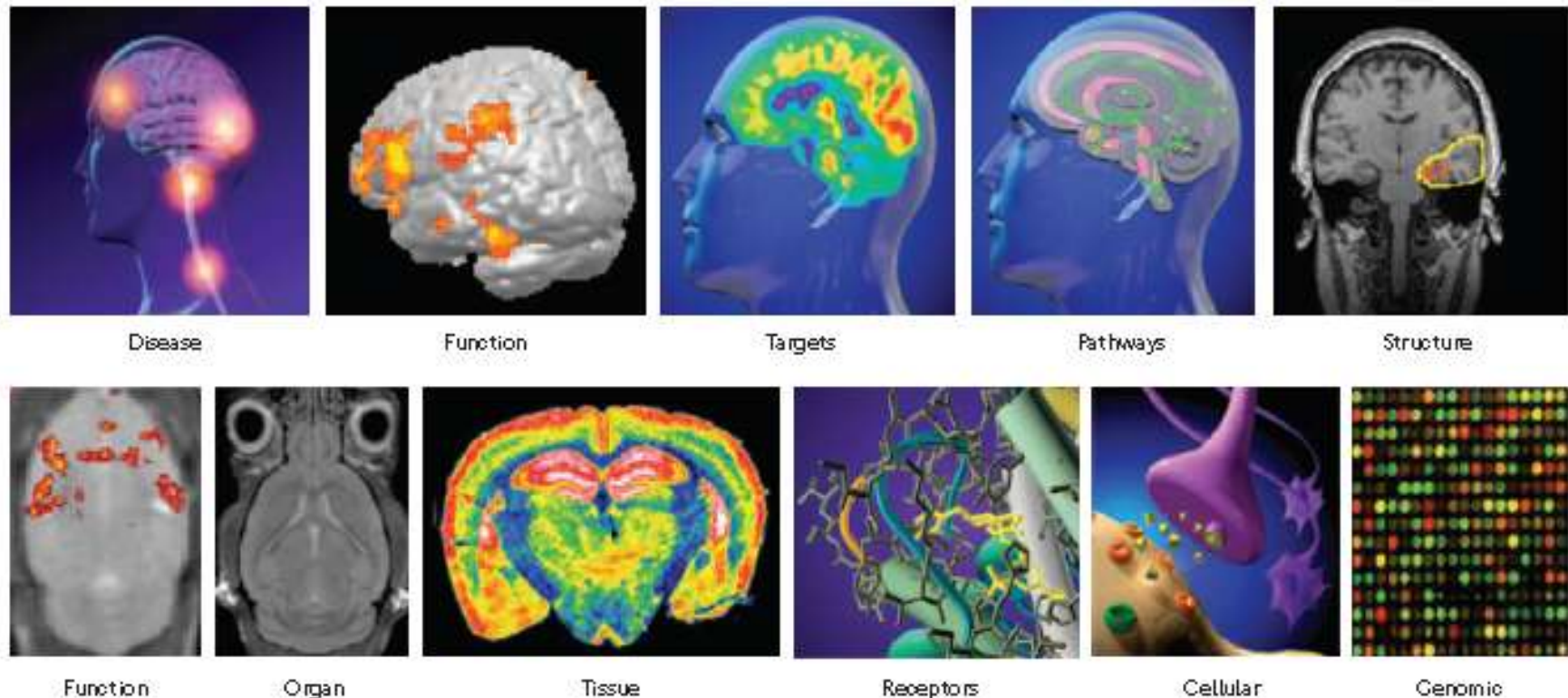


Figure 1 | Locus of imaging in drug development — from disease to genomics and back again. The figure shows the spectrum that imaging covers from molecular (for example, positron emission tomography (PET)) and functional (for example, fMRI) imaging to its use in molecular diagnostics and a link to genomic profiling in the clinical and preclinical domains. Potential targets (circuits or specific regions) can be evaluated in the context of the role they play in functional pathways, and can be studied in the preclinical domains using more invasive processes, including molecular and cellular imaging, before detailed evaluation using high-field and high-resolution functional, neuroanatomical and microimaging techniques. (Thanks to K. Moldoff for permission to use some of these graphics)

Biodistribution et évaluation du taux d'occupation

Exemple d'application pour le développement de médicaments en neurologie



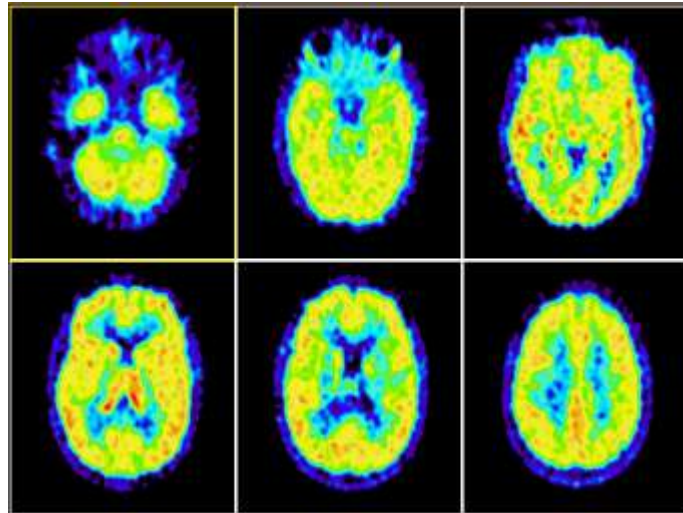
Biodistribution

- Measures brain uptake of the radiolabelled drug candidate
- Requires radiolabelling of the drug candidate with C-11 or F-18, often feasible

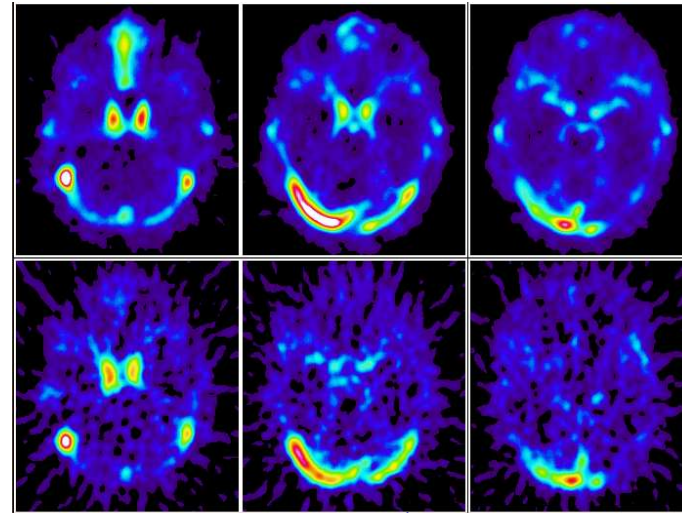
Occupancy

- Measures the effect of a drug candidate on the binding of a radiolabeled ligand to a specific target.
- Provides direct measure of occupancy.
- Requires the existence or development of a radioligand, not always feasible

Passage de médicaments à travers la BHE



Good uptake SUV=3

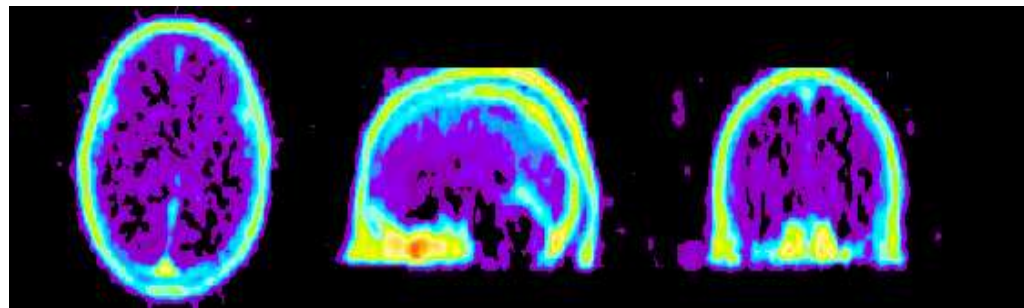


No uptake

no extra-vascular component

Drug

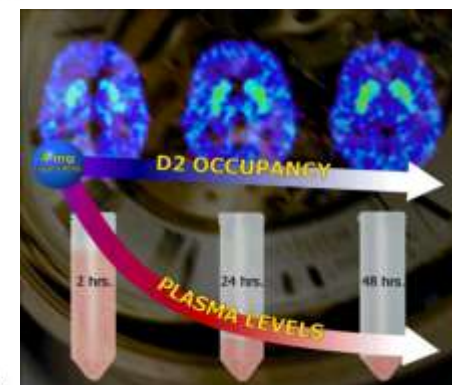
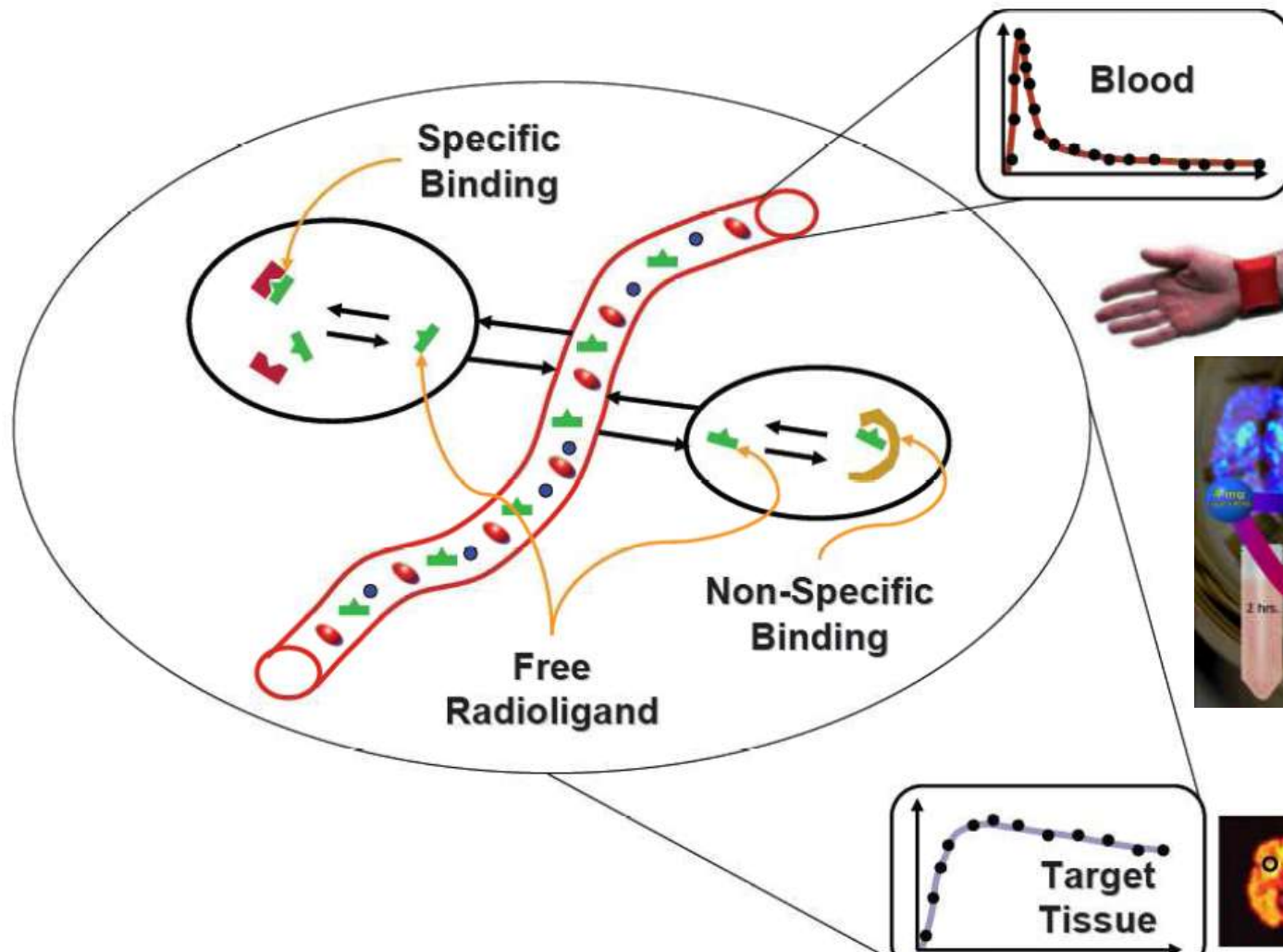
Blood
volume



Some uptake in brain

extravascular component SUV= 0.1

Modélisation Données PET : Quantification

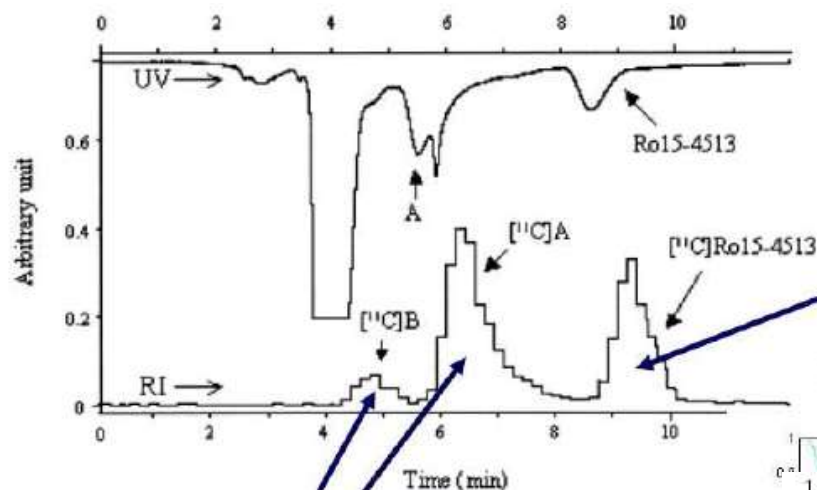


*J Tauscher & al
Molecular Psychiatry (2002)*

Estimation des paramètres physiologiques

Quantification Traceur PET

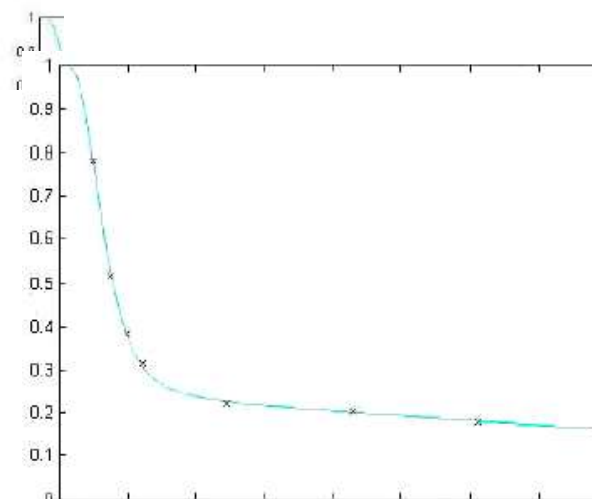
Prise en compte du métabolisme dans les données d'entrée



parent

metabolites

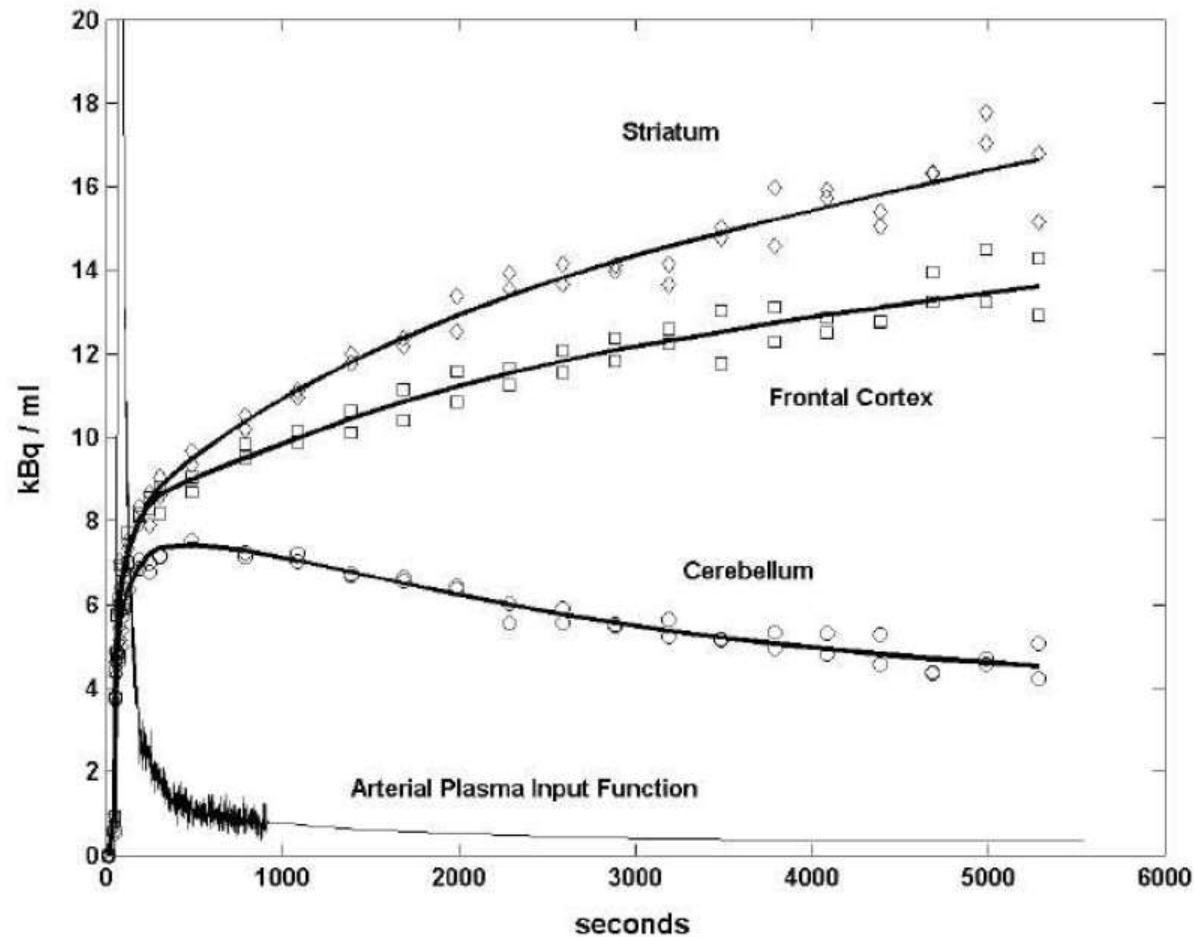
parent fraction in plasma



PET Radiotracer biodistribution : Quantification



- Time-activity curves (TACs) in Region of Interest (ROI)



