

# Tecovirimat Resistance in Mpox Patients, United States, 2022–2023

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During the 2022 multinational outbreak of monkeypox virus (MPXV) infection, the antiviral drug tecovirimat (TPOXX; SIGA Technologies, Inc., <https://www.siga.com>) was deployed in the United States on a large scale for the first time. The MPXV F13L gene homologue encodes the target of tecovirimat, and single amino acid changes in F13 are known to cause resistance to tecovirimat. Genomic sequencing identified 11 mutations previously reported to cause resistance, along with 13 novel mutations. Resistant phenotype was determined using a viral cytopathic effect assay. We tested 124 isolates from 68 patients; 96 isolates from 46 patients were found to have a resistant phenotype. Most resistant isolates were associated with severely immunocompromised mpox patients on multiple courses of tecovirimat treatment, whereas most isolates identified by routine surveillance of patients not treated with tecovirimat remained sensitive. The frequency of resistant viruses remains relatively low (<1%) compared with the total number of patients treated with tecovirimat.

In May 2022, an outbreak of mpox disease, caused by infection with monkeypox virus (MPXV) clade IIb (formerly West Africa clade), was identified in the United States (1). Since that time, >30,000 cases and 46 deaths associated with the outbreak have been identified in the United States. As a result of effective education, vaccination, and case identification, US cases

peaked the first week of August 2022 at 459 cases per week. The United States has identified more cases than any other country in the global outbreak (2).

The FDA licensed the therapeutic agent TPOXX (SIGA Technologies, Inc., <https://www.siga.com>) containing the drug tecovirimat (i.e., ST-246) under the animal rule for smallpox treatment in 2018 (3). Tecovirimat has been tested extensively in cell culture (4–6) and within many orthopoxvirus (OPXV) animal models (7–15), including the nonhuman primate variola virus (VARV) model (16,17). After MPXV clade IIb emerged in 2022, Warner et al. demonstrated tecovirimat efficacy against the outbreak strain (lineage B.1) in a nonlethal mouse model (18). Although tecovirimat has shown efficacy against multiple OPXVs, researchers have noted that nucleotide alterations to the orthopoxviral F13L gene homologue leading to amino acid substitutions in the F13 protein (also known as VP37) allow for resistance (4,19). In addition, resistance emerged during use of tecovirimat in an extended treatment course of a patient with progressive vaccinia (20).

Because TPOXX is only licensed for treatment of smallpox, the Centers for Disease Control and Prevention (CDC) holds an expanded access investigational new drug protocol for treatment of nonvariola OPXV infections, including mpox. Since May 2022, at least 7,563 patients have received tecovirimat for mpox treatment in the United States; a fraction of those have been severe cases where patients have moderate to severe immunocompromise usually caused by uncontrolled HIV infection (21). To test for resistance among patients who received tecovirimat, we collected specimens from 435 patients who received tecovirimat for whom resistance was possible or suspected based on clinical data (Table 1). We genotyped

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and phenotyped specimens from 68 patients and confirmed a resistant phenotype in 46 of those patients. The geographic distribution of tecovirimat resistance has conformed to the geographic distribution of the larger mpox outbreak (Figure 1). Here, we describe our investigation and findings. The activities in this report were reviewed by the Human Subjects Advisor in the National Center for Emerging and Zoonotic Diseases at the Centers for Disease Control and Prevention and determined that it does not meet the regulatory definition of research under provision 45 CFR 46.102(l); the activities fall under public health surveillance and do not require IRB review.

**Methods**

During the mpox outbreak, whole-genome metagenomic sequencing and, more recently, amplicon-based sequencing targeting the F13L gene have been used to screen for changes in the MPXV F13L homologue (Appendix 1, <https://wwwnc.cdc.gov/EID/article/29/12/23-1146-App1.pdf>). A total of 3,247 CDC-generated sequences have been screened by either passive genomic surveillance (n = 3,101) or targeted F13L sequencing (n = 146). Only genomic sequencing completed at CDC was included because the raw data were required to find minor variants. The primary outbreak strain (MPXV clade IIb lineage B.1) has a substitution, E353K, in the F13 protein that is not present in the secondary outbreak strain (lineage A.2), historical clade IIb sequences from Nigeria, or MPXV clade IIa (22). Because the E353K substitution was not previously described in other OPXV, the effect on tecovirimat phenotype was unknown.

We adapted a cytopathic effect (CPE) assay, used at CDC to test VARV sensitivity to tecovirimat, to use for MPXV as described previously (22). In brief, we used clinical specimens that were decoded but not anonymous to culture MPXV on Vero (African green monkey) cell lines (either BSC-40 or E6). We then used

