Association of the Endobiont Double-Stranded RNA Virus LRV1 With Treatment Failure for Human Leishmaniasis Caused by *Leishmania braziliensis* in Peru and Bolivia

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*Leishmania* are widespread protozoan parasites transmitted by phlebotomine sand flies. *Leishmania* infections afflict >12 million people worldwide, with 1.2 million new cases/year [1]. The true incidence is likely far higher, as most infections are persistent and asymptomatic, only emerging as disease following immune compromise [2, 3]. Leishmaniasis can be viewed as a spectral disease, with a range of manifestations, including tegumentary disease (cutaneous leishmaniasis [CL] or mucosal leishmaniasis [ML]) and visceral disease (visceral leishmaniasis [VL]); these manifestations are typically associated with different parasite species [4, 5]. Among the different species, *Leishmania braziliensis* is considered one of the most important in North and South America because of its prevalence, the difficulty of curing the disease it causes, and its public health importance. Notably, *L. braziliensis* is the most frequent cause of ML, which typically manifests first as CL and progresses to ML in up to 10% of the cases [6]. The factors responsible for the progression from CL to ML are not well understood and likely involve both host and parasite factors [5].
As yet there is no effective vaccination against *L. braziliensis*, and treatment depends on diagnosis and chemotherapy. Penta-valent antimonials (SbV), typically sodium stibogluconate (Pentostam) or meglumine antimoniate (Glucantime), are presently the primary treatment. However, in Latin America, SbV treatment is characterized by a variable outcome, with treatment failure rates reaching 39% [7, 8]. While in some *Leishmania* species, SbV resistance has been linked to intrinsic changes in parasite susceptibility, this does not appear to be the case in *L. braziliensis* in Peru [9, 10]. Risk factors identified thus far include the presence of concomitant distant lesions and factors associated with the immunological response [11, 12]. For example, the persistence of high levels of interleukin 10 in parasite susceptibility, this does not appear to be the case in *L. braziliensis* [23] or *L. guyanensis* (unpublished data). There, LRV1 can act as an immunomodulator through the interactions of its dsRNA genome with the host Toll-like receptor 3 (TLR3), leading to a hyperinflammatory response [24]. Similar responses in humans likewise would be expected to result in increased disease severity, as well, but currently the clinical impact of LRV1 is uncertain. Two studies reported little association of LRV1 with disease severity, as manifesting CL lesions, ML lesions, or both CL and ML (MCL) lesions. Patients underwent standard supervised treatment with intravenous or intramuscular meglumine antimoniate (Glucantime; Sano) or generic sodium stibogluconate (Viteco, Colombia; or Albert-David, India), depending on availability; both drugs are considered equally effective [35]. We used dosages of 20 mg/kg/day for 20 days (for CL) or 30 days (for ML or MCL) or conventional amphotericin B (Fungizone, Bristol-Myers Squibb) at dosages of 0.6 mg/kg/day for 30–41 days [9]. Follow-up visits were scheduled at 1, 2, 3, 6, and 12 months after treatment ended. The clinical outcomes were as follows: cure, defined as complete reepithelialization with a characteristic scar and no inflammation at the time point of follow-up assessment (3–12 months after treatment, which depended on patients returning for their medical evaluation); primary unresponsive, defined as the absence or incomplete scarring of lesion(s) and/or the persistence of inflammatory signs at 3 months after treatment or the worsening of existing lesion(s) or the appearance of new lesion(s) ≤ 3 months after treatment; and relapse, defined as the reappearance of an ulcer or nodule and/or local signs of inflammation after initial cure [9]. Cured patients were still observed until 12 months after treatment, to detect possible relapses. Patients with treatment failure received either a repeat course of antimonials with or without topical imiquimod (Aldara; 3 M Pharmaceuticals) or intravenous treatment failure); the work conformed to all relevant European regulations. The research was also reviewed and approved by the ethics committees of the Universidad Peruana Cayetano Heredia (Lima, Peru), the Hospital Nacional Cayetano Heredia (Lima), and the Universidad Mayor de San Simón (Cochabamba, Bolivia). All human strains of *Leishmania* had been isolated from patients as part of normal diagnosis and treatment, with no unnecessary invasive procedures, and with written and/or verbal informed consent recorded at the time of clinical examination. Data on human isolates were coded and anonymized.

