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National Institutes of Health
Department of Health and Human Services

National Institute of Allergy and Infectious Diseases

IV BCG Immunization Prevents Infection and Disease in NHP

NVAC Panel Presentation
February 13, 2020

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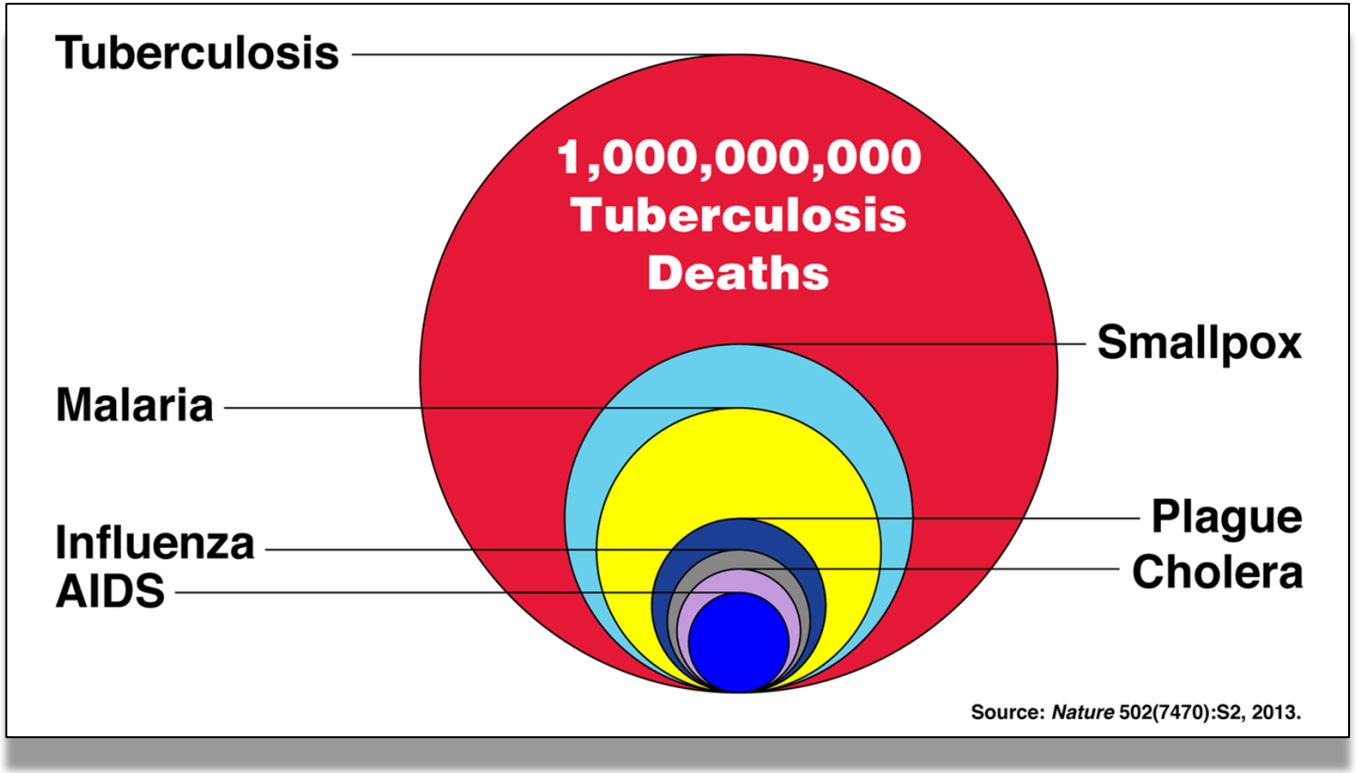
TB Remains the Leading Cause of Death Among Infectious Diseases

In 2017:

- **10 million new TB cases**
- **1.6 million deaths**
- **450,000 new cases of MDR-TB**



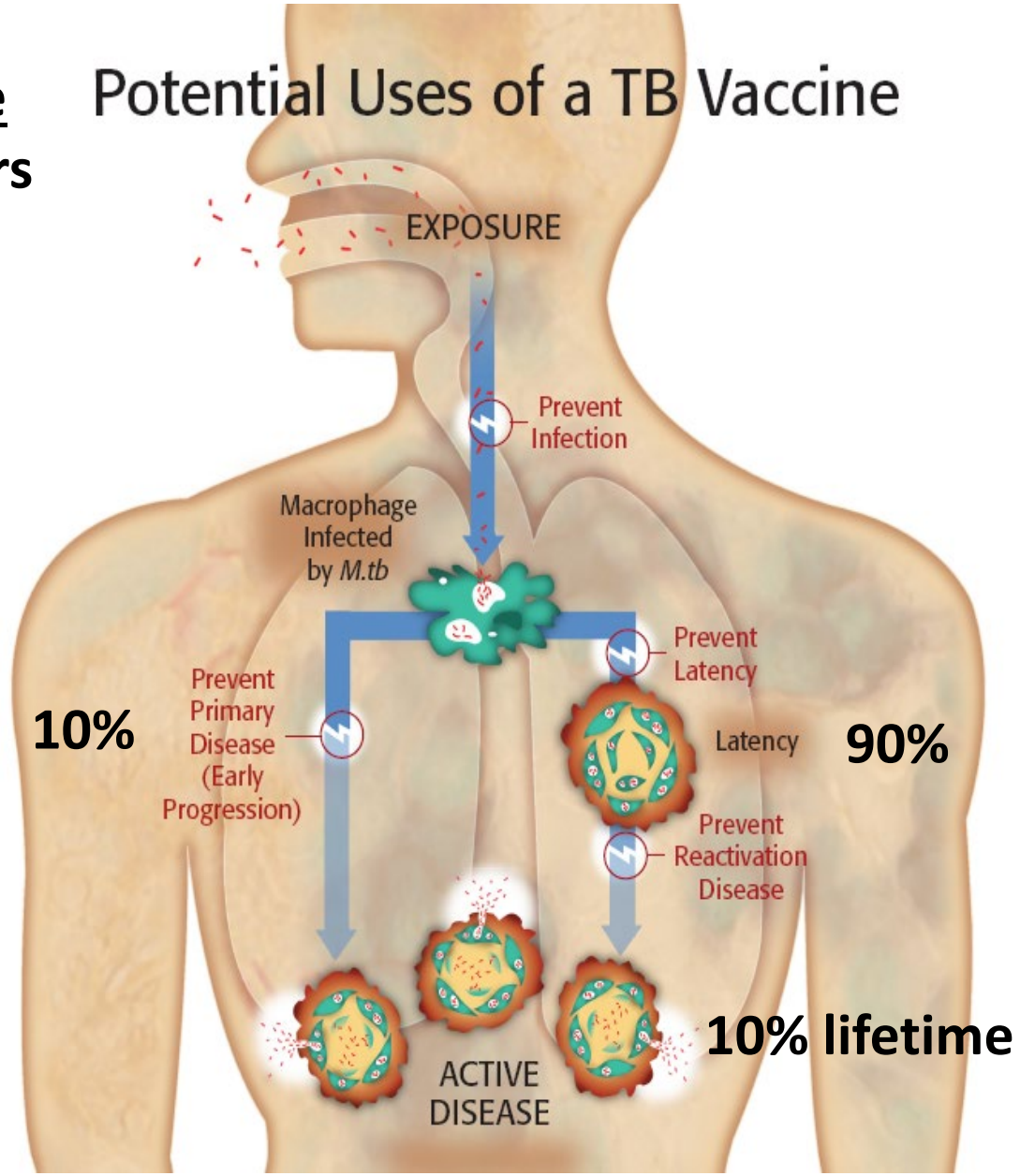
Source: WHO Global Tuberculosis Report 2018

Vaccination to Prevent TB Infection and Disease

A vaccine that is ~50% protective against <u>TB disease</u> for 10 years could reduce TB cases by 40% over 25yrs
*(17 million cases; India; 2025-2050)**

Primary Goal: Prevent primary disease

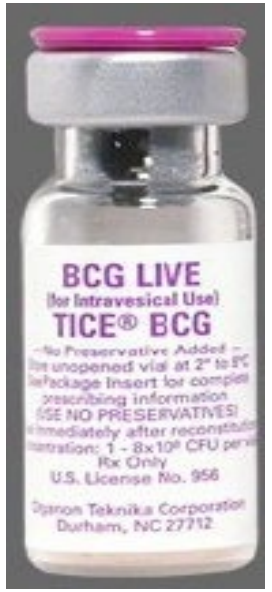
Ultimate Goal: Prevent infection



*White RG, Harris RC. 2019. *Lancet*. 7:204.
*Knight GM, et al. 2014. PNAS. 111:15520.

Requirements for a Vaccine-Elicited T Cell Response to Protect Against TB

- **Magnitude:** Frequency of CD4 and CD8 T cells
- **Quality:** Function (cytokines), phenotype, transcriptional
- **Breadth:** Diversity of antigens
- **Durability:** T cell memory
- **Location:** Tissue resident T cells (Trm) in the lung



Delivery: Elicit high frequency Trm in lung and systemic responses in blood with antigenic breadth

BCG Vaccine

- **BCG (Bacille Calmette Guerin, live attenuated *Mycobacterium bovis*) given by the ID route at birth is the only approved vaccine**
- **BCG ID provides protection against severe forms of disease in infants/young children**
- **BCG has variable to limited efficacy in preventing adolescent/adult pulmonary disease, the most transmissible form of the disease**

Albert Calmette
(1863-1933)

Camille Guerin
(1872-1961)

Presentation Overview

<u>Goal of Study</u>: Will altering the *route* or *dose* of BCG immunization influence immunity and protection in a highly pathogenic non-human primate model of Mtb infection?

- **Protection and immunogenicity after BCG immunization in NHP**
- **Clinical Development of IV BCG**

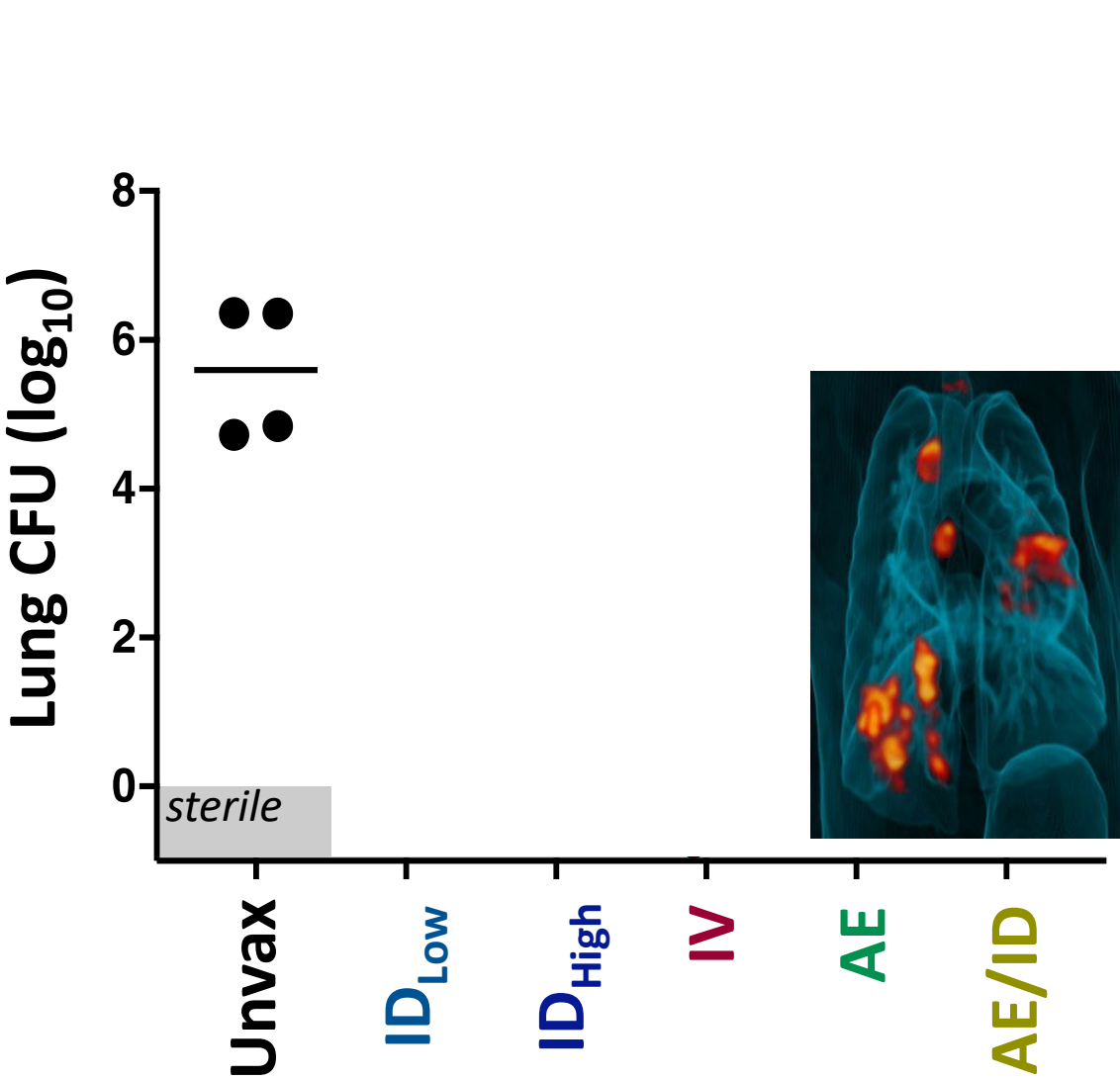
BCG Efficacy Study in Rhesus Macaques

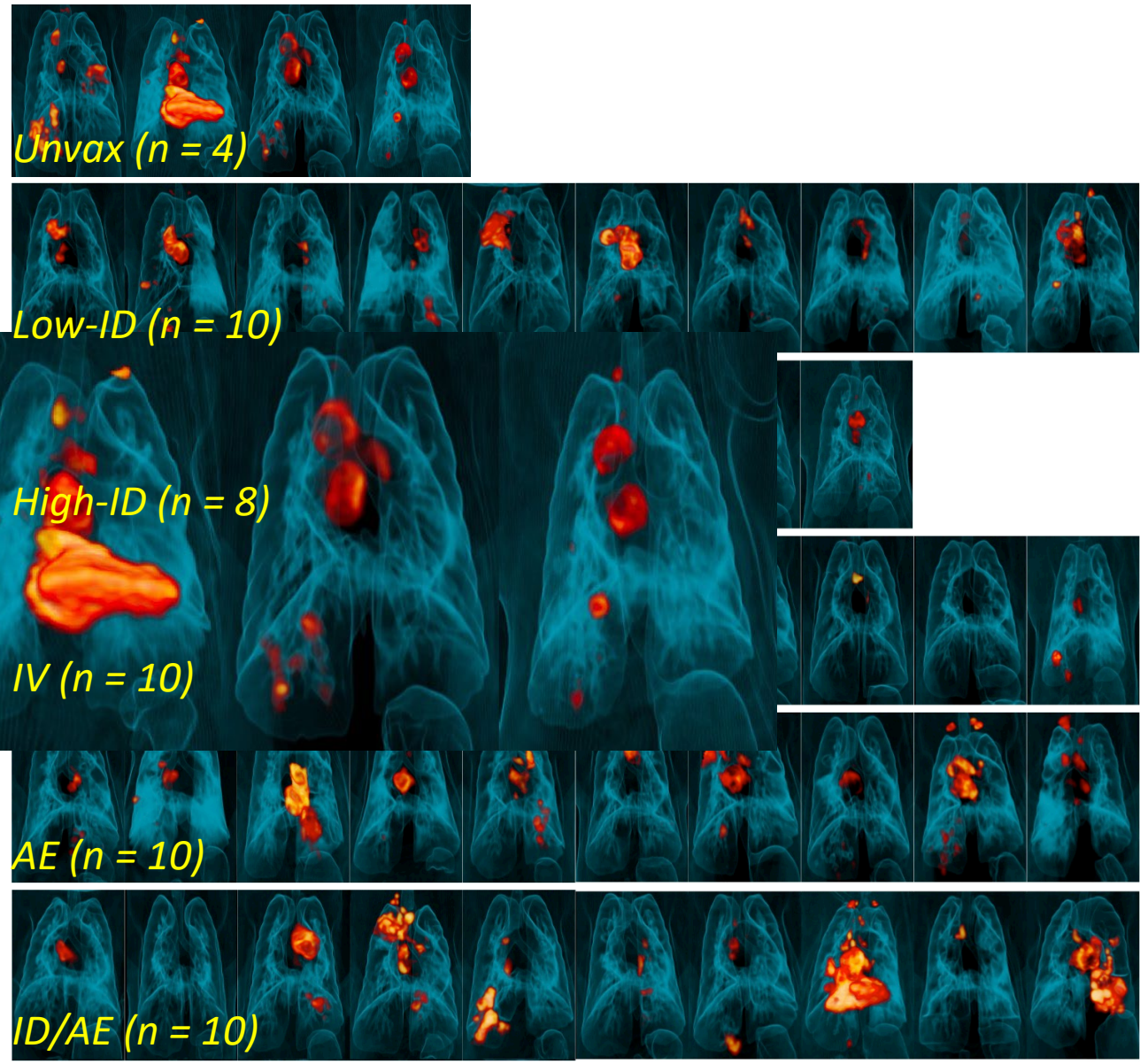
Vaccine	Route	Dose
BCG	ID (low)	$5x10^5$
	ID (high)	$5x10^7$
	AE	$5x10^7$
	IV	$5x10^7$
	ID + AE	$5x10^5 + 5x10^7$

Dose Route

6 months after BCG: Low-dose Mtb challenge (bronchoscope)

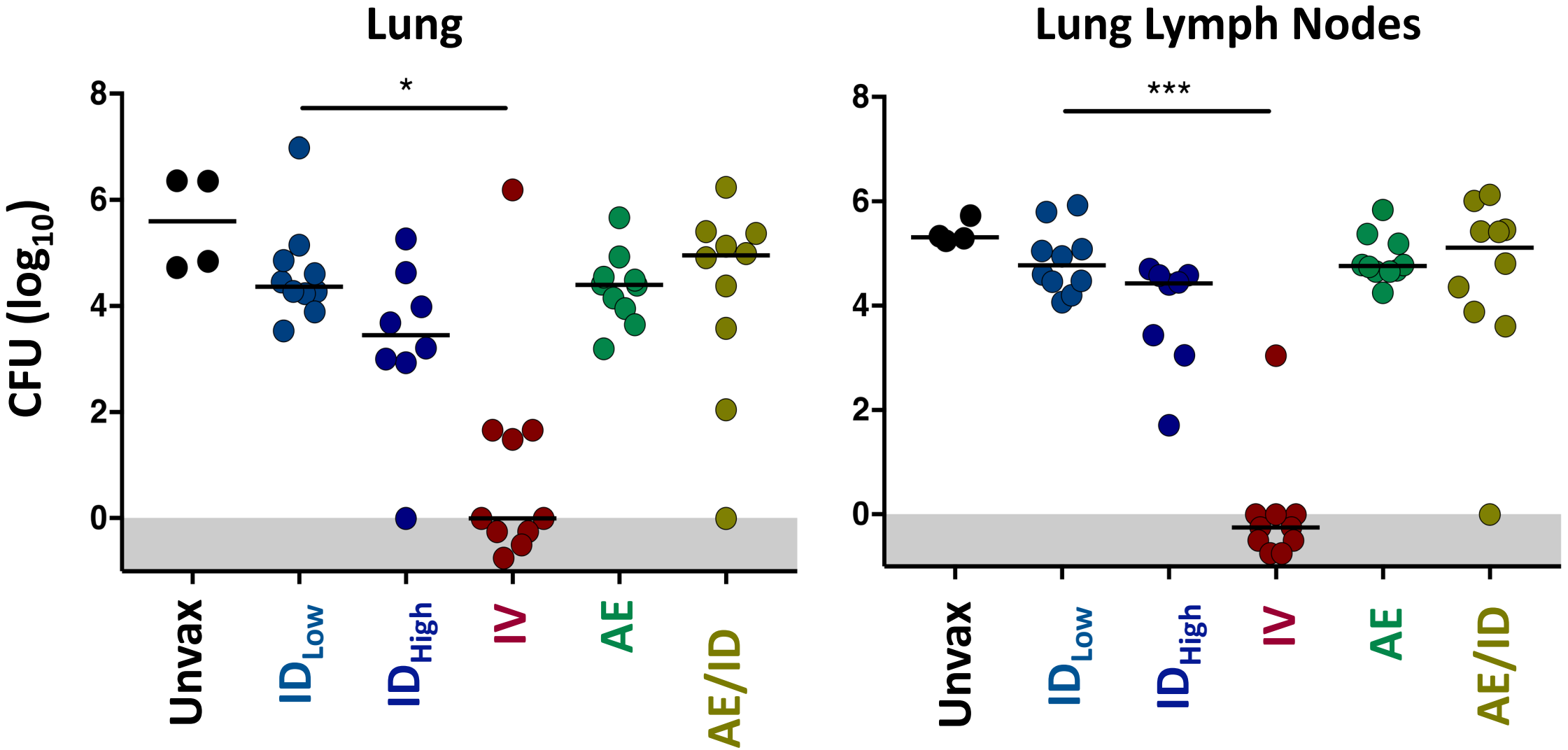
Mtb Burdens and PET Scans at Necropsy





Darrah PA, et al. 2020. *Nature*. 577:95-102.

