

Mucosal BCG Delivery

Enhancing adaptive and innate immunity towards efficacious revaccination

Frank Verreck

10 October 2024

7th Global Forum | Rio de Janeiro



Biomedical Primate Research Centre

Rijswijk • The Netherlands • www.bprc.nl

Committed to health research and alternatives

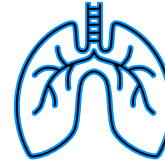
BCG: The Shortcoming of Successful Vaccine

ever since 1921 ...



Mycobacterium bovis
bacille Calmette-Guérin
nowadays, intradermally
injected

pulmonary
tuberculosis



disseminated
meningitis



BCG efficacy

BONUS: heterologous 'trained innate' immunity

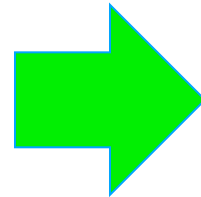
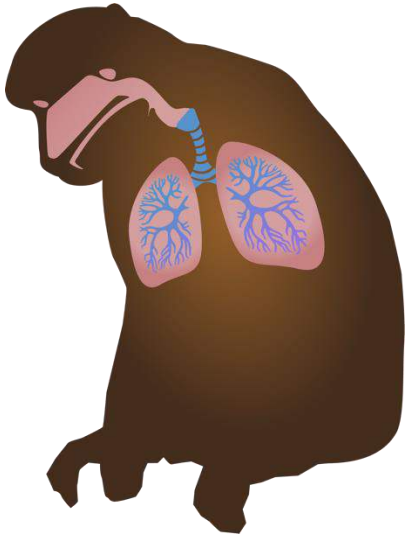


over 4000
deaths today!

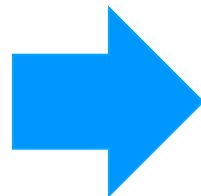


Today's Presentation & Take-Away

Respiratory mucosal BCG delivery shows superior efficacy
in naive and in id.BCG primed animals



showing signatures of protective immunity
AS AN EXPLORATORY POSITIVE CONTROL



suitable for improved revaccination
AS AN ENCOURAGING VACCINE R&D PERSPECTIVE

The TRINITY of infectious disease pathogenesis

issues to consider towards NHP TB or any infectious disease modelling



Intrinsic pathogen factors

virulence, drug resistance

Mtb Erdman K01



Intrinsic host factors

genetic susceptibility

Rhesus macaques



Extrinsic host factors

environmental & disease risk factors

Clinical grade BCG

- **Pre-exposure** | naive, BCG, LTBI, NTM,
- **Coinfection** | HIV
- **Comorbidity** | diabetes
- **Others** | malnutrition, ageing, smoking, alcohol, immunosuppression, immunotherapy (anti-TNFa)

Single High Dose Challenging

Evaluating candidates against the standard of intradermal BCG (01)



Rhesus

Naïve | adolescent,
Chinese-type ♀

Mtb Erdman

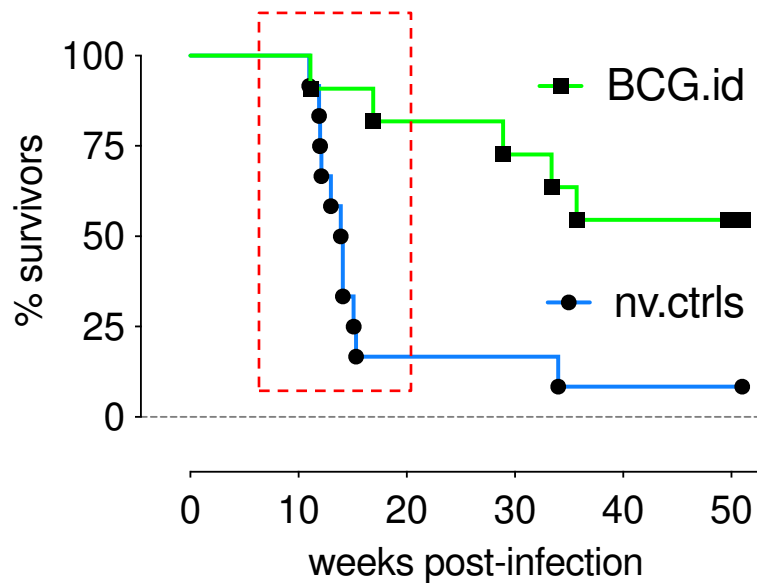
single high dose
(500 CFU) eb.

BCG

Danish 1331,
standard human
dose

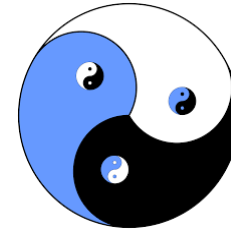


50% Vaccine Efficacy



1 year follow up,
towards reaching a humane endpoint

FAW Verreck et al (unpublished)



Rhesus

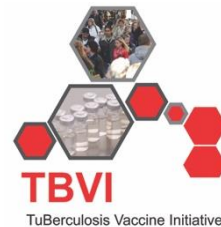
Naïve | adults,
Chinese- & Indian-
type ♀

Mtb Erdman

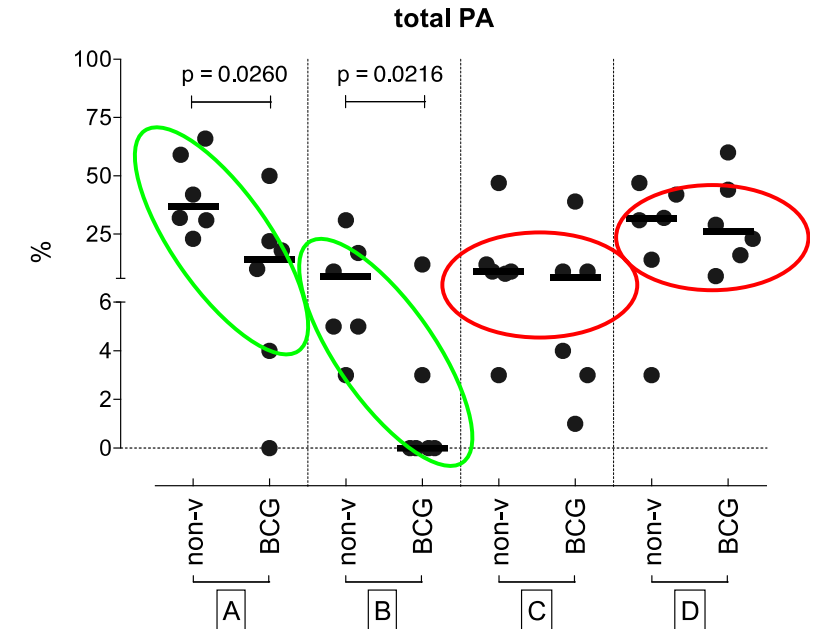
single high dose
(500 CFU) eb.

BCG

Danish 1331,
standard human
dose



BCG Variability | in retrospect

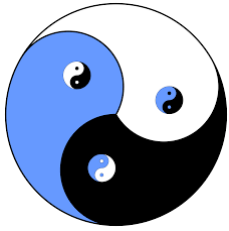


3-4 months follow up,
fixed endpoint

FAW Verreck et al Tuberculosis 2017



Evaluating candidates against the standard of intradermal BCG (02)



Rhesus

Naïve | adults,
Chinese- & Indian
type ♂

Mtb Erdman

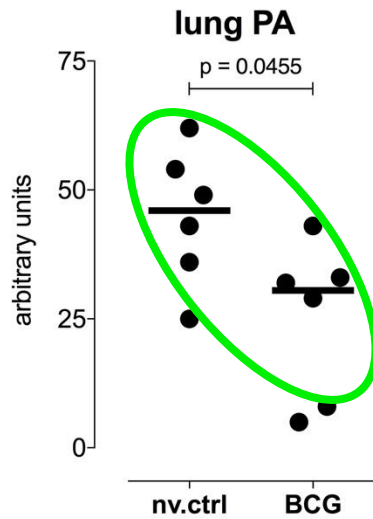
single high dose
(500 CFU) e.b.

BCG

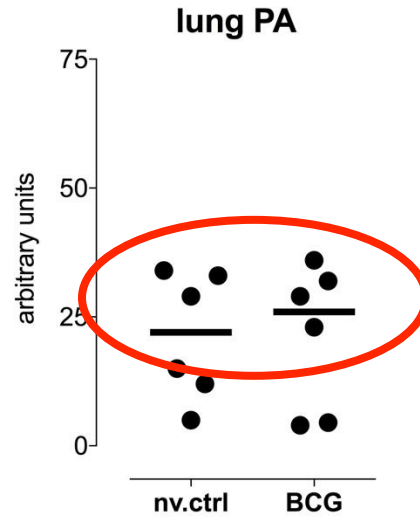
Danish 1331,
standard human
dose



BCG Variability | in prospect



Cohort X



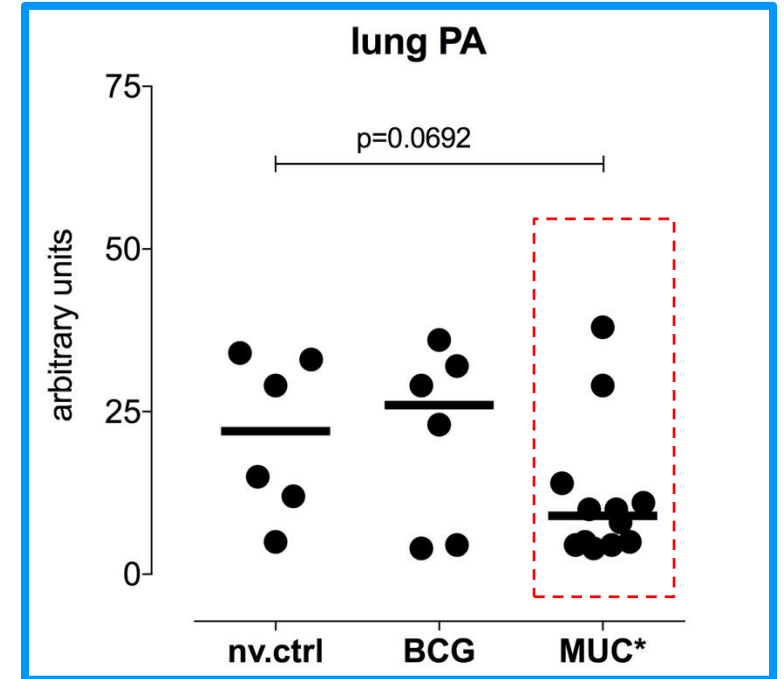
Cohort Y

a fully randomised, prospective study

FAW Verreck et al Tuberculosis 2017



Mucosal BCG 'protects' where id fails



* single dose endobronchially ('eb'; n=6)
or half a dose each 'id' and 'eb' (n=6)

