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## *Leishmania* Resistance to Miltefosine Associated with Genetic Marker

**To the Editor:** During 2000–2010, serial *Leishmania* isolates obtained from an HIV-infected patient who was not responding to treatment showed a gradual decrease in in vitro miltefosine susceptibility. We performed *L. donovani* miltefosine transporter (*Ldmt*) gene analysis to identify an association between miltefosine resistance of reference *L. donovani* lines and variability in miltefosine response of *L. infantum* isolates. A new single-nucleotide polymorphism (SNP), L832F, was identified, which might be a marker of miltefosine resistance in leishmaniasis.

The patient, a 46-year-old woman, had lived in France since 1994 but regularly returned to Algeria, her country of birth. HIV-1 infection was diagnosed in 1991. Antiretroviral therapy was initiated in 1993, leading to undetectable viral load and a CD4+ T-cell count of 185 cells/mm<sup>3</sup> (reference >450/mm<sup>3</sup>). Concurrent conditions were thoracic herpes zoster in 1996, hairy leukoplakia of tongue, oropharyngeal candidiasis, and chronic renal failure of unknown cause since 2000.

Visceral leishmaniasis was diagnosed in 1998 by culture of a bone marrow smear, which showed intracellular amastigotes. Use of meglumine antimonate (Glucantime; Sanofi, Paris, France), a drug of choice for the treatment of leishmaniasis, was contraindicated because of pancreatitis in the patient and in vitro isolate susceptibility variation; therefore, induction therapy consisted of liposomal amphotericin B (AmB [AmBisome; Astellas Pharma US, Deerfield, IL, USA]) at a dose of 3 mg/kg/d for 5 consecutive days, then 1× week for 5 weeks (total dose 30 mg/

kg) during 1998–2000 (Table). The same medication was administered for relapses at 4 mg/kg/d for 5 days, then 4 mg/kg 1× week for 5 weeks (total dose 40 mg/kg) during 2001–2010. Given the adverse effects of AmB and the availability of oral miltefosine (Impavido; AEterna Zentaris Inc., Quebec City, Quebec, Canada), the latter drug was used for maintenance treatment during 2001–2007 at 50 mg 2×/d. Leishmaniasis was monitored by leukocytoconcentration and culture of blood samples on Novy-Nicolle-McNeal medium.

When signs of biological and clinical relapse appeared, bone marrow was aspirated for parasite detection. After culture of the aspirate and isoenzyme determination, the strain was identified as *L. infantum*, zymodeme MON-24. Eleven relapses were documented; all were confirmed by positive direct examination of bone marrow or blood, but cultures of only 7 samples yielded positive results (Table).

The susceptibility of 4 cryopreserved isolates (S<sub>1</sub>, S<sub>3</sub>, S<sub>4</sub>, and S<sub>6</sub>; Table) to AmB and to miltefosine was studied in the in vitro promastigote and axenic amastigote form by determining the concentrations inhibiting parasite growth by 50% (I<sub>2</sub>). The 50% inhibitory concentration (IC<sub>50</sub>) was determined in parallel for the following reference *L. donovani* lines: a wild-type *L. donovani* LV9 (MHOM/ET/67/HU3) line (LV9 WT), a wild-type *L. donovani* DD8 (MHOM/IN/80/DD8) line (DD8 WT), a laboratory miltefosine-resistant line obtained from LV9 WT (LV9 miltefosine-R, resistant to 90 μmol/L miltefosine), and the laboratory AmB-resistant line obtained from DD8 WT (DD8 AmB-R, resistant to 1.4 μmol/L AmB) on promastigote and axenic amastigote forms (3,4).

The AmB susceptibility of the isolates did not change notably over time; IC<sub>50</sub> values ranged from 0.09

Table. Comparisons of IC<sub>50</sub> for AmpB and miltefosine against promastigotes and axenic amastigotes and distribution of *LdMT* SNPs in *Leishmania infantum* isolates and reference strains\*

