



Inhibition of HCV Replication by PSI-6130: Characterization of Activity in the HCV Replicon System

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β -D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) is a specific and potent inhibitor of HCV RNA replication

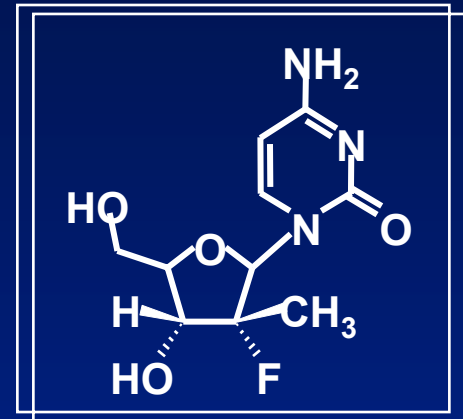
J. Clark et al. 2005. J. Med. Chem. 48(17):5504
W.-R. Jiang et al. 2005. 12th HCV Meeting #P293

- Potent, non-cytotoxic inhibitor of the HCV replicon

$$IC_{50} = 0.61 \pm 0.04 \mu\text{M}, CC_{50} > 300 \mu\text{M}$$

- Highly selective inhibitor of HCV replication
Only weak activity vs. other viruses
- PSI-6130-TP inhibits NS5B RdRp and acts as a chain-terminator

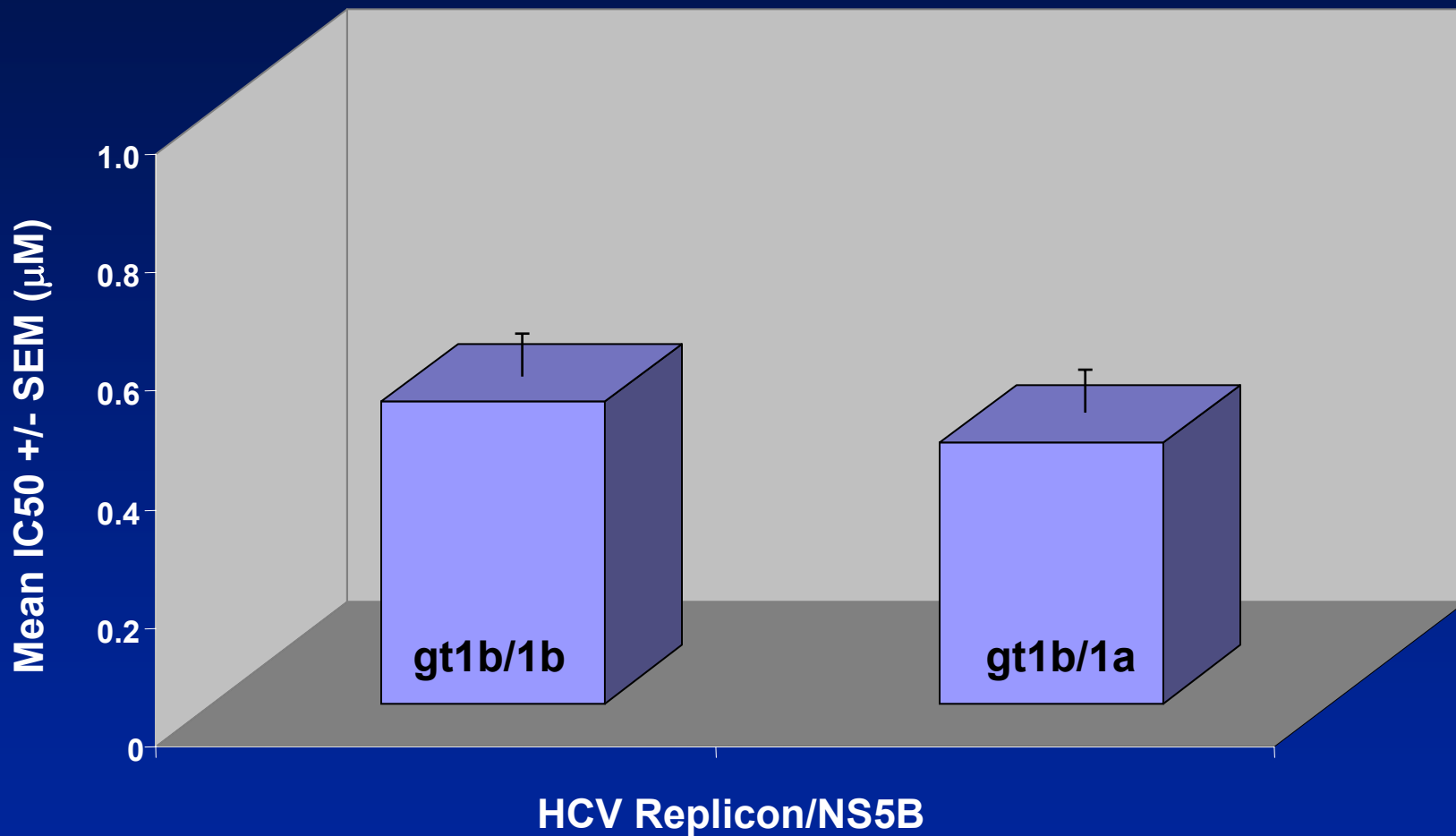
$$IC_{50} = 0.128 \pm 0.013 \mu\text{M} \text{ (NS5B570n -Con1 enzyme)}$$



