

Immunotherapy of leishmaniases

(First a bit about vaccine development)

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Challenges of leishmaniasis vaccine

Why should it be difficult to produce a vaccine against leishmaniasis?

- Infection induces immunity
 - Leishmanization (LZ)
 - Problems
- Genetic manipulation system was developed a decade ago
- *Leishmania* genome is sequenced
- Can grow *Leishmania* easily
- Have good vaccines for mice! (even Balb/c)
- Can protect monkeys against VL!
- Can protect hamsters against disease
- Why not humans???

Challenges of leishmaniasis vaccine

1- Funding

Estimates

For prophylactic vaccine development \$150 – 500 Million, 10-15 years

Discovery

Preclinical

Clinical

Registration

Post-registration

5-10%

10-30%

60-80%

0.5%

1.5-3%

10 M

20 M

165 M

1 M

4 M

35 years



For leishmaniasis vaccine DEVELOPMENT: (TDR, Brazil, Venezuela, Ecuador, Colombia, NIH (USA))..... **\$10 - 12 M**

For discovery.....30 Labsx35yrsx0.5M/yr = **525 M**

Gates \$15 +\$34M (S. Reed) for one vaccine..... (49M)

Solution

- Scientists should be left alone, but encouraged to address questions relevant to control
- Advocacy to raise funds for DEVELOPMENT (sensitize donors) – (need specialized experts)
- Involve industries in endemic countries India, Brazil, Mexico, etc.

Immunotherapy

Problems

- Resistance to antimonials
- Expected emergence of resistant *L. donovani* to Miltefosine and Paromomycin
- Toxicity and/or cost of full doses of drugs
- Unresponsiveness of ACL, *L. aethiopica*

Need alternate treatment modalities:

Solutions

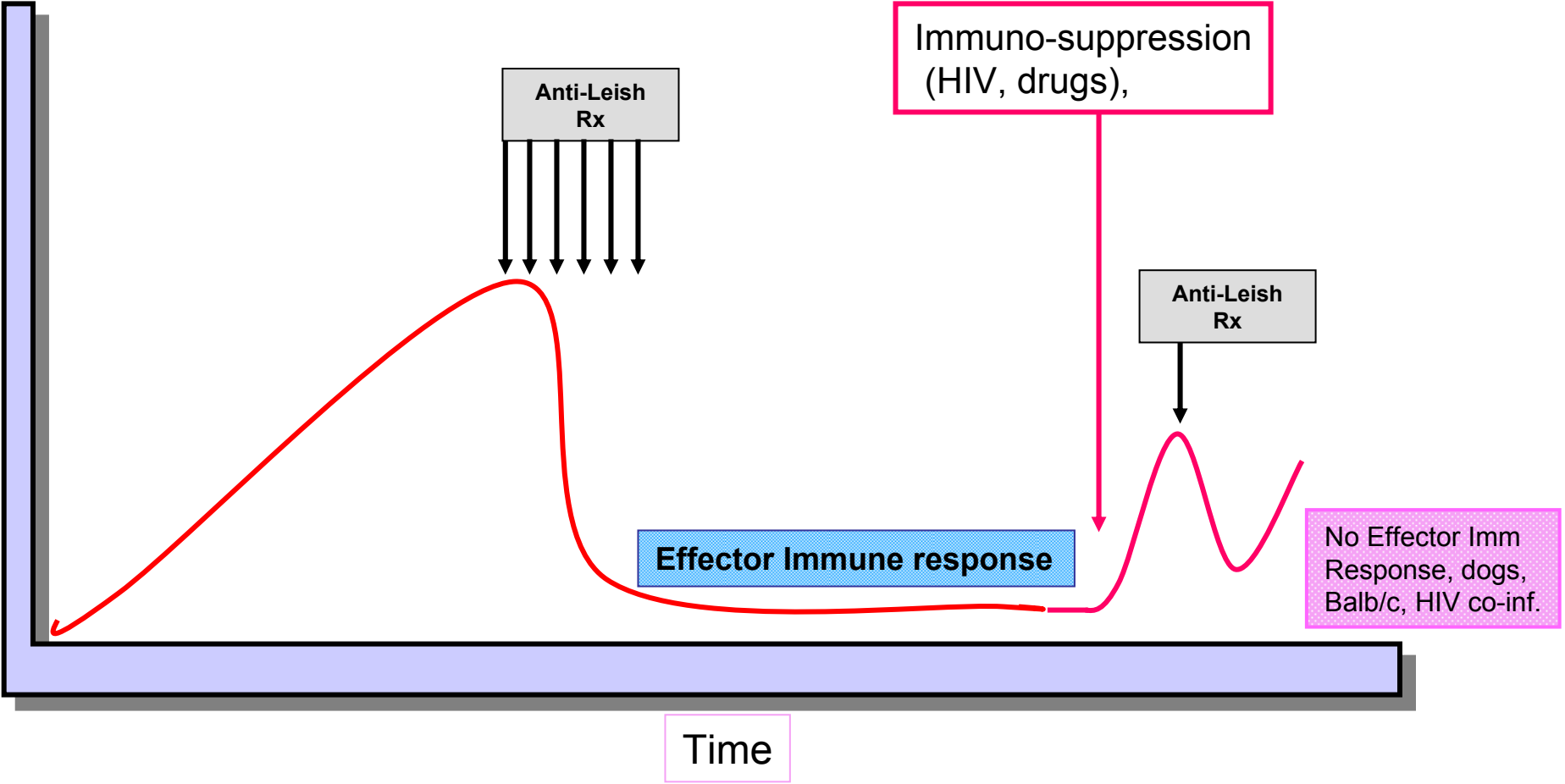
- Combined therapy with available drugs:
 - Shorter course
 - Lower cost
 - Prevent/delay emergence of resistant organisms
 - Sundar, Olliaro *et al.*
- Immuno (+chemo) therapy
 - All the above + possible response in refractory ACL and *L. aethiopica*

