

# Anti-HBV Activity of In Vitro Combinations of Tenofovir with Nucleoside Analogs

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- \* I have financial relationship with Gilead that is relevant to my presentation.**
- \* My presentation includes discussion of investigational use.**

**57th Annual Meeting of the American Association for the Study of Liver Diseases  
October 27 – 31, 2006  
Boston, Massachusetts  
Oral Presentation # 172**

# Introduction

- Antiviral treatment for CHB is primarily monotherapy which can lead to resistance
- Combination therapy may improve response rates and minimize resistance
- Tenofovir is active against WT and LAM-R HBV
  - LAM-R HBV is cross resistant to most other drugs
- Tenofovir DF (approved for HIV) is in phase III trials for CHB

# Background

## Possible Antiviral Drug Interactions:

- **Additivity:** Combination activity equals that predicted by the individual activities of the drugs
- **Synergy:** Combination activity is greater than additive
- **Antagonism:** Combination activity is less than additive

# Analysis and Interpretation of Data

Evaluated with both accepted models for in vitro drug interaction:

- Bliss independence ( $E(xy) = E_x \cdot E_y$ )
  - MacSynergy II
- Loewe additivity ( $dx/Dx + dy/Dy = 1$ )
  - Isobologram analysis

# Objective

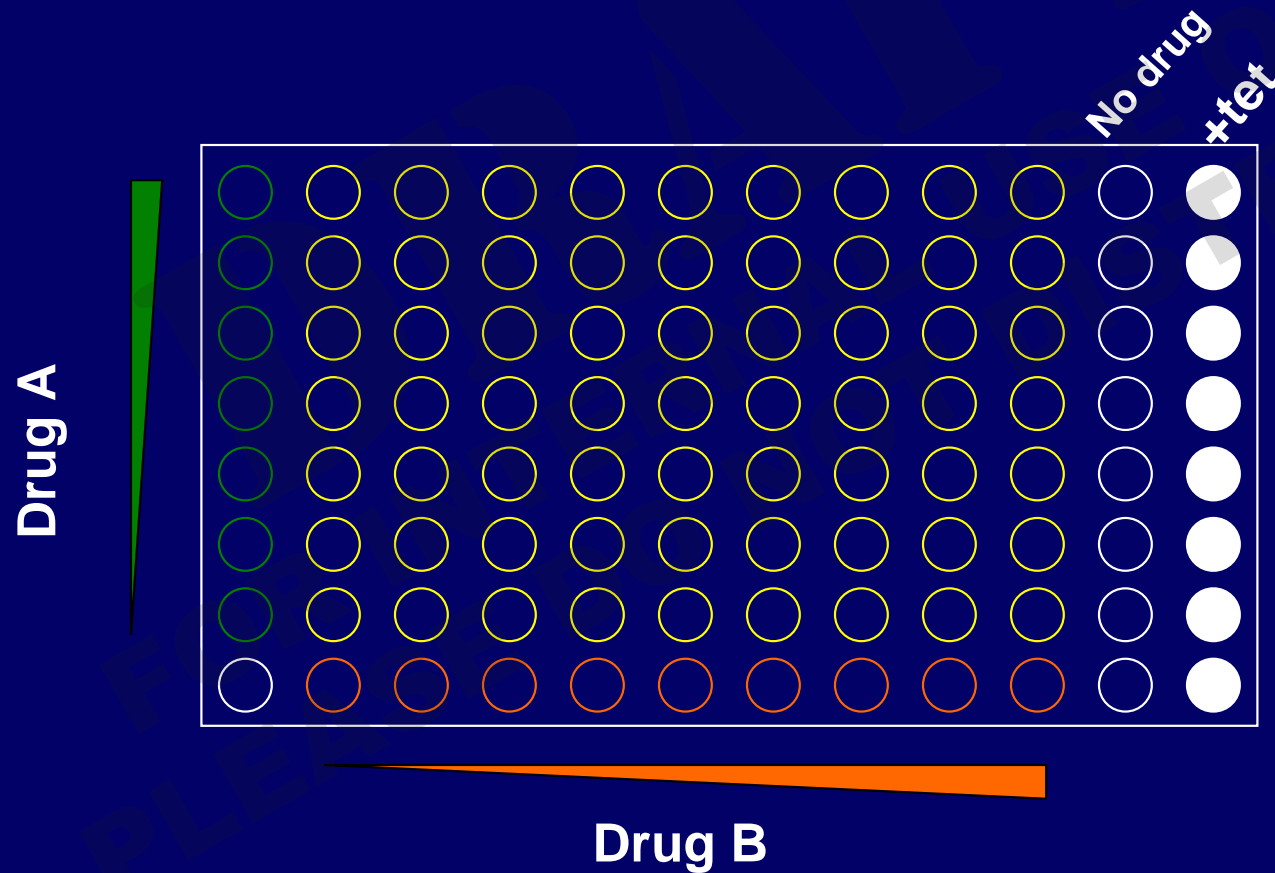
- To evaluate the in vitro anti-HBV activity of tenofovir (TFV) combined with:
  - Emtricitabine (FTC)
  - Lamivudine (3TC)
  - Entecavir (ETV)
  - Telbivudine (LdT)

