ANTIVIRAL ACTIVITY, COMBINATION AND RESISTANCE OF ACH-1625, A POTENT HCV NS3 PROTEASE INHIBITOR

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Abstract 492

Background

HCV NS3 protease is a clinically validated target. Analysis of structural information of the target and its ligands led to design and synthesis of linear, competitive reversible inhibitors including the clinical development candidate ACH-1625. ACH-1625 displays good human metabolic stability and high human hepatoselectivity *in vitro*. ACH-1625 demonstrates favorable pharmacokinetic and good safety profiles in animals (Abstract 172). ACH-1625 is very well tolerated after administration of single and multiple doses up to 2000 mg in healthy volunteers and displays a mean maximal viral load of 4.25 log10 after administration of 500 mg BID in HCV genotype-1 infected patients (LB 2012).

Methods & Results

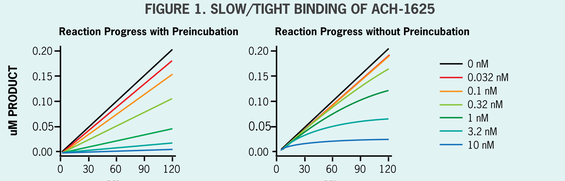
1 ACH-1625 IS A POTENT INHIBITOR OF HCV NS3 PROTEASES

TABLE 1. INHIBITORY ACTIVITY AGAINST GT-1 NS3 PROTEASES

NS3 Protease	Kinetic Parameters			ACH-1625		Telaprevir	
Isoform	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m (min^{-1} $μM^{-1}$)	K_i (nM)	IC_{50} (nM)	K_i (nM)	IC_{50} (nM)
1a	53 ± 6	<0.5 ± 0.0	>110	<0.3	0.91± 0.06	<12	36 ± 3
1b	35 ± 7	1.1 ± 0.6	31	1.1	2.1 ± 0.5	33	62 ± 2

Following 15 minute preincubation of NS3 protease/NS4A cofactor complex with inhibitor, Fam-QXL substrate was added. The increase in fluorescence was monitored in real time to determine the protease activity.

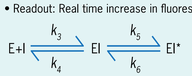
FIGURE 1. SLOW/TIGHT BINDING OF ACH-1625



Kinetic Parameters	
K_i^*	0.061 nM
K_i	>47 nM
k_5	>1.6 min^{-1}
k_6	<0.0020 min^{-1}
$T_{1/2}$	>5.9 hr

Assay

- Preformed NS3 protease (genotype 1b) and NS4A cofactor complex
- Fam-QXL substrate
- With preincubation (steady state): reaction starts by adding substrate post preincubation of enzyme with inhibitor
- Without preincubation: reaction starts by adding enzyme to substrate and inhibitor
- Readout: Real time increase in fluorescence



Initial affinity: $K_i = k_4 / k_3$
Steady state "true" affinity: $K_i^* = K_i (k_6 / (k_5 + k_6))$

- *ACH-1625 displays potent activity against both genotype-1a and -1b NS3 proteases.*
- *ACH-1625 potent activity is retained across genotypes 2a; 4a; 5a and 6a:*
  - *Reduced susceptibility against genotype 3a is likely due to the natural variation at the amino acid 168 of NS3 protease (D vs Q).*
- *ACH-1625 displays slow binding to NS3 protease as indicated by biphasic progress curves:*
  - *Inhibition likely occurs by a two-step model in which an initial inhibitor-enzyme collision complex (EI) isomerizes slowly to a highly stable complex (EI*).*
  - *The accompanying slow dissociation of ACH-1625 from EI* (estimated $T_{1/2}$ > 5.9 hr) results in persistent inhibition of NS3 protease.*

2 ACH-1625 DISPLAYS POTENT ACTIVITY AGAINST HCV REPLICONS

TABLE 2. POTENCY AND HUMAN SERUM PROTEIN EFFECT ON POTENCY

Compound	Potency in Replicon–Containing Cells (nM)				Fold Increase in Presence of 40% HS	
	DMEM + 10% FBS		DMEM + 40% HS			
	EC_{50}	EC_{90}	EC_{50}	EC_{90}	EC_{50}	EC_{90}
ACH-1625	11±4.8	31±9.0	37±13	88±13	3.2	3.8
Telaprevir	408±132	1843±527	3600±2048	8673±2008	8.8	4.7

Huh-luc/neo cell line (harboring a luciferase-encoding GT-1b/Con-1 replicon) was treated with inhibitor for 3 days and anti-replicon activity was determined with luciferase endpoint.