Study Goals

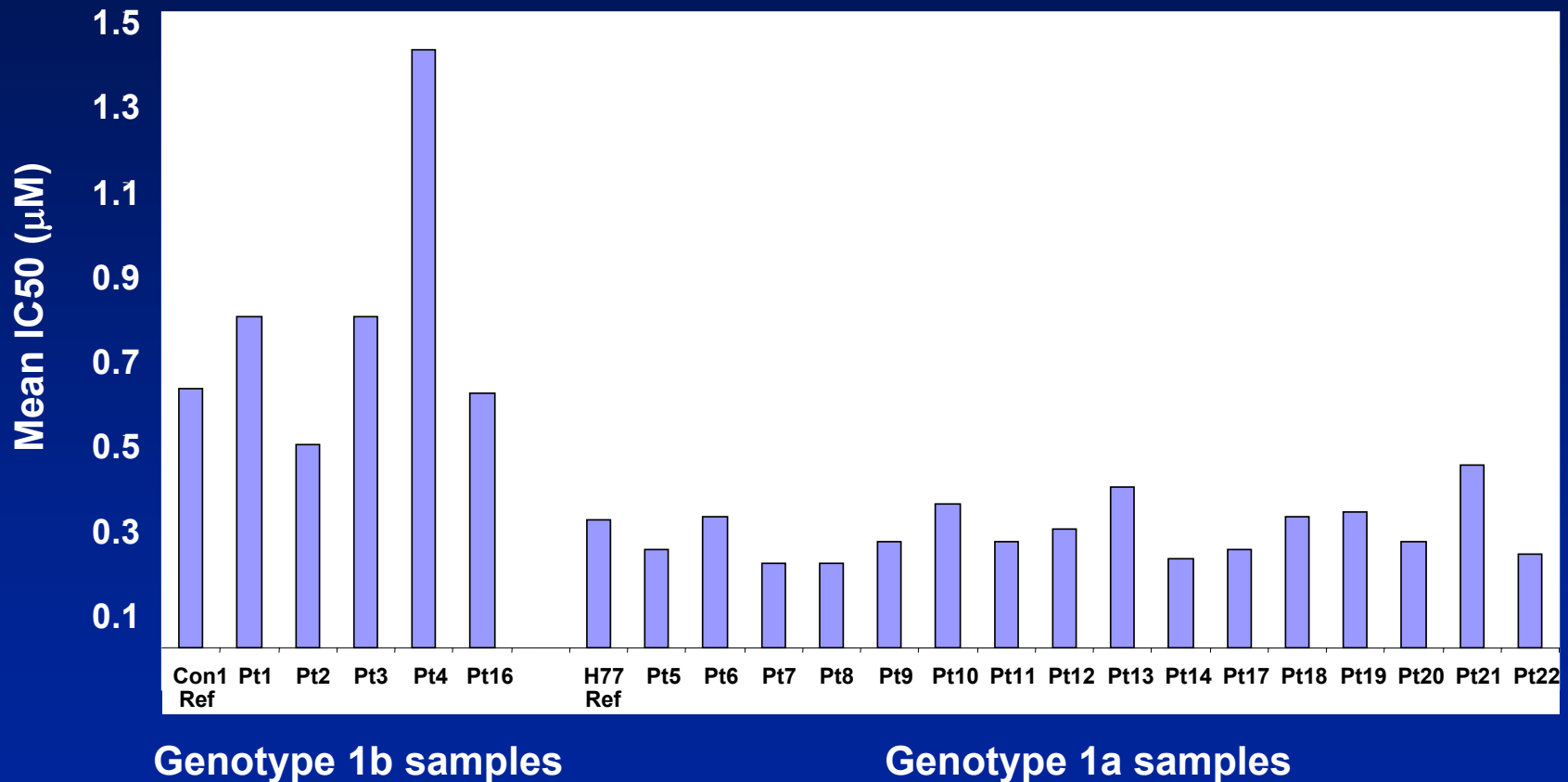
- Investigate the activity of PSI-6130 against HCV replicons containing NS5B genes derived from genotype 1a and 1b subtypes
- Assess the potential drug-drug interactions between PSI-6130 with other HCV inhibitors using the HCV replicon assay

