

Combination of IDX184, a Nucleotide Prodrug Polymerase Inhibitor, with Other Classes of HCV Inhibitors is Additive to Synergistic in the HCV Replicon *in vitro*

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INTRODUCTION

- The current standard-of-care for the treatment of hepatitis C virus (HCV), a combination of pegylated interferon and ribavirin, is effective in approximately 40-50% of patients infected with HCV genotype 1 and is frequently associated with significant side effects.¹ Thus, there remains a need for new, more effective and tolerable HCV treatment options.
- IDX184 is a liver-targeted nucleotide prodrug, which provided significant anti-HCV activity and safety in a proof-of-concept study and is currently in Phase II clinical trials (see poster LB18).²
- This *in vitro* study examined the potential for combining IDX184 with IDX375 (a non-nucleoside, palm-binding polymerase inhibitor), IDX316 (a protease inhibitor) or standard-of-care agents to reduce *in vitro* HCV viral load and the emergence of resistance (as demonstrated by suppression of breakthrough colonies).

METHODS

HCV replicon assay: Replicon cells (genotype 1b) were seeded onto 96-well plates, cultured for 3 days in the presence of compound(s) and subjected to ELISA or luciferase assay. The effect of drug combination was evaluated using MacSynergy software (Bliss Independence Model) and CalcuSyn software (Combination Index). Compound(s) cytotoxicity was measured in parallel using MTS.

Long-term treatment assay: Replicon cells (genotype 1b) were treated with compound(s), without G418, for 14 days and the level of replicon RNA was measured at multiple time points. At the end of the 14-day treatment, cells were cultured in the absence of compound ± G418 in 10 cm dishes for 21 days, whereupon the surviving cell colonies were stained with crystal violet and counted.

Colony formation assay: Replicon cells (genotype 1b) were treated with compound(s) and G418 for 3 weeks; compound(s) was replaced every 3-4 days. Surviving cell colonies were then stained with crystal violet.

RESULTS

IDX184 two-drug combinations give additive to synergistic activity in short-term assays

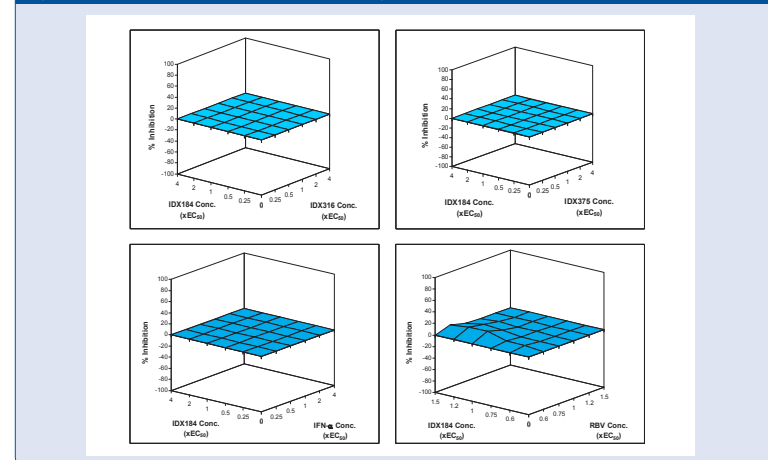
- As seen in Table 1 and Figure 1, additive to synergistic activity was observed when IDX184 was combined with IDX375, IDX316, IFN-α or RBV in short-term (3-day) replicon studies.
- The combination of IDX184 with RBV showed a synergistic effect in both analyses.

Table 1: Combination effects of IDX184 with other agents

IDX184 +	Bliss Independence	Combination Index
IDX375	Additive	Moderate Synergy
IDX316	Additive	Additive
IFN-α	Additive	Moderate Synergy
RBV	Weak Synergy	Strong Synergy

ELISA assay. No cytotoxicity was observed.

Figure 1: Effect of IDX184 combinations using the Bliss Independence model



IDX184 triple combination is synergistic in short-term assays

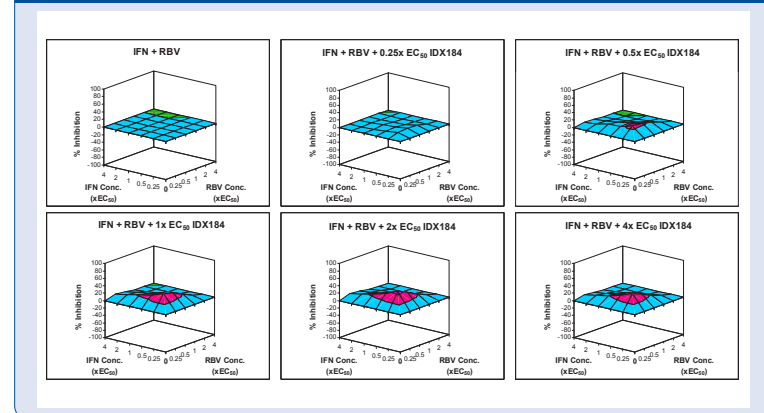
- A triple combination of IDX184 with IFN-α and RBV showed synergy in both analyses across all IDX184 concentrations tested (Table 2 and Figure 2).
- The strength of synergy was dose-dependent with respect to IDX184.
- This is the only combination that we have observed to date to give strong synergy *in vitro*.

Table 2: Combination effects of IDX184 with IFN-α and RBV

IFN-α + RBV + x EC ₅₀ IDX184	Bliss Independence	Combination Index
0	Additive	Moderate Synergy
0.25x	Significant Synergy	Synergy
0.5x	Strong Synergy	Synergy
1x	Strong Synergy	Synergy
2x	Strong Synergy	Strong Synergy
4x	Strong Synergy	Strong Synergy

Luciferase assay. No cytotoxicity was observed.

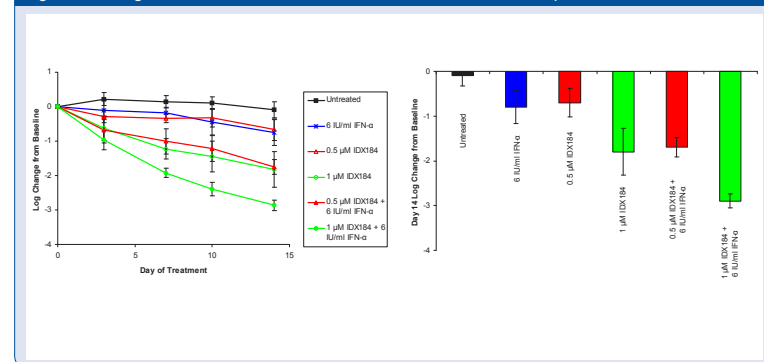
Figure 2: Effect of IDX184/IFN-α/RBV triple combination using the Bliss Independence model



Additive activity of IDX184 after long-term treatment with IDX375 or IFN-α

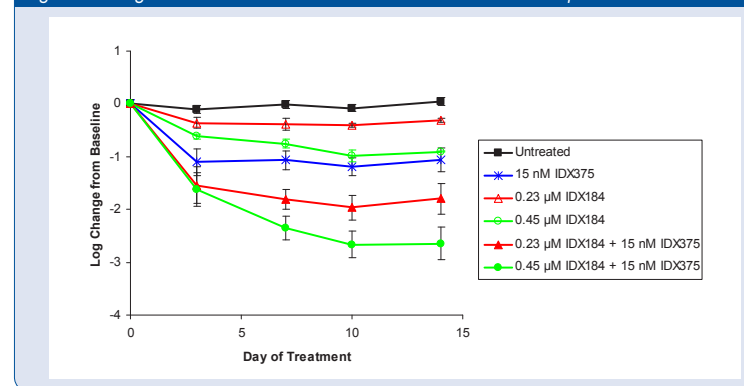
- During 14-day treatments of replicon cells, a sharper decline in replicon RNA was observed when IDX184 was combined with IFN-α (Figure 3) or IDX375 (Figure 4 and Table 3) as compared with single treatment, consistent with an additive antiviral effect.
- A continual decline in replicon RNA was observed over 14 days when replicon cells were treated with a combination of IDX184 and IFN-α (Figure 3).
- In contrast, replicon inhibition reached a plateau after 3 to 7 days of IDX184/IDX375 combination treatment; this suppression was maintained for the remaining treatment duration (Figure 4).
- No RNA rebound or cytotoxicity was observed in these studies.

Figure 3: Long-term combination effect of IDX184 and IFN-α on replicon RNA



Replicon RNA quantitated by RT-qPCR of 5'-UTR

Figure 4: Long-term combination effect of IDX184 and IDX375 on replicon RNA



Replicon RNA quantitated by RT-qPCR of 5'-UTR

Table 3: Effect of 14-day treatment with IDX184 and IDX375 on replicon RNA

IDX184 Conc. (μM)	IDX375 Conc. (nM)	Log ₁₀ reduction in replicon RNA ^a	Number of colonies following G418 selection ^b
0	0	-0.1	>400
0.23	0	0.3	>400
0.45	0	0.9	>400
0	7.5	0.6	>396
0	15	1.1	>220
0	37.5	1.5	248
0.23	7.5	1.3	>255
0.45	7.5	2.3	34
0.23	15	1.8	130
0.45	15	2.6	32
0.23	37.5	2.1	66
0.45	37.5	3.0	6

^a Values represent the mean log₁₀ reduction values across three independent experiments.

The log₁₀ reduction was calculated by subtracting the average log₁₀ HCV replicon copies/ng RNA of the sample at Day 14 from the average log₁₀ copies/ng RNA of the untreated control at Day 0.

^b Values represent the mean number of colonies counted across three independent experiments.

