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## EXPERIMENTAL STUDIES OF URANIUM RENAL TOXICOLOGY IN LOW DOSES EXPOSURES

Yann Guéguen, Marie Frerejacques, Annabelle Manoury, David Suhard, Olivier Claude, Gillot Clara, V. Monceau, Christelle Elie, Finet Clémence, Chrystelle Ibanez, et al.

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Égalité  
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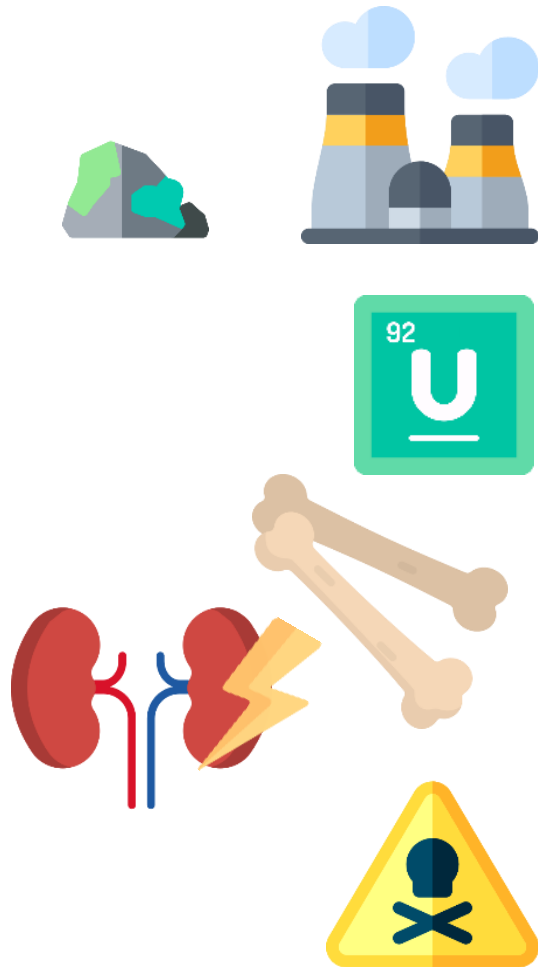
INSTITUT DE RADIOPROTECTION  
ET DE SÛRETÉ NUCLÉAIRE

# EXPERIMENTAL STUDIES OF URANIUM RENAL TOXICOLOGY IN LOW DOSES EXPOSURES

Yann GUEGUEN

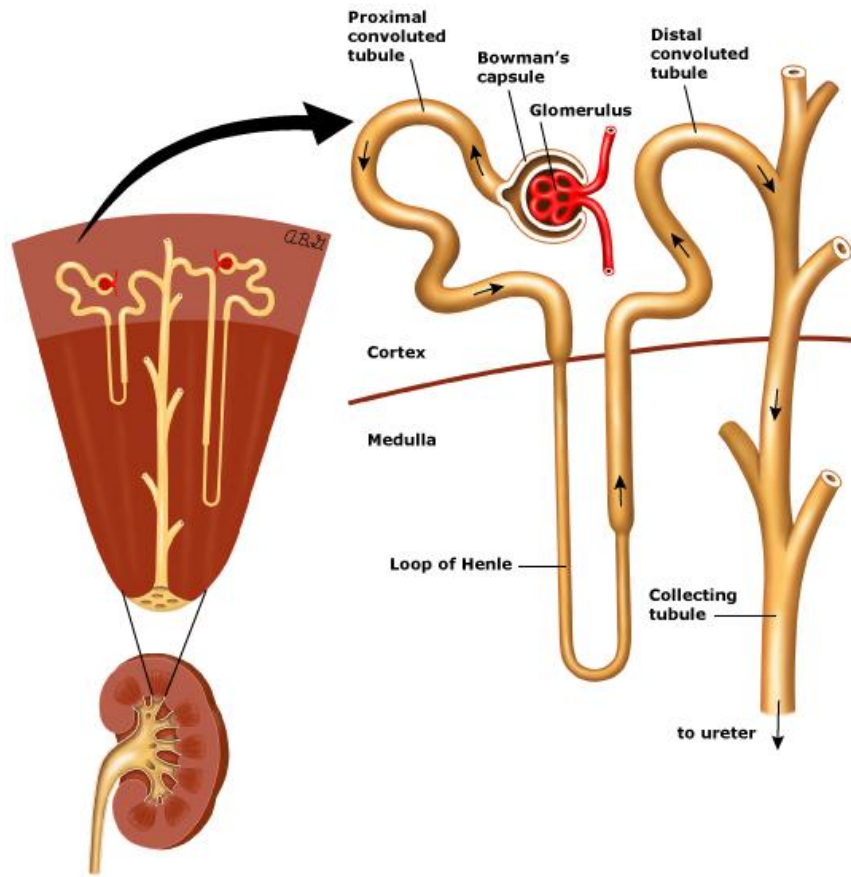
Institut de Radioprotection et de Sûreté Nucléaire (IRSN)  
PSE-SANTE, SESANE

## What we already know about uranium exposures and toxicities?



- Natural and anthropogenic presence (mine, fuel production, military uses)
- Chemical (metal-ion) and radiological ( $\alpha$ -emitting radionuclide) toxicities
- Affect human health mainly after internal contamination depending on the route of exposure, the chemical form, the isotopic composition [Stradling et al, 1984; Guéguen et al, 2017; Ansoborlo et al, 2006]
- Preferential accumulation in the kidneys and bones with highly heterogenous distribution in tissues and cells [Poisson et al, 2014; Guéguen et al, 2015; Suhard et al, 2018]
- Nephritis is the first symptom of uranium poisoning
- Known nephrotoxic agent : renal tubular damages associated with impairment

# Renal function and nephrotoxicity



## Mains renal functions :

- Homeostasis of the organism (electrolytes, water, osmotic pressure)
- Elimination of metabolic degradation products and xenobiotics
- Biosynthesis of hormones (vitamin D, EPO)

## Iatrogenic or environmental nephrotoxicity:

- Most highly perfused organ (/g tissue), therefore highly exposed to circulating toxins
- Toxic substances altered filtration, reabsorption and secretion
- Proximal convoluted tubules (PCTs) are the target of toxic substances
- Acute or chronic renal failure

➤ Cancer or non-cancer renal diseases?

## Experimental studies on going in the lab on uranium

**UKCAN**

Uranium Kidney  
CANcerous effect in mice

Collaborations : Gustave  
Roussy institute, Bicêtre  
Hospital

Co-funding Orano

**ARMOR**

AOP study of the renal  
toxicity of a radionuclide  
through a kidney organoid  
model

Collaboration:  
INSERM-IRMETIST

**BIOCUBK**

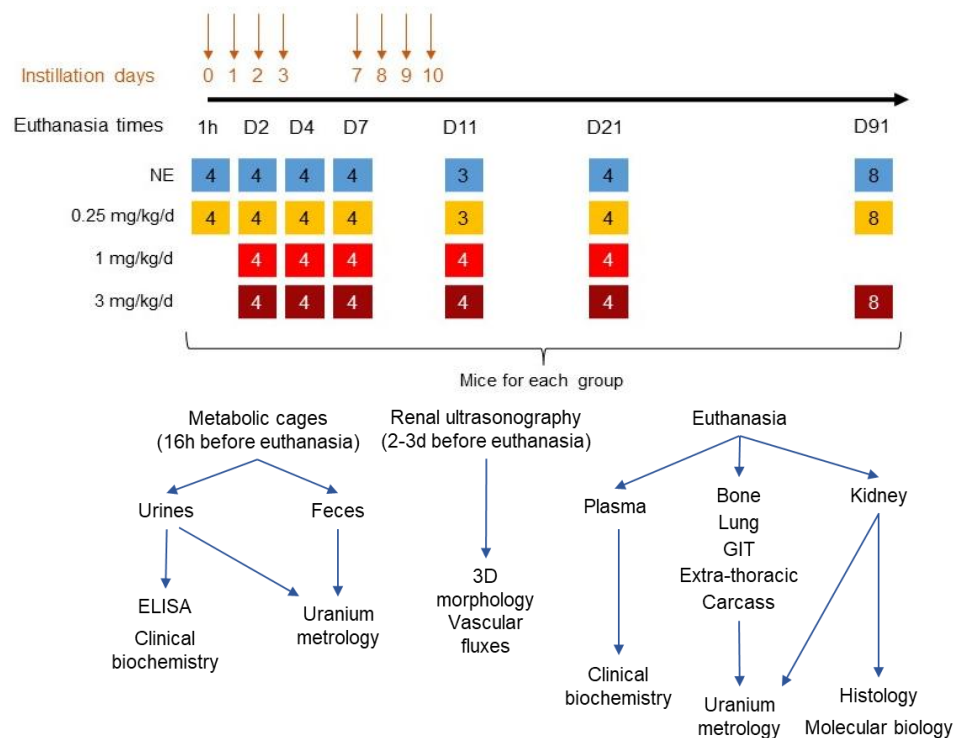
Biochemical Cycle of  
Uranium in Bone and  
Kidney

