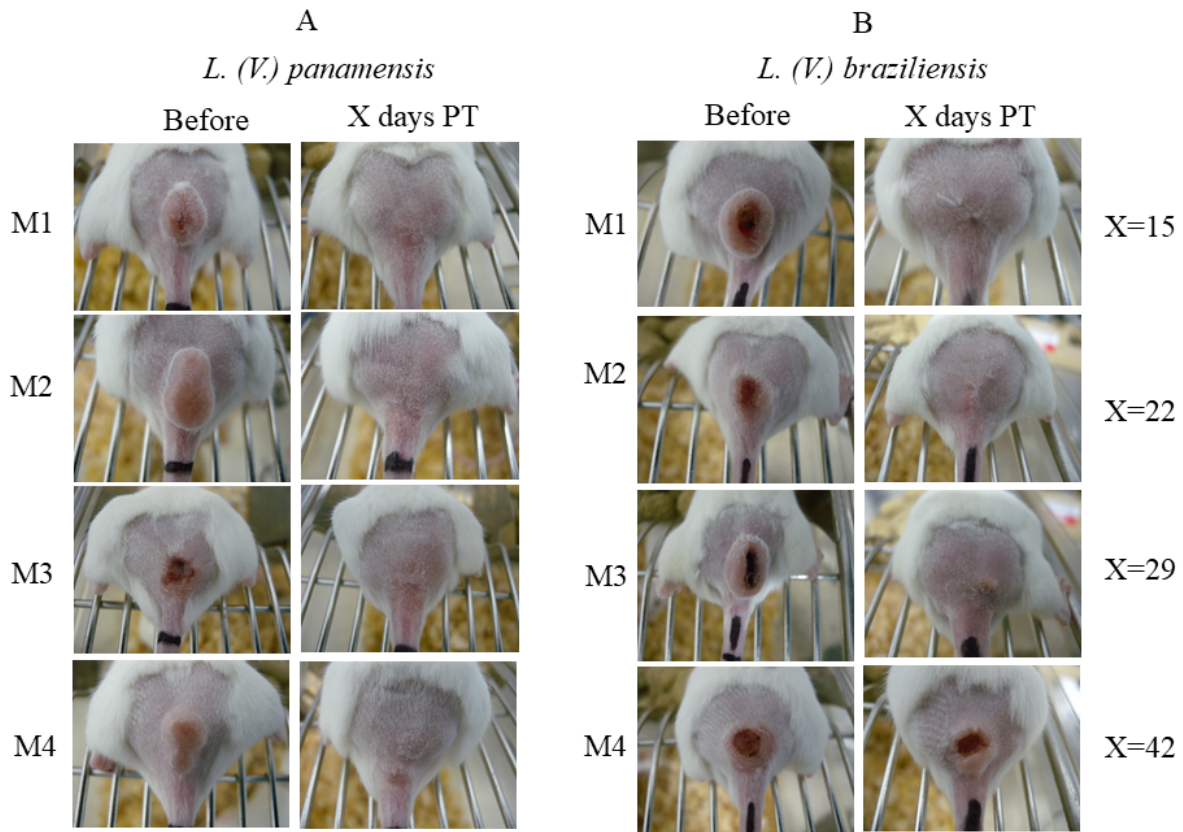


APPENDIX 1

Efficacy of 29 doses of IL-Sb<sup>V</sup> (150 mg/kg/day) at various time points



Mice M1, M2, M3, and M4 were treated with IL-Sb<sup>V</sup> and sacrificed after 15, 22, 29, and 42 days pt. Panels show samples from mice infected with each *Leishmania* species: A, *L. (V.) panamensis* and B, *L. (V.) braziliensis*) with CL-lesions before (right column) and after (left column) treatment on different days pt. (X = 15, 22, 29, 42 days pt.) (N = 1).