Inhibitory activity of PSI-6130 against a gt-1b and gt-1b/1a chimeric transient replicon



PSI-6130 demonstrates activity against HCV replicons containing an NS5B gene from both gt 1a and gt 1b

Inhibitory activity of PSI-6130 against gt1b/1a transient replicons containing NS5B genes from clinical isolates

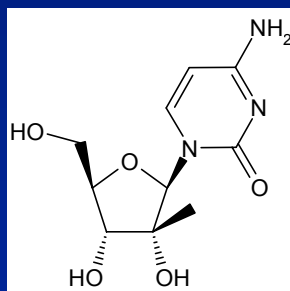


PSI-6130 demonstrates consistent activity against multiple genotype 1a and 1b clinical isolates

Assessment of potential drug-drug interactions between PSI-6130 with other HCV inhibitors

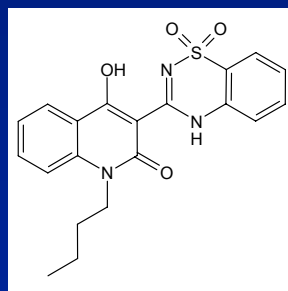
- Current therapies: IFN- α 2a, Ribavirin
- Investigational anti-HCV agents

HCV Pol inhibitor Nucleoside

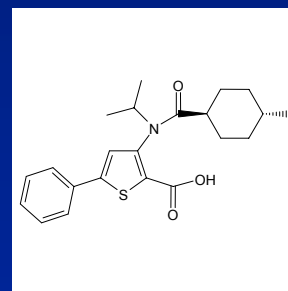


2'-C-Me-C

HCV Pol inhibitor Non-nucleoside

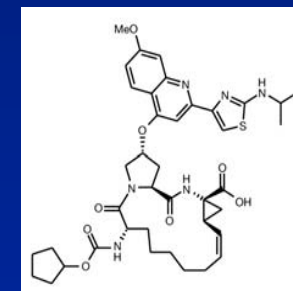


NNI-1
benzo-1,2,4-
thiadiazine
Palm-binding



NNI-2
thiophene-2
carboxylic acid
Thumb-binding

NS3 protease

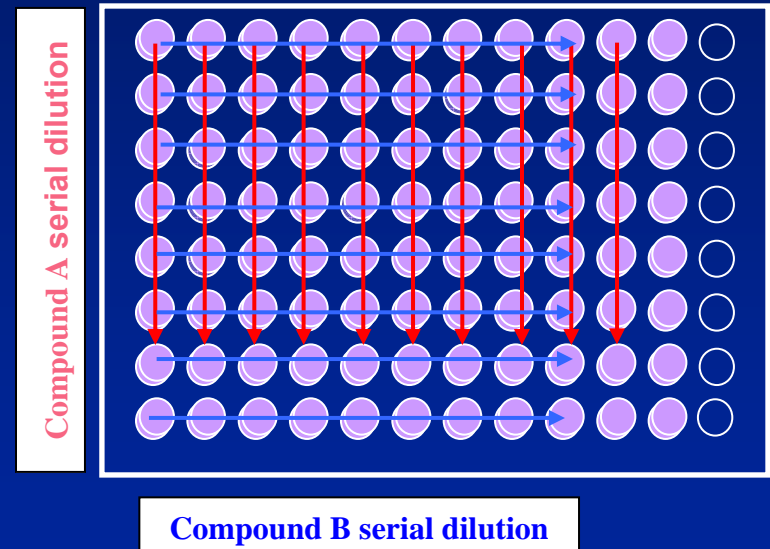


BILN 2061

Combination study using the replicon system



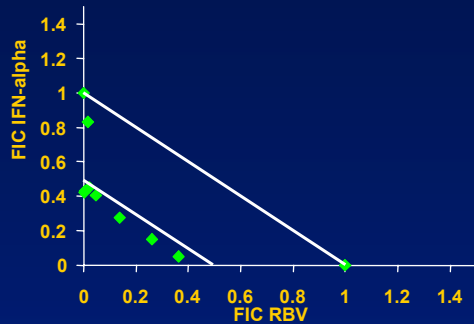
- Cells treated with inhibitors in a checkerboard, pair-wise manner
