

**ANTI-HCV ACTIVITY AND CELLULAR
PHARMACOLOGY OF 2'-METHYLCYTIDINE ALONE
AND IN COMBINATION WITH NON-TOXIC
CONCENTRATIONS OF RIBAVIRIN IN LIVER CELLS
AND THE HCV REPLICON SYSTEM**

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HCV Therapy

☀️ peg-IFN- α + Ribavirin (RBV)

- ☀️ Current standard therapy

- ☀️ Poorly tolerated

- ☀️ Sustained viral response (SVR) in treated subjects

 - ~ 50% genotype 1

 - ~ 80% genotype 2 or 3

☀️ Oral drug candidates (Phase 2)

- ☀️ NM283 [Polymerase Inhibitor, prodrug of NM107 (2'-MeC)]

- ☀️ R1626 (Polymerase inhibitor, prodrug of R1479)

- ☀️ HCV-796 (Non-nucleoside polymerase inhibitor)

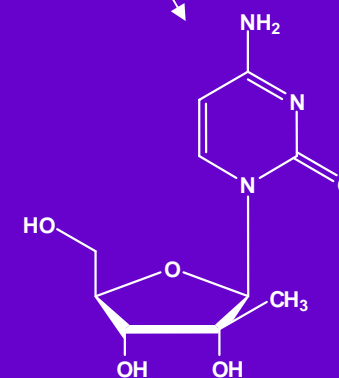
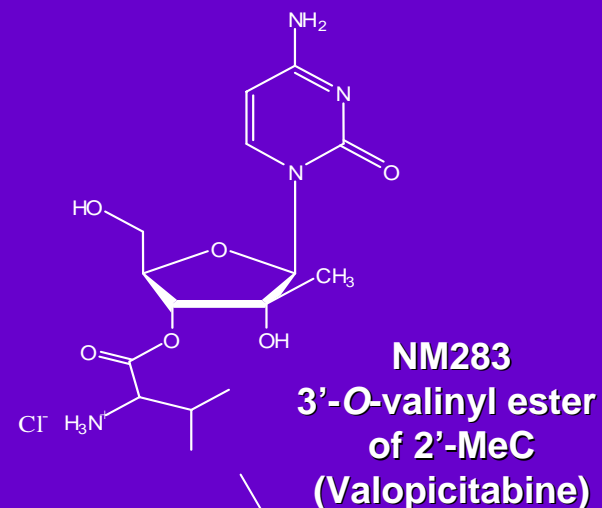
- ☀️ VX-950 (Protease inhibitor)

- ☀️ SCH-503034 (Protease inhibitor)

NM283 [prodrug of NM107 (2'-MeC)]

☀ *In vitro*, selectively inhibits HCV replication by interfering with HCV RNA synthesis.

☀ *In vitro* studies indicated that protease inhibitor resistance mutations (R155Q, A156T, D168A, D168V and D168Y) were fully sensitive to 2'-MeC with EC_{50} values similar to the wild type replicon. (Bichko, *J Hepat.* 46: S163, 2006.)



NM107
(2'-MeC)

NM283 Combination Therapy

- ☀ Preclinical data demonstrated enhanced replicon activity with no cross-resistance with the combination of:

2'-MeC + SCH 503034 or IFN- α

- ☀ NM283 + peg-IFN- α

- ☀ **In treatment-naïve patients**

- At dose of 200 mg/d NM283 + 180 μ g/QW peg-IFN- α
- Markedly suppresses viremia ($\sim 4 \log_{10}$ drop) at week 36
- Good tolerability

- ☀ **In previously experienced non-responders to peg-IFN- α /RBV**

- HCV RNA suppression at week 48 with the combination of NM283 (400-800 or 800 mg/d) + peg-IFN- α was approximately 0.8 \log_{10} (~ 6 -fold) greater than the standard treatment of peg-IFN- α /RBV
- 42% fully suppressed HCV replication, but 0% attained SVR
- **Thus, RBV should be important to prevent relapse.**

Bassit, L., et al., *J. Hepatol.* 46 Suppl 1: S217, 2007.

Ralston, R. et al. *J. Hepatol.* 46 Suppl 1: S298, 2007.

Lawiz, E., et al. *J Hepatol.* 46 Suppl 1: S8, 2007.

Afdhal, N., *J Hepatol.* 46 Suppl 1: S5, 2007.

Ribavirin

- ☀ Is a nucleoside analog that has broad-spectrum antiviral activity.
- ☀ Suggested mechanisms (none confirmed):
 - ☀ Depletion of intracellular GTP pools (by inhibition of IMP dehydrogenase).
 - ☀ Inhibition of HCV polymerase activity by RBV-TP at high concentrations.
 - ☀ Immunomodulation (Enhancing T_H1 and decreasing T_H2 cytokine production).
 - ☀ Induction of error catastrophe as a result of accumulation of mutations in the viral genome.

Dixit, NM., et al. Cell. Mol. Life Sci. 63: 832- 842, 2006.

