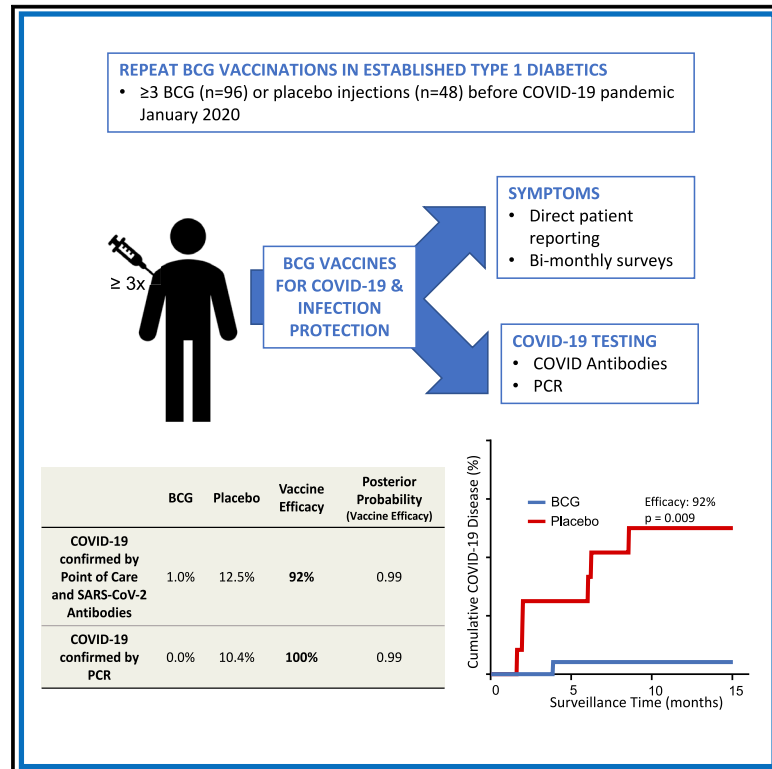**Article**

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

Graphical abstract

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In brief

In a randomized, double-blinded, placebo-controlled phase 2/3 trial, Faustman et al. investigate the safety and efficacy of multiple doses of the more than 100-year-old Bacillus Calmette-Guérin (BCG) vaccine against COVID-19. They also determine vaccine efficacy against other infectious diseases, which, if present, would suggest platform protection against new SARS-CoV-2 variants.

Highlights

- We report on our randomized trial of multi-dose BCG for protection against COVID-19

- BCG is safe and has 92% efficacy versus placebo against COVID-19

- Findings also suggest platform protection against additional infectious diseases

- Efficacy takes 1–2 years to manifest, but the protection may last decades

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

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SUMMARY

There is a need for safe and effective platform vaccines to protect against coronavirus disease 2019 (COVID-19) and other infectious diseases. In this randomized, double-blinded, placebo-controlled phase 2/3 trial, we evaluate the safety and efficacy of a multi-dose Bacillus Calmette-Guérin (BCG) vaccine for the prevention of COVID-19 and other infectious disease in a COVID-19-unvaccinated, at-risk-community-based cohort. The at-risk population is made of up of adults with type 1 diabetes. We enrolled 144 subjects and randomized 96 to BCG and 48 to placebo. There were no dropouts over the 15-month trial. A cumulative incidence of 12.5% of placebo-treated and 1% of BCG-treated participants meets criteria for confirmed COVID-19, yielding an efficacy of 92%. The BCG group also displayed fewer infectious disease symptoms and lesser severity and fewer infectious disease events per patient, including COVID-19. There were no BCG-related systemic adverse events. BCG's broad-based infection protection suggests that it may provide platform protection against new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants and other pathogens.

INTRODUCTION

The Bacillus Calmette-Guérin (BCG) vaccine is a more than 100-year-old vaccine originally developed for tuberculosis protection. It is heralded as the safest vaccine ever developed, with 3–4 billion people already vaccinated, and an annual 120 million newborns vaccinated.[1] It is highly affordable at about 10–75 cents/dose. Over the last 17 years, randomized clinical trials and epidemiology studies have shown that the BCG vaccine protects humans from a multitude of infections, including upper respiratory tract infections, leprosy, malaria, viral, and bacterial infections.[2–16] The first indication of the broad infectious disease protective abilities of this vaccine came 100 years ago when Albert Calmette, the vaccine's co-inventor, noted a 4-fold decline in child mortality (unrelated to tuberculosis) in vaccinated children, presumably from broad infectious disease protection.[17] These protective effects appear also when adolescents are re-vaccinated with BCG after the typical newborn dose.[10] The BCG vaccine may also protect humans from immune diseases such as type 1 diabetes and multiple sclerosis.[18–21] The mechanisms behind these wide-ranging benefits are a topic of active scientific discovery.

With the onset of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, some epidemiology studies

began to uncover an association between neonatal BCG vaccination and reduced morbidity and mortality from COVID-19, even in older adults decades after the typical neonatal vaccinations, on a country-by-country basis.[22–40] In some other global populations with varying neonatal exposures, different BCG strains, and other populations, benefits were not observed.[24,41–47] Because the United States has never vaccinated newborns or adults with BCG, a randomized trial of BCG for possible protection from SARS-CoV-2 infection offers a clean comparison in a vaccine-naive adult United States population. This parallel trial was adapted from an ongoing double-blinded, randomized trial of BCG for treatment of long-standing adult type 1 diabetes, so all cohorts were fully vaccinated with 3 BCG or placebo vaccinations at the start of the coronavirus 2019 (COVID-19) pandemic in the United States on January 1, 2020. Those with type 1 diabetes represent one of the most vulnerable populations in the United States for morbidity and mortality from COVID-19.[48] Patients with type 1 diabetes also have well-known increased infectious disease risk, including COVID-19.[49]

As the COVID-19 pandemic worsens, vaccine development assumes center stage. But antigen-specific vaccines, the focus of most clinical programs, are struggling to keep pace with new viral variants. The ideal vaccine should be safe, efficacious, affordable, and offer durable protection against ever-changing

viral variants and future infectious disease pandemics. We conducted a randomized, double-blinded, placebo-controlled platform trial over 15 months (January 1, 2020 to April 2021) to determine whether the BCG vaccine might offer a platform vaccine technology for broad-based infectious disease protection, including protection against COVID-19 in the vulnerable type 1 diabetic population.

RESULTS

We assessed the impact of multi-dose BCG vaccinations for protection from SARS-CoV-2 infections and other infections (Figure 1A). All participants at the start of this parallel study had 3 BCG vaccinations or 3 placebo vaccinations over a 2-year time period preceding the pandemic.

The trial, which had no dropouts, found that multi-dose BCG was safe

All 144 participants were United States citizens not previously vaccinated with BCG and had long-standing type 1 diabetes, a co-morbid condition for worse COVID-19 disease and symptoms (Figure 1B).[49] None of the participants was lost to follow up or dropped out during the current 15-month parallel study. A total of 150 participants were enrolled for the first study on type 1 diabetes; at the start of this parallel clinical trial (January 2020) evaluating the effects of BCG multi-dosing on overall infections and COVID-19-specific disease, there were 96 participants treated with BCG and 48 treated with placebo. These recipients were all vaccinated with BCG or placebo in the 2 years prior. Unlike antigen-specific vaccines, no BCG-related systemic adverse events occurred in any of the participants during the vaccination time period. BCG vaccines do cause local skin reactions that usually appear at 2–4 weeks. No excess local reactions were reported as adverse events. During the time period of this trial, other COVID-19 vaccines were not yet available and therefore had no influence on this study.

