Leishmaniasis: Immunology and vaccination

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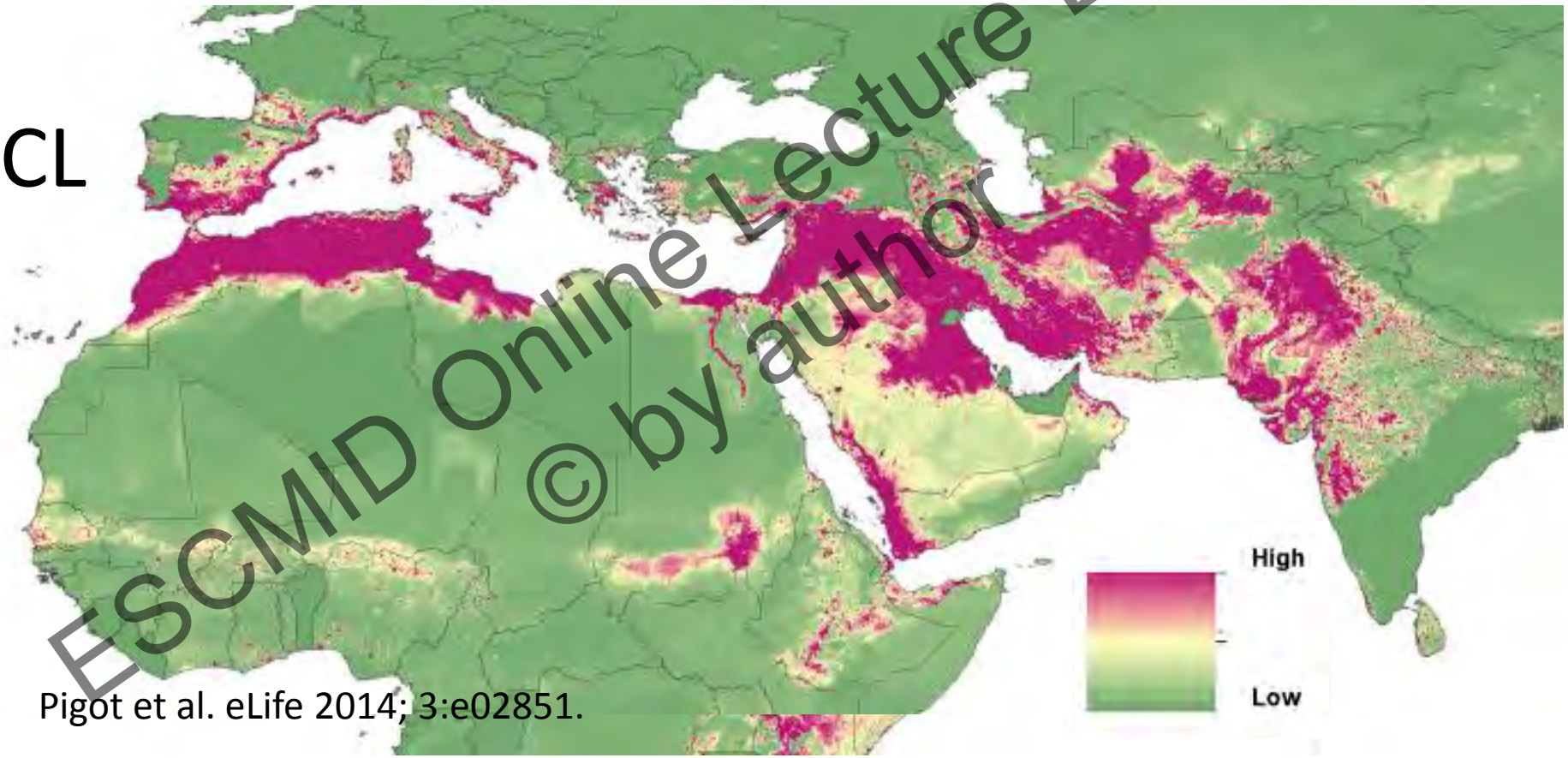
Predicted risk of CL and VL Old World

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

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*"

