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Case report

Use of tafenoquine to treat a patient with relapsing babesiosis with clinical and molecular evidence of resistance to azithromycin and atovaquone

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ABSTRACT

Tafenoquine is a highly effective treatment for *Babesia microti* infections in animal models. An immunocompromised patient infected by a strain of *B. microti* that was at least partially resistant to both azithromycin and atovaquone was treated with tafenoquine. Systematic clinical studies using tafenoquine for treating other patients with babesiosis should be considered.

Tafenoquine is an 8-aminoquinoline primaquine analogue that received United States Food and Drug Administration approval in 2018 for two indications: prophylaxis of malaria for up to 6 months in total duration and prevention of relapse of *Plasmodium vivax* malaria [1,2]. Because of the long half-life of the drug of approximately 14–17 days in humans, a single dose of the drug can be administered once per week to prevent malaria [1–5].

Experimental data from 3 different studies conducted using hamsters or mice [6–8], including highly immunocompromised mice (severe combined immunodeficiency [SCID] mice [7], have demonstrated that tafenoquine can rapidly clear *Babesia microti* parasites. Therefore, this drug may have a potential role in the treatment of patients with babesiosis, particularly for patients who are highly immunocompromised, such as those who have been treated with the drug rituximab [9], who can require many months of antiparasitic drug therapy before a cure is achieved [10–12].

To begin to understand the potential therapeutic role for tafenoquine, an immunocompromised adult patient with multiple relapses of a *B. microti* infection was eventually treated with a 6 week course of tafenoquine alone. Prior to starting tafenoquine, the *B. microti* parasite causing this patient's infection was found to be at least partially resistant to both azithromycin and atovaquone. Tafenoquine was well tolerated and over the course of the nearly 19 months of follow-up since completion of the 6 week drug regimen of tafenoquine the patient has remained well.

Case summary

A 36 year old male was hospitalized on January 9, 2019 because of unexplained fevers for approximately 2 weeks. He was diagnosed with babesiosis based on a positive blood smear (8.5%). His nadir hemoglobin level was 5.7 g/dL, and he was transfused 3 units of blood. He had a history of granulomatosis with polyangiitis diagnosed in 2001. He had been remotely treated with methotrexate and cyclophosphamide, and he had received two doses of rituximab, the last of which was administered in January of 2017. Since the patient was only being treated with 7.5–20 mg of prednisone per day at the time of hospitalization, he was not regarded as being immunocompromised. The dose of prednisone was 7.5 mg during the first hospitalization, which he continued to receive during the first course of treatment for babesiosis.

The patient's initial treatment for *B. microti* infection was with a combination of atovaquone and azithromycin for 10 days (Table 1) with symptom resolution. However, the dosage of atovaquone was lower than usual [13]. Blood smears on both 1/11/19 and 3/6/19 were

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Table 1
Summary of Babesiosis Treatment Courses (all oral except where indicated): January 2019-April 2020.

Initiation date and duration	Reason for treating with antiparasitic drug therapy	Azithromycin (dose)	Atovaquone[a] (dose)	Clindamycin (dose)	Malarone®[b] (dose)	Tafenoquine (dose)
1/9/19 for 10 days	Active babesiosis with +blood smear (8.5%)	500 mg IV x 1 dose, then 500 mg QD x5 days, then 250 mg QD x4 days	750 mg QD			
3/29/19 for 84 days	Fevers, night sweats, myalgias and chills with +blood smear (1.5%)	500 mg QD	750 mg BID			
8/28/19 for 20 days	Recurrent fever with +blood smear (0.1%)	500 mg QD	750 mg BID			
9/17/19 for 14 days	Persistent +blood smear	500 mg QD	750 mg BID	300 mg TID		
10/1/19 for 45 days (until 11/14/19)	Continuation of the regimen started on 8/28/19	500 mg QD	750 mg QD			
12/13/19 for 47 days (through 1/28/20)	Recurrent fevers with + blood smear (0.08%)	500 mg QD	750 mg BID			
1/29/20 for 41 days[c]	Night sweats and fatigue with +blood smear (0.08%)	1000 mg QD	750 mg once/day	450 mg TID	4 pills/day	
3/10/20 for 42 days	The rationale was to provide a curative treatment regimen either by prolonging the duration of treatment with an effective therapy or based on the high degree of efficacy expected from tafenoquine per se					200 mg daily x 3 days then 200 mg once per week

