KEMENTERIAN KESEHATAN REPUBLIK INDONESIA

Vaksin TB Protein Sub-Unit sebagai Booster Vaksinasi BCG di Indonesia

Konsorsium Riset Vaksin TB
Center for Biomedical and Basic Technology of Health
National Institute of Health Research and Development

Solo, 26-27 Oktober 2017
Seminar Nasional Riset TB

Outline

- Vaksin BCG
- Pilihan protein sub-unit sebagai kandidat booster vaksin BCG
- Capaian Riset Vaksin TB
- Kolabrasi – produk tambahan

Konsorsium Riset Vaksin TB

Riset Prioritas Nasional - BAPPENAS

Koordinator
PBTDK

Sinergi
Efisiensi
No duplikasi

RISTEKDIKTI

Anggota
13 Institusi

Riset Dasar
- *M.tb* "dorman"
- Model Granuloma in vitro
- Interaksi human - patogen

PT Bio Farma
PT Pakar Biomedika Indonesia

Riset → Produk
- Vaksin
- Kit Diagnostik
- Produk Biosimilar / Model

2012 - 2020

KEMENTERIAN KESEHATAN REPUBLIK INDONESIA

Vaksin BCG Pasteur

2013; 8(1): 53-58

MÆDICA - a Journal of Clinical Medicine

EDITORIALS

BCG vaccine strain.

However, the majority of the world's population is supplied with BCG vaccine procured by UNICEF (The United Nations Children's Fund) on behalf of the Global Alliance for Vaccines and Immunization. UNICEF uses only four BCG vaccine suppliers who produce only three different BCG vaccine strains: BCG-Denmark produced by the Statens Serum Institute in Denmark, BCG-Russia (genetically identical to BCG-Bulgaria) produced by Bulbio (BB-NCIPD) in Bulgaria and by the Serum Institute in India, and BCG-Japan produced by the Japan BCG Laboratory.

In humans, there have been three studies investigating protective efficacy induced by different BCG vaccine strains (insert ref). In two studies (with between 4- and 50-yr follow-up), BCG Pasteur was associated with statistically significantly better protective efficacy than BCG-Phipps or BCG-Glaxo (30). In the third study (with 15-yr follow-up), BCG-Denmark had a greater protective efficacy than BCG-Pasteur (25 and 17%, respectively) (31).

History of BCG Vaccine

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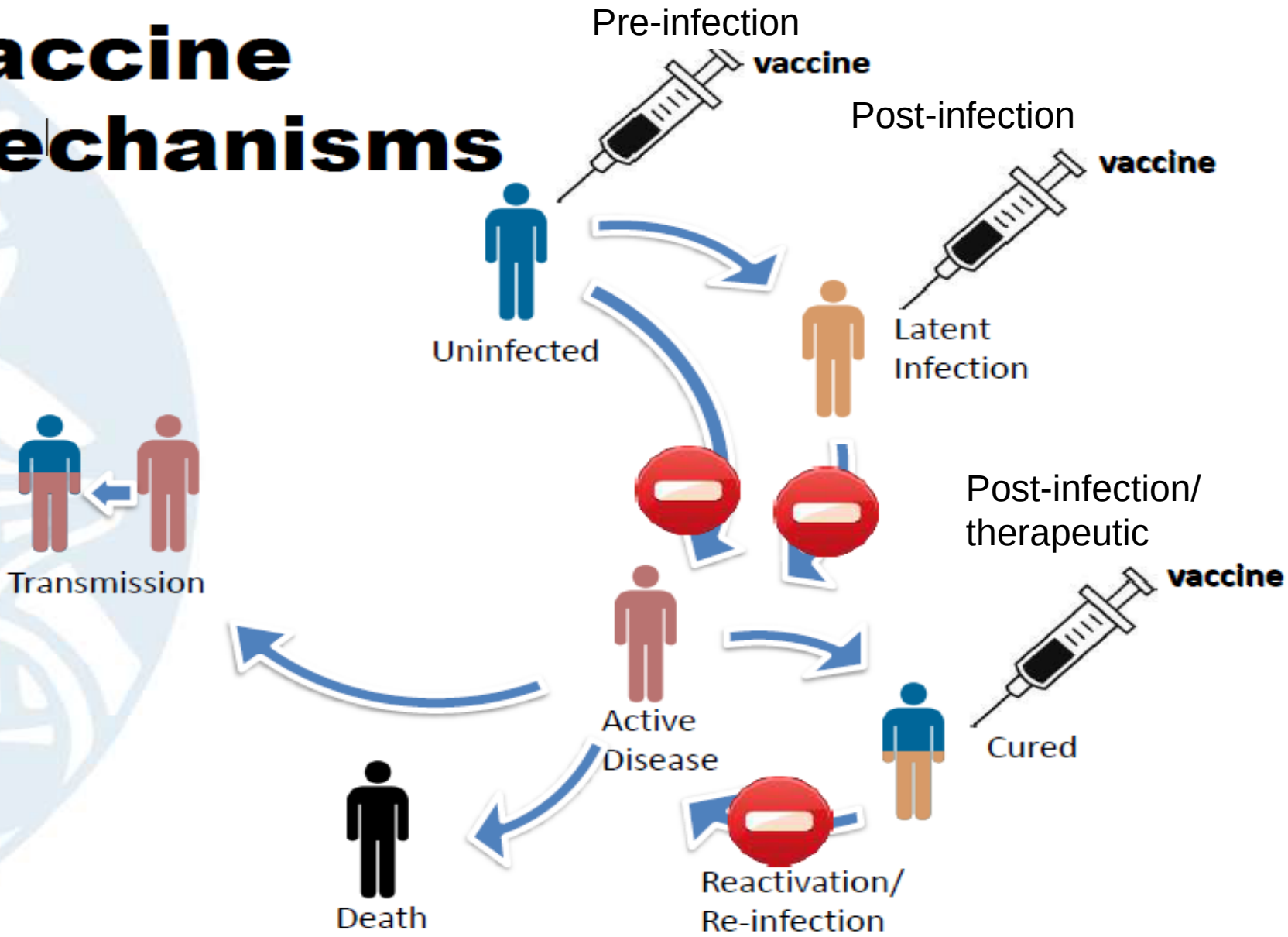
BCG VACCINES today