- The cells treated with IDX184 and IDX375 were further cultured without compound(s) ± G418 to quantitate the remaining replicon-bearing cells.
- In the absence of G418 selection, a lawn of cells was observed after treatment with all combinations of IDX184 and IDX375, indicating that the treatments did not affect cell viability (data not shown).
- In the presence of G418, a dose-dependent reduction in replicon-bearing colonies was observed after single and combination treatment, but suppression was greater after combination treatment (Table 3).

Combination treatment suppresses the emergence of breakthrough colonies

- Upon 3-week combination treatment with IDX184 and IDX375 (Figure 5), IDX316 (Figure 6), or IFN-α (Figure 7), in the presence of G418, the suppression of replicon-bearing cells was greater than each treatment alone.
- This observation is consistent with results obtained from 3-day combination treatment of luciferase replicon-bearing cells showing that combination treatment of IDX184 with IDX375, IDX316, or IFN-α was additive (Table 1 and Figure 1).
- The combination of IDX184 with IDX375, IDX316, or IFN-α more effectively suppressed the emergence of breakthrough colonies than treatment with each drug alone.

Figure 5: Colony formation assay following combination treatment with IDX184 and IDX375

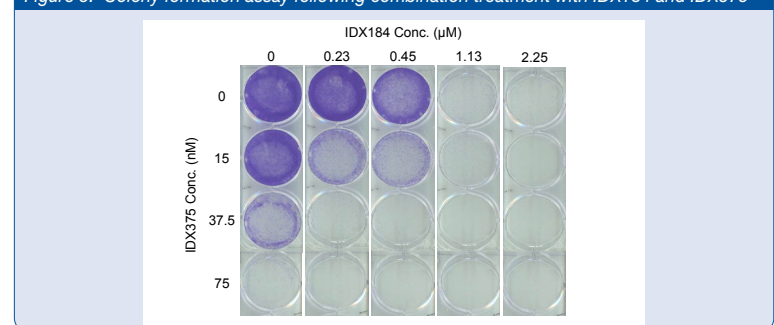


Figure 6: Colony formation assay following combination treatment of with IDX184 and IDX316

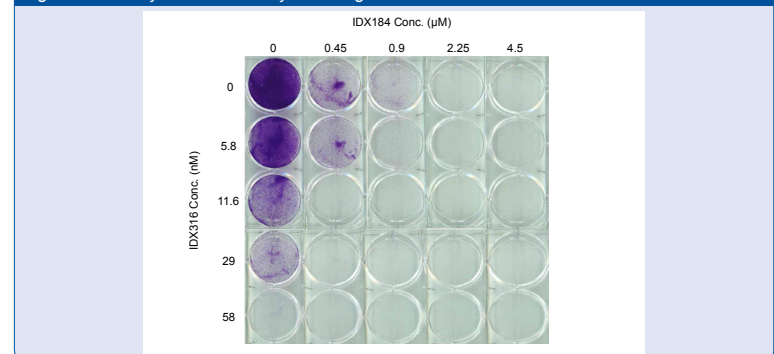
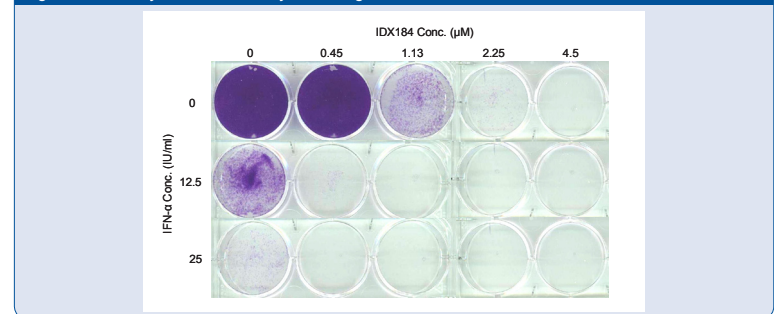


Figure 7: Colony formation assay following combination treatment of with IDX184 and IFN-α



CONCLUSIONS

- Two-drug combinations of IDX184 with IDX375 (non-nucleoside inhibitor), IDX316 (protease inhibitor), or IFN-α resulted in at least additive antiviral effects after 3-day treatment. Longer term combinations of IDX184 with IDX375 or IFN-α indicated that these effects were maintained over 14 days.
- The combination of IDX184 and RBV was synergistic *in vitro*.
- A triple combination of IDX184 with standard-of-care agents (IFN-α and RBV) exhibited strong synergy in *in vitro* short-term assays; the strength of synergy was dose-dependent with respect to IDX184.
- Combination treatment with IDX184 and IDX375, IDX316, or IFN-α yielded fewer breakthrough colonies after a 3-week treatment. The presence of resistance mutations in these colonies is currently being evaluated.
- The combination of IDX184 with other direct antivirals or standard-of-care agents may provide an effective strategy to suppress HCV viral replication and prevent emergence of drug resistance.

References

- Zeuzem S, et al (2009) J. Viral Hepat. 16:75-90.
- Cretton-Scott E, et al (2008) Journal of Hepatology 48, S220.

Acknowledgments

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