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Pyrazinamide prevents the clinical signs of cutaneous leishmaniasis

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Leishmaniasis in a box

- A group of diseases caused by more than 20 leishmanial species

- Vector borne: sandflies

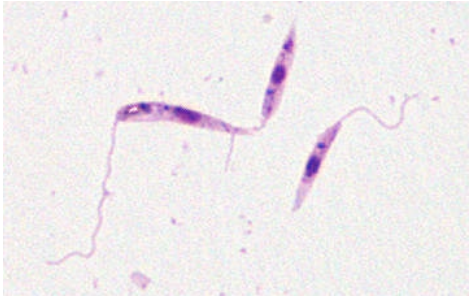
- Emerging: enhanced traveling, vector expansion, mass migration. Prevalence grossly underestimated (not reportable).

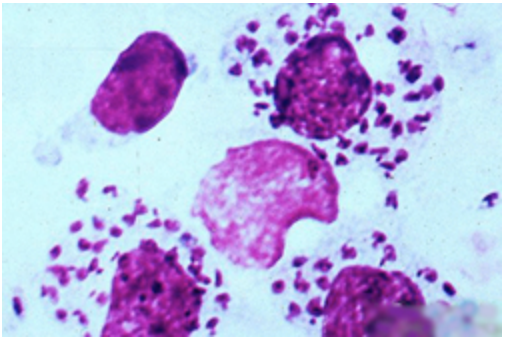
- Worldwide distribution

- Zoonotic: many animals act as natural hosts or reservoirs (some spp. anthroponotic)

- Chronic: immunity is not sterile (reactivation, PKDL)

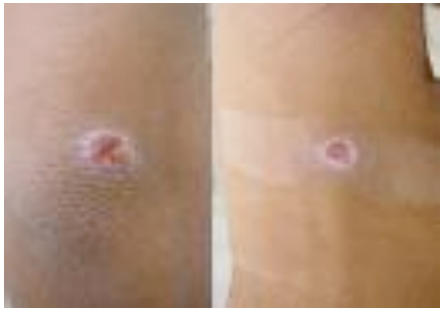
- Opportunistic: Leishmaniasis/HIV (70% coinfections Europe, 30% Africa)



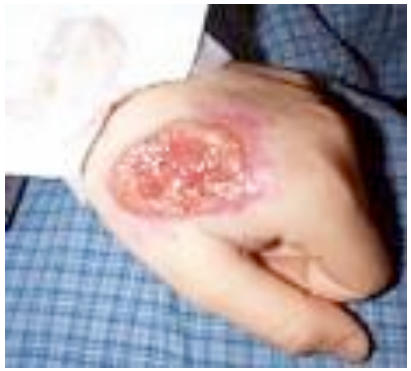


- No vaccine

Complex clinical manifestations, some overlapping

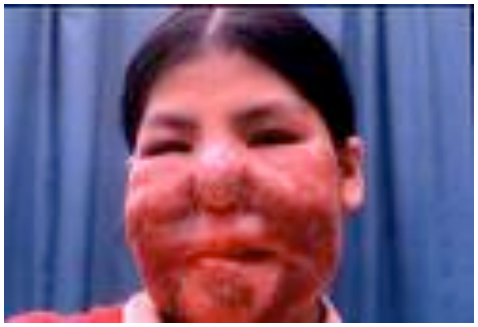


Cutaneous (mild)



Cutaneous (severe)

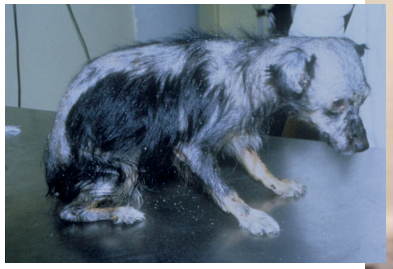
Visceral
(kala-azar)