### Patients

Patients were recruited at the Instituto de Medicina Tropical Alexander von Humboldt (Lima) and the Universidad Mayor de San Simón (Cochabamba, Bolivia) as part of 2 prospective studies: LeishBolPe (Bolivia and Peru, 1994–1998), an epidemiological study aiming to discriminate factors underlying clinical variability in infection and disease; and LeishNatDrug-R (Peru, 2001–2004), a case-control study of incident cases to understand risk factors of treatment failure. Here we focused on *L. braziliensis*, owing to its prevalence and association with a higher risk of treatment failure [11]. From both prior studies, 290 isolates were typed, and all 97 *L. braziliensis* isolates with adequate clinical and epidemiological documentation were considered (Table 1). Of these, 54 had been monitored for treatment outcomes for up to 1 year and were included in our analysis (Table 1 and Figure 3). Patients were classified clinically as manifesting CL lesions, ML lesions, or both CL and ML (MCL) lesions. Patients underwent standard supervised treatment with intravenous or intramuscular meglumine antimoniate (Glucantime; Sanofi Aventis) or generic sodium stibogluconate (Viteco, Colombia; or Albert-David, India), depending on availability; both drugs are considered equally effective [35]. We used dosages of 20 mg/kg/day for 20 days (for CL) or 30 days (for ML or MCL) or conventional amphotericin B (Fungizone, Bristol-Myers Squibb) at dosages of 0.6 mg/kg/day for 30–41 days [9]. Follow-up visits were scheduled at 1, 2, 3, 6, and 12 months after treatment ended. The clinical outcomes were as follows: cure, defined as complete reepithelialization with a characteristic scar and no inflammation at the time point of follow-up assessment (3–12 months after treatment, which depended on patients returning for their medical evaluation); primary unresponsive, defined as the absence or incomplete scarring of lesion(s) and/or the persistence of inflammatory signs at 3 months after treatment or the worsening of existing lesion(s) or the appearance of new lesion(s) ≤ 3 months after treatment; and relapse, defined as the reappearance of an ulcer or nodule and/or local signs of inflammation after initial cure [9]. Cured patients were still observed until 12 months after treatment, to detect possible relapses. Patients with treatment failure received either a repeat course of antimonials with or without topical imiquimod (Aldara; 3 M Pharmaceuticals) or intravenous

### MATERIALS AND METHODS

#### Ethics Statement

Research in this study was subject to ethical review by the European Commission and was approved as part of contract negotiation for LeishBolPe (an epidemiological study in Bolivia and Peru) and LeishNatDrug-R (a multicenter study on SbV.
Table 1. Properties of *Leishmania braziliensis* Isolates From Peru and Bolivia, Including LRV1 Status

<table>
<thead>
<tr>
<th>International Code</th>
<th>Origin (Department, Province)</th>
<th>Lesion Type</th>
<th>Treatment Outcome</th>
<th>Classification</th>
<th>LRV1 Present</th>
<th>Reference</th>
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<td>International Code</td>
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<td>Treatment Outcome</td>
<td>Classification</td>
<td>LRV1 Present</td>
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<td>–</td>
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<td>CL</td>
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<td></td>
<td>–</td>
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<td>Cure/scar</td>
<td>Cure</td>
<td>+</td>
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<td>+</td>
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<td>MHOM/BO/94/CUM24</td>
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<td>[30]</td>
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</tbody>
</table>
amphotericin B deoxycholate (Bristol-Myers Squibb) [9, 11]. Some patients had previously been treated for leishmaniasis; these were classified retrospectively as primary unresponsive if the second treatment led to cure or as secondary unresponsive if it did not. For statistical analysis, treatment failure was defined as either unresponsiveness or relapse (Table 1 and Supplementary Figures 1 and 2).

Parasite Isolates
The LRV1-positive *L. guyanensis* strain Lg5313 (World Health Organization code WHI/BR/78/M5313) and the LRV1-deficient line Lg17 [23] were obtained from Nicolas Fasel (University of Lausanne, Switzerland). Ninety-seven isolates of *L. braziliensis* (62 from Peru, 35 from Bolivia) were available ed retrospectively as primary unresponsive if it did not. For statistical analysis, treatment failure was defined as “cured” because previous studies showed that the cure rate assessed at 3 months was very nearly that seen at 12 months [11].

