Leishmaniasis in Europe & the Middle East:

Vaccine trials

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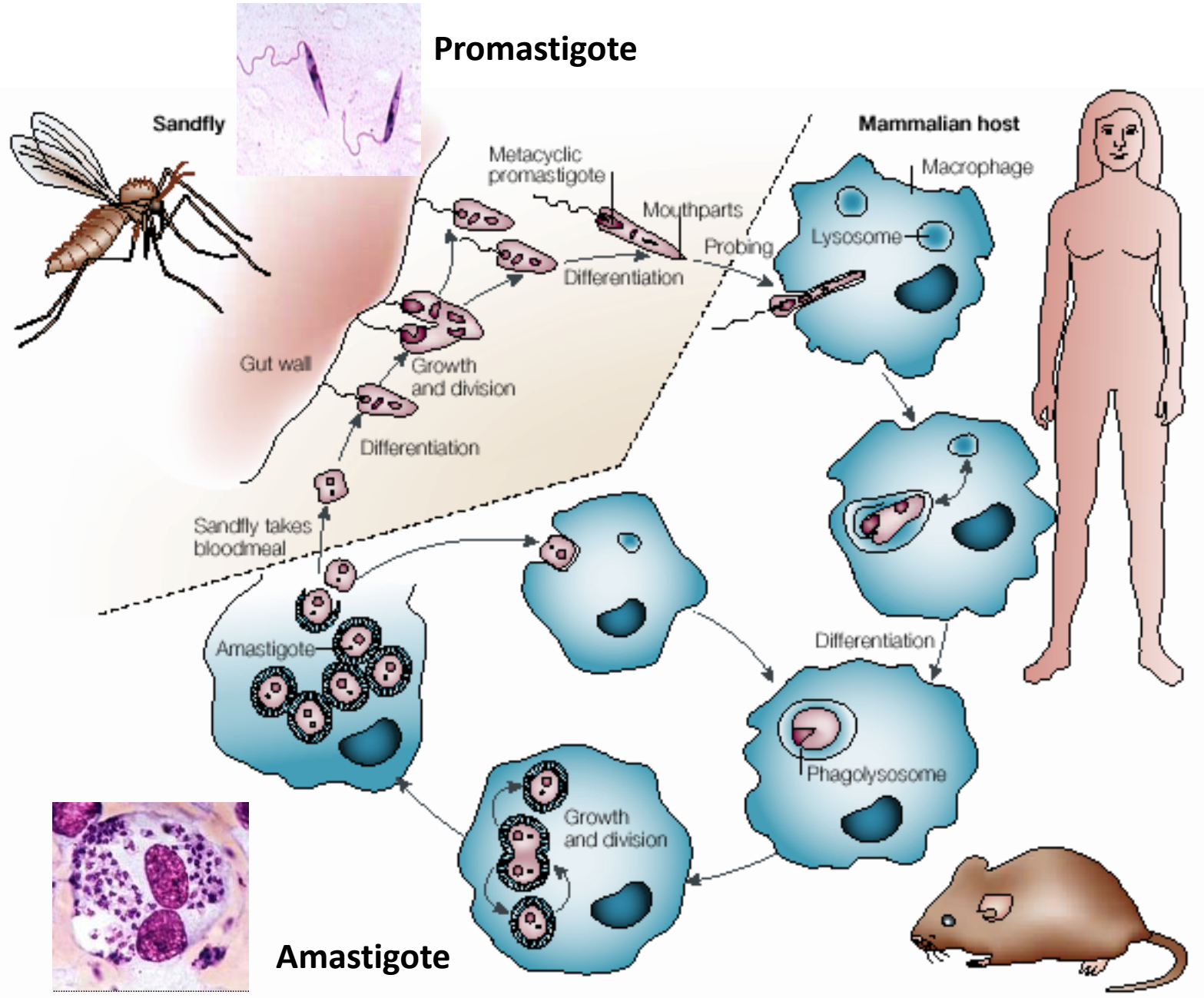
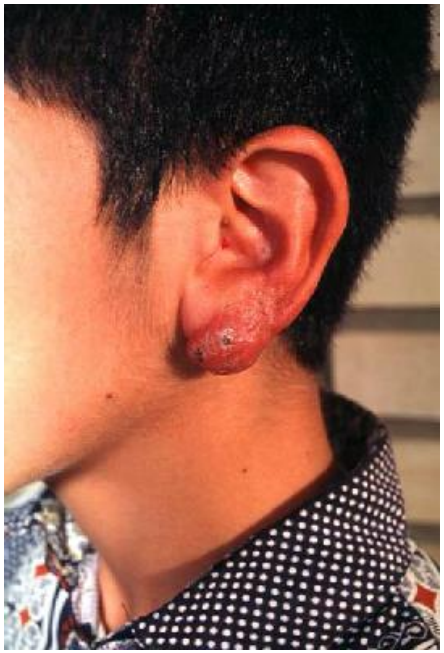
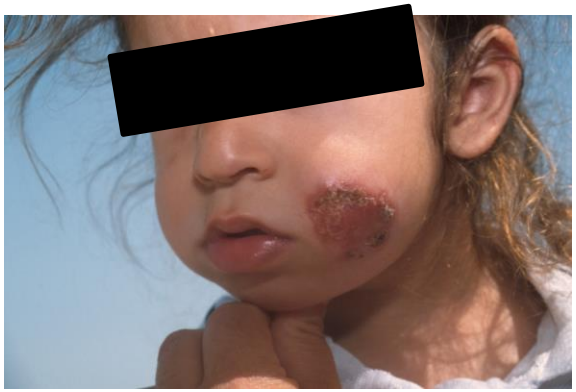
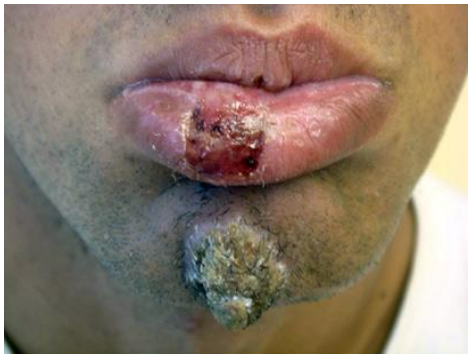

Promastigote
Sandfly
Mammalian host
Metacyclic promastigote
Mouthparts
Probing
Macrophage
Lysosome
Differentiation
Gut wall
Growth and division
Differentiation
Sandfly takes bloodmeal
Amastigote
Differentiation
Phagolysosome
Growth and division
Amastigote

Cutaneous

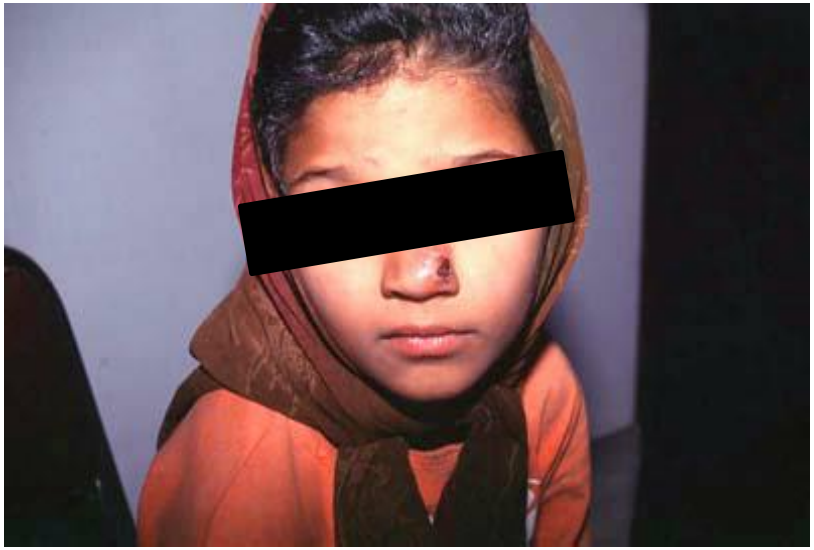




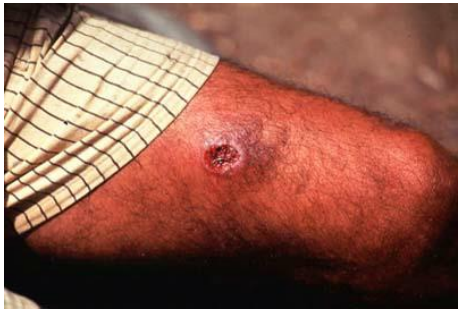
Israel



Iran



Brazil



Reservoirs

L. major is zoonotic

small rodents like -

Psammomys obesus (fat sand rat),
Meriones crassus (gerbil),
Microtus guentheri (vole),
others

L. tropica both zoonotic & anthroponotic

Hyraxes
Rodents (Gundi, rats?)

