**Introduction:** Standard intradermal BCG vaccination fails to curb the ongoing TB pandemic. New vaccine strategies are urgently needed. Exploring alternative routes of vaccination in NHP, we have shown previously that pulmonary mucosal delivery of BCG shows improved protection against TB, associated with unique local adaptive immune signatures. We explored a new Mtb-derived vaccine candidate MTBVAC, with a broadened antigenic repertoire. In the present study we have analyzed both adaptive and innate immune response – trained immunity – profiles after mucosal delivery of MTBVAC in comparison with BCG and standard intradermal vaccination.

**Diagram: Study design**

**Results:** A) Unlike BCG, MTBVAC broadens immune response to ESAT6-CFP10; B) Pulmonary MTBVAC delivery confers immune signature correlating with TB protection; C) This signature disseminates to the non-targeted lung lobe; D) Local vaccine-induced T cells have increased mucosal homing and tissue residency markers CCR5, CD103 and CXCR3

**Methods:** Adult rhesus macaques were vaccinated with intradermal (.id) or mucosal (.muc) BCG or MTBVAC (N=6/group). Three animals were vaccinated intravenously with BCG for the evaluation of trained immunity in NHP. Local and peripheral Ag specific (PDP or ESAT6-CFP10) immune responses were analyzed. For trained immunity CD14+ blood and bone marrow derived monocytes were isolated and stimulated with lipopolysaccharide (LPS), before and after vaccination.

**Ethics Statement.** This study was performed in compliance with Dutch legislation and European directives on animal experimentation.

**Acknowledgements:** Work supported by the BPRC and as a research effort of the TRANSVAC2 Consortium, under the Horizon 2020 program, grant agreement 730964. ESAT6-CFP10 fusion protein was kindly provided by Kees Franken, from the lab of Prof. Dr. Ottenhoff.


---

**Mucosal vaccination with MTBVAC displays improved induction of trained immunity and broadened local adaptive immune signatures (Dijkman et al. & Vierboom et al., Cell Reports Medicine, 2021)