### Table 1 continued.

<table>
<thead>
<tr>
<th>International Code</th>
<th>Origin (Department, Province)a</th>
<th>Lesion Type</th>
<th>Treatment Outcome</th>
<th>Classification</th>
<th>LRV1 Present</th>
<th>Reference</th>
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<tr>
<td>Amphotericin B treatment</td>
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<td></td>
<td></td>
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<td>MCL</td>
<td>Cure/scar</td>
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<td>CL</td>
<td>Cure/scar</td>
<td>Cure</td>
<td>–</td>
<td>This work</td>
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<td>MHOMPE/02/PER011</td>
<td>Huanuco, Huanuco</td>
<td>MCL</td>
<td>NA</td>
<td>. . .</td>
<td>+</td>
<td>[9]</td>
</tr>
<tr>
<td>MHOMPE/03/PER136</td>
<td>Ucayali, Coronel Portillo</td>
<td>ML</td>
<td>Cure (12 mo)</td>
<td>Cure</td>
<td>–</td>
<td>[9]</td>
</tr>
</tbody>
</table>

Unless otherwise indicated, ‘Cure’ signifies that the patient was monitored for 12 months. In some cases, patients could only be monitored for 3 or 6 months. For analysis, these were classified as “cured” because previous studies showed that the cure rate assessed at 3 months was very nearly that seen at 12 months [11]. Abbreviations: CL, cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis; ML, mucosal leishmaniasis; NA, not available; p, prospective (within the LeishNatDrug-R study); re, retrospective (previous leishmaniasis episode).

### RNA Purification
Parasites were thawed and grown in Schneider’s medium containing fetal bovine serum until reaching late log/early stationary phase. A total of $3 \times 10^8$ promastigotes were washed with ice-cold phosphate-buffered saline, collected by centrifugation, and resuspended and lysed in 1 mL of Trizol (Invitrogen). Cell lysates were stored at $-80^\circ$C prior to shipment or processing. Total RNA was isolated according to the manufacturer’s instructions (Life Technologies, California). Residual DNA was removed by treatment with DNase I (Life Technologies, California) at 37°C for 45 minutes, and RNA was further purified with the Zymo RCC-25 kit, using the manufacturer’s instructions (Zymo Research, Irvine, California). The integrity of the purified RNA was verified by electrophoresis in 0.8% agarose gels in TAE buffer (40 mM Tris, 20 mM acetic acid, and 1 mM ethylenediaminetetraacetic acid, pH 8) at 4°C.

### LRV1 Detection and Sequencing
These methods have been reported previously [17, 36, 37]. Complementary DNA (cDNA) was prepared from total RNA by priming with random hexamers and was then subjected to 30 cycles of PCR, using universal LRV degenerate primers that amplified a 488-nucleotide segment within the LRV1 capsid gene. Controls included buffer only, mock cDNA lacking reverse transcriptase, and both LRV1-positive and LRV1-negative strains of *L. guyanensis*. Reverse transcription–PCR (RT-PCR) products were analyzed on a 1.5% native agarose gel in TAE buffer, and LRV1 amplicons were purified and subjected to automated sequencing. The sequence was obtained from both strands, assembled and trimmed to remove low quality bases and primer sequences, and edited and aligned using DNAStar Lasergene software. Molecular phylogenies were constructed on a 299-nucleotide segment, using MEGA 6 analysis software [38]. The final LRV1 data set has been deposited in GenBank (KP682453-KP682484).

The presence of LRV1 was confirmed independently by the presence of an appropriately sized dsRNA following digestion with single-stranded nucleases (data not shown) [37, 39]. Following a recent proposal to the International Committee on the Taxonomy of Viruses, LRVs are referred to as “LRV1” or “LRV2,” followed by a species and then strain designation [40].
Thus, LRV1-Lguy-M4147 is the preferred name for M4147
LRV1–4, and LRV1-Lbr-CUM24 is the preferred name for the
LRV occurring within strain MHOM/BO/94/CUM24.

