

# HEP DART 2005

frontiers in drug development for viral hepatitis

Possible roles of virus replication rates, hepatocyte death rates, trans-complementation and super-infection in the emergence of drug resistant virus strains: theoretical considerations

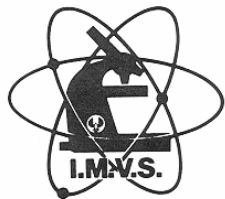
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# Roles of virus replication rates, hepatocyte death rates, polymerase trans-complementation & super-infection in the emergence of drug resistant virus strains in healthy carriers: Computer modeling

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## Resistance to Monotherapy (lamivudine)

- Antiviral therapy with lamivudine works best in individuals with immunologically active liver disease (hepatocyte turnover)
- Treatment failure after 1 yr occurs in ~25% of patients due to emergence of drug resistant mutants
- After 4 yrs treatment failure occurs in >70% of patients
- Drug resistant mutants replicate (synthesize viral DNA) in cell culture at near wild-type rates (>10% of wild-type)

## Problem

- Based on reported rates of mutation that occur during hepadnavirus replication, lamivudine resistant mutants should be present at a frequency of  $10^{-8}$  to  $10^{-4}$  prior to therapy
- Therefore, intuitively at least, it would seem that as hepatocytes are cleared of wild-type virus in healthy carriers they should immediately be infected with drug resistant mutants
- However, this is not the case!

For example, clevudine therapy of chronically infected woodchucks induces a decline in the percentage of infected hepatocytes.

In some animals, after an 80% decline in infected hepatocytes, WHV infection rebounds, starting after 5-6 months of antiviral therapy.

In others rebound may take >12 months

## Question

- Why does antiviral therapy work at all in healthy carriers, even for a short time?
- What determines the durability of the response? (time to emergence of drug resistant mutants)

## Approach

- Design a computer model that tracks the fate of wild-type & drug resistant mutant viruses & infected hepatocytes (only include assumptions that are testable experimentally)

## The computer model

(J. Clin. Virol. 34 suppl. 1, S96-S107, 2005)

Simulates a liver of  $3 \times 10^{10}$  hepatocytes

Tracks in each hepatocyte:

- Infection status
- cccDNA copy number
- Viral genotypes (wild-type, mutant)
- Virus production

as a function of the number of rounds of hepatocyte death & proliferation during antiviral therapy

## Assumptions in initial modeling

cccDNA is stable & distributed to progeny cells during mitosis. Average copy number = 30

Rate of build up of cccDNA is the same as rate of virus production & release

No virus is released until cccDNA reaches its final (assigned) copy number

All hepatocytes have an equal probability of dividing to maintain liver mass

Wild-type replication is inhibited 10,000-fold during antiviral therapy

## The computer model

1. Divides the liver into compartments (groups of cells) with different cccDNA copy numbers (aver. = 30)
2. Distributes cccDNA to progeny cells at division
3. Allows a latency period for cccDNA amplification before cells produce virus
4. Assumes virus is produced by cells in proportion to their cccDNA content & rate of DNA replication
5. User assigns rates of wild-type & mutant virus replication in the presence & absence of drug
6. Determines at start-up a rate constant for production of “infectious” virus based on user assigned infected cell doubling time
7. Allows separate rate constants for the death of infected ( $K_d$ ) & uninfected hepatocytes ( $K_u$ )

## Experimental Issue 1: Rate of wild-type virus replication

- Jilbert & Summers labs both found that wild-type DHBV has an infected cell doubling time of about 1 day or less following *de novo* infection of neonatal ducks

## Experimental Issue 2: Hepatocyte turnover

- The rate of hepatocyte turnover is uncertain
- Estimates of hepatocyte turnover in chronically WHV-infected woodchucks measured by incorporation of bromodeoxyuridine & PCNA staining are about 2% per day
- Uninfected hepatocytes appear to have a turnover rate of 0.05% per day

Conclusions from computer modeling using the DHBV replication rate (infected cell doubling time) of 1 day & infected-cell death rate of 2% per day