- 3-day assay for the replicon inhibition and cell viability
- Concentration ranges well below the cytotoxic concentrations
- Data analyzed using 3 independent methods



Drug interaction analyzed by 3 models

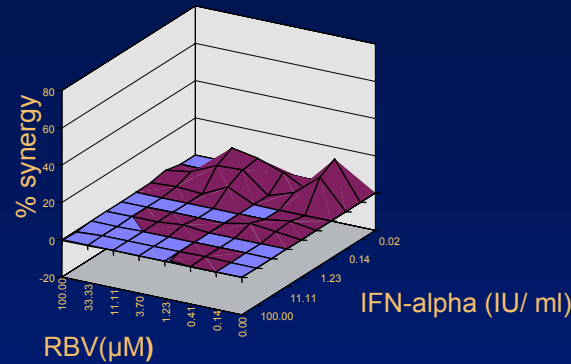


Isobologram & combination index



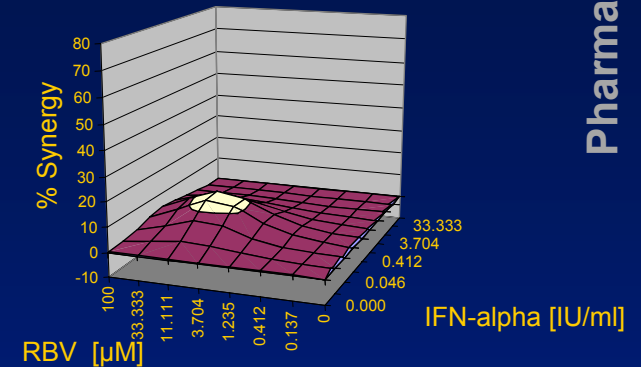
CI ~ 1 additivity
Moderate Synergism
 CI < 0.5 synergism
 CI > 1 antagonism

Prichard's MacSynergy II™



Vol: ≈ -100 ~ 100 , additivity
Moderate synergism
 Vol: ≥ 100 , synergism
 Synergy Vol: = 116
 Vol: ≤ -100 , antagonism
 % Synergy = 16
 % Synergy -20 ~ 20 , reduced significance

Greco's Model (Interact™)



α -1 ~ 1 , 95%CL inc.0, additivity
Synergism
 $\alpha > 1$, 95%CL not inc.0, synergism
 $\alpha < -1$, 95%CL not inc.0, antagonism
 $\alpha = 2.33 \pm 1.28$
 % Synergy > 10

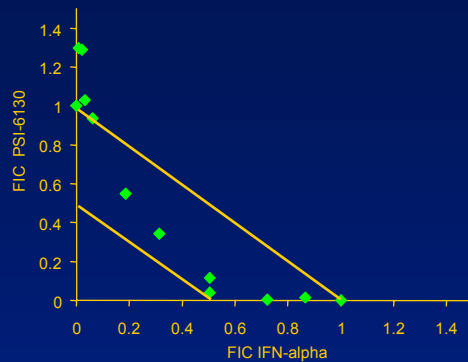
- Isobologram: combination index at 90% replicon inhibition
- MacSynergy II™: non-parametric response surface model
Combination of IFN- α and RBV is moderately synergistic
 based on Bliss-independence approach
- Interact™: parametric response surface approach based on Loewe additivity theory