Statistical Analysis
The type of lesion was treated as a nonordered 3-level categorical
variable. Simple exact logistic regression was used to indepen-
dently model the total (unadjusted) effect of the presence of
LRV1 and lesion type on the probability of treatment failure in
scenarios involving a small sample size. Multiple exact logistic re-
gression was used to evaluate the direct effect on the probability
of treatment failure of the presence of LRV1, after adjustment for
the type of lesion. Statistical tests were performed under a 5%
significance level, using the statistical software Stata 13.

RESULTS
We analyzed a collection of 97 isolates of L. braziliensis obtained
from patients exhibiting CL, ML, or MCL in Peru and Bolivia
(Table 1 and Supplementary Figures 1 and 2). Axenic proma-
stigote cultures were established in vitro following biopsy. Subse-
csequently, patients were treated with SbV (93) or amphotericin B
(4), and we were able to monitor the treatment outcome of 54
patients for up to 1 year. The patient response was classified as
described previously [9] and, for statistical analysis, was further
classified as “cure” or “failure,” with the latter including both
instances of unresponsiveness and relapses (Table 1 and Supple-
mentary Figures 1 and 2). Parasites were confirmed as L. brazili-
ensis by molecular typing, and RT-PCR with universal LRV
primers was used to detect LRV1 (Figure 2 and Table 1).

LRV1 is Associated With a Significant Increase in the Risk of
Treatment Failure
We next examined the association of LRV1 with treatment out-
come for all patients. Extensive clinical data were available for
54 patients, including treatment history and outcome; all but
4 patients had been treated with SbV. The association of
LRV1 and treatment outcome for the entire data set is shown
in Figure 3; findings subdivided by country are shown in

Figure 1. Geographical distribution of LRV1-positive Leishmania brazili-
ensis isolates from Peru and Bolivia. The origins of L. braziliensis lines
summarized in Table 1 are displayed on a map of Peru and Bolivia, created
using the software package Quantum GIS, version 2.0.1 (available at:
http://www.qgis.org/en/site/forusers/download.html), and the latitude
and longitude coordinates of each locality. Both LRV-positive (star) and
LRV-negative (circle) isolates occurred in the same geographic areas; in
Peru, mostly in the jungle. Most Bolivian L. braziliensis isolates (33 of
35) originated from the Indigenous Territory and National Park Isiboro Sé-
cure (municipality of Villa Tunari), and 2 isolates (CUM67 and CUM68) origi-
nated from the town of Shinahota (municipality of Tiraque), all located in
the department of Cochabamba.

Figure 2. Reverse transcription–polymerase chain reaction (PCR) detec-
tion of LRV1 in Leishmania braziliensis. Agarose gel electrophoresis of PCR
products obtained using the LRV universal primers SMB4647 and SMB4648
with randomly primed complementary DNA derived from RNA from the
species/strains is shown, as described in "Materials and Methods" section.
M, double-stranded DNA (dsDNA) molecular weight marker (1 kb plus; Life
Technologies, CA). Lanes 1–11: L. braziliensis isolates LC2143 (lane 1),
LC2147 (lane 2), LC2176 (lane 3), LC2177 (lane 4), LC2284 (lane 5),
LC2289 (lane 6), LC2321 (lane 7), LC2353 (lane 8), LC2367 (lane 9),
LC2398 (lane 10), and LC2318 (lane 11). Lanes 12 and 13, L. guyanensis iso-
lates: Lg17 (LRV1 negative; lane 12) and Lg5313 (LRV1 positive; lane 13).