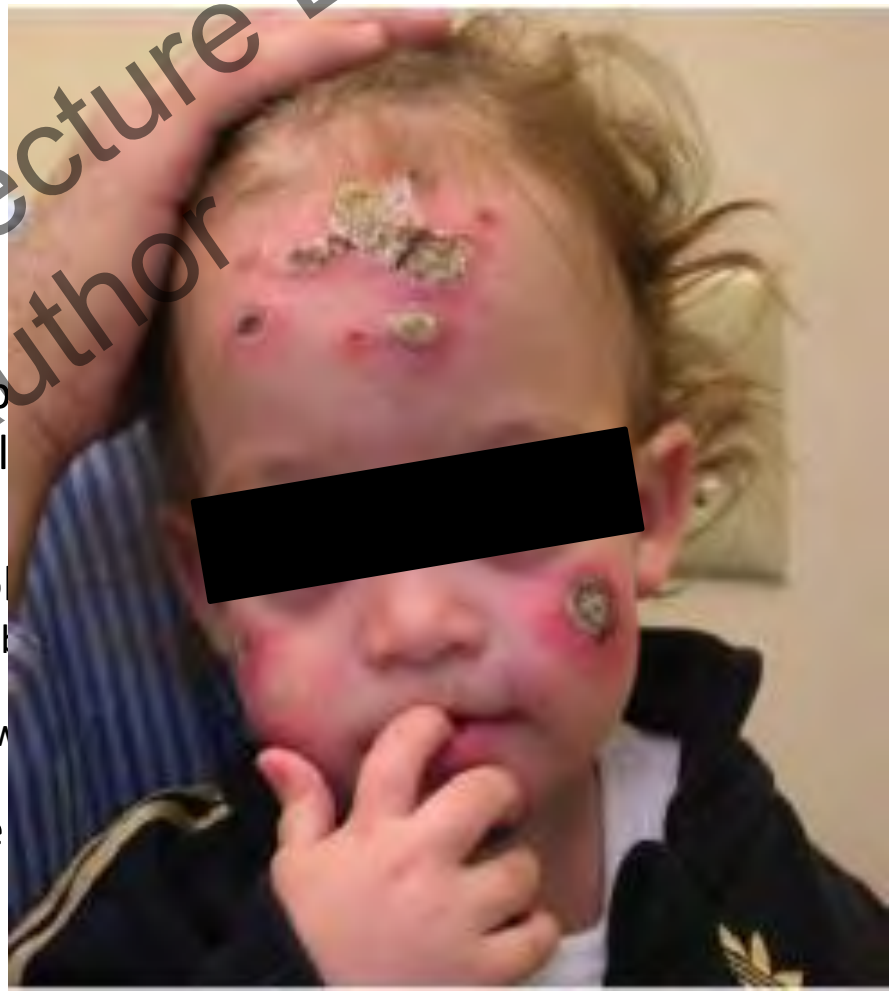
*CDC, AMA, PMA - canada

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

Ideal Leishmaniasis Vaccine

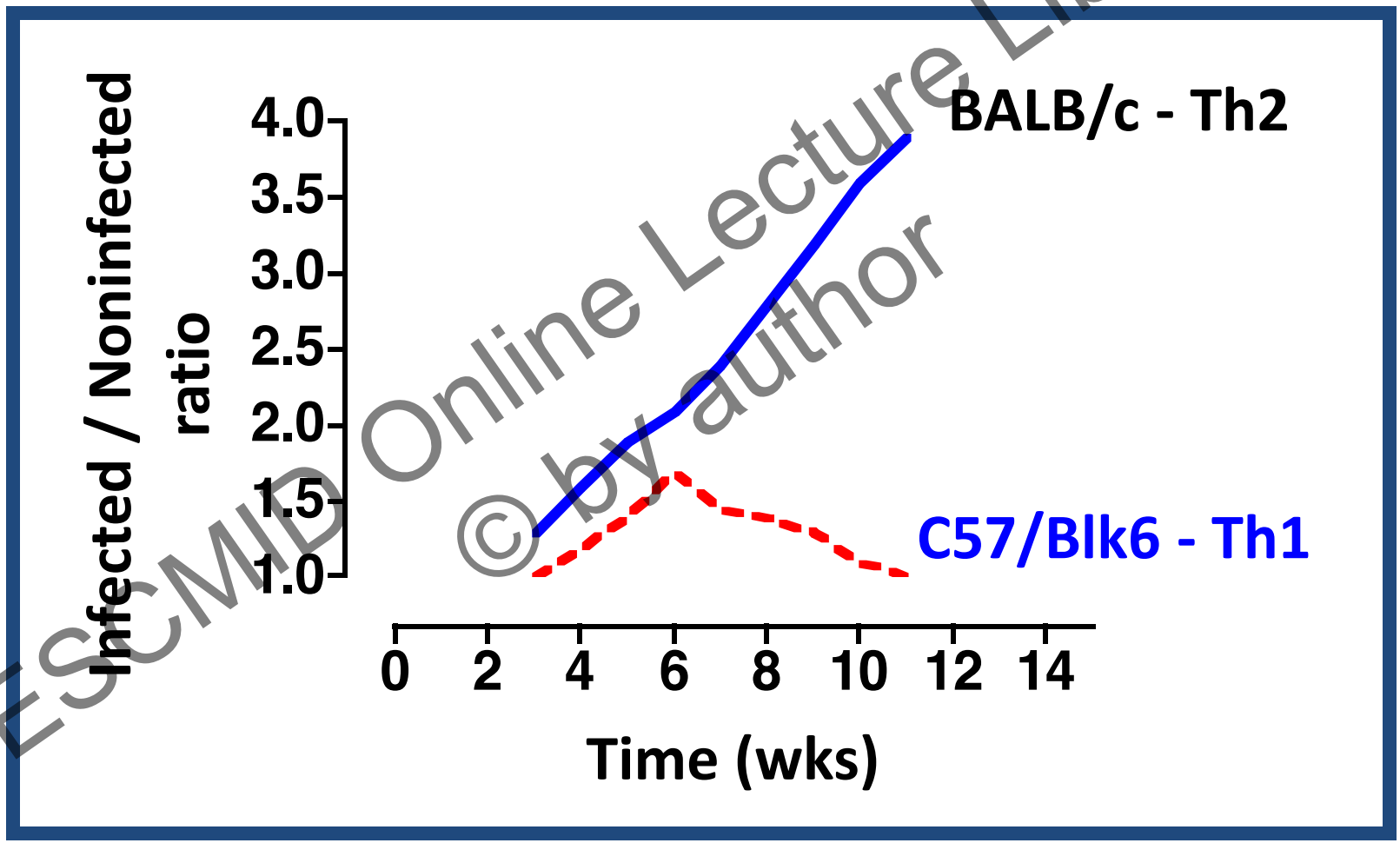
- Effective T-cell response
- Long-term immunity
- Prophylactic and therapeutic activity
- Multiple species
- Safe, Stable, Cost-effective

T-cell immune response

- Th1 response
- Cell mediated
- Macrophage activation
- Intracellular pathogens
- IL-2, IFN-γ & LT-α
- Mice - IgG2a, IgG3
- Neutrophil activation

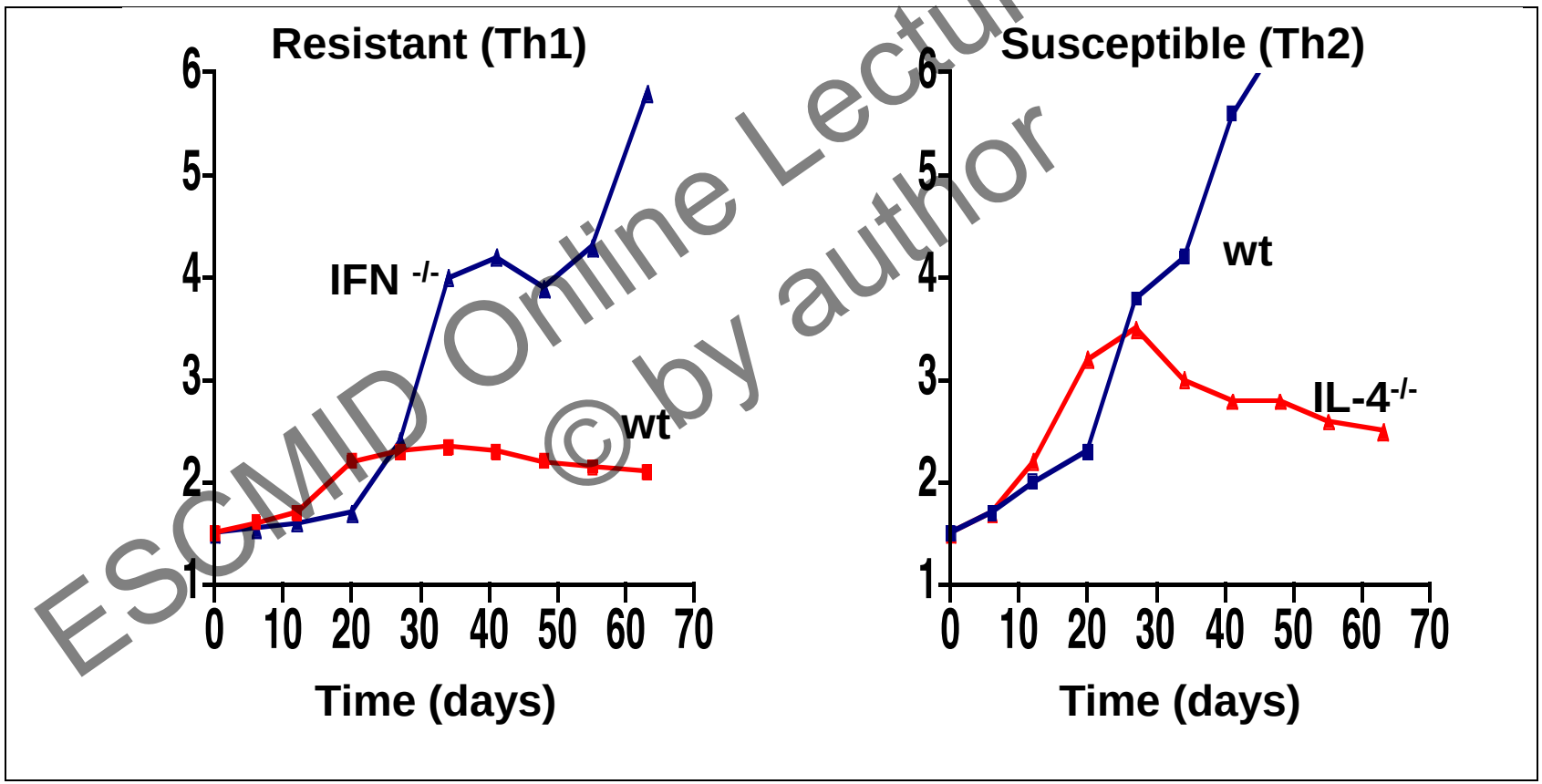
- Th2 response
- Antibodies
- Allergic reaction, IgE
- Extracellular pathogens
- IL-4, -5, -6 & -13
- Mice - IgG1, IgE
- Macrophage inhibition
- Mast cells / Eos