FAW Verreck et al Tuberculosis 2017



aerosol inhaled & intravenous BCG provide superior protection



1973 WR Barclay et al
Am Rev Respir Dis

Protection of Monkeys against Airborne Tuberculosis
by Aerosol Vaccination with Bacillus Calmette-Guerin^{1,2}

WILLIAM R. BARCLAY,² WILLIAM M. BUSEY, DAN W. DALGARD,
ROBERT C. GOOD, BERNARD W. JANICKI,² JOHN E. KASIK,² EDGAR RIBI,²
CHARLES E. ULRICH, and EMANUEL WOLINSKY²

TABLE 3
PATHOLOGIC EVIDENCE OF TUBERCULOSIS

Abnormalities	id	iv	ae	nv	E
Granulomas in lungs	3/10 ¹	0/10	1/10	0/10	0/10
Liquefaction necrosis in	0/10	0/10	0/10	2/10	0/10
Mycobacteria in lungs	5/10	0/10	2/10	8/10	0/10
Granulomas in tracheobron	5/10	0/10	0/10	8/10	0/10
Mycobacteria in tracheobron	5/10	1/10	1/10	7/10	0/10
Granulomas in spleen	0/10	0/10	0/10	2/10	0/10
Mycobacteria in spleen	0/10	0/10	0/10	1/10	0/10
Granulomas in liver	0/10	0/10	0/10	2/10	0/10
Mycobacteria in liver	0/10	0/10	0/10	0/10	0/10
Granulomas in kidneys	0/10	0/10	0/10	1/10	0/10
Mycobacteria in kidneys	0/10	0/10	0/10	0/10	0/10

¹See table 1 for definition of groups.
²Numerator represents number of animals showing the abnormality; denominator represents the total number of animals in the group.

2017 FAW Verreck et al
Tuberculosis



1970

1980

1990

2000

2010

2020



Alternative Routing of BCG in NHP

> 40 years after Anacker and Barclay and colleagues

Mucosal delivery :

2016 | D Kaushal et al. Nat Comm

2017 | FAW Verreck et al Tuberculosis

2019 | K Dijkman et al Nat Medicine

.... and more

Dhiraj K Singh
Presentation OA-18
at this meeting



2010

2020

Intravenous delivery:

2016 | S Sharpe et al. Tuberculosis

2020 | PA Darrah et al Nature

2021 | Edward Irvine et al Nat Immunology

.... and more

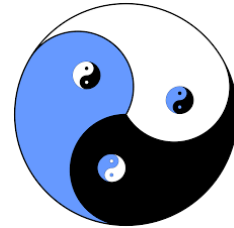
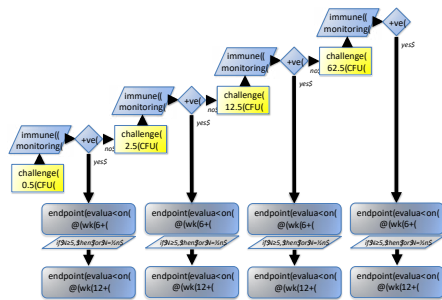
With finding no candidate vaccine better than id.BCG
can we refine our NHP challenge model?

... and further investigate muc.BCG

Superior Efficacy of muc.BCG over Standard id.BCG

repeated limiting dose (RLD) challenging suggests a Prevention of Infection signal by delayed IGRA conversion

Mtb dose escalation



Rhesus macaques

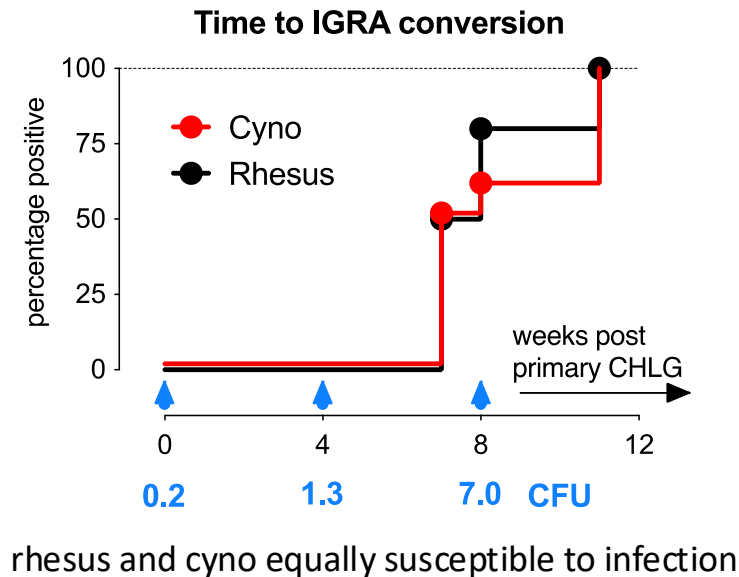
naive, adult, Indian-type males

Mtb Erdman

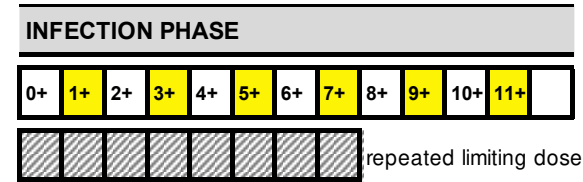
repeated limiting dose | 8x 1 CFU endobronchially