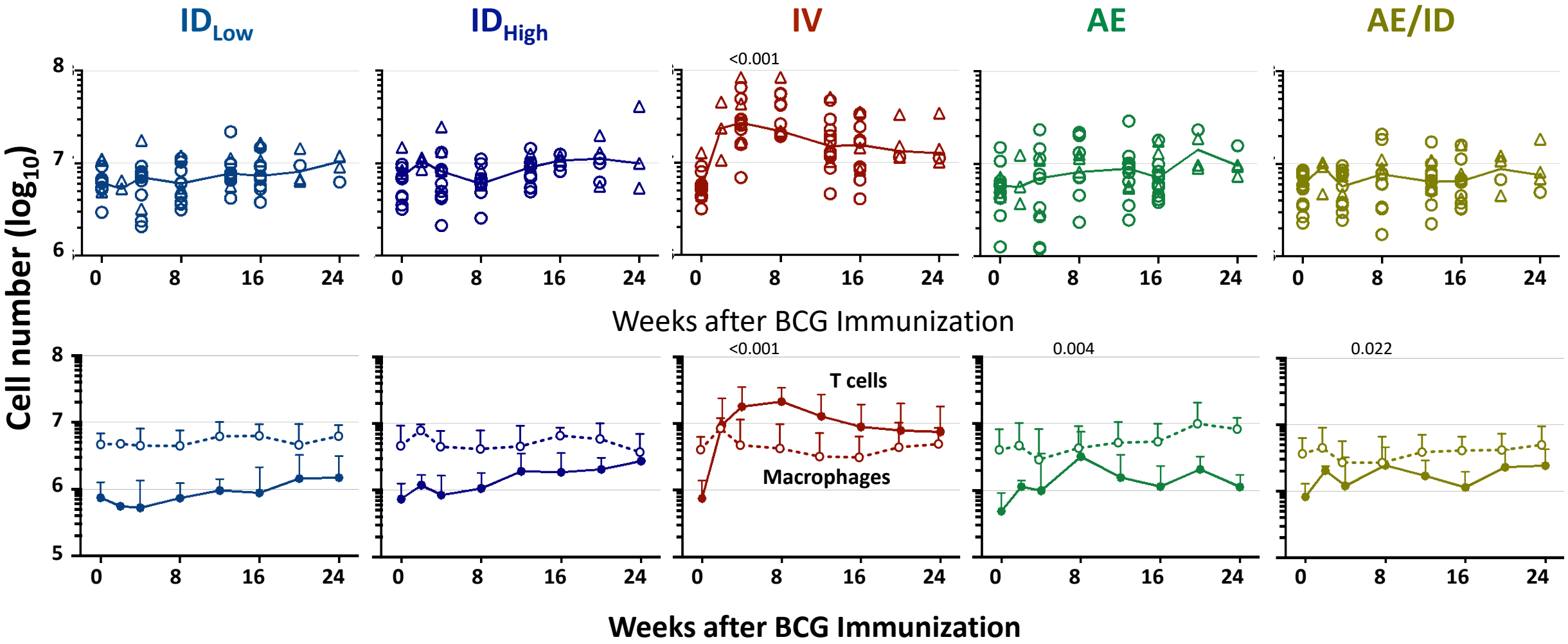
Mtb Burdens at Necropsy



Summary of Protection

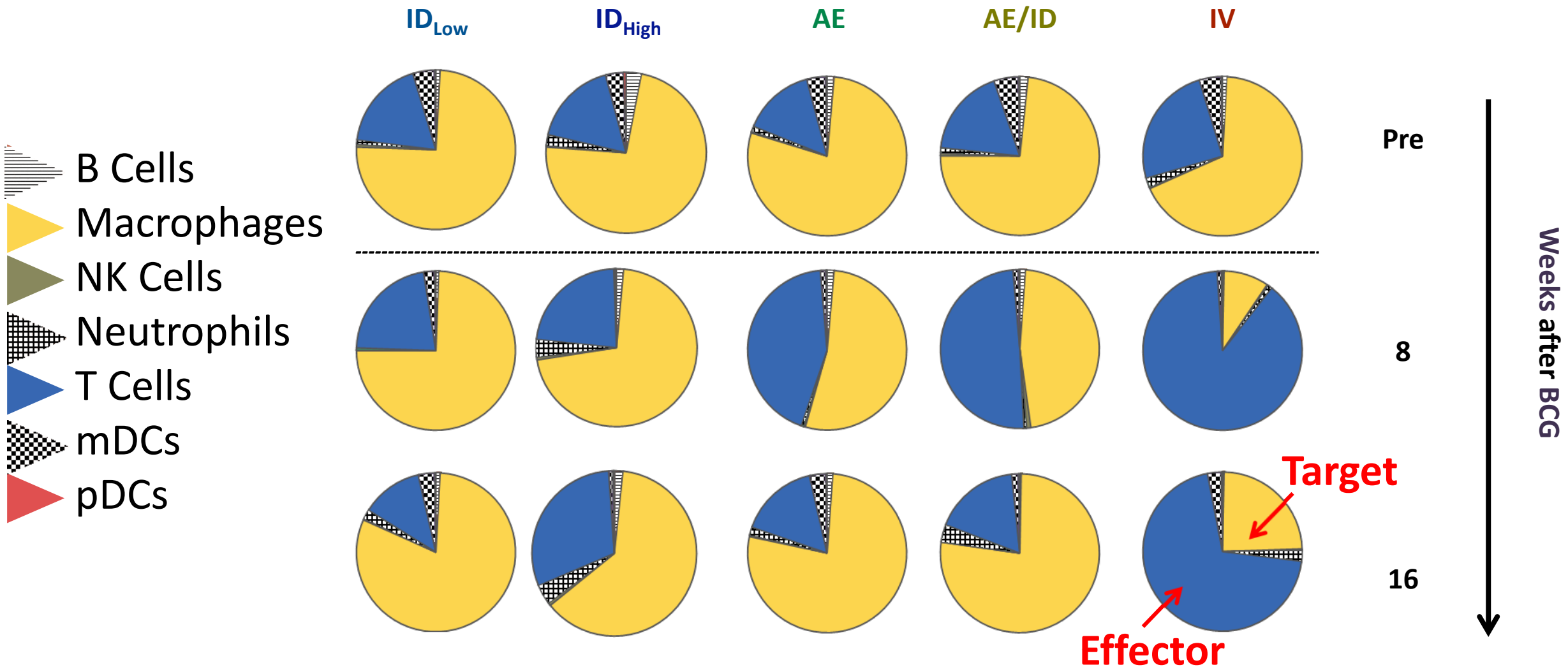
- **BCG IV prevented detectable infection and disease in 6/10 NHP**
  - 3/10 animals had ~50 total CFU (4-logs less than BCG ID)
  - 9/10 animals highly protected
- **Standard Low-Dose BCG ID conferred 1-log protection**
  - BCG High-Dose ID: trend toward increased protection
- **BCG AE or AE/ID are not significantly better than Low-Dose ID**

Cell Number in the BAL after BCG

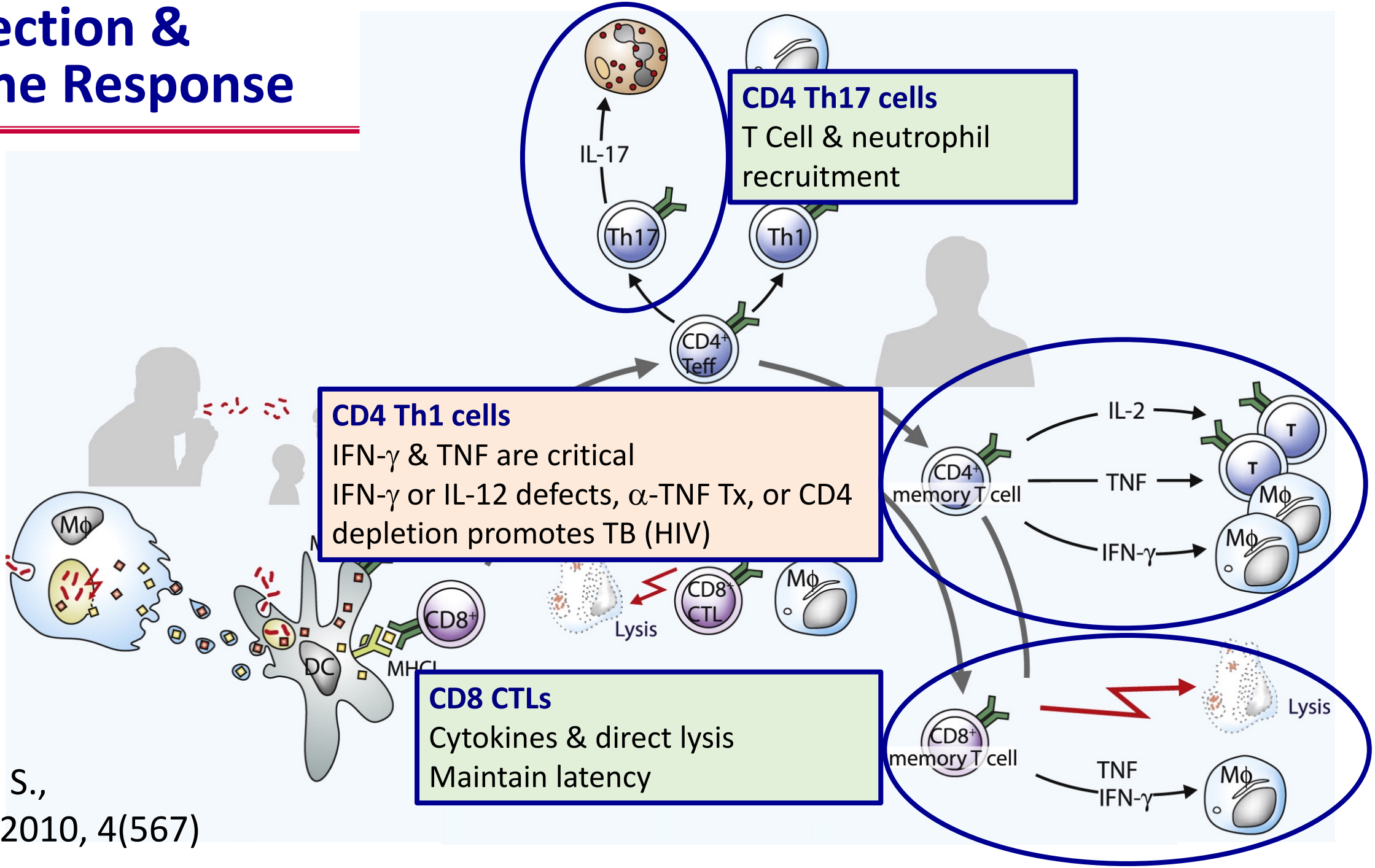


Darrah PA, et al. 2020. *Nature*. 577:95-102.

Proportion of Macrophages and T cells in the BAL after BCG

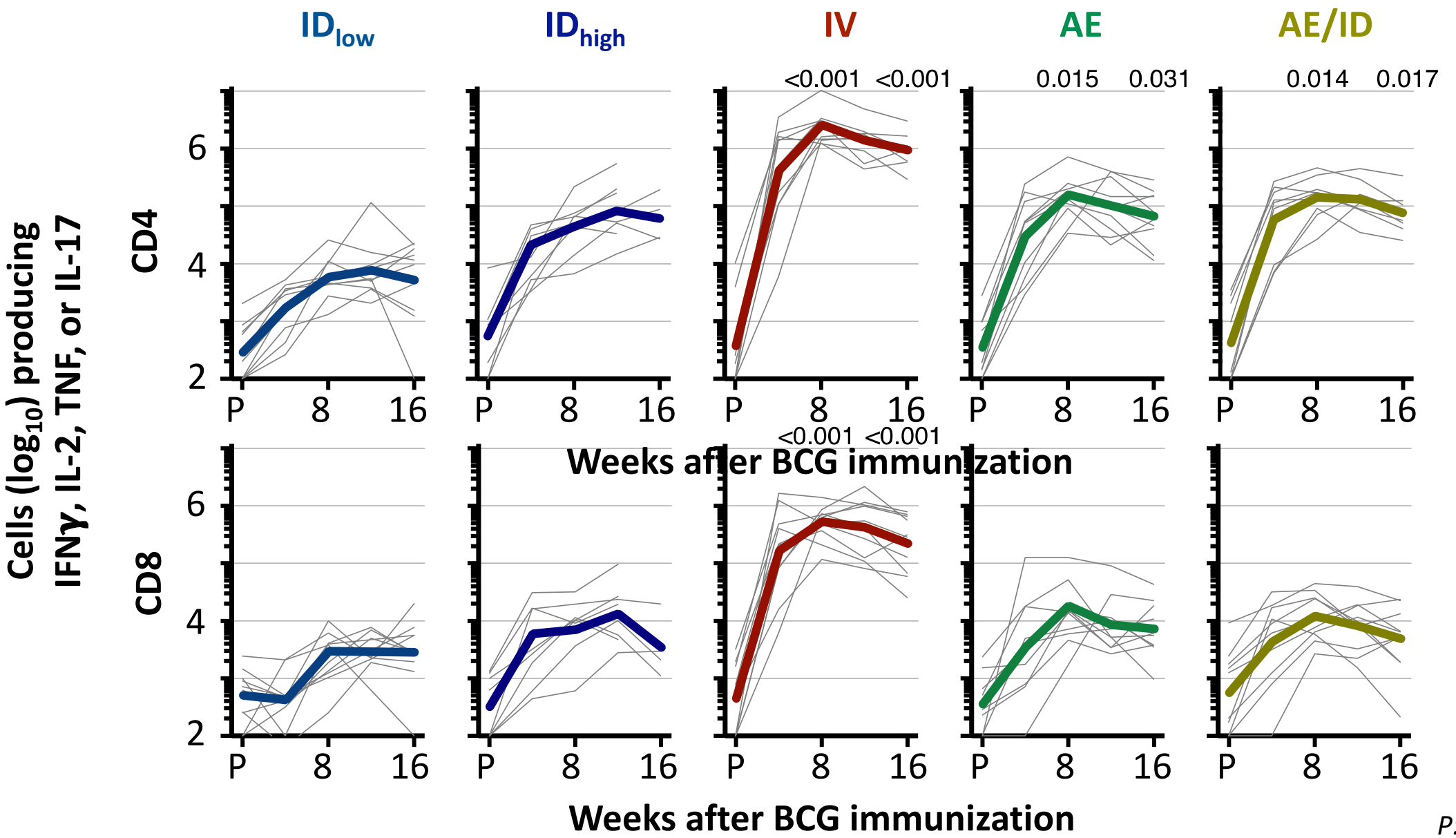


TB Infection & Immune Response



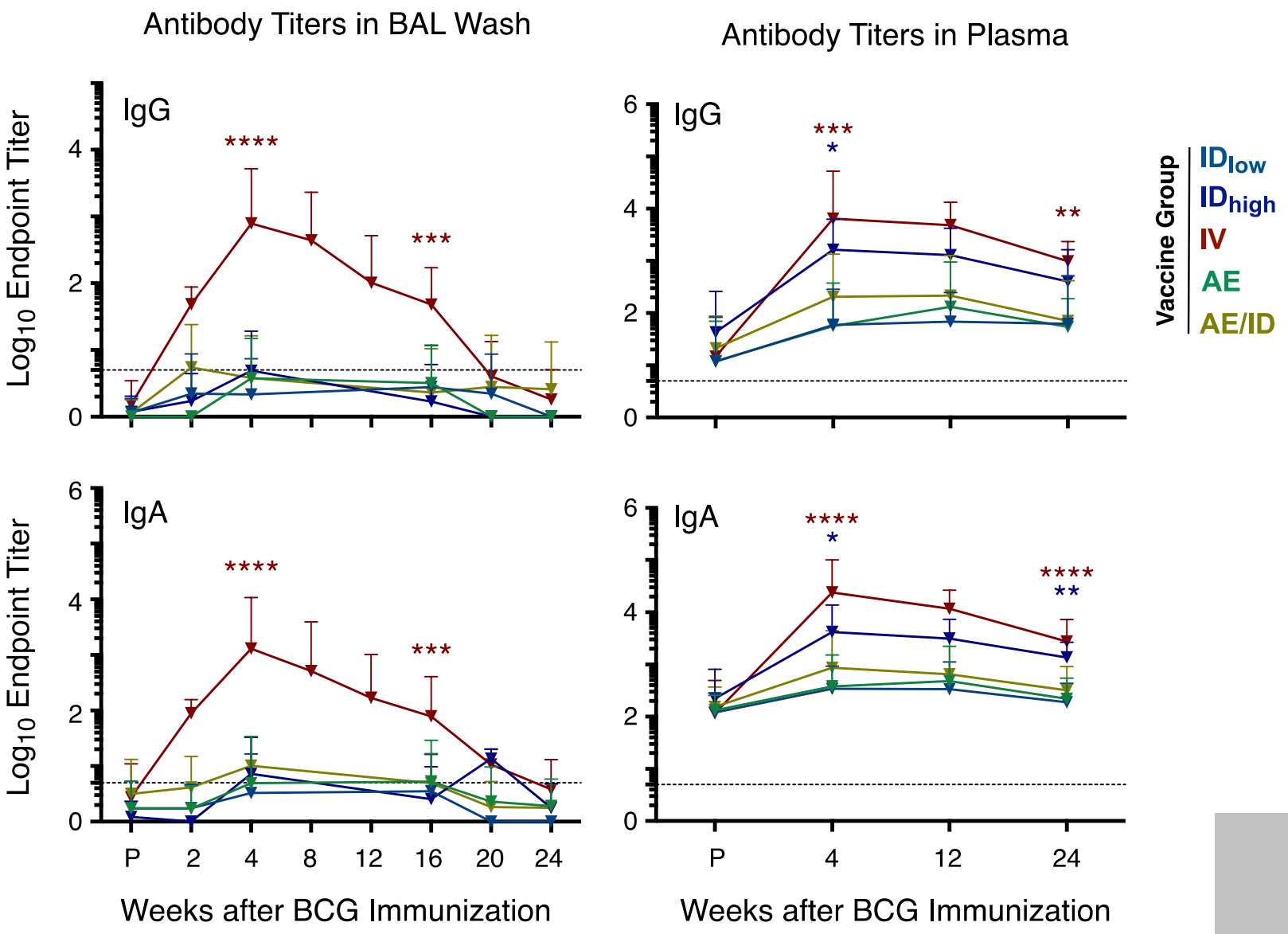
Kaufmann, S.,
Immunity, 2010, 4(567)

Ag-Specific T cell Responses in the BAL after BCG



P: compared to IDlow

IV BCG Induces Higher TB-specific Antibody Responses



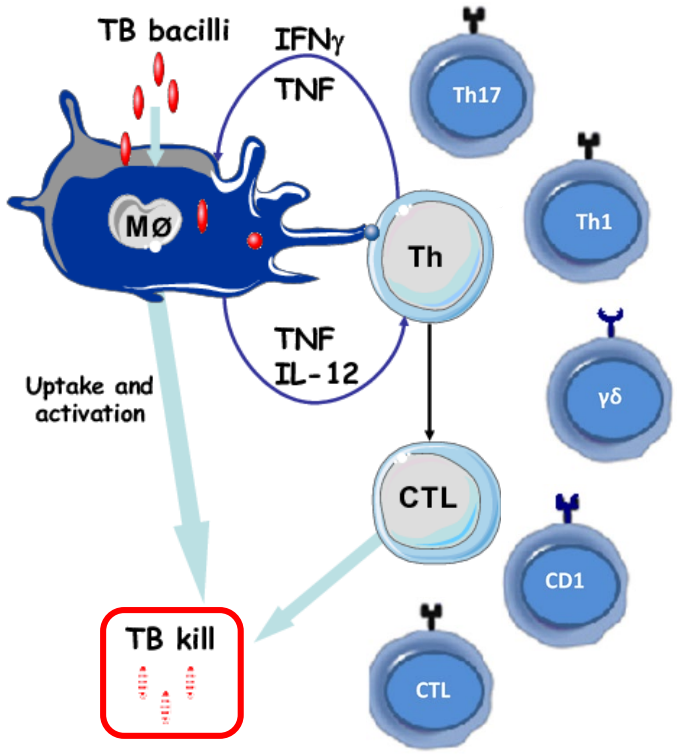
Systems serology analysis
Galit Alter
Eddie Irvine

Summary of T Cell Responses after BCG

- **Magnitude/Location**: In BAL, IV BCG elicited highest number of Ag-specific CD4 and CD8 T cells and dramatically alters ratio of T cells to macrophages
- **Quality:** Th1/Th17 CD4 T cells
- **Breadth:** Ongoing efforts at mapping BCG responses

Potential Mechanisms of Protection

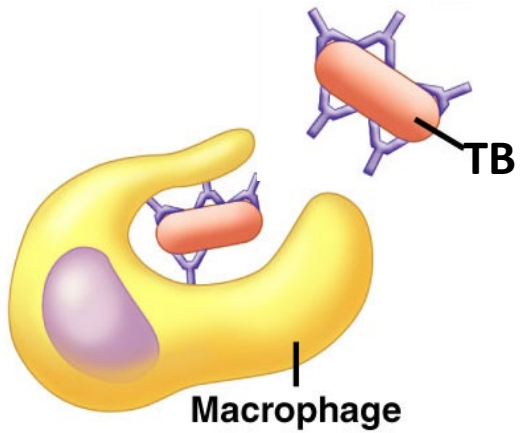
T Cells



T cell depletion

Harriff MJ… Lewinsohn DM; *Sci Rep* 2017, Jul
Greene JM…Sacha JB; *Mucosal Immunol* 2017 May
Seshadri C…Moody DB; *J Immunol* 2013 Jan

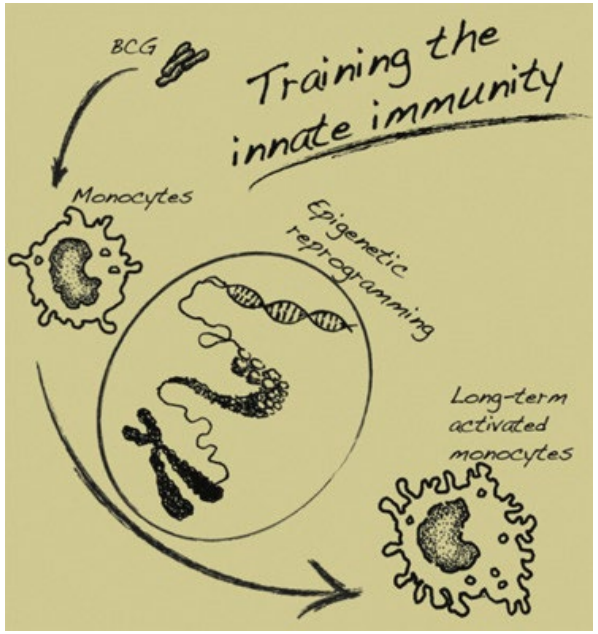
Antibody



Passive transfer Rituximab Tx

Lu LL…Fortune SM, Alter G; *Cell* 2016 Oct
Phuah J…Flynn JL; *Infect Immun* 2016 April

