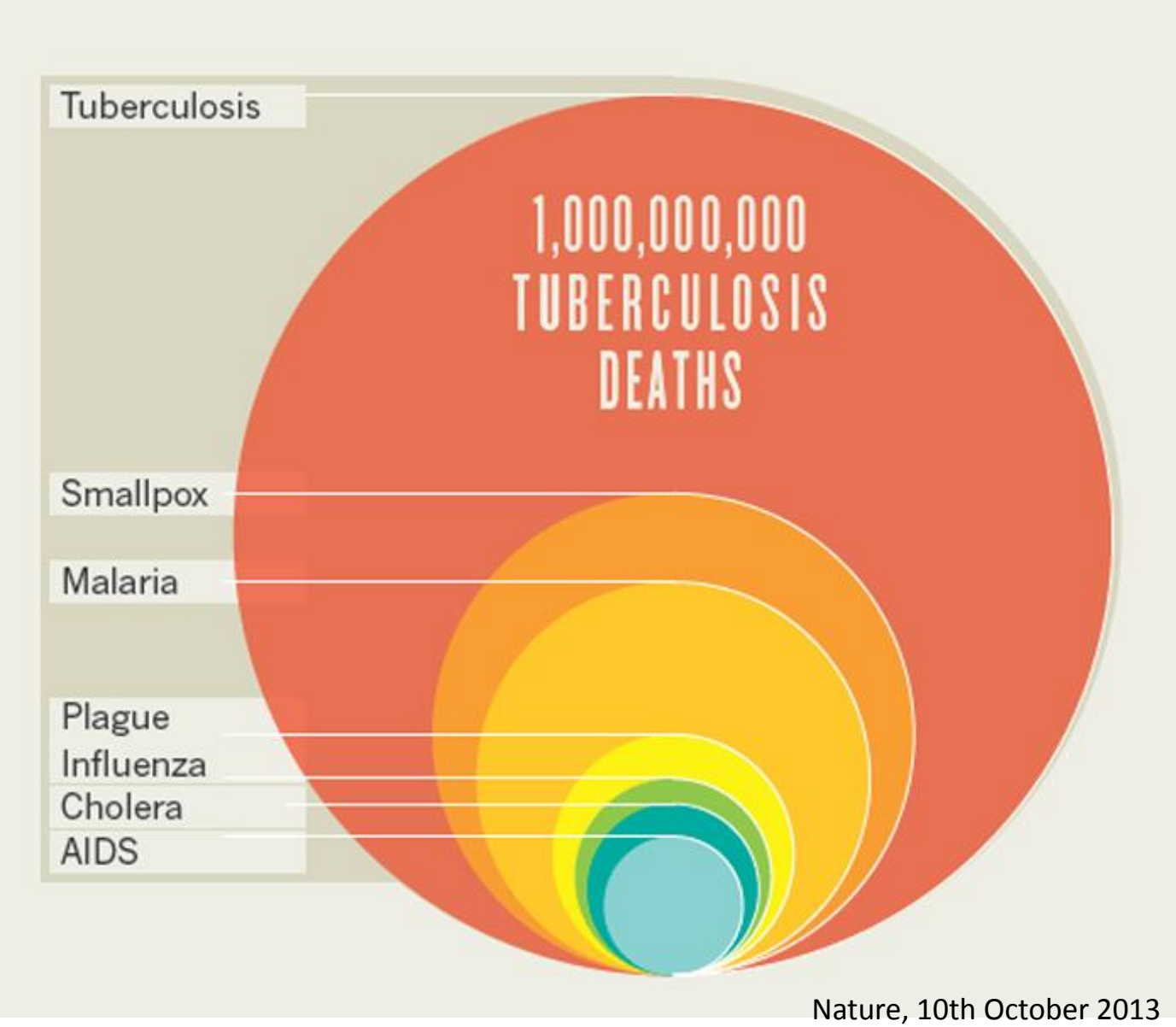
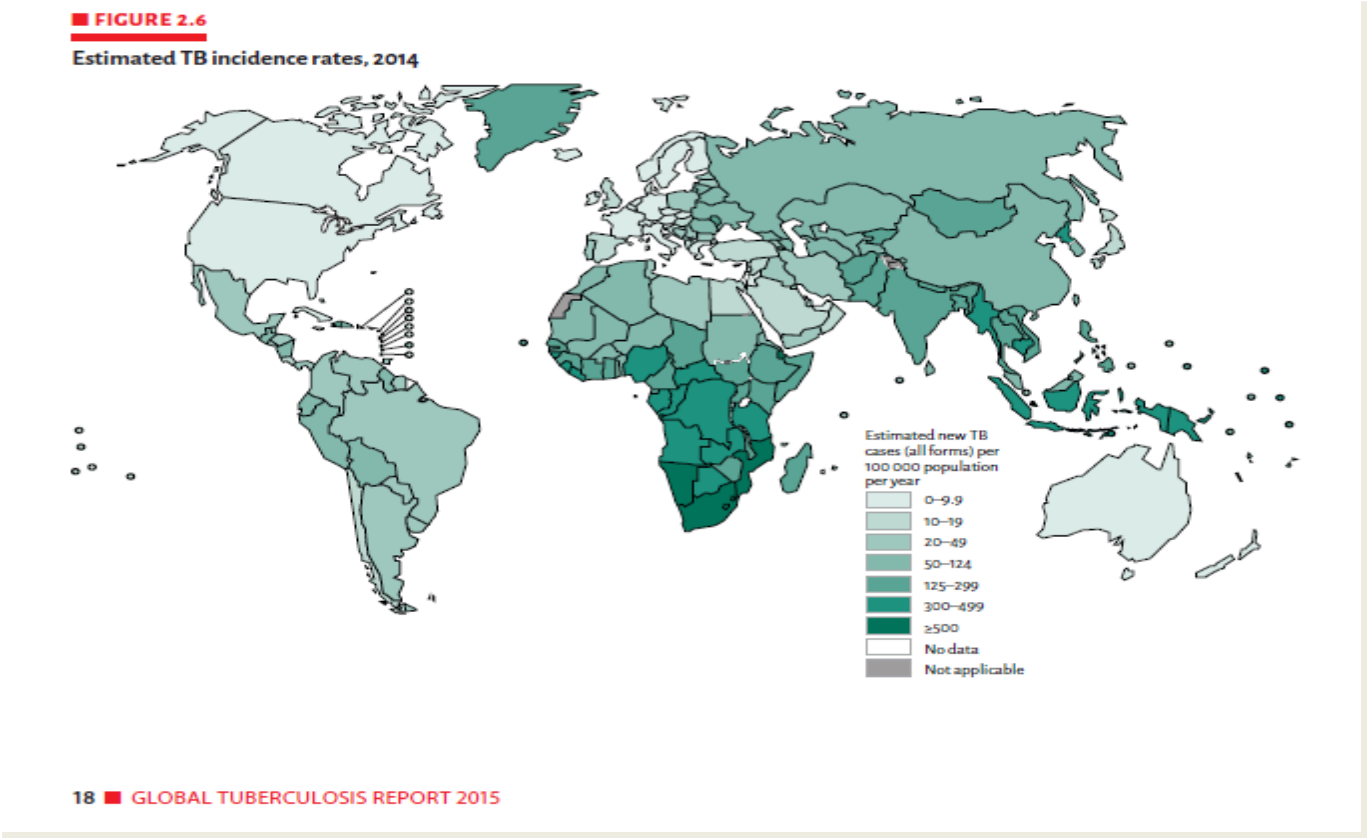
TB has killed more people than any other infectious disease the past 200 years



