Oral Miltefosine as Salvage Therapy for Refractory *Acanthamoeba* Keratitis

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• PURPOSE: **To report a case series of patients with treatment-resistant *Acanthamoeba* keratitis (AK) using oral miltefosine, often as salvage therapy.**

• DESIGN: **Descriptive, retrospective multicenter case series.**

• METHODS: **We reviewed 15 patients with AK unresponsive to therapy who were subsequently given adjuvant systemic miltefosine between 2011 and 2017. The main outcome measures were resolution of infection, final visual acuity, tolerance of miltefosine, and clinical course of disease.**

• RESULTS: **All patients were treated with biguanides and/or diamidines or azoles without resolution of disease before starting miltefosine. Eleven of 15 patients retained count fingers or better vision, and all were considered disease free at last follow-up. Eleven of 15 patients had worsening inflammation with miltefosine, with 10 of them improving with steroids. Six patients received multiple courses of miltefosine. Most tolerated oral miltefosine well, with mild gastrointestinal symptoms as the most common systemic side effect.**

• CONCLUSIONS: **Oral miltefosine is a generally well-tolerated treatment adjuvant in patients with refractory AK. The clinician should be prepared for a steroid-responsive inflammatory response frequently encountered during the treatment course. (Am J Ophthalmol 2021;223:75–82. © 2020 Elsevier Inc. All rights reserved.)**

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ACANTHAMOEBA KERATITIS (AK), FOUND PRIMARILY but not exclusively in contact lens wearers, is an often severe, debilitating infection of the cornea. Recent ongoing outbreaks of AK in the United States and elsewhere have refocused efforts on its prevention and management. Estimated incidence remains an order of magnitude higher than previously described in the United States and is rising in other regions of the world as well.[1–4] In the early stages, traditional compounded drugs such as chlorhexidine 0.02% and polyhexamethylene biguanide 0.02% showed good efficacy. For clinically resistant cases, several novel therapies were introduced, including various antifungals and systemic diamidines but with variable success.[5-16] Adjunctive therapies, such as collagen crosslinking and various forms of corneal transplantation, often failed in advanced recalcitrant or resistant disease, especially when some semblance of medical control was not achieved.[17-19] Furthermore, our own personal experience was in line with other investigators, in that recent cases became dramatically more resistant to traditional therapy, affecting treatment duration and outcomes.[20] A critical need for additional options for medical therapy for AK exists.

Unfortunately, identifying and evaluating candidate drugs is challenging in a rare disease like AK, in which none of the myriad of described laboratory sensitivity testing schemes have currently been correlated with clinical outcomes. Analogously, the evaluation of antifungal compounds include their use as salvage therapy for infections in which other therapy has failed to support their efficacy.[21] Miltefosine (Profounda Pharmaceuticals, Orlando, Florida, USA) is a unique alkylphosphocholine anti-amoebic drug, first approved by the Food and Drug Administration (FDA) for the treatment of visceral leishmaniasis, that has also been demonstrated to have good *in vitro* activity against *acanthamoeba* species.[22] In combination with other medications, it has been used successfully for systemic *acanthamoeba* infections, such as granulomatous amoebic encephalitis, a disease with few other options.[23-27] It was initially made available from the Centers for Disease Control and Prevention for the treatment of systemic free-living amoeba infections in humans but not for eye infections.[28] Although successful use of oral miltefosine in AK has been described in

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TABLE 1. Demographic and Clinical Characteristics and Outcomes in Patients With *Acanthamoeba* Keratitis Treated With Oral Miltefosine