There are several BCG vaccines in use today. The major producers for the international market are Pasteur-Merieux-Connaught, the Danish Statens Serum Institute, Evans Medeva (which has taken over the old Glaxo vaccine), and the Japan BCG Laboratory in Tokyo. Each of these BCG vaccines is produced in a different manner, and they are recognized to differ in various qualities, such as the proportion of viable cells per dose (22). BCG strains derived from the original Paris strain after 1925 (e.g., the current Pasteur, Copenhagen, Glaxo-Evans strains) lack a region of the genome known as the RD-2, which is still present in strains derived earlier than that date [represented by the current Brazilian (Moreau), Japanese and Russian strains] (28,29).

Vaccine Mechanisms

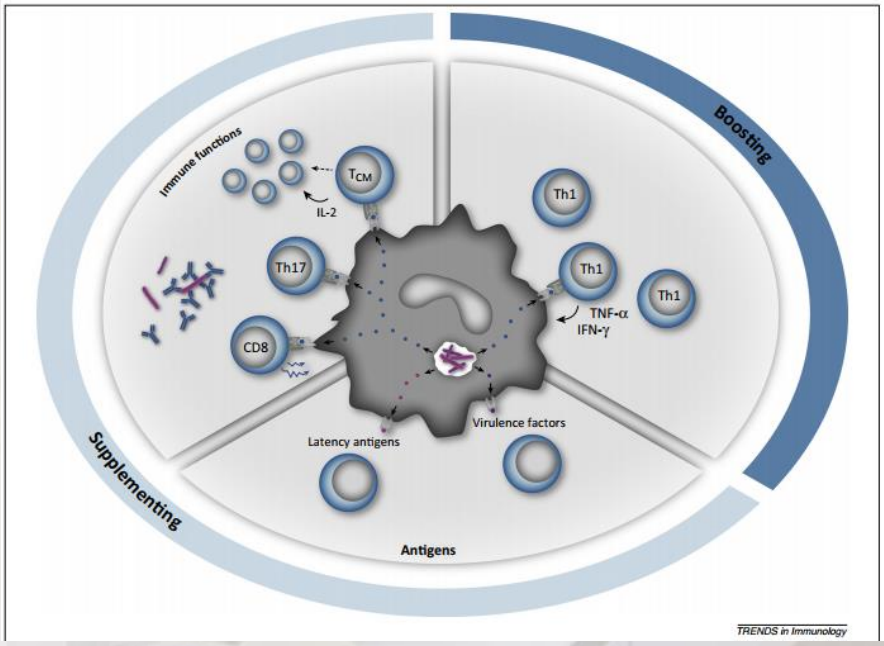


TB Vaccine → rethinking

- The life cycle of TB infection/disease and its impact on vaccination strategies
- Host–pathogen interaction in various stages of TB infection
  - Cellular dynamics in different stages of TB infection
  - Antigen expression in different stages of infection
- Immunological memory and TB protection
- Vaccine strategies against TB
  - Pre-exposure vaccines
    - Viable mycobacteria are designed to replace BCG as prime vaccines
    - **Subunit vaccines comprise MTB protein antigens** expressed in viral vectors or **delivered in adjuvant** and are designed as BCG boosters
  - Post-exposure vaccines target adolescents and adults with LTBI

