

Supplemental materials for “Preclinical Characterization of BMS-791325, an Allosteric Inhibitor of Hepatitis C Virus NS5B Polymerase” (Lemm J, *et al.*)

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Table S1. Triple Combination Using BMS-791325 ('325) With IFN α and Ribavirin (RBV)

Expt	'325 EC ₅₀ , nM	IFN α EC ₅₀ , U/mL	RBV EC ₅₀ , nM	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	4.4	1.6	24566	0.97 (0.85, 1.09)	0.97 (0.80, 1.14)	1.02 (0.74, 1.29)	Additivity
2	4.3	7.1	45323	1.04 (0.95, 1.13)	0.95 (0.84, 1.06)	0.88 (0.73, 1.04)	Additivity
3	4.1	9.1	53362	0.93 (0.84, 1.01)	0.82 (0.72, 0.93)	0.76 (0.61, 0.91)	Additivity/ Synergy

Table S2. Combination of BMS-791325 ('325) With the NS3 Protease Inhibitor Asunaprevir (ASV)

Expt	'325 EC ₅₀ , uM	ASV EC ₅₀ , nM	ASV:'325	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	0.003	0.7	1:1	1.05 (0.98, 1.12)	0.96 (0.86, 1.06)	0.88 (0.74, 1.02)	Additivity
			2.5:1	0.95 (0.87, 1.03)	0.99 (0.88, 1.11)	1.04 (0.84, 1.23)	Additivity
			1:2.5	0.98 (0.91, 1.06)	0.99 (0.88, 1.09)	0.99 (0.82, 1.15)	Additivity
2	0.003	0.7	1:1	1.04 (0.96, 1.12)	0.95 (0.84, 1.05)	0.86 (0.71, 1.01)	Additivity
			2.5:1	0.97 (0.89, 1.05)	1.00 (0.88, 1.12)	1.03 (0.84, 1.22)	Additivity
			1:2.5	0.97 (0.89, 1.05)	0.98 (0.87, 1.10)	1.00 (0.82, 1.18)	Additivity
3	0.004	0.9	1:1	1.00 (0.92, 1.07)	0.96 (0.86, 1.06)	0.92 (0.77, 1.08)	Additivity
			2.5:1	1.07 (1.00, 1.14)	1.00 (0.91, 1.09)	0.93 (0.80, 1.07)	Additivity
			1:2.5	1.02 (0.93, 1.11)	1.03 (0.90, 1.16)	1.04 (0.84, 1.24)	Additivity

Table S3. Combination of BMS-791325 ('325) With the NS5A Inhibitor Daclatasvir (DCV)

Expt	DCV EC ₅₀ , nM	'325 EC ₅₀ , uM	'325:DCV	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	0.010	0.015	1:1	0.88 (0.81, 0.95)	0.83 (0.73, 0.93)	0.79 (0.64, 0.94)	Synergy
			1:2.5	0.80 (0.72, 0.88)	0.79 (0.68, 0.89)	0.78 (0.62, 0.94)	Synergy
			2.5:1	0.97 (0.90, 1.04)	0.97 (0.87, 1.07)	0.96 (0.80, 1.13)	Additivity
2	0.007	0.013	1:1	0.81 (0.76, 0.86)	0.88 (0.80, 0.95)	0.95 (0.82, 1.08)	Synergy / Additivity
			1:2.5	0.66 (0.62, 0.70)	0.65 (0.59, 0.72)	0.65 (0.55, 0.75)	Synergy
			2.5:1	0.95 (0.88, 1.01)	0.98 (0.90, 1.07)	1.02 (0.88, 1.16)	Additivity
3	0.006	0.010	1:1	0.85 (0.77, 0.93)	0.88 (0.77, 0.98)	0.90 (0.73, 1.08)	Synergy / Additivity
			1:2.5	0.84 (0.79, 0.90)	0.81 (0.72, 0.91)	0.79 (0.64, 0.93)	Synergy
			2.5:1	0.92 (0.85, 0.99)	0.92 (0.82, 1.01)	0.92 (0.77, 1.06)	Synergy / Additivity

Table S4. Combination of BMS-791325 ('325) With the Palm Site 2 NS5B Inhibitor HCV-796

Expt	'325 EC ₅₀ , uM	HCV-796 EC ₅₀ , uM	HCV- 796:'325	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	0.005	0.013	1:1	0.81 (0.77, 0.85)	0.76 (0.70, 0.81)	0.70 (0.63, 0.78)	Synergy
			2.5:1	0.82 (0.78, 0.87)	0.79 (0.73, 0.84)	0.75 (0.66, 0.83)	Synergy
			1:2.5	0.88 (0.84, 0.91)	0.78 (0.73, 0.84)	0.70 (0.63, 0.78)	Synergy
2	0.004	0.008	1:1	0.79 (0.75, 0.83)	0.66 (0.61, 0.71)	0.55 (0.48, 0.62)	Synergy
			2.5:1	0.79 (0.75, 0.84)	0.70 (0.65, 0.76)	0.62 (0.54, 0.70)	Synergy
			1:2.5	0.76 (0.72, 0.81)	0.69 (0.64, 0.75)	0.63 (0.55, 0.71)	Synergy
3	0.003	0.011	1:1	0.83 (0.76, 0.90)	0.73 (0.64, 0.82)	0.64 (0.52, 0.77)	Synergy
			2.5:1	0.74 (0.68, 0.80)	0.71 (0.63, 0.80)	0.69 (0.57, 0.82)	Synergy
			1:2.5	0.88 (0.80, 0.95)	0.84 (0.74, 0.93)	0.80 (0.66, 0.94)	Synergy

Table S5. Combination of BMS-791325 ('325) With the NS5B Nucleoside Inhibitor NM-283

Expt	'325 EC ₅₀ , uM	NM-283 EC ₅₀ , uM	NM- 283:'325	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	0.003	1.2	1:1	0.95 (0.85, 1.05)	0.88 (0.75, 1.01)	0.81 (0.63, 1.00)	Additivity / Synergy
			2.5:1	0.77 (0.68, 0.86)	0.84 (0.70, 0.98)	0.92 (0.68, 1.15)	Synergy / Additivity
			1:2.5	0.80 (0.71, 0.89)	0.82 (0.69, 0.95)	0.85 (0.64, 1.06)	Synergy / Additivity
2	0.003	1.3	1:1	0.53 (0.47, 0.59)	0.59 (0.50, 0.69)	0.67 (0.50, 0.83)	Synergy
			2.5:1	0.44 (0.39, 0.50)	0.53 (0.44, 0.62)	0.64 (0.47, 0.80)	Synergy
			1:2.5	0.43 (0.37, 0.49)	0.59 (0.47, 0.70)	0.80 (0.56, 1.04)	Synergy / Additivity
3	0.003	1.0	1:1	0.76 (0.68, 0.84)	0.76 (0.65, 0.87)	0.75 (0.58, 0.93)	Synergy
			2.5:1	0.77 (0.69, 0.85)	0.75 (0.64, 0.86)	0.73 (0.57, 0.90)	Synergy
			1:2.5	0.60 (0.54, 0.66)	0.63 (0.54, 0.71)	0.66 (0.52, 0.80)	Synergy

Table S6. Triple Combination Using BMS-791325 ('325) With the NS5A inhibitor Daclatasvir (DCV) and the NS3 Protease Inhibitor Asunaprevir (ASV)

Expt	'325 EC ₅₀ , nM	DCV EC ₅₀ , nM	ASV EC ₅₀ , nM	Combination Indices (confidence interval)			Overall Result
				50% effective	75% effective	90% effective	
1	5.4	0.003	1.2	1.00 (0.92, 1.08)	1.05 (0.93, 1.17)	1.11 (0.91, 1.30)	Additivity
2	2.3	0.002	0.7	0.93 (0.85, 1.01)	1.01 (0.88, 1.14)	1.10 (0.88, 1.31)	Additivity
3	2.8	0.002	0.8	1.01 (0.94, 1.08)	0.97 (0.88, 1.06)	0.93 (0.79, 1.07)	Additivity

'325, BMS-791325; DCV, daclatasvir; ASV, asunaprevir.

Database Consensus Sequences for GT 2b, 3a, 4a, 5a and 6a NS5B Proteins

GT-2b

MSTSYSWTGALITPCGPEEEKLPINPLSNLSLMRFHNKVYSTTSRSASQRAKKVTFDRVQVLDTHYDSVLQDVKRA
ASKVSARLLSVEEACALTPPHSAKSRYGFGAKEVRSLRRAVNHRSVWEDLLEDQHTPIDTTIMAKNEVFCVDPA
KGGKKSARLIVYDGLGVRVCEKMALYDIAQKLPKAVMGSSYGFQYSPAERVDFLLKAWGSKKDPMGFSYDTRCF
DSTVTERDIRTEESIQACSLPQEARTVIHSLTERLYVGGPMINSKGQSCGYRRCRASGVFTTSMGNTMTCYIKAL
AACKAAGIVDPIMLVCGDDLVIIVISESQGNEEDERNLRAFTEAMTRYSAPPGDLPRPEYDLELITSCSSNVSVLDP
RGRRRYYLTRDPTTPISSRAAWETVRHSPVNSWLGNIQYAPTIWVRMVIMTHFFAILLAQDTLNQNLNFEMYGAVY
SVNPLDLPALIERIHGLDAFSLHTYSPHELRSVAATLRKLGAPPLRAWKSRARAVRASLIAQGGGRASVCGRYLFNW
AVRTKCLKLTPLPEASRLDLSG WFTVGAGGGDIFHSVSHARPRLLL

GT-3a

MSMSYSWTGALITPCSAEEEEKLPISPLSNLSLLRHHNLVYSTSSRSASQRQKKVTFDRLQVLDDHYKTVLQEVKER
ASRVKARTLTIEEACALVPPHSARSKFGYSAKDVRSLSSKAINQIRSVWEDLLEDTTTTPIPTTIMAKNEVFCVDPTK
GGRKPTRLIVYDGLGVRVCEKRALYDVIQKLSIETMGPAYGFQYSPQQRVERLLKMWTSKKTPLGFSYDTRCFDS
TVTEQDIRVEEIIYQCCNLEPEARKVISSLTERLYCGGPMFNSKGAQCCGYRRCRASGVLPSTFGNTITCYIKATAA
ARAAGLRNPEFLVCGDDLVIIVAESDGVDEDRATLRAFTEAMTRYSAPPGDAPQPTYDLELITSCSSNVSVARDDK
GKRYYYLTRDATTPLARAAWETARHTPVNSWLGNIIMYAPTIWVRMVMMTHFFSILQSQEILDRPLDFEMYGATYS
VTPLDLPALIERLHGLSAFTLHSPVELNRVAGTLRKLGCPLRAWRHRARAVRAKLIQGGKAKICGLYLFNWA
VRTKTKLTPLPATGQLDLSSWFTVGVGNDIYHSVSRARTRYLL

GT-4a

MSMSYSWTGALVTPCAAEEKLPISPLSNLSLLRHHNMVYATTTSAVTRQKKVTFDRLQVVDNHYNELTKEIKAR
ASRVKARLLTTEEACDLTPPHSAKSKFGYGAKDVRSHSRKAINHINSVWEDLLEDNNTPIPTTIMAKNEVFAVNPA
KGGKPARLIVYDGLGVRVCEKRALHDVINQLPKAVMGAAAGFYSPAQRVEFLLTSWKSCKTPMGFSYDTRCF
DSTVTEKDIRTEEEVYQCCDLEPEARKVIAALTERLYVGGPMHNSKGDLCGYRRCRASGVYTTTSGNTLTTCYLKA
TAAIKAAGLRDCTMLVCGDDLVIIVAESDGVVEDNRALRAFTEAMTRYSAPPGDAPQPAYDLELITSCSSNVSVAH
DATGKKVYYLTRDPETPLARAAWETVRHTPVNSWLGNIIVYAPTIWVRMVLMTTHFFSILQSQEALDFDYMVG
TYSITPLDLPALIIQRLHGLSAFTLHGYSPELNRVAGSLRKLGVPLRAWRHRARAVRAKLIQGGKAKICGIYLFN
WAVKTKLKLTPLPAAANLDLSSWFTVGAGGGDIYHSVSHARPRYLL

GT-5a

MSMSYSWTGALITPCSADEEKLPINPLSNLTLRHHNLVYSTSSRSAGLRQKKVTFDRLQVLDDHYREVVDKMKRL
ASKVKARLLPLEEACGLTPPHSARSKYGYGAKEVRSLDKKALNHKGVWQDLDLSDTPIPTTIMAKNEVFAVEPS
KGGKPARLIVYDGLGVRVCEKRALYDVAQKLPTALMGPSYGFQYSPAQRVEFLLKAWKSKKTPMAFSYDTRCF
DSTVTEHDIMTEESIQSCDLQPEARAAIRSLTQRLYCGGPMYNSKGQCCGYRRCRASGVFTTSMGNTMTCYIK
ALASCRAAKLRDCTLLVCGDDLVAICESQGTHEDEASLRAFTEAMTRYSAPPGDPPVPAYDLELVTSCSSNVSV
RDASGNRVYYLTRDPQVPLARAAWETAKHSPVNSWLGNIIMYAPTLWARIVLMTTHFFSVLQSQEQLKALAFEMY
GSVYSVTPDLPALIIQRLHGLSAFSLHSPSEINRVASCLRKLGVPLRAWRHRARAVRAKLIQGGRAAICGIYL
FNWAVKTKRKLTPADADRLDLSSWFTVGAGGGDIYHSMSRARPRYLL

GT-6a

MSMSYSWTGALITPCAAEEEEKLPINPLSNLSLRHHNMVYSTTSRSASLRQKKVTFDRVQVFDQHYQDVLKEIKLRA
STVQAKLLSIEEACDLTPSHSARSKYGYGAQDVRSHASKAVNHRSVWEDLLEDSDTPIPTTIMAKNEVFCVDPSK
GGRKPARLIVYDGLGVRVCEKMALYDVTRKLPQAVMGSAAYGFQYSPNQRVEYLLKMWRSKKVPMPGFSYDTRCF

DSTVTERDIRTENDIYQSCQLDPVARRAVSSLTERLYVGGPMVNSKGGQSCGYRRCRASGVLPTSMGNTLTCYLK
AQAACRAANIKDCDMLVCGDDL VVICESAGVQEDTASLRAFTDAMTRY SAPPGDAPQPTYDLELITSCSSNV SVA
HDGNGKRYYYLTRDCTT PLARAAWETARHTPVNSWLGNII MFAPTIWVRMVL MTHFFSILQSQEQLKALDFDIYG
VTYSVSPLDLP AIIQRLHGMAAFSLHGYS PVELNRVGACLRKLGVPPLRAW RHRARAVRAKLI AQGGKAAICGKYL
FNWAVKTKLKL TPLVSASKLDLSGWFVAGYDGGDIYHSVSQARPRLLL