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Identification of Tecovirimat Resistance-Associated Mutations in Human Monkeypox Virus - Los Angeles County

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Tecovirimat (also known as TPOXX or ST-246) is a drug available for the treatment of mpox through the Centers for Disease Control and Prevention's Expanded Access Investigational New Drug "compassionate use" protocol (https://www.cdc.gov/poxvirus/monkeypox/clinicians/Tecovirimat.html). In Los Angeles County, a fatal case of mpox with tecovirimat resistance was previously reported (1). Epidemiologic surveillance in Los Angeles County has since identified additional cases of severe mpox that did not improve after multiple rounds of tecovirimat treatment, including one involving a person who succumbed to infection (Table 1). Consistent with reports describing severe manifestations of mpox within the current global outbreak (1, 2), the identified cases involved host immunodeficiency due to advanced HIV infection.

Tecovirimat targets the conserved orthopoxvirus VP37 envelope protein required for extracellular virus particle generation, and previous studies from monkeypox virus (MPXV) and other orthopoxviruses identified more than 20 mutations in VP37 associated with tecovirimat resistance (https://www.fda.gov/emergency-preparedness-and-response/mcm-issues/fda-mpox-response#therapeutics) (1, 3). To determine whether these identified cases involved tecovirimat resistance, MPXV specimens were subjected to whole-genome sequencing and examined for mutations in the homolog of the vaccinia virus Copenhagen *F13L* gene that encodes VP37 protein (1, 4). All specimens encoded lysine at amino acid position 353, which is prevalent within the current outbreak and does not affect tecovirimat sensitivity (5). Specimens from six cases harbored a cumulative total of eight VP37 mutations associated with resistance in prior studies: H238Q, P243S, N267D, A288P, A290V, D294V, A295E, and I372N (Table 2). Five previously undescribed mutations of unknown significance were also identified (T220A/I, T245I, A265D, and T289A), which may represent novel tecovirimat-interacting residues. Allele frequency analysis demonstrated resistance mutation heterogeneity within single lesion specimens, and different mutations were identified among distinct lesion specimens from the same person, suggesting mutations were acquired during treatment. Consistent with a *de novo* process, comparison of specimens collected before and after treatment in a single person (Patient F) showed resistance-associated mutations in VP37 only after treatment exposure (Table 2).

Phenotypic testing of MPXV cultured from a subset of specimens (1, 5) displayed a wide range of tecovirimat resistance levels *in vitro* compared to wild-type isolates (Table 2), arguing these mutations confer resistance within currently circulating strains. For heterogenous cultures with multiple VP37 mutations, it is unclear how much individual mutations contribute to resistance and whether resistance is due to single or multiple mutations, especially in cases with low allele frequencies. It is also possible that uncharacterized mutations outside of VP37 enhance or diminish tecovirimat resistance.

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TABLE 1 Clinical data for mpox cases