Collaborations:  
UCA/CEA/CNRS,  
University Lyon/ISA,  
ICN/RHE

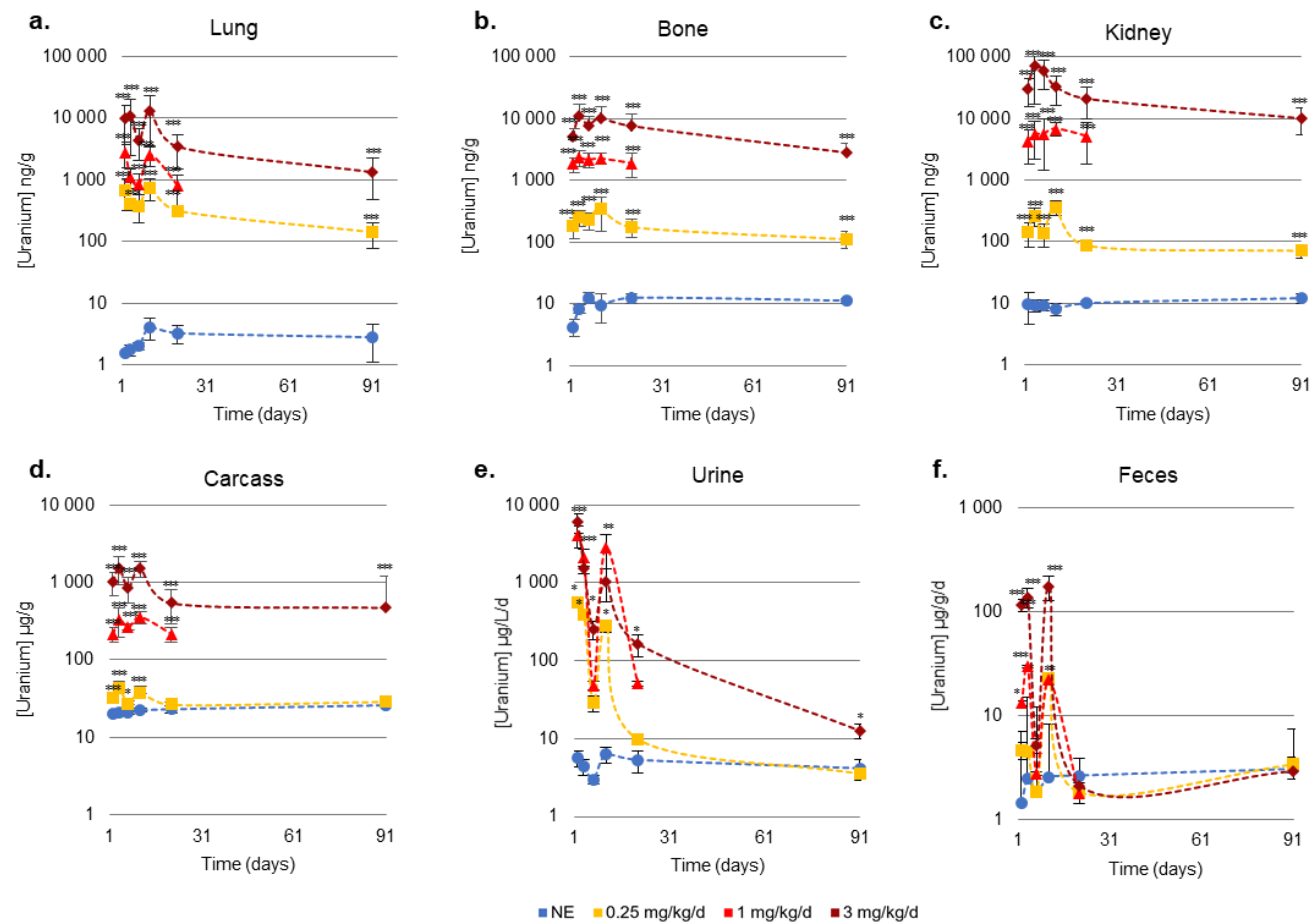
# Pre-UKCAN project

## Uranium induced nephrotoxicity via inhalation

### Biokinetics in organs



- Mice were exposed to the repeated instillation of uranium in a dose–response study to mimic inhalation
- Consistent with the established ICRP model for inhalation except for renal uranium retention for the highest uranium dose

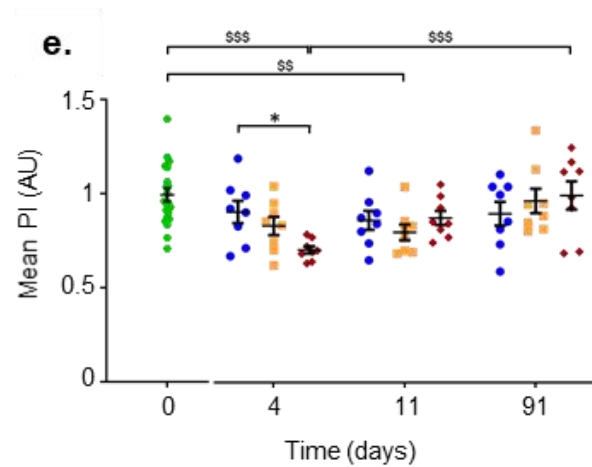
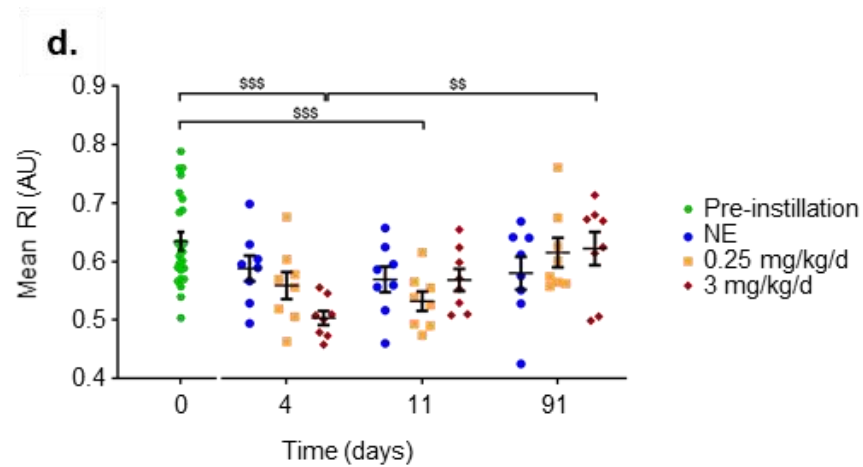
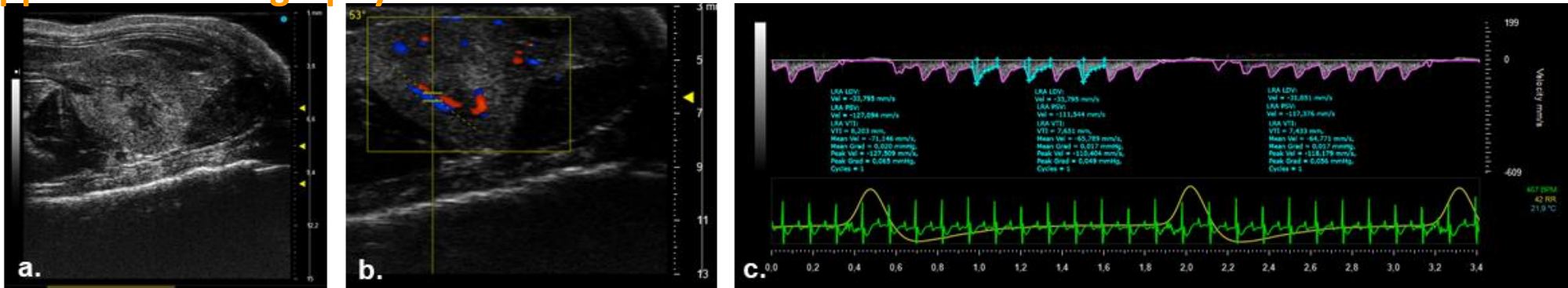


*De Castro et al, Sci Reports 2023*

# Pre-UKCAN project

## Uranium induced nephrotoxicity via inhalation

### Doppler-ultrasonography

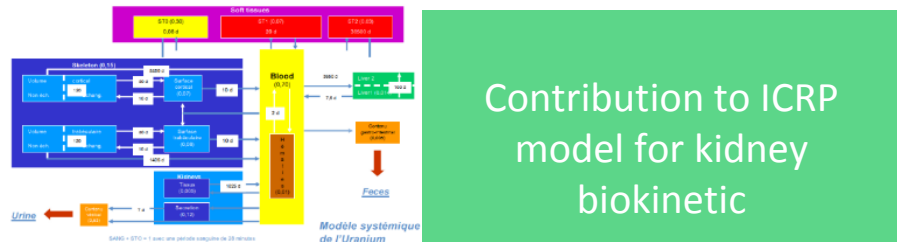
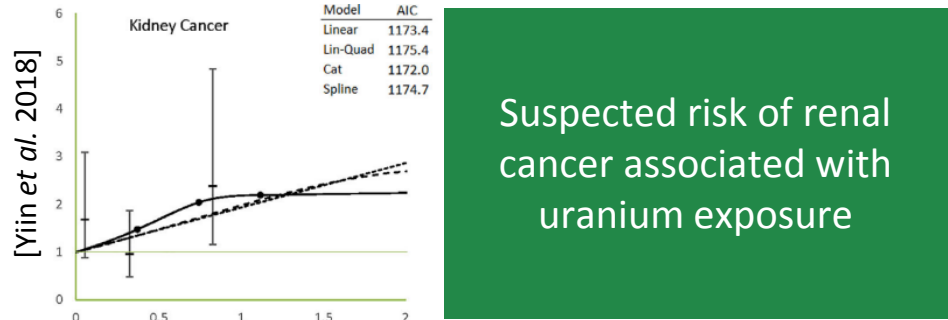
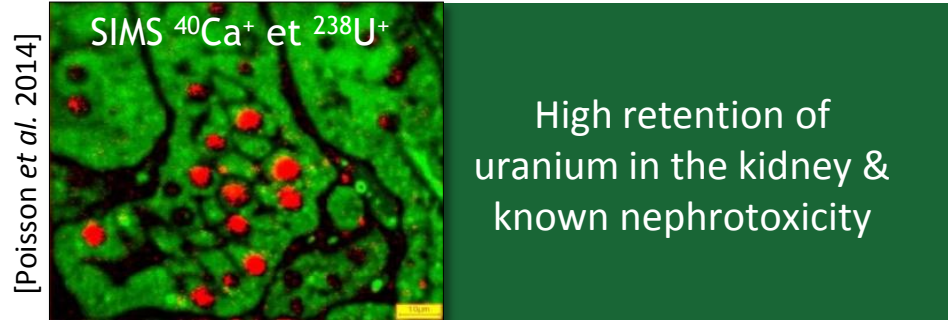


- Early (D4) decrease vascular parameters (resistivity and pulsatility indexes for the highest dose (3 mg/kg/d)
- Late decrease at D11 for the lowest dose (0.25 mg/kg/d)
- Transient acute renal failure for dose less than 3 mg/kg/d

*De Castro et al, 2023*

# UKCAN project

## Uranium kidney cancer effect in GEEM models (genetically-engineered mouse)



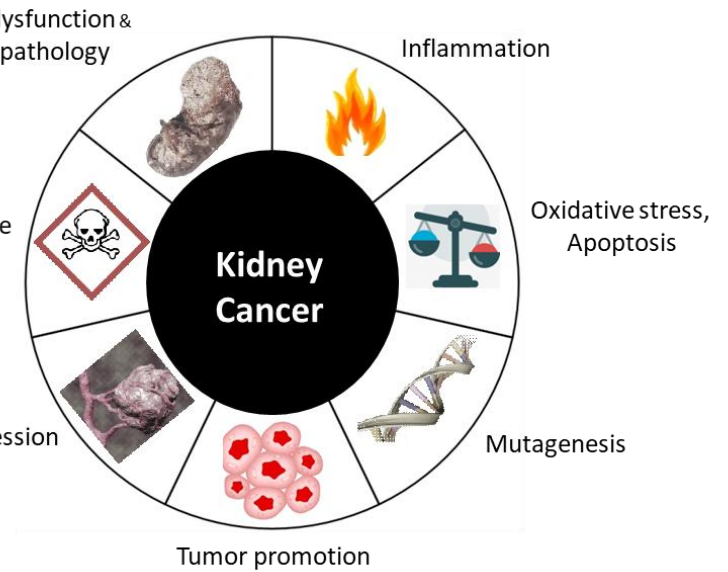
GEEM models representative of human kidney cancer



