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# The Investigation of Virus-Driven Intrahepatic Cholangiocarcinoma through Dysregulated Hepatic Differentiation

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# Introduction



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HCV as a risk factor for iCCA



Plasticity within the hepatic compartment and possible cell of origin of HCV driven iCCA



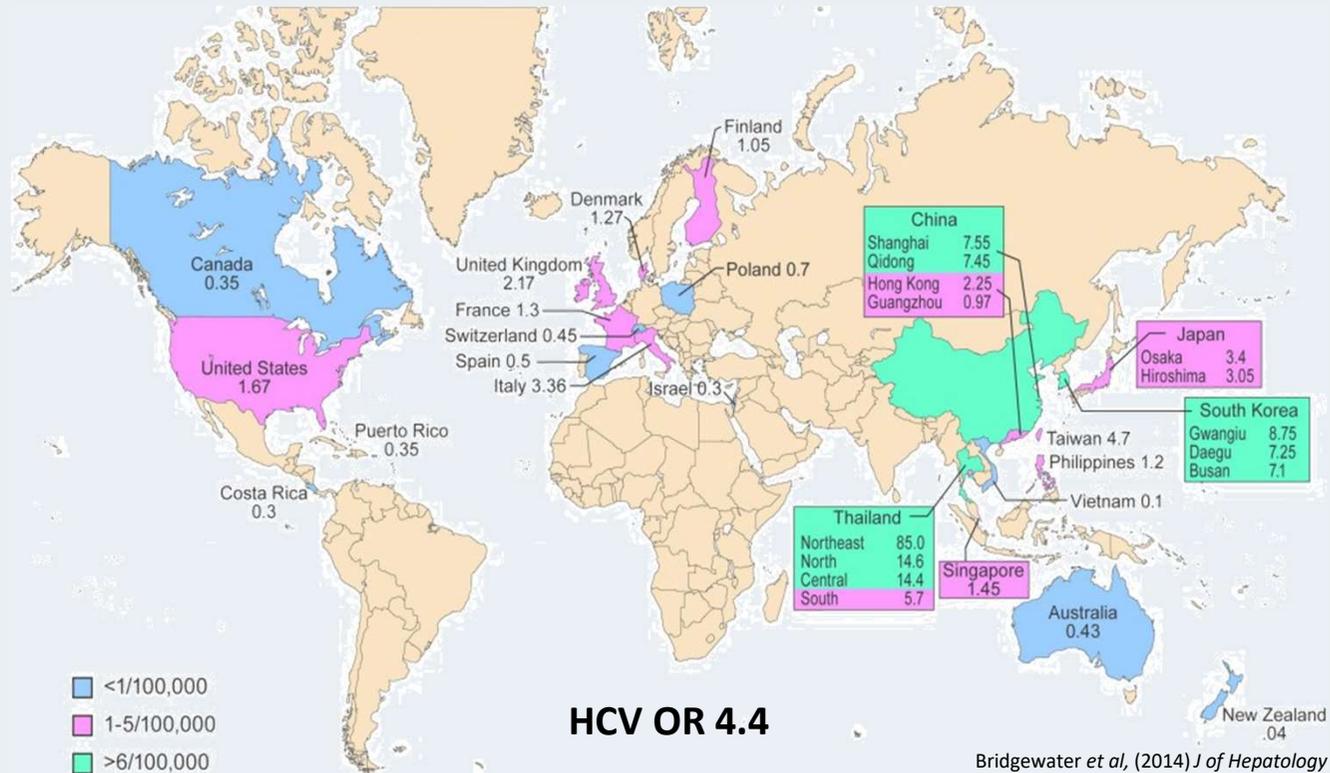
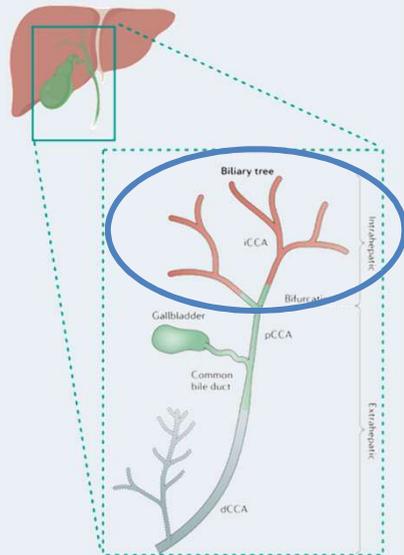
Pathway that may be involved

# Hepatitis C virus as a risk factor for iCCA



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## CCA cases worldwide

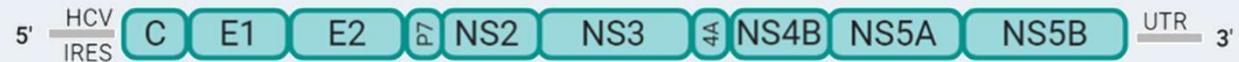


↑iCCA in the westernised world → could this be associated with epidemiological pattern of chronic liver diseases such as viral hepatitis?



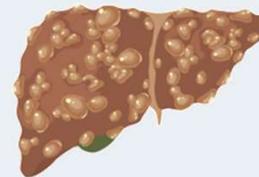
# Hepatitis C virus as a risk factor for iCCA

## Hepatitis C Virus Genome



### HCV Class A Carcinogen

HCV treated with DAAs = 90% 'clinically cured'  
≠ ↓ PLC risk unlike previous interferon based treatments



**Indirect**  
-Inflammation



**Direct**  
-Viral oncogenes  
-'hit and run'

# HCV and Epigenetic Imprinting



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Hamdane *et al*, (2019) *Gastro*

**HCV-Induced Epigenetic Changes Associated With Liver Cancer Risk Persist After Sustained Virologic Response**

Perez *et al*, (2019) *PLoS One Gen*

Hepatitis C virus leaves an epigenetic signature post cure of infection by direct-acting antivirals

Demonstrate epigenetic changes as a result of HCV associated with HCC risk persisting post cure