Pendekatan Pengembangan Vaksin Sub-Unit

- **Early secreted protein**
  - RD1 deletion (ESAT6, CFP10 dll)
- PE/PPE family
  - Highly antigenic – function?
  - 10% of genome



- Gap analysis of BCG Vaccine Pasteur vs Tokyo sequences (results of WGS approach)
- Proteomics analysis of “granuloma in vitro” medels

Output I - Riset TB

- Protein Sub-Unit/Antigen Kandidat Vaksin

  - Ag85B-Rv2660c
  - Ag85B
  - Esat6-Cfp10

  - Esat6-Mtb32c-Cfp10
  - RpfB & RpfD
  - PPE41 & PPE17
  - PE-PGRS 14, 24, 32, 35, 45
  - LipY
  - ManLam

→ **Diserahkan ke PT Bio Farma**
- **Protein/Klon**
- **MTA**
- ***Seed history***

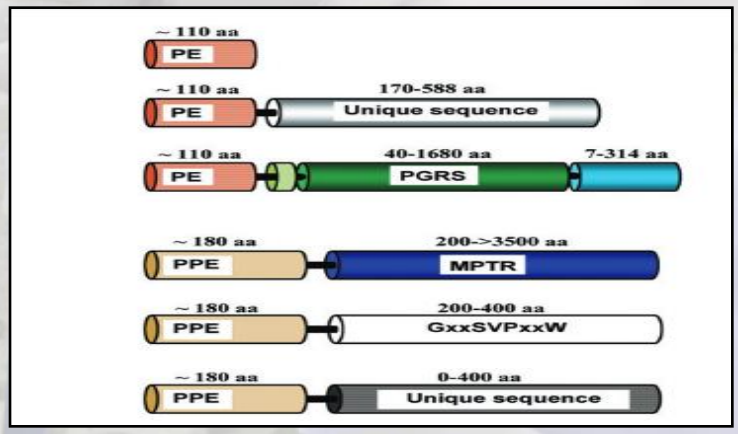
↓ QUICK WIN

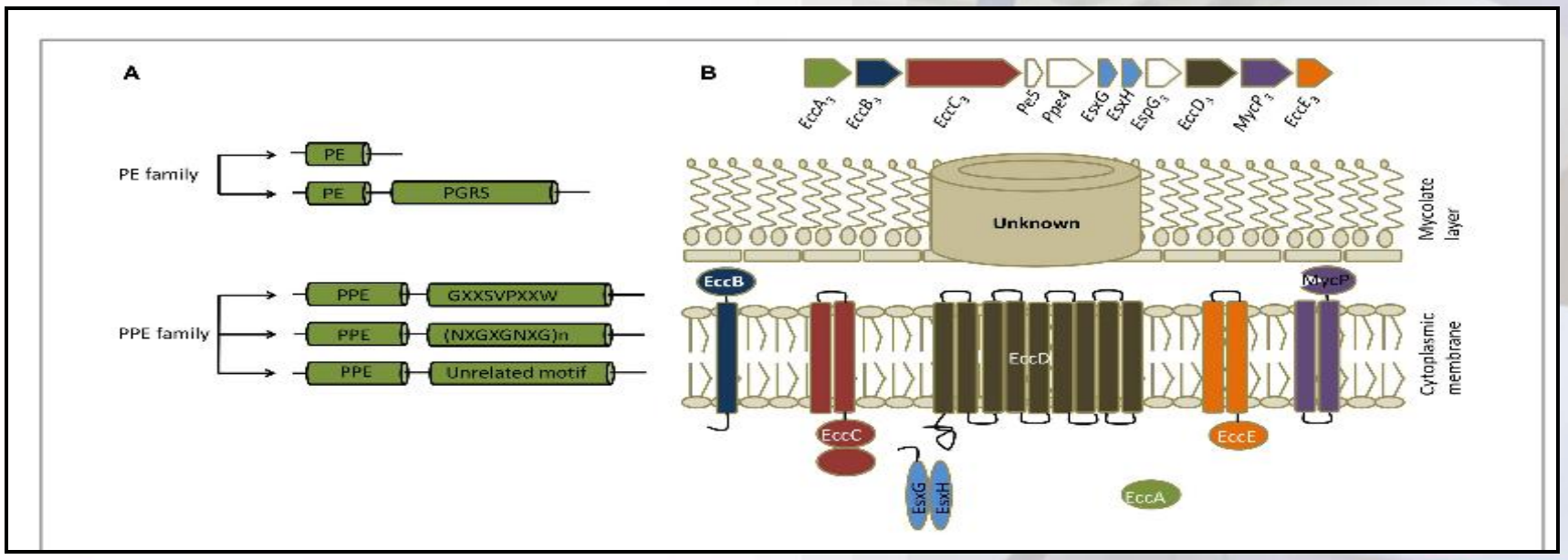
Hasil Purifikasi

Pelaksana: PBTDK, ITB, UI, UH, UM, UGM, LIPI

PE/PPE Protein

- PPE41
  - Antigenik, disekresi bersama sistem Esx sebagai kandidat vaksin TB. Optimasi dg PPE25 (esx system) →soluble protein
  - Dormancy
- PPE17 - Diagnostic tools

