

***IN VITRO* EVALUATION OF  
COMBINATION TREATMENT OF HCV:  
ACH-806 WITH IFN, VX-950 AND NM-107**

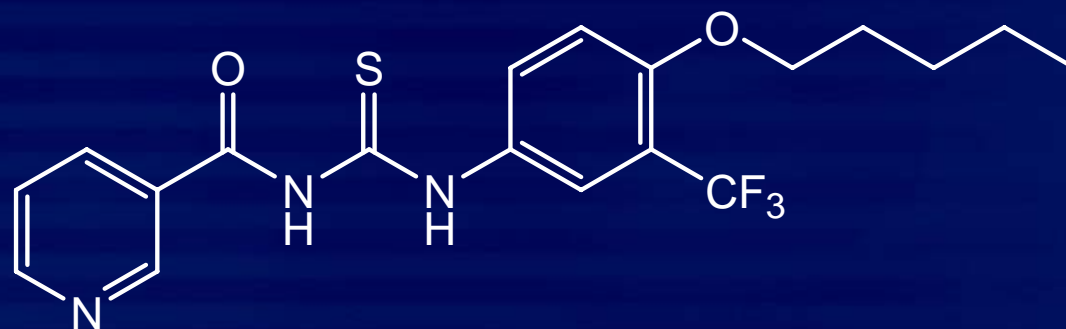
**Mingjun Huang, Wengang Yang, Joanne  
Fabrycki, Xingtie Nie, Avinash Phadke and  
Milind Deshpande**

**ACHILLION PHARMACEUTICALS, INC.**

## ACH-806 (GS-9132): INHIBITOR ACTIVITY

**$EC_{50} = 14 \text{ nM}$  against HCV replicon (subtype 1b/con-1);  
Therapeutic Index  $>1,000$**

**$EC_{50} > 40 \text{ }\mu\text{M}$  against RSV, BVDV, HIV-1 and HSV-1**



**ACH-806 is a potent and specific inhibitor against HCV.**

## ACH-806: MECHANISM OF ACTION (MOA)

**ACH-806 selectively binds to NS4A protein.**

**NS4A binds and is a cofactor for NS3 protease.**



Alters the viral protein composition as well as viral protein interaction in the replication complex, machinery for viral RNA synthesis.