Chevaliez, S., et al. J. Virol. ahead pub: May 2007.

RBV in combination with nucleoside analogs

☀ *In vitro* studies

- ☀ RBV enhances the antiviral effect of 2',3'-dideoxynucleoside purines^{a,b}
- ☀ RBV antagonizes the effect of: ZDV^c, 2',3'-dideoxynucleoside pyrimidines^a and 2'-MeC^d
- ☀ Reduced the d4T phosphorylation in PBM and U937 cells^e

☀ *In vivo* study

- ☀ In HIV/HCV co-infected patients

RBV in combination with peg-IFN- α 2a does not significantly affect the intracellular phosphorylation or plasma pharmacokinetics of 3TC, d4T and ZDV^f.

^aBaba, M., et al. AAC. 31: 1613-1617, 1987; ^bVogt, M., et al. Science 235: 1376-1379, 1987

^cBalzarini, et al. JBC 266: 21509-21514, 1991; ^dCoelmont, L. et al. AAC. 50: 3444-3446, 2006

^eHoggard, et al. AAC 41: 1231-1236, 1997; ^fRodriguez-Torres, et al. AAC. 49: 3997-4008, 2005

AIM

To study a possible drug-drug interaction between 2'-MeC and RBV

- ✿ by measuring **2'-MeC-TP** levels in liver cells and
- ✿ by determining the **antiviral drug interaction** in HCV Replicon (Clone B) Huh-7 cell line

Cytotoxicity assay of 2'-MeC and RBV alone and in combination in liver cells (5 days assay)

Compound	Concentration (μM)	CC ₅₀ in Huh-6 cells (μM)	CC ₅₀ in Huh-7 cells (μM)
2'-MeC	1	> 100	> 100
	10		
	100		
RBV	1	> 100	40.2
	10		
	100		
2'-MeC + RBV	10: 1	> 100	53.1
	10:10		
	10:100		

Mitochondrial toxicity assay in liver cells (7 day assay)

Compound	Concentration (μM)	CC ₅₀ in Huh-6 cells (μM)		CC ₅₀ in Huh-7 cells (μM)	
		MitCoxII	rDNA	MitCoxII	rDNA
No drug	0	0.0	0.0	0.00	0.0
ddC	10	< 10	> 10	< 10	> 10
3TC	10	> 10	> 10	> 10	> 10
2'-MeC	10	> 10	> 10	> 10	> 10
RBV	1	36.3	29.0	38.0	29.6
	10				
	33				
	50				
	100				
2'-MeC + RBV	10:1	43.5*	28.1*	34.5*	33.1*
	10:10				
	10:33				
	10:50				
	10:100				

*CC₅₀ of RBV only. Data represent average of three replicates in cell culture.

Stuyver, L., et al. AAC. 46: 3854- 3860, 2002.

As expected ddC (positive control) was toxic at concentration less than 10 μM in both cell lines. RBV alone was toxic at concentration above 33 μM. The combination of 2'-MeC with RBV was not markedly more toxic than RBV alone.

2'-MeC-TP intracellular concentration after [³H]2'-MeC (10 μM; 250 - 500 dpm/pmol) incubation alone or in combination with RBV (1 - 100 μM) in liver cells for 4 hr

	[³H]2'-MeC-TP intracellular concentration (pmol/10⁶ cells ± SD)		
	Huh-6 cells	PLC/SH2 cells	PLC/SH2 + RBV
2'-MeC (10 μM)	9.6 ± 1.7	1.1 ± 0.2	1.1 ± 0.2
2'-MeC (10 μM) + 50 μM Cyd	BLD^{*a}	1.1 ± 0.2	1.1 ± 0.2
2'-MeC (10 μM) + 1 μM RBV	6.9 ± 0.8	1.1 ± 0.2	1.1 ± 0.2
2'-MeC (10 μM) + 10 μM RBV	3.4 ± 0.6^a	1.1 ± 0.2	1.1 ± 0.2
2'-MeC (10 μM) + 100 μM RBV	2.7 ± 0.2^a	1.1 ± 0.2	1.1 ± 0.2

BLD* = Below limit of detection (0.01 pmol/10⁶ cells).

T-test (two-sample assuming equal variance) was used to determine statistical significance (p < 0.05)^a.

**Cyd prevent 2'-MeC phosphorylation
At 1:1 and 1:10 ratio (2'-MeC:RBV) a 65% and 72% inhibition of
2'-MeC-TP was observed respectively.**

2'-MeC-TP intracellular concentration after [³H]2'-MeC (10 μM; 250 - 500 dpm/pmol) incubation alone or in combination with RBV (1 - 100 μM) in liver cells for 4 hr

	[³H]2'-MeC-TP intracellular concentration (pmol/10⁶ cells ± SD)		
	Huh-6 cells	Huh-7 cells	Control
2'-MeC (10 μM)	9.6 ± 1.7	8.1 ± 0.6	
2'-MeC (10 μM) + 50 μM Cyd	BLD^{*a}	BLD^a	
2'-MeC (10 μM) + 1 μM RBV	6.9 ± 0.8	6.3 ± 1.4	
2'-MeC (10 μM) + 10 μM RBV	3.4 ± 0.6^a	3.3 ± 0.3^a	
2'-MeC (10 μM) + 100 μM RBV	2.7 ± 0.2^a	2.6 ± 1.1^a	

BLD* = Below limit of detection (0.01 pmol/10⁶ cells).