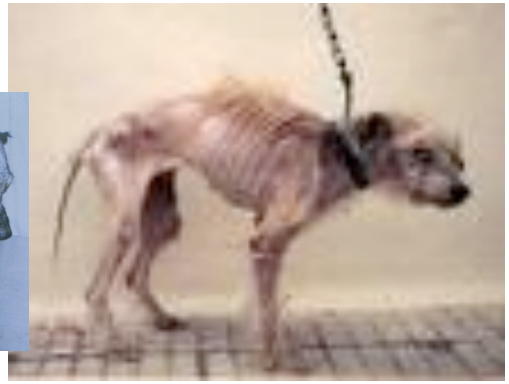


Mucocutaneous

Mucocutaneous

PKDL





Canine

There is no perfect antileishmanial drug, and there are few

Pentavalent antimonials
Amphotericin B
Miltefosine
Paromomycin

Hallmarks:

- Parenteral use (not Miltefosine)

- Hospitalization required

- Adverse side effects

- Costly

- Development of resistance

- Some need competent immune system

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- Developed in vitro and in vivo models for drug testing (rodent, dogs)
- Tested polyamine inhibitors, cystatins and many “classified” compounds

“The most fruitful basis for the discovery of a new drug is to start with an old drug”

(Chong & Sullivan, 2007)

2007- Begin collaboration with Michael Cynamon (SUNY Upstate, Syracuse) and John Welch (SUNY at Albany) to test PZA in trypanosomatids (*Leishmania*)

Antimycobacterial effect of PZA and analogs has been established (Cynamon, Welch and Zimhony's lab)

Ngo, S. C., Zimhony, O., Chung, W. J., Sayahi, H., Jacobs, W. R., Jr. and Welch, J. T. *Antimicrob Agents Chemother* 2007. **51**: 2430-2435.

Zimhony O, Cox JS, Welch JT, Vilchèze C, Jacobs WR Jr. Nat Med.2000. **6**: 1043-7.

Speirs, R. J., Welch, J. T. and Cynamon, M. H. *Antimicrob Agents Chemother* 1995. **39**: 1269-1271.

Cynamon, M. H., Klemens, S. P., Chou, T. S., Gimi, R. H. and Welch, J. T. *J Med Chem* 1992. **35**: 1212-1215.

Why Leishmania?

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2005, p. 808–812 Vol. 49, No. 2
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.2.808–812.2005

In Vitro Antileishmanial Activity of Nicotinamide

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U R008, Pathogénie des Trypanosomatidés, Centre IRD de Montpellier, Montpellier, France,[1] and Department of Biochemistry, Faculty of Pharmacy, and Institute of Cellular and Molecular Biology, University of Porto, Porto, Portugal[2]

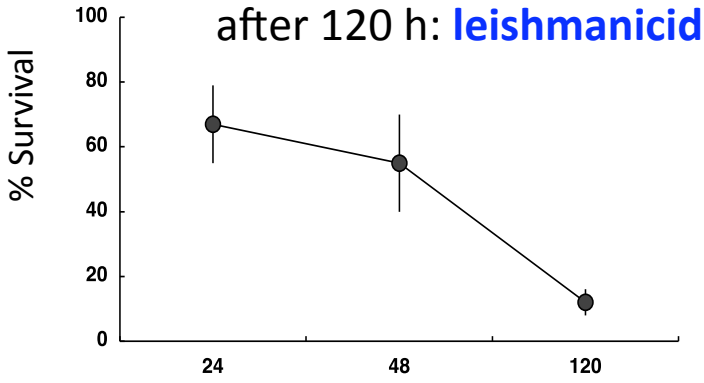
In vitro: PZA has activity against *L. major*

Survival 48 h after treatment

	PZA (µg/ml)							Amphotericin B
	1	12.5	50	100	200	1000	MIC_{50} (µg/ml and µM)	
Promastigote	88 ± 18	50 ± 18	38 ± 14	-	20 ± 10	10 ± 12	16.2 and 16.1	0
Amastigote	89 ± 12	47 ± 12	-	23 ± 10	-	20 ± 15	10.2 and 8.2	10 ± 5
Uninfected J774 cells	100 ± 11	100 ± 10	100 ± 12	100 ± 15	85 ± 12	0	524.8 and 425.6	95 ± 16

-: Not determined

Parasites eliminated from culture after 120 h: **leishmanicidal**



Good news: SI=26 (safe)