Nature, 10th October 2013

Tuberculosis anno 2016

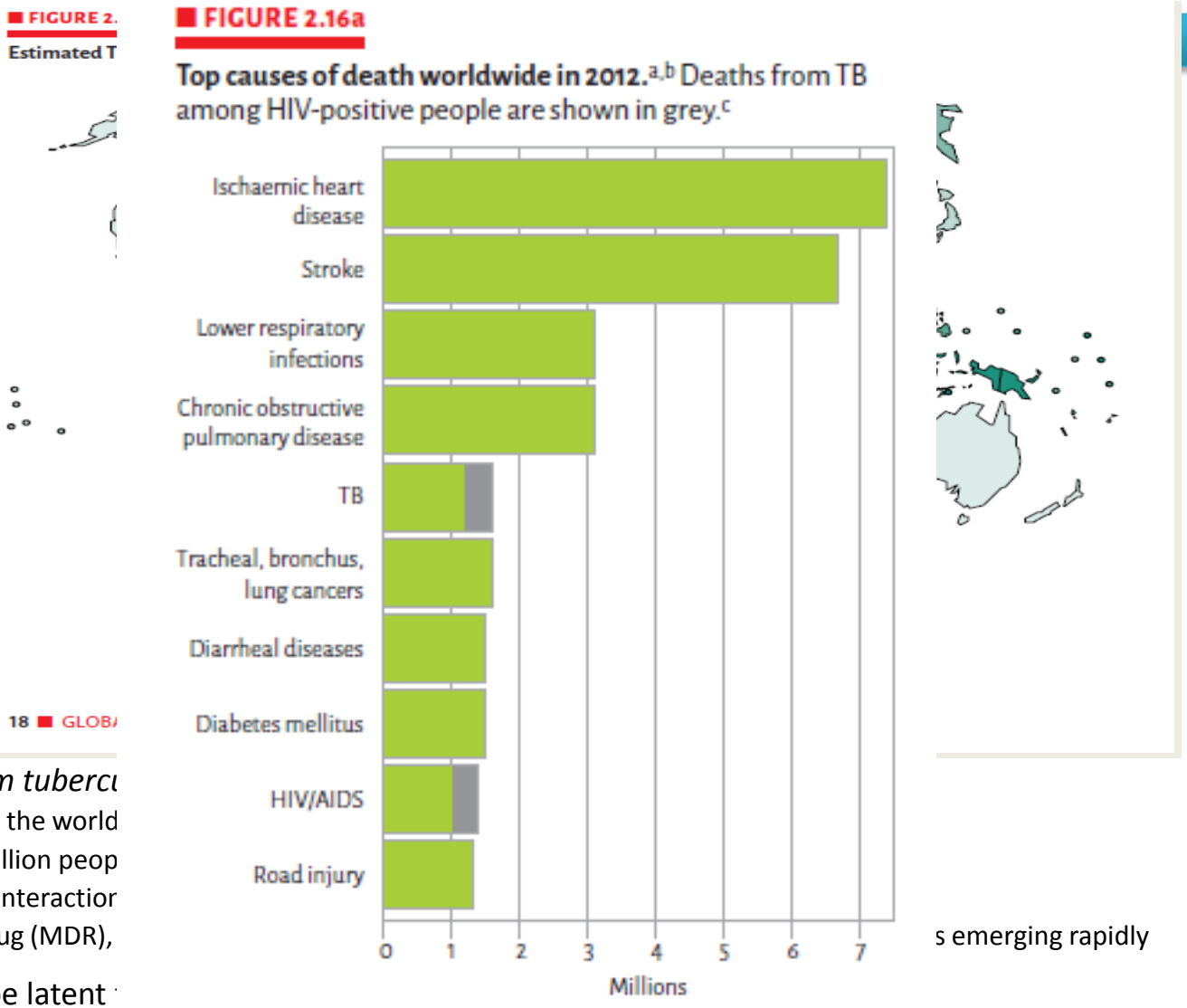


WHO, 2015

- *Mycobacterium tuberculosis* (Mtb) infection:
  - > 1/3 of the world population is infected
  - > 9,6 million people develop TB disease every year, ~1,5 million die
  - Deadly interaction with HIV infection
  - Multidrug (MDR), extensively drug resistant (XDR) and totally drug resistant (TDR) strains emerging rapidly
- Infection can be latent for decades
- Reactivation disease develops in 3-10% of latently infected persons

Tuberculosis anno 2016

WHO, 2015



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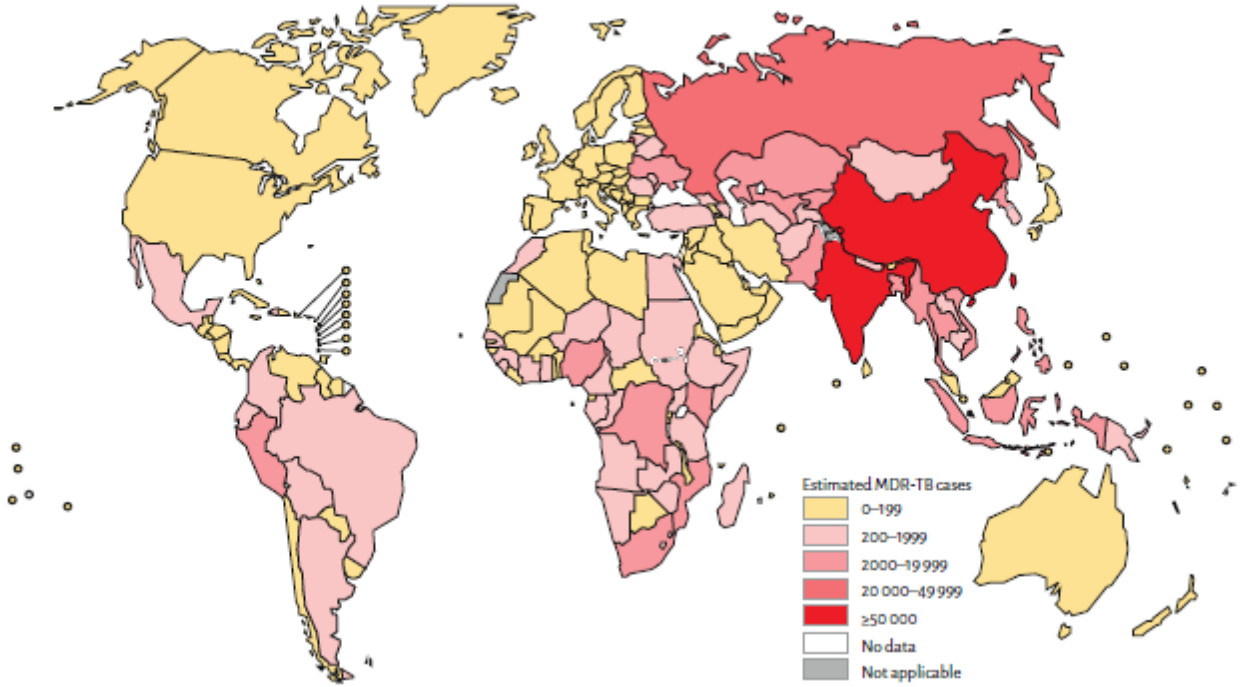
The increasing threat of MDR and XDR TB at the borders of the EU

FIGURE 4.6

Number of MDR–TB cases estimated to occur among notified pulmonary TB cases, 2014

WHO, 2015



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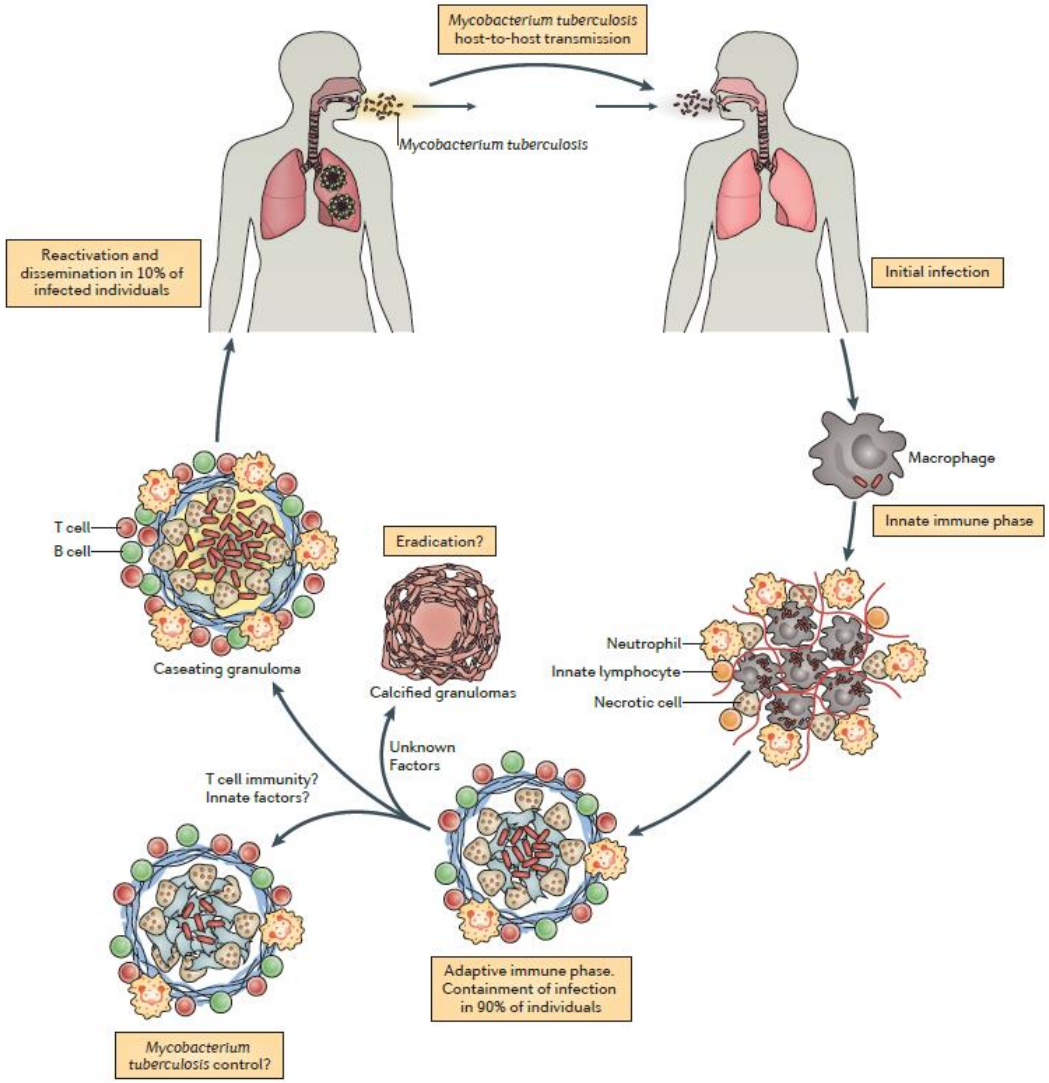
Tuberculosis: Infection, Immunity and Pathogenesis



Nunes Alves C et al, Nat Rev Microbiol, 2014

We have BCG so why worry?

- most used global vaccine (> 4 billion doses)
- protects against severe TB in childhood

but:

- it fails to protect adults consistently against pulmonary TB
- it does not protect against reactivation of latent TB
- it does not induce immunity against several relevant antigens of Mtb, including latency antigens