Prichard MN 1990 Antiviral Res.14:181
 Greco WR 1990 Cancer Res. 50:5318

Combination of PSI-6130 with IFN- α 2a

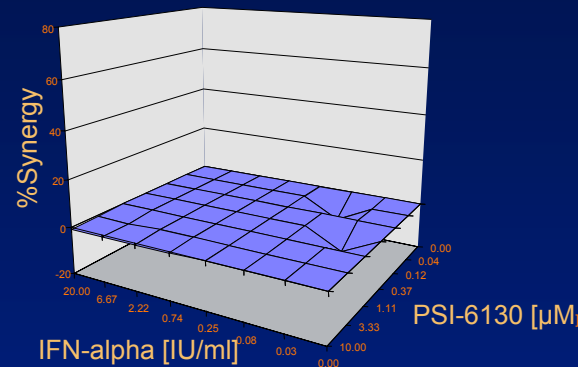


Isobologram & combination index



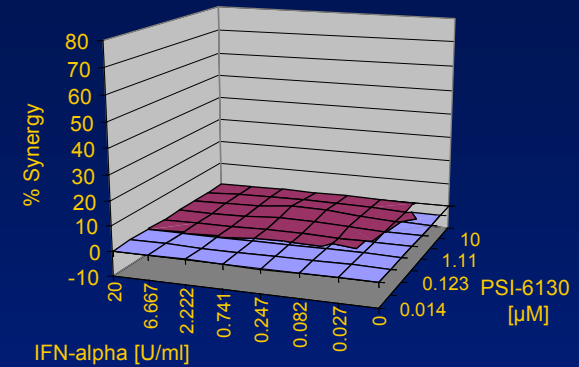
Additive
CI=0.5 -1

Prichard's Mac Synergy II™



Additive
Synergy Vol.=0
Antagonism Vol.= -21

Greco's Model (Interact™)



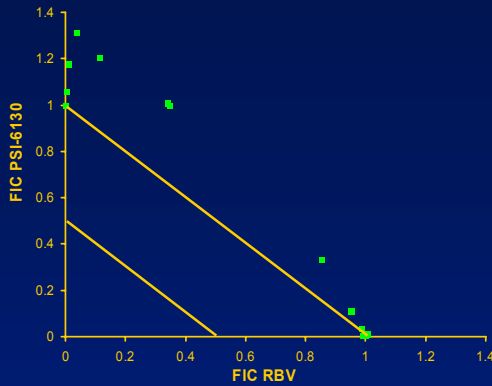
Additive
 $\alpha = 0.17 \pm 0.47$

•Combination of PSI-6130 and IFN- α 2a is additive in the replicon system using all three analysis models

