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Challenges and advances in the medical treatment of granulomatous amebic encephalitis

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***Abstract*:** Granulomatous amebic encephalitis, caused by the free-living amebae *Balamuthia mandrillaris* or *Acanthamoeba* species, is a rare and deadly infectious syndrome with a current mortality rate of >90%. Much work remains to define the optimal treatment for these infections. Here, we provide a comprehensive overview of the supporting evidence behind antimicrobials currently recommended by the Centers for Disease Control and Prevention (CDC) with updated statistics on survival rates and medication usage from the CDC Free-Living Ameba Database. We also discuss promising treatments, especially the emerging therapeutic agent nitroxoline, and provide recommendations for the next steps in this area.

Keywords: ameba, Balamuthia, Acanthamoeba, drug repurposing, encephalitis

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The problem of granulomatous amebic encephalitis

Granulomatous amebic encephalitis (GAE) is an uncommon, highly morbid, and poorly understood infectious syndrome. The two known etiologic agents of GAE are the free-living amebae (FLA) *Balamuthia mandrillaris* and *Acanthamoeba* species. GAE is an infection of the central nervous system (CNS) characterized by encephalitis or meningoencephalitis, often including the development of space-occupying lesions. GAE caused by *B. mandrillaris* or *Acanthamoeba* has a mortality rate of >90%, making it one of the deadliest infectious syndromes.[1,2] The optimal treatment for these infections is currently not well defined.

The first case of human *Acanthamoeba* infection was reported in 1972.[3] Multiple species of *Acanthamoeba* have been implicated in human infection, and the term *Acanthamoeba* will be used in this article to describe any of the known pathogenic species.[4] A total of 122 cases of *Acanthamoeba* GAE have been identified in the United States between 1956 (diagnosed nearly 20 years post-mortem) and 2020.[2] *Acanthamoeba* GAE disease generally presents as an encephalitis syndrome with gradual onset.[2] *Acanthamoeba* primarily causes invasive disease in immunocompromised patients, especially those living with human immunodeficiency virus (HIV), malignancy, or who have undergone solid organ or stem cell transplant.[2] In addition to GAE, *Acanthamoeba* can cause rhinosinusitis, cutaneous disease, or disseminated disease, involving multiple organ systems. More commonly, it causes a destructive form of keratitis that affects immunocompetent hosts who use contact lenses.[2] *Acanthamoeba* has a worldwide distribution and is found throughout the environment including soil and tap water samples.[5] In the United States, most non-keratitis *Acanthamoeba* cases have been reported in California, Texas, New York, and Georgia.[2]

B. mandrillaris was identified in 1990 in a neurologically devastated female mandrill at the San Diego Zoo, and noted to be distinct from *Acanthamoeba* based initially on morphology and antigenic analysis.[6] However, cases in the United

States were found to date back to 1974 upon retrospective review.[1] *Acanthamoeba* and *B. mandrillaris* have since been found to be closely related by ribosomal ribonucleic acid (RNA) analyses.[7] Both of these FLA have a proliferative, motile trophozoite stage, and a thick-walled, dormant cyst form. The cyst form is thought to be more resistant to antimicrobials than the trophozoite.

Since its discovery, *B. mandrillaris* has been reported in more than 200 human cases worldwide, in every continent except Antarctica. Similar to *Acanthamoeba*, GAE due to *B. mandrillaris* usually presents as the subacute onset of neurological symptoms.[1] Besides GAE, *B. mandrillaris* can cause cutaneous disease.[1] In rare case reports, it has also been described as causing pulmonary infection, endophthalmitis, and disseminated disease.[8,9] *B. mandrillaris* can also be found in soil and water, and while the mechanism of infection in most cases is not known, a history of soil exposure is common.[1] Humans are usually a dead-end host for the ameba, except in rare cases of *B. mandrillaris* transmission *via* organ transplant.[10,11] *B. mandrillaris* is also known to cause fatal infections in a variety of non-human mammals, including tigers,[12] great apes,[13] and dogs.[14] Case incidence and clinical presentation vary by geographic location. Most cases of *B. mandrillaris* infections in the United States have been in California, Texas, and Arizona.[1] Outside the United States, the largest case series have been reported from Peru[15] and China.[16] Patients diagnosed outside North America are more apt to present with a cutaneous lesion than patients in the United States, who typically present with GAE without a preceding skin lesion.[1,15] The first case of *B. mandrillaris* GAE in Africa was reported in 2022.[17]

Naegleria fowleri is another pathogenic FLA that infects humans. It causes primary amebic meningoencephalitis (PAM), a rapidly progressive and highly fatal CNS infection with only seven well-characterized survivors reported among 182 laboratory-confirmed cases (4% survival).[18,19] Of the three FLA species, only *N. fowleri* has a clearly defined mode of acquisition. This ameba infects humans when water forcefully enters the nose, through participation in aquatic activities or nasal rinsing with contaminated tap water. Because PAM cases are readily distinguished from GAE, both by epidemiology and clinical presentation, this article focuses solely on the treatment of *B. mandrillaris* and *Acanthamoeba* CNS infections. A recent review of PAM was published in 2021.[19]

The treatment for GAE is based largely on case studies of survivors. The Centers for Disease Control and Prevention (CDC) currently suggests an antimicrobial regimen for GAE comprised of five or six drugs: pentamidine, sulfadiazine, flucytosine, fluconazole, and miltefosine; for *B. mandrillaris* GAE, a macrolide (azithromycin or clarithromycin) is added (Table 1).[1,2,20] In addition to this core regimen, survivors of GAE have received many other empiric therapies, including acyclovir, albendazole, amphotericin B, doxycycline, ethambutol, ketoconazole, isoniazid, minocycline, pyrazinamide, posaconazole, rifampin, trifluoperazine, thioridazine, trimethoprim–sulfamethoxazole, voriconazole, and/or various steroid formulations.[1,21] The wide variety of treatments used may reflect both delay in the diagnosis (and therefore empiric treatment for hypothesized viral, bacteria, mycobacterial, or fungal etiologies) and a paucity of available evidence to guide treatment.

Mortality remains high despite therapy, and many of the recommended medications have significant toxicities, especially pentamidine, sulfadiazine, and flucytosine. Large-scale *in vitro* drug screens have identified new drugs that may be more clinically effective and less toxic than those historically used. This article reviews the clinical, pharmacological, and laboratory data underlying the current antimicrobial agents used for GAE, novel studies in this area, and future directions.