Exposure to repeated U-exposure at low-doses



Longitudinal follow-up during 12 months by echography & metabolic cages





# UKCAN project

## Uranium kidney cancer effect in GEEM models

**Pax8-cre, Vhl<sup>f/f</sup>**  
**Pax8-cre, Vhl<sup>f/f</sup>, Pbrm1<sup>f/f</sup>**  
 [Gu et al. 2017]

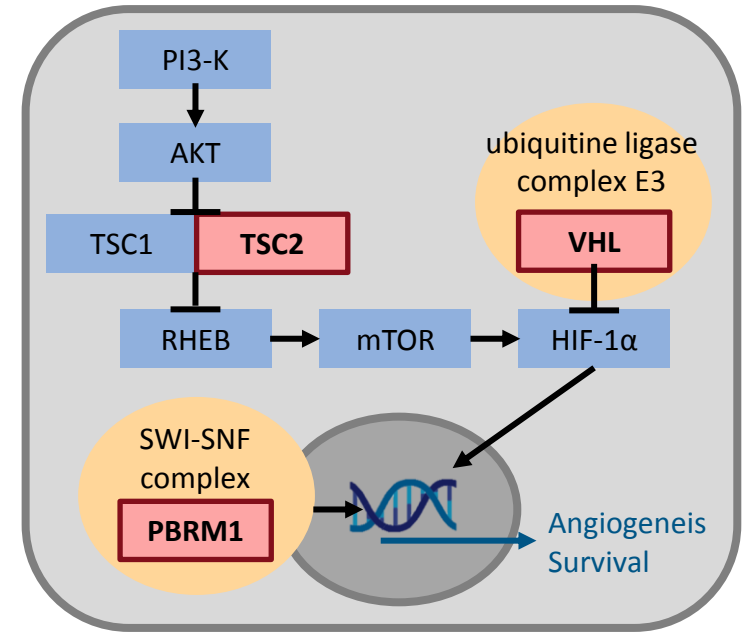
C57BL6



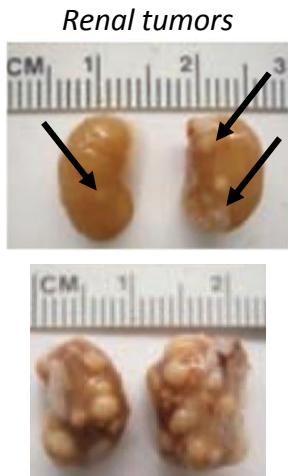
**Tsc2<sup>+/-</sup>**  
 [Onda et al. 1999; Yang et al. 2015; Surshkumar et al. 2019]

Renal carcinogenesis similar to human ccRCC

Benign and malignant renal lesions



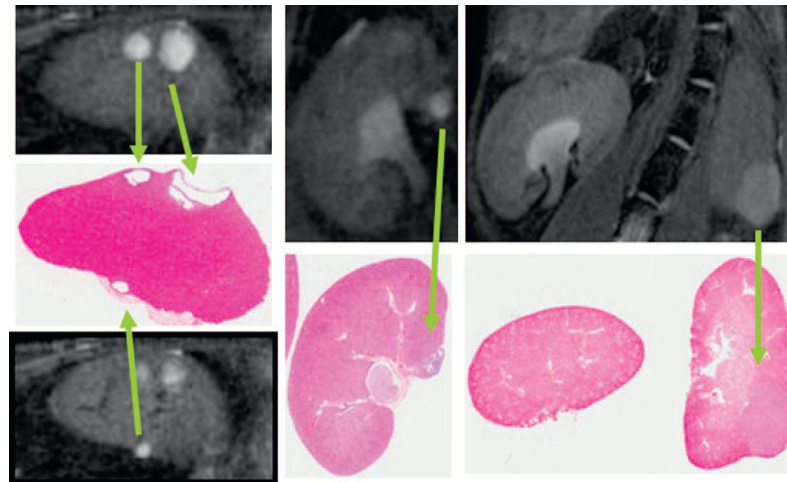
Signaling pathways involved in kidney cancer



7 to 9 months  
 First tumors  
 Survival >75%

10 to 14 months  
 Multiples tumors  
 Survival <50%

After 15 months  
 Tumors invasion  
 Survival <20%



Kystes

Adénome papillaire

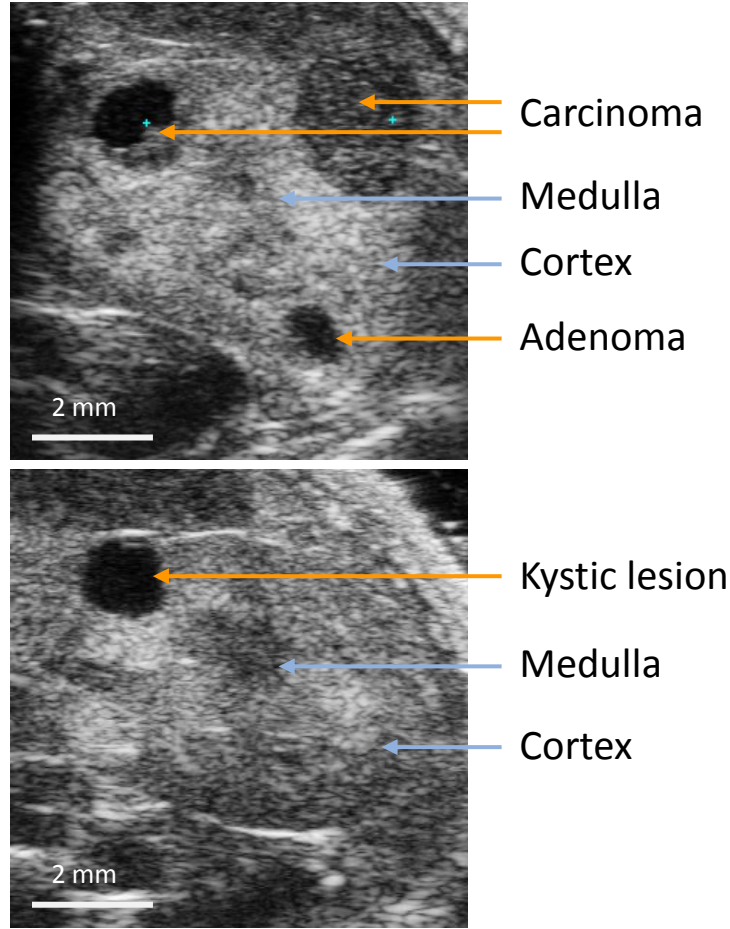
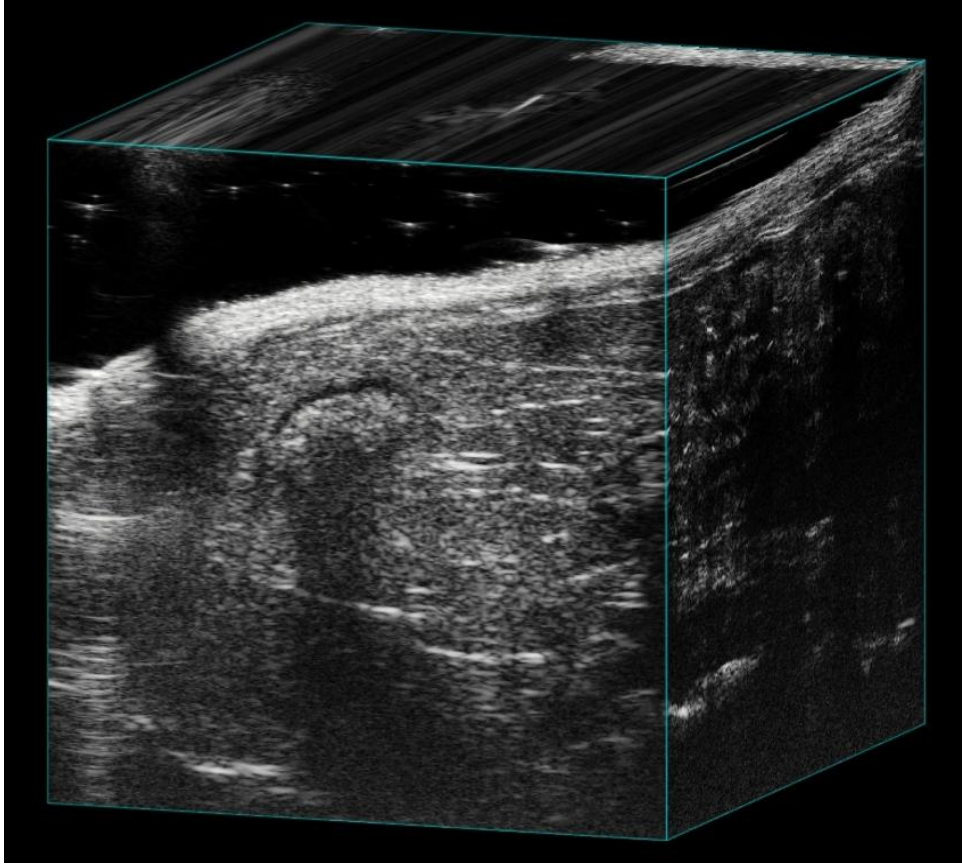
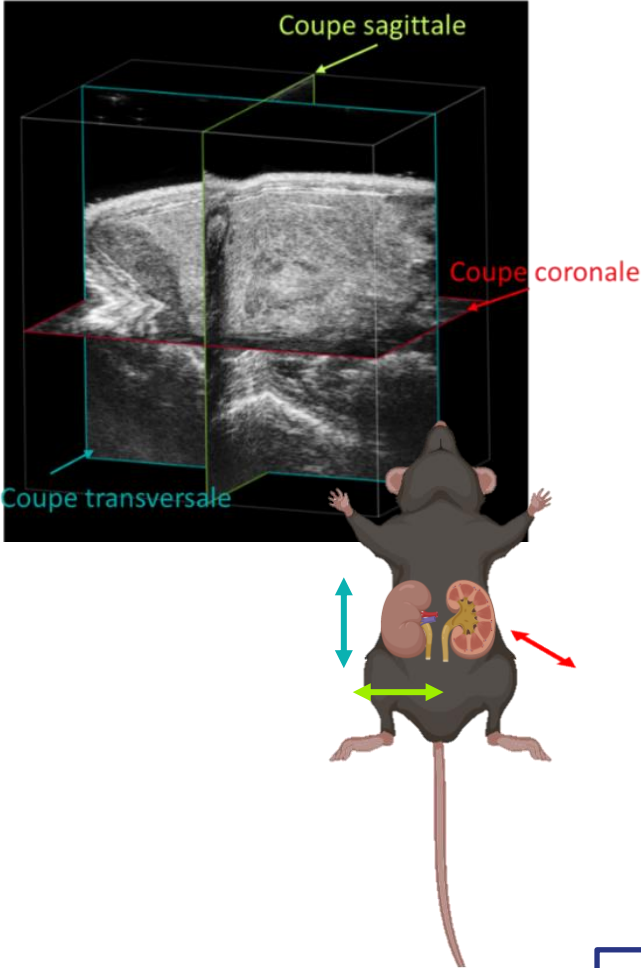
Carcinome solide