Immuno-chemotherapy

- Control of *Leishmania* growth in a host is immunologically mediated:
 - HIV & *Leishmania* co-infection
 - Patients on immunosuppressive drugs
 - Relapse in previously infected (symptomatic or not) by suppression of immune response
 - Animal models (why can't cure dogs, Balb/c)

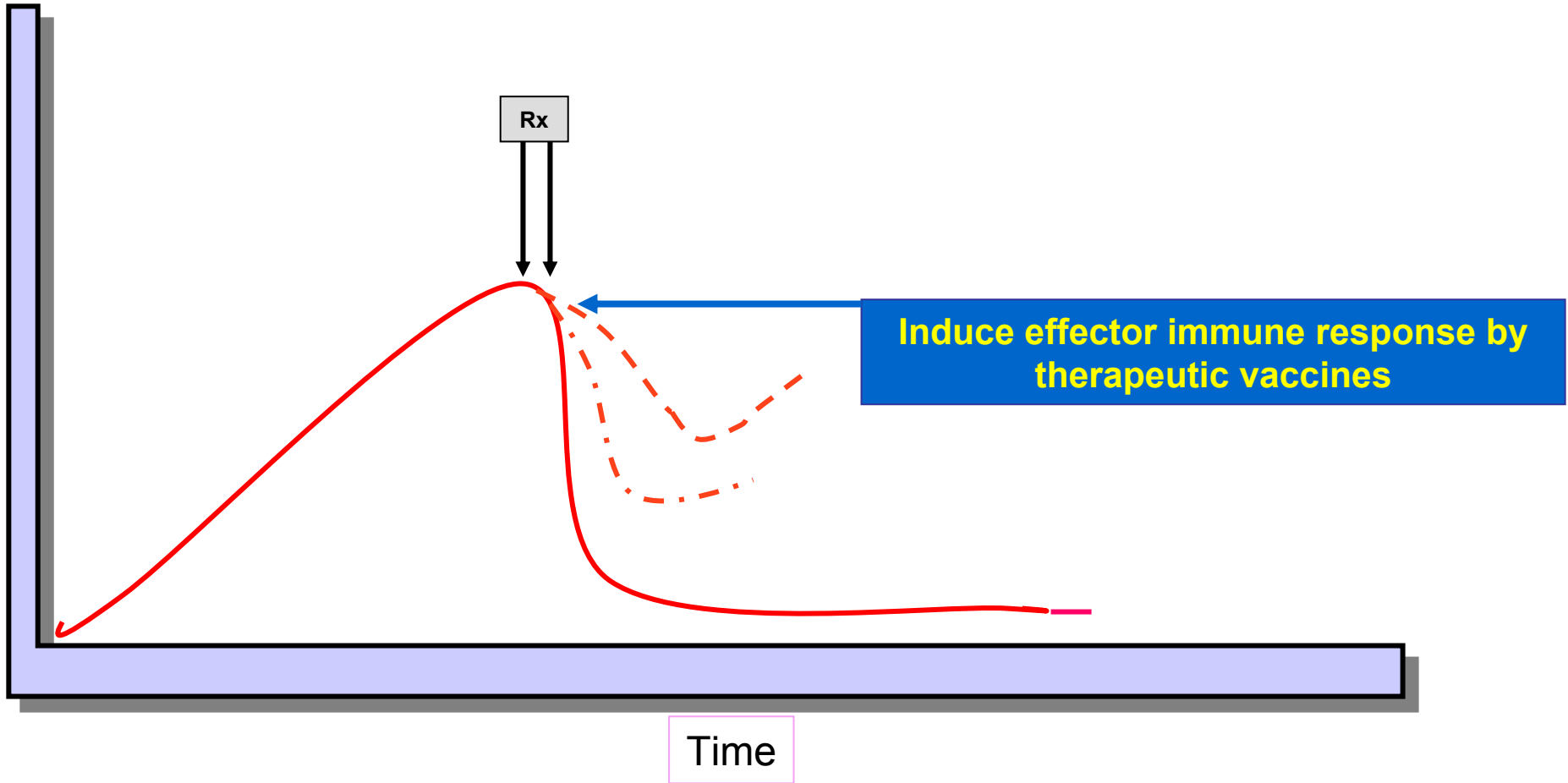
VL Model of Leishmaniasis

Parasite #



Immuno-chemotherapy of Leishmaniasis

Parasite #



Immuno-chemotherapy

- Enhancement/induction of an effector immune response during drug therapy would:
 - Facilitate recovery,
 - Prevent relapse,
 - Reduce total drug dose (side effects)
 - Shorten the duration of treatment (cost/side effects)
 - Prevent emergence of resistance
 - Revert drug resistance
 - Reduce patient/health worker contact