Combination of PSI-6130 with Ribavirin

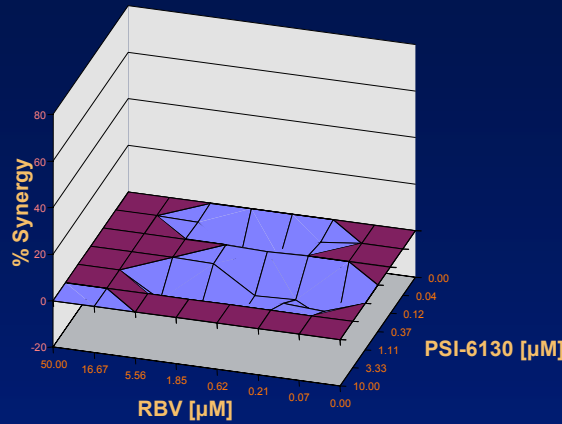


Isobologram & combination index



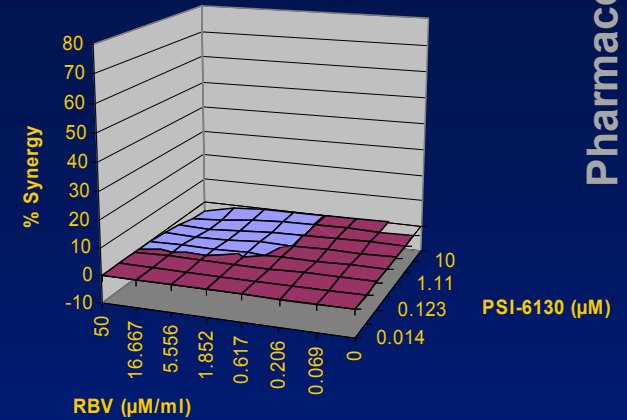
Additive-to-weak antagonism
CI = 1-1.4

Prichard's Mac Synergy II™

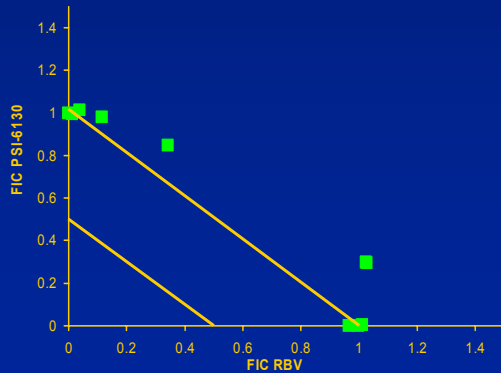


Moderate antagonism
Antagonism Vol. = -177
% Antagonism = -18

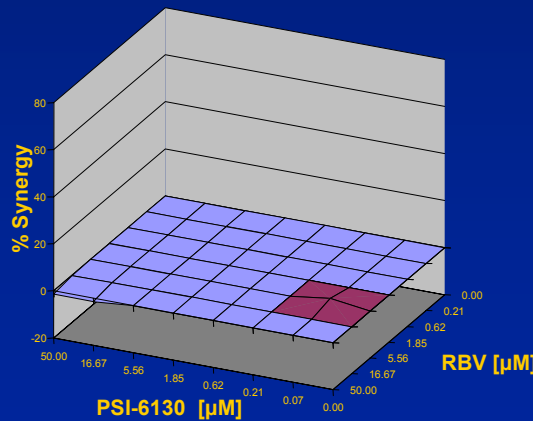
Greco's Model (Interact™)



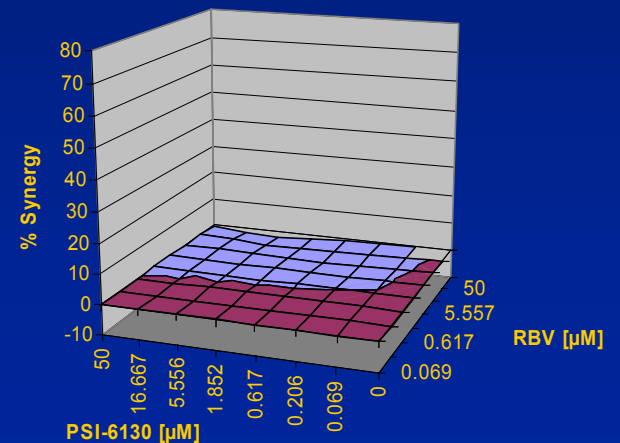
Additive
 $\alpha = -0.15 \pm 0.36$



Additive-to-weak antagonism
CI = 1-1.2



Additive
Synergy Vol. = 2
Antagonism Vol. = -3



Additive
 $\alpha = -0.09 \pm 0.14$

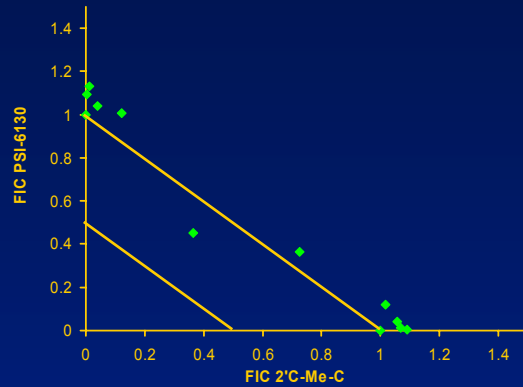
Combination of PSI-6130 with Ribavirin (Continued)

- Combination of PSI-6130 with Ribavirin produced different results depending on the concentration range analyzed and the analysis method used
- Overall analysis suggests that combination of PSI-6130 with Ribavirin has an additive-to-weak antagonistic effect on replicon replication