Not so good= activity only moderate

Ryan Translavina

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The analogs 5-Cl and 5-Fl PZA has greater antileishmanial activity than PZA

Table I. ***MIC_{50} for PZA on amastigotes of Leishmania***

***Leishmania* strain**	**Origin**	**MIC_{50} (μM)**
L. major V1	Human, Israel	10.1
L. major 173	Human, Israel	7.8
L. major LV39	Human, Sudan	3.0
L. chagasi (infantum) #2	Human, Brazil	22.2
L. infantum #9	Canine, Spain	39.2
L. donovani 1S	Human, India	26.1

Table II. ***MIC_{50} for 5-Cl PZA on amastigotes of Leishmania***

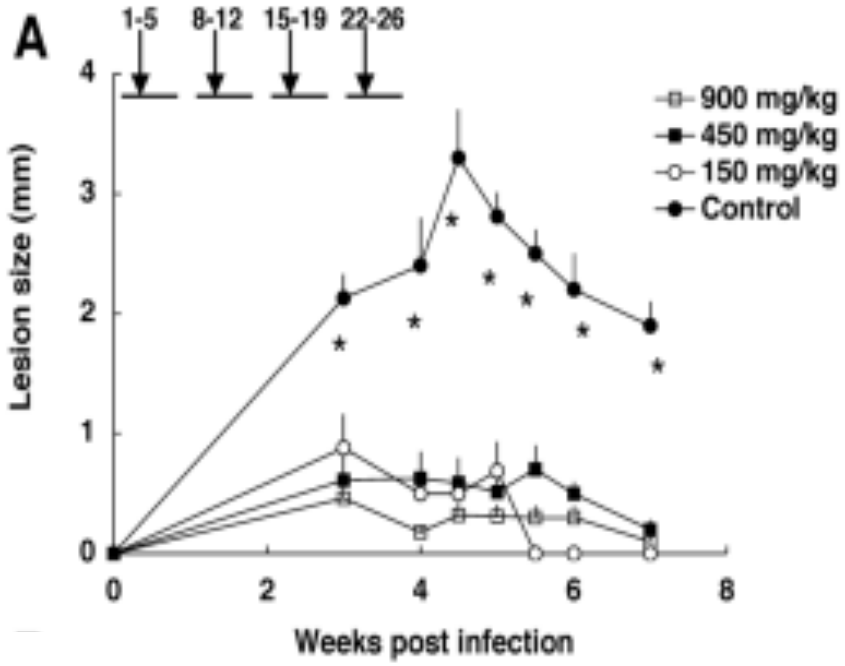
***Leishmania* strain**	**Origin**	**MIC_{50} (μM)**
L. major V1	Human, Israel	1.5
L. major 173	Human, Israel	1.1
L. major LV39	Human, Sudan	0.45
L. chagasi (infantum) #2	Human, Brazil	9.3
L. infantum #9	Canine, Spain	13.4
L. donovani 1S	Human, India	8.7

Table III. ***MIC_{50} for 5-Fl-PZA on L. major***

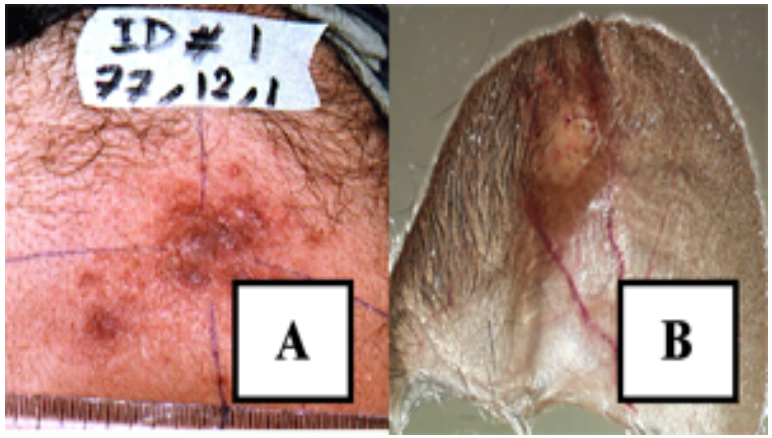
Drug	**MIC_{50} (μM)**
PZA	9.5
5-Cl PZA	2.1
5-Fl PZA	0.08

PZA shows relatively modest activity, analogs are more encouraging (as in TB!!!)