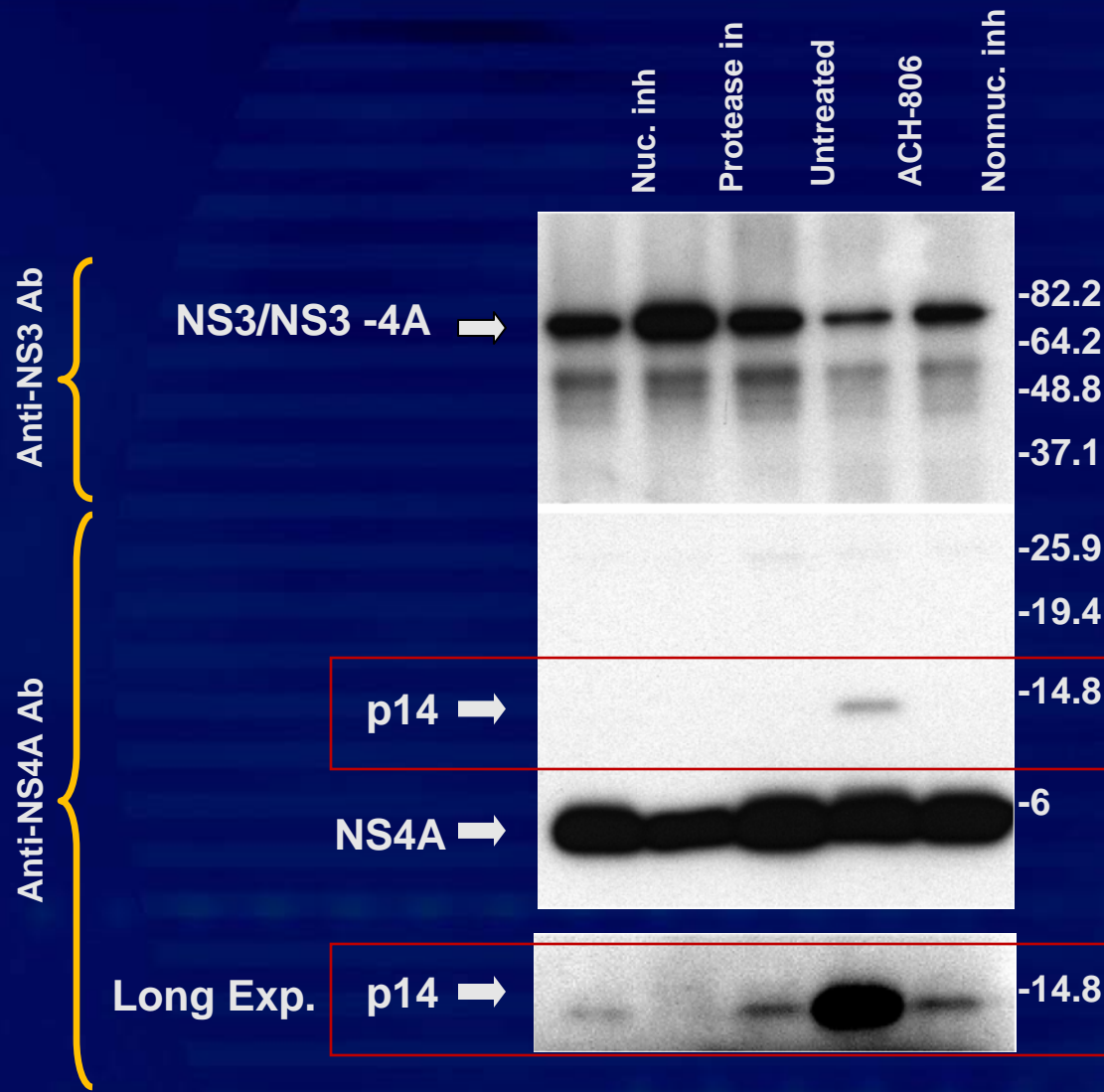


Inhibits replicon replication.