# Materials and Methods

- AD38 cells which express high levels of HBV
- Combinations tested: TFV plus FTC, 3TC, ETV or LdT
- Antiviral assay:
  - Five replicate 96-well plates treated with drug combinations for 4 days
  - Extracted intra-cellular HBV DNA quantified with TaqMan PCR assay
- Cytotoxicity assay: cell viability following drug treatments assessed with XTT cleavage as marker

# Materials and Methods (cont.)

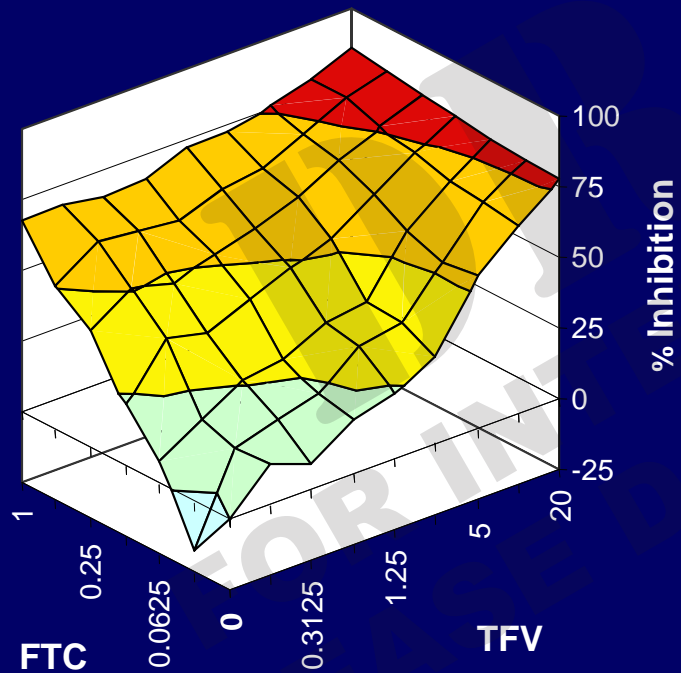
Experimental design of two drug combination matrix