The BCG group had fewer cases of confirmed COVID-19 than the placebo group, with vaccine efficacy at 92%

The cumulative incidence of confirmed COVID-19 in the BCG cohort was 1 out of 96 (1%), whereas it was 6 out of 48 (12.5%) in the placebo group, at the end of the 15-month COVID-19 trial. The criteria for a confirmed case of COVID-19 were developed early in the pandemic prior to widespread availability of PCR. Therefore, the criteria required (see STAR Methods and Figure S1) were reporting at least 1 FDA-defined symptom of COVID-19 plus positive findings on ≥5 out of 10 assays (9 SARS-CoV-2 immunoglobulin G [IgG] antibody binding assays and a 10th assay that included PCR testing for viral RNA). Because of the limited PCR availability, several subjects were diagnosed on the basis of symptoms plus multiple positive serologies only. The efficacy of BCG (versus placebo) for preventing a confirmed case of COVID-19 was 92% (Figure 2). Monte Carlo statistics resulted in a posterior probability (with vaccine efficacy >30%) of 0.99. Because the current standard of diagnosis for COVID-19 is based on PCR, we also calculated BCG efficacy based on at least one FDA-defined symptom of COVID-19 plus a positive PCR alone. We found no symptomatic subjects that were PCR positive in

the BCG group (0%), whereas 5 symptomatic and PCR-positive subjects were in the placebo group (10.4%). These results indicate an efficacy of 100% at 0.99 posterior probability if only PCR results are considered. This can also be readily visualized in the PCR cumulative graph (Figure 3B), which shows the first detected COVID-19 case based on PCR at 7 months into the trial, whereas serology testing for SARS-CoV-2 antibodies revealed the first positive cases as early as 2 months (Figure 2B).

There were no deaths from COVID-19 in either the BCG group or the placebo group.

Heatmap comparison shows higher SARS-CoV-2 antibody-level presence and intensity in the placebo group over the BCG group

Heatmap comparisons of SARS-CoV-2 antibody-level presence and intensity (Z scores versus pre-COVID baseline) show a significant increase in infections and the intensity of the antibody response in symptomatic subjects in the placebo over the BCG group (Figure 3A). These data are in symptomatic subjects. For completeness, we also present the COVID-19 antibodies in all trial subjects, with and without symptoms. The heatmap of all trial participants again shows the presence of COVID-19 infections almost exclusively in the placebo subjects (Figure S2).

Cumulative graphs for the anti-COVID-19 antibody tests to different regions of the virus are shown in Figure 2B. Number at risk data are shown in Figure S4. The identity of the SARS-CoV-2 protein region eliciting an antibody response, mostly the spike protein, is denoted by the Roman numerals listed in Figure S1. Six protein regions eliciting an antibody response were from viral spike protein 1, two were located in spike protein 2, and one viral region was specific for antibodies against viral RNA polymerase. Almost all COVID-19 viral regions included in these tests show significantly increased accumulation of antibody reactivity in the placebo group compared with the BCG group, except for COVID-19 viral epitope II.

The BCG group (versus placebo group) exhibited fewer infections per patient and fewer infectious disease symptoms and severity assessed by the total and average infectious disease index

We analyzed infectious disease events collected as adverse events by the Medical Dictionary for Regulatory Activities (MedDRA) classification coding (Figure S3B). Prior clinical trial data suggest that BCG vaccination exhibits "off-target" effects, i.e., viral and bacterial infectious disease prevention (and, in this study, prevention of SARS-CoV-2 viral infections) beyond its intended use in tuberculosis protection.[22–25,27,37–39,50] Using the time period of the current COVID-19 trial, we analyzed infectious disease symptoms including COVID-19 and other infections. The cumulative infectious diseases reported by the subjects and represented as cumulative infections per patient were significantly fewer in the BCG versus placebo group (Poisson model comparing adverse events rates p = 0.004; Figures 4A and S3B). These findings are likely to represent the minimum protection. Some clinical trial data for other off-target effects of the BCG vaccine suggest that the time period for BCG clinical effectiveness is often in the 2- to 3-year range after vaccinations begin.[19,20] To look at this time period of BCG onset of vaccine

A   Study Diagram
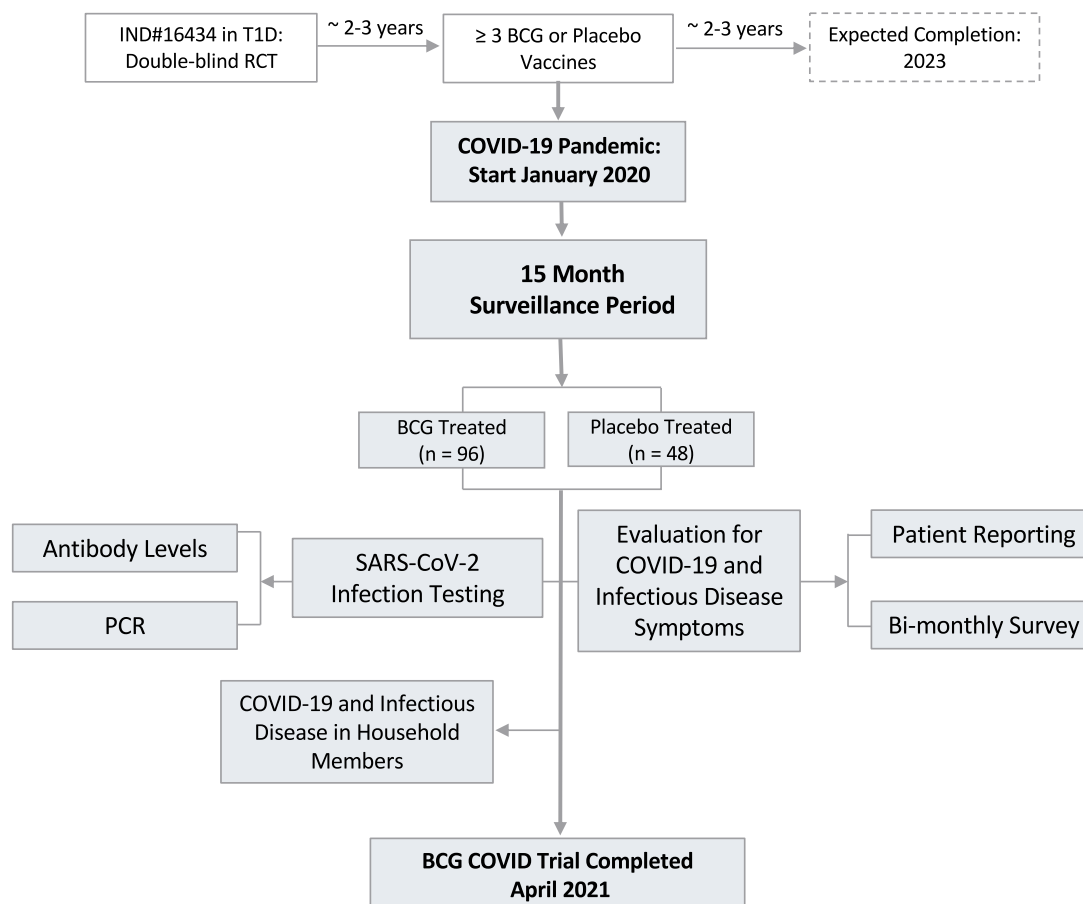
B   Characteristics of Participants

	BCG (N = 96)	Placebo (N= 48)	Total (N = 144)
Sex – no. (%)			
Male	55 (38.2)	29 (20.1)	84 (58.3)
Female	41 (28.5)	19 (13.2)	60 (41.7)
Country – no. (%)			
United States	96 (66.7)	48 (33.3)	144 (100)
Current Age – yr			
Average (Median)	44.5 ± 1.3 (43.1)	43.6 ± 1.9 (40.7)	43.8 ± 1.1 (42)
Age at Baseline Injection – yr			
Average (Median)	39.3 ± 1.3 (38)	38.4 ± 1.9 (35.5)	39 ± 1.0 (37)
Age of Diabetes Onset – yr			
Average (Median)	26.8 ± 1.2 (26.3)	25.1 ± 1.9 (23.4)	25.8 ± 1.0 (25)
Duration of Diabetes – yr			
Average (Median)	17.7 ± 0.7 (17)	18.6 ± 1.0 (16.5)	18 ± 0.6 (17)