Trained Innate Immunity



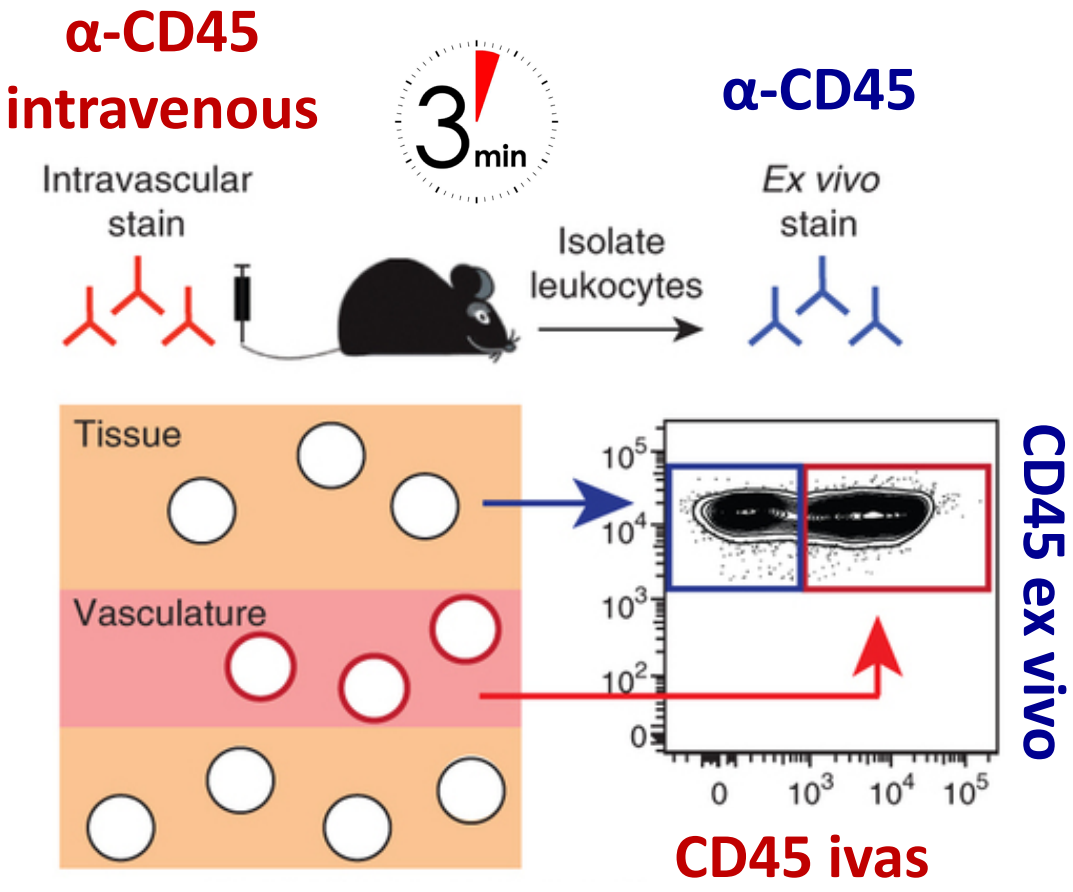
Monocyte Analysis

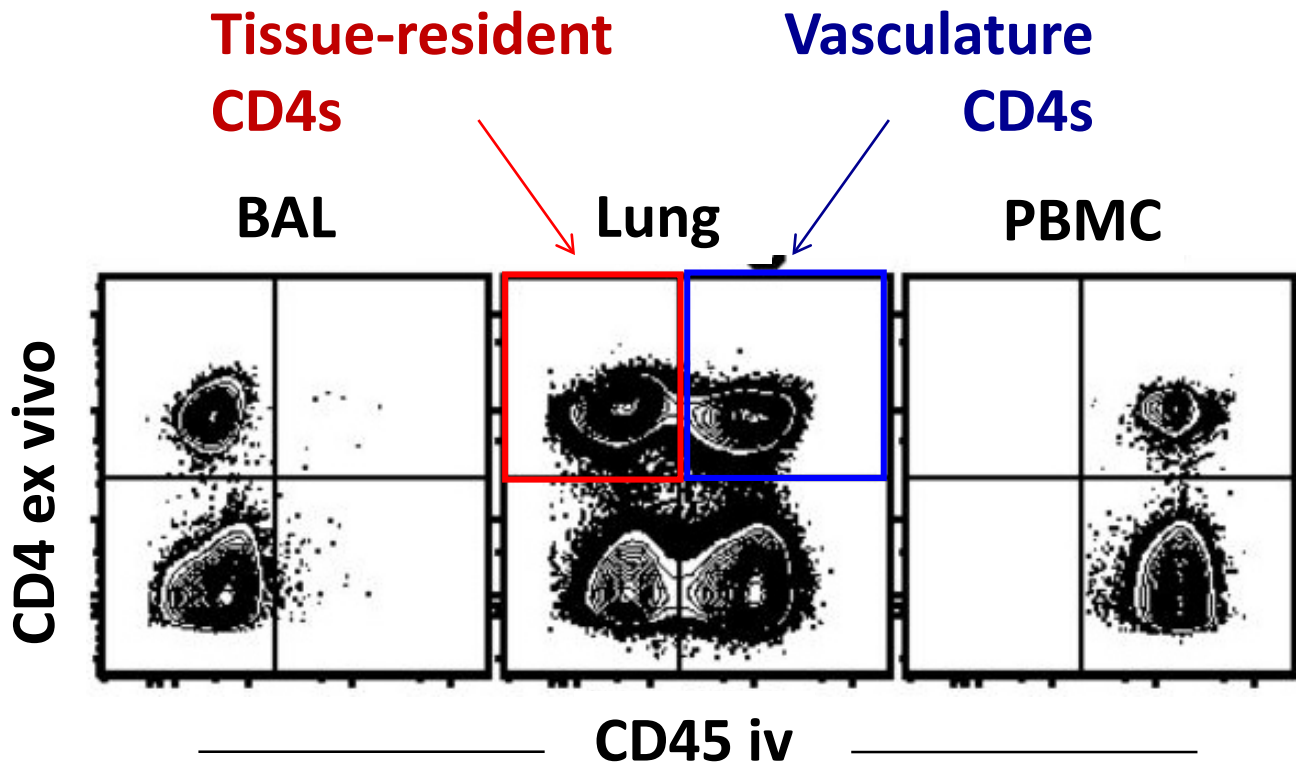
Kaufmann E…Divangahi M; *Cell* 2018 Jan
Arts RJW…Netea MG; *Cell Host Microbe* 2018 Jan
Joosten SA…Ottenhoff TH; *J Clin Invest* 2018 April

Does Route of BCG Influence T cell Responses in Lung?

- Does IV BCG elicit high lung-resident T cells?

CD45 IV Staining to Discriminate Tissue & Blood Cells

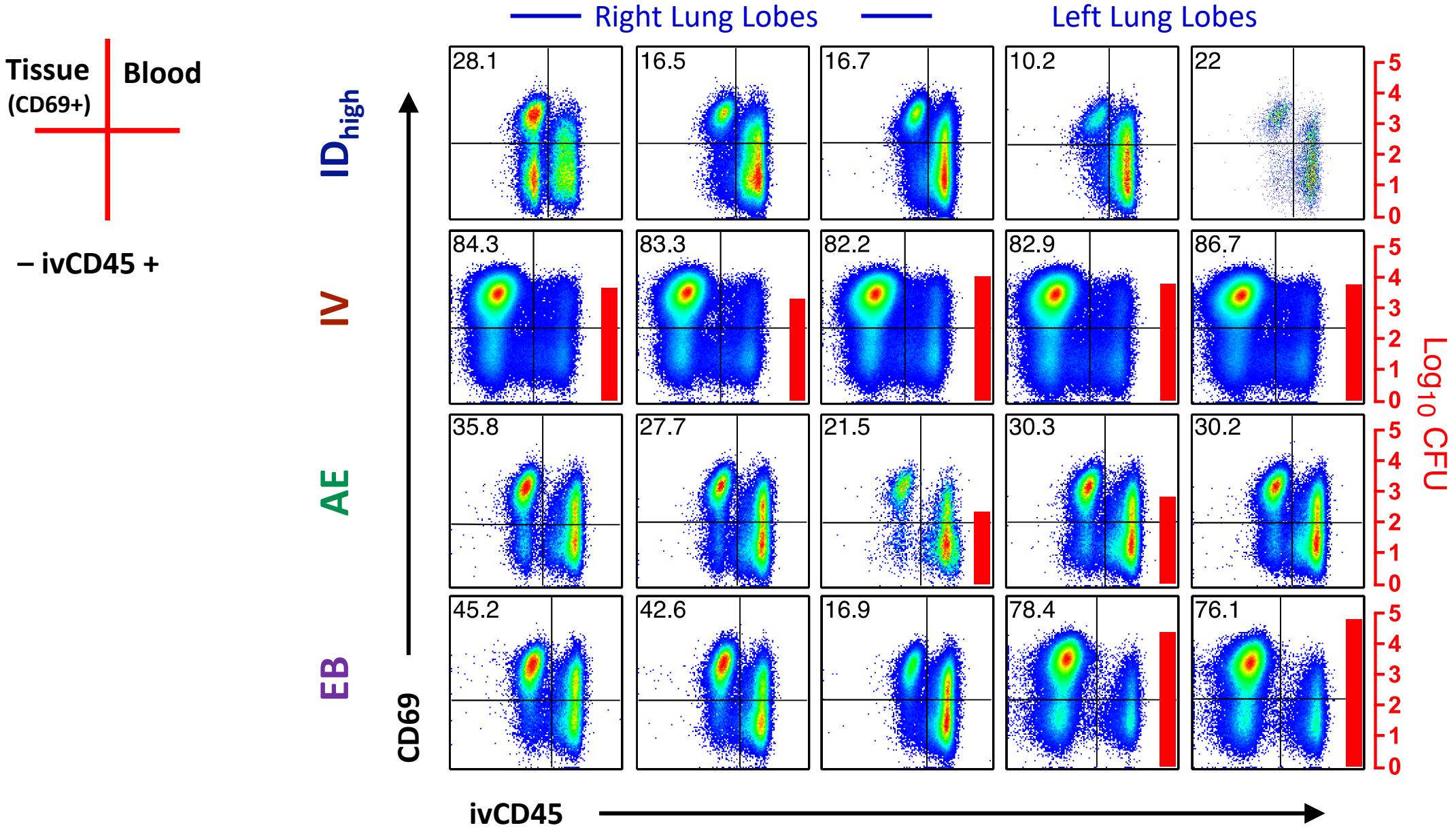




Anderson & Masopust, et al.
Nat Prot 2014; 9: 209

Sakai & Barber, et al.
J Immunol 2014;

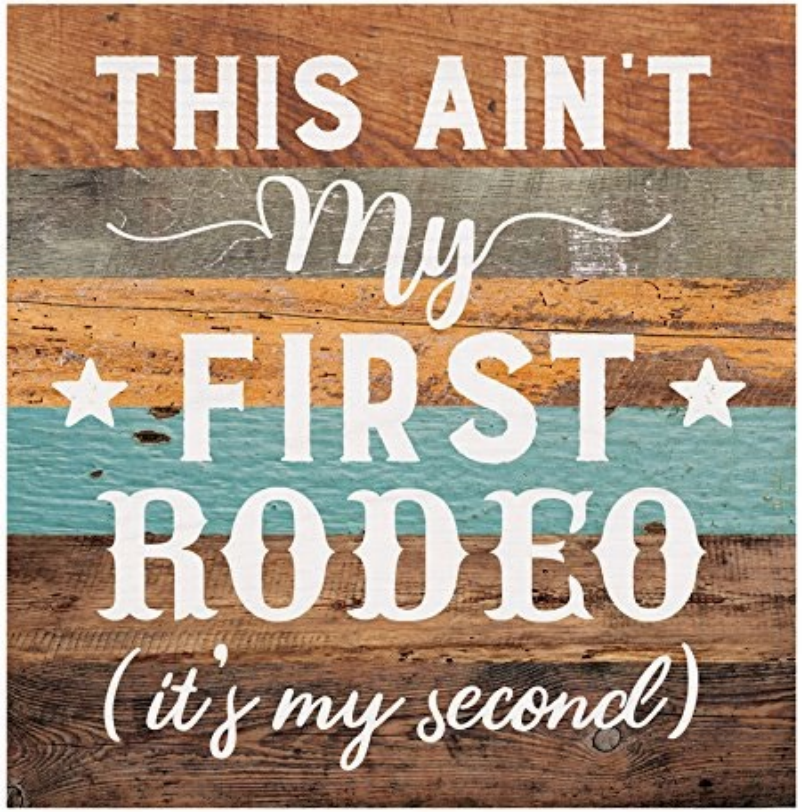
BCG IV Recruits T Cells into Lung Tissue



Key Follow-Up Studies of BCG IV in NHP

- **Immune Correlates of Protection**
  - Define immune threshold by varying dose of BCG IV
  - High level protection achieved at 50-100 fold less BCG IV
- **Mechanism of Protection**
  - Deplete CD4 or CD8 T cells prior to challenge following BCG IV
- **Durability of Protection**
- **Requirement for BCG persistence**

IV BCG for a TB Vaccine in Humans

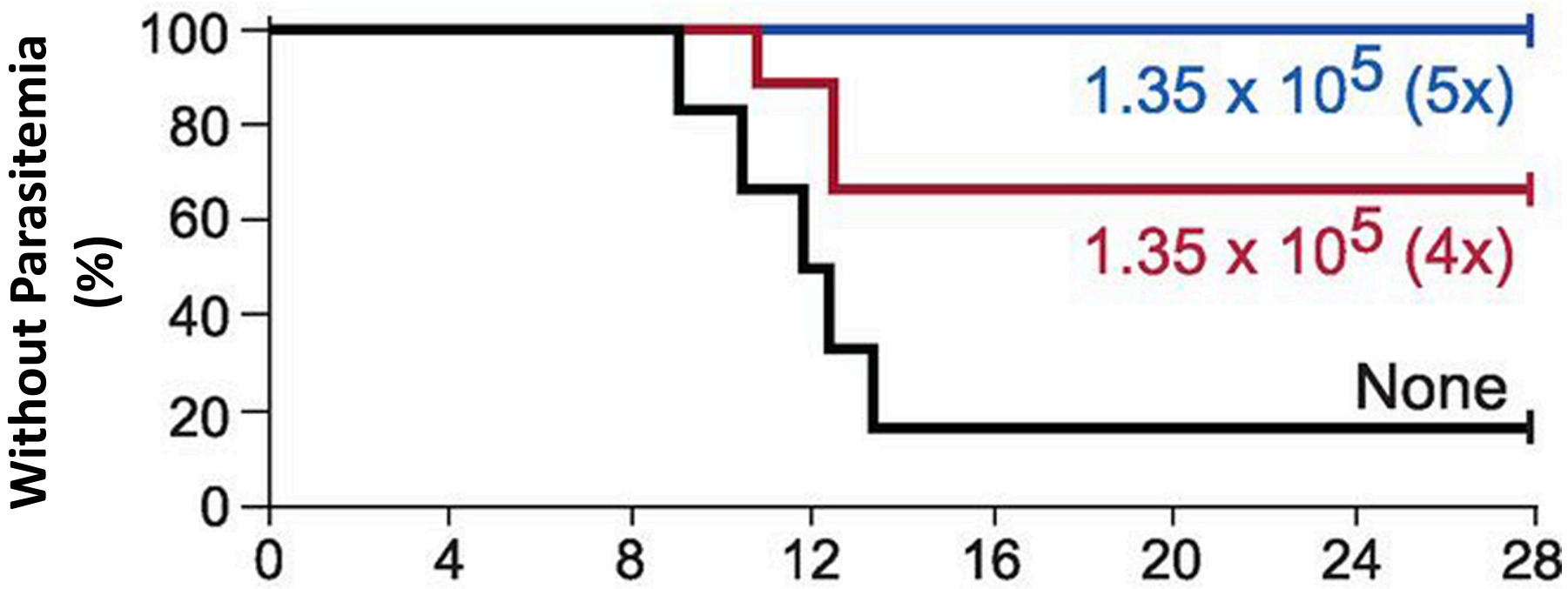


- **Feasibility and Safety of IV vaccination**

Attenuated Malaria Vaccine is More Protective IV than SQ Against Malaria Infection in Humans

Vaccine Regimen 135,000 PfSPZ (4 or 6 doses)

Controlled Human Malaria Challenge ~3 Weeks After Final Immunization



IV Immunization (12/15 protected)

SQ Immunization (0/17 protected)

Epstein J, *et al. Science* (2011)
Seder R, *et al*. *Science* (2013)

Clinical Development of IV BCG for Adolescents/Adults

- **Feasibility of IV delivery: Multiple clinical trials in Africa (10 studies in 6 different countries showing direct venous inoculation with attenuated malaria vaccine is well tolerated and safe)**

- **Clinical development plan for VPM1002 underway (DMID & VRC)**
  - **Evaluate safety/efficacy of IV VPM1002 (ΔUre rBCG-hly) in NHP**

Acknowledgments

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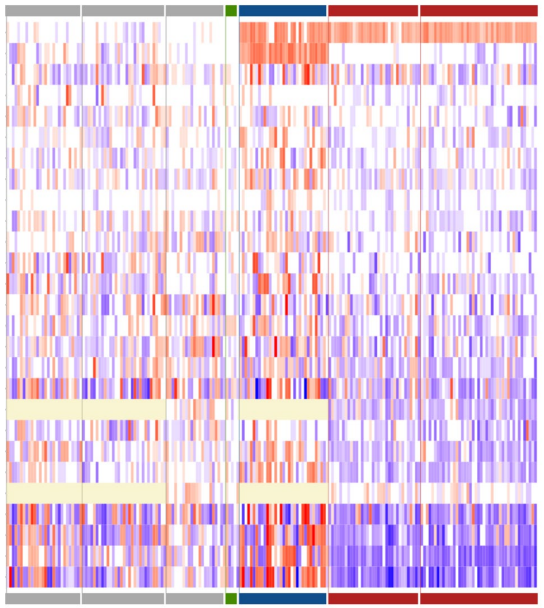
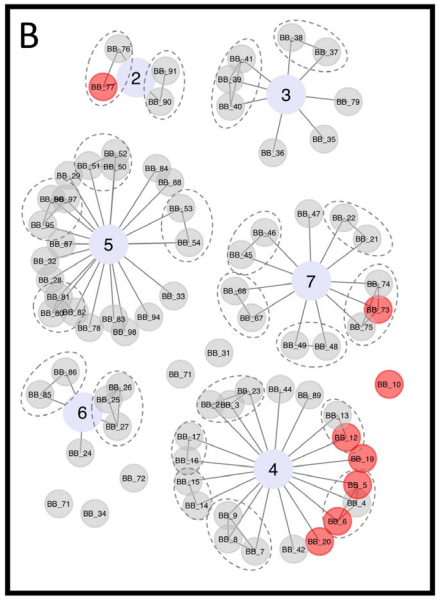
HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH

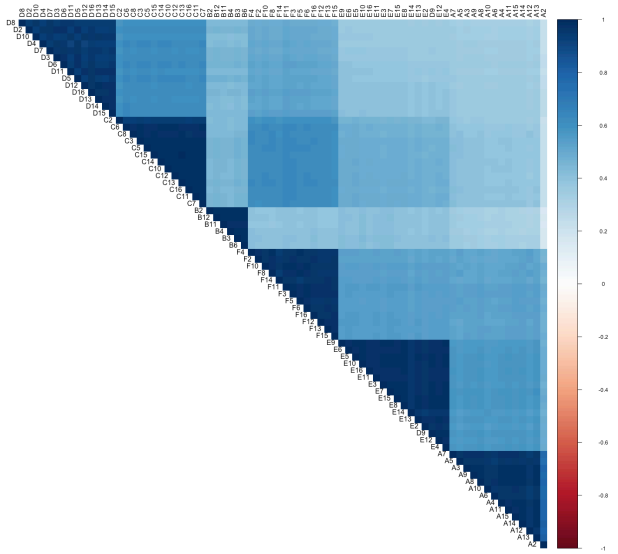
HARVARD MEDICAL SCHOOL

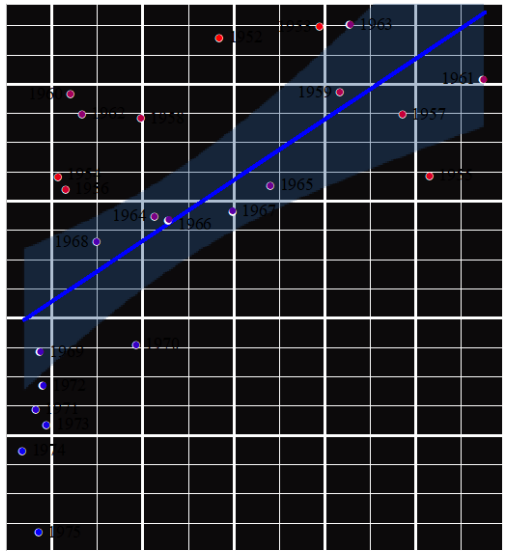
Brigham & Women's Hospital

What's old is new again: a new twist on measles vaccines and reduced overall childhood mortality









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Feb 13, 2020
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CENTER *for* COMMUNICABLE DISEASE DYNAMICS

What I want you to take away

- Measles infection **increases risk** of **other infectious diseases** for **2-3 years**
- Measles virus may have been associated with up to **50% of childhood infectious diseases deaths**
- **Measles vaccines** may therefore have led to **large reductions** (30%-50%) in childhood infectious disease **deaths**
- Measles does this by deleting pre-existing immune memory, causing "**immune-amnesia**"

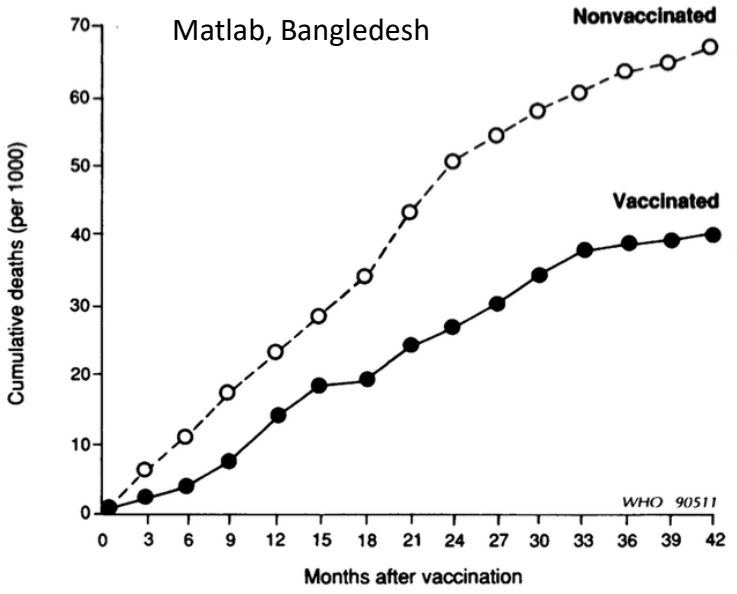
Outline of talk

- Motivation
- Evidence that :
  - Measles causes 2-3 year increases in infectious disease mortality
  - Measles causes Immune amnesia
- Conclude that measles vaccines are a ‘best buy’ in public health and serve to preserve immunity from other infectious diseases

Motivation:
Reduced mortality following vaccine introductions

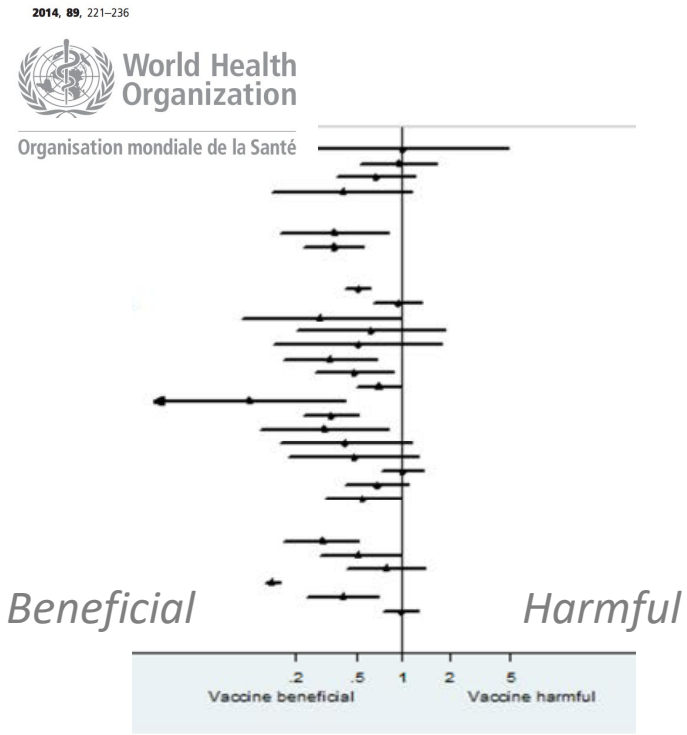
- **Measles vaccine** introduction → sustained **reductions** in overall mortality
- Often **>30-50%** reductions in childhood mortality in low income regions

Fig. 2. **Cumulative risk of death, by vaccination status, Matlab intervention area, 1982–85.**