[a] Liquid suspension
[b] Each malarone® tablet contains 100 mg of proguanil plus 250 mg of atovaquone
[c] This treatment regimen was discontinued due to adverse effects.

negative; polymerase chain reaction (PCR) testing was not done. His first relapse was documented on March 29, 2019 after having 4 days of fevers, night sweats, chills and myalgias. The blood smear was now positive (1.5%), and he was hospitalized (the hemoglobin level was 11.3 g/dL). He was treated with azithromycin plus atovaquone for 12 weeks (Table 1), and he had 2 consecutive negative blood smears on 4/10/19 and 5/23/19.

At the end of August 2019, the patient became symptomatic again, and the blood smear was positive (0.1%) (second relapse). Atovaquone plus azithromycin was restarted on 8/28/19, but because of a persistently positive blood smear, on 9/17/19 oral clindamycin was added to the azithromycin-atovaquone regimen for 14 days; then the azithromycin plus atovaquone regimen was administered without clindamycin for another 45 days starting on 10/1/19 and ending on 11/14/19 (Table 1). Blood smear and PCR testing (LabCorp, Burlington, NC, USA) for *B. microti* were both negative on 10/25/19 and 11/9/19.

The third relapse began in December of 2019, and again there was a positive blood smear (0.08%). Testing for IgG and IgM antibody to *B. microti* was negative twice (4 weeks apart) during this third relapse (prior babesia antibody testing had not been performed). In addition, a blood sample was tested for genetic evidence of drug resistance to either azithromycin or atovaquone, and at least partial resistance to both azithromycin and atovaquone was found, although this was not known until August 2020.

However, while on azithromycin plus atovaquone the patient again developed fatigue and night sweats, along with a positive blood smear on 1/28/20 (0.08%). Therefore, on 1/29/20 the patient was started on a malarone®-based 4 drug regimen that included high dose azithromycin at 1000 mg per day, plus clindamycin orally at 450 mg three times per day, plus a 750 mg dose of atovaquone (in addition to the 1000 mg/day of atovaquone received as part of the malarone® drug therapy) for 41 days (Table 1). Blood smears became negative by 2/14/20, but PCR testing remained positive until at least 2/28/20. Of note, the patient was found to have a high IgG antibody titer to *B. microti* on 1/28/20 before starting the new drug regimen ($\geq$1:1024); this was of relevance since he was seronegative on 11/9/19.

Because the patient developed nausea and diarrhea with this 4 drug regimen, the decision was made to find an alternative therapeutic option to complete therapy. On 3/10/20 he was started on a 6 week regimen of tafenoquine alone (the patient did not have a history of a psychotic disorder, nor did he have current psychotic symptoms). The dose was 200 mg daily for three consecutive days and then 200 mg per week, i.e., the regimen FDA approved for malaria prophylaxis [1]. The blood smear was negative when tafenoquine was initiated. During tafenoquine treatment PCR testing was negative on 3 different dates, and continued to be negative after completion of treatment (the last date of testing was on 5/22/20, approximately 1 month after completion of the tafenoquine treatment).

Prior to initiation of tafenoquine the patient tested negative for glucose-6-phosphate dehydrogenase deficiency. An EKG showed a QTc interval of 409 ms; repeated electrocardiograms while taking tafenoquine did not show any QT interval changes. The patient tolerated the tafenoquine drug regimen well with no adverse effects. The patient has remained asymptomatic through 11/9/21 and is considered cured.

Resistance testing: methods

Babesia microti DNA was isolated from a blood sample collected on December 18, 2019 (parasitemia 0.08%) from the patient described. Nucleic acid amplification and direct Sanger DNA sequencing of the *cytb* and *rpl4* genes were performed using methods described previously [14]. Point mutations were found in each gene

leading to amino acid changes previously correlated with treatment failure, CYTb (Y272C) and RPL4 (R86C) [15]. Cytochrome *b* (CYT*b*) is a highly conserved mitochondrial protein and a well described target of atovaquone in apicomplexan parasites including *B. microti* [14,15]. The mutation at position 272 is at a highly conserved region of the CYTb ubiquinol binding pocket. Likewise, mutations in RPL4 have been described in relapsed disease in patients who have been treated with azithromycin, and position 86 is highly conserved with alternative amino acid changes being reported at this site [14,15]. No testing was available to determine if the strain of *B. microti* was resistant to clindamycin. As there is no in vitro system for testing the inhibitory effects of drugs against *B. microti*, there is no way to directly measure a shift in drug sensitivity. Some patients with reported resistance mutations were eventually successfully treated with atovaquone and azithromycin, albeit at higher than usual doses [12,14]; therefore, the clinical relevance of these mutations needs to be more extensively studied.