APPENDIX 2

Response of mice infected with *L. (V.) panamensis* to treatment with low doses of IL-Sb<sup>V</sup>. A, 29 doses, 50 mg/kg/day. B, 29 doses, 16.6 mg/kg/day

A. IL-Sb <sup>V</sup> -50 mg/kg, <i>L. (V.) panamensis</i> infected mice													
Mice	Before mm <sup>2</sup>	end treatment			15-day pt.			60-day pt.				Parasites imprints biopsies	iE
		mm <sup>2</sup>	LS <sub>red</sub> (%)	eE (%)	mm <sup>2</sup>	LS <sub>red</sub> (%)	eE (%)	mm <sup>2</sup>	LS <sub>red</sub> (%)	eE (%)			
M1	50.0	24.3	51.4	33.3	20.8	58.4	50	59.9	0	0	+++	+++	0
M2	40.5	29.3	27.6		22.8	43.7		132	0		+++	+++	

<b>M3</b>	59.2	26.4	55.4	<b>26.4</b>	<b>55.4</b>	70.8	0	+++	+++	_____
<b>M4</b>	<b>33.9</b>	<b>0</b>	<b>100</b>	<b>0</b>	<b>100</b>	11.6	Re <sup>a</sup>	+++	+++	
<b>M5</b>	<b>50.9</b>	18.0	64.6	<b>5.3</b>	<b>89.5</b>	17.3	66.0	+++	+++	
<b>M6</b>	<b>32.1</b>	<b>0</b>	<b>100</b>	<b>0</b>	<b>100</b>	3.5	Re	+++	+++	

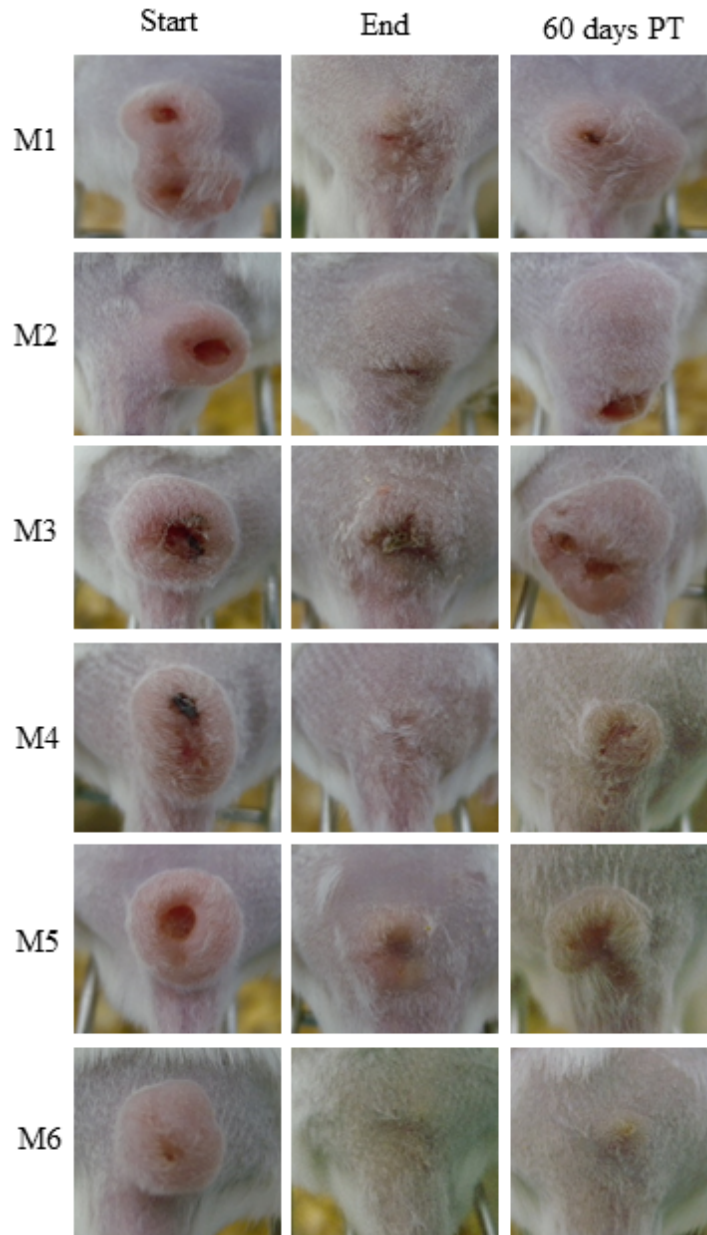
B. IL-Sb<sup>V</sup>-16.6 mg/kg, *L. (V.) panamensis* infected mice