In vivo: treatment prevents lesion development and reduces parasite burden



Ear injection model



Patricia Green

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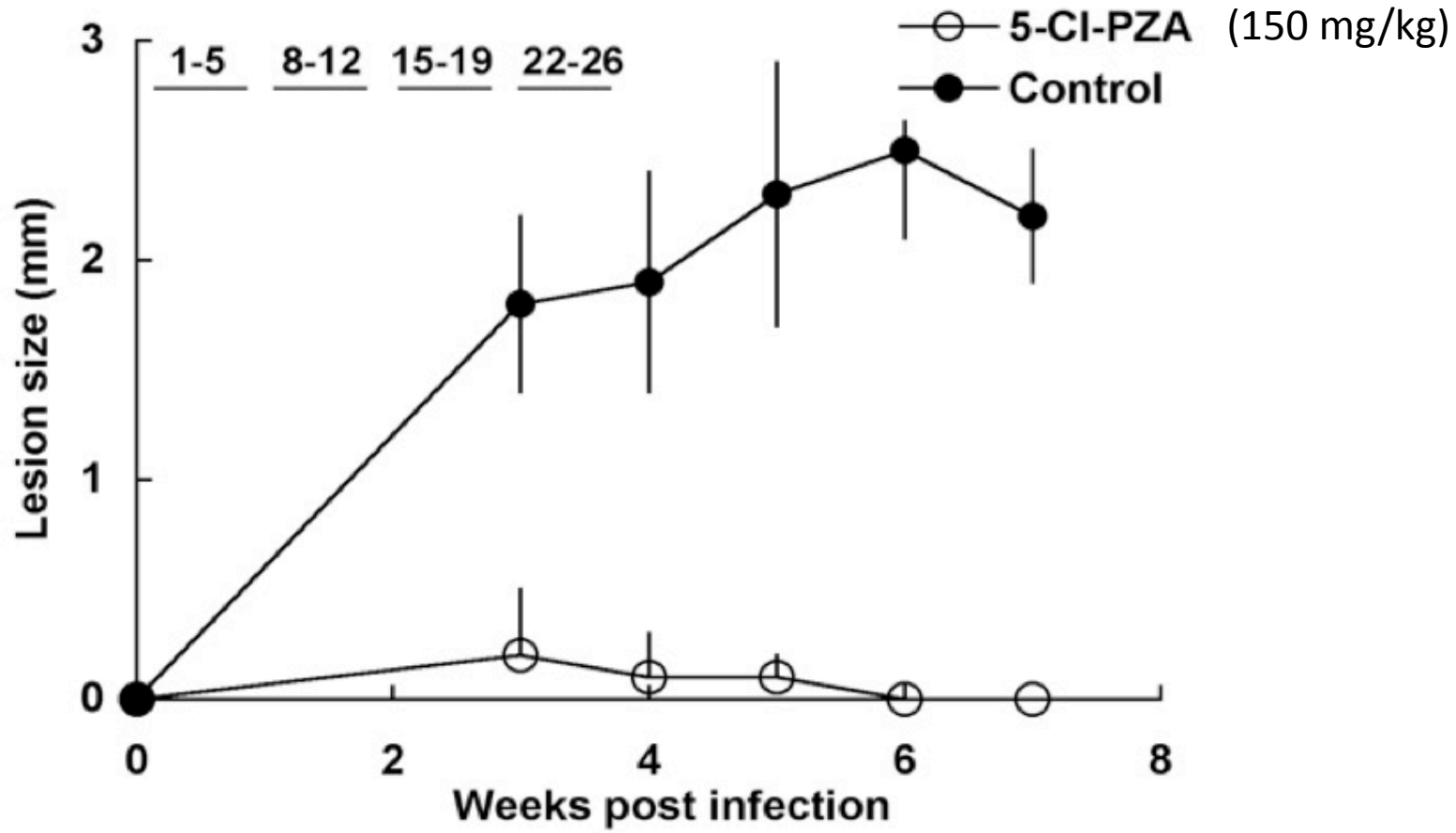
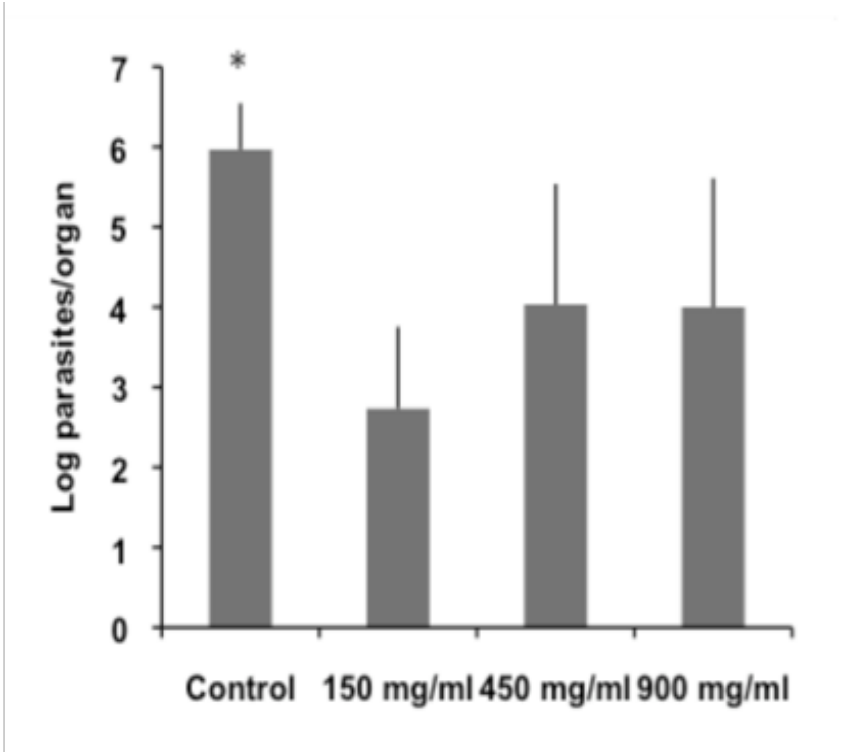
5-Cl PZA treatment also decreases pathology in mice



