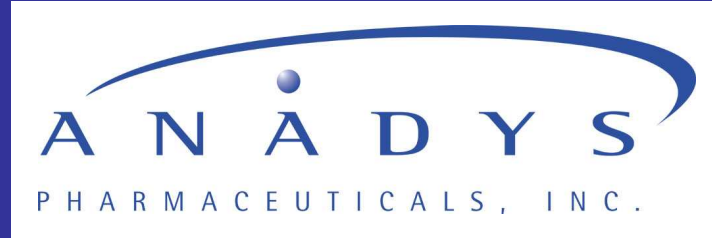


# IN VITRO COMBINATION STUDIES OF ANA598 WITH ANTI-HCV AGENTS DEMONSTRATE ENHANCED ANTI-VIRAL ACTIVITY

Rupal A. Patel, Peggy A. Thompson, Richard E. Showalter, James R. Appleman

Anadys Pharmaceuticals, Inc, San Diego, CA, USA



ICAR 2010

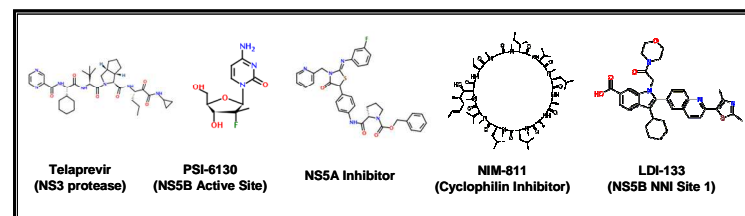
## BACKGROUND

- ANA598 is a potent non-nucleoside polymerase inhibitor in clinical development for the treatment of genotype 1 (GT-1) HCV infections
- Nanomolar antiviral potency was observed against multiple clinical isolates: EC<sub>50</sub> for GT-1b is 0.5 – 15 nM; (n=18) and EC<sub>50</sub> for GT-1a is 1.1 – 52 nM (n=19)
- ANA598 retains activity against replicons bearing mutations that confer resistance to other direct anti-HCV agents
- ANA598 demonstrated potent antiviral activity in a Phase 1b study in treatment naïve GT-1 patients (EASL 2009 LB1055)
- In an ongoing Phase II study of ANA598 with SOC in treatment naïve GT-1 patients, ANA598 200mg BID plus SOC resulted in 73% of patients achieving undetectable levels of virus at week 12 (cEVR). ANA598 400 mg BID plus SOC resulted in 72% of patients achieving undetectable levels of virus at 8 weeks (12 week data pending) (EASL 2010 LB2009)
- No patient receiving ANA598 has experienced viral breakthrough

## Methods

Combination studies were performed in Huh-7 cells bearing either the wild-type 1b Luc replicon or the 1b dicistronic replicon containing either the M414T or G554D mutation. Cells were cultured in the presence of compounds for 72 hours. The Luc replicon cells were lysed and luciferase activity was measured. The HCV NS3 and cellular GAPDH RNA levels for the mutant cell lines were determined by bDNA assay. The ratio of combination agents was either kept fixed or varied across the dosing range to explore a wide combination surface. Combination data were analyzed using Loewe Additivity (CalcuSyn software) and the Bliss Independence models (Anadys proprietary software).

## Structures



Reference for NSSA Inhibitor:  
International Patent Publication Number WO2004/014852 A2

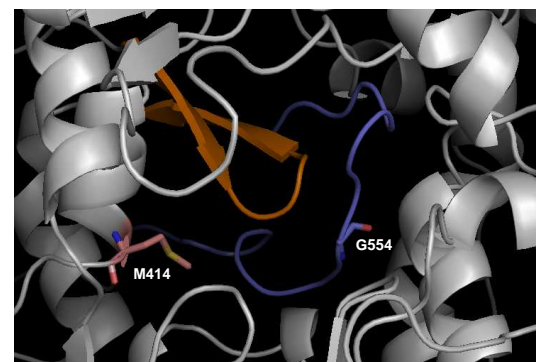
## RESULTS

### Combination of ANA598 with DAVs in WT and G554D Mutant Replicons

Agent/ANA598 Ratio (Fold EC <sub>50</sub> )	NS5B Mutation	Compound Class	Loewe Additivity (CalcuSyn) CI* @ EC <sub>50</sub> ± 95% Confidence Interval	Bliss Independence
<b>Telaprevir/ANA598</b>				
10x/10x	WT	NS3 PI	Synergistic 0.7 ±0.4	Additive/Synergistic
10x/10x	G554D		0.8 ±0.6	
<b>PSI-6130/ANA598</b>				
5x/5x	WT	NS5B NI	Synergistic 0.5 ±0.4	Additive/Synergistic
5x/10x	G554D		0.8 ±0.2	
10x/5x	G554D		0.7 ±0.1	
<b>NSSA Inhibitor/ANA598</b>				
5x/10x	WT	NS5A INH	Synergistic 0.7 ±0.4	Additive/Synergistic
5x/5x	WT		0.6 ±0.4	
<b>LDI-133/ANA598</b>				
10x/10x	WT	NS5B NNI (Site I)		Additive
<b>ANA598/ANA598</b>				
10x/10x	WT	NS5B NNI (Site III)	Additive 0.9 ±0.01	Additive
5x/5x	WT		1.1 ±0.01	