Th1 / Th2 T-cell response



Challenge with *L. major*

Modification of Th1 or Th2 Response



Adapted from Kopf etal (1996) J. Exp. Med. 184: 1127

So what's stopping us

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

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

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

Vaccines that induce CD8+ responses

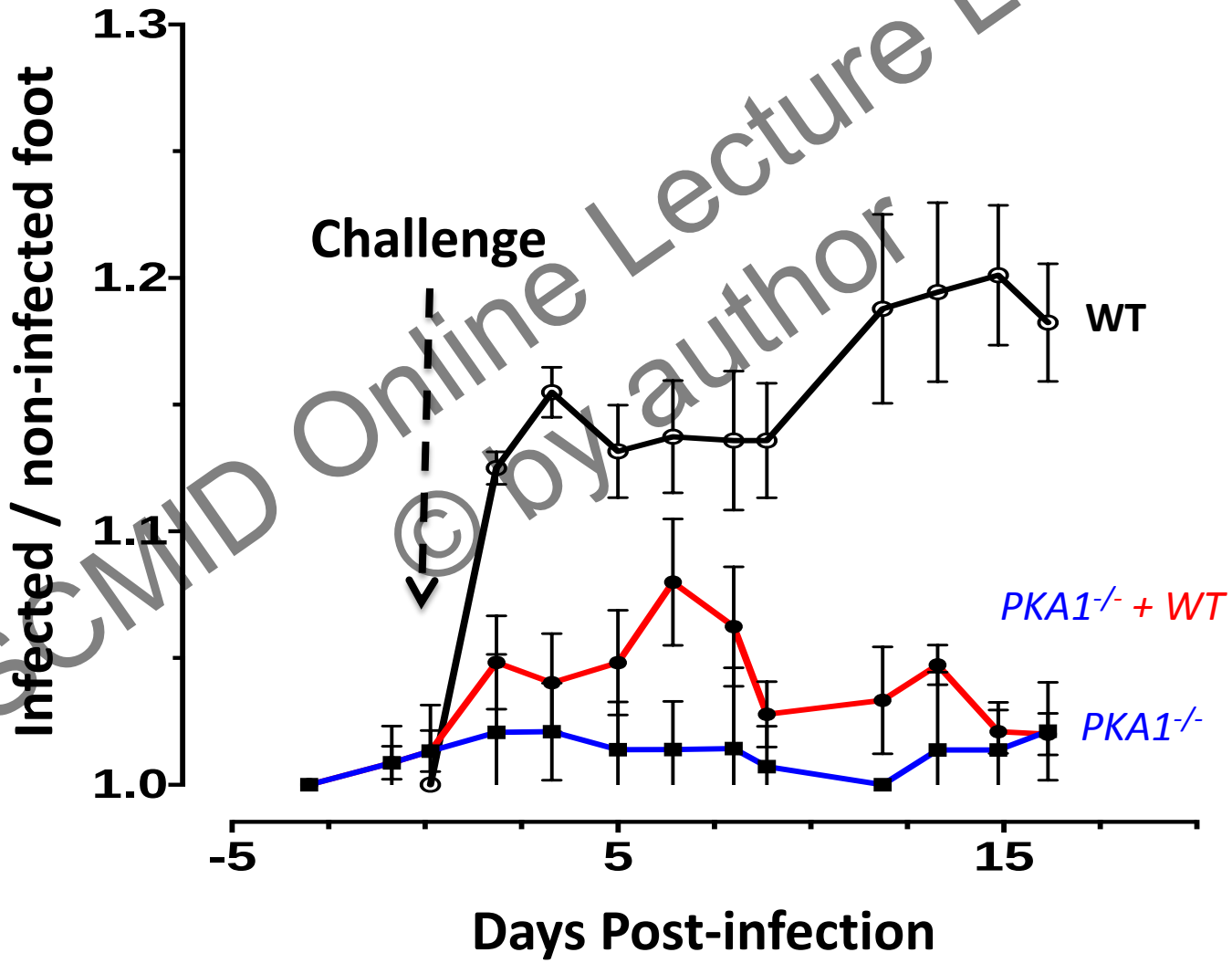
- Live attenuate
  - Low dose, no boosting or adjuvants needed. *Risk reversion*
- Recombinant antigens
  - High dose, boosting, **need adjuvants**. LOW RISK
- Recombinant virus or bacteria
  - Replication competent. *Risk reversion*
    - Low dose, no boosting or adjuvants needed
  - Replication defective. LOW RISK
    - High dose, ± boosting, no adjuvants
- DNA
  - High dose, boosting, adjuvants. LOW RISK
- Prime boost heterologous
  - High dose, boosting, adjuvants. Risk Varies

Adapted from Robinson and Amara (2005) Nature Medicine 11: S25.

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. 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

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

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

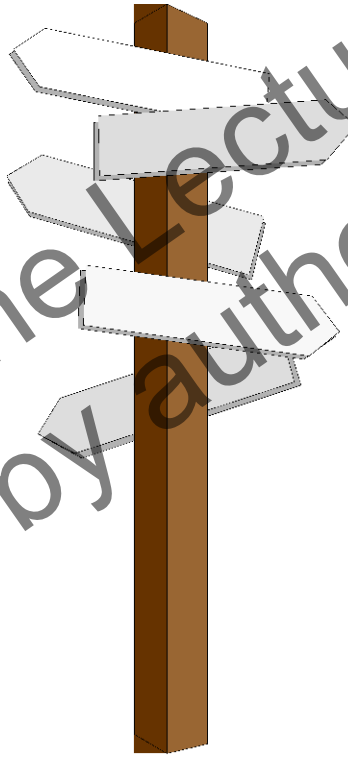
Defined Vaccine Antigens

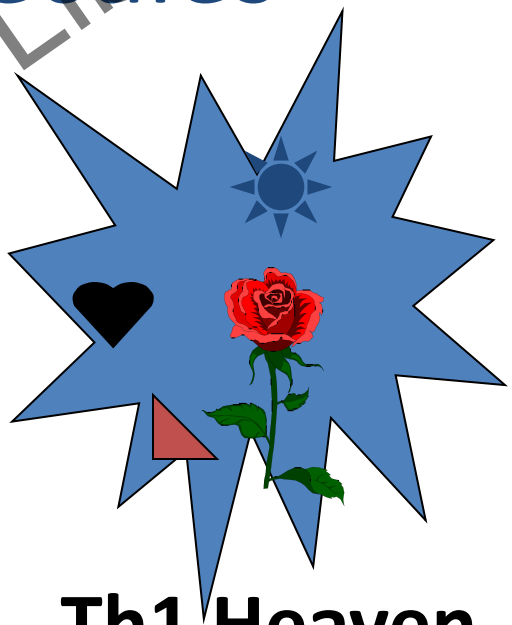
- **Leishmanolysin (gp63)**
- **PAS-2 (Promastigote Surface Antigen 2 Complex)**
- **Dp72**
- **LACK (Leishmanial Receptor for Activated C Kinase)**
- **LeIF (Leishmanial Eukaryotic Ribosomal Protein)**
- **Lcr1(Leishmanial Flagellar Antigen)**
- **Ld23**
- **KMP-11 (Lipophosphoglycan associated protein)**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

