

HEP DART 2005

frontiers in drug development for viral hepatitis

Development of a novel mouse model to evaluate drug candidates against wild type and mutant hepatitis B virus

Mark Feitelson
Thomas Jefferson University, USA

Development of a Novel Mouse Model to Evaluate Drug Candidates Against Wild Type and Mutant Hepatitis B Virus

**Mark A. Feitelson^{1,2}, Marcy M. Clayton¹,
Bill Sun¹, and Raymond F. Schinazi³**

¹Department of Pathology, Anatomy and Cell Biology, and

²Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA USA;

³Emory University/VA Medical Center, Decatur, GA, USA

Background (I)

Although there are culture systems available to evaluate putative drugs against HBV, and WHV infected woodchucks are preferred for preclinical drug development, there is no simple, *in vivo* model to evaluate small amounts of compounds that directly target wild type or mutant HBV.

Currently available systems include:

- HBV transgenic mice
- Transplantation of human hepatocytes into RAG-2, uPA/RAG2 or NOD/SCID mice
- Trimeric mice (infected human liver fragments transplanted into lethally irradiated, SCID bone marrow reconstituted mice)

However, the current challenge is to develop drugs against clinically significant HBV mutants.

Background (II)

The model developed herein is based upon the use of HepAD38 cells. HepAD38 cells consist of HepG2 (human hepatoblastoma) that were stably transfected with an expression vector in which HBV (subtype ayw) replication is regulated by tetracycline (tet). In the presence of tet, virus transcription and replication is suppressed, while in the absence of tet, virus replicates to very high levels and is secreted (Ladner et al., *Antimicrob Agents Chemother* 41:1715-20, 1997). Given that HepG2 cells grow out as subcutaneous tumors in nude mice, preliminary experiments were conducted to test the hypothesis that this would result in high levels of viremia, and that viremia would be regulated by adding tetracycline to the drinking water.

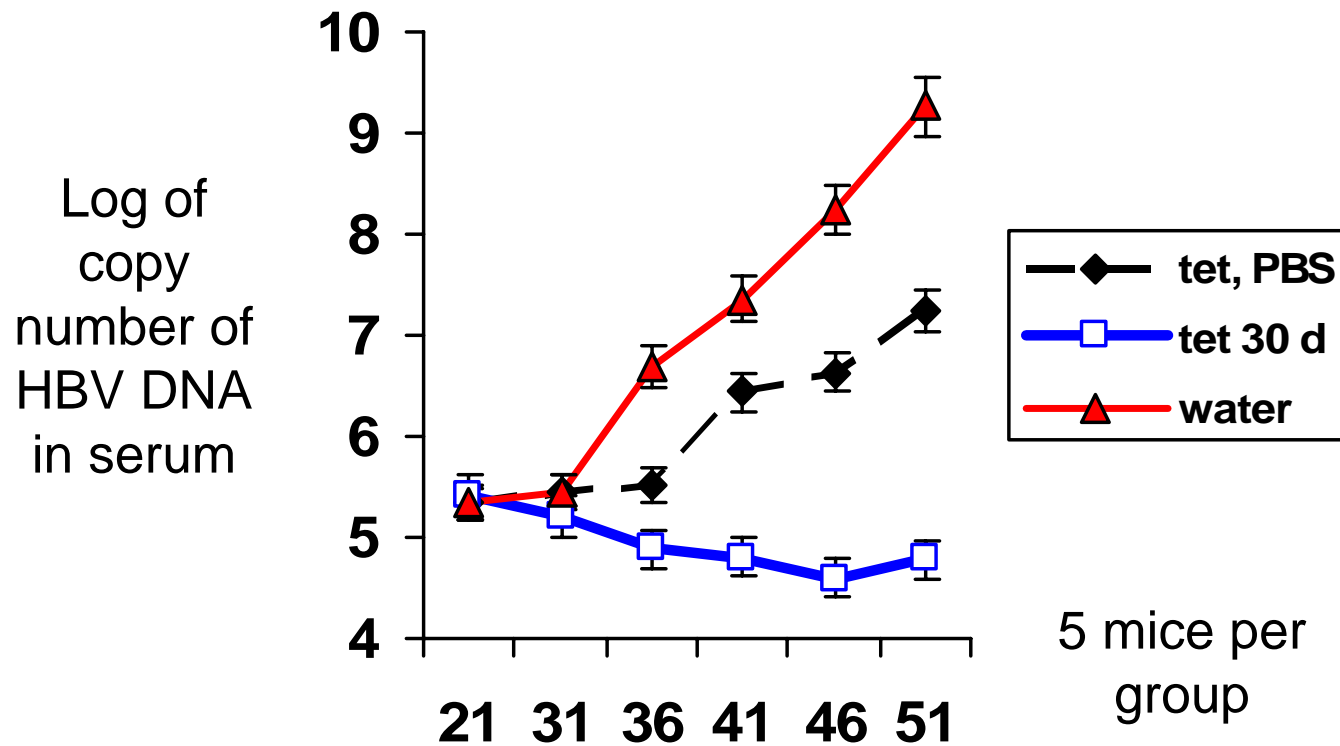
Establishment of Nude Mouse Model for Preclinical Antiviral Drug Testing Against HBV