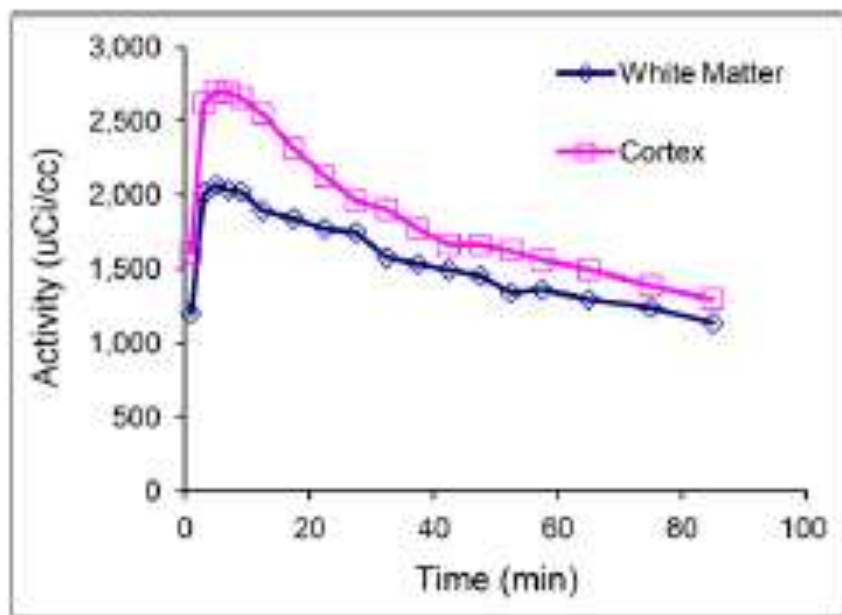
Imagerie des plaques amyloïdes : Cinétique de captation cérébrale et wash out



Preclinical Properties of ¹⁸F-AV-45: A PET Agent for A β Plaques in the Brain

Seok Rye Choi¹, Geoff Golding¹, Zhiping Zhuang¹, Wei Zhang¹, Nathaniel Lim¹, Franz Hefti¹, Tyler E. Benedum¹, Michael R. Kilbourn², Daniel Skovronsky^{1,3}, and Hank F. Kung^{3,4}

¹Avid Radiopharmaceutical Inc., Philadelphia, Pennsylvania; ²Department of Radiology, University of Michigan, Ann Arbor, Michigan; ³Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania; and ⁴Department of Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania



Metabolite analysis of [¹⁸F]Florbetaben (BAY 94-9172) in human subjects: a substudy within a proof of mechanism clinical trial

M. Patt · A. Schildan · H. Barthel · G. Becker ·
M. H. Schultze-Mosgau · B. Rohde ·
C. Reininger · O. Sabri

J Radioanal Nucl Chem (2010) 284:557–562

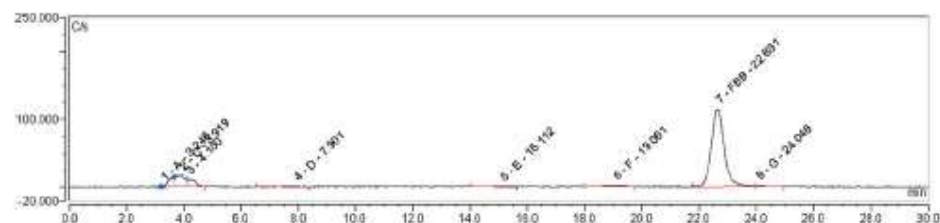


Fig. 5 Radiochromatogram of a protein-free plasma sample taken 3 min p.i. of 300 MBq [¹⁸F]Florbetaben, using HPLC (column PRP-1, 305 × 7 mm, 10 μm, flow 3 mL/min, injection volume 5 mL, gradient: Table 1)

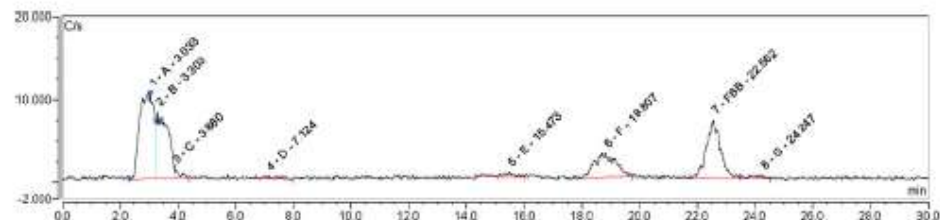
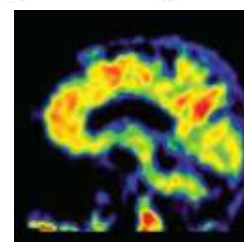


Fig. 6 Radiochromatogram of a protein-free plasma sample taken 20 min p.i. of 300 MBq [¹⁸F]Florbetaben, using HPLC method B (column PRP-1, 305 × 7 mm, 10 μm, flow 3 mL/min, injection volume 5 mL, gradient: Table 1)



Barthel P
Lancet Neurol 2011

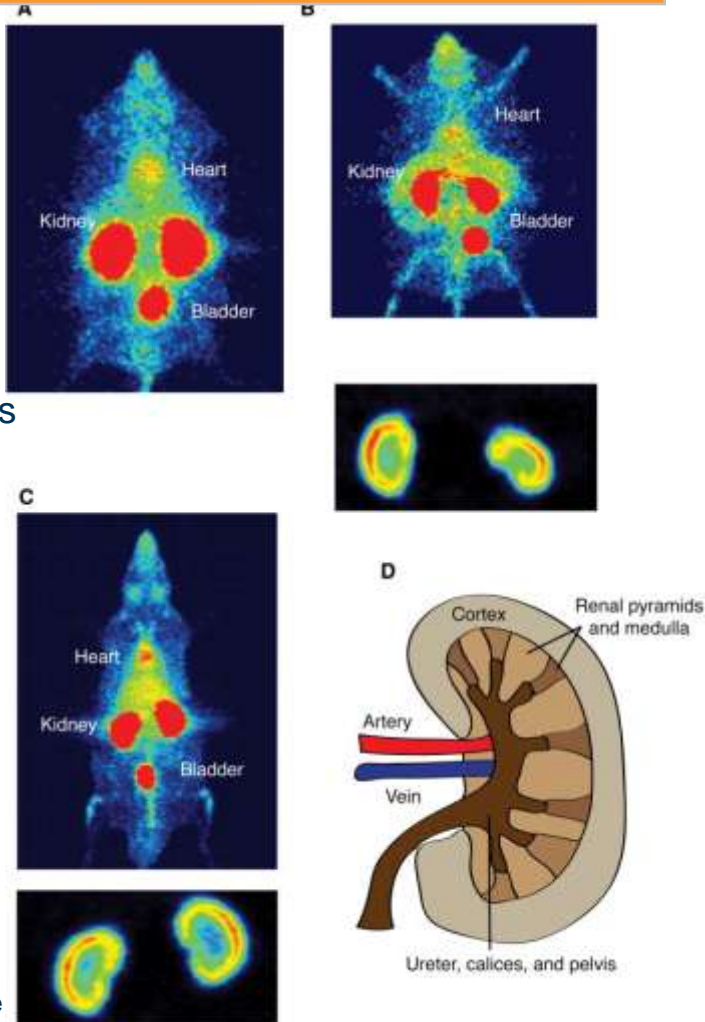
Guerbet

PET Imaging of Leptin Biodistribution and Metabolism

in Rodents and Primates Ceccarini G & al, Cell Metab 2009, 10(2):148-159



Leptin is taken up in the cortex of the kidney

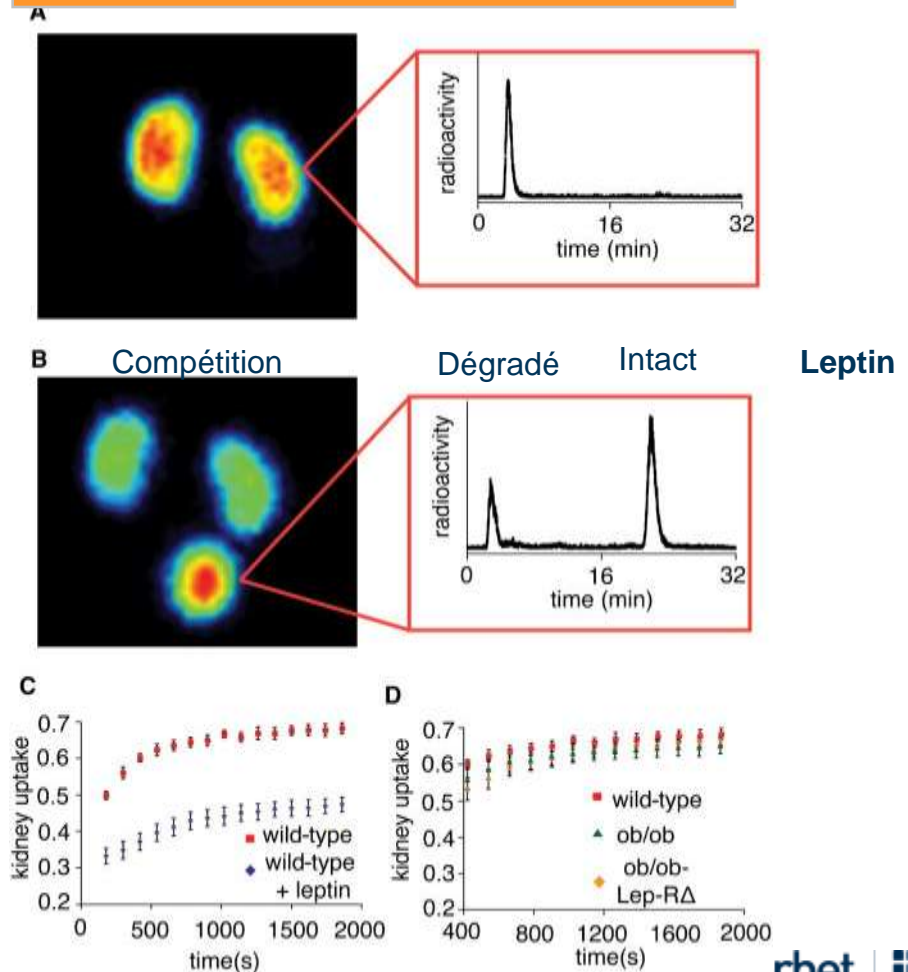


Souris

Rat

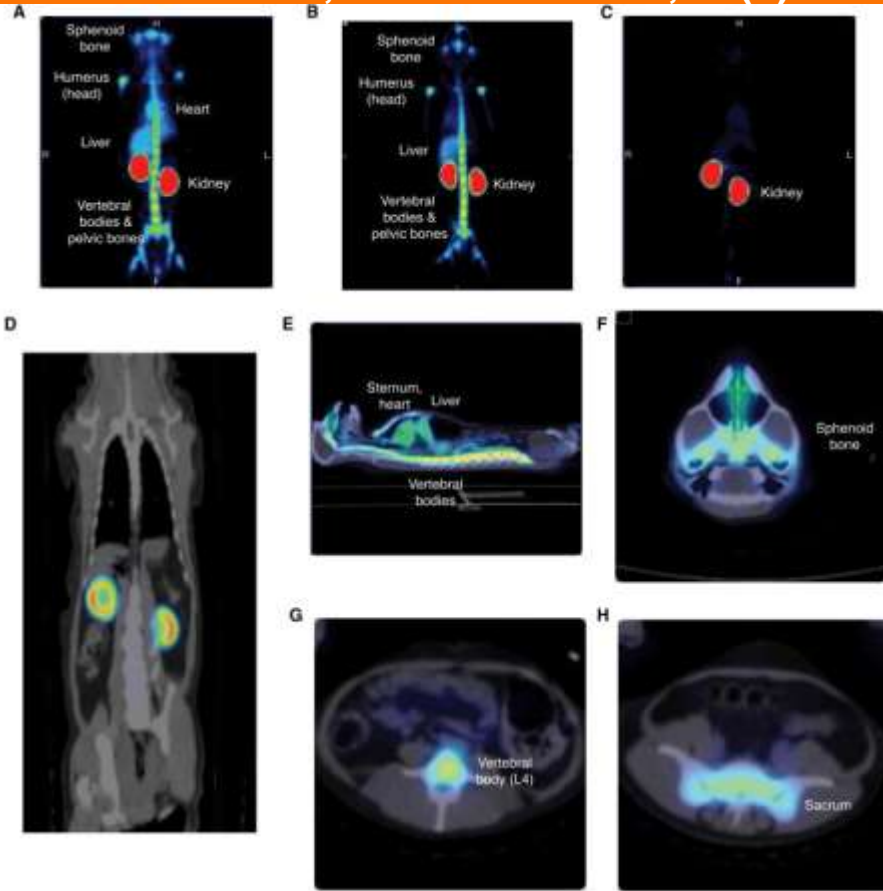
Page

Blocking experiments of leptin uptake in the kidney in wild type and Lep-R deficient animals



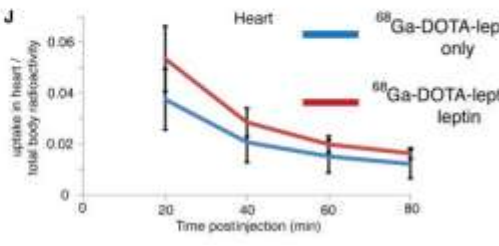
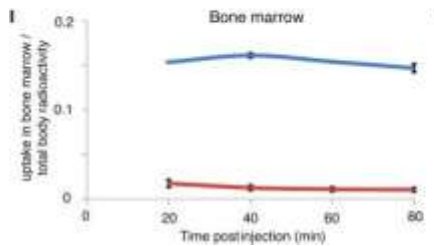
PET Imaging of Leptin Biodistribution and Metabolism in Rodents and Primates

