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:

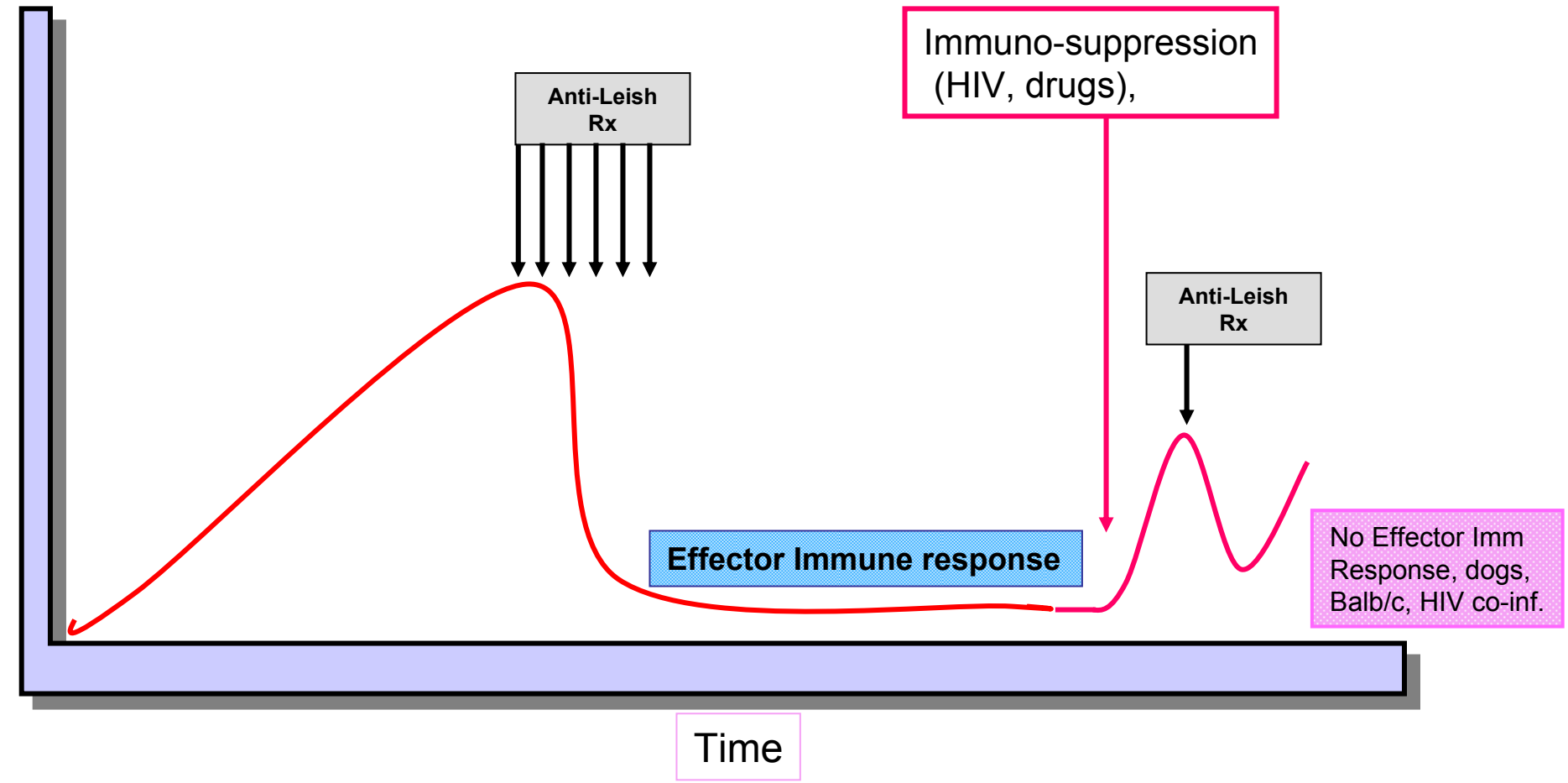
Solutions

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

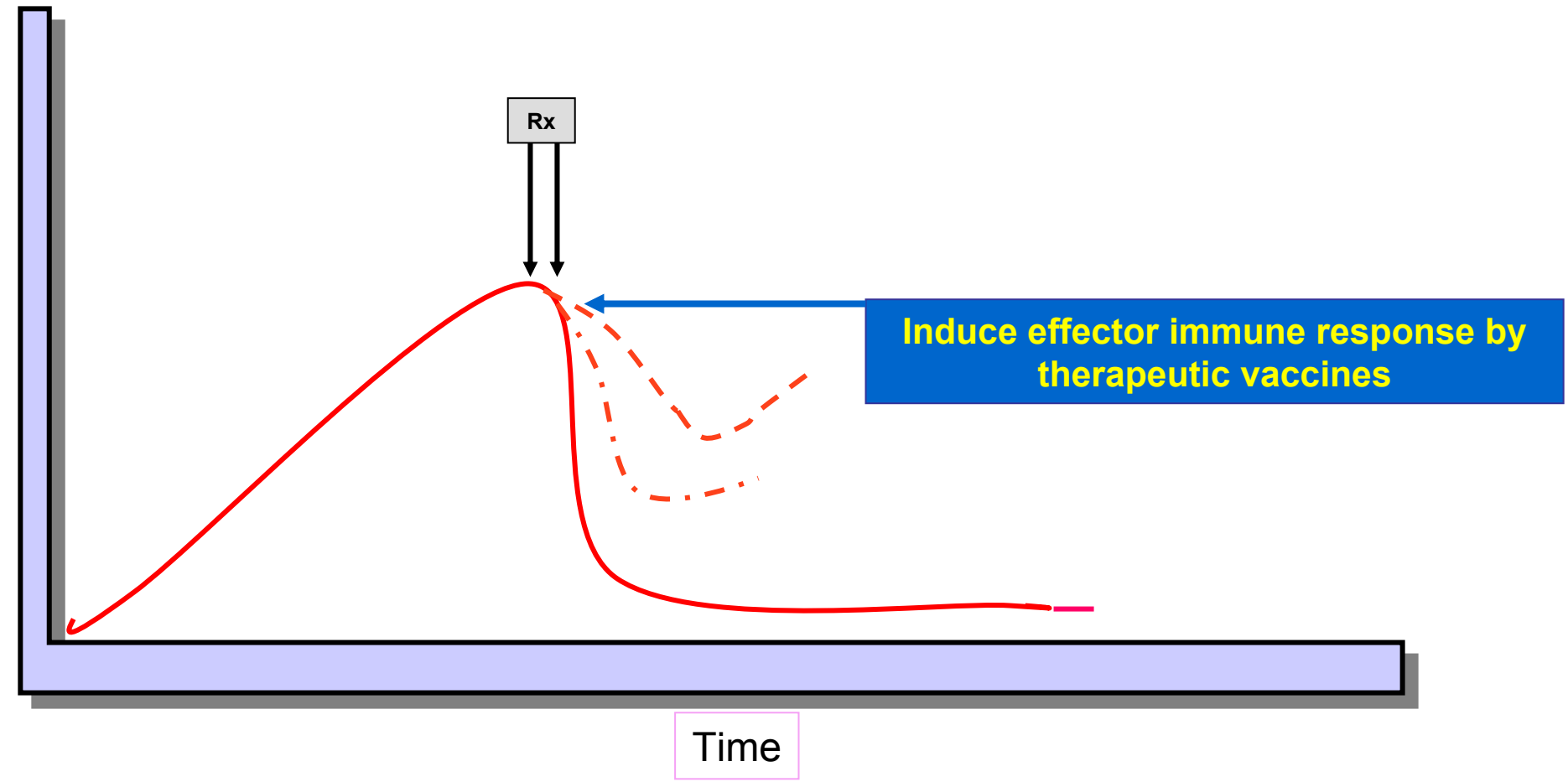
Immuno-chemotherapy

- 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



Immuno-chemotherapy of Leishmaniasis



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 ?)

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- 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