Immunotherapy of leishmaniasis

Animal models

- With DNA vaccines and CpG ODN's (Long-term prophylaxis and therapeutic effects)
 - Gurunathan, S. *et al.* 1997 *Journal of Experimental Medicine* 186:1137-1147. LACK-DNA +CpG.
 - Gurunathan, S., *et al.* 2000. *Cur. Opin. In Immunol* 12:442-447.
 - Walker, P. S., *et al.* 1999. *Proc.Natl.Acad.Sci.U.S.A* 96:6970-75
- With Hybrid Cell Vaccination
 - Basu. ... Walden,P & Roy, S. HCV Kmp-11 (2007)
- With FML (Leishmune) + 2X saponin in dogs (mechanism ?)

- Many more... What is emerging as mechanism:
- Not a simple Th-2 vs Th-1 shift (IL-4 vs. IL-10 & IL-13)
- Probably, IL-12- and IFN- γ -dependent mechanism is important

Immunochemotherapy

Humans

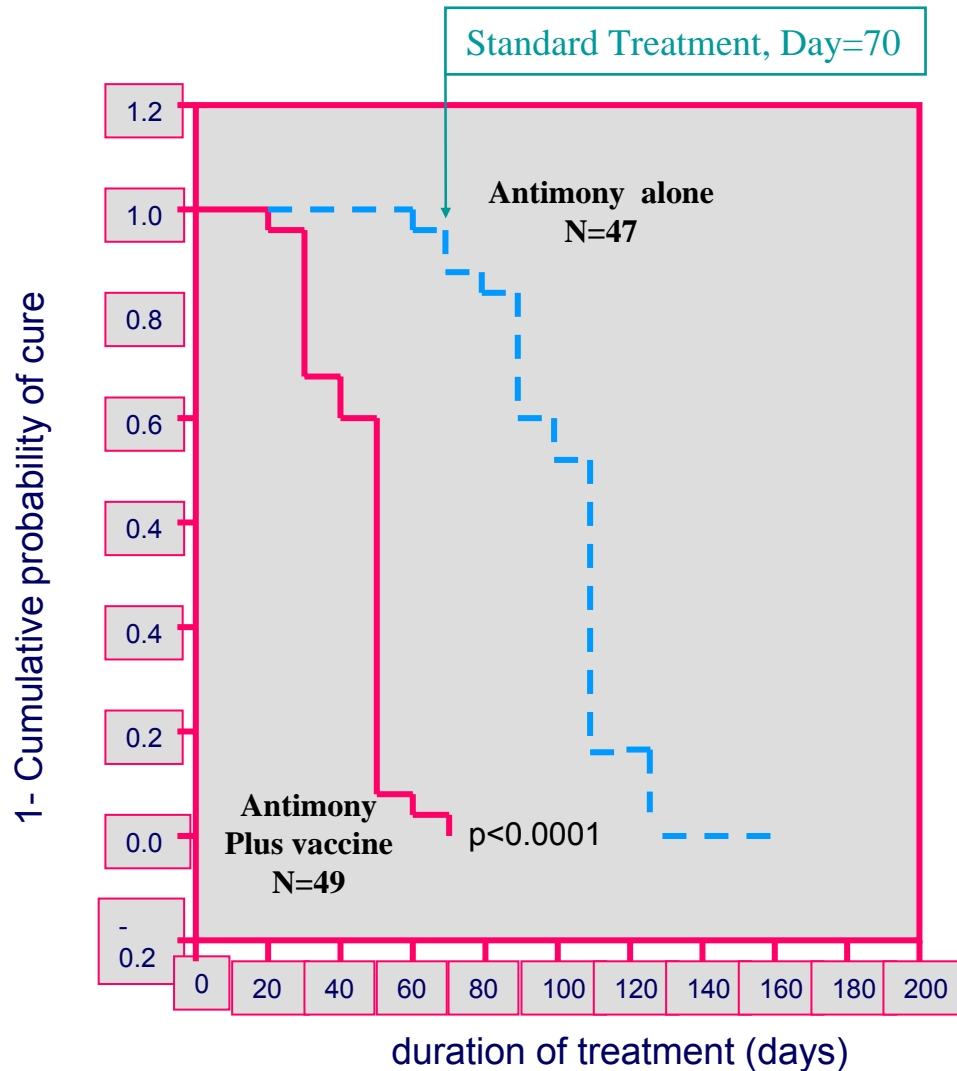
- Four Therapeutic vaccines:
 - Convit, et al. Autoclaved *L. mexicana* + BCG
 - Venezuela (thousands of patients, a few controlled trials)
- Three proof of Principle trials
 - Mayrink's vaccine + low dose antimonial for treatment of *L. braziliensis* CL in Brazil
 - Machado-Pinto et al
 - Alum-ALM +BCG with full dose antimonial for treatment of persistent PKDL in Sudan
 - Musa, Khalil et al
 - Recombinant proteins + GM-CSF and antimonial for treatment of ML, Brazil
 - Badaro, ...Reed, et al

