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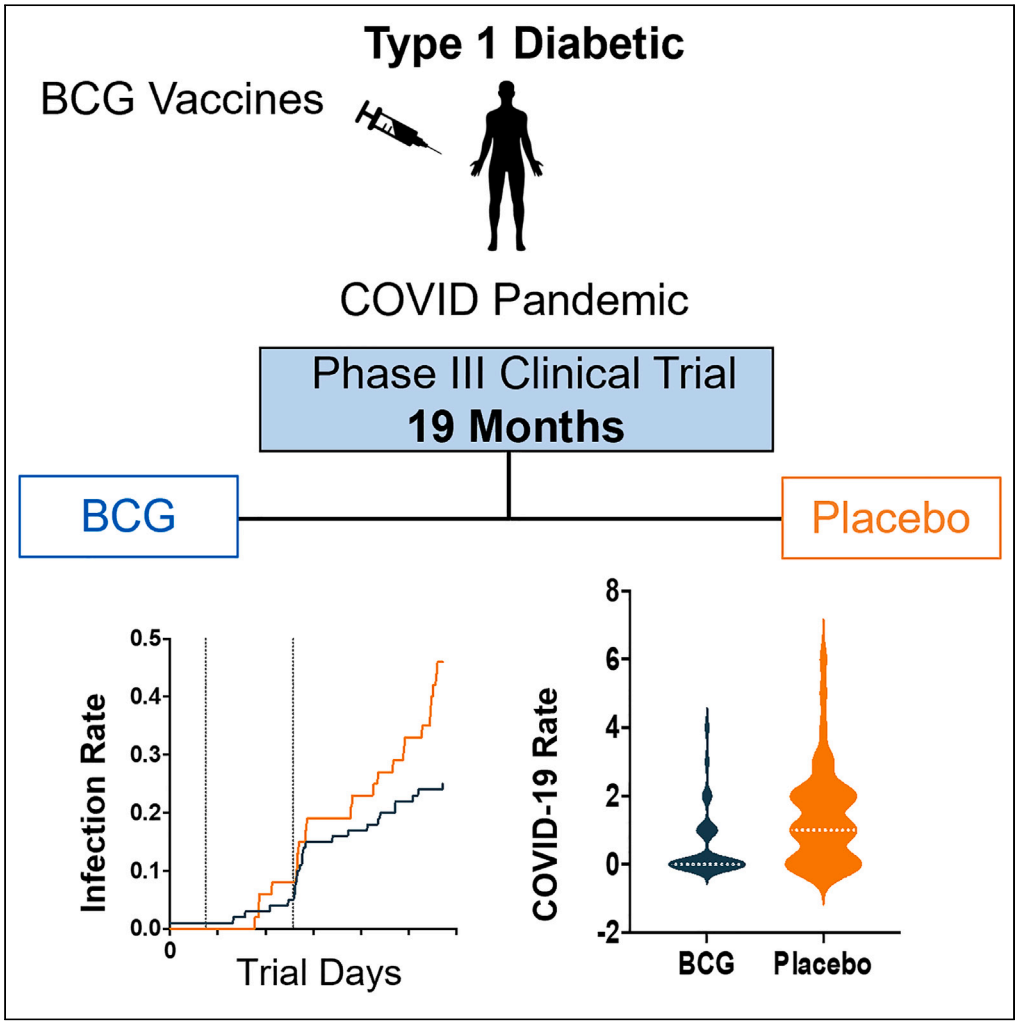

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Highlights

A randomized Phase III trial of BCG vaccines for COVID-19 and infection protection

Trial conducted late in US pandemic in infectious vulnerable type 1 diabetics

BCG vaccines provide platform infectious disease protection including COVID-19

Commercial mRNA vaccines in type 1 diabetics do not appear to protect from COVID-19

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Late in the US pandemic, multi-dose BCG vaccines protect against COVID-19 and infectious diseases

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SUMMARY

The Bacillus Calmette-Guérin vaccine has many off-target benefits, including protection from diverse infectious diseases. As SARS-CoV-2 evolved, COVID-19 disease became more transmissible and less lethal. In this Phase III double-blinded, placebo-controlled trial conducted late in the pandemic, we tested at-risk US adults with type 1 diabetes if multi-dose BCG protected against COVID-19 and other infectious disease, co-primary outcomes. From April 2021 to November 2022, Tokyo-strain BCG vaccines provided significant protection against COVID-19 disease ($p = 0.023$) and strong platform protection against all infectious diseases ($p < 0.0001$). Over the course of the study, commercial COVID-19 vaccines were rolled out, most of which were mRNA-based. In contrast to the protection afforded by BCG, as reported by others, COVID-19 mRNA vaccine alone provided no protection from COVID-19 disease ($p = 0.43$). BCG vaccination efficacy was unaffected by concurrent COVID-19 vaccinations; COVID-19 vaccines neither helped nor hindered BCG protection.