Second Generation

- Defined antigenic molecules

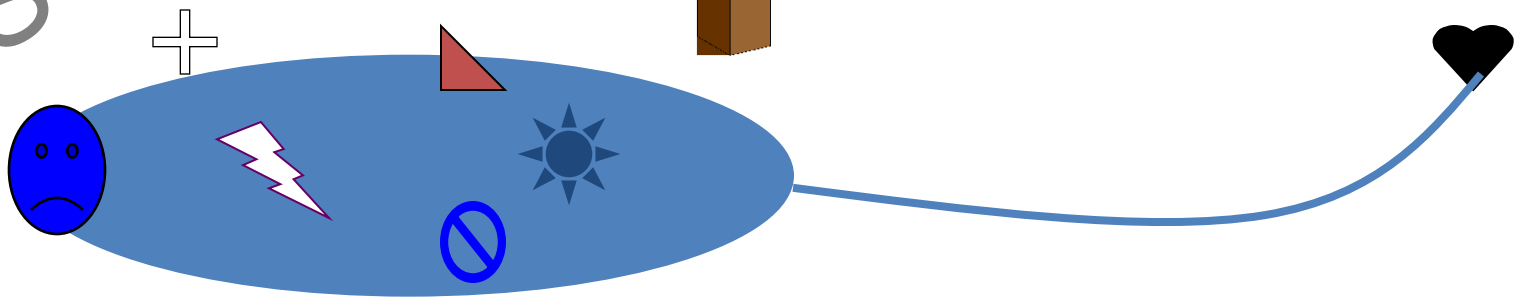
Which antigen & how to get the right response?



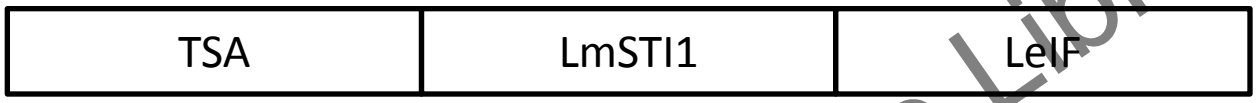


Th2 Trash

Th1 Heaven



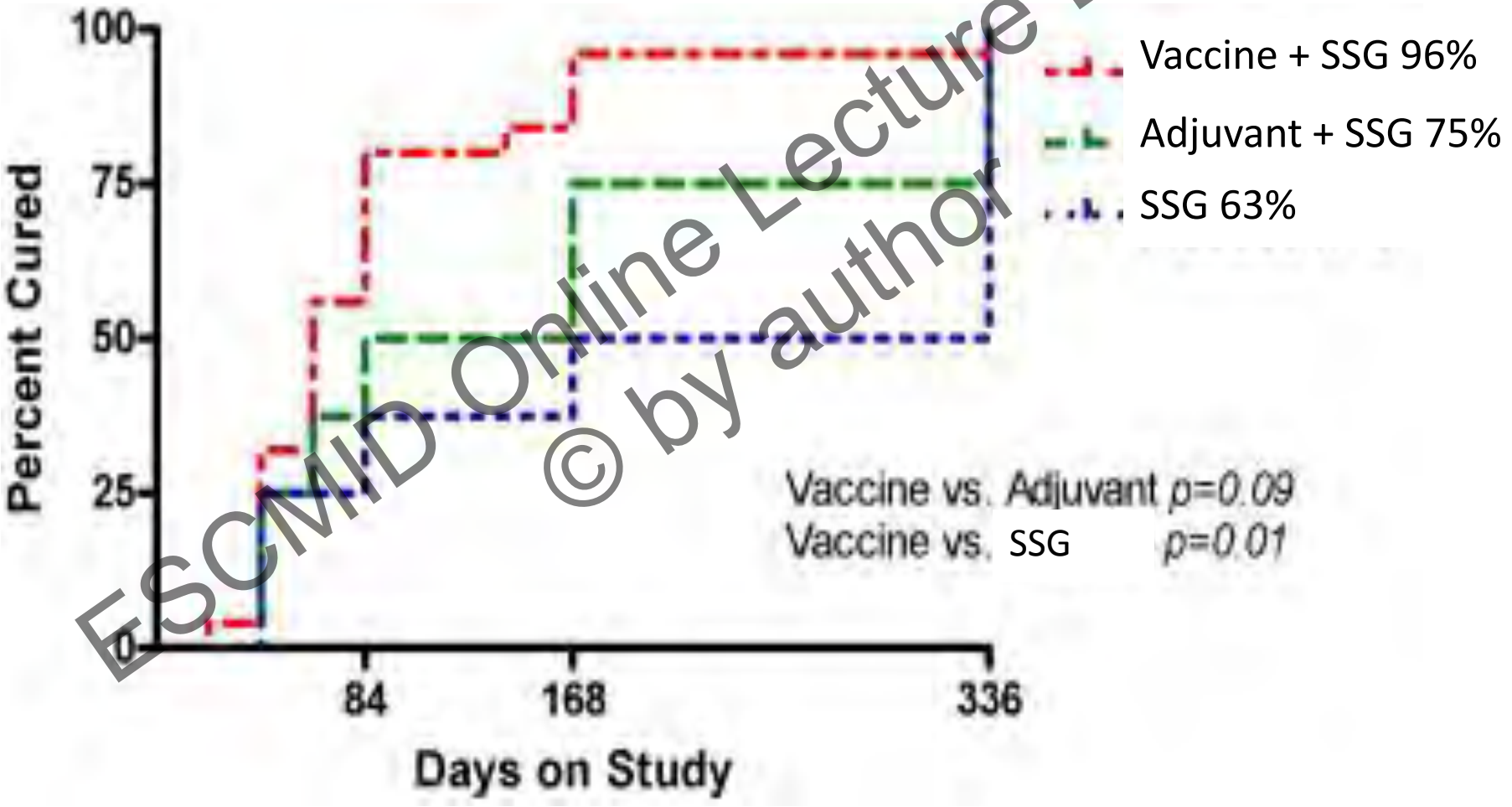
IDRI - LeishF1/F2 + MPL-SE



- 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

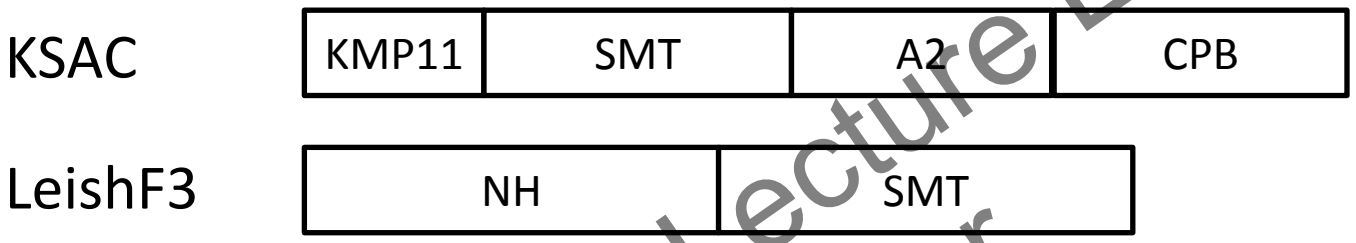
Therapeutic CL Trial - Brazil

LeishF1 + suboptimal chemotherapy (SSG)