BCG

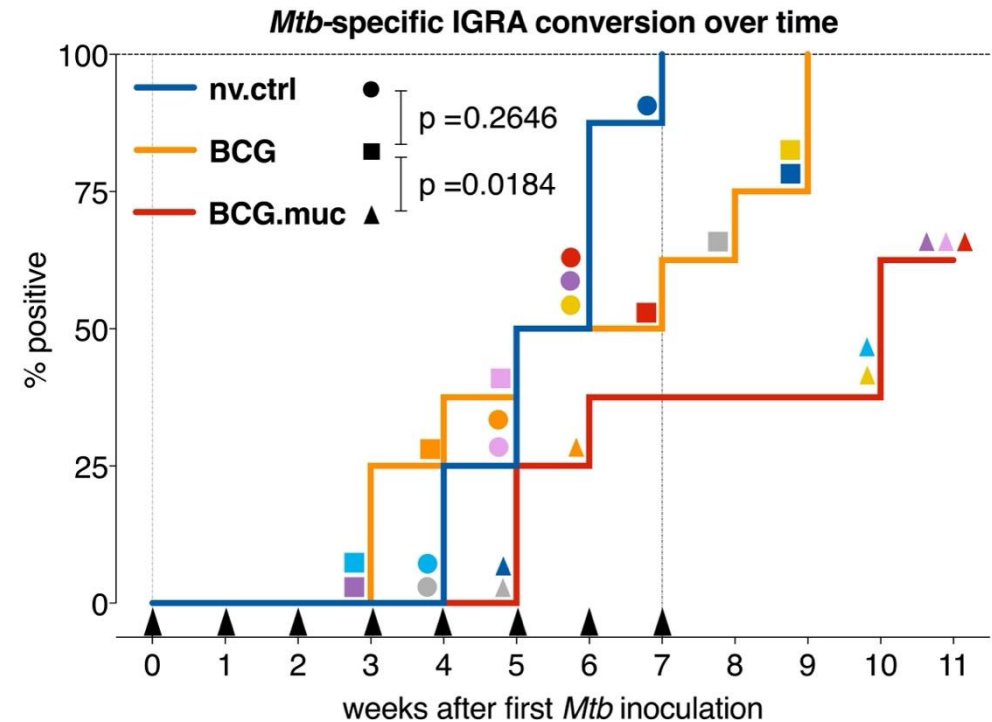
strain Sofia



K Dijkman et al FIMMU 2019



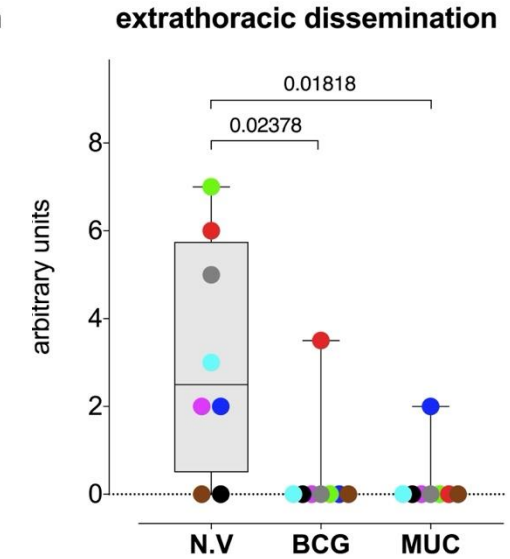
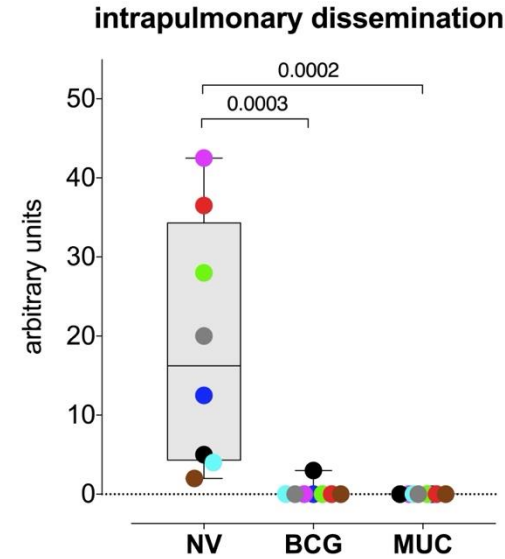
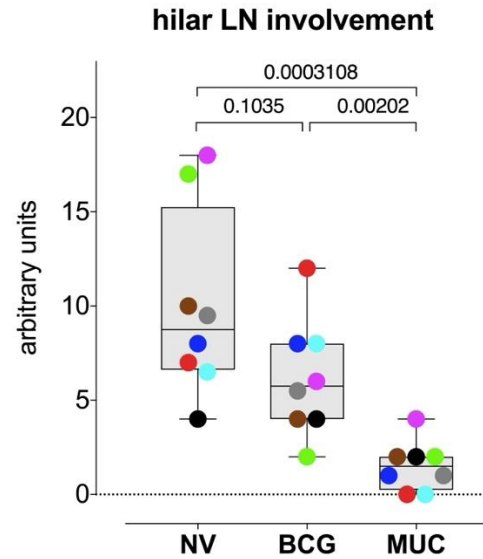
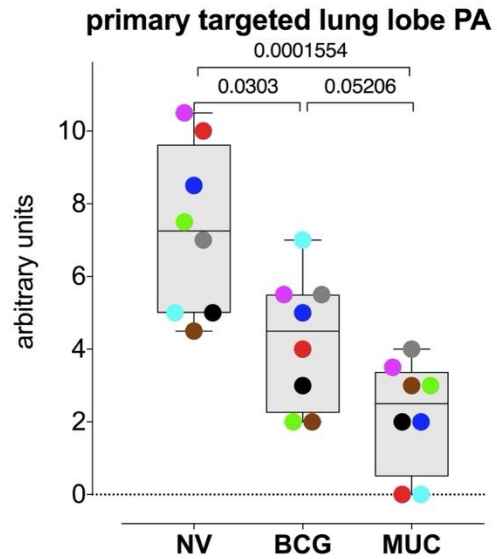
- non-vaccinated ctrls
- id.BCG ctrls
- muc.BCG



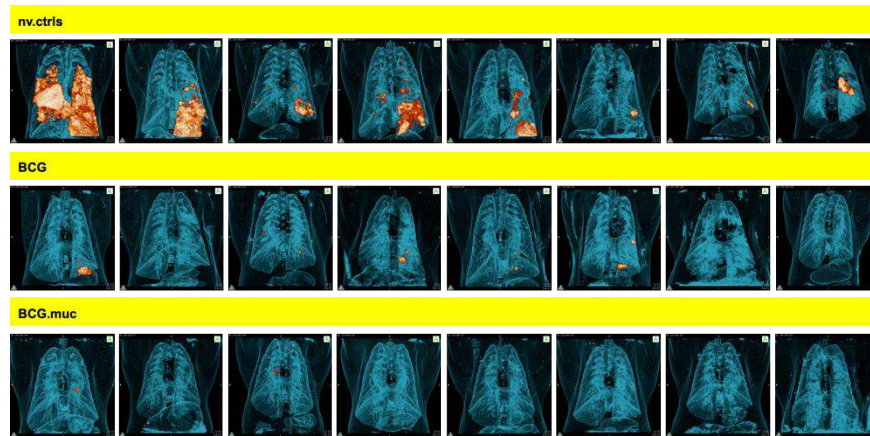
K Dijkman et al NMED 2019



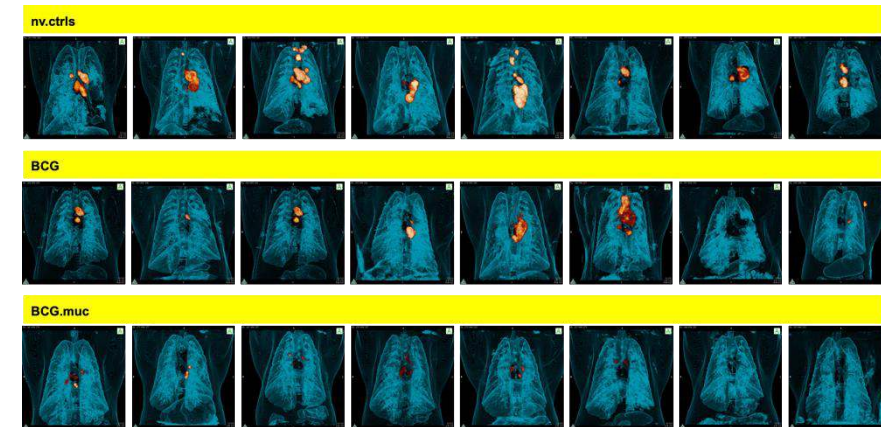
Pulmonary mucosal BCG significantly reduces pulmonary TB disease



Lung PET-CT
1 wk after RLD.Mtb



hil.LN PET-CT
1 wk after RLD.Mtb



K. Dijkman
et al. NMED
2019

Mucosal BCG Induces Strong, Distinctive Th1/Th17 Responses in the Airways

endobronchial instillation of BCG versus standard intradermal injection | repeated limiting dose *M.tb* challenge

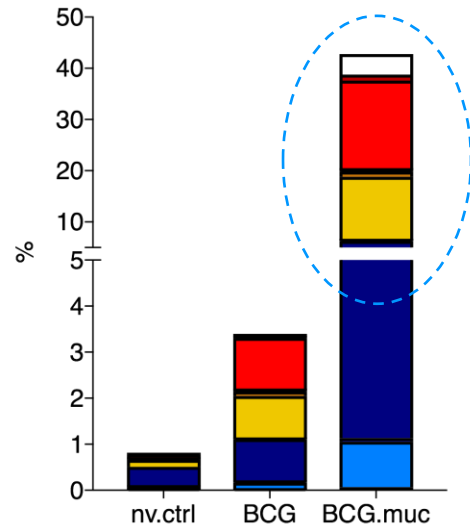
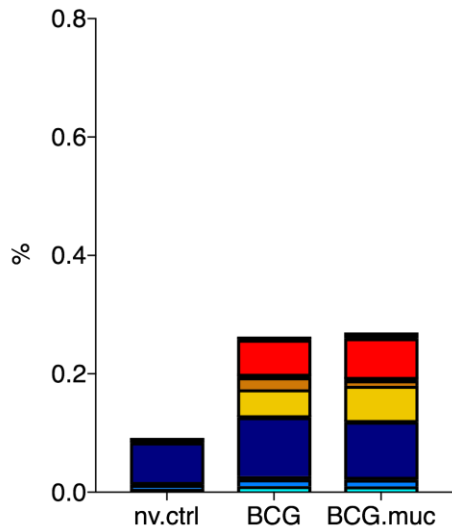
after vaccination

Blood

Airway

CD4+ cytokine production PBMC

CD4+ cytokine production BAL



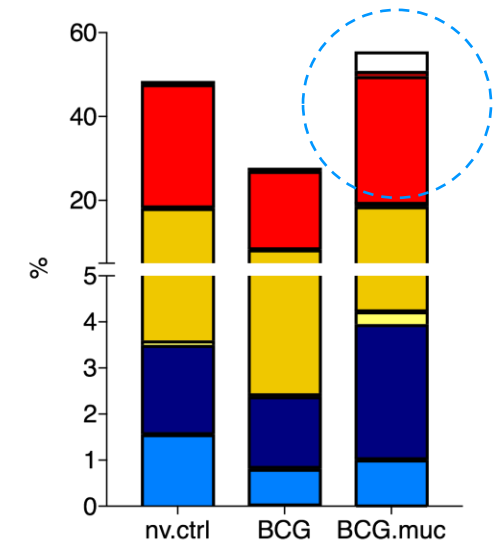
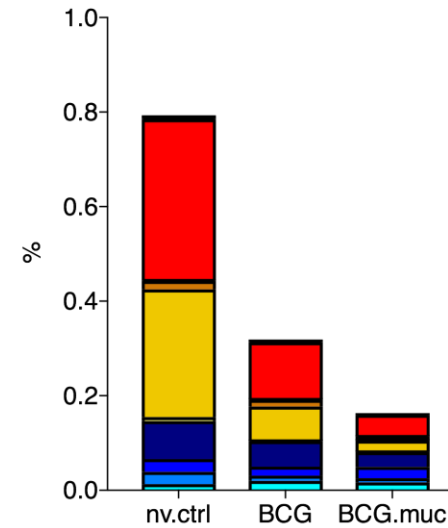
after infection

