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Supplemental information

Multiple BCG vaccinations for the prevention of COVID-19 and other infectious diseases in type 1 diabetes

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SUPPLEMENTARY INFORMATION

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Supplemental Figure S1.

COVID-19 Detection Methods

I-IX. COVID-19 Directed Antibodies		Antibody Binding Regions
I-VIII. IgG Antibodies	Protein array	SARS-CoV-2 Amino Acid (AA) regions[a]
I. Spike 1		115-QSLLIVNNATNV-126
II. Spike 1		145-YHKNNKSWMESE-156
III. Spike 1		307-TVEKGIYQTSNF-318
IV. Spike 1		319-RVQPTESIVRFP-330
V. Spike 1		457-RKSNLKPFERDI-468
VI. Spike 2		710-NSIAIPTNFTIS-721
VII. Spike 2		1088-HFPREGVFVSNG-1099
VIII. RNA Polymerase		RNA polymerase
IX. IgG Antibodies	ELISA	Spike 1 Receptor Binding Domain[b]
X. Point of Care		
Nasal Swab	RT-PCR	Nucleocapsid RNA
Rapid Antigen Test	Antigen Test	Majority nucleocapsid protein
Antibody Test	ELISA	Nucleocapsid or Spike antibodies

[a] SARS-CoV-2 Protein Microarray: 2-in-1 protein and peptide assay from CDI Labs, Baltimore, Maryland (CDICOV2-001.0)
[b] AntiCoV-ID™ IgG ELISA from Akston BioSciences, Beverly, Massachusetts (SKU: 600016)
I-X represent the same numbers and thus diagnostic tests shown in Figure 2

Figure S1: Molecular COVID-19 Detection Methods Indicating Current or Past Infection

Related to Figure 2.

The table gives an overview of all the analytical methods for COVID-19 detection described in this trial (Fig. 2). For confirmation of SARS-CoV-2 infection the presence of subject antibodies to the virus were sought and confirmed through multiple methods (I-VIII). For SARS-CoV-2 antibodies detected, the average and standard deviation of antibody levels prior to the onset of COVID-19 pandemic (Pre-2020) was determined. The average of the levels after the start of the pandemic (2020 and 2021 data) was also calculated. Using these averages and the Pre-2020 standard deviation the Z-score per patient was then calculated. The Z-score thus represents the difference in average pre-COVID and average COVID signal levels, expressed as the number of standard deviations of pre-COVID. A Z-score of ≥3 was considered to represent a statistically significant difference. Efficacy was calculated from % patients in BCG and placebo groups that had a Z-score ≥3 using the formula: (p1 – p2)/p1 x 100, where p1 is the % COVID-positive in the placebo group and p2 is the % COVID-positive in the BCG group. We also used an ELISA assay specific to antibodies against the Receptor Binding Domain (RBD) portion of the S1 spike subunit (IX). Patients in their community locations also received a diagnosis of COVID-19 infections through a variety of methods, including PCR on nasal samples (X).