PZA treatment decreases liver parasite burden in *L. donovani* infected mice

Parasite burdens in livers 3 weeks post infection (IV, 10^5 L. donovani)



Meleana Hinchman

Discrepancy between modest activity in vitro and striking efficacy in vivo

PZA activates bone marrow-derived macrophages and dendritic cells from mice to produce proinflammatory cytokines

Concentration (pg/ml)	Unstimulated	*L. major*	PZA (100 μM)	*L. major*/PZA (100 μM)	LPS/ IFN-γ
Macrophage					
IL-12	56 ± 11	34 ± 6	867 ± 546*	921 ± 445*	3,678 ± 456*
IL-10	15 ± 13	30 ± 22	155 ± 21*	199 ± 120*	321 ± 156*
TNF-α	35 ± 22	104 ± 89	758 ± 246*	921 ± 345*	2,678 ± 625*
Nitric Oxide	30 ± 3	12 ± 21	543 ± 221*	699 ± 112*	1,240 ± 516
Dendritic cell					
IL-12	46 ± 31	114 ± 26	956 ± 145*	1,035 ± 785*	3,365 ± 789*
IL-10	35 ± 33	160 ± 52	185 ± 63*	203 ± 60*	621 ± 102*
TNF-α	102 ± 45	637 ± 67	1,654 ± 546*	1,856 ± 125*	3,456 ± 768*
Nitric Oxide	156 ± 39	243 ± 71	545 ± 221*	699 ± 212*	806 ± 506*