➔ Representative of human renal cancerogenesis and its pathological mechanisms

# UKCAN project

## Uranium kidney cancer effect in GEEM models

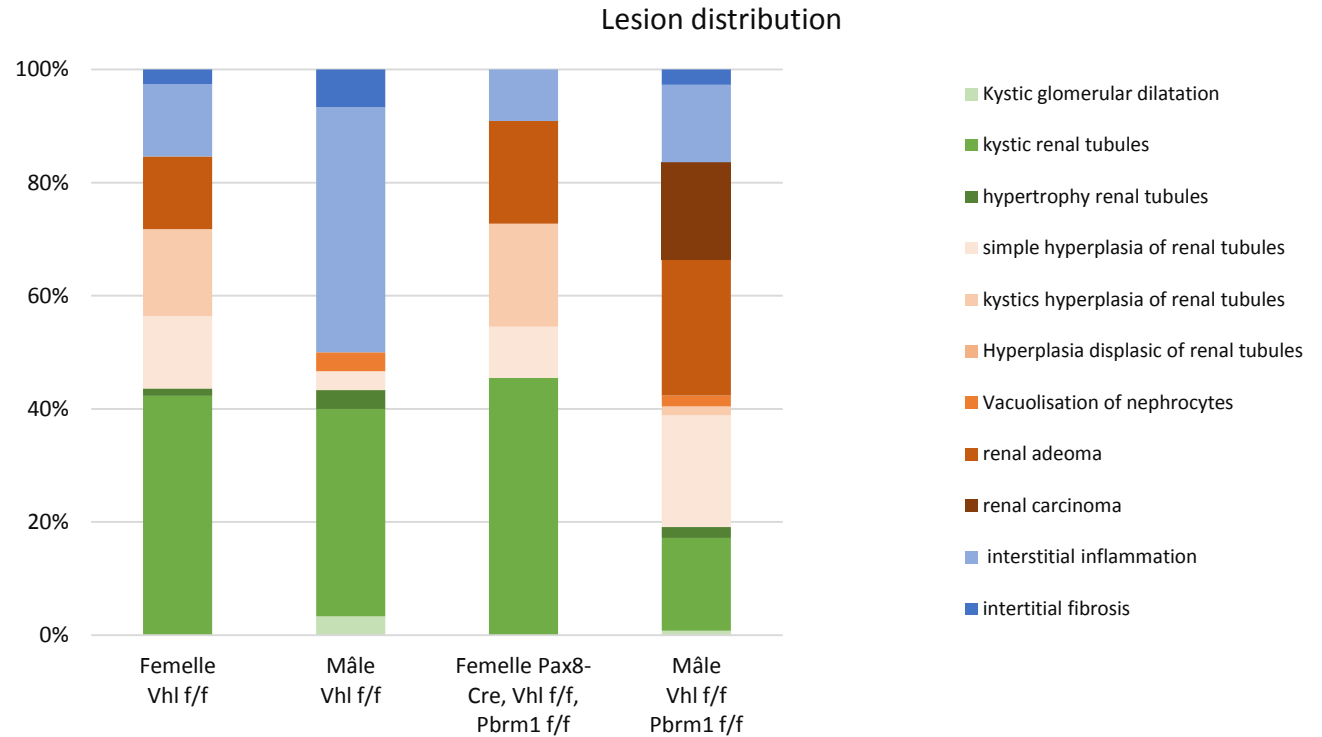
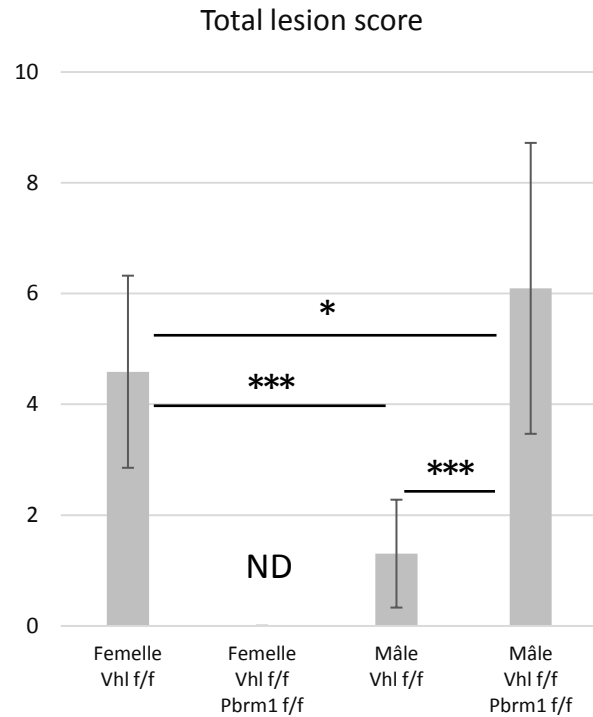
### Visualisation des lésions



Correlation of echography analysis to histological analyses

# UKCAN project

## Histological scoring of lesions



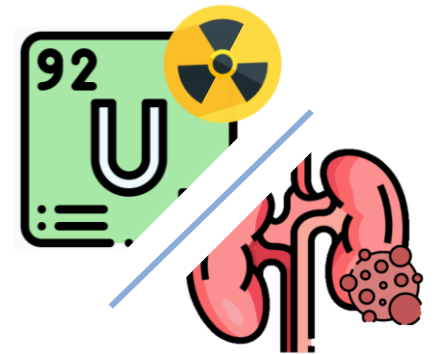
Lesions scores depends on sex and genotype

Wilcoxon test, \* p < 0,05; \*\*\* p < 0,001  
 Females *Vhl*<sup>f/f</sup> n = 17  
 Males *Vhl*<sup>f/f</sup> n = 23  
 Males *Vhl*<sup>f/f</sup> *Pbrm1*<sup>f/f</sup> n = 43

## On going projet

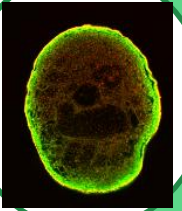
Study of potential renal cancerous effect of uranium exposure and its correlation to heterogenous distribution in the tissue

1. Evaluation of tumor incidence as a function of time and dose
2. Study of the hallmarks and mecanisms of tumorigenesis

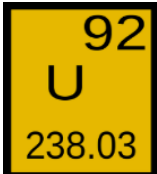


# ARMOR project

## AOP (Adverse Outcome Pathway) study of uranium-induced kidney toxicity in iKO



Development of kidney organoid model from stem cells, representative of a human organ



Improvement of knowledge for renal impairment due to nephrotoxicants such as Uranium

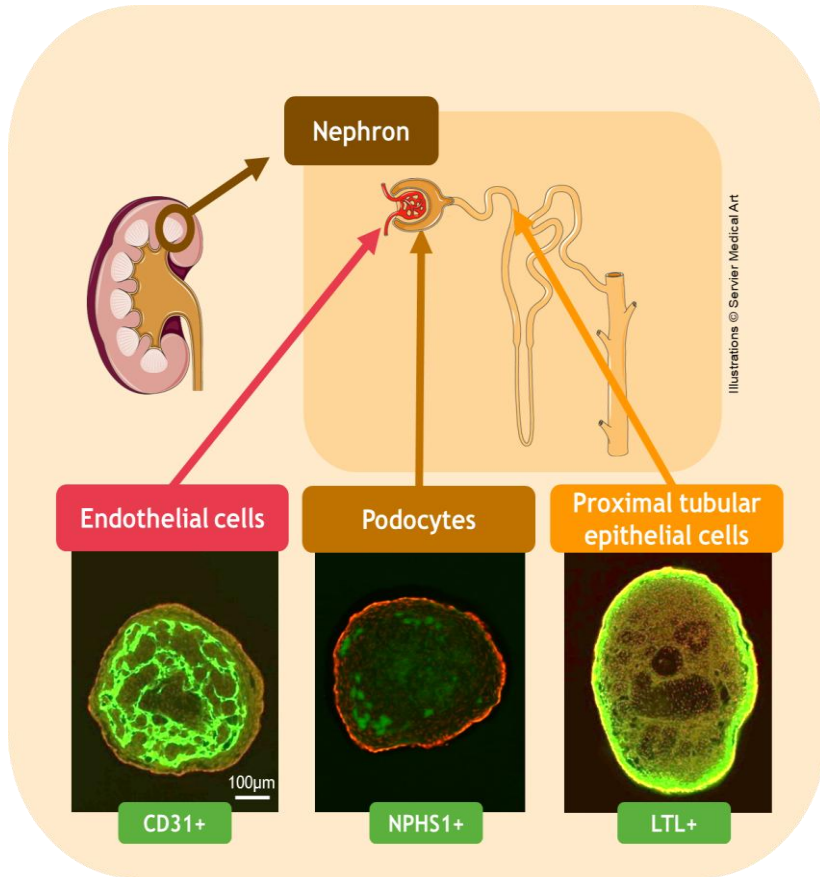
AOP Wiki

Construction of AOP for risk assessment

- **AOP (Adverse Outcome Pathway)** : characterization of detailed toxicity pathways leading to deleterious effect
- **Ionizing radiation** and AOP : kidney toxicity induced by uranium identified as a priority by Rad/Chem expert group
- Objectives:
  - ✓ **Identify biological key events** that are causally and sequentially linked in comparison to other stressors by molecular and protein assays
  - ✓ **Study of morphological and functional deleterious effects** by immunohistology and enzymatic assays
  - ✓ **Localize and quantify uranium** in the kidney organoid culture model by SIMS and ICP-MS

# ARMOR project

## AOP (Adverse Outcome Pathway) study of uranium-induced kidney toxicity in iKO

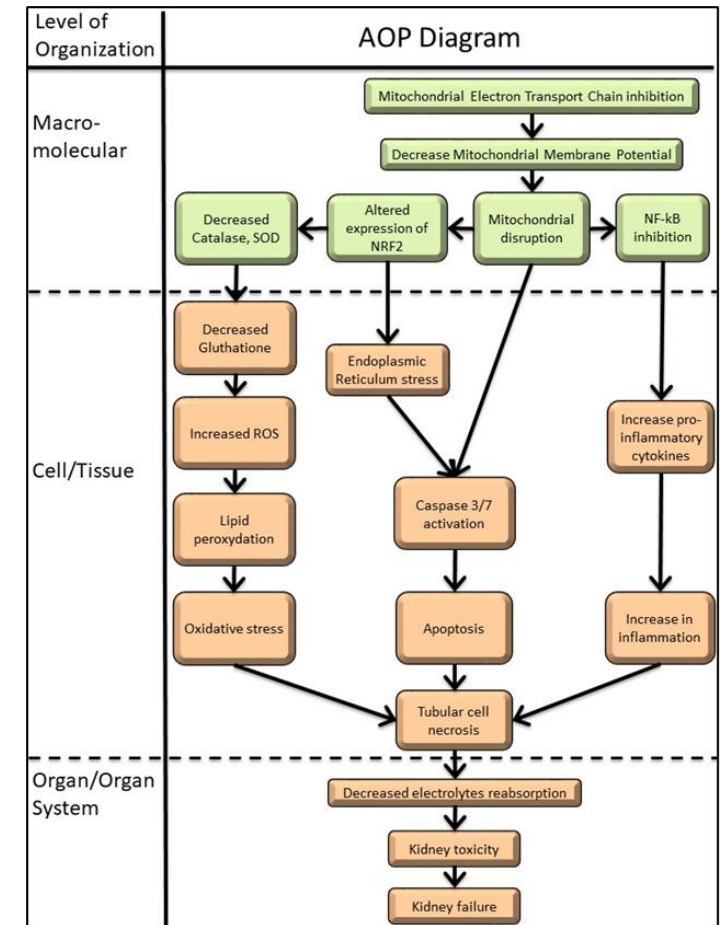


### Induced Kidney Organoids (iKO)

- Generation from human **Induced Pluripotent Stem cells (iPS)**
- Induction of **cell differentiation** with growth factors following fetal development
- **Immunolabeling cell population** by sheet light microscopy
- Follow up of **nephron structure** development by histology (HES staining)

