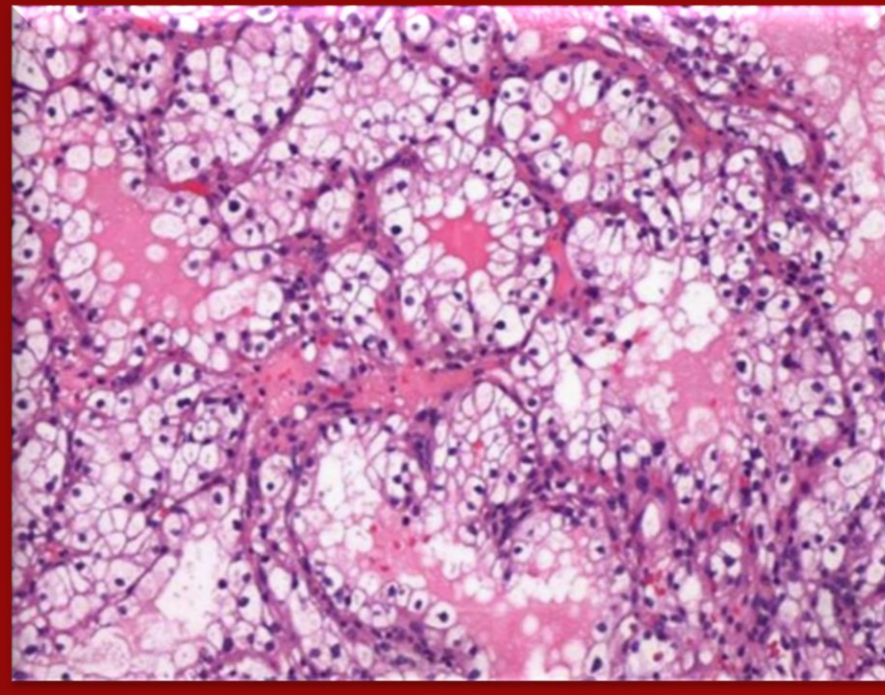
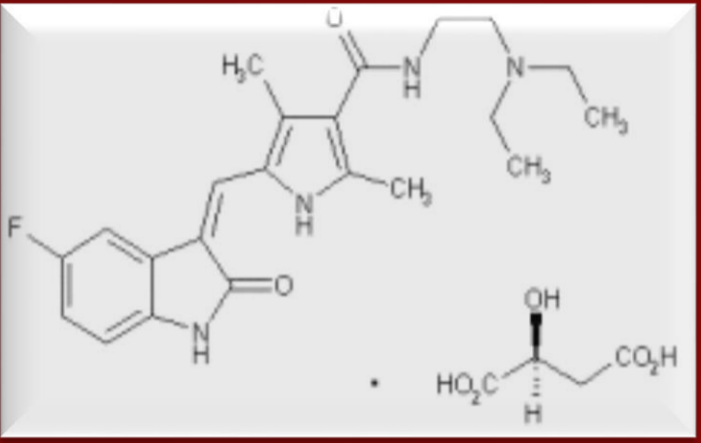


Renal cell carcinoma and Sunitinib :

Renal cell carcinoma (RCC) is one of the most common cancer in developed countries and affects preferentially men {1}. This carcinoma, whose incidence increases sharply in the fourth decade of life, shows a high rate of metastasis and a strong resistance to conventional chemotherapy and radiotherapy {2}. Different types of renal cell carcinoma exist and the most common is the clear cell RCC representing itself 75% of all RCC {3}



