Challenge 29: ImmuLiver

Launch Meeting: 06 September 2018

SANOFI PASTEUR

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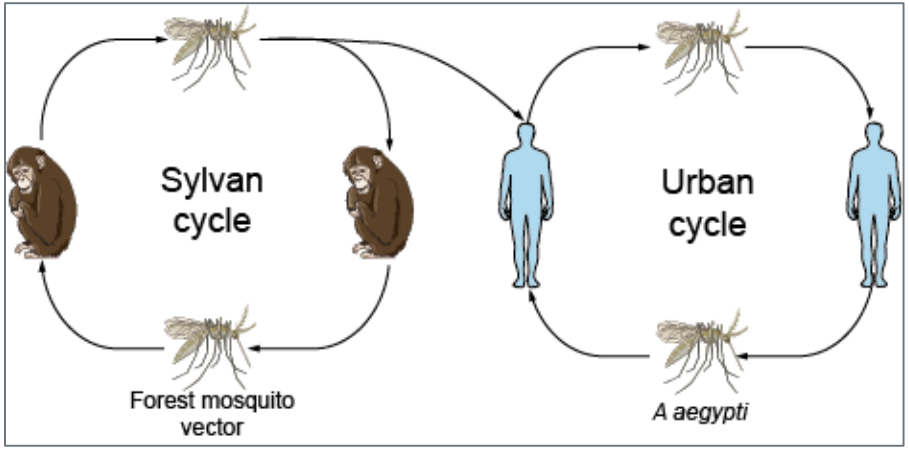
The challenge

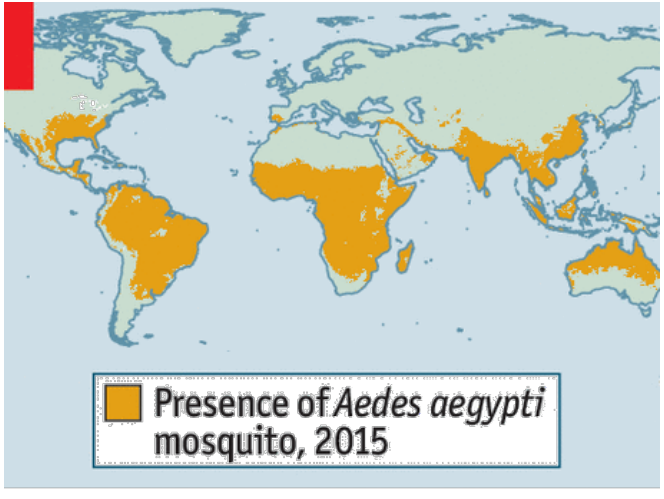
Provide an immunologically-competent liver model to assess attenuation of yellow fever live-vaccines

- **Provide a cellular model capable of reproducing major metabolic and immunological functions of the liver**
  - .. utilizing some combination of human cell lines equivalent to primary liver cells
- **Provide a device or platform which is amenable to use with viruses**
  - .. in biosafety confinement level 2 and 3 laboratories and in validated assays.
- **The device should be medium throughput and compatible with standard equipment and measurement platforms**
  - ..e.g. microscopy, biochemical analysis, robotics.

Context: Yellow Fever

- **Mosquito-borne infection**
- **Urban cycle almost eliminated since the introduction of vector control**
- **Persistence of a sylvatic cycle and recurrent epidemics in Africa and South America**
- **Disease not eradicable**