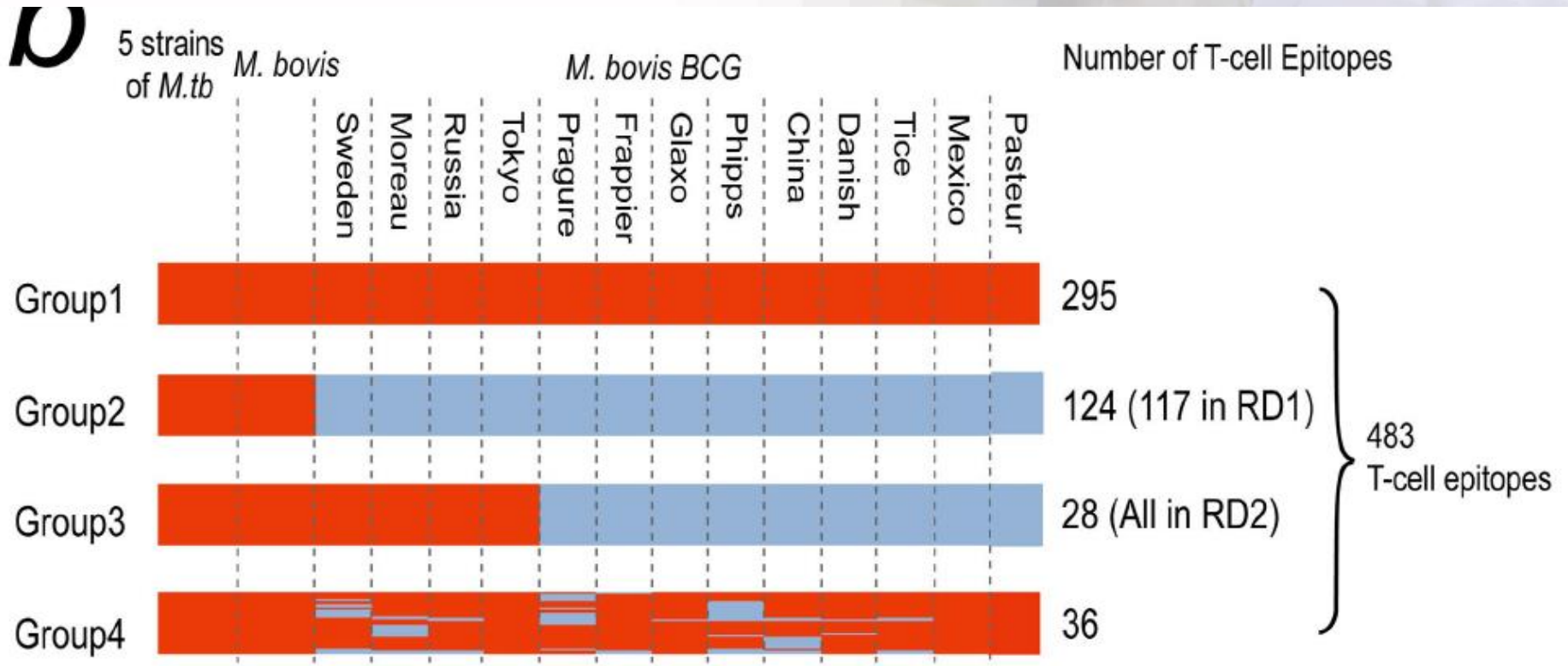




PLOS ONE

Genome Sequencing and Analysis of BCG Vaccine Strains

Wen Zhang[1,2☯], Yuanyuan Zhang[1,2☯], Huajun Zheng[3☯], Yuanlong Pan[4☯], Haican Liu[1], Pengcheng Du[1], Li Wan[1], Jun Liu[6], Baoli Zhu[4*], Guoping Zhao[3,5,7,8*], Chen Chen[1,2*], Kanglin Wan[1,2*]



Whole Genome Sequencing Vaksin BCG Pasteur

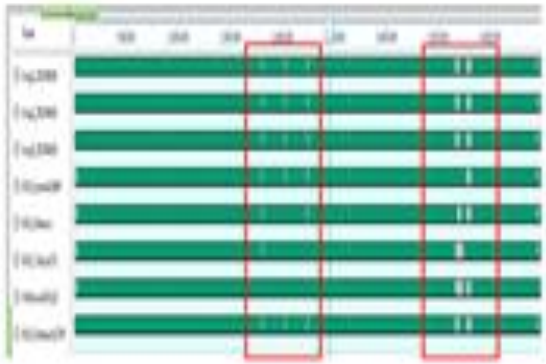


Fig 2. Maximum Parsimony Analysis. Phylogenetic tree developed using maximum parsimony method via MEGA6 software. Consistency index of .99 with retention index of .88 and composite index of .87 for all sites tested. Samples (bcg1, bcg2 and bcg5) formed a cluster with BCG Pasteur 1173P2 reference (red box). All samples and BCG reference originates from *M.bovis* and have high similarities with BCG Tokyo.

BCG Pasteur Biofarma sesuai Parent seed

Terdapat 5 gen yang berbeda antara BCG Pasteur dengan BCG Tokyo

Pemilihan antigen kandidat vaksin TB sesuai dengan BCG Tokyo yang lebih efektif