Clear cell renal cell carcinoma

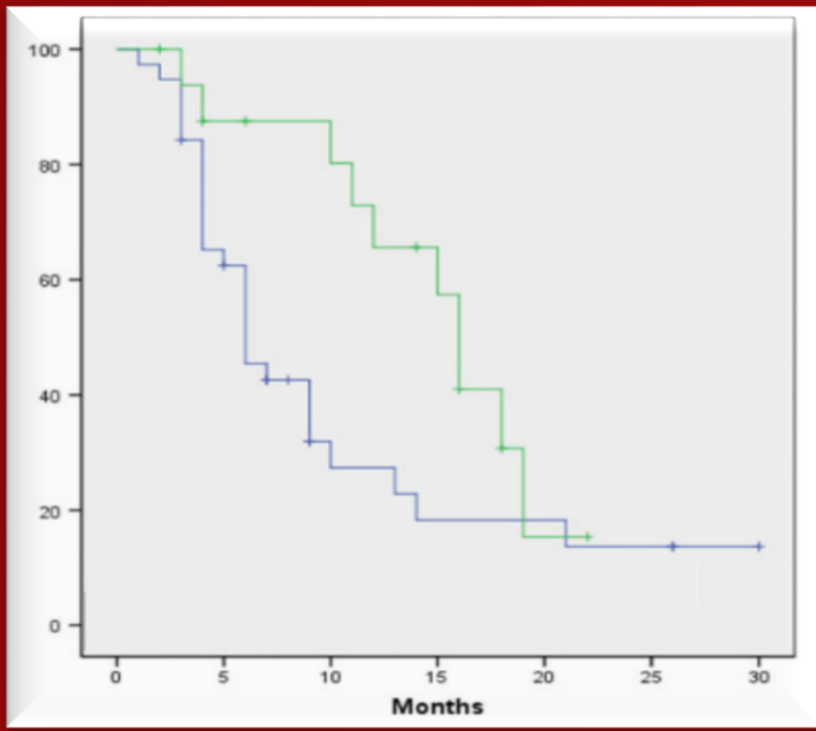


Sunitinib (SUTENT® - Pfizer)

Currently, Sunitinib is the first-line choice in the treatment of renal cell carcinoma. This multiple inhibitor of tyrosine kinase receptors has anti-angiogenic activity effective against this type of tumor via its actions on receptors VEGFR-1, -2 and -3 {4}. Sunitinib causes a restriction or even blocks tumoral progression.

Among the many side effects of Sunitinib, the onset of hand-foot syndrome, hypertension or hypothyroidism is correlated with increased treatment efficacy, synonymous with longer tumor-free survival and overall survival {5}.

Survival time without tumoral progression : Hypothyroid vs normothyroid patients.