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Summary

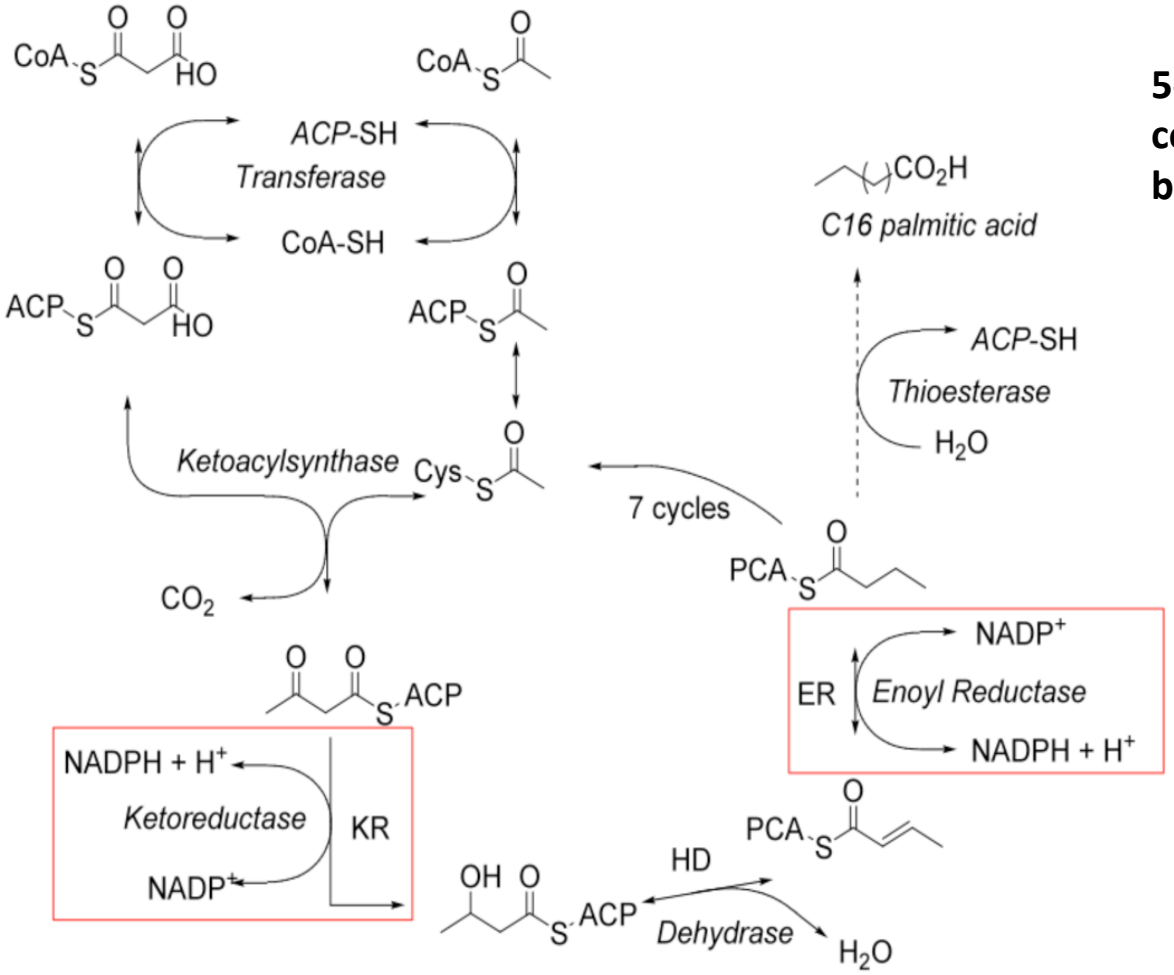
- PZA and analogs have anti-leishmanial activity
- PZA and analogs protect mice against the development of disease and reduce parasite burden
- The apparent discrepancy between in vivo and in vitro results could be explained by the ability of PZA to increase cell activation

PZA works because:

- Is toxic for the parasite (mechanism of action?)