**Table 1.** Surveillance for tecovirimat resistance in mpox cases, United States, 2023\*

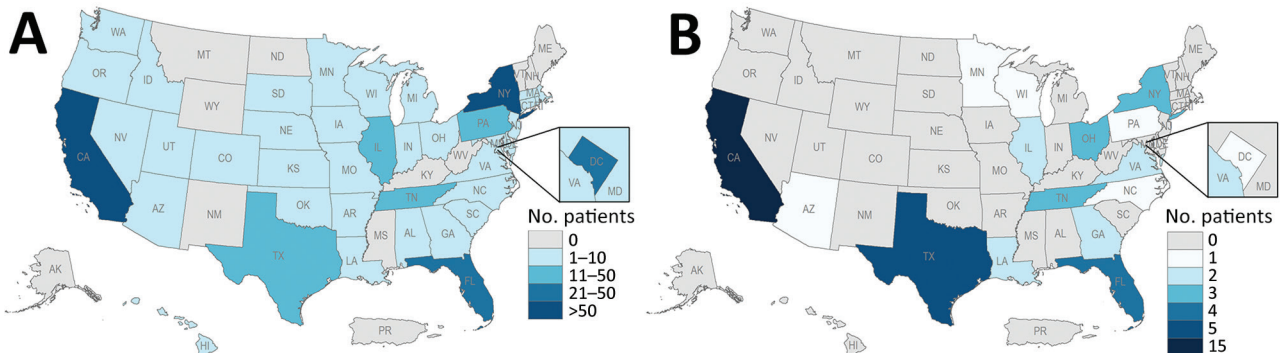
Category	No. samples or isolates	No. patients
<b>Genomic testing</b>		
Sequences analyzed	3,247	
F13 substitutions other than E353K found (Table 2)	130	76
<b>Phenotype testing</b>		
Submitted to CDC	801	435
MPXV isolated	164	83
Phenotype testing complete	124	68
Tecovirimat resistant	96	46
<b>HIV-positive</b>		
CD4+ T-cell count <350 cell/μL		31
CD4+ T-cell count <200 cell/μL		28
<b>Deceased</b>		
		10
<b>Hospitalized</b>		
		34
<b>Tecovirimat treated</b>		
		39

\*CDC, Centers for Disease Control and Prevention; MPXV, monkeypox virus.

the isolated MPXV to infect confluent Vero E6 cell monolayers pretreated for 1 h with different concentrations of tecovirimat. We incubated infected cells for 72 h at 35°C with 6% CO<sub>2</sub>. We fixed and stained wells with formalinized crystal violet and measured absorbance at 570 nm; intact cell monolayers having a high absorbance indicated that the drug was protective. We used the CPE assay to show that MPXV isolates with the E353K mutation remained sensitive to tecovirimat (18,22–24).

**Results**

In total, 130 samples from 76 patients produced sequences with amino acid changes other than E353K in the F13 protein relative to MPXV clade IIb variant B.1 reference strain (GenBank accession no. ON563414), collected in the United States in 2002 (Table 2). Isolates with amino acid substitutions D100N, D217N, D248N, D256N, and S369L identified by routine sequencing of samples from patients not treated with tecovirimat have remained sensitive (Table 2). We confirmed 11 amino acid mutations



**Figure 1.** Geographic distribution of patients with mpox who had samples received for tecovirimat resistance testing (A) and who had samples confirmed resistant (B) June 2022–July 2023, United States.

**Table 2.** MPXV F13 mutations identified from 76 patients with mpox, United States, 2023\*

Amino acid substitution	Isolates	Patients	EC <sub>50</sub> , μmol/L	Fold change†
A288P (25,26)	6	4	0.5 to >500	29 to >29,000
A288P, A290V, D294V (26)	3	1	0.66 to >500	38 to >29,000
A288P, A290V, L297ins (26)	1	1	>500	>29,000
A288P, A290V, I372N	1	1	15	880
A288P, D294V, A295E	1	1	1.4	83
A288P, D294V, D301del (26)	1	1	>500	>29,000
A288P, I372N	1	1	>150	>8,600
A290V (25,26)	9	9	0.17–43	10–2,500
A290V, I372N (25)	5	5	30–32	1,700–1,800
A295E	3	3	2.0–3.3	110–190
D100N	2	2	0.008	–2
D217N	11	11	0.007–0.012	–2.4 to –1.3
D248N	1	1	0.007	–2.4
D256N	3	3	0.009	–1.8
D283G	2	1	7.1–7.3	404–420
D294V (25)	8	7	0.23–1.4	13–78
D294V, A295E	1	1	1	59
H238Q (25)	4	4	0.54–0.6	28–34
H238Q, A288P, D294V, I372N (25)	1	1	≈5.2	≈290
H238Q, N267D, A295E	1	1	24	1,400
I372N (25)	12	9	0.04–>150	2.3 to >8600
K174N, N267D	1	1	12	720
N267D (25)	3	3	10–11	570–630
N267D, A288P (25,26)	4	3	1.2–16	71–900
N267D, A290V	1	1	2.0	110
N267D, D294V	1	1	12	680
N267D, A288P, A290V, D294V (26)	1	1	>500	>29,000
N267D, A288P, A290V, A295E, L297ins (26)	1	1	>500	>29,000
N267D, A288P, A290V, A295E, I372N	1	1	>500	>29,000
N267del (27)	8	7	1.5–4.0	85–230
N267del, N267D	1	1	Not tested	
N267del, N267D, A295E	2	2	2.9–18	160–1,000
N267del, N267D, A288P, A295E	1	1	Not tested	
N267del, N267D, D294V, A295E	1	1	2.5	140
N267del, A288P, A295E	1	1	>500	>29,000
N267del, T289A, A295E	1	1	0.26	15
N267del, A290V	1	1	0.13	7.5
N267del, A290V, I372N	1	1	3.1	180
P243S, A288P, A290V (26)	1	1	0.56	32
S215F, T289A, A290V, I372N	1	1	Not tested	
S369L	3	3	0.006	–2.9
T245I, A290V	1	1	0.17	10
T289A	3	3	0.078–0.14	3.7–7.8
T289A, I372N	1	1	Not tested	
T289A, R291K	1	1	1.7	98
Y258C	1	1	18	1,000
Y285H, I372N	1	1	0.045	2.6

\*All specimens belong to MPXV clade IIb lineage B.1 and contain E353K substitution in addition to the listed substitutions. All substitutions detected from a specimen are listed regardless of their proportion in the viral population. Insertions (ins) and deletions (del) were detected in addition to substitutions. EC<sub>50</sub>, 50% effective concentration; MPXV, monkeypox virus.