Economist.com

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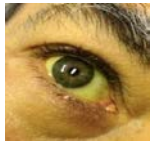
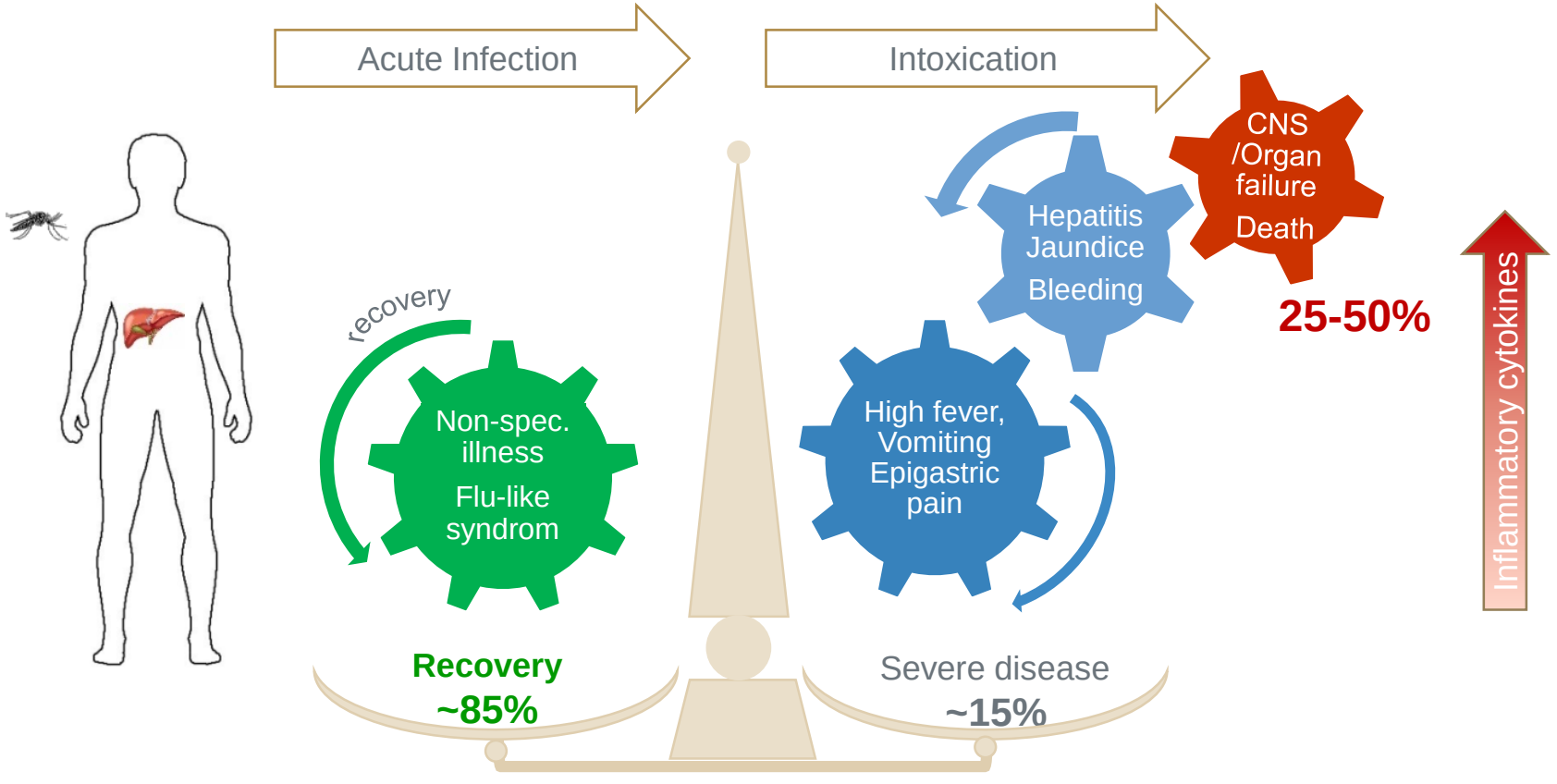
YF pathogenesis: strong liver involvement





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Yellow Fever vaccine

- **1937: Isolation of YF attenuated viruses by amplification passages of wild-type YFV isolates strain through mouse tissues**
  - Attenuation criteria: viruses no longer able to cause hepatic disease in monkeys
  - Two initial lineages: Asibi strain ->YF17D & Dakar strain -> FNV (withdrawn in 1996)
    - 17D: Attenuation-related mutations identified, but molecular determinants for virulence attenuation poorly understood

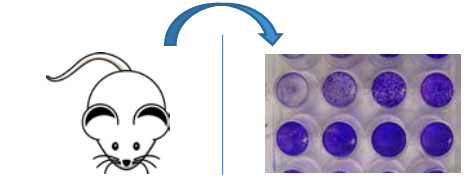
- **1945: Introduction of the monkey neurovirulence test in in the control of yellow fever 17D vaccine safety***
  - Some early FNV lots associated with a high incidence of encephalitis in humans
  - Increased neurovirulence also observed in macaques inoculated by the intra-cerebral (IC) route with these lots (J.P. Fox & H.A. Penna, 1943)