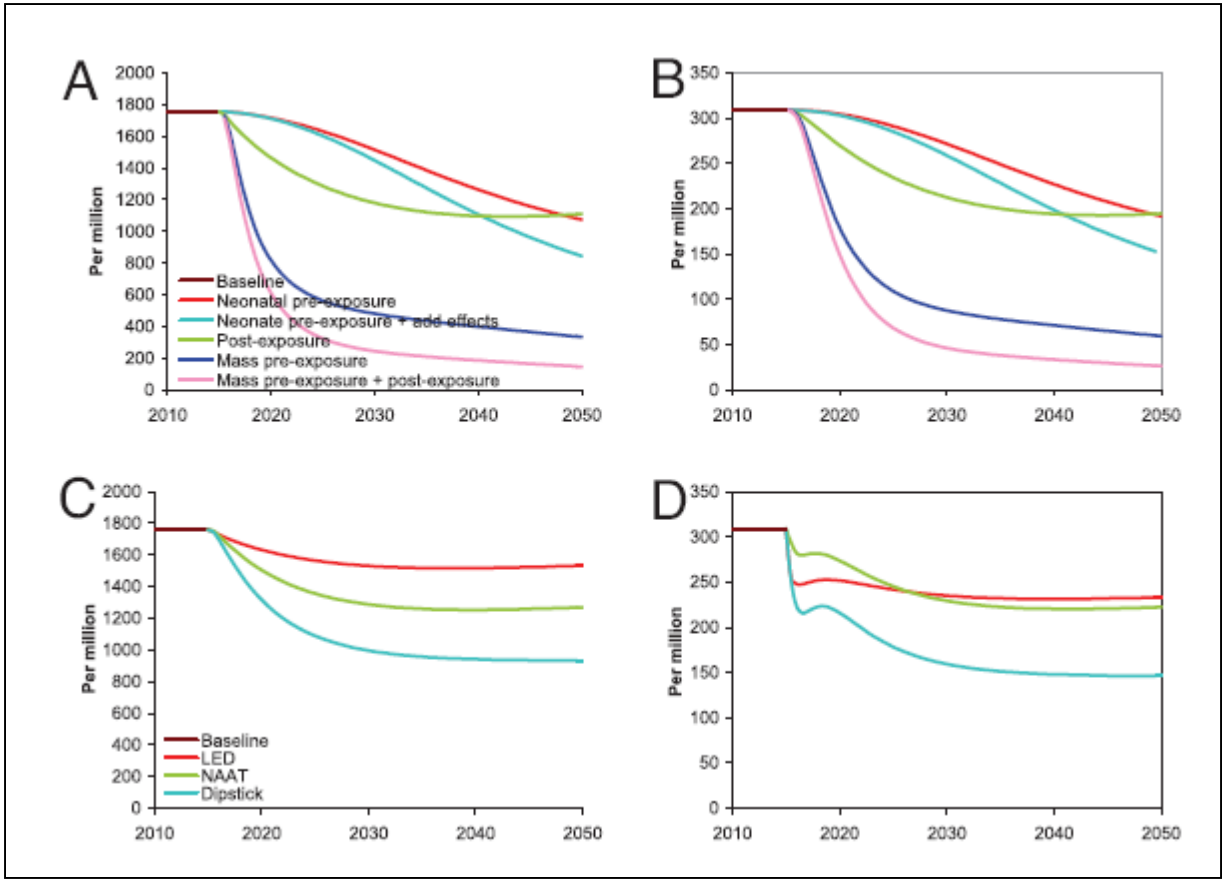
-> BCG vaccination has limited impact on TB pandemic

High global health benefit from more effective TB vaccination

TB incidence **TB mortality**

vaccines

diagnostics

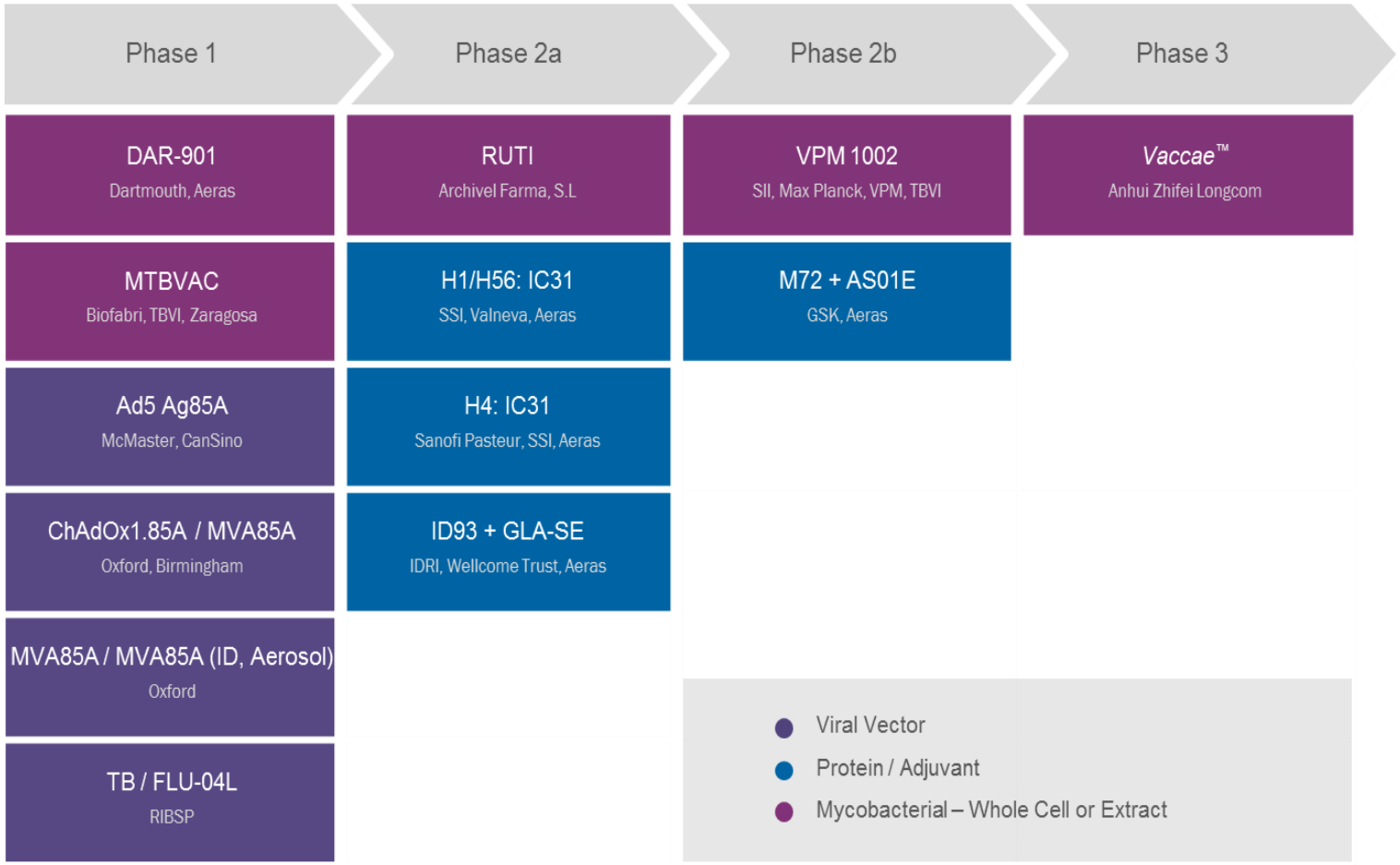


Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics

Laith J. Abu-Raddad[a,1], Lorenzo Sabatelli[a], Jerusha T. Achterberg[a,b,c], Jonathan D. Sugimoto[a,b], Ira M. Longini, Jr.[a,d], Christopher Dye[e], and M. Elizabeth Halloran[a,d,2]

13980–13985 | PNAS | August 18, 2009 | vol. 106 | no. 33

The Current Global Clinical Portfolio of TB Vaccine Candidates



Last updated December 2015

A recent phase IIb trial showed no efficacy of a new TB vaccine

Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial

Michele D Tameris, Mark Hatherill*, Bernard S Landry, Thomas J Scriba, Margaret Ann Snowden, Stephen Lockhart, Jacqueline E Shea, J Bruce McClain, Gregory D Hussey, Willem A Hanekom, Hassan Mahomed†, Helen McShane†, and the MVA85A 020 Trial Study Team*

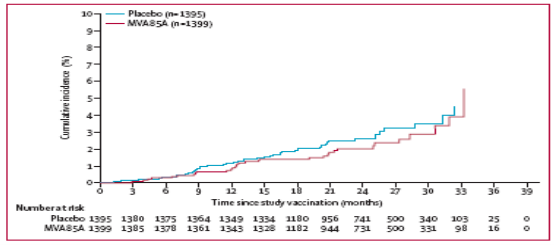
Summary

Background BCG vaccination provides incomplete protection against tuberculosis in infants. A new vaccine, modified Vaccinia Ankara virus expressing antigen 85A (MVA85A), was designed to enhance the protective efficacy of BCG. We aimed to assess safety, immunogenicity, and efficacy of MVA85A against tuberculosis and *Mycobacterium tuberculosis* infection in infants.

	Placebo (n=1395)	MVA85A (n=1399)	Vaccine efficacy
Endpoint 1 (primary efficacy endpoint)	39 (3%)	32 (2%)	17·3% (−31·9 to 48·2)
Endpoint 2 (exploratory efficacy endpoint)	52 (4%)	55 (4%)	−6·9% (−56·1 to 26·9)
Endpoint 3 (exploratory efficacy endpoint)	177 (13%)	196 (14%)	−12·1% (−37·4 to 8·5)

Data are n (%) or % (95% CI). Participants with more than one diagnosis were analysed in each level of diagnosis attained. Vaccine efficacy and corresponding 95% CI was estimated with the Cox regression model (1– estimated hazard ratio).

Table 2: Primary and secondary efficacy endpoints



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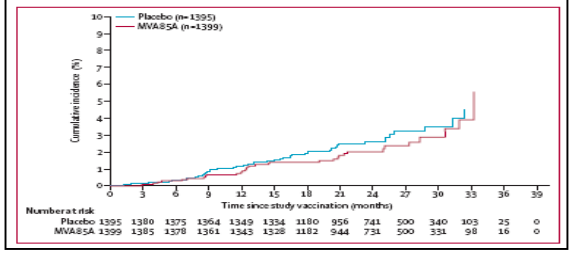
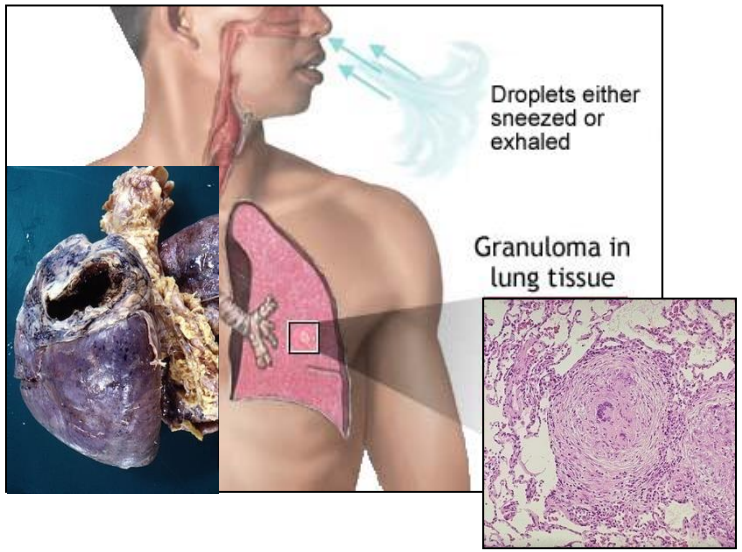