Figure 3. Geographical distribution of LRV1-positive Leishmania brazili-
ensis isolates from Peru and Bolivia. The origins of L. braziliensis lines
summarized in Table 1 are displayed on a map of Peru and Bolivia, created
using the software package Quantum GIS, version 2.0.1 (available at:
http://www.qgis.org/en/site/forusers/download.html), and the latitude
and longitude coordinates of each locality. Both LRV-positive (star) and
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Peru, mostly in the jungle. Most Bolivian L. braziliensis isolates (33 of
35) originated from the Indigenous Territory and National Park Isiboro Sé-
cure (municipality of Villa Tunari), and 2 isolates (CUM67 and CUM68) origi-
nated from the town of Shinahota (municipality of Tiraque), all located in
the department of Cochabamba.
Supplementary Figure 3. Overall, 33% (18 of 54) were classified as failures; importantly, the percentage of failure was less in the LRV-negative isolates than in the LRV-positive isolates (24% [9 of 37] vs 53% [9 of 17]). Exact logistic regression showed this difference to be significant (P = .043) and having a notably high odds ratio (OR) of 3.5, associating the risk of failure with LRV1. This finding was seen within both the Peruvian and Bolivian isolates, although the number of treatment failures among the latter group was too few for statistical significance. Exclusion of the 3 patients treated with amphotericin B (all with LRV1-negative isolates) caused the overall significance to decrease (OR, 3.12; P = .067).

We further assessed the impact of LRV1 after adjustment for lesion type (both variables showing no significant interaction), using multiple exact logistic regression analysis. Again the presence of LRV1 was associated with an increased risk of treatment failure (OR, 3.99; P = .05). Interestingly, patients with CL showed a higher risk of treatment failure than those showing mucosal involvement (ie, patients with ML or MCL; OR, 18.5; P = .009). This was unexpected, as prior studies had not revealed a consistent difference [41–46]. In our studies, patients with ML or MCL received a longer course of SbV treatment than patients with CL (30 vs 20 days [9]), perhaps accounting for this outcome. Given the implications for the success of SbV treatments, this warrants further controlled studies in the future.

**LRV1 Does Not Confer Intrinsic Parasite Antimony Resistance in Infected Macrophages**

We considered the hypothesis that, in some manner, the presence of LRV1 conferred intrinsic drug resistance to the parasites. In a previous study, 26 of the Peruvian isolates had been examined for in vitro resistance to SbV as intracellular amastigotes in macrophage infections [9]. Of the SbV-resistant lines, 10 were LRV1 positive, while 12 were LRV1 negative; of the SbV-sensitive lines, 2 were LRV1 positive, while 2 were LRV1 negative. Thus, LRV1 was not significantly associated with SbV resistance directly (P = .43).

**LRV1 Subtypes Are Not Associated With Treatment Outcome**

We considered the possibility that the association between LRV1 and treatment failure arose not from the presence of LRV1, but from other parasite genetic factors. LRV1, like most other Totiviridae, are not shed or infectious and are transmitted only during cell division; thus by coinheritance, isolates that bear closely related LRV1s are closely related at the nuclear DNA level [47, 48]. If observed, clustering of treatment failures by LRV1 and, presumably, nuclear DNA relationship could signify that shared ancestry of other genetic factors was responsible, rather than LRV1. Differences in LRV1 sequence are unlikely to play a role, as it is the viral dsRNA itself (rather than any specific sequence motif) that serves to mediate virulence through interactions with TLR3 [23].

We constructed a dendrogram depicting LRV1 sequence relationships, onto which we displayed drug treatment outcomes where available (Figure 4). It was clear that treatment failures (Figure 4) did not cluster preferentially by the degree of LRV1 relationship. Instead, failures were interspersed among cures in most LRV1 lineages, including 2 bearing identical LRV1s (PER012 and PER010). Where known from microsatellite typing [31], the relationships of LRV1s were consistent with those of the underlying parasite genomes, including the close relationship of PER010 and PER012. While these data cannot rule out a direct contribution of other genetic factors, they suggest that the LRV1 effect seen here is independent of these, if present. Future studies using high resolution methods to probe the relationships of the isolates studied here may further test and extend these findings.

**Figure 3.** Treatment failure versus LRV1 prevalence among Leishmania isolates. The number of cures (open bars) and treatment failures (closed bars) following chemotherapy is shown for the complete data set (n = 54). Within each grouping, the number of isolates positive or negative for LRV1 are shown. Data are taken from Table 1.
We examined the LRV1 phylogeny for geographic associations; however, there was no clear cline of LRV1 across Peru or Bolivia. This is perhaps best illustrated by closer examination of 2 sympatric populations, one occurring in Pilcopata (the Amazonian foothills, Cusco) in Peru and the other in Parque Isiboro (the Amazonian lowlands) in Bolivia. Both populations displayed considerable LRV1 diversity, spanning (or nearly so) the limits of the evolutionary tree (Figure 5). These data further

