# Of mice and monkeys: generating mice repopulated with non-human primate hepatocytes as a predictive small animal model

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## INTRODUCTION

Liver-humanized **FRG** KO mice have proven to be of great use for **in vivo** studies in many application areas including infectious diseases, NAFLD/NASH, gene editing/therapy, and metabolism.
The **FRG** KO mice can be repopulated with hepatocytes from different species. This allows the replacement with "lower" species by, instead of using non-human primates, for example, using a mouse transplanted with primate hepatocytes to achieve the same results.

## AIM

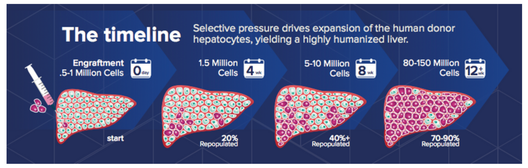
Building on the liver-humanized **FRG** KO model[1], we set out to generate **in vivo** liver-xenograft models for different species and of different human liver-related diseases.

## MATERIAL & METHODS

**FRG** KO mice were transplanted with hepatocytes from a rhesus macaque.
The level of repopulation correlated to the level of NHP-specific albumin concentration in the blood. Alternatively, the repopulation can be measured by IHC with a species-specific staining or by staining for FAH expression.

## BACKGROUND: HUMAN HEPATOCYTE ENGRAFTMENT IN FRG KO MICE

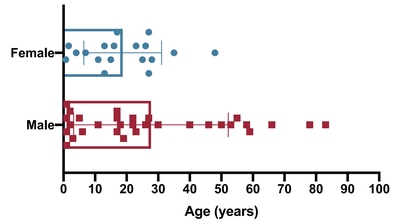
### Liver repopulation




The liver-humanized **FRG** KO model is generated by transplanting healthy hepatocytes into immunodeficient mice with a controllable liver disease. The healthy hepatocytes replace the damaged mouse hepatocytes.
Xenograft repopulation can be correlated to proteins secreted by the hepatocytes into the blood. Albumin (the most abundant protein in blood) can be easily measured using species-specific ELISA kits.
Animals remain highly engrafted for the remainder of their life.

### Human Hepatocyte Donor Characteristics

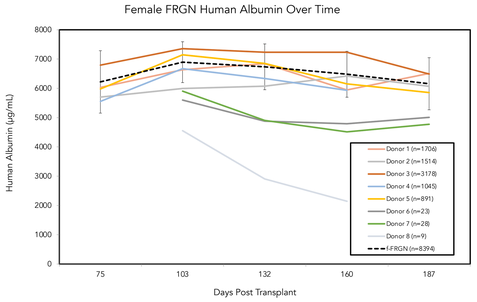
Almost any healthy human donor can be used to repopulate **FRG** KO mice.
**Cryopreserved** hepatocytes are first tested to determine repopulation efficacy and survival rates.




More than 50 human donors have been used to successfully generate liver-humanized **FRG** KO mice. Donors had an average age of 18 (F) and 27 (M) years old. While donor age has some influence on the success rate, engraftment is not limited to pediatric hepatocytes.

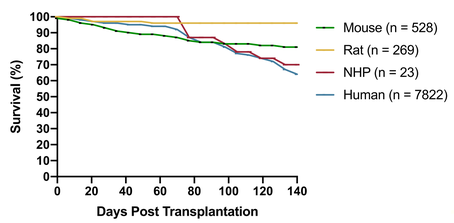
### Transplant Success Rate

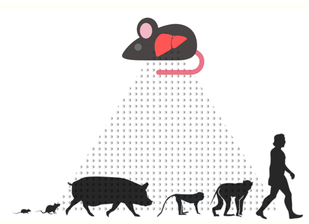
A new donor is evaluated by comparing human albumin in blood and survival rates of liver-humanized **FRG** KO mice transplanted with human hepatocytes from previous successful donors.




## NHP ENGRAFTMENT in FRG KO MICE: MONKEYNIZED MICE

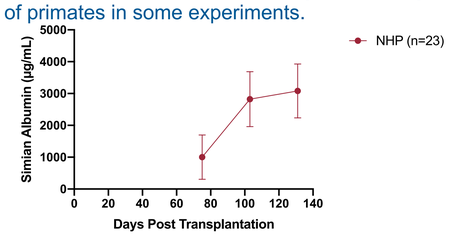
**FRG** KO mice can be repopulated with hepatocytes from any species.
Animals have been repopulated successfully with **mouse**, **rat**, **cat**, **dog**, **pig**, or **non-human primate** hepatocytes.



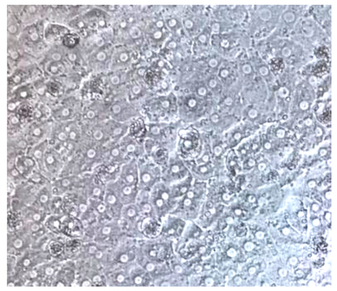



**Murinized FRG** KO mice are used frequently as a control for liver-humanized **FRG** KO mice.
Transplantation with mouse hepatocytes from a specific genetic model generates animals with the mutation only present in the liver[2].

**FRG** KO mice transplanted with **non-human primate hepatocytes (NHP)** have replaced the use of primates in some experiments.




The **liver-monkeynized mice** show a metabolism profile of the original NHP species. This allows the testing of novel compounds in a small animal model.
For gene therapy or delivery, the liver-monkeynized or humanized FRG mice can be used to predict which formulations (for example AAV or LNP) will work in NHP studies or in human clinical trials, which has been an issue when using wildtype mice.
Moreover, due to their smaller size, the amount of viral or nonviral vector needed per animal is much lower than when using larger NHP models.



Fresh NHP hepatocytes in vitro

For infectious disease research, freshly harvested hepatocytes are needed to reach high levels of infection in vitro. **FRG** KO mice transplanted with **cryopreserved NHP** hepatocytes can be used to generate **fresh NHP** hepatocytes on site, without the need of a specialized primate vivarium.

## CONCLUSION

**FRG** KO mice can be repopulated with hepatocytes from **non-human primates**. These **monkeynized FRG mice** can be used either for in vivo studies, or their hepatocytes can be harvested and used fresh for in vitro applications. By using **monkeynized FRG mice**, larger cohorts can be used without the need for specially adapted facilities or regulatory requirements.

## FRG MODEL

**FRG** KO mice have a knockout of the **F**ah, **R**ag2, and Il2**rg** genes. Fah deficiency kills mouse hepatocytes, while the immune deficiency (Rag2 and IL2rg) allows the mice to be rescued with hepatocytes from any species including human. Hepatocytes from nearly **any donor of interest** can be engrafted in **FRG** mice, including those with specific genotypes. Liver-humanized animal models are useful for in vivo studies in many application areas including infectious diseases, NAFLD/NASH, gene editing/therapy, metabolism, and toxicology.

## REFERENCES

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