- **Ag85A may not be the "right" antigen: gene expression analysis shows its expression is regulated (e.g. Commandeur et al, JI 2013)**
- **Cells other than Th1 may be needed for full protection**
- BCG may have masked possible protective MVA85A effect
- MVA85A might have shown protection against severe forms of TB (endpoint was mild TB)
- Possible protective MVA85A effect may depend on population setting

Antigenic phase variation during *Mtb* infection

in vitro and in vivo



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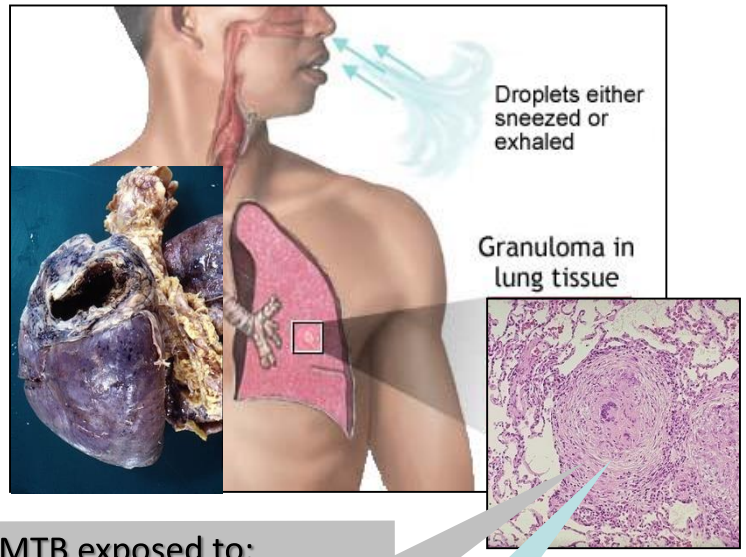


MTB exposed to:

- ✓ low O_2, NO
- ✓ lack of nutrients
- ✓ low pH

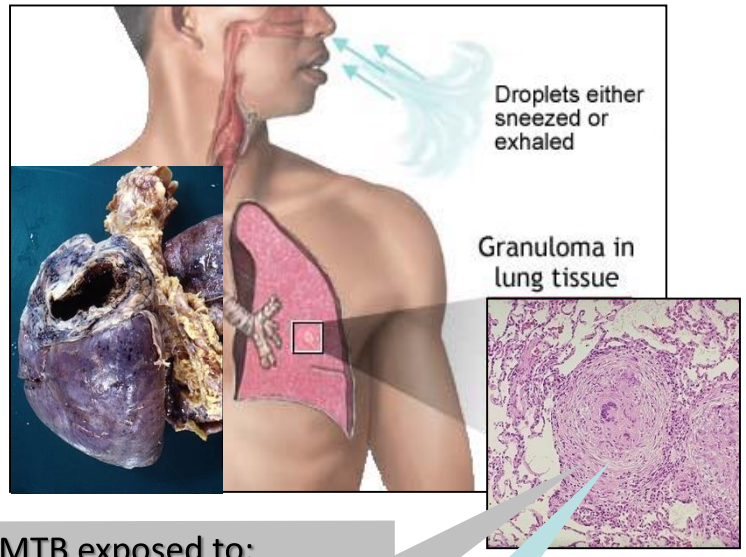
- ✓ macrophages
- ✓ epithelioid cells
- ✓ lymphocytes

↓

physical containment of bacilli

Antigenic phase variation during *Mtb* infection

in vitro and in vivo



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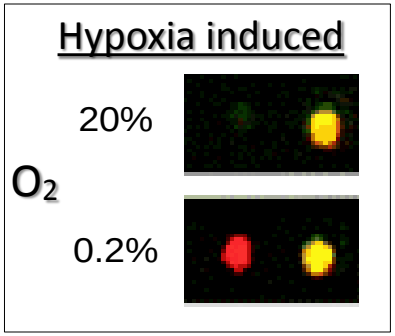
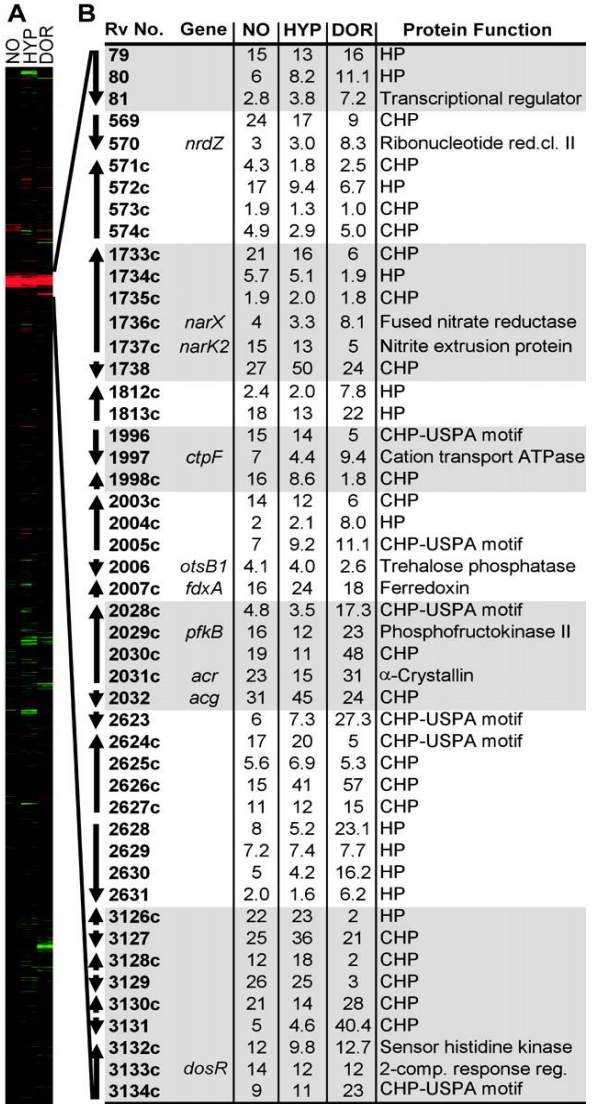


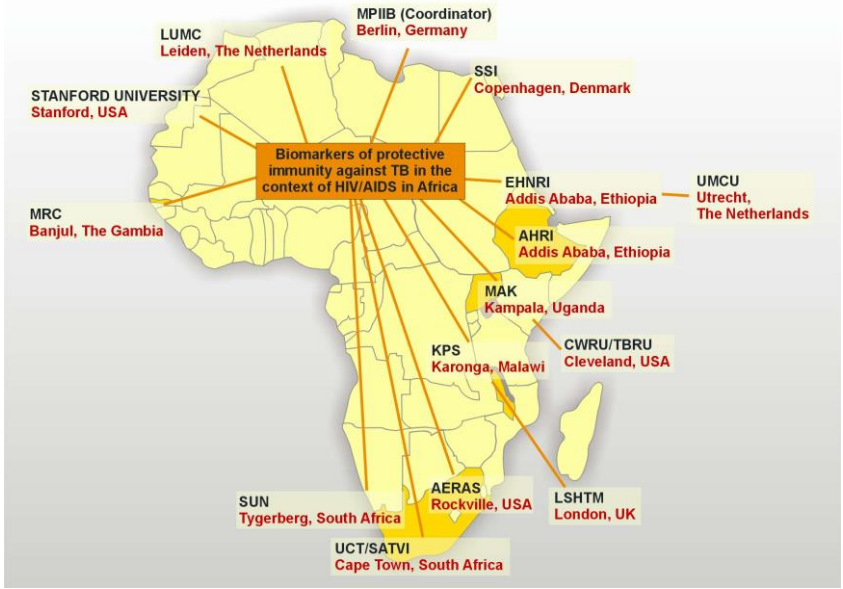


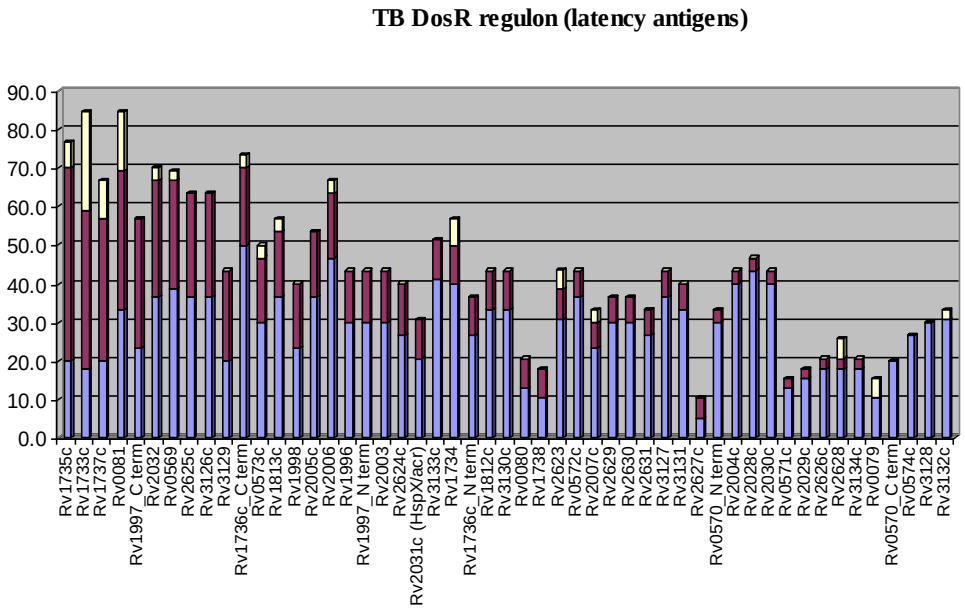
Rv No.	Gene	NO	HYP	DOR	Protein Function
79		15	13	16	HP
80		6	8.2	11.1	HP
81		2.8	3.8	7.2	Transcriptional regulator
569		24	17	9	CHP
570	*nrdZ*	3	3.0	8.3	Ribonucleotide red.cl. II
571c		4.3	1.8	2.5	CHP
572c		17	9.4	6.7	HP
573c		1.9	1.3	1.0	CHP
574c		4.9	2.9	5.0	CHP
1733c		21	16	6	CHP
1734c		5.7	5.1	1.9	HP
1735c		1.9	2.0	1.8	CHP
1736c	*narX*	4	3.3	8.1	Fused nitrate reductase
1737c	*narK2*	15	13	5	Nitrite extrusion protein
1738		27	50	24	CHP
1812c		2.4	2.0	7.8	HP
1813c		18	13	22	HP
1996		15	14	5	CHP-USPA motif
1997	*ctpF*	7	4.4	9.4	Cation transport ATPase
1998c		16	8.6	1.8	CHP
2003c		14	12	6	CHP
2004c		2	2.1	8.0	HP
2005c		7	9.2	11.1	CHP-USPA motif
2006	*otsB1*	4.1	4.0	2.6	Trehalose phosphatase
2007c	*fdxA*	16	24	18	Ferredoxin
2028c		4.8	3.5	17.3	CHP-USPA motif
2029c	*pfkB*	16	12	23	Phosphofructokinase II
2030c		19	11	48	CHP
2031c	*acr*	23	15	31	α-Crystallin
2032	*acg*	31	45	24	CHP
2623		6	7.3	27.3	CHP-USPA motif
2624c		17	20	5	CHP-USPA motif
2625c		5.6	6.9	5.3	CHP
2626c		15	41	57	CHP
2627c		11	12	15	CHP
2628		8	5.2	23.1	HP
2629		7.2	7.4	7.7	HP
2630		5	4.2	16.2	HP
2631		2.0	1.6	6.2	HP
3126c		22	23	2	HP
3127		25	36	21	CHP
3128c		12	18	2	CHP
3129		26	25	3	CHP
3130c		21	14	28	CHP
3131		5	4.6	40.4	CHP
3132c		12	9.8	12.7	Sensor histidine kinase
3133c	*dosR*	14	12	12	2-comp. response reg.
3134c		9	11	23	CHP-USPA motif