* Recommendations to assure the quality, safety and efficacy of live attenuated yellow fever vaccines. WHO, 2013. *http://www.who.int/biologicals/areas/vaccines/TRS_978_Annex_5.pdf* “

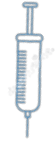
Current Approach to YF vaccine safety assessment

Non-Human Primate (NHP) Neurovirulence Test

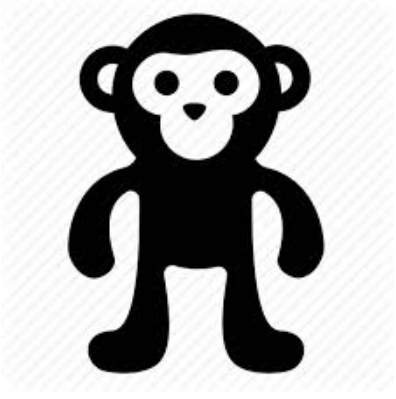
3.3 log_{10} Lethal Dose 50% (LD_{50}): mouse neurovirulence assay
OR Equivalent infectious titer in International Units (IU)



I.C. injection



Neurovirulence	• Clinical and histopathological scores
Immunogenicity	• 90% seroconversion 30 days after inoculation
Viscerotropism	• <500 IU in 0.03 mL of D2, D4 and D6 sera



Rationale for developing an *in vitro* hepatic model

1- As part of a strategy aiming to replace the NHP neurovirulence test

- **Test conducted on each new vaccine seed lot, as per regulatory guidelines**
  - Control and Test group: 2 x 10 macaques, animals cannot be re-used
- **Neurovirulence**
  - Promising *in vitro* model (minibrain) developed by Pasteur Institute/SP (Da Costa et al. 2018)
  - Would replace both mouse and monkey NV assays and could be used to evaluate neurovirulence and neurotropism
- **Immunogenicity and viscerotropism: not translatable to human (i.c. route)**
  - Immunogenicity can be evaluated in small animal models (mouse, hamster)
  - Viscerotropism: blood viremia is only a surrogate marker, does not assess liver damages
    - **No substitution assay available yet**

Animal models for Yellow Fever

- **Macaque**
  - Only animal model that reproduces human YF pathogenesis
  - Model used to define the surrogate of protection

- **Hamster**
  - Develop fatal viscerotropic infection resembling the human disease when inoculated SC with a hamster-adapted strain
  - Develop YF virus-specific antibodies

- **Immunocompetent mouse**
  - Used for neurovirulence studies (i.c. route)
  - Does not replicate YF viruses injected by SC or IM route
  - Poor responders to YF vaccines

- **IFNR1-deficient mouse**
  - Neurotropic disease after SC inoculation with YF: lethal model
  - Replication in the liver

1-Thibodeaux *et al.* 2012. Vaccine 30, 3180-3187
2-WHO 2012 World health organ tech rep ser, 1-228 Back cover.
3-Meier *et al.* 2009 PLoS Pathog 5, E10000614
4-Julager et al. 2016

Rationale for developing an *in vitro* hepatic model

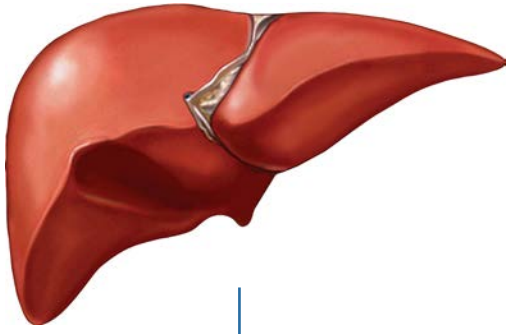
2- As a proof-of- concept for other vaccines, or other hepatic pathogens