Patient	Subject (Age, y)	Anti-amoebic Treatment Before Miltefosine	Time to Diagnosis From Symptom Onset (days)	Surgical Interventions During Course of Disease	Final Vision
1	16WM	PHMB	18	PK	20/50
2	19AM	PHMB, hexamidine	65		20/40
3	22WF	PHMB	13		20/30
4	24WF	PHMB, CHX, oral voriconazole, oral posaconazole	25	PK × 2	20/60
5	25HF	PHMB, propamidine	31		20/60
6	40WF	CHX, PHMB, propamidine, oral voriconazole	16	PK, CE/IOL, enucleation	NLP
7	43WF	PHMB, propamidine	10	CE/IOL	20/50
8	59AAF	Oral posaconazole	85	PK, CE/IOL, glaucoma shunt	HM
9	59WF	PHMB, CHX, propamidine, oral voriconazole	35	PK × 2	20/80
10	61WF	PHMB, propamidine, oral voriconazole	11	PK × 3, AMT, CE, secondary IOL, PPV	HM
11	63WF	PHMB, CHX, oral voriconazole	54	PK × 2, CE/IOL, glaucoma shunt, CPC	20/80
12	64WF	PHMB, CHX, oral voriconazole	8		CF
13	65WF	PHMB, propamidine	28	PK, CE, PPV	LP
14	71WM	PHMB, propamidine, oral voriconazole	65	PK, glaucoma shunt, PPV	20/30
15	80WM	CHX, oral voriconazole	98	PK, CE/IOL, PPV	20/500

AC = anterior chamber; AMT = amniotic membrane transplant; CE = cataract extraction; CPC = cyclophotocoagulation; CHX = chlorhexidine; HM = hand motion; IOL = intraocular lens implantation; LP = light perception; NLP = no light perception; PHMB = polyhexamethylene biguanide; PK = penetrating keratoplasty; PPV = pars plana vitrectomy.

published case reports,[15,29-31] a descriptive analysis of patients' response and tolerance of the drug has not been published. We present a retrospective case series of patients treated with adjuvant systemic miltefosine, often as salvage therapy, for treatment-resistant AK.

METHODS

THIS IS A DESCRIPTIVE, RETROSPECTIVE, MULTICENTER case series of patients with AK treated with systemic miltefosine from 2011 to 2017. Justification for the use of miltefosine was determined by the managing ophthalmologist. Centers with relevant cases were identified by author E.Y.T. from consultations regarding recalcitrant or resistant AK and inquiry concerning alternate treatment options. Subsequently, all cases in whom miltefosine was used in the treatment of AK were identified and included at these centers during this period. The study was approved by the University of Illinois Institutional Review Board with individual data use agreements approved by each participating center. All data were collected after obtaining the institutional review board approval in accordance with the Declaration of Helsinki and were transmitted de-identified to the University of Illinois Eye and Ear Infirmary. Demographic data, risk factors and mode of diagnosis, previous treatment options, treatment course, surgical interventions, and final visual outcomes were recorded. The primary outcome of interest was resolution of *acanthamoeba* ocular disease. Secondary outcomes included final visual acuity, incidence of miltefosine-related inflammation, clinical course, and need for additional interventions during active infection.

RESULTS

FIFTEEN PATIENTS WERE IDENTIFIED FROM 8 CLINICAL CENters (University of Illinois Eye and Ear Infirmary, Massachusetts Eye and Ear Infirmary, University of Minnesota, Wills Eye Hospital, University of Iowa, Berg-Feinfield Vision Correction, and Geisel School of Medicine at Dartmouth, and Montefiore Medical Center). Two patients were treated before FDA approval of miltefosine, for any indication, in 2014, under approval from the University of Illinois Institutional Review Board and individual FDA emergency use of investigational new drugs applications. The remaining patients used the drug either off-label after its approval for visceral leishmaniasis or after its FDA approval for AK in 2016. Table 1 summarizes demographic and clinical characteristics of patients treated with miltefosine. Patients ranged from ages 16 to 80 years, with contact lens use being the most common risk factor. All patients were diagnosed either by tissue diagnosis

TABLE 2. Miltefosine Dosage, Tolerability, and Side Effects in patients with *Acanthamoeba* Keratitis