**ACH-806 is an NS4A antagonist.**

*For details, see Poster 2713.*

# SIGNATURE CHANGE IN REPLICATION COMPLEXES

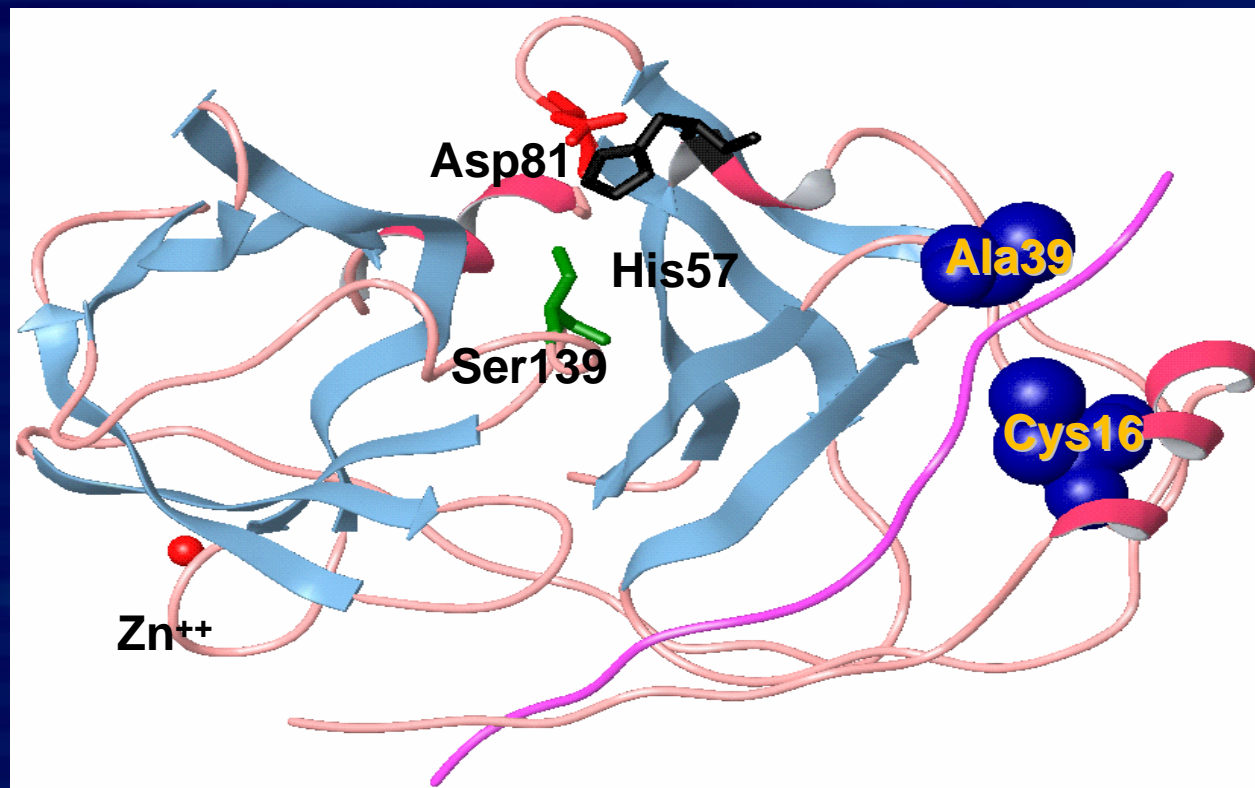


- p14 is detected in untreated cells, and also in cells treated with NS5B inhibitors.
- p14 is not detected in cells treated with NS3 protease inhibitor.
- p14 accumulates in ACH-806 treated cells.

## ACH-806 RESISTANCE PROFILE

**No cross resistance with other inhibitors**

**Resistance mutations mapped to the very N-terminus of NS3 where NS4A binds**



## PROOF OF CONCEPT STUDY

**A Phase 1b/2 proof of concept clinical trial demonstrated antiviral activity of ACH-806 in patients infected with HCV genotype 1a and 1b.**

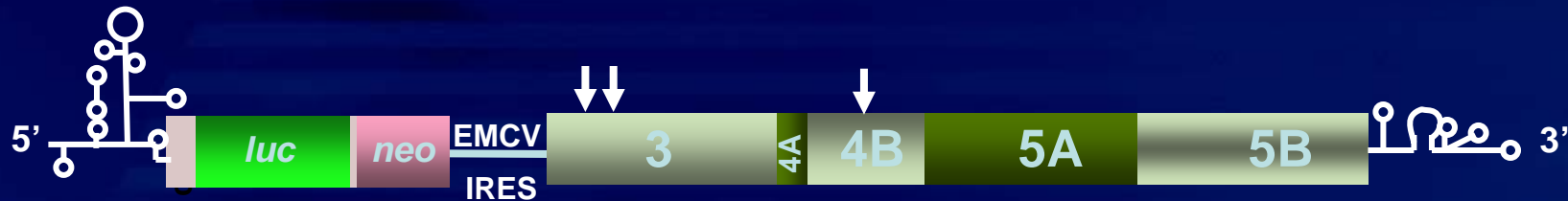
**Details will be presented on April 14<sup>th</sup>  
(LB\_OR\_3024).**

## EVALUATION OF DRUG-DRUG INTERACTION OF ACH-806 *IN VITRO*

### Assessment of ACH-806 in combination with:

- IFN- $\alpha$ -2b
- Other investigational agents:
  - NM-107 (active component of NM-283)  
NS5B polymerase nucleoside inhibitor  
 $EC_{50} = 0.8 \mu\text{M}$
  - VX-950  
NS3 serine protease inhibitor  
 $EC_{50} = 0.3 \mu\text{M}$

## SHORT TERM COMBINATION STUDIES



Replicon-containing cells were treated with varying concentrations of ACH-806 in combination with IFN; VX-950; or NM 107 in a checker-board manner.