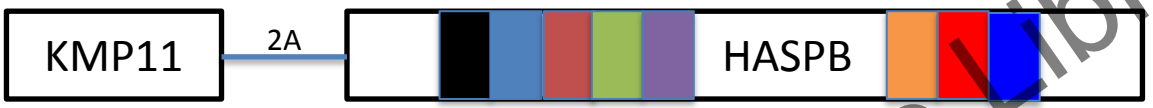
Nascimento et al 2010. Vaccine 28: 6581

Improving the IDRI vaccine for VL



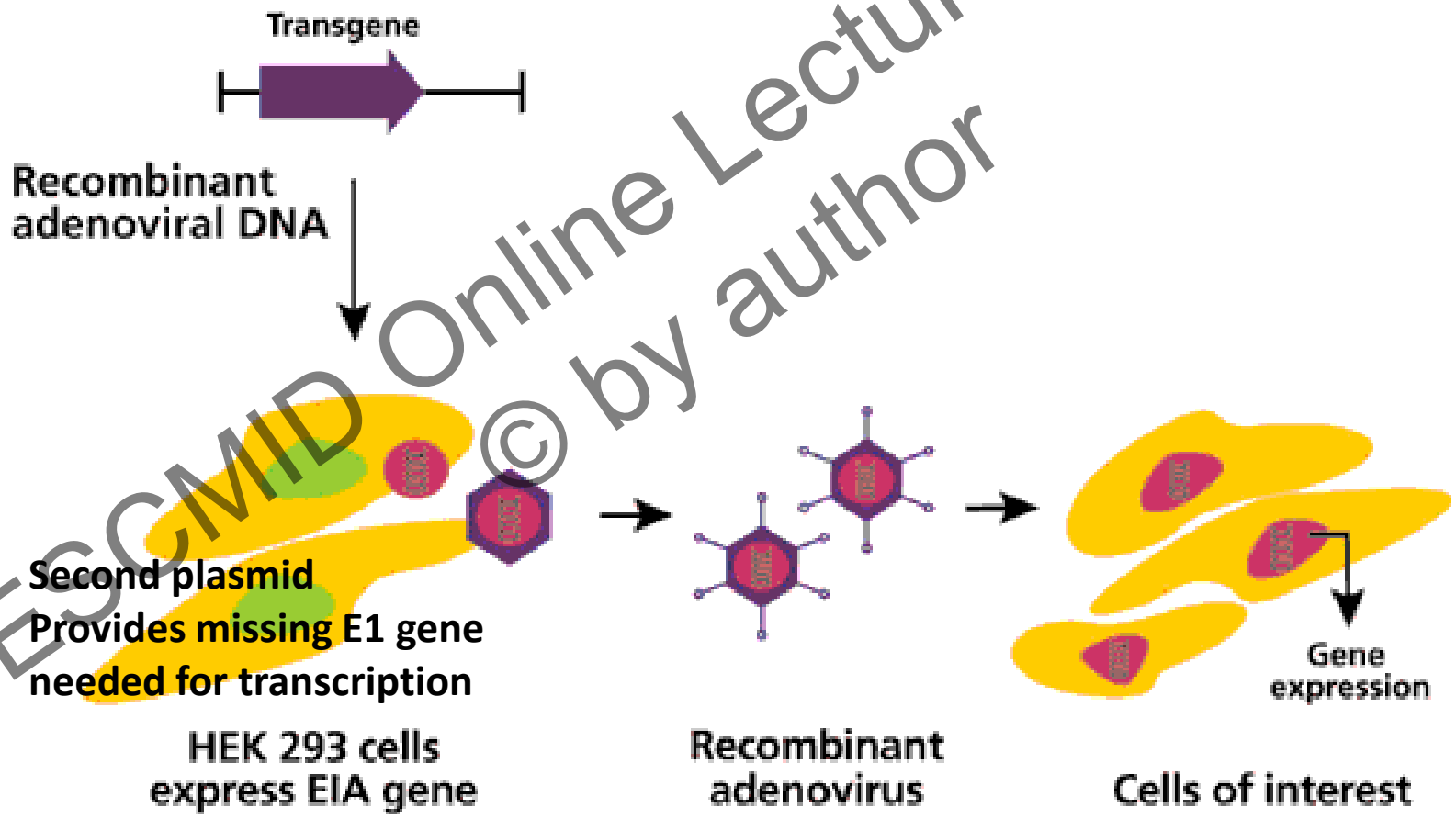
- KSAC works better than LeishF1/F2 in BALB/c mice using sandfly challenge (Gomes et al 2012. PLoS NTD 6:e1610)
- Conserved antigens in Leishmania species causing CL and VL
  - Fusion of nucleoside hydrolase (NH36) and sterol 24-c-methyltransferase (SMT)
  - 84 – 99.7% AA identity; 86 – 99.7% aa identity *L. donovani* protein
- Single antigens protect in mouse or hamster models
- Pre-clinical screening
- *Ex vivo* screening for T-cell responses

YORK –Therapeutic Adenovirus vaccine



- Synthetic *Leishmania* vaccine gene
- KMP11 and HASPB
  - Engineered to reflect strain diversity East Africa/Asia
  - Human codon usage
- Preclinical data using human viral vector Ad5
  - Maroof et al 2012. J Inf Dis 205:853.

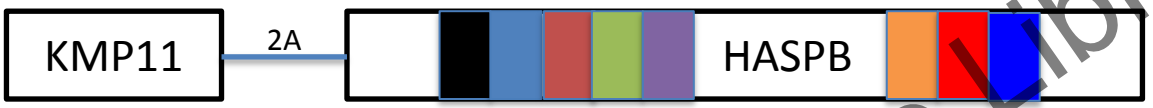
Packaging of Gene in replication deficient Adenovirus (Ad5)