40% reduction
Childhood mortality in MV vaccinated

Koenig et al. Bull. WHO 1990 (68)4



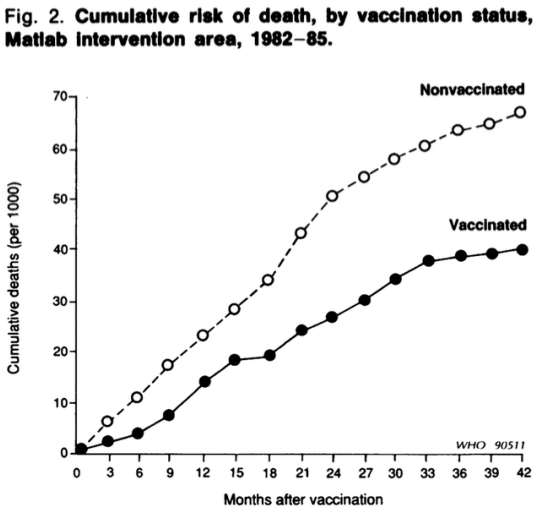
- **But measles is not known to have caused 40% of all childhood deaths…**
- **How could the vaccine improve survival so much?**

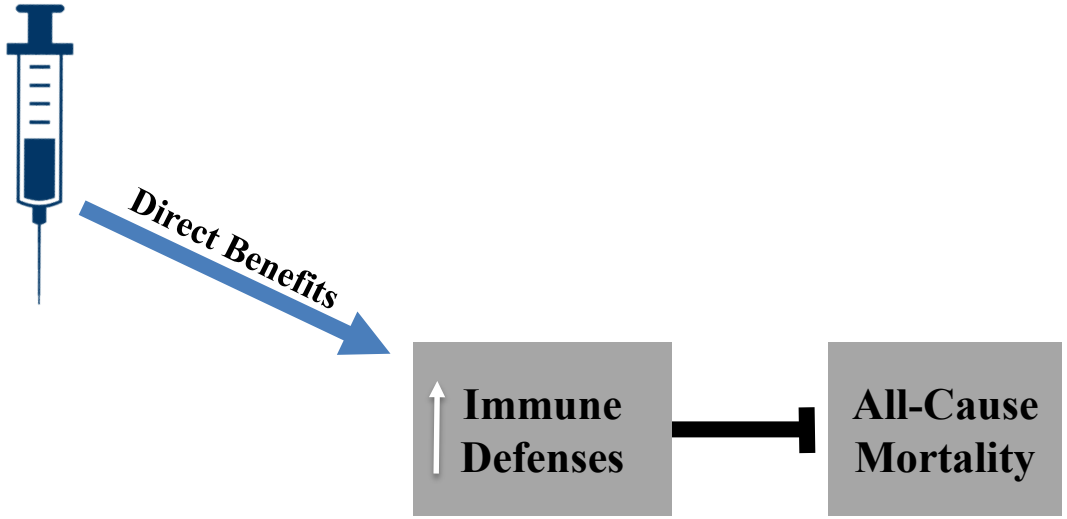
Michael Mina: mjmina@bwh.harvard.edu

Reduced Mortality post-vaccination

- **Hypothesis 1 - Direct Effect**

  Measles Vaccination “boosts” immunity for years via “non-specific immunologic effects”



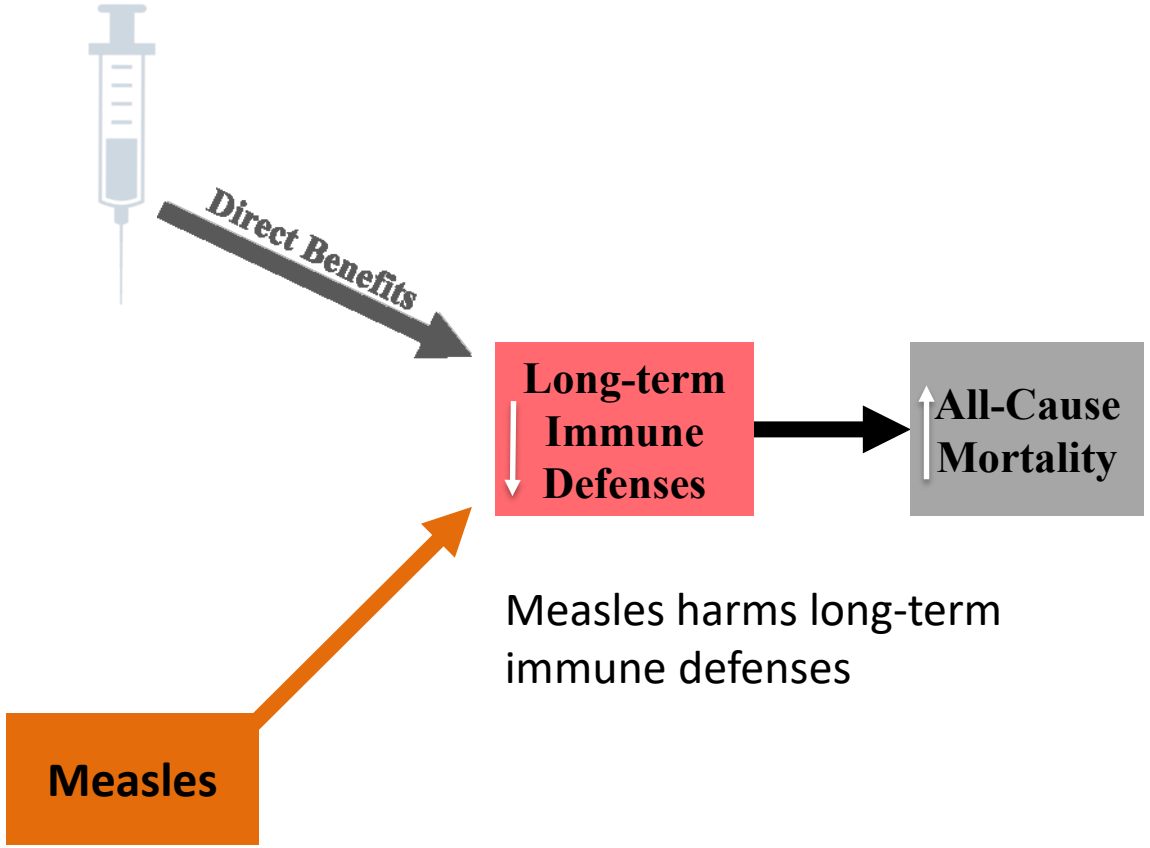


?? Benefits appeared to **increase over time** after vaccination

Thus, not consistent with a general ‘boosting’ effect

Reduced Mortality post-vaccination

- **Hypothesis 2 - Indirect effect**
  Measles has long lasting adverse effects on immunity



Reduced Mortality post-vaccination

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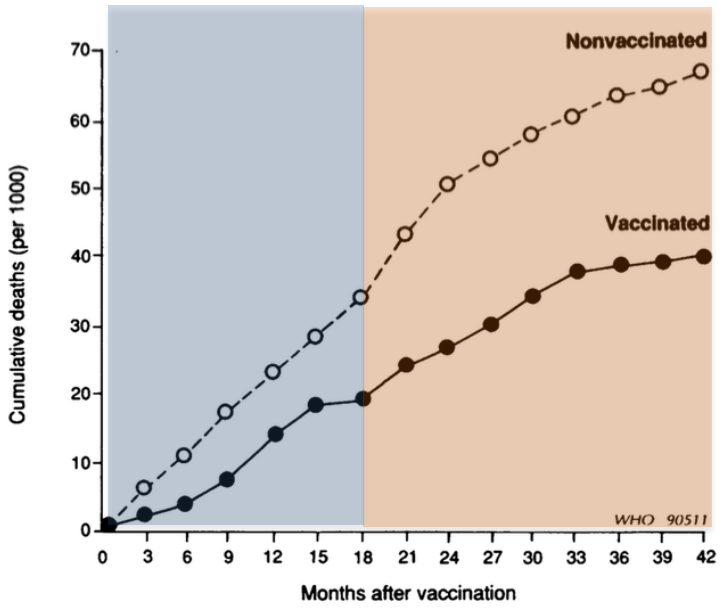
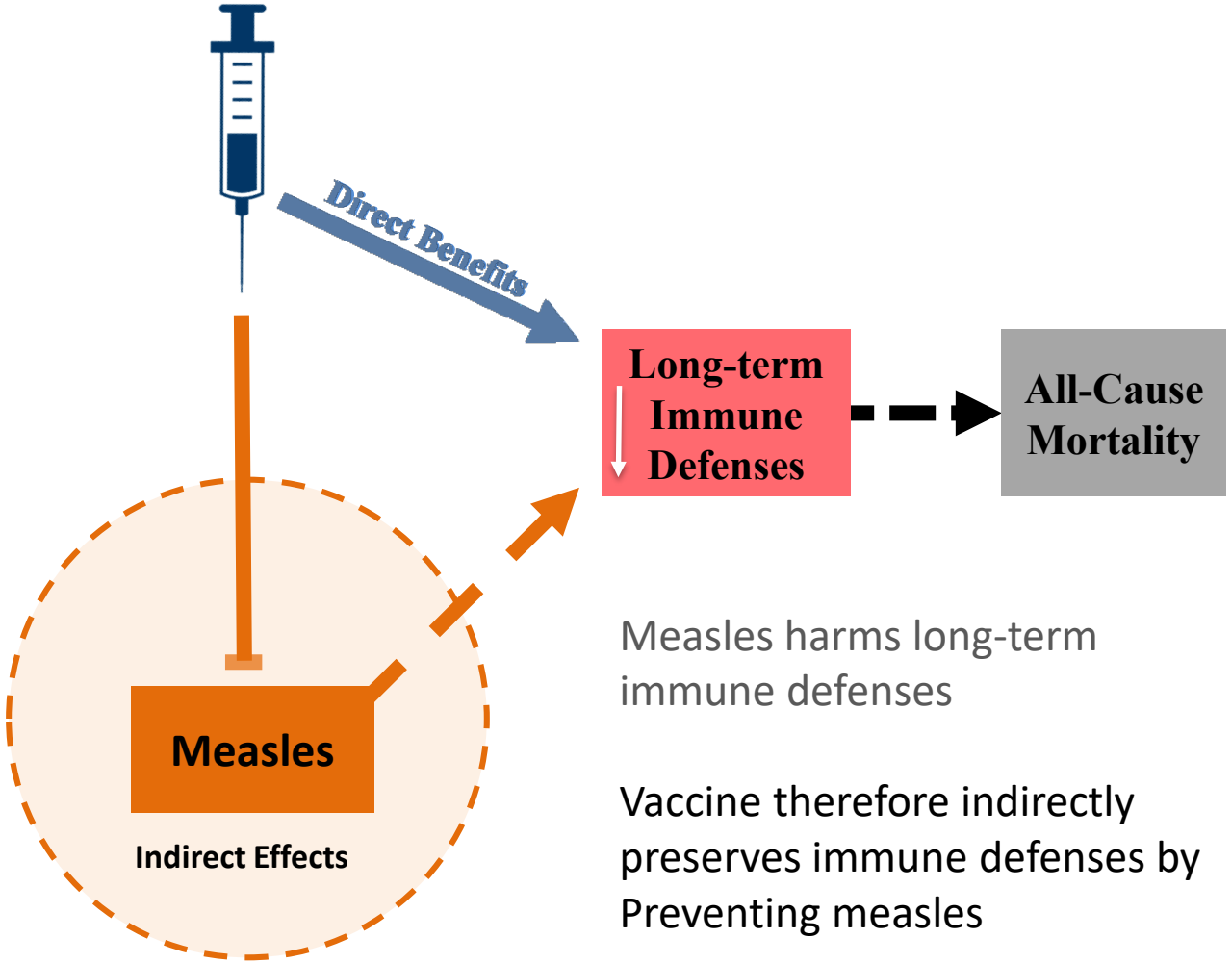
Fig. 2. **Cumulative risk of death, by vaccination status, Matlab intervention area, 1982–85.**





Measles harms long-term immune defenses

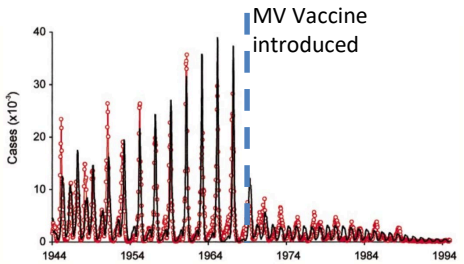
Vaccine therefore indirectly preserves immune defenses by Preventing measles

When measles was common a long-term effect of measles to increase childhood mortality should be detectable in population data.

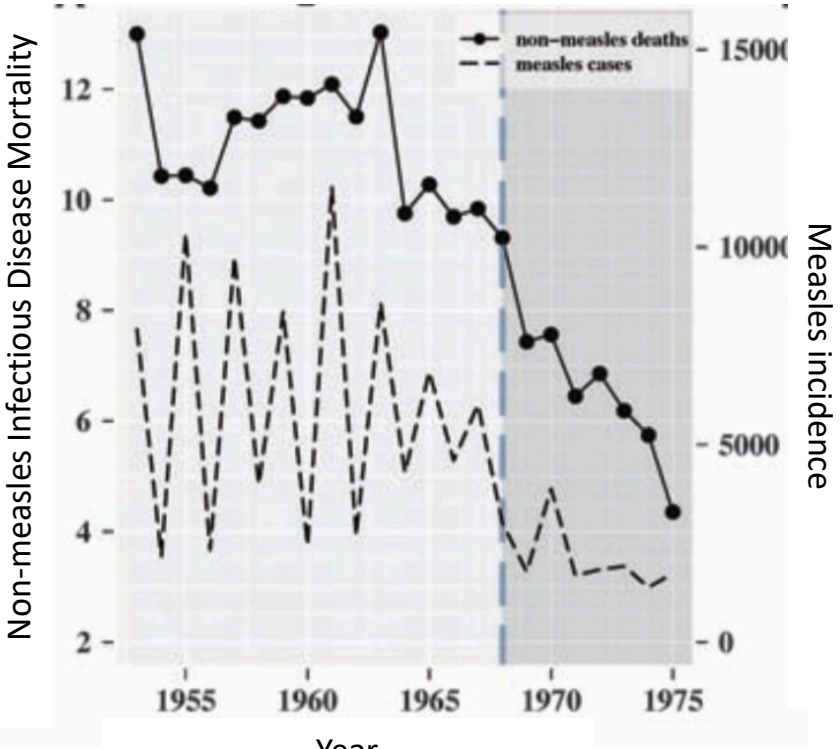
Test: does measles incidence predict non-measles mortality when incidence is aggregated over a longer time period

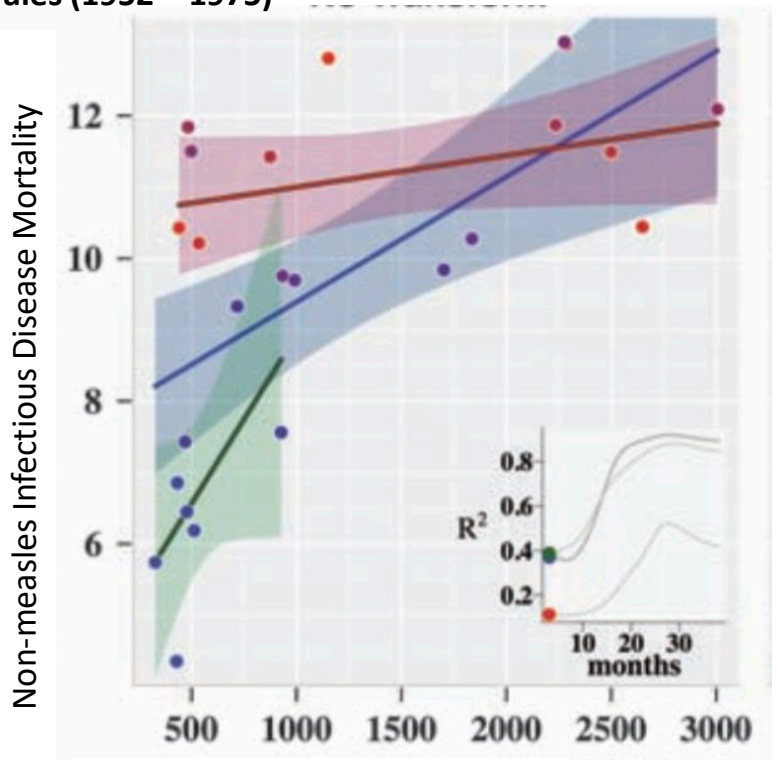
Population Evidence: Measles increases risk of death from other infectious diseases for 2-3 years

Compare:
Measles incidence vs.
non-measles infectious disease mortality

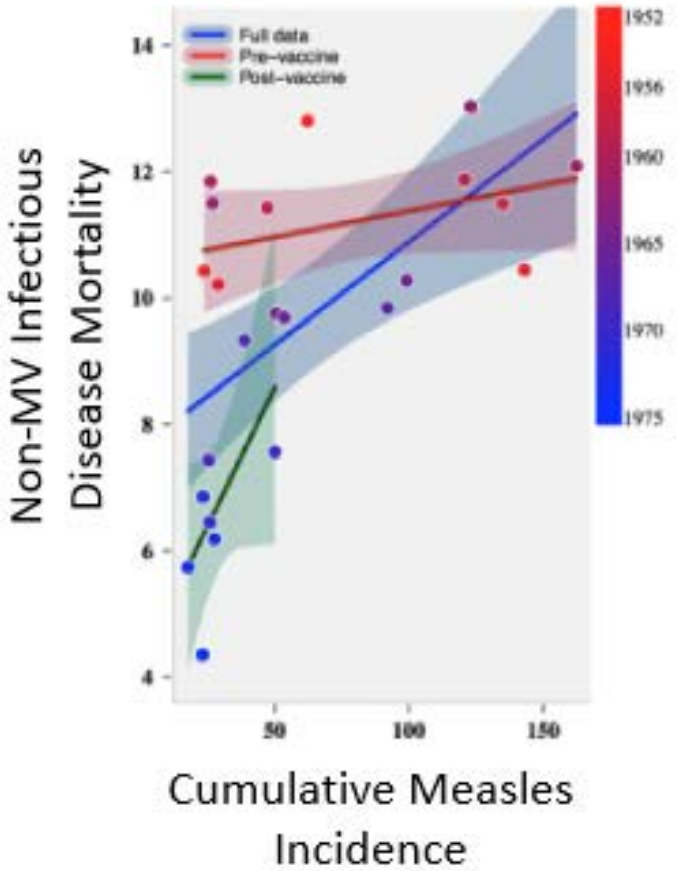


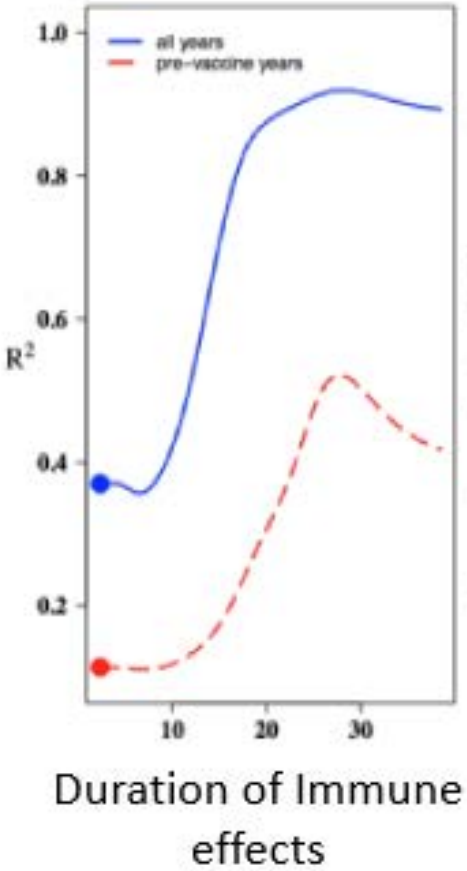
England & Wales (1952 – 1975)

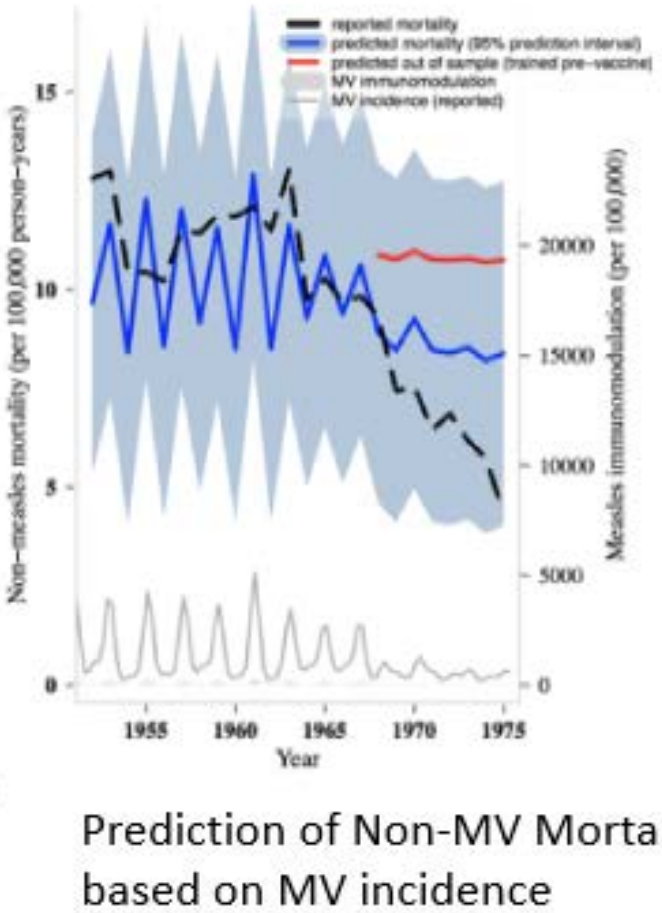




Mina, Science, June 2015
Mina, Science, July 2019







England & Wales 1950 – 1975

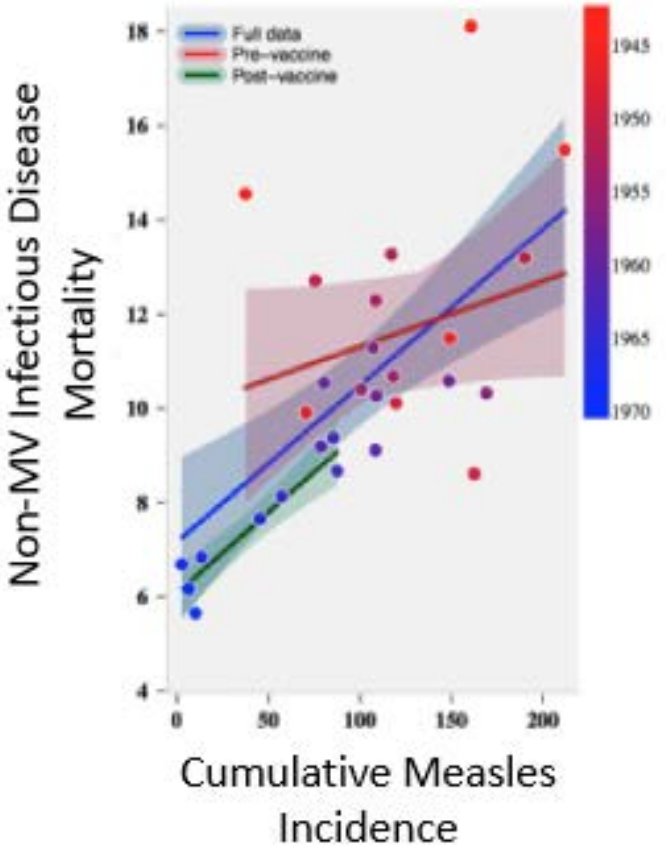
1. Measles incidence should predict to non-measles infections / deaths