### AOP447:

Proposed AOP diagram of kidney failure induced by inhibition of mitochondrial electron transfer

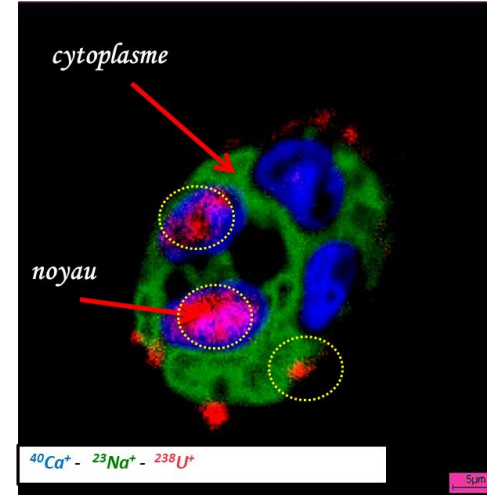


Guéguen & Frerejacques, *IJMS* (2022)  
[AOP-Wiki \(aopwiki.org\)](http://AOP-Wiki.org)

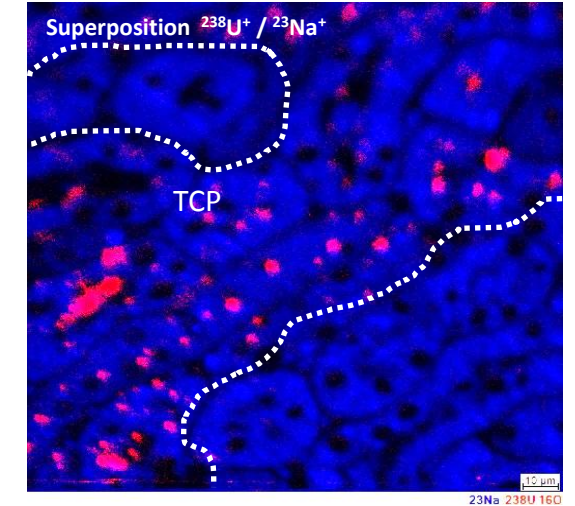
# ARMOR project

## Biodistribution of uranium in iKO

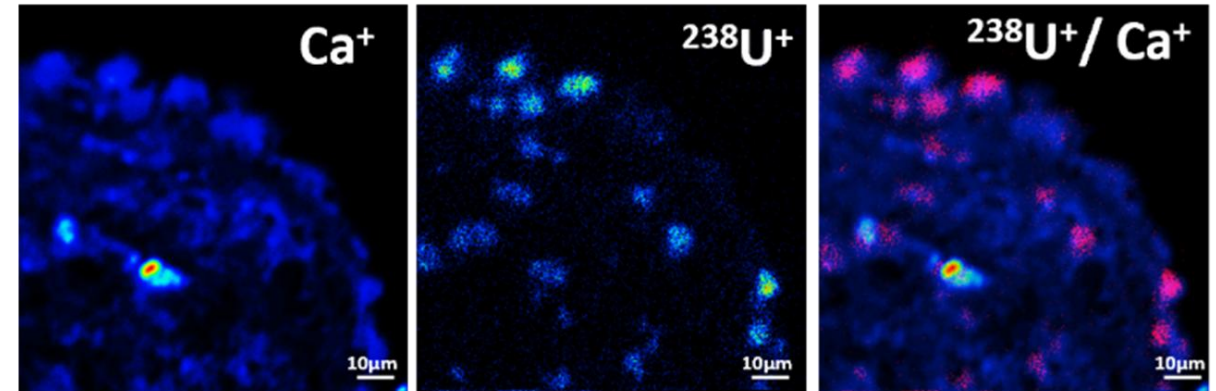
- The **SIMS (Secondary Ion Mass Spectrometry)** technique allows to obtain ionic images of endogenous or exogenous elements in a biological tissue
- Heterogeneous distribution of uranium, preferentially located in the nuclei at low concentration (<100µM)
- Uranium enters into the cells of the kidney organoid model
- Heterogenous distribution of uranium at cellular and subcellular level at 100µM and 1000µM



**Hepatocytes** exposed to 100µM UA  
(**uranium, calcium, sodium**)  
Guéguen et al, Toxicol In Vitro 2015



**Kidney tissue** of mouse exposed to 3mg/kg UA (**uranium, sodium**)  
Unpublished data IRSN – David Suhard



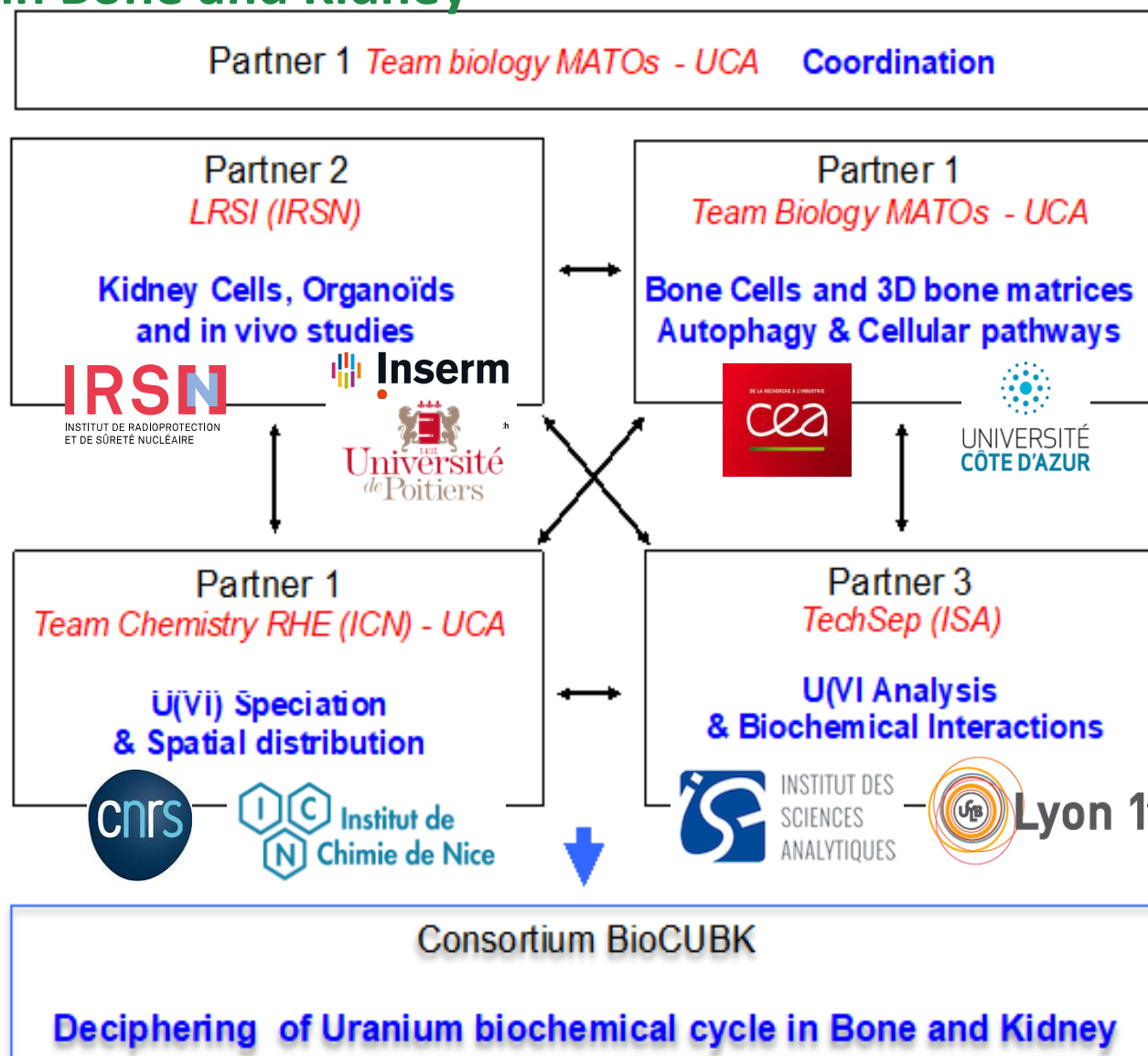
**Kidney organoid** exposed to 100µM UA 24h (J22)  
(**uranium, calcium**)  
Unpublished data IRSN – David Suhard





# BioCUBK : Biochemical Cycle of Uranium in Bone and Kidney

- Funding ANR-22—CE44-0027
- Consortium of specialist on uranium
- Interdisciplinary research (radiochemistry, biochemistry et radiotoxicology)
  - MATOs, Mecanisms of bone tissue alteration
  - RHE, Radiochemistry in human and environment
  - TechSep, Separative technics
  - LRSI, Nephrotoxicology mecanisms of radioactivity
  - IRMETIST, Ischemia-reperfusion en transplantation



# Acknowledgements

## IRSN

**Radiotoxicology, cancerology,  
imaging**

LRSI :

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David Suhard

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LRTOX :

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Virginie Monceau

Chrystelle Elie

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Amandine Sache

Frédéric Voyer

LEDI:

Estelle Davesne



**Génétique des cancers du rein**

Sophie Gad-Lapiteau

Flore Renaud

Sophie Couvé



**Anatomopathologie clinique**

Sophie Ferlicot



**Tissu osseux & mécanismes cellulaires**

Georges Carle

Sabine Santucci



**MTA souris Pax8-cre/Vhl/Pbrm1**

James Brugarolas



**Organoïd model**

Clara Steichen

Thierry Hauet



**Anatomopathologie vétérinaire**



**Génotypage des animaux**



**Co-financement UKCAN**



**Co-financement BioCUBK**

# ETUDES EXPÉRIMENTALES DE LA TOXICOLOGIE DE L'URANIUM LORS D'EXPOSITIONS À DE FAIBLES DOSES

Yann GUEGUEN

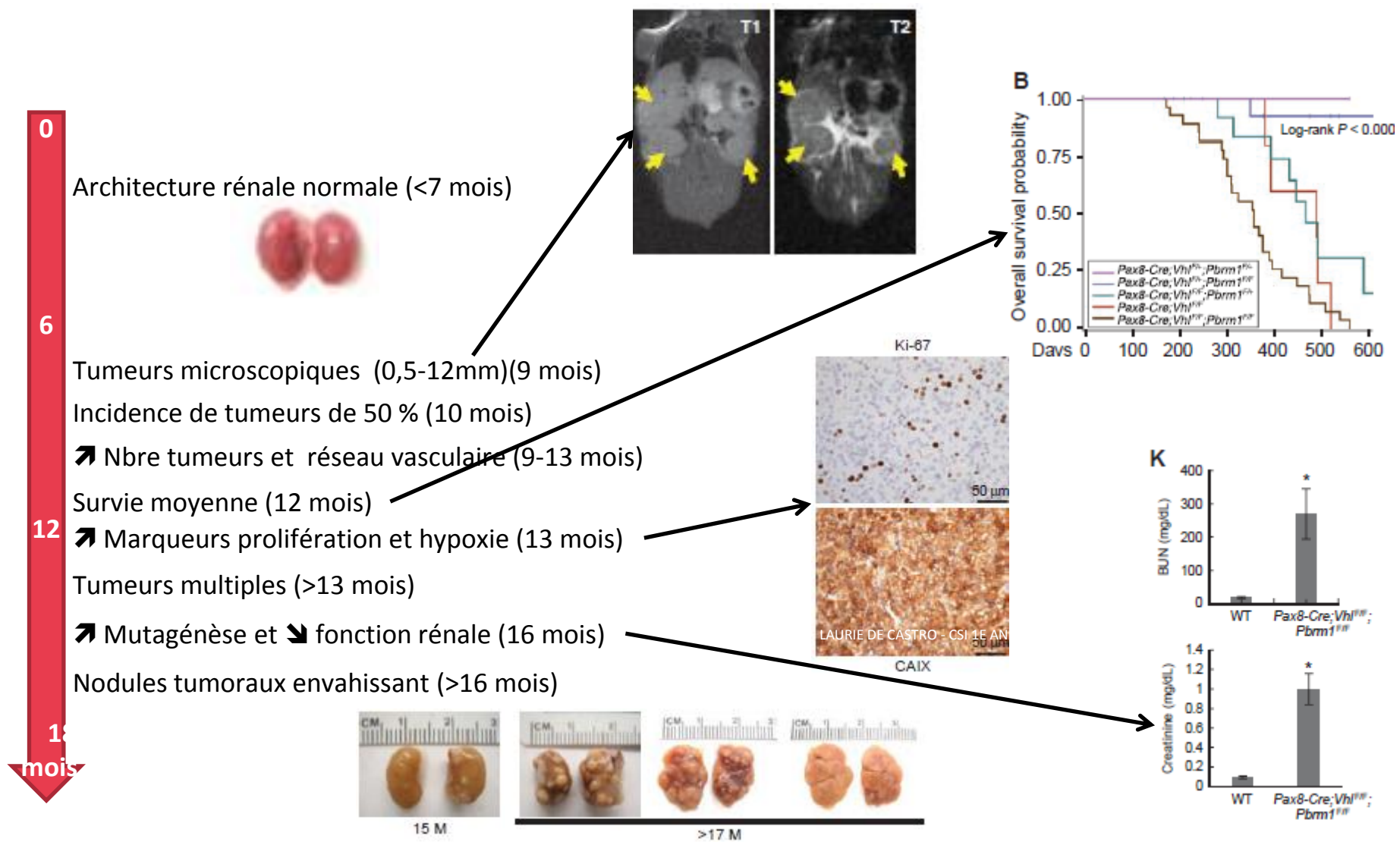
Pôle Santé et Environnement

Service de recherche sur les effets biologiques et sanitaires des rayonnements ionisants



# Développement du cancer rénal chez les souris Pax8-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/F</sup>

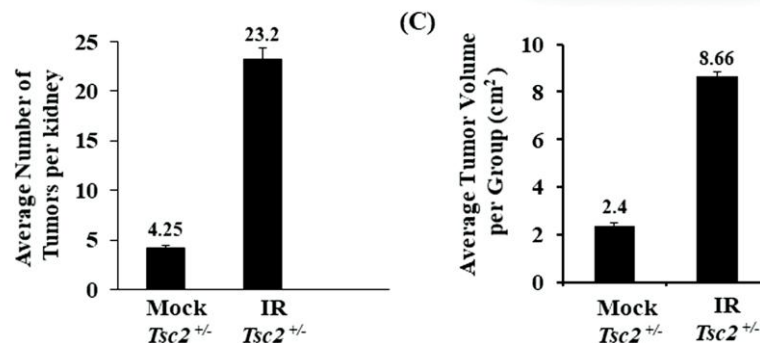
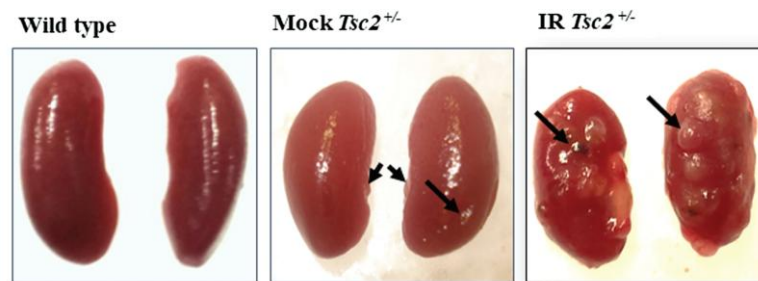
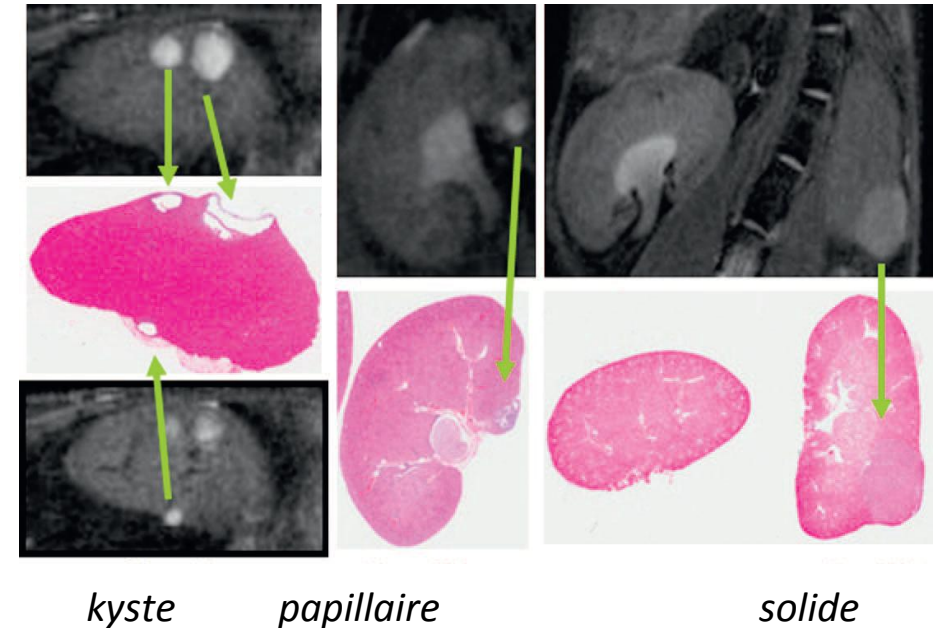
ccRCC de grade faible



# Développement du cancer rénal chez les souris $Tsc2^{+/-}$

## Phénotype

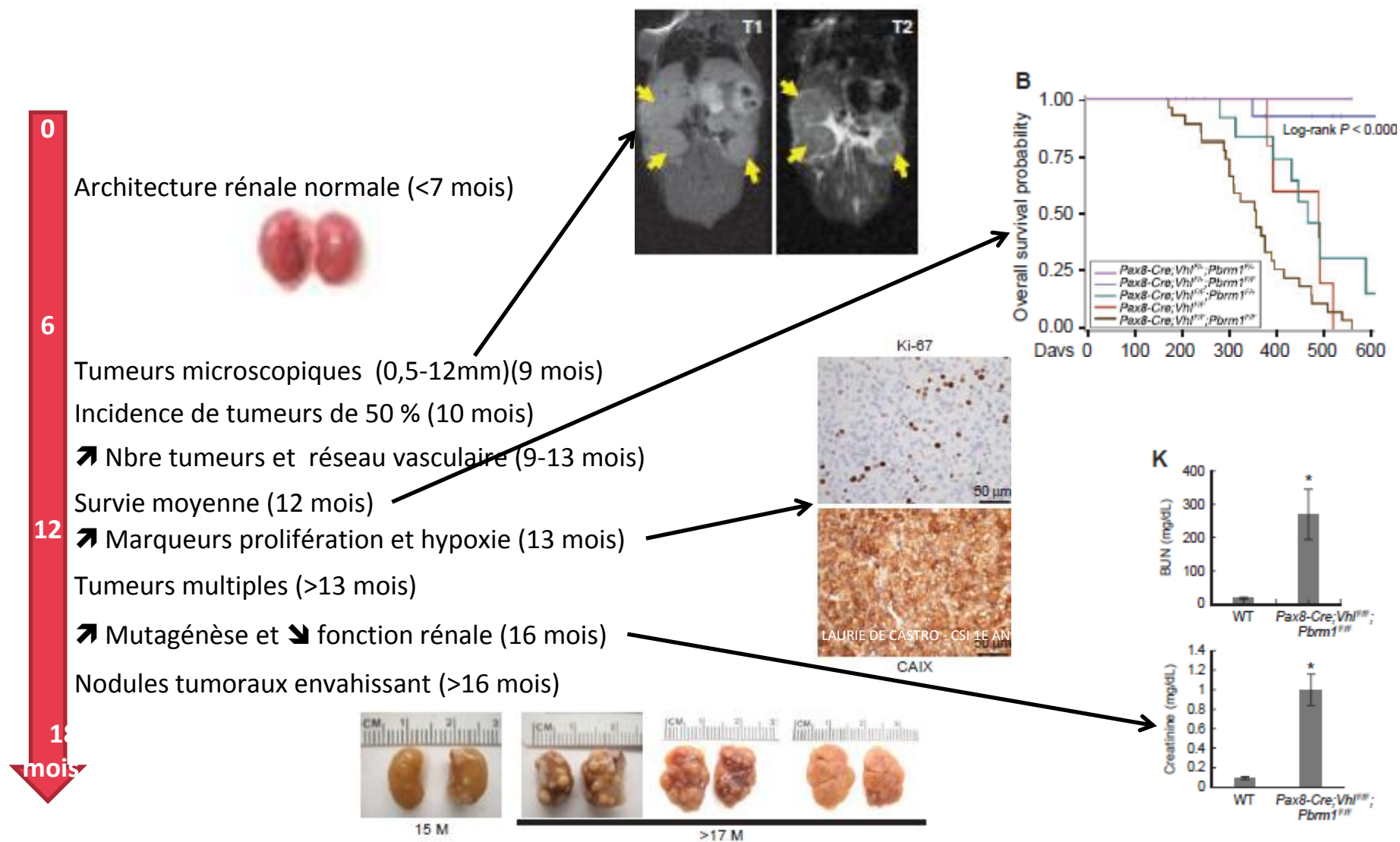
- Tuberosus sclerosis (TSC) : syndrome génétique mutation de TSC1 ou TSC2
- TSC1 & 2: gènes suppresseur de tumeurs (inhibition de la voie mTOR)
- Lésions rénales multiples : kyste, néphroblastome bénin, adénome solide, carcinome