Schoolnik et al. RNA-microarray: dormancy regulon (**DosR**)

LUMC

Mtb DosR regulated latency Ags tested all across Africa. Strongly recognized across different African populations



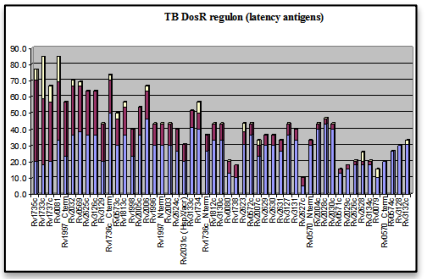


Grand Challenges in Global Health #6-74

Black et al., B4TB GC6 consortium, CVI 16: 1203, 2009

Rv1733 as candidate

Amino acid sequences of Rv1733c synthetic long peptides (SLPs).



MIATTRDREGATMITFRLRLPCRTILRV **p1-28**

FRLRLPCRTILRVFSRNPLVRGTDRLEA **p16-43**

FSRNPLVRGTDRLEAVVMLLAVTVSLLT **p29-56**

AVVMLLAVTVSLLTIPFAAAAGTAVQDS **p43-70**

IPFAAAAGT**A**VQ**D**S**R**SHVYAHQAQTRHP **p57-84**

RSHVYAHQAQTRHPATATV**I**DH**E**GVIDS **p71-98**

ATATVIDHEGVIDSNTTATSAPPRTKIT **p85-112**

NTTATSAPPRTKITVPARWVVNGIERSG **p99-126**

VPARWVVNGIERSGEVNAKPGTKSGDRV **p113-140**

SGEVNAKPGTKSGDRVGIWVDSAGQLVD **p125-152**

GIWVDSAGQLVDEPAPPARAIADAALAA **p141-168**

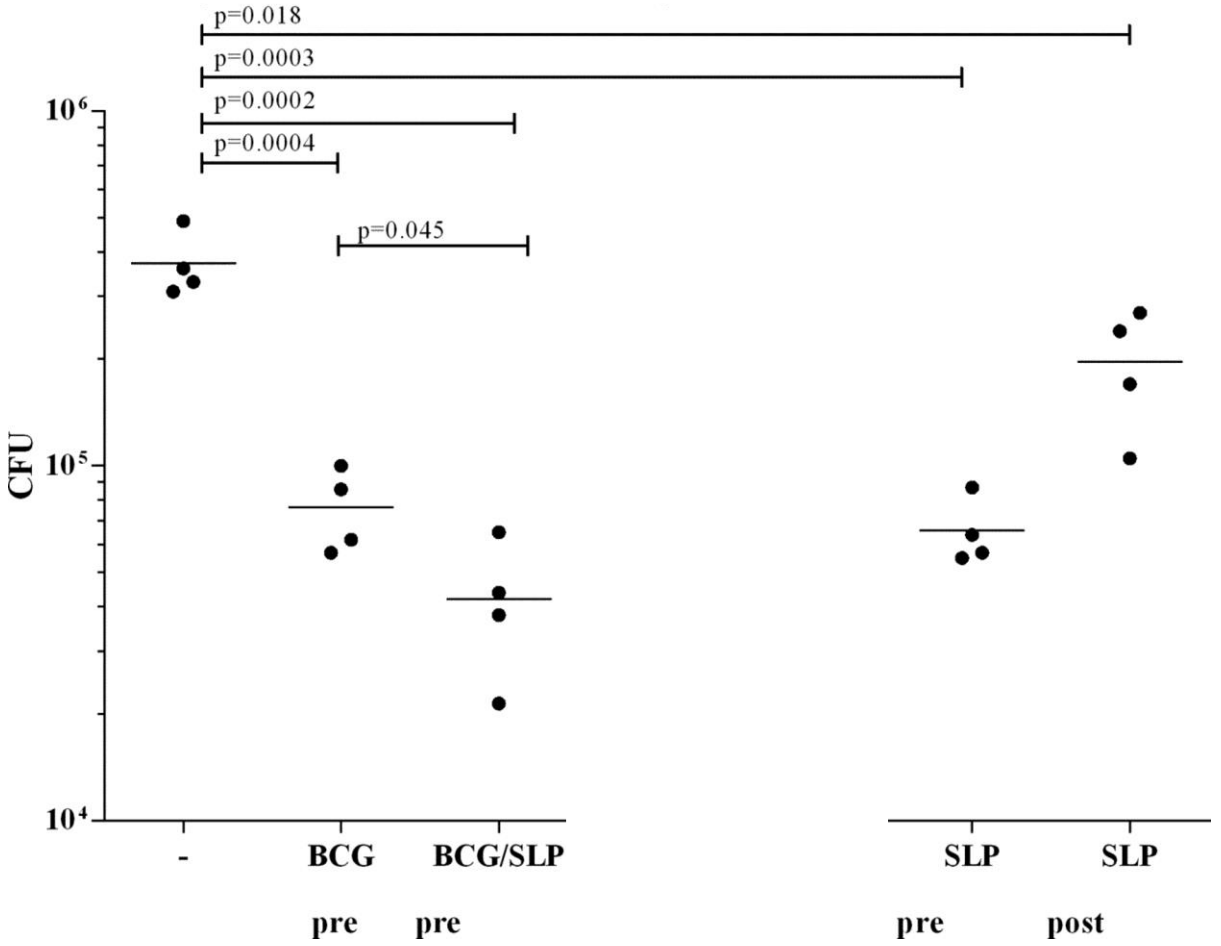
APPARAIADAALAALGLWLSVAAVAGAL **p155-182**

LGLWLSVAAVAGALLALTRAILIRVRNA **p169-196**

Mariateresa Coppola et al. Clin. Vaccine Immunol. 2015;22:1060-1069

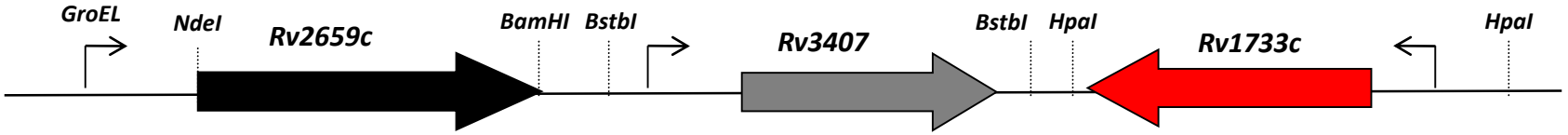
Clinical and Vaccine Immunology

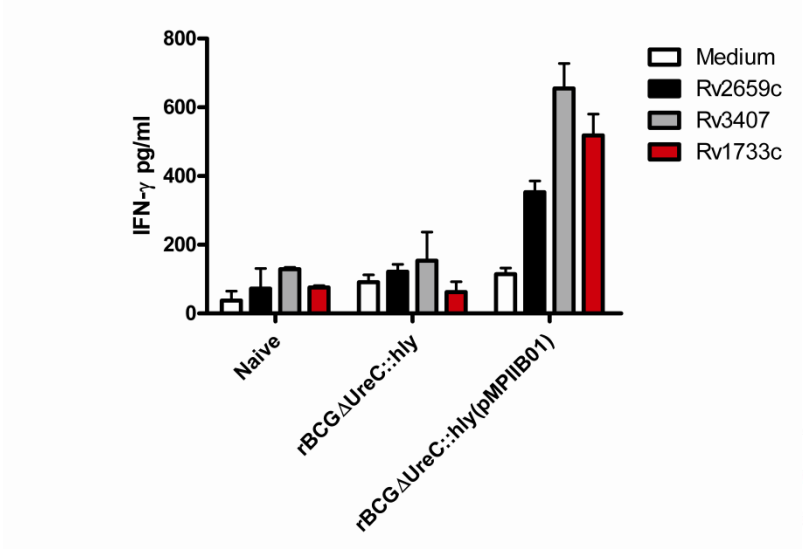
The addition of a single peptide based vaccine significantly improves BCG efficacy



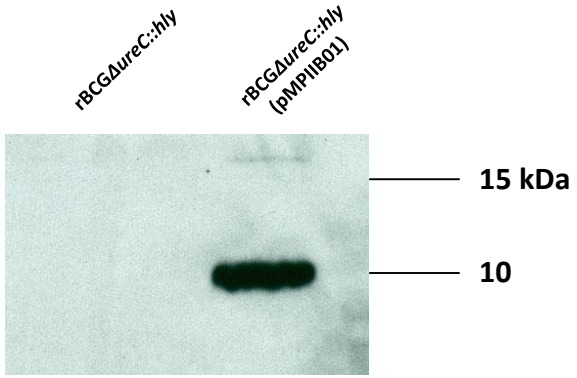
Mariateresa Coppola et al. Clin. Vaccine Immunol. 2015;22:1060-1069