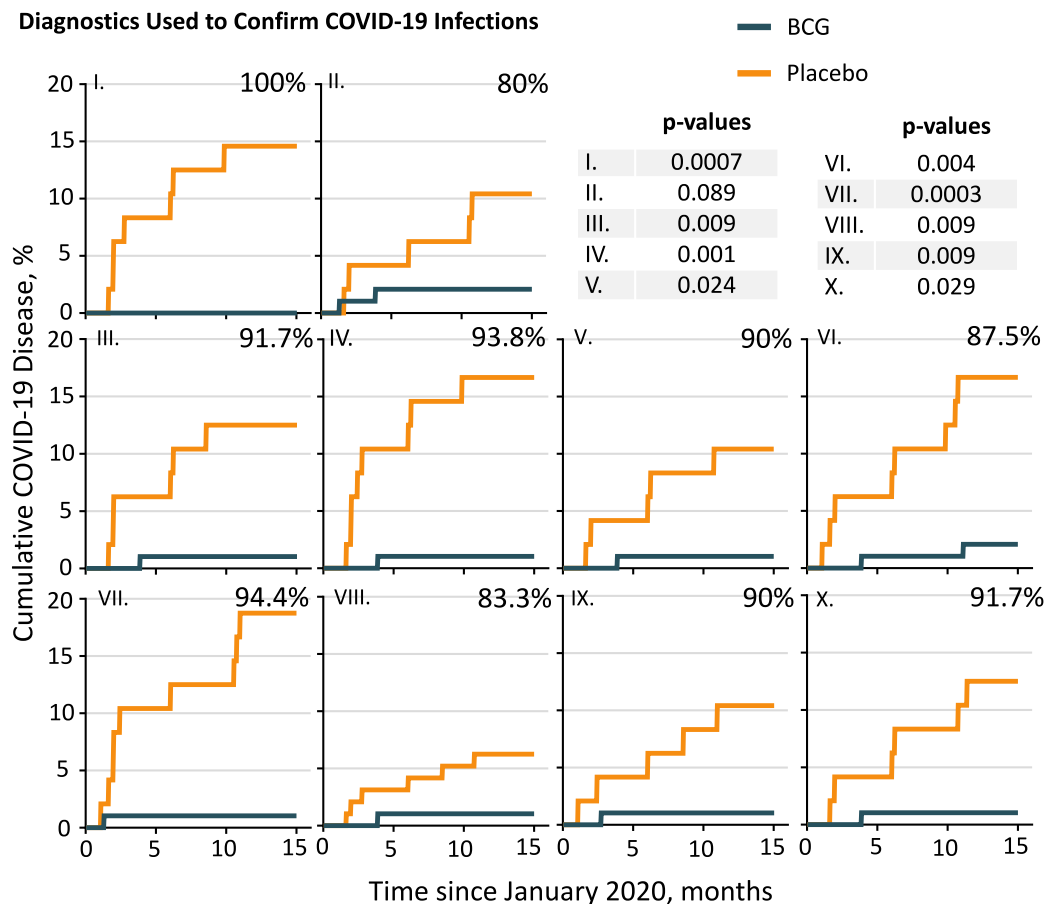**Figure 1. Flow diagram and characteristics of participants**
(A) Flow diagram representing all enrolled participants from January 1, 2020 to April, 2021 for this double-blinded, randomized clinical trial testing repeat Tokyo-172 BCG vaccination versus placebo for COVID-19 protection. All 144 subjects were followed for 15 months with a 2:1 randomization and no dropouts. Data collection for this trial ended on April 2, 2021, the date when subjects started to receive provisionally approved COVID-19-specific vaccines. The geographical locations of the participants within the United States are shown in Figure S5.
(B) Table of participant characteristics.

A BCG Efficacy Against Symptomatic COVID-19

	BCG (n=96)	Placebo (n=48)	Surveillance time (months)	Vaccine Efficacy	Posterior Probability (Vaccine Efficacy >30%)
COVID-19 confirmed by Point of Care and SARS-CoV-2 Antibodies	1 (1.0%)	6 (12.5%)	15	**92%**	0.99
COVID-19 confirmed by PCR only	0 (0.0%)	5 (10.4%)	15	**100%**	0.99

B Diagnostics Used to Confirm COVID-19 Infections

|	p-values			p-values
I.	0.0007	VI.	0.004	
II.	0.089	VII.	0.0003	
III.	0.009	VIII.	0.009	
IV.	0.001	IX.	0.009	
V.	0.024	X.	0.029	

Figure 2. BCG vaccine efficacy and diagnostic confirmation of COVID-19

(A) Shown is the cumulative incidence of confirmed COVID-19 as a primary endpoint. During the 15-month surveillance time, one BCG recipient out of 96 (1.0%) met our criteria for molecularly confirmed COVID-19. In contrast, 6 out of 48 placebo recipients met the criteria (12.5%). Fisher's exact testing showed a significant difference (two-tailed p = 0.006). Calculated vaccine efficacy was 92%, and the posterior probability (vaccine efficacy >30%) was 0.99. This was calculated using the Monte Carlo method. Vaccine efficacy was defined as (p1 − p2)/p1 × 100, where p1 is the percentage of COVID-positive subjects in the placebo group and p2 is the percentage of COVID-positive subjects in the BCG group. Our criteria for confirmed COVID-19 (see main text) required a combination of COVID-19 symptom(s) and ≥5 of 10 positive antibody assays including PCR, if available. Since many current clinical trials only define confirmed COVID-19 by symptom(s) and positive PCR testing, the PCR-only group was studied separately for vaccine efficacy (A and B).

(B) Cumulative findings from each of the 10 diagnostic tests used to confirm COVID-19 (along with positive symptoms). These tests included the presence of COVID-19-specific antibodies to various SARS-CoV-2 virus epitopes through protein display (I–VIII), antibodies to the receptor-binding domain with an ELISA test (IX), and point-of-care testing (X) that included PCR. Our criteria for having confirmed COVID-19 required at least 5 of 10 detection methods to be positive, along with symptom(s). For the antibody assays, a patient was considered positive when the test resulted in a Z score of ≥3. In the cumulative graphs, the x axis data show the time period of the 15-month trial. The y axis shows the cumulative percentage of positive subjects. Except for the point-of-care graph (X), all other

(legend continued on next page)

A Change in antibody levels for subjects reporting COVID-like symptoms (Z-scores)

B COVID-19 confirmed by RT-PCR only
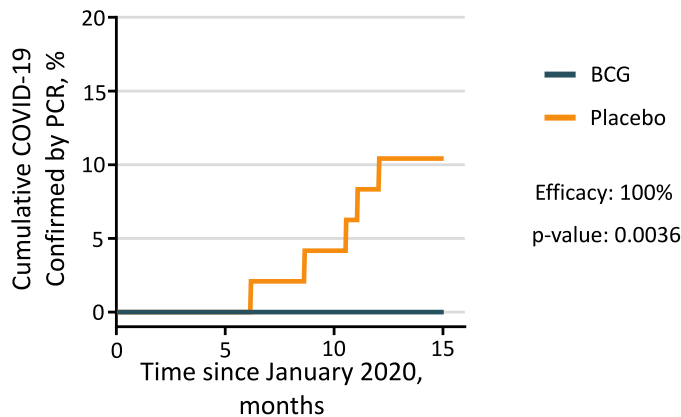

Efficacy: 100%

p-value: 0.0036
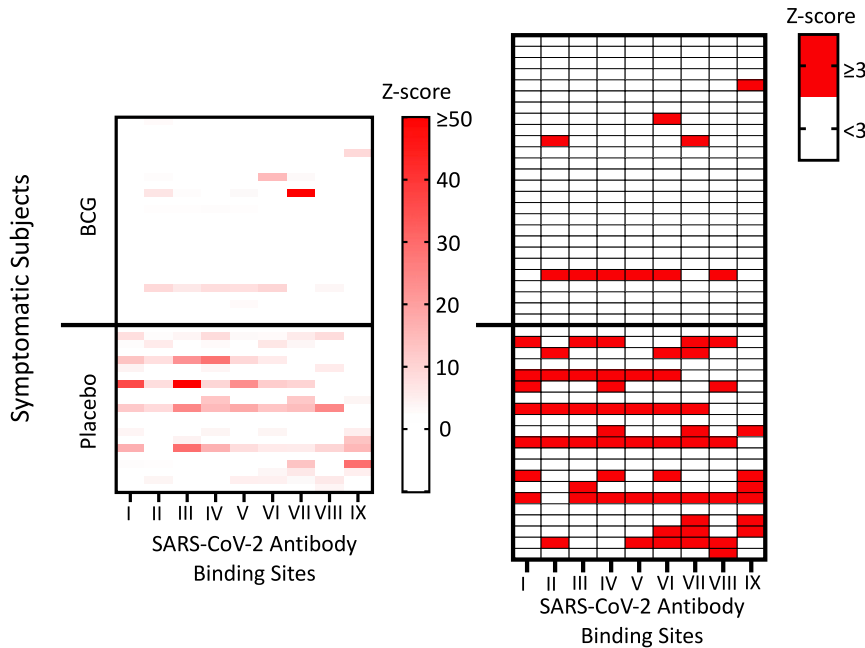
Figure 3. Reduced COVID-19 disease markers after BCG vaccination