#	Disease Duration Before Miltefosine (days)	Dose of Miltefosine (mg)	Duration of Miltefosine Use (days)	Improved Pain Upon Starting Miltefosine	Worsened Inflammation	Improvement Responsive to Steroids?	Side Effects of Treatment
1	255	150 qd, 150 qd	28, 28	Y	Y	Y	None
2	410	50 TID	150	N	Y	Y	None
3	26	100 qd, 100 TID	28	Y	Y	Y	None
4	142	50 BID, 50 BID	28, 26	Y	Y	Y	None
5	41	50 TID, 50 BID	12, 21	Maybe	Y	Y	GI upset
6	398	50 BID, 50 BID	28, 14	Maybe	Y	Y	Nausea
7	118	50 BID	28	N	N	N/A	Nausea
8	60	50 BID	35	N	N	n/a	None
9	408	50 BID, 50 BID	25, 34	Maybe	Y	Y	None
10	363	50 BID, 50 BID	56, 28	Maybe	Y	Y	Nausea, elevated LFTs
11	116	50 BID	40	Y	Y	Y	None
12	36	50 TID	59	Y	Y	Y	Nausea
13	129	50 TID	28, 79, 23	Y	Y	Y	Nausea, elevated LFTs
14	211	50 BID	28	Y	N	N/A	None
15	532	50 TID	59	Y	N	N/A	Nausea, malaise, weight loss

BID = twice a day; GI = gastrointestinal; LFT = liver function tests; qd = every day; TID = 3× a day.

(culture or histology) (n = 12) and/or by confocal microscopy (n = 10). Treatment failed with a biguanide, with or without a diamidine, in all patients except 1. Previous therapy also included 9 patients who received oral voriconazole or posaconazole. Five patients (patients 6, 9, 10, 14, 15) in whom therapeutic keratoplasty also failed before starting miltefosine had recurrent infection within the graft.

Table 2 summarizes patients' side effects to miltefosine and their clinical responses. Most patients tolerated miltefosine well, with gastrointestinal disturbances being the most common systemic side effect, for which only 1 patient needed dose reduction from 50 mg 3× a day to 50 mg twice a day (patient 5). Two patients had elevated liver function tests after multiple and extended administration of miltefosine and needed to discontinue the drug (patients 10 and 13). Patient 13 also developed pneumocystis pneumonia with resultant acute kidney injury, believed to be secondary to oral corticosteroids used to control acanthamoeba-related scleritis.

Six patients were administered multiple courses of miltefosine (patients 1, 4, 6, 9, 10, 13). Five of these 6 patients (patients 1, 4, 6, 9, 10) underwent a penetrating keratoplasty in between treatment courses - 4 patients (patients 1, 4, 6, 9) had cysts on pathologic examination of the excised corneal button, prompting a second course of miltefosine. Patient 10 had no cysts on pathologic examination but had significant postoperative inflammation that resolved with restarting miltefosine and polyhexylmethylene biguanide (PHMB). Patient 13 had recurrent keratitis within 2 months of completing the first course, prompting a second course of miltefosine. She eventually underwent a penetrating keratoplasty 6 months later. The corneal button showed residual cysts, and she was treated with PHMB without any evidence of recurrent disease.

All but 1 patient retained some degree of vision, with 11 of 15 (73.3%) patients retaining count fingers or better visual acuity. One patient (patient 6) progressed to enucleation for a secondary complication of therapeutic keratoplasty (epithelial downgrowth). All other patients were clinically disease free at their last follow-up.

Eleven patients (73.3%) showed worsening of inflammation after starting miltefosine (Table 2). The onset of inflammation was usually at 3 weeks (range: 2-5 weeks) and lasted an average of 2 weeks (range: 1-3 weeks). All patients responded to the addition or increase of topical and/or oral corticosteroids. One patient (patient 1) had significant thinning after resolution of this inflammation, contributing to a need for penetrating keratoplasty 2.5 months later. Another patient (patient 4) underwent penetrating keratoplasty after a course of miltefosine and had progressive ectasia at the graft-host junction, believed to be due to uncontrolled intraocular pressures, and required a second penetrating keratoplasty. Ten patients (66.7%) showed either significant or possible improvement in pain within a few days after starting miltefosine.