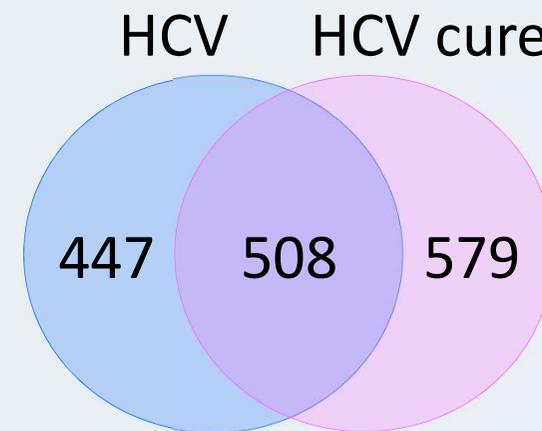
# HCV and Epigenetic Imprinting

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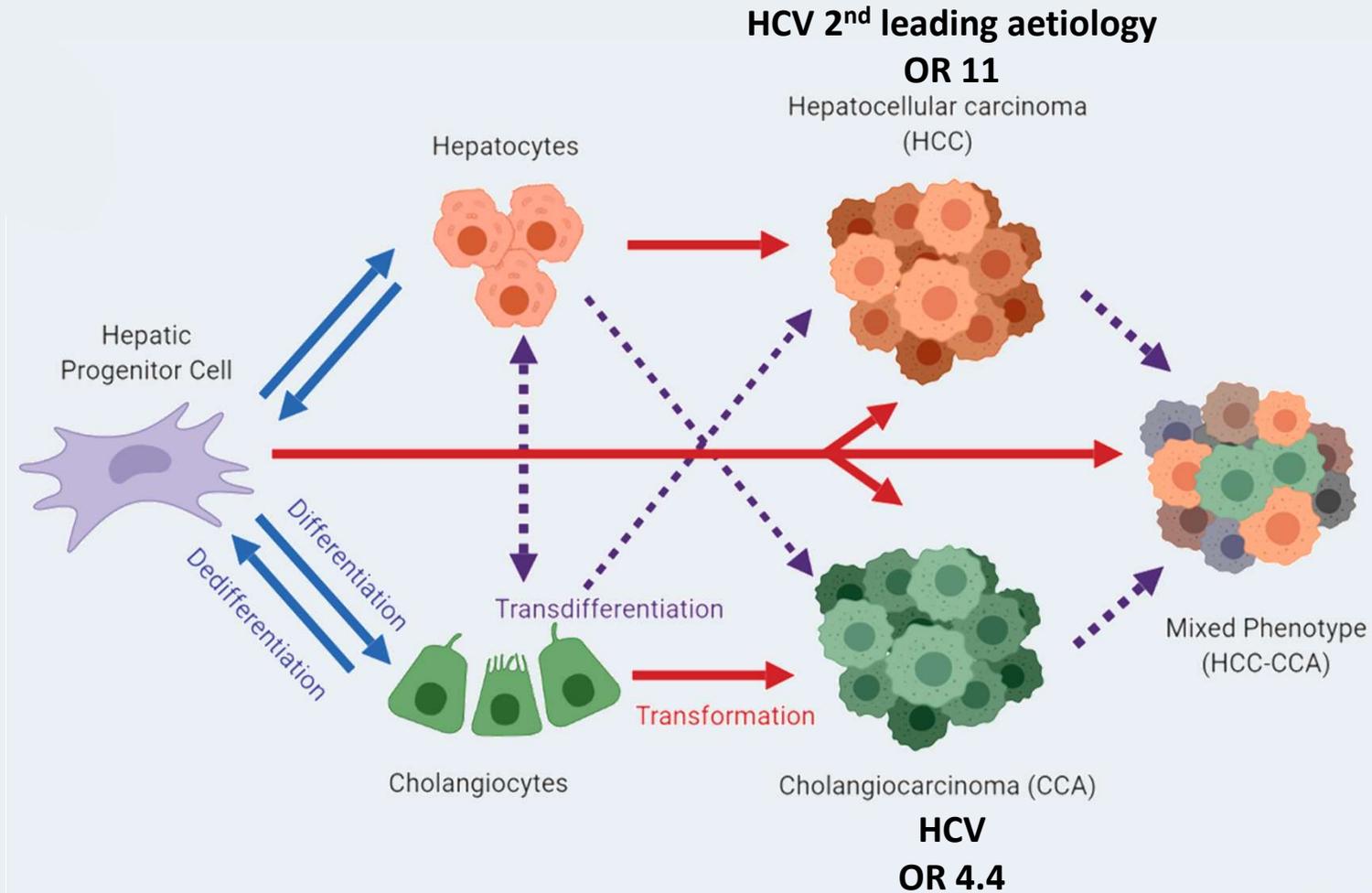


Demonstrate epigenetic changes as a result of HCV associated with HCC risk persisting post cure

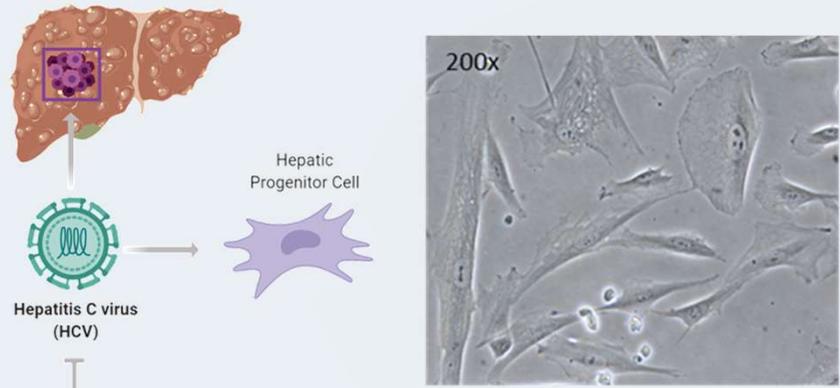
# High Plasticity within the Hepatic Compartment



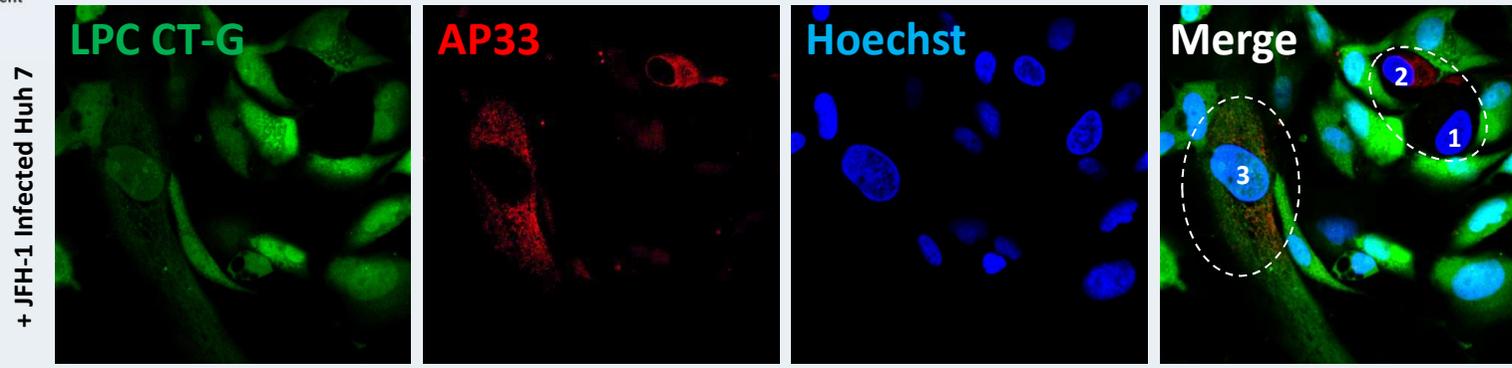
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# Can HCV infect HPCs?