Isolate	Year	AmpB	Miltefosine	IC <sub>50</sub> , µmol/L ± SEM				<i>Ldmt</i> SNP
				AmpB		Miltefosine		
				Promastigotes	Axenic amastigotes	Promastigotes	Axenic amastigotes	
–	1998	3 mg/kg/d × 5 d; then 1×/wk × 5 wk	–	–	–	–	–	–
S <sub>1</sub>	2000	3 mg/kg/d × 5 d; then 1×/wk × 5 wk	–	0.09 ± 0.04†	0.10 ± 0.03	7.14 ± 0.56†	5.00 ± 0.7†	L832
–	2001	4 mg/kg/d × 5 d; then 1×/wk × 5 wk	50 mg 2×/d	–	–	–	–	–
S <sub>3</sub>	2005	4 mg/kg/d × 5 d; then 1×/wk × 5 wk‡	–	0.13 ± 0.03	0.20 ± 0.03	25.93 ± 1.46†	21.00 ± 1.50†	832 <sub>L</sub> /F
S <sub>4</sub>				0.24 ± 0.01†	0.15 ± 0.02	27.89 ± 1.76†	31.90 ± 1.60†	
–	2007	4 mg/kg/d × 5 d; then 1×/wk × 5 wk	–	–	–	–	–	–
S <sub>6</sub>	2008	4 mg/kg/d × 5 d; then 1×/wk × 5 wk	–	0.16 ± 0.03	0.11 ± 0.03	44.30 ± 3.70†	50.10 ± 1.00†	832F
S <sub>7</sub>	2010	4 mg/kg/d × 5 d; then 1×/wk × 5 wk	–	–	–	–	–	L832
Reference strain								
		LV9 WT		0.03 ± 0.02	0.02 ± 0.05	4.46 ± 0.29†	6.20 ± 0.3	L832
		LV9 Miltefosine-R		0.22 ± 0.04	0.70 ± 0.09	45.84 ± 2.40†	54.20 ± 2.20†	832F
		DD8 WT		0.06 ± 0.02†	0.05 ± 0.03†	17.40 ± 1.70	12.40 ± 1.50	L832
		DD8 AmpB-R		1.42 ± 0.06†	1.00 ± 0.07†	15.20 ± 1.00	10.30 ± 1.20	L832

\*IC<sub>50</sub>, 50% inhibitory concentration; AmpB, amphotericin B; *Ldmt*, *Leishmania donovani* miltefosine transporter gene; SNP, single-nucleotide polymorphism; –, assay not performed because sample unavailable or not culturable; WT, wild type; R, resistant.

†Significance was analyzed by using the nonparametric Mann-Whitney U test to compare the IC<sub>50</sub> of the isolates with the IC<sub>50</sub> of reference strains; p<0.01 was considered significant. IC<sub>50</sub> of AmpB and miltefosine was compared with IC<sub>50</sub> of reference strains and S<sub>1</sub>/S<sub>3</sub>, S<sub>1</sub>/S<sub>4</sub>, and S<sub>1</sub>/S<sub>6</sub>. Miltefosine: S<sub>1</sub>/S<sub>3</sub>, S<sub>1</sub>/S<sub>4</sub>, S<sub>1</sub>/S<sub>6</sub>; p<0.01. AmpB: S<sub>1</sub>/S<sub>4</sub> significant p<0.01; S<sub>1</sub>/S<sub>3</sub>, S<sub>1</sub>/S<sub>6</sub> not significant.

‡For each relapse.

µmol/L to 0.24 µmol/L, regardless of parasite form, similar to those of wild-type reference strains (Table). In contrast, the IC<sub>50</sub> values of miltefosine increased greatly over time, from 5.00 µmol/L to 50.10 µmol/L. During the 6 years of follow-up with miltefosine maintenance therapy, the susceptibility of the isolate (S<sub>6</sub>) obtained 6 months after miltefosine treatment withdrawal in 2008 was 6-fold higher than that of the first isolate (S<sub>1</sub>) obtained in 2000.

The *L. donovani* miltefosine transporter protein (LdMT) promotes miltefosine translocation (5), and LdMT inactivation in *L. donovani* promastigotes leads to miltefosine resistance at the promastigote and amastigote stages (6). In 2003 and 2006 studies, several mutations were linked to the inability of parasites to take up miltefosine and to miltefosine resistance (5,7). In a 2009 study, the weak expression of LdMT and its β subunit LdROS3 in *L. braziliensis* isolates was linked to diminished sensitivity (8). We sequenced the

entire *Ldmt* gene (3,294 bp) in the reference strains and the clinical isolates for SNP analysis (5,7). Only 1 new SNP, L832F, was found in the miltefosine-resistant reference strain (LV9 miltefosine-R) and in clinical isolate S<sub>6</sub>. The L832 wild-type allele was found in isolate S<sub>1</sub> and in the miltefosine-sensitive reference lines (LV9, DD8, and DD8 AmpB-R), whereas both alleles were found in isolates S<sub>3</sub> and S<sub>4</sub>, with a decrease in the wild-type allele (Table). The last isolate, which was obtained 3 years after miltefosine withdrawal and could not be subcultured, had reverted to the wild-type allele (L832).

