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Supplemental Information

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ACTIVATE: RANDOMIZED CLINICAL TRIAL OF BCG VACCINATION AGAINST INFECTION IN THE ELDERLY

SUPPLEMENTARY MATERIAL

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Data S1/Methods S1: Statistical plan for interim analysis of the ACTIVATE trial. Related to STAR Methods

The trial sample size was calculated on the assumption that the median time to new infection would be 4 months in the placebo group and 7 months with BCG vaccination. To achieve so with 90% power at the 5% level of significance, 100 patients were allocated to each arm. Under these prerequisites, the study is sufficiently powered to prove that differences in the first time incidence of infection between the placebo and the BCG group of the order of 20% will be statistically significant.

The primary outcome was the time, in weeks, of the appearance of first new infection, censored at twelve months after vaccination. Differences between the placebo and BCG vaccination groups were assessed with the hazard ratio (HR), of the Cox proportional hazards regression model with its 95% confidence intervals (CI). The corresponding p-values were also reported. The effects of other confounders, both at the univariate and the multivariate Cox model were also assessed with the corresponding HR. Only variables found to be significant in the univariate analysis entered in the stepwise multivariate analysis and they were retained in the model only if they had a significant effect after adjusting for the other effects. The proportionality of the hazard function at different levels throughout the follow-up period was assessed with the Schoenfeld residuals method (Xue et. al., 2013). Since this was an interim analysis, a sensitivity analysis was also performed for the total number of participants with the primary aim to show that individuals that were censored had the same probability of experiencing a subsequent event as individuals that remain in the study

Between groups differences were assessed depending on the nature of the parameters: for continuous variables following the normal distribution the independent samples t-test was employed, while in the absence of normality in the distribution the Mann-Whitney U-test was employed. The number of infections in each group was expressed as patient-infections per year. The same analysis was done for the secondary endpoints. The frequency of adverse events was compared by the Fisher's exact test.

The interim analysis included only patients with completed 12-month of follow-up. In order to preserve the overall Type I error rate at 5%, an adjustment of the level of significance of the interim and final analysis was done by O'Brien-Fleming strict alpha adjustment. This adjustment provides significance a=0.0054 at interim and a=0.0492 at final (deMets, & Gordon Lan, 1994). The purpose of using this seemingly unattainable level of significance at interim analysis was to allow the study to conclude, at the same time providing evidence that the required level of significance will be attained at the final stage. Statistical analysis was performed with the IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA) and corroborated with the R statistical package (R Core Team, 2013).

Cytokine data were expressed for each group of vaccination as the ratio of the cytokine production at month 3 versus the production at month 0 (before vaccination). Receiver operator characteristic curve analysis was done to discriminate the ratio of each cytokine that can better differentiate the two groups of vaccination. The best cut-off of this ratio was selected by the co-ordinate points of the curve using the Youden index. Patients above and below this cut-off were compared between groups by the Fisher's exact test. Analysis was conducted using IBM SPSS Statistics v. 25.0.

All p values were two-sided and any p value <0.05 was considered as statistically significant.

SUPPLEMENTARY TABLES

Table S1. Causes of hospital admission before study enrolment. Related to Table 1.

Cause of hospital admission	Placebo (N=78)	BCG (N=72)	p-value
Lower respiratory tract infection — no. (%)	20 (25.6)	23 (31.9)	0.471
Biliary tract infection — no. (%)	8 (10.3)	7 (9.7)	1.000
Ischemic stroke — no. (%)	10 (12.8)	12 (16.7)	0.645
Acute kidney injury — no. (%)	4 (5.1)	4 (5.6)	1.000
Gastrointestinal tract bleeding — no. (%)	8 (10.3)	6 (8.3)	0.783
Anemia — no. (%)	4 (5.1)	2 (2.8)	0.683
Electrolyte disturbance — no. (%)	2 (2.5)	4 (5.6)	0.428
Pulmonary embolism — no. (%)	2 (2.6)	2 (2.8)	1.000
Cerebral hemorrhage — no. (%)	1 (1.3)	1 (1.4)	1.000
Acute pyelonephritis — no. (%)	6 (7.7)	2 (2.8)	0.279
ABSSI — no. (%)	4 (5.1)	0 (0)	0.121
Other disease — no. (%)	9 (11.5)	8 (11.1)	1.000

Abbreviation: ABSSSI: acute bacterial skin and skin structure infection

Table S2. Sensitivity analysis of the incidence of new infections. Related to Figure 2.