After 3 days, inhibitory activity was evaluated with luciferase assay and toxicity evaluated with MTS assay.

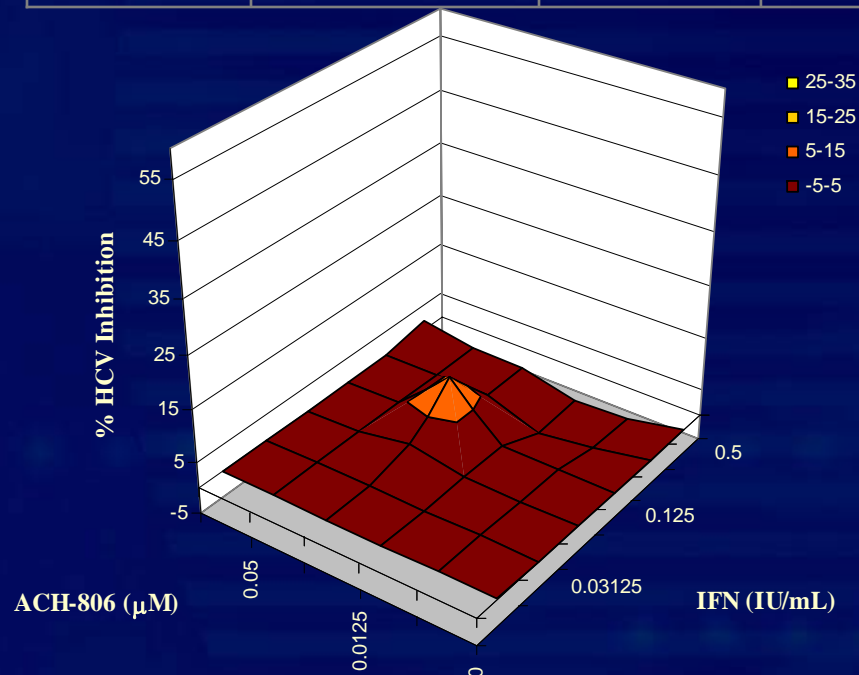
Concentration ranges tested bracketed the  $EC_{50}$ , and were well below  $CC_{50}$  values.

Data analyzed by two independent methods:

- Chou and Talalay method: Combination Index (CI)
  - CI value  $<0.9$ : synergistic;  $>1.1$ : antagonistic; in between: additive
- Prichard and Shipman: Volume of Synergy or Antagonism
  - Absolute volume value  $<50$ : additive;  $>100$ : synergistic or antagonistic; in between: moderately synergistic or moderately antagonistic

# COMBINATION OF ACH-806 WITH IFN

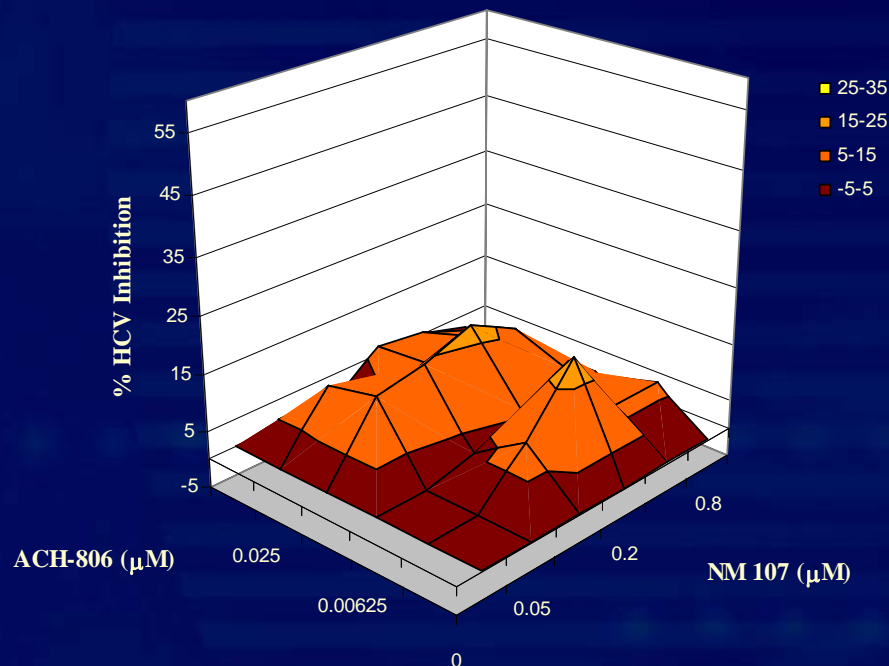
VOLUME $\mu\text{M}^2$ %		MOLAR RATIO	CI AT REPLICON INHIBITION OF:			OVERALL
Synergy	Antagonism		50%	75%	90%	
4	5	1:10	1.12	0.93	0.81	ADDITIVE
		1:20	1.04	0.85	0.73	