**HCV can infect iPSC derived HPCs and ex vivo HPCs**  
→ Could HPCs be the origin of HCV driven iCCA?

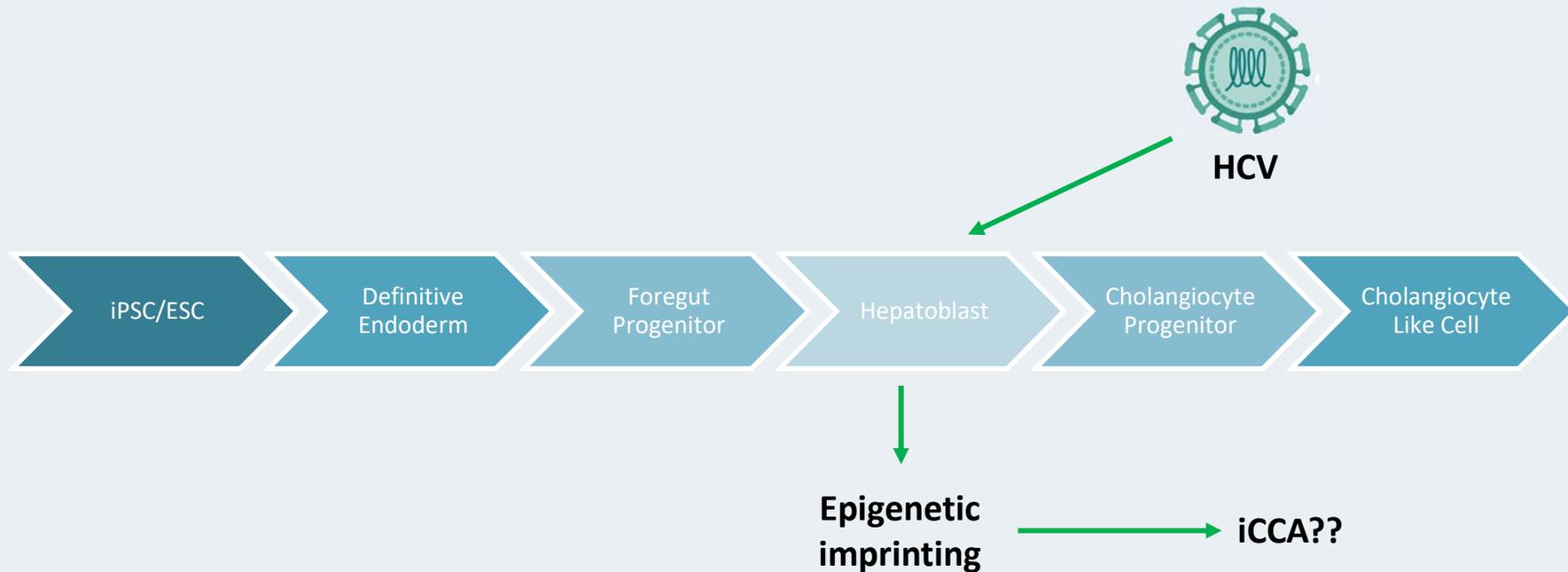


Co-culture with control/HCV-infected Huh7 cells – 1:Uninfected Huh7 cell; 2: Infected Huh7 cell; 3: Infected LPC

# Developing a stem cell derived model



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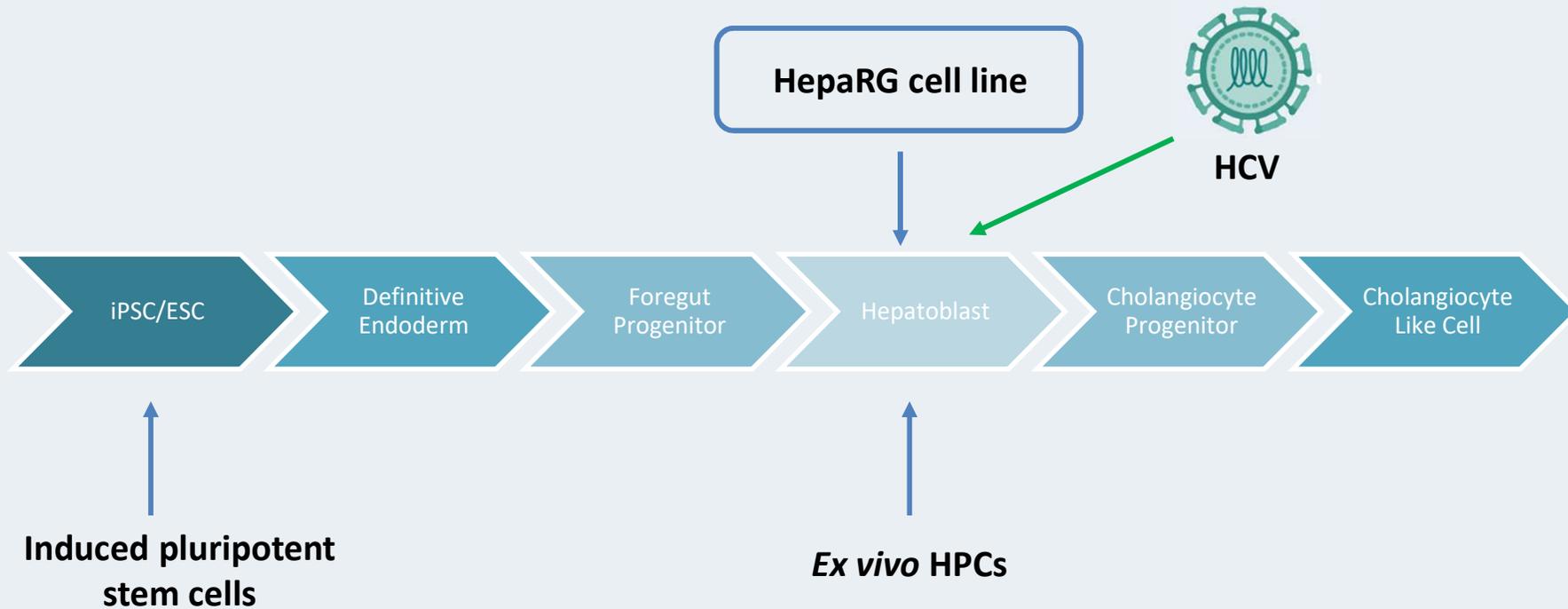


