To overcome some limitations of existing models, CIEA developed a novel experimental in vivo humanized model. To do this, a herpes simplex virus type 1 thymidine kinase (HSVtk) transgene was expressed within the liver of highly immunodeficient NOG mice (TK-NOG). Mouse liver cells expressing this transgene were ablated after a brief exposure to a non-toxic dose of ganciclovir (GCV), and transplanted human liver cells are stably maintained within the liver (humanized TK-NOG) without exogenous drug. We have shown that the reconstituted liver is mature and functional and could generate: 
- A human-specific profile of anti-cancer drug metabolism. The humanization of the liver of TK-NOG mice modified the pharmacokinetic profile of the sorafenib anti-cancer agent. We were also able to detect the N-oxyde metabolite of sorafenib in humanized mice with a ratio of 8% of the non-metabolized sorafenib, in comparison to a 10% ratio in patients and 0% (not detectable) in non-humanized mice.
- An efficient environment for metastatic cell homing in patient-derived xenograft (PDX) model of Uveal melanoma. In two PDX Uveal melanoma models orthotopically xenografted in humanized TK-NOG mice, we were able to detect liver metastases, ranging from 10 to 50% of animals, whereas metastases have never been detected in non-humanized mice. This novel in vivo system provides an optimized platform for increasing our predictivity of patient anti-cancer drug metabolism, potential toxicology, and efficacy.

**Material and Methods**

- **TK-NOG transgenic mice (CIEA, Japan)** expressing a herpes simplex virus type 1 thymidine kinase (HSVtk) transgene within murine hepatocytes of severely immunodeficient NOG mice.
- **Humanization protocol:**
  - OT implantation of one PDX model of Uveal melanoma (MP55), supplied by Institut Curie.
  - Sorafenib metabolism study:
    - 20 TK-NOG mice (10 humanized + 10 non humanized)
    - Sorafenib treatment: 80 mg/kg - PO - 1Qdx1
    - Blood sampling: 0.25, 1, 3, 6 and 24 hours after dosing (3 mice / time point)
    - Quantification of sorafenib and 2 metabolites (N-oxyde-sorafenib and sorafenib glucuronide) by HPLC-MSE/MS.

> **Liver-humanization**:

- **Circulating level of human albumin correlates with chimerism of liver.**
- **Liver humanization increases plasma concentration of sorafenib and its N-oxyde-conjugated derivative.**
- **Higher rate of sorafenib conversion to the active N-oxyde metabolite in humanized mice.**

> **PK/metabolism profile of Sorafenib**

- **Known metabolization of sorafenib in human:**
  - Sorafenib is metabolized by UGT1A9 to sorafenib glucuronide.
  - Sorafenib is metabolized by CYP3A4 to the active metabolite N-oxyde-sorafenib (reported to represent approximately 10% of circulating sorafenib concentration in humanized TK-NOG mice).
- **Sorafenib glucurono-conjugated derivative in humanized TK-NOG mice:**
  - Glucuron-conjugated derivative is highly detectable in humanized liver.
  - Liver humanization of TK-NOG mice leads to detect sorafenib glucurono-conjugated derivative.

> **Conclusions and perspectives**

- Liver-humanization modifies the PK profile and the metabolism of sorafenib in mice.
- Humanized mice have higher circulating level of sorafenib and its N-oxyde conjugate of sorafenib.
- Chimeric TK-NOG mice constitutes a preclinical tool for detection of deadly drug side effects and for improvement of tumor dissemination rate.