Visceral leishmaniasis

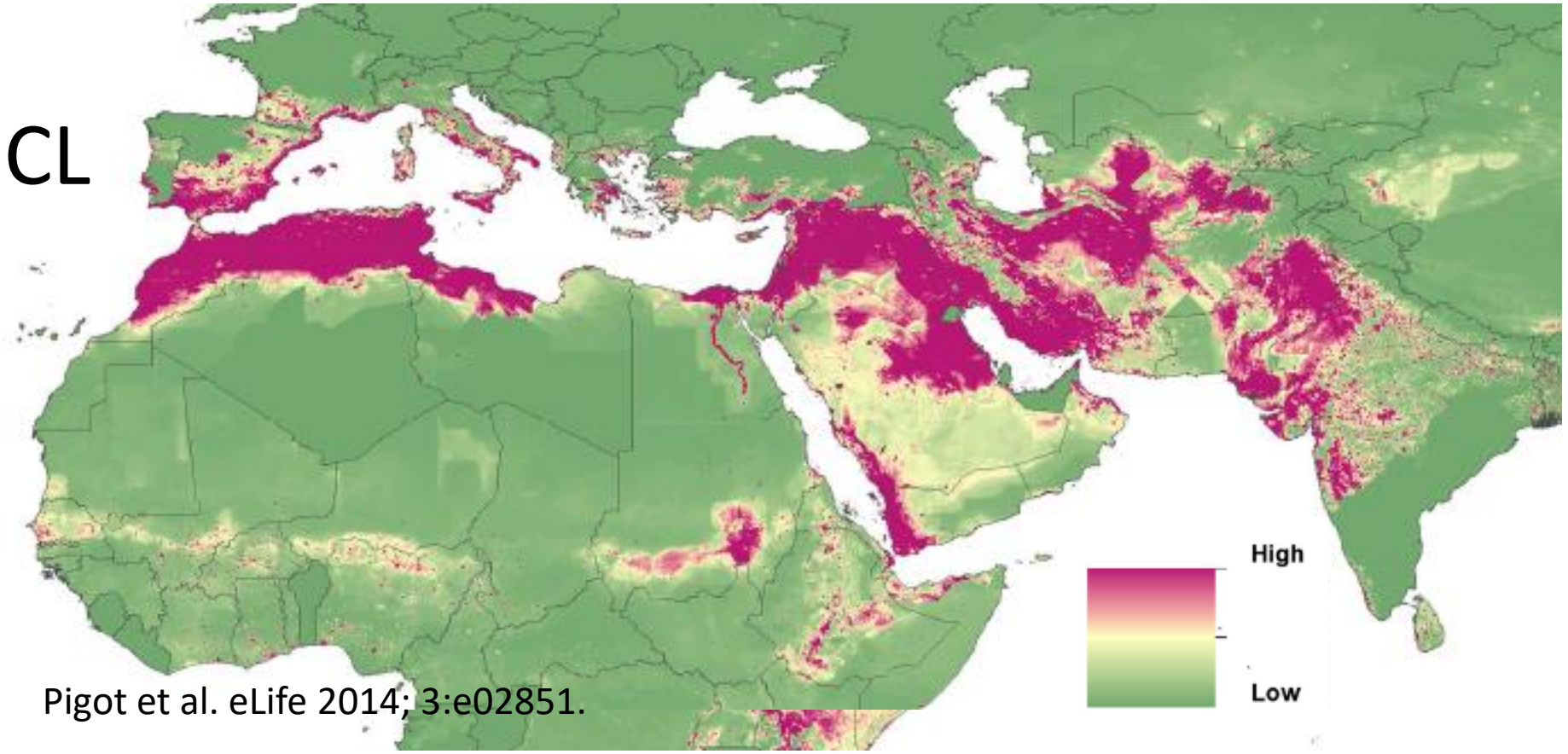
Reservoirs

L. Infantum - zoonotic

Dogs, foxes and jackals

Predicted risk of CL and VL Old World

CL



Pigot et al. eLife 2014; 3:e02851.

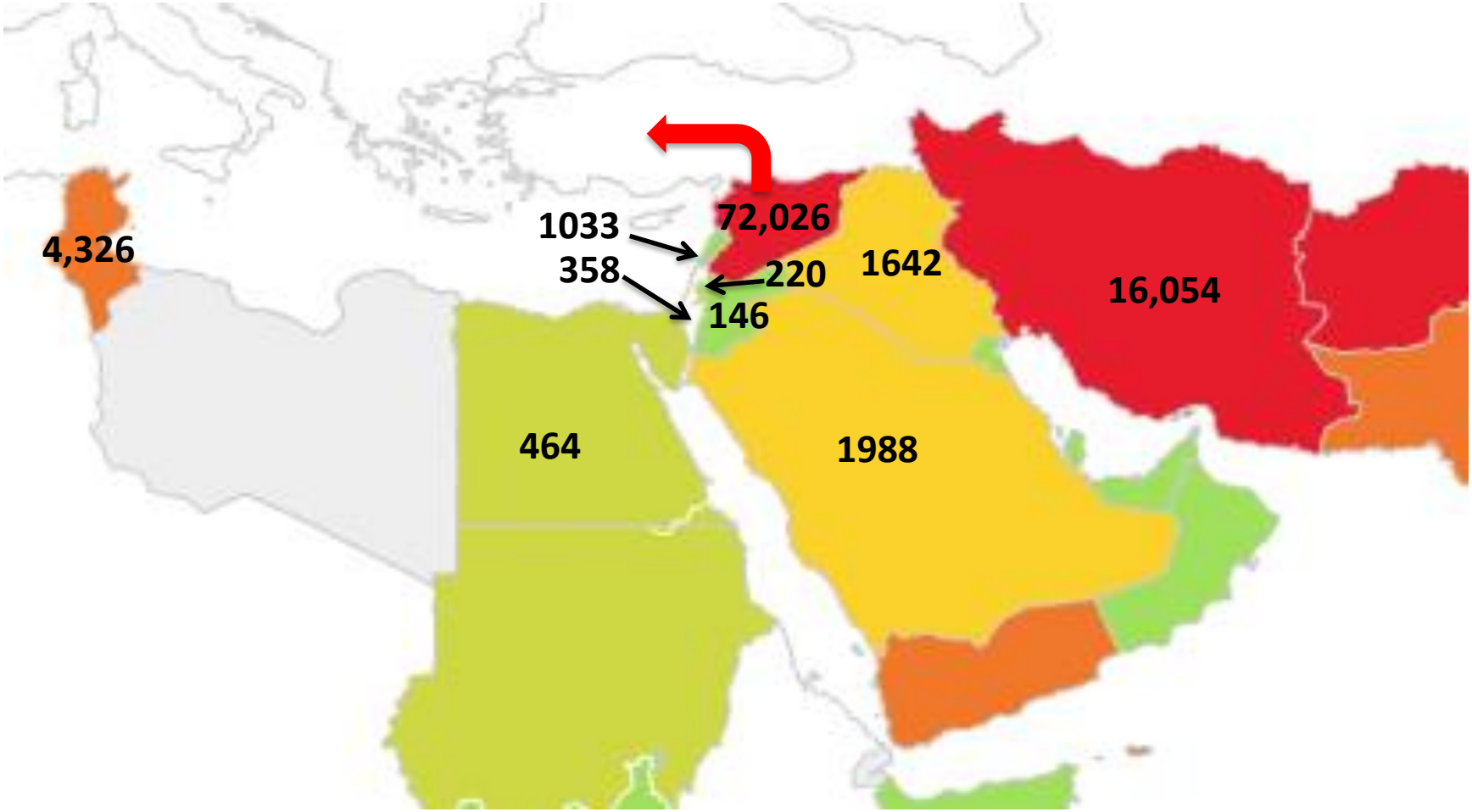
Est. annual incidence Med, ME and Central Asia: VL 6200-12,000; CL 465,000-810,000

Alvar et al 2012. PLoS ONE 7:e35671

Upsetting the balance

- Environmental
  - climate
    - (Killick-Kendrick 1996. Bul Trop Med Int Health 4: 5)
  - reservoir hosts
    - Hares (Molina et al 2012. Vet Parasitol 190: 268)
    - Wild rabbits (Diaz-Saez et al 2014. Vet Parasitol 202: 119)
  - sandfly density
    - Italy (Maroli et al 2008. Trop Med Int Health 13: 256)
- Socio-economic
  - development
  - travel
  - Other diseases
- Political
    - Syria est. 100,000 cases (http://www.uossm.org/index.php/the-outbreak-of-leishmania/)

Endemic Countries for Cutaneous Leishmaniasis - 2012



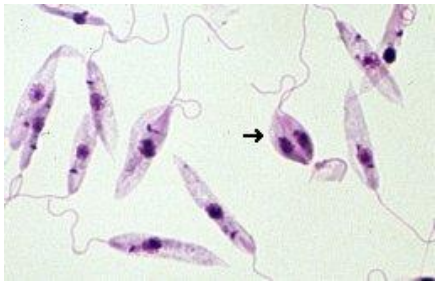
http://gis.emro.who.int/leishmanya/atlas.html;

Why Vaccination?