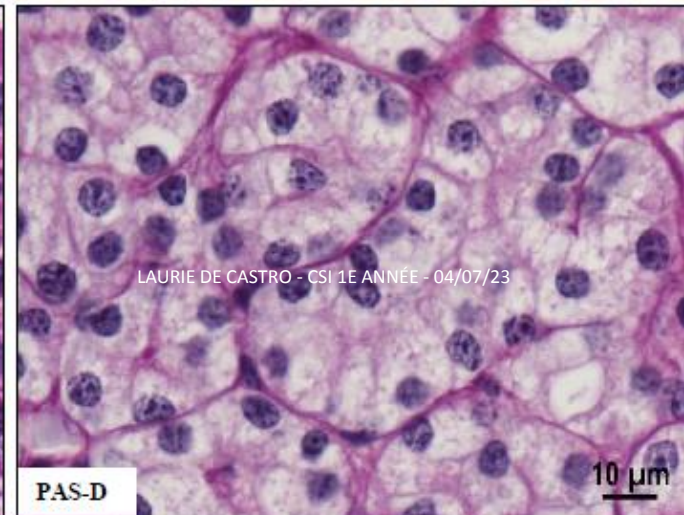
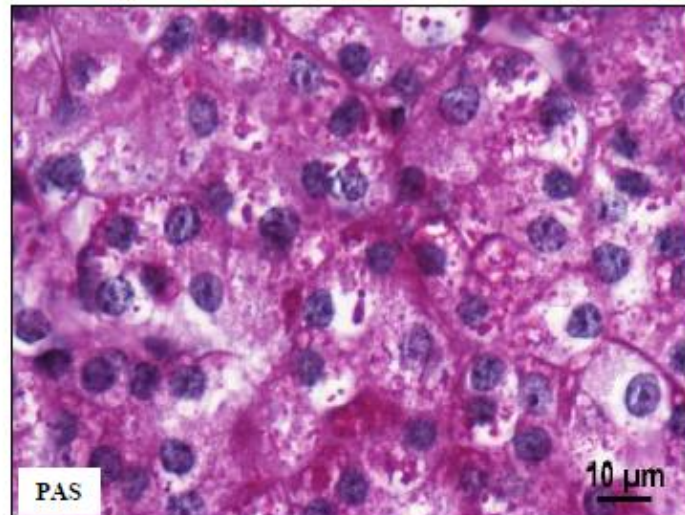
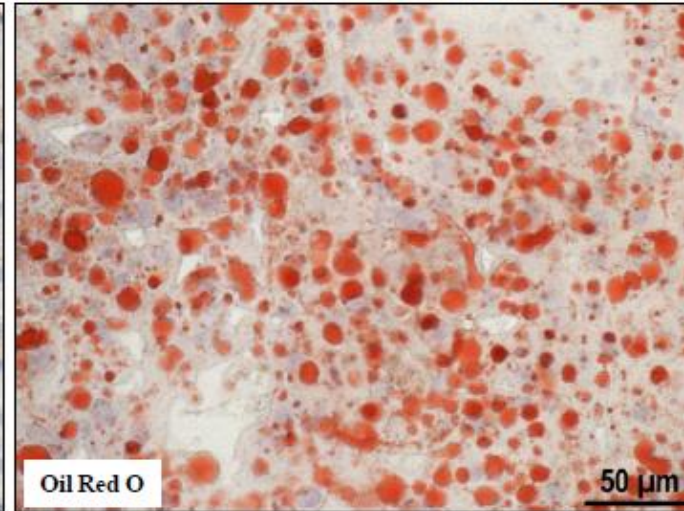
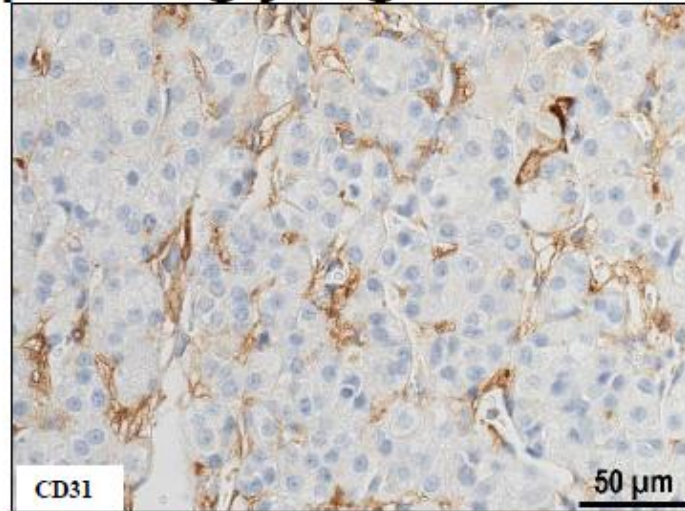
- Induction de tumeurs rénales chez les souris  $Tsc2^{+/-}$  après exposition IR faibles doses répétées (*Sureshkumar et al, 2019*)

# Développement du cancer rénal chez les souris Pax8-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/F</sup>

ccRCC de grade faible

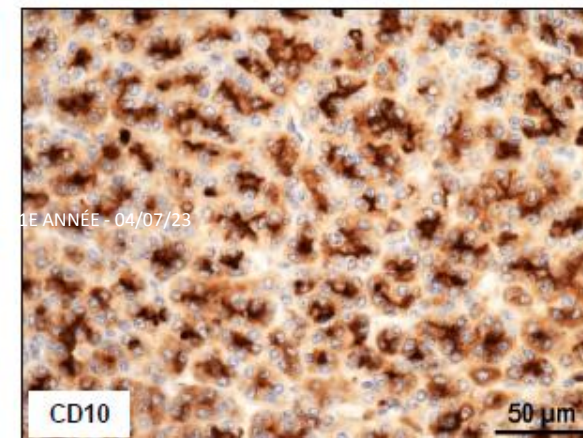
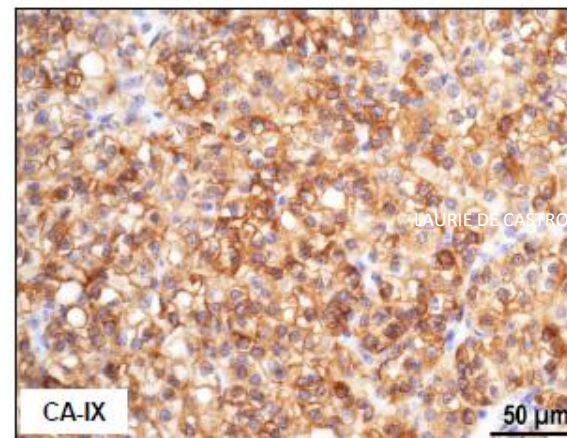
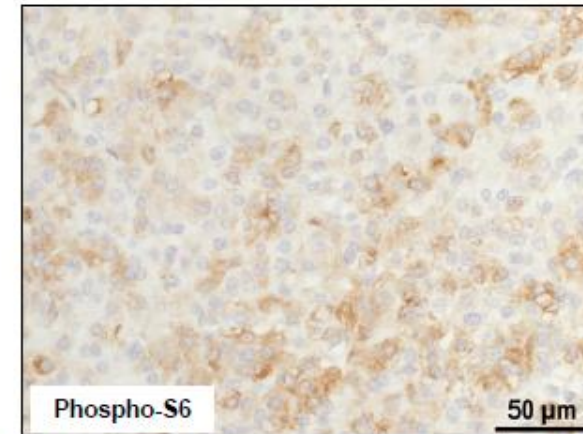
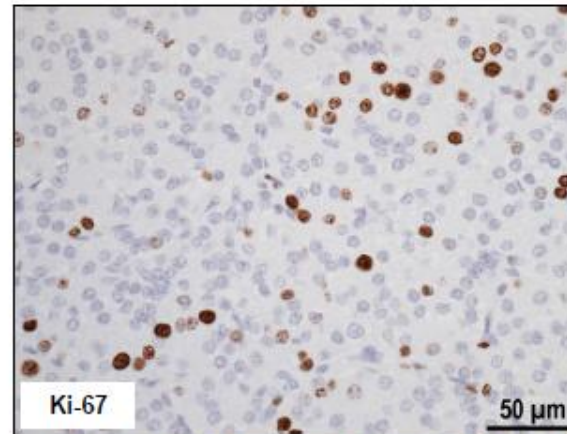
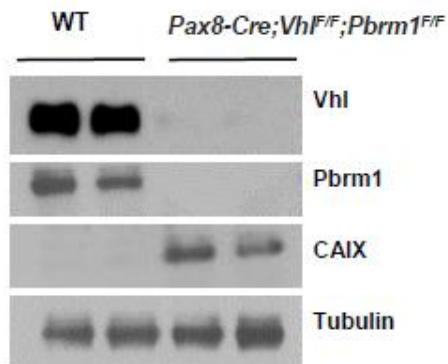
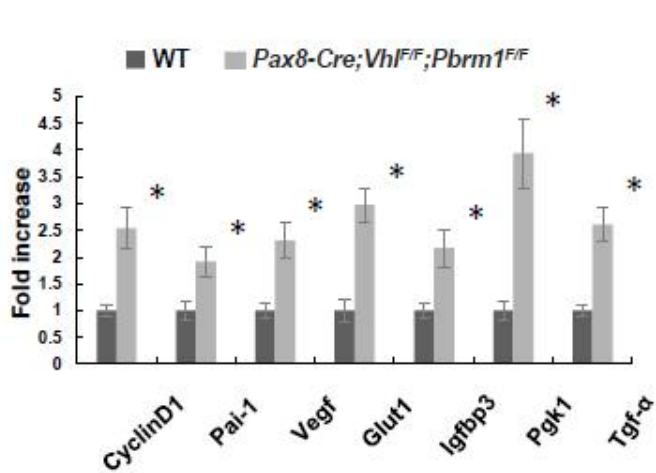


Tumors in *Pax8-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/F</sup>* mice show vascular pattern, neutral lipids & glycogen accumulate akin to human ccRCC