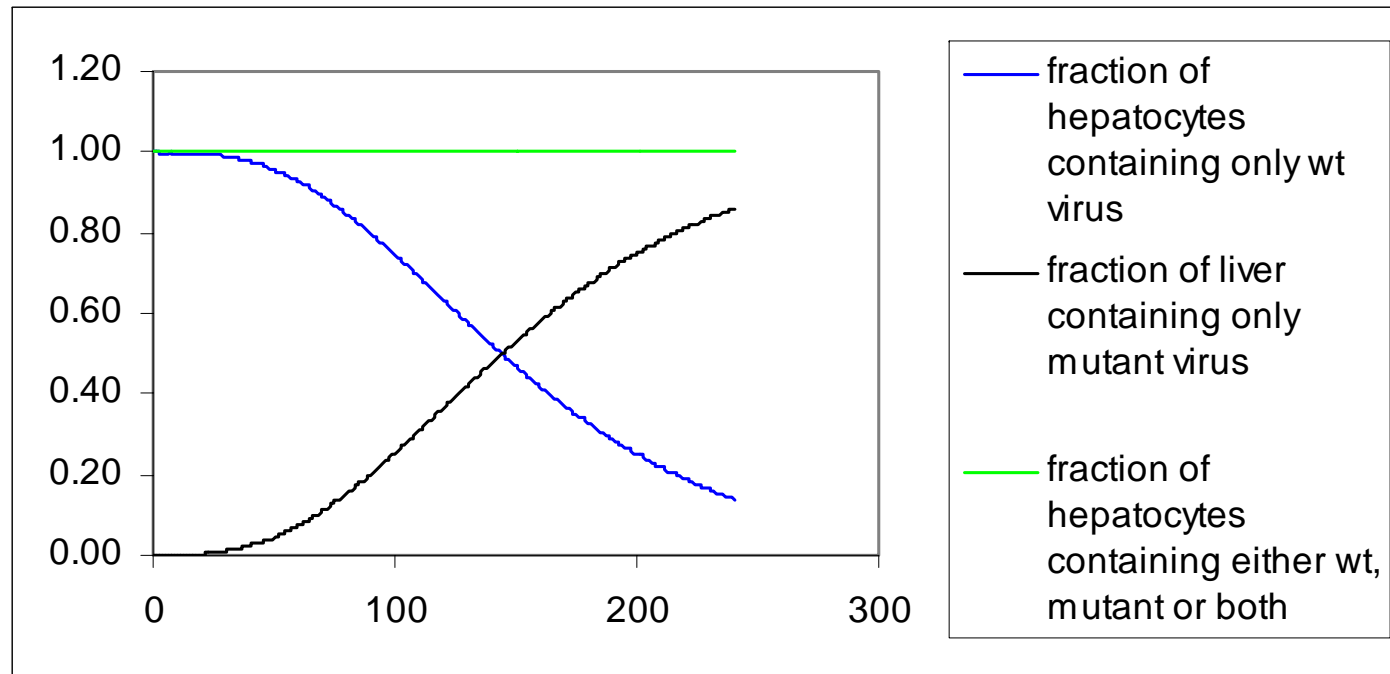
- Resistant mutants with a replication rate of 10% or more of the wild-type will emerge to fill the liver nearly as fast as uninfected hepatocytes become available
- Reduction to 2% the wild-type rate is needed for a durable response (3 yrs). (Permanent clearance occurs with a replication rate that is 1% of the wt)
- This low a mutant replication rate seems incompatible with *in vitro* assays

One way to reduce the replication rate of drug resistant mutants that is compatible with *in vitro* studies is to reduce the uninhibited wild-type rate, since rate estimates from the *in vitro* studies are relative

- In particular the infected cell doubling times of HBV & WHV, as well as DHBV in adult ducks, may be much greater than DHBV in neonatal ducks (1 day)
- Some evidence to suggest an infected cell doubling time of 3-4 days for WHV & HBV in adult hosts

No significant decline in the fraction of infected cells occurs if the doubling time is 3 days, the mutant replicates at 100% the uninhibited wild-type rate & the initial mutant frequency is  $10^{-4}$  ?