Blood

Airway

CD4+ cytokine production PBMC

CD4+ cytokine production BAL



IFN γ + IL2+ IL17A+ TNF α +

IL17A+ TNF α +

TNF α +

IFN γ + IL17A+ TNF α +

IL2+ TNF α +

IL17A+

IFN γ + IL2+ TNF α +

IL2+ IL17A+

IFN γ +

IFN γ + IL2+ IL17A+

IFN γ + TNF α +

IL2+

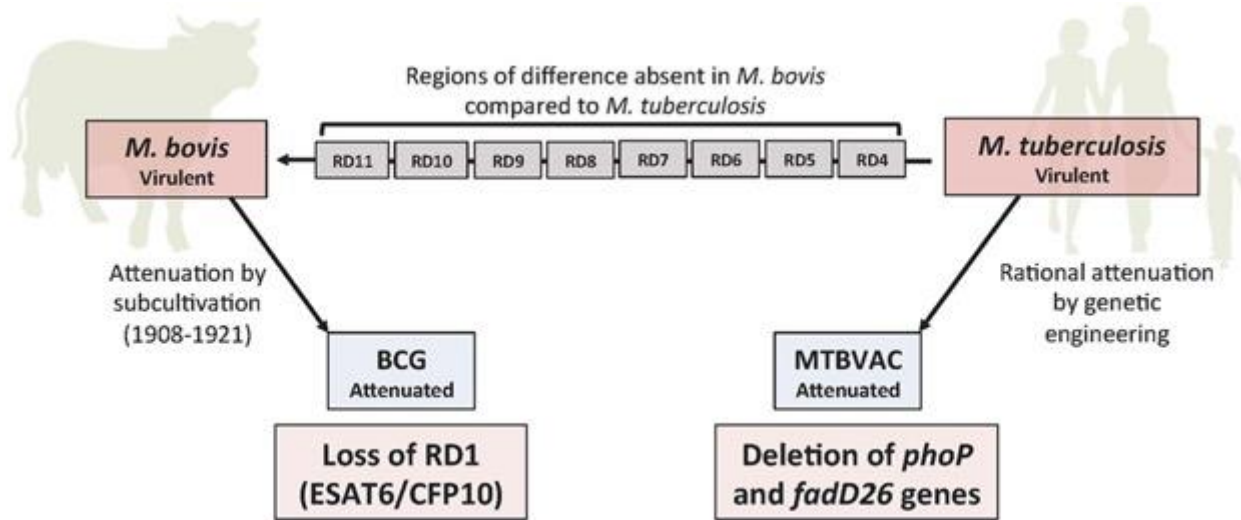
IL2+ IL17A+ TNF α +

IFN γ + IL17A+

MTBVAC, a live attenuated *Mtb* candidate vaccine

a genetically modified, *phoP*-/*fad26*-deficient strain of *M. tuberculosis*

MTBVAC



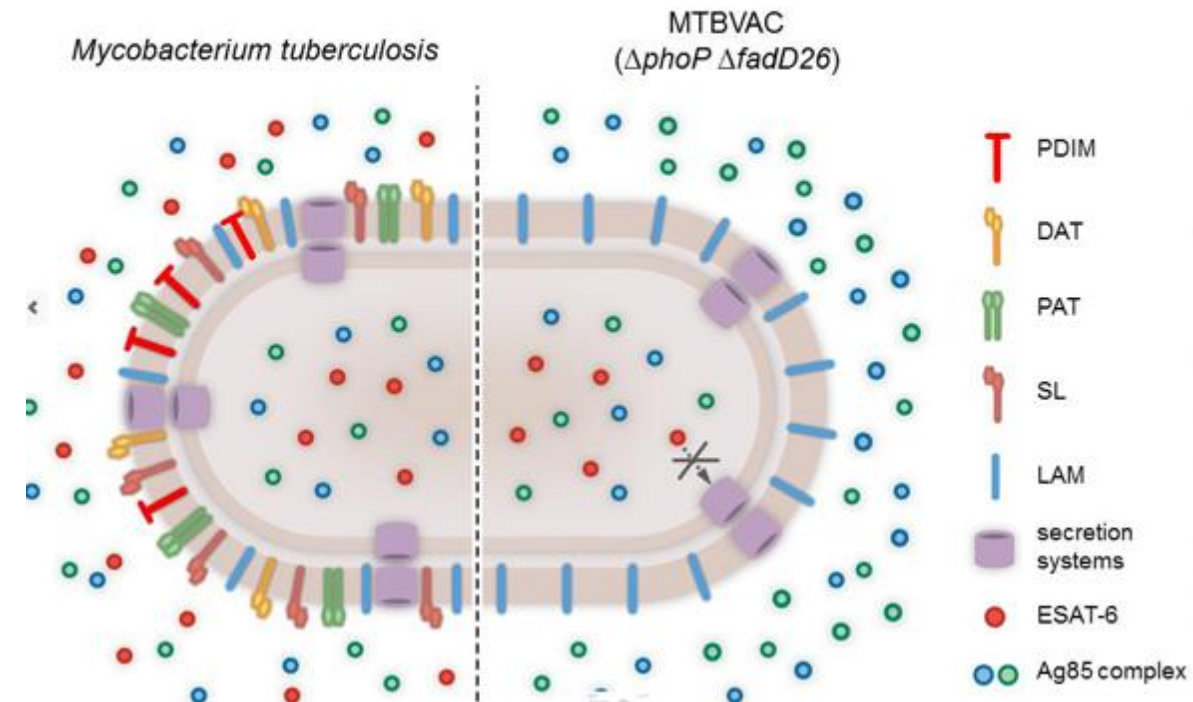
J Gonzales-Asensio et al FIMMU 2017

- Safe & immunogenic in infants and adults
- At least as protective as or better than BCG in preclinical models (mouse, guinea pig and NHP)



Universidad
Zaragoza

BIOFABRI



Immunogenicity of MTBVAC in NHP

A comparative study of MTBVAC vs BCG, by pulmonary mucosal (MUC) vs standard intradermal (ID) vaccination



Rhesus macaques

naive, Indian-type, adult ♂

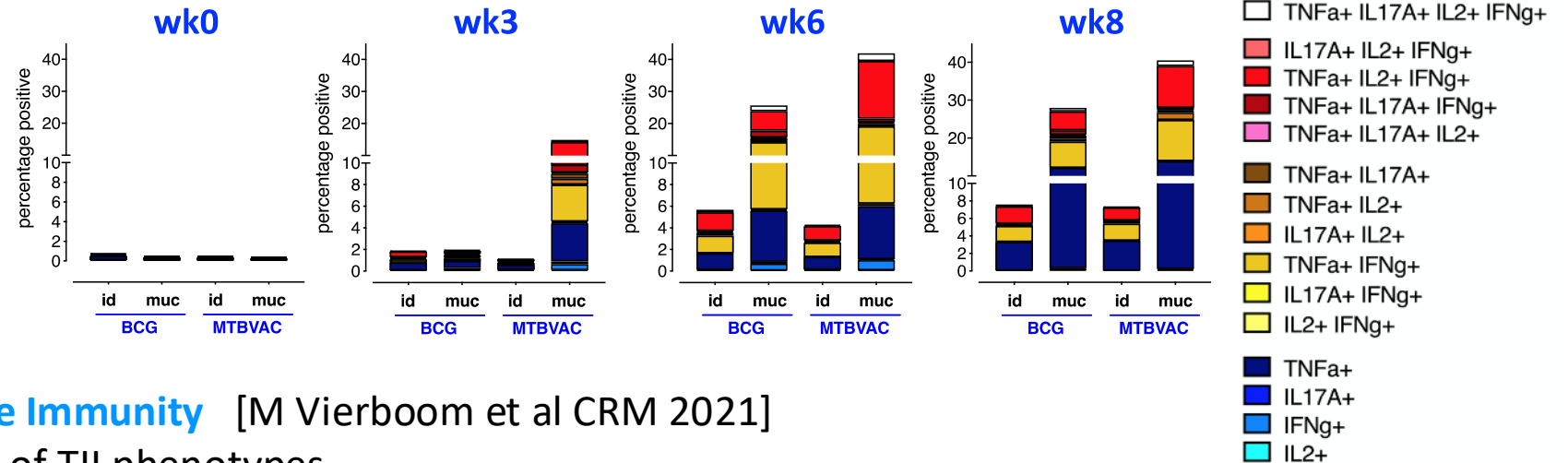
Mtb Erdman

does not apply

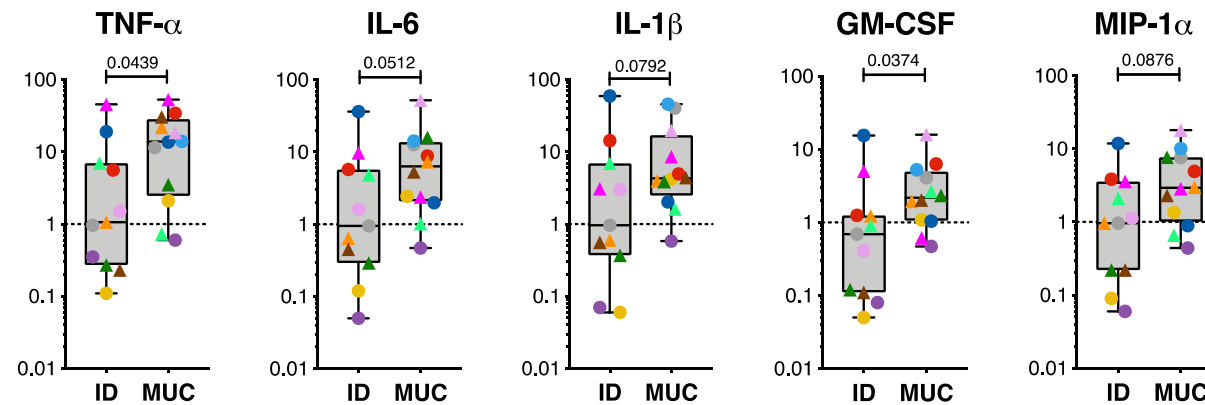
BCG

Danish 1331

Tissue Resident Memory [K Dijkman et al CRM 2021] Induction of polyfunctional CD4+ in the airways (BALC)



Trained Innate Immunity [M Vierboom et al CRM 2021] Enhancement of TII phenotypes