Immuno-chemo therapy of CL Brazil

- Objective: Safety and efficacy of Mayrink's vaccine added to low dose Sb^{+5} (8mg/kg/d) for treatment of CL in Brazil
 - *Single blind, sequential, controlled trial vs antimony alone*
- *49 patients 8 mg Sb^{+5} X10 + Vaccine X10*
Followed by 10 day rest, repeated until complete cure
- *47 Patients 8 mg Sb^{+5} X 10 followed by 10 day rest, repeated until rescued on day 70*
- *Marink's vaccine = killed *L. amazonensis**

Immunotherapy of CL in Brazil Proof of Principle Trial

Machado—Pinto J. *et al. Int. J. Dermatol.* 41:73-8, 2002

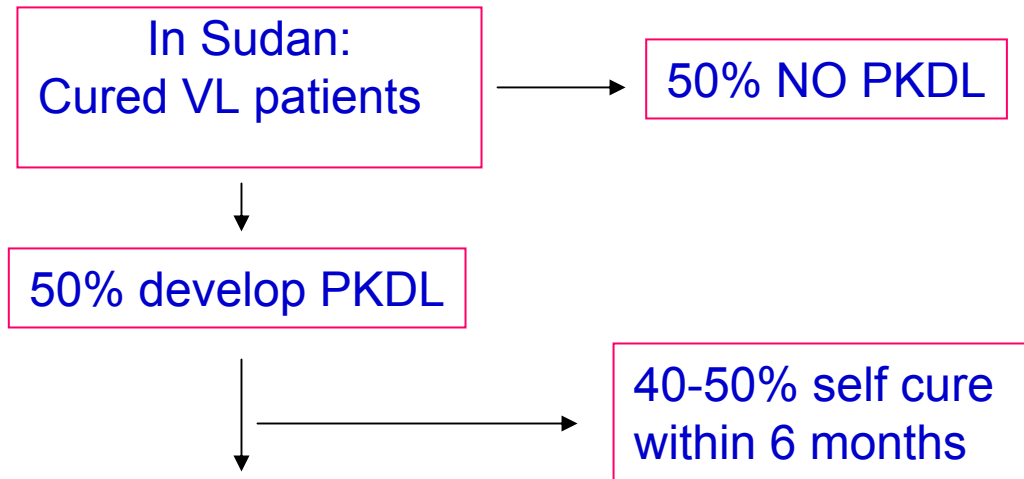


Mayrink's vaccine
registered as adjunct to
low -dose antimony
In Brazil

Kaplan-Meier plot of the probability of cure according to therapeutic regimen

Immuno-chemotherapy of PKDL

Proof of principle (2) in humans



- 20-25% become persistent PKDL, hard to cure, require months of treatment
- Many are LST – negative, parasite +3
- Are sources of parasite transmission
- Are active and do not seek treatment
- Those who are LST+ tend to respond to SSG treatment better

Immuno-chemotherapy with Alum-ALM+BCG

A Phase-1/2 trial

Rationale:

- 1- PKDL patients with LST⁺ tend to respond better to Sb⁺⁵ treatment
- 2- Alum-ALM + BCG converts 80-90 % of LST-negative healthy volunteers after a single injection (in a non-endemic focus)

Hence induction of a cellular response might enhance cure by Sb⁺⁵

Primary Objective:

Safety and immunogenicity of Alum-ALM + BCG as an adjunct to Sb⁺⁵ for treatment of persistent PKDL patients in Sudan.

(As part of safety, evolution of disease was monitored to rule out exacerbation, hence efficacy was also evaluated).

Secondary objective:

Immunological responses associated with cure (LST, IL-10 and γ -IFN)

Immuno-chemotherapy with Alum-ALM+BCG

A Phase-1/2 trial

- Clinical screen, transfer to Khartoum.
- Select 30 patients: inclusion criteria:
 - PKDL > 6 months
 - Age > 6 (following safety/immunogenicity trials in healthy adults)
 - No previous treatment with anti-leishmanials
 - Consent of parents (also transferred to Khartoum)
 - Baseline signs within acceptable range
 - No diagnosed/known chronic concomitant infection

Protocol Design

•Double Blind, BCG controlled, Randomized to:

Group-1

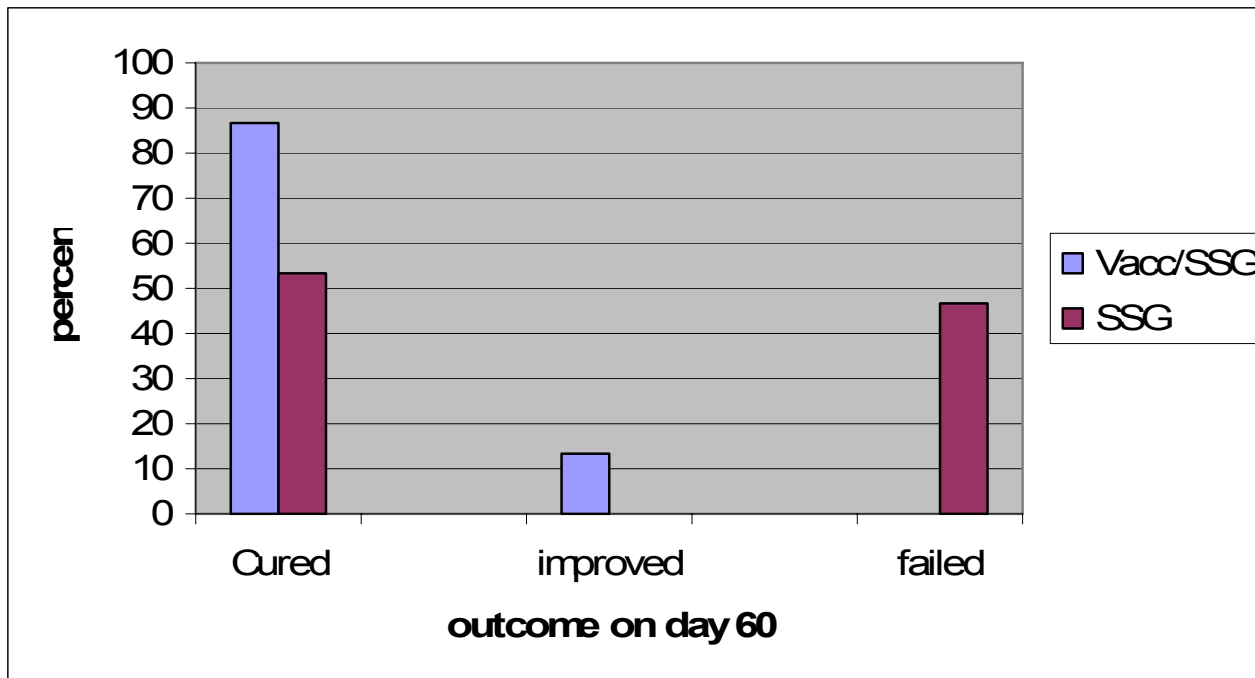
Alum-ALM (100ug) +
BCG weekly (x4) +
Sb⁺⁵ (20 mg/kg/d x40)

Group-2

Placebo +
Sb⁺⁵ (20 mg/kg/d x40)

Safety/immunogenicity followed for 60 days in hospital, then in the field.
Efficacy evaluation day 60 and 6 months after end of treatment

Additional treatment with Sb⁺⁵ for non-responders until day 60,
then rescued with AmBisome



Day	Chem	Chemo + Vaccine
60	8/15 (53%)	13/15 (87%)
<hr/>		
180 (Final)	6/15 (40%) 2 relapsed	15/15 (100%) All cured
p < 0.0000		