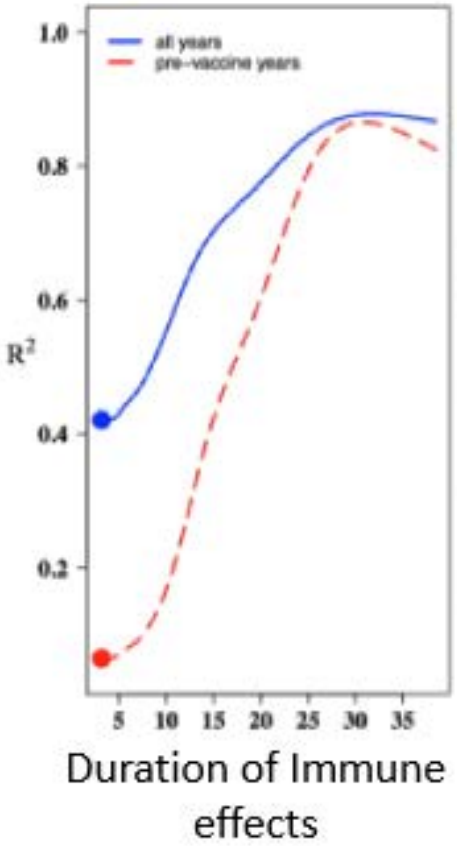
2. The strongest fit should occur **when measles cases are accumulated over the average duration of immune effects**

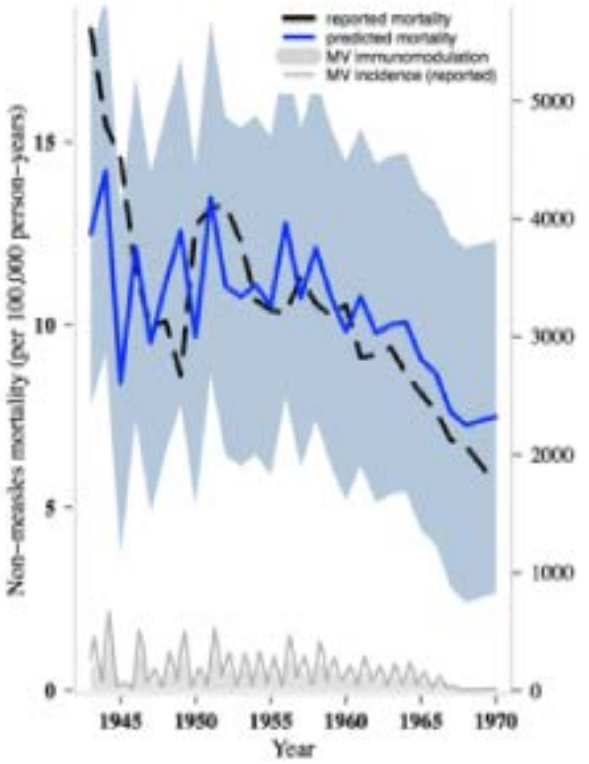
**As movie progresses, measles cases are being accumulated at each time point from increasing durations back in time… This duration reflects duration of expected immune effects **

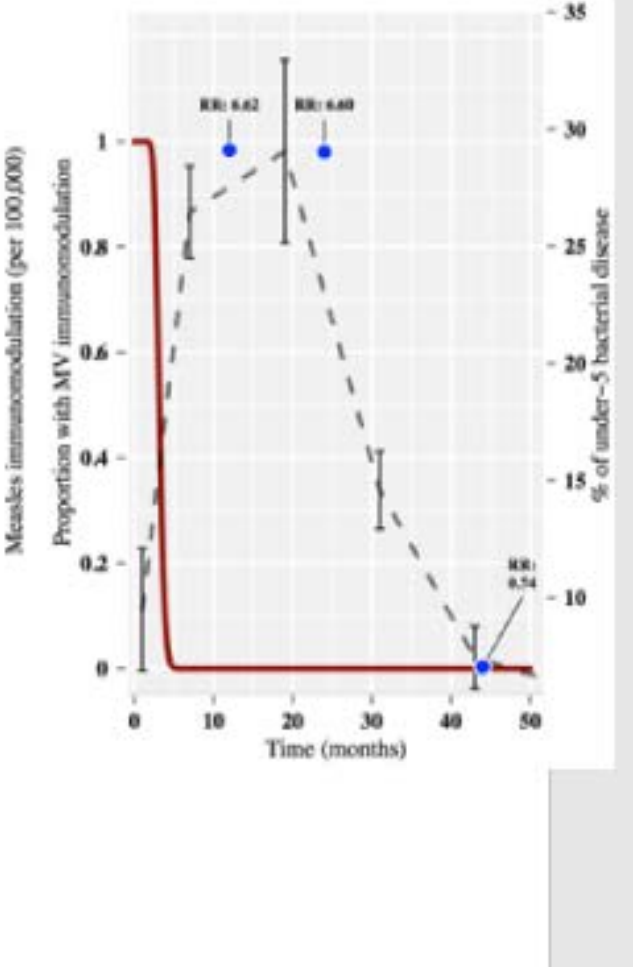
Mina et al. Science 2015

Michael Mina: mjmina@bwh.harvard.edu







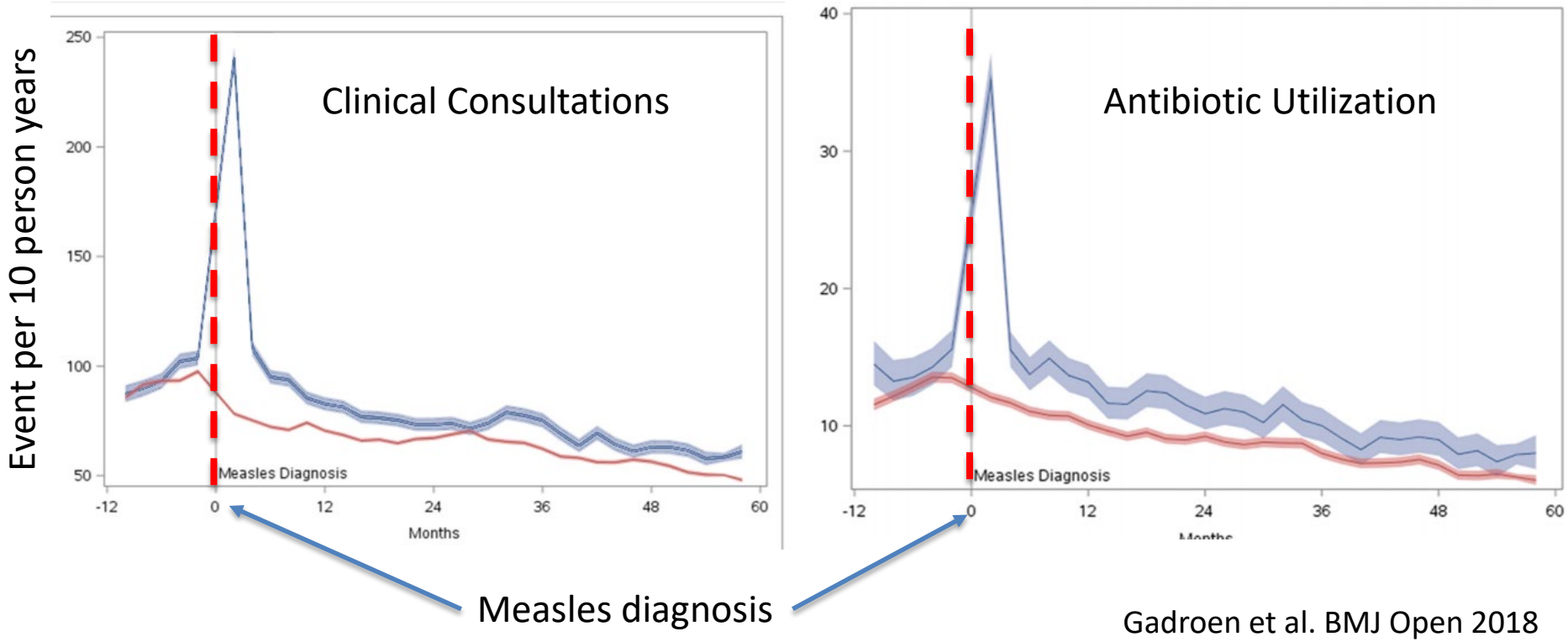


USA Data
1943-1972

Recent Evidence from Clinical Reports

UK THIN database:
Evaluated 2228 measles infections in UK since 1990

Up to 5 years post measles
- Increases Risk for non-measles infections after measles:
- 43% increase in month 1
- 20% increase in yr 1
- 10% overall years 1-5



Gadroen et al. BMJ Open 2018

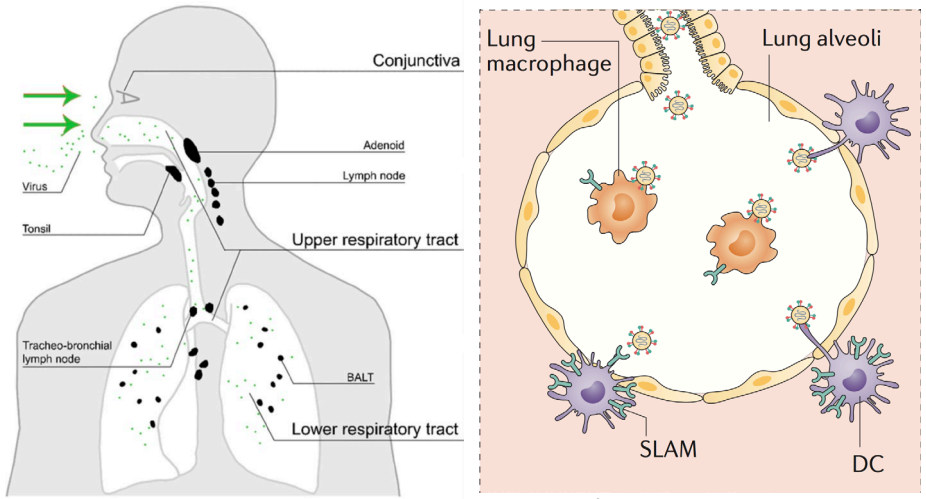
When effects of measles are assumed to last for ~2-3 years, measles predicts childhood infectious disease mortality

But… measles is the textbook acute infection

How could it have such long-lasting effects?

Measles – Entry (CD150)

Entry – CD150+



Rota et al. Nature Reviews 2016

nature International weekly journal of science

Nature **406**, 893-897 (24 August 2000) | doi:10.1038/35022579; Received 12 May 2000; Accepted 10 June 2000

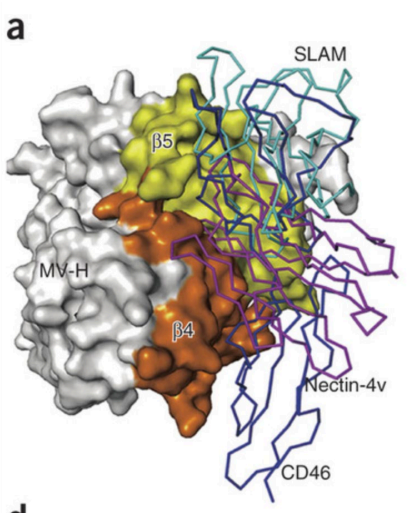
SLAM (CDw150) is a cellular receptor for measles virus

Hironobu Tatsuo[1], Nobuyuki Ono[1], Kotaro Tanaka & Yusuke Yanagi

CD150 = SLAM-F1 = Signaling Lymphocytic Activating Molecule

On Memory B, T and long-lived plasma cells that produce antibody

MV HA binds CD150 on lymphoid cells as sole mode of entry into human cells (Trojan Horse)

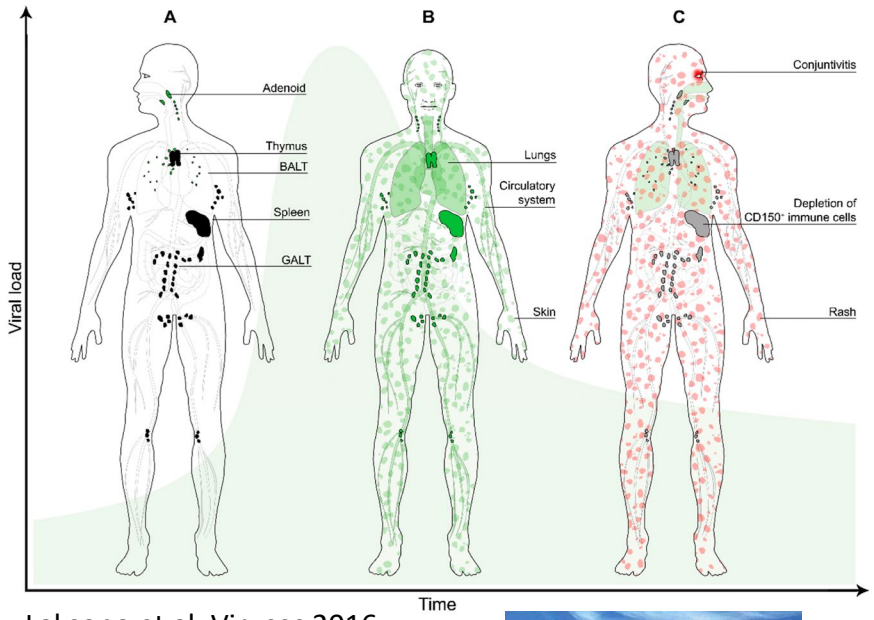


Zhang et al. Nat Struct & Mol Bio (2013)

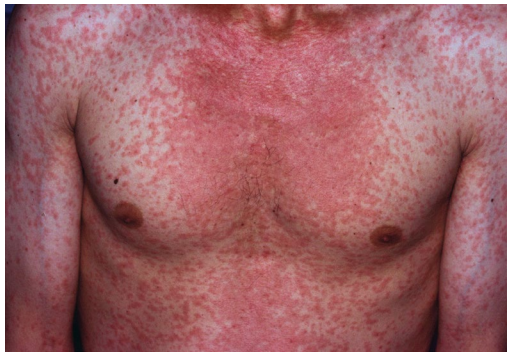
Michael Mina: mimina@bwh.harvard.edu

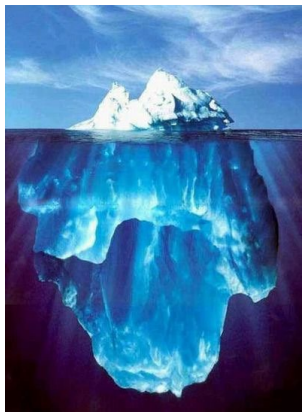
Measles infects and destroys immune cells

Dissemination
(Trojan Horse)

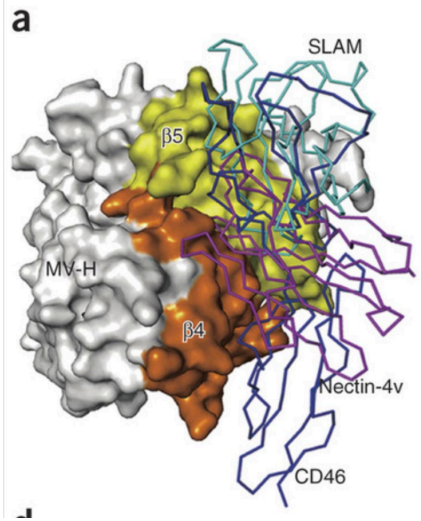


Laksono et al. Viruses 2016



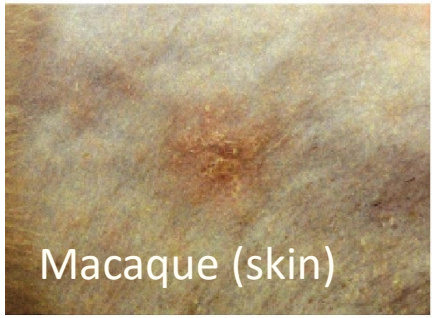


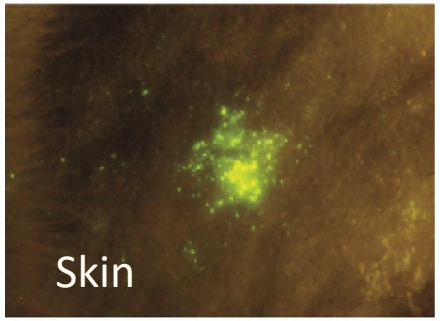
The MV receptor CD150
or SLAMF-1

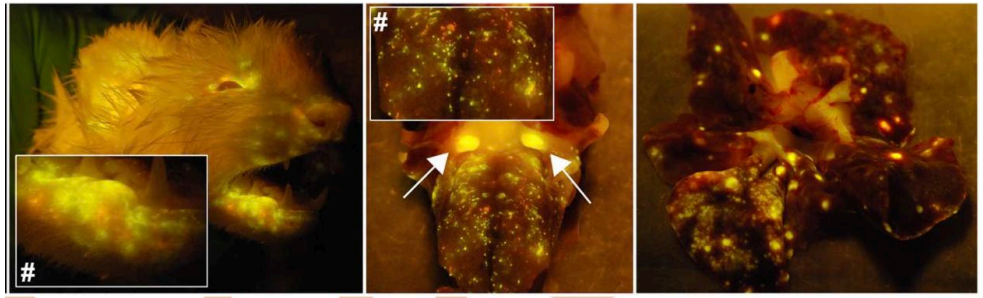


Primarily expressed on
immune memory cells

B-, T-, Plasma cells



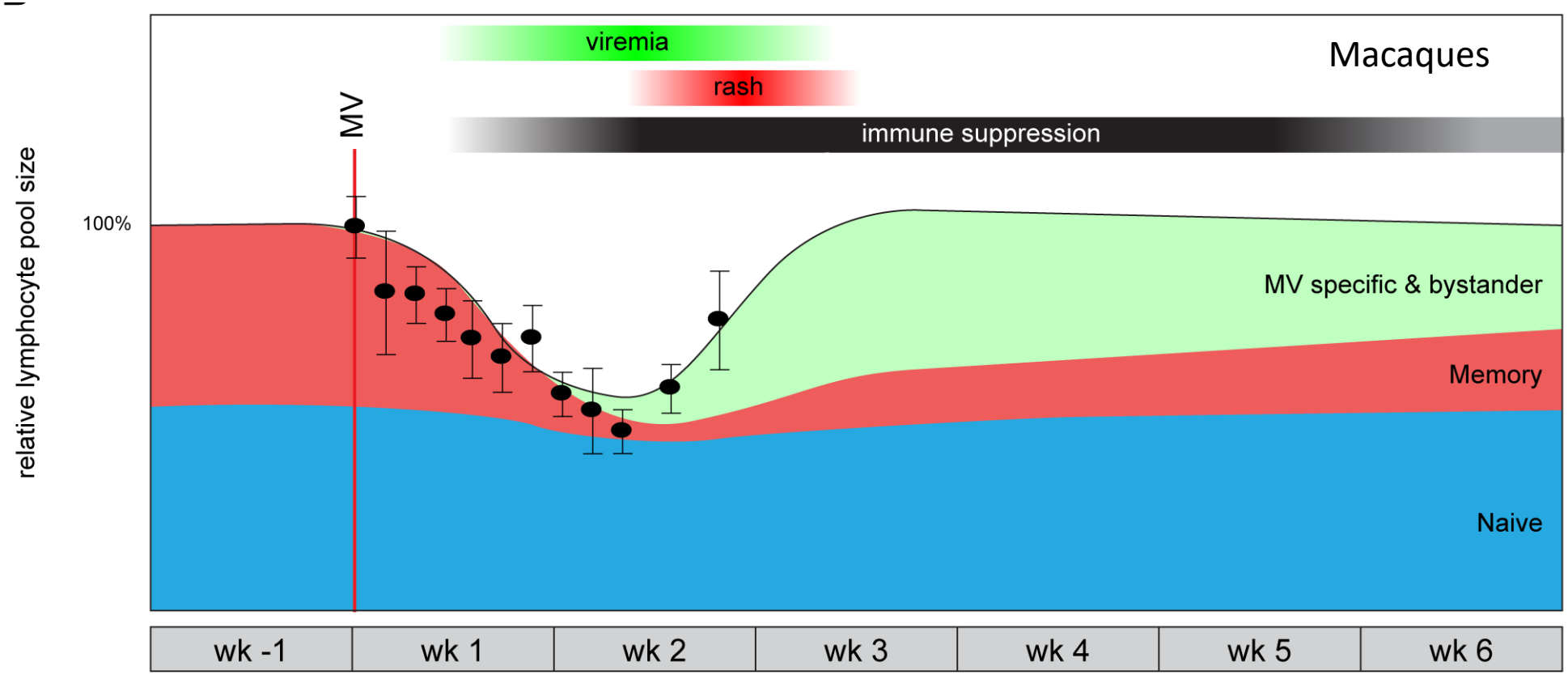




De Vries et al, PLoS Pathog (2012, 2017), &
Courtesy of Duprex, De Swart

Zhang et al. Nat Struct & Mol Bio (2013)

Immune depletion effects

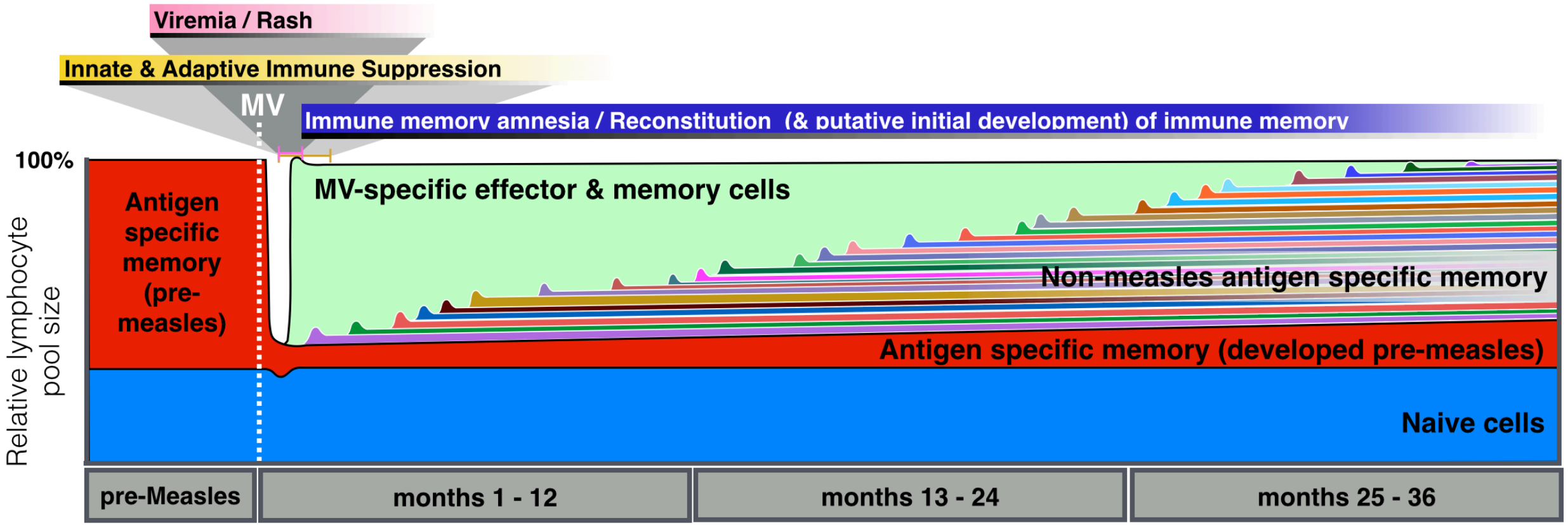


- MV Depletes Memory Cell Pool
- Massive expansion of lymphocytes 2-3 weeks later
- What happens to pre-existing memory repertoire?