- **Possibility to extend the use of this model to other vaccines**
  - The NHP neurovirulence test is also requested for YF17D-based vaccines
    - Chimeric vaccines: CYD-TDV Dengue, JE-CV
- **Many human hepatropic pathogens are species-specific**
  - Robust model systems that can faithfully replicate human hepatotropic infections are needed
    - Improvement of existing 2D- and 3D- models by addition of a robust immune component would be a considerable progress
  - Clinically relevant pathogens that target the liver:
    - Plasmodium spp : malaria, mosquito bite transmission
    - Hepatitis A (HAV) and hepatitis E (HEV) viruses: acute infections, oro-fecal transmission
    - Hepatitis B (HBV) and hepatitis C (HCV) viruses: chronic infections, blood-borne disease
    - Hepatitis D (HDV): HBV satellite virus: co-exists with HB and follows its infection pathway

Hepatic injury in YF infection

Liver Biology

- ↗ ALT/AST
- ↗ CRP levels
- ↘ Coagulation factors



Histology

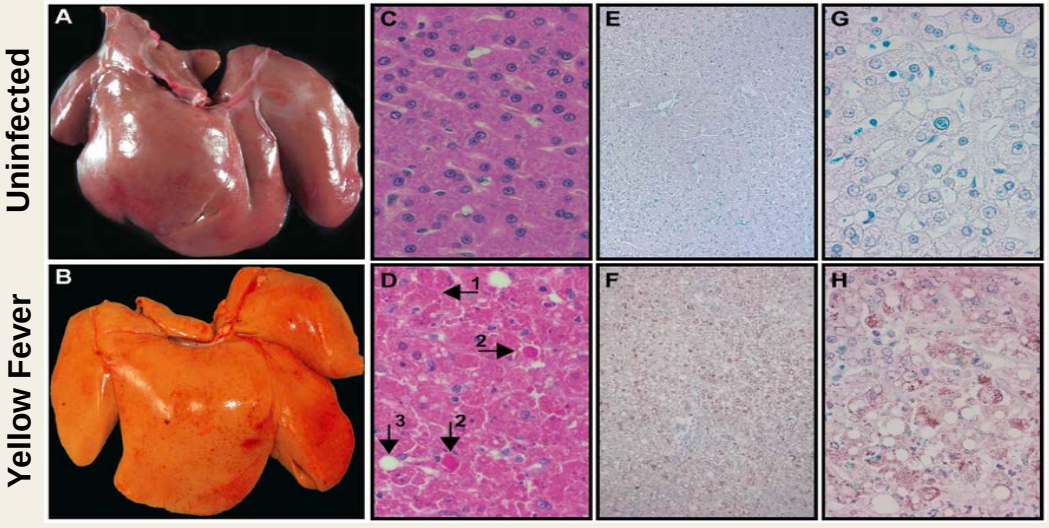
- Mid-zonal lesions
- Councilman bodies
- Inflammatory infiltrates
- Steatosis

Antigen detection

- Hepatocytes (+dsRNA)
- Kupffer cells

Evidence of direct liver involvement in YF infection

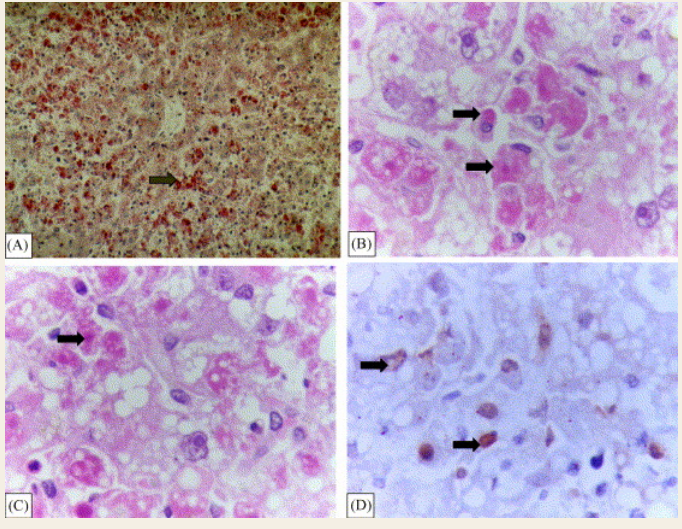
Macaque



Uninfected **(A)** and YFV-DakH1279-infected **(B)** macaque liver. The infected liver is discolored with signs of hemorrhagic foci.
(C–D): H&E staining of liver sections (200×). (**1),** extensive hepatocytes necrosis; (**2**), eosinophilic degeneration of liver cells (Councilman bodies), (**3**) fatty changes
(E–H): Histological analysis of YFV antigen (200× and 400×)