Mice	Before mm <sup>2</sup>	end treatment		15-day pt.			60-day pt.						
		mm <sup>2</sup>	LS <sub>red</sub>	eE	mm <sup>2</sup>	LS <sub>red</sub>	eE	mm <sup>2</sup>	LS <sub>red</sub>	eE	Parasites		fE
			(%)	(%)		(%)	(%)		(%)	(%)	(%)	imprints	
M1	39.7	19.2	51.6		24.5	38.2		78.8	0		+++	+++	
M2	40.2	34.2	14.9		59.9	0		121.7	0		+++	+++	
M3	42.9	38.9	9.3		40.1	6.5		125.8	0	0	+++	+++	0
M4	42.8	24.2	43.4	0	25.0	41.5		140.9	0		+++	+++	
M5	28.7	26.3	8.3		25.1	12.5		41.3	0		+++	+++	
M6	23.6	19.8	16.1		18.1	23.3		29.9	0		+++	+++	

The table shows individual lesion sizes (mm<sup>2</sup>) before and at the end of treatment, and at 15 and 60 days post-treatment (pt.). Percentage lesion size reduction (LS<sub>red</sub>) in each mouse, aesthetic efficacy (eE), and final efficacy (fE) were calculated (N = 6). Parasite loads were scored at the end of treatment. Before treatment, mean lesion sizes were 44.4 mm<sup>2</sup> ± 9.7 mm<sup>2</sup> and 36.3 mm<sup>2</sup> ± 7.4 mm<sup>2</sup>. %LS<sub>red</sub>, percentage lesion size reduction; % eE, aesthetic efficacy (% mice with LS<sub>red</sub> between 75 %-100 %); fE, final efficacy. No = 0, scarce = 1-5 (+), moderate = 6-10 (++) , abundant > 11 (+++) parasites. Mice presenting with a complete aesthetic response or improvement (LS<sub>red</sub> > 75 %) during follow-up are highlighted. <sup>a</sup> Reactivation.

APPENDIX 3

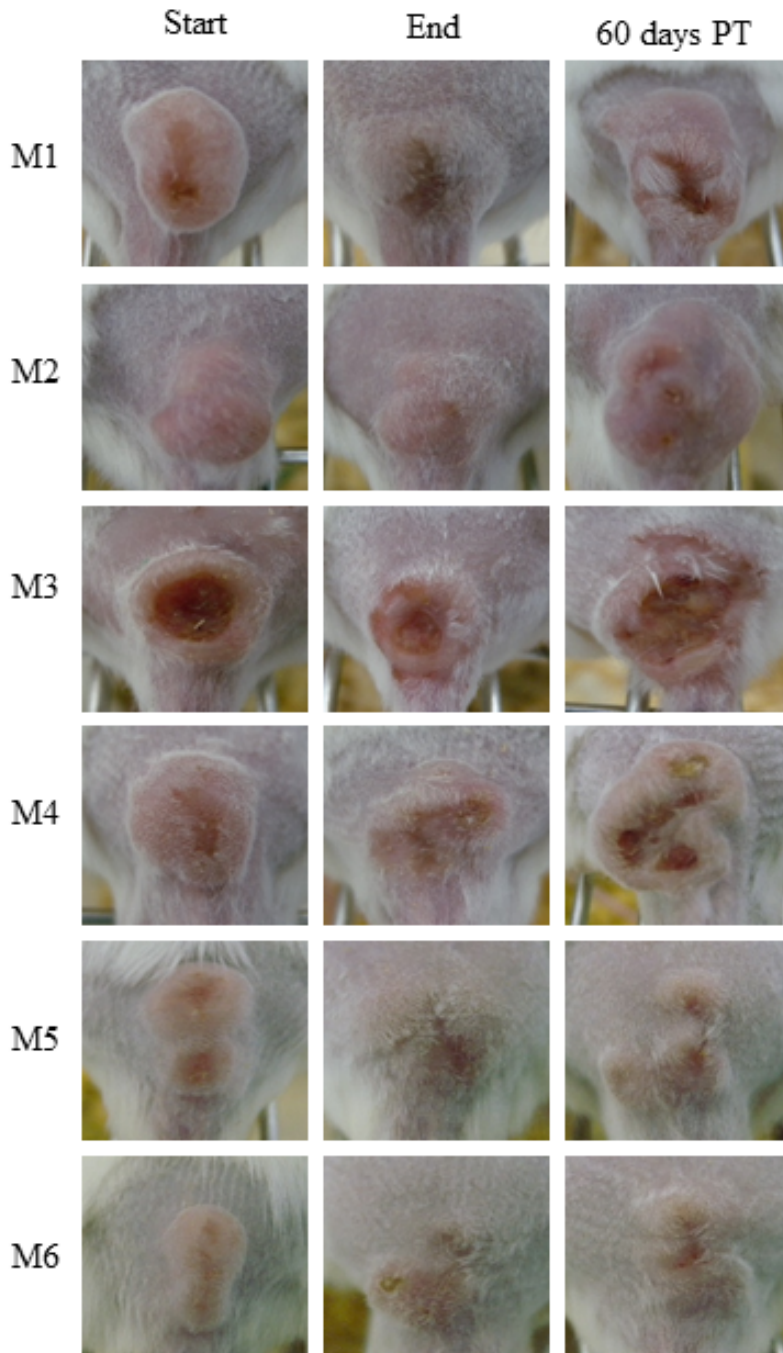
Response of mice infected with *L. (V.) panamensis* to treatment with 29 doses of IL-Sb<sup>V</sup> (50 mg/kg/day)