†Fold change was calculated based on the EC<sub>50</sub> of the reference strain MPXV clade IIa (U.S., 2003), which was 0.0175 μmol/L.

(H238Q, Y258C, N267D, N267del, D283G, A288P, A290V, D294V, A295E, L297ins, I372N) that were previously identified in other OPXV (19,20,25–28) as resistant by phenotypic testing (Table 2). One confirmed resistance mutation, T289A, had not been described before the 2022 mpox outbreak (26). T289A resulted in up to an 8-fold increase in the 50% effective concentration when compared with the MPXV clade IIa reference strain. This position is part of the predicted tecovirimat binding site and adjacent to A288P and A290V, which both confer resistance (19). We identified 7 other amino acid substitu-

tions (K174N, S215F, P243S, T245I, Y285H, R291K, D301del) but have not yet determined the effects of those mutations (25,26). Those mutations have been observed and tested only in combination with other resistance mutations.

Eight of 27 nonsynonymous mutations observed in F13L were GA to AA or TC to TT, which may suggest they arose through APOBEC3 editing. All the APOBEC3 motif mutations produced amino acid changes that did not affect tecovirimat resistance in culture. Resistance phenotype is currently unknown for R291K, S215F, and P243S.

For phenotype testing, we considered an isolate resistant if the increase in 50% effective concentration was  $\geq 2$ -fold compared to the 2003 MPXV clade IIa reference strain. Isolates with 2-fold to 9-fold change were considered partially resistant, and isolates with  $\geq 10$ -fold change were considered resistant (25). A total of 83 isolates from 41 patients were resistant, and 16 isolates from 11 patients were partially resistant. Four patients with partially resistant isolates also had  $\geq 1$  other isolate that was resistant. The clinical relevance of partially resistant and resistant isolates remains unknown.

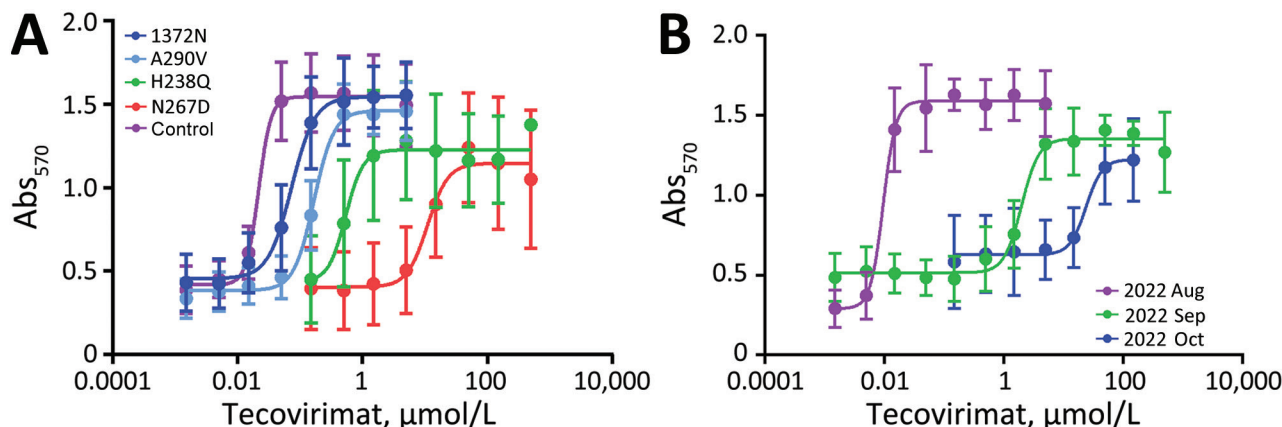
## Discussion

Multiple lines of evidence point to tecovirimat resistance developing during drug treatment in most patients. First, genome sequencing has revealed unique mutational profiles from different sample sites from the same patient (Figure 2, panel A), indicating different viral subpopulations were selected at different sites during treatment. Second, longitudinal sampling was investigated for 4 of the 46 patients with a resistant isolate and showed samples before tecovirimat treatment were sensitive, whereas later samples were resistant (Figure 2, panel B). An exception was found for 1 patient; T289A was detected in 58% of reads, along with minor populations of A295E (9%) and N267del (22%), from a sample the day before the patient started tecovirimat treatment. A second sample from the same patient after tecovirimat treatment showed the T289A mutation was selected (93%), and a new variant R291K was also detected (31%). In addition, N267del was detected in a cluster of cases in California with no known tecovirimat treatment (27).

Whether those drug-resistant infections were acquired from another person treated with tecovirimat is unknown but is a viable hypothesis. Such rare cases show that viruses with mutations in F13L resulting in tecovirimat resistance can be transmitted from person to person.

For patients that had  $\geq 1$  specimen with confirmed tecovirimat resistance, 39/46 had HIV infection; HIV status was unknown for the remaining 7 patients. Of the 39 patients with HIV infection, 31 had a CD4+ T-cell count available; all 31 were  $< 350$  cells/ $\mu\text{L}$ , and 28 were  $< 200$  cells/ $\mu\text{L}$ . Ten of the 39 patients died (25,26), and all 10 deceased patients had CD4+ T-cell counts  $< 200$  cell/ $\mu\text{L}$ . In the United States, 46 deaths associated with mpox have been reported (2). In those patients, severe immunocompromise is resulting in severe disease and death as well as tecovirimat resistance.