Engelmann *et al*. PLoS Neglected Tropical Diseases (2014)
Pathophysiologic and Transcriptomic Analyses of Viscerotropic Yellow Fever in a Rhesus Macaque Model

Human



(A) Imunohistochemistry. Arrow: viral antigen in hepatocytes of the lobules (200×).
(B and C) H&E staining. Councilman bodies (400×).
(D) Imunohistochemistry for apoptosis (APOPTAG) , marking of the hepatocytes (400×).

Quaresma *et al.* Acta Tropica 94 (2005)
Reconsideration of histopathology and ultrastructural aspects of the human liver in yellow fever

In vitro human hepatic models

Variability of YF17D replication efficiency

- **In humans: low-level of short-lived 17D viremia 3-6 days post-vaccination**
  - Viremia possibly lead to hepatocytes infection (Monath 2002; Reinhardt 1998; Wheelock 1965).

Cells	Type	Source	Peak Titer (log_{10} IU/mL)	Day	Reference
HepG2	Hepatocyte	Hepatocarcinoma	**7.9**	3	Brandler 2005
Huh7	Hepatocyte	Hepatocarcinoma	**8.0**	4	*Id*
THLE-3	Hepatocyte	Liver cells transformed with SV40 large T Ag	**5.6**	4	*Id*
PH5CH8	Hepatocyte	SV40 large T Ag- immortal, nonneoplastic hepatocytes	**4.0**	2	Woodson 2011a
Kupffer	Macrophage	Primary	**4.0**	2	Woodson 2013
U-937	Macrophage	Cell line	**6.0**	3	Linardi 1983
HUVEC	Endothelial	Umbilical vein	**4.3**	4	Khaiboullina 2005

Different type of cells likely needed to mimic YFV liver infection

HepG2, Huh-7 cells

(Lefeuvre et al., 2006; Fernandez-Garcia,2016)

- Replication: 17D > YF
  - **Earlier apoptosis**
- Antiviral, cytokine-mediated response: 17D>Asibi

PH5CH8

(Woodson & Holbrook, 2011)

- Replication: 17D < Asibi
- Cytokines with role in disease progression: YF 17D > YF Asibi
  - **IL1-β, IL-4, IL-6, IL-8, IL-10, TNF-α**

Kupffer cells

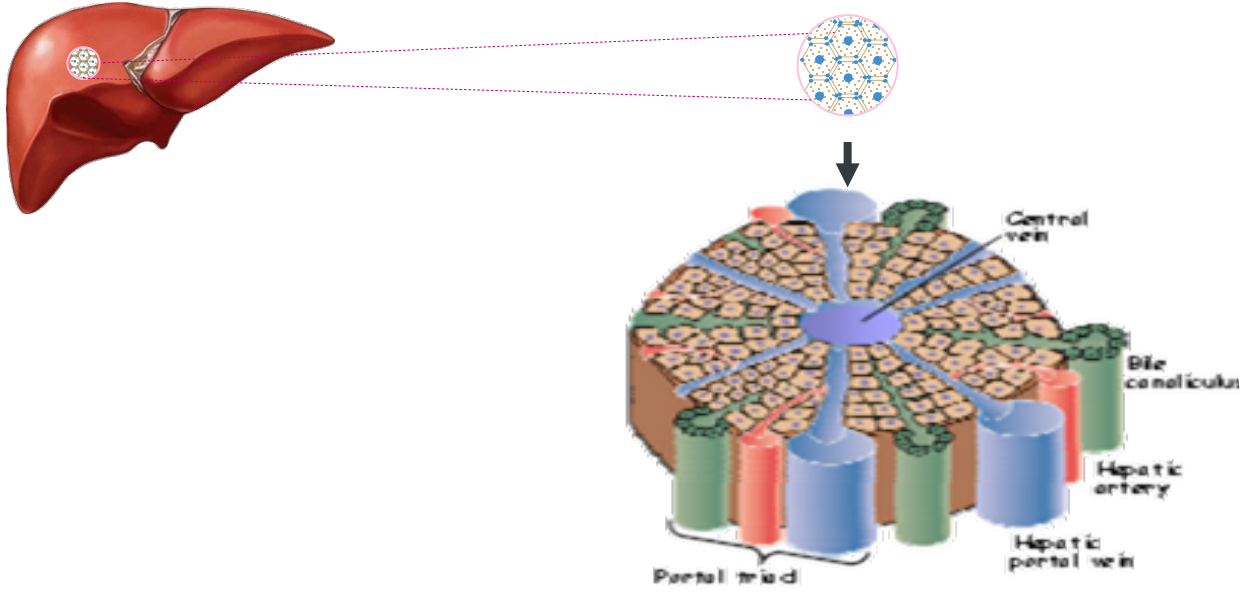
(Woodson et al. 2011)