Challenges of evaluating therapies for rare and deadly infections

There has never been a randomized, controlled medication trial for GAE, nor is one realistically feasible. Cases are exceptionally rare, and the randomized allocation of potentially lifesaving medications is impossible in the face of a condition with mortality exceeding 90%. Thus, therapeutic advances for this condition have relied primarily on case studies of survivors, despite their known potential for bias, supplemented by *in vitro* studies.

Table 1. Association of medications received and survival in patients with granulomatous amebic encephalitis in the United States diagnosed antemortem and reported to CDC, 1955–2020.

	***Acanthamoeba* GAE[a]**			***B. mandrillaris* GAE[b]**		
Total survivors (%)	7/112 (6%)			12/123 (10%)		
Total survivors among those diagnosed antemortem (%)	7/42 (17%)			12/76 (16%)		
Total survivors among those diagnosed antemortem who received any recommended treatment (%)	7/30 (23%)			11/48 (23%)		
	Patients with *Acanthamoeba* GAE diagnosed with antemortem who received at least one recommended medication (*n* = 30)			**Patients with *B. mandrillaris* GAE diagnosed antemortem who received at least one recommended medication (*n* = 48)**		
Drug	**Received drug (*n* survived, % survival)**	**Did not receive drug (*n* survived, % survival)**	***p* Value[c]**	**Received drug (*n* survived, % survival)**	**Did not receive drug (*n* survived, % survival)**	***p* Value[c]**
Currently recommended regimen						
Pentamidine	3/13 (23%)	4/17 (24%)	1.00	8/31 (26%)	3/17 (18%)	0.72
Sulfadiazine	5/14 (36%)	2/16 (13%)	0.20	9/32 (28%)	2/16 (13%)	0.29
Flucytosine	6/13 (46%)	1/17 (6%)	0.02	10/30 (33%)	1/18 (6%)	0.04
Fluconazole	5/16 (31%)	2/14 (14%)	0.40	10/36 (28%)	1/12 (8%)	0.25
Miltefosine	6/15 (40%)	1/15 (7%)	0.08	6/22 (27%)	5/26 (19%)	0.73
Azithromycin[d]	4/11 (36%)	3/19 (16%)		9/32 (26%)	2/16 (13%)	0.29
Candidate drugs						
Nitroxoline[e]	No data	No data		No data	No data	
Voriconazole	1/5 (20%)	6/25 (24%)	1.00	1/5 (20%)	10/43 (23%)	1.00
Isavuconazole	No data	No data		No data	No data	
Posaconazole	No data	No data		No data	No data	
Itraconazole	1/3 (33%)	6/27 (22%)	1.00	0/4 (0%)	11/44 (25%)	0.56
Plicamycin	No data	No data		No data	No data	
Ponatinib	No data	No data		No data	No data	

[a]10 patients were excluded due to unknown survival outcomes.
[b]7 patients excluded due to unknown survival outcome.
[c]Fisher exact test was used, as ⩾25% of the cells had expected counts of ⩽5 for all comparisons.
[d]Azithromycin is not currently part of the recommended regimen for *Acanthamoeba* GAE.
[e]Data presented in this table includes patients diagnosed through 2020. The two patients treated with nitroxoline were diagnosed in subsequent years.
CDC, Centers for Disease Control and Prevention; GAE, granulomatous amebic encephalitis.

Patients with confirmed or suspected GAE infection are typically treated with multiple medications simultaneously.[1,2,22] It is challenging to distinguish the clinical effects of individual medications of a multiple-agent anti-amebic regimen and identify the most active compound[s]. Publication bias is also a substantial concern; case reports describing survival are more likely to be published than those describing death. CDC maintains records of all reported U.S. GAE cases, but FLA infections, are not nationally notifiable; hence, some cases may be unreported. Furthermore, patients who receive early diagnoses and prompt, guideline-directed therapy may systematically differ from those who do not. Large medical centers are more likely to have had experience in treating GAE patients and therefore accumulated the institutional expertise and resources to expedite diagnosis, involve public health officials, and manage the expected toxicities of anti-amebic regimens.

Given the limitations of case studies, *in vitro* studies of potential anti-amebic medications have been key to identifying potential treatments for GAE; however, challenges and limitations also exist for *in vitro* data. First, culturing FLA is technically challenging. Also, early studies of anti-amebic medications in the 1970s, 1980s, and 1990s often equated amebic death in culture to the cessation of movement, an experimental outcome likely susceptible to error. Finally, different FLA isolates are thought to have potentially different drug susceptibility patterns, potentially limiting the findings of the FLA cultured in any single laboratory study.[23]

Classically, drug discovery progresses from *in vitro* compound identification, through chemical optimization by structure activity relationship experiments, and pre-clinical safety and efficacy testing in animal models of disease, and culminates in human trials. Yet for GAE, there is limited *in vivo* animal data. *Acanthamoeba* keratitis has been simulated in rodent models,[24] and a small number of studies have trialed intranasal instillation of *Acanthamoeba* in mice to simulate rodent GAE.[25] A model of *B. mandrillaris* was developed in immunocompetent[26] and immunodeficient mice[27] but has not been widely used to assess drug efficacy.

Clinical data of anti-amebic medications in GAE

The Infectious Disease Society of America encephalitis guidelines, released in 2008, initially recommended either a combination of trimethoprim–sulfamethoxazole, rifampin, and ketoconazole, or a combination of fluconazole, sulfadiazine, and pyrimethamine for the treatment of *Acanthamoeba* GAE.[28] Since then, CDC experts have formalized a recommended five-drug regimen consisting of pentamidine, flucytosine, fluconazole, sulfadiazine, and miltefosine.[2] Among patients with non-keratitis *Acanthamoeba* infection, receipt of those five drugs has been significantly associated with survival.[2]

The first two U.S. patients reported to survive *B. mandrillaris* GAE were diagnosed in 1996 and 2000. These patients were treated with a regimen of pentamidine, flucytosine, fluconazole, a macrolide (azithromycin or clarithromycin), and sulfadiazine.[29,30] This five-drug regimen remained the standard of care until 2009 when miltefosine was added after it was used in several patients with *B. mandrillaris* GAE who survived.[31,32] Miltefosine was initially available under investigational new drug (IND) status for each patient; it was then made available through CDC[33] and is now commercially available in the United States, primarily as a treatment for leishmaniasis.