Combination of ACH-806 and IFN- $\alpha$  for 3 days led to an additive effect in activity.

# COMBINATION OF ACH-806 WITH NM-107

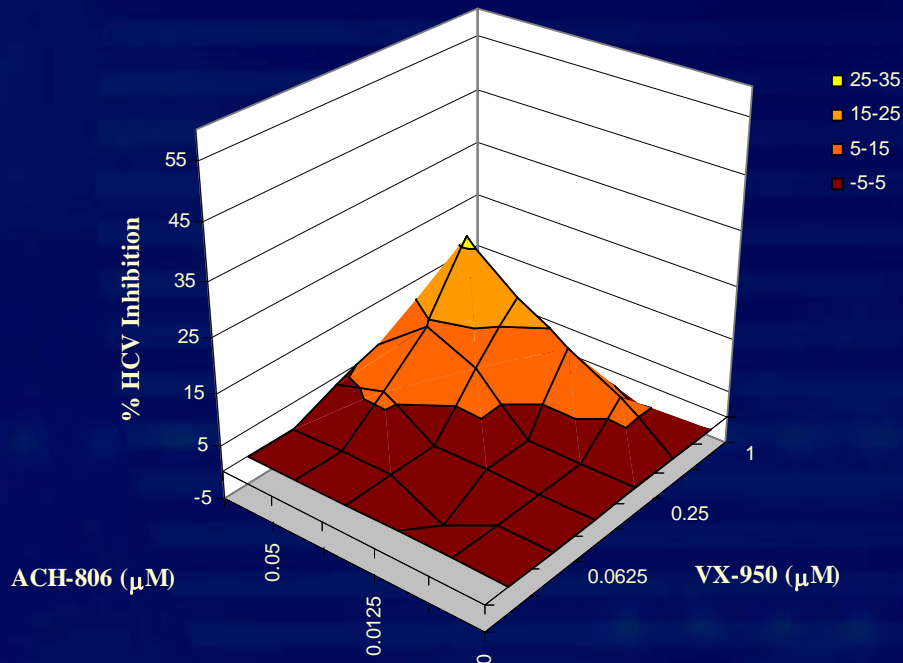
VOLUME $\mu\text{M}^2$ %		MOLAR RATIO	CI AT REPLICON INHIBITION OF:			OVERALL
SYNERGY	ANTAGONISM		50%	75%	90%	
116	2	1:10	0.69	0.72	0.79	SYNERGISTIC
		1:20	0.67	0.71	0.83	



Combination of ACH-806 and NM-107 for 3 days led to a synergistic effect in activity.

# COMBINATION OF ACH-806 WITH VX-950

VOLUME $\mu\text{M}^2$ %		MOLAR RATIO	CI AT REPLICON INHIBITION OF:			OVERALL
Synergy	Antagonism		50%	75%	90%	
119	8	1:10	0.86	0.82	0.83	SYNERGISTIC
		1:20	0.79	0.61	0.50	



Combination of ACH-806 and VX-950 for 3 days led to a synergistic effect in activity.