- Replication: 17D < Asibi
- INF response: YF 17D > YF Asibi
  - **IL-8, TNF-α and RANTES/CCL5**
  - **Little control by IL-10**

HUVEC cells

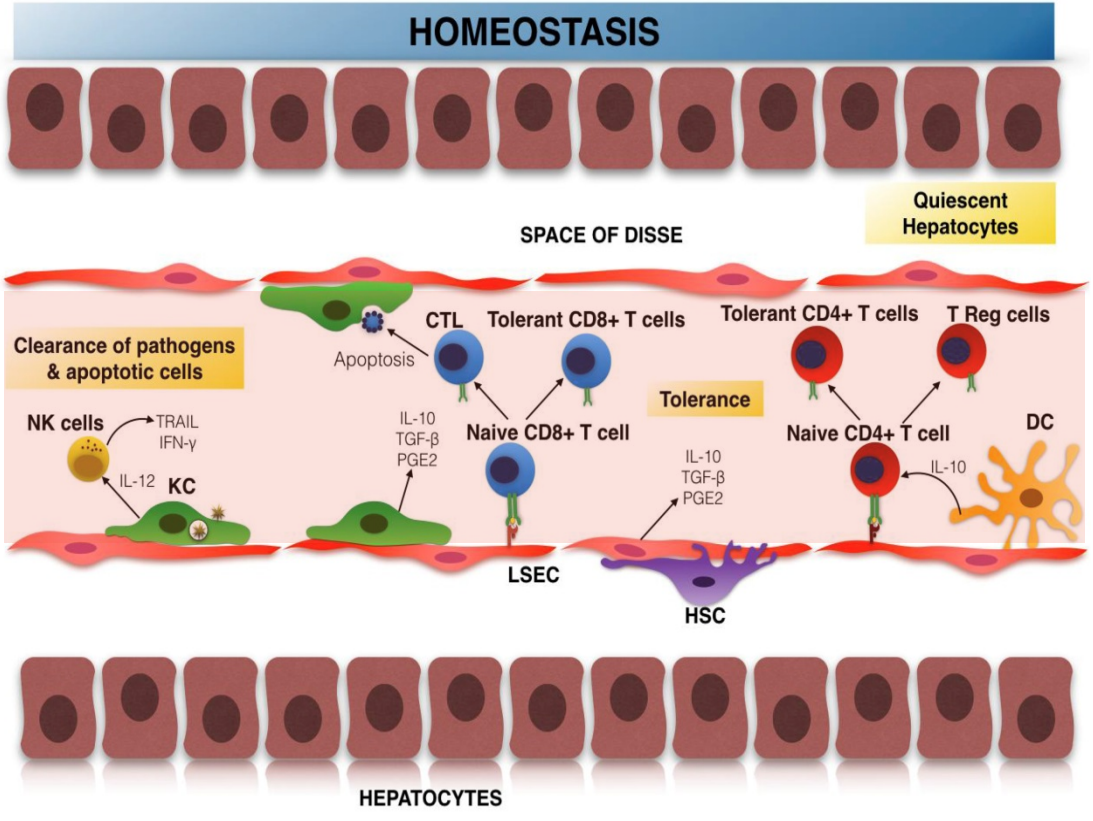
(Khaiboullina et al., 2005)

- Replication: Asibi = 17D
- Cytokines response: Asibi>17D
  - **IL-6, BCL-2 and RANTES/CCL5**



MODELING AN IMMUNOCOMPETENT LIVER MODEL

Sinusoid composition



Immune cell regulation of liver regeneration and repair. D. Markose et al. Journal of Immunology and Regenerative Medicine, Sep 2018.