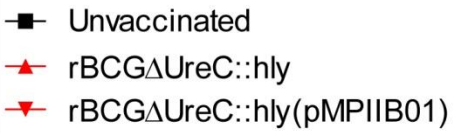
Mtb latency antigens Rv3407 + Rv1733c expressed in rBCG induce superior protection in mice

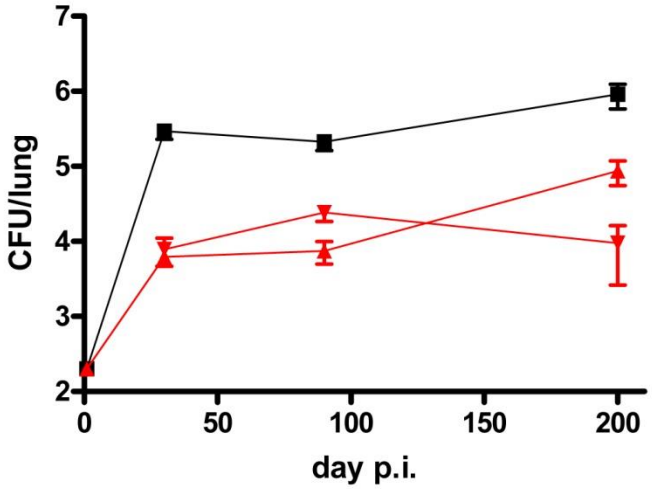




d)







Effect latency antigens on improving rBCG

Reece et al, JID 2011

Model

Phase of infection	Primo infection	Latent infection	Reactivation of infection
Phase specific Ag expression by MTB	Ag set # 1: secreted Ag, ..	Ag set #2: latency / starvation Ag	Ag set #3: reactivation-Ag
BCG priming	++	-	?
new vaccines (prime/boost)		+	+

This first example demonstrates:

based on a fundamental understanding of host pathogen interactions in TB, it is possible to identify candidate vaccine antigens that can improve BCG

Leyten et al 2006; Lin et al 2007; Commandeur et al 2011a; Schuck et al 2009; Goletti et al 2009; Black et al, 2009; Coppola et al, CVI 2015.

A second example, applicable to many bacterial infectious diseases.

Starting with a very simple hypothesis:

Mtb antigens targeted by vaccination must be expressed *during infection* in the primary target organ, which is the *lungs* of *susceptible* individuals

Susanna Commandeur, Krista E. van Meijgaarden, Corine Prins, Alexander V. Pichugin, Karin Dijkman, Susan J. F. van den Eeden, Annemieke H. Friggen, Kees L. M. C. Franken, Gregory Dolganov, Igor Kramnik, Gary K. Schoolnik, Fredrik Oftung, Gro Ellen Korsvold, Annemieke Geluk and Tom H. M. Ottenhoff

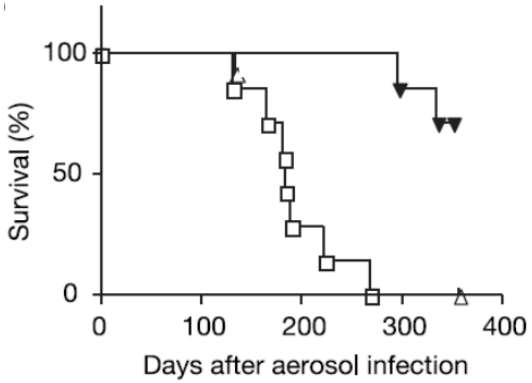
J. Immunology 190:1659-1671 (2013)

LUMC

Super TB susceptible (sst1) mice

C57BL/6J (B6)

Resistant to TB



C3HeB/FeJ

Highly susceptible to TB

Super-susceptibility to tuberculosis 1 locus (sst1) (chr 1)

C3H-sst1 locus generates caseous necrosis in *Mtb* lung granulomas

Intracellular pathogen resistance 1 **(Ipr1)(Pan et al, Nature 2005)**

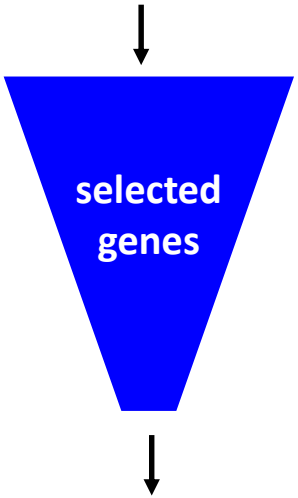
A genome wide unbiased antigen discovery approach

1. Mtb RNA isolated directly from the lungs of infected mice
2. Four congenic mouse strains, ranging from hyper susceptible (sst1) to genetically resistant
3. qRT-PCR perfomed on all first genes of all predicted Mtb operons (n=2170)
4. Data sets -> analysis:
5. Selection of genes that were *persistently and highly expressed*

   (in multiple different genetic backgrounds)
6. Recombinant antigen / peptide production and testing in human T cell assays

Classification of newly identified IVE TB antigens

2170 Mtb genes



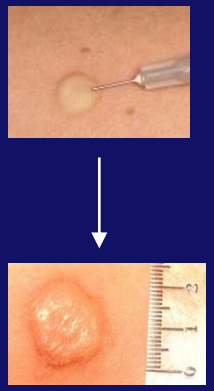
19 genes selected

<u>Previously identified</u> (*in vitro*)	
* Dos Regulon genes	**n = 2**
* Enduring hypoxic response (EHR)	**n = 6**
/ Starvation	
<u>Newly identified</u>	**n = 11**

Immunogenicity of IVE-TB antigens (human)

IFN-γ responses to TB disease associated antigens in mantoux positive individuals

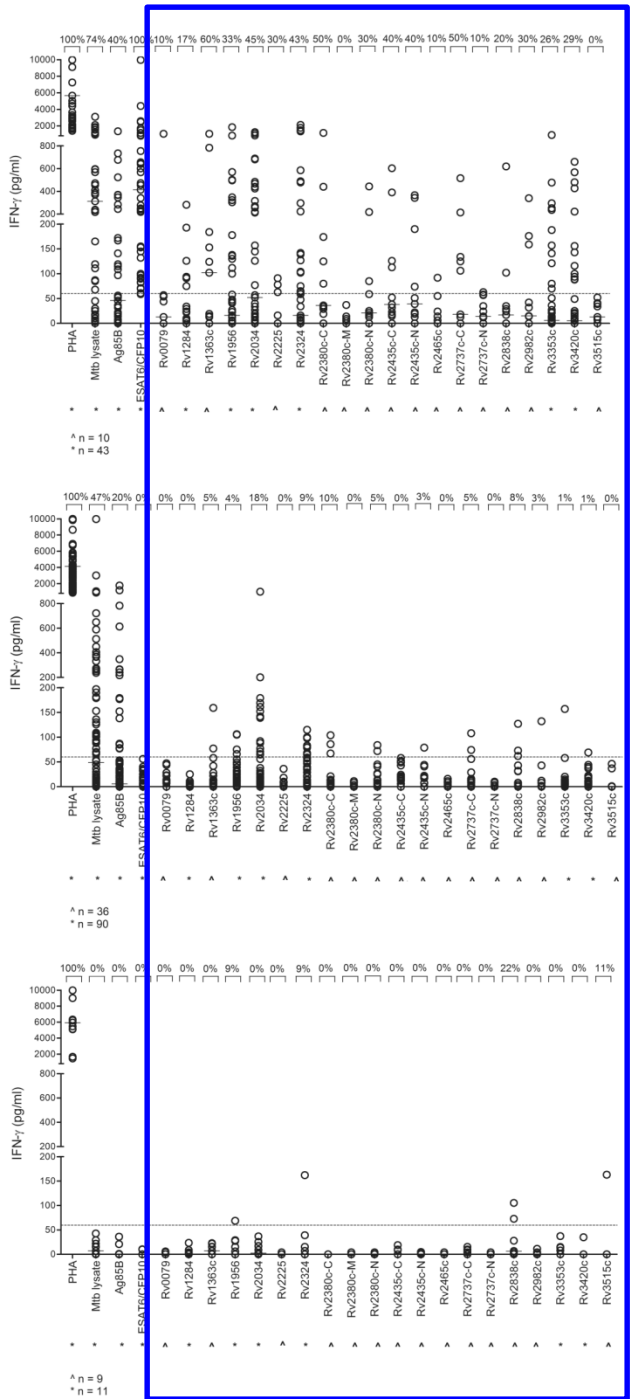
Exposed to TB index case ⟶ positive mantoux



Follow up 2 years (0, 3, 6, 12 and 24 months)

Whole blood diluted 1:10 incubated with recombinant IVE-TB proteins for 6 days

Immunogenicity of IVE-TB antigens (human)



