

# Combination of the NS3/4A Protease Inhibitor ITMN-191 (R7227) with the Active Moiety of the NS5B Inhibitors R1626 or R7128 Enhances Replicon Clearance and Reduces the Emergence of Drug Resistant Variants

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## Abstract

**Background:** ITMN-191 is an inhibitor of HCV NS3/4A protease activity, and R1626 and R7128 are nucleoside inhibitors of the polymerase activity of HCV NS5B. All three compounds promote multi-log<sub>10</sub> reductions in circulating HCV RNA in chronic HCV patients when administered for short durations as monotherapy. Here, to support future clinical studies that would combine ITMN-191 with R1626 or R7128, we investigated the combined antiviral effect of these compounds.

**Methods:** In the HCV clearance assay, cells harboring an HCV genotype 1b replicon were treated for 2 weeks with ITMN-191, the active moiety of R1626 (R1479), the active moiety of R7128 (PSI 6130), or a combination of inhibitors in the absence of G418 selection for replicon retention. Cells were counted and aliquots harvested for RT-PCR-based quantification of replicon RNA levels under G418 selection over 4 subsequent weeks. In the colony formation assay, cells were treated with either one or two compounds at 1X, 10X, or 15X their respective EC<sub>50</sub>. After 3 weeks in culture with G418, cells were fixed and stained with crystal violet or total cellular RNA extracted. For drug-drug interaction studies, HCV replicon cells were treated for 3 days with a pair of inhibitors in checkerboard dilution and percent reductions of reporter gene activity obtained.

**Results:** In the HCV clearance assay, 18 nM ITMN-191 and low μM concentrations of the active moiety of R1626 and R7128 (18 μM & 27 μM, respectively) eliminated HCV replicon in the absence of G418 selective pressure for replicon retention. Addition of the lowest tested concentration of ITMN-191 (6 nM) to the lowest tested concentration of the active moiety of R1626 or R7128 (0.3 μM & 0.45 μM, respectively) resulted in replicon clearance, demonstrating significant combined antiviral effect. In the colony formation assay in the presence of G418 selective pressure for replicon retention, NS5B inhibitors at 10X or 15X EC<sub>50</sub> supported replicon clearance and did not result in drug resistant colonies. Similar treatment with ITMN-191 selected resistant colonies, but these were suppressed by an NS5B inhibitor at 1X EC<sub>50</sub>. Drug-drug interaction studies over a 3 day incubation period demonstrated additive to slightly synergistic interactions between the two inhibitor classes.

**Conclusions:** The combination of ITMN-191 with the active moiety of either R1626 or R7128 results in enhanced antiviral activity and suppression of ITMN-191 resistant variants. These findings suggest that the combination of ITMN-191 with R1626 or R7128 may confer significantly greater antiviral activity than has been observed with these agents in previous monotherapy trials.

## Direct antiviral targets

**NS3/4A protease/helicase**  
NS3 is a bifunctional protein that possesses both serine protease and nucleic acid unwinding activity. Protease activity is responsible for cleavage of several linkages in the HCV poly protein and is essential for the viral life cycle. Inhibitors of serine protease activity have shown promise in clinical studies. Due to the high tolerance for substitutions in the serine protease substrate binding site, short duration monotherapy with protease inhibitors that bind to this site has been reported to lead to viral breakthrough.

**NS5B RNA-dependent RNA polymerase**  
NS5B is the viral polymerase. Polymerase activity is essential for the viral life cycle. NS5B inhibitors can bind either to the polymerase active site or allosteric sites. Nucleoside analogs which induce premature termination of the RNA have shown promising activity in clinical studies. The active site of NS5B is relatively intolerant to substitution. Short duration monotherapy with RNA elongation inhibitors is not associated with viral breakthrough.

## Inhibitors used in these studies

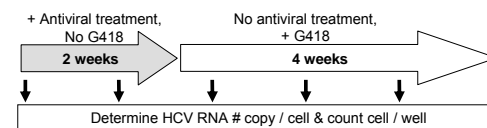
**ITMN-191 (R7227)**  
Inhibitor of NS3/4A protease  
EC<sub>50</sub> ≈ 1.8 nM (con1-genotype 1b)  
Very low plasma exposure may contribute to a favorable safety profile in clinical studies conducted to date  
14 day monotherapy EOT median viral load reductions of 3.1 and 3.8 log<sub>10</sub> promoted by 200 mg at 12 hr and 8 hr intervals, respectively (Poster 1847)  
Currently under clinical study in combination with SOC

**R7128 active moiety (PSI-6130)**  
Terminator of NS5B RNA elongation  
EC<sub>50</sub> = 0.6 μM (con1-genotype 1b)  
14 day monotherapy resulted in HCV RNA reductions ranging from 0.9 to 2.7 log<sub>10</sub> following administration of 750 mg or 1500 mg at 12 hr and 24 hr intervals  
Phase 2a development completed: 1000 mg BID in combination with Peginterferon alfa-2a + ribavirin resulted in 88% PCR negativity (<15 IU/mL) in 4 weeks (RVR)