Patient	CD4+ T-cell count (cells/mm³)[a]	HIV viral load (copies/mL)[a]	Mpox treatment and Specimen Collection History	Clinical outcome
A	<35	130,565	Tecovirimat PO[c] 600 mg: BID[d], 1 wk Tecovirimat PO 600 mg: BID, 1 wk Tecovirimat IV[e] 200 mg: 3 doses Tecovirimat PO 600 mg: BID, 2 wks Tecovirimat IV 200 mg: q[f] 12 hours, 2 days **Specimen A.1 collected** VIGIV[g] (unknown dose): single infusion Tecovirimat IV 200 mg: q 12 hours, 2 wks **Specimens A.2-A.26 collected**	Deceased
B	73	<20	Tecovirimat PO 600 mg: BID, 2 wks Tecovirimat PO 600 mg: BID, 2 wks **Specimens B.1-B.2 collected** Tecovirimat IV 200 mg: q 12 hours, 2 wks VIGIV (unknown dose): single infusion Brincidofovir PO (unknown dose): single dose	Unknown
C	136	178,982	Tecovirimat PO 200-600 mg: QD[h]-BID, 2 wks Tecovirimat IV 200 mg: q 12 hours, 3 wks **Specimens C.1-C.2 collected** VIGIV (unknown dose): single infusion	Ongoing lesions (after 6 mo)
D	96	2,620	Tecovirimat PO 600 mg: BID, 2 wks Tecovirimat PO 600 mg: BID, 11 days **Specimen D.1 collected**[b] Cidofovir IV 5 mg/kg: single infusion Tecovirimat PO 600 mg: BID, 4 days VIGIV 9000 units/kg: single infusion Tecovirimat IV 200 mg: q 12 hours, 2 wks Cidofovir IV 5 mg/kg: single infusion	Recovered
E	191	606	Tecovirimat PO 600 mg: BID, 2 wks Tecovirimat PO 600 mg: BID, 4 wks **Specimens E.1-E.8 collected** Tecovirimat PO 600 mg: BID, 6 wks Tecovirimat PO 600 mg: BID, 10 wks **Specimens E.9-E.14 collected**	Ongoing lesions (after 6 mo)
F	54	485,298	**Specimen F.1 collected** Tecovirimat PO 600 mg: BID, 1 wk Tecovirimat PO 600 mg: BID, 2 days Tecovirimat IV 200 mg: q 12 hours, 4 days **Specimens F.2-F.5 collected**	Ongoing lesions (after 7 mo)

[a]Results are most proximal to case identification and management. Specimen collection history is in bold text.
[b]Specimen D.1 was collected 5 days into preceding tecovirimat treatment course.
[c]PO = Per Oral.
[d]BID = Twice daily.
[e]IV = Intravenous.
[f]q = every.
[g]VIGIV = Vaccinia Immune Globulin Intravenous.
[h]QD = Once daily.

These findings demonstrate that tecovirimat resistance can arise in the setting of prolonged treatment exposure with host immunodeficiency and underscore the need for combined therapeutics with different viral targets (including VIGIV and cidofovir/brincidofovir) (6, 7) when treating mpox in certain people (8), as is the case with progressive vaccinia (9). Clinical management should ensure effective drug delivery/dosing (e.g., making sure oral tecovirimat is taken with a fatty meal) and optimization of immune function when treating with tecovirimat to prevent resistance from developing. Additionally, this work suggests that sequencing of MPXV to identify tecovirimat resistance could serve as a valuable clinical tool.

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TABLE 2 VP37 mutation and tecovirimat resistance data for human monkeypox virus specimens[a]