(A) The heatmaps show Z scores for antibodies against SARS-CoV-2 in BCG- and placebo-treated symptomatic subjects (BCG n = 26, placebo n = 21). Since some of the Z scores in the left map are faint, the right map shows which Z scores are ≥3. The Roman numerals at the bottom of each lane denote antibodies against SARS-CoV-2 protein regions as listed in Figure S1. The maximum Z score in the left heatmap was 126.

(B) Cumulative incidence of confirmed COVID-19 defined by symptomatic subjects testing positive solely by PCR. The BCG group (n = 96) had no symptomatic subjects who were PCR positive (0%), whereas the placebo group (n = 48) had 5 symptomatic and PCR-positive subjects (10.4%). This difference was significant (Fisher's exact p = 0.0036) and translated to a vaccine efficacy of 100% with 0.99 posterior probability. There was low availability of positive PCR tests at point-of-care locations during the first 7 months of the trial. This, together with the need to perform the PCR test in a narrow window of about 2 weeks to be positive, is the reason why we designed the 9 non-PCR methods shown in Figures 2A, 2B, and 3A.

adverse events in BCG versus placebo groups during the current trial had a significant Poisson distribution (p = 0.004), there was no significant difference in all infections during the pre-COVID trial period (Poisson p = 0.46). These data suggest that, just like other reported off-target events of the BCG vaccine, it might similarly take about 2 years after the first vaccine for maximal effectiveness for the platform infectious disease protection.

Our intent was to evaluate the severity of COVID-19 symptoms with BCG treatment compared with placebo in confirmed COVID-19 patients, but this was not possible because only one subject in the BCG group fit our criteria for confirmed COVID-19. We therefore analyzed the symptoms in symptomatic BCG- and placebo-treated participants—regardless of whether they were confirmed COVID positive—to understand the impact of BCG on overall infectious disease severity.

Using data from symptom surveys based on FDA guidelines[51] that were completed every 2 months, we calculated a total and
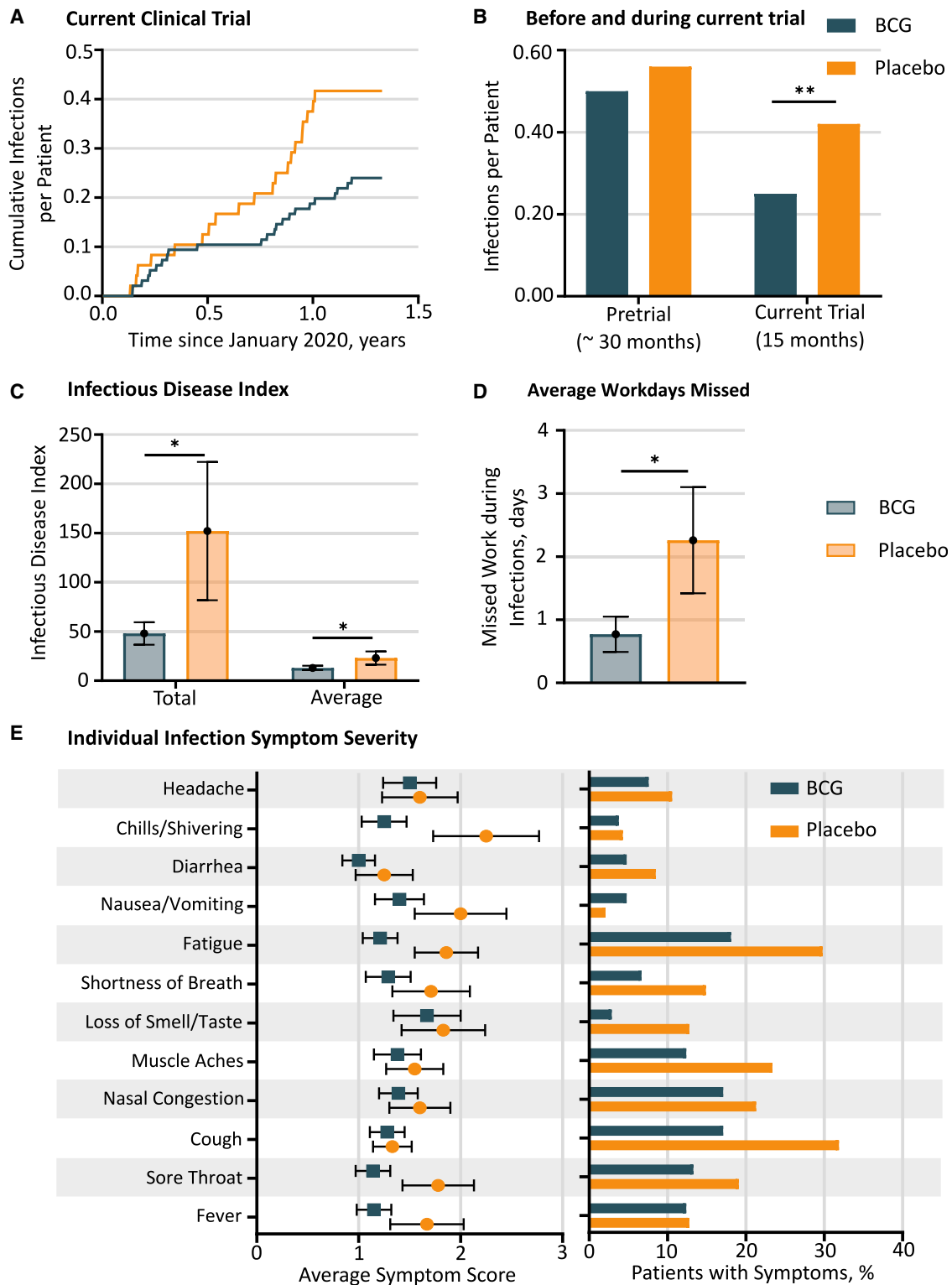
effectiveness but in the setting of infection protection, the clinical trial infectious disease adverse events were broken into the pre-COVID trial period, during which the three BCG vaccines were administered, versus the subsequent 15-month time period of this current clinical trial (Figure 4B). While comparison of infectious

graphs represent the percentage of BCG and placebo patients with a Z score of ≥3 for the anti-SARS-CoV-2 antibody binding to a given protein region of the virus, i.e., the average antibody level during the COVID trial period was at least 3 standard deviations greater than the average level in the period preceding the COVID trial (baseline). The percentiles at the top right of each graph represent the calculated vaccine efficacy if this test alone was used to diagnose COVID-19 disease. Respective efficacy and Fisher's exact p values for each COVID-19 antibody test were as follows: (I) 100%, p = 0.0007; (II) 80%, p = 0.089; (III) 91.7%, p = 0.009; (IV) 93.8%, p = 0.001; (V) 90%, p = 0.024; (VI) 87.5%, p = 0.004; (VII) 94.4%, p = 0.0003; (VIII) 83.3%, p = 0.009; (IX) 90%, p = 0.009; and (X) 91.7%, p = 0.029. For all graphs, BCG n = 96, placebo n = 48.

Figure S1 shows the viral protein regions for each anti-COVID antibody tested. Number at risk data for each cumulative graph are shown in Figure S4.