# Infected Hepatocytes

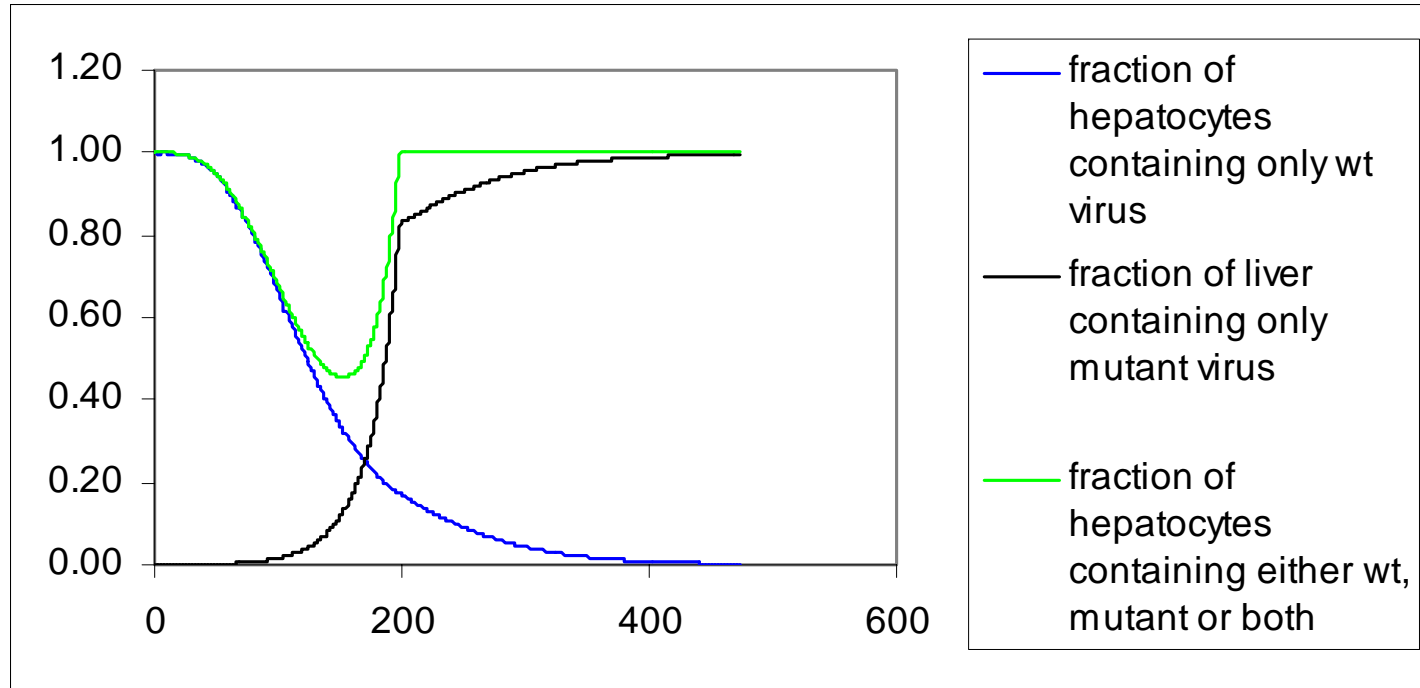


Days

Wild-type doubling time = 3 days  
Mutant replication rate = 100% of wt  
Initial Mutant Frequency =  $10^{-4}$   
 $K_d = 2\%$

What if the wild-type doubling time is 3 days, the mutant replicates at 20% the uninhibited wild-type rate & the initial mutant frequency is  $10^{-4}$  ?