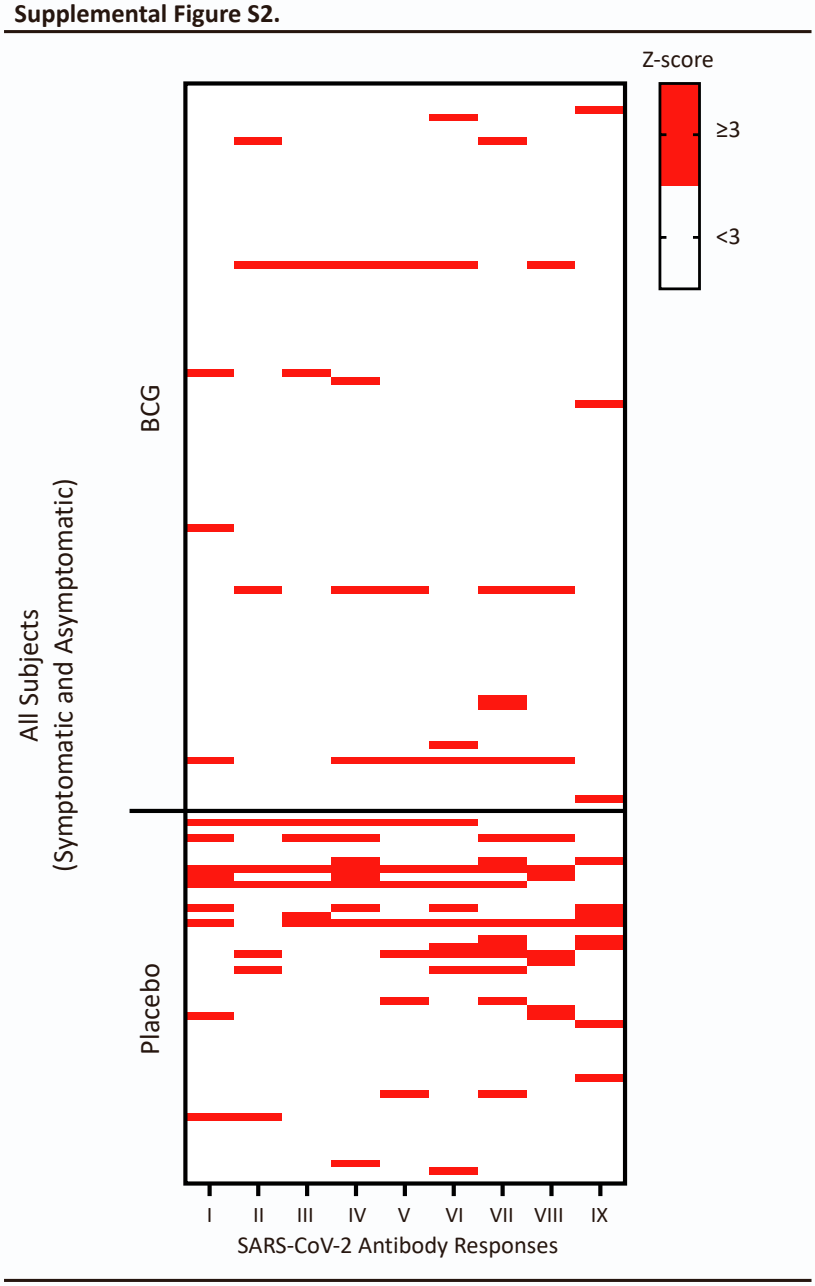
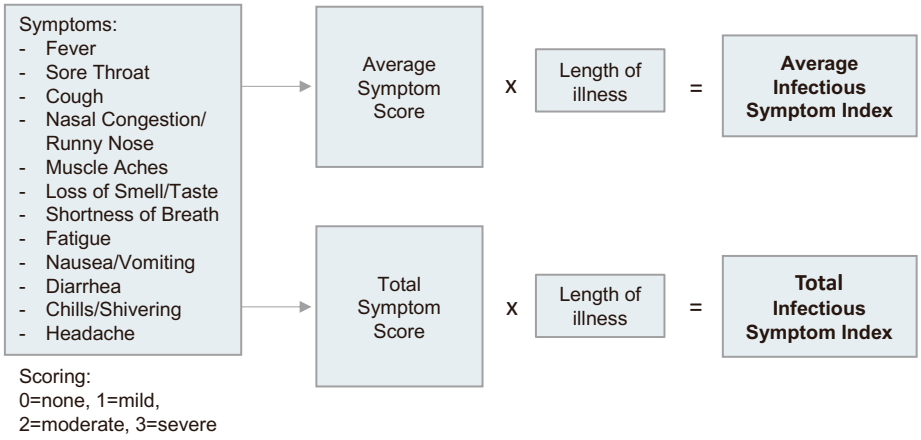


Figure S2. Heat Map Display of SARS-CoV-2 Epitopes that Tested Positive (Z-score ≥3) for All Trial Participants (Symptomatic as well as Asymptomatic)

Related to Figure 3A

Supplemental Figure S3.