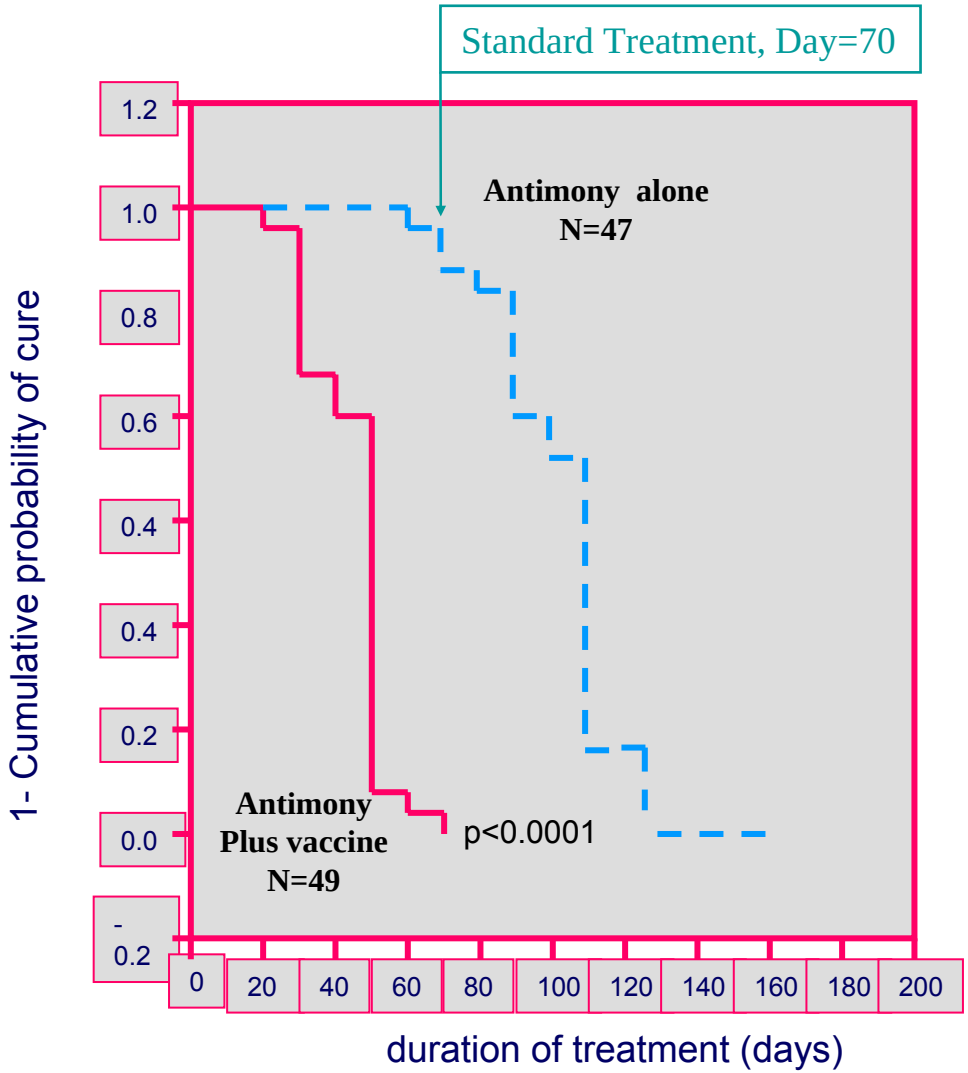
- Objective: Safety and efficacy of Mayrink's vaccine added to low dose Sb^{+5} (8mg/kg/d) for treatment of CL in Brazil
  - *Single blind, sequential, controlled trial vs antimony alone*
- *49 patients 8 mg Sb^{+5} X10 + Vaccine X10*

  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

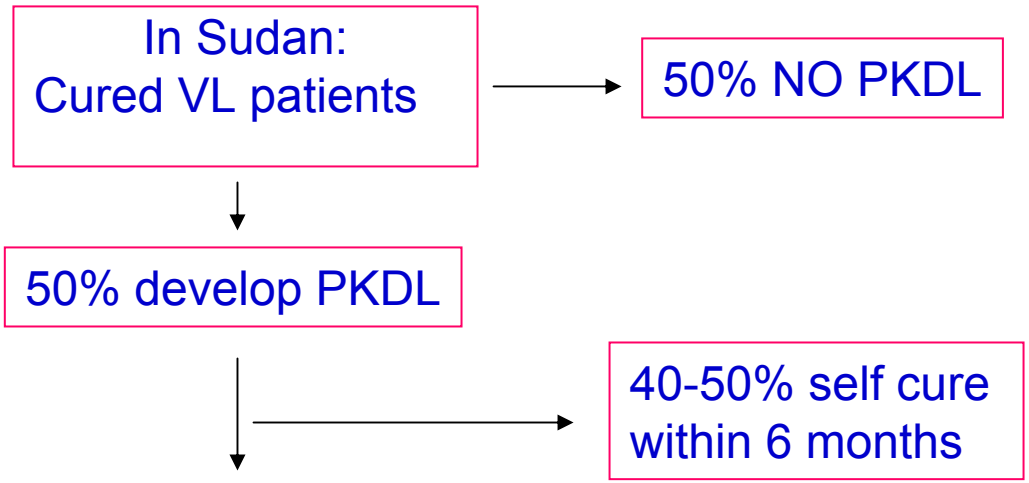
Machado—Pinto J. *et al. Int. J. Dermatol.* ***41***:*73-8, 2002*