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**Figure 4.** LRV1 molecular phylogeny and drug treatment outcomes. The figure shows a molecular tree based on comparisons of a 299-nucleotide region of LRV1 (described in “Materials and Methods” section). When known for a given isolate, the clinical outcome of pentavalent antimonials therapy is shown (no patients treated with amphotericin B yielded strains bearing LRV1). The tree was constructed using the neighbor-joining algorithm, based on the uncorrected number of nucleotide differences and uniform rate assumptions. The scale shows a branch length of 5-nucleotide differences. Bootstrap values calculated from 5000 replicas are shown at each node. Cure, open boxes; treatment failure, dark boxes (see Table 1 for data and classification).
suggest considerable diversity of *L. braziliensis* parasite populations in these localities.

### LRV1 is Not Preferentially Associated With MCL or ML

For CL presentations, 28 of 67 (42%) were LRV1 positive; for MCL and ML presentations, 1 of 13 (8%) and 7 of 17 (41%), respectively, were positive; and for ML/MCL combined, 8 of 30 (27%) were positive. These values did not differ significantly when analyzed separately or after combining the ML and MCL groups (*P* = .29 and *P* = .57, respectively). These findings are consistent with studies of other *Leishmania* populations [19, 20]; there was no significant association between disease status at the time of biopsy and the presence of LRV1 in axenic cultured parasites.

### DISCUSSION

Here we examined a large panel of isolates of *L. braziliensis* and probed for associations between the presence of LRV1 and response to treatment or disease manifestations. Our data show a significant association between the presence of LRV1 and treatment failure (Figure 3). We ruled out the possibility that this arose by intrinsic LRV1-mediated SbV resistance, as there was no correlation between the presence of LRV1 and parasite SbV resistance that manifested during infections of macrophages in vitro. Similarly, we ruled out a significant contribution from other parasite genetic factors, using LRV1 sequence relationships as a surrogate measure of parasite genetic relatedness (due to LRV1-parasite coevolution [48]), rather than the presence of LRV1 itself, to assess whether the treatment failures were clustered preferentially into one or a few lineages. This analysis provided no evidence for preferential genetic clustering of treatment failures, further pointing to the presence of LRV1 itself as key risk factor.

Importantly, a companion article by Bourreau et al reports a similar association of LRV1 with pentamidine treatment failures in cases of *L. guyanensis* infection, in the absence of intrinsic parasite resistance [18]. Thus, current data suggest that LRV1 may act across species and drug classes to thwart efforts to treat leishmaniasis. These remarkable findings prompted us to consider potential mechanisms by which this occurs.
In the *L. guyanensis* murine model, LRV1-bearing parasites induce the expression of a distinctive set of macrophage inflammatory markers constituting a hyperinflammatory response, resulting ultimately in a TLR3-dependent increase in parasite numbers and disease severity [23, 24]. Correspondingly, many studies have shown a critical role for the host immune system in mediating SbV activity [10, 13, 14]. Thus, LRV1-mediated changes in the human host response could potentially serve to dampen the efficacy of SbV action. A second and nonexclusive model suggests the elevated parasite burden associated with LRV1 would act to compromise the efficacy for any given drug treatment regimen, even in the absence of intrinsic parasite resistance or drug-specific host interactions. Indeed, this may be especially likely for most antileishmanial compounds, whose efficacy and selective index is far from optimal [49, 50]. One key prediction of the higher parasite load model is independence from the specific mode of drug action and/or drug-specific involvement of host metabolism, which differ considerably among SbV, amphotericin B, and pentamidine. It also provides a potential framework for viewing the preferential association of treatment failures in CL (if this finding is confirmed in the future), as parasite numbers are generally much higher in this form of the disease than in chronic forms of ML.

The evidence presented here and in the companion work by Bourreau et al [18] provides a strong rationale implicating LRV1 in important aspects of human–parasite biology. Current data do not permit a firm determination of the mechanism by which the presence of LRV1 leads to treatment failures, and further studies will be required to unravel this process. Regardless of the mechanism, these findings have important implications for antileishmanial therapy, as they suggest that knowledge of the LRV1 status in *L. braziliensis* and *L. guyanensis* could support prognostics and follow-up. Our findings should also guide further research on new options for combination therapy, including targeting LRV1.