For patients who had  $\geq 1$  specimen with confirmed tecovirimat resistance, at least 34 of 46 patients were hospitalized. No medical history was available for 6 patients, and the medical history concerning hospitalization was not clear or did not mention hospitalization for another 6 patients. Of the 46 patients, 39 patients received tecovirimat either oral or IV; 5 patients did not receive tecovirimat (27), and 2 patients' tecovirimat status was unknown. Exact data on length of tecovirimat exposure is difficult to obtain because of possible noncompliance with oral administration and multiple rounds of treatment in which drug administration stops and starts. We estimated the average length of tecovirimat treatment using the reported start date of tecovirimat treatments. Dates were available for 28 of the 39 patients that received



**Figure 2.** Examples of tecovirimat resistance in mpox patients, United States, 2022–2023. Patient samples were sequenced, cultured, and subjected to tecovirimat sensitivity testing in a cytopathic effect assay. A) Different samples from the same patient showed different F13 amino acid substitutions that result in different levels of resistance compared with the wild-type control (MPXV clade IIa, collected in the United States in 2003 [GenBank accession no. ON563414]). B) Samples from the same patient at different times before and after starting tecovirimat treatment in August 2022, showing sensitivity before drug treatment and increasing resistance after drug treatment. Abs<sub>570</sub>, absorbance at 570 nm.

tecovirimat (Appendix Table). The average length of tecovirimat treatment was 39 days (range 14–167 days); a standard regimen is 14 days.

A tecovirimat-resistant phenotype was previously published for 6 patients from Los Angeles County, California, USA (25,26). The previous case reports were limited in geographic scope, whereas our study is an overview for the entire United States. The 6 patients previously reported are included in this report for completeness. The larger dataset reported herein is complementary to the previously published data and supports the conclusions of the previous reports. In addition, 1 other case report found MPXV with a tecovirimat-resistant phenotype that was linked to selection of the N267D mutation during tecovirimat treatment (29). Of note, despite detection of drug resistance from 1 anatomic site, the patient improved clinically (29). Other case reports have suspected tecovirimat resistance on the basis of deteriorating clinical status after tecovirimat treatment (30,31). Treatment with cidofovir was successful in those cases and should be considered when tecovirimat resistance is suspected.

The first limitation of our study is that the phenotype assay is culture-based, which is labor intensive and of low throughput. As of July 2023, we had phenotyped 124 specimens from 68 patients. However, the lag in testing means all the specimens that have been tested are from September 2022–April 2023, so results only give a retrospective sample of possible drug resistance. Because submission of samples for tecovirimat sensitivity is voluntary and cannot be used to inform clinical care, sampling bias may exist for certain physicians, hospitals, or states and may make it appear that certain states have more drug resistance than others (Figure 1). As genomic sequencing has increased, we have prioritized samples with predicted resistance mutations for phenotype testing. Mixed populations of cultured virus were tested to meet the need for efficiency for a public health emergency. In the future, we will begin plaque purification for selected samples to test clonal populations.

Our results confirm that tecovirimat resistance mutations are being selected in human mpox patients by tecovirimat treatment. Resistance has been confirmed in a small percentage of cases, currently <1% of the total number of patients that have received tecovirimat. Characteristics of patients with resistant isolates are very similar: uncontrolled HIV infection with very low CD4+ T-cell counts and potential for extensive tecovirimat exposure while hospitalized. The frequency of tecovirimat resistance may be higher in persons with uncontrolled HIV infection. In rare cases,

a drug-resistant virus appeared to have been transmitted to another person. Genomic and phenotype testing are ongoing. Our results may be useful when considering treatment for patients that match the clinical profile we described; aggressive early dosing and combination therapy regimens could be considered in those instances (21). Results will also provide critical knowledge to potentially build a genomic assay for early detection of resistance mutations which could be used to inform clinical care decisions. For clinicians concerned about tecovirimat resistance, we encourage enrolling patients in the CDC VIRISMAP study (<https://www.cdc.gov/poxvirus/mpox/clinicians/virismap.html>) and the STOMP (Study of Tecovirimat for Mpox) trial (<https://stomptpox.org>).

In conclusion, we describe a large number of tecovirimat-resistant MPXV isolates from humans and provide crucial data on the amino acid changes leading to resistance in MPXV paired with clinical outcomes; these combined data may inform decisions on tecovirimat use in the future. Our findings also highlight the need for additional, well-tolerated OPXV therapeutics with different modes of action, particularly for use with immunocompromised patients.

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Health and Mental Hygiene; Ohio Department of Health Laboratory; Pennsylvania Department of Health, Bureau of Laboratories; Rhode Island State Health Laboratories; Tennessee Division of Laboratory Services; Texas Department of State Health Services, Lab Services Section; Texas-Houston Health Department Laboratory; and Virginia Division of Consolidated Laboratory Services.

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Dr. Smith is a microbiologist in the Poxvirus and Rabies Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Infectious Diseases, Centers for Disease Control and Prevention, in Atlanta, Georgia. His primary research interest is in smallpox preparedness.