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

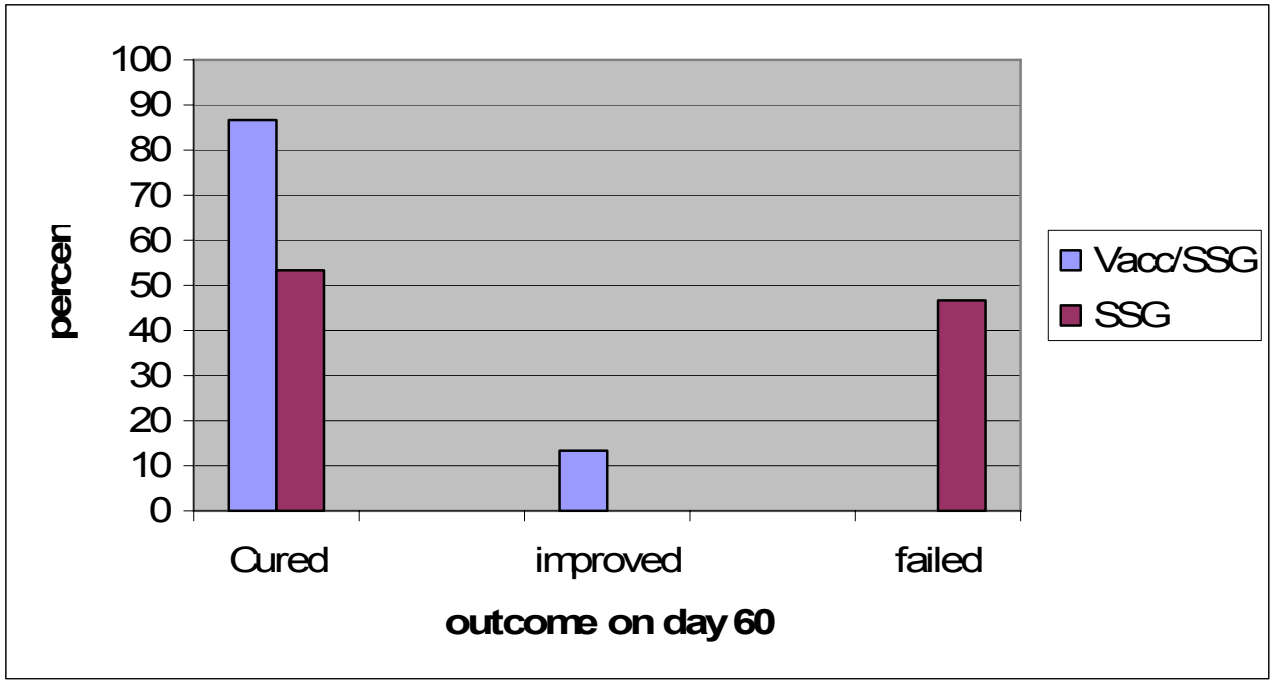
Alum-ALM (100ug) +
BCG weekly (x4) +
Sb^{+5} (20 mg/kg/d x40)

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

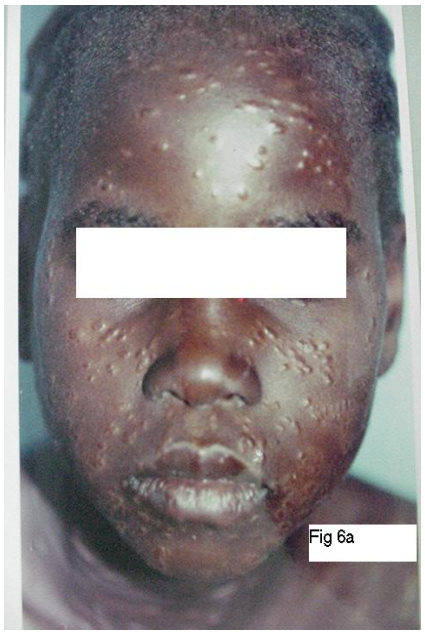


Day	Chem	Chemo + Vaccine
60	8/15 (53%)	13/15 (87%)
180 (Final)	6/15 (40%) 2 relapsed	15/15 (100%) All cured