Final outcome: safe, AE related to BCG, final efficacy 100% vs 40% at 6 months

Immunotherapy in Leishmaniasis

Proof of Principle Trials 1- Persistent PKDL



Before



After



Fig 8b



Fig 5a



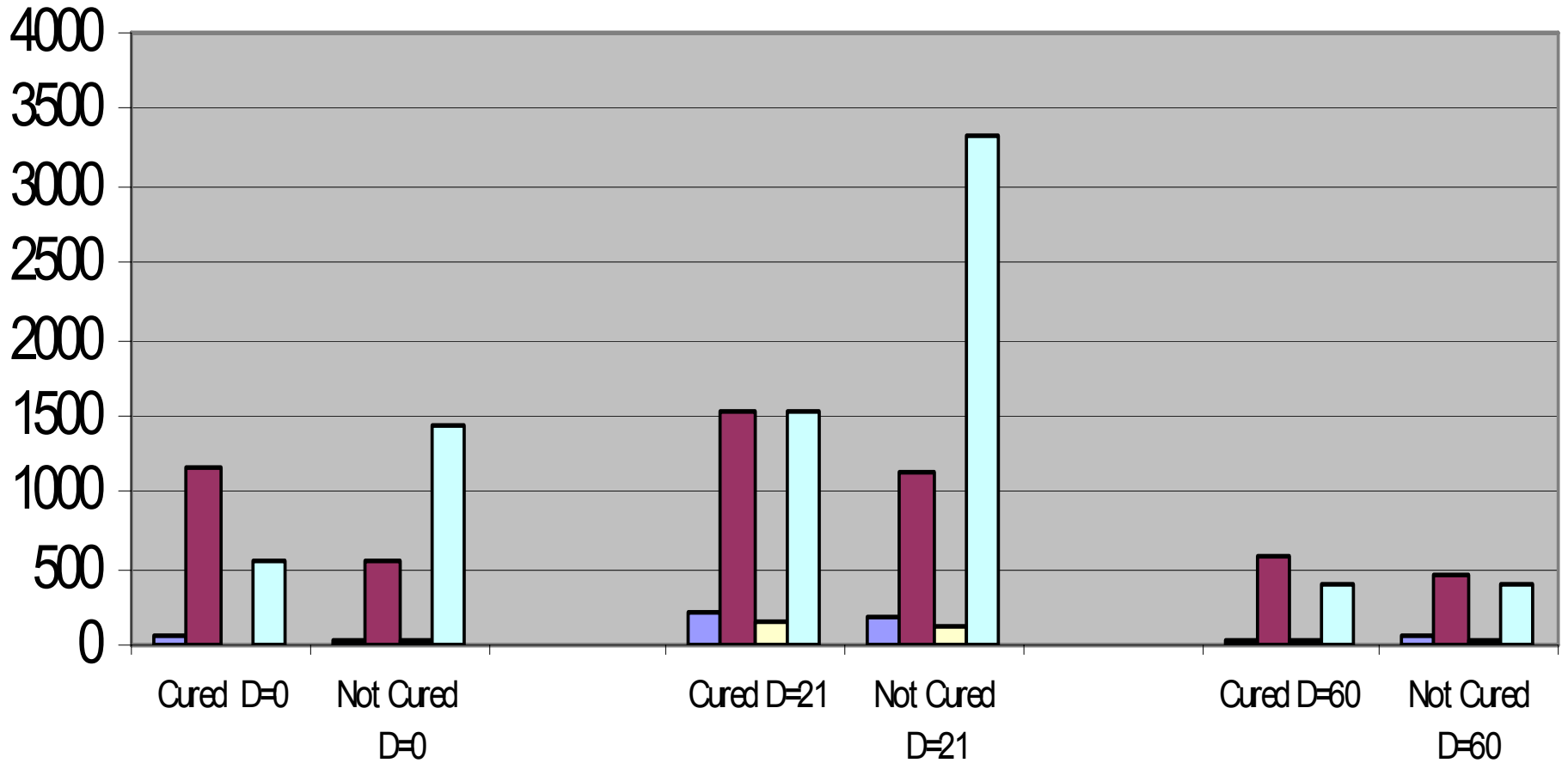
Fig 5b

Before

After

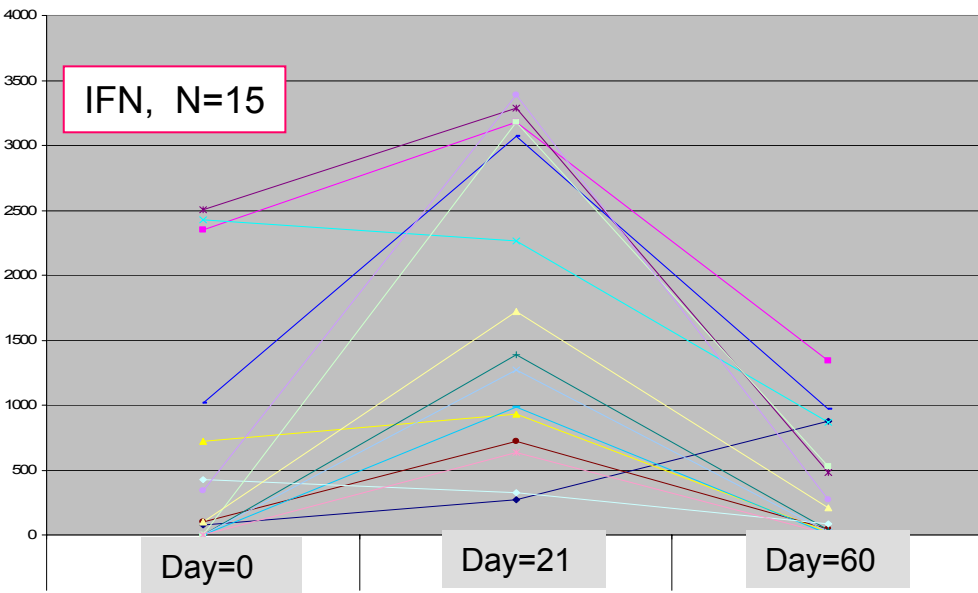
Musa AM, Khalil EAG, Mahgoub FAE, Elgawi SHH, Modabber F, Elkadaru AEMY, Aboud MH, Noazin S, et al. *Trans Roy Soc. Trop. Med.* Oct. 2007 (in press).