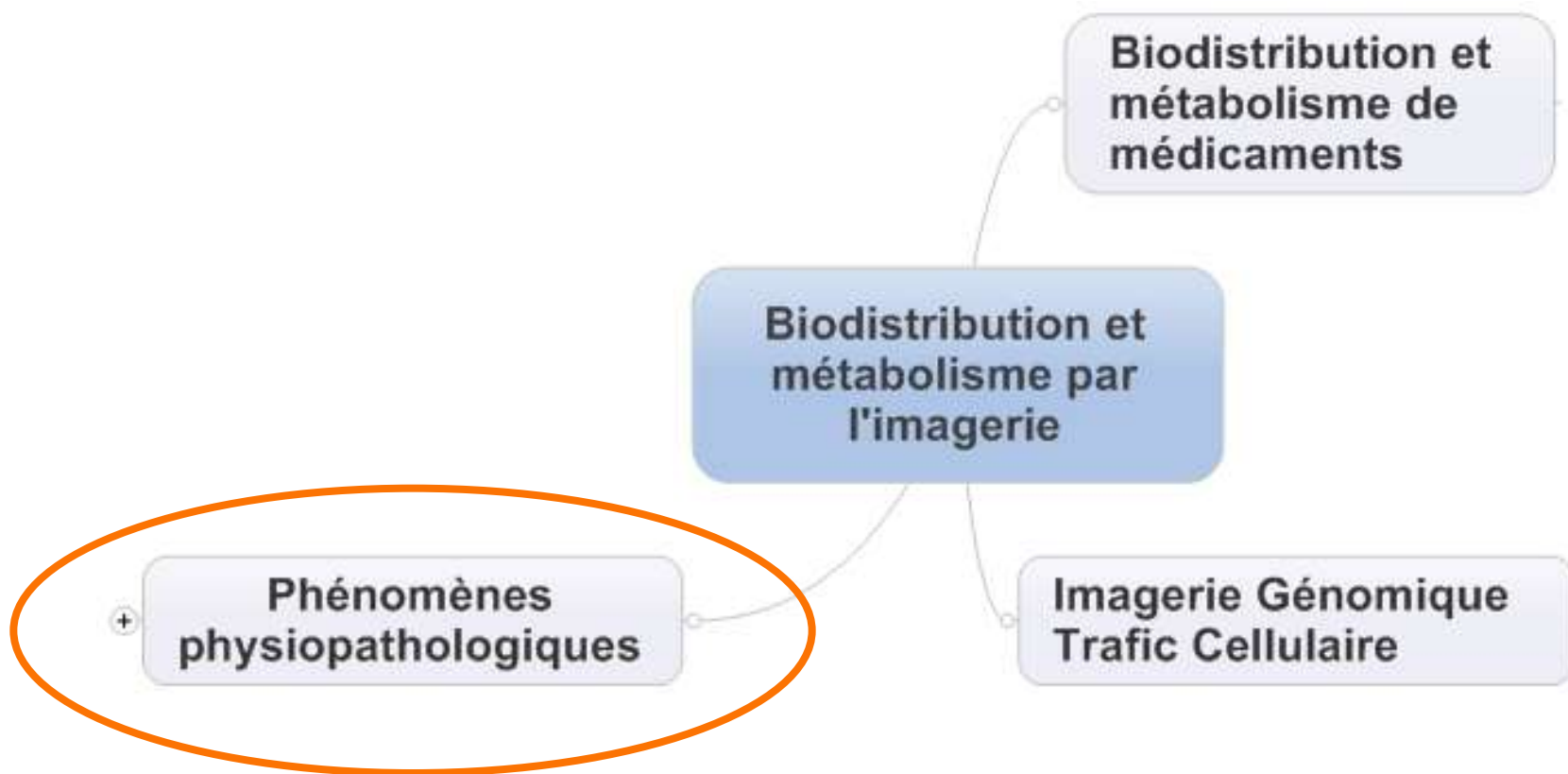
Ceccarini G & al, Cell Metab 2009, 10(2):148-159



Primate PET imaging

- Images were acquired 10 minutes post injection of 15 MBq ^{68}Ga -DOTA-leptin or ^{18}F -FBA-leptin. A-B) Coronal MIP acquired 10 minutes post injection
- In addition to kidney
- substantial leptin binding to red bone marrow in rhesus macaques
- 15.8 and 16.4% of ^{68}Ga -DOTA-leptin localized to and was retained in the bone marrow in a saturable manner



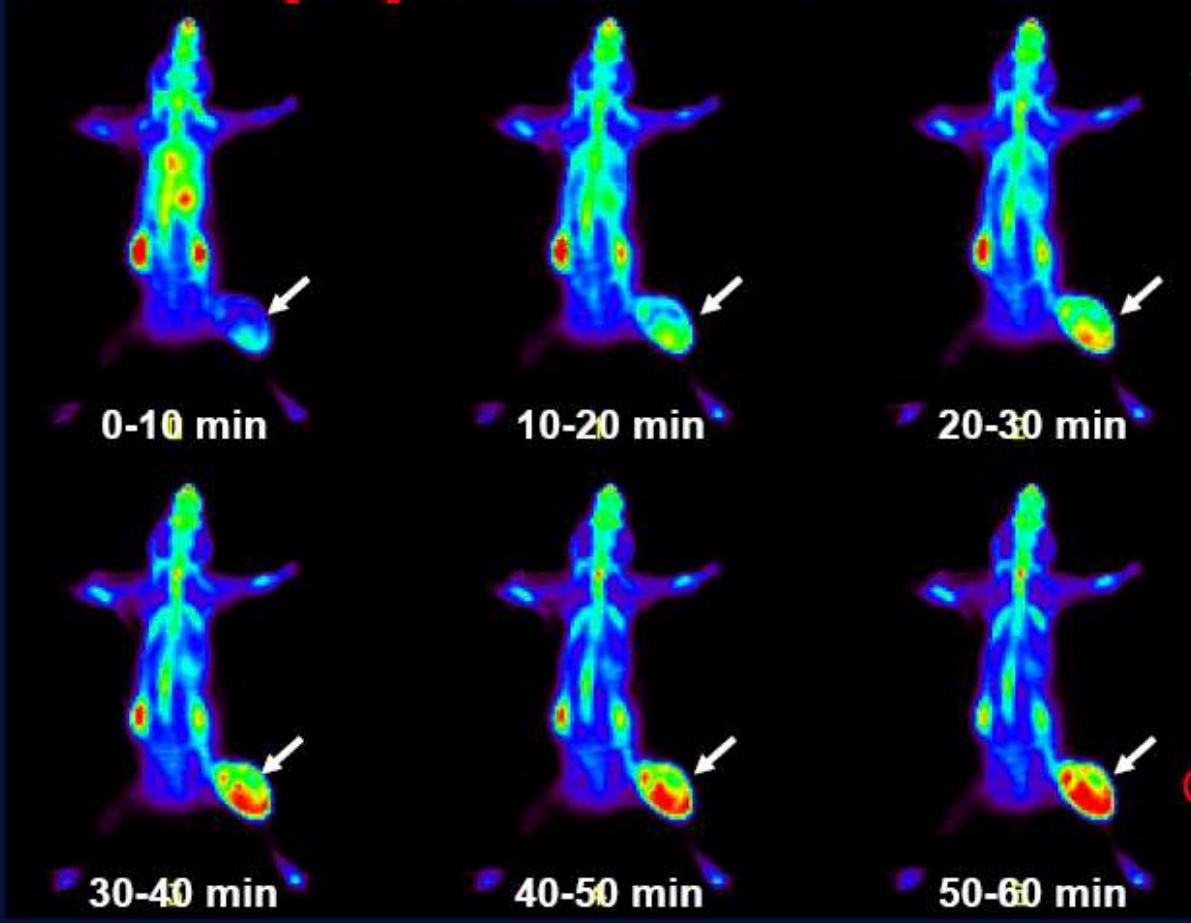


¹⁸F-FDG : Traceur TEP de la consommation du glucose

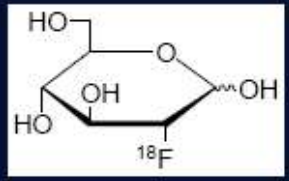


Whole Body FDG-PET Imaging of WSU-DLCL-2 Xenograft

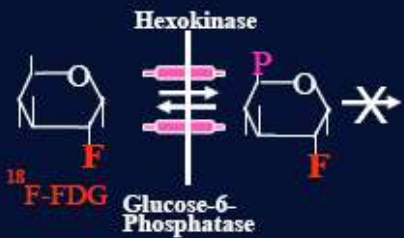
[¹⁸F]FDG-PET Time course



18F-Fluoro-2-deoxy-D-glucose (FDG)



GLUT 1 or GLUT 3

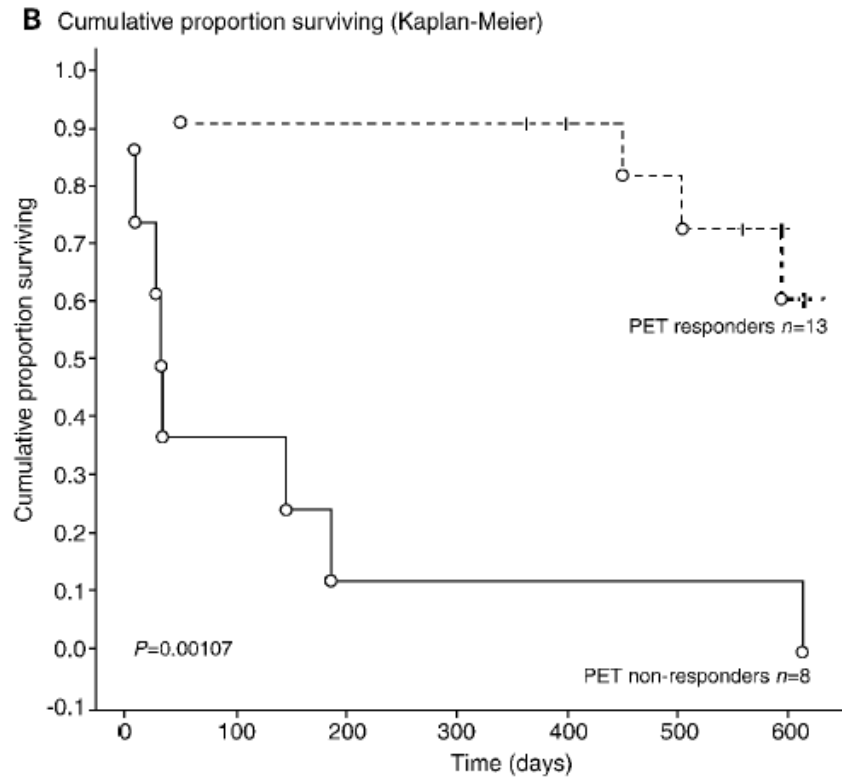
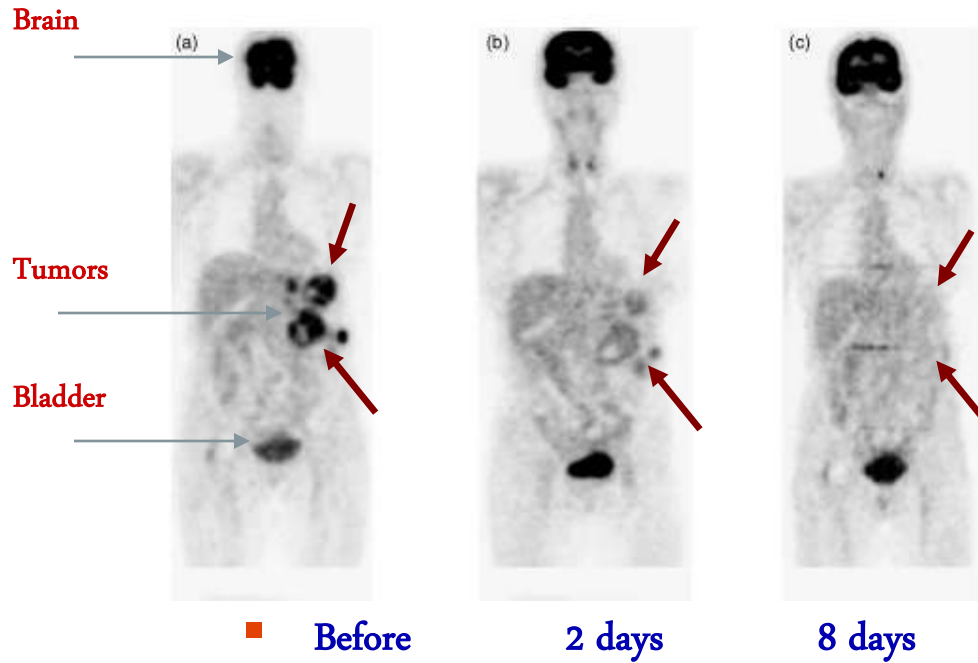


Glucose Metabolism Imaging

Early detection of the effect of drug treatment?



- GIST response to Glivec measured by ^{18}F FDG



- Select for treatment

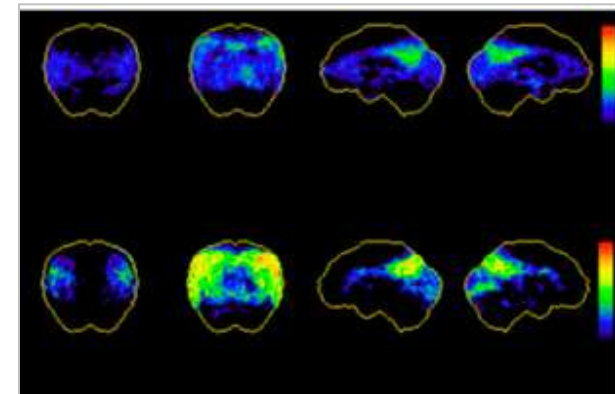
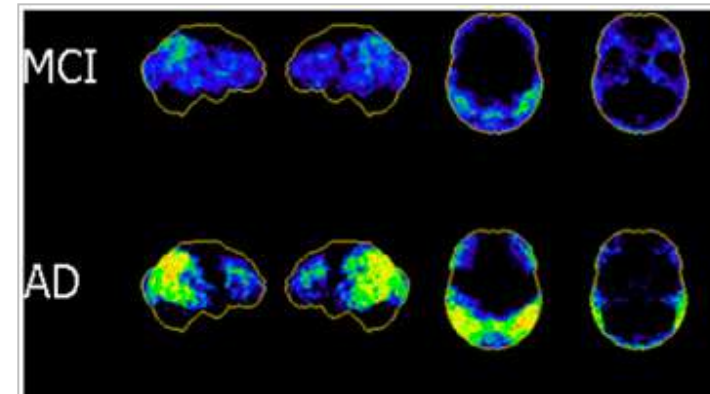
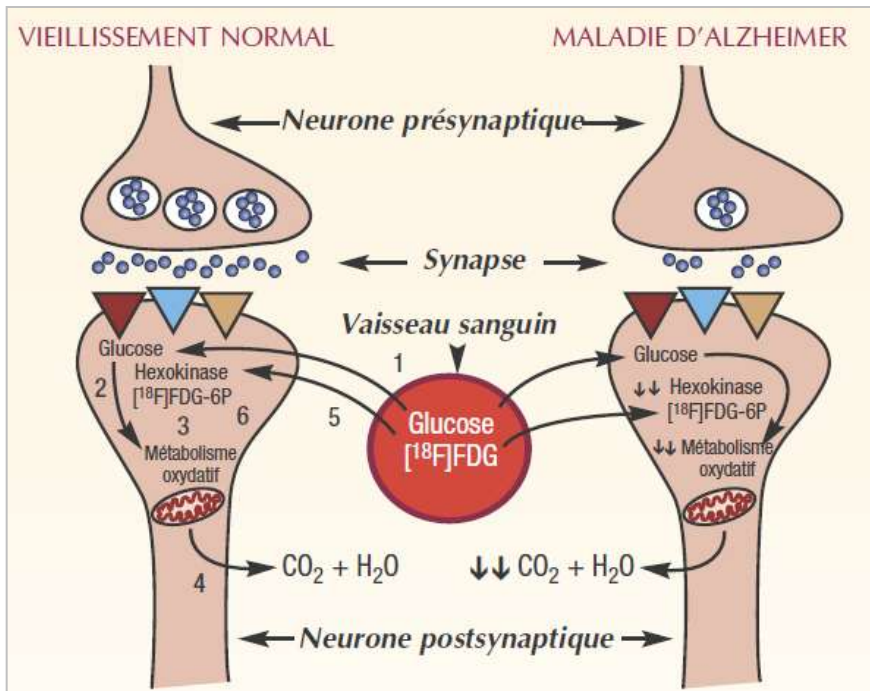