## References

- Minhaj FS, Ogale YP, Whitehill F, Schultz J, Foote M, Davidson W, et al.; Monkeypox Response Team 2022. Monkeypox outbreak—nine states, May 2022. *MMWR Morb Mortal Wkly Rep.* 2022;71:764–9. <https://doi.org/10.15585/mmwr.mm7123e1>
- Centers for Disease Control and Prevention. 2022 mpox outbreak global map. 2023 [cited 2023 Aug 16]. <https://www.cdc.gov/poxvirus/mpox/response/2022/world-map.html>
- Merchlinsky M, Albright A, Olson V, Schiltz H, Merkeley T, Hughes C, et al. The development and approval of tecovirimat (TPOXX), the first antiviral against smallpox. *Antiviral Res.* 2019;168:168–74. <https://doi.org/10.1016/j.antiviral.2019.06.005>
- Yang G, Pevear DC, Davies MH, Collett MS, Bailey T, Rippen S, et al. An orally bioavailable antipoxvirus compound (ST-246) inhibits extracellular virus formation and protects mice from lethal orthopoxvirus Challenge. *J Virol.* 2005;79:13139–49. <https://doi.org/10.1128/JVI.79.20.13139-13149.2005>
- Smith SK, Olson VA, Karem KL, Jordan R, Hruby DE, Damon IK. In vitro efficacy of ST246 against smallpox and monkeypox. *Antimicrob Agents Chemother.* 2009;53:1007–12. <https://doi.org/10.1128/AAC.01044-08>
- Duraffour S, Snoeck R, de Vos R, van Den Oord JJ, Crance JM, Garin D, et al. Activity of the anti-orthopoxvirus compound ST-246 against vaccinia, cowpox and camelpox viruses in cell monolayers and organotypic raft cultures. *Antivir Ther.* 2007;12:1205–16. <https://doi.org/10.1177/135965350701200802>
- Berhanu A, King DS, Mosier S, Jordan R, Jones KF, Hruby DE, et al. ST-246 inhibits in vivo poxvirus dissemination, virus shedding, and systemic disease manifestation. *Antimicrob Agents Chemother.* 2009;53:4999–5009. <https://doi.org/10.1128/AAC.00678-09>
- Grosenbach DW, Berhanu A, King DS, Mosier S, Jones KF, Jordan RA, et al. Efficacy of ST-246 versus lethal poxvirus challenge in immunodeficient mice. *Proc Natl Acad Sci U S A.* 2010;107:838–43. <https://doi.org/10.1073/pnas.0912134107>
- Jordan R, Goff A, Frimm A, Corrado ML, Hensley LE, Byrd CM, et al. ST-246 antiviral efficacy in a nonhuman primate monkeypox model: determination of the minimal effective dose and human dose justification. *Antimicrob Agents Chemother.* 2009;53:1817–22. <https://doi.org/10.1128/AAC.01596-08>
- Zaitseva M, Shotwell E, Scott J, Cruz S, King LR, Manischewitz J, et al. Effects of postchallenge administration of ST-246 on dissemination of IHD-J-Luc vaccinia virus in normal mice and in immune-deficient mice reconstituted with T cells. *J Virol.* 2013;87:5564–76. <https://doi.org/10.1128/JVI.03426-12>
- Smith SK, Self J, Weiss S, Carroll D, Braden Z, Regnery RL, et al. Effective antiviral treatment of systemic orthopoxvirus disease: ST-246 treatment of prairie dogs infected with monkeypox virus. *J Virol.* 2011;85:9176–87. <https://doi.org/10.1128/JVI.02173-10>
- Sbrana E, Jordan R, Hruby DE, Mateo RI, Xiao SY, Siirin M, et al. Efficacy of the antipoxvirus compound ST-246 for treatment of severe orthopoxvirus infection. *Am J Trop Med Hyg.* 2007;76:768–73. <https://doi.org/10.4269/ajtmh.2007.76.768>
- Santos-Fernandes É, Beltrame CO, Byrd CM, Cardwell KB, Schnellrath LC, Medaglia ML, et al. Increased susceptibility of Cantagalo virus to the antiviral effect of ST-246. *Antiviral Res.* 2013;97:301–11. <https://doi.org/10.1016/j.antiviral.2012.11.010>
- Quenelle DC, Buller RM, Parker S, Keith KA, Hruby DE, Jordan R, et al. Efficacy of delayed treatment with ST-246 given orally against systemic orthopoxvirus infections in mice. *Antimicrob Agents Chemother.* 2007;51:689–95. <https://doi.org/10.1128/AAC.00879-06>
- Nalca A, Hatkin JM, Garza NL, Nichols DK, Norris SW, Hruby DE, et al. Evaluation of orally delivered ST-246 as postexposure prophylactic and antiviral therapeutic in an aerosolized rabbitpox rabbit model. *Antiviral Res.* 2008;79:121–7. <https://doi.org/10.1016/j.antiviral.2008.03.005>
- Mucker EM, Goff AJ, Shamblin JD, Grosenbach DW, Damon IK, Mehal JM, et al. Efficacy of tecovirimat (ST-246) in nonhuman primates infected with variola virus (smallpox). *Antimicrob Agents Chemother.* 2013;57:6246–53. <https://doi.org/10.1128/AAC.00977-13>
- Huggins J, Goff A, Hensley L, Mucker E, Shamblin J, Wlazlowski C, et al. Nonhuman primates are protected from smallpox virus or monkeypox virus challenges by the antiviral drug ST-246. *Antimicrob Agents Chemother.* 2009;53:2620–5. <https://doi.org/10.1128/AAC.00021-09>
- Warner BM, Klassen L, Sloan A, Deschambault Y, Soule G, Banadyga L, et al. In vitro and in vivo efficacy of tecovirimat against a recently emerged 2022 monkeypox virus isolate. *Sci Transl Med.* 2022;14:eade7646. <https://doi.org/10.1126/scitranslmed.ade7646>
- Duraffour S, Lorenzo MM, Zöller G, Topalis D, Grosenbach D, Hruby DE, et al. ST-246 is a key antiviral to inhibit the viral F13L phospholipase, one of the essential proteins for orthopoxvirus wrapping. *J Antimicrob Chemother.* 2015;70:1367–80. <https://doi.org/10.1093/jac/dku545>
- Lederman ER, Davidson W, Groff HL, Smith SK, Warkentien T, Li Y, et al. Progressive vaccinia: case description and laboratory-guided therapy with vaccinia immune globulin, ST-246, and CMX001. *J Infect Dis.* 2012;206:1372–85. <https://doi.org/10.1093/infdis/jis510>
- Rao AK, Schrodtt CA, Minhaj FS, Waltenburg MA, Cash-Goldwasser S, Yu Y, et al. Interim clinical treatment considerations for severe manifestations of mpox—United States, February 2023. *MMWR Morb Mortal Wkly Rep.* 2023;72:232–43. <https://doi.org/10.15585/mmwr.mm7209a4>
- Gigante CM, Korber B, Seabolt MH, Wilkins K, Davidson W, Rao AK, et al. Multiple lineages of monkeypox virus detected