Inject 1×10^7 AD38 cells ↓	tumors palpable; virus titers $2-4 \times 10^5$ ↓	sacrifice, remove liver (weigh + H&E stain liver sections) ↓			
day: 0	21	31	36	41	51
bleed:	*	*	*	*	*
group:					
1	<u>drinking water</u>				
2	<u>drinking water + tet (2.5 mg/ml) for 30 days</u>				
3	<u>water + tet for 10 days, then PBS injections*</u>				

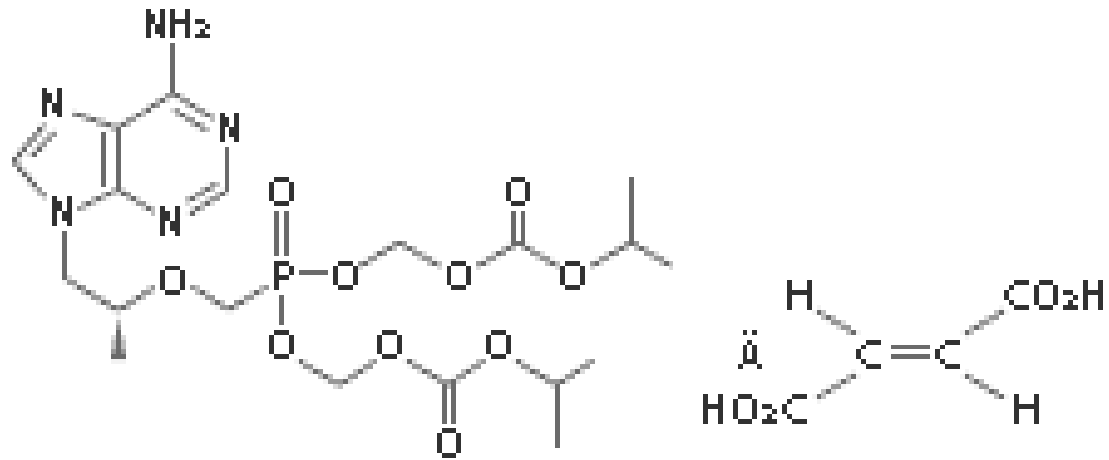
* each injection was given i.p. from days 31-36

HBV DNA was quantitated at each time point by real-time PCR. (Cepheid Smartcycler)

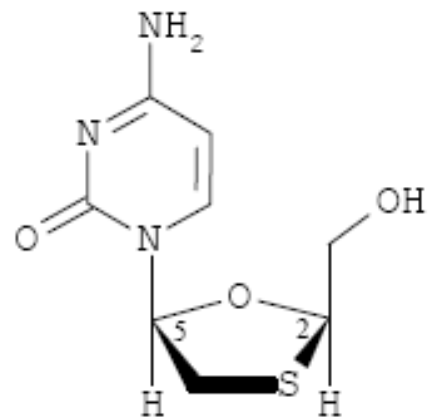
HBV DNA titers (assayed by real-time PCR) in serum samples from nude mice injected subcutaneously with AD38 cells.