**Supplementary Data**

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

**Acknowledgments.** We thank Catherine Ronet and Nicolas Fasel (University of Lausanne, Switzerland), for discussions and communicating unpublished results; F. Matthew Kuhlmann, for advice on the presentation of clinical data; and Jonathan Berman, for discussions concerning SbV treatment failures.

**Disclosure.** The sponsors had no role in study design, data collection, data analysis, data interpretation, or report writing.

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**References**

Supplementary Figures

Supplementary Figure 1. Detailed patient treatments and outcomes in Peru.

Supplementary Figure 2. Detailed patient treatments and outcomes in Bolivia.

Supplementary Figure 3. Treatment failure vs. LRV1 prevalence in Peru or Bolivia.

The data shown in Fig. 3 has been subdivided by country for presentation (Peru, left panel; Bolivia, right panel).
62 Peruvian patients infected with *L. braziliensis*

- Excluded: 32 patients: Treatment & follow-up data not available
- 2 patients: Follow-up data incomplete

28 patients with documented treatment outcome

14 patients LRV1-

14 patients LRV1+

First leishmaniasis episode (N = 12)

- Cured (C) after 1 course
  - SSG: 8 patients
  - Amphoto B: 1 patient
- Failure (F) after 1 course (∗)
  - SSG: 1 patient
  - MA: 2 patients

  - Cured after MA + imiquimod: 2 patients
  - Failure after MA + imiquimod: 1 patient

Relapse previous episode (drug unknown) (N = 1)

- Failure (F) after 1 course (∗∗)
  - SSG

  - Cured after SSG

Unresponsiveness previous episode (SSG) (N = 1)

- Failure (F) after 1 course (∗∗∗)
  - SSG

  - Cured after Amphoto B

Unresponsiveness previous episode (MA) (N = 5)

- Failure (F) after 1 course (****)
  - SSG: 1 patient
  - MA: 1 patient
  - AmphoB: 1 patient

- Cured after SSG + imiquimod: 1 patient

Unresponsiveness previous episode (SSG) (N = 1)

- Failure (F) after 1 course (****)
  - SSG

  - Cured after Amphoto B

SSG = sodium stibogluconate; MA = meglumine antimoniate; AmphoB = amphoterich B

Classification at enrollment before treatment onset within LeishNatDrug-R study

Classification of failure (F) outcomes during follow-up of first course treatment

- (*) primary unresponsiveness
- (∗∗) relapse (re) + secondary unresponsiveness
- (∗∗∗) secondary unresponsiveness (re + p)
- (∗∗∗*) relapse (p)
- (****) primary unresponsiveness (re)

p = prospective (within LeishNatDrug-R study); re = retrospective (previous episode)

'C' (cured) and 'F' (failure) indicate the final classification used for statistical analysis

Second course of treatment during follow-up
35 Bolivian patients infected with *L. braziliensis*

- Excluded:
  - 7 patients: Treatment & follow up data not available
  - 2 patients: Follow up data incomplete

26 patients with documented treatment outcome

- 23 patients LRV1-
- 3 patients LRV1+

First leishmaniasis episode (N = 22)
- Cured (C) after 1 course
  - MA: 11 patients
  - AmphoB: 2 patients
  - MA + 1 course AmphoB: 6 patients
- Failure (F) after 1 course (***)
  - MA: 3 patients

First leishmaniasis episode (N = 3)
- Cured (C) after 1 course
  - MA: 1 patient
  - MA + 1 course AmphoB: 1 patient
- Failure (F) after 1 course (*)
  - MA: 1 patient

Unresponsiveness previous episode (drug unknown) (N = 1)
- Failure (F) after 1 course (***)
  - MA
- Cured after AmphoB

MA = meglumine antimoniate; AmphoB = amphotericin B
N.A. = not available

Classification at enrollment before treatment onset
Classification of failure (F) outcomes during follow-up:
(*) primary unresponsiveness
(**) secondary unresponsiveness

"C" (cured) and "F" (failure) indicate the final classification used for statistical analysis
Second course of treatment during follow-up