Photographs of CL lesions in mice M1–M6 at the beginning and end of treatment, and at 60 days pt.

APPENDIX 4

Response of mice infected with *L. (V.) panamensis* to treatment with 29 doses of IL-Sb<sup>V</sup> (16.6 mg/kg/day)



Photographs of CL lesions in mice M1–M6 at the beginning and end of treatment, and at 60 days pt.

APPENDIX 5

Response of mice infected with *L. (V.) braziliensis* to treatment with low doses of IL-Sb<sup>V</sup>. A. 29 doses, 50 mg/kg/day. B. 29 doses, 16.6 mg/kg/day

A. IL-Sb <sup>V</sup> -50 mg/kg, <i>L. (V.) braziliensis</i> infected mice													
Mice	Before	end of treatment			15-day pt.			60-day pt.				fE	
	mm <sup>2</sup>	mm <sup>2</sup>	LS <sub>red</sub> (%)	eE (%)	mm <sup>2</sup>	LS <sub>red</sub> (%)	eE (%)	mm <sup>2</sup>	LS <sub>red</sub> (%)	eE (%)	Parasites imprints    biopsies		
M1	42	18.1	56.9		11.3	73.0		34.8	17.1		+++	+++	
<b>M2</b>	<b>36.4</b>	<b>0</b>	<b>100</b>		<b>0</b>	<b>100</b>		7.8	Re		+++	+++	
M3	45.3	21	53.6	33.3	20	55.8	66.6	39.4	13.0		+++	+++	
<b>M4</b>	50.2	22	56.1		<b>10</b>	<b>80.0</b>		<b>41</b>	18.3	16.6	+++	+++	
<b>M5</b>	45.8	20	56.3		<b>0</b>	<b>100</b>		<b>0</b>	<b>100</b>		-	+++	
<b>M6</b>	<b>48.9</b>	<b>9</b>	<b>81.5</b>		<b>0</b>	<b>100</b>		20	Re		+++	+++	

