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)	E23K	5	RG	(1)
	A36V	4	RG	(1)
	I38F	8–11	RG	(1)
	I38L	6	RG	(2)
	I38M	13	RG	(1)
	I38N	24	RG	(2, 3)
	I38S	12	RG	(2)
	I38T	22–54	RG; Cell/BXA	(1, 4, 5)
	I38V	2	RG	(1)
	E119D	7	RG	(1)
A(H1N1)pdm09	E23G	4–7	Sur	(6, 7, 22)
	E23K	7–9	Sur/No	(8, 22)
	K34R	2–5	Sur	(22)
	A37T	5	Sur/BXA	(23)
	I38F	7–17	RG	(9, 10)
	I38L	7–9	Sur/No	(6, 7, 11)
	I38M	7– 9	RG; Mice/BXA	(9, 10, 12)
	I38S	31–112	Cell/BXA; Clin/BXA; Sur/BXA	(8, 11, 13, 22)
	I38T	11–124	RG; Cell/BXA; Clin/BXA; Sur/BXA	(8–11, 14, 15, 22)
	I38V	2–4	Sur/No	(6, 22)
	E199G	1–4	Mice/BXA; Sur	(12, 22)
A(H3N2)	L28P	1–3	RG; Sur	(1, 22)
	E23G[c]	2–3	RG	(2, 3)
	E23K	6	RG	(1)
	K34R	4	Sur	(22)
	A36V	6	RG	(1)
	A37T	8	RG	(1)
	I38F	16–20	RG	(1, 9, 10)
	I38L	2–8	RG; Sur	(2, 22)
	I38M	4–24	RG; Sur/No; Sur/BXA	(1, 3, 6, 7, 9–11, 22)
	I38N	10	RG	(2)
	I38S	6	RG	(2)
	I38T	20–14	RG; Clin/BXA; Cell/No; Sur/BXA; Sur/No	(1, 3, 10, 11, 13–20, 22)
	I38V	1–2	RG; Sur/No	(1, 6)
	E119D	5	RG	(1)
	E199G	5	RG	(1)
B	E23K	1–3	RG	(1, 21)
	M34I	1	Sur	(22)
	F36V[d]	1	RG	(1)
	I38F	2–8	RG	(1, 10)
	I38M	2–8	RG	(1, 9, 10, 21)
	I38T	5–15	RG	(1, 9, 10, 21)
	I38V	1–2	Sur/No	(6, 22)
	E120D[e]	2	RG	(1)

[*] 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 matched control virus or type/subtype-specific median EC_{50}.
[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.

References

1. Omoto S, Speranzini V, Hashimoto T, Noshi T, Yamaguchi H, Kawai M, Kawaguchi K, Uehara T, Shishido T, Naito A, Cusack S. 2018. Characterization of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil. Sci Rep 8:9633.
2. Hashimoto T, Baba K, Inoue K, Okane M, Hata S, Shishido T, Naito A, Wildum S, Omoto S. 2020. Comprehensive assessment of amino acid substitutions in the trimeric RNA polymerase complex of influenza A virus detected in clinical trials of baloxavir marboxil. Influenza Other Respir Viruses doi:10.1111/irv.12821.
3. Ince WL, Smith FB, O'Rear JJ, Thomson M. 2020. Treatment-Emergent Influenza Virus Polymerase Acidic Substitutions Independent of Those at I38 Associated With Reduced Baloxavir Susceptibility and Virus Rebound in Trials of Baloxavir Marboxil. J Infect Dis 222:957-961.
4. Noshi T, Kitano M, Taniguchi K, Yamamoto A, Omoto S, Baba K, Hashimoto T, Ishida K, Kushima Y, Hattori K, Kawai M, Yoshida R, Kobayashi M, Yoshinaga T, Sato A, Okamatsu M, Sakoda Y, Kida H, Shishido T, Naito A. 2018. In vitro characterization of baloxavir acid, a first-in-class cap-dependent endonuclease inhibitor of the influenza virus polymerase PA subunit. Antiviral Res 160:109-117.