INTRODUCTION

Clinical trial and epidemiologic investigations conducted over the past decade have shown that the Bacillus Calmette-Guérin (BCG) vaccine, originally developed for tuberculosis protection, appears to have wide-ranging off-target benefits in the form of platform infectious disease protection.[1–14] The BCG vaccine, a live attenuated version of the mycobacterium that causes tuberculosis in cattle (*Mycobacterium bovis*), confers durable protection. When administered to neonates, it can protect against tuberculosis for more than 40 years, and it can benefit glucose metabolism for more than 70 years.[15,16] Heralded as the safest vaccine in continuous use globally, the BCG vaccine is designated an essential medicine by the World Health Organization.[17] mRNA vaccines developed for COVID-19 have limitations due to narrow efficacy against specific viral variants as well as short duration of protection against infection.[18]

As the SARS-CoV-2 pandemic unfolded, a profusion of international investigations sought to test whether BCG could protect against development of COVID-19. Five randomized trials showed efficacy,[13,19–23] whereas seven randomized trials showed no efficacy.[24–29] . Furthermore, looking at the hard endpoint of death after COVID-19 infections, a post-hoc analysis of eight of the recent clinical trials found that BCG groups had statistically significant less death than among placebo recipients.[30] It is important to note the differences in the designs of these trials.[31] All of the clinical trials showing no benefit had one or more features that might have diminished the likelihood of finding a BCG effect: the trial enrolled subjects that had previous received neonatal BCG vaccination or previous exposure to tuberculosis (TB), all of which confer long-term protection; the trials used a less potent strains of BCG; the trials used a single BCG dose, rather than multiple dose benefits; or the trial had insufficient follow-up time from the vaccination date to the infection exposure data and therefore could not capture BCG's efficacy (i.e., trial was weeks to months instead of the needed two or more years for many off-target effects). When the BCG vaccine was used successfully against autoimmune diseases in BCG-naïve adults in Europe and the US, full efficacy was not reached until at least two years. The time lag may be due to BCG's potential mechanism of action involving the multi-year slow epigenetic reset of critical genes in the adult human innate and adaptive immune system, at least in adults.[13,16,32–34]

We now report on the second of two trials of multi-dose BCG protection against COVID-19 in a US population of infection vulnerable type 1 diabetics. The earlier Phase II trial was conducted early in the US pandemic.[13] Studying a US population has multiple advantages: it has not been vaccinated with neonatal BCG; it has low rates of endemic tuberculosis; and it is highly vulnerable to infectious diseases in general and to COVID-19 in particular.[35–37] In our earlier BCG study, an RCT Phase II trial, we excluded patients with a history of tuberculosis exposure and with a history of neonatal BCG vaccination.[13] The same exclusions apply to the current Phase III trial conducted late in the US pandemic, also a

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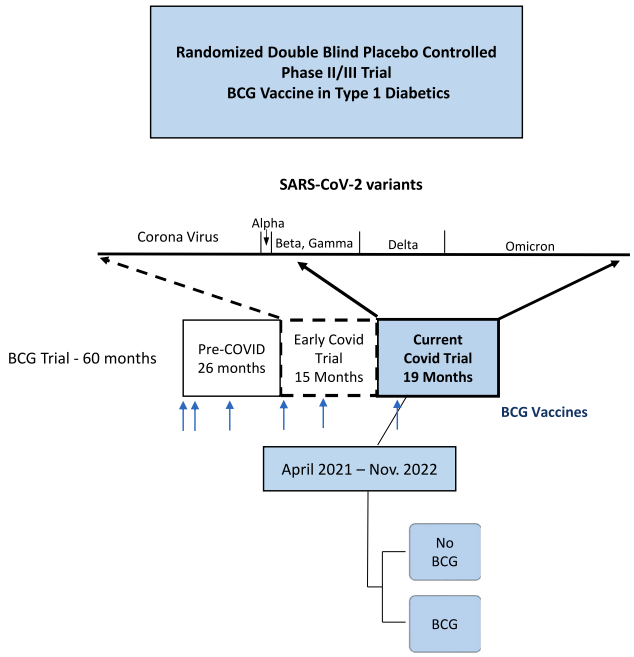

Figure 1. Flow Diagram of the current Phase III BCG trial

The current Phase III clinical trial started on April 1, 2021 and continued for 19 months until near the end of the COVID-19 public health emergency in the United States on May 11, 2023, as defined by the US Centers for Disease Control and Prevention. The co-primary outcomes were BCG vaccine protection from COVID-19 disease and from other infectious diseases.

randomized, double-blinded, placebo-controlled evaluation. While the patient population and outcome measures are similar across the two trials, there are three key differences: non-overlapping, consecutive time periods of study with different circulating SARS-CoV-2 variants (early vs. late pandemic); different diagnostics (antibody profiling vs. standard of care diagnostics); and different US availability of commercial COVID-19 vaccines (none vs. multiple vaccines) (Figures 1, S1, and S2).