FDG (PET) : fonctionnement synaptique

Dégénérescence neuronale



Métabolisme du glucose \leftrightarrow ^{18}F -FDG en PET



Alzheimer : Les études cliniques actuelles longitudinales montrent une conversion plus importante vers la maladie d'alzheimer des patients amyloïde-PET positif



Cognitif OK
PIB Negatif
MRI Negatif

Cognitif OK
PIB Positif
MRI Negatif

Cognitif Déficit
PIB Positif
MRI Positif

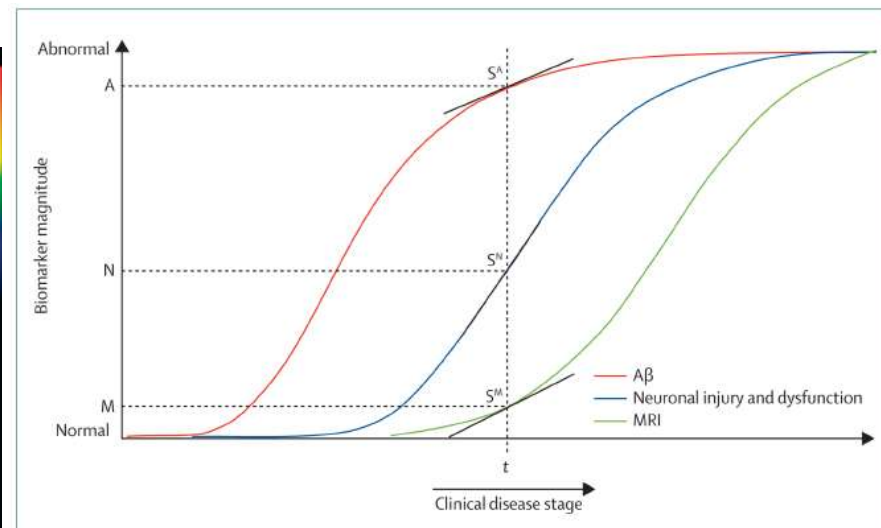
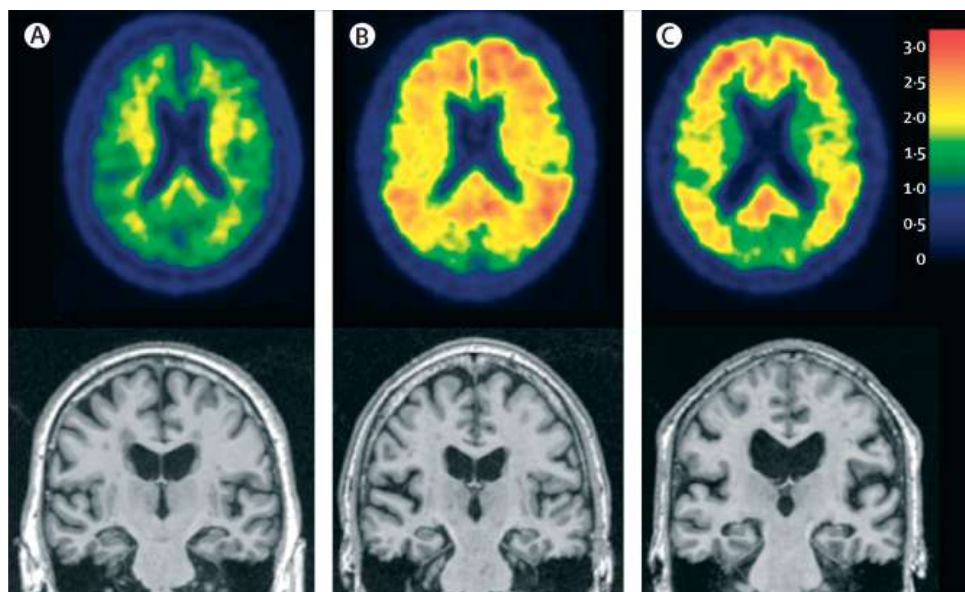
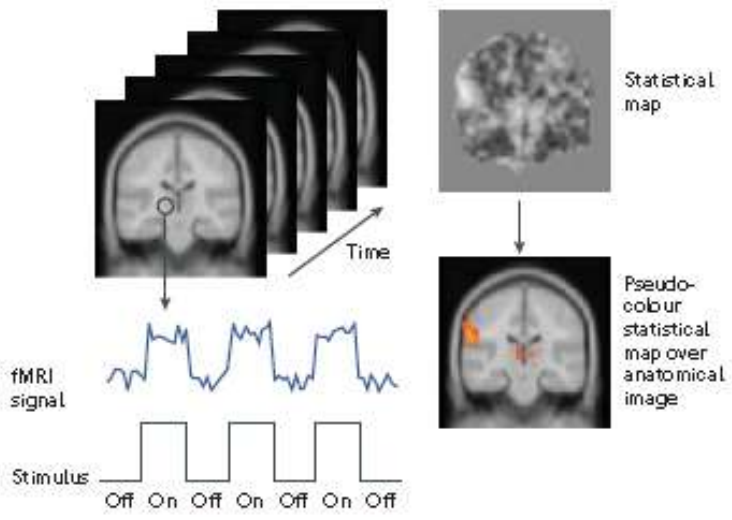
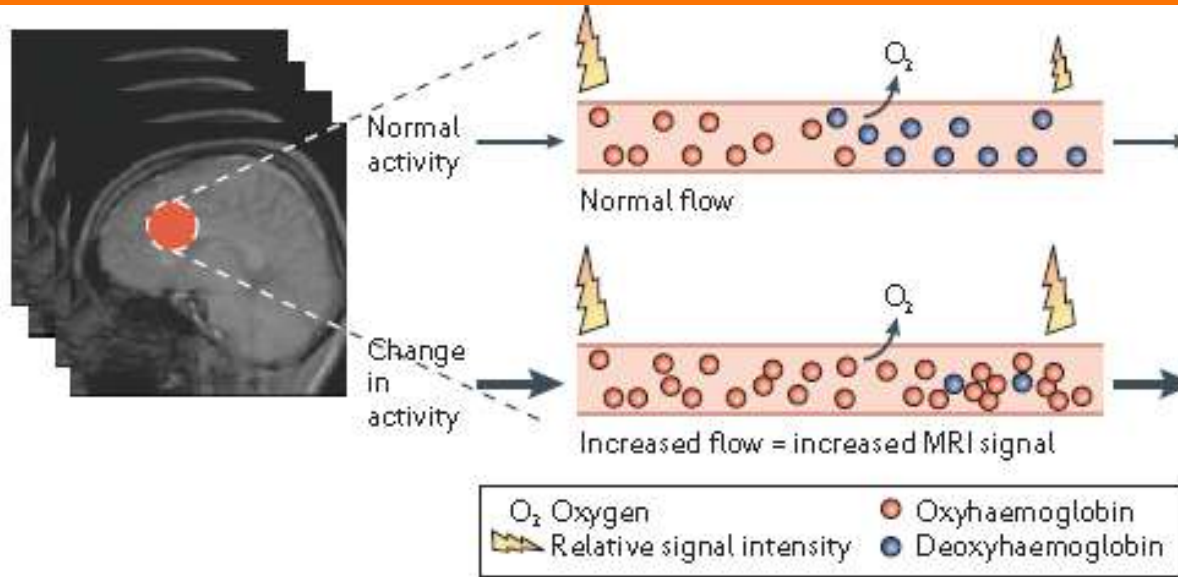


Figure 1. Illustration of biomarker staging of Alzheimer's disease

Three elderly individuals are placed in order from left to right by use of our proposed biomarker staging scheme. (A) A cognitively normal individual with no evidence of $A\beta$ on PET amyloid imaging with PiB and no evidence of atrophy on MRI. (B) A cognitively normal individual who has no evidence of neurodegenerative atrophy on MRI, but has significant $A\beta$ deposition on PET amyloid imaging. (C) An individual who has dementia and a clinical diagnosis of Alzheimer's disease, a positive PET amyloid imaging study, and neurodegenerative atrophy on MRI. $A\beta$ = β -amyloid. PiB=Pittsburgh compound B.

Jack C et al
Lancet Neurol, 2010

fMRI : Evaluation du fonctionnement cérébral par mesure de la consommation d'oxygène



Neuron, Vol. 32, 565-577, November 20, 2001, Copyright ©2001 by Cell Press

Visual Motion Processing Investigated Using Contrast Agent-Enhanced fMRI in Awake Behaving Monkeys

Neurotech

Wim Vanduffel,^{1,4} Denis Fize,¹
Joseph B. Mandeville,³ Koen Nelissen,¹
Paul Van Hecke,² Bruce R. Rosen,³
Roger B.H. Tootell,³ and Guy A. Orban¹

Until now, several reports have demonstrated that fMRI is feasible in monkeys (Logothetis et al., 2001; Dubowitz et al., 1998; Stefanacci et al., 2000; Vanduffel et al., 1998; Disbrow et al., 2000; He

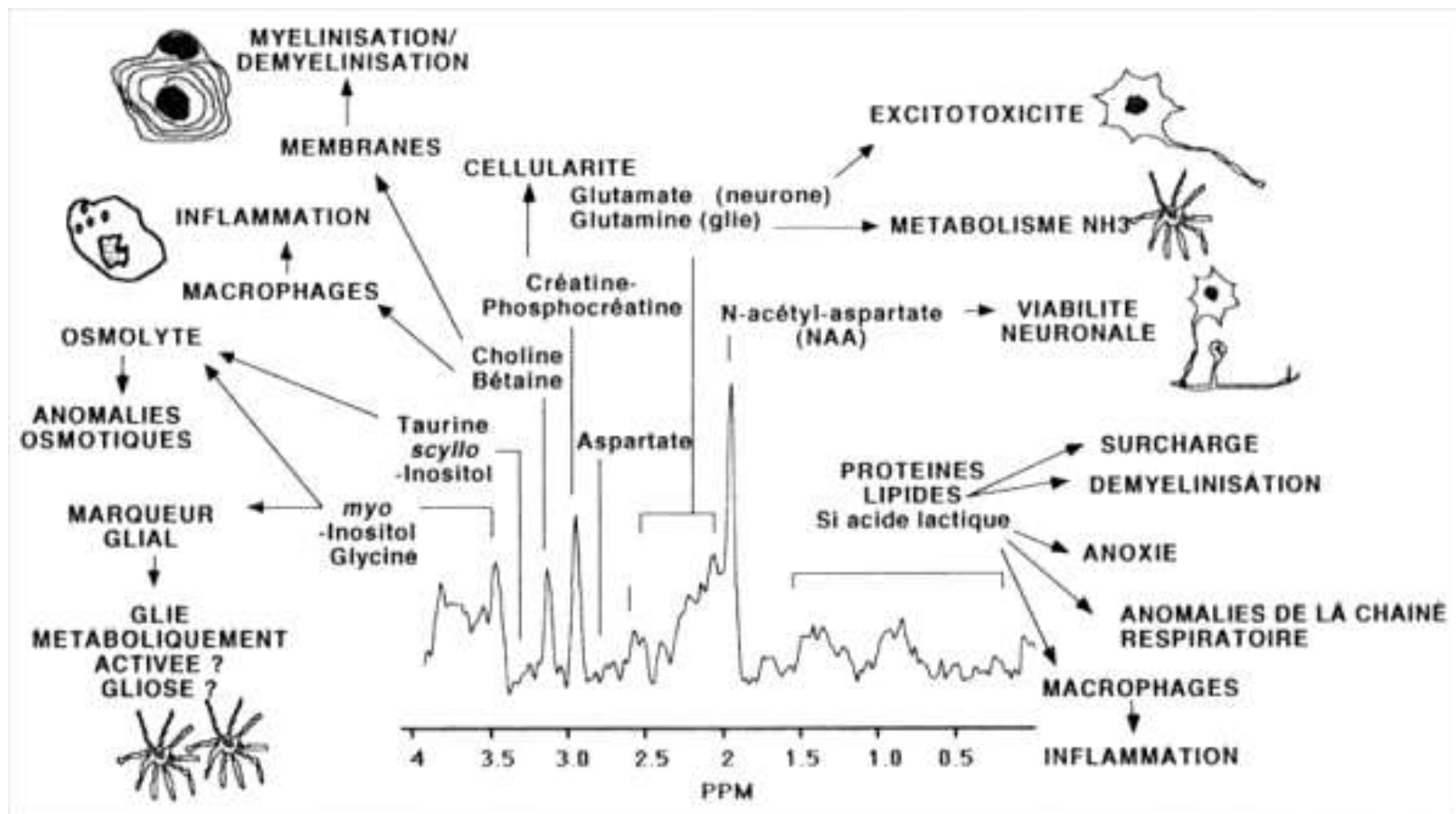
MR spectroscopie : métabolisme in vivo



Nucleus	γ [MHz/T]	Sensitivity of detection	Applications
^1H	42.58		<ul style="list-style-type: none"> • Total choline+* • Lactate+* • Lipid+* • N-acetyl-aspartate+ • Citrate+ • Extracellular pH (pHe)+ • Treatment efficacy+* • Detection of metastasis+* • pO2+
^{19}F	40.08		<ul style="list-style-type: none"> • Drug pharmacokinetics+* • pHe+ • pO2+ • Enzyme activity+ • Labeled substrate utilization+
^{31}P	17.25		<ul style="list-style-type: none"> • Energy metabolism (ATP, PCr, Pi)+* • Intracellular pH (pHi)+* • Phospholipid metabolism+*
^{13}C	10.71		<ul style="list-style-type: none"> • Labeled substrate utilization to evaluate drug pharmacokinetics and metabolic pathways+*

Glunde K et al, 2010

Spectroscopie IRM – Métabolisme et physiopathologie



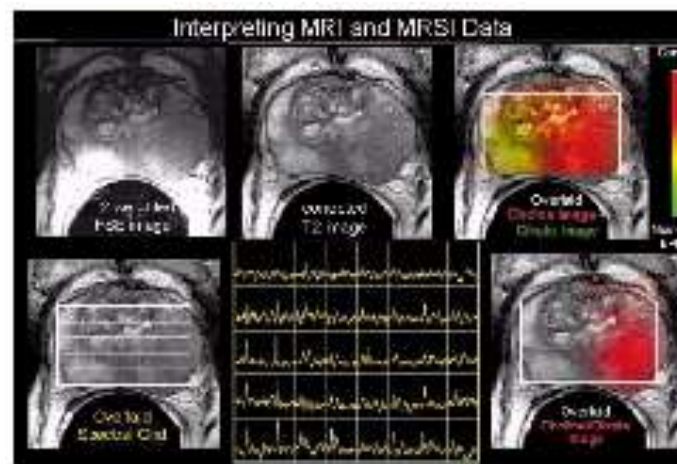
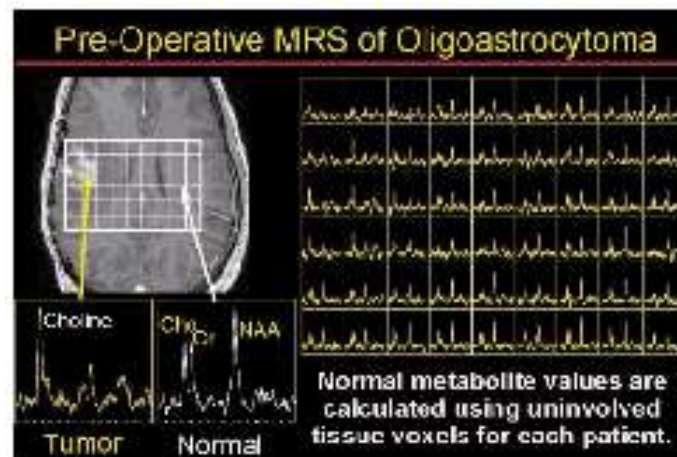
Glunde K et al, 2010



Applications limitées car faibles sensibilité et résolution spatiale

Cartographie des métabolites : ATP, phosphocreatine, choline ...