DISCUSSION

ACANTHAMOEBA RESISTANCE TO TRADITIONAL THERAPEUTIC options has risen dramatically in recent years, and combined with persistent outbreak levels here in the

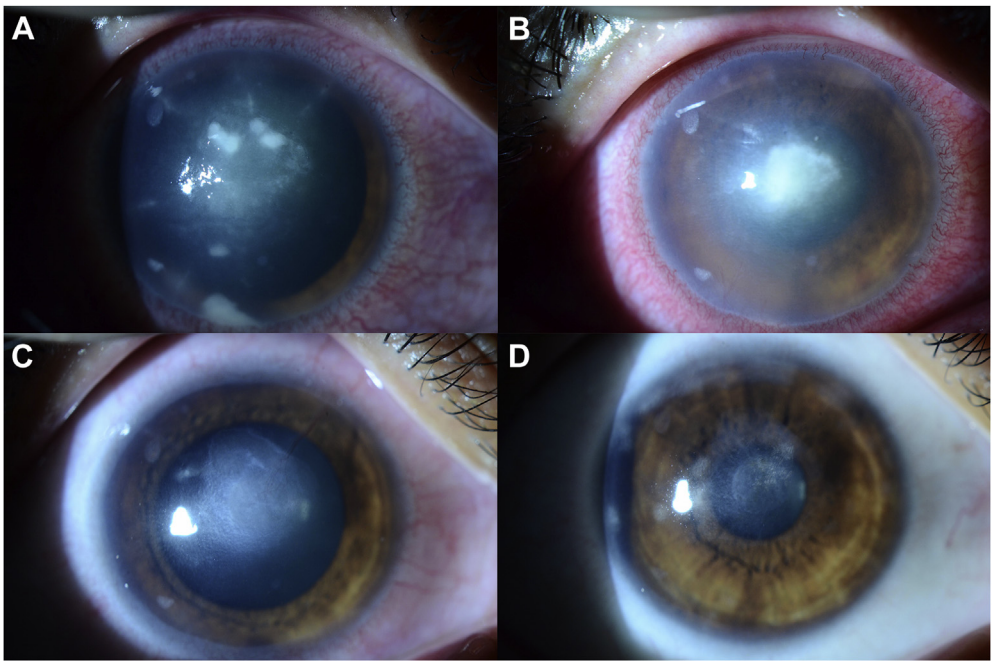

FIGURE 1. Representative case (patient 5), (A) 1 month after onset of symptoms, (B) 11 days after starting miltefosine, (C) 2 weeks, and (D) 7 months after completing oral miltefosine therapy, with resolution of *acanthamoeba* keratitis.

United States and abroad, presents a critical challenge for its ongoing management.[20] To date, few alternative treatment options have been described, each demonstrating highly variable rates of success. We presented 15 cases of clinically resistant AK treated with adjuvant oral miltefosine that demonstrated a high rate of microbiologic or clinical cure (Figure 1). Most of the patients in our series were given miltefosine as salvage therapy, which highlighted the severity of the infection before starting miltefosine. The series also characterized the occurrence of significant ocular inflammation within a few weeks of receiving the drug and discussed its potential mechanism and management (Figure 2).

The patients in this study reflected the well-established broader clinical experience with AK: patients are often contact lens wearers, are often treated with multiple other antibiotic and antiviral therapies, and have a delay in diagnosis of AK. All but 1 patient was treated with traditional biguanide therapy without success, and those with long delays in diagnosis also had other systemic drugs (n = 9) and surgical interventions (n = 5) that failed to cure the infection. Conventional therapy failed in every patient enrolled in our study, as judged by their treating physician, and miltefosine was often used an adjuvant for salvage therapy, after prolonged treatment with other medications (216 days, on average).