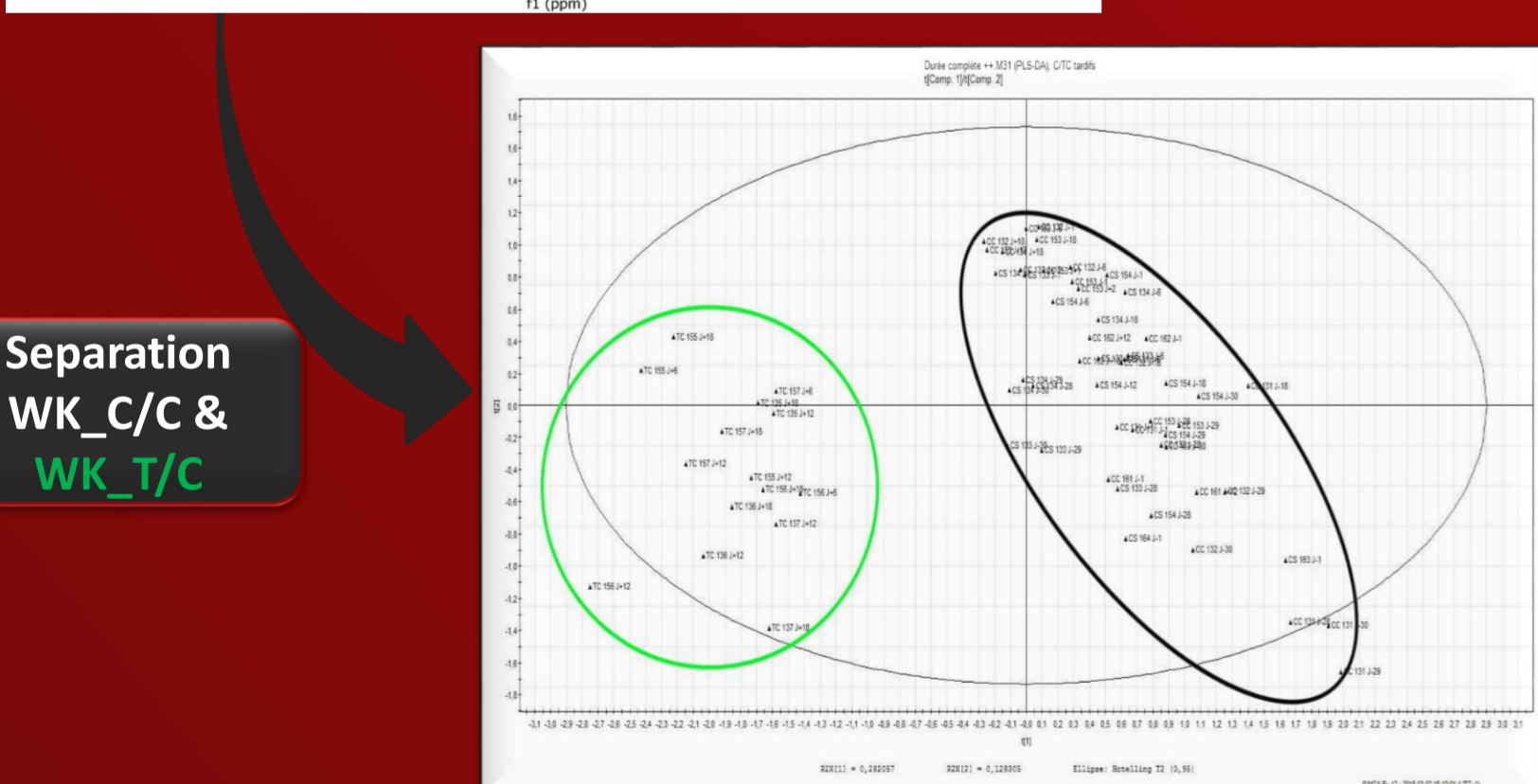