- *ACH-1625 shows potent activity against genotype 1b/lab strain (Con-1) replicon.*
- *Human serum has only a moderate effect on ACH-1625's activity.*
- *ACH-1625 displays similar activity against genotype 1a/lab strain (H77) replicon.*

3 ACH-1625 RETAINS ACTIVITY AGAINST HCV NS3 PROTEASE NATURAL VARIANTS

TABLE 3. IMPACT OF NS3 PROTEASE POLYMORPHISM ON POTENCY

Compound	EC_{50} against GT-1a (nM)						EC_{50} against GT-1b (nM)						EC_{50} (nM)		
	H77	P1	P2	P3	P4	P5	Con-1	P6	P7	P8	P9	P10	Range	Mean	Median
ACH-1625	15	4	3	28	8	30	5	3	20	8	30	2	2-30	13	7
Telaprevir	213	133	63	190	246	63	301	355	7225	82	300	400	63-7225	851	230

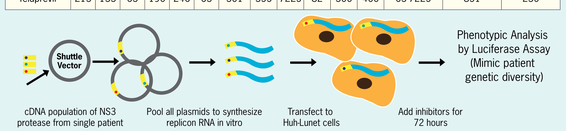
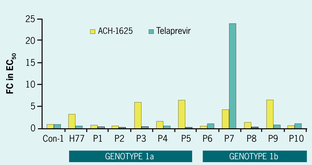


FIGURE 2. COMPARISON OF POTENCY BETWEEN REFERENCE REPLICON (CON-1) AND CHIMERIC REPLICONS



- *Relative to Con-1 replicon, the highest EC_{50} value is no more than 7 fold higher against chimeric replicons carrying NS3 protease from genotype –1a and 1b clinical isolates.*
- *These results demonstrate broad coverage of natural variants with ACH-1625.*

4 ACH-1625 IS SPECIFIC FOR INHIBITION OF HCV

TABLE 4. ACTIVITY OF ACH-1625 AGAINST OTHER MAMMALIAN VIRUSES

Virus	Description	Host Cells	Endpoint	EC_{50} (μM)	CC_{50} (μM)
HSV-1	Herpes virus (dsDNA)	Vero	CPE	>100	>100
RSV	Paramyxovirus (-ve ssRNA)	Hep-2	CPE	>100	>100
HIV	Retrovirus (RNA reverse transcribing)	CEM-SS	CPE	>18	18
HBV	Hepadnavirus (DNA reverse ranscribing)	HEPAD38/Huh–7	Viral DNA	>10	>10
BVDV	Flavivirus (+ve ssRNA)	MDBK	CPE	15	>100
HCV	Flavivirus (+ve ssRNA)	Huh–7	Viral DNA	0.01	>10