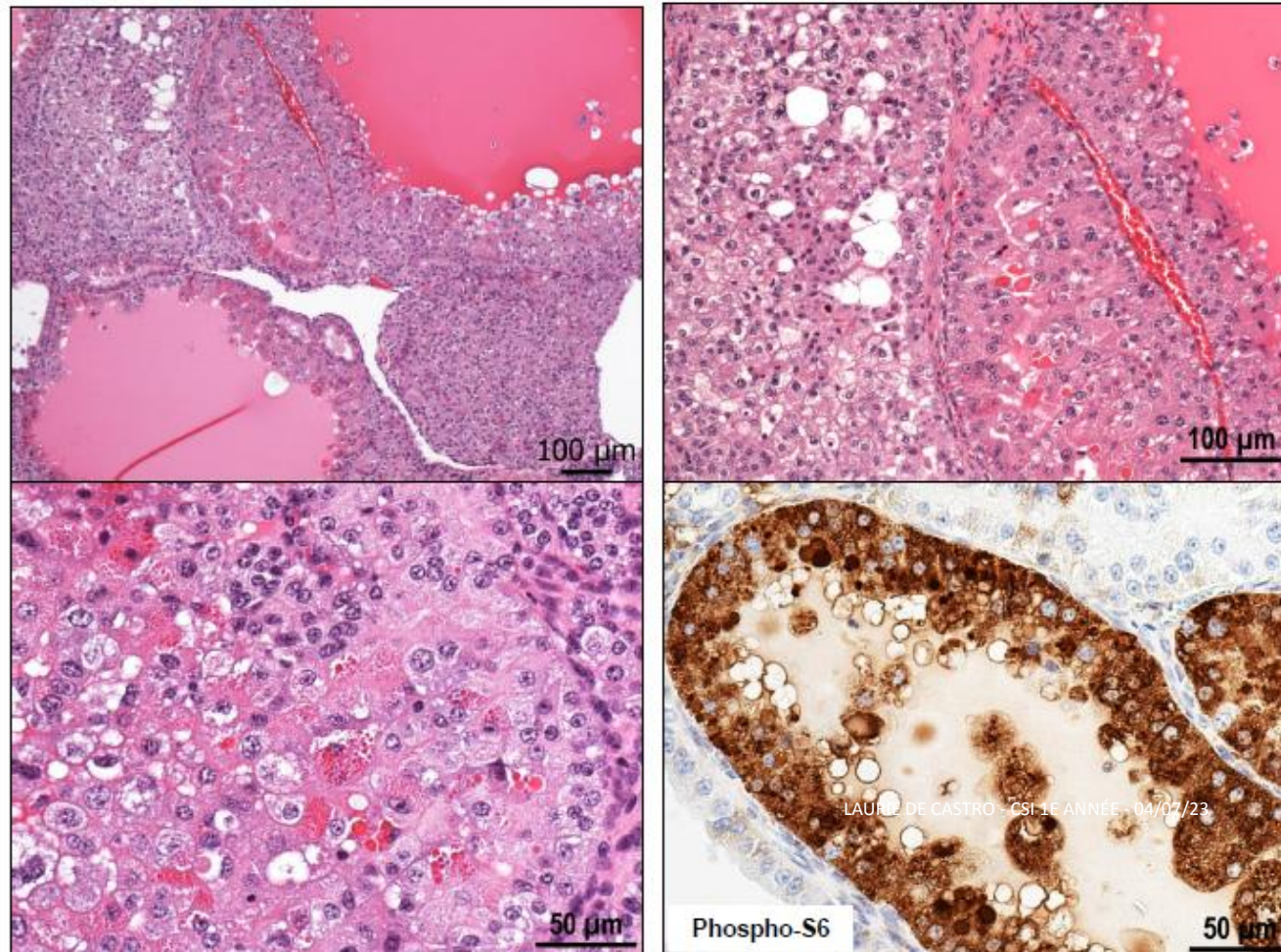


# Tumors in *Pax8-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/F</sup>* mice show expression profile similar to human ccRCC



LAURIE DE CASTRO - 1<sup>RE</sup> ANNÉE - 04/07/23

## Rare high grade tumors in *Pax8-Cre;Vhl<sup>F/F</sup>;Pbrm1<sup>F/F</sup>* mice



High grade tumors were seen in 20% (5/26) of mice and constituted 4% (11/253) tumors observed. They showed strong diffuse pS6 expression suggesting mTORC1 may contribute to higher grade.

# VEVO 3100 – Echographie haute résolution / Doppler (ultrasonographie)

Chercheurs responsables de la plateforme : Y. Guéguen & V. Monceau (PSE-SANTE/SESANE)

## Vevo® 3100 imaging platform (Fuji-Visualsonic)

### Transducers used:

- MX550D – Bandwidth 25-55 MHz, Axial Resolution: 40 µm, Image range 14x15x7 mm
- MX250 – Bandwidth 13-24 MHz, Axial Resolution: 75 µm, Image range 23x36x15 mm

### Imaging Modes:

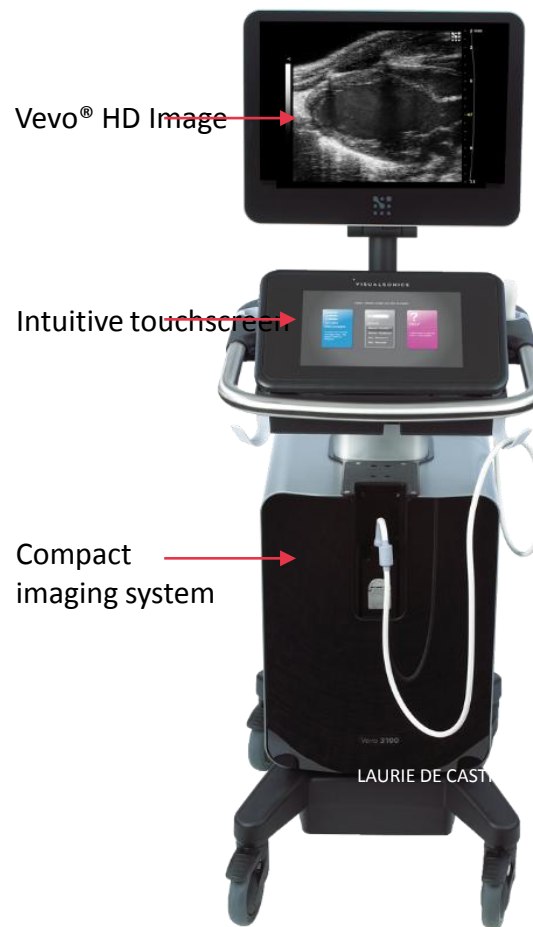
- Contrast/Molecular Imaging (B-Mode, M-Mode, Non-Linear Contrast Mode)
- Tumor imaging, cardiac cycle (3D-Mode, 4D-Mode)
- Measure Physiological parameters (ECG, heart rate, core temperature, respiration, blood pressure)
- Blood flow velocity, percent vascularization (Power Doppler Mode, Color Doppler Mode)

### Animal model:

- Kidney Cancer mouse model
- Atherosclerosis mouse model

### Applications:

- UKCAN1-3: Renal blood flow
- UKCAN5-6: Kidney tumors imaging
- ARARAT: Atherosclerosis plaque imaging
- CREDO
- RADIOMETRY
- SIROCCO



Vevo® HD Image

Intuitive touchscreen

Compact imaging system

LAURIE DE CAST

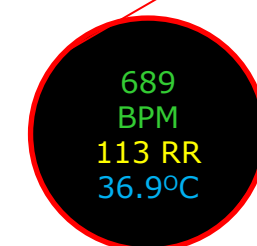
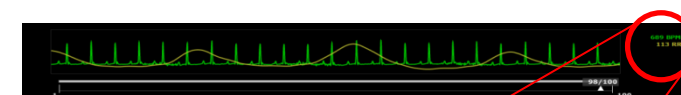
Vevo 3100

## Vevo Imaging Station



Transducer mounting system

Warmed platform

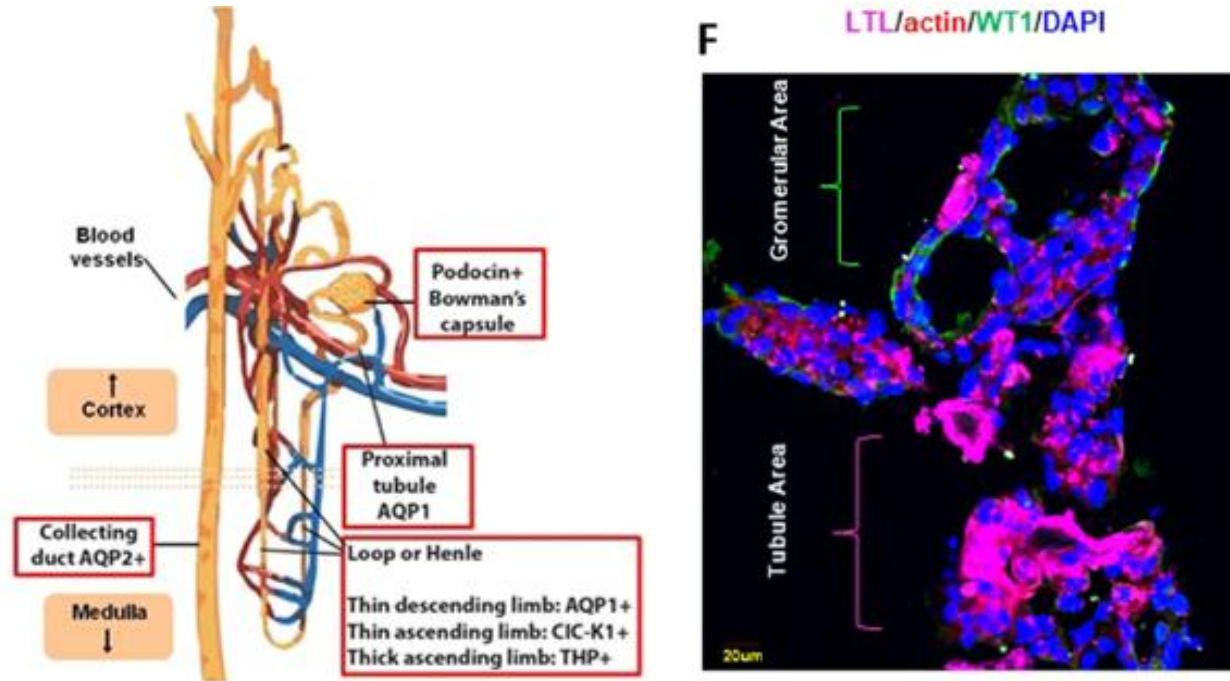


Monitoring physiological parameters

# Projet ARMOR

## ETUDE AOP DE LA TOXICITÉ RÉNALE D'UN RADIONUCLÉIDE À L'AIDE D'UN MODÈLE ORGANOÏDE RÉNAL

Grassi et al., 2019



Un organoïde rénal reproduit :

- La structure (présence de tubules proximaux, distaux, glomérules et anses de Henlé)
- La fonctionnalité (capacité d'endocytose, expression de transporteurs fonctionnels...)

Steichen et al., 2019