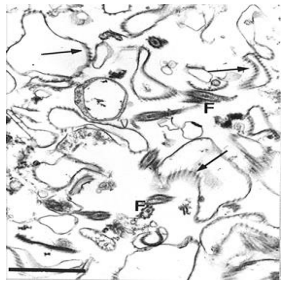
- Control difficult or impossible
  - Vectors, Reservoirs
- Drugs unsatisfactory
  - Toxic
  - Expensive
  - Resistance
  - Don’t work
- “Vaccines are the most effective method of preventing infectious diseases*”

*CDC, AMA, PMA - canada

Types of Vaccines

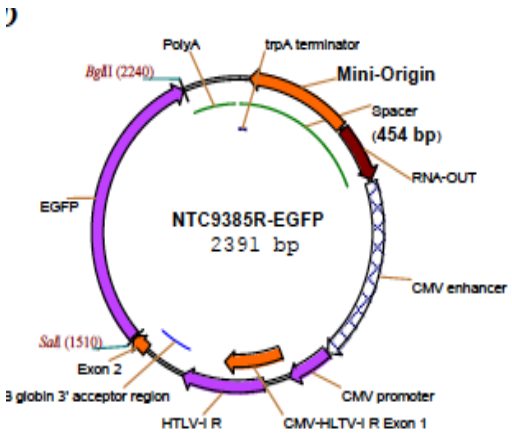


Live; Dead; Attenuated

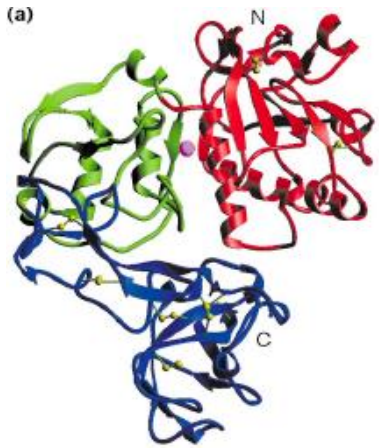


Subcellular; secreted

Leishmaniasis



DNA



Recombinant proteins

Vaccines for human leishmaniasis

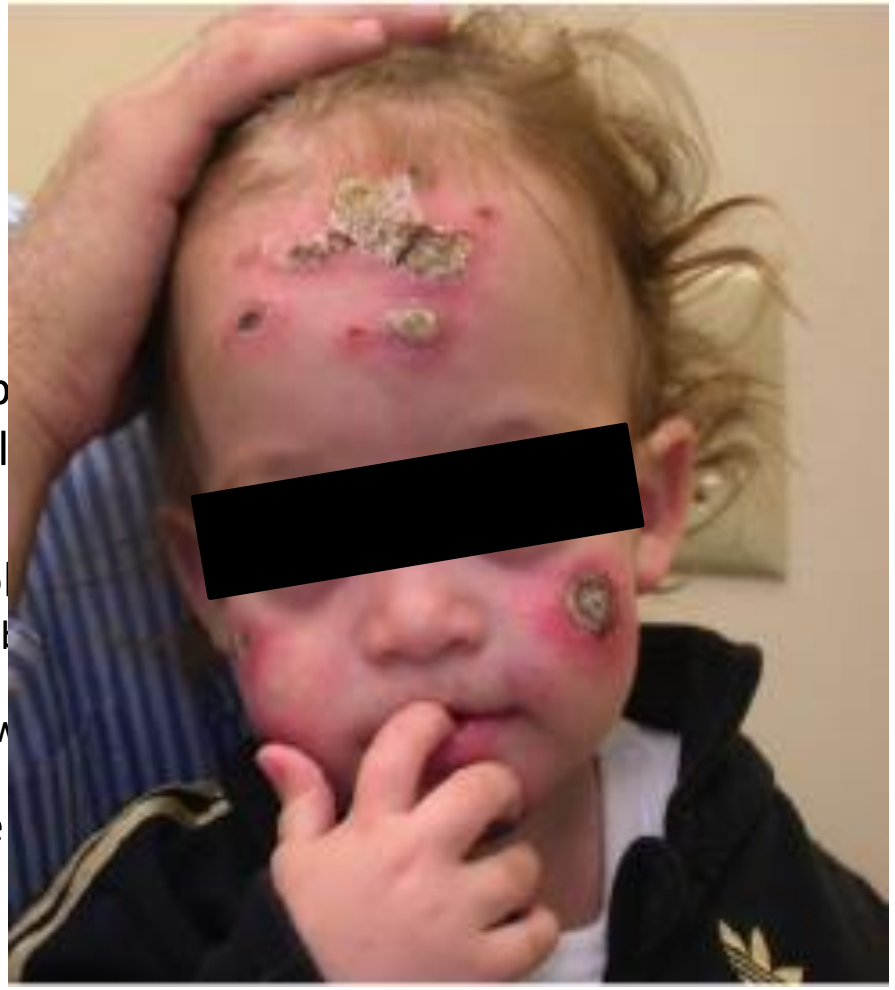
- Prophylactic vaccine
  - Vaccine to prevent disease
  - 1st and 2nd generation
- Therapeutic vaccine
  - Immuno (+chemo) therapy
  - Combine “vaccine antigens” with drugs
  - Cure active disease

Why is a vaccine feasible?

- Protection in animal models
- Humans
  - Strong immunity after recovery
  - Protection against re-infection
  - Many infected people don’t develop disease
- Immunological requirements
- Genome sequenced
- Technology available

Vaccination works!!
At least for cutaneous leishmaniasis

- Leishmanization
  - Prehistory
  - Inoculation with live parasites
    - Russia 1937 - 1970's
    - Israel - 1970's
    - Iran - 1982 -1989 ~ 2 million peop
    - Uzbekistan – licensed mixture of l
  - Protects against new infection
    - Nadim et al (1983) Bull Soc Pathol
      - 250 people, 47% had lesions; incul months
      - Incidence of natural disease afterw naïve controls
    - Khamesipour et al (2005) Vaccine
      - 11/11 protected



Problems

- **Have the disease, lasts ~8-9 months**
- 5-10% develop large sores >2 cm dia
- 3% of lesions last > 1year
- 0.02% of the lesions don't heal
- Up to 25% develop secondary infections
- Hypersensitivity, cheloid formation, psoriasis, immunosuppression re: other vaccines
- Need to make sure isolate is recent and virulent

Killed prophylactic vaccines

- 1940's
  - Failure in Middle East
  - Success in Brazil, 82% protection
- 1990's studies +/- BCG as adjuvant
  - Ecuador - 72% efficacy
  - Iran Overall no protection, but see association between protection & LST conversion
  - Sudan same as Iran