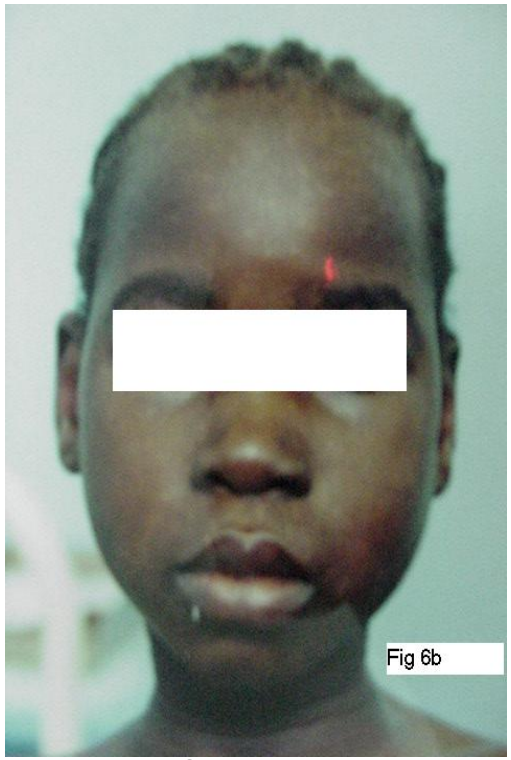
p<0.0000

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

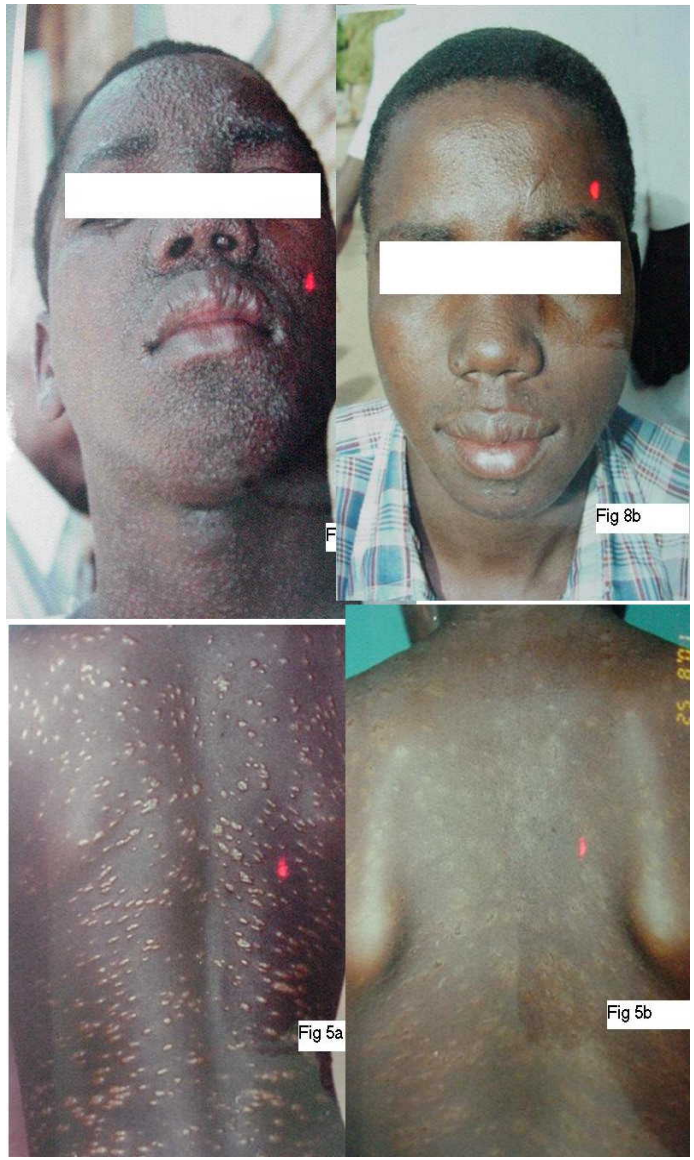
Immunotherapy in Leishmaniasis
Proof of Principle Trials 1- Persistent PKDL