E/C positive, Mtb positive donors

E/C negative, Mtb positive donors

Mtb negative donors

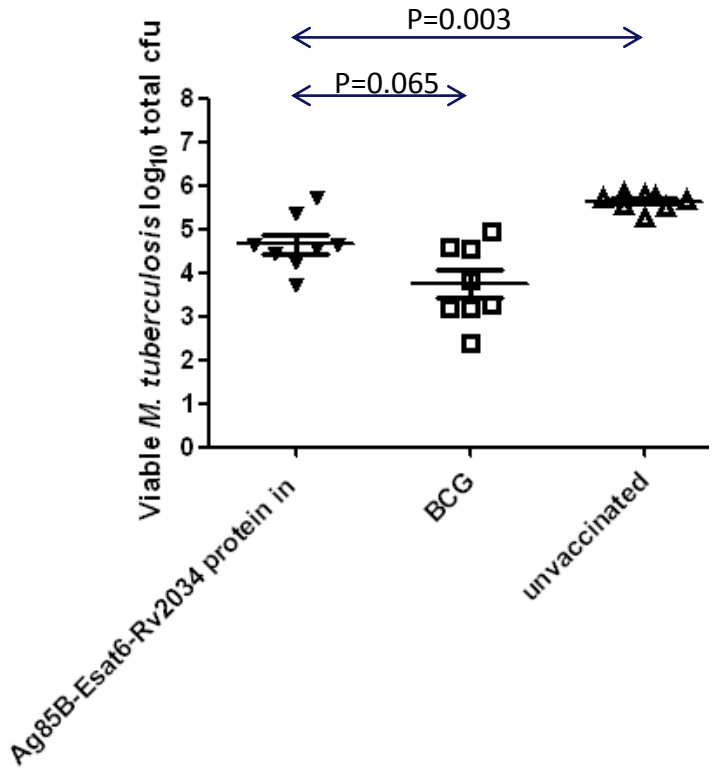
WBA assay

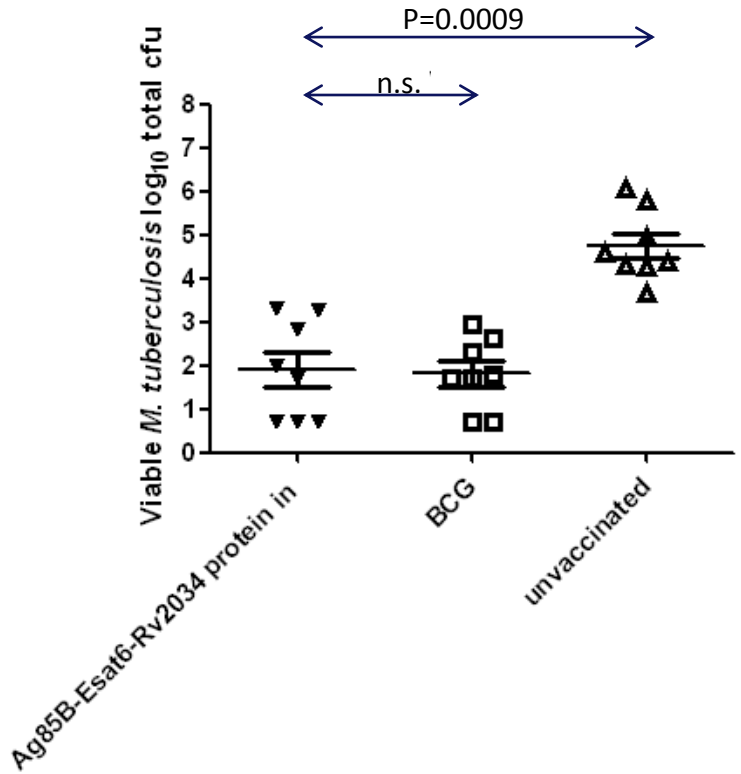
Public Health England

Vaccine evaluation in guinea pigs

Ag85B-esat6-Rv2034/Caf09 equivalence to BCG

Bacterial load





Commandeur et al, Vaccine 2015

Summary of *Mtb* T-cell antigen discovery in humans

Candidate gene

1. Mtb Latency antigens
   - *Mtb* DosR regulon encoded antigens:
     - Leyten et al 2006; Lin et al 2007; Commandeur et al 2011a; Schuck et al 2009; Goletti et al 2009; Black et al, 2009; Arroyo et al, 2015; other papers
2. Mtb Rpf antigens
   - Commandeur et al 2011b; Schuck et al 2009; …
3. Mtb secreted antigens
   - *Mtb* esx antigens (SSI group's papers)

Genome wide

4. Mtb HLA-class Ia presented CD8 T-cell epitopes
   - *Mtb* genome wide algorithm-based inquiry for HLA class Ia ligands
     - Tang et al, J. Immunol. 2010; library approach Lewinsohns lab
5. Mtb HLA-class Ib presented CD8 T-cell epitopes
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Summary of *Mtb* T-cell antigen discovery in humans

Candidate gene

Genome wide

1. Mtb Latency antigens
   - *Mtb* dosR Ag are potently recognized by human T-cells across different TB exposed populations
     - Multiple epitopes, presented by diverse HLA class I and II molecules
     - Mono- as well as multi-functional CD4 and CD8 T-cell responses
   - Recognition is invariably associated with latent infection in humans and mice
     - -> Vaccine antigens
     - -> Biomarkers of immune associated infection control
   - No response to latency antigens induced by BCG (humans, mice)
     - Partial explanation for BCG's inefficiency? -> possibilities for repair by boosting

   - *Leyten et al 2006; Lin et al 2007; Commandeur et al 2011a; Schuck et al 2009; Goletti et al 2009; Black et al, 2009; Coppola et al, CVI 2015*

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3. Mtb secreted antigens
   - *Mtb* esx antigens (SSI group's papers)

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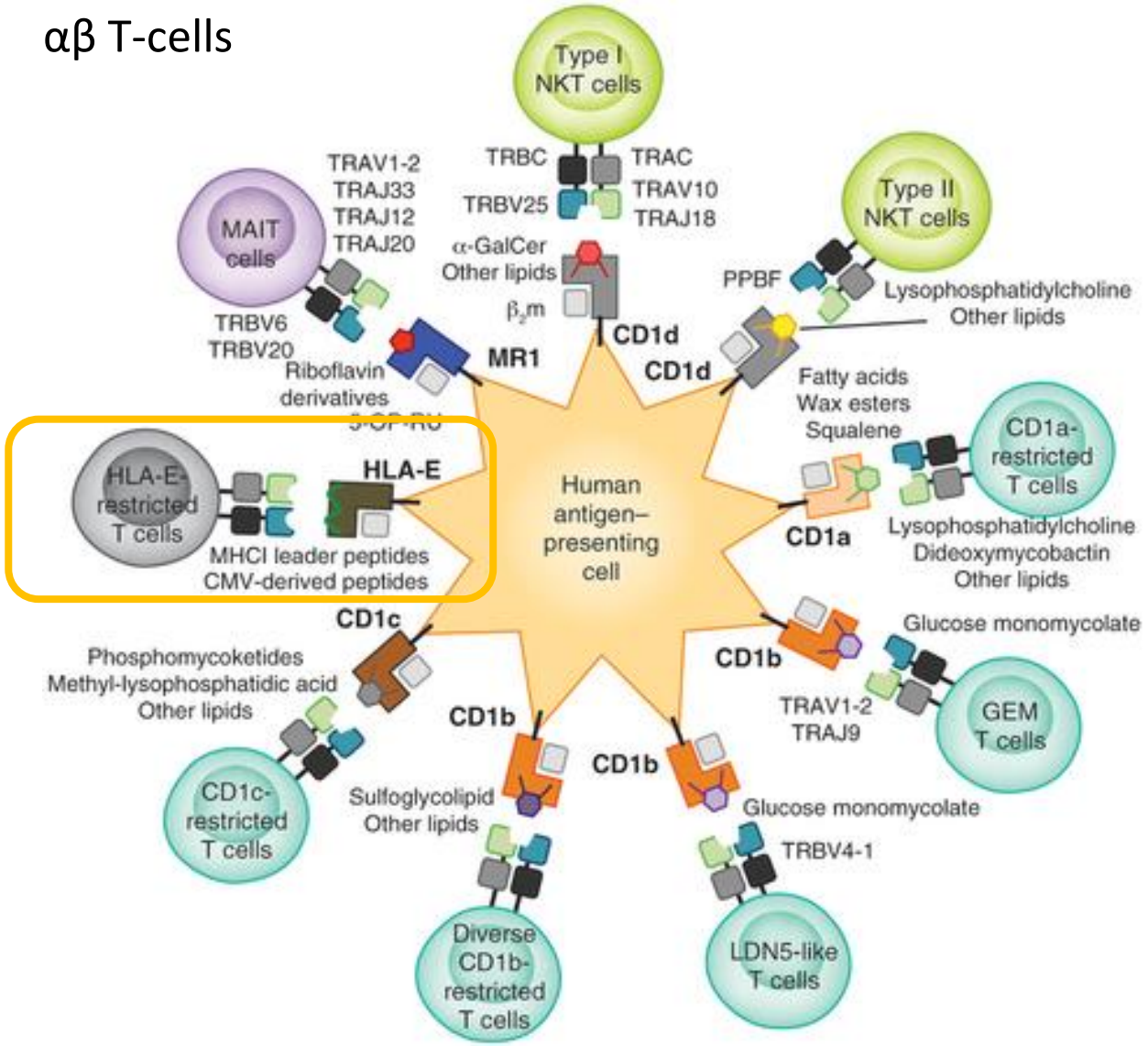
Unconventional immunity – donor unrestricted T-cells

αβ T-cells



DI Godfrey, AP Uldrich, J McCluskey, J Rossjohn & DB Moody
Nature Immunology 16, 1114–1123 (2015) doi:10.1038/ni.3298