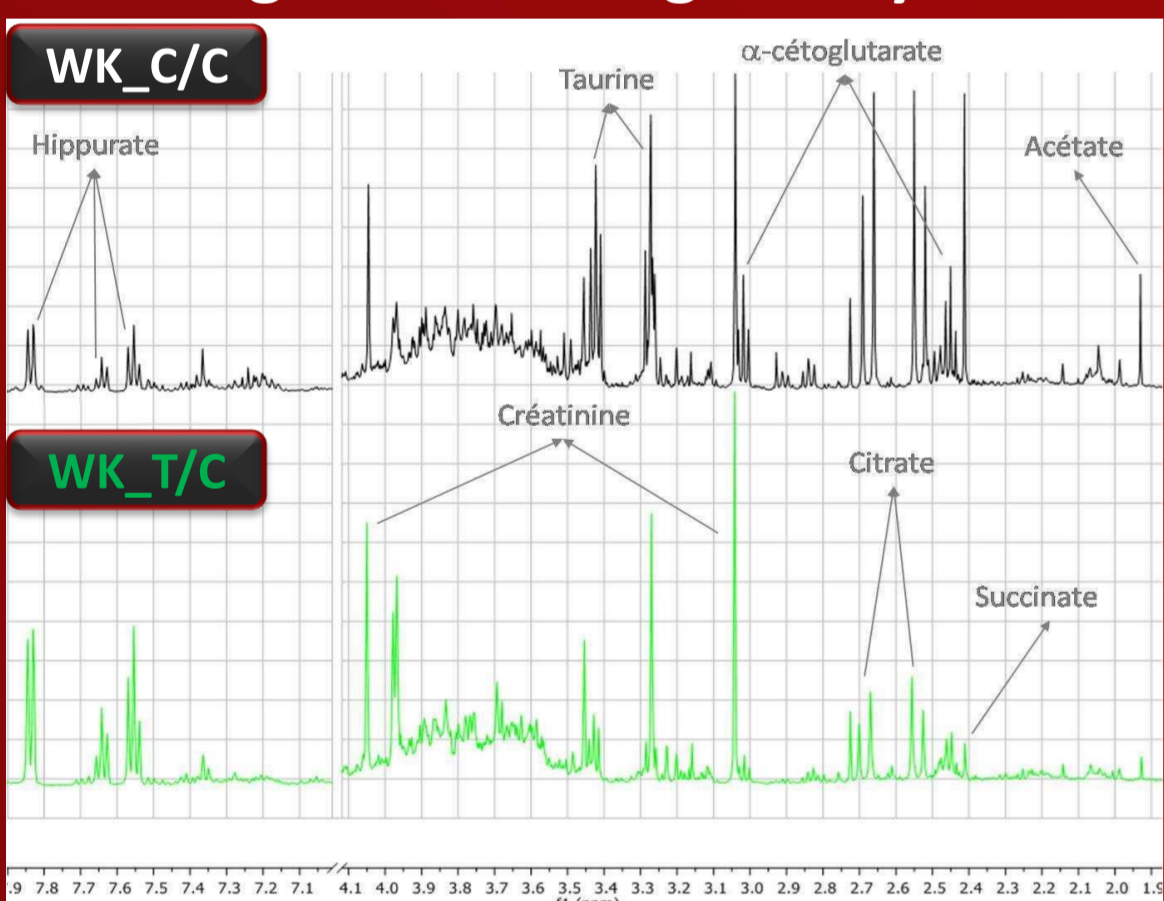