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

YORK –Therapeutic Adenovirus vaccine



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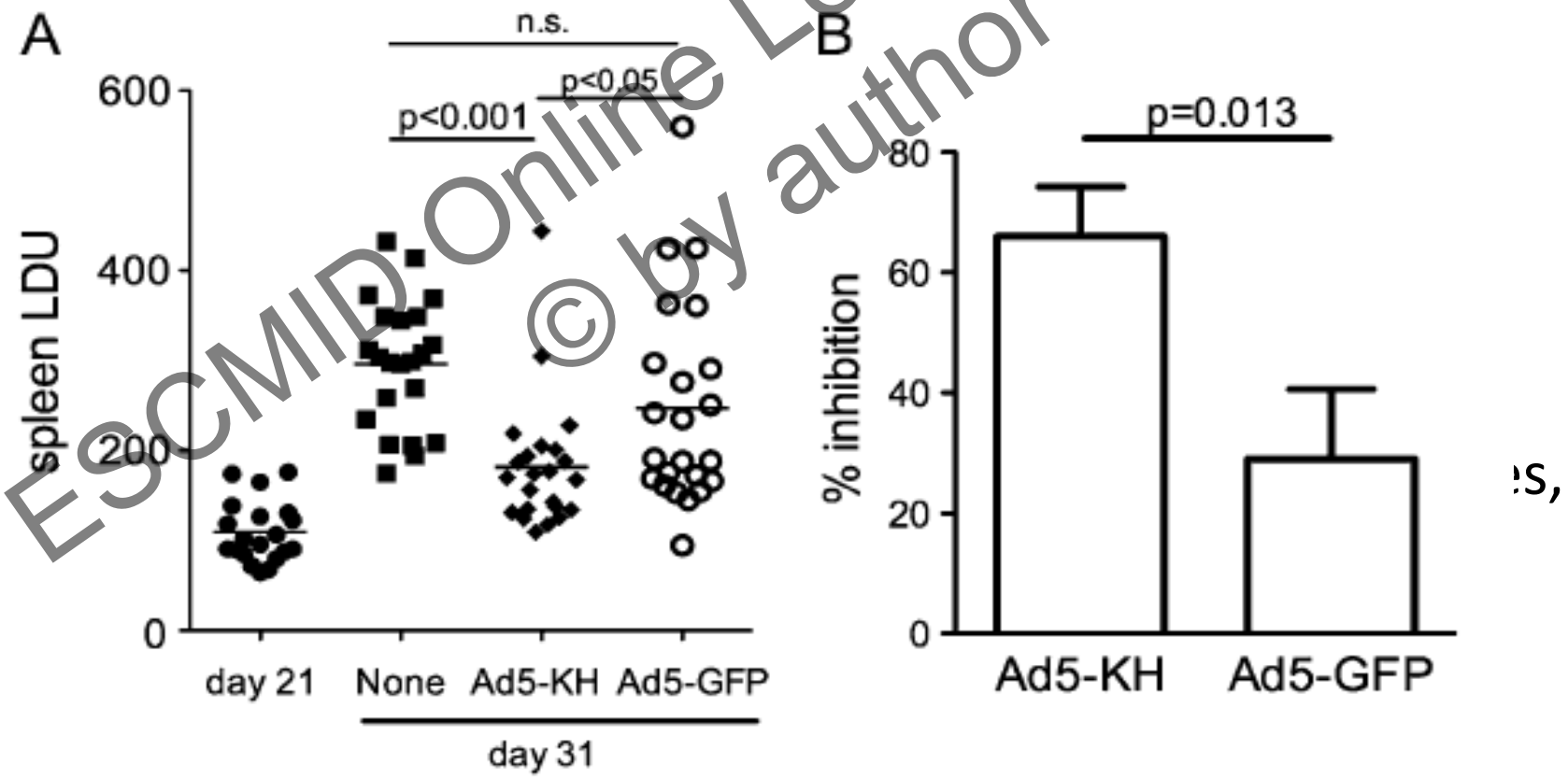
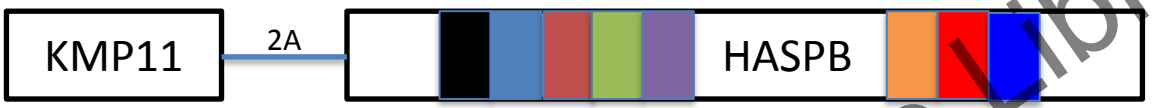
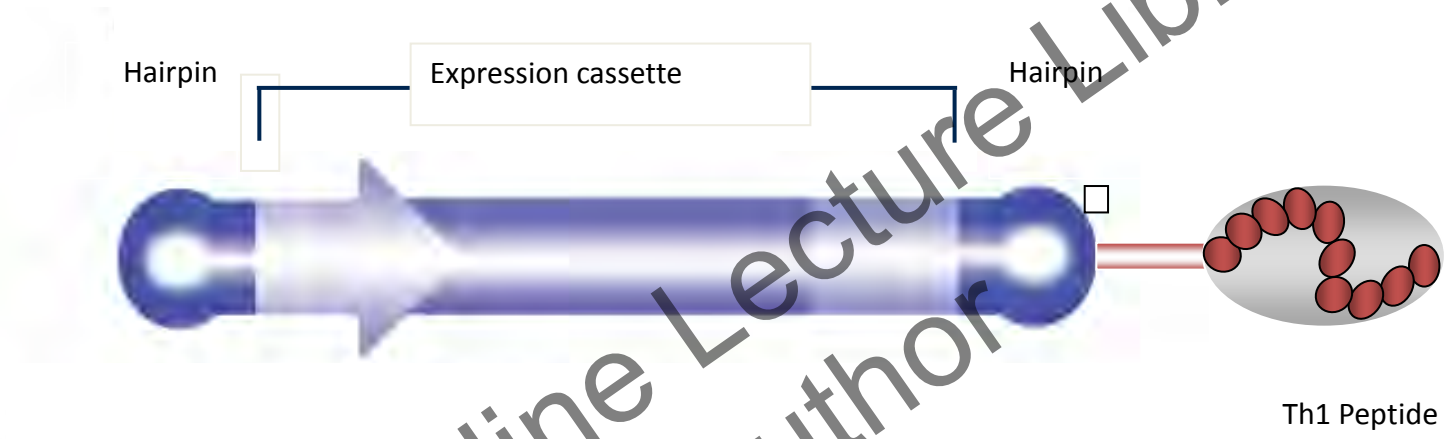


YORK –Therapeutic Adenovirus vaccine



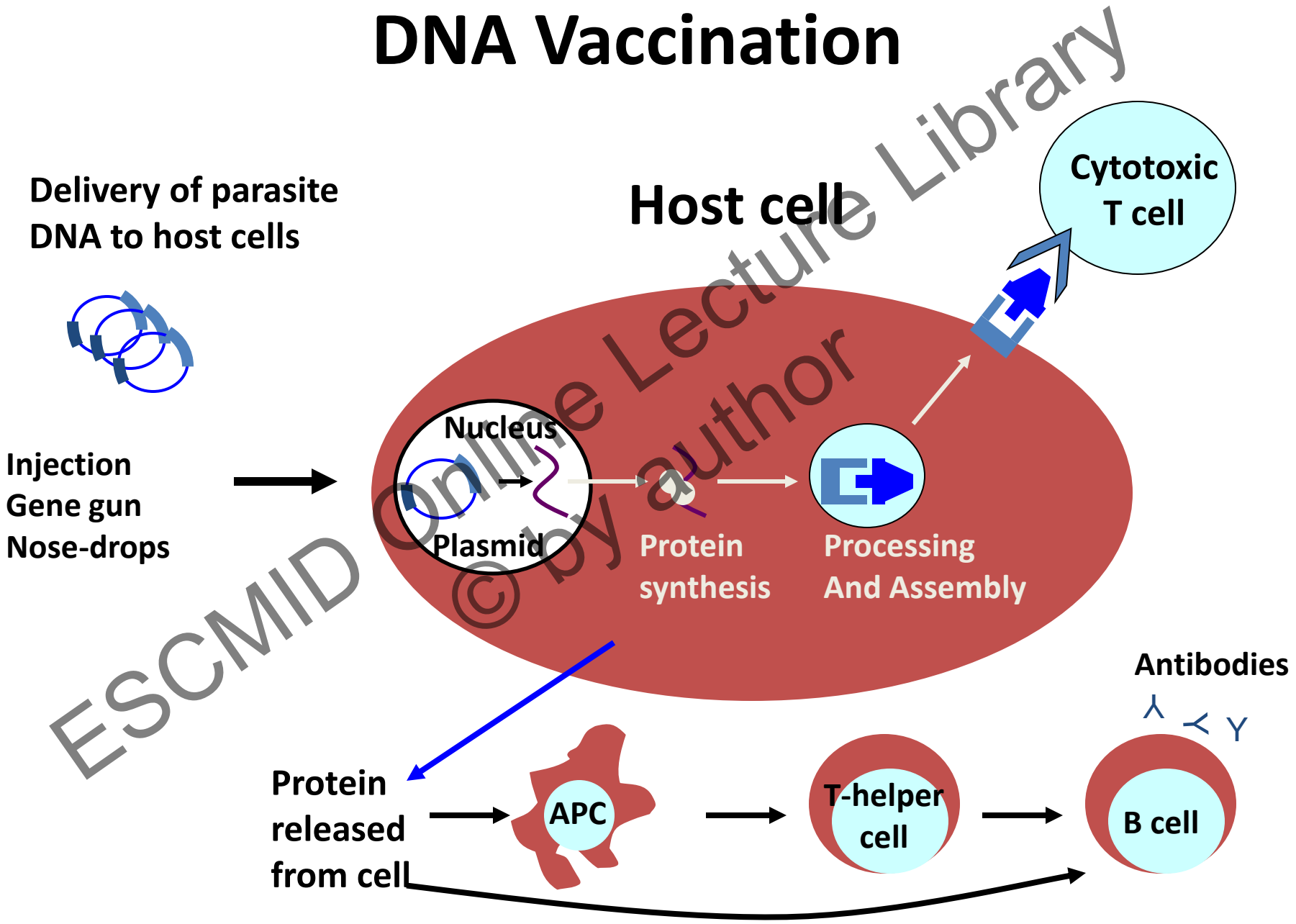
- Synthetic *Leishmania* vaccine gene
- KMP11 and HASPB
  - Engineered to reflect strain diversity East Africa/Asia
  - Human codon usage
- Phase I trial in humans Simian Ad vector AD63
  - Excellent safety profile confirmed
  - Immunogenicity: good levels of $CD8^+$ T cell responses, % responders