- *ACH-1625 shows weak or no activity against other viruses.*

5 ACH-1625 ENHANCES ANTI-HCV ACTIVITY OF INTERFERON-α AND RIBAVIRIN

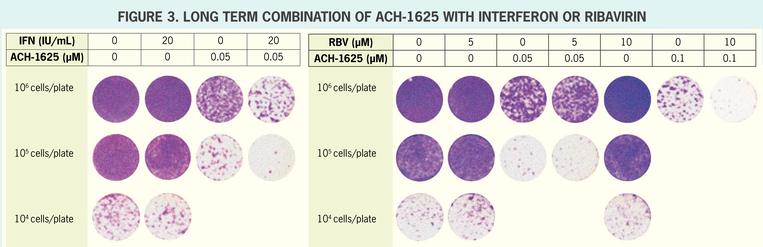
TABLE 5. SHORT-TERM COMBINATION OF ACH-1625 WITH INTERFERON OR RIBAVIRIN

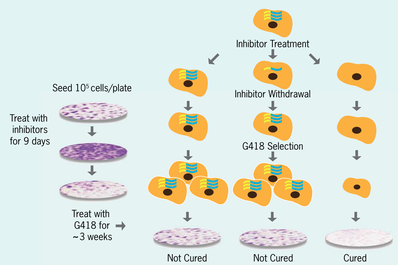
ACH-1625 Combined with	Volume μM²%		Molar Ratio	CI at Replication Inhibition of			Overall
	Synergy	Antagonism	μM vs. μM or μM vs. IU/mL	50%	75%	90%	
IFN	55±7.7	0±0.0	1:100 1:50	1.0±0.001 0.96±0.073	0.89±0.15 0.85±0.086	0.81±0.27 0.79±0.21	Additive to Synergistic
RBV	47±28.2	1.5±2.1	1:2400 1:1200	0.69±0.020 0.83±0.35	0.62±0.11 0.75±0.36	0.56±0.17 0.69±0.38	Additive to Synergistic

Huh-luc/neo cell line was treated for 3 days with inhibitors in a checkerboard manner, anti-replicon activity was determined with luciferase endpoint and analysis was conducted with Prichard and Shipman method (Volume of Synergy or Antagonism) and Chou and Talalay method (Combination Index, CI).

- *ACH-1625 displays an additive to synergistic effect when combined with interferon or ribavirin for 3 days.*
- *ACH-1625 significantly enhances the curing effect (percent cells rid of replicons) when used either with interferon or ribavirin for 9 days.*
- *These data indicate that studies of drug combination therapy including ACH-1625 would be appropriate.*

FIGURE 3. LONG TERM COMBINATION OF ACH-1625 WITH INTERFERON OR RIBAVIRIN





6 ACH-1625 HAS LOW POTENTIAL FOR OFF-TARGET ACTIVITY

TABLE 6. ACTIVITY OF ACH-1625 AGAINST HOST PROTEASES

Enzyme	Family	% Inhibition @ 10 μM	IC_{50} (μM)
Chymase	Serine Protease	4	-
Chymotrypsin	Serine Protease	6	-
Factor VIIa	Serine Protease	2	-
Factor Xa	Serine Protease	26	-
Kallikrein (plasma)	Serine Protease	–1	-
Plasmin	Serine Protease	6	-
Urokinase	Serine Protease	–19	-
Thrombin	Serine Protease	5	-
tPA	Serine Protease	2	-
Trypsin	Serine Protease	4	-
Tryptase	Serine Protease	5	-
Elastase	Serine Protease	5	-
Human Neutrophil Elastase	Serine Protease	–10	-
Cathepsin G	Serine Protease	29	-
Cathepsin L	Cysteine Protease	–1	-
Cathepsin S	Cysteine Protease	81	4.5
HCV NS3 Protease	Serine Protease	100	0.001

- *ACH-1625 displays > 4000-fold selectivity against human proteases.*
- *CC_{50} > 32 μM in all primary cells and cell lines tested.*
- *ACH-1625 was investigated at a concentration of 10 μM in a panel of 39 mammalian receptors, transporters, and ion channels and 16 mammalian enzymes. ACH-1625 shows no significant activity.*
- *All together, ACH-1625 displays low potential for off-target activity.*

7 SELECTION OF RESISTANCE REPLICON VARIANTS

- *The dominant resistance variants that emerged under ACH-1625 selection in Huh-luc/neo cell line carry a mutation at the amino acid residue 168 of NS3.*
- *Reverse genetic studies conducted with mutant NS3 proteases and replicons confirmed that these mutations are responsible for the replicon resistance to ACH-1625.*
- *All variants that emerged under ACH-1625 selection are not cross resistant with other classes of inhibitors including interferon. In addition, the D168 variants remain sensitive to Telaprevir.*

Conclusions

- ACH-1625 displays potent activity against NS3 proteases representing genotypes 1, 2, 4, 5 and 6.
- Activity against NS3 protease translates into potent inhibition of genotypes 1a and 1b HCV replicons including natural variants.
- The effect of human serum on the potency of ACH-1625 is moderate (~3 fold).
- ACH-1625 displays additive to synergistic effect in combination with pegylated interferon or ribavirin.
- ACH-1625 is a specific inhibitor for HCV NS3 protease and displays minimal off target activity.
- Potent and selective antiviral activity, favorable pharmacokinetic profile, and demonstrated safety, tolerability and excellent efficacy in HCV-infected patients warrant further development of ACH-1625.

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