Attenuated *M.tb* - i.c. MTBVAC - Delivered by Aerosol

a translational pilot study in the NHP RLD *M.tb* Challenge model



Rhesus macaques

naive, Indian-type, adult males

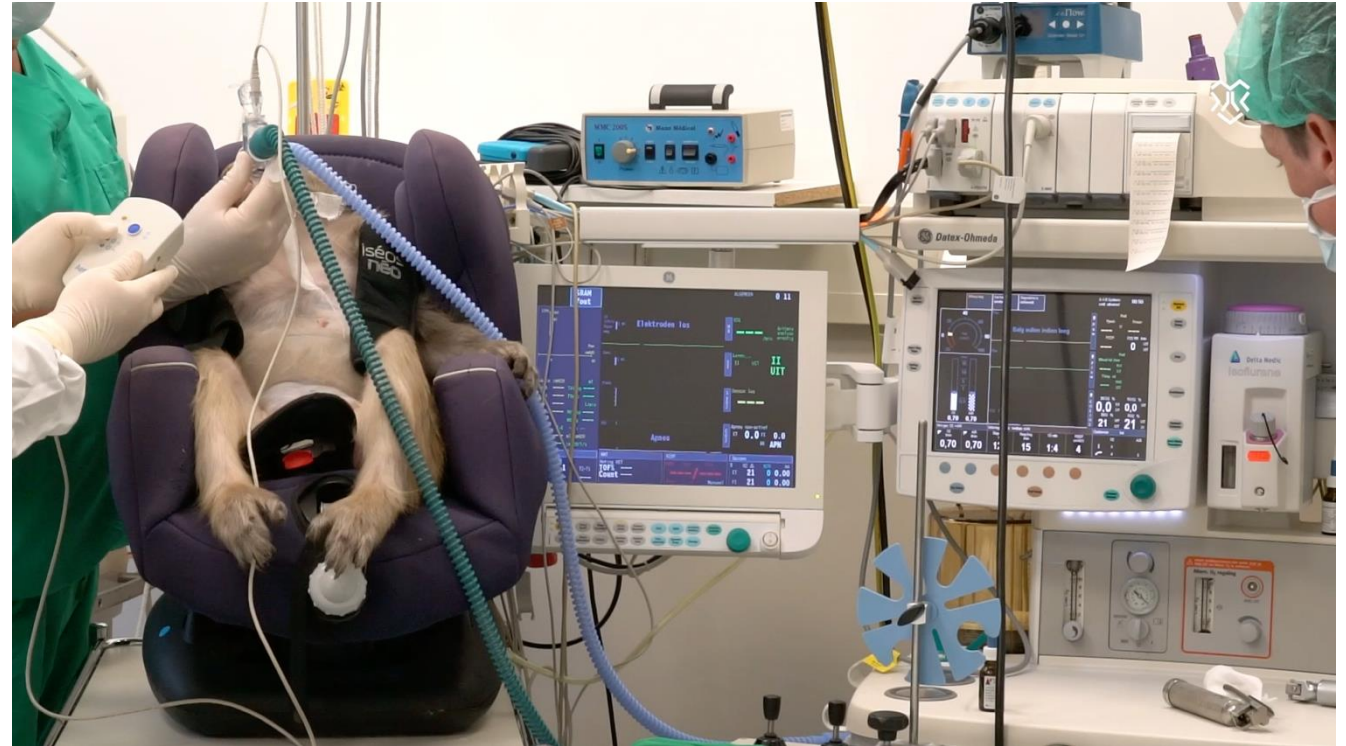
Mtb Erdman

repeated limiting dose | 8x 1 CFU endobronchially

MTBVAC

GMP batch 1917678 (by Biofabri)

Aerogen™ Solo
using a mesh nebuliser for aerosol delivery



Attenuated *M.tb* - i.c. MTBVAC - Delivered by Aerosol

a translational pilot study in the NHP RLD *M.tb* Challenge model



Rhesus macaques

naive, Indian-type, adult males

Mtb strain Erdman

repeated limiting dose

8x a single CFU
endobronchially

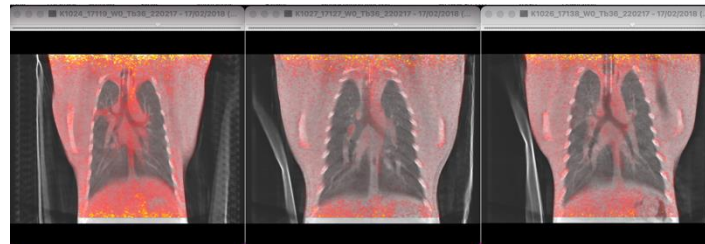
K01 harmonisation stock,
BEI Resources

MTBVAC

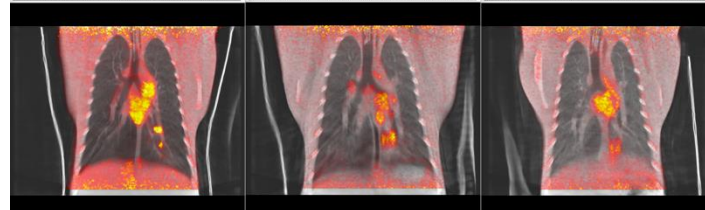
GMP Batch 1917678,
by Biofabri

non-vaccinated controls

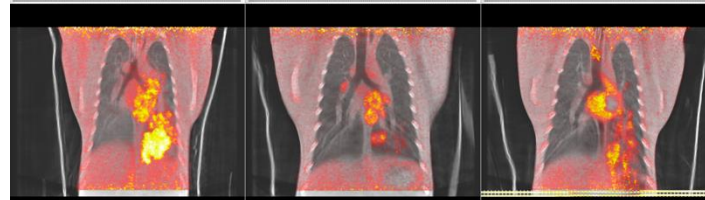
pre



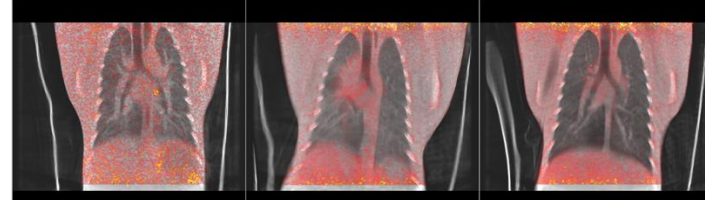
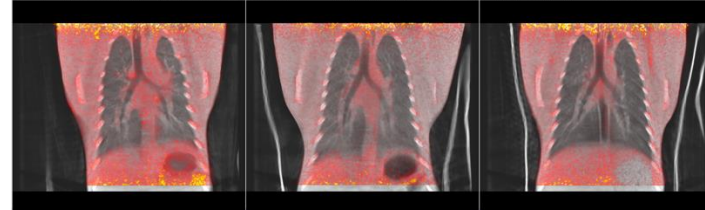
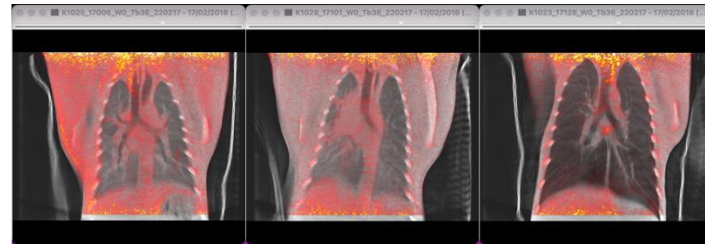
wk6+



wk8+



aerosol MTBVAC



with a prominent post-vaccination
TRM response in the airways



How does pulmonary mucosal BCG perform in the face of a pre-existing id.BCG response?

Considering the specific local immune profile associated with mucosal BCG delivery we hypothesized we can circumvent any possible blocking effect!

Respiratory Mucosal BCG Vaccination in the Face of an Existing id.BCG Response

a repeat study, using qTag.Mtb for mapping infection dynamics

a collaborative study with the Sarah Fortune lab, Harvard TH Chan School of Public Health, Harvard, Boston