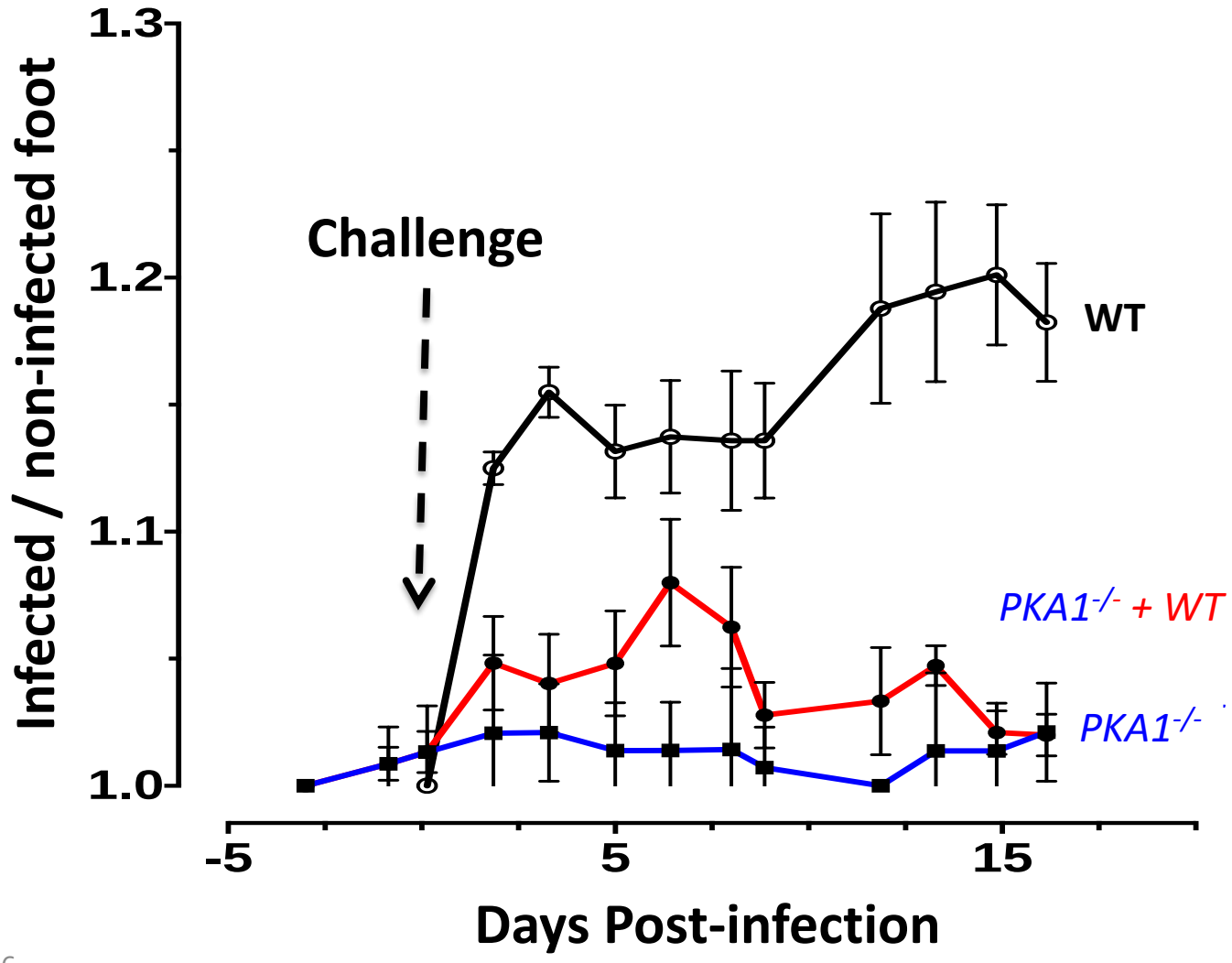
Need better adjuvants, multiple injections

Some success for therapeutic applications

Live Leishmaniasis Vaccines

- Genetically attenuated parasites
  - Protein kinase A (*LmPKA1*$^{-/-}$)
  - Dihydrofolate reductase thymidylate synthase (*dhfr-ts* $^{-/-}$)
  - Lipophosphoglycan (*lpg*$^{-/-}$)
  - Cysteine protease A and B (*cys* A$^{-/-}$ or B$^{-/-}$)
  - A2 gene cluster (A2 $^{-/-}$)
  - Amastigote specific Centrin gene (*LdCen*$^{-/-}$)
  - Amastigote specific cytochrome c oxidase (*Ldp27*$^{-/-}$)
  - *L. tarantolae*

L. major pka1$^{-/-}$ null mutants are not virulent in BALB/c mice



Requirements for live vaccine

- Safe - Normal & Immune compromised patients
  - No disease or minimal side effects
- Stable, defined alteration and no revertants
- Not transmitted by sandfly
- Complete and long-term protection
  - No boosting needed
- No drop potency or efficacy with time or lots
- Problem GMP production
  - No human or animal by products

Live Leishmaniasis Vaccines

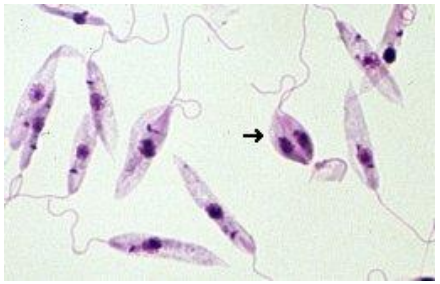
- Hira Nakhasi, FDA
  - Amastigote specific Centrin gene (*LdCen*$^{-/-}$)
  - Amastigote specific cytochrome c oxidase (*Ldp27*$^{-/-}$)
  - *L. donovani* mutants show cross species protection
    - Dey et al J Immunol Aug 25
  - LdCen$^{-/-}$ parasites trigger stronger immune response than commercial CanL vaccine Leishmune®
    - >antibodies; >IFN-gamma; >$CD8^{+}$ T-cell activation & <IL-4
    - Fiuza et al (2013) Vaccine 31:1785

What have we learned from preclinical and human studies?

So what's stopping us

- Leishmaniasis protective response mediated via
  - Th1 T-cell response
  - Cytotoxic $CD8^+$ T-cells
- Almost all licensed vaccines to date mediate protection via
  - Antibodies i.e., B-cell based

Types of Vaccines



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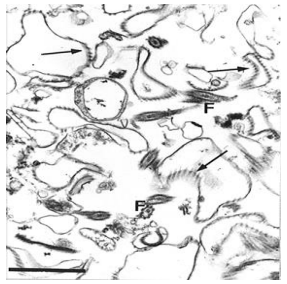
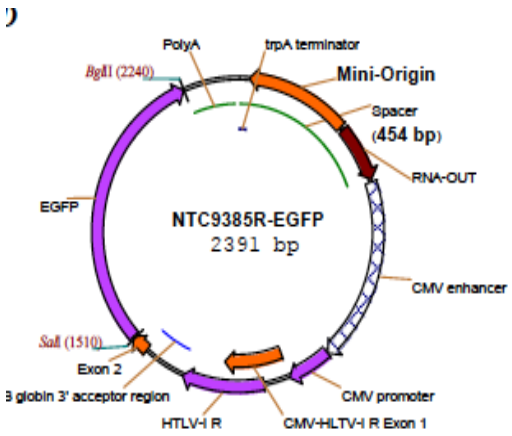
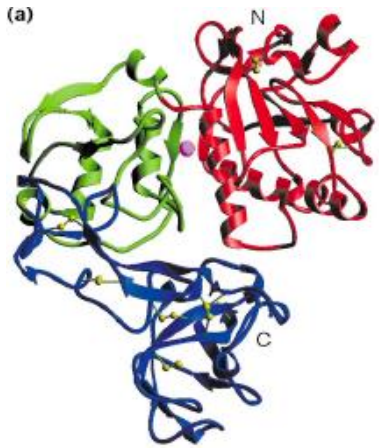