A



B

Pre-Trial

MedDRA Code:	Adverse Event	Adverse Events per Patient:	
		BCG	Placebo
10006451	Bronchitis	0.02	0.02
10010106	Common cold	0.06	0.06
10011224	Cough	0.01	0.02
10014011	Ear infection	0.09	0.04
10021789	Infection	0.03	0.06
10034839	Strep throat	0.02	0.04
10040745	Sinus infection	0.21	0.21
10046571	Urinary tract infection	0.04	0.08
10062352	Upper respiratory infection	0.01	0.02
	Total:	0.50	0.56
	Poisson distribution p	**0.46**	

Current Trial

MedDRA Code:	Adverse Event	Adverse Events per Patient:	
		BCG	Placebo
10016791	Flu-like symptoms	0.18	0.19
10040745	Sinus infection	0.02	0.04
10046571	Urinary tract infection	0.02	0.06
10084268	COVID-19	0.03	0.13
	Total:	0.25	0.42
	Poisson distribution p	**0.004**	

Figure S3: Infectious Disease Index and Analyzed Infections

Related to Figure 4 and 5.

(A) Average and Total Infectious Disease Index are calculated as shown.

(B) Infections were documented (through adverse events [AEs] reporting and surveys) during the 2½ year Pretrial period and during the 15-month Current Trial period. Listed are only the infections for which multiple events were documented in both BCG and placebo groups. Poisson distribution analysis shows that there was no significant difference between BCG and placebo AEs during the Pretrial Period (p=0.46), whereas during the Current Trial period the difference was significant (p=0.004).

Supplemental Figure S4.

Number at risk data for Figure 2B

Diagnostic	No. at Risk	0 months	5 months	10 months	15 months
I	BCG	96	96	96	96
	Placebo	48	44	41	41
II	BCG	96	94	94	94
	Placebo	48	46	45	43
III	BCG	96	95	95	95
	Placebo	48	45	42	42
IV	BCG	96	95	95	95
	Placebo	48	43	40	48
V	BCG	96	95	95	95
	Placebo	48	46	44	43
VI	BCG	96	95	95	94
	Placebo	48	45	42	40
VII	BCG	96	95	95	95
	Placebo	48	43	42	39
VIII	BCG	96	95	95	95
	Placebo	48	45	43	42
IX	BCG	96	95	95	95
	Placebo	48	46	44	43
X	BCG	96	95	95	95
	Placebo	48	48	44	42

Number at risk data for Figure 5B

Symptom	BCG Cohort Score (%)				Placebo Cohort Score (%)				Household Members Score (%)			
	0	1	2	3	0	1	2	3	0	1	2	3
Headache	85	15	0	0	57	29	14	0	75	10	15	0
Chills/Shivering	92	0	8	0	71	14	14	0	95	5	0	0
Diarrhea	100	0	0	0	86	0	14	0	100	0	0	0
Nausea/Vomiting	92	0	8	0	100	0	0	0	95	5	0	0
Fatigue	62	38	0	0	29	14	0	57	60	15	20	5
Shortness of Breath	85	15	0	0	57	43	0	0	90	5	5	0
Loss of Smell/Taste	100	0	0	0	71	0	14	14	80	15	5	0
Muscle Aches	85	15	0	0	57	14	29	0	70	15	15	0
Nasal Congestion	46	23	31	0	43	29	14	14	65	15	15	5
Cough	54	38	8	0	57	14	29	0	55	25	15	5
Sore Throat	69	23	8	0	86	0	14	0	75	10	15	0
Fever	92	8	0	0	71	29	0	0	75	10	10	5

Figure S4. Number at risk data for Figure 2B and 5B

Related to Figure 2B and 5B. Number at risk data for the graphs are shown

Supplemental Figure S5.