To examine clinical evidence underlying the currently recommended GAE regimens, data from the CDC Free-Living Ameba database were analyzed. Comparative analyses were restricted to U.S. patients reported to the database from 1955 to 2020 with GAE (with or without disease at other sites) who were diagnosed with antemortem, and for whom treatments and survival outcomes were reported. All *B. mandrillaris* GAE cases were laboratory confirmed. *Acanthamoeba* cases included those with confirmed GAE and those with suspected GAE in patients with confirmed *Acanthamoeba* in another organ system.[2] Fisher exact tests were used to assess the association between receipt of medication and survival. Data regarding dosage, duration, and timeliness of starting each drug were not available. Significance was defined as $p < 0.05$. This activity was reviewed by CDC and was conducted consistent with applicable federal law and CDC

policy (see e.g. 45 C.F.R. part 46.102(I)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 33 U.S.C §3501 et seq.).

A total of 122 patients with *Acanthamoeba* GAE were reported to the CDC during this time period and 42 (34%) of those received antemortem diagnoses. Seven patients survived, which represents 6% of those with known outcomes, and 17% of those who were diagnosed with antemortem (Table 1). Treatment with any anti-amebic medications was associated with an increased likelihood of survival. For patients with *Acanthamoeba* GAE, survival improved to 23% among those diagnosed with antemortem who received at least one recommended medication.

Between 1974 and 2020, 130 U.S. cases of *B. mandrillaris* GAE were reported, 76 (58%) of which were diagnosed as antemortem. Survival among patients with *B. mandrillaris* GAE was also rare. Twelve of 123 (10%) patients with a known outcome survived, representing 16% of patients diagnosed with antemortem. Of patients with *Balamuthia* GAE who received any anti-amebic medications, 23% survived.

To avoid confounding due to the association of receipt of any anti-amebic medication and survival, only patients who received at least one recommended medication were included in the analyses that evaluated the association between survival and receipt of each medication. For patients with *Acanthamoeba* GAE ($n=30$ who received at least one medication), the percentage of patients who survived was higher among those who received flucytosine (46%) compared with those who did not (6%) ($p=0.02$). A non-statistically significant association was noted between survival and receipt of miltefosine ($p=0.08$). Pentamidine was not associated with survival, with 23% survival among those who received the drug, and 24% among those who did not ($p=1.0$). Among patients with *B. mandrillaris* GAE ($n=48$ who received at least one medication), flucytosine was statistically associated with survival, with 33% survival in those patients who received it, as compared with 6% in those who did not ($p=0.04$). Other recommended medications did not show a statistically significant association with survival, including pentamidine ($p=0.72$).

There are several limitations to consider with this analytic approach. First, surveillance data are observational and therefore may contain missing, incomplete, or inaccurate information. Also, while confounding due to treatment with any medication was addressed, the survival analysis does not account for many other potential confounders, including duration of therapy, stage of illness at the time of therapy initiation, age, and comorbidities. Furthermore, drug combinations and a number of drugs received were not assessed given the very low number of patients available for analysis, but may be relevant to survival.

Of note, no clinical data were available in this database for GAE candidate drugs identified *in vitro* screens and discussed subsequently, including nitroxoline, isavuconazole, posaconazole, plicamycin, and ponatinib. Data available for voriconazole and itraconazole were limited.

Surveying *in vitro* data underlying the medication regimens used to treat GAE

To identify studies that had tested the core antimicrobials used to treat GAE, including pentamidine, sulfadiazine, fluconazole, flucytosine, miltefosine, and azithromycin or clarithromycin; we performed a literature search for the terms 'Acanthamoeba' or 'Balamuthia' and the drug of interest. We selected studies that included *in vitro* and/or mouse model experiments. Articles were first screened by title and abstract, then a full-text review was performed. Articles were included if effective amebicidal concentration was reported after the treatment of trophozoites with the given drug. Studies that examined the effects of drugs on the cyst life stage only were excluded, and rodent experiments that focused exclusively on keratitis models were also excluded. When more than five articles were available for an ameba/drug combination (applicable to *B. mandrillaris*/pentamidine only), only five references were cited; preference was given to larger-scale studies performed in recent years.

The studies were heterogeneous, varying by FLA species, strain, and time the amebae were subjected to drug treatment (Supplemental Table 1). The endpoints of these studies also varied. Early studies described minimal motility inhibitory concentration, defined as the amount of drug needed to stop the subjective determination of trophozoite motility. Most recent studies report results as the minimum concentration of drug needed to reduce trophozoite growth to 50% of control. This is reported as minimal inhibitory concentration (MIC), inhibitor concentration

needed for 50% effect (IC_{50}), or half-maximal concentration as otherwise defined.[34]

We report here (Table 2) the 50% maximal effect against *Acanthamoeba* or *B. mandrillaris* trophozoites according to the authors' definition (MIC, IC_{50}, or half-maximal concentration as defined) in different studies. If authors reported drug concentrations in grams per milliliter, we converted this using the compound molar weight to micromolar equivalent and reported the result as micromolar. If the authors performed tests only at fixed drug concentrations, or if they described their results as 'no effect at the limit of assay' or '100% amebic inhibition', we characterized growth at a given drug concentration as <50% or >50% inhibition at the concentration tested.

A key limitation of this approach is that given the heterogeneity in experimental assays and reporting, we do not believe that these numbers can be considered direct comparisons but we believe it is useful to tabulate the approximate inhibitory concentrations of each drug across studies. We also gathered from the literature the estimated serum and cerebrospinal fluid (CSF) concentrations of each drug.

Acanthamoeba in vitro studies

Pentamidine was found in multiple studies to be effective *in vitro* against *Acanthamoeba* species, with anti-trophozoite effects generally reported in the low micromolar range (<5 μM, Table 2).[38,37] Pentamidine, however, is not thought to penetrate the CNS well and may only achieve CSF concentrations that are lower than the effective amebicidal range.[36,74]

A study from 1993 found an IC_{50} for sulfadiazine of 6.0 μM against *Acanthamoeba*.[46] A study from 1974 examined the effects of flucytosine on *Acanthamoeba in vitro* and *in vivo* and found that flucytosine had no reliable effect *in vitro* at their limit of assay. The same authors found a positive effect of flucytosine dosing on survival in a mouse model of *Acanthamoeba* infection but only when flucytosine was given on the same day as amebic inoculation.[25] The studies that tested the effects of fluconazole on *in vitro* trophozoite growth found no or negligible effects of fluconazole at the highest doses tested, which amply exceeded the maximum estimated CSF fluconazole concentrations.[51,52] Miltefosine *in vitro* had moderate micromolar efficacy, at concentrations less than its maximum estimated serum concentrations (CSF/plasma ratio is not known), and varying by ameba strain.[23,55–57]