Rhesus macaques

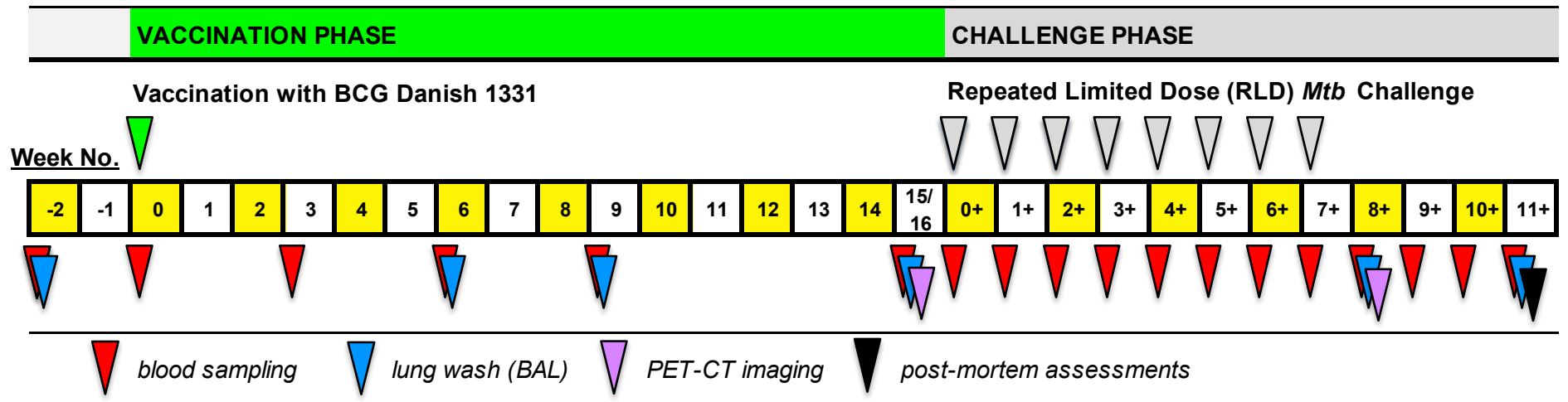
naive, Indian-type, adult females

Mtb Erdman

repeated limiting dose | 8x 1 CFU endobronchially | w. molecular tag

BCG

Danish 1331



- nv.ctrls
- BCG.id ctrls
- BCG.muc Priming
- **BCG.muc Boosting**

after BCG.id vaccination
14 weeks earlier

using **8 distinctive tagged Mtb Erdman stocks** for RLD chg



unpublished

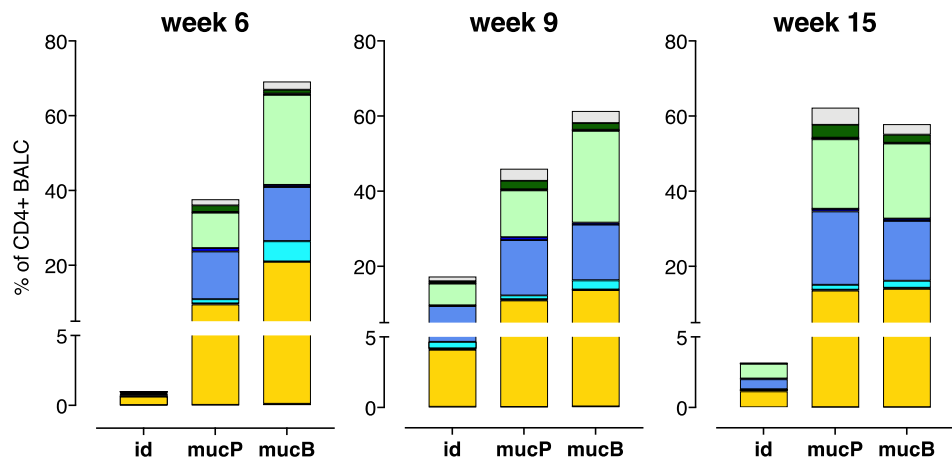
manuscript in preparation



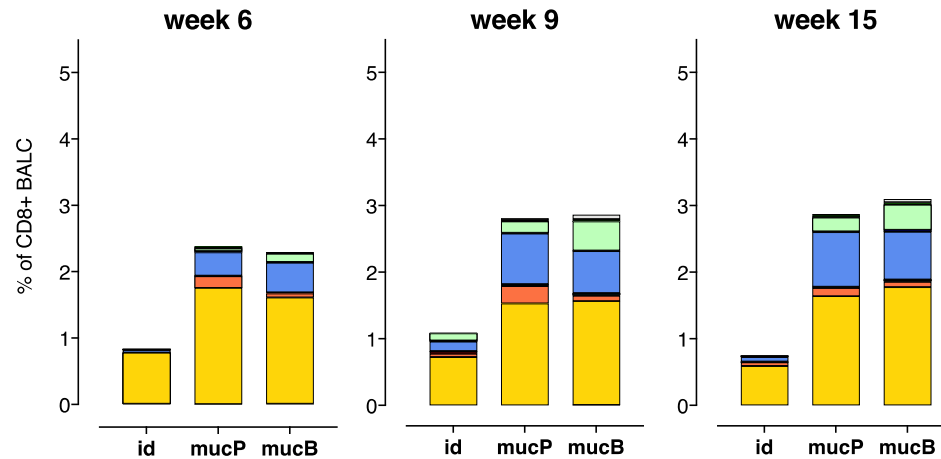
Similar Polyfunctional T Cells Profiles upon BCG.muc Boosting

characterised by IFN γ and IL17 secretion (Th1/Th17)

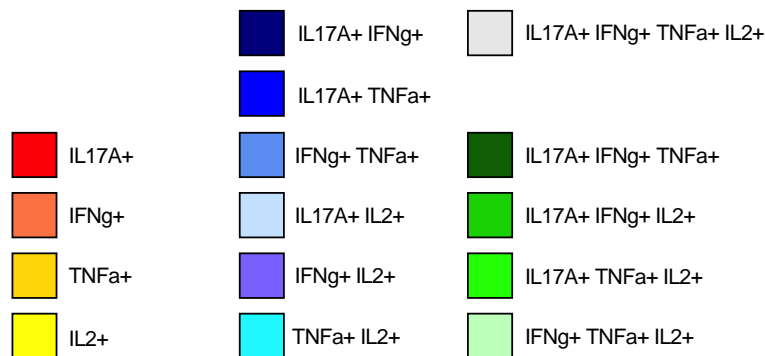
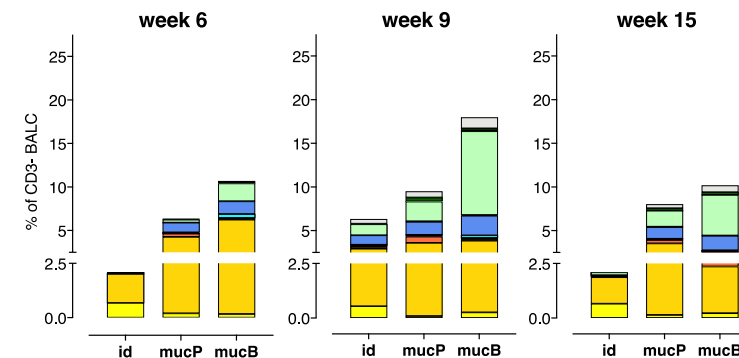
CD4+



CD8+



CD3- ILC/NK

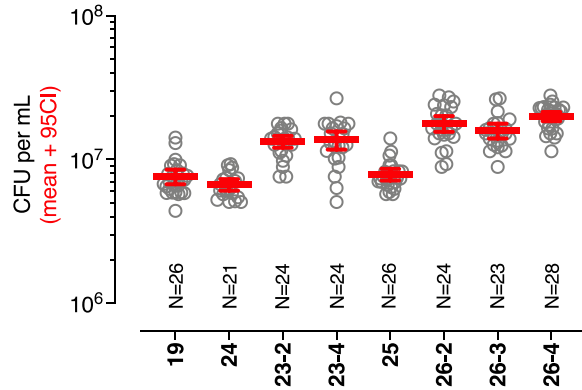



unpublished
manuscript in preparation



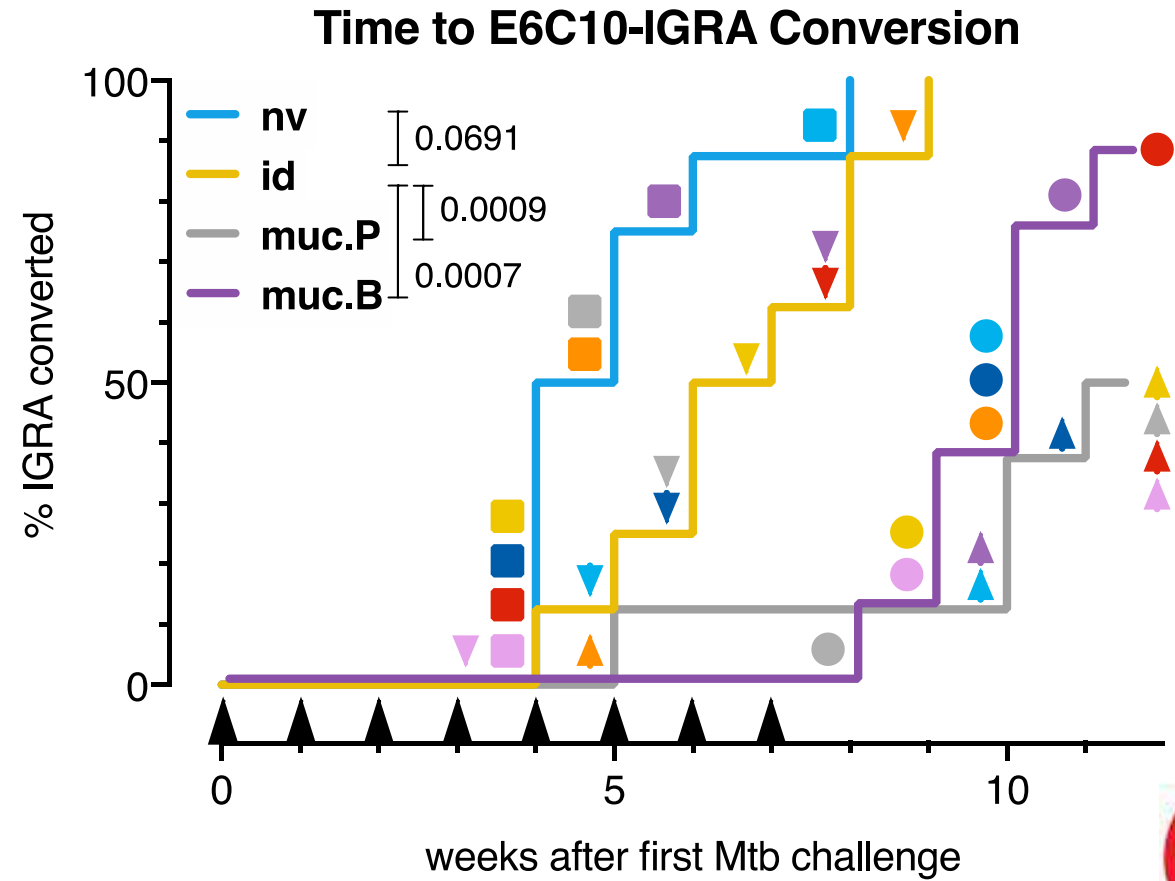
BCG.muc priming as well as boosting delay IGRA conversion

8 distinctive, tagged *Mtb* Erdman stocks



Applying 9 CHLG rounds on 2 stacks with even representation of all treatment groups!
 qTAG.19 is used twice, both in the 1st and 9th CHLG round

		CHALLENGE PHASE											
Weeks:	15	0+	1+	2+	3+	4+	5+	6+	7+	8+	9+	10+	11+
Stack 1		1st	2nd	3rd	4th	5th	6th	7th	8th				
Stack 2			2nd	3rd	4th	5th	6th	7th	8th	9th			
qTAG.ID:	19	24	23-2	23-4	25	26-2	26-3	26-4	19				