More commonly than other corneal infections, medically resistant AK augurs a poor prognosis not only for corneal transplantation but also for extracorneal involvement and loss of the eye. Even in this setting of extended time and treatment failure, 14 of the 15 eyes treated with adjuvant miltefosine achieved eventual clinical cure and 9 of 15 eyes had preserved 20/80 vision or better.

Although all patients were considered clinically disease-free at last follow-up, not all patients were considered treatment successes. For example, patient 1 required additional treatment after completing miltefosine therapy and was characterized as disease free, but the contribution of miltefosine, if any, to the eventual cure, was unclear. Patient 6 developed epithelial downgrowth that led to a decision to enucleate the eye. In this patient, a mixture of anterior segment membranes and inflammation made it difficult to discern whether residual amoebic infection persisted, although an extensive pathologic examination of the enucleated specimen failed to demonstrate residual *acanthamoeba* organisms. Patient 13 had improvement with their first course of miltefosine, but the disease recurred 2 months later, prompting a second course. However, this patient had to discontinue the second course due to other systemic complications (*pneumocystis* pneumonia from oral steroid use) and eventually needed a keratoplasty and additional topical biguanide therapy to be disease free.

Multiple therapies failed in the first patient treated with miltefosine in 2012 (patient 9), with 2 recurrences after successive penetrating keratoplasty and was offered miltefosine as a last resort. This patient developed a significant anterior chamber coagulum at week 3 (Figure 2). Assuming a therapeutic effect as the cause, topical corticosteroids were added to modify the inflammatory response, which then subsided and completely resolved after a 28-day course of miltefosine therapy. A similar effect was seen in subsequent patients,

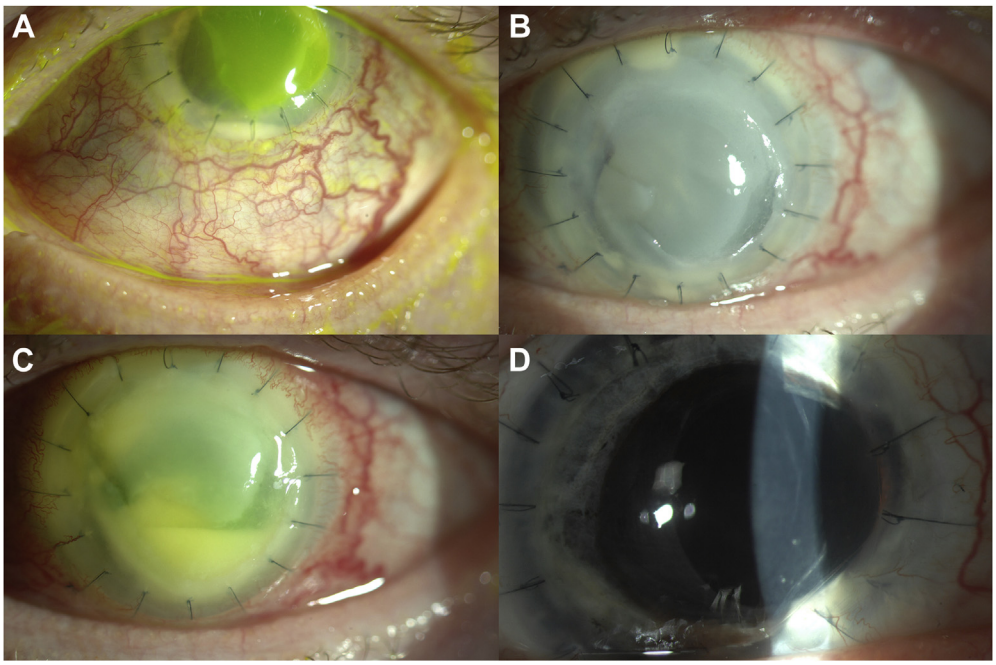

FIGURE 2. The index patient's disease course (patient 9); topical anti-amoebic therapy and oral voriconazole failed in this patient, necessitating a penetrating keratoplasty. (A) The infection recurred within the graft, prompting the use of miltefosine at this time. (B) This led to worsening inflammation, onset 2 weeks after starting miltefosine, and (C) worsening over the next week (week 3). The worsening inflammation improved with the addition of topical steroids. (D) This patient underwent a repeat keratoplasty and a second course of miltefosine for prophylaxis and remained disease free after stopping all anti-amoebic therapy.