B. mandrillaris in vitro studies

Multiple studies report *in vitro* activity of pentamidine against *B. mandrillaris* with IC_{50} estimates below 20 μM,[30,40–42] or inhibition >50% at a low concentration,[30,42,43] although in several studies the effective IC_{50} concentration exceeded the estimated maximum CSF concentrations of the drug.[40,41] Studies that tested *in vitro* effects of sulfadiazine on *B. mandrillaris* found no effect at the highest concentration tested,[40,42] although the maximum of these assays was lower than the estimated maximum CSF concentrations of sulfadiazine.[45] One study reported that flucytosine was amebicidal at IC_{50} 86 μM,[41] which is lower than its estimated CSF concentrations.[48,49] Other reports found minimal effect but tested only to a maximum concentration that was lower than the CSF concentrations that flucytosine is capable of achieving.[40,42] Fluconazole had poor efficacy, with all studies reporting no or minimal (<50%) effect at the highest concentrations tested, including at levels that exceed estimated CSF concentrations.[30,40–42] Two studies found that miltefosine was effective with IC_{50} values of approximately 65 μM,[40,55] one study noted the effect at a concentration below 250 μM,[43] while one study found no effect at the limit of the assay.[41] Finally, azithromycin was active at a high concentration (IC_{50} 244 μM) in one study,[40] which exceeds the estimated CSF concentrations. Other studies found no activity.[30,34,41]

Evaluating the current treatments for GAE

A limited number of patients have survived GAE after treatment with the currently recommended agents. However, the mortality rate remains high.[1,2] More efficacious and less toxic regimens may help to improve the prognosis of patients with GAE. We therefore summarize the existing evidence for the current anti-amebic regimens, and then turn to discussing potential novel agents.

Of the current antimicrobial regimen for GAE, clinical data are most supportive of an association with survival with flucytosine. *In vitro*, flucytosine

Table 2. *In vitro* studies of anti-amebal agents against *Acanthamoeba* and *B. mandrillaris*. Theoretical maximum CSF concentration is calculated by multiplying known serum maximum concentration (μM) by the maximum reported CSF/plasma ratio. If the CSF/plasma ratio is not known (relevant for miltefosine, nitroxoline, and plicamycin), a ratio of 1 is assumed, acknowledging that the true penetration of the three agents may be lower.

Agent	Original indication	Serum maximum concentration (μM)	Reported CSF/plasma ratio	Theoretical maximum CSF concentration (μM)	Acanthamoeba species *in vitro* drug concentration to reduce trophozoite growth to 50% (μM)	Balamuthia mandrillaris *in vitro* drug concentration to reduce trophozoite growth to 50% (μM)
Currently recommended regimen						
Pentamidine	*Antiparasitic*: trypanosomiasis, leishmaniasis, pneumocystis	1.80[35]	0.5–0.8[36]	1.4	3.4[37] 0.6[38] <183.6–734.4[39,a]	9.1[40] 18.4[41] <2.9[30] <2.9[42] <500.0[43]
Sulfadiazine	*Antiparasitic*: toxoplasmosis	241.3[44]	0.27–0.33[45]	79.6	6.0[46]	>40.0[42] >20.0[40]
Flucytosine	*Antifungal*: cryptococcal meningitis	542.2[47]	0.74–0.84[48,49]	455.3	>309.8[25]	>77.5[42] >20.0[40] 86.3[41]
Fluconazole	*Antifungal*: Candida, cryptococcus, and others	21.9[50]	0.5–1[49]	21.9	>50.0[51] >218.1[52]	>32.6[30] >32.6[42] >163.3[41] >20.0[40]
Miltefosine	*Antiparasitic*: leishmaniasis	91.3[53]	Unknown[54]	91.3	3.9–62.5[23,a] <80.0[55] <80.0[56] 31.3–250[57,a]	65.0[55] <250.0[43] 62.2[40] >122.7[41]
Azithromycin[b]	*Antibacterial*: pneumonia, others	2.3[58]	0–0.015[59]	0.035	2.2[34]	>12.8[30] 244.1[40] >66.8[41] >10.0[34]
Candidate drugs						
Nitroxoline	*Antibacterial*: urinary tract infections	30.0[60]	Unknown	30.0	11.2[61]	2.8[40] 7.8[61]
Isavuconazole (isavuconazonium)	*Antifungal*: endemic fungi, aspergillus, others	16.9[62]	0.23–0.38[63]	6.4	0.001–0.03[51,a] 0.001–0.04[64,a] 0.09[65] 0.9[34]	–
Voriconazole	*Antifungal*: endemic fungi, aspergillus, others	6.6[66]	0.22–0.88[67]	5.8	2.7[34] 2.8–5.7[52,c] 0.6[51]	>143.1[41] >20.0[40]

(Continued)

Table 2. (Continued)

Agent	Original indication	Serum maximum concentration (μM)	Reported CSF/plasma ratio	Theoretical maximum CSF concentration (μM)	Acanthamoeba species *in vitro* drug concentration to reduce trophozoite growth to 50% (μM)	Balamuthia mandrillaris *in vitro* drug concentration to reduce trophozoite growth to 50% (μM)
Posaconazole	*Antifungal*: endemic fungi, aspergillus, others	4.0[68]	0.01–2.25[67]	9.0	0.003–0.07[51,a] 0.6[38]	49.6[41] >20.0[40]
Itraconazole	*Antifungal*: endemic fungi, aspergillus, others	2.8[69,70]	0–0.12[49,67]	0.34	22.7[52] 50.0[51]	49.2[41] >20.0[40]
Plicamycin (mithramycin)	*Antibiotic/ oncology*: testicular cancer	1.6[71]	Unknown	1.6	6.1[61]	11.3[61] 1.5[34] >20.0[40]
Ponatinib	*Oncology*: tyrosine kinase inhibitor	0.14[72]	0[73]	0	1.6[61]	0.3[61]

[a]Varies by strain or species tested.
[b]Azithromycin is currently recommended for *B. mandrillaris* only. Searches were also performed for clarithromycin.
[c]Reported as a range.
CSF, cerebrospinal fluid.

has been shown in a single study to have effectiveness against *B. mandrillaris* but only at high concentrations (one study found an IC_{50} of 86 μM; others stated greater than the limit of assay). However, flucytosine has excellent CNS penetration and may achieve high CSF concentrations that exceed the amounts tested *in vitro*. *In vitro* efficacy of flucytosine against *Acanthamoeba* has not yet been demonstrated, but only one study was reported, which also did not extend to the high concentrations that flucytosine may achieve. Miltefosine also may be associated with survival, although with borderline clinical significance. It has moderate *in vitro* activity against both *Acanthamoeba* and *B. mandrillaris* in most reported studies and a favorable side effect profile.