A **Current Clinical Trial**

B **Before and during current trial**
- BCG
- Placebo

C **Infectious Disease Index**

D **Average Workdays Missed**

- BCG
- Placebo

E **Individual Infection Symptom Severity**

- BCG
- Placebo

Figure 4. Cumulative infections and infection severity

(A) Cumulative total infectious diseases during the 15 months of surveillance. This cumulative figure shows all infections per patient, including all COVID-19 events, within the BCG group (blue) compared with the placebo group (red). Included are the infections for which multiple subject events were documented in both BCG and placebo groups. Comparison by means of a Poisson model yields a significant difference with p = 0.004 (BCG n = 24 out of 96 T1D total, placebo n = 20 out of 48 T1D total).

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an average infectious disease index (Figure S3A). Indexes for each individual patient were calculated separately and then averaged across all subjects in the BCG and placebo cohorts (Figure 4C). Comparing only symptomatic patients, the total covid index in the BCG cohort (48 ± 11, n = 31) was significantly reduced versus the placebo group (152 ± 70, n = 20; p = 0.04). The average infectious disease index also showed a significant decrease (BCG 13 ± 2, n = 31 versus placebo 23 ± 7, n = 20; p = 0.04). This indicates that BCG vaccination reduced the severity and duration of all infectious disease symptoms compared with placebo. Symptomatic patients in the BCG group also reported significantly fewer numbers of days of missed work compared with the placebo group (Figure 4D; BCG 0.77 ± 0.28; placebo 2.26 ± 0.84, p = 0.02). All individual average symptom scores (12 out of 12) were more severe in the placebo group versus the BCG group (Figure 4E, left panel). For 11 out of 12 symptoms, there also was a higher percentage of patients in the placebo group versus the BCG group (Figure 4E, right panel).

BCG recipients had comparable or milder infectious disease symptoms compared with their household members, whereas most placebo recipients had more severe symptoms compared with their household members

We, on an exploratory basis, studied infectious disease severity of trial participants compared with non-diabetic adults living in the same household. We collected infectious disease symptom information for trial participants and for co-habitating adult partners in 20 households (13 BCG and 7 placebo) and determined the differences in total infectious index for each household (Figures 5A and S3A). The data show that BCG recipients had comparable or milder symptoms compared with their household members, whereas most placebo recipients had more severe disease compared with their household members. The symptom scores between participants and household members were compared between the BCG-treated group and the placebo-treated group using a two-sample Wilcoxon test (p = 0.049, two-tailed). The stacked horizontal bars in Figure 5B show the distributions of the infectious disease symptom scores in each group. BCG recipients overall had milder symptoms compared with placebo recipients and even compared with non-diabetic household member controls. Number at risk data are shown in Figure S4.

These data support previously published data that type 1 diabetics not only have more severe COVID-19 but also have more severe and more infectious disease events.[49] Lastly, the distribution of symptoms in Figure 5B shows once again that for BCG-treated trial participants, individual symptoms were minimized with vaccinations compared with placebo.

DISCUSSION

This randomized, double-blinded, placebo-controlled trial shows that multi-dose BCG vaccination is safe and prevents COVID-19 with an efficacy of 92%–100% (depending on case definition) relative to placebo. This trial was conducted in the United States in previously unvaccinated at-risk type 1 diabetic subjects. BCG-vaccinated adults also had reduced incidence and severity of all infections. This randomized, double-blinded study is now a rigorous manner of formal testing and substantiates a large body of epidemiological, observational, and clinical trial evidence that BCG vaccines might, in some settings, provide a broad platform for infectious disease protection. These data are from a study conducted in the United States as a multi-dose BCG vaccine therapy and administered 2 years prior to the pandemic to allow sufficient time for full effectiveness.[2–16]

This clinical trial has several strengths. First, this trial is, to our knowledge, the first peer-reviewed randomized double-blinded trial of multi-dose BCG for COVID-19 protection and infection protection in a United States population. A prior trial of single-dose BCG, with immediate COVID-19 disease monitoring, showed neither protection from COVID-19 infections nor protection from disease severity.[7] This trial was likely too short in duration to find an effect based on the evidence from other off-target effects (see below) indicating years to show clinical efficacy. Second, our trial uses a very potent strain of BCG, Tokyo-172. BCG strain differences for other off-target indications are important, and this strain of BCG exhibits some of the highest *in vitro* potency and is highly immunogenic.[52–57] Indeed, it is now appreciated that Japan, as a country with mandatory BCG vaccines and as one of the oldest populations in the world, has remarkable resistance to COVID-19.[58] Third, the diabetic study population is high risk for infections, suggesting that the vaccine is efficacious in populations susceptible to infections. Fourth, the study uses rigorous molecular methods to define a case of COVID-19 based on both

(B) Cumulative infections for two different time periods: pre-COVID-19 pandemic (the 2.5-year period prior to this trial, i.e., the pretrial) and during the COVID-19 pandemic (the current trial period of 15 months). The pretrial time period was when all clinical trial subjects received their ≥3 BCG vaccines or placebo vaccines; the current trial was when the subjects were monitored during the 15-month observation during the COVID-19 pandemic (total patients BCG n = 96, placebo n = 48). The lack of a statistical difference in the number of infections between BCG and placebo groups in the pretrial period suggests that a longer length of time than 2.5 years is necessary to realize BCG's maximal infectious disease protection. It appears that during the entire period of 15 months, prior BCG vaccinations were protecting from COVID-19 and other infections. **p < 0.01.

(C) Infectious disease index for symptomatic patients. Symptomatic patients in the BCG-treated group had significantly reduced total infectious disease symptom index (placebo 152 ± 70 [n = 20] versus BCG 48 ± 11 [n = 31], p = 0.04, single tail and unpaired) as well as average infectious disease symptom index (placebo 23 ± 7 [n = 20] and BCG 13 ± 2 [n = 31], p = 0.04, single tail and unpaired). We first calculated total and average symptom scores per patient and then calculated average and SEM of each of these for BCG and placebo cohorts separately (*Student's t test p < 0.05, one-tailed, unpaired).

(D) Patients in the BCG cohort reported significantly fewer days of missed work during infections compared with the placebo group (*p = 0.02). Missed work was reported by 7 out of 32 BCG patients and by 8 out of 17 placebo patients.

(E) The number of patients that reported at least one symptom were 20 out of 48 in the placebo group and 33 out of 96 in the BCG group. The placebo group had more severe average symptoms compared with the BCG group for 12 out of 12 symptoms (left panel). The number of patients in BCG and placebo groups that reported each symptom was then expressed (right panel). For 11 out of 12 symptoms, there was a higher percentage of symptomatic patients in the placebo group compared with the BCG group. Statistical analysis by Student's t test (one-tailed, unpaired, *p < 0.05).