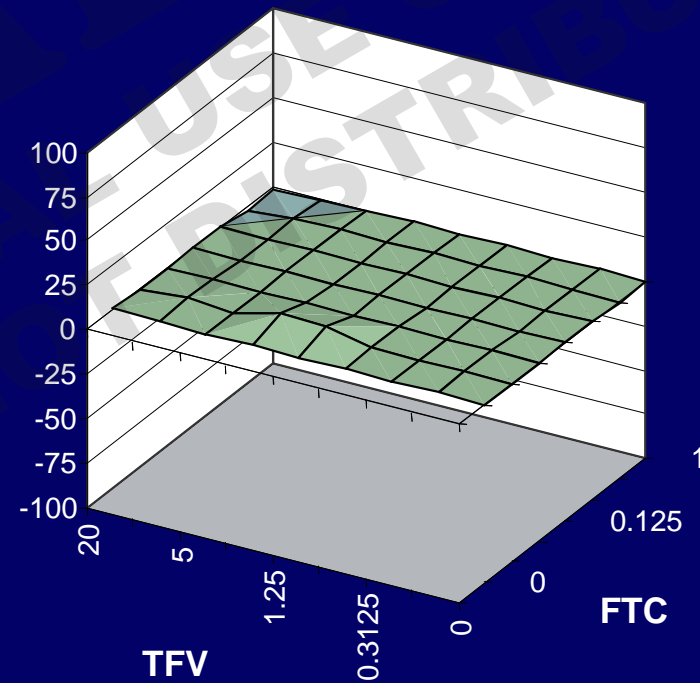
# TFV + FTC Combination

## MacSynergy Analysis of Anti-HBV Effects

Inhibition Plot

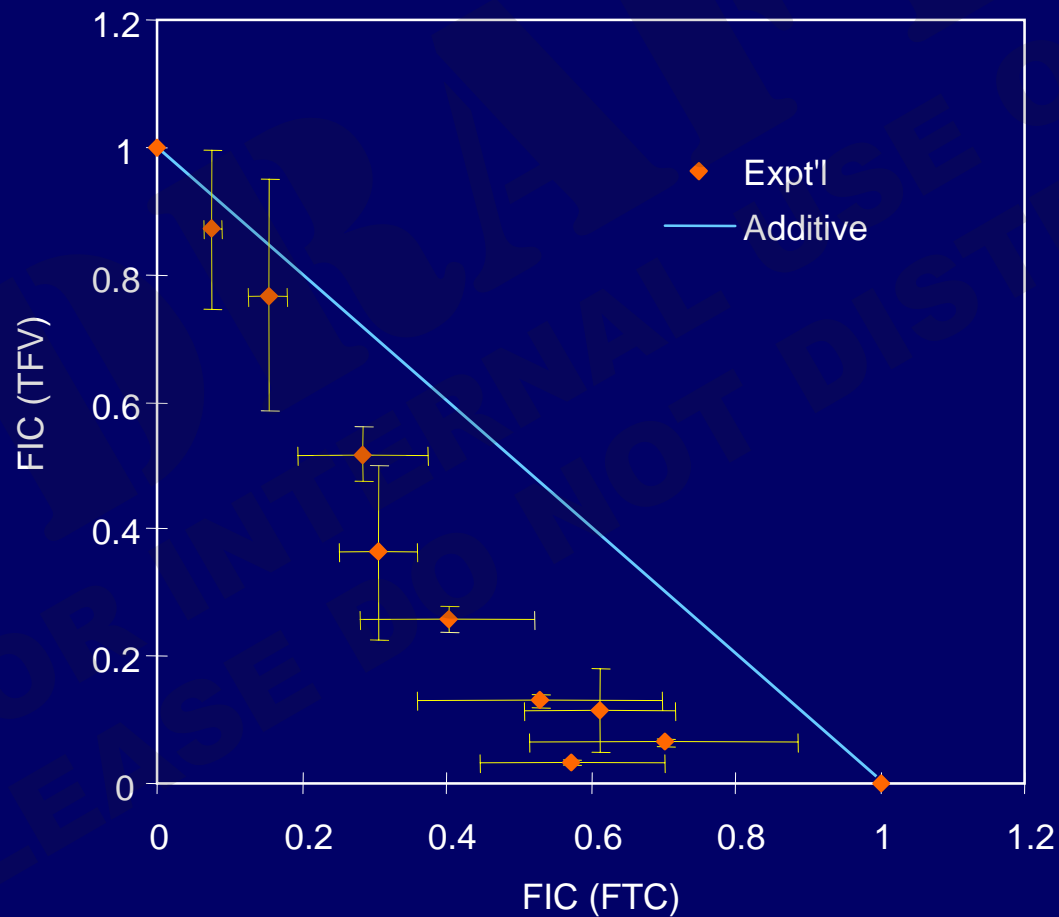