Goal :

Therefore, this work aimed at determining the metabonomic signature of one of these effects, hypothyroidism, to try to understand the mechanisms and to ensure the faster choice of an adequate treatment for the patient.

Results :

Exposition to Propylthiouracil 0,05% in drinking water during 48 days

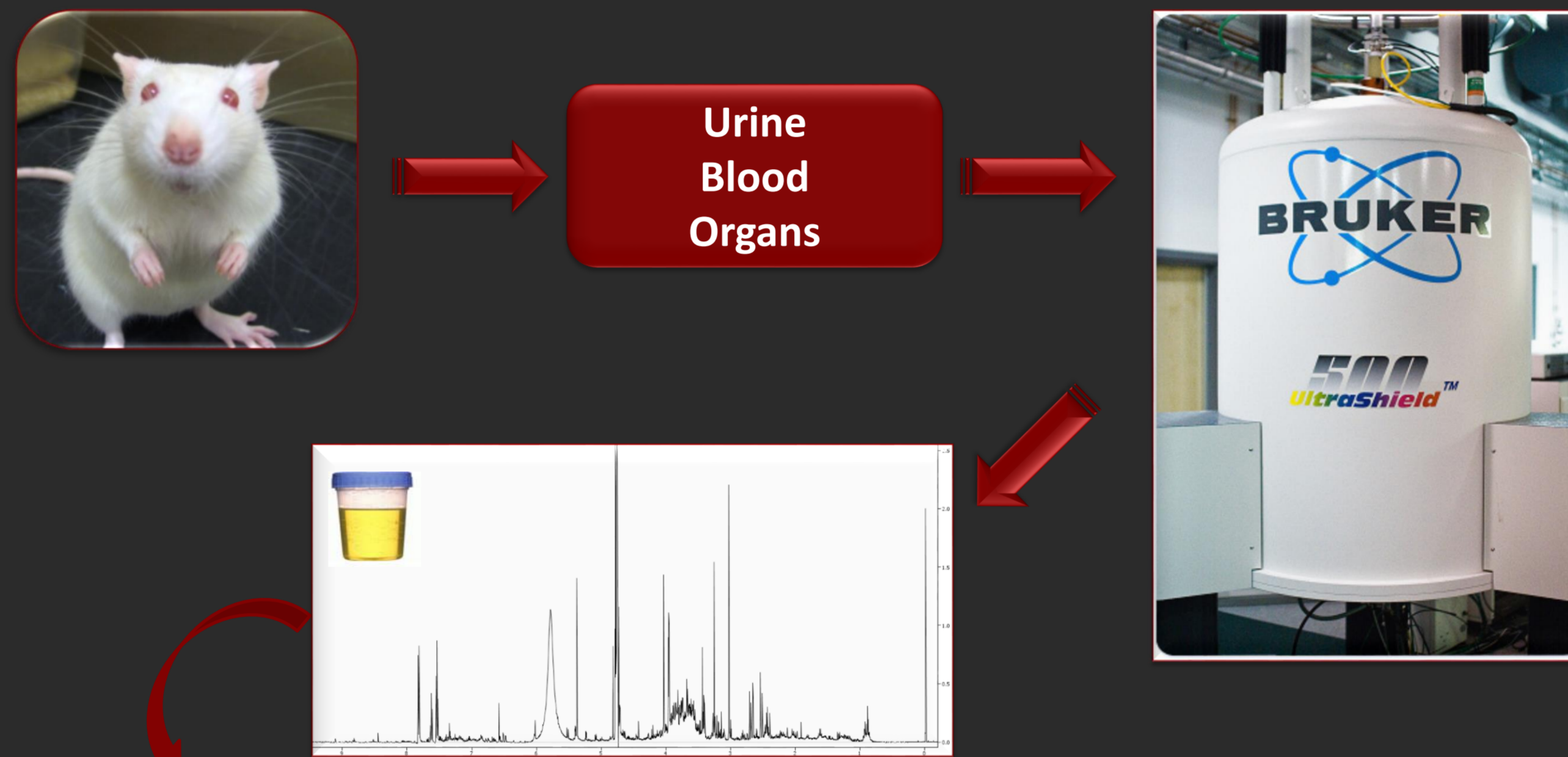


Separation WK_C/C & WK_T/C

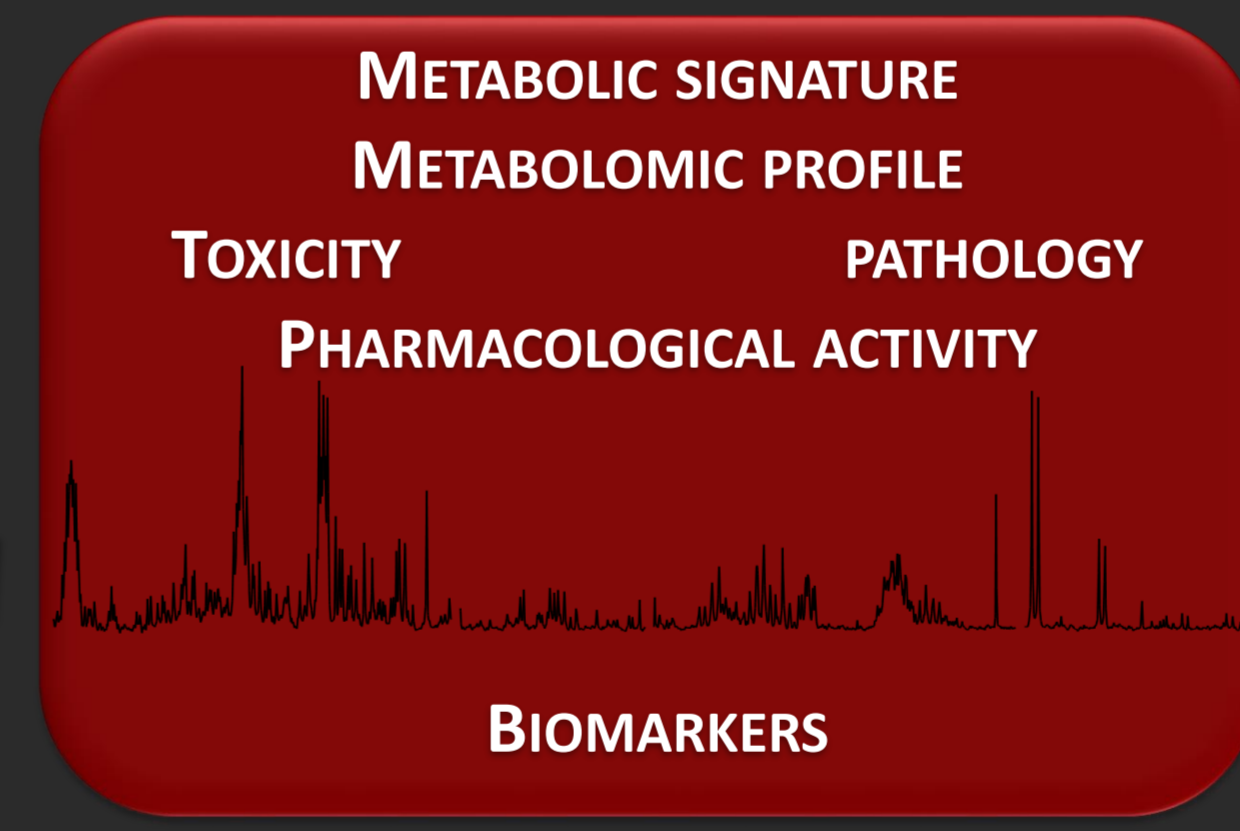