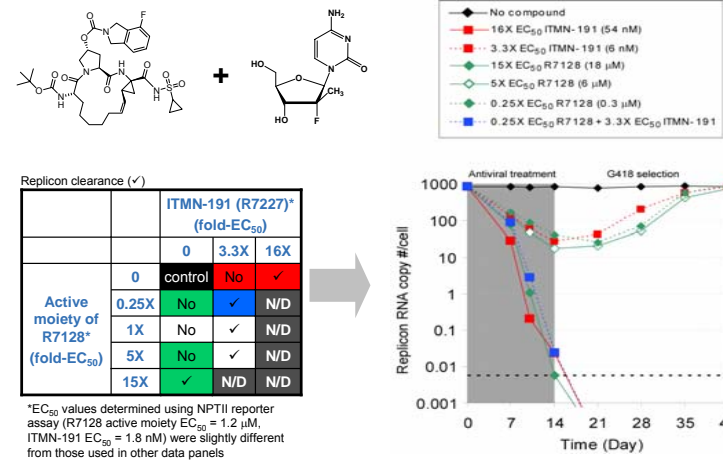
**R1626 active moiety (R1479)**  
Terminator of NS5B RNA elongation  
EC<sub>50</sub> = 1.28 μM (con1-genotype 1b)  
14 day monotherapy responses ranging from 0.3 to 3.7 log<sub>10</sub> following BID doses of 500 up to 4500 mg.  
Phase 2a development completed: 1500 mg in combination with Peginterferon alfa-2a + ribavirin resulted in 5.2 log<sub>10</sub> reduction in 4 weeks with 81% PCR negativity (RVR). Neutropenia observed

## Results

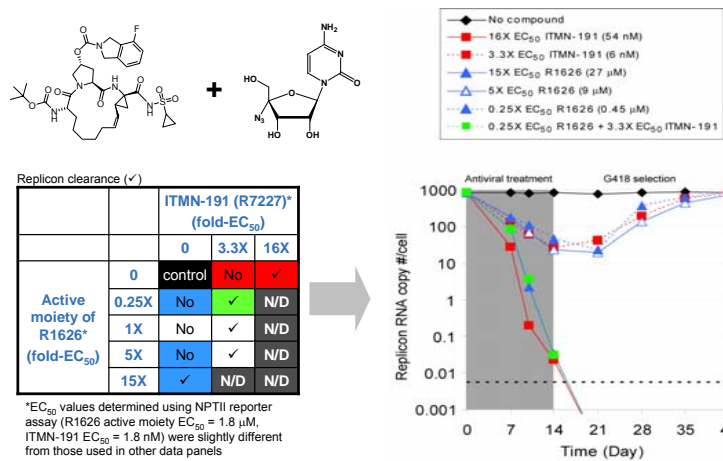
### HCV replicon clearance



#### ITMN-191 (R7227) + R7128

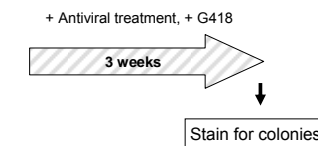


#### ITMN-191 (R7227) + R1626



R7128 or R1626 concentrations near their respective EC<sub>50</sub> promote replicon clearance when the NS3 protease inhibitor ITMN-191 is present at a concentration near its EC<sub>50</sub>

### Colony formation assay



Selection (X EC <sub>50</sub> )	Number of Colonies (Mean ± SEM)
10X ITMN191	138 ± 35
15X ITMN-191	78 ± 23
10x OR 15X R7128	0
10X ITMN-191/1X R7128	8 ± 2
10X ITMN-191/10X R7128	0
10X ITMN-191/15X R7128	0
15X ITMN-191/1X R7128	2 ± 1
15X ITMN-191/10X R7128	0
15X ITMN-191/15X R7128	0
10X OR 15X R1626	0
10X ITMN-191/1X R1626	52 ± 18
10X ITMN-191/10X R1626	0
10X ITMN-191/15X R1626	0
15X ITMN-191/1X R1626	17 ± 6
15X ITMN-191/10X R1626	0
15X ITMN-191/15X R1626	0

Treatment of the HCV replicon with ITMN-191 (up to 15X EC<sub>50</sub>) alone resulted in the selection of drug resistant colonies after 3 weeks incubation

In combination with low concentration (1X EC<sub>50</sub>) of either R7128 or R1626, the number of resistant colonies is reduced dramatically

In combination with either nucleoside inhibitor at concentrations 10X EC<sub>50</sub> or higher, the replicon is cleared.

## Summary and Conclusions

» In vitro combination of the NS3/4A protease inhibitor ITMN-191 with the active moiety of the NS5B inhibitor R7128 or R1626:

- Reduces compound concentrations required for cellular elimination of HCV replicon
- Suppresses the emergence of drug resistant HCV replicons

» These studies suggest that the combined antiviral effect of ITMN-191 (R7227) and either R7128 or R1626 in chronic HCV patients would be greater than the antiviral effect displayed by these agents when administered as monotherapy



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