Subcellular; secreted

Leishmaniasis



DNA

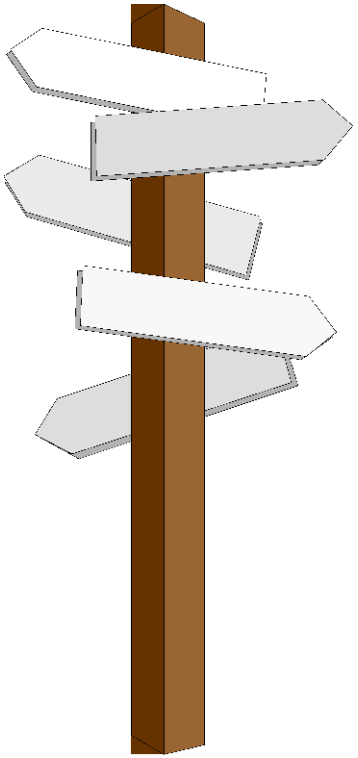


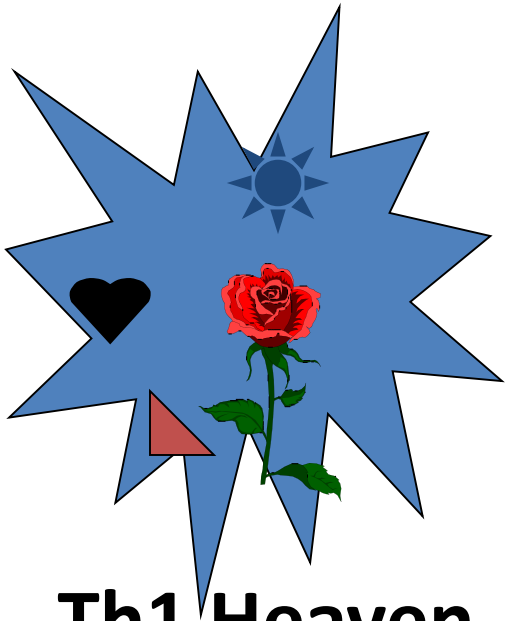
Recombinant proteins

Second Generation

- Defined antigenic molecules

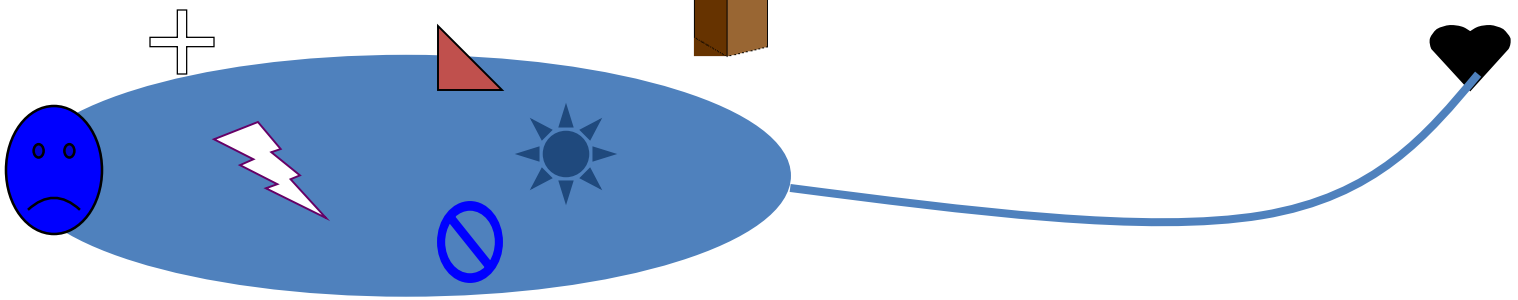
Which antigen & how to get the right response?





Th2 Trash

Th1 Heaven



Defined Vaccine Antigens

- **Leishmanolysin (gp63)**
- **PAS-2 (Promastigote Surface Antigen 2 Complex)**
- **Dp72**
- **LACK (Leishmanial Receptor for Activated C Kinase)**
- **LeIF (Leishmanial Eukaryotic Ribosomal Protein)**
- **Lcr1(Leishmanial Flagellar Antigen)**
- **Ld23**
- **KMP-11 (Lipophosphoglycan associated protein)**
- **A2 (Amastigote stage specific protein family)**
- **Many Others**

Vaccines for human leishmaniasis

- Prophylactic vaccine
  - Vaccine to prevent disease
- Therapeutic vaccine
  - Vaccine to treat active disease
  - Historic use
    - Convit Venezuela autoclaved *L. mexicana* + BCG
    - Mayrink Brazil ; killed *L. amazonensis* ± chemo

Second Generation Vaccines

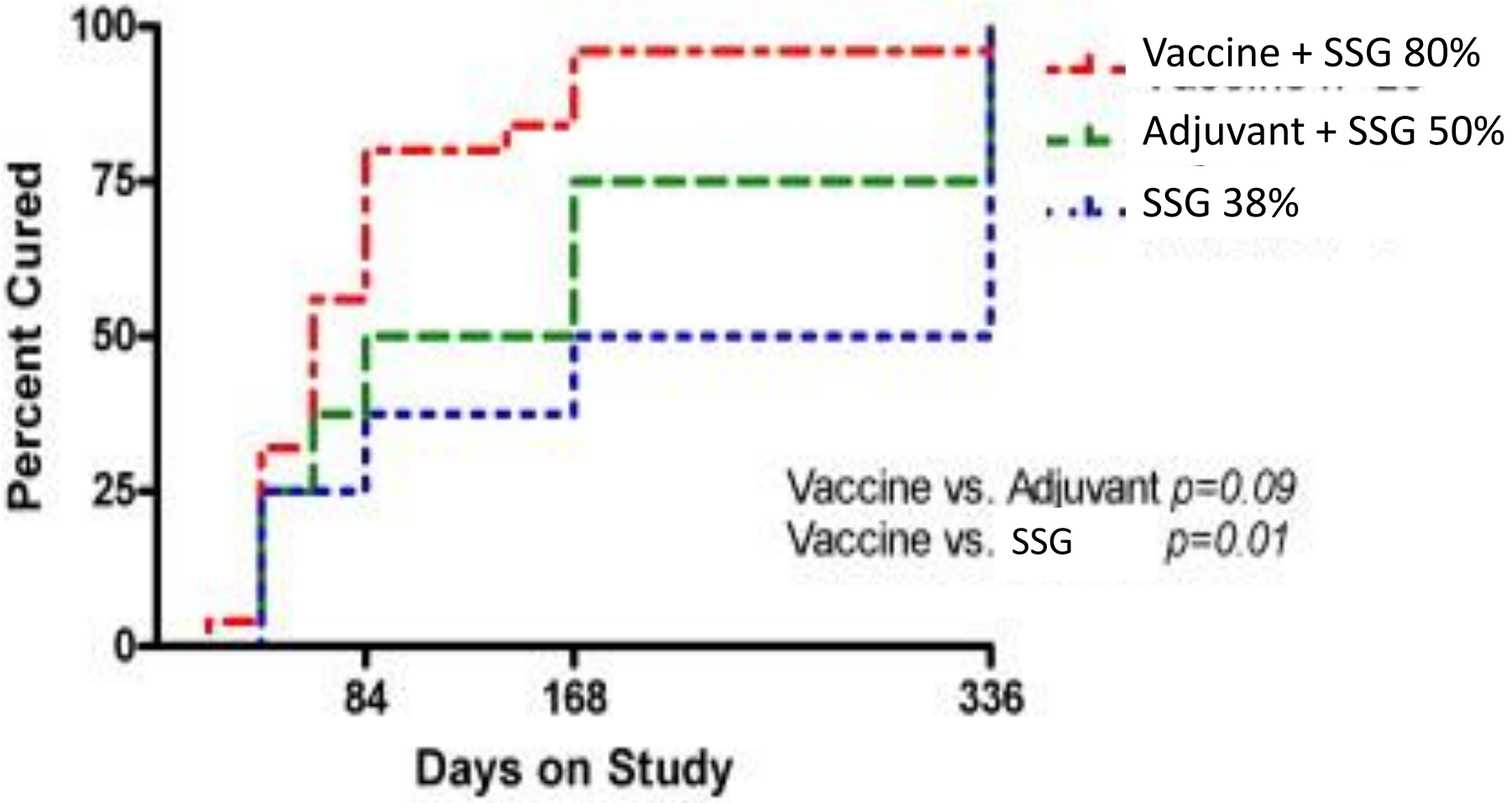
- IDRI - recombinant fusion polypeptide
  - LeishF1/F2 (Leish-111f or Leish-110f) + MPL-SE adjuvant
    - Preclinical protection, and Phase I/II completed
  - LeishF3 + MPL-SE or GLA-SE adjuvant for VL
    - Preclinical and phase I in India and USA (2012)
- York - Adenovirus vector
  - 2 *Leishmania* genes
  - Preclinical and Phase I completed
- Mologen - DNA vaccine
  - LeishDNAVac - 5 plasmids
  - Preclinical, no adjuvant, Ready for Phase I

IDRI - LeishF1/F2 + MPL-SE

- Protection
  - Preclinical
    - Prophylactic CL in mice & monkeys; VL in mice, hamsters
    - Therapeutic VL in dogs
  - Multifunctional $CD4^+$ Th1-cell responses; IgG, IFN-γ , TNF and IL-2; T-cell epitopes in TSA and LmSTI1
  - Phase I
    - Prophylactic - CL & VL (Velez et al 2009. Vaccine 28: 329 & Chakravarty et al. 2011. Vaccine 29: 3531)
    - Therapeutic – CL & MCL (Nascimento et al 2010. Vaccine 28: 6581 & Llanos-Cuentas et al 2010. Vaccine 28: 7427)
    - Safe, immunogenic (IgG, IFN-γ & DTH) and well tolerated
  - Didn't protect dogs against VL (Gradoni et al 2005. Vaccine 23: 5245)

Therapeutic CL Trial - Brazil

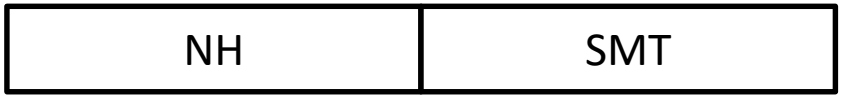
LeishF1 + suboptimal chemotherapy (SSG)



Nascimento et al 2010. Vaccine 28: 6581

Improving the IDRI vaccine for VL

Leish-F3



- Conserved in *Leishmania* species causing CL and VL
  - AA identity across species
    - nucleoside hydrolase (NH36) 84 – 99.7%;
    - sterol 24-c-methyltransferase (SMT) 86 – 99.7%
- Single antigens protect in mouse or hamster models
  - Polyfunctional $CD4^+$ T-cells secrete IFN-γ, TNF, IL-2)
- Recognized by Human T-cells, *Ex vivo* responses
  - Asymptomatic $CD4^+$ T-cells secrete IFN-γ, TNF, IL-2
- Phase I trial – safe and immunogenic (Coler et al Clin Trans Immunol 2014. 4: e35.)