	Placebo n/ total (%)	BCG n/total (%)	Difference in proportions % (95% CI)	HR	95% CI	p-value
Interim (150)	33/78 (42.3)	18/72 (25.0)	17.3 (2.1-31.3)	0.55	0.31-0.97	0.035
Total (198)	41/98 (41.8)	27/100 (27.0)	14.8 (1.6-27.4)	0.59	0.36-0.95	0.029

Abbreviations: CI: confidence interval; OR: odds ratio

Table S3. Overview of analyzed circulating inflammatory markers. Related to Methods Details.

	FDR_OVERALL.D14	FDR_OVERALL.D90
IL8_P10145	0.996582031	0.996582031
VEGFA_P15692	0.996582031	0.996582031
CD8A_P01732	0.996582031	0.996582031
MCP.3_P80098	0.996582031	0.996582031
GDNF_P39905	0.996582031	0.996582031
CDCP1_Q9H5V8	0.996582031	0.996582031
CD244_Q9BZW8	0.996582031	0.996582031
IL7_P13232	0.996582031	0.996582031
OPG_O00300	0.996582031	0.996582031
LAP.TGF.beta.1_P01137	0.996582031	0.996582031
uPA_P00749	0.996582031	0.996582031
IL6_P05231	0.996582031	0.996582031
IL.17C_Q9P0M4	0.891113281	0.996582031
MCP.1_P13500	0.996582031	0.996582031
IL.17A_Q16552	0.996582031	0.996582031
CXCL11_O14625	0.996582031	0.996582031
AXIN1_O15169	0.996582031	0.996582031
TRAIL_P50591	0.996582031	0.996582031
CXCL9_Q07325	0.996582031	0.996582031
CST5_P28325	0.996582031	0.996582031
OSM_P13725	0.996582031	0.996582031
CXCL1_P09341	0.996582031	0.996582031
CCL4_P13236	0.996582031	0.996582031
CD6_Q8WWJ7	0.996582031	0.996582031
SCF_P21583	0.996582031	0.996582031
IL18_Q14116	0.996582031	0.996582031
SLAMF1_Q13291	0.996582031	0.996582031
TGF.alpha_P01135	0.996582031	0.996582031
MCP.4_Q99616	0.996582031	0.996582031
CCL11_P51671	0.996582031	0.996582031
TNFSF14_O43557	0.996582031	0.996582031
FGF.23_Q9GZV9	0.996582031	0.996582031
IL.10RA_Q13651	0.996582031	0.996582031
MMP.1_P03956	0.996582031	0.996582031
LIF.R_P42702	0.996582031	0.996582031
FGF.21_Q9NSA1	0.996582031	0.996582031
CCL19_Q99731	0.996582031	0.996582031
IL.10RB_Q08334	0.996582031	0.996582031
IL.18R1_Q13478	0.996582031	0.996582031
PD.L1_Q9NZQ7	0.996582031	0.996582031
Beta.NGF_P01138	0.996582031	0.996582031
CXCL5_P42830	0.996582031	0.996582031