## LONG TERM COMBINATION STUDIES

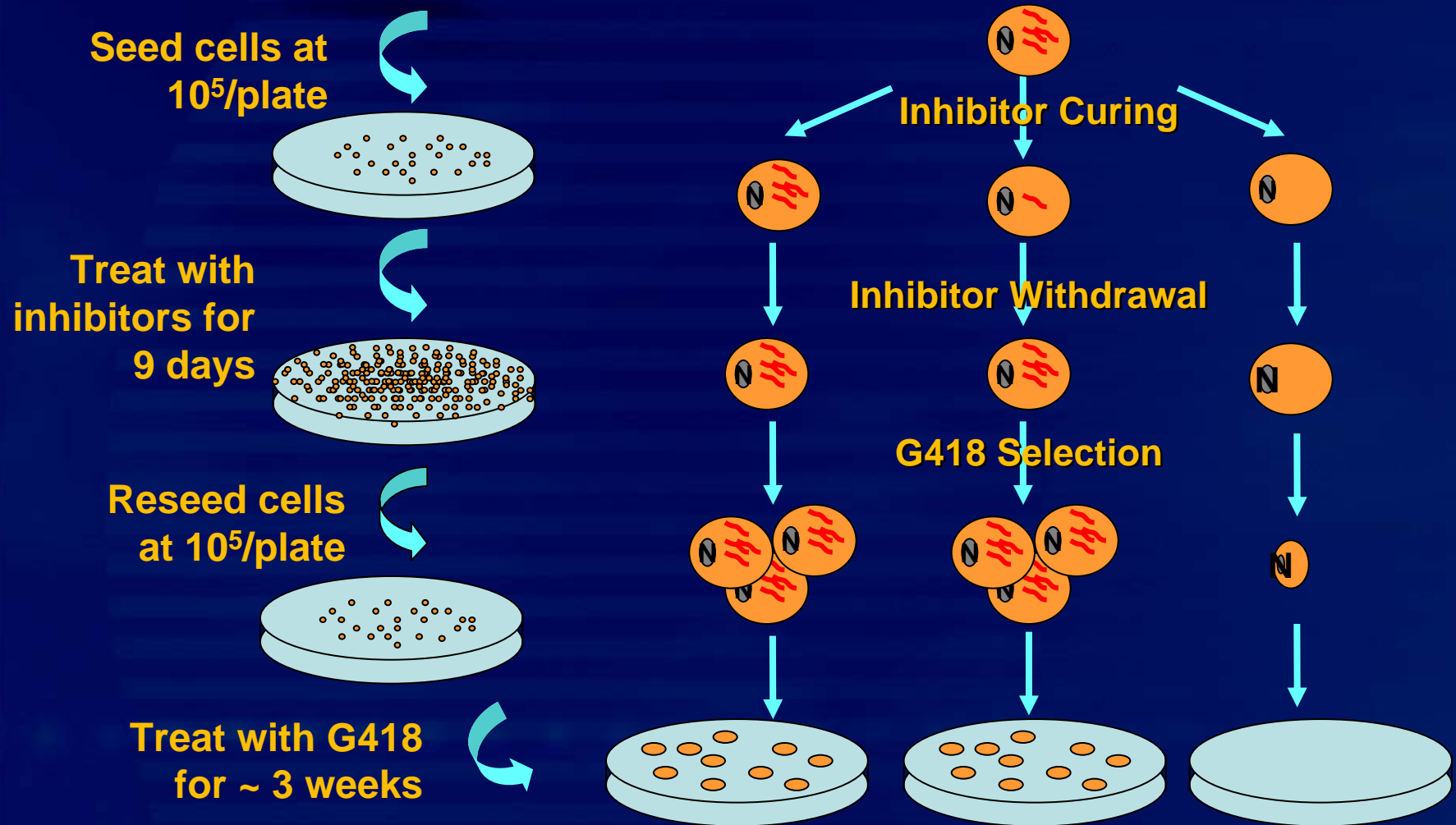


Replicon-containing cells were seeded at  $10^5$  cells/10-cm plate and were treated with ACH-806, IFN, VX-950, or NM-107 alone or in combination at concentrations of 3 – 5 X  $EC_{50}$  for 9 days.