Hepatocytes: 80% liver mass

Kupffer cells, KC

Liver sinusoidal EC, LSEC

Hepatic stellate cells, HSC

Circulating monocytes and DC

Modeling liver-specific YF virus infection

Limits of current models

- **Exhaustible cell sources (e.g. primary cells)**
  - Not compatible with routine testing for the lifetime of the vaccine (usually >30 years)
- **No consensus definition of a “healthy” liver model**
  - Primary cells: limited supply from “healthy donors”, most donors have medical history of liver pathology (diabetes, cancer, alcohol abuse).
  - No biological signature for identification of a “healthy” liver, or young versus aged
- **Lack of a robust immune component**
- **Culture conditions**
  - Cell medium ± ECM components not optimized for viral infection
    - e.g. interference with receptor fixation, reduced virus half-time, viral particle dissociation
- **Devices**
  - Pathogen containment (protection of the operator and the environment)
  - Insufficient assay throughput and limited number of readouts for assay validation.

Phase I deliverables

Proposal and Gap Analysis

White paper describing the proposed model

Cells

- Hepatocytes, Kupffer cells, endothelial cells ± stellate cells, at least
- Human iPSC-derived cells or cell lines
- List of technical tools to routinely identify the cell populations and to monitor the evolution of their functionality over time.
  - Quantitative assays are preferred
- Healthy liver signature definition

Platform / Device

- Amenable to use with viruses, in BSL2 /3 laboratories and in validated assays.
  - Minimal risk of contamination during handling, for the operator and the environment
- Medium throughput, compatible with standard equipment and measurement platforms
- Absence of known viral inhibitors in culture media

Preliminary data

- Normal cells: physiological parameters stable for 12-15 days, minimum
- Evidence that the model is able to support YF virus productive infection

Phase II deliverables

Evaluation phase

- **To address the potential gaps identified in the 1st phase and to set-up the model**
  - Establish and characterize the cell banks
  - Run the platform in mock-infected conditions
  - Set-up the analysis tools and check the cell characterization markers, in normal conditions and after activation of the hepatic metabolism (drugs)

- **To evaluate YF17D infection**
  - Set-up infection read-outs
  - Characterize the model , qualitatively and quantitatively
    - Reproducibility
    - Hepatic metabolism dysregulations and innate immune responses

Sanofi Pasteur in-kind contribution

- **Scientific expertise on YFV (and viruses in general).**
  - Feedback on previous internal experiments with YFV infection of microliver tissue and iPSC-derived hepatocytes.
- **Technical support**
  - Infection protocols, qPCR primers for detection.
  - In-house viral testing with the wild-type YFV (BSL3 confinement).
- **Premises: BSL2 and BSL3 laboratories are available onsite, training is needed for access.**
- **Equipment available on site: Luminex (multiplex ELISA), BioMark (gene profiling by qPCR), digital PCR, confocal microscopy.**

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THANK YOU

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The challenge

Provide an immunologically-competent liver model to assess attenuation of yellow fever live-vaccines

- **Provide a cellular model capable of reproducing major metabolic and immunological functions of the liver, utilizing some combination of human cell lines equivalent to primary liver cells**
  - Major cell types components and resident immune liver cells like Kupffer cells
  - Non-exhaustible, documented source of cells
- **Provide a device or platform which is amenable to use with viruses, in biosafety confinement level 2 and 3 laboratories and in validated assays.**
  - Absence of viral inhibitors in culture media
  - Minimal risk of contamination during handling, for the operator and the environment
- **The device should be medium throughput and compatible with standard equipment and measurement platforms (e.g. microscopy, biochemical analysis, robotics).**
  - Dynamic studies over a 3-5 day period