A Phase III randomized, double-blinded placebo-controlled trial was conducted in the United States over a 19-month period to test if multi-dose, intradermal BCG offered protection from COVID-19 disease and protection from overall infectious disease, the co-primary outcomes (Figure 1). Randomization was 2:1 with two subjects receiving BCG for every enrolled placebo subject (Figure 1). The Phase III trial was conducted late in the COVID-19 pandemic during sequential dominance of Beta, Gamma, Delta, and Omicron variants. As compared to the original SARS-CoV-2 strain, these later variants are more transmissible and, in some cases, less virulent.[38] During this trial, the US rollout of the commercial COVID-19 vaccines unfolded gradually, consisting largely of mRNA vaccines. We therefore evaluated the efficacy of COVID-19 vaccines with and without concurrent BCG vaccination as an observational study.

RESULTS

Type 1 diabetic subjects' baseline characteristics were similar in the BCG ($n = 93$) and placebo ($n = 48$) groups (Table 1). Participants were adult men and women with similar sex distributions in the BCG arm (43% female and 57.0% male) and placebo arm (39.6% female and 60.4% male). All participants were US citizens and, at the time of original enrollment, were negative for prior BCG vaccines, and were negative for tuberculosis as confirmed with a negative QuantiFERON Gold TB test.

The retention rate was excellent, with 100% of subjects ($n = 141$) completing the Phase III trial of 19 months' duration. At the launch of this Phase III clinical trial, all subjects had been randomized and vaccinated with 6 BCG or placebo vaccine (70% of subjects) or vaccinated with 5 BCG or placebo vaccines (30%) (Figure 1). The average number of days until the sixth and final vaccine was 124 $\pm$ 16 days. The trial used the Tokyo-174 strain of BCG. The median age at diabetes onset for all subjects was 26.2 $\pm$ 1 year, and the average duration of type 1 diabetes was 17.9 years (Table 1).

BCG protects against COVID-19 disease, a co-primary outcome

Subjects with confirmed COVID-19 disease were identified over the 19 months of observations. The subjects were determined to have a confirmed case of COVID-19 disease when *all* of the following conditions were met: reporting at least one COVID-19 symptom (as defined by the FDA Guidelines for Industry document;[39] at least one day of illness; and testing positive in point of care testing for COVID-19 infection using commercial assays (PCR or Rapid Antigen testing).

Table 1. Characteristics of participants in phase III clinical trial

Characteristics of Participants	BCG (*N* = 93)	Placebo (*N* = 48)	Total (*N* = 141)
Sex – no. (%)			
Male	53 (57.0)	29 (60.4)	82 (58.2)
Female	40 (43.0)	19 (39.6)	59 (41.8)
Country – no. (%)			
United States	93 (66.0)	48 (34.0)	141 (100)
Tuberculosis Status – (%)			
Negative	100%	100%	141 (100)
History of BCG Vaccinations			
Un- Vaccinated	100%	100%	141 (100)
Current Age – yr			
Average (Median)	44.3 ± 1.3 (43.3)	43.4 ± 1.9 (40.5)	44.0 ± 1.1 (42.3)
Age at Baseline Injection – yr			
Average (Median)	40.7 ± 1.3 (39.6)	39.8 ± 1.9 (36.7)	40.4 ± 1.1 (38.9)
Age of Diabetes Onset – yr			
Average (Median)	26.6 ± 1.2 (25.9)	25.1 ± 1.9 (23.4)	26.1 ± 1.0 (25.0)
Duration of Diabetes – yr			
Average (Median)	17.7 ± 0.7 (17.2)	18.4 ± 1.0 (16.2)	17.9 ± 0.6 (17.2)
Vaccinations – no. (%)			
Pfizer	52 (55.9)	22 (45.8)	74 (52.5)
Moderna	23 (24.7)	11 (22.9)	34 (24.1)
Johnson & Johnson	1 (1.1)	6 (12.5)	7 (5.0)
None	17 (18.3)	9 (18.8)	26 (18.4)

During the 19 months of this clinical trial, COVID-19 disease occurred in 22 of 93 participants in the BCG group (23.7%) and in 20 out of 48 participants in the placebo group (41.7%) (Figure 2). A one-tailed Fisher's exact test was significant at $p = 0.023$, showing that multiple BCG vaccines protected type 1 diabetic subjects. BCG vaccine efficacy versus placebo, according to the formula in the STAR methods section, was 43.2%.