Synergy 95% Plot



# TFV + FTC Combination

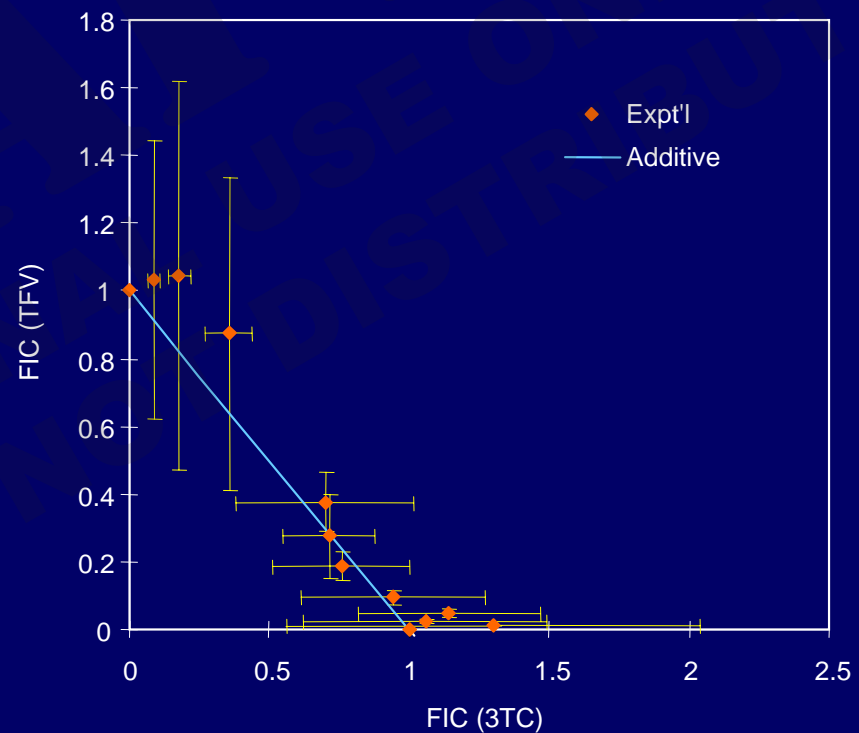
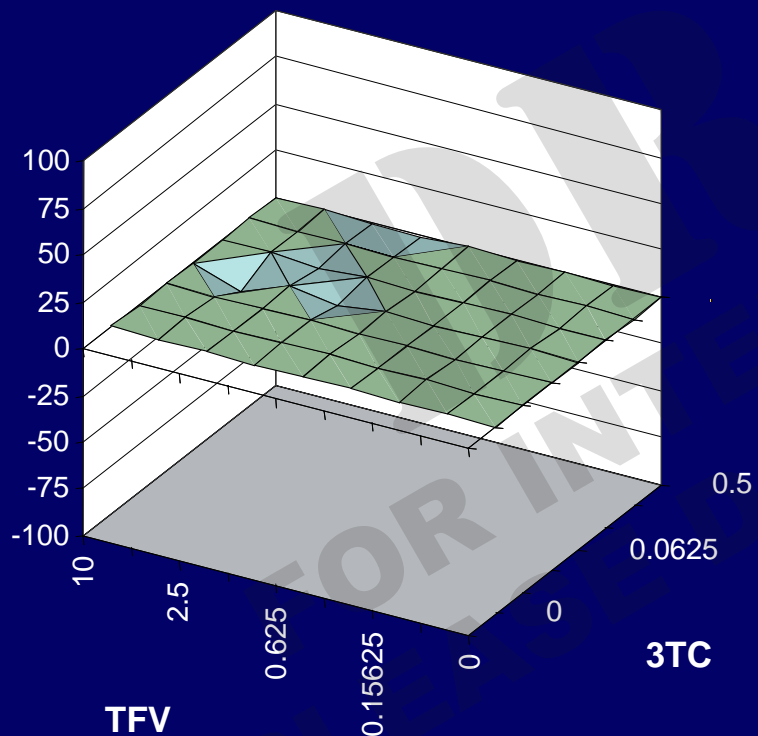
## Isobologram Analysis of Anti-HBV Effects