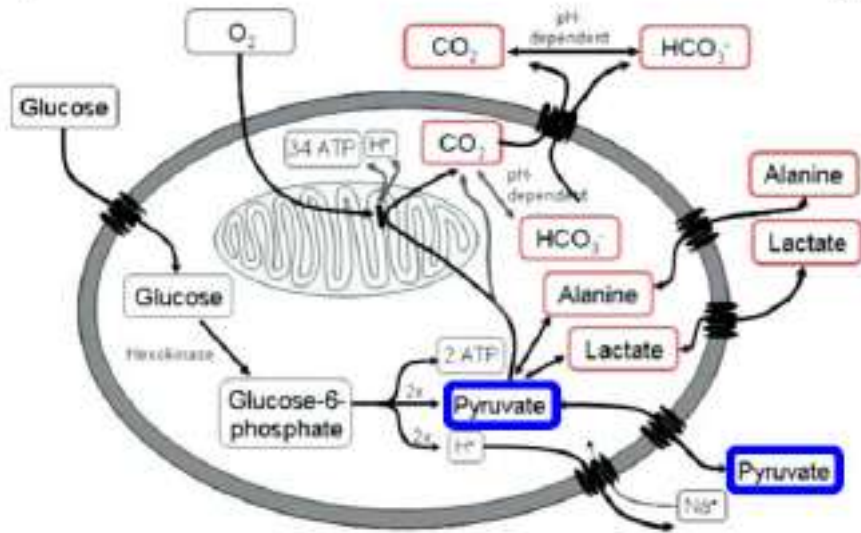
- Neuro: tumeurs, démence
- Cancer de la prostate
- Myopathies



13C hyperpolarisation MRI : Cycle Krebs in vivo Demi-vie très courte de l'hyepolarisation

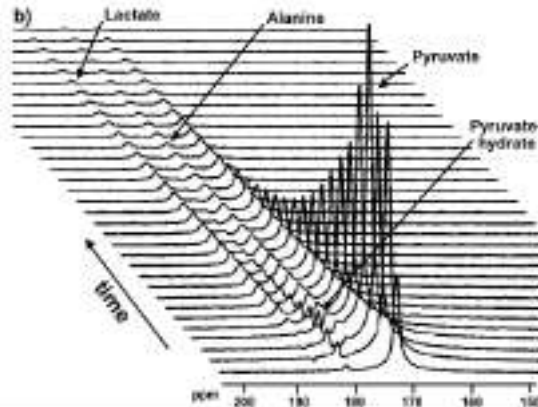
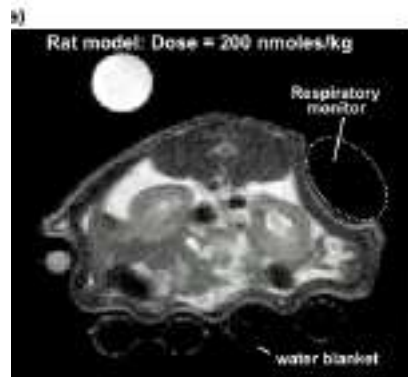


Hyperpolarized 13C-labeled Pyruvate



Applications limited to small molecular weight compounds

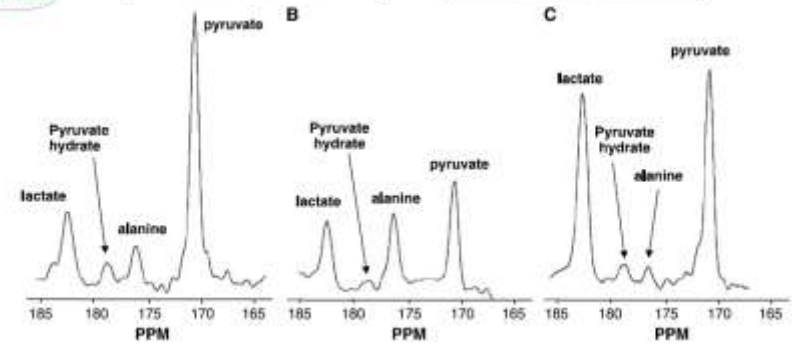
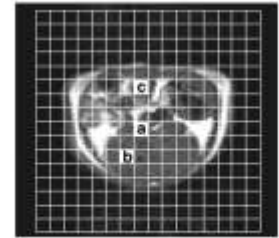
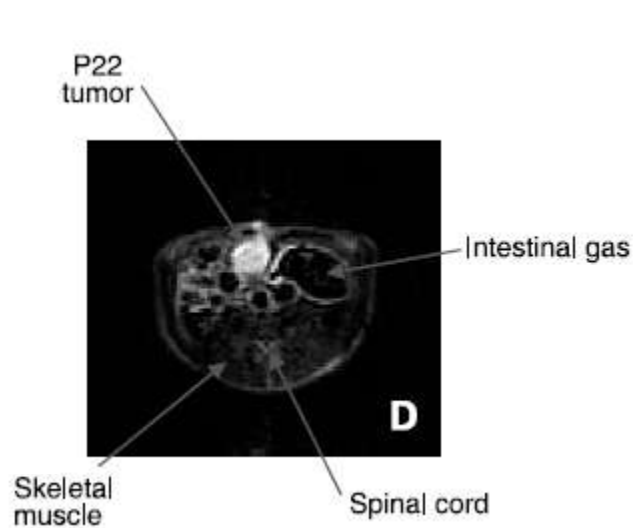
- 1-[13C]-pyruvate
- 2-[13C]-pyruvate
- 3-Br-1-[13C]-pyruvate
- [13C]-bicarbonate
- 1-[13C]-acetate
- 1-[13C]-alanine
- 5-[13C]-glutamine
- 1-[13C]-lactate
- 1-[13C]-ethylpyruvate
- 1,4-[13C]-fumarate
- 1-[13C]-acetyl-amino acids (several)
- [6Li]
- [15N]-choline



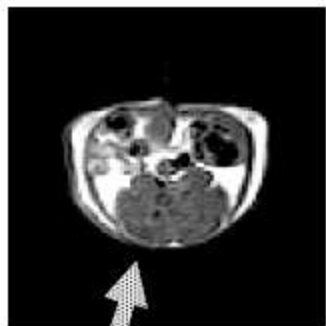
Time course of 13C-MRS signals following the bolus injection of hyperpolarized 13C-labeled pyruvate (samples taken every 3 s)

13C Hyperpolarisation MRI

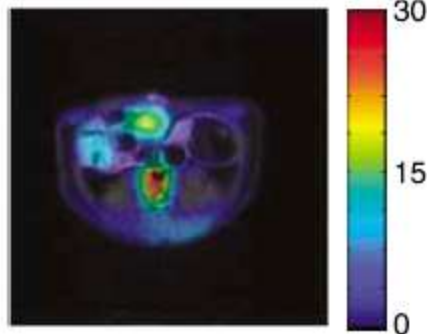
13C(HP)-pyruvate imagerie tumorale



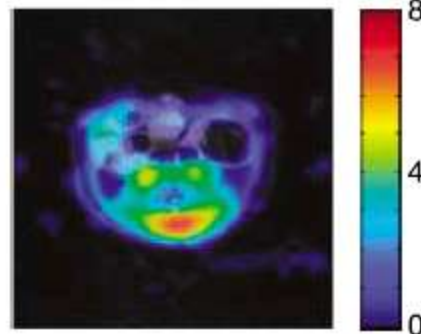
Anatomical 1H image



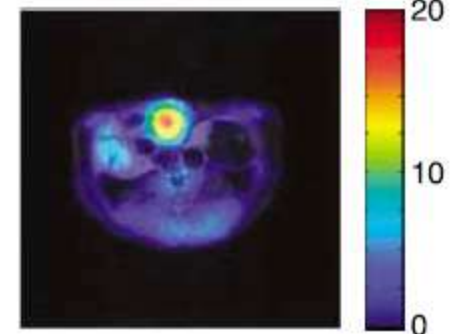
¹³C-pyruvate



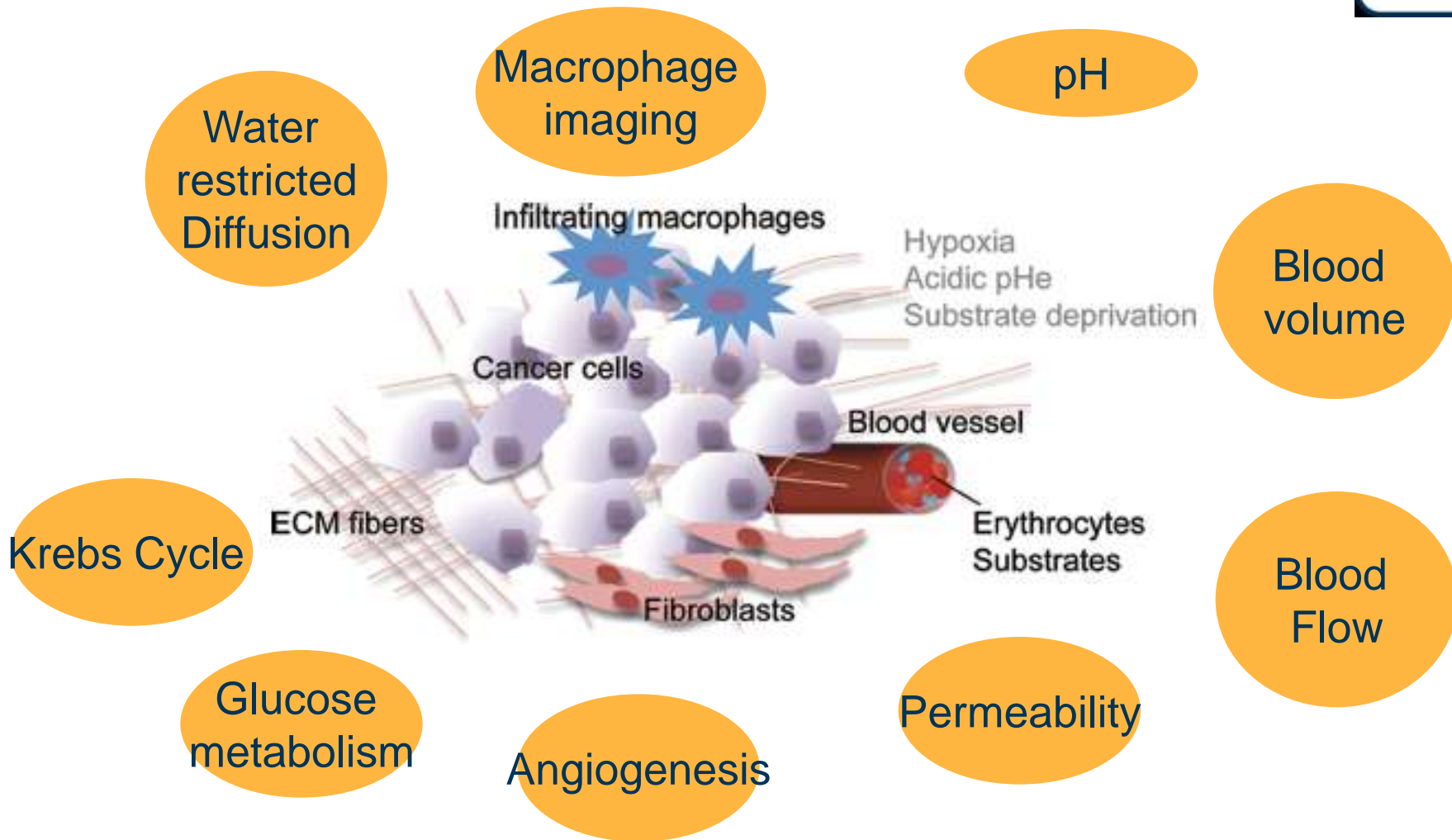
¹³C-alanine



¹³C-lactate



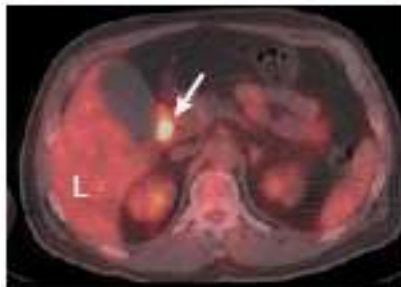
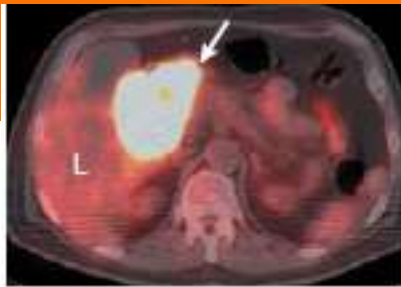
Imagerie fonctionnelle tumorale



Glunde K et al, 2010

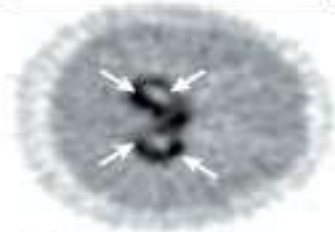
Caractérisation tumorale

PET-CT



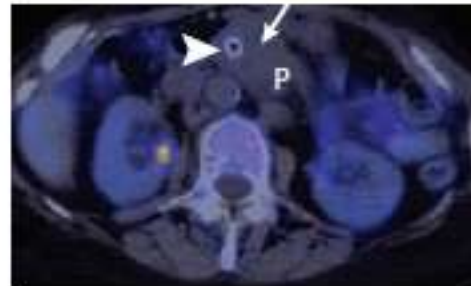
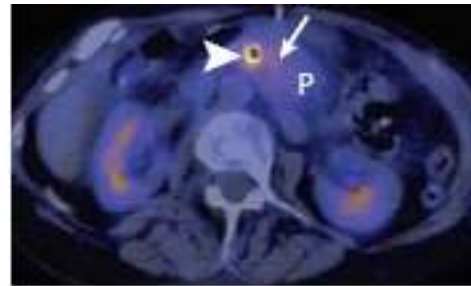
a Tumour metabolism

- Radiolabelled glucose, amino acids, choline



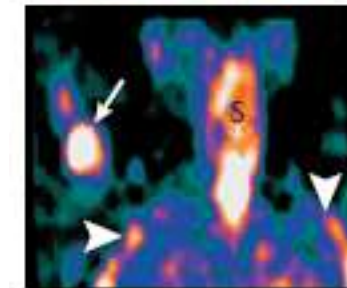
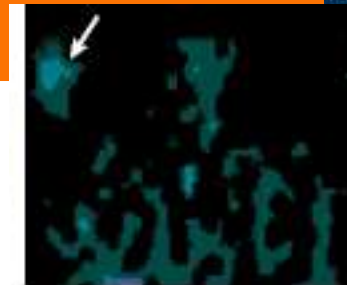
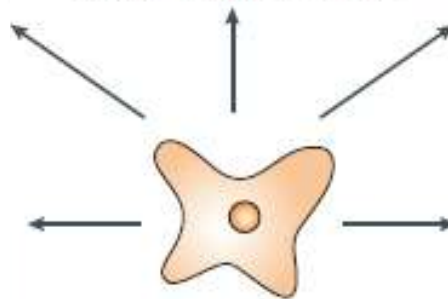
e Tumour hypoxia

- Radiolabelled MISO, ATSM, FAZA



b Tumour proliferation

- Radiolabelled thymidine analogues: JdR, FMAU, FLT



c Apoptosis in tumour

- Radiolabelled annexin V
- Caspase



d Tumour angiogenesis

- Radiolabelled RGD, $\alpha_v\beta_3$ -targeted paramagnetic nanoparticles
- $\alpha_v\beta_3$ /VEGFR2-targeted microbubbles

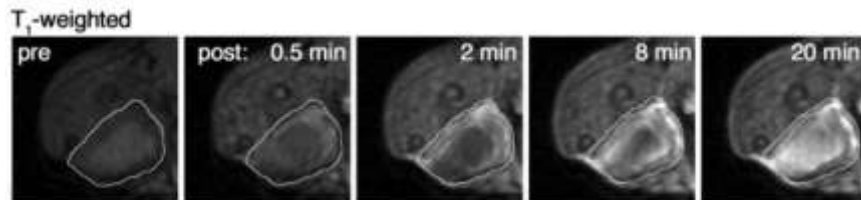
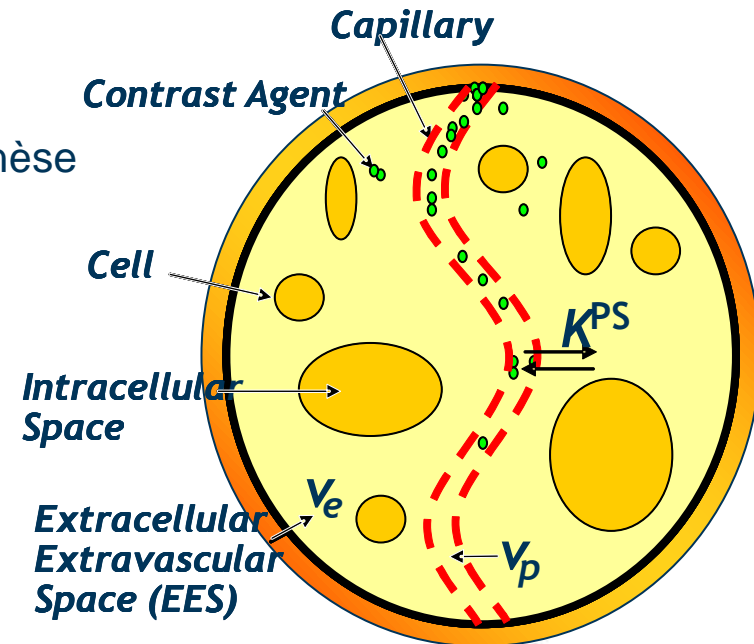