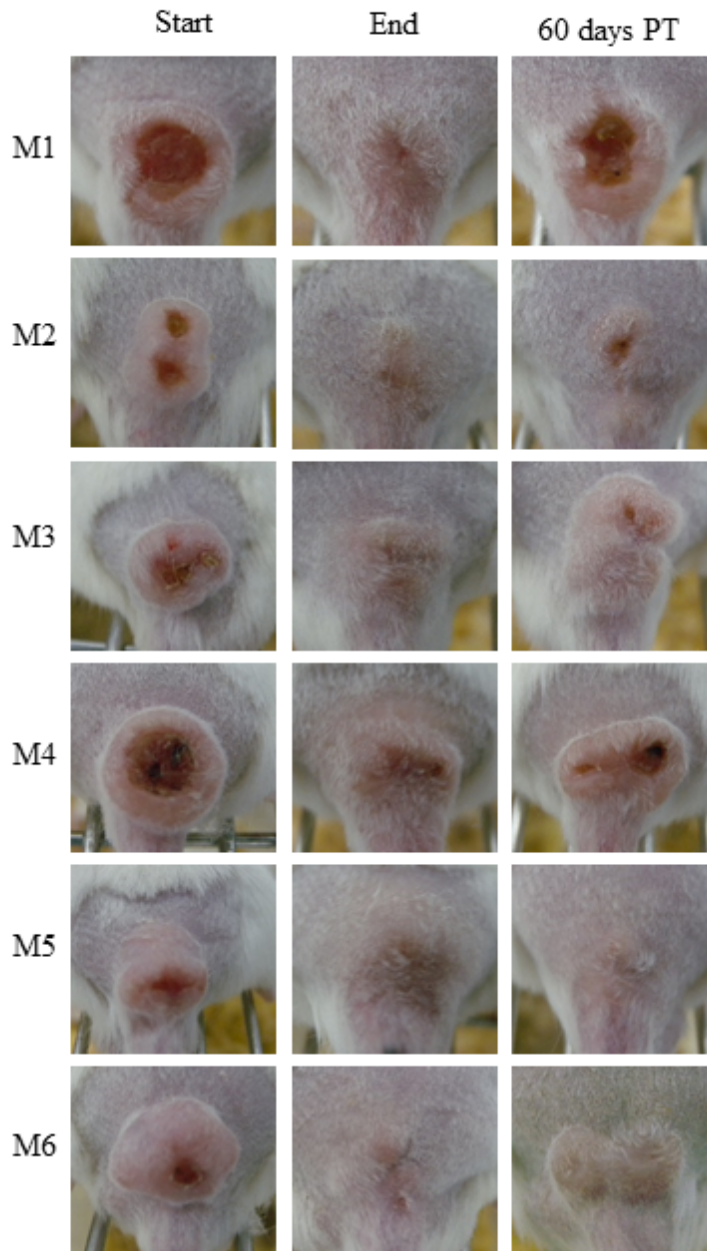
  

B. IL-Sb <sup>V</sup> -16,6 mg/kg, <i>L. (V.) braziliensis</i> infected mice													
Mice	Before	end of treatment			15-day pt.			60-day pt.				fE	
	mm <sup>2</sup>	mm <sup>2</sup>	LS <sub>red</sub> (%)	eE (%)	mm <sup>2</sup>	LS <sub>red</sub> (%)	eE (%)	mm <sup>2</sup>	LS <sub>red</sub> (%)	eE (%)	Parasites imprints    biopsies		
M1	42.8	33.3	22.1		33.3	22.1		53.3	0		+++	+++	
M2	38.9	32.0	17.7		22.0	43.4		68.5	0		+++	+++	
M3	38.1	44.7	0	16.6	54.6	0	16.6	125.5	0	0	+++	+++	
M4	40.6	42.7	0		30.1	25.8		120.4	0		+++	+++	
M5	44.7	48.0	0		37.8	154		78.0	0		+++	+++	
<b>M6</b>	<b>40.7</b>	<b>8.7</b>	<b>78.6</b>		<b>5.2</b>	<b>87.2</b>		29.8	26.7		+++	+++	

The table shows individual lesion areas (mm<sup>2</sup>) before and at the end of treatment, and at 15 and 60 days post treatment (pt.). Percentage lesion size reduction (LS<sub>red</sub>) in each mouse, aesthetic efficacy (eE), and final efficacy (fE) were calculated (N = 6). Parasite loads were scored at the end of treatment. Before treatment, mean lesion size was 44.7 mm<sup>2</sup> ± 4.5 mm<sup>2</sup> and 40.9 mm<sup>2</sup> ± 2.3 mm<sup>2</sup>. %LS<sub>red</sub>, percentage lesion size reduction; % eE, aesthetic efficacy (% mice with LS<sub>red</sub> between 75 %-100 %); fE, final efficacy. No = 0, scarce = 1-5 (+), moderate = 6-10 (++), abundant > 11 (+++) parasites. Mice presenting with a complete aesthetic response or improvement (LS<sub>red</sub> > 75 %) during follow-up are highlighted. <sup>a</sup> Reactivation.