- Rv0490&Rv0491,
- Rv1189,
- Rv1441&Rv1325

Model *"granuloma in vitro"*

- M.tuberculosis dorman in vitro
  - Kultur – deplesi O2, minimal nutrition

- Interaksi human-pathogen
  - Model granuloma *in vitro*
    - PBMC - *M.tb*
    - Ditambah matriks extraseluler

- Indikator Model Granuloma
  - CD68 (macrophage) & CD3 & CD63 (sel T)
  - Mikroskopik
- Analisis Proteomiks (LC MS/MS)
  - Hari 1 dan hari 7, dengan dan tanpa *M.tb*

Indikator

Penilaian *M.tb* dorman in vitro pada pembentukan granuloma

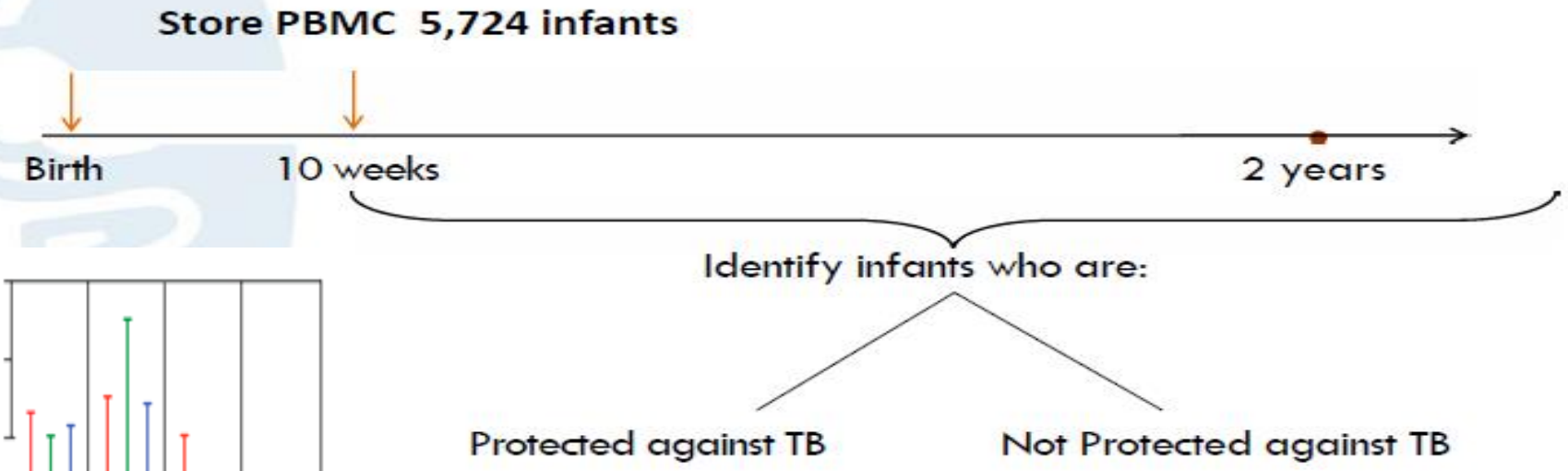
- Agregasi sel – Granuloma like:
  - Dibuat dari PBMC ditambahkan M.tb H37Rv → inkubasi selama 3 hari (waktu penilaian)
    - Fenotipik: Gambaran terbentuknya granuloma dengan indeks kepadatan sel (index score) dibuat kriteria nya menggunakan mikroskop inverted. Morfologi MO dan limfosit dengan HE → mikroskop cahaya
    - Respon seluler: mikroskop fluorescence & confocal. Ekspresi CD68 (merupakan marker permukaan MO). Marker limfosit: CD4 & CD8