Combination of PSI-6130 with 2'C-Me-C

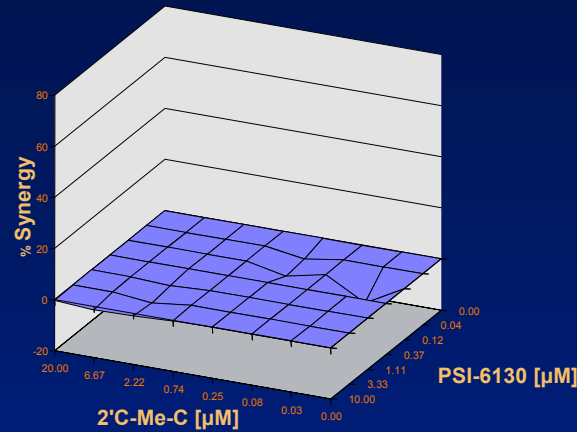


Isobologram & combination index



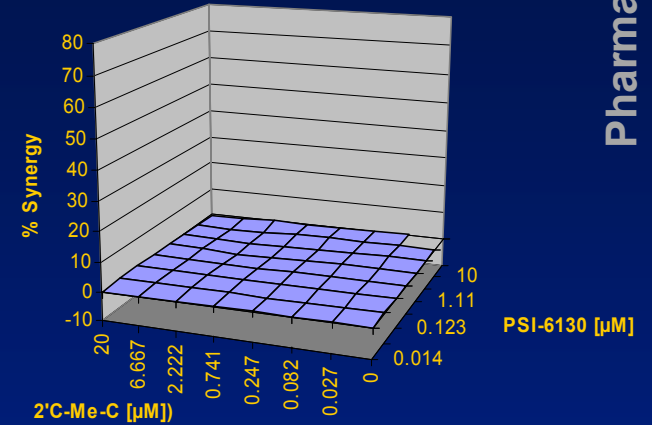
Additive
CI ~1

Prichard's Mac Synergy II™



Additive
Synergy.Vol.=0
Antagonism Vol.= -34

Greco's Model (Interact™)



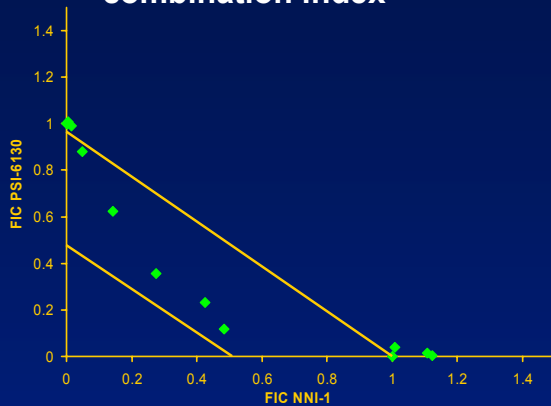
Additive
 $\alpha = -0.08 \pm 0.18$

•PSI-6130 is additive with 2'C-Me-C in the replicon system using all three analysis models