De Vries et al, PLoS Pathog (2012)

Michael Mina: mjmina@bwh.harvard.edu

Hypothesis – Immune amnesia



Mina et al. Science May 2015
Mina et al. Science July 2019

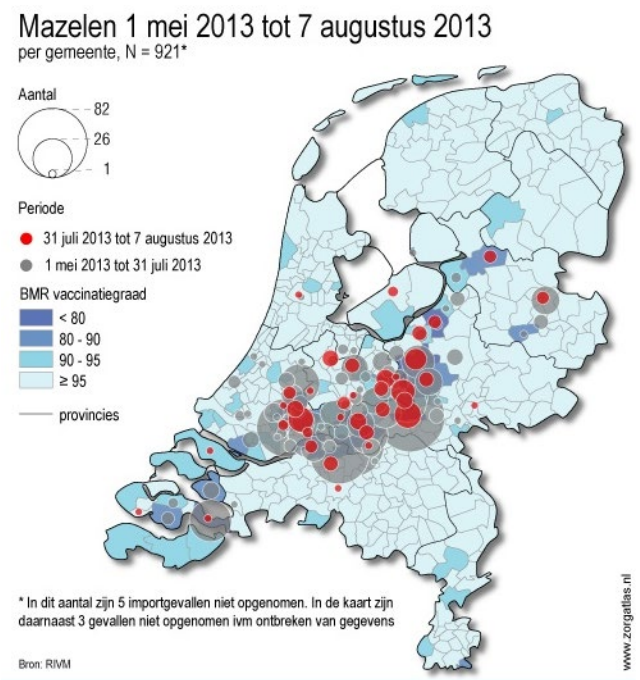
Hypothesis

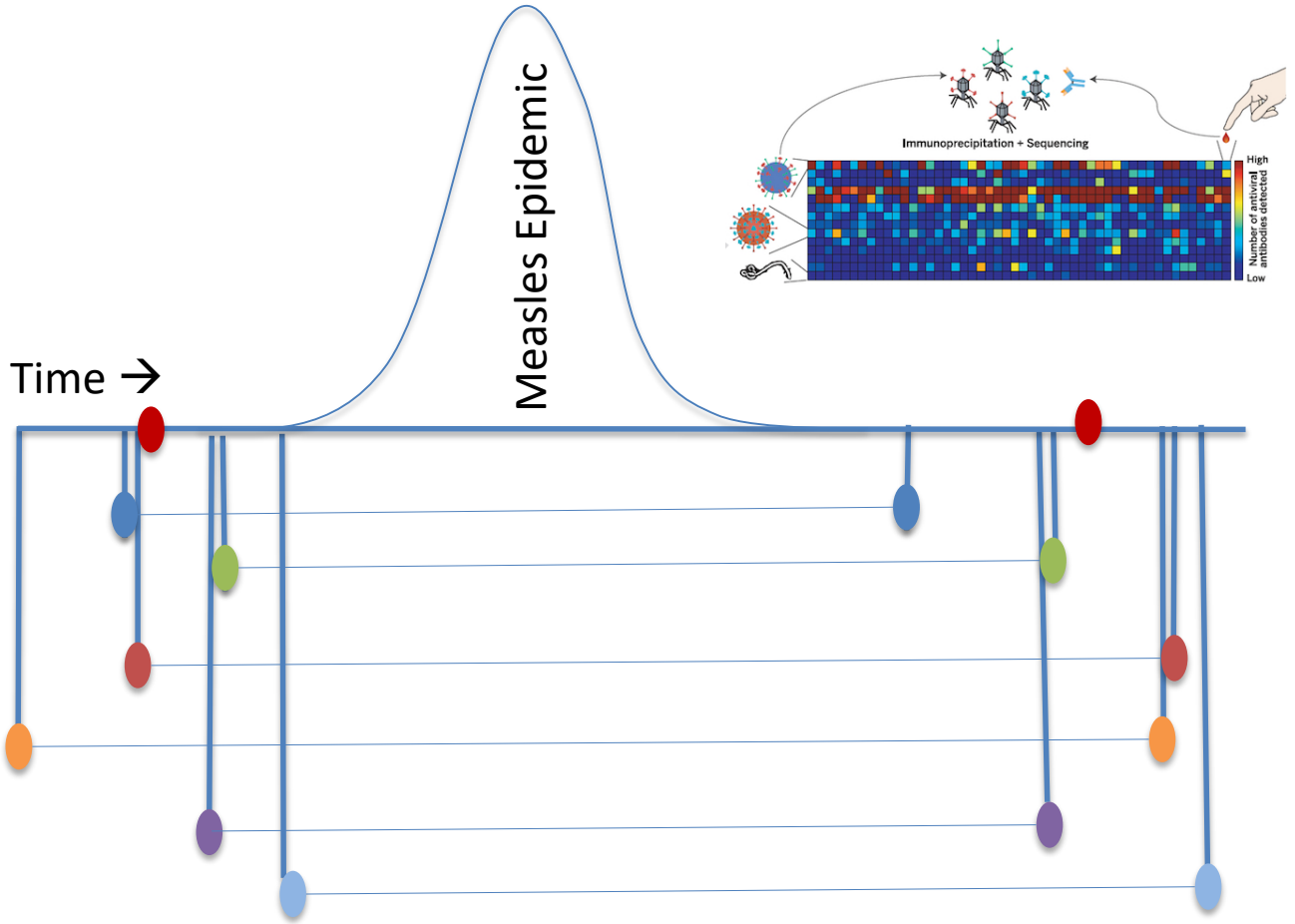
- Measles deletes pre-existing immunity
- Children have to build it back – but this takes a few years
- During this time, they are at increased risk of disease and death from other infections

Testing MV Immune-Amnesia

- 2013 Dutch Measles epidemic
- Collected **Pre** & **Post** measles plasma from 77 MV infected children and controls
- Analyzed their immune repertoire using VirScan phage-display system

Dutch MV Outbreak





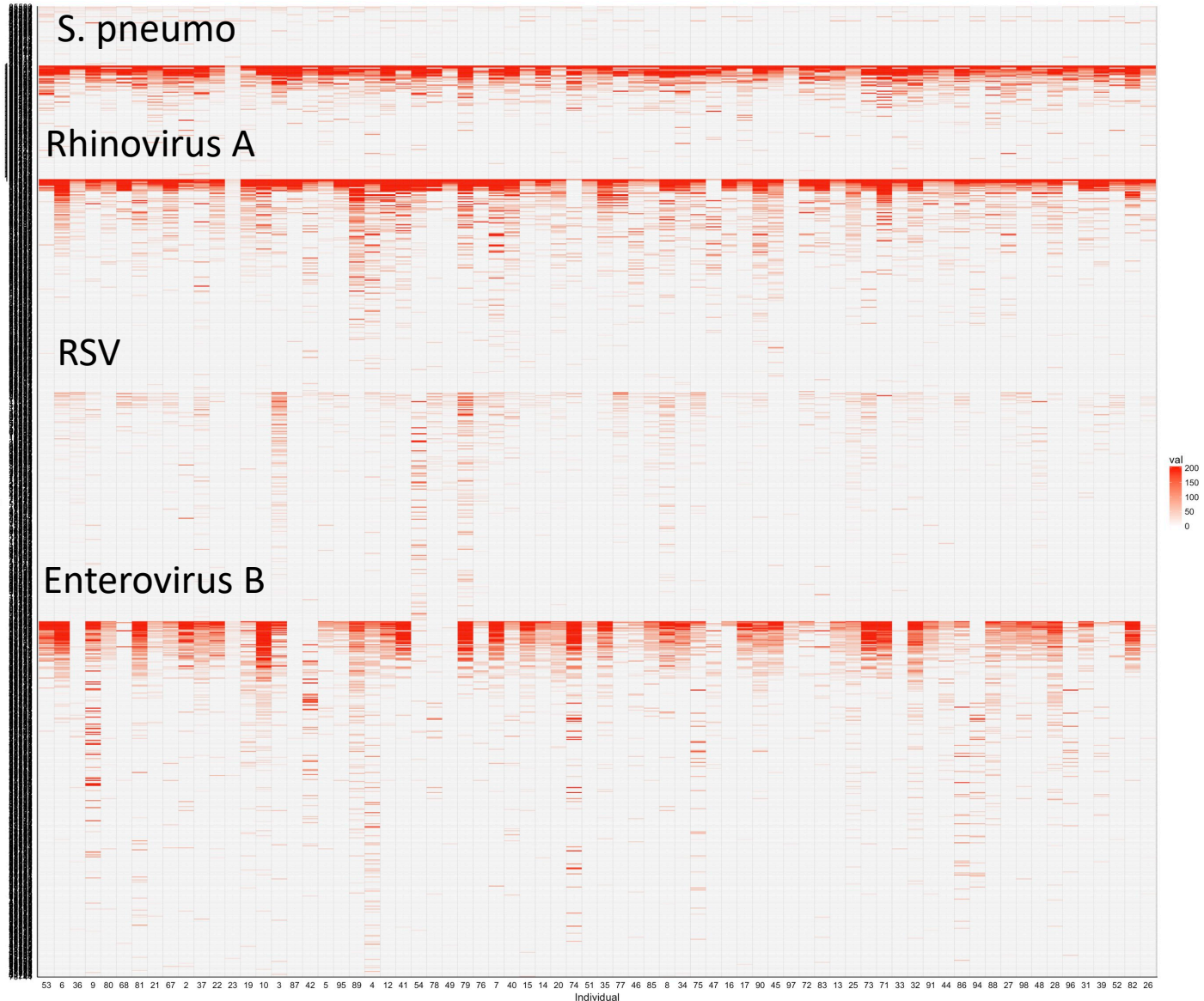
Measure 1000's of antibodies against hundreds of pathogens simulatnously

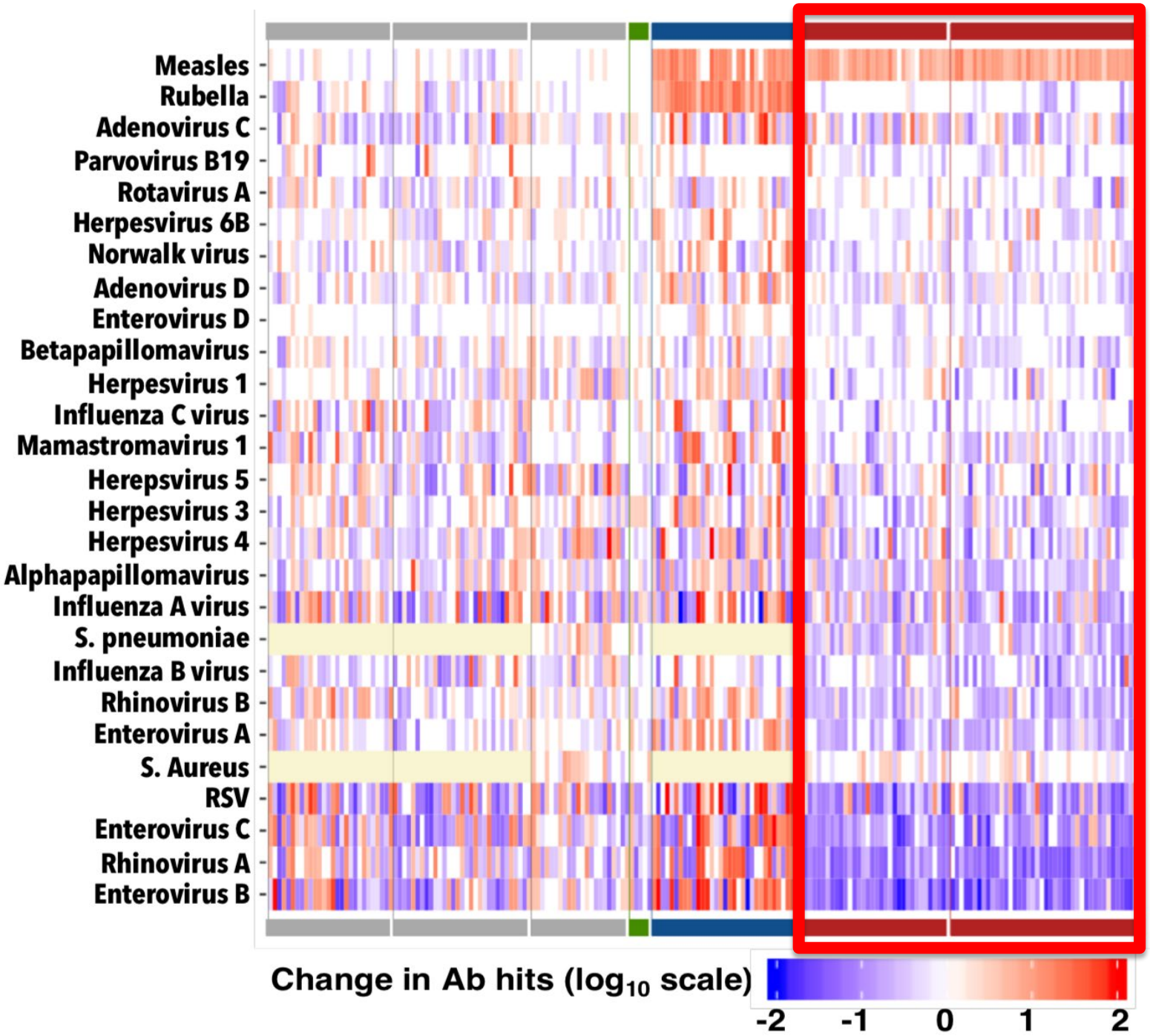
Michael Mina: mmina@hsph.harvard.edu

VirScan antibody data

Measure 10,000's of antibodies per individual - simultaneously

Only 4 of ~400 pathogens are shown here

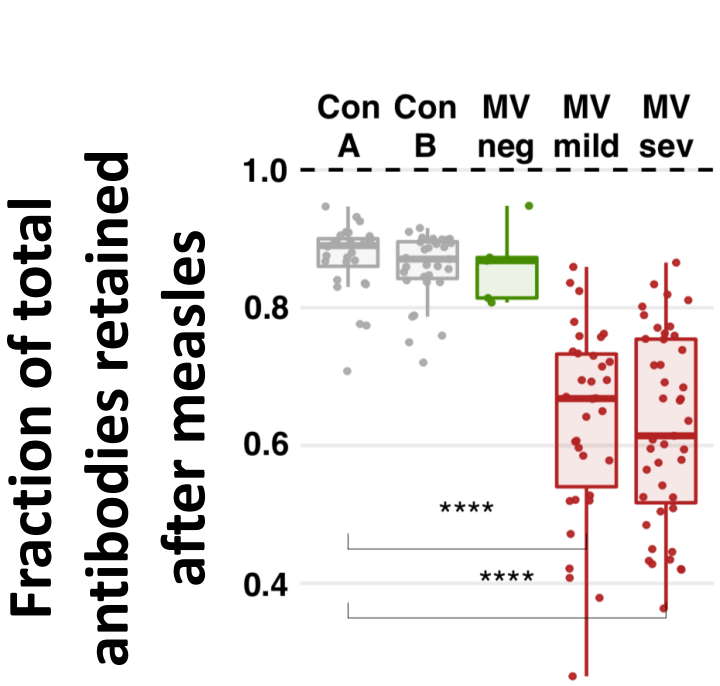


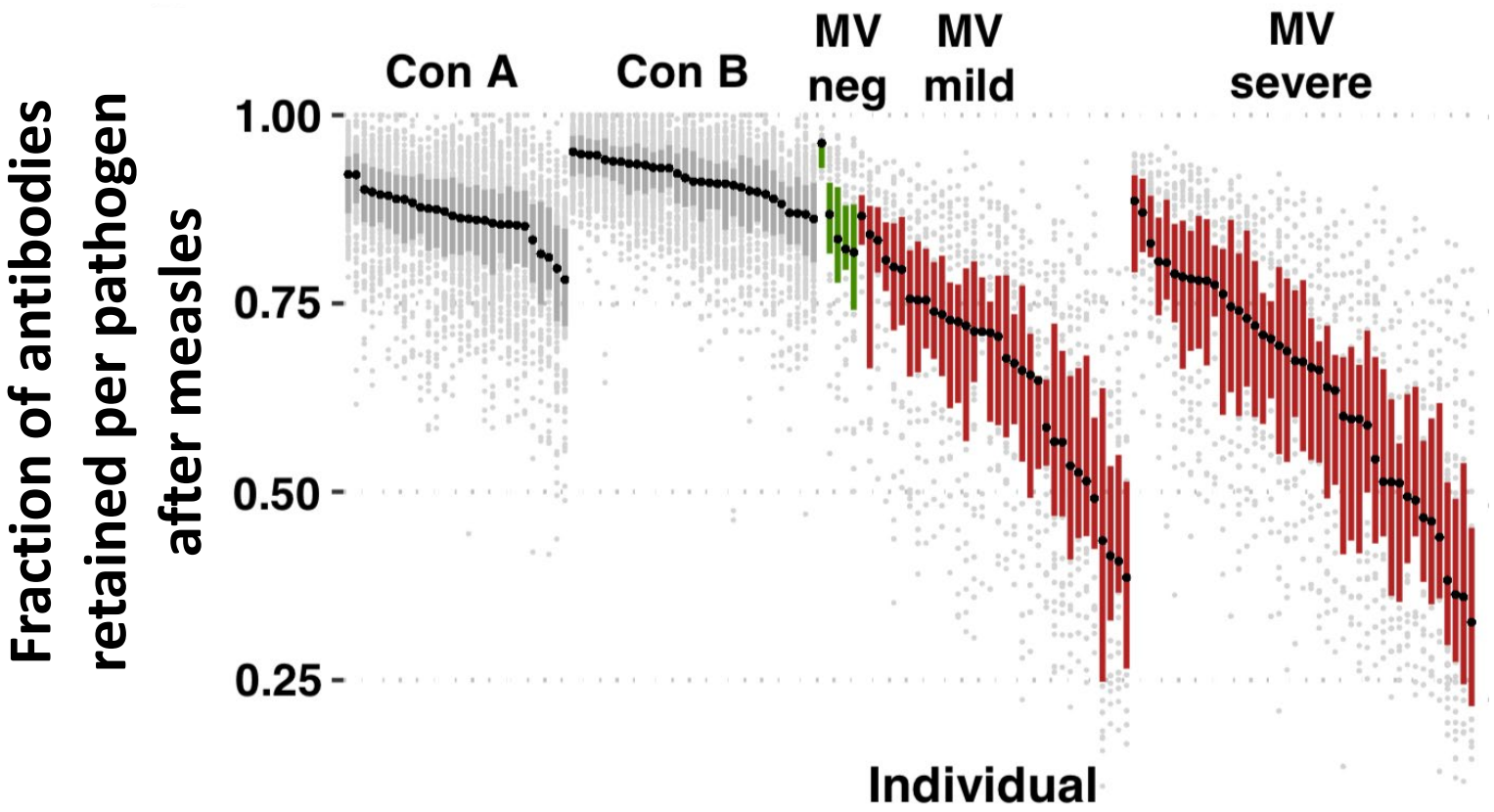


Colors indicate change in numbers of individual antibodies detected

Mina et al. Science Nov 1, 2019

Measles deletes 10-60% of pre-existing immunological memory

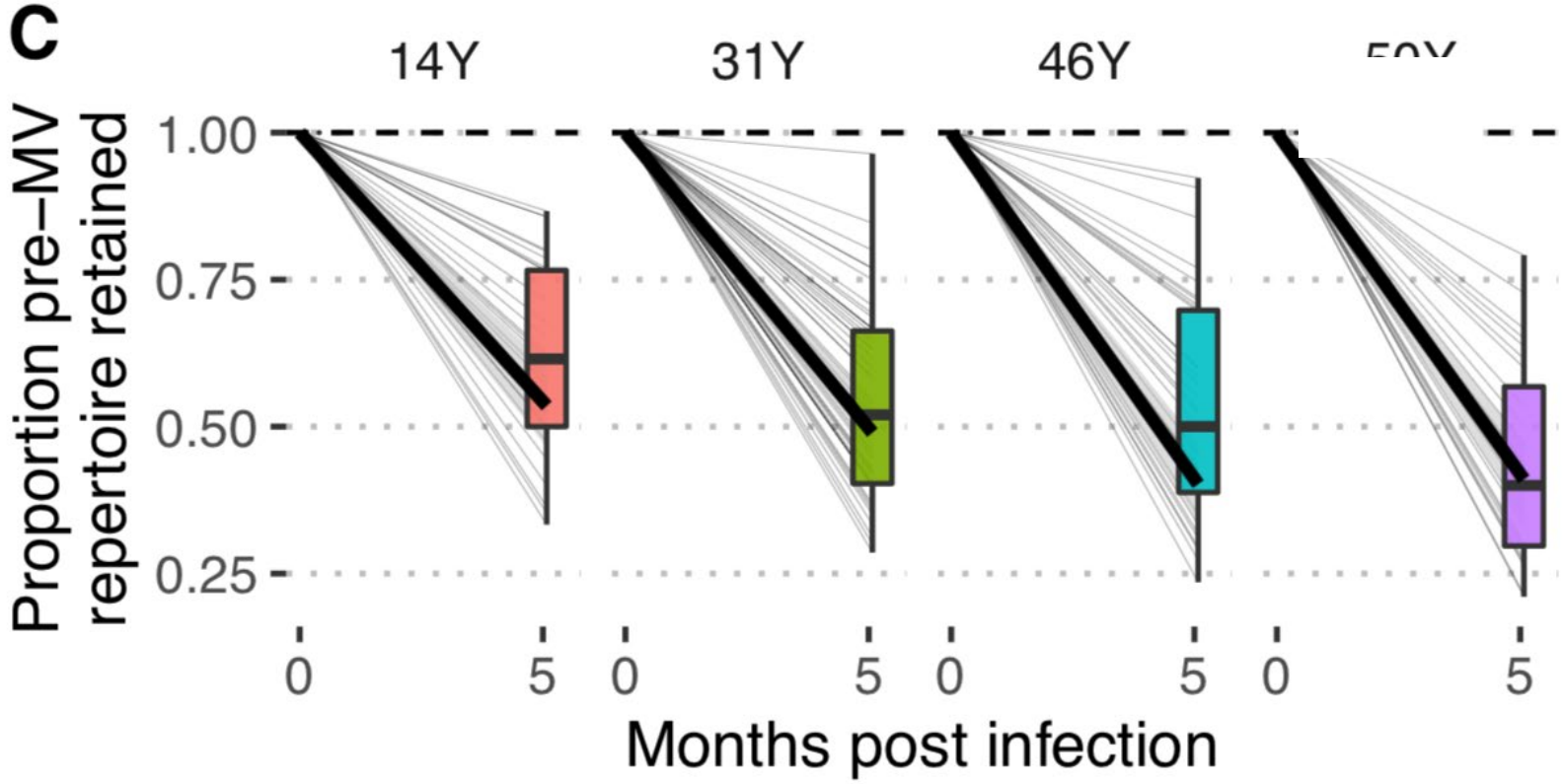




IQR: 30-50% loss in pre-existing antibodies

Mina et al. Science Nov 1, 2019

Measles infected macaques: **5 months** post measles



40%-60% Reduction in antibodies at 5 months post measles

Mina et al. Science Nov 1, 2019

Summary

- Measles deletes previously acquired immunological memory – causing **‘immunological-amnesia’**
- **Explains population evidence large reductions in mortality after MV vaccine introductions**
- **Together suggests that measles was once associated with ~50% of all childhood deaths due to infectious diseases**
- Measles vaccines may thus have unintentionally reduced global childhood mortality by as much as **30%-50%**
- **Suggest measles is a ‘best buy’ in public health**

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HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH

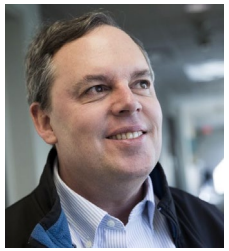
Brigham & Women's Hospital

HARVARD MEDICAL SCHOOL

Princeton University


Bryan Grenfell, PhD
Jess Metcalf, PhD

Harvard Medical School



Stephen Elledge, PhD
Tomasz Kula
Ellen Shrock

Erasmus MC **NEDIL/BU**


Rik de Swart, PhD
Paul Duprex, PhD

Johns Hopkins

Diane Griffin

Value of Vaccine Research Network (VoVRN)

BILL & MELINDA GATES foundation

hhmi Howard Hughes Medical Institute

NATIONAL INSTITUTES OF HEALTH

DARPA

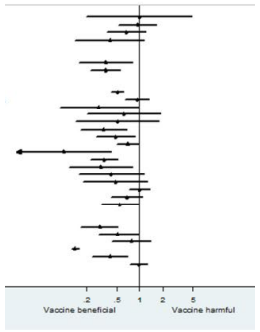
U.S. DEPARTMENT OF HOMELAND SECURITY

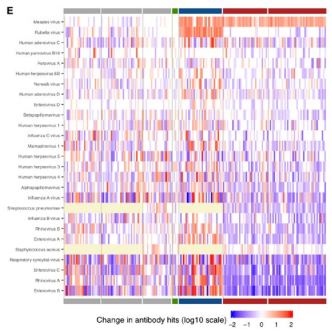
CENTER for COMMUNICABLE DISEASE DYNAMICS

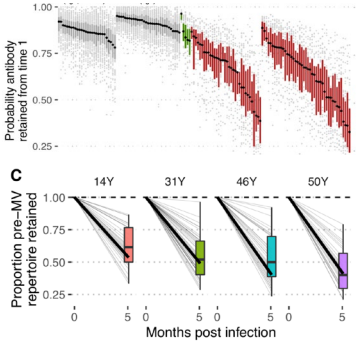
Many families who donate their blood!