Days post injection of AD38 cells, where day 21 is the first day of treatment



tenofovir disoproxil fumarate (TDF)



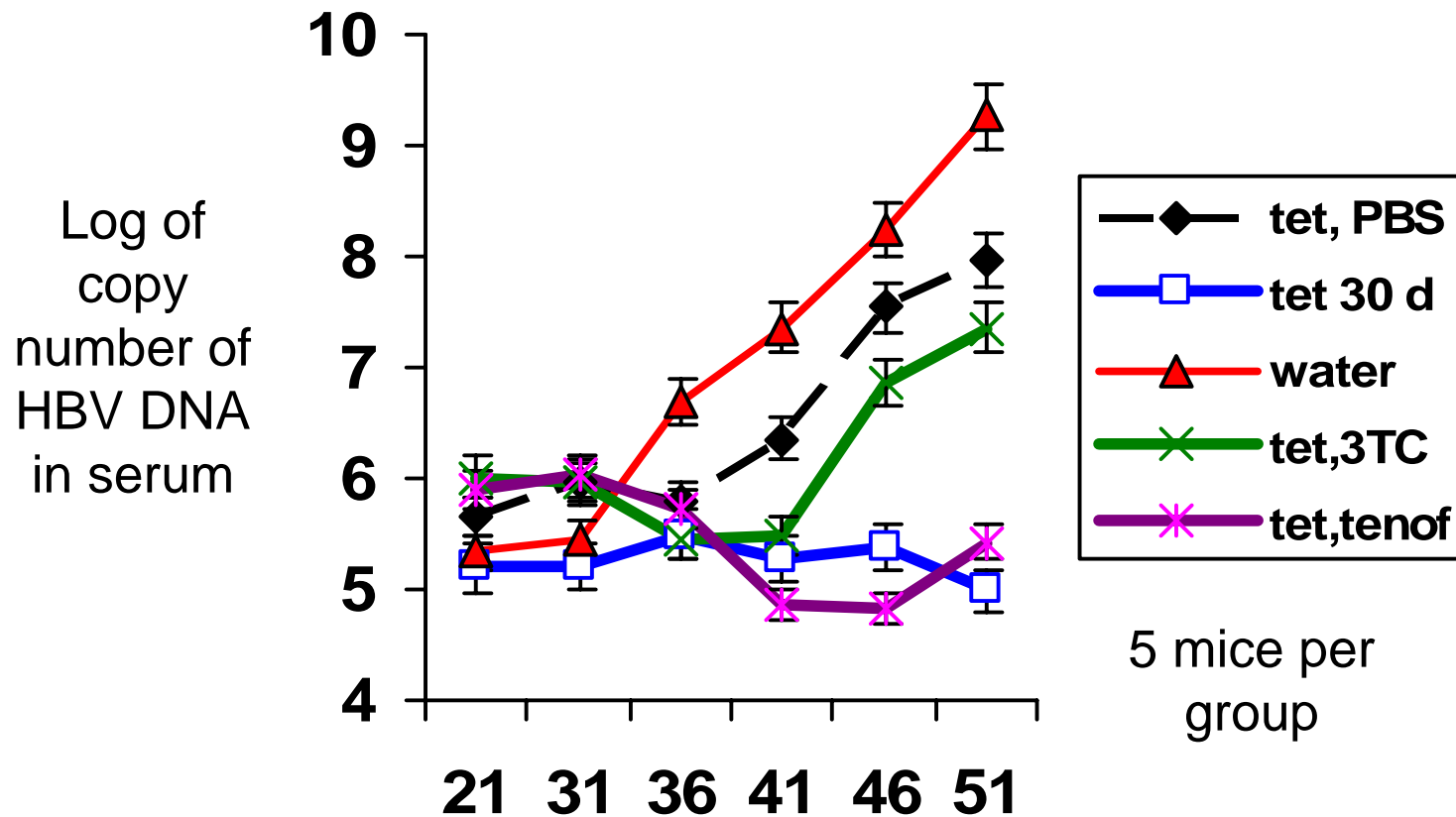
lamivudine (3TC)

Establishment of Nude Mouse Model for Preclinical Antiviral Drug Testing Against HBV