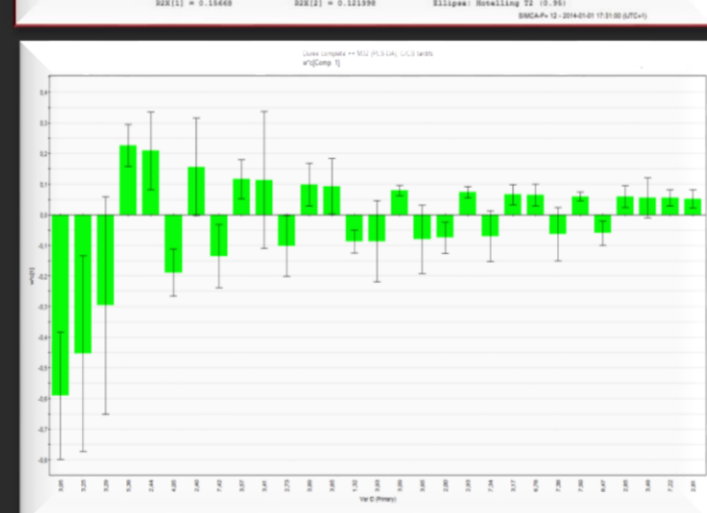
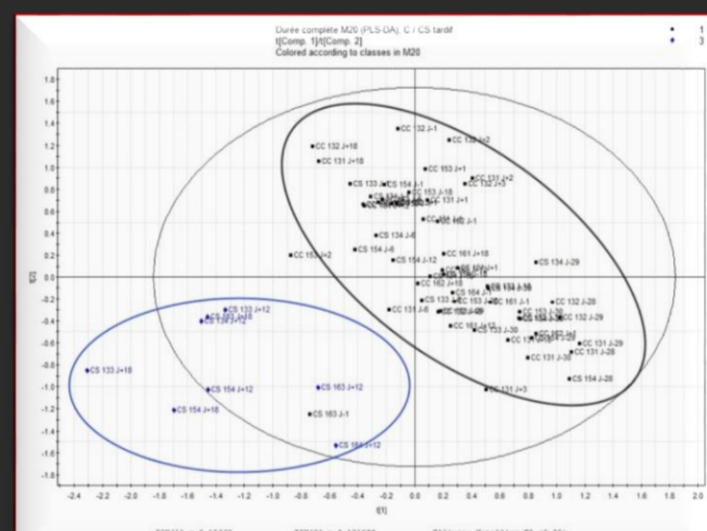
Metabolites	Indications
Citrate	Krebs cycle
Aconitate	
α-ketoglutarate	
Succinate	Choline metabolism
Choline	
Betaine	
Sarcosine	
Glycine	
Taurine	Antioxidant
Creatine	Liver injury + creatin synthesis decreased
Guanidoacetate	
Allantoine	Reg./Food/S.O.
TMAO	Osmoprotection
Hippurate	Intact renal function
Creatinine	
Serine	Sphingolipides
Glucose	Food