# TFV + 3TC Combination

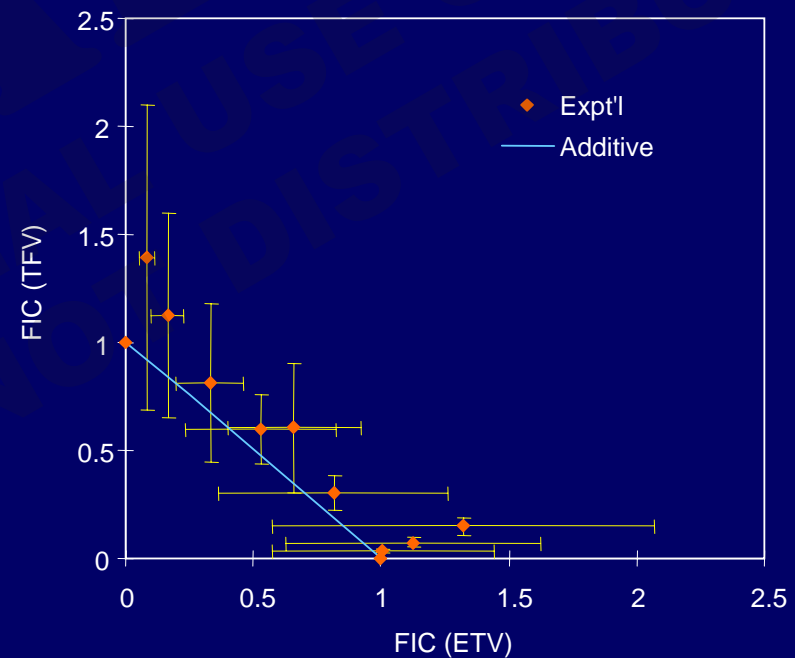
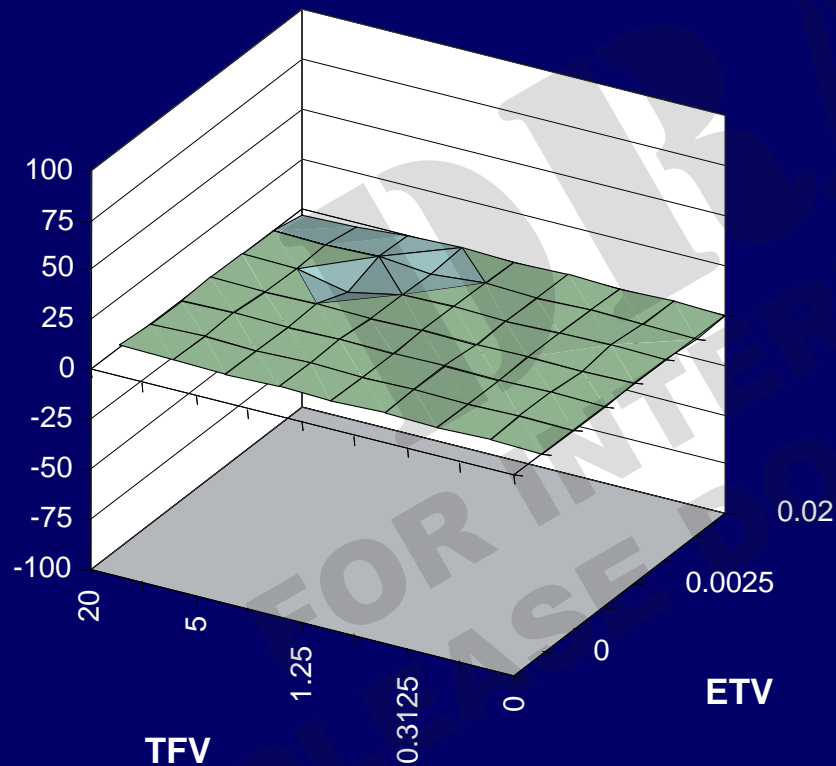
## MacSynergy and Isobologram Analysis