# Infected Hepatocytes

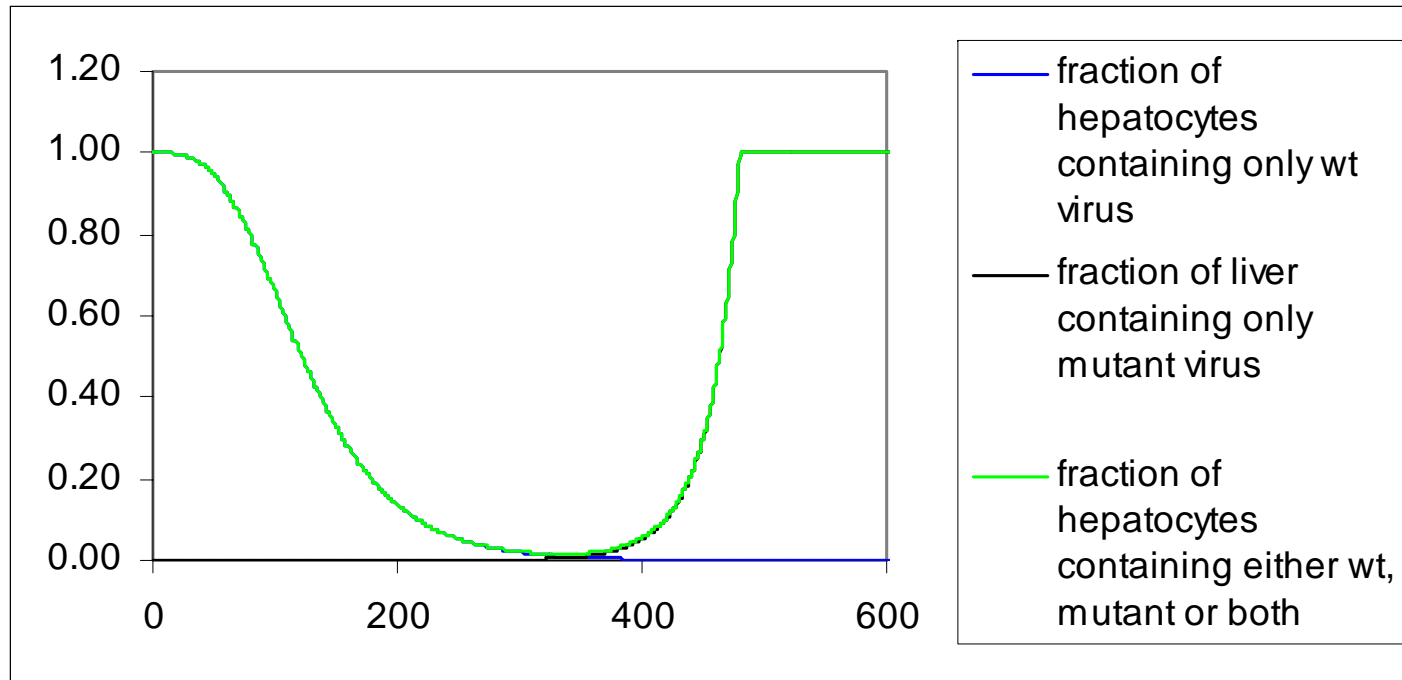


Days

Wild-type doubling time = 3 days  
Mutant replication rate = 20% of wt  
Initial Mutant Frequency =  $10^{-4}$   
 $K_d = 2\%$

What if the wild-type doubling time is 3 days, mutant replication rate is 20% of wt, & initial frequency of mutants is  $10^{-8}$ ?

# Infected Hepatocytes



Days

Wild-type doubling time = 3 days  
Mutant replication rate = 20% of wt  
Initial mutant frequency =  $10^{-8}$   
 $K_d = 2\%$

Overall conclusions from computer modeling using  
an infected cell doubling time of 3 days with an  
infected cell death rate of 2% per day

- Mutant viruses present at a frequency of  $10^{-4}$  prior to therapy & that replicate at 10% of the wild-type rate will emerge to fill the liver after 2 yrs (durable response)
- Durability is increased from 2 to 6 yrs when the initial frequency of mutant virus is reduced from  $10^{-4}$  to  $10^{-8}$
- Mutants that replicate at 5% of wild-type will not emerge
- As the infected cell doubling time approaches the rate needed to replace hepatocytes killed by the immune response, small differences in virus replication rate have major effects on efficacy

- Increasing the rate of destruction of infected hepatocytes can have conflicting results on durability depending on the relative rate of mutant virus replication, since hepatocyte turnover shortens the time until the appearance of uninfected hepatocytes, which allows the mutants to begin to spread earlier in therapy.
- If cccDNA loss occurs at mitosis, the rate of loss of infected hepatocytes is accelerated, which also allows the mutants to begin spreading earlier in therapy (and rebound from a durable response does not necessarily return to 100%)

The current model does not explain  
co-emergence of wild-type with mutant viruses