Patient	Specimen (source)	VP37 mutations detected (allele frequency[b])	GISAID[c] ID	GenBank ID	SRA[d] ID	EC^{e}_{50} (μM)	Fold change[f]
A	A.1 (Unknown)	**A290V (0.97)**	EPI_ISL_15597062	OP748968	SRR22116985	0.2032	12
	A.2 (Unknown)	N267D (0.24), **A288P (0.76)**	EPI_ISL_15896341	OP890565	SRR22398844	Pending	
	A.3 (Penis)	N267D (0.38), A288P (0.37), A295E (0.31), I372N (0.22)	EPI_ISL_15896342	OP890566	SRR22398843	Culture contaminated	
	A.4 (Scrotum)	**A288P (0.95)**	EPI_ISL_15896343	OP890567	SRR22398842	Culture contaminated	
	A.5 (Lt[g] Thigh)	**A288P (1.0)**	EPI_ISL_15896344	OP890568	SRR22398841	Pending	
	A.6 (Rt[h] Sole)	**A295E (0.82)**	EPI_ISL_15896345	OP890569	SRR22398840	Culture contaminated	
	A.7 (Rt Heel)	**I372N (0.84)**	EPI_ISL_15896346	OP890570	SRR22398839	Culture negative	
	A.8 (Lt Back Knee)	N267D (0.55), A288P (0.18), D294V (0.29)	EPI_ISL_15896347	OP890571	SRR22398838	Pending	
	A.9 (Unknown)	**N267D (0.91)**	EPI_ISL_15896348	OP890572	SRR22398837	Culture contaminated	
	A.10 (Unknown)	T220A (0.44)[i], A265D (0.50)[i], A295E (0.51)	EPI_ISL_15896349	OP890573	SRR22398836	Culture contaminated	
	A.11 (Lt 2nd Finger)	**A288P (0.62),** I372N (0.10)	EPI_ISL_15896350	OP890574	SRR22398835	Culture negative	
	A.12 (Lt Medial Thigh)	N267D (0.37), **A288P (0.63)**	EPI_ISL_15896351	OP890575	SRR22398833	Culture contaminated	
	A.13 (Rt Eyelid)	**A288P (1.0)**	EPI_ISL_15896352	OP890576	SRR22398832	Pending	
	A.14 (Tongue)	**A288P (1.0)**	EPI_ISL_15896353	OP890577	SRR22398831	Pending	
	A.15 (Rt Forehead)	**I372N (0.96)**	EPI_ISL_15896329	OP890553	SRR22398858	Pending	
	A.16 (Lt Forehead)	**N267D (0.88),** D294V (0.11)	EPI_ISL_15896330	OP890554	SRR22398857	Culture contaminated	
	A.17 (Between Eyes)	**A288P (0.88)**	EPI_ISL_15896331	OP890555	SRR22398855	10.28	590
	A.18 (Lt Ear)	**I372N (0.94)**	EPI_ISL_15896332	OP890556	SRR22398854	Pending	
	A.19 (Lt Nose)	A288P (0.19), A290V (0.25), I372N (0.35)	EPI_ISL_15896333	OP890557	SRR22398853	Pending	
	A.20 (Rt Cheek)	A288P (0.12), **I372N (0.87)**	EPI_ISL_15896334	OP890558	SRR22398852	Culture negative	
	A.21 (Lower Lip)	**N267D (0.70),** A288P (0.22)	EPI_ISL_15896335	OP890559	SRR22398851	Pending	
	A.22 (Lt Neck)	**A288P (0.85)**	EPI_ISL_15896336	OP890560	SRR22398850	Culture negative	
	A.23 (Lt Chest)	T220I (0.49)[i], P243S (0.50), A295E (0.51)	EPI_ISL_15896337	OP890561	SRR22398849	Culture negative	
	A.24 (Rt Hand)	**A290V (0.88)**	EPI_ISL_15896338	OP890562	SRR22398848	Culture negative	
	A.25 (Lt Elbow)	**D294V (1.0)**	EPI_ISL_15896339	OP890563	SRR22398847	Pending	
	A.26 (Lt Shoulder)	**H238Q (0.99)**	EPI_ISL_15896340	OP890564	SRR22398846	0.5803	33
B	B.1 (Unknown)	T245I (0.12)[i], **D294V (0.91)**	EPI_ISL_15325434	OP615280	SRR21864633	0.3820	22
	B.2 (Rt Ear)	H238Q (0.27), A288P (0.21), D294V (0.15), I372N (0.33)	EPI_ISL_15417996	OP680496	SRR21966131	~5.150	~290
C	C.1 (Unknown)	**A290V (1.0)**	EPI_ISL_15896305	OP890529	SRR22398845	Culture negative	
	C.2 (Unknown)	**A290V (1.0)**	EPI_ISL_15955339	OP920682	SRR22459664	Culture negative	
D	D.1 (Penis)	**N267D (0.68),** A288P (0.29)	EPI_ISL_17582852	OQ888843	SRR24326324	15.89	910
E	E.1 (Lt Foot Between Toes)	**I372N (1.0)**	EPI_ISL_15418000	OP680500	SRR21966127	0.07286	3.5
	E.2 (Rt Cheek)	**D294V (1.0)**	EPI_ISL_15418001	OP680501	SRR21966126	0.3364	19
	E.3 (Lt Hand by Thumb)	**A290V (1.0)**	EPI_ISL_15418002	OP680502	SRR21966125	0.1670	7.9
	E.4 (Lt Foot)	A290V (0.30), **I372N (0.86)**	EPI_ISL_15418003	OP680503	SRR21966124	0.05551	2.6
	E.5 (Lt Ring Finger)	**H238Q (1.0)**	EPI_ISL_15418004	OP680504	SRR21966123	0.5390	28
	E.6 (Rt Foot)	**N267D (0.97)**	EPI_ISL_15418005	OP680505	SRR21966122	11.05	480
	E.7 (Lt Foot, Scab)	A290V (0.56), **I372N (0.77)**	EPI_ISL_15418007	OP680507	SRR21966119	77.37	4400
	E.8 (Lt Chin, Scab)	**D294V (1.0)**	EPI_ISL_15418008	OP680508	SRR21966118	0.2344	13
	E.9 (Lt Great Toe)	**I372N (1.0)**	EPI_ISL_16997407	OQ503839	SRR23580660	Pending	
	E.10 (Lt 4th Finger)	**H238Q (1.0)**	EPI_ISL_16997408	OQ503840	SRR23580659	Pending	
	E.11 (Rt Medial Foot)	**N267D (1.0)**	EPI_ISL_16997409	OQ503841	SRR23580658	Pending	
	E.12 (Lt Great Toe, Tissue)	**I372N (1.0)**	EPI_ISL_16997410	OQ503842	SRR23580657	Pending	
	E.13 (Rt Medial Foot, Tissue)	N267D (0.45), I372N (0.56)	EPI_ISL_16997411	OQ503843	SRR23580656	Pending	