Combination of PSI-6130 with NNI-1

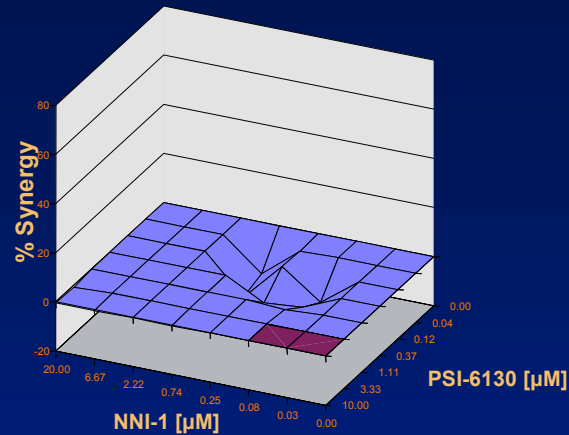


Isobologram & combination index



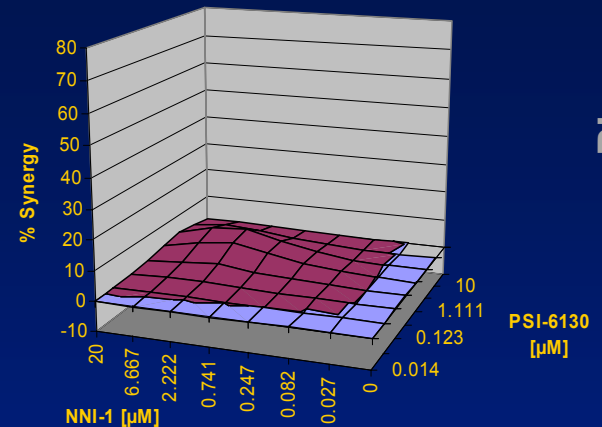
Additive
CI = 0.5-1

Prichard's Mac Synergy II™



Additive
Antagonist Vol. = -76
% antagonism = -15

Greco's Model (Interact™)



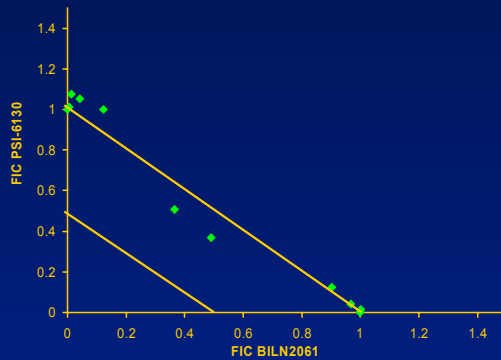
Additive
 $\alpha = 0.76 \pm 0.68$
% Synergy < 10

- PSI-6130 is additive with a palm-binding NNI in the replicon system using all three analysis models
- Additive effect also observed with a thumb-binding NNI

Combination of PSI-6130 with the HCV protease inhibitor BILN 2061

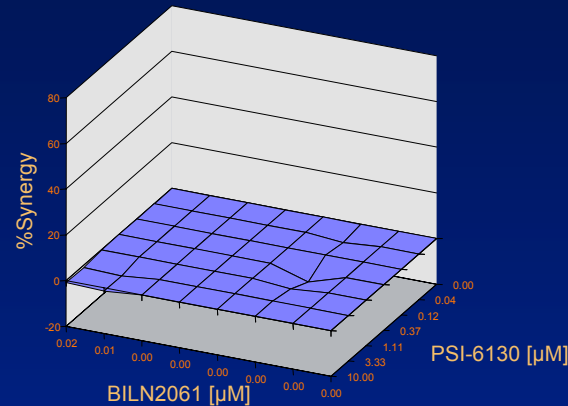


Isobologram & combination index



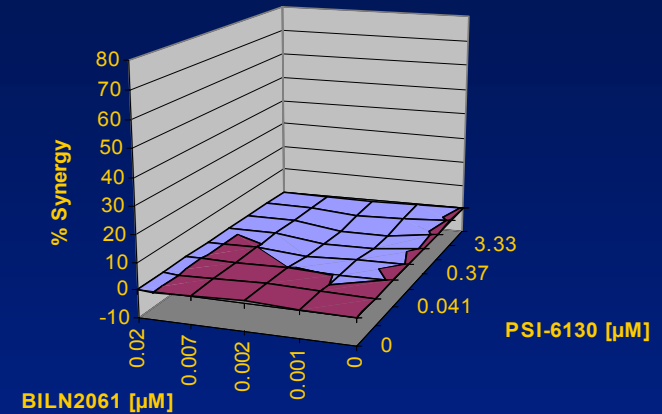
Additive
CI ~1

Prichard's Mac Synergy II™



Additive
Synergy.Vol.=29
Antagonism Vol.= -14

Greco's Model (Interact™)



Additive
 $\alpha = -0.17 \pm 0.02$
% Antagonism <10

•Combination of PSI-6130 with the protease inhibitor BILN 2061 is additive in the replicon system using all three analysis models

Summary



- PSI-6130 demonstrates potent activity against HCV replicons containing NS5B genes derived from multiple genotype 1a and 1b clinical isolates
- Combinations of PSI-6130 with other HCV inhibitors are predominantly additive with no strong synergistic or antagonistic effects observed
- PSI-6130 therefore represents a potentially useful compound for the combination treatment of patients with chronic hepatitis C

Acknowledgements

- Biology
 - Samir Ali
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