Cytokine Assays

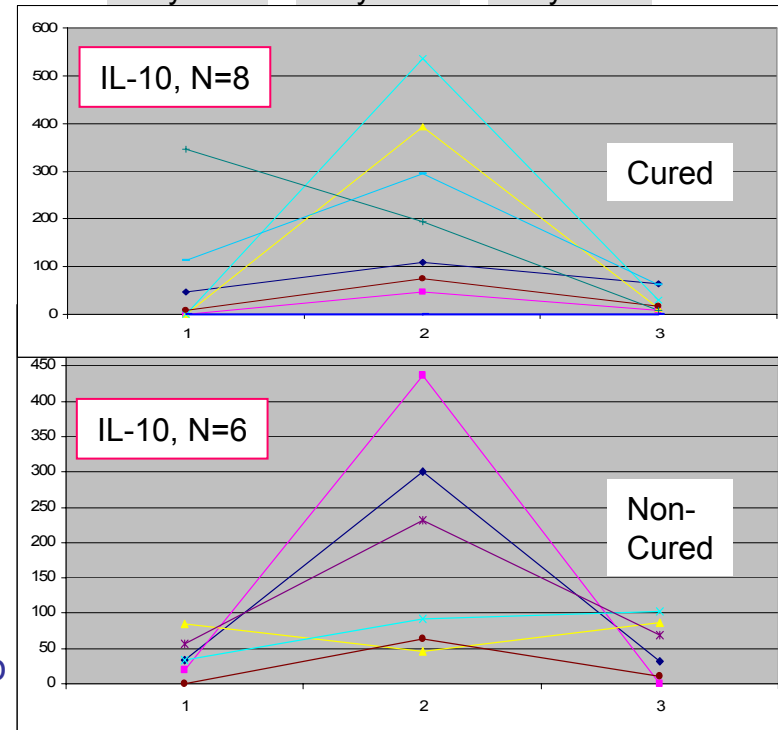
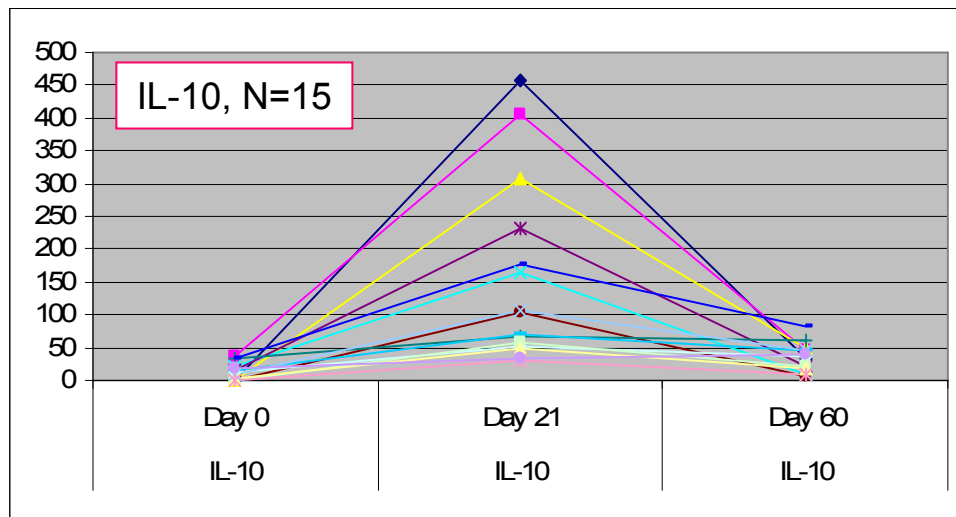
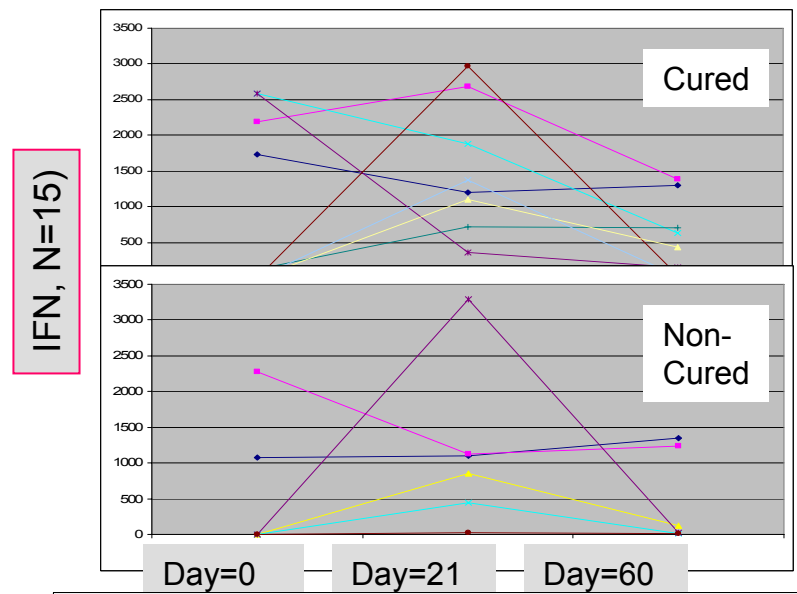


■ Placebo Mh IL-10 ■ Placebo Mh IFN ■ Immuno Chemo Mh IL-10 ■ Immuno Chemo Mh IFN