ChAd63-KH: a third generation therapeutic vaccine for VL/PKDL

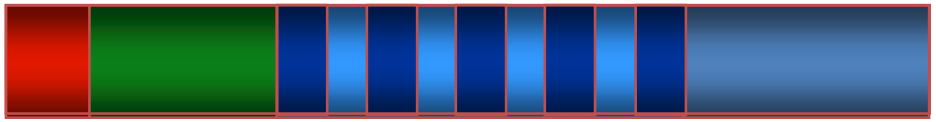
General properties

KMP-11

1. Highly conserved
2. CD8 T cell recognition across multiple HLA class I alleles in target population.

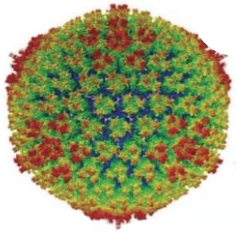
2A

synthetic HASPB



1. Conserved N and C termini contain human $CD4^+$ and $CD8^+$ T cell epitopes
2. Designed to cover spectrum and preserve native arrangements of repeats found within Indian / East African isolates

Rich in $CD4^+$ and $CD8^+$ T cell epitopes
No significant homologies to human proteins
No known biological functions leading to potential adverse events



Chimpanzee adenoviral-based vaccines

- have been in clinical trials in over 1000 volunteers in seven countries
- have an excellent safety profile alone and in prime-boost combination
- induce highly potent immune responses ($CD8^+$, $CD4^+$ and Ab) in man

ChAd63

- produced in suspension culture Procell 92 cell line for scalable manufacture
- Safety and immunogenicity data available from other humans vaccine trials

UNIVERSITY of York

ROBERT KOCH INSTITUT

okairos
from genes to vaccines

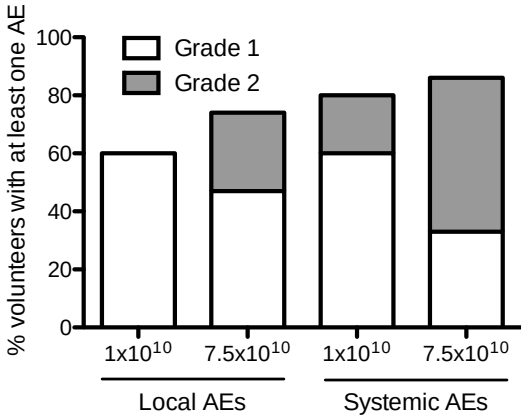
wellcome

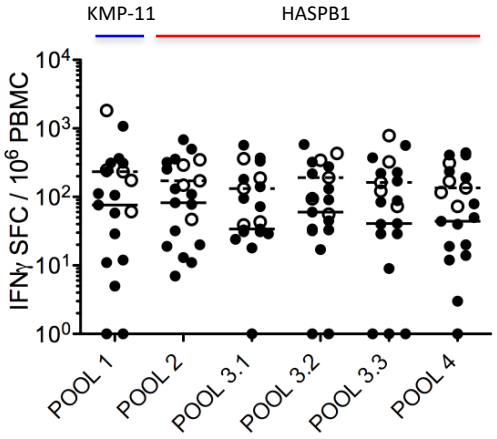
Adenovirus vectors

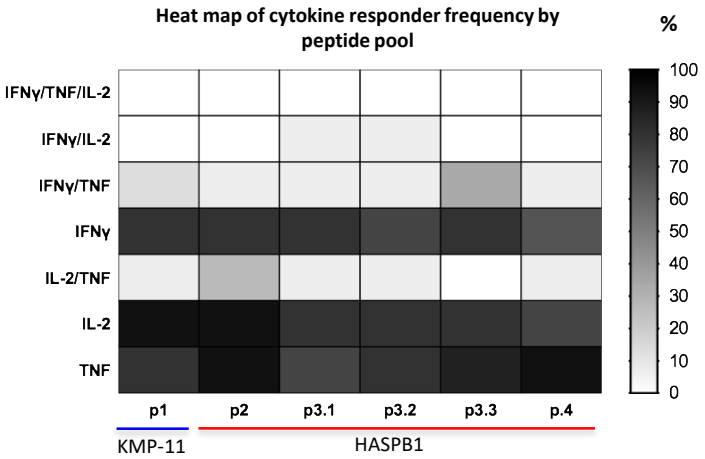
- Advantages
  - Elicit > cell mediated immunity, cytotoxic T-cells
  - Infection of APC such as dendritic cells
  - Induce circulating and mucosal immunity
  - Ease of manufacture
  - Doesn't integrate into host DNA
  - Large number of human vaccines in clinical trials or development (safety and immunogenicity data)
- Disadvantages
  - Pre-existing immunity to wild type virus; 40-60% people in USA have Abs
  - Can't give two injections (get anti-Ad responses)
  - Limited number of genes can be incorporated
  - Dose related toxicity

ChAd63-KH: clinical development

LEISH1: *dose escalation prime only Phase 1 first-in-human, n=20 UK healthy volunteers*







Safety: *on par with other viral vaccines in development; average 2-3 Grade 1 or 2 AEs per subject; no grade 3/4 AEs.*

CD8 IFNγ ELISpot: *overall response rate 85%; 40% with peak summed responses >1200 spots / 10^6 PBMC*

CD8 ICS: *overall response rate 100% for 1 or more cytokines in at least 1 pool; response dominated by single cytokine producing cells*

Osman et al, submitted

LEISH2a: *dose escalation prime only Phase IIa safety study; n=24 Sudanese PKDL patients*
Trial start date: Nov. 2016

LEISH2b: *prime only placebo controlled Phase IIb RCT; n=90 Sudanese PKDL patients*
Trial start date: late 2017 TBC