5. Takashita E, Morita H, Ogawa R, Nakamura K, Fujisaki S, Shirakura M, Kuwahara T, Kishida N, Watanabe S, Odagiri T. 2018. Susceptibility of Influenza Viruses to the Novel Cap-Dependent Endonuclease Inhibitor Baloxavir Marboxil. Front Microbiol 9:3026.
6. Gubareva LV, Mishin VP, Patel MC, Chesnokov A, Nguyen HT, De La Cruz J, Spencer S, Campbell AP, Sinner M, Reid H, Garten R, Katz JM, Fry AM, Barnes J, Wentworth DE. 2019. Assessing baloxavir susceptibility of influenza viruses circulating in the United States during the 2016/17 and 2017/18 seasons. Euro Surveill 24: 1800666.
7. Takashita E, Daniels RS, Fujisaki S, Gregory V, Gubareva LV, Huang W, Hurt AC, Lackenby A, Nguyen HT, Pereyaslov D, Roe M, Samaan M, Subbarao K, Tse H, Wang D, Yen HL, Zhang W, Meijer A. 2020. Global update on the susceptibilities of human influenza viruses to neuraminidase inhibitors and the cap-dependent endonuclease inhibitor baloxavir, 2017-2018. Antiviral Res 175:104718.
8. Takashita E, Abe T, Morita H, Nagata S, Fujisaki S, Miura H, Shirakura M, Kishida N, Nakamura K, Kuwahara T, Mitamura K, Ichikawa M, Yamazaki M, Watanabe S, Hasegawa H, Influenza Virus Surveillance Group of J. 2020. Influenza A(H1N1)pdm09 virus exhibiting reduced susceptibility to baloxavir due to a PA E23K substitution detected from a child without baloxavir treatment. Antiviral Res 180:104828.
9. Koszalka P, Tilmanis D, Roe M, Vijaykrishna D, Hurt AC. 2019. Baloxavir marboxil susceptibility of influenza viruses from the Asia-Pacific, 2012-2018. Antiviral Res 164:91-96.
10. Jones JC, Pascua PNQ, Fabrizio TP, Marathe BM, Seiler P, Barman S, Webby RJ, Webster RG, Govorkova EA. 2020. Influenza A and B viruses with reduced baloxavir susceptibility display attenuated in vitro fitness but retain ferret transmissibility. Proc Natl Acad Sci U S A 117:8593-8601.
11. Chesnokov A, Patel MC, Mishin VP, De La Cruz JA, Lollis L, Nguyen HT, Dugan V, Wentworth DE, Gubareva LV. 2020. Replicative Fitness of Seasonal Influenza A Viruses With Decreased Susceptibility to Baloxavir. J Infect Dis 221:367-371.
12. Kiso M, Yamayoshi S, Murakami J, Kawaoka Y. 2020. Baloxavir Marboxil Treatment of Nude Mice Infected With Influenza A Virus. J Infect Dis 221:1699-1702.
13. Sato M, Takashita E, Katayose M, Nemoto K, Sakai N, Hashimoto K, Hosoya M. 2020. Detection of Variants With Reduced Baloxavir Marboxil Susceptibility After Treatment of Children With Influenza A During the 2018-2019 Influenza Season. J Infect Dis 222:121-125.
14. Checkmahomed L, M'Hamdi Z, Carbonneau J, Venable MC, Baz M, Abed Y, Boivin G. 2020. Impact of the Baloxavir-Resistant Polymerase Acid I38T Substitution on the Fitness of Contemporary Influenza A(H1N1)pdm09 and A(H3N2) Strains. J Infect Dis 221:63-70.