- Has immunostimulatory properties (immunostimulation highly desirable-i.e. AIDS)

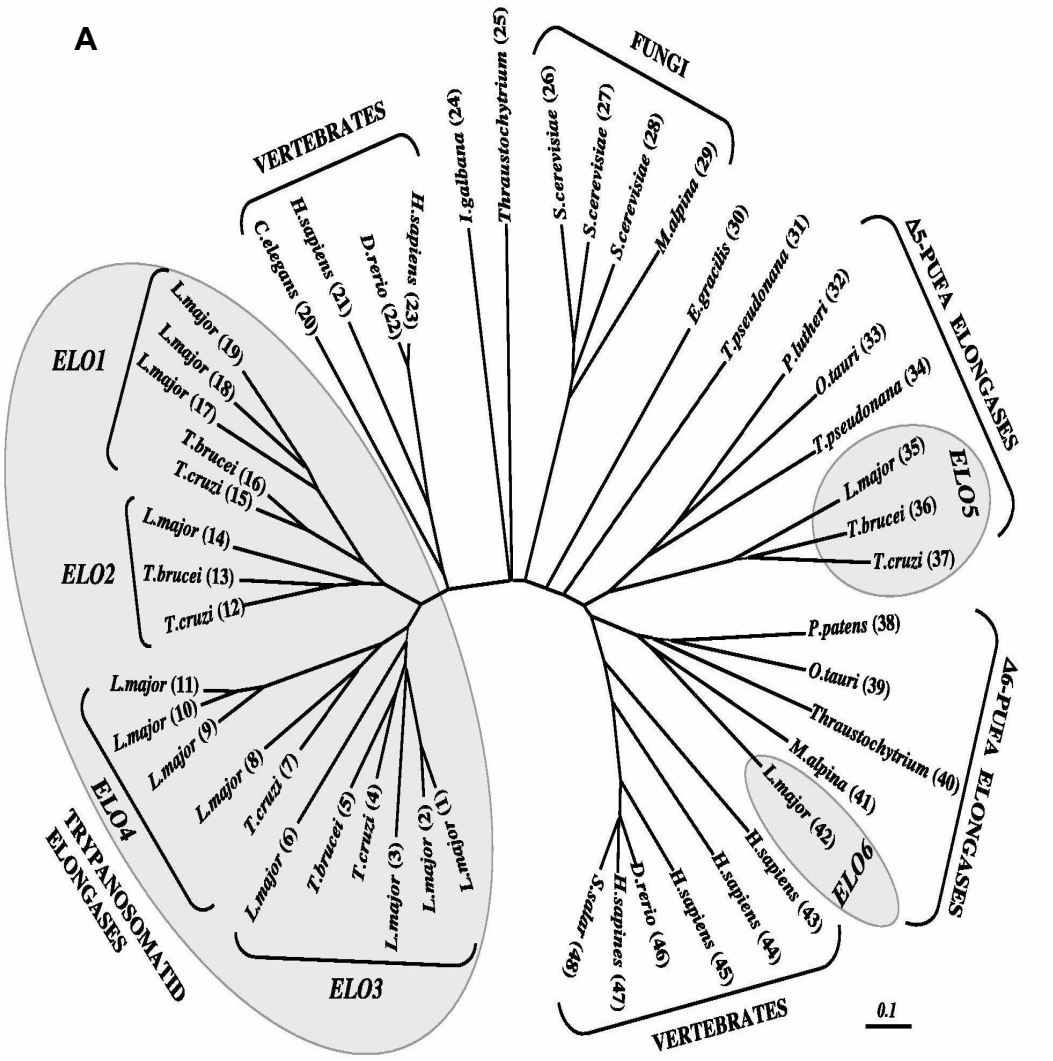
PZA acts on the FASI system in mycobacteria (Welch/Zimhony)



5-Cl PZA and PZA inhibit FAS I by competitive inhibition of binding sites (K_d= 90μM and 250μM)