Synergy 95% Plot



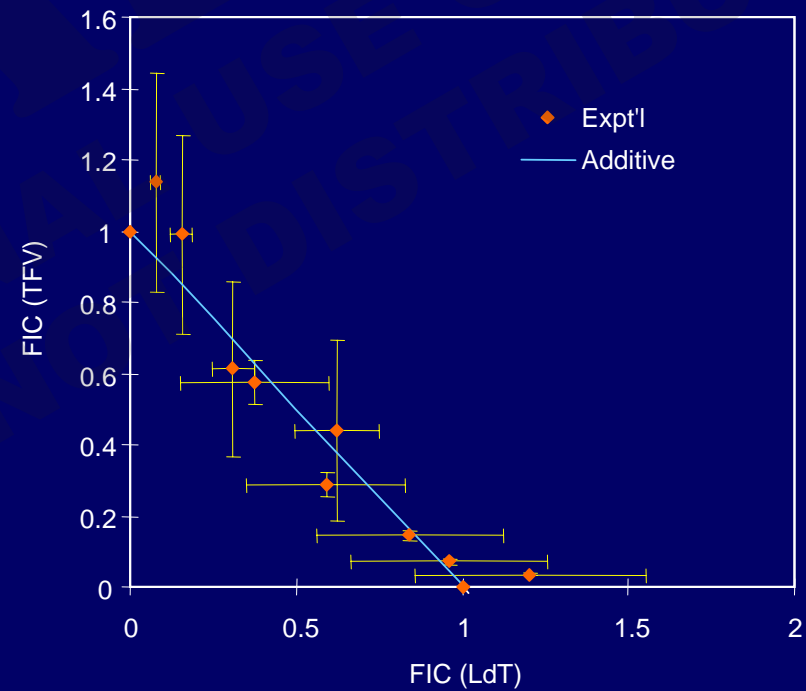
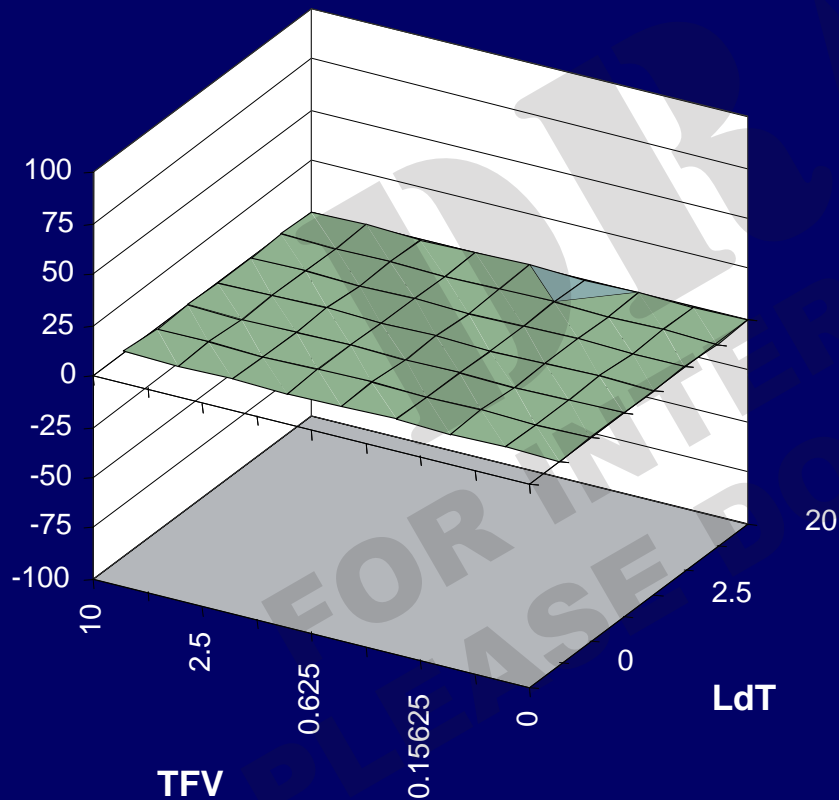
# TFV + ETV Combination MacSynergy and Isobologram Analysis

Synergy 95% Plot



# TFV + LdT Combination MacSynergy and Isobologram Analysis

Synergy 95% Plot



# Summary of MacSynergy Analysis Results

Drug Combination	Synergy <sup>a</sup> Vol $\pm$ std	Antagonism <sup>a</sup> Vol $\pm$ std	Net Effect
TFV + FTC	5.88 $\pm$ 6.67	-1.88 $\pm$ 3.26	Additive
TFV + 3TC	7.16 $\pm$ 12.41	-11.35 $\pm$ 4.64	Additive
TFV + ETV	0.4 $\pm$ 0.69	-9.53 $\pm$ 4.95	Additive
TFV + LdT	3.5 $\pm$ 4.95	-8.81 $\pm$ 11.22	Additive

a. Synergy and antagonism volumes at confidence interval (CI) 95% ( $\mu\text{M}^2\%$ )

# Summary of Isobologram Analysis Results

Drug Combination	D Value Range	Net Effect
TFV + FTC	-0.25 to -0.12	Synergistic
TFV + 3TC	-0.17 to 0.12	Additive
TFV + ETV	-0.036 to 0.24	Additive
TFV + LdT	0.066 to 0.047	Additive

# Conclusions

- **The combination of tenofovir and emtricitabine resulted in additive to synergistic activity against HBV**
- **Tenofovir in combination with lamivudine, entecavir or telbivudine produced additive antiviral effects**
- **These in vitro results support the use of tenofovir in combination therapy of chronic hepatitis B**