Mologen – LEISHDNAVAX



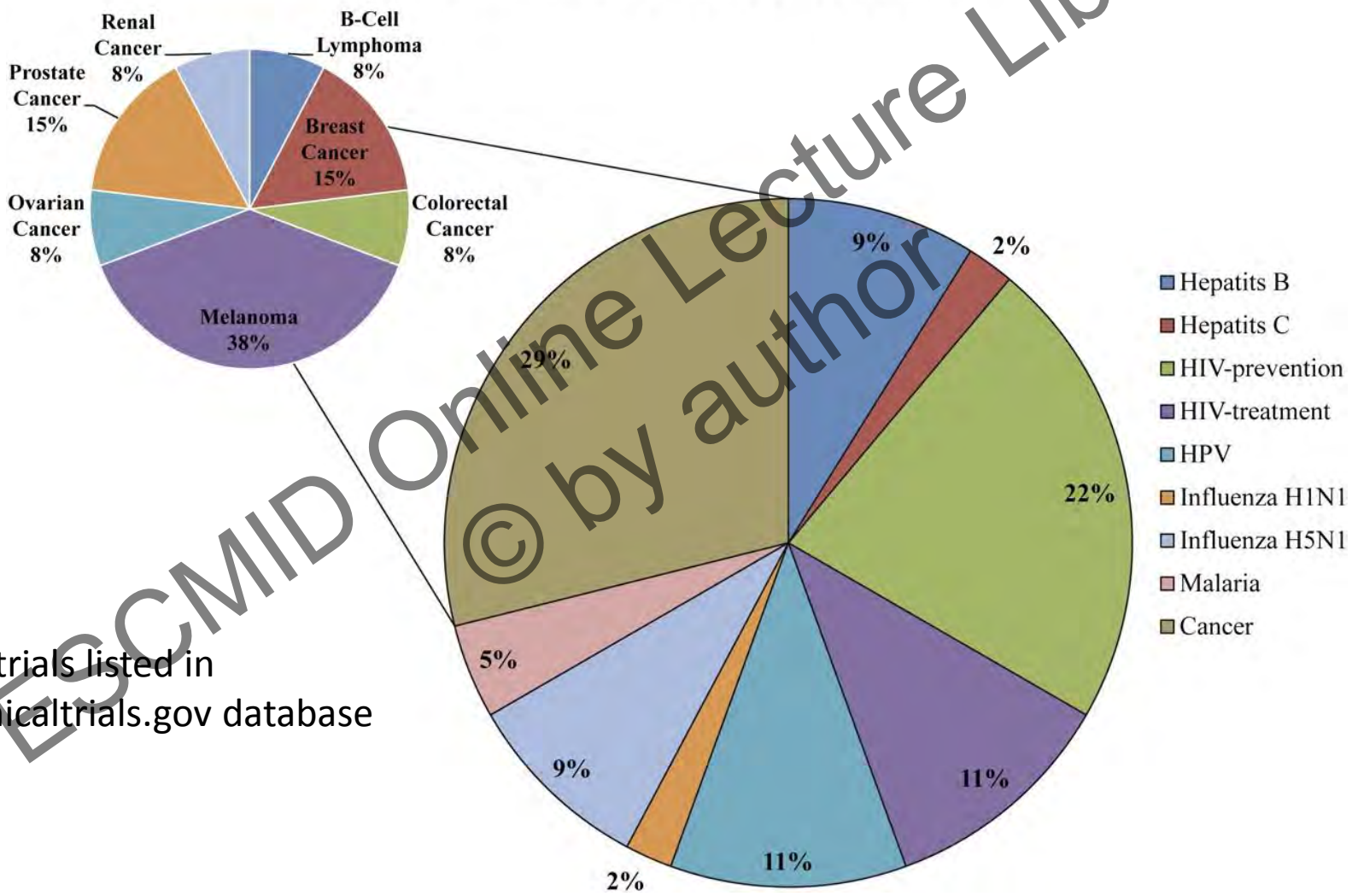
- **M**inimalistic **I**mmunogenically **D**efined **G**ene **E**xpression with **T**-**h**elper **1** peptide vector (MIDGE-Th1)
  - No antibiotic resistance genes
  - Minimum bacterial backbone

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

Mologen – LEISHDNAVAX

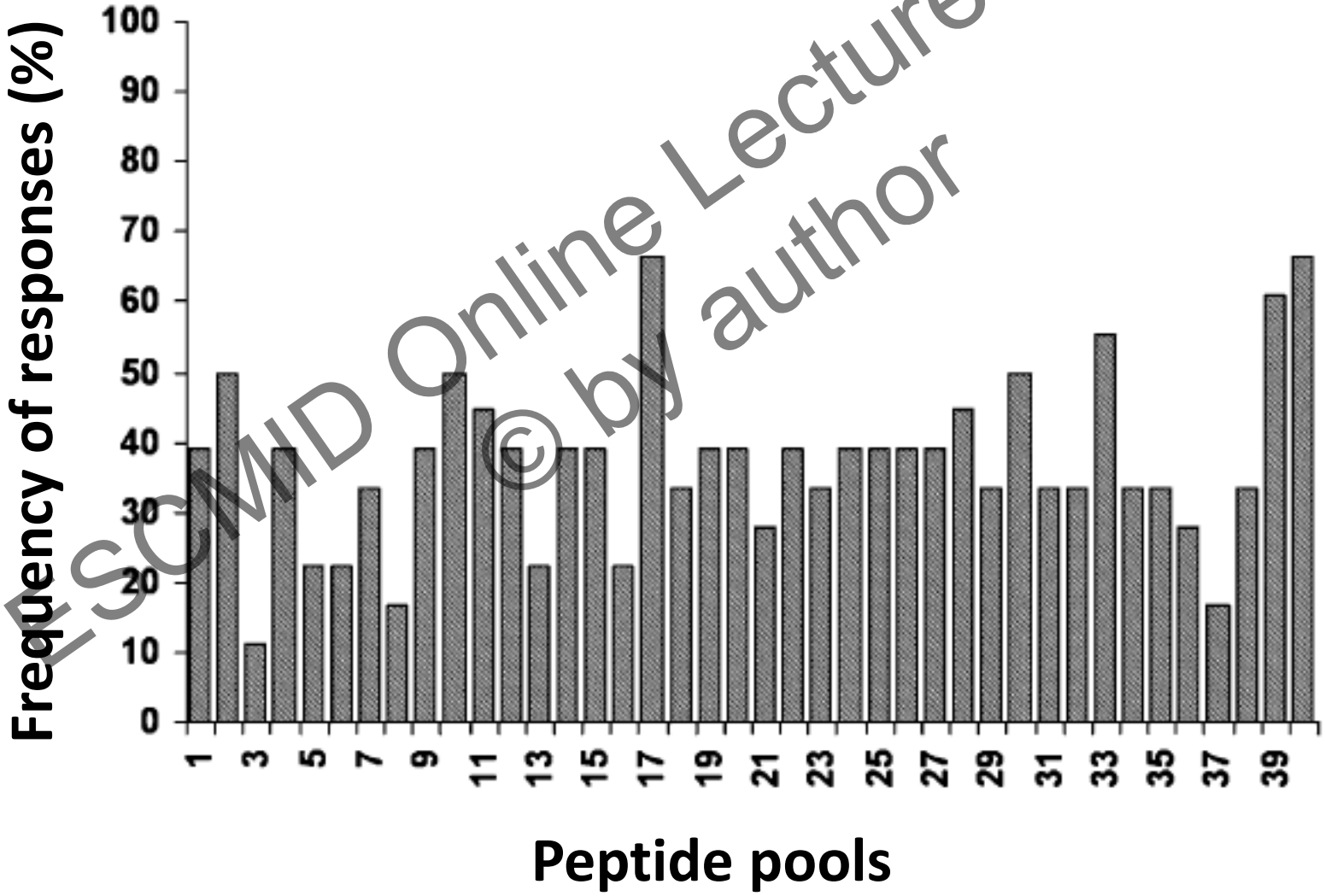
- Antigen selection criteria
  - Conserved between different *Leishmania* species
  - "Antigenic complexity" i.e., different HLAs
  - Induce antigen specific T cell responses
  - Immunogenic in humans
  - Presented by cells of vaccinated individuals
- Start by looking a list of vaccine candidates
  - Excluded highly variable antigens