Principe : caractérisation de la tumeur via la dynamique de rehaussement d'un produit de contraste

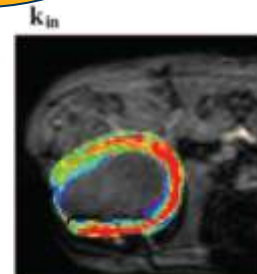
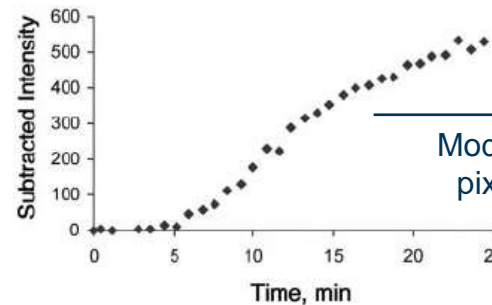
- Volume sanguin tumoral / perfusion ↔ angiogénèse
- Perméabilité des microvaisseaux tumoraux ↔ angiogénèse

Suivi du rehaussement

- Résolution temporelle (TEP : 1-2 min, IRM : 1-10 s)
- Injection bolus
- ± fonction d'entrée artérielle → quantification absolue



Dadiani, Cancer Research, 2004



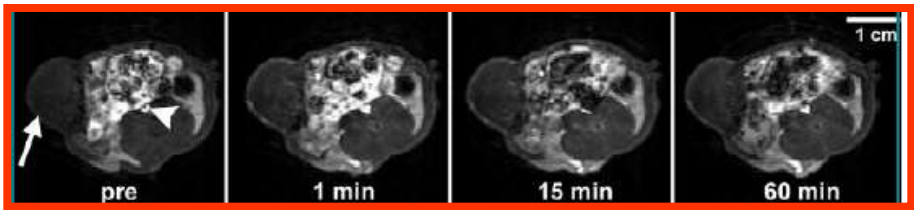
DCE MRI suivi thérapeutique

Vascular Permeability during Antiangiogenesis Treatment: MR

Imaging Assay Results as Biomarker
for Subsequent Tumor Growth in Rats¹

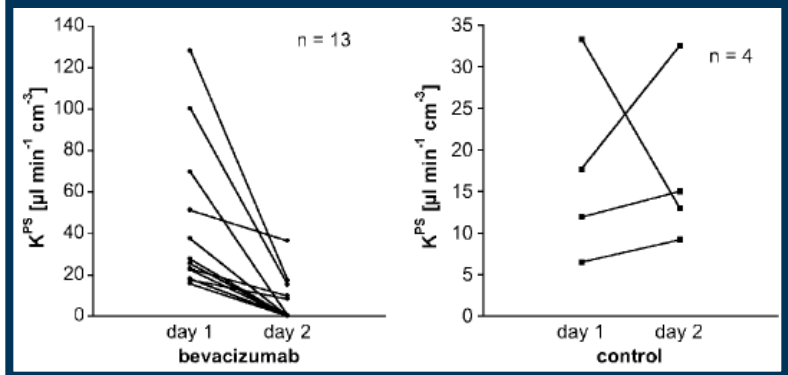
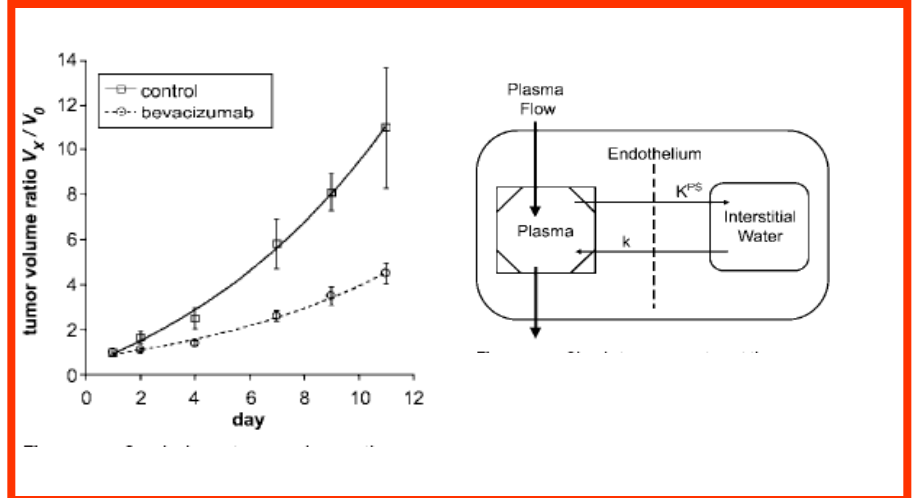


Hans-Juergen Raatschen, MD
Gerhard H. Simon, MD
Yanjun Fu, PhD
Barbara Sennino, PhD
David M. Shames, MD
Michael F. Wendland, PhD
Donald M. McDonald, MD, PhD
Robert C. Brasch, MD



Dose levels:

- 0 mg bevacizumab (n 4 [control rats])
- 0.1 mg bevacizumab (n 3)
- 0.25 mg bevacizumab (n 2)
- 0.5 mg bevacizumab (n 5)
- 1.0 mg bevacizumab (n 3)

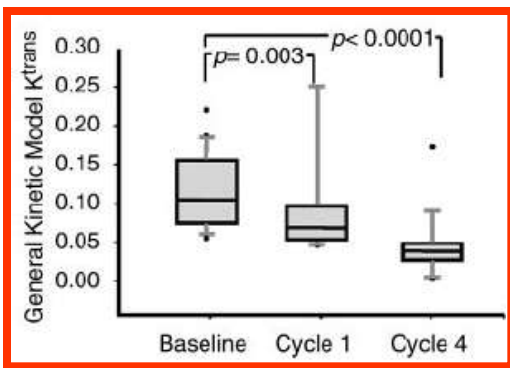
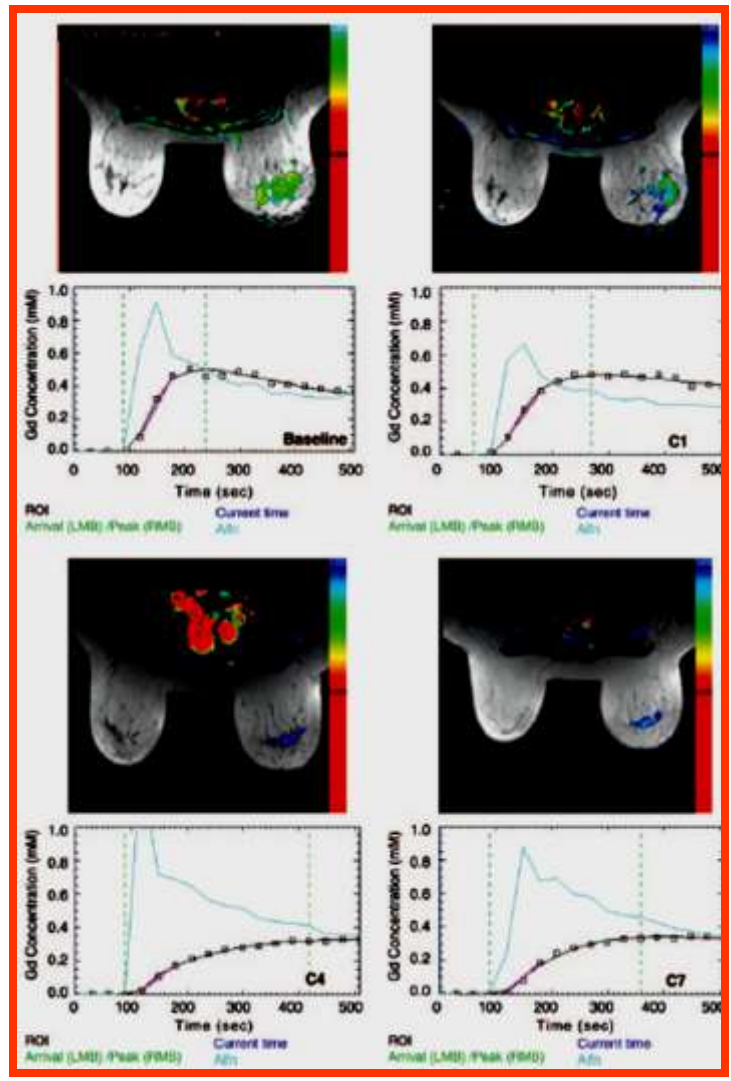


DCE MRI suivi thérapeutique

Inflammatory Breast Cancer: Dynamic Contrast-enhanced MR in Patients Receiving Bevacizumab— Initial Experience¹



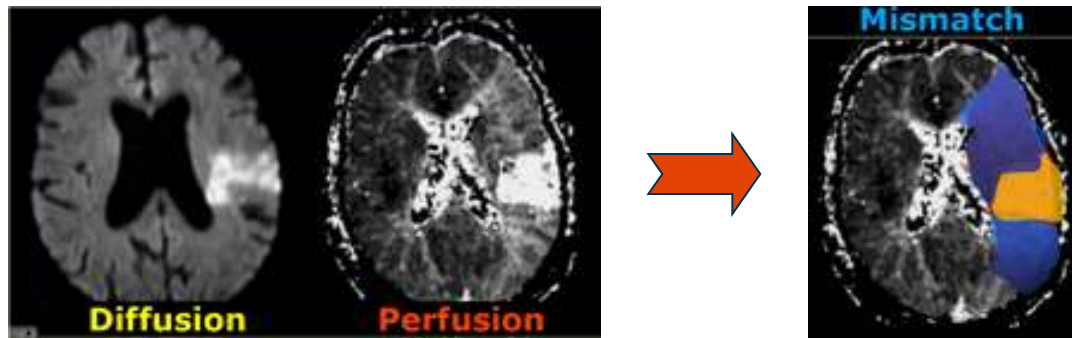
Arpi Thukral, MD
David M. Thomasson, PhD
Catherine K. Chow, MD
Reyes Eulate, MD
Suparna B. Wedam, MD
Sandeep N. Gupta, PhD
Betty J. Wise, BS, RT(R)MR
Seth M. Steinberg, PhD
David J. Liewehr, PhD
Peter L. Choyke, MD
Sandra M. Swain, MD²



Parameter	Cycle 1 to Cycle 4		Cycle 4 to Cycle 7	
	Median Percentage Change*	P Value†	Median Percentage Change*	P Value†
Brix amplitude‡	-33 (-100 to 65)	.003	2 (-100 to 120)	.46
Brix K_{ep}	-45 (-100 to 78)	.002	-13 (-100 to 203)	.86
GKM k^{trans}	-58 (-97 to 80)	.01	-12 (-85 to 240)	.76
GKM K_{ep}	-47 (-84 to 134)	.05	-14 (-96 to 296)	.89
Slope wash-in	-47 (-81 to 245)	.02	-10 (-83 to 180)	.39
Slope washout	-28 (-1017 to 2806)	.96	-38 (-519 to 228)	.36
IAUGC 90 ^S	-50 (-100 to 200)	.12	-35 (-100 to 50)	.13
IAUGC 180 ^S	-44 (-94 to 71)	.007	0 (-67 to 50)	.52



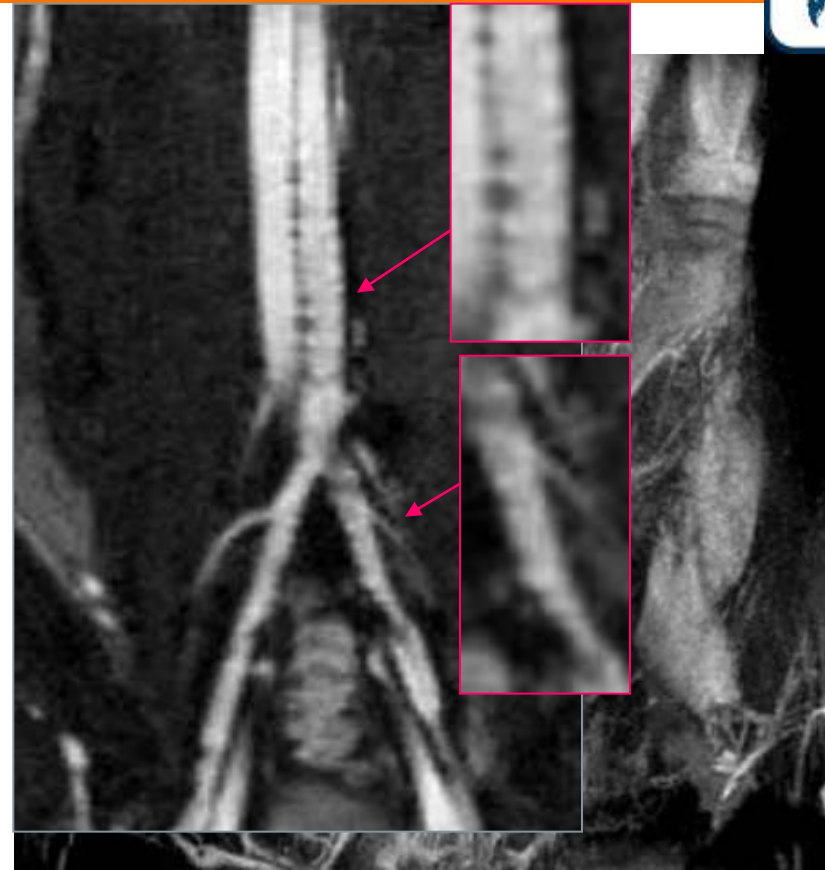
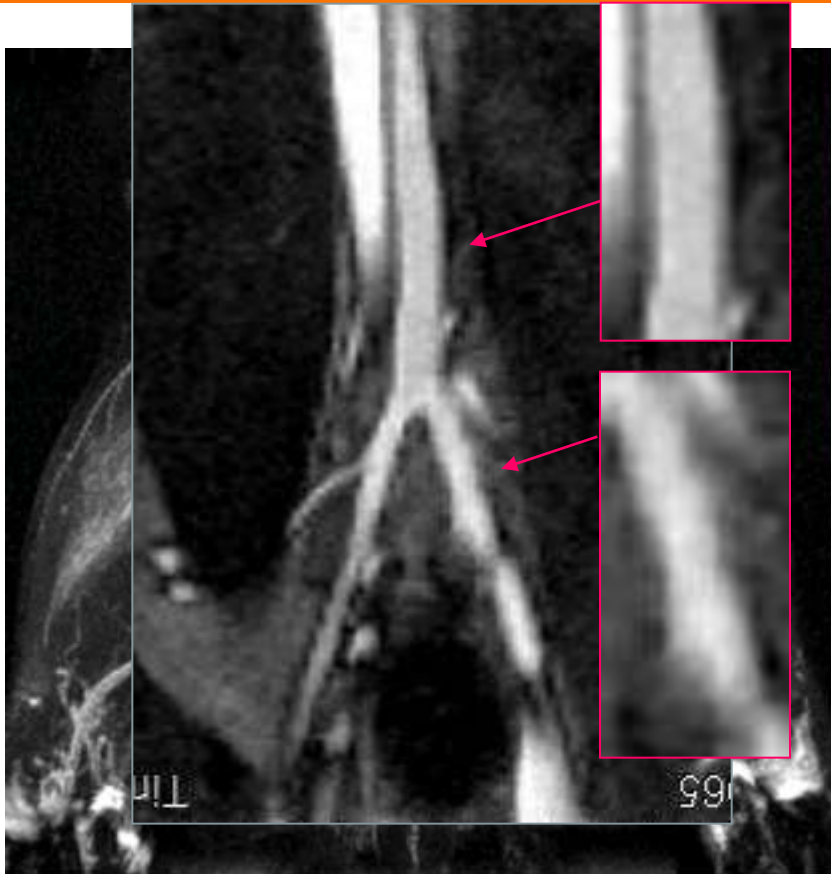
- **Modifications précoces après un accident**
 - Détection précoce de la zone en souffrance dès 30 min post ischémie (vs plusieurs heures en CT ou en IRM conventionnelle)
 - Chute de l'ADC liée à l'oedème cytotoxique (gonflement cellulaire)
 - Permet de "dater" l'événement ischémique
- **Notion de mismatch diffusion / perfusion**
 - Association avec l'imagerie de perfusion au premier passage (pondération T2*)
 - Plus le mismatch est important, meilleur est le pronostique