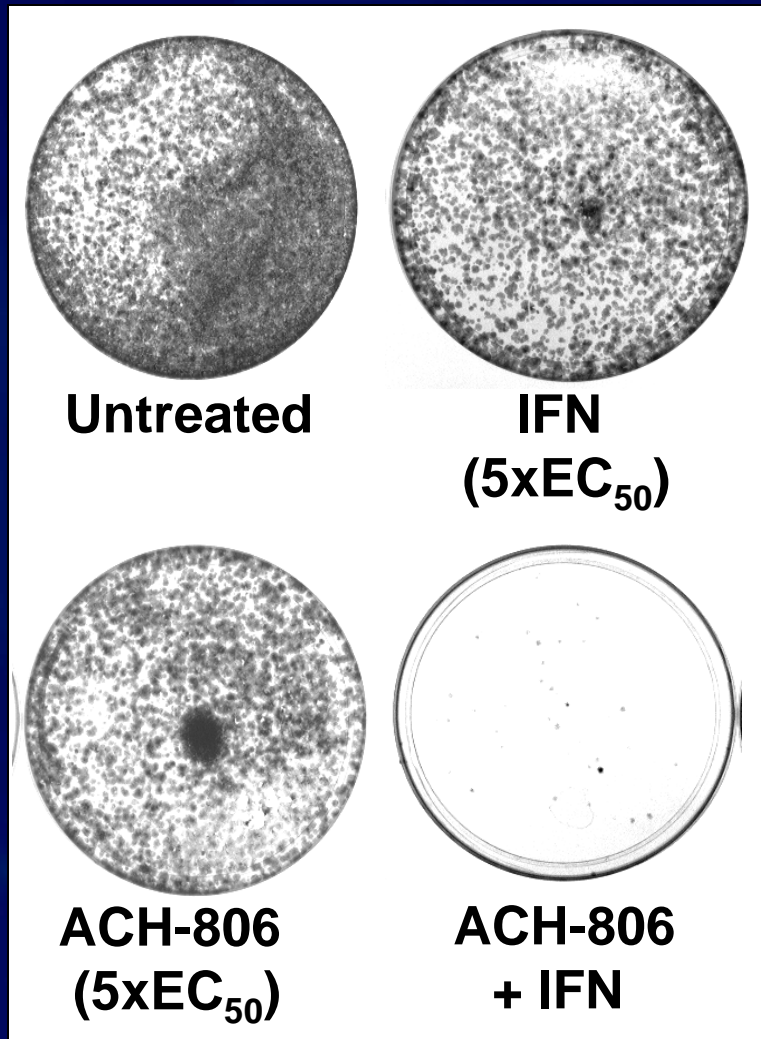
Post inhibitor treatment, the cells were reseeded at  $10^5$  cells/10-cm plate and were cultured in the presence of G418.

About 3 weeks later when the colonies formed, the plates were fixed and stained.

# MEASURING CURING EFFECTS OF HCV INHIBITORS



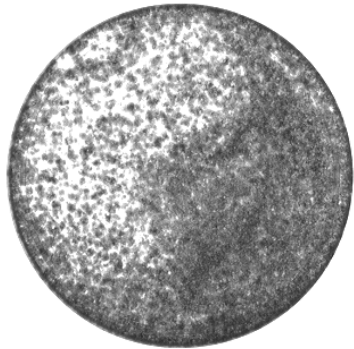
## COMBINATION OF ACH-806 WITH IFN



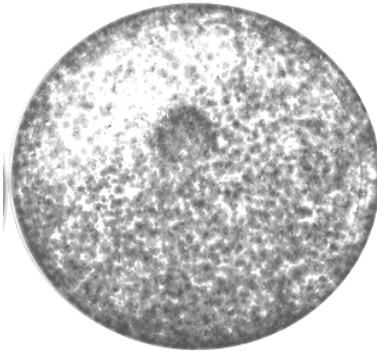
Use of IFN or ACH-806 as single agents for 9 days, at concentrations = 5 X EC<sub>50</sub>, resulted in modest curing of replicon-containing cells.

However, combination of ACH-806 with IFN cured almost all replicon-containing cells.