**Hypothesis:** HCV infection results in an imprinted pro-tumourigenic transcriptional phenotype that persists following DAA treatment

# Developing a stem cell derived model

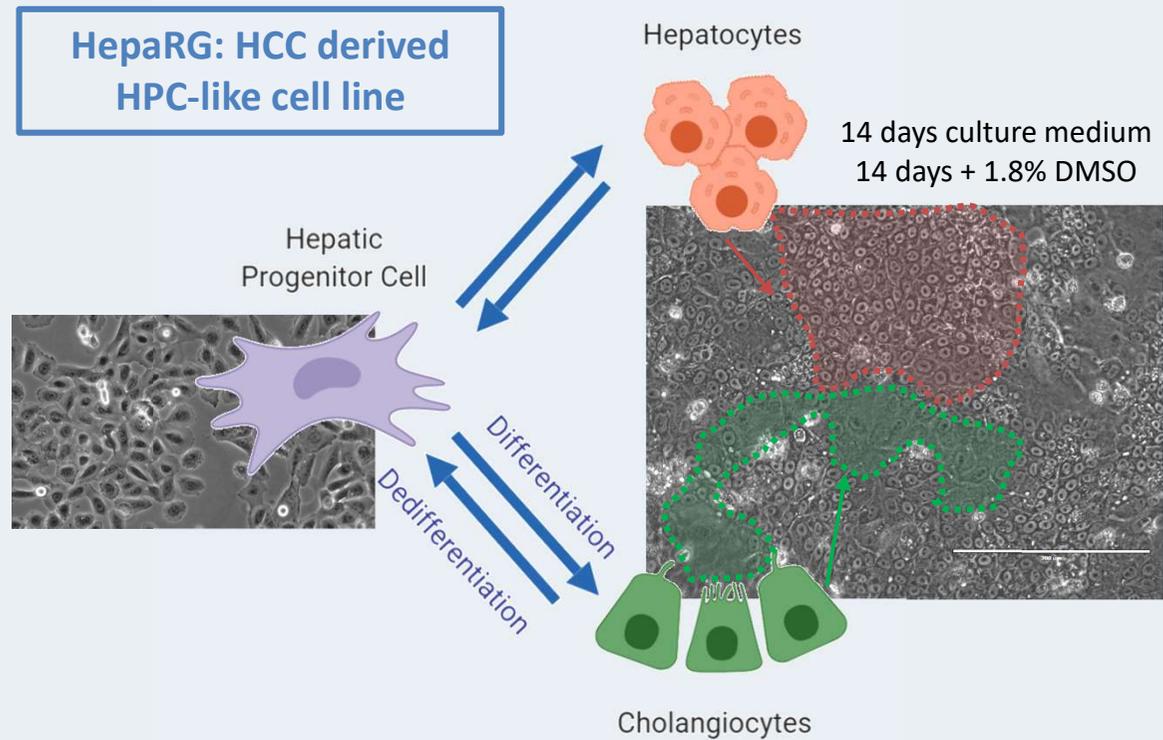


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1. Can HCV infect our cell line of choice?
2. Can this cell line be differentiated towards a cholangiocyte specific population?

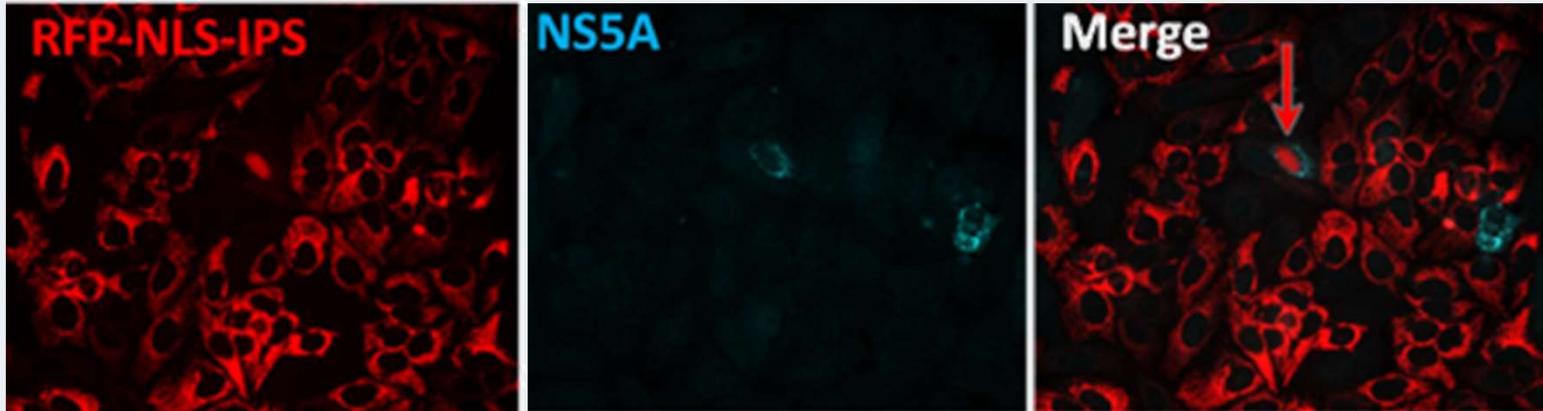
# HepaRG cell line



Parent *et al*, (2004) *Gastroenterology*  
Cerec *et al*, (2007) *Hepatology*

# HepaRG HCV infection

## 1. Are HepaRG cells permissive to HCV?



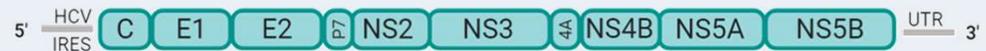
J6-JFH1 HCV + HepaRG RFP-NLS-IPS-1 cells