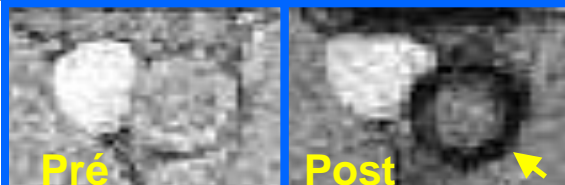
Imagerie de l'inflammation par IRM

Nanoparticules d'oxyde de fer ciblant les macrophages

Modèles de plaques d'athérome



Lapin
Athérome



In vivo MRI



Perl's : Iron
staining



Enhanced Perl's
DAB



Macrophages

Souris

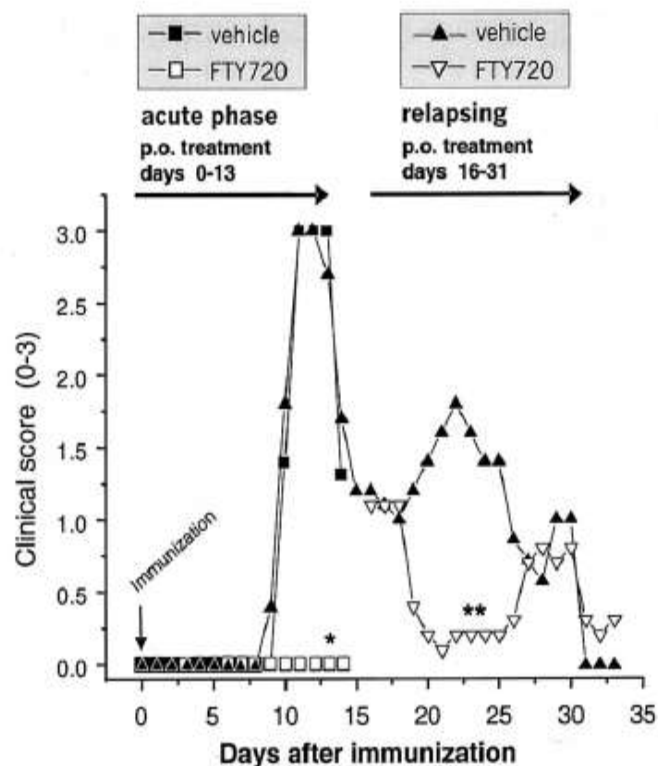
Sclérose en plaque : suivi de traitement

Imagerie de l'inflammation par IRM

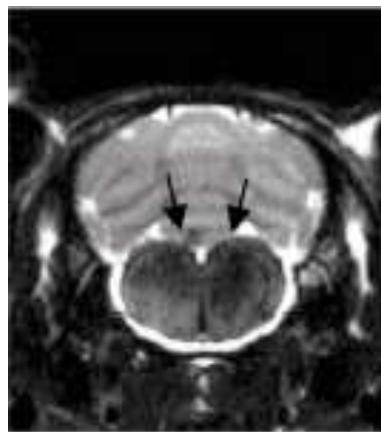
Nanoparticules d'oxydes de fer ciblant les macrophages



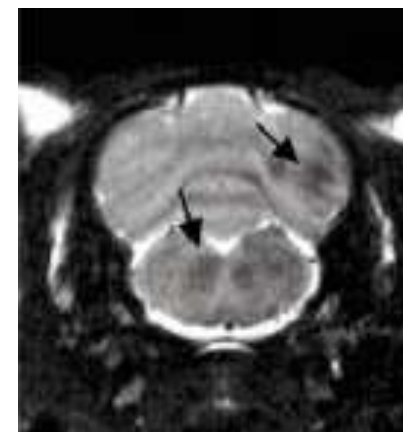
Modèle de sclérose en plaque chez le rat



Véhicule

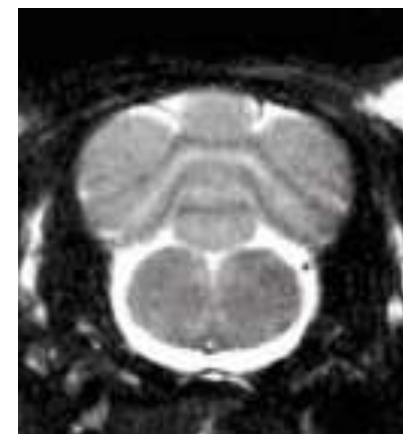


Aigue

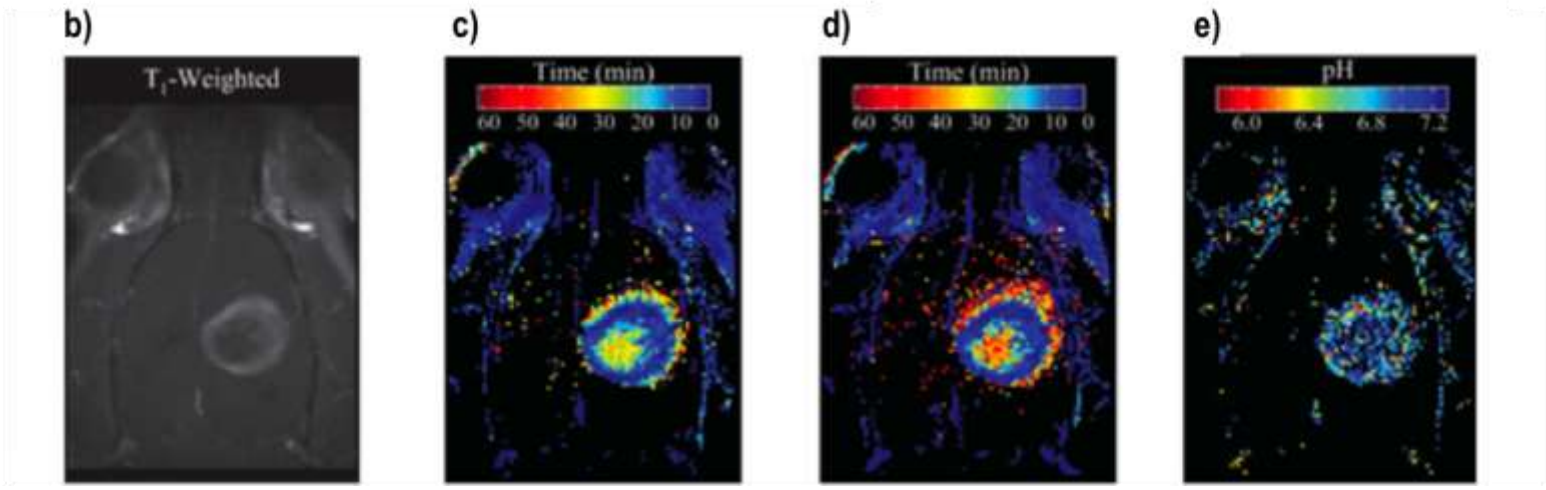
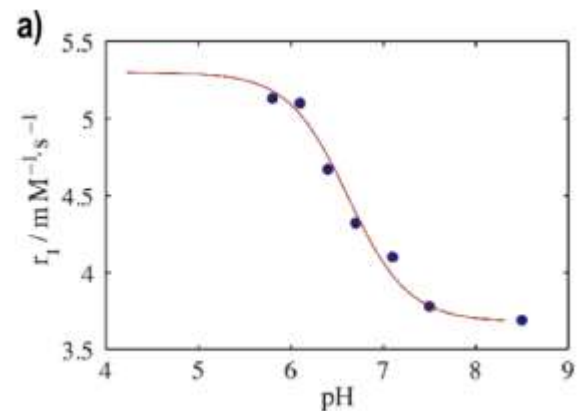
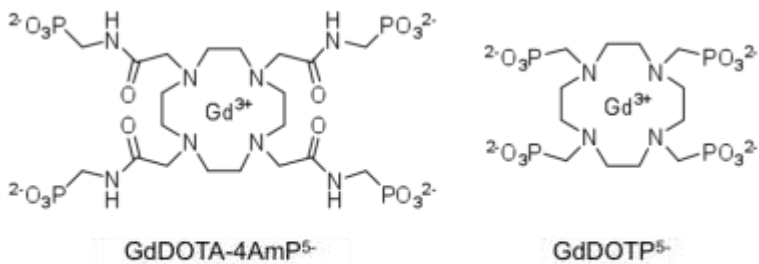


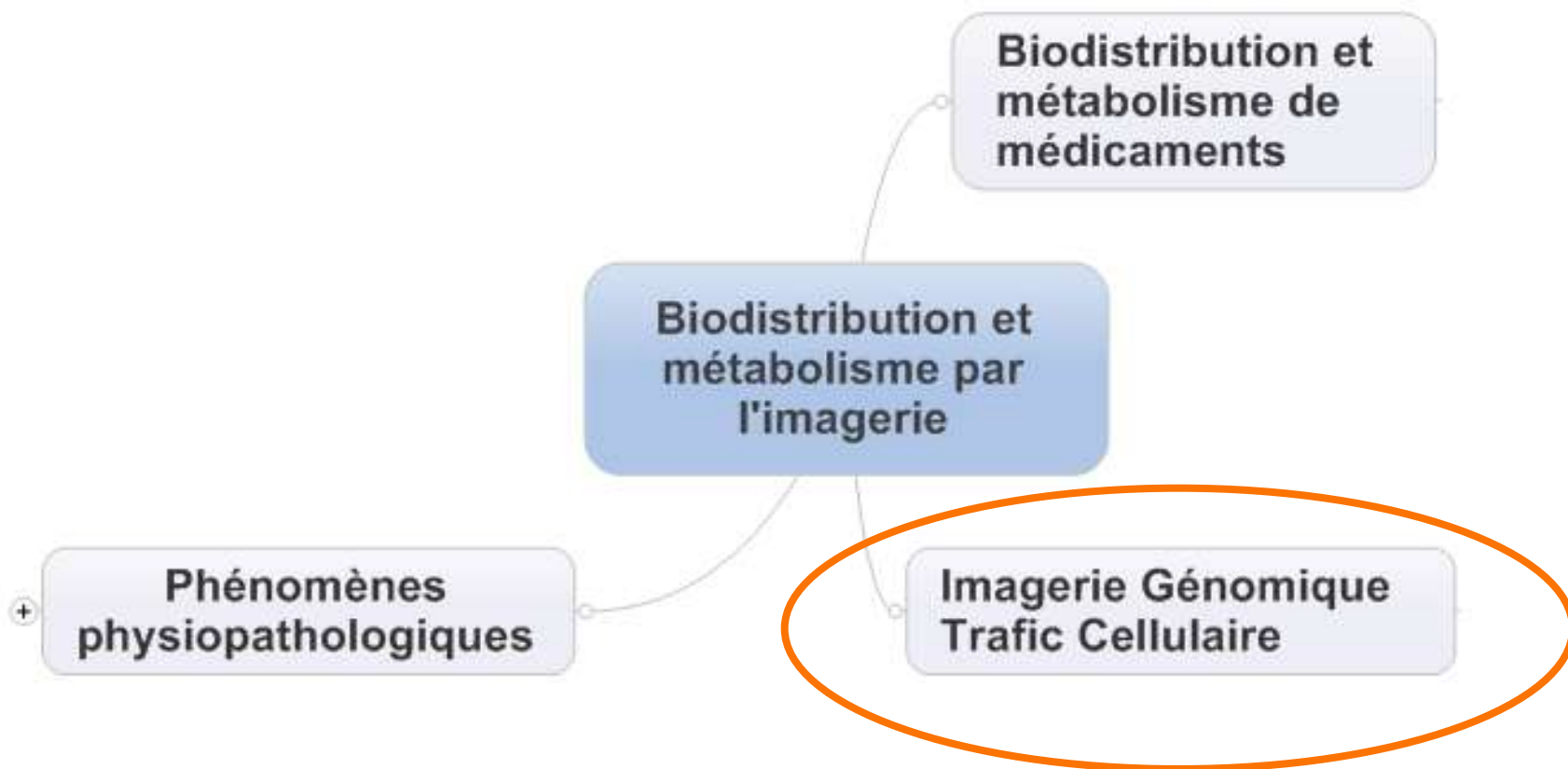
Rechute

Traitement FTY20



Mesure du pH in vivo Produits de contraste IRM dont l'effet paramagnétique varie en fonction du pH





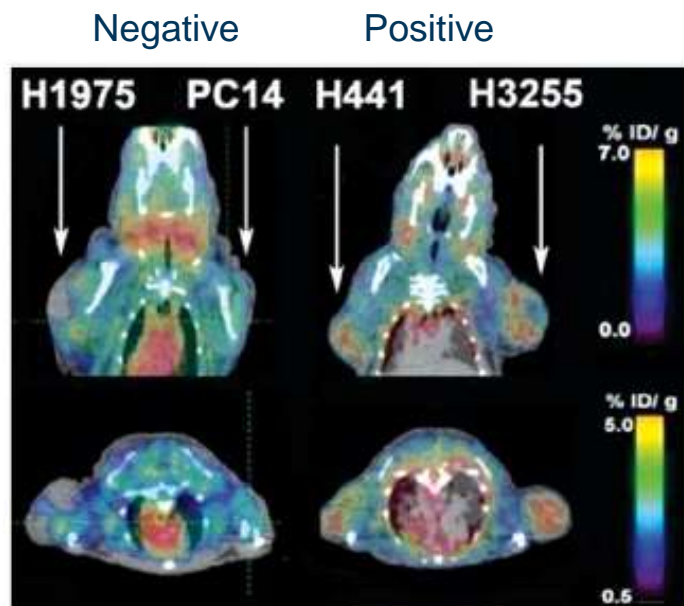
Imagerie moléculaire du récepteur EGFR muté

Ciblage néo-angiogenesis en Imagerie expérimentale



PET Tracer

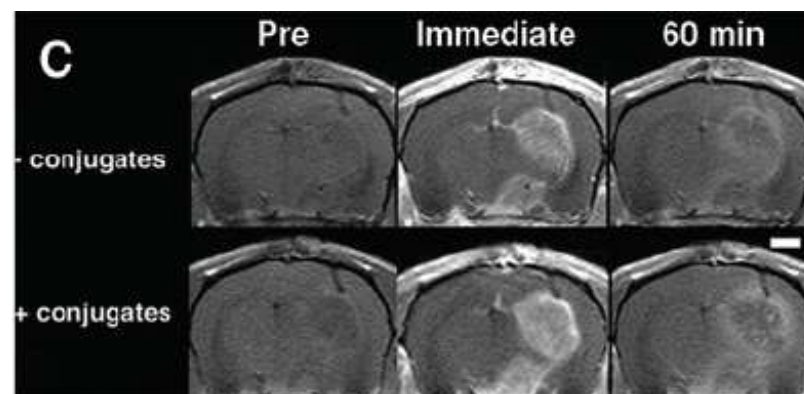
Molecular imaging of active mutant L858R EGF receptor (EGFR) kinase-expressing nonsmall cell lung carcinomas using PET/CT



Yel & al, PNAS, 2011

MRI Tracer

Targeted Signal-Amplifying Enzymes Enhance MRI of EGFR Expression in an Orthotopic Model of Human Glioma



Shazeeb & al, Cancer Research, 2011

PET Imaging in mice : transgenic expression



Thymidine kinase gene expression

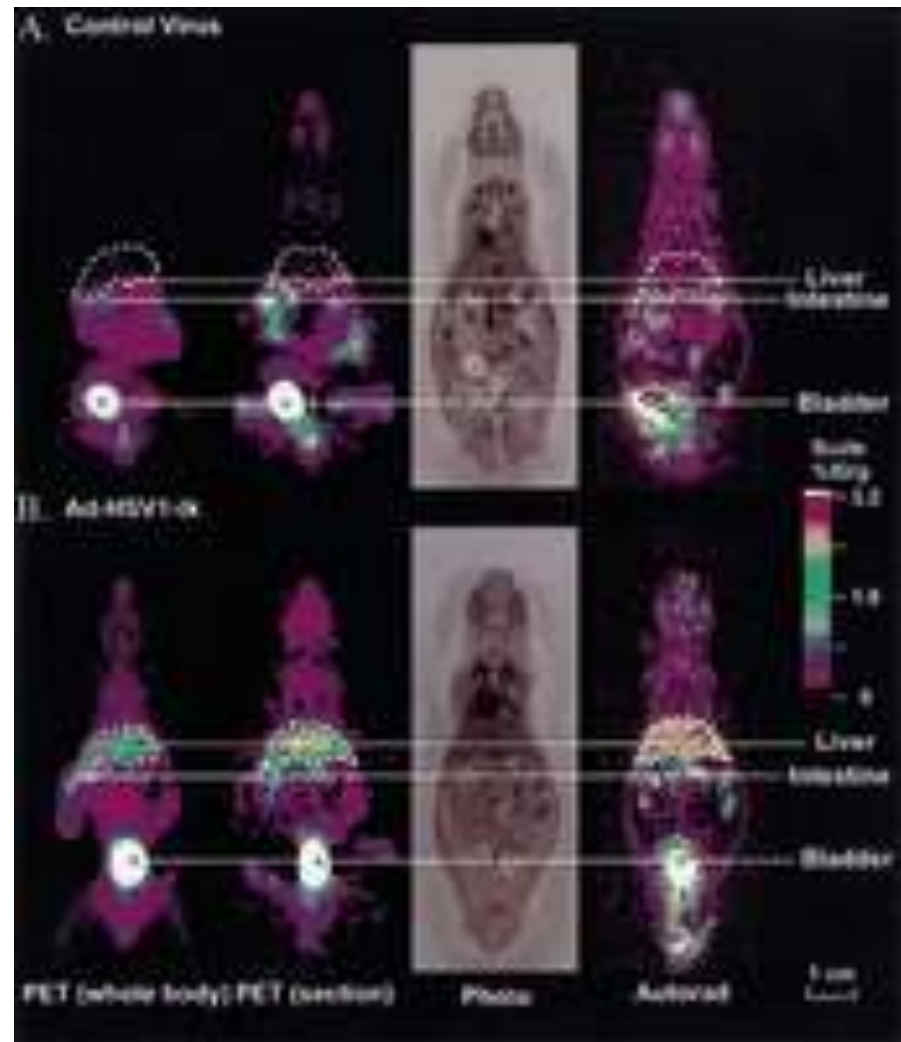
A, Injection of 1.53×10^9 plaque-forming units of control virus.