A Total Infectious Symptom Index

B Symptom Severity Comparison
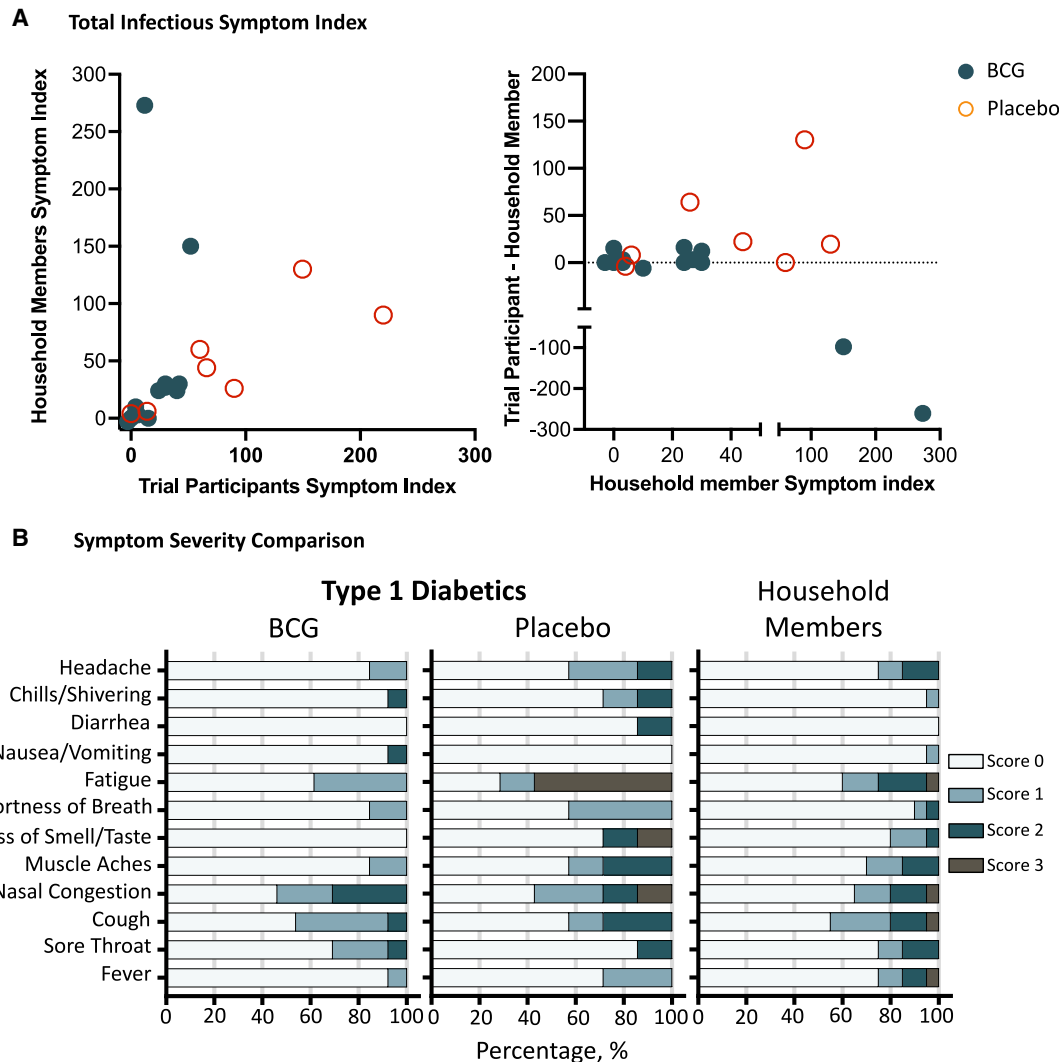
Figure 5. Infection symptoms of trial participants versus adult household members

(A) Infection symptoms in BCG and placebo groups compared with non-diabetic adult partners living in the same households. We collected surveys of symptoms of infectious diseases from all trial participants and household members of 13 BCG families and 7 placebo families. The BCG-treated trial participants had comparable or lower total infectious symptoms indexes compared with their partners living in the same household, whereas most placebo-treated trial participants had more severe symptoms compared with their partners. Statistical analysis of the differences by two-sample Wilcoxon testing was significant (two-tailed; p = 0.049; BCG n = 13 and placebo n = 7). Left panel: xy plot of symptom index of trial participants versus their household members. Right panel: the same data are also shown as an xy plot of household members versus the difference of trial participants and their household members. This plot makes it easier to visualize which groups had milder or more severe disease by introducing positive and negative differences.

(B) Distribution of individual infectious disease symptoms in BCG- and placebo-treated participants and infected household members across the four scoring possibilities (0: no symptoms; 1: mild; 2: moderate; 3: severe symptoms). Number at risk data are shown in Figure S4.

PCR and/or SARS-CoV-2-directed immunoglobulin production to very specific regions of the virus to confirm current or past infection. Fifth, there was no patient attrition. Sixth, the subjects are from the United States. This is important because all subjects, prior to enrollment, were confirmed by diagnostics and by history to be unexposed to tuberculosis and lacking prior BCG vaccinations. The United States has never had a country policy of neonatal BCG vaccinations. Two properties of the vaccine are especially noteworthy. BCG's 100-year-strong safety record and its international use as a neonatal vaccine should help to over-

come vaccine hesitancy. Seventh, this trial was conducted in subjects who all had negative serology for prior COVID-19 infections and also had not yet received the approved Moderna and Pfizer vaccines that could confound the effect of BCG's protection alone. Finally, the BCG vaccine is affordable, which is especially important in the developing world, where it offers almost immediate availability.

In conclusion, the BCG vaccine effectively protects against COVID-19 and provides broad infectious disease protection as now tested with a formal double-blinded and randomized clinical

trial. Also, the BCG vaccine is safe, effective, affordable, and potentially protective against every changing viral variant of the COVID-19 pandemic, based on its broad-based protection against other infections.

Limitations of the study

The most important limitation of our trial is that off-target effects take time to systemically manifest, but, once they do appear, they may offer broad-based infectious disease protection over the long term. BCG does not work as fast as the antigen-specific COVID-19 vaccines and also likely requires multiple treatments over time to be effective, at least in BCG-naive adults. Antigen-specific vaccines generally take weeks to show an effect, although protection is limited by a specific strain and then, downstream, by short durability. In the case of the BCG vaccine, the off-target platform protection from infections has a slower onset (months to years) but offers perhaps life-long durability. The other off-target effects of BCG are in its reset of autoimmune diseases, benefits that take 2 years to show an effect.[18–21] Nonetheless, the benefits may last for decades thereafter as well, at least 8 years in randomized clinical trials. Many of the epidemiologic studies show off-target protection after neonatal or adult vaccination, including one study showing >60 years of protection from a neonatal BCG vaccine.[19,59] This "long immunity" is both long to start and perhaps long lasting. The slow onset of therapeutic effects and perhaps lifelong durability could be due to the gradual migration of the BCG organism from the vaccine injection site to the bone marrow, where it may infect resident stem cells, a process that is hastened by the intravenous (i.v.) administration of BCG.[19,60,61] Longitudinal mechanistic data from our lab show that BCG, over time, alters key metabolic and immune signaling pathways via methylation of genes over a 2- to 3-year time period.[61–64] Others have shown that more acute changes are brought about by chemical modifications (methylation, acetylation, etc.) of the histones in the promoters and enhancers of the genes.[65,66] The slow reset of key immune genes by BCG-driven methylation and demethylation of select host genes and in almost all lymphoid cell types, such as T cells and monocytes, shows that this long immunity is multi-lineage. This could be due to possible stem cell-driven re-modeling of the human immune system by this very synergistic organism. This randomized, double-blinded clinical trial documents, in humans, the broad infectious benefits of host microbe interactions.

Other potential limitations for this BCG study, as well as other studies that test COVID-19 vaccines in specialized patient populations, such as type 1 diabetic subjects, healthcare workers, or the elderly, is that the translation of vaccine efficacy to the general public, in countries with diverse populations and different neonatal vaccination histories, may not always be applicable.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
BCG vaccine (Bacillus Calmette-Guérin)	Japan BCG Laboratory, Tokyo, Japan	N/A
Critical commercial assays		
AntiCoV-ID™ IgG ELISA	Akston BioSciences, Beverly, MA	SKU: 600016
SARS-CoV-2 Protein Microarray: 2-in-1 protein and peptide assay	CDI Labs, Baltimore, MD. (http://cdi.bio)	CDICOV2-001.0
Point of Care SARS-CoV-2 testing (PCR, Rapid Antigen test, Antibody test)	Quest Diagnostics, Secaucus, NJ	N/A
Software and algorithms		
StudyTrax Electronic Data Capture System for Clinical Research	StudyTrax, Macon, GA, USA	StudyTrax
Other		
BD Vacutainer Serum Blood Collection Tubes (Red Top)	Becton and Dickinson, Franklin Lakes, NJ	Ref. 366430
BD Vacutainer Push Button Blood Collection Set	Becton and Dickinson, Franklin Lakes, NJ	Ref. 367344

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Denise Faustman (dfaustman@mgh.harvard.edu).

Materials availability
This study did not generate new unique reagents.