(Continued on next page)

TABLE 2 (Continued)

Patient	Specimen (source)	VP37 mutations detected (allele frequency[b])	GISAID[c] ID	GenBank ID	SRA[d] ID	EC$^{e}_{50}$ (μM)	Fold change[f]
	E.14 (Lt 4th Finger, Tissue)	**H238Q (1.0)**	EPI_ISL_16997412	OQ503844	SRR23580655	Pending	
F	F.1 (Unknown)#	none	EPI_ISL_16679205	OQ330972	SRR23238068	0.007144	0.41
	F.2 (Face)	**A290V (0.68)**	EPI_ISL_16997464	OQ503820	SRR23580586	Culture contaminated	
	F.3 (Lt Ear)	T289A (0.41)[i], **I372N (0.81)**	EPI_ISL_16679238	OQ331005	SRR23238051	Pending	
	F.4 (Lt Buttock)	**A290V (1.0)**	EPI_ISL_16679239	OQ331006	SRR23238050	0.2257	13
	F.5 (Rt Foot)	A290V (0.52)	EPI_ISL_16679240	OQ331007	SRR23238049	Culture negative	

[a]All specimens are from lesion swabs unless noted otherwise. Consensus mutations with an allele frequency of 0.6 or greater are in bold text. Specimens with a 2- to 9-fold increase in resistance are considered partially resistant, while a 10-fold or greater increase is considered resistant.
[b]Alelle frequency = proportion of mapped reads containing the corresponding mutation at each site.
[c]GISAID = Global Initiative on Sharing Avian Influenza Data.
[d]SRA = Sequence Read Archive.
[e]EC_{50} = Half-maximal effective concentration.
[f]Fold change = observed EC_{50} compared to EC_{50} for MPXV Clade IIa (US, 2003 strain).
[g]Lt = Left.
[h]Rt = Right.
[i]Previously unassociated with tecovirimat resistance; #Tecovirimat-naïve specimen.

of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention or their institutions, or the County of Los Angeles Department of Public Health.

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The Los Angeles County Department of Public Health Institutional Review Board (IRB) determined that this study met the criteria for not research per 45 CFR 46.102(e). Therefore, IRB review was not needed.

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