B, Injection of 1.53×10^9 plaque-forming units of the replication-deficient adenovirus.

Left : whole-body mean coronal projection PET scan of the ^{18}F activity distribution was obtained. The location of the liver (dotted white outline) was determined from both the 8- ^{18}F -fluoroganciclovir signal and the cryostat slices (second from right).

Coronal micro-PET sections (second from left) are approximately 2-mm thick.

After PET, the mice were sectioned (second from right), and autoradiography (Autorad) was performed (right).



CEST Chemical Exchange Shift Transfer Lysin rich reporter gene

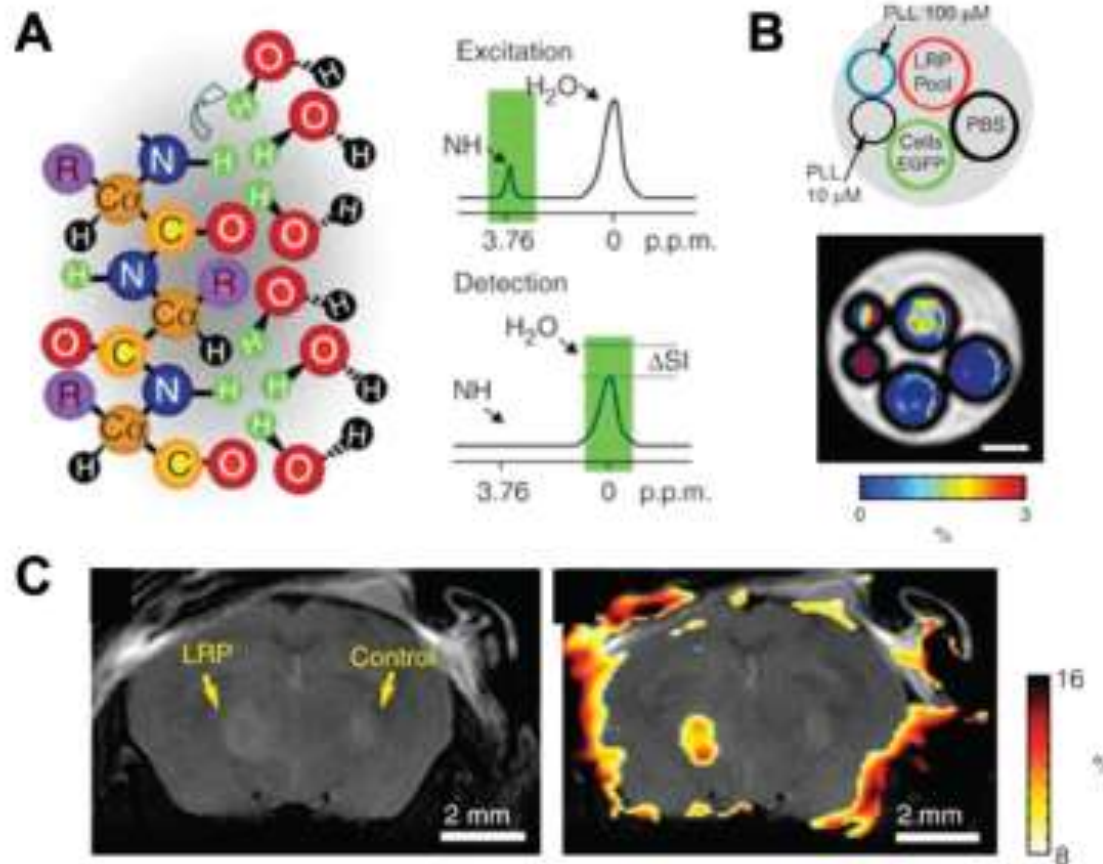
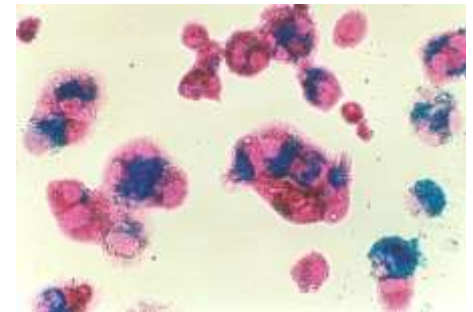


Figure 3. CEST imaging of lysine rich-protein (LRP) reporter. (A) Frequency-selective radiofrequency pulses excite the amide protons. These protons exchange with water protons, thereby reducing the MRSI signal intensity (SI) of the water signal by ΔSI . (B) Ex vivo proof-of-principle MRSI of the LRP reporter protein in phantoms demonstrated that the LRP-containing phantom displayed significantly higher ΔSI when excited at ± 3.76 ppm as compared to poly-L-lysine, phosphate-buffered saline, or green fluorescent protein as controls (scale bar, 1 mm). (C) Anatomical image (left) and CEST signal intensity-difference map overlaid on the anatomical image (right) was able to distinguish the LRP-expressing and control tumor xenografts. Adapted from ref 114.



■ Marquage ex vivo avec nanoparticules d'oxyde de fer

- Réinjection IV des cellules marquées
 - Adressage cellulaire
 - Suivi des cellules transplantées (migration)
- Réinjection in situ des cellules marquées
 - Migration cellulaire : cellules neurales
 - Division cellulaire



■ Marquage in vivo

- Endocytose des nanoparticules d'oxyde de fer par les cellules sanguines et suivi de leur migration dans les tissus pathologiques (ex monocyte/macrophage)

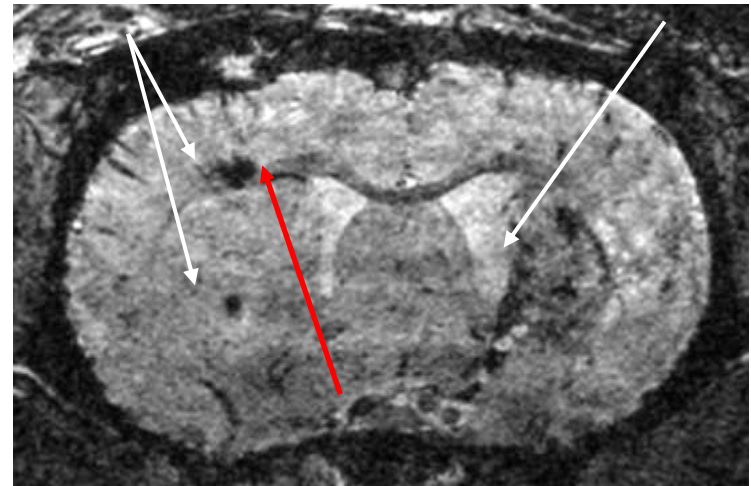
Ischémie cérébrale : migration de cellules souches



- **Ischémie cérébrale chez le rat**
- **Cellules souches implantée après marquage magnétique (USPIO)**
- **Imagerie @ 7 T (Bruker BioSpec)**
- **Résolution : $78 \times 49 \times 78 \mu\text{m}^3$**

Sites d'implantation

Ischémie



Migration des cellules souches



■ **Imagerie expérimentale chez l'animal**

- Etudes pharmacologiques : évaluation anatomique et fonctionnelle non invasive
- Etudes Toxicologiques : Suivi dynamique d'anomalies tissulaires (nécrose, métabolisme, perfusion....)
- Recherche en génomique, thérapie cellulaire

■ **Pas de techniques d'imagerie universelle**

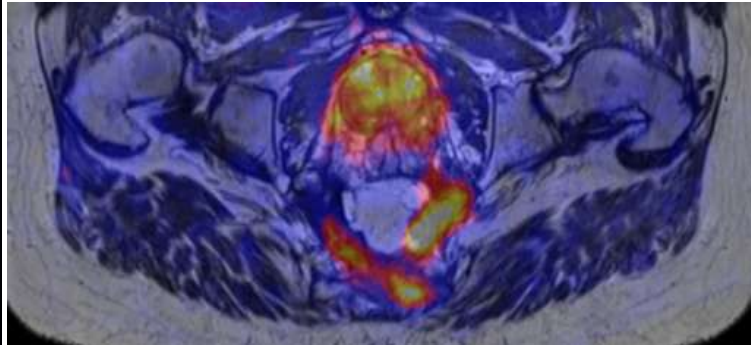
- Résolution spatiale
- Résolution temporelle
- Contraste
- Coût

■ **Agents de contraste**

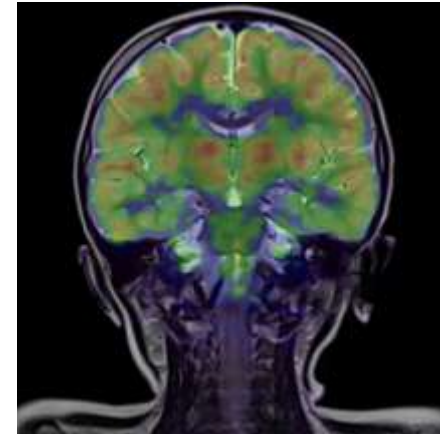
- Nécessaires pour chaque modalités d'imagerie
- Nouveaux développements => Specificité



- **Preuve de concept chez l'animal**
 - Accessibilité
 - Binding
 - Effet biologique / pharmacologique
- **Examens répétés chez le même animal permettant des études longitudinales**
- **Quantification d'un effet pharmacologique**
- **Identification de métabolisme**
 - De médicaments
 - D'effets physiopathologiques
- **Méthodologie transférable chez l'homme**
- **Biomarqueurs / surrogates**

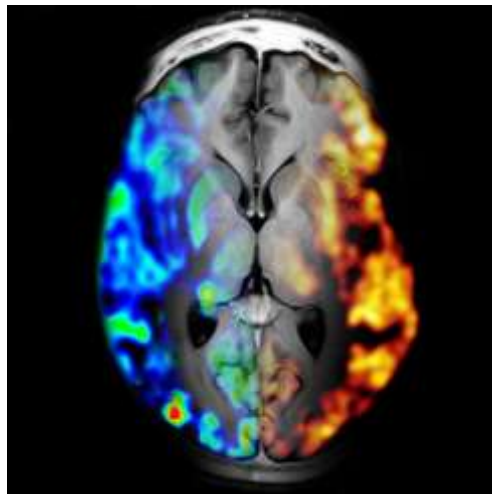


Temporal co-registration

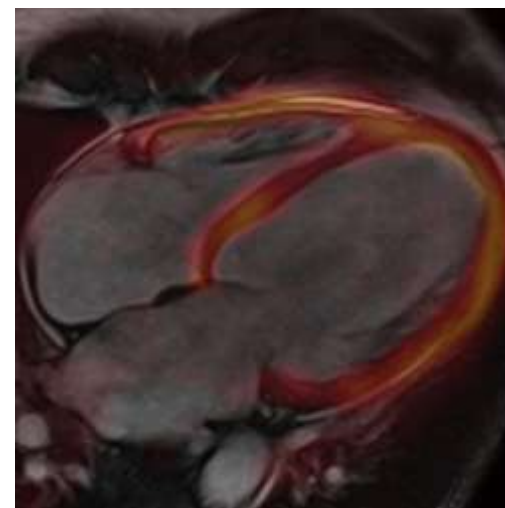


Pediatric oncology : minimize radiation dose (vs PET/CT)

Spatial co-registration



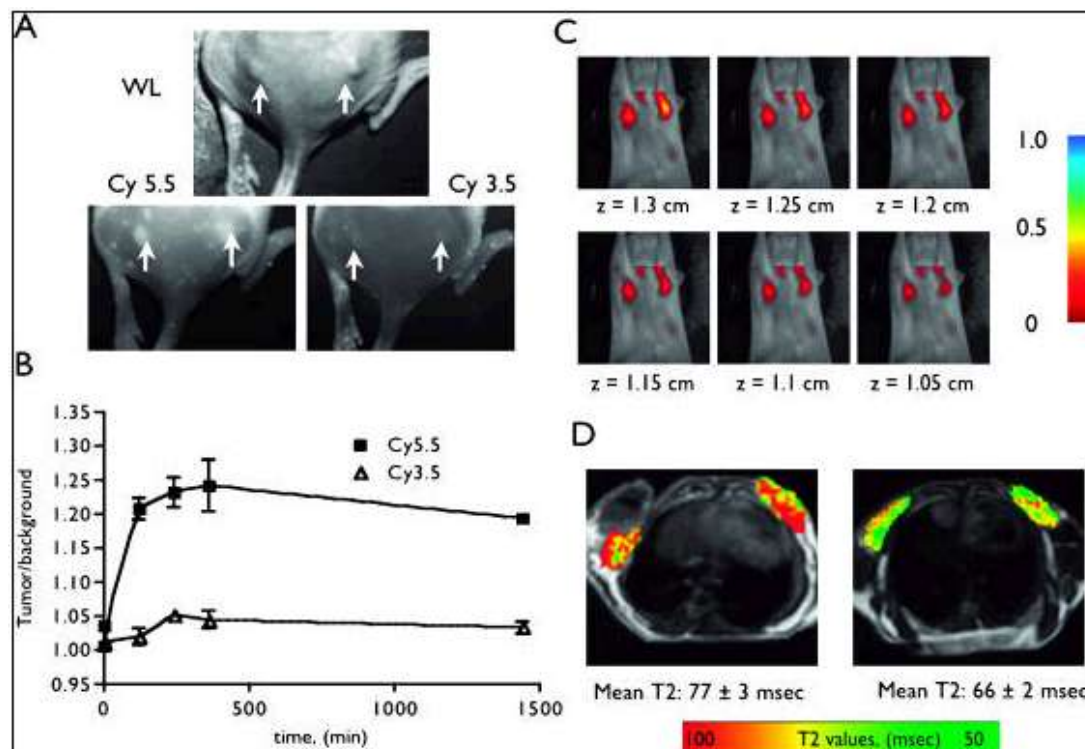
Minimise effect of motion





▪ Sondes bimodales (MRI + Optical)

Imagerie de l'accumulation du cRGD-CLIO- (Cy5.5) Nanoparticule par fluorescence et IRM.



Montet X et al . Neoplasia (2006) ; 8: 214 (Weissleder Group)

Recherche Guerbet – Une équipe pluridisciplinaire