Before



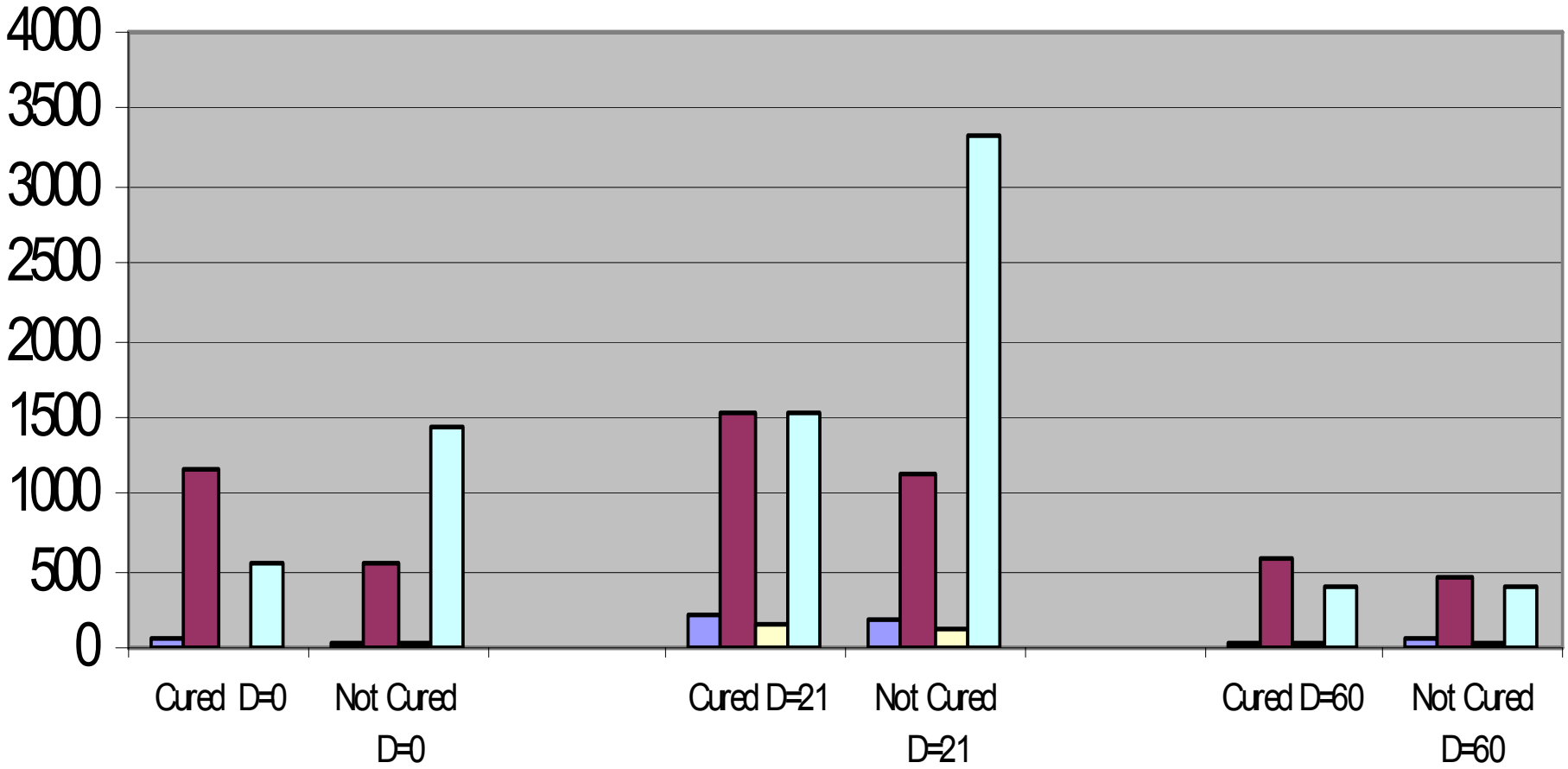
After



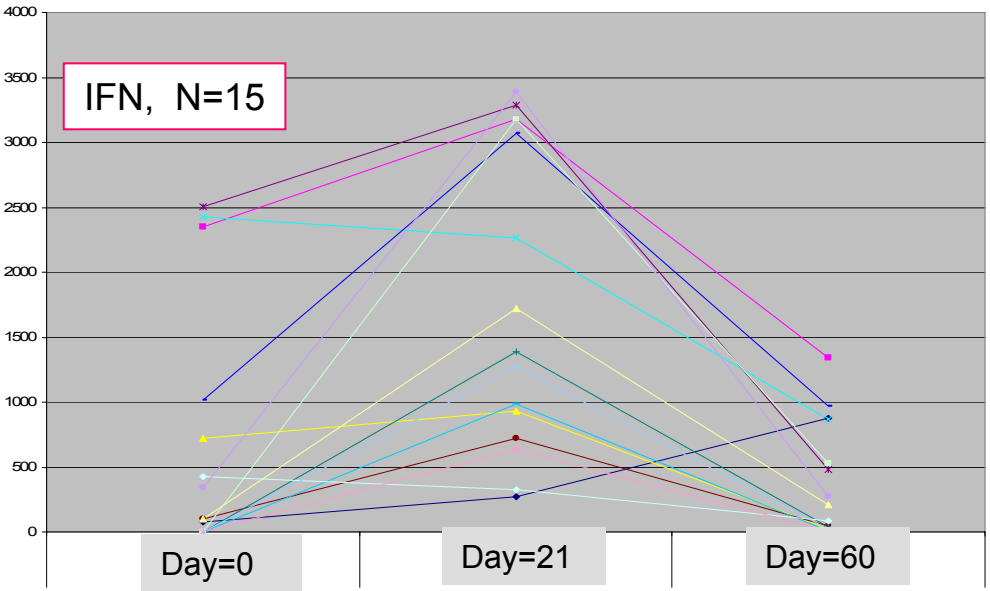
Before **After**

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).