Vaccine+ Sb



Placebo+ Sb



November 2007

LeishRisk-Antwerp

From studies of Musa et al, 2007

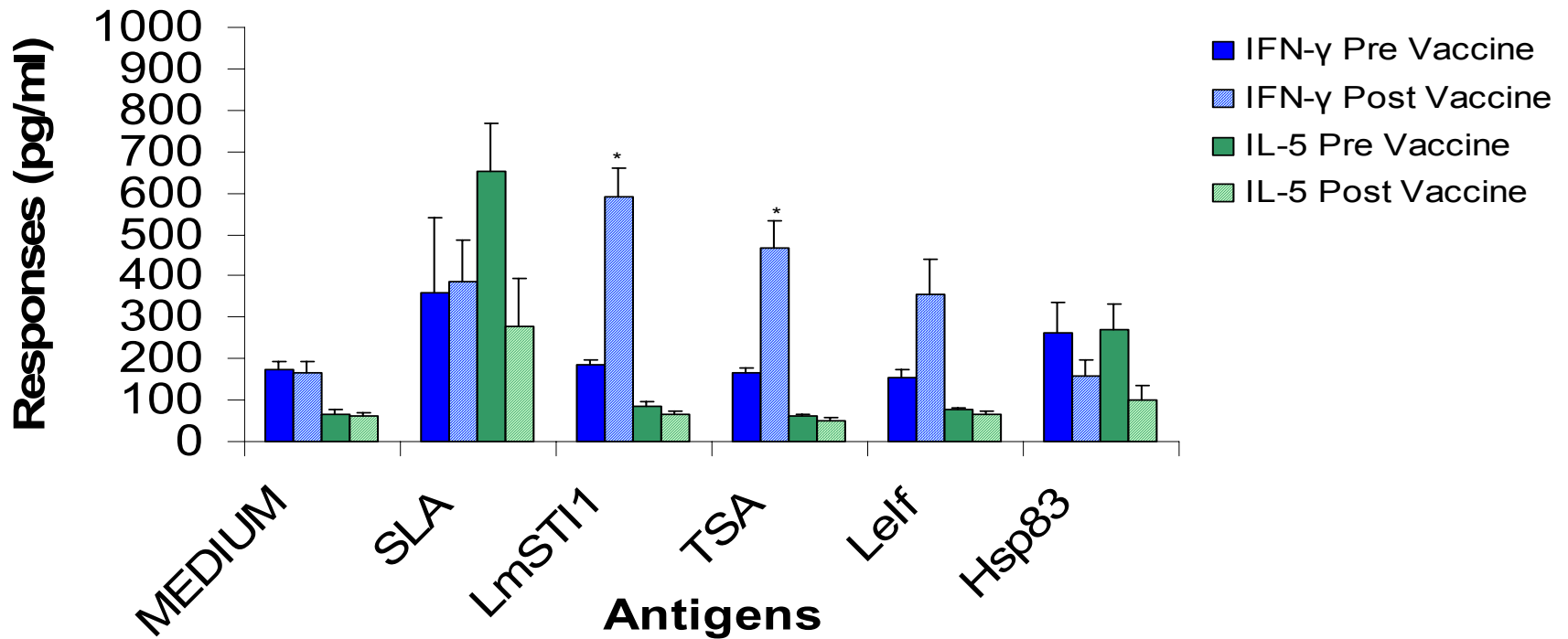
Immuno-chemotherapy of Refractory Mucosal Leishmaniasis with Antimony plus second Generation Vaccines

- Badaro, et al. Case report Braz J Infect Dis. 2001; **5**:223-32.
- Badaro, et al. J Infect Dis. 2006; **194**:1151-9.
- Recombinant antigens plus GM-CSF
- *Antigen individual doses*
TSA (5µg), LmSTI1 (5µg), Lbhsp83 (5µg), LeIF (10µg)
Adjuvant
ruHGM-CSF 50µg (Leukine®)
- *Vaccine Schedule*
- *Three doses 30 day intervals*
 - Booster (if required)
- *Route of Injection*
 - Subcutaneous

Table 2- Overall clinical responses and follow-up of the mucosal leishmaniasis patients with previous antimony experience , than treated with immunotherapy.

Patient CRF #	Previous antimony Time / Courses	One month after 3 doses of vaccine	2 nd Re-treatment 2 nd Vaccine booster	6 months follow-up	3 rd Re-treatment 3 rd Vaccine booster	9 month follow-up	12 month follow -up	18 month follow-up	>24 month follow-up	5 years follow-up
01	1 yr/1 crs	CC	None	CC	None	CC	CC	CC	CC	CC
02	5 yrs/10 crs	F	1 course Sb ^v 3 doses vaccine	R	1 course Sb ^v 3 doses vaccine	CC	CC	CC	CC	CC
03	1 yr/4 crs	F	1 course Sb ^v 3 doses vaccine	R	1 course Sb ^v 3 doses vaccine	CC	CC	CC	CC	CC
04	3 yrs/1 crs	CI	None	CC	None,	CC	CC	CC	CC	CC
05	5 yrs/15 crs	F	1 course Sb ^v 3 doses vaccine	R	1 course of Sb ^v	CI	CI	CC	CC	CC
06	1 yr/2 crs	CI	None	CI	None	CC	CC	CC	CC	CC

Footnote; CC =clinically cured; CI= clinical improvement; R= relapsed; Sb^v = pentavalent antimonial;
1 course of Sb^v = 20 days / 20 mg of Sb^v IV



Immuno-chemotherapy of Refractory Mucosal Leishmaniasis with Antimony plus second Generation Vaccines and GM-CSF

Badaro et al. See Ghalib H & Modabber F. Kinetoplastid Biol Dis.; **6**:7. Aug. 2007

In Summary

- Immuno-chemotherapy is an approach worthy of pursuing
 - The first generation vaccines, useless for prophylaxis, show activity as therapeutic vaccines
 - Safe, affordable and efficacious therapeutic vaccines
 - should prevent or delay the emergence of resistant parasites
 - Reduce drug dose and duration of treatment
- Thereby increase compliance, reduce cost and side effects associated with chemotherapy.

Thank you