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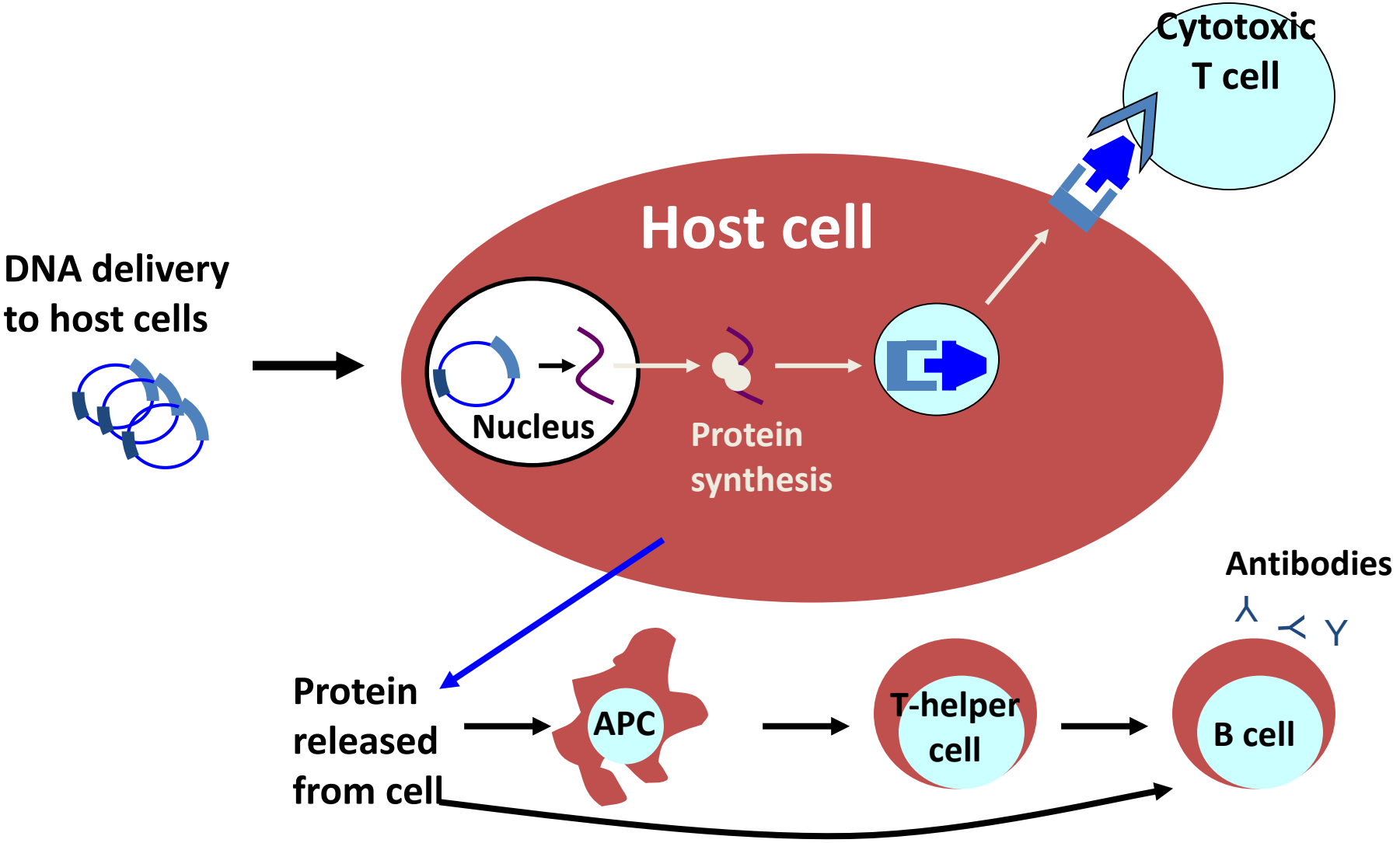
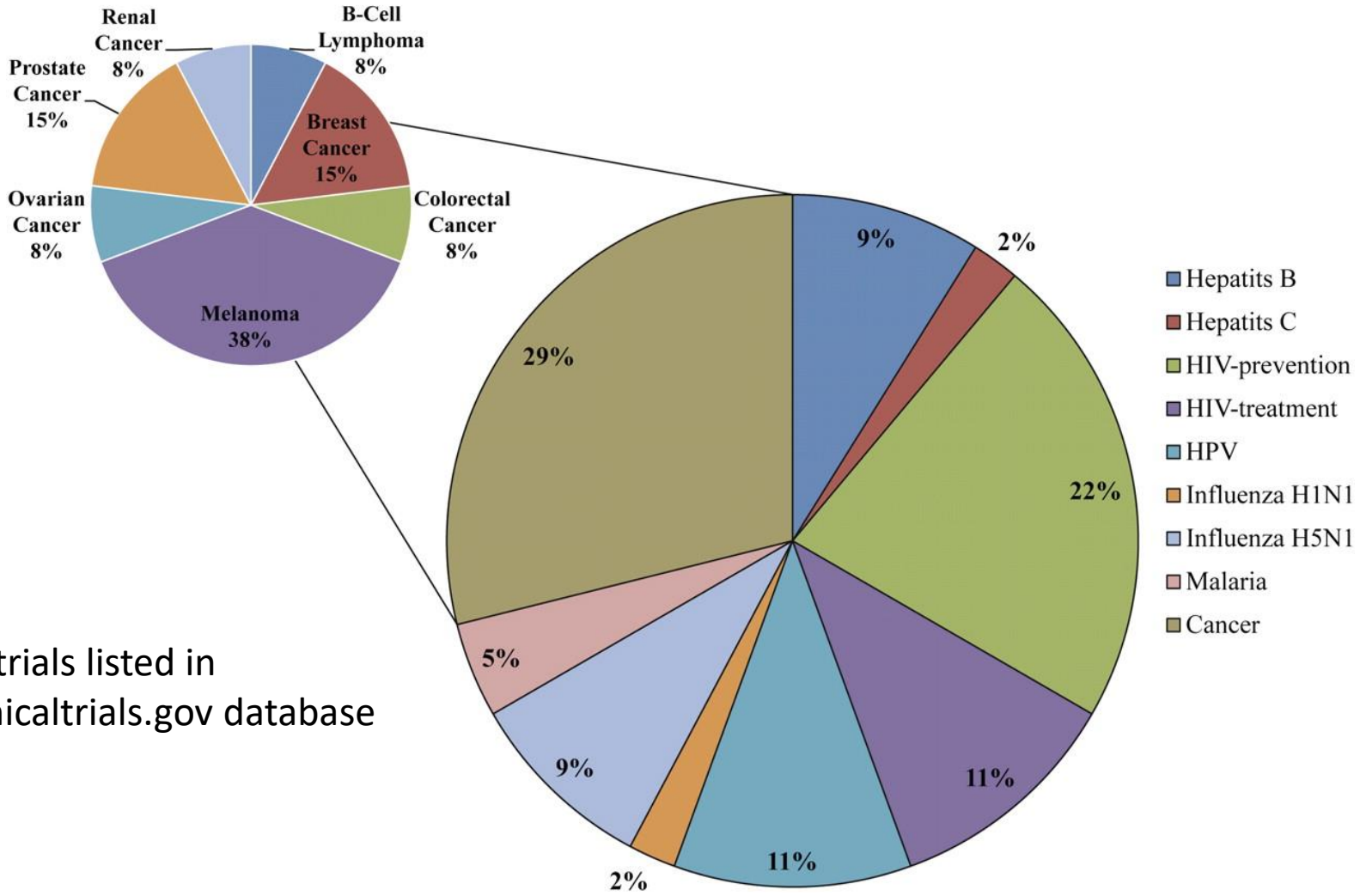
DNA Vaccination



Adapted from Waine & McManus Parsitol. Today (1995) 11:113.

Current DNA Vaccine Clinical Trials



43 trials listed in Clinicaltrials.gov database

Ferraro B et al. Clin Infect Dis. 2011;53:296-302

DNA vs Protein

- Advantages
  - Stable, inexpensive & easily produced
  - Expression in native form & long lasting
  - CD4+ and CD8+ T-cell responses
  - Humoral immunity
- Potential disadvantages
  - Integration, little or no evidence
  - Autoimmunity, little or no evidence
  - Induction or breaking of tolerance to host proteins
  - Need to improve responses in humans - delivery techniques and adjuvant inclusion
  - Antibiotic resistance on plasmid

LEISHDNAVAX Vaccine

Science Translational Medicine
AAAS

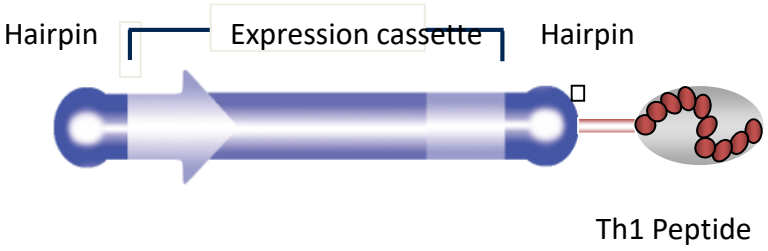
2014. 6: 234ra56

RESEARCH ARTICLE

LEISHMANIA

Modular Multiantigen T Cell Epitope–Enriched DNA Vaccine Against Human Leishmaniasis

Shantanabha Das,[1]* Anja Freier,[2]* Thouraya Boussoffara,[3]* Sushmita Das,[4]* Detlef Oswald,[5] Florian O. Losch,[2] Melanie Selka,[2] Nina Sacerdoti-Sierra,[6] Gabriele Schönian,[2] Karl-Heinz Wiesmüller,[7] Karin Seifert,[8] Matthias Schroff,[5] Christiane Juhls,[5†] Charles L. Jaffe,[6†] Syamal Roy,[1†] Pradeep Das,[4†] Hechmi Louzir,[3†] Simon L. Croft,[8†] Farrokh Modabber,[9†] Peter Walden[2†‡]