- in the United States, 2021–2022. *Science*. 2022;378:560–5. <https://doi.org/10.1126/science.add4153>
23. Bojkova D, Bechtel M, Rothenburger T, Steinhorst K, Zöller N, Kippenberger S, et al. Drug sensitivity of currently circulating mpox viruses. *N Engl J Med*. 2023;388:279–81. <https://doi.org/10.1056/NEJMc2212136>
  24. Frenois-Veyrat G, Gallardo F, Gorgé O, Marcheteau E, Ferraris O, Baidaliuk A, et al. Tecovirimat is effective against human monkeypox virus in vitro at nanomolar concentrations. *Nat Microbiol*. 2022;7:1951–5. <https://doi.org/10.1038/s41564-022-01269-8>
  25. Garrigues JM, Hemarajata P, Karan A, Shah NK, Alarcón J, Marutani AN, et al. Identification of tecovirimat resistance-associated mutations in human monkeypox virus—Los Angeles County. *Antimicrob Agents Chemother*. 2023;67:e0056823. <https://doi.org/10.1128/aac.00568-23>
  26. Alarcón J, Kim M, Terashita D, Davar K, Garrigues JM, Guccione JP, et al. An mpox-related death in the United States. *N Engl J Med*. 2023;388:1246–7. <https://doi.org/10.1056/NEJMc2214921>
  27. Garrigues JM, Hemarajata P, Espinosa A, Hacker JK, Wynn N, Gigante CM, et al. Community spread of a human monkeypox virus variant with a tecovirimat resistance-associated mutation. *Antimicrob Agents Chemother*. 2023;e0097223.
  28. Food and Drug Administration. FDA mpox response. 2022 [cited 2023 Apr 12]. <https://www.fda.gov/emergency-preparedness-and-response/mcm-issues/fda-mpox-response>
  29. Mertes H, Rezende AM, Brosius J, Naesens R, Michiels J, deBlock T, et al. Tecovirimat resistance in an immunocompromised patient with mpox and prolonged viral shedding. *Ann Intern Med*. 2023;176:1141–3. <https://doi.org/10.7326/L23-0131>
  30. Dammann F, Raja M, Camargo JF. Progression of human monkeypox infection despite tecovirimat in an immunocompromised adult. *Transpl Infect Dis*. 2023;25:e14022. <https://doi.org/10.1111/tid.14022>
  31. Stafford A, Rimmer S, Gilchrist M, Sun K, Davies EP, Waddington CS, et al. Use of cidofovir in a patient with severe mpox and uncontrolled HIV infection. *Lancet Infect Dis*. 2023;23:e218–26. [https://doi.org/10.1016/S1473-3099\(23\)00044-0](https://doi.org/10.1016/S1473-3099(23)00044-0)

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# etymologia revisited

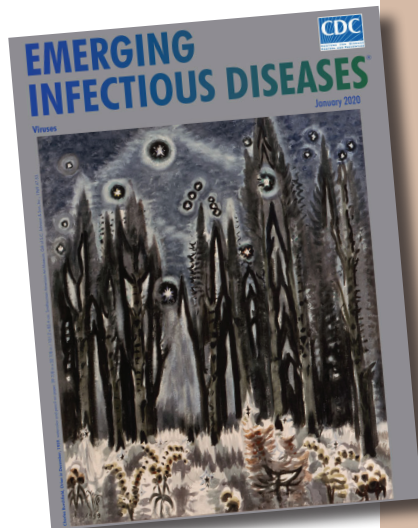
## *Picobirnavirus* [pi-ko-burr'nə-vi"rəs]

*Picobirnavirus*, the recently recognized sole genus in the family *Picobirnaviridae*, is a small (*Pico*, Spanish for small), bisegmented (*bi*, Latin for two), double-stranded RNA virus. Picobirnaviruses were initially considered to be birna-like viruses, and the name was derived from birnavirus (bisegmented RNA), but the virions are much smaller (diameter 35 nm vs. 65 nm).

Picobirnaviruses are reported in gastroenteric and respiratory infections. These infections were first described in humans and black-footed pigmy rice rats in 1988. Theafter, these infections have been reported in feces and intestinal contents from a wide variety of mammals with or without diarrhea, and in birds and reptiles worldwide.

### Sources

1. Delmas B, Attoui H, Ghosh S, Malik YS, Mundt E, Vakharia VN; ICTV Report Consortium. ICTV virus taxonomy profile: Picobirnaviridae. *J Gen Virol*. 2019;100:133–4. <https://doi.org/10.1099/jgv.0.001186>
2. Malik YS, Kumar N, Sharma K, Dhama K, Shabbir MZ, Ganesh B, et al. Epidemiology, phylogeny, and evolution of emerging enteric Picobirnaviruses of animal origin and their relationship to human strains. *BioMed Res Int*. 2014;2014:780752. <https://doi.org/10.1155/2014/780752>
3. Pereira HG, Flewett TH, Candeias JA, Barth OM. A virus with a bisegmented double-stranded RNA genome in rat (*Oryzomys nigripes*) intestines. *J Gen Virol*. 1988;69:2749–54. <https://doi.org/10.1099/0022-1317-69-11-2749>
4. Smits SL, van Leeuwen M, Schapendonk CM, Schürch AC, Bodewes R, Haagmans BL, et al. Picobirnaviruses in the human respiratory tract. *Emerg Infect Dis*. 2012;18:1539–40. <https://doi.org/10.3201/eid1809.120507>



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# Tecovirimat Resistance in Mpox Patients, United States, 2022–2023

## Appendix

### Additional Methods

DNA was extracted from lesion swabs using EZ-1 DNA tissue kit (Qiagen) followed by heat inactivation at 56°C for  $\geq 1$  hour. Monkeypox virus infection was confirmed by real-time PCR using a clade II-specific monkeypox virus real-time PCR assay as described in Li et al. (1).