Fluconazole and azithromycin are two medications with relatively favorable side effect profiles but little evidence of efficacy. We were unable to find any *in vitro* studies that identified fluconazole as amebicidal for either *B. mandrillaris* or *Acanthamoeba* nor do the clinical data presented here support an association of fluconazole and survival among patients who were treated. Similarly, azithromycin (recommended currently for *B. mandrillaris* but not *Acanthamoeba*) appears to have weak *in vitro* activity against both FLA species, poor CNS penetration, and no clear link to survival in our analyses.

Pentamidine and sulfadiazine are two agents with minimal reported evidence of efficacy and significant toxicities. In our bivariate analysis, pentamidine was not associated with survival in GAE of either etiology. It has *in vitro* effective amebicidal concentrations that are the lowest of the current regimen but the limited studies that have characterized pentamidine CSF concentrations suggest that it may reach concentrations that are comparable to, or even lower than, its amebicidal *in vitro* concentrations.[36,74] Moreover, pentamidine is the most toxic medication of the core antimicrobials used, with one study noting adverse events in 71.7% of patients treated intravenously, primarily nephrotoxicity, dysglycemia, and hepatotoxicity.[75]

Similarly, sulfadiazine was not found to be associated with survival in this bivariate analysis. There is no evidence supporting sulfadiazine as effective against *B. mandrillaris*, and efficacy against *Acanthamoeba* has only been demonstrated in one small study from 1993 that has not been replicated. Moreover, sulfadiazine has significant toxicities, including renal failure, which may preclude the use of other anti-amebal agents.

Taking these data together, and acknowledging significant uncertainties due to limitations in all data, the cumulative *in vitro*, pharmacologic, and clinical evidence of efficacy is most supportive of the agents miltefosine and flucytosine for the treatment of GAE. Of the remaining four currently recommended agents, pentamidine has such significant and frequent toxicities that it should be used with caution and potentially stopped if toxicities arise. Sulfadiazine, similarly, has minimal *in vitro* or clinical evidence of efficacy, and its primary toxicity (renal failure) can preclude the use of other anti-amebic agents, as was our clinical experience.[76] Therefore, this agent should also be used with caution.

Identification and evaluation of leading candidate drugs for GAE

Prior to the 2010s, most studies reviewed were designed to test a small number of compounds. The development of drug screens against *Acanthamoeba* and *B. mandrillaris* permitted larger-scale analyses. Using this approach, new compounds of potential efficacy were identified and tested in comparison to medications in the current core regimen. We highlight here the results of larger-scale studies (>50 compounds) that tested compound libraries against *in vitro* amebic cultures.

We identified seven published papers that fit this criterion, including one drug screen that tested a 400 compound library against *Acanthamoeba* only,[38] three drug screens that examined compound libraries (85, 800, and 2,177 compounds) against *B. mandrillaris* only,[40,41,77] and three drug screens (159, 400, and 12,000 compounds) that looked at both *B. mandrillaris* and *Acanthamoeba* isolates.[34,61,65] We highlight a limited number of drug candidates here (Table 2) that fulfill the following criteria: (1) approved by the U.S. Food and Drug Administration (FDA) or internationally for systemic administration and (2) *in vitro* efficacy against one or both FLA.

The quinolone metal-chelating antibiotic nitroxoline was identified in a large-scale screen as having activity against *B. mandrillaris* trophozoites at low concentration (IC_{50} 2.8 μM)[40] relative to its known serum concentrations in humans. Nitroxoline is in use in Europe and China for the treatment of urinary tract infections and has a robust safety and tolerability profile after decades of use.[78] A study comparing drug efficacy across all three pathogenic FLA species confirmed efficacy in *B. mandrillaris*, *Acanthamoeba* (IC_{50} 11.2 μM), and *N. fowleri* (IC_{50}~1.5 μM).[61]

Voriconazole and isavuconazole, which are non-fluconazole medications in the azole class, had robust *in vitro* activity against *Acanthamoeba*,[34,51,52,64,65] but not against *B. mandrillaris*.[40,41] Posaconazole was effective at low concentration in *Acanthamoeba* but less so in a study of *B. mandrillaris* (half-maximal activity at 0.003–0.63 and 49.5 μM, respectively).[41] Itraconazole showed moderate activity against both *Acanthamoeba*[51,52] and *B. mandrillaris*.[41]

Of other potential candidate drugs, two studies found that plicamycin (mithramycin) had activity against *B. mandrillaris*,[34,61] and in one study, also *Acanthamoeba*[61] but at higher concentrations than it likely achieves in serum.[71] This drug is not currently available in the United States. Ponatinib, a tyrosine kinase inhibitor, was identified in a single screen to have activity against both FLA but its CNS penetration is thought to be poor.[61]

Experience with nitroxoline: from *in vitro* identification in an unbiased screen to clinical use

As described by Spottiswoode *et al* (2022),[76] a patient with *B. mandrillaris* GAE was initially started on the core regimen of pentamidine, fluconazole, flucytosine, azithromycin, miltefosine, and sulfadiazine. Albendazole was also included.[76] His lesions initially demonstrated mild improvement by magnetic resonance imaging (MRI) but the patient suffered significant medication-related toxicities, including bone marrow suppression, renal failure, and severe hypoglycemia, necessitating the cessation of pentamidine and sulfadiazine and dose reduction of flucytosine. On fluconazole, azithromycin, miltefosine, albendazole, and dose-reduced flucytosine, his lesions enlarged. The decision was made to treat with nitroxoline based on *in vitro* data,

the medication's long safety record, and urgent clinical need.[40] Nitroxoline was obtained under an emergency IND protocol approved by the FDA. After the addition of nitroxoline, the patient had a marked, sustained improvement clinically and radiologically. Flucytosine, fluconazole, and albendazole were stopped after 14 months, and nitroxoline, azithromycin, and miltefosine were stopped after an additional 7 months. He is now living independently 2 years post-initial presentation.

The potential identification of a novel anti-amebic agent with *in vitro* activity and minimal toxicity sparked interest among physicians caring for other GAE patients. A second, pediatric patient with *B. mandrillaris* GAE received nitroxoline in 2022 due to the progression of the disease, evidenced by the increasing number and size of brain lesions on brain MRI, despite approximately 9 months of standard therapy as well as surgical excision of the primary lesion. Preliminary data suggest radiographic and clinical improvement after the addition of nitroxoline to the drug regimen, despite toxicities necessitating the discontinuation of miltefosine, fluconazole, flucytosine, and pentamidine over time. The patient has been on nitroxoline for approximately 1 year and continues to show improvement [unpublished].