improving or subsiding with corticosteroid therapy and completion of the drug course. Patients who received multiple courses of miltefosine had a significantly milder inflammatory response during the second course, which suggested that those with higher disease burden might develop a more robust inflammatory response.

The inflammation manifested as a sticky anterior chamber coagulum, infiltrative keratitis, subepithelial infiltrates, corneal thinning, or scleritis and/or limbitis. A similar inflammatory response and resolution with steroids was seen in patients with non-ocular leishmaniasis treated with oral miltefosine.[32,33] Although the origin of the inflammatory response remained unclear, our observations suggested that this most likely represented an immune response to the parasite. It was possible that as the parasites were killed by miltefosine, they became more antigenic and elicited a prominent inflammatory reaction. Analogous reactions were seen in other infections, for instance, the Mazzotti reaction in onchocerciasis and the Jarisch–Herxheimer reaction with syphilis or Lyme disease. Regardless, pretreatment with corticosteroids in patients who receive miltefosine might be able to modify the response substantially, and consideration should be given to pausing or reducing the frequency of the drug after the first month of treatment if the inflammation persists or becomes severe. Consideration should also be given to the possibility of treatment failure and worsening disease if inflammation persists.

Another interesting observation in our case series was the effect of miltefosine on pain. Ten of 15 patients reported an almost immediate improvement in pain that was noticeable within the first few days of treatment. This was often seen before any clinical improvement was apparent, regardless of whether the patients were being treated with additional steroids. Again, the mechanism of this effect was unclear, but it was not unique to miltefosine. An analogous improvement in pain was seen in patients with other bacterial keratitis, especially *pseudomonas* keratitis, in which patients often reported improved pain even as they had contemporaneous worsening inflammation.

The most frequent dosing schedule of 50 mg twice a day was well tolerated, with minor gastrointestinal upset as the most common side effect. For Leishmaniasis, adult dosing is based on body weight of <45 kg (50 mg twice daily) or >45 kg (50 mg 3× daily), but its pharmacokinetics are poorly understood, and optimal dosing for AK is unknown. None of the patients discontinued therapy secondary to gastrointestinal side effects, although 1 patient needed dose reduction to twice a day from 3× a day. Two patients discontinued miltefosine due to laboratory abnormalities noted after multiple courses of treatment.

Introduced abroad for the treatment of Leishmaniasis in the 1990s, miltefosine was first approved for this purpose in the United States in 2014 and was made commercially available shortly thereafter. In December 2016, the FDA

approved its use for AK as an orphan drug. Its mechanism of action is unclear but likely involves facilitation of apoptosis, lipid membrane structural interference, and interruption of phospholipid signaling, among others.[34] Studies in *acanthamoeba* have identified signs of membrane disruption and organism death. As part of a multidrug regimen, miltefosine has been effective in granulomatous amoebic encephalitis and animal models of AK. Although a trial of a topical formulation of miltefosine for AK was deemed unsuccessful,[35] individual case reports of systemic use were associated with cure. Animal studies demonstrated fetal harm and transient effects on reproductive function in both males and females, which led to pregnancy as a strict contraindication for its use.[36] It is also recommended that effective birth control be practiced during and for 4-5 months after completion of a 28-day treatment course.[37] In our series, congruent with its use for leishmaniasis, the most common side effect (7/15 patients) was nausea and vomiting (probably from direct mucosal toxicity from oral administration), and the most frequent laboratory abnormality was abnormal liver function tests. These side effects all resolved with discontinuation of the medication.