The model was therefore modified to

- 1) allow random sharing of polymerase in hepatocytes co-infected by wt & drug resistant mutants
- 2) allow circulating virus to multiply infect uninfected hepatocytes that arise during therapy
- 3) allow circulating virus to super-infect hepatocytes in which cccDNA copy number has declined due to passage through mitosis

None of these changes explains co-emergence of wt

To try to explain co-emergence of wild-type we are attempting to model super-infection of “fully” infected hepatocytes

Problems:

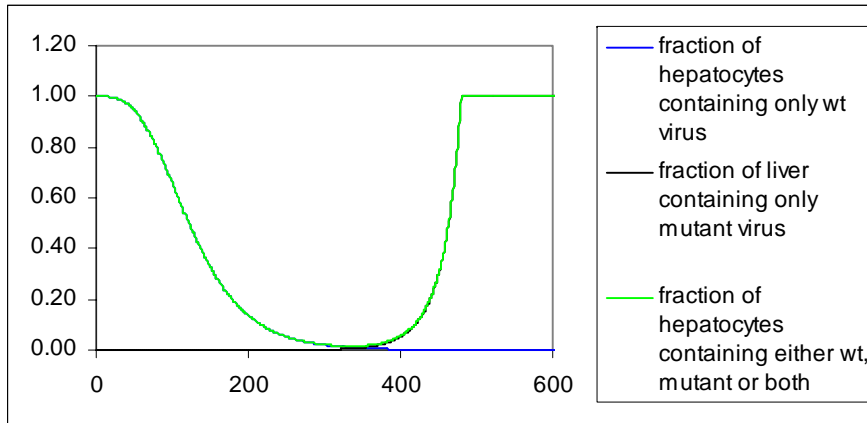
- Super-infection of fully infected hepatocytes would increase cccDNA copy number. In theory this could lead to a gradual buildup of cccDNA levels to cytotoxic levels
- If super-infection is a common event, then cccDNA copy number buildup is either compensated by hepatocyte death (viral cytotoxicity) or intracellular cccDNA decay

## Summary

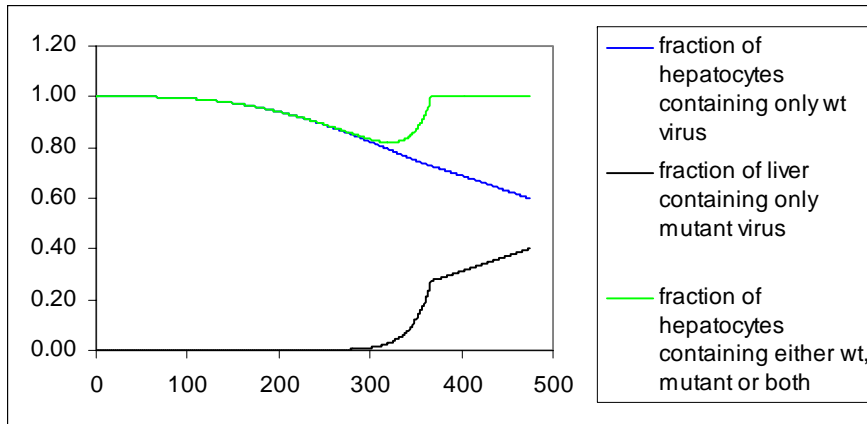
- Therapy of healthy carriers may work if the mutant frequency is lowered to  $<10^{-8}$  by combination therapy provided there is a reasonably high rate of destruction of infected hepatocytes (e.g., 2% per day)
- Sequential therapy is much less likely to work
- Successful therapy depends on a substantial death rate of infected hepatocytes; e.g., in the above simulations, lowering the rate to 0.5% substantially reduces efficacy

What if the mutant replication rate is 20% of wt & initial frequency of mutants is  $10^{-8}$  and  $K_d = 0.5$ ?

# Infected Hepatocytes



$K_d = 2\%$



$K_d = 0.5\%$

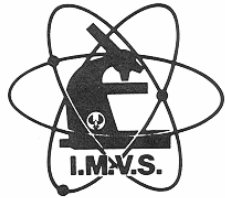
Days

Wild-type doubling time = 3 days  
 Mutant replication rate = 20% of wt  
 Initial Mutant Frequency =  $10^{-8}$

Ultimately, successful antiviral therapy depends on a significant rate of death of infected hepatocytes

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