**Supplementary File**

**Modeling hepatitis C virus kinetics during liver transplantation highlights the role of the liver in virus clearance**

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**Cell Culture Experiments**

JFH-1 HCVcc virus stock collected in complete Dulbecco's modified Eagle's medium (cDMEM) (HyClone, Logan, UT) with 10% fetal bovine serum (FBS) (HyClone), 100 units/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine (Gibco Invitrogen, Carlsbad, CA) was prepared by amplification in Huh7 cells as previously described in detail [1].

To measure HCVcc half-life in the absence of cells, JFH-1 HCVcc stock was diluted to 104 ffU/mL, which is the level observed in culture media during steady state HCV infection, and incubated at 37°C simulating conditions during HCV infection in cell culture. At indicated time points, two aliquots were frozen at -80°C until encapsidated HCV RNA was isolated for quantification.

To measure HCVcc half-life in the presence of chronically HCV infected cells, non-growing Huh7 cells [1] were infected with JFH-1 HCVcc at a low MOI (0.01) and cultured for 12 days until HCV levels were at steady state. Cells were then treated with 1nM daclatasvir (provided by Bristol-Myers Squibb NYC, NY) to stop HCV replication and infectious virus secretion or 200M Naringenin to block HCV secretion. At indicated times, medium and cell lysates were harvested from triplicate wells. Total RNA was isolated and 1 μg of RNA was used for cDNA synthesis using Fermentas reverse transcriptase reagents (ThermoScientific), followed by real-time PCR quantification using an Applied Biosystems 7300 real-time thermocycler (Applied Biosystems). Thermal cycling consisted of an initial denaturation step for 10 min at 95°C followed by 40 cycles of denaturation (15 s at 95°C) and annealing/extension (1 min at 60°C). HCV levels were determined relative to an HCV standard curve and normalized to carrier RNA levels (as a control for recovery efficiency). The PCR primers used to detect HCV were 5’-CGACACTCCACCATAGATCACT-3’\_ (sense) and 5’-GAGGCTGCACGACACTCATACT-3’\_ (antisense).

**Table S1:** Characteristics of five HCV infected patients who underwent liver transplantation (LT) and donors

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **Gender** | **Weight (kg)** | **BMI** | **Age at LT** | **Donor age** | **Donor gender** | **HCV genotype** |
| 1 | M | 88 | 30 | 68 | 72 | M | 1b |
| 2 | F | 70 | 31 | 66 | 33 | M | 1b |
| 3 | M | 87 | 34 | 56 | 69 | M | 3 |
| 4 | F | 43 | 19 | 60 | 73 | F | 1b |
| 5 | M | 90 | 29 | 49 | 29 | M | 1b |
| Median (range) |  | 87 (43-90) | 30 (19-34) | 60 (49-68) | 69 (29-73) |  |  |

**Table S2: HCV kinetics during AH**

|  |  |  |
| --- | --- | --- |
| **Phase** | **Case** | **Decrease slope (log/day)** |
| AH | 1 | VP |
| 2 | VP |
| 3 | VP |
| 4 | 7.6 |
| 5 | 3.7 |
| **Median (range)** |  | **5.6 (3.7-7.6)** |

AH, anhepatic phase; VP, viral plateau (slope ~0).

**Table S3: HCV kinetics during PR**.

|  |  |  |  |
| --- | --- | --- | --- |
| **Phase** | **Case** | **Initial decrease slope (log/day)\***  **and [duration, min]** | **Final# Decrease slope (log/day)\*\*** |
|  | 1 | 66 [16] | 6.0 |
| RP  **Median (range)** | 2 | 36 [22] | 8.4 |
| 3 | 50 [14] | 5.3 |
| 4 | 49 [11] | 8.8 |
| 5@ | - | 1.4 |
|  | **50**  **(36-66)** | **6.0**  **(1.4-8.8)** |

@, For Case 5 during graft reperfusion (RP) only one slope was estimated from the last point of AH phase until 4 hr post RP; #, For Cases 1-4 during RP, an initial rapid decrease is estimated, followed by a slower/final decrease;**\* ,** Slopes were calculated by taking the measured viral load (VL) of the last point of AH phase and the first 3 points of RP phase;**\*\*** **,** VL after the first 3 points of RP until 4 hr post RP were used.



**Figure S1**: Virus kinetics of five HCV infected patient before, during and after liver transplantation. Surgery was initiated at *t = 0*. In all patients, virus levels decreased after graft reperfusion. A nadir was reached after a median time of 15 h (range 9-87 h) post RP. HCV RNA resurged within 7 days after RP, with 3 of 5 Cases reaching greater or comparable viral levels to those observed at baseline. For the remaining 2 Cases, the corresponding viral loads were 0.87 and 0.43 log10 IU/mL lower than those just before surgery. Interestingly, HCV RNA levels in Case 5 remained in a lower plateau from 11 hours post-surgery until the end of follow up period (slope < 0.001, p = 0.76), with a corresponding viral load of 2.45 ± 0.002 log10 IU/mL.

|  |  |
| --- | --- |
|  | Equation 1 |
|  | Equation 3 |
|  | Equation 4 |
|  | Not Modelled  Note that Case 5 maintained plateau at this stage, while Cases 1-4 experienced a resurgence. |

**Figure S2**: **Visual description of the procedure and modeling assumptions**. **(A)** Prior to the anhepatic (AH) phase viral load is at steady state in which virus is being produced and balanced by viral clearance (Eq. 1 in the main text). **(B)** During the AH phase, the liver is not present and therefore there is no viral production. However, virus may appear to be cleared by the input of fluids that dilute the virus in circulation (see F in Eq. 2 in the main text) and through unknown virus *physiological* clearance. **(C)** At 4 hr post RP there is no viral production as the new liver has not yet been productively infected (i.e., is not releasing new virions). Clearance still occurs both via fluid balances but is mainly predicted via a time-dependent function (see c(t) in Eq. 4) that may represent a rapid clearance phase (possibly due to viral binding/entry into hepatocytes) immediately after graft reperfusion and a final/slower clearance phase (*physiological*). **(D)** Longer after RP (over 4 hours), the new liver begins producing new virions (as evident in Figure S1).

**Cited literature**

1. Yu, X. and S.L. Uprichard, *Cell-based hepatitis C virus infection fluorescence resonance energy transfer (FRET) assay for antiviral compound screening.* Curr Protoc Microbiol, 2010. **Chapter 17**: p. Unit 17 5.