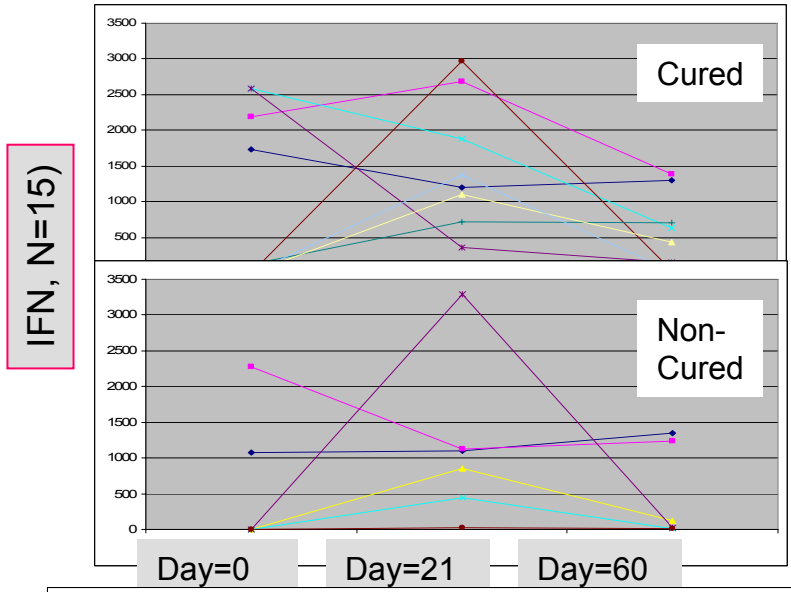
Cytokine Assays

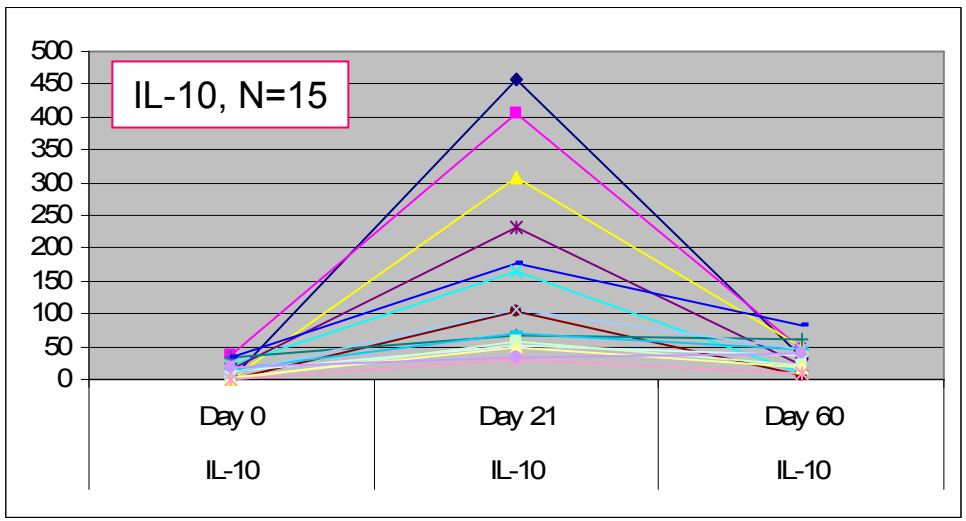


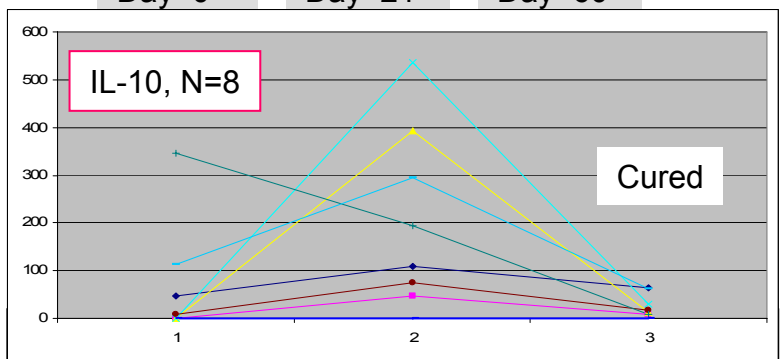
Vaccine+ Sb

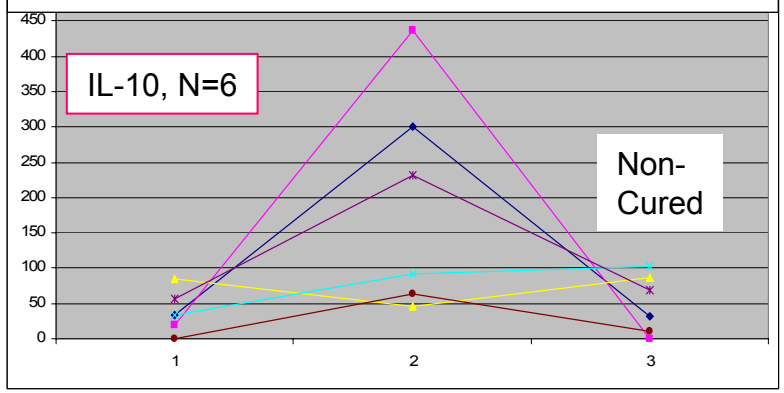


Placebo+ Sb









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)