### RFP-NLS-IPS-1 lenti-construct



Cleaved by NS3-4A  
RFP → Localised to nucleus

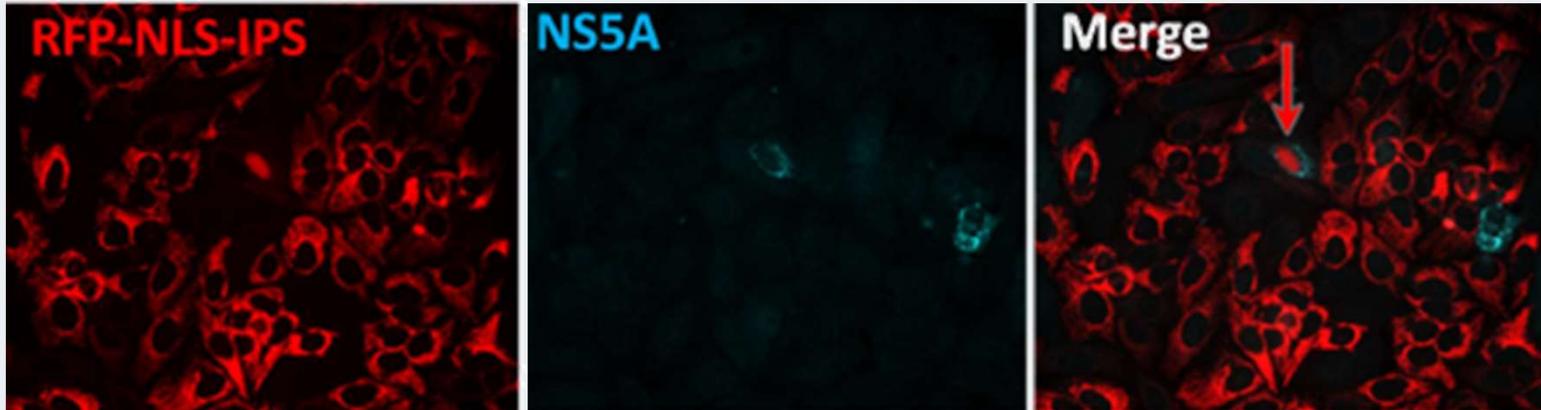
### Hepatitis C Virus Genome



Full length virus

# HepaRG HCV infection

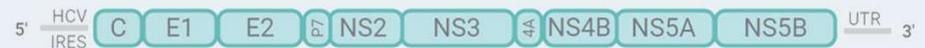
## 1. Are HepaRG cells permissive to HCV?



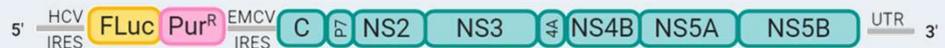
J6-JFH1 HCV + HepaRG cells

Currently optimising for transfection with HCV subgenomic replicons → puromycin selection luciferase assay detection

### Hepatitis C Virus Genome



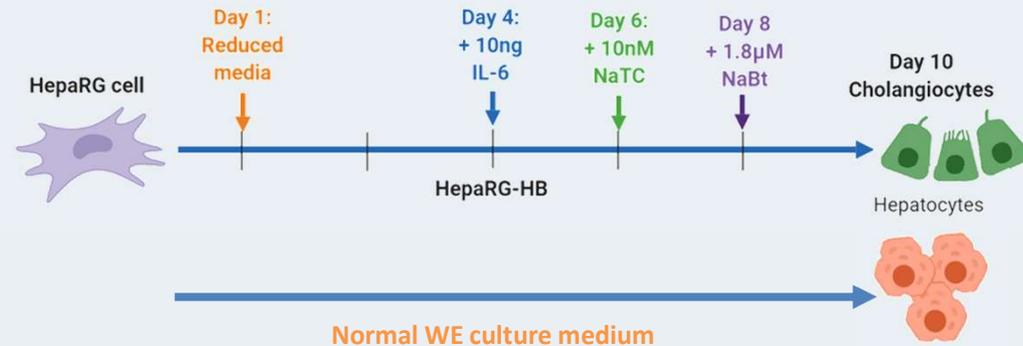
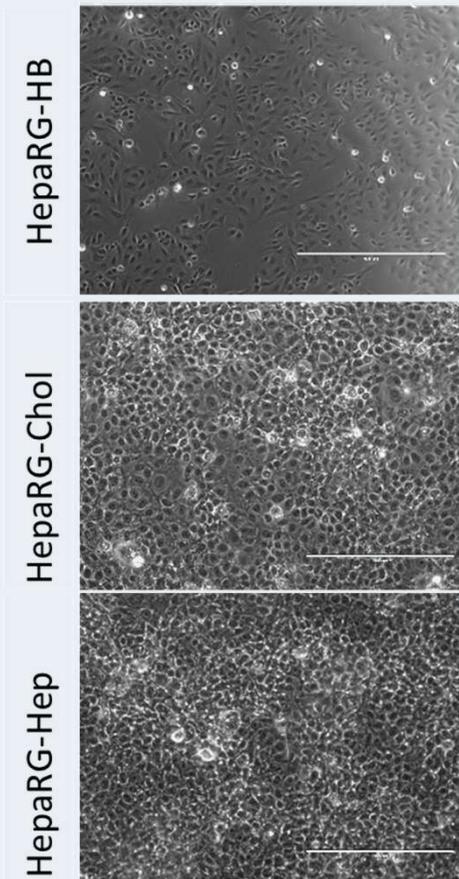
### N17/JFH1 subgenomic replicon



# Cholangiocyte differentiation

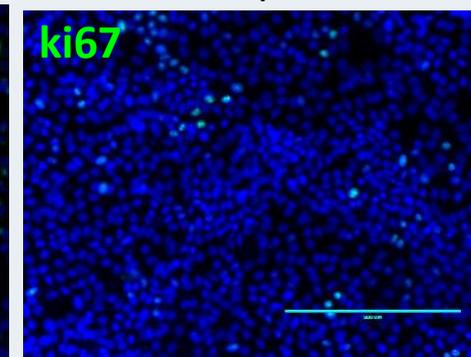
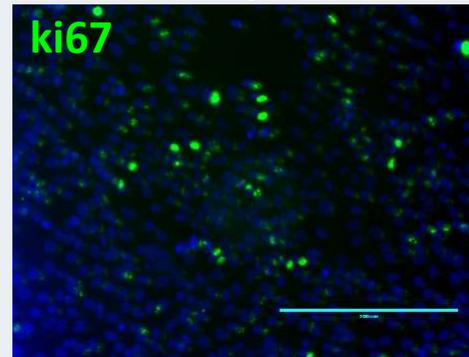
## 2. Can HepaRG cells be differentiated towards a cholangiocyte-like cell specific population?