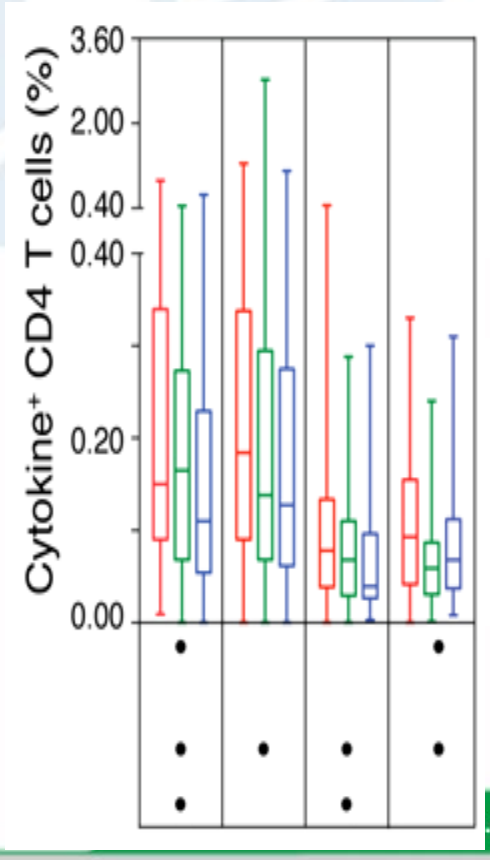
Indikator

Penambahan ekstraseluler matriks (bersama dengan S2)

- Pembentukan “granuloma” dengan 3 jenis ECM
- Parameter penilaian:
  - Granuloma
    - Fenotipik:
      - Gambaran terbentuknya granuloma dengan indeks kepadatan sel (index score) dibuat kriteria nya menggunakan mikroskop inverted
      - Morfologi MO dan limfosit dengan HE → mikroskop cahaya
    - Respon seluler: mikroskop fluorescence & confocal
      - Ekspresi CD68 (merupakan marker permukaan MO) →
      - Marker limfosit: CD4 & CD8
    - Ekskresi sitokin: IFNg, TNFa, IL10 (respon imun host) - Elisa
    - Reaktivasi dengan menilai faktor virulensi bakteri berdasarkan enzim metabolik: ICL (isocitrate lyase) (Rv0467) – upregulasi pada jalur metabolisme lemak merupakan marker dormancy
  - Dorman
    - Pembentukan granuloma: TNFα dan IFNγ (minimal)

Biomarkers of protection against TB, following BCG vaccination





From 5,724 enrolled infants:

- TB cases (n=29)
- Community controls (n=55)
- Household controls (n=55)

IL-2
IL-17
IFN-γ
TNF

Classical Th1 responses do not associate with risk of TB disease, after BCG

*BCG given at birth. Infants followed for 2 years to assess protection; Results shown from whole blood incubation with BCG at 10 weeks of age.

Kolaborasi/Kontribusi

- Sudi efektivitas BCG
  - Masyarakat
  - Faktor yang mempengaruhi
- Mycobacterial Growth Inhibitor Assay (MGIA)
- Clinical trial – kandidat vaksin

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