Novel GAE medications and areas of further investigation

Much remains to be learned about the optimal treatment of GAE. Priorities include characterization of potential and current anti-amebic agents, coupled with rigorous collection of clinical data. We suggest several areas for further study.

First, ongoing studies of nitroxoline may offer clinical promise. Nitroxoline remains the only agent identified in an unbiased screen to have been used clinically to date,[76] and preliminary clinical data are promising, although only a small number of cases have been treated with this agent. The combination of compelling *in vitro* activity,[40] limited but positive clinical data, and well-defined safety profile[78] makes nitroxoline an attractive option for future use for GAE for at least *B. mandrillaris* and, potentially, *Acanthamoeba*. Key areas of future study include assessing the effectiveness of nitroxoline against *Acanthamoeba* and exploring the mechanism of action, which may help generate more effective compounds. Finally, nitroxoline concentrations in the CSF or brain parenchyma of patients on nitroxoline, for GAE or any other indication, have not been reported. If brain tissue or CSF becomes available from a patient treated with nitroxoline, measuring drug levels may help to inform future use.

While nitroxoline is commercially available in several countries, it is not available in the United States nor is it FDA approved. Hence, to use nitroxoline to treat *B. mandrillaris* GAE patients, it must be obtained under an emergency use authorization. This single-patient approval process is labor-intensive and time-consuming, and thus too slow for many critically ill patients.

Non-fluconazole azole agents, such as isavuconazole and voriconazole, have robust efficacy against *Acanthamoeba in vitro* but not for *B. mandrillaris*. Given that fluconazole has minimal effects on both amebae *in vitro*, it is possible that substitution of another azole, such as isavuconazole or voriconazole, may improve outcomes in *Acanthamoeba* GAE specifically. Of the other potential agents highlighted in this article, most have unfavorable CNS penetration data, unfavorable side effect profiles, or both.

Finally, ongoing close communication with experts in the field and the detailed collection of clinical data are essential to advancing our understanding of this disease. We recommend that clinicians treating any patient with suspected or confirmed GAE contact the CDC Emergency Operations Center promptly at 770-488-7100 to request clinical guidance. Other platforms that can help clinicians to archive and disseminate information about rare diseases and repurposed treatments include CURE ID, a website and app developed by the FDA and the National Institutes of Health initially to share information about treatments for SARS-CoV-2, and now maintained for sharing information about repurposed treatments for neglected infectious diseases.[79]

Declarations

Disclaimer

The findings and conclusions of this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control (CDC).

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Not applicable.

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Availability of data and materials
Data presented here from the CDC Free-Living Ameba database are not publicly available. These national surveillance data are summarized periodically for public dissemination.

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References
1. Cope JR, Landa J, Nethercut H, *et al.* The epidemiology and clinical features of Balamuthia mandrillaris disease in the United States, 1974–2016. *Clin Infect Dis* 2019; 68: 1815–1822.
2. Haston JC, O'Laughlin K, Matteson K, *et al.* The epidemiology and clinical features of non-keratitis Acanthamoeba infections in the United States, 1956–2020. *Open Forum Infect Dis* 2023; 10: ofac682.
3. Jager BV and Stamm WP. Brain abscesses caused by free-living amoeba probably of the genus Hartmannella in a patient with Hodgkin's disease. *Lancet* 1972; 2: 1343–1345.
4. Centers for Disease Control and Prevention. Acanthamoeba: pathogen and environment, https://www.cdc.gov/parasites/acanthamoeba/pathogen.html (2023, accessed 3 June 2023).
5. Stockman LJ, Wright CJ, Visvesvara GS, *et al.* Prevalence of Acanthamoeba spp. and other free-living amoebae in household water, Ohio, USA-1990–1992. *Parasitol Res* 2011; 108: 621–627.
6. Visvesvara GS, Schuster FL and Martinez AJ. Balamuthia mandrillaris, N. G., N. Sp., agent of amebic meningoencephalitis in humans and other animals. *J Eukaryot Microbiol* 1993; 40: 504–514.
7. Booton GC, Schuster FL, Carmichael JR, *et al.* Balamuthia mandrillaris: identification of clinical and environmental isolates using genus-specific PCR. *J Eukaryot Microbiol* 2003; 50: 508–509.
8. Wilson MR, Shanbhag NM, Reid MJ, *et al.* Diagnosing *Balamuthia mandrillaris* encephalitis with metagenomic deep sequencing. *Ann Neurol* 2015; 78: 722–730.
9. Schafer KR, Shah N, Almira-Suarez MI, *et al.* Disseminated *Balamuthia mandrillaris* infection. *J Clin Microbiol* 2015; 53: 3072–3076.
10. Farnon EC, Kokko KE, Budge PJ, *et al.* Transmission of *Balamuthia mandrillaris* by

organ transplantation. *Clin Infect Dis* 2016; 63: 878–888.