Dianat *et al*, (2014) *Hepatology*



Day 4

Day 10

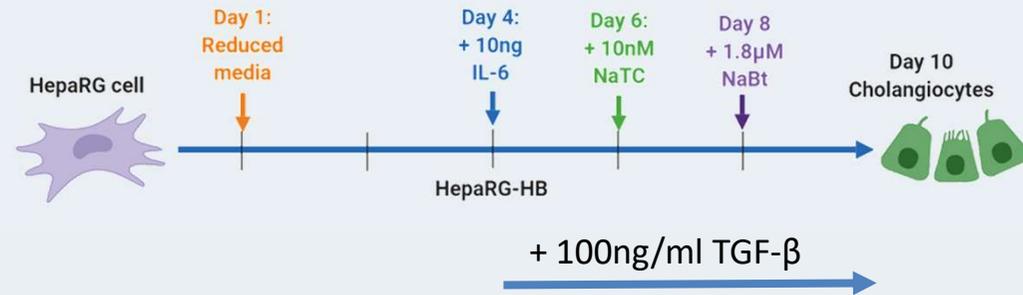
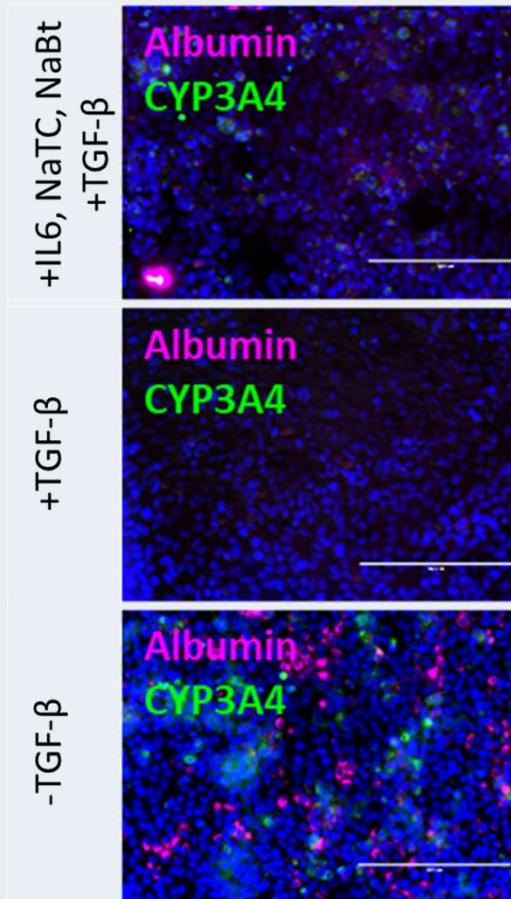


+ IL-6, NaTC & NaBt

# Cholangiocyte differentiation

## 2. Can HepaRG cells be differentiated towards a cholangiocyte-like cell specific population?

Dianat *et al*, (2014) *Hepatology*

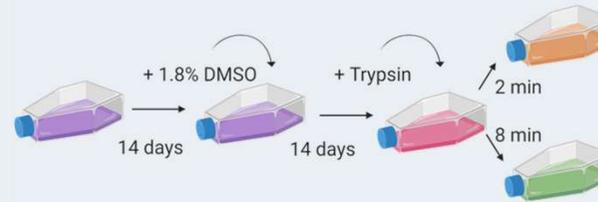


+/- TGF- $\beta$   $\rightarrow$  prevention of hepatic differentiation

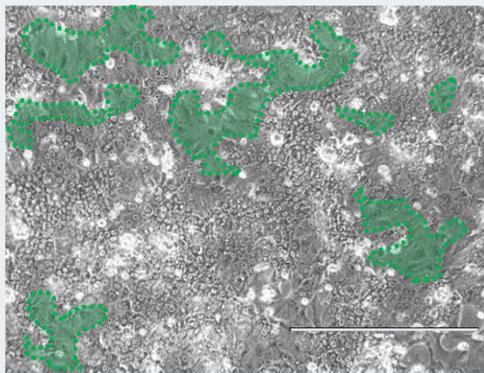
Currently optimising

# HepaRG cells are heterogeneous

## 2. Can HepaRG cells be differentiated towards a cholangiocyte-like cell specific population?

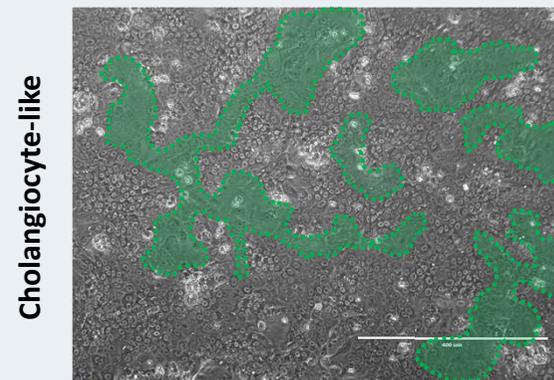


D28



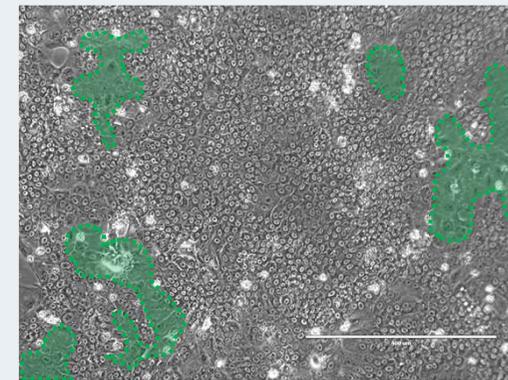
14 days culture medium  
14 days + 1.8% DMSO

D56



Dedifferentiate  
28 days + 1.8% DMSO

D56



Dedifferentiate  
28 days + 1.8% DMSO

We can select HepaRG clones predisposed towards cholangiocyte-like cells shown by morphology