	Inject 1×10^7 AD38 cells ↓	tumors palpable; virus titers $2-4 \times 10^5$ ↓	sacrifice, remove liver (weigh + H&E stain liver sections)			↓
day:	0	21	31	36	41	51
bleed:		*	*	*	*	*
group:						
1		<u>drinking water</u>				
2		<u>drinking water + tet (2.5 mg/ml) for 30 days</u>				
3		<u>water + tet for 10 days, then PBS injections*</u>				
4		<u>water + tet for 10 days, then 3TC injections*</u>				
5		<u>water + tet for 10 days, then tenofovir injections*</u>				

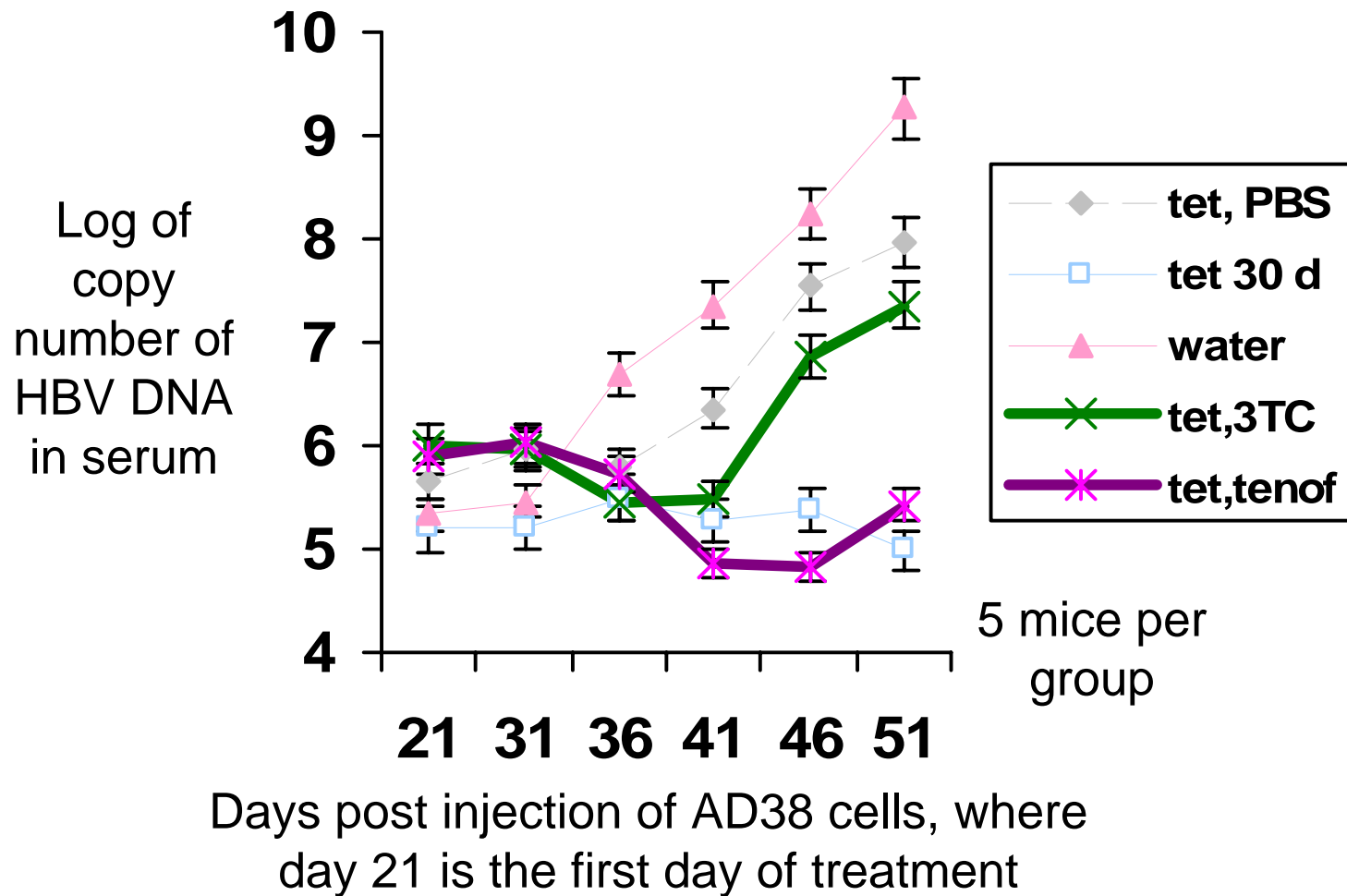
* each injection was given i.p. (100 mg/kg/day) from days 31-36

HBV DNA titers (assayed by real-time PCR) in serum samples from nude mice injected subcutaneously with AD38 cells.