APPENDIX 6

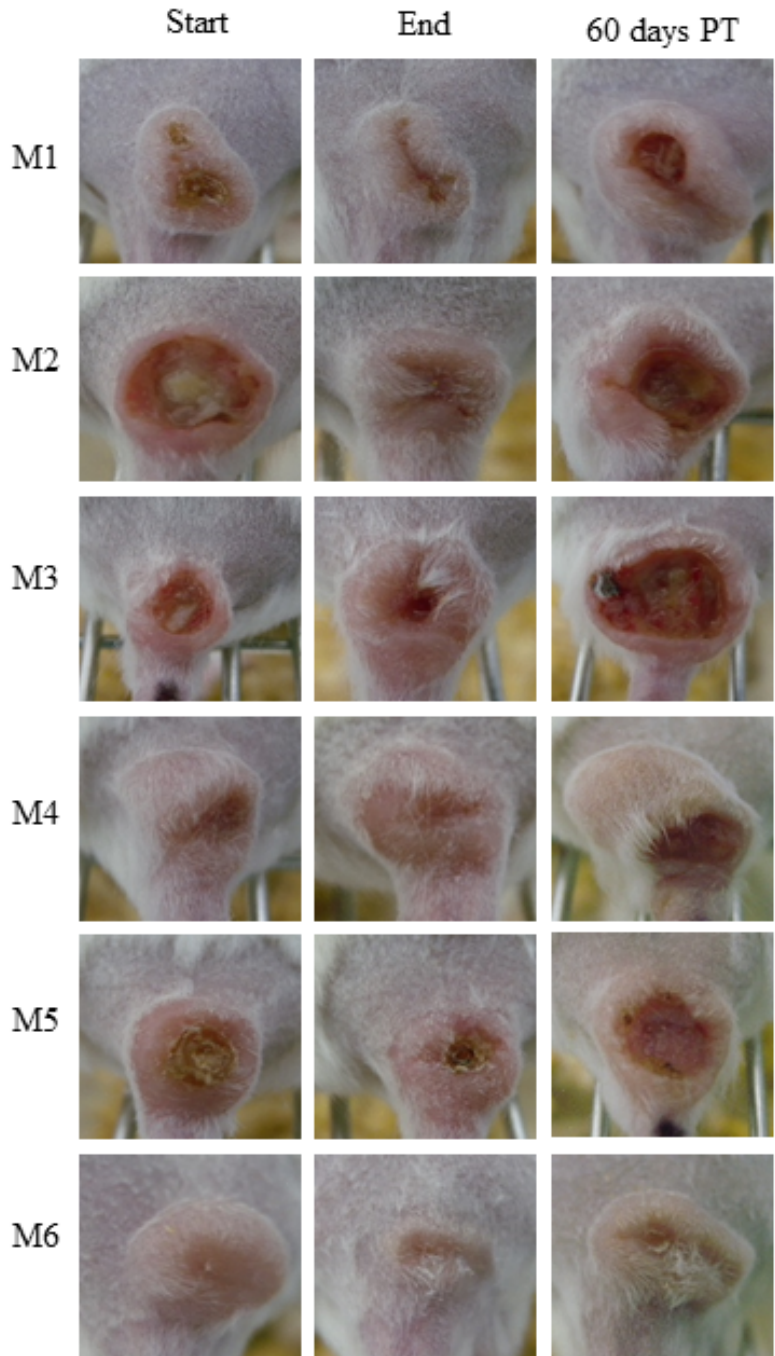
Response of mice M1–M6 infected with *L. (V.) braziliensis* to low dose treatment with 29 doses of IL-Sb<sup>V</sup> (50 mg/kg/day)



Photographs of CL lesions in mice M1–M6 at the beginning and end of treatment, and at 60 days pt.

APPENDIX 7

Response of mice M1–M6 infected with *L. (V.) braziliensis* to low dose treatment with 29 doses of IL-Sb<sup>V</sup> (16.6 mg/kg/day)



Photographs of CL lesions in mice M1–M6 at the beginning and end of treatment, and at 60 days pt.

APPENDIX 8

Histopathological features induced by 29 doses of IL-Sb<sup>V</sup> (150 mg/kg/day) in skin (epidermis and dermis) at 60 days  
pt