11. Gupte AA, Hocevar SN, Lea AS, *et al.* Transmission of *Balamuthia mandrillaris* through solid organ transplantation: utility of organ recipient serology to guide clinical management. *Am J Transplant* 2014; 14: 1417–1424.
12. Niedringhaus KD, Gordon M, Yabsley MJ, *et al.* Fatal balamuthosis in a Siberian tiger and a literature review of detection options for free-living amoebic infections in animals. *J Vet Diagn Invest* 2023; 35: 311–316.
13. Hawkins SJ, Struthers JD, Phair K, *et al.* Diagnostic evaluation of fatal Balamuthia mandrillaris meningoencephalitis in a captive Bornean orangutan (Pongo pygmaeus) with identification of potential environmental source and evidence of chronic exposure. *Primates* 2021; 62: 51–61.
14. Foreman O, Sykes J, Ball L, *et al.* Disseminated infection with Balamuthia mandrillaris in a dog. *Vet Pathol* 2004; 41: 506–510.
15. Bravo FG and Seas C. *Balamuthia mandrillaris* amoebic encephalitis: an emerging parasitic infection. *Curr Infect Dis Rep* 2012; 14: 391–396.
16. Wang L, Cheng W, Li B, *et al. Balamuthia mandrillaris* infection in China: a retrospective report of 28 cases. *Emerg Microbes Infect* 2020; 9: 2348–2357.
17. Tootla HD, Eley BS, Enslin JMN, *et al.* *Balamuthia mandrillaris* Granulomatous Amoebic Encephalitis: the first African experience. *J Pediatric Infect Dis Soc* 2022; 11: 578–581.
18. Centers for Disease Control and Prevention. *Naegleria fowleri. Primary Amebic Meningoencephalitis (PAM)*. Treatment 2023 [updated 2023]. https://www.cdc.gov/parasites/naegleria/treatment.html#:~:text=Primary%20amebic%20meningoencephalitis%20(PAM)%20is,to%20treat%20patients%20who%20survived
19. Gharpure R, Bliton J, Goodman A, *et al.* Epidemiology and clinical characteristics of primary amebic meningoencephalitis caused by Naegleria fowleri: a global review. *Clin Infect Dis* 2021; 73: e19–e27.
20. Centers for Disease Control and Prevention. Balamuthia mandrillaris: Granulomatous amoebic encephalitis. *Treatment* 2023.
21. Brondfield MN, Reid MJ, Rutishauser RL, *et al.* Disseminated Acanthamoeba infection in a heart transplant recipient treated successfully with a miltefosine-containing regimen: case report and review of the literature. *Transpl Infect Dis* 2017; 19: 10.1111/tid.12661.
22. Haston JC and Cope JR. Amebic encephalitis and meningoencephalitis: an update on epidemiology, diagnostic methods, and treatment. *Curr Opin Infect Dis* 2023; 36: 186–191.
23. McBride J, Ingram PR, Henriquez FL, *et al.* Development of colorimetric microtiter plate assay for assessment of antimicrobials against Acanthamoeba. *J Clin Microbiol* 2005; 43: 629–634.
24. Costa AO, Furst C, Rocha LO, *et al.* Molecular diagnosis of Acanthamoeba keratitis: evaluation in rat model and application in suspected human cases. *Parasitol Res* 2017; 116: 1339–1344.
25. Stevens AR and O'Dell WD. *In vitro* and *in vivo* activity of 5-fluorocytosine on Acanthamoeba. *Antimicrob Agents Chemother* 1974; 6: 282–289.
26. Janitschke K, Martinez AJ, Visvesvara GS, *et al.* Animal model *Balamuthia mandrillaris* CNS infection: contrast and comparison in immunodeficient and immunocompetent mice: a murine model of "granulomatous" amebic encephalitis. *J Neuropathol Exp Neurol* 1996; 55: 815–821.
27. Kiderlen AF, Laube U, Radam E, *et al.* Oral infection of immunocompetent and immunodeficient mice with Balamuthia mandrillaris amebae. *Parasitol Res* 2007; 100: 775–782.
28. Tunkel AR, Glaser CA, Bloch KC, *et al.* The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2008; 47: 303–327.
29. Deetz TR, Sawyer MH, Billman G, *et al.* Successful treatment of Balamuthia amoebic encephalitis: presentation of 2 cases. *Clin Infect Dis* 2003; 37: 1304–1312.
30. Schuster FL and Visvesvara GS. Axenic growth and drug sensitivity studies of Balamuthia mandrillaris, an agent of amebic meningoencephalitis in humans and other animals. *J Clin Microbiol* 1996; 34: 385–388.
31. Martinez DY, Seas C, Bravo F, *et al.* Successful treatment of Balamuthia mandrillaris amoebic infection with extensive neurological and cutaneous involvement. *Clin Infect Dis* 2010; 51: e7–e11.
32. Vollmer ME and Glaser C. A Balamuthia survivor. *JMM Case Rep* 2016; 3: e005031.
33. Jennifer R. Cope. Investigational drug available directly from CDC for the treatment of infections

with free-living amebae. *MMWR Morb Mortal Wkly Rep* 2013; 62: 666.

34. Rice CA, Colon BL, Chen E, *et al.* Discovery of repurposing drug candidates for the treatment of diseases caused by pathogenic free-living amoebae. *PLoS Negl Trop Dis* 2020; 14: e0008353.
35. PENTAM (package insert). Lake Zurich, Il.: Fresenius Kabi USA, LLC. (2022, accessed 8 July 2023)
36. Bronner U, Doua F, Ericsson O, *et al.* Pentamidine concentrations in plasma, whole blood and cerebrospinal fluid during treatment of Trypanosoma gambiense infection in Cote d'Ivoire. *Trans R Soc Trop Med Hyg* 1991; 85: 608–611.
37. Ondarza RN, Iturbe A and Hernandez E. *In vitro* antiproliferative effects of neuroleptics, antimycotics and antibiotics on the human pathogens Acanthamoeba polyphaga and Naegleria fowleri. *Arch Med Res* 2006; 37: 723–729.
38. Sifaoui I, Reyes-Batlle M, Lopez-Arencibia A, *et al.* Screening of the pathogen box for the identification of anti-Acanthamoeba agents. *Exp Parasitol* 2019; 201: 90–92.
39. Alizadeh H, Silvany RE, Meyer DR, *et al.* In vitro amoebicidal activity of propamidine and pentamidine isethionate against Acanthamoeba species and toxicity to corneal tissues. *Cornea* 1997; 16: 94–100.
40. Laurie MT, White CV, Retallack H, *et al.* Functional assessment of 2,177 U.S. and international drugs identifies the quinoline nitroxoline as a potent amoebicidal agent against the pathogen *Balamuthia mandrillaris. mBio* 2018; 9: 02051-18.
41. Phan IQ, Rice CA, Craig J, *et al.* The transcriptome of Balamuthia mandrillaris trophozoites for structure-guided drug design. *Sci Rep* 2021; 11: 21664.
42. Dunnebacke TH, Schuster FL, Yagi S, *et al.* Balamuthia mandrillaris from soil samples. *Microbiology (Reading)* 2004; 150: 2837–2842.
43. Ahmad AF, Heaselgrave W, Andrew PW, *et al.* The *in vitro* efficacy of antimicrobial agents against the pathogenic free-living amoeba *Balamuthia mandrillaris. J Eukaryot Microbiol* 2013; 60: 539–543.
44. SULFADIAZINE (package insert). Laurelton, NY: Eon Labs, Inc. (2020, accessed 8 July 2023)
45. Nau R, Sorgel F and Eiffert H. Penetration of drugs through the blood-cerebrospinal fluid/ blood-brain barrier for treatment of central nervous system infections. *Clin Microbiol Rev* 2010; 23: 858–883.
46. Mehlotra RK and Shukla OP. In vitro susceptibility of Acanthamoeba culbertsoni to inhibitors of folate biosynthesis. *J Eukaryot Microbiol* 1993; 40: 14–17.
47. ANCOBAN (package insert). Bridgewater, NJ: Valeant Pharmaceuticals North America LLC (2017, accessed 8 July 2023)
48. Stott KE, Ahmadu A, Kajanga C, *et al.* Population pharmacokinetics and CSF penetration of flucytosine in adults with HIV-associated cryptococcal meningoencephalitis. *J Antimicrob Chemother* 2023; 78: 1015–1022.
49. Felton T, Troke PF and Hope WW. Tissue penetration of antifungal agents. *Clin Microbiol Rev* 2014; 27: 68–88.
50. DIFLUCAN (package insert). NY, NY: Pfizer. (2011, accessed 7 July 2023)
51. Shing B, Singh S, Podust LM, *et al.* The antifungal drug isavuconazole is both Amebicidal and Cysticidal against *Acanthamoeba castellanii. Antimicrob Agents Chemother* 2020; 64: e02223-19.
52. Lamb DC, Warrilow AG, Rolley NJ, *et al.* Azole antifungal agents to treat the human pathogens Acanthamoeba castellanii and Acanthamoeba polyphaga through inhibition of sterol 14alpha-demethylase (CYP51). *Antimicrob Agents Chemother* 2015; 59: 4707–4713.
53. IMPAVIDO (package insert). Wilmington, DE: Paladins Therapeutics Inc. (2014, accessed 7 July 2023)
54. Monogue ML, Watson D, Alexander JS, *et al.* Minimal cerebrospinal fluid concentration of miltefosine despite therapeutic plasma levels during the treatment of amebic encephalitis. *Antimicrob Agents Chemother* 2019; 64: e01127-19.
55. Schuster FL, Guglielmo BJ and Visvesvara GS. *In vitro* activity of miltefosine and voriconazole on clinical isolates of free-living amebas: *Balamuthia mandrillaris*, *Acanthamoeba* spp., and Naegleria fowleri. *J Eukaryot Microbiol* 2006; 53: 121–126.
56. Walochnik J, Duchene M, Seifert K, *et al.* Cytotoxic activities of alkylphosphocholines against clinical isolates of *Acanthamoeba* spp. *Antimicrob Agents Chemother* 2002; 46: 695–701.
57. Mrva M, Garajova M, Lukac M, *et al.* Weak cytotoxic activity of miltefosine against clinical