Data and code availability
- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

We assessed the safety and efficacy of ≥ 3 BCG vaccinations (Tokyo-172 strain) versus placebo for prevention of COVID-19 and other infectious diseases in a community-based, single-center, Phase $2/3$ randomized, double-blind, placebo-controlled clinical trial. The trial duration was 15 months, starting at the beginning of the SARS-CoV-2 pandemic in the US (01/0½020) and ending with the US launch of COVID-19 vaccines in April 2021 (Figure 1A). All participants only received the placebo or BCG vaccine and did not receive COVID-19 antigen specific vaccines. Clinical trial participants were volunteers ages 18–50 years old with a co-morbid condition, type 1 diabetes (Figure 1B). The current trial was a parallel study using already enrolled double-blinded participants in a multi-dose BCG vaccine trial assessing its 5-year impact on other outcomes unrelated to infections (US FDA Investigational New Drug [IND] #16434; Massachusetts General Hospital [MGH] protocol #2013P002633). At the three-year time point of the original study, this parallel study on COVID-19 protection was initiated, while the original trial continued in a double-blinded fashion. The original clinical trial and this parallel clinical trial were pre-registered in the clinicaltrials.gov NCT site as: NCT02081326.

The protocol for the current COVID-19 prevention trial was approved as an amendment to the original FDA IND and MGH protocols as well as a new MGH protocol #2020P001462. A study synopsis is provided (Figure S6). The current trial and the original trial were performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonization (ICH)/Good Clinical Practice, applicable regulatory requirements, and the MGH policy on Bioethics. Informed consent was written for the intervention; informed consent could be oral for the COVID-19 symptom surveys.

A total of 150 subjects were recruited for the original trial. All subjects were recruited with a requirement to have no previous or current tuberculosis and no history of BCG vaccinations, even during childhood. All subjects were US born and followed from the beginning of the original trial for all infection diseases (viral, bacterial, parasitic, etc) and any sort of antibiotic usage through a direct

MedDra documentation system. The US has never offered newborn or childhood BCG vaccinations. The original trial recruited 150 subjects (2:1 BCG:placebo) and at the start of this COVID-19 trial two years later, 144 subjects were enrolled for this parallel trial design. The parallel infectious disease study design studied the off-target effects of repeat BCG vaccinations for COVID-19 and infectious disease protection (96 BCG treated, 48 placebo treated). There was no patient attrition over the 15-month time course of the COVID-19 study. The original exclusion criteria included positive purified protein derivative (PPD) test, positive T-spot test for tuberculosis, or born in a foreign country with mandatory BCG vaccinations. The goal of these exclusion criteria was to prevent prior ongoing and durable protection from *Mycobacterium bovis* (the origin of the BCG vaccine) or *Mycobacterium tuberculosis* (TB) exposures causing long-term protection. Exclusion criteria also included no active glucocorticoids treatment, chronic immunosuppressive medications, or currently living with an immunosuppressed individual to prevent an adverse event from the administration of this live vaccine. All subjects lived within the United States (Figure S5). The largest contingent of participants, 36, was from Massachusetts, the second largest, 16, was from New York, and the next largest, 11, was from Texas. During this time in the US for this COVID-19 study (January 2020 - April 2021) the Delta variant was identified in May 2021 so this study addresses BCG protection from all infectious diseases and resistance to predominantly the Alpha variant of the SARS-CoV-2 pandemic. Importantly both the diagnostic PCR testing and serology testing performed to confirm COVID-19 disease, detected all genetic variants of the virus.

This trial had three types of oversight. At 6-month intervals, this trial had audits from either Massachusetts General Brigham (MGB) Division of Quality Management or from outside auditors (Advanced Clinical Trials, Deerfield, IL). All trial data was processed by two unblinded statisticians (D.S., H.Z.), and an independent data and safety monitoring board (DSMB) met at 6-month intervals to monitor subject safety and reporting compliance.

The MGH Research Pharmacy generated the randomization code for the administration to study participants of either the BCG or the saline placebo vaccine. This randomization code was held in the MGH pharmacy. All drug within the syringe had an identical appearing fluid for the BCG and placebo vaccine in the 0.1 cc volume. With this vaccine there are absolutely no systemic symptoms after administration so blinding of study staff and also subjects collecting post-vaccine adverse systemic events is easier than antigen specific vaccines that frequently yield multi-day systemic reactions and sometimes missed workdays. A separate third party study nurse collected systemic reactions to the vaccine (none reported) and also consulted with participants for any skin reactions. Skin reactions were kept in separate locked storage center and not within the Studytrax or EPIC data management center. All investigators and study staff remained blinded when studying both the primary and parallel study outcomes of COIVID-19 and infectious diseases. Subject and staff unblinding will occur at the end of the primary study.

METHOD DETAILS

Procedures

The BCG vaccine or saline placebo (both 0.1 mL volume per dose) was administered intradermally over a period of 2.5–3 years prior to the start of this parallel trial. Two BCG doses were given 4 weeks apart in the beginning of year 1, and an identical booster dose was given in year 2. Site staff were responsible for reporting all drug- and non-drug-related safety information and were unaware of group assignments.

Monitoring for COVID-19 or infectious disease over the 15-month surveillance period included emailed surveys (every 2 months), as well as surveys during every visit to our clinic. The survey asked about COVID-19 and any other infections subjects had over the previous three months. It was completed by the patient via e-mail, over the phone with research staff at the 6 months visit time or online through Studytrax. Studytrax is an FDA compliant electronic data capture system for clinical research. At each 6-month clinic visit 10 mL of blood was drawn. The survey was also completed by the patient for any adult household member with infectious symptoms. Participants also directly reached out to the clinic to report infections. Household members' blood for confirmation of SARS-CoV-2 infection was locally obtained by a national based chain of blood drawing centers (Quest Diagnostics, Secaucus, NJ). The symptoms questionnaire for study participants and infected household members was in compliance with FDA Guidance for Industry.[51] For each symptom, the participants provided a severity score of 0 (none), 1 (mild), 2 (moderate) or 3 (severe). The length of the infectious illness in days was also reported. From the individual symptom scores a Total Symptom Score and an Average Symptom Score were tabulated. Then, multiplying by the length of illness, the Total Infectious Symptom Index or the Average Infectious Symptom Index was assessed (Figure S3A).

For SARS-CoV-2 antibodies detected, the average and SD 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 SD 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.

Outcomes

This trial had the following co-primary efficacy endpoints on potential benefits of multi-dose BCG: prevention of symptomatic and molecularly-confirmed COVID-19 (see case criteria below); and reduction of infectious disease (including COVID-19) symptomatology and severity by the Total or Average Infectious Disease Index. The study synopsis is provided (Figure S6). These outcomes conformed to other primary US based trials for the antigen-specific vaccines (Pfizer-BioNTech and Moderna trials). Secondary

outcomes were average workdays missed, and individual infection symptom severity, and household members' total infectious symptom index.

COVID-19 is defined according to FDA by the presence of at least one of 12 symptoms[51] (headache, chills/shivering, diarrhea, nausea/vomiting, fatigue, shortness of breath, loss of smell or taste, muscle aches, nasal congestion, cough, sore throat and fever) and molecular confirmation. These symptoms, other than the loss of smell or taste, are symptoms of most infectious diseases, so the survey was used for both purposes.

Our criteria for symptomatic and molecularly confirmed COVID-19 had to be rigorous while overcoming the problem that PCR testing was not available at the origin of this trial and/or not equally accessible in all parts of the US. PCR testing only became widely available seven months into the trial. In addition, PCR detects SARS-CoV-2 virus in a smaller time window than molecular antibody confirmation through the measurement of host direct SARS-CoV-2 antibodies. Therefore for symptomatic and molecularly confirmed COVID-19 we required subjects to exhibit one or more FDA-defined symptoms of COVID-19[1] together with positive findings on ≥ 5 out of 10 assays (9 SARS-CoV-2 IgG antibody binding assays and a 10th assay that included PCR testing for viral RNA) (Figure S1). A subject was deemed positive for a particular SARS-CoV-2 antibody assay if the serology antibody Z score was ≥ 3, i.e., at least 3 standard deviations higher than during the pre-COVID period (baseline). Serum was obtained in all cases within 3 months of the infection for the detection of IgG antibodies.