### F13L Amplicon Sequencing

5  $\mu$ L of MPXV DNA was used as input to the primary PCR reaction with tagged primers (F13L Forward: ont\_tag-GACCTTCTTCATTTTCGTGCCA, F13L: Reverse ont\_tag-AATGTGGCCATTTGCATCGG), where ont\_tag was added as described by the manufacturer (Oxford Nanopore Technologies, EXP-PBC096). Reaction contained 10  $\mu$ L 2x GC Buffer I (Takara, RR02AG), 0.2  $\mu$ L long amplicon Taq polymerase (Takara, RR02AG), 0.5  $\mu$ L each of F13L forward and reverse primers at 20  $\mu$ M, 2  $\mu$ L dNTPs (Takara, RR02AG), and 1.8  $\mu$ L nuclease-free water. PCR reaction was run for 2 minutes at 94°C followed by 25 to 35 cycles of 30 s at 94°C, 30 s at 56°C, and 1 minute at 72°C, followed by 5 minutes at 72°C. Cycle number was determined by Clade II-specific Ct value: 25 cycles for Ct 20 – 25, 30 times for Ct 25 – 30, and 35 cycles for Ct >30. Samples with Ct <20 were diluted 100-fold then run for 25 cycles. PCR reactions were cleaned up with 0.65x AMPure XP beads (Beckman). Barcoding PCR was performed according to the manufacturer's instructions (Oxford Nanopore Technologies, EXP-PBC096) using 20  $\mu$ L reactions, Takara LA taq with GC buffers as above (Takara, RR02AG), 1 minute extension time and 12 cycles of PCR. PCR reactions were cleaned up with 0.65x AMPure XP beads (Beckman). Samples concentrations were estimated using a Qubit and pooled at equal concentrations. Library preparation was performed using the SQK-LSK109 kit for sequencing on the Flongle device, according to the manufacturer (Oxford Nanopore



Technologies). Basecalling was performed using guppy 6.1.2 (Oxford Nanopore Technologies) and flags `-kit SQK-LSK109 -flowcell FLO-FLG001 -barcode_kits EXP-PBC096 -trim_barcodes -require_barcodes_both_ends`. Nanopore reads were trimmed to remove 55 bp from each end (seqtk 1.0, <https://github.com/lh3/seqtk>) and all reads below 50 bp were removed (trimmomatic 0.39, <https://github.com/timflutre/trimmomatic>) before mapping to MPXV Nigeria reference MT903344 with 6,000 bp removed from the left terminus using minimap2 2.16 (<https://github.com/lh3/minimap2>). Variants were called using ivar 1.3.1 (<https://andersen-lab.github.io/ivar/html/manualpage.html>) and samtools 1.7 (<https://github.com/samtools/samtools>) using the command `samtools mpileup -aa -A -B -Q 0 -d 80000 mappingfile.bam | ivar variants -p sampleName -t 0.05 -m 5 -q 20`.

### **Illumina Metagenomics Sequencing**

Extracted DNA (15 µL) was used as input for the Illumina DNA Prep method according to the manufacturer's protocol except one half reagent volumes were used throughout. Libraries were visualized using an Agilent Fragment Analyzer instrument and an HS NGS Fragment Kit (Agilent Technologies Inc., Santa Clara, CA). Forty-eight samples were pooled at approximately equal molarity generating 200 pM final loading concentration and sequenced on an Illumina NovaSeq 6000 instrument using the 300 cycle SP sequencing components. Orthopoxvirus reads were filtered using Kraken2 v2.1.2 (2) run with default settings, using a database that included human genome for negative selection and MPXV genomes for positive selection. We used seqtk v1.3 `subseq` (3) to subsample our reads to orthopoxvirus with default settings and the `--no-name` flag, then used fastp v0.23.2 (4) with to trim and clean our filtered reads. Reads were aligned to MPXV Clade IIb reference genome (UK-P2; MT903344.1) using bwa mem v0.7.17 (H. Li et al., unpub. data, <https://doi.org/10.48550/arXiv.1303.3997>) then sorted using Samtools v.1.15.1 (5). F13L variants were called using iVar v.1.3.1 (6) with the following parameters: `samtools mpileup -aa -A -d 600000 -B -Q 0 $PREFIX.BAM | ivar variants -p $PREFIX -r $REFERENCE -q 20 -t 0.05 -m 5`. We converted from tsv to vcf format using a custom python script ([https://github.com/jts/ncov-tools/blob/master/workflow/scripts/ivar\\_variants\\_to\\_vcf.py](https://github.com/jts/ncov-tools/blob/master/workflow/scripts/ivar_variants_to_vcf.py)), filtering for an allele frequency of 0.05 and >5 supporting reads. For both ONT and Illumina data, only variants with allele frequency >10% are reported here (Appendix Table).