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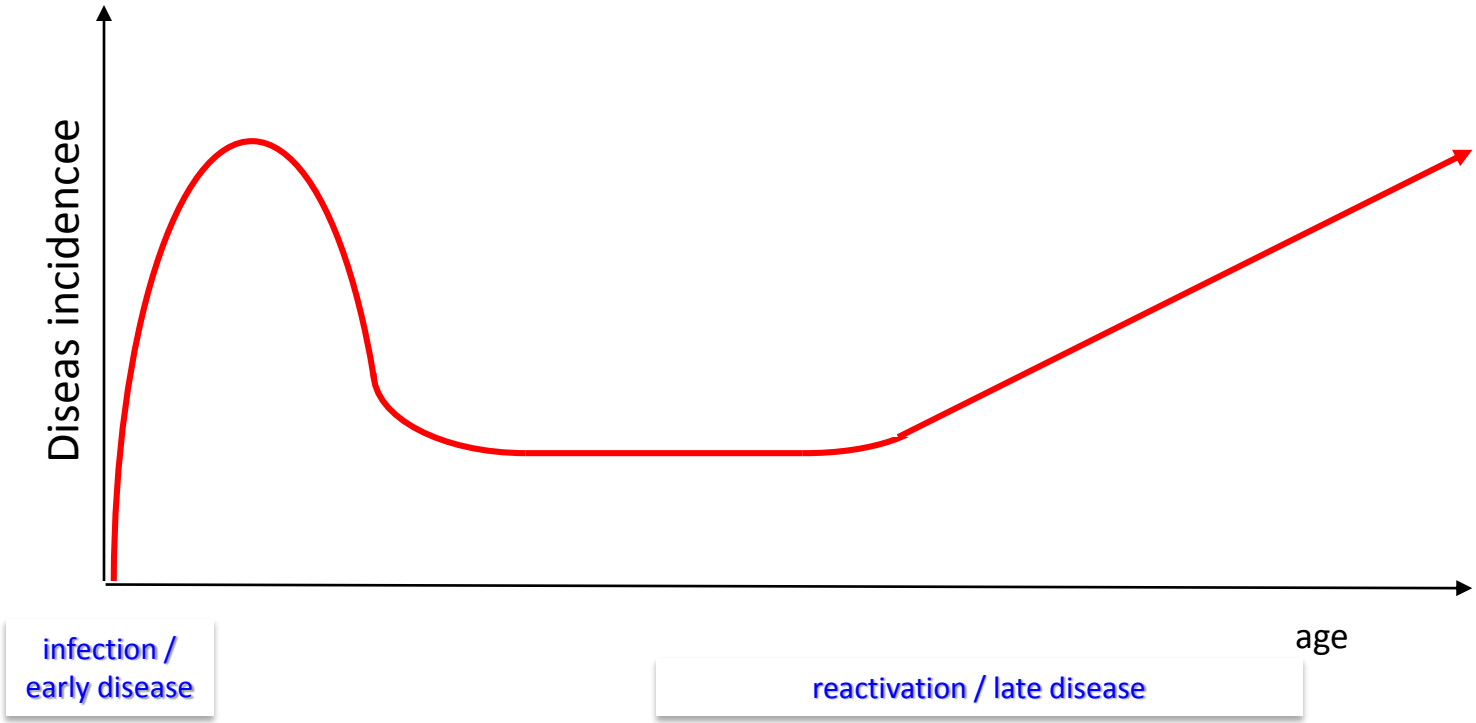
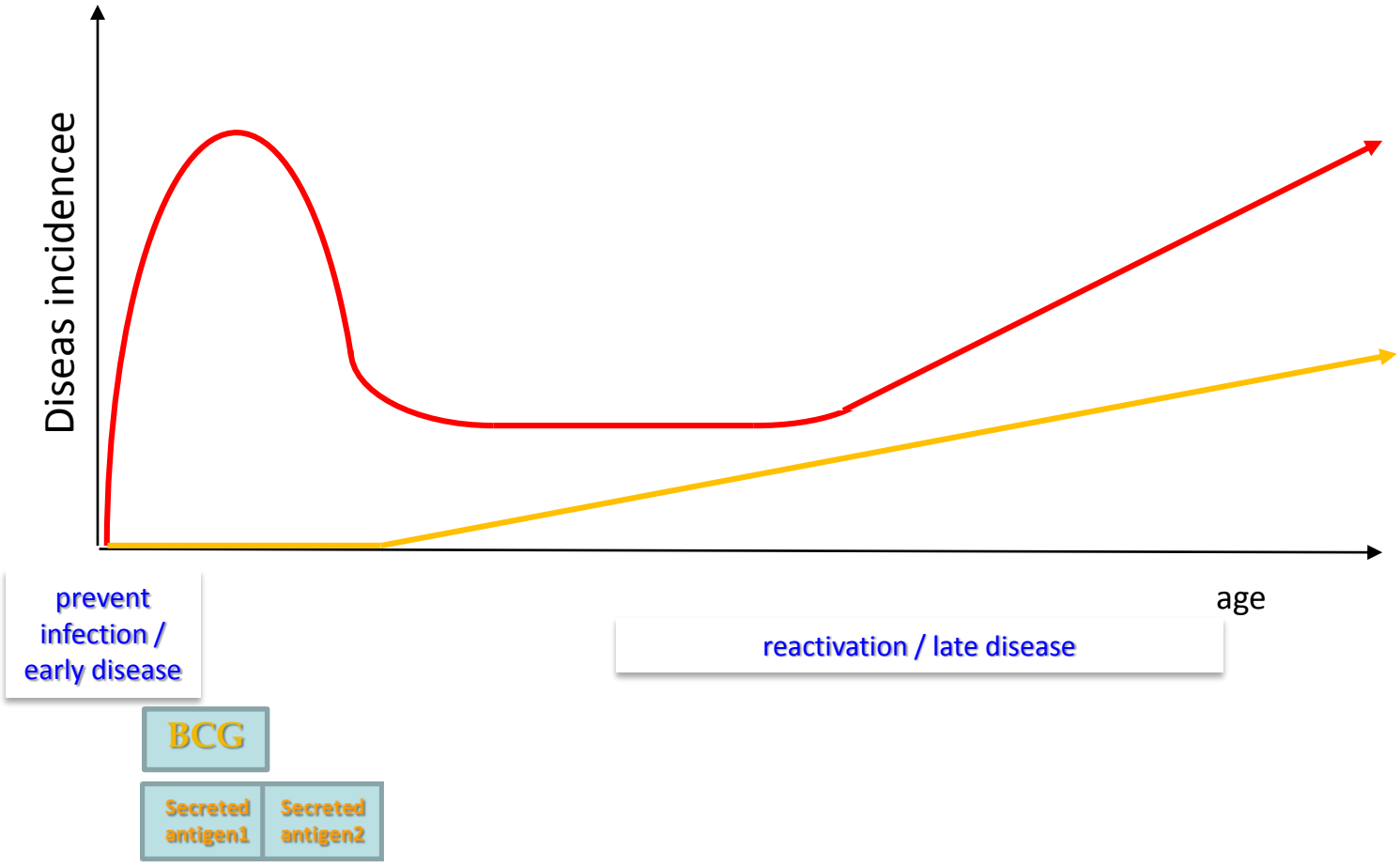
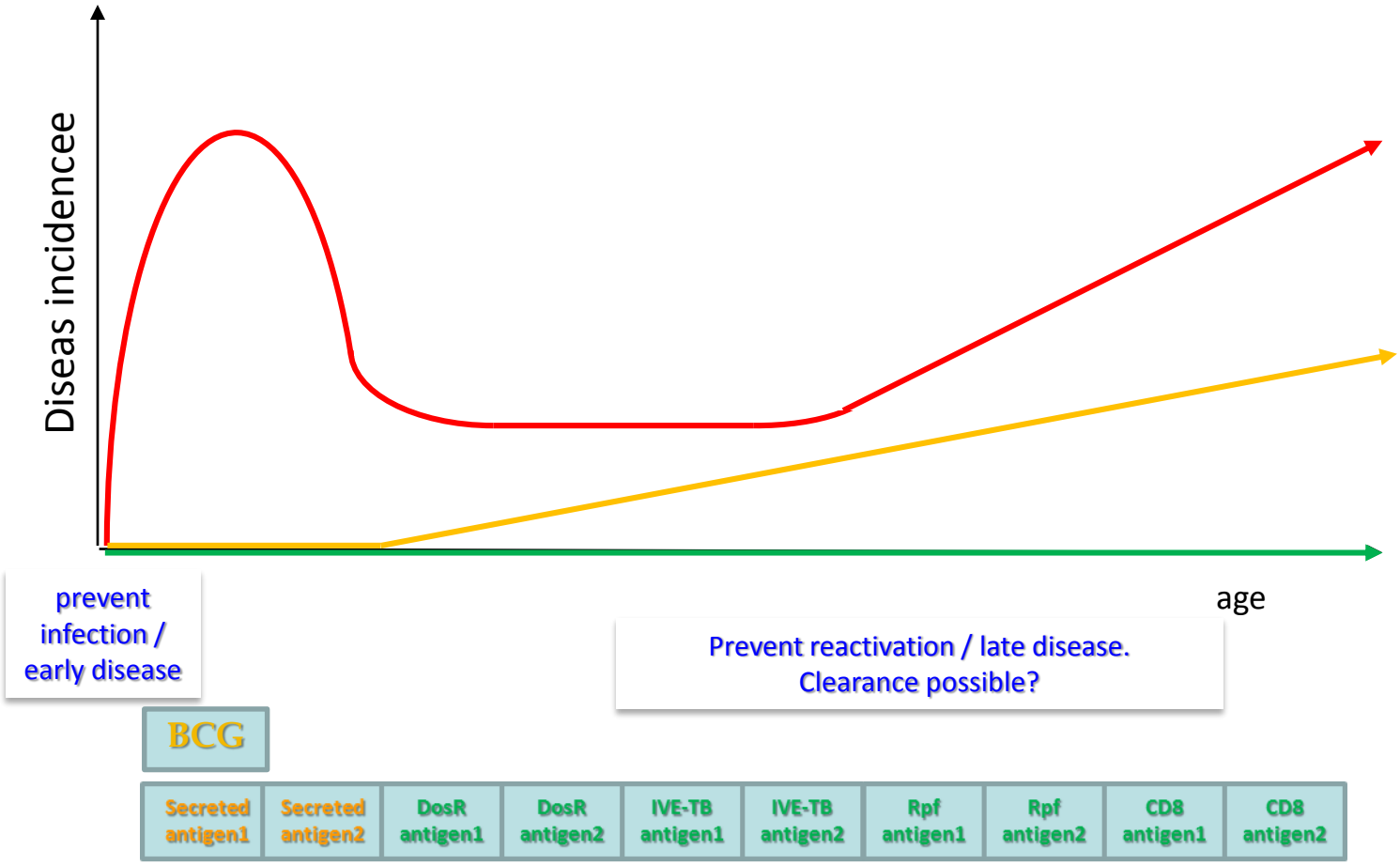
Multi-phase, multi-component TB vaccines



Multi-phase, multi-component TB vaccines



Multi-phase, multi-component TB vaccines



Conclusions

There are no effective vaccines against TB yet

The biology of TB infection is complex and involves extensive immune evasion and inflammation, both compromising host immunity

New antigen discovery approaches are yielding novel classes of Mtb antigens, with promising vaccine potential. This is much needed after the MVA85A failure

Latency as well as IVE-TB antigens are promising. Can induce protection in humanized mice and guinea pigs

IVE-TB approaches would have predicted MVA85A vaccine failure

IVE antigen discovery approaches can be applied to other infectious diseases

HLA-E restricted CD8+ T cells can inhibit Mtb in macrophages, despite being Th2 like and helping B cells through IL4

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Mars

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