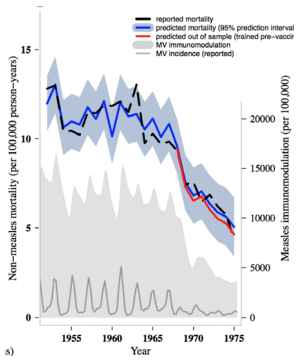
Michael Mina: mjmina@bwh.harvard.edu

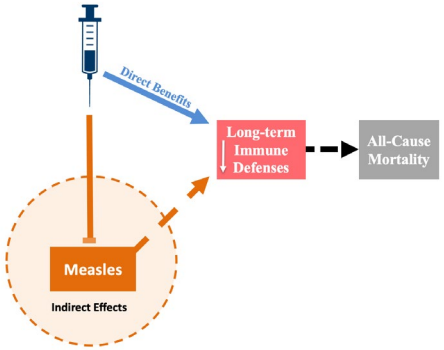
Questions?









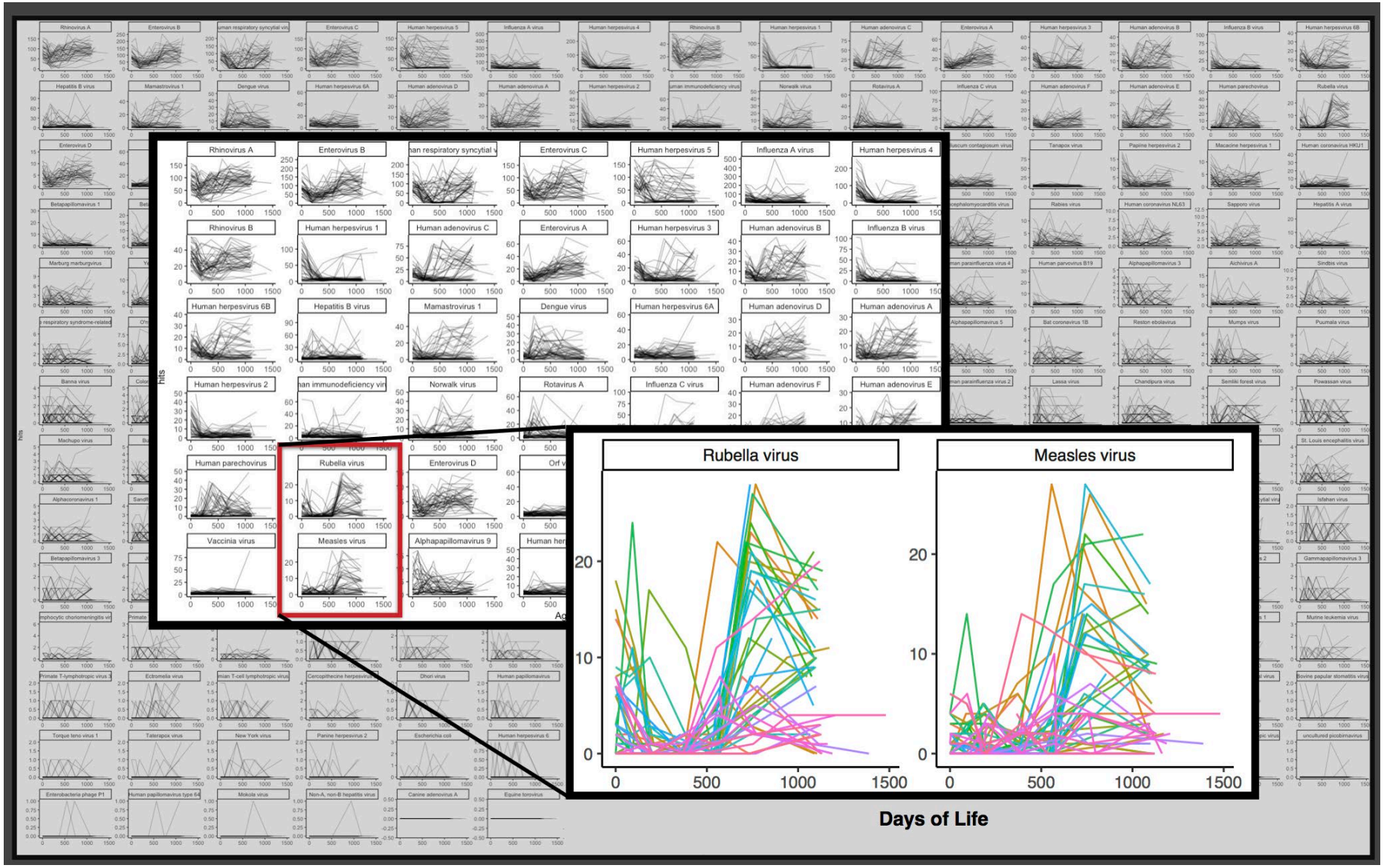


Email: mmina@hsph.Harvard.edu
Twitter: Michaelmina_lab

Center *for* Communicable Disease Dynamics

HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH

One drop of blood
Allows to track exposures to hundreds of pathogens, simultaneously for < $10/sample



Reconstruct attack rates, epidemic histories & infectious disease timelines

Towards Global Serological Surveillance

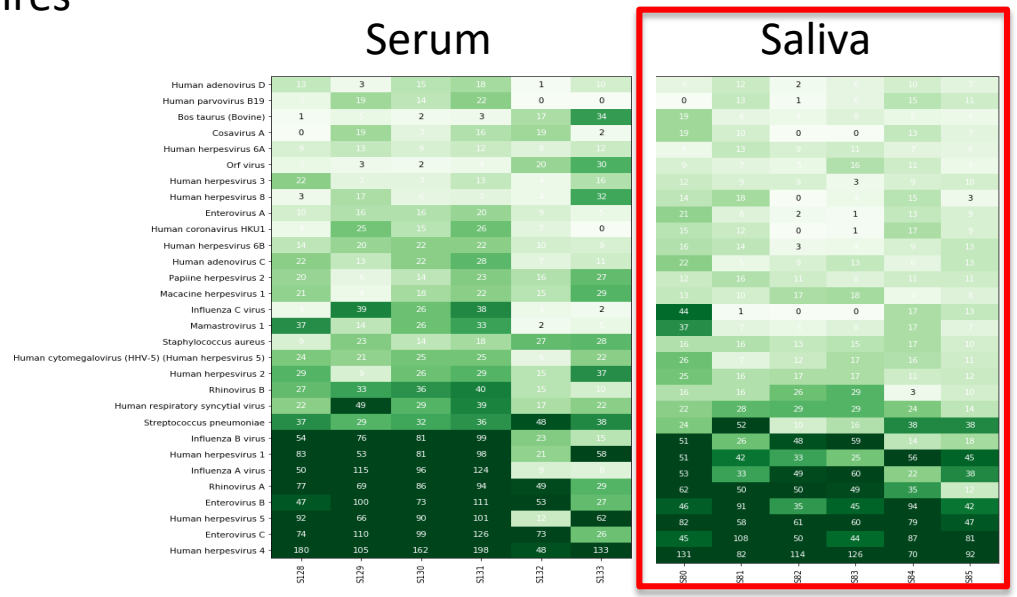
Requires easily accessible samples

Immune repertoires from saliva!

Spit ‘n Drop

Serum

Saliva



< $0.01

Global Immunologic Observatory

To understand the global landscape of immunity and infections

Sentinel and Affiliated Observatories

Sample Collection
Specimen short and long-term storage
Sample Preparation and Shipment to BioRepository Core

BioRepository Core

Sample Collection, Processing and Registration
Rapid and Focused Specimen Requisition as Needed
Long-term Specimen Archiving
Specimen Distribution to
Immunologic Core & Specialized Labs

Data, Statistics, and Modeling Core

Database Maintenance
Sample Enrollment
Clinical & Demographic Data Entry
Geographic / Spatial Immune Landscape Modeling
Epidemic / Pandemic Detection and Forecasting

Immunology Core

High throughput, massively parallel measurement of anti-microbial antibodies
High throughput pathogen detection
B- and T-cell Receptor / Repertoire Sequencing
Acute phase and inflammatory reactants

Data scientists / modelers

Outside scientists and modelers can request access to biorepository data.
Fees can be used to offset lab costs incurred by the immunologic core and laboratories for specialized diagnostics

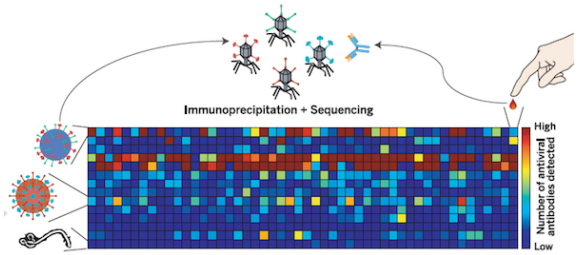
Laboratories for Novel Methods Development

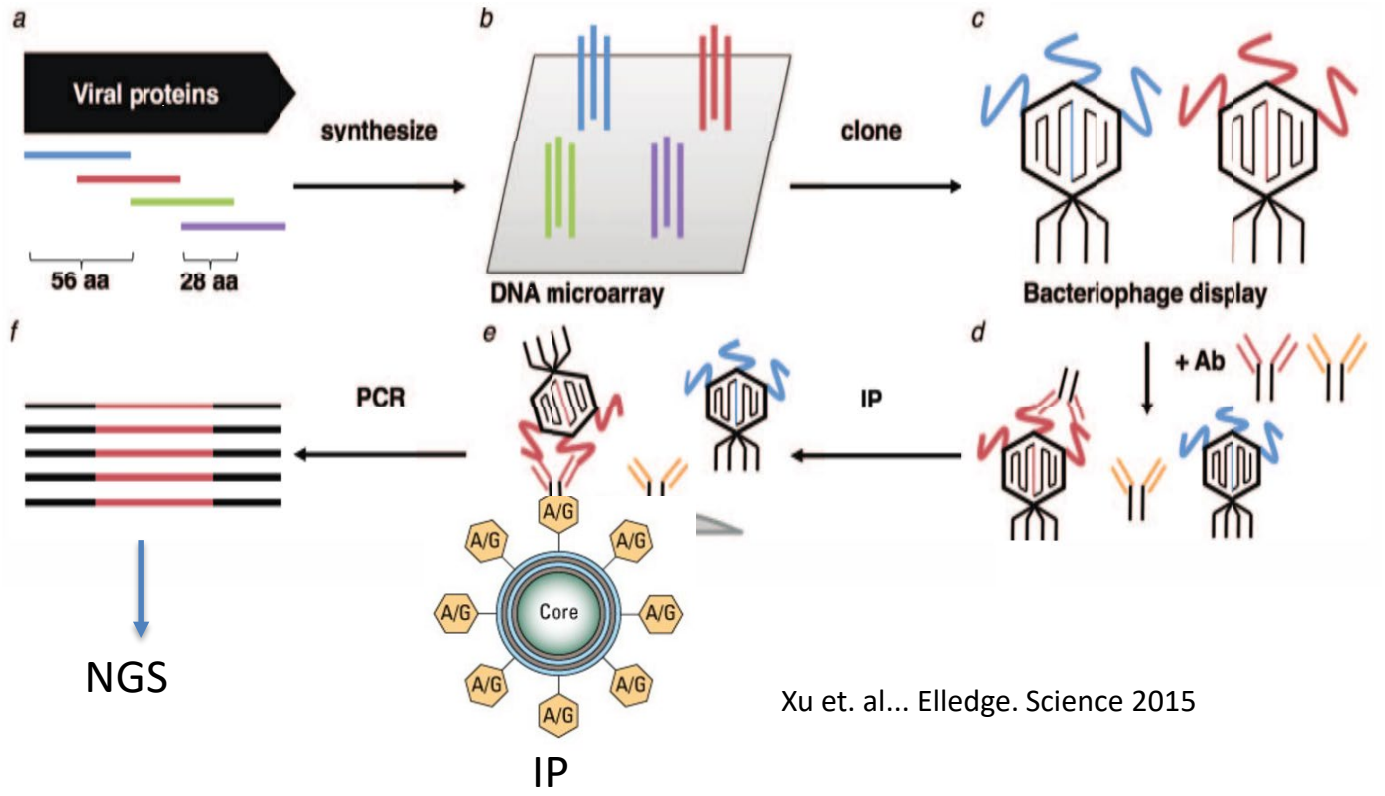
Outside laboratories can request access to biorepository specimens for novel methods development and sample analysis.
Results will feed back into the central database

Laboratories for Specialized Diagnostics

Outside laboratories may be consulted for specialized diagnostic assays that fall outside the scope of the immunology core on an as-needed basis.

Phage display for comprehensive immunological repertoire analysis





Xu et. al... Elledge. Science 2015

- **VIRSCAN**
- **Bioinformatically combine full proteomes of all viruses that infect humans + many bacterial genes…**
- **Synthesize ~200K unique 56aa peptides (28aa overlap) covering full proteomes**
- Display on T7 bacteriophage
- **Mix library + < 1µl (~2ug) serum**
- **Immunoprecipitate & NGS**
- **Align** to unique IDs **to identify precise epitopes that Abs detected**
- Readout is **NGS counts per epitope** and **#'s of epitopes recognized per virus**

Using Synthetic Biology to Improve Vaccines

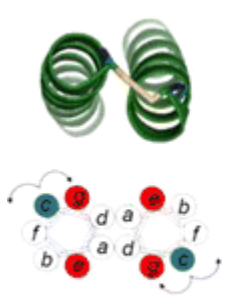
Gigi Kwik Gronvall, PhD

Senior Scholar, Johns Hopkins Center for Health Security

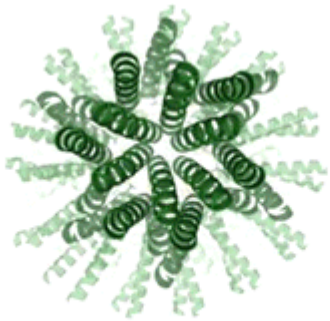
Associate Professor, Department of Environmental Health and Engineering

Synthetic biology and biosecurity

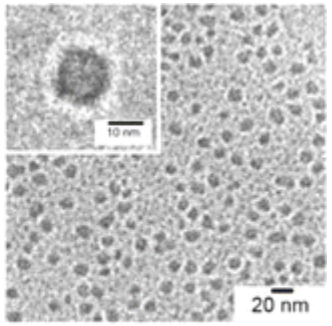
- Synthetic biology refers to any work that manipulates the genome, and subsequent phenotype, of an organism, nucleic acid, or peptide sequence
- Synthetic biology drives biomedical advances, including
  - Reverse genetics in virology
  - CRISPR/Cas9 techniques
- With these technology advances come biosecurity concerns:
  - Synthesis or modification of existing pathogens
  - Deliberate misuse of biotechnology tools



self-assembling subunit



virus-like topology



peptide shells as synthetic virions

Synthesis of virus-like particles for delivery of genetic material

Noble et al 2016

Using synthetic biology to tailor vaccine efforts– new tools for old problems

Challenges in vaccine development

- Vaccines must
  - Elicit immune responses
  - Present the correct antigen to immune cells
  - Have minimal adverse effects
- Vaccines often
  - Have significant costs ($200-500 million - +++)
  - Take years to develop (>10 years)

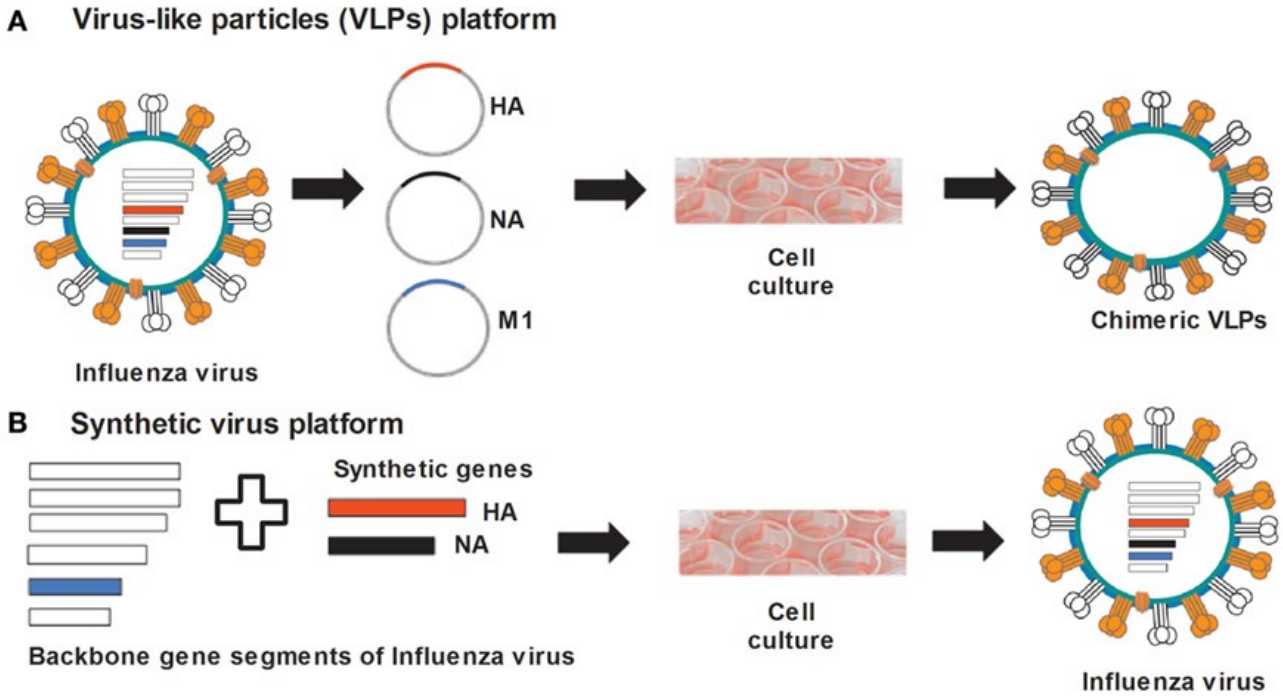
Promises with synthetic biology tools

- New synthetic biology approaches can improve
  - Production of antigen in animal, plant, or insect cells
  - Proper protein processing
  - Reduced costs (~40%)
  - Immune responses in the host
  - Faster development with genetic modification tools (weeks)

Recent development in vaccine formulations

- Virus-like particle (VLP) vaccines and vaccine platforms
- Selection of cell lines for improved protein yield
- Improved protein folding in plant systems
- Microbes as living vaccines

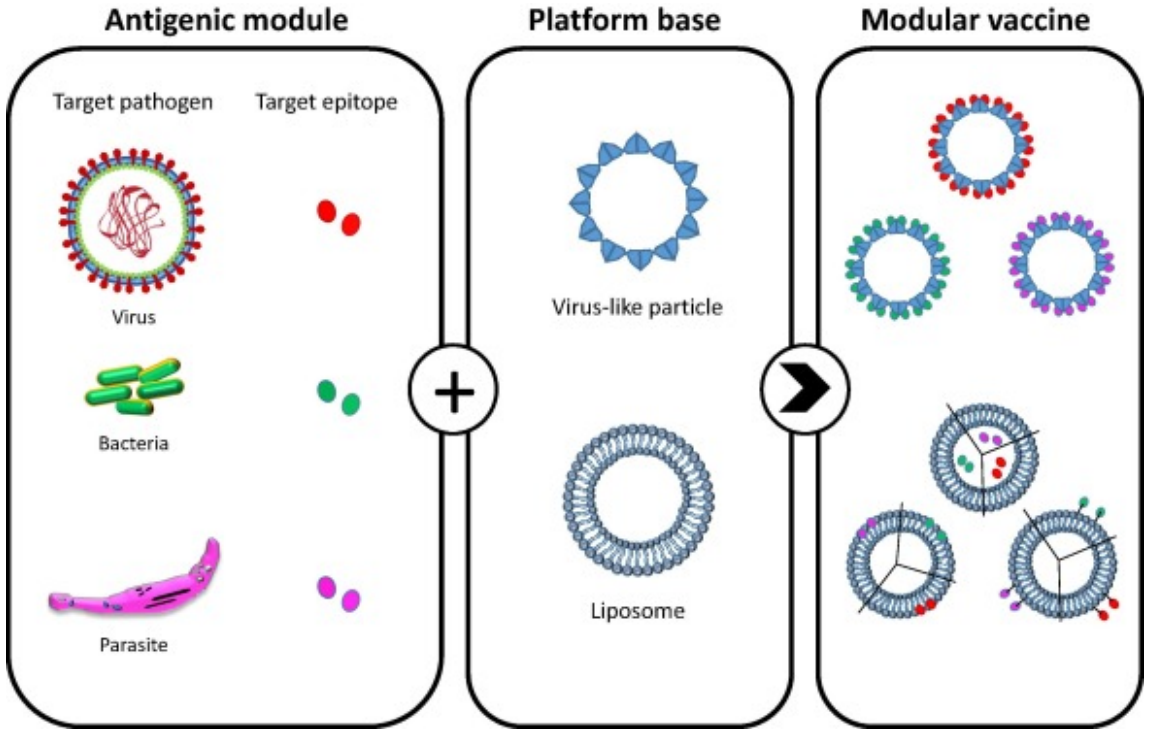
Synthetic biology fuels all of these advances