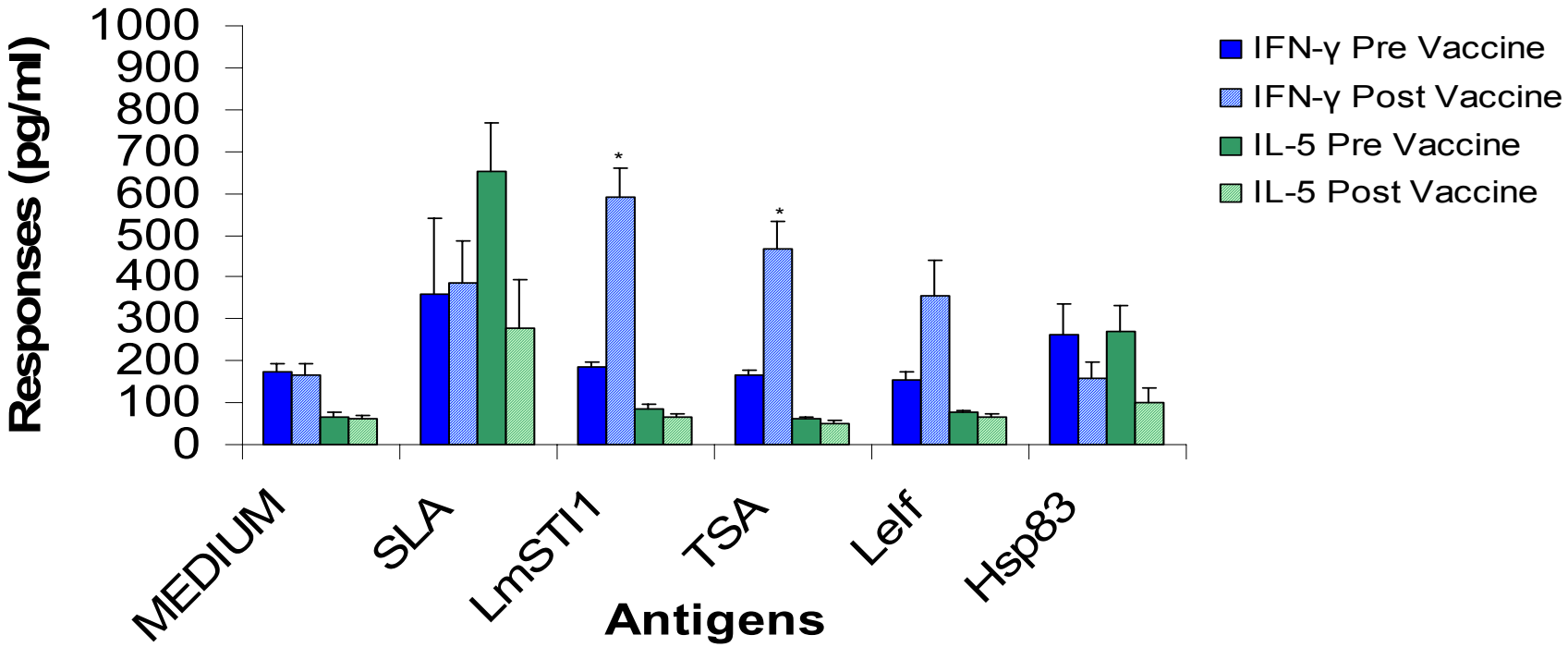
  Adjuvant

  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	2nd Re-treatment 2nd Vaccine booster	6 months follow-up	3rd Re-treatment 3rd 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