manuscript in preparation

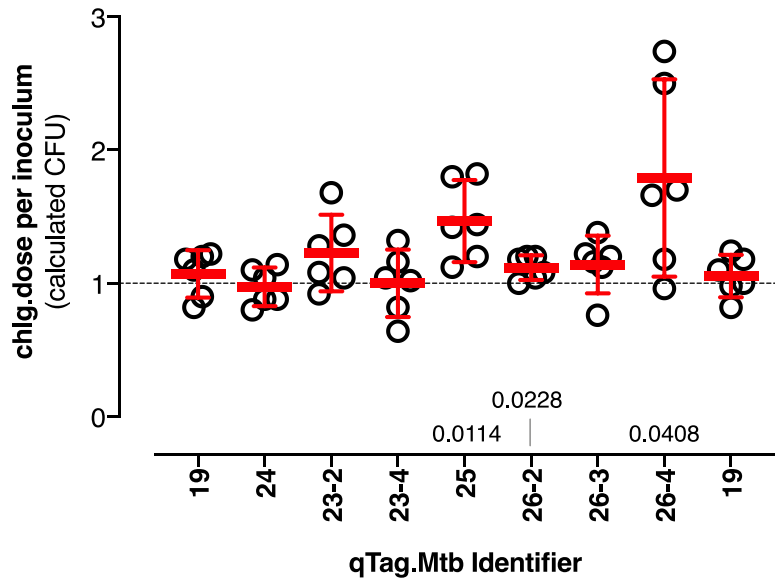


unpublished

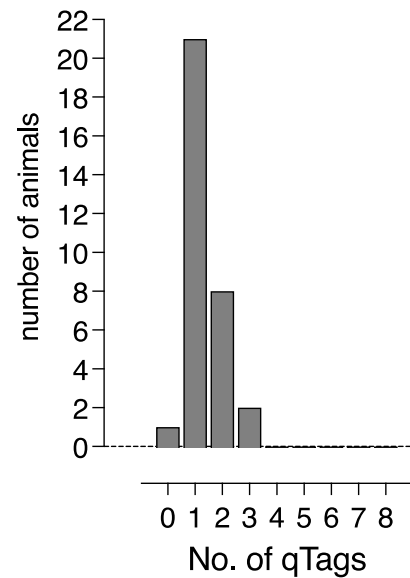


qTag.Mtb analysis, however, shows no difference in time to infection

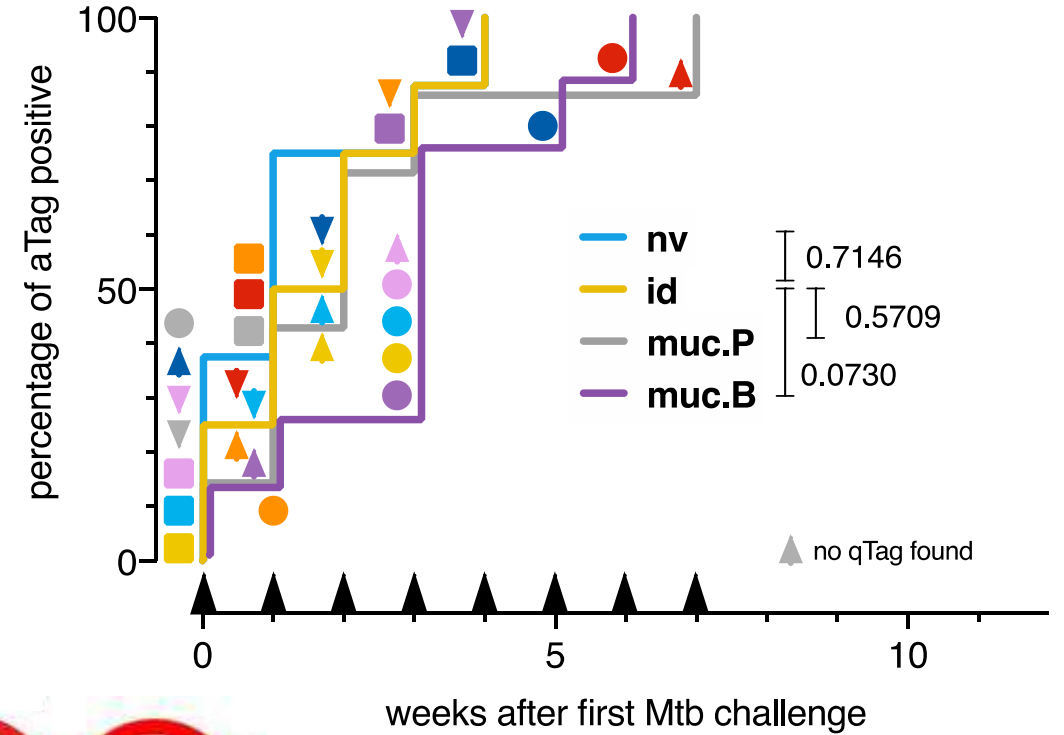
QC on the inocula



qTag.Mtb Retrieval



Challenge Take over Time (by qTag analysis)



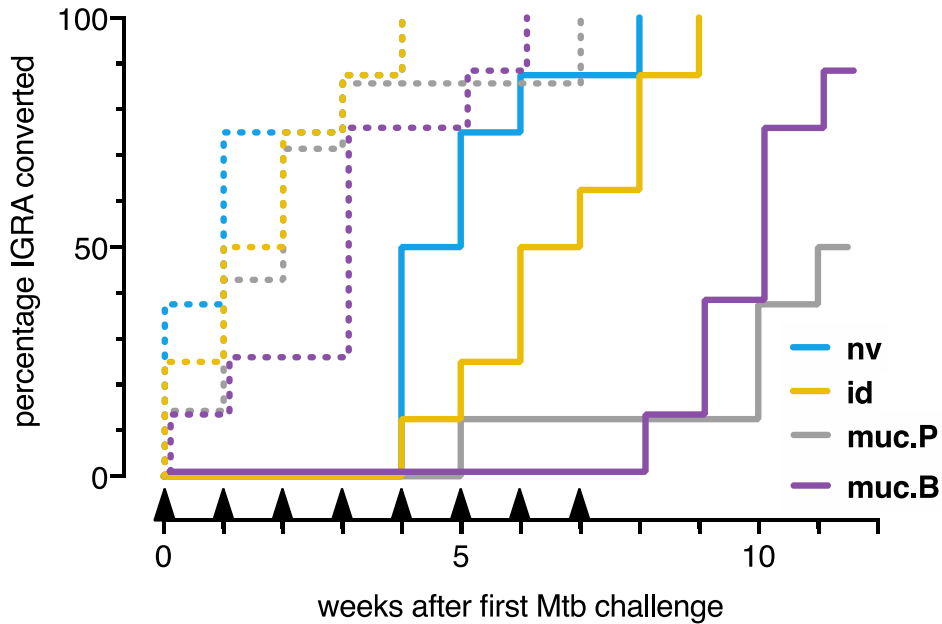
unpublished

manuscript in preparation

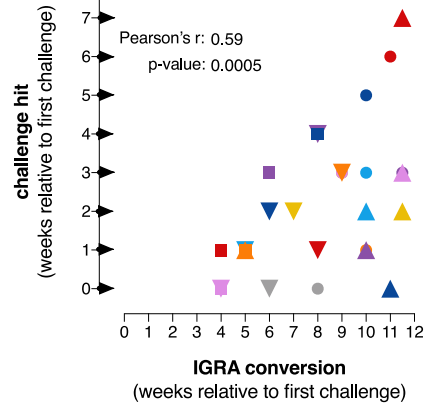


qTag.Mtb analysis: correlating *IGRA conversion* to *Time to CHLG Hit*

Time to IGRA Conversion / Chlg Take (overlay)



Correlation between IGRA+ en qTag.Mtb+

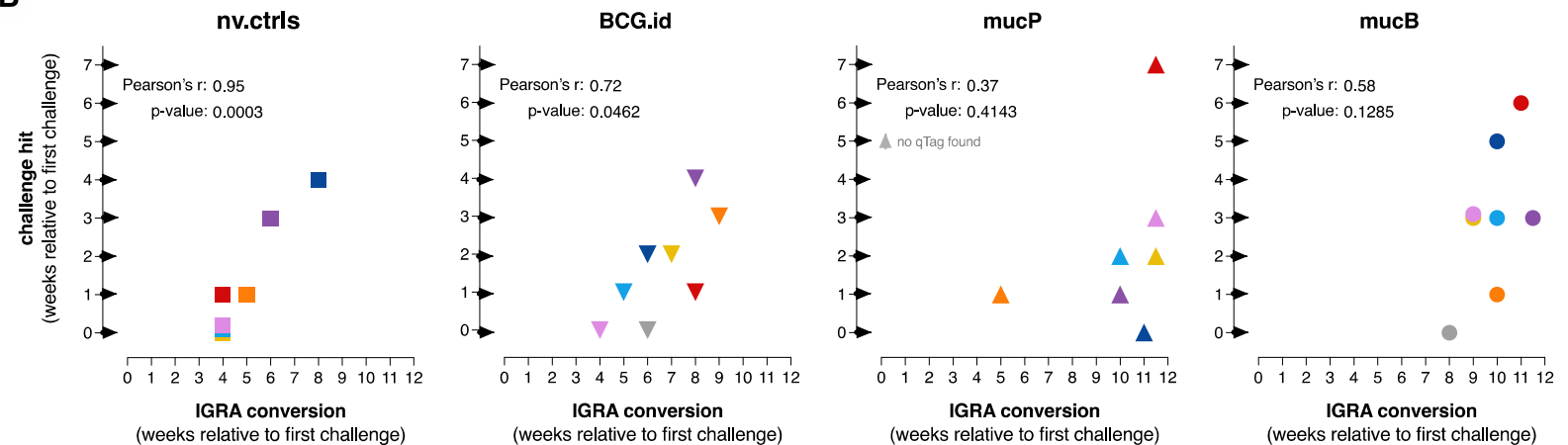


A strong and highly significant correlation in the non-vaccinated controls ($\rho = 0.95$, $p = 0.0003$) is lost upon prior mucosal BCG priming or boosting