T-test (two-sample assuming equal variance) was used to determine statistical significance (p < 0.05)^a.

Results were similar in Huh-6 and Huh-7 cells.

2'-MeC-TP intracellular concentration after [³H]2'-MeC (10 μM; 250 - 500 dpm/pmol) incubation alone or in combination with RBV (1 - 100 μM) in liver cells for 4 hr

	[³H]2'-MeC-TP intracellular concentration (pmol/10⁶ cells ± SD)		
	Huh-6 cells	Huh-7 cells	Primary human hepatocytes
2'-MeC (10 μM)	9.6 ± 1.7	8.1 ± 0.6	0.5 ± 0.2
2'-MeC (10 μM) + 50 μM Cyd	BLD^a	BLD^a	BLD^a
2'-MeC (10 μM) + 1 μM RBV	6.9 ± 0.8	6.3 ± 1.4	0.7 ± 0.3
2'-MeC (10 μM) + 10 μM RBV	3.4 ± 0.6^a	3.3 ± 0.3^a	0.8 ± 0.1
2'-MeC (10 μM) + 100 μM RBV	2.7 ± 0.2^a	2.6 ± 1.1^a	0.4 ± 0.0

BLD* = Below limit of detection (0.01 pmol/10⁶ cells).

T-test (two-sample assuming equal variance) was used to determine statistical significance (p < 0.05)^a.

No reduction in 2'-MeC-TP formation was observed in human hepatocytes. As expected, Cyd reduced 2'-MeC phosphorylation

Antiviral effect of 2'-MeC and RBV (at non-toxic concentrations) combination in HCV replicon (Clone B) in Huh-7 cells (analyzed by CalcuSyn®)

Treatment (ratio)	Parameter ^a		Combination Index (CI) ^b values at inhibition of:			
	EC ₅₀ (μM)	EC ₉₀ (μM)	50%	75%	90%	95%
2'-MeC	1.3	6.5	-	-	-	-
RBV	21.5	>33	-	-	-	-
2'-MeC + RBV (1:5)	6.8	78.9	1.2 ± 0.2	1.3 ± 0.3	1.5 ± 0.6	1.6 ± 0.8

^a EC₅₀ is the median effective concentration and EC₉₀ is the effective concentration at 90% inhibition in μM as determined from the median effect plot. ^bC.I. values (CalcuSyn® program) were determined for a mutually exclusive interaction ± standard deviation; CI <1, equal to 1 or > 1 indicates synergy, additivity and antagonism, respectively.

Stuyver, L., et al., AAC. 48: 651-654, 2004.

RBV did not antagonize the 2'-MeC activity in HCV replicon (Clone B) system at 1:5 ratio (2'-MeC + RBV) - Additive interaction observed

Results

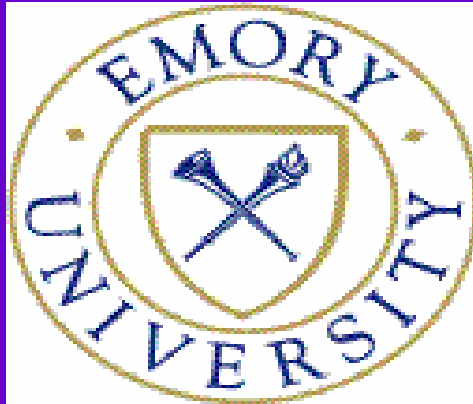
- ☀ No cytotoxicity of 2'-MeC in Huh-6, Huh-7 and at the mitochondrial level were observed.
- ☀ RBV showed modest cytotoxicity in Huh-7 cells. We also observed mitochondrial toxicity in Huh-6 and Huh-7 cells with RBV alone and in combination (above 33 μM).
- ☀ 2'-MeC-TP levels decreased in the presence of RBV (10 and 100 μM) in Huh-6 and Huh-7 cells, ***but NOT in primary human hepatocytes.***
- ☀ 2'-MeC in combination with RBV (10-100 μM) in Huh-6/7 cells produced levels of 2'-MeC-TP higher than the K_i reported for viral RNA polymerases (0.16 to 1.6 μM).
- ☀ Combination between 2'-MeC and RBV at clinically relevant ratios resulted in no antagonistic effect at non-toxic concentrations of RBV.

Conclusions

Based on these cellular pharmacological and virological results of 2'-MeC (and NM283 or valopicitabine) with RBV, it is unlikely that these drugs will have negative drug-drug interactions in the clinic when used in combination for the treatment of HCV in liver cells.

Recent clinical data on this combination released by Idenix 24 hr ago indicate that this is indeed the case.

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