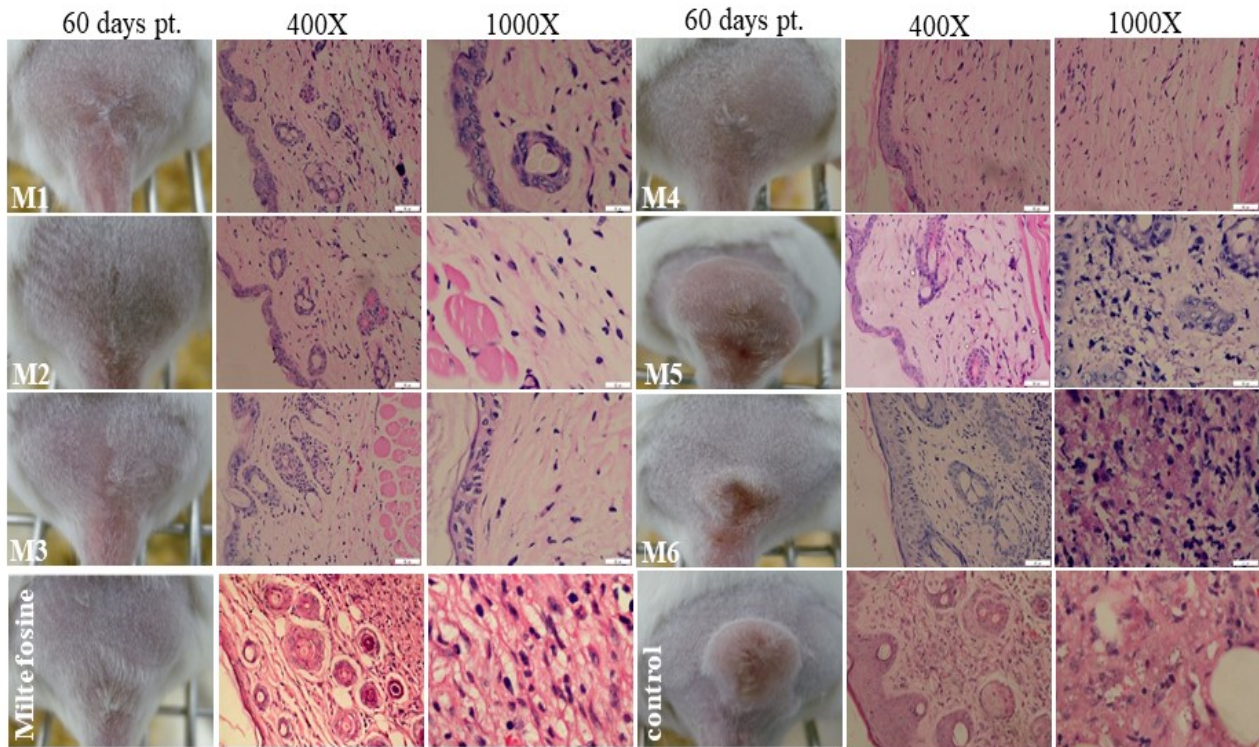
Histopathological Characteristic	<i>L. (V.) panamensis</i>						<i>L. (V.) braziliensis</i>					
	M1 cure	M2 cure	M3 cure	M4 cure	M5 fail <sup>a</sup>	M6 re <sup>b</sup>	M1 cure	M2 fail	M3 cure	M4 re	M5 re	M6 re
Acanthosis	-	-	+	-	+	++	-	-	-	+++	+	+++
Hyperkeratosis	-	-	+	+	+	+	-	-	-	+	+	+
Parakeratosis	-	-	+	-	+	-	-	-	-	+	+	++
Spongiosis	-	-	-	-	+	++	-	-	-	+	+	++
Necrosis	-	-	-	-	+	-	-	++	-	+	-	++
Cellular infiltrate	+	+	+	+	+++	+++	-	++	+	++	++	+++
Lymphocytes	+	+	+	+	+++	+++	-	+++	+	+	+++	+++
Macrophages	+	+	+	+	+++	+++	-	+	+	+++	+++	+++
Neutrophils	+	+	+	+	+++	+++	-	+	+	+++	+++	+++
Giants cells	-	-	-	-	+	+++	-	+	+	+	++	++
Amastigotes	-	-	-	-	+++	+++	-	++	-	+++	+++	+++

Values were obtained from the most dominant histopathological areas and scored as -, absent; +, mild; ++, moderate; and +++ severe. Observations were from 50 microscopic fields, 1000× magnification. M<sub>cure</sub>, cured mice; M<sub>fail</sub>, mice with treatment failure; M<sub>Re</sub>, mice exhibiting reactivation. <sup>a</sup> Failure; <sup>b</sup> reactivation. Histological parameters evaluated (-, absence, +, mild; ++, moderate; +++ severe). The values were obtained from the most dominant histopathological areas. Observation in 50 microscopic fields, 1 000X magnification.