TRANCE_O14788	0.996582031	0.996582031
HGF_P14210	0.996582031	0.996582031
IL.12B_P29460	0.996582031	0.996582031
MMP.10_P09238	0.996582031	0.996582031
IL10_P22301	0.996582031	0.996582031
CCL23_P55773	0.996582031	0.996582031
CD5_P06127	0.996582031	0.996582031
CCL3_P10147	0.996582031	0.996582031
Flt3L_P49771	0.996582031	0.996582031
CXCL6_P80162	0.996582031	0.996582031
CXCL10_P02778	0.996582031	0.996582031
XE.BP1_Q13541	0.996582031	0.996582031
SIRT2_Q8IXJ6	0.996582031	0.996582031
CCL28_Q9NRJ3	0.996582031	0.996582031
DNER_Q8NFT8	0.996582031	0.996582031
EN.RAGE_P80511	0.996582031	0.996582031
CD40_P25942	0.996582031	0.996582031
FGF.19_O95750	0.996582031	0.996582031
MCP.2_P80075	0.996582031	0.996582031
CASP.8_Q14790	0.996582031	0.996582031
CCL25_O15444	0.996582031	0.996582031
CX3CL1_P78423	0.996582031	0.996582031
TNFRSF9_Q07011	0.996582031	0.996582031
NT.3_P20783	0.996582031	0.996582031
TWEAK_O43508	0.996582031	0.996582031
CCL20_P78556	0.996582031	0.891113281
ST1A1_P50225	0.996582031	0.996582031
STAMPB_O95630	0.996582031	0.996582031
ADA_P00813	0.996582031	0.996582031
TNFB_P01374	0.996582031	0.996582031
CSF.1_P09603	0.996582031	0.996582031

Table S4. Synopsis of ongoing interventional clinical trials studying the protective effect of BCG vaccination on Covid-19 (as of July 10 2020; source: Clinicaltrials.gov). Related to STAR Methods and to Discussion

Registration number	Acronym	State	Study title	Population	N	Design and Groups	Primary endpoint
NCT04328441	BCG-CORONA	Recruiting	Reducing health care workers absenteeism in Covid-19 pandemic through BCG vaccine	HCWs	1500	RCT Placebo/BCG	HCW absenteeism
NCT04379336		Recruiting	BCG vaccination for healthcare workers in COVID-19 pandemic	HCWs	500	RCT Placebo/BCG	Incidence of HCWs hospitalized due to COVID-19
NCT04327206	BRACE	Recruiting	BCG vaccination to protect healthcare workers against COVID-19	HCWs	10078	RCT Placebo/BCG	COVID-19 disease incidence
NCT04414267	ACTIVATE II	Recruiting	Bacillus Calmette-guérin vaccination to prevent COVID-19	COPD, CHD, CCI>3	900	RCT Placebo/BCG	Composite on COVID-19 incidence
NCT04348370	BADAS	Recruiting	BCG vaccine for health care workers as defense against COVID 19	HCWs	1800	RCT Placebo/BCG	Incidence of COVID 19 Infection
NCT04362124		Not yet recruiting	Performance evaluation of BCG vaccination in healthcare personnel to reduce the severity of SARS-COV-2 Infection	HCWs	1000	RCT Placebo/BCG	Incidence of confirmed or probable COVID-19 cases

NCT04417335		Not yet recruiting	Reducing COVID-19 related hospital admission in elderly by BCG vaccination	Elderly	2014	RCT Placebo/BCG	SARS-CoV-2 related hospital admission
NCT04350931		Not yet recruiting	Application of BCG Vaccine for immune-prophylaxis among Egyptian healthcare workers during the pandemic of COVID-19	HCWs	900	RCT Placebo/BCG	Incidence of confirmed COVID-19
NCT04461379		Not yet recruiting	Prevention, efficacy and safety of BCG vaccine in COVID-19 among healthcare workers	HCWs	908	RCT Placebo/BCG	Cumulative incidence of infection in 6 months
NCT04373291		Not yet recruiting	Using BCG vaccine to protect health care workers in the COVID-19 pandemic	HCWs	1500	RCT Placebo/BCG	Number of days of unplanned absenteeism for any reason
NCT04384549	COVID-BCG	Not yet recruiting	Efficacy of BCG vaccination in the prevention of COVID19 via the strengthening of innate immunity in health care workers	HCWs	1120	RCT Placebo/BCG	Incidence of documented COVID-19

Abbreviations CCI: Charlson's comorbidity index; CHD: coronary heart disease; COPD: chronic obstructive pulmonary disease; HCW: heath care worker; RCT: randomized clinical trial