We also stratified BCG protection from COVID-19 disease according to calendar year (2021 and 2022) (Figure 2B). For 2021, there were 10 out of 93 unique confirmed COVID-19-positive subjects in the BCG group (10.8%) and 7 out of 48 (14.6%) in the placebo group. BCG vaccine efficacy was 26.3%, but the difference between groups was not significant (one-tailed Fisher's exact test $p = 0.342$). For 2022 there were 13 out of 93 confirmed COVID-19-positive subjects in the BCG group (14.0%) and 14 out 48 (29.2%) in the placebo group. BCG vaccine efficacy was 52.1%, and the difference was significant (one-tailed Fisher's exact test $p = 0.015$). Therefore, during predominance of Omicron, the most transmissible SARS-CoV-2 variant, BCG vaccinations provided more protection than placebo.

This is the second US trial of BCG's efficacy in the type 1 diabetic population as it relates to infectious disease protection and COVID-19 protection. Although these were two separate trials with separate clinical trial protocols, separate consents, separate data and safety monitoring board (DSMB), separate time periods with different COVID-19 genetic variant exposures and with different primary outcomes, both trials used the same randomized type 1 diabetic subjects residing in the United States. A cumulative timeline of COVID-19 events across the two trials is presented in Figure 3. The Phase II trial (January 2020 to April 2021) covered 15 months, with BCG efficacy of 92% ($p = 0.004$) (Figure 3A). The current Phase III trial (April 2021 - November 2022) shows BCG efficacy of 43.2% ($p = 0.023$) (Figure 3B). In this phase the placebo group accumulated more COVID-19 events and at a faster rate during the latter part of the trial (Figure 3B). There was a clear step-up of cumulative events during Omicron-dominance, presumably corresponding to its higher transmissibility as compared to earlier variants.[38] The statistical difference between the BCG and the placebo groups persisted ($p = 0.023$) (Figure 3B). Over the full 34 months of the US COVID-19 pandemic, BCG shows an efficacy of 54.3% ($p = 0.0007$) (Figure 3C). The numbers at risk across the two trials are presented in Figure S2B.

BCG protects against infectious diseases, the co-primary outcome

We analyzed all infectious disease events collected as adverse events by the MedDRA classification coding (Figure S3). For this clinical trial the observed infectious diseases included viral, bacterial, and fungal events as well as COVID-19 disease itself. BCG provides broad-based protection against infectious diseases from the global literature, and we have previously shown this to be the case for type 1 diabetic subjects during the Phase II trial.[1–3,13]

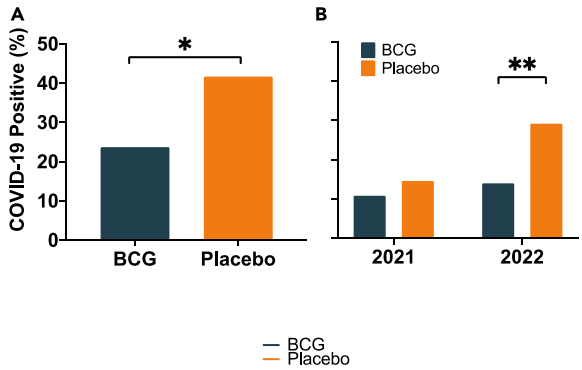

Figure 2. BCG vaccines protect type 1 diabetics from COVID-19 disease

(A) During the 19-month trial, multi-dose BCG vaccines protected type 1 diabetics against COVID-19 disease. During the Phase III trial period (April 1, 2021–November 3, 2022), 22 of 93 subjects (23.7%) in the BCG group acquired COVID-19 disease (i.e., became COVID-19 positive), whereas 20 of 48 subjects (41.7%) in the placebo group became COVID-19 positive. The one-tailed Fisher's exact test shows that this difference is significant ($p = 0.023$). BCG vaccine efficacy was 43.2%, with efficacy 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.

(B) Percent of type 1 diabetics patients who became COVID-19-positive, according to year. In 2021 10 of 93 subjects (10.8%) in the BCG group became COVID-19 positive, whereas in the placebo group, 7 of 48 (14.6%) did. For 2022,13 (14.0%) in the BCG group became COVID-19-positive and 14 (29.2%) in the placebo group did. A one-tailed Fisher's exact test for the 2021 data was not significant ($p = 0.342$), but for 2022 the difference between groups was significant ($p = 0.015$).

We therefore studied whether BCG vaccines in this Phase III trial period of 19 months continued to show broad protection after introduction of commercial COVID-19 vaccines late in the US pandemic. In the BCG group there were 44 infectious events, whