Summary of polymerase acidic protein (PA) amino acid substitutions assessed for their effects on PA inhibitor (PAI) baloxavir susceptibility*

Type/subtype	Amino acid substitution	Baloxavir susceptibility by phenotypic assay (EC_{50} fold-change)[a]	Source of viruses[b]	References
A(H1N1)	E18G	**10**	RG	(32)
	E23K	**5−17**	RG	(1, 32)
	A36V	**3.6**	RG	(1)
	I38F	**11**	RG	(1)
	I38L	**6**	RG	(2)
	I38M	**13**	RG	(1)
	I38N	**24**	RG	(2, 3)
	I38S	**12**	RG	(2)
	I38T	**27−54**	RG; Cell/BXA	(1, 4, 5, 32)
	I38V	2.2	RG	(1)
	E119D	**6**	RG	(1)
	E198K	2.5	RG	(6)
A(H1N1)pdm09	E23G	**3.7−7**	Sur; RG	(7-10)
	E23K	**7−13**	Sur/No; RG	(9-11)
	E23R	**13**	RG	(12)
	K34R	1.6−**5**	Sur	(9)
	A37T	**5**	Sur/BXA	(13)
	I38F	**7−17**	RG	(14, 15)
	I38L	**7−12**	Sur/No; RG	(7, 8, 16, 17)
	I38M	**7−29**	RG; Mice/BXA	(14, 15, 18)
	I38S	**31−112**	Cell/BXA; Clin/BXA; Sur/BXA	(9, 11, 16, 19)
	I38T	**11−124**	RG; Cell/BXA; Clin/BXA; Sur/BXA	(9, 11, 14-16, 20, 21)
	I38V	2.2−**3.7**	Sur/No	(7, 9)
	E198K	1.8	RG	(6)
	E199D	2.9	RG	(17)
	E199G	0.5−**3.7**	Mice/BXA; Sur	(9, 18)
A(H3N2)	L28P	0.4−2.6	RG; Sur	(1, 9)
	E23G[c]	1.8−2.4	RG	(2, 3, 10)
	E23K	**6**	RG	(1, 10)
	E23R	**19**	RG	(12)
	K34R	**3.7−4**	Sur	(9)
	A36V	**6**	RG	(1)
	A37T	**8**	RG	(1)
	I38F	**16−20**	RG	(1, 14, 15)
	I38L	2.2−**8**	RG; Sur	(2, 9)
	I38M	**3.7−24**	RG; Sur/No; Sur/BXA	(1, 3, 7-9, 14-16)
	I38N	**10**	RG	(2)
	I38S	**6**	RG	(2)
	I38T	**20−614**	RG; Clin/BXA; Cell/No; Sur/BXA; Sur/No	(1, 3, 9, 15, 16, 19-26)
	I38V	0.4−1.8	RG; Sur/No	(1, 7)
	E119D	**5**	RG	(1)
	E198K	**6**	RG	(6)
	E199G	**3.4−7**	RG; Sur/No	(1, 29)
A(H5N1)	A37T	**5−6**	Sur/No	(30)
	I38F	**24**	RG	(31)
	I38M	**6−16**	Sur/No; RG	(30, 31)

	I38T	**48−108**	Sur/No; RG	(30, 31)
B	E23K	0.8−2.6	RG	(1, 27)
	M34I	0.6	Sur	(9)
	F36V[d]	0.8	RG	(1)
	I38F	2.4−**8**	RG	(1, 15)
	I38M	1.7−**8**	RG	(1, 14, 15, 27)
	I38T	**5−15**	RG	(1, 14, 15, 27)
	I38V	0.9−1.9	Sur/No	(7, 9)
	E120D[e]	2.0−2.6	RG	(1, 28)
	G199R	2.0	RG	(17)

[*] Additional amino acid substitutions in PA, which conferred no change in baloxavir susceptibility, were investigated in references #1 (Omoto S et al., 2018) and #2 (Hashimoto T et al., 2020).

[a] Assessed by cell culture-based assays (focus, plaque or yield reduction assays, high-content imaging neutralization (HINT) and ViroDot assay). EC_{50} fold-change was calculated compared to sequence-matched control virus or type/subtype-specific median EC_{50}. Fold-change values < 4 are shown as reported (to one decimal point [no rounding up]), while fold-change values > 4 are rounded up. A fold-change value > 3-fold is provisionally considered as reduced susceptibility to baloxavir. Values of 3 and above are shown in bold.

[b] Cell, Cell culture; Clin, Clinical trial; Mice, mouse model; RG, Reverse Genetics; Sur, Surveillance studies; BXA, Substitution selected under baloxavir pressure; No, baloxavir not used.

[c] E23G in A(H3N2) subtype was detected in a baloxavir-treated patient in a clinical trial (T0831). RG virus with E23G was tested by phenotypic assay.

[d] Corresponds to A36V in influenza type A PA.

[e] Corresponds to E119D in influenza type A PA.

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