Increase (red) decrease (green)

Metabonomic approach :

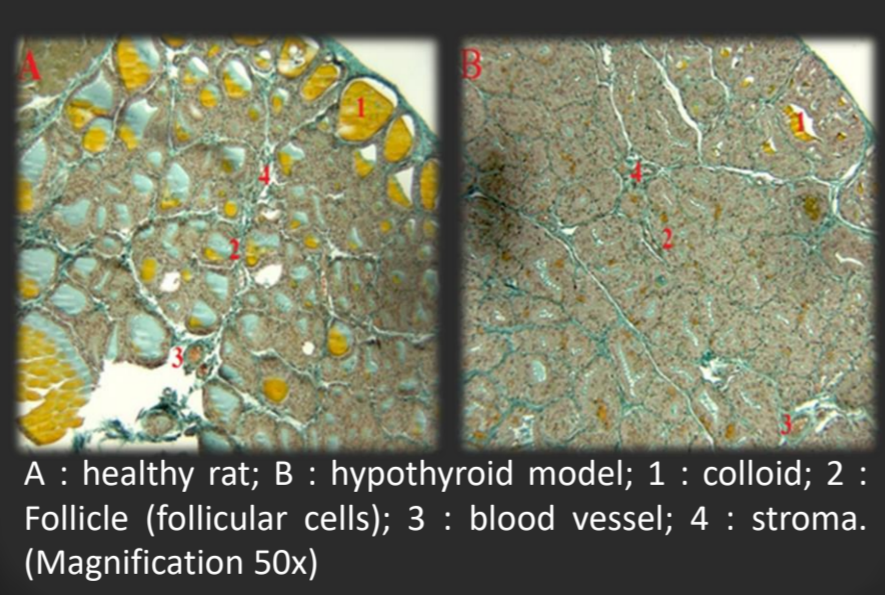


Multivariate analysis (PCA, PLS)



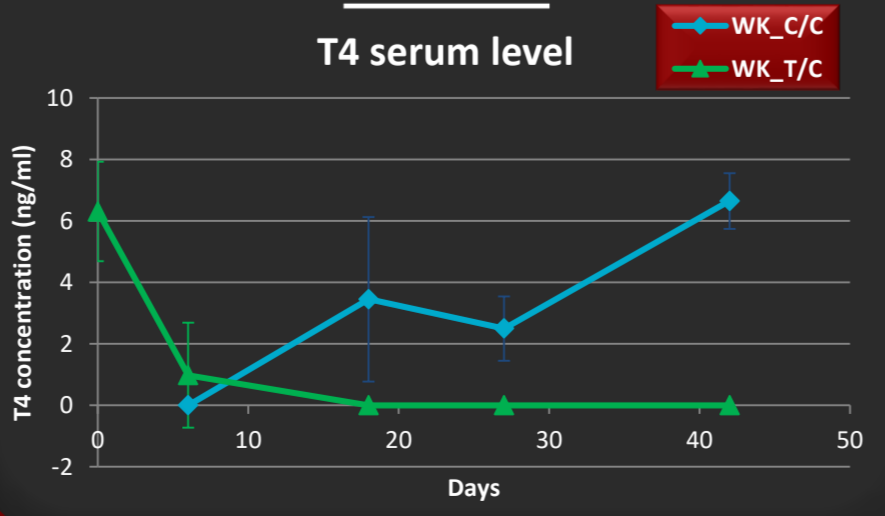
Model validation :

Histology :



A : healthy rat; B : hypothyroid model; 1 : colloid; 2 : Follicle (follicular cells); 3 : blood vessel; 4 : stroma. (Magnification 50x)

ELISA :



Material and methods :