QUANTIFICATION AND STATISTICAL ANALYSIS

The sample size and power analysis of this trial were based on the primary randomized Phase II clinical trial that started 2–3 years prior to this parallel trial focusing on COVID-19. Vaccine efficacy is defined by (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. For comparison to published Covid vaccine trials such as the mRNA trials, we also calculated the posterior probability that the vaccine efficacy is greater than 30% using a Bayesian Beta binomial model fitted using WinBUGS.[67] We used a non-informative beta prior with parameters (1,1) and took 5000 MCMC samples to compute the posterior probability for efficacy to be greater than 30%. The 95% credible interval for the posterior probability for efficacy to be >30% is 98.9–100%. Further details are provided in the Figure legends and these method mirror past studies on COVID-19 vaccine efficacy.[68] Average antibody levels were compared using Student's T-testing in Prism (Graphpad Software, San Diego, CA) or Microsoft Excel. The number of patients positive in the BCG versus the Placebo cohort were compared using Fisher Exact Test (available online at https://www.graphpad.com/quickcalcs/contingency1/). Differences in the symptoms scores between participants and household members were compared between the BCG-treated group and the placebo-treated group using a Two-Sample Wilcoxon test. Statistics were considered significant at p < 0.05.

ADDITIONAL RESOURCES

This trial is listed on clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT02081326).

Supplemental information

Multiple BCG vaccinations for the prevention

of COVID-19 and other infectious diseases

in type 1 diabetes

Denise L. Faustman, Amanda Lee, Emma R. Hostetter, Anna Aristarkhova, Nathan C. Ng, Gabriella F. Shpilsky, Lisa Tran, Grace Wolfe, Hiroyuki Takahashi, Hans F. Dias, Joan Braley, Hui Zheng, David A. Schoenfeld, and Willem M. Kühtreiber

SUPPLEMENTARY INFORMATION

Denise L. Faustman, Amanda Lee, Emma R. Hostetter, Anna Aristarkhova, Nathan C. Ng, Gabriella F. Shpilsky, Lisa Tran, Grace Wolfe, Hiroyuki Takahashi, Hans F. Dias, Joan Brale , Hui Zheng1, David A. Schoenfeld, Willem M. Kühtreiber.

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

Cell Reports Medicine

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: $(p1 – p2)/p1 \times 100$, 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).
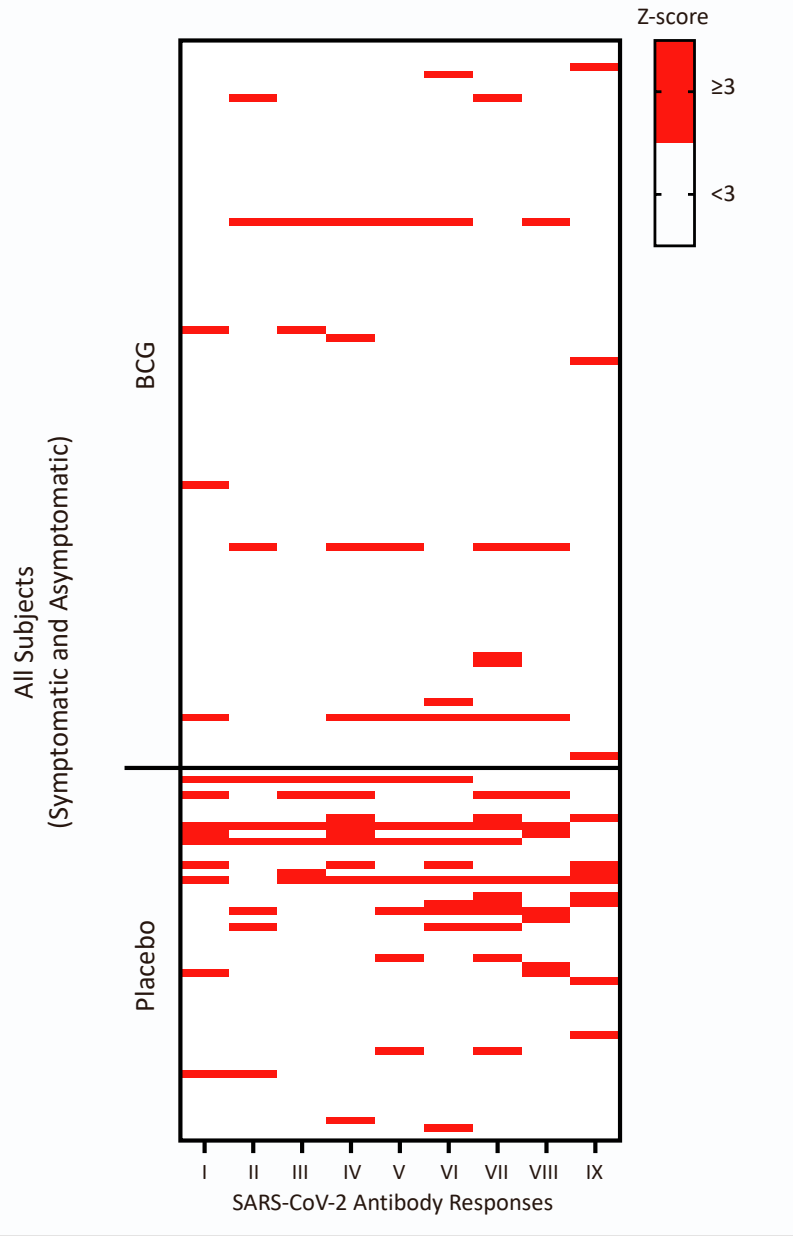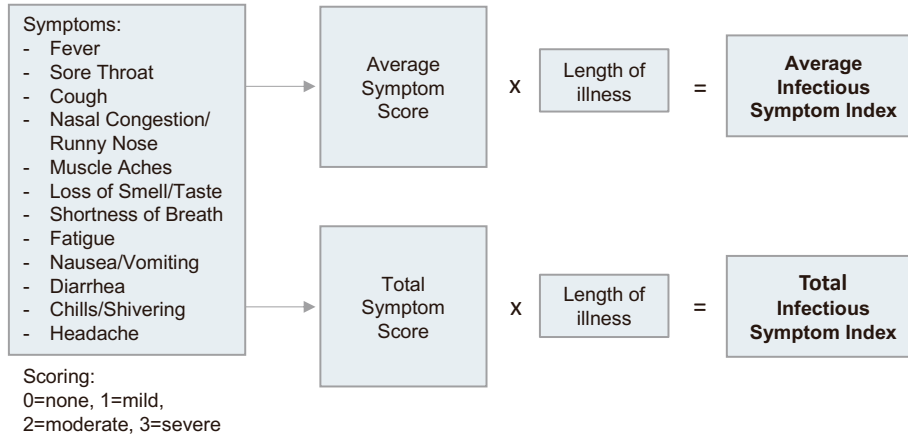
Figure S2. Heat Map Display of SARS-CoV-2 Epitopes that Tested Positive (Z-score ≥3) for All Trial Participants (Symptomatic as well as Asymptomatic)
Related to Figure 3A

Supplemental Figure S3.

A

Symptoms:
- Fever
- Sore Throat
- Cough
- Nasal Congestion/Runny Nose
- Muscle Aches
- Loss of Smell/Taste
- Shortness of Breath
- Fatigue
- Nausea/Vomiting
- Diarrhea
- Chills/Shivering
- Headache

Average Symptom Score X Length of illness = **Average Infectious Symptom Index**

Total Symptom Score X Length of illness = **Total Infectious Symptom Index**

Scoring:
0=none, 1=mild, 2=moderate, 3=severe

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
I	Placebo	48	44	41	41
II	BCG	96	94	94	94
II	Placebo	48	46	45	43
III	BCG	96	95	95	95
III	Placebo	48	45	42	42
IV	BCG	96	95	95	95
IV	Placebo	48	43	40	48
V	BCG	96	95	95	95
V	Placebo	48	46	44	43
VI	BCG	96	95	95	94
VI	Placebo	48	45	42	40
VII	BCG	96	95	95	95
VII	Placebo	48	43	42	39
VIII	BCG	96	95	95	95
VIII	Placebo	48	45	43	42
IX	BCG	96	95	95	95
IX	Placebo	48	46	44	43
X	BCG	96	95	95	95
X	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.

Subject Location Distribution

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