• **STUDY LIMITATIONS:** There were significant limitations to this report, which included the small number of patients and the variability in treatment duration and regimen. Selection bias was also a possibility because cases were identified by 1 author (E.Y.T.) through consultations with various centers on treatment-resistant AK cases. However, our aim was to report the results of all patients treated at these centers during this time with miltefosine. As a new, costly drug, it was used only in patients in whom the individual treating physicians judged traditional therapy to have failed, making the inclusion characteristics nonuniform but not unexpected of a retrospective study. The time from onset to introduction of miltefosine ranged widely, from 26 to 532 days with an average of 216 days, which supported our suggestion that other therapies had failed in the preponderance of cases. In many cases, the goal with miltefosine was for salvage therapy, which further highlighted the severity of most of our patients. Furthermore, the drug was used as an adjunct because patients were continued on topical anti-amoebic treatment, which made it difficult to evaluate individual drug efficacy. AK, despite its increased incidence, remains a rare disease with other topical therapeutic options, which would make a prospective randomized trial difficult.

Although *in vitro* and systemic responses are promising, much needs to be evaluated regarding miltefosine's use in AK. The cytotoxic activities are variable *in vitro* between stains of *acanthamoeba*, and there is no information on optimal dosing for use in ocular infections.[22,38,39] Our patients were treated with some variance in dosing. Similarly, its interactions with other anti-amoebic therapy needs to be elucidated, although there is some suggestion of a synergistic effect when used with a biguanide.[40] There are anecdotal reports of resolution of treatment-resistant AK with miltefosine, but there are no systematic studies of these cases. Regardless, the high rate of microbiologic cure when used as an adjuvant is encouraging in this subset of treatment-resistant cases. Our study did not address its use in other stages of AK that have effective treatment options. Nevertheless, its role in earlier stages of disease should be investigated, either as a sole or adjunctive treatment.

CONCLUSIONS

IN SUMMARY, WE REPORTED ON 15 CASES OF CLINICALLY resistant AK on topical anti-amoebic therapy treated with adjuvant systemic miltefosine with a high rate of disease resolution. Miltefosine treatment can result in significant inflammatory sequelae that may be due either to its therapeutic effect or to an idiopathic drug reaction that can be managed with topical corticosteroids and/or discontinuing the drug, as most of the cases noted here. Although most patients experienced tolerable or no systemic side effects, ophthalmologists considering using miltefosine in patients with AK should be prepared to recognize and manage both ocular and systemic complications. Strong consideration should be given to partnering with an internist or infectious disease specialist because of the systemic and reproductive risks associated with miltefosine. Further study of the optimal timing and dose of miltefosine, as well as the management of the resultant corneal inflammation, is necessary as we move forward in assessing its usefulness in AK, but its success as salvage therapy provides support for its efficacy. Its role as a front-line drug in earlier, milder disease is unknown, but will be easier to explore now that it is more widely available. Although difficult in a rare disease such as AK, further studies will better define the efficacy and role of the drug as part of our armamentarium against this devastating infection.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

PRANEETHA THULASI: FORMAL ANALYSIS, INVESTIGATION, Data curation, Writing - original draft, Writing - review & editing. **Hajirah N. Saeed:** Investigation, Writing - review & editing. **Christopher J. Rapuano:** Investigation, Writing - review & editing. **Joshua H. Hou:** Investigation, Writing - review & editing. **Alpheus B. Appenheimer:** Investigation, Writing - review & editing. **James Chodosh:** Investigation, Writing - review & editing. **Joann J. Kang:** Investigation, Writing - review & editing. **Amber M. Morrill:** Investigation, Writing - review & editing. **Neil Vyas:** Investigation, Writing - review & editing. **Michael E. Zegans:** Investigation, Writing - review & editing. **Richard Zuckerman:** Investigation,

Writing - review & editing. **Elmer Y. Tu:** Conceptualization, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Supervision.

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