manuscript in preparation



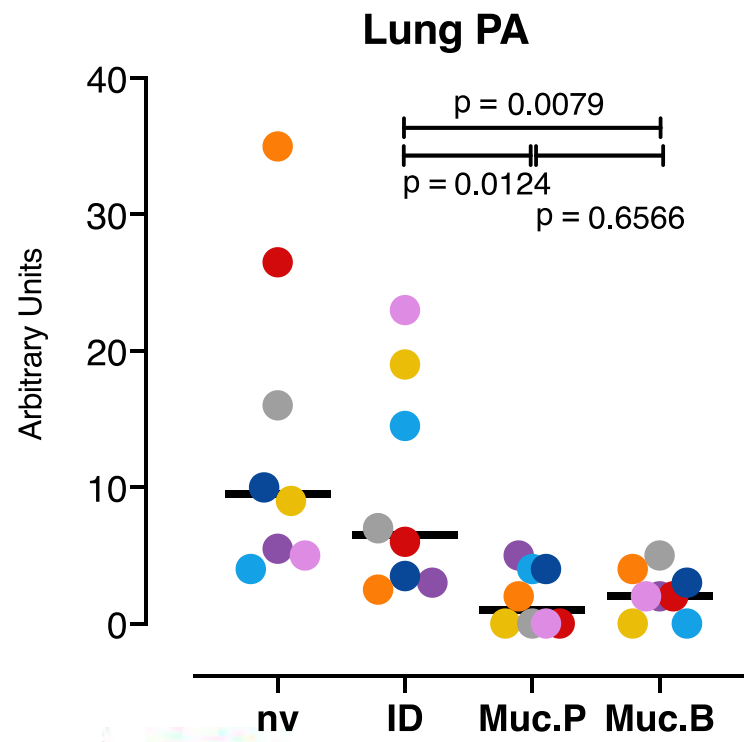
unpublished



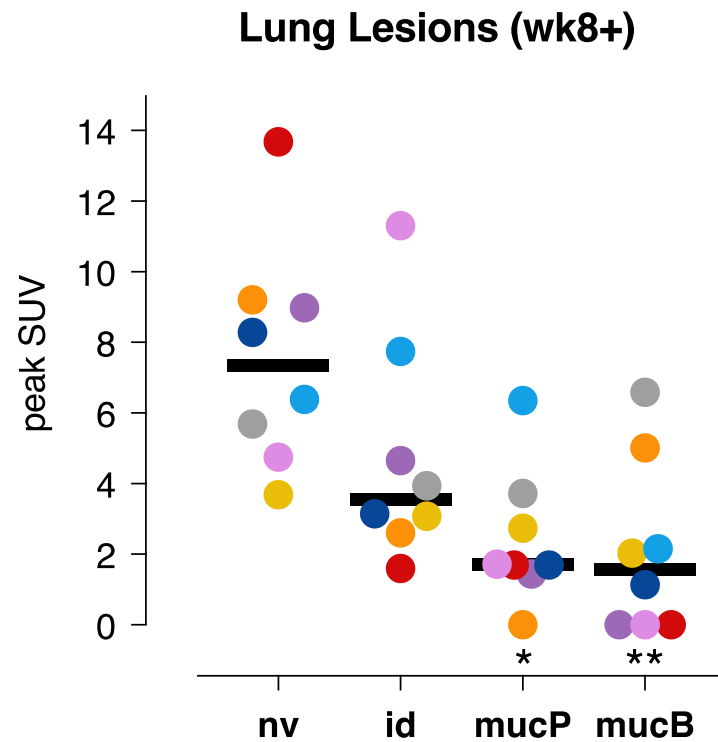
Respiratory Mucosal BCG Boosting Appears Equally Potent against Pulmonary TB

by gross pathology, FDG-PET imaging and enumeration of bacterial tissue burden

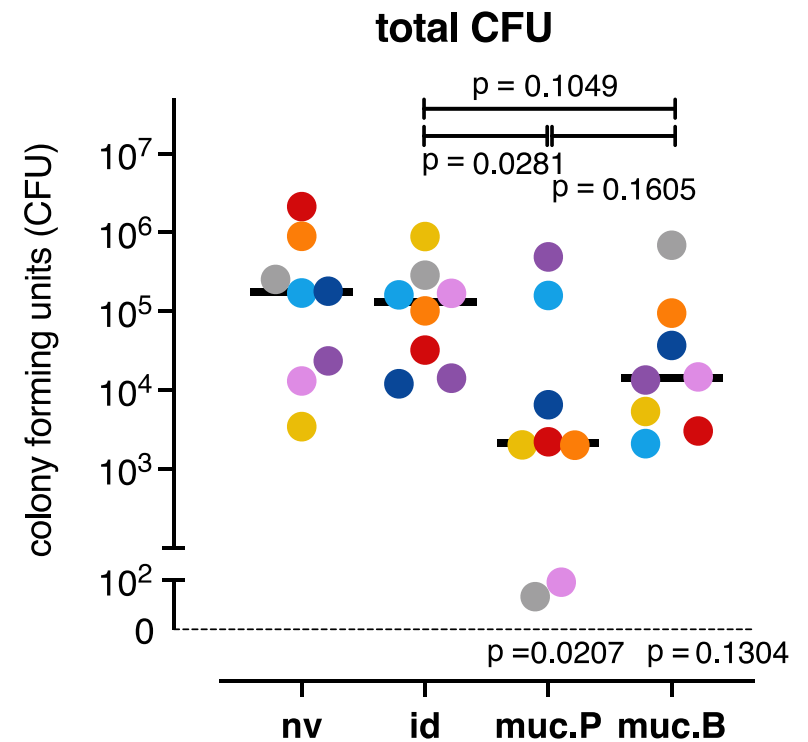
pathology



FDG-PET



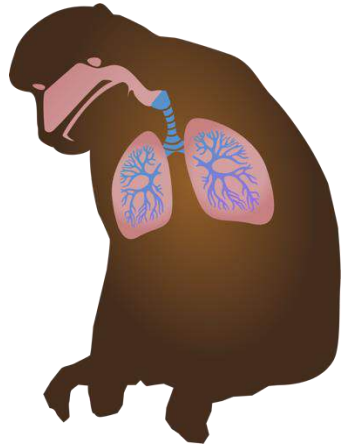
bacteriology



manuscript in preparation



Take Home | Summary



Repeated Limiting Dose (RLD) *Mtb* challenge in rhesus macaques

provides for a **refined & robust** model for TB vaccine research

Proof of Concept for efficacious pulmonary mucosal vaccination

provided both for **primary** and/or **revaccination** approaches

for BCG by instillation, and **for translatable MTBVAC aerosol inhalation** (pilot data so far!)

Identified *Correlates of Protection* from pulmonary TB

hallmarked by increased lymphocytes in the airways, Th1/Th17, IL10 secretion, elevated (functional) immunoglobulin levels (not shown)

Enhanced trained immunity signals by mucosal delivery of live attenuated vaccine

underpinned by increased monocyte cytokine secretion capacity (epigenetics, metabolic rewiring)

FAW Verreck et al Tuberculosis 2017

K Dijkman et al. NMED 2019

K Dijkman et al CRM 2021

MMP Vierboom et al CRM 2021

and unpublished data

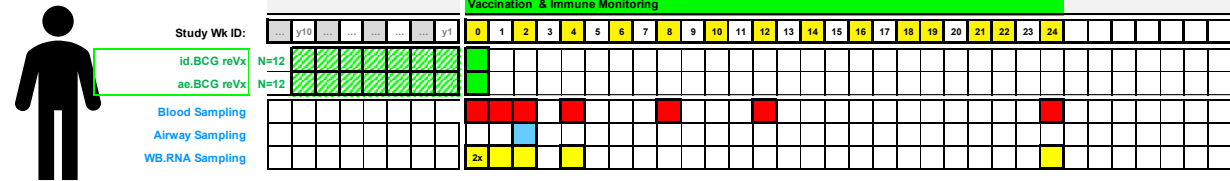
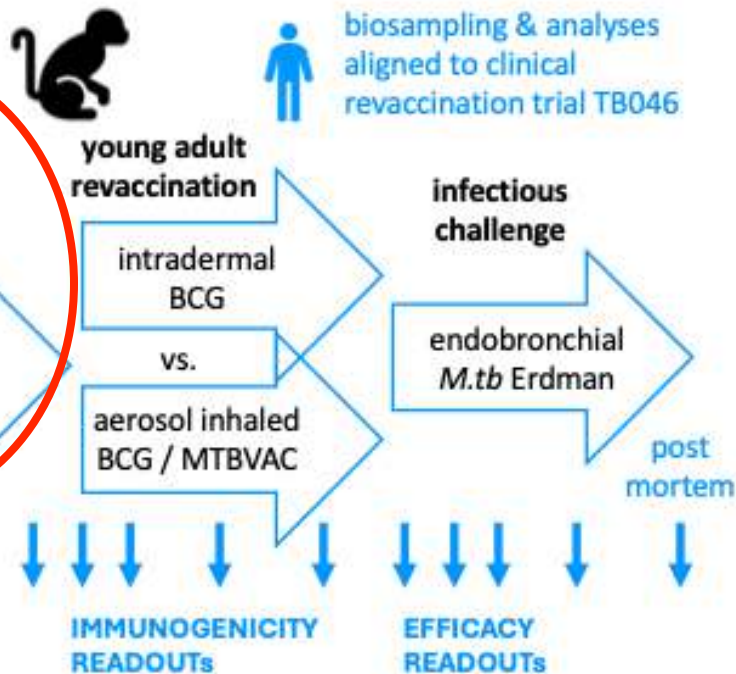
WP2 | Aerosol BCG Revaccination as an Exp Med Approach

aligned study design between man and monkey | harmonised host response analyses in WP3

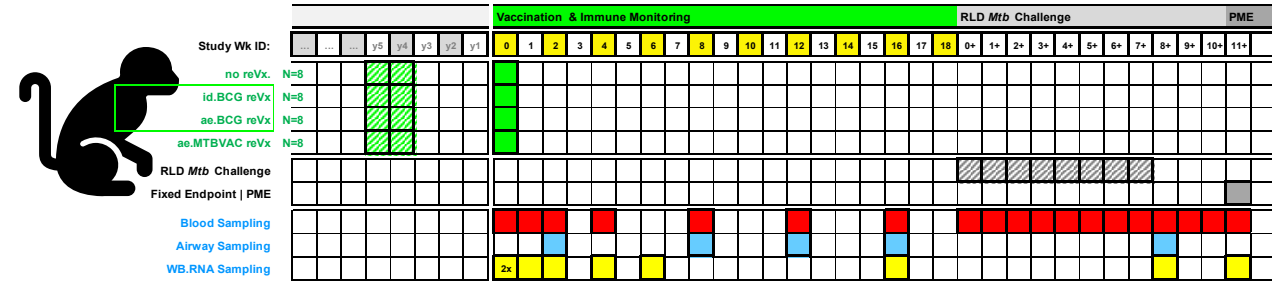


Aerosol Inhaled BCG / MTBVAC Revaccination after neonatal id.BCG priming

UOXF | TB.046



BPRC | TB.039



Alignment of Study Design, Sampling & Analyses as much as practically feasible



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Thank you for your attention !

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Essentially, all models are wrong, but some are useful.

(George E. P. Box)