IN VIVO TK-NOG LIVER-HUMANIZED MODEL TO PREDICT PATIENT PHARMACOLOGICAL PROFILE OF ANTI-CANCER AGENTS

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 # 1452

Abstract

To overcome some limitations of existing models, CIEA developed a novel experimental in vivo liver-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:

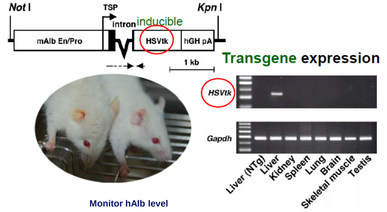
- A human-specific profile of anti-cancer drug metabolism. The humanization of the liver of TK-NOG mice modified the pharmacokinetic profile of the sorafenib anti-cancer agent. We were also able to detect the N-oxyde metabolite of the sorafenib in humanized mice with a ratio of 8% of the non-metabolized 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 liver-humanized TK-NOG mice, we were able to detect liver metastasis, 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.

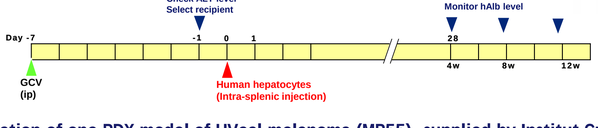
Material and Methods

- **TK-NOG transgenic mice (CIEA, Japan) expressing a herpes simplex virus type 1 thymidine kinase (HSVtk) within murine hepatocytes of severely immunodeficient NOG mice.**

(M. Hasegawa and all.; "The reconstituted humanized liver in TK-NOG mice is mature and functional"; Biological and Biophysical Research Communications (2011))



- **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 – Q1Dx1
  - 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-MS/MS.

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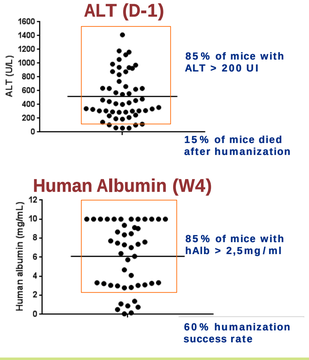
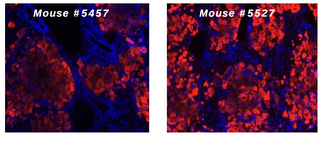
Animal housing and experimental procedures were realized according to the French and European Regulations and NRC Guide for the Care and Use of Laboratory Animals. Animal facility is authorized by the French authorities (Agreement N° A21231011EA). All procedures using animals were approved by the Animal Care and Use Committee of Oncodesign (Oncomet) agreed by French authorities (CNREEA agreement N° 91).
Human biological resources were collected by approved Oncodesign Biological Research Center.

Liver humanization



Mouse	% CK8/18 positive cells	Human albumin level (mg/ml)
5457	48 ± 28	8.48
5527	50 ± 18	9.12
5549	57 ± 35	>10
5577	54 ± 29	>10

Human CK 8/18 positive cells (W4)



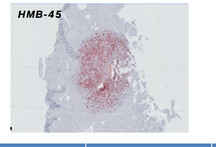
- Circulating level of human albumin correlates with chimerism of liver.

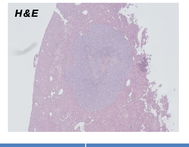
Optimized Mice (*n*)	hAlb (mg/mL)	Chimerism (%)
5	0.8	13.4
14	1.6	22.5
8	3.3	41.9
10	4.5	55.7
1	6.9	83.1
5	7.8	93.4
Total, 43	Average, 3.3***	Average, 42.5

Modified from Table 1 – M. Hasegawa and all. (2011)

PDX metastases

- Histological analyses of reconstituted liver from MP55 tumor bearing TK-NOG mice confirm the presence of metastases.





Model ID	Mean survival time ± SD (days)	% of mice with metas	Ranged nb of metas *per* mouse
MP55	106 ± 62	33 %	1-17

- Macroscopic liver metastases were never observed in SCID mice bearing OT MP55 tumors.

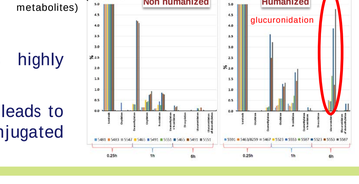
Sorafenib metabolization

PK/metabolism profile of Sorafenib

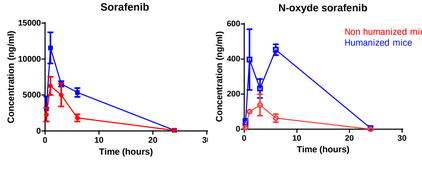
- **Known metabolization of sorafenib in human:**
  - Sorafenib is metabolized by UGT1A9 to sorafenib glucuronide.
  - Sorafenib is metabolized by CYP3A4 to the active metabolite N-oxyde-sorafenib (reported to represent approximately 10% of circulating sorafenib concentration in healthy subjects and cancer patients).

Sorafenib glucurono-conjugated derivate in humanized TK-NOG

- Glucurono-conjugated derivate is highly detectable in humanized mice.
- Liver humanization of TK-NOG mice leads to detect sorafenib glucurono-conjugated derivate.



Plasma concentration of N-oxyde-sorafenib



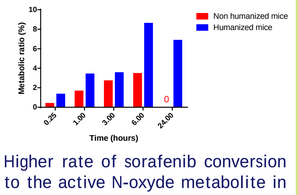
Mice	AUC Sorafenib	AUC N-Oxyde sorafenib
Humanized	90544	5951
Non humanized	41840	1147

x5

AUC in h.mg/mL)

- Liver humanization increases plasma concentration of both sorafenib and its N-oxyde active metabolite.

Metabolic ratio N-oxyde-sorafenib / sorafenib



- Higher rate of sorafenib conversion to the active N-oxyde metabolite in humanized liver mice.
- Ratio is 8.5% in humanized mice (*vs* ≈ 10% in Human).

Conclusions and perspectives

- Liver-Humanization modify the PK profile and the metablism of sorafenib in mice.
- Metabolism profile of sorafenib in liver-humanized mice is close to Human.
- Chimeric TK-NOG mice constitutes a preclinical tool for detection of deadly drug side effects and for improvement of tumor dissemination rate.

H. Kamimura and all.; « Formation of the Accumulative Human Metabolite and Human-specific Glutathione Conjugate of Diclofenac in TK-NOG Chimeric Mice with Humanized Livers »; ASPET Journals (2015)
D. Xu and all.; « Fialuridine Induces Acute Liver Failure in Chimeric TK-NOG Mice: A Model for Detecting Hepatic Drug Toxicity Prior to Human Testing »; PLOS Medicine (2014)
M. Kim and all.; « Generation of Humanized Liver Mouse Model by Transplant of Patient-Derived Fresh Human Hepatocytes »; Transplantation Proceedings (2014)
H. Yamazaki and all.; « In Vivo Drug Interactions of the Teratogen Thalidomide with Midazolam: Heterotropic Cooperativity of Human Cytochrome P450 in Humanized TK-NOG Mice »; Chemical Research in Toxicology (2013)
A. Tsukada and all.; « Plasma concentrations of melengestrol acetate in humans extrapolated from the pharmacokinetics established in *in vivo* experiments with rats and chimeric mice with humanized liver and physiologically based pharmacokinetic modeling »; Regulatory Toxicology and Pharmacology (2013)