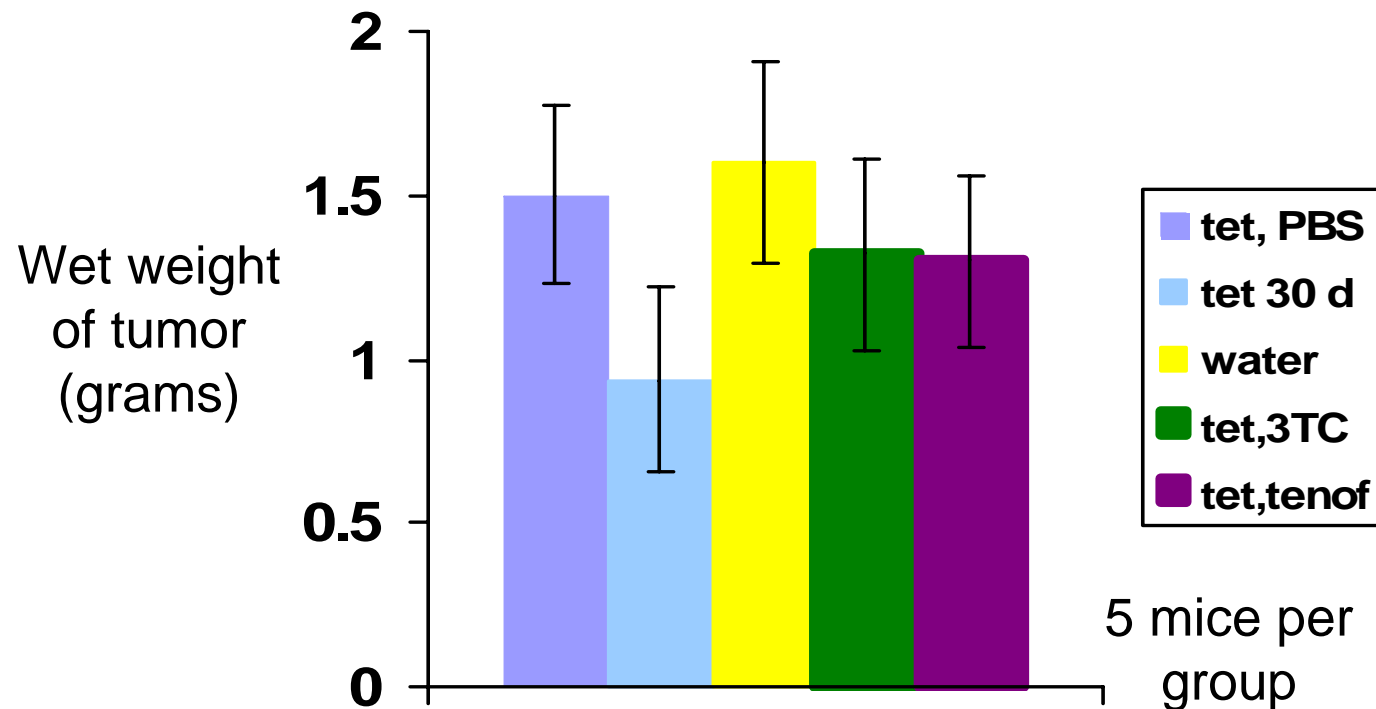


Days post injection of AD38 cells, where day 21 is the first day of treatment

HBV DNA titers (assayed by real-time PCR) in serum samples from nude mice injected subcutaneously with AD38 cells.