## References

1. Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. *J Virol Methods*. 2010;169:223–7. [PubMed https://doi.org/10.1016/j.jviromet.2010.07.012](https://doi.org/10.1016/j.jviromet.2010.07.012)
2. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. *Genome Biol*. 2019;20:257. [PubMed https://doi.org/10.1186/s13059-019-1891-0](https://doi.org/10.1186/s13059-019-1891-0)
3. Li H. Toolkit for processing sequences in FASTA/Q formats. GitHub. 2012 [cited 2023 Apr 12]. <https://github.com/lh3/seqtk>
4. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. 2018;34:i884–90. [PubMed https://doi.org/10.1093/bioinformatics/bty560](https://doi.org/10.1093/bioinformatics/bty560)
5. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al.; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25:2078–9. [PubMed https://doi.org/10.1093/bioinformatics/btp352](https://doi.org/10.1093/bioinformatics/btp352)
6. Castellano S, Cestari F, Faglioni G, Tenedini E, Marino M, Artuso L, et al. IVar, an interpretation-oriented tool to manage the update and revision of variant annotation and classification. *Genes (Basel)*. 2021;12:384. [PubMed https://doi.org/10.3390/genes12030384](https://doi.org/10.3390/genes12030384)
7. Alarcón J, Kim M, Terashita D, Davar K, Garrigues JM, Guccione JP, et al. An Mpox-Related Death in the United States. *N Engl J Med*. 2023;388:1246–7. [PubMed https://doi.org/10.1056/NEJMc2214921](https://doi.org/10.1056/NEJMc2214921)
8. Garrigues JM, Hemarajata P, Karan A, Shah NK, Alarcón J, Marutani AN, et al. Identification of Tecovirimat Resistance-Associated Mutations in Human Monkeypox Virus - Los Angeles County. *Antimicrob Agents Chemother*. 2023;67:e0056823. [PubMed https://doi.org/10.1128/aac.00568-23](https://doi.org/10.1128/aac.00568-23)

**Appendix Table. F13 mutations identified in 15 mpox cases, United States, 2022\***

Patient	Specimen	Mutation	Illumina	ONT	Difference	Illumina depth	ONT depth	Days of treatment	Sample day
1	1	N267D	100.00%	98.90%	1.10%	37	366	28	51
2†	1	A288P	26.90%	29.30%	-2.40%				
		D294V	26.10%	18.00%	8.10%	2831	1617	28	31
		D301del	23.60%	17.10%	6.50%				
2†	2	A288P	18.59%	19.41%	-0.82%				
		A290V	26.88%	26.50%	0.38%	1926	4670	28	31
		D294V	24.80%	16.64%	8.16%				
2†	3	A288P	13.97%	14.78%	-0.81%				
		A290V	20.61%	22.44%	-1.83%	1446	5723	28	31
		L297ins	37.59%	33.22%	4.36%				
3	1	D294V	100.00%	96.60%	3.40%	75	70	36	38
4	1	T289A	100.00%	100.00%	0.00%	71	162	NA	NA
5	1	D294V	99.20%	98.20%	1.00%	118	148	19	56
6	1	N267del	22.20%	20.00%	2.20%				
		T289A	58.20%	61.00%	-2.80%	90	5962	0	0
		A295E	9.46%	11.20%	-1.74%				
6	2	T289A	92.90%	89.90%	3.00%	114	4461	18	18
		R291K	30.70%	34.10%	-3.40%				
7	1	N267D	59.11%	66.59%	-7.48%	291	317	75	91
		D294V	29.51%	17.02%	12.49%				
7	2	N267del	89.81%	72.85%	16.97%	373	1563	75	91
8	1	A295E	100.00%	98.52%	1.48%	37	2456	35	26
8	2	N267del	47.79%	30.68%	17.11%				
		A288P	22.06%	28.22%	-6.16%	66	138	35	26
		A295E	15.15%	27.11%	-11.96%				
9	1	N267D	25.42%	29.78%	-4.35%				
		A288P	ND	11.76%	missed				
		A290V	16.24%	12.00%	4.24%	118	131	28	65
		A295E	21.01%	11.61%	9.40%				
		I372N	17.53%	15.26%	2.26%				
10	1	A290V	95.00%	84.29%	10.71%	20	5377	77	87
		T245I	ND	11.75%	missed				
11‡	1	A290V	100.00%	97.80%	2.20%	161	3373	52	60
11‡	2	I372N	100.00%	85.72%	14.28%	334	5176	52	60
12	1	N267D	18.32%	28.07%	-9.75%	198	418	30	43
		A295E	60.32%	56.01%	4.31%				
12	2	N267del	78.40%	67.94%	10.46%	125	4824	30	43
13‡	1	A288P	54.94%	53.75%	1.19%	134	1241	28	32
		N267D	43.28%	48.72%	-5.44%				
14	1	Y285H	10.13%	8.83%	1.30%	60	4092	56	84
		I372N	90.00%	77.04%	12.96%				
15	1	D217N	100.00%	98.97%	1.03%	19	91	NI	NI

\*DNA extracted from each specimen was sequenced by direct DNA sequencing on an Illumina NovaSeq 6000 or targeted F13L amplicon sequencing on an Oxford Nanopore MinIon. Percent of reads with each mutation is shown for the two methods. Two minor alleles were not detected by the direct DNA sequencing method (ND not detected). Allele frequencies less than 10% were not reported unless it was detected at >10% by the other method. Average read depth is included. For some cases, multiple specimens collected from different anatomic sites yielded different mutational patterns. Amino acid deletion (del) and insertion (ins) mutations are included. Length of treatment indicates the potential number of days of tecovirimat exposure. Sample day indicates the number of days from initial mpox diagnosis to resistant sample collection. One patient's medical history was not available (NA), and one was not investigated (NI) because the sample was sensitive to tecovirimat.

†Previously published by Alarcón, et al. (7).

‡Previously published by Garrigues, et al. (8).