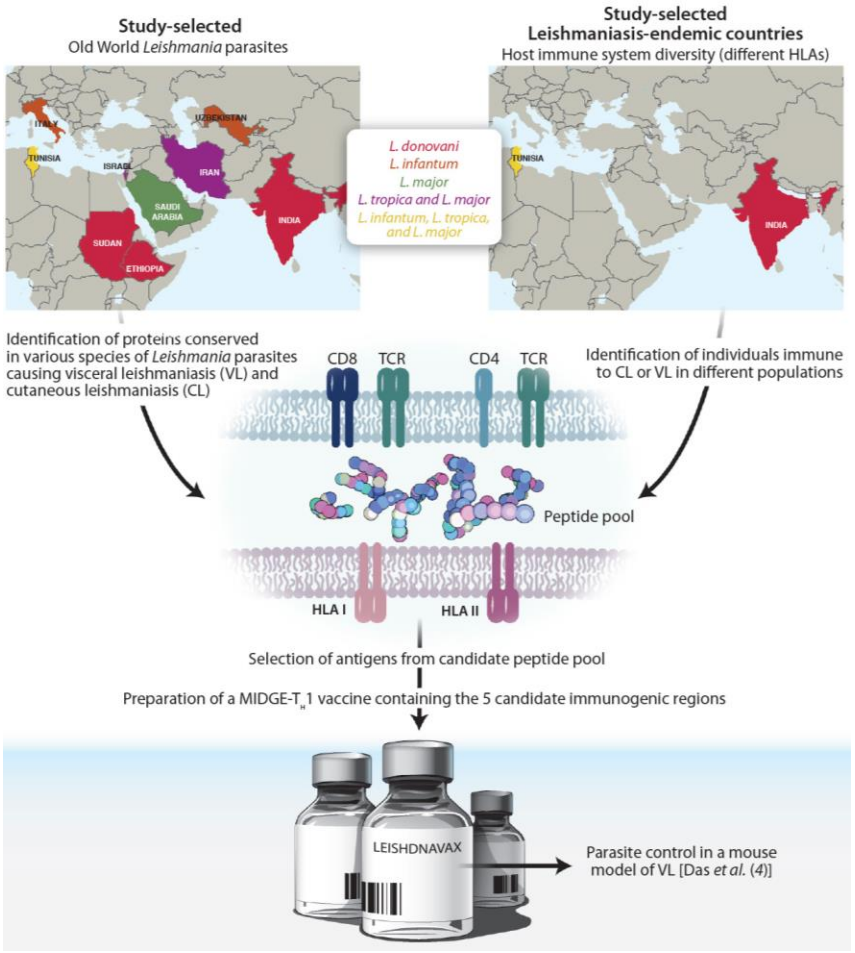
MIDGE – Th1 vector

No antibiotic resistance genes

Minimum bacterial backbone

LEISHDNAVAX Vaccine

- Selection criteria
- KMP11, TSA, P74, CPA & tCPB
  - Conserved between different *Leishmania* species
  - "Antigenic complexity" i.e., different HLAs
  - Induce antigen specific T cell responses
  - Immunogenic in humans
  - CD4 and CD8 T cell responses, IFN-γ detected in cured VL and CL patients
  - Safe in animals



Mologen – LEISHDNAVAX

- Prophylactic protection in
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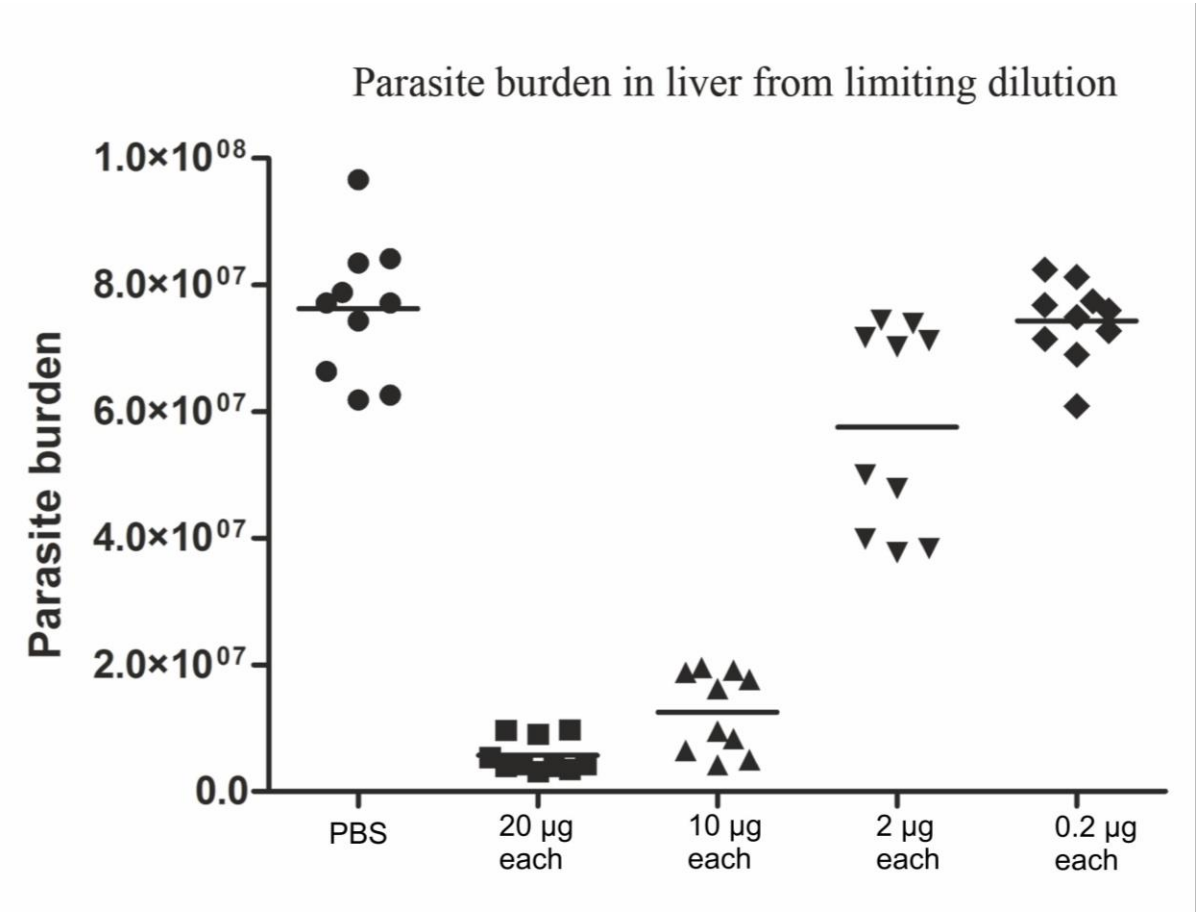

Vaccines for Canine leishmaniasis

- Leishmune® (Fernades et al 2014 Vaccine 32: 128)
  - Licensed Brazil: fucose-mannose ligand glycoprotein (FML) + saponin-based adjuvant
  - >87.8% protection, adverse effects 2.2%, xenodiagnosis 5.1% +
- Leish-Tec® (Fernades et al 2014 Vaccine 32: 128)
  - Licensed Brazil: recombinant amastigote stage protein A2 + saponin-based adjuvant
  - >81.1% protection, AE - 13.0%, xenodiagnosis 5.4% +
- CaniLeish® (Oliva et al 2014 PLoS NTD 8:e3213
  - Licensed in Europe: excreted-secreted proteins + saponin-based adjuvant
  - 92.7% protection, xenodiagnosis reduced transmission, minor AE - 20%
- Letifend®
  - Licensed in Europe: protein Q recombinant, no adjuvant
  - 58% protection, xenodiagnosis – not done, minor AE – 10%

Problems

- Host responses vary (HLA-I and -II)
  - Variation immunogenicity
- Different requirements, different species
  - Gene variation & polymorphism in parasites
- Not understood what is needed for protective response in humans
- What to measure, no easy assays to monitor correlates of protection
- Potential drive selective pressure away from vaccine molecules
- Protection in animal models -

Final steps

- Phase I - Is it safe, side-effects, immunogenicity
  - Completed - LeishF1/F2, ChAd63-KH; In progress – LeishF3
- Phase II - expanded safety + immunogenicity
  - ChAd63-KH
- Phase III - double-blind study of efficacy
- **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

“little evidence that any positive results in animals correlates with efficacy in human beings”

Moorthy and Kieny (2010) Lancet Inf. Dis 10:204

Initiative for Vaccine Research, WHO