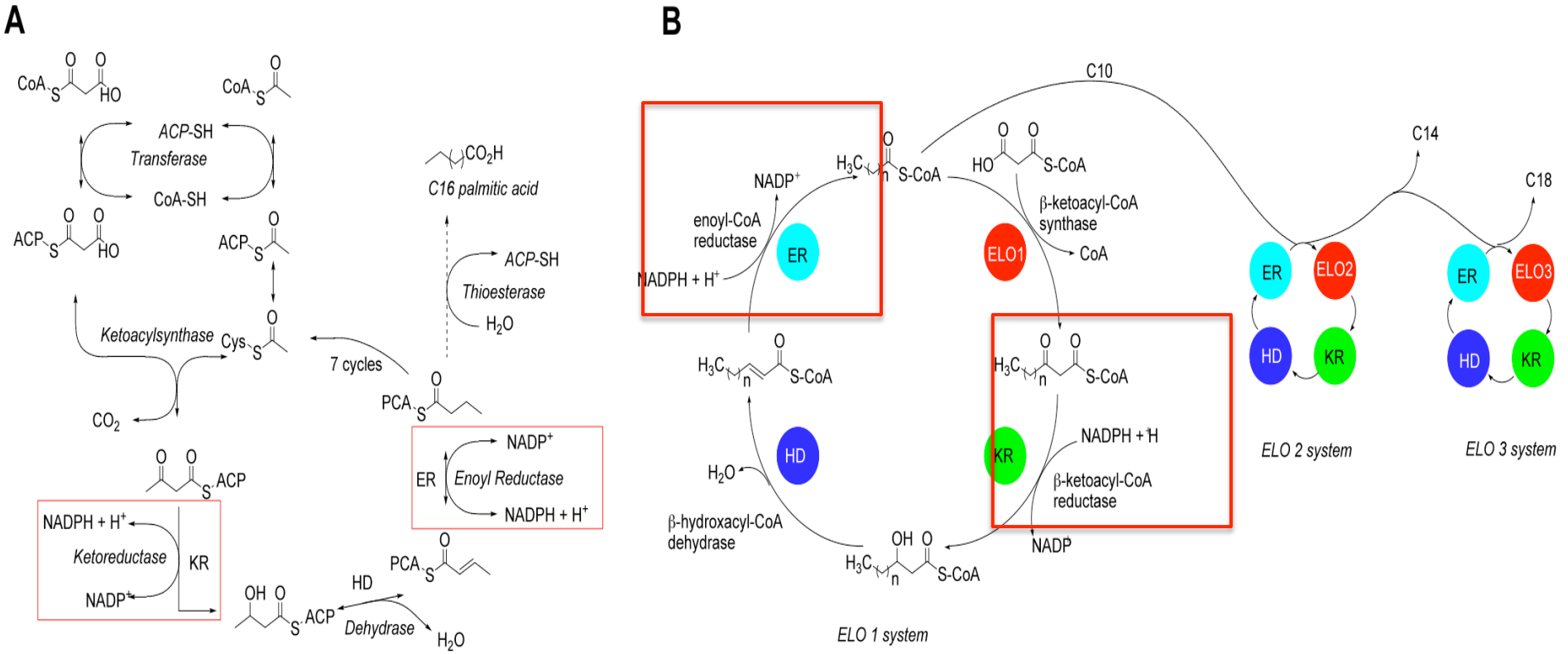
PZA does not have activity against any other bacteria
***Leishmania* is an eukaryotic organism**
***Leishmania* does not have FASI**

Kinetoplastids employ endoplasmic reticulum-based elongases (ELOs) to synthesize long and very long chain fatty acids *de novo*



Livore *et al*., 2007

The chemistry of the ELO system resembles mycobacterial FASI



Our hypothesis: PZAs inhibit enoyl-CoA and B-ketoacyl-CoA reductases in a manner similar to mycobacteria.

Alternative hypothesis (Ouassi's group): **Nicotinamide is an inhibitor of SIR2-like proteins**

Do the PZAs modulate the immune system of the host?

Nicotinamide is able to:

- **prevent immunosuppression** caused by ultraviolet irradiation
- development of **cancer**
- prevent **apoptosis**

Is this via interference with fatty acid (or ELO) metabolism?

Alterations in fatty acid metabolism may:

- change plasma membrane fluidity (phagocytosis and chemotaxis)
- affect the synthesis of modulatory factors (eicosanoids, cytokines) and NO
- influence the function of signaling molecules generated from membrane phospholipids

(*i.e.*, ceramide, that inhibits proinflammatory cytokine production by disrupting signal transduction)

The missing link? How is the mouse responding to the treatment?

Thanks!

To Mendez's lab, collaborators, mentors and AUDIENCE

Questions?