15. Imai M, Yamashita M, Sakai-Tagawa Y, Iwatsuki-Horimoto K, Kiso M, Murakami J, Yasuhara A, Takada K, Ito M, Nakajima N, Takahashi K, Lopes TJS, Dutta J, Khan Z, Kriti D, van Bakel H, Tokita A, Hagiwara H, Izumida N, Kuroki H, Nishino T, Wada N, Koga M, Adachi E, Jubishi D, Hasegawa H, Kawaoka Y. 2020. Influenza A variants with reduced susceptibility to baloxavir isolated from Japanese patients are fit and transmit through respiratory droplets. Nat Microbiol 5:27-33.
16. Takashita E, Kawakami C, Morita H, Ogawa R, Fujisaki S, Shirakura M, Miura H, Nakamura K, Kishida N, Kuwahara T, Mitamura K, Abe T, Ichikawa M, Yamazaki M, Watanabe S, Odagiri T, On Behalf Of The Influenza Virus

Surveillance Group Of J. 2019. Detection of influenza A(H3N2) viruses exhibiting reduced susceptibility to the novel cap-dependent endonuclease inhibitor baloxavir in Japan, December 2018. Euro Surveill 24:1800698.
17. Takashita E, Kawakami C, Ogawa R, Morita H, Fujisaki S, Shirakura M, Miura H, Nakamura K, Kishida N, Kuwahara T, Ota A, Togashi H, Saito A, Mitamura K, Abe T, Ichikawa M, Yamazaki M, Watanabe S, Odagiri T. 2019. Influenza A(H3N2) virus exhibiting reduced susceptibility to baloxavir due to a polymerase acidic subunit I38T substitution detected from a hospitalised child without prior baloxavir treatment, Japan, January 2019. Euro Surveill 24:1900170.
18. Takashita E, Ichikawa M, Morita H, Ogawa R, Fujisaki S, Shirakura M, Miura H, Nakamura K, Kishida N, Kuwahara T, Sugawara H, Sato A, Akimoto M, Mitamura K, Abe T, Yamazaki M, Watanabe S, Hasegawa H, Odagiri T. 2019. Human-to-Human Transmission of Influenza A(H3N2) Virus with Reduced Susceptibility to Baloxavir, Japan, February 2019. Emerg Infect Dis 25:2108-2111.
19. Uehara T, Hayden FG, Kawaguchi K, Omoto S, Hurt AC, De Jong MD, Hirotsu N, Sugaya N, Lee N, Baba K, Shishido T, Tsuchiya K, Portsmouth S, Kida H. 2020. Treatment-Emergent Influenza Variant Viruses With Reduced Baloxavir Susceptibility: Impact on Clinical and Virologic Outcomes in Uncomplicated Influenza. J Infect Dis 221:346-355.
20. Yano T, Ochiai H, Akachi S, Matsumura Y. 2020. Polymerase Acidic Subunit I38T Mutant Influenza A(H3N2) Virus Isolated from a Pediatric Patient without Prior Baloxavir Marboxil Treatment in Mie Prefecture (November 2018). Jpn J Infect Dis 73:383-385.
21. Abed Y, Fage C, Checkmahomed L, Venable MC, Boivin G. 2020. Characterization of contemporary influenza B recombinant viruses harboring mutations of reduced susceptibility to baloxavir marboxil, in vitro and in mice. Antiviral Res 179:104807.
22. Govorkova EA, Takashita E, Daniels RS, Fujisaki S, Presser LD, Patel MC, Huang W, Lackenby A, Nguyen HT, Pereyaslov D, Rattigan A, Brown SK, Samaan M, Subbarao K, Wong S, Wang D, Webby RJ, Yen HL, Zhang W, Meijer A, Gubareva LV. 2022. Global update on the susceptibilities of human influenza viruses to neuraminidase inhibitors and the cap-dependent endonuclease inhibitor baloxavir, 2018-2020. Antiviral Res 200: 105281.
23. Sato M, Takashita E, Katayose M, Nemoto K, Sakai N, Fujisaki S, Hashimoto K, Hosoya M. 2021. Detection of variants with reduced baloxavir marboxil and oseltamivir susceptibility in children with influenza A during the 2019-2020 influenza season. J Infect Dis 224:1735-1741.