isolates of *Acanthamoeba* spp. *J Parasitol* 2011; 97: 538–540.

58. ZITHROMAX (package insert). New York, NY: Pfizer. (2013, accessed 7 July 2023)
59. Jaruratanasirikul S, Hortiwakul R, Tantisarasart T, *et al*. Distribution of azithromycin into brain tissue, cerebrospinal fluid, and aqueous humor of the eye. *Antimicrob Agents Chemother* 1996; 40: 825–826.
60. Bergogne-Berezin E, Berthelot G and Muller-Serieys C. [Present status of nitroxoline]. *Pathol Biol (Paris)* 1987; 35: 873–878.
61. Kangussu-Marcolino MM, Ehrenkaufer GM, Chen E, *et al*. Identification of plicamycin, TG02, panobinostat, lestaurtinib, and GDC-0084 as promising compounds for the treatment of central nervous system infections caused by the free-living amebae Naegleria, *Acanthamoeba* and *Balamuthia*. *Int J Parasitol Drugs Drug Resist* 2019; 11: 80–94.
62. CRESEMBA (package insert). Northbrook, IL: Astellas Pharma US, Inc. (2022, accessed 5 July 2023)
63. Davis MR, Chang S, Gaynor P, *et al*. Isavuconazole for treatment of refractory coccidioidal meningitis with concomitant cerebrospinal fluid and plasma therapeutic drug monitoring. *Med Mycol* 2021; 59: 939–942.
64. Shing B, Balen M and Debnath A. Evaluation of amebicidal and cysticidal activities of antifungal drug isavuconazonium sulfate against acanthamoeba T4 strains. *Pharmaceuticals* 2021; 14: 1294.
65. Rice CA, Troth EV, Russell AC, *et al*. Discovery of anti-amoebic inhibitors from screening the MMV pandemic response box on *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Acanthamoeba castellanii*. *Pathogens* 2020; 9: 476.
66. VFEND (package insert). New York, NY: Pfizer Inc. (2022, accessed 5 July 2023)
67. Wirth F and Ishida K. Antifungal drugs: an updated review of central nervous system pharmacokinetics. *Mycoses* 2020; 63: 1047–1059.
68. NOXAFIL (package insert). Rahway, NJ: Merck Sharp & Dohme LLC. (2022, accessed 5 July 2023)
69. SPORANOX Capsules (package insert). Titusville, NJ: Janssen Pharamceuticals, Inc., 2022.
70. SPORANOX Oral Solution (package insert). Titusville, NJ: Janssen Pharamceuticals, Inc. (2022, accessed 5 July 2023)
71. Fang K, Koller CA, Brown N, *et al*. Determination of plicamycin in plasma by radioimmunoassay. *Ther Drug Monit* 1992; 14: 255–260.
72. ICLUSIG (package insert). Lexington, MA: Takeda Pharmaceuticals America, Inc. (2022, accessed 5 July 2023)
73. Tanimura K, Yamasaki K, Okuhiro Y, *et al*. Monitoring ponatinib in a child with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Case Rep Oncol* 2021; 14: 24–28.
74. Donnelly H, Bernard EM, Rothkotter H, *et al*. Distribution of pentamidine in patients with AIDS. *J Infect Dis* 1988; 157: 985–989.
75. O'Brien JG, Dong BJ, Coleman RL, *et al*. A 5-year retrospective review of adverse drug reactions and their risk factors in human immunodeficiency virus-infected patients who were receiving intravenous pentamidine therapy for Pneumocystis carinii pneumonia. *Clin Infect Dis* 1997; 24: 854–859.
76. Spottiswoode N, Pet D, Kim A, *et al*. Successful treatment of *Balamuthia mandrillaris* granulomatous amebic encephalitis with nitroxoline. *Emerg Infect Dis* 2023; 29: 197–201.
77. Rice CA, Lares-Jimenez LF, Lares-Villa F, *et al*. In vitro screening of the open-source medicines for malaria venture malaria and pathogen boxes to discover novel compounds with activity against *Balamuthia mandrillaris*. *Antimicrob Agents Chemother* 2020; 64: e02233-19.
78. Naber KG, Niggemann H, Stein G, *et al*. Review of the literature and individual patients' data meta-analysis on efficacy and tolerance of nitroxoline in the treatment of uncomplicated urinary tract infections. *BMC Infect Dis* 2014; 14: 628.
79. CURE ID. CURE ID: Aggregating and Analyzing COVID-19 Treatments from EHRs & Registries Globally https://cure.ncats.io/home (2020, accessed 13 June 2023)