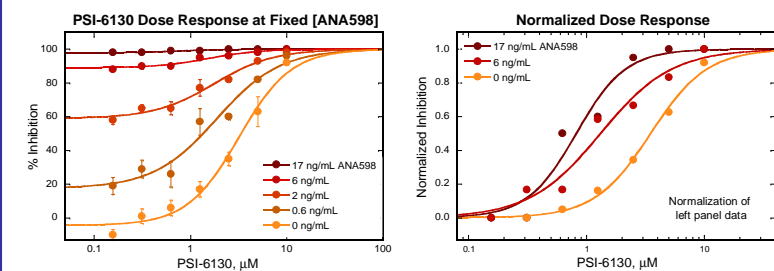
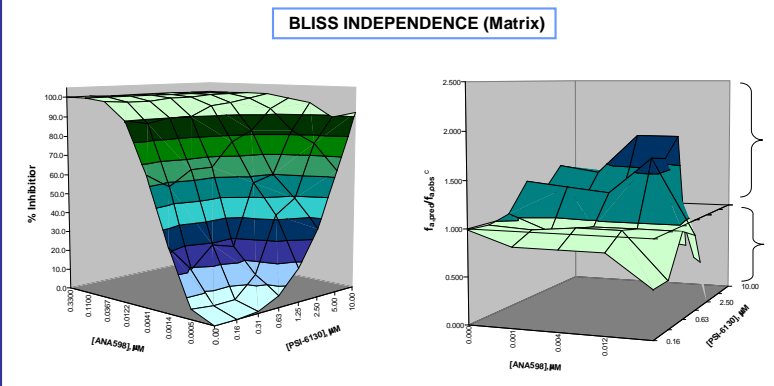
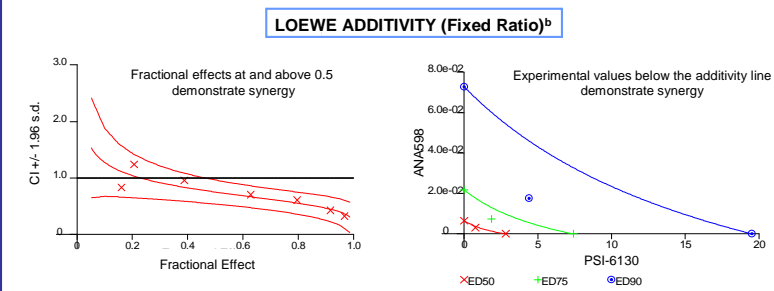
### NS5B Polymerase Palm Site

Two representative mutations conferring resistance to ANA598 (AASLD 2008 1908)



**Footnotes:**  
\* Combination Index (CI) values <0.90 = synergism, between 0.90 and 1.10 = additive, and >1.10 = antagonism  
† Conservative isobologram for mutually non-exclusive drugs that have independent modes of action  
‡  $CI = (FA_{obs}/FA_{add}) = (100 - \%InH_{obs}) / (100 - \%InH_{add})$   
§ When adjusted for protein binding

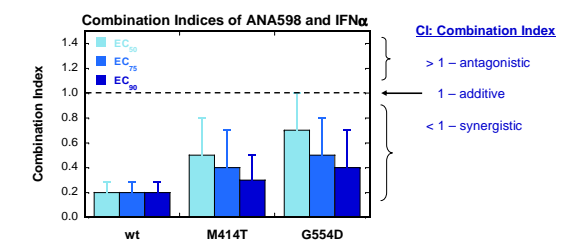
### Combination of ANA598 and PSI-6130 (Representative Data Analysis)



Physiologically relevant concentrations of ANA598 increase the potency of PSI-6130 by at least 4 fold

### Combination of ANA598 with Host Factors

Agent/ANA598 Ratio (Fold EC <sub>50</sub> )	NS5B Mutation	Loewe Additivity (CalcuSyn) CI* @ EC <sub>50</sub> ± 95% Confidence Interval	Bliss Independence
<b>IFN/ANA598</b>			
6x/25x	WT	Synergistic 0.7 ±0.5	Additive/Synergistic
10x/25x	WT	0.2 ±0.08	
25x/50x	M414T	0.7 ±0.4	
10x/20x	M414T	0.5 ±0.4	
50x/5x	G554D	0.7 ±0.3	
5x/5x	G554D	0.4 ±0.06	
<b>NIM-811/ANA598</b>			
10x/10x	WT		Additive



Combination of IFNα and ANA598 is synergistic against mutations conferring resistance to ANA598

## SUMMARY

- Synergistic/additive interactions were observed in *in vitro* combination studies of ANA598 with other antiviral agents against WT and mutant variants
- At concentrations achieved in Phase I and Phase II clinical trials, ANA598<sup>d</sup> increases the *in vitro* potency of other antiviral agents

## CONCLUSIONS

- Preclinical *in vitro* results support the exploration of clinical studies combining ANA598 with other classes of anti-HCV agents (NS3 protease, NS5A, NS5B nucleoside, NS5B non-nucleoside, and cyclophilin inhibitors)

**Acknowledgements**  
Data for ANA598 with LDI133 and NIM811 was kindly provided by Kai Lin of Novartis Pharmaceuticals Corporation.