Wistar Kyoto rats, 4 groups :

- Control (WK_C/C)
- Sunitinib (WK_C/S)
- Hypothyroidism (WK_T/C)
- Hypothyroidism + Sunitinib (WK_T/S)



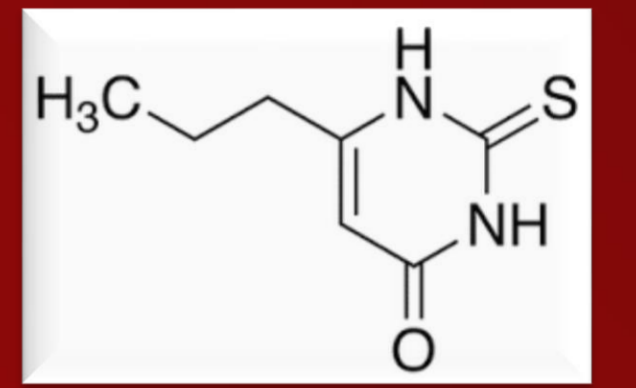
Metabolism cage

Exposure and samples :

	-30	-29	-28	-27	-26	-25	-24	-23	-22	-21	-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18											
WK_C/C	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U				
WK_C/S	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U		
WK_T/C	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U		
WK_T/S	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U

Manipulation calendar (in days; 11 is the first exposition to Sunitinib ; U - Urine collect; B - Blood sample

- Sunitinib by daily gavage (20mg / kg) from day 1 to 18
- Propylthiouracil (thyroperoxidase inhibitor) for the induction of hypothyroidism at a rate of 0.05% in drinking water of the day -30 to 18.



Propylthiouracil

Proton Nuclear Magnetic Resonance :

The urine was analyzed by 1H NMR spectroscopy after centrifugation, addition of phosphate buffer and a reference. The measurements were performed on a Bruker Avance 500 NMR spectrometer.

Clinical Chemistry (Spotchem EZ Sp-4430, Arkay®):

Tests performed on plasma samples to evaluate liver enzymes (AST, ALT), total cholesterol, glucose, triglyceride, creatinine, albumin, etc.

Histology :

Histological examination of collected organs during euthanasia (liver, kidney, heart, thyroid).

Discussion :

The signature obtained during exposure to Sunitinib shows many similarities with the metabonomic profile of the hypothyroid model. Sunitinib induces hypothyroidism in these animals after only 18 days of administration as confirmed by the determination of thyroxine (T4) serum level and histological examination of the thyroid. In addition to the different markers of toxicity, the urinary profile of hypothyroidism corresponds to a significant alteration of the Krebs cycle with passage to β-oxidation and a rise in serine urinary level. The latter increases because it is no longer converted into pyruvate and the sphingolipid pathway is altered.

Perspectives :

To refine the study of thyroid function by addition of triiodothyronine (T3) and thyroid stimulating hormone (TSH) may be of interest

One of the hypotheses put forward to explain the presence of hypothyroidism would be a very strong reduction in the vascularization of the thyroid. An immunohistochemical analysis to evaluate the vascular density of the thyroid gland, by immuno-labeling directed against CD-34 epithelial cells, could provide some answers to this question.

References :

- {1} Parkin D. M. and al.. Global cancer statistics, 2002. CA Cancer J Clin. 2005 Mar-Apr;55(2):74-108.
- {2} Grimm M-O. Advances in renal cell carcinoma treatment. Ther Adv Urol. 2010 February; 2(1): 11-17
- {3} Linehan W. M. and al. Hereditary kidney cancer. Urol Clin North Am. 2003 Nov;30(4):831-42
- {4} Stein M. N. Flaherty K.T. CCR drug updates : sorafenib and sunitinib in renal cell carcinoma. Clin cancer Res. July 1,2007 13;3765
- {5} Riesenbeck L. M. and al. Hypothyroidism correlates with a better prognosis in metastatic renal cancer patients treated with sorafenib or sunitinib. World J Urol (2011) 29:807-813