These results point to a relation between the 832F allele and diminished susceptibility to miltefosine. Analysis of this case of miltefosine resistance in a patient co-infected with *Leishmania* sp. and HIV strongly suggests that an SNP (L832F) in the *Ldmt* gene could represent a molecular marker of miltefosine resistance in *L. infantum* and *L. donovani*.

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DOI: <http://dx.doi.org/10.3201/eid1804.110841>

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## Prolonged KI Polyomavirus Infection in Immunodeficient Child

**To the Editor:** Two novel polyomaviruses (PyVs), KIPyV and WUPyV, were identified in respiratory and fecal specimens from children with signs and symptoms of respiratory tract infection (1,2). A review of literature on emerging viruses in transplant recipients indicated that up to 80% of patients harboring these PyVs are co-infected with another respiratory virus, complicating interpretation of positive findings (3). Seroprevalence of KIPyV and WUPyV in healthy blood donors in Germany have been reported to be 67% and 89%, respectively (4).

The effect of these viruses in immunocompromised patients is unknown. Some studies report a higher frequency of KIPyV DNA detection in hematopoietic stem cell transplant (HSCT) recipients (5–7) than in immunocompetent patients. In fact, HSCT recipients might be more prone to productive infection with KIPyV and WUPyV than to infection with PyVs JC and BK (BKPyV) (5).

We report prolonged detection of KIPyV DNA in the respiratory tract of an immunocompromised child. A 12-year-old girl with severe combined immunodeficiency was admitted to the Freiburg University Medical Center, Germany, in November 2009 for treatment of progressive respiratory problems and cytomegalovirus (CMV) disease. Although the molecular basis of the immune disorder was unknown, HSCT was indicated because of uncontrolled CMV infection and progressive clinical deterioration.

Allogenic HSCT was performed in February 2010. Pretransplant treatments included thyothepa (day –7; 8 mg/kg), fludarabine (days –6 to –3; 120 mg/m<sup>2</sup>), treosulfan (days –6 to –4; 42 g/m<sup>2</sup>), and antithymocyte

globulin (days –4 to –2; 45 mg/kg). The patient received bone marrow cells ( $4.2 \times 10^6$  CD34-positive cells/kg) from an 8/10 human leukocyte antigen-matched, CMV-positive, unrelated donor. Graft-versus-host disease prophylaxis consisted of cyclosporine A (from day –1) and methotrexate (days +1, +3, +6; 10 mg/m<sup>2</sup>). Leukocyte, granulocyte, and platelet engraftment occurred on days +18, +19, and +32, respectively. Full donor chimera was detected by day +62 (Figure, panel A).

Before hospitalization, the child had had several pulmonary infections. At admission, chest radiograph showed middle lobe atelectasis but no visible infiltrates. On day –83, human bocavirus was detected. On day –27, the occurrence of bilateral infiltrates was assessed, and pneumonia was diagnosed. On day +55, fever and hypoxia were monitored; chest radiograph revealed regressive infiltrates in the lower lobes but central infiltrates in the upper lobes. Rhinovirus RNA was detected at this time and persisted in the respiratory tract until day +98 (Figure, panel A). Retrospectively, KIPyV DNA was detected in 6 nasopharyngeal aspirate specimens, 4 throat swab specimens, and 1 bronchoalveolar lavage specimen collected between days –103 and +98 (Figure, panel B). No KIPyV was detected in EDTA-treated blood samples at any time. Stool samples were not available. The highest level of KIPyV DNA ( $10^9$  copies/mL) was detected on day +16. Starting from day +43, a steady decrease in KIPyV viral load was observed. Phenotypical analysis of blood leukocytes on day +55 showed normal CD56<sup>+</sup>/16<sup>+</sup> natural killer cells and good T-cell engraftment but no B cells. On day +108, viral clearance had occurred. Sequencing of the small t antigen amplified from all available samples was performed (8) and showed 100% nucleotide identity (GenBank accession no. JN874415).