Shantanabha et al 2014. Sci Transl Med 6: 234ra56

Mologen – LEISHDNAVAX

- 5 antigens chosen
  - KMP11, TSA, P74, truncated cysteine protease-B (tCP-B) and CP-A
  - High sequence conservation between strains over time
  - Immunogenic in target population
- Synthetic genes designed for each antigen
  - Consensus amino acid sequences
  - Maximum number of epitopes
- Optimization of DNA sequences
  - Expression, mRNA stability, GC content
  - Codon usage

T-cell responses in cured VL patients



Mologen – LEISHDNAVAX

- Selection criteria
  - ✓Induce antigen specific T cell responses
  - ✓Immunogenic in humans
    - CD4 and CD8 T cell responses, IFN-γ detected in cured VL and CL patients
  - ✓Conserved between different *Leishmania* species
  - ✓"Antigenic complexity" i.e., different HLAs
  - ✓Safe in animals
  - – Presented by cells of vaccinated individuals

Mologen – LEISHDNAVAX

- Prophylactic protection in
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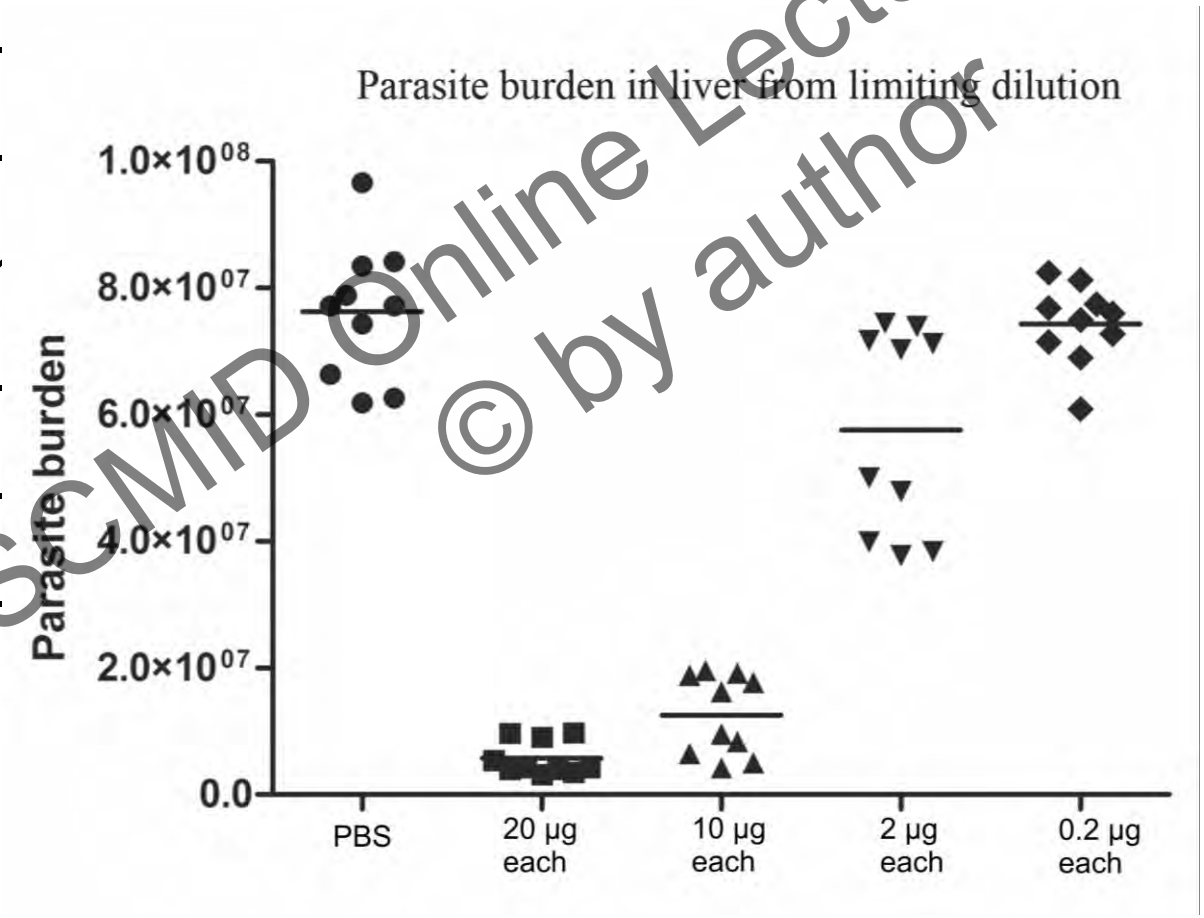

Vaccines for Canine leishmaniasis

- Leishmune®
  - Licensed Brazil: fucose-mannose ligand glycoprotein (FML) + saponin-based adjuvant
  - >87.8% protection, adverse effects 2.2%, xenodiagnosis 5.1% +
- Leish-Tec®
  - Licensed Brazil: recombinant amastigote stage protein A2 + saponin-based adjuvant
  - >81.1% protection, adverse effects 13.0%, xenodiagnosis 5.4% +
- CaniLeish®
  - Licensed in Europe: excreted-secreted proteins + saponin-based adjuvant
  - 4-fold reduction in risk infection, xenodiagnosis, minor adverse effects, 20%

Final steps

- Phase I - Is it safe, side-effects, immunogenicity
  - Completed - LeishF1/F2, Ad63-KH; In progress – LeishF3
- Phase II - expanded safety + immunogenicity
- Phase III - double-blind study of efficacy
- Phase IV- expanded trial

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

Problems

- Host responses vary (HLA-I and -II)
- Different requirements, different species
- Not understood what is needed for protective response in humans
- What to measure, no easy assays to monitor correlates of protection
- Protection in animal models -

"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

Thanks

- PKA1
  - Laura Malki-Feldman
  - Michelle Siman Tov
- LEISHDNAVAX -
  - Nina Sacerdoti-Sierra
  - Mologen Christiane Juhls & Matthias Schroff
  - LSTMH Karin Seifert & Simon Croft
  - IPT Thouraya Boussoffara & Hechmi Louzir
  - Charite Universitatsmedizin Berlin Anja Freier, Florian Losch, Melanie Selka, Gabriele Schonian & Peter Walden
  - IICB Shantanabha Das & Syamal Roy
  - RMRIMS Sushmita Das & Pradeep Das
  - DNDi Farrokh Modabber