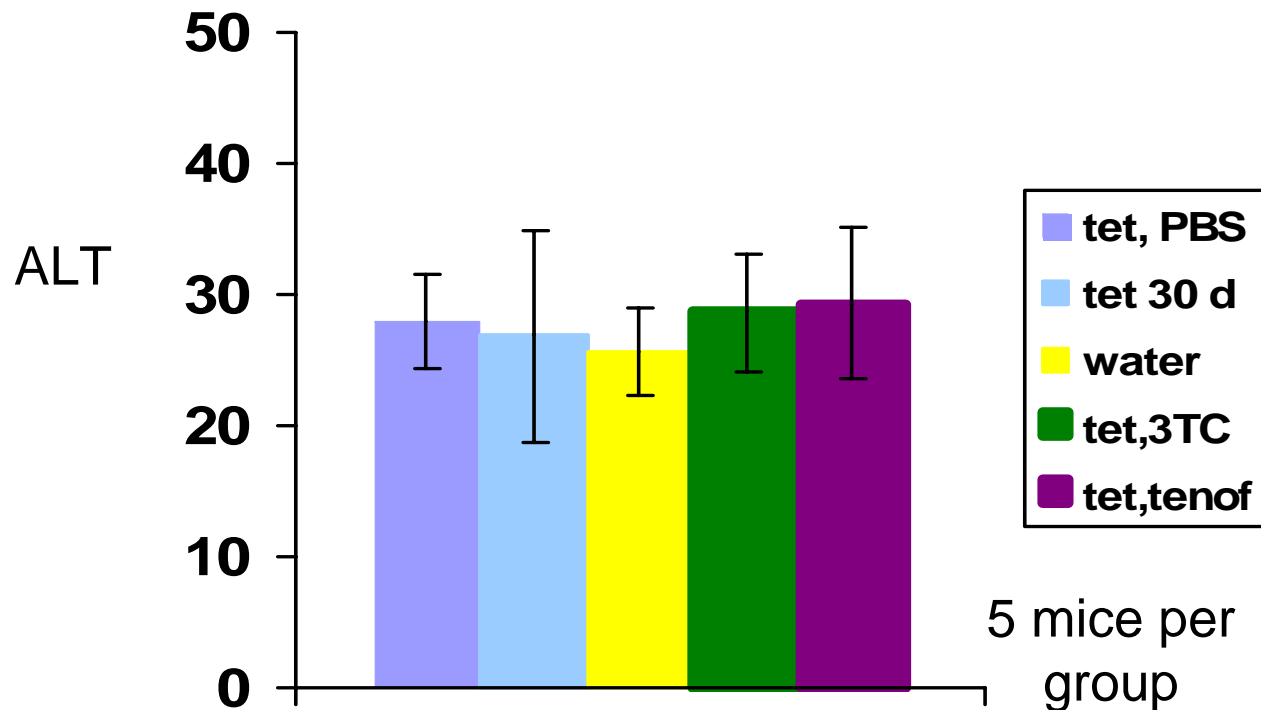


Tumor weights from nude mice injected subcutaneously with AD38 cells at day 51.



All mice survived and appeared healthy at the end of the experiment; water consumption was similar between test and control mice.

ALT values from nude mice injected subcutaneously with AD38 cells at day 36.



H&E staining of tumors showed little evidence of necrosis

Summary/Conclusions

- HepG2-AD38 cells, in which wild type HBV is under control of the tet promoter, develop subcutaneous tumors in nude mice, and as the tumors grow, all mice become viremic.
- Injection of 1×10^7 AD38 cells/mouse results in a palpable tumors by 3 weeks postinjection. At this time, virus titers range from $2-6 \times 10^5$ vge/ml, and by week 7 postinjection, titers regularly reach $> 10^9$ vge/ml.
- Addition of tetracycline to the drinking water when tumors first become palpable (day 21) partially inhibits virus production despite increase in tumor size.

Summary and Conclusions (II)

- Replacement of tetracycline with PBS treatment after 10 days results in a continual increase in virus titers over 30 days.
- Replacement of tetracycline with lamivudine or tenofovir after 10 days results in continued suppression of virus during the period of drug treatment. Virus titers increased after the end of lamivudine treatment, but after the end of tenofovir treatment, continued suppression of virus for > 3 weeks was observed.
- Treatment with tetracycline, lamivudine or tenofovir were not accompanied by any significant change in the mean weight of tumors recovered at the end of the experiment. In addition, elevated ALT was not observed at any time point in any mouse. This suggests little or no toxicity.

Prospects

- Based upon these data, the system will be used to conduct parallel experiments with different clinically relevant HBV mutants cloned into HepG2 cells. Experiments are now underway to assay new drugs (or drug combinations) against a lamivudine resistant mutant replicating in HepG2 cells (HepAD79).