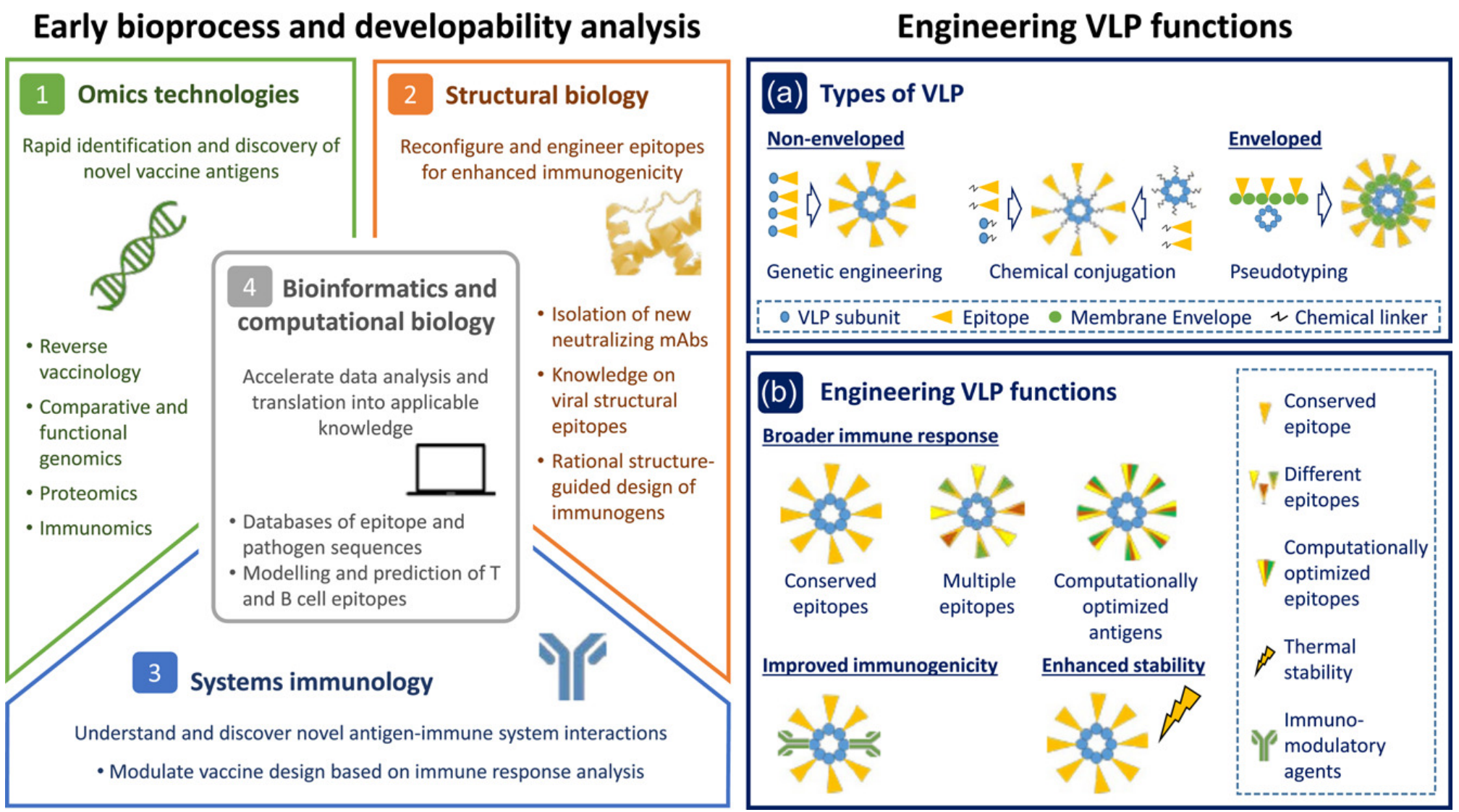


Improving universal influenza vaccine through synthetic biology techniques

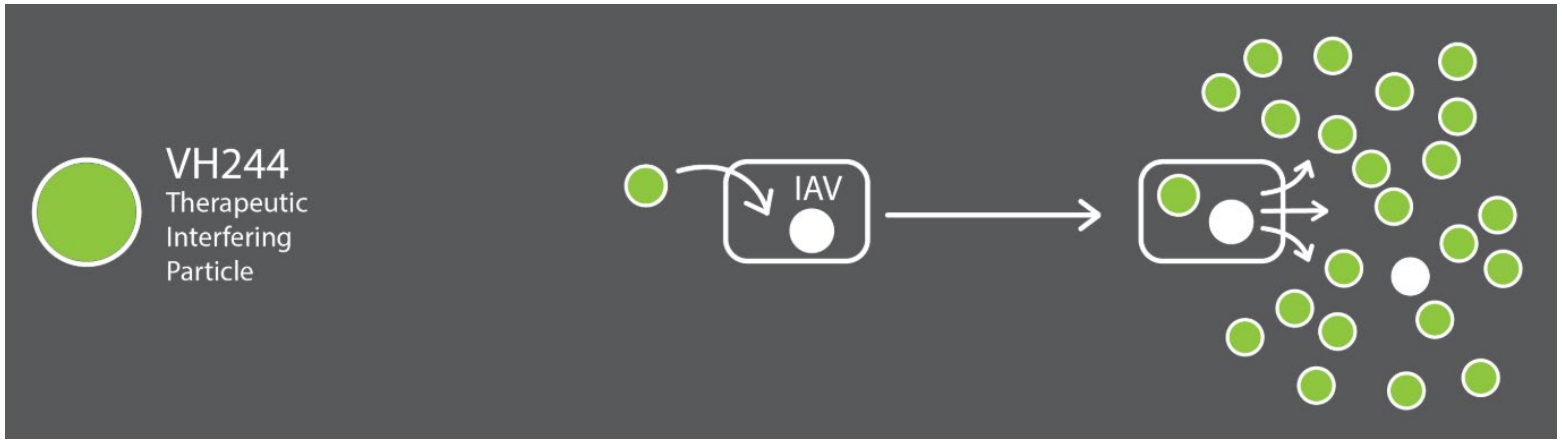
VLPs as vaccine platforms

- Because VLPs are so easily modified, especially by CRISPR, these offer an efficient vaccine platform
- This can allow for precise integration and presentation of an antigen(s) of interest
- Current VLP targets include:
  - Malaria (bacteriophage AP250 platform)
  - Influenza (murine polymavirus platform)
  - Dengue (HepB platform)




Early bioprocess and developability analysis
1 Omics technologies
Rapid identification and discovery of novel vaccine antigens
• Reverse vaccinology
• Comparative and functional genomics
• Proteomics
• Immunomics
2 Structural biology
Reconfigure and engineer epitopes for enhanced immunogenicity
• Isolation of new neutralizing mAbs
• Knowledge on viral structural epitopes
• Rational structure-guided design of immunogens
4 Bioinformatics and computational biology
Accelerate data analysis and translation into applicable knowledge
• Databases of epitope and pathogen sequences
• Modelling and prediction of T and B cell epitopes
3 Systems immunology
Understand and discover novel antigen-immune system interactions
• Modulate vaccine design based on immune response analysis
Engineering VLP functions
(a) Types of VLP
Non-enveloped
Enveloped
Genetic engineering
Chemical conjugation
Pseudotyping
VLP subunit
Epitope
Membrane Envelope
Chemical linker
(b) Engineering VLP functions
Broader immune response
Conserved epitopes
Multiple epitopes
Computationally optimized antigens
Improved immunogenicity
Enhanced stability
Conserved epitope
Different epitopes
Computationally optimized epitopes
Thermal stability
Immuno-modulatory agents

Therapeutic interfering particles

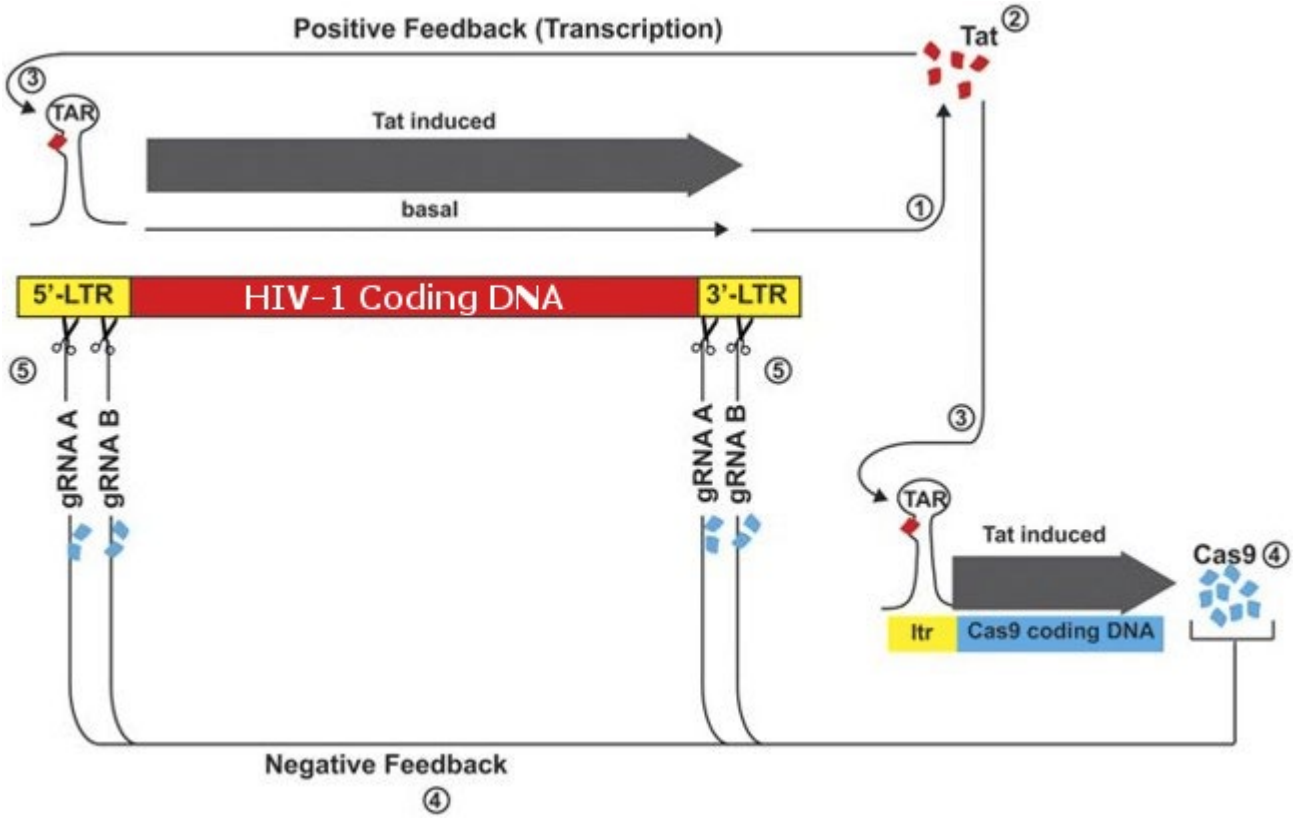


Virion Biotherapeutics' TIP technology against influenza A virus

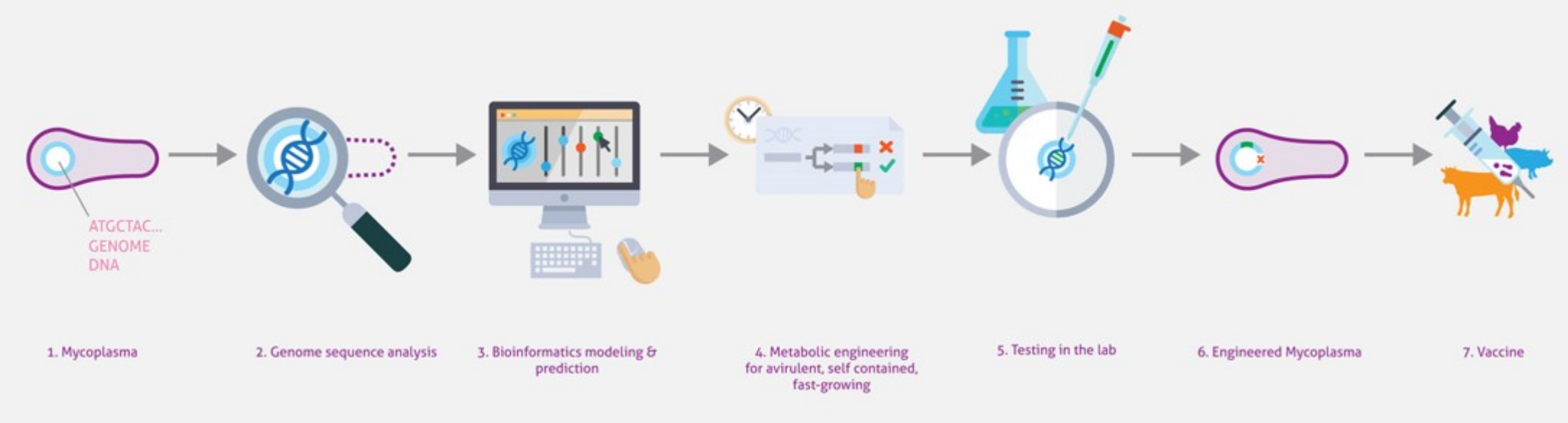
- These particles are similar to VLPs, but are non replicating, and take over the virus replication machinery
- When in the same cell as an infectious virus, such as influenza A, over 99% of the virus that the cell produces will be non-infectious
- DARPA was recently accepting proposals for this technology

CRISPR in Vaccines: targeting the pathogen

- Researchers used CRISPR/Cas9 to create a feedback loop of cleavage of HIV viral genome to create a “vaccine” for HIV
- The CRISPR essentially inactivates any viral genomes that arise

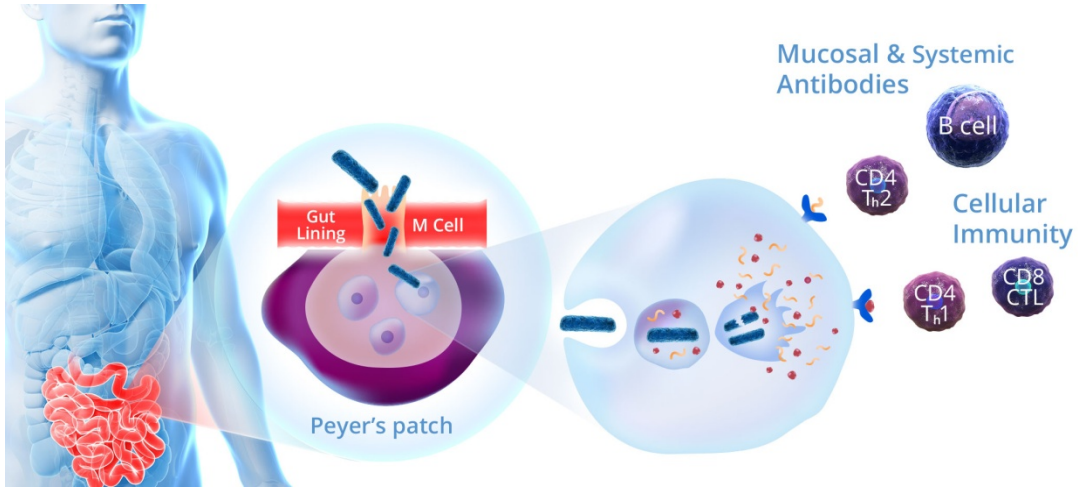


Recombinant attenuated bacterial vaccines



- The EU is funding a project for attenuated *Mycoplasma* vaccines
- Engineering existing bacteria to replicate and present antigen to the immune system
- Created a transposon system to more easily transform *Mycoplasma,* SynMyco
- Primarily intended to treat veterinary infections

Engineering the microbiome for disease prevention

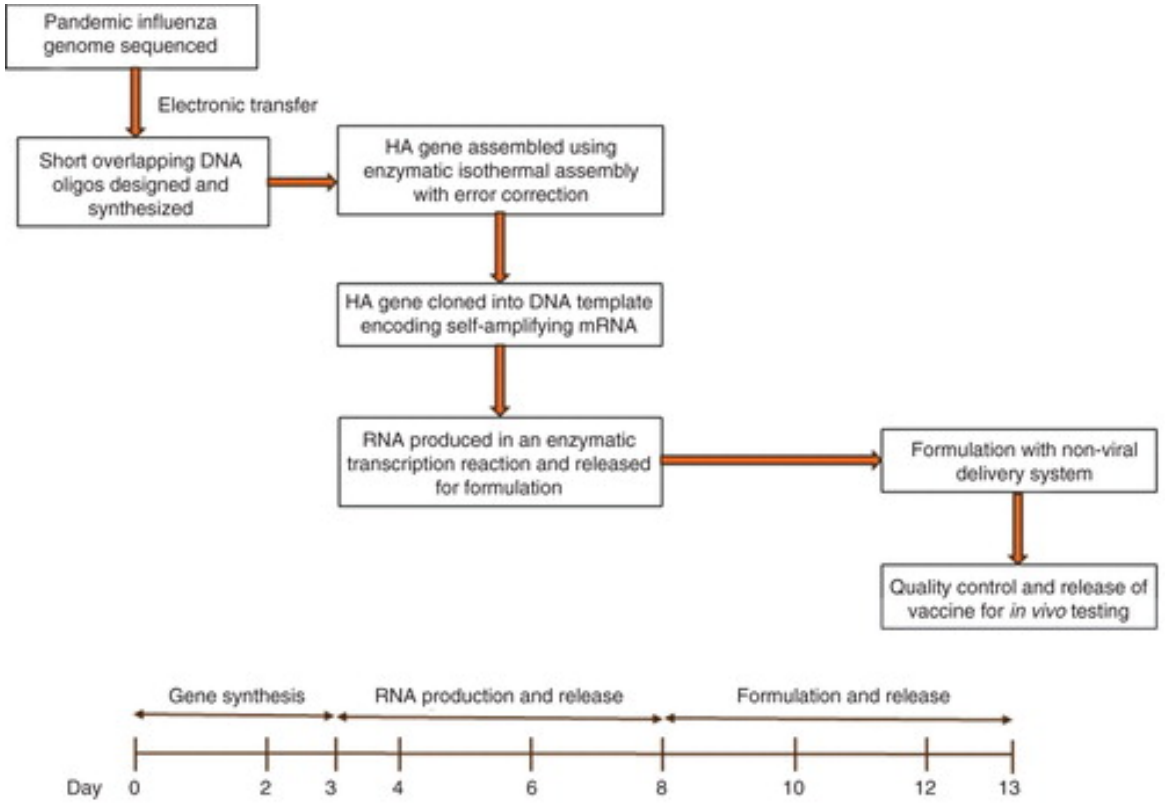


Vaxonella system for treatment of enteric fever

- As the bacteria are processed by immune cells, the immune cells present the antigen of interest to elicit an immune response.
- One such effort uses *Salmonella enterica* serovar Typhi strain ZH9 to present Enterotoxigenic *E. coli* antigens
- No adjuvants are necessary

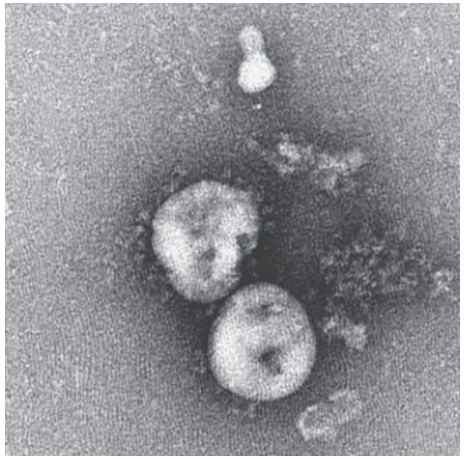
Nucleic-acid based vaccines strategies: SAMs

- Novel methods, such as GSK's Self-Amplifying mRNAs (SAM), rely on the body's processing of mRNA to produce viral antigens
- No adjuvants would be necessary, as the body becomes a "vaccine factory"
- Reverse vaccinology
- Rapid production of an exact, tailored vaccine

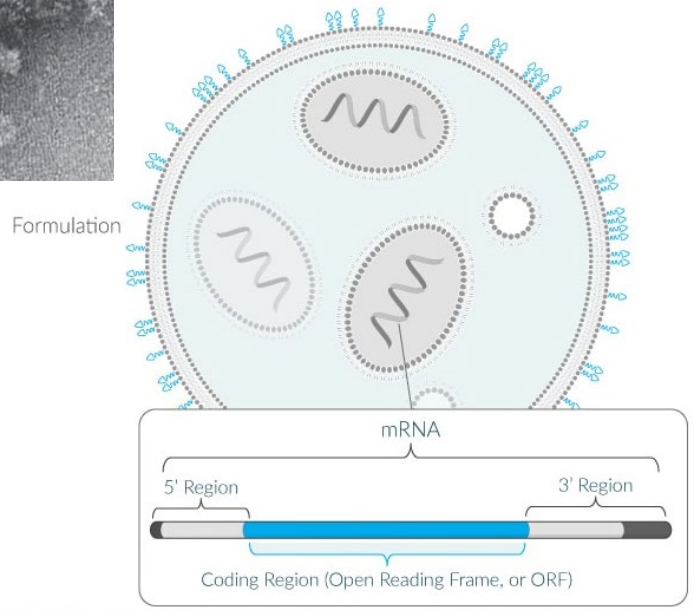


Nucleic-acid based vaccines for 2019-nCoV

- The current outbreak (as of January 2020) of 2019-nCoV originating in Wuhan, China has shown human-human transmission
- Moderna is now developing an mRNA based vaccine for the coronavirus, based on the published genome
  - Funded by CEPI and NIAID



moderna



Within a given modality, the base components are generally identical across development candidates - formulation, 5' region and 3' region. Only the coding region varies based on the protein/s the potential medicine is directing cells to produce.

The current landscape of synthetic biology and vaccines in the industry

Emerging infectious diseases

Meissa Vaccines

inovio

Reverse vaccinology

CODAGENIX INC.

VLP based vaccines

NOVAVAX

CaroGen Corporation

Creative Biostructure

medicago

Living vaccines

Prokarium

Upcoming opportunities for innovation

- Using VLPs to respond to pandemics in real time
- Computational methods to identify antigens of emerging infectious diseases
- CRISPR as a tool to modify the pathogen, and host, for better vaccine production
- More cost-effective methods of vaccine production in plants