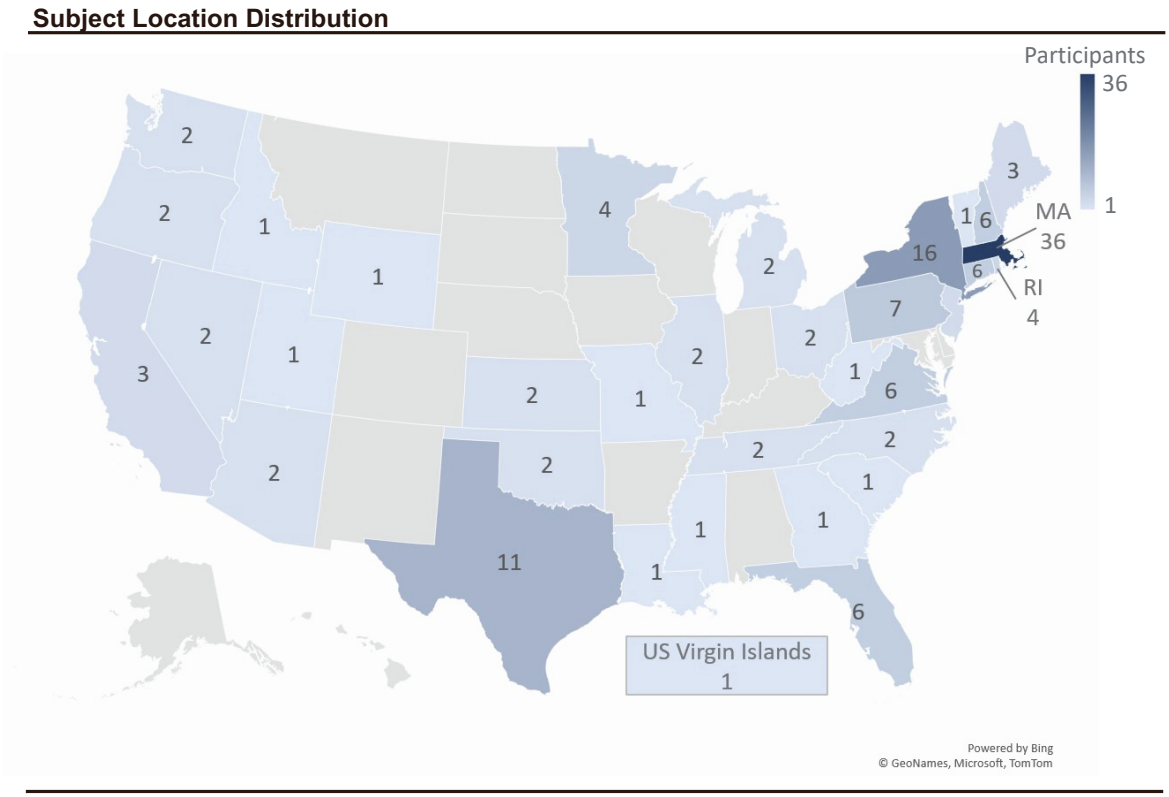


Figure S5. Geographical Location of Participants within the US as of January 2020
Related to Figure 1 and section "Method Details - Study design and participants".

Supplemental Figure S6.

Study Synopsis

Title	**USE OF BCG-JAPAN VACCINATIONS TO PREVENT COVID-19 AND LIMIT SYMPTOMS IN AT RISK SUBJECTS**
Short Title	**BCG to Prevent COVID-19**
Clinical Phase	Phase III
Number of Sites	1 enrollment site; US based recruitment
IND Sponsor	Massachusetts General Hospital, Boston, MA
Study Objectives	To determine if multi-dosing BCG-Japan (Tokyo-172) can protect high risk subjects from COVID-19 symptomatic infection. To determine the impact (severity, duration of symptoms, absence from work) of BCG-Japan on Covid-19 symptoms. To determine if BCG-Japan can also protect from other infectious diseases.
Study Design	Randomized, double blind, placebo-controlled clinical trial of three doses of BCG-Japan
Co-primary Outcomes	1. Prevention of Covid-19 antibody conversion of symptomatic COVID-19 disease 2. Prevention of infections that are not COVID-19

Figure S6. Study Synopsis for BCG COVID-19 Prevention Trial
Related to Figure 1