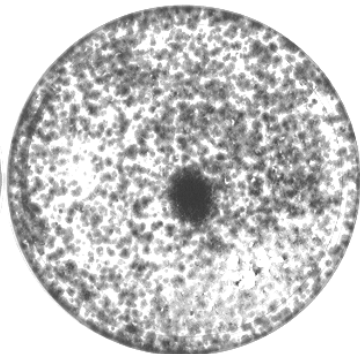
## COMBINATION OF ACH-806 WITH NM-107



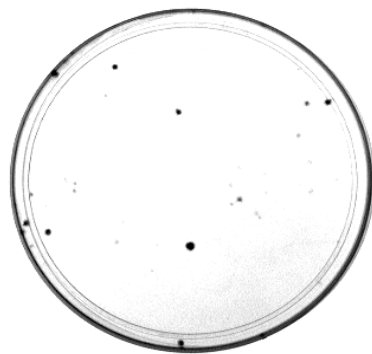
**Untreated**



**NM-107  
(5xEC<sub>50</sub>)**



**ACH-806  
(5xEC<sub>50</sub>)**

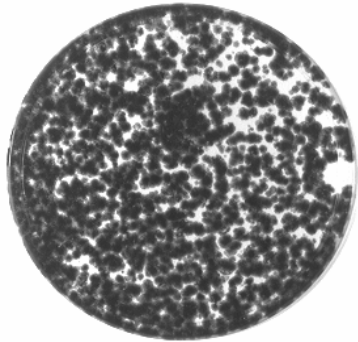


**ACH-806  
+ NM-107**

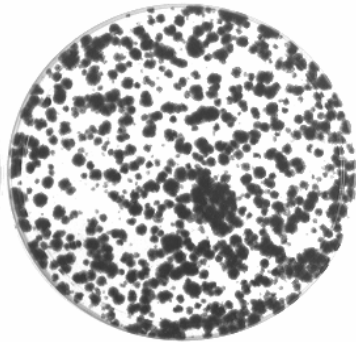
**Use of NM-107 or ACH-806 as single agents for 9 days resulted in modest curing of replicon-containing cells.**

**However, combination of ACH-806 with NM-107 cured almost all replicon-containing cells.**

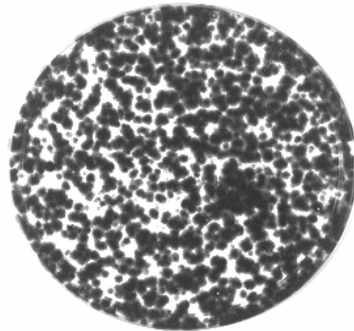
## COMBINATION OF ACH-806 WITH VX-950



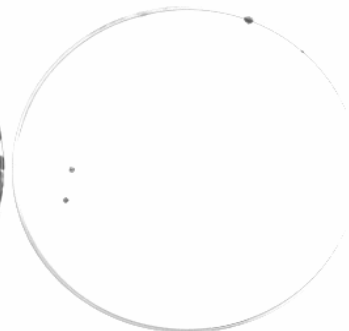
**Untreated**



**ACH-806  
(5xEC<sub>50</sub>)**



**VX-950  
(3xEC<sub>50</sub>)**



**ACH-806  
+VX-950**

**Use of VX-950 or ACH-806 as single agents for 9 days resulted in modest curing of replicon-containing cells.**

**However, combination of ACH-806 with IFN cured almost all replicon-containing cells.**

## SUMMARY

**The anti-HCV activity of ACH-806 in combination with IFN- $\alpha$ , NM-107 and VX-950 was assessed *in vitro* using HCV replicon-containing cells.**

**Combination treatment of ACH-806 with any of the inhibitors for short duration resulted in additive to synergistic effects. No antagonism was observed.**

**None of the combinations were synergistic for cytotoxicity.**

**Combination treatment of ACH-806 with any of the inhibitors for longer duration cured almost all replicon-containing cells although many remained uncured when each inhibitor was used alone.**

**The results support a combination therapy of NS4A antagonists with other inhibitors for treatment of hepatitis C patients.**