## APPENDIX 9

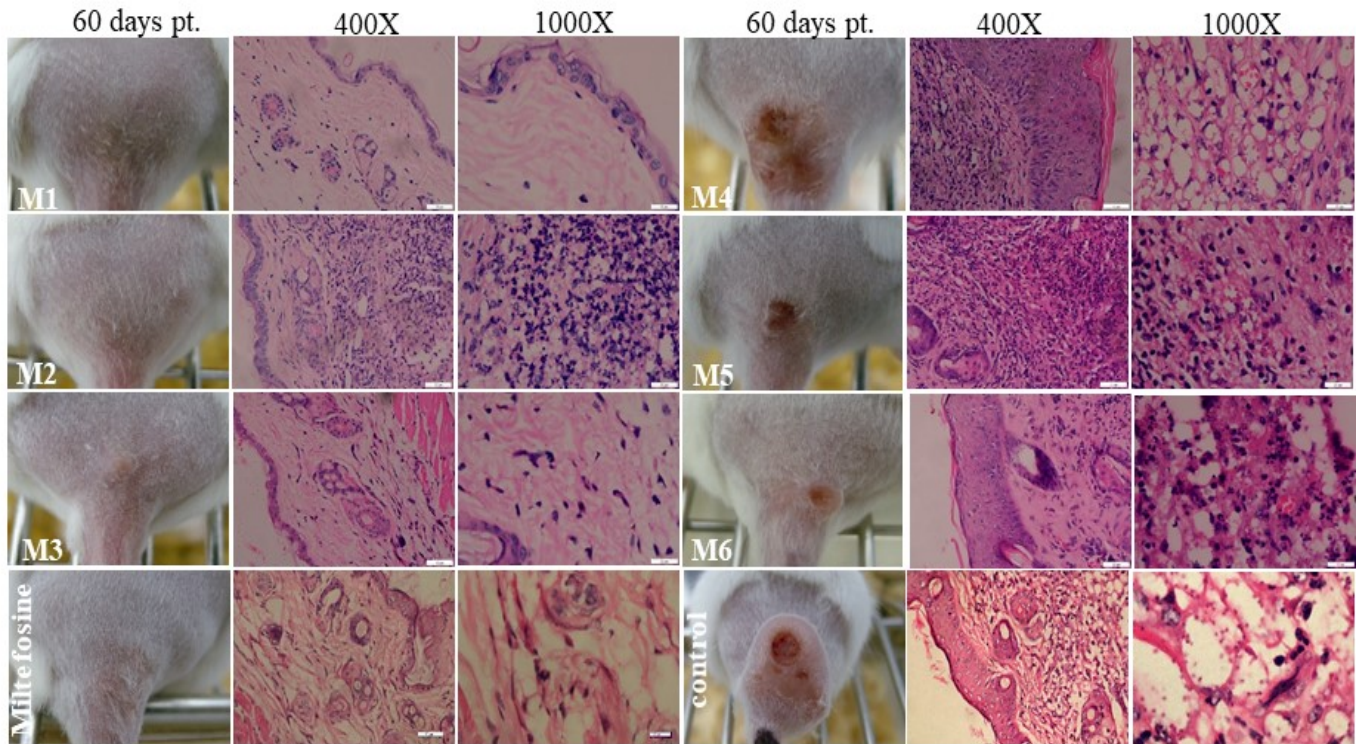
Histopathological features after 29 doses of IL-Sb<sup>V</sup> (150 mg/kg/day) in skin biopsies of *L. (V.) panamensis*-infected mice, at 60 days post treatment (pt.)



The first column (60 days pt.) shows the response of mice M1–M6 to IL-Sb<sup>V</sup>-150, topical miltefosine (positive control) and IL-saline solution (negative control). The microphotographs were taken at different magnifications (400X and 1000X) and showed epidermal/dermal changes such as parakeratosis, spongiosis, hyperkeratosis, acanthosis, and inflammatory infiltrate. Intra and extracellular amastigotes are observed on mice M5, M6 and control.

APPENDIX 10

Histopathological features after 29 doses of IL-Sb<sup>V</sup> (150 mg/kg/day) in skin biopsies of *L. (V.) braziliensis*-infected mice, at 60 days post treatment (pt.)



The first column (60 days pt.) shows the response of mice M1–M6 to IL-Sb<sup>V</sup>-150, topical miltefosine (positive control) and IL-saline solution (negative control). The microphotographs were taken at different magnifications (400X and 1000X) and showed epidermal/dermal changes such as parakeratosis, spongiosis, hyperkeratosis, acanthosis, and inflammatory infiltrate. Intra and extracellular amastigotes are observed on mice M5, M6 and control.