Preclinical characterization of CC-31244, a pan-genotypic, potent NS5B non-nucleoside inhibitor for the treatment of chronic hepatitis C

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INTRODUCTION

- Hepatitis C virus is the major cause of chronic liver disease, leading to cirrhosis, liver failure, hepatocellular carcinoma. HCV viruses are classified into 6 genotypes, and 130-180 million people are infected worldwide.
- NS5B non-nucleoside inhibitors are a distinct class of direct acting agents (DAA) for the treatment of HCV. We have developed a novel, pan-genotypic nonnucleoside inhibitor (NNI),CC-31244, which can be used as part of combination DAA therapies. We present here our recent *in vitro* characterization of CC-31244, binding mode, drug resistance profiles, and pharmacokinetic data.

AIM

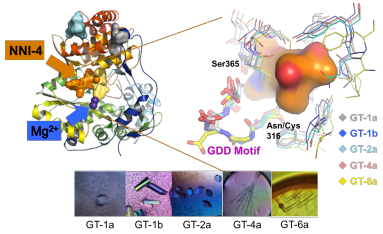
- Design and develop novel structure-guided pan-genotypic NS5B NNI leads.
- Demonstrate excellent activity toward known NS5B NNI drug resistant variants.
- Demonstrate good pharmacokinetic and *in vitro* safety profiles.
- Demonstrate liver targeting activity.

METHODS

Genotype HCV NS5B polymerases (GT1-6) and drug resistant NS5B polymerases were purified for protein crystallization and IC_{50} determination. NS5B polymerase crystals (GT1, 2, 4, and 6) and cocrystals diffracted to 1.7 – 2.2 Å. Antiviral activity was determined using HCV replicon and chimeric replicon assays. The genetic barrier to resistance in HCV GT1b replicon was measured in resistant colony selection assays using inhibitor concentrations at various multiples over the EC_{50} value of CC-31244. Safety pharmacology and pharmacokinetic profiles of CC-31244 were determined. X-ray data collection was done at BCSB (ALS), LS-CAT (APS), and SMB (SSRL).

RESULTS

Figure 1. HCV NS5B polymerase NNI sites



- Various genotype NS5B polymerases were crystallized and diffracted at 1.7 -2.2 Å
- NS5B NNI-4 site: highly conserved drug binding pocket

Figure 2. Binding mode of novel pan-genotypic NNI, CC-31244 – extends from NNI-4 to active site.

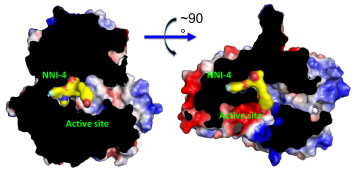
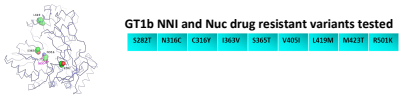


Table 1. CC-31244 is a potent HCV inhibitor exhibiting broad genotype coverage *in vitro*.

Genotype	Sofosbuvir-TP EC_{50}, uM	EC_{50} fold change	CDI-31244 EC_{50}, uM	EC_{50} Fold change
1b	0.042	1	0.005	1
1a	0.034	0.8	0.009	1.8
2b	0.028	0.66	0.026	5.2
3a	0.136	3.2	0.011	2.2
4a	0.047	1.1	0.021	4.2
5a	0.075	1.7	0.002	0.4

- CC-31244 demonstrates excellent activity against HCV replicons containing NS5B genes from genotype 1a, 1b, 3a, 4a, and 5a.
- CC-31244 also exhibits broad genotype coverage with purified GT1-6 NS5B polymerases (IC_{50} fold change <5-fold)
- No cytotoxicity observed : therapeutic index >10,000. (data not shown)

Figure 2. (A) NNI and Nuc drug resistant variants



(B) IC50 determination with common NNI and Nuc NS5B polymerase drug resistant variants

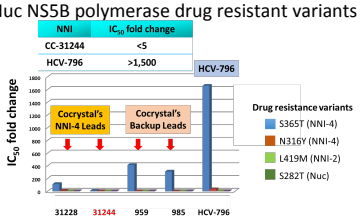


Table 2. (A) HCV GT1b replicons containing NS5B variants identified by CC-31244 resistant colony selection.

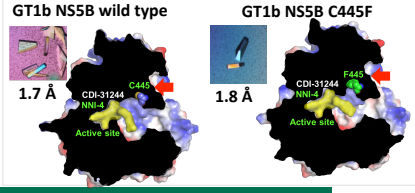
Clone	C316	I363	S365	M414	F430	D444	C445	Q446	Q514	S549
1							C445F			
2				M414V			C445F			
3					F430Y		C445F			
4							C445F			
5							C445F	Q446R		

(B) Activity of CC-31244 in the GT1b major replicon, C445F/S549G and GT1b NS5B C445F mutant polymerase.

Inhibitor	GT1b C445F/S549G EC_{50}, µM	GT1b EC_{50}, µM	EC_{50} fold change
CC-31244	0.08	0.005	16

Inhibitor	GT1b, C445F IC_{50}, µM	GT1b IC_{50}, µM	IC_{50} fold change
CC-31244	0.23	0.24	0.94

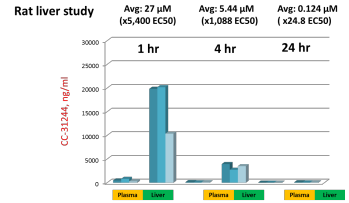
Figure 3. CC-31244 binding mode in NS5B C445F



RESULTS

Table 3. Pharmacokinetic and ADMET properties of CC-31244

Pan-genotypic	Yes	hERG	>10 uM
Genotype 1 -5, EC_{50} nM	2-26	Intrinsic clearance (liver microsomes)	Rat 54 min; Dog >60 min, Human 41 min
Activity against common NNI resistant variants	Excellent IC_{50} <5-fold	CYP3A CYP2D	>10 uM >10 uM
Drug binding pocket	NNI 4	Time-dependent CYP3A Time-dependent CYP2D	>10 uM
Binding to target	Slow	Rat PK, dose	PO 5mg/kg IV 1mg/kg
HepG2 cytotoxicity (Cell number, nuclear size, Mitochondrial membrane potential, intracellular free calcium)	No	Oral bioavailability (%)	44.5
		Cl (ml/min/kg)	11.7
Cytotoxicity against proliferating/non-proliferating cell lines	No	Vd (L/kg)	6.88
		t1/2 (h)	6.85
Solubility in PBS	189 uM	Cmax (ng/mL)	2794 (4,450 nM, > 200x EC50)
Human plasma binding	99 %	$AUC_{0\text{-}last}$ (ng•h/ml)	3089
Caco2 A-B, 10^{-6} cm/s Caco2 B-A, 10^{-6} cm/s	7.1 44.4	Liver targeting	>3,000 x EC50
Safety (off-target) profile	Excellent		



CONCLUSION

- CC-31244 demonstrates pan-genotypic activity against NS5B polymerases of GT 1-6.
- GT1b replicons bearing the NS5B C445F mutation were selected as a major drug resistant variant.
- CC-31244 exhibits good potency against common NNI and Nuc drug resistant variants.
- Based on its favorable preclinical activity and pharmacokinetic characteristics, CC-31244 was selected as a clinical candidate and regulatory submission is scheduled for early 2016.

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