

Supplementary Materials

Supplementary Table 1: Activity of ASV in combination with BMS-791325, an allosteric NS5B inhibitor targeting site I

Expt	ASV EC ₅₀ , nM	BMS-791325 EC ₅₀ , nM	Ratio, BMS-791325 to ASV	Combination Indices (Confidence Interval)			Overall Result
				50% Effective	75% Effective	90% Effective	
1	0.7	3	4:1	1.05 (0.98, 1.12)	0.96 (0.86, 1.06)	0.88 (0.74, 1.02)	Additivity
			8:5	0.95 (0.87, 1.03)	0.99 (0.88, 1.09)	1.04 (0.84, 1.23)	Additivity
			10:1	0.98 (0.91, 1.06)	0.99 (0.88, 1.09)	0.99 (0.82, 1.15)	Additivity
2	0.7	3	4:1	1.04 (0.96, 1.12)	0.95 (0.84, 1.05)	0.86 (0.71,1.01)	Additivity
			8:5	0.97 (0.89, 1.05)	1.00 (0.88, 1.12)	1.03 (0.84, 1.22)	Additivity
			10:1	0.97 (0.89, 1.05)	0.98 (0.87, 1.10)	1.0 (0.82, 1.18)	Additivity
3	0.9	4	4:1	1.00 (0.92, 1.07)	0.96 (0.86, 1.06)	0.92 (0.77, 1.08)	Additivity
			8:5	1.07 (1.00, 1.14)	1.00 (0.91, 1.09)	0.93 (0.80, 1.07)	Additivity
			10:1	1.02 (0.93, 1.11)	1.03 (0.90, 1.16)	1.04 (0.84, 1.24)	Additivity

Supplementary table 2: Activity of ASV in combination with HCV-796, an allosteric inhibitor targeting site II

Expt	ASV EC ₅₀ , nM	HCV-796 EC ₅₀ , nM	Ratio, HCV-796 to ASV	Combination Indices (Confidence Interval)			Overall Result
				50% Effective	75% Effective	90% Effective	
1	0.001	0.187	1:160	0.85 (0.77, 0.93)	0.88 (0.76, 0.99)	0.91 (0.72, 1.10)	Synergy/Additivity ^a
			1:400	0.99 (0.90, 1.08)	0.93 (0.81, 1.05)	0.88 (0.70, 1.05)	Additivity
			1:1000	1.03 (0.93, 1.12)	0.91 (0.80, 1.02)	0.81 (0.65, 0.97)	Synergy/Additivity ^a
2	0.0013	0.393	1:160	0.92 (0.85, 0.99)	0.88 (0.79, 0.98)	0.85 (0.71, 0.99)	Synergy
			1:400	0.92 (0.85, 0.99)	0.90 (0.8, 1.00)	0.88 (0.73, 1.04)	Synergy/Additivity ^b
			1:1000	1.08 (0.99, 1.16)	1.07 (0.95, 1.19)	1.06 (0.88, 1.25)	Additivity

^aIn experiment 1, synergy observed at 50% and 75% effective level at 1:160 ratio of ASV and HCV-796 and at 90% at the 1:1000 ratio

^bIn experiment2, synergy observed at 50%, additivity observed at 75% and 90% effective levels

Supplementary Table 3: Activity of ASV in combination with NM-107, a NS5B nucleoside inhibitor

Exp	ASV EC ₅₀ , μM	NM-107 EC ₅₀ , μM	Ratio, NM-107 to ASV	Combination Indices (Confidence Interval)			Overall Result
				50% Effective	75% Effective	90% Effective	
1 ^a	0.0015	1.375	1:80	0.82 (0.75, 0.89)	0.90 (0.79, 1.01)	1.00 (0.81, 1.18)	Synergy/Additivity
			1:200	0.85 (0.78, 0.93)	0.90 (0.79, 1.01)	0.96 (0.78, 1.14)	Synergy/Additivity
			1:500	0.88 (0.81, 0.96)	0.89 (0.78, 0.99)	0.91 (0.74, 1.08)	Synergy/Additivity
2	0.0007	1.094	1:80	0.85 (0.78, 0.91)	0.90 (0.80, 1.00)	0.96 (0.79, 1.12)	Synergy/Additivity ^b
			1:200	1.01 (0.93, 1.09)	1.04 (0.93, 1.15)	1.07 (0.89, 1.25)	Additivity
			1:500	0.95 (0.87, 1.02)	0.93 (0.83, 1.02)	0.91 (0.76, 1.06)	Additivity

^aIn experiment 1, synergy observed at 50% effective level at all drug ratios and at 75% at the 1:500 ratio of ASV and NM-107

^bIn experiment 2, synergy was observed at 50% and 75%, additivity was observed at 90% effective level

Supplementary Table 4: Triple combination studies using ASV, daclastavir and rIFN α

Exp ^a	ASV EC ₅₀ , nM	DCV EC ₅₀ , nM	rIFN α EC ₅₀ , units per mL	Combination Indices (Confidence Interval)			Overall Result
				50% Effective	75% Effective	90% Effective	
1	1.5	0.005	6.4	0.99 (0.89, 1.08)	0.87 (0.76, 0.98)	0.79 (0.64, 0.95)	Additivity/Synergy ^b
2	1.3	0.005	7.0	0.93 (0.85, 1.02)	0.81 (0.71, 0.91)	0.72 (0.58, 0.85)	Additivity/Synergy ^b
3	2.2	0.006	5.9	0.88 (0.77, 0.99)	0.81 (0.69, 0.94)	0.79 (0.61, 0.97)	Synergy
4	1.9	0.005	2.3	0.98 (0.86, 1.10)	0.83 (0.68, 0.97)	0.75 (0.55, 0.96)	Additivity/Synergy ^b

^aCompounds tested at molar ratio of 250:1:500, ASV to daclastavir to rIFN α

^bIn experiments 1, 2, and 4 additivity observed at 50%, synergy observed at 75% and 90% effective levels

Supplementary Table 5: Triple combination studies using ASV, BMS-791325 and rIFN α

Exp ^a	ASV EC ₅₀ , nM	'325 EC ₅₀ , nM	rIFN α EC ₅₀ , units per mL	Combination Indices (Confidence Interval)			Overall Result
				50% Effective	75% Effective	90% Effective	
1	2.5	4.6	4.5	0.92 (0.82, 1.02)	0.94 (0.80, 1.07)	0.98 (0.76, 1.20)	Additivity
2	2.4	6.5	6.5	0.98 (0.90, 1.07)	0.91 (0.81, 1.02)	0.88 (0.72, 1.04)	Additivity

^aCompounds tested at molar ratio of 1:4:2, ASV to '325 (BMS-791325) to rIFN α

Supplementary Figure 1. Three-dimensional synergy plot representing the MacSynergy combination analysis of ASV with ribavirin. Representative graph from three independent experiments depicting the interaction between ASV and ribavirin using the MacSynergy II software developed by Pritchard and Shipman (University of Michigan, 2003) for data analysis.