Discussion

The patient described in this report experienced multiple relapses of *B. microti* infection and was eventually found to be infected with a *B. microti* strain regarded as at least partially resistant to both azithromycin and atovaquone [12,13]. His relapsing clinical course was likely because he was still immunosuppressed from the prior treatment with rituximab, in conjunction with an initial anti-babesia drug regimen of just 10 days duration using azithromycin combined with a lower than usual dose of atovaquone (Table 1); a 10-day treatment regimen is primarily intended for treating non-immunocompromised patients [13].

Although the patient was only receiving low dose prednisone at the time he became ill (7.5 mg/day which was later transiently increased to 20 mg/day when he experienced exacerbations of symptoms of his underlying autoimmune disease), he had received a dose of rituximab approximately 2 years before he was diagnosed with babesiosis. Consistent with the long-term effects of rituximab, the patient was found to be seronegative for antibodies to *B. microti* as late as 11/9/19 [10,11]. On 1/29/20 he was begun on a malarone®-based treatment regimen that included high doses of azithromycin and atovaquone, plus clindamycin (Table 1). malarone®-based treatment regimens have been previously used successfully to treat babesiosis patients, who had relapsed despite other drug regimens [10,12]. The patient's last positive babesia blood smear was found on 1/30/20 and the last positive PCR test on 2/28/20.

After excluding that the patient had glucose-6-phosphate dehydrogenase deficiency [1,4,16], he was additionally treated with tafenoquine as a single agent starting on 3/10/20 for 6 weeks. He received 200 mg per day for 3 consecutive days followed by 200 mg once per week. Blood smears in 2020 performed on 3/23, 3/31, 4/8, 4/20, and 5/22 were all negative, as was PCR testing performed on 3/23, 3/31, 4/20, and 5/22. As of November 9, 2021, the patient has remained completely well. Given that patient became seropositive for antibodies to *B. microti* by 1/28/20, arguably indicating that the effects of the rituximab on the humoral immune system had largely disappeared, and that he was prescribed an intensive malarone-based treatment regimen after documentation of seroconversion, it is impossible to conclude that the drug tafenoquine provided any clinical benefit. What can be stated is that it was well tolerated, with, as expected, no QTc prolongation on serial electrocardiograms [17]. It should be emphasized that a well-tolerated, single-drug treatment regimen, administered on a once per week basis, is unprecedented in the management of patients with babesiosis. Therefore, this single drug regimen may be of potential clinical importance, especially for treating highly immunocompromised patients with babesiosis, who require a minimum of at least 6 weeks of treatment, often extending into many months [10,13]. Systematic clinical studies using tafenoquine for treating patients with *B. microti* infections should be considered.

Declaration Section

Funding

None.

Conflict of interest

Dr. Marcos does not have any disclosures. Dr. Kirkman does not have any disclosures. Annie Leung does not have any disclosures. Dr. Wormser reports receiving research grants from the Institute for Systems Biology and Pfizer, Inc. He has been an expert witness in malpractice cases involving babesiosis; and is an unpaid board member of the non-profit American Lyme Disease Foundation.

Ethics approval

Stony Brook University IRB approval # 1210472 "Biomarkers for diagnosis and prognosis for Babesia". Label-off use of tafenoquine for babesiosis in this case was a shared-decision between the treating physician and the patient as salvage therapy.

CRediT authorship contribution statement

Luis Marcos: Conceptualization, Writing – review & editing. **Gary Wormser:** Conceptualization, Writing – review & editing. **Laura Kirkman:** Susceptibility testing, Writing – review & editing. **Annie Leung:** Susceptibility testing, Writing – review & editing.

Consent to participate

Informed consent was obtained for study IRB #1210472.

Consent for publication

Patient consented for publication for this brief report.

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