## Differentiation

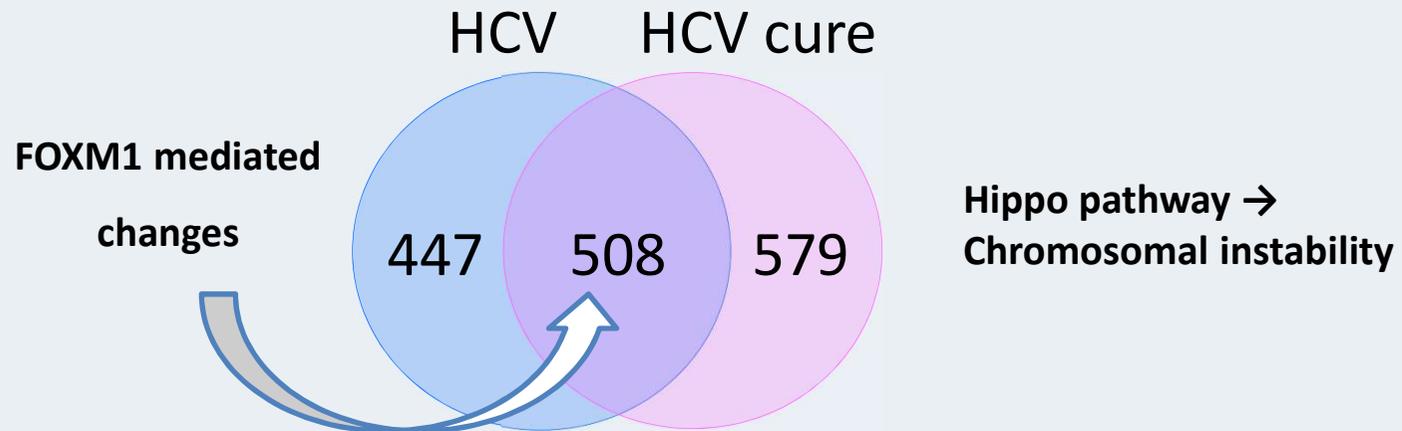
- PCR → cholangiocyte differentiation
- Trypsin isolation creates ↑cholangiocyte-like cells → isolate further using flow cytometry
- Move to iPSCs → established methods differentiation and literature on HCV infection

Model → determine the role of HCV in HPC differentiation and a iCCA pre-cancerous state.

# Which pathways could be involved?



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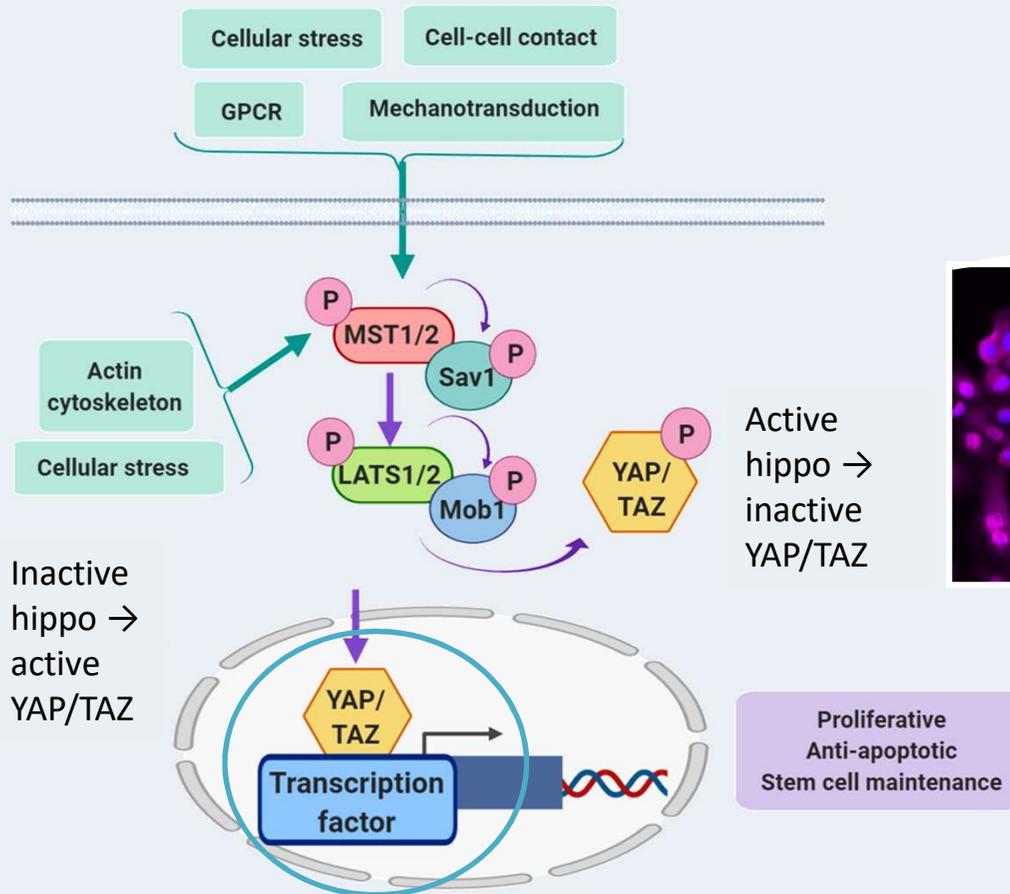
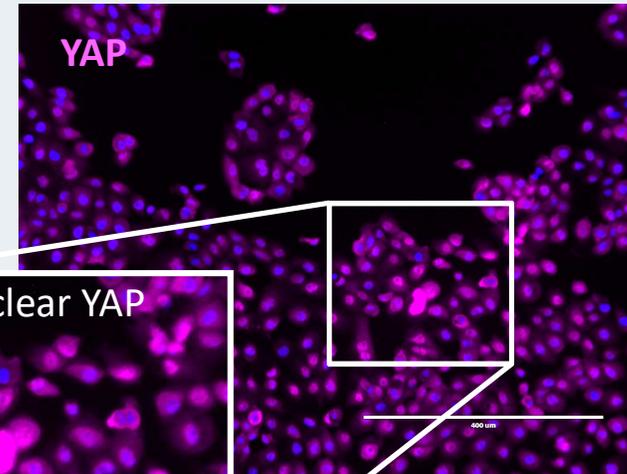


# The hippo pathway



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HuCCT1: metastatic CCA cell line

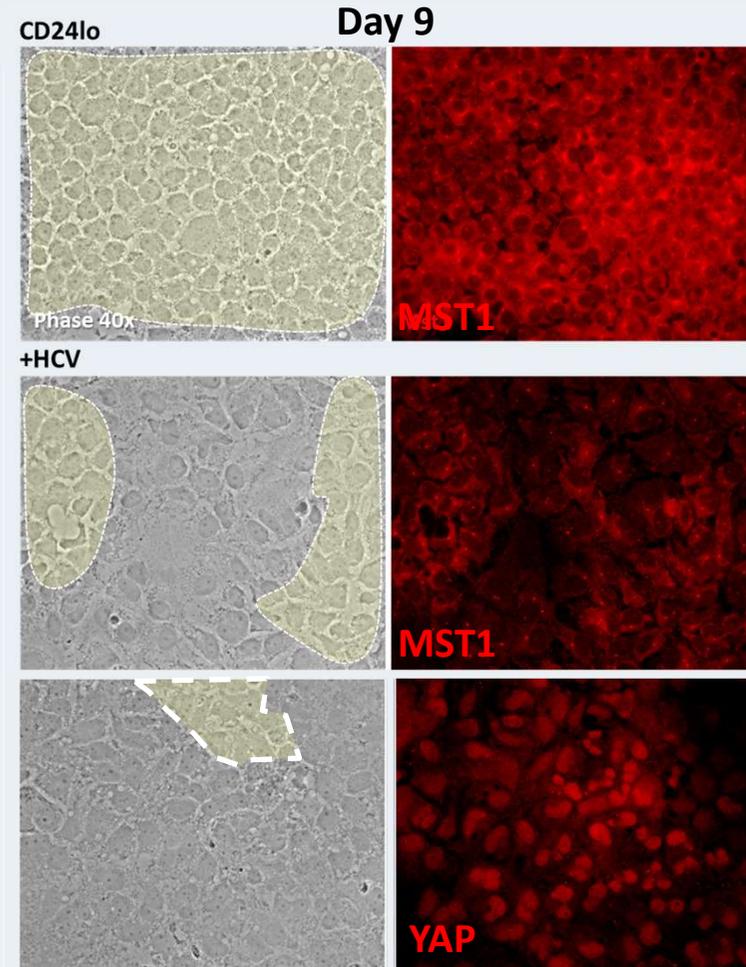
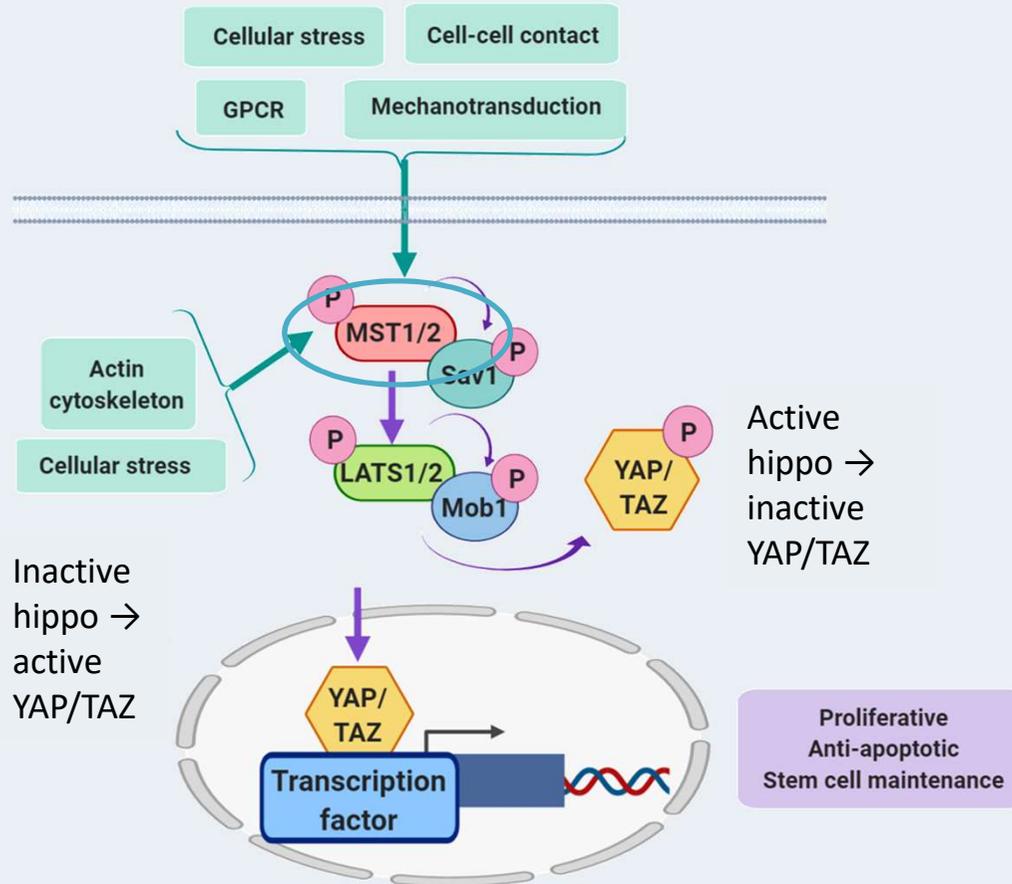


Inactive in various cancer types  
→ known driver of iCCA

# The hippo pathway



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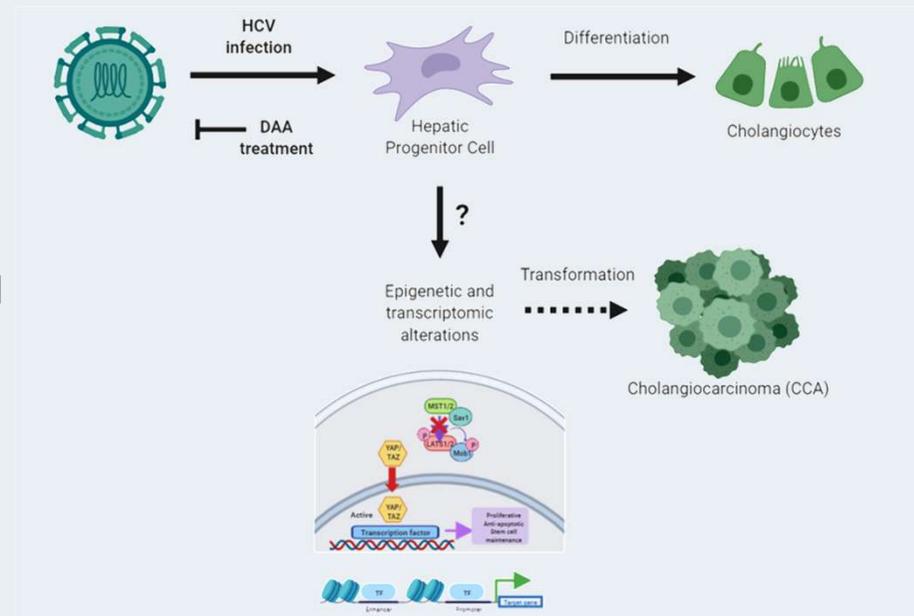


Huh7 CD24lo hepatocyte differentiation cell model



# Summary

- ↑iCCA in westernized world → related to HCV infection?
- DAAs do not eliminate the risk of developing PLCs → HCV epigenetic ‘hit and run’
- HPCs can be infected with HCV → origin of HCV driven iCCA?
- Cholangiocyte-differentiation model using HepaRG cells → currently optimising
- Use this model to investigate the pathways linked to HCV epigenetic changes
- Hippo pathway involved?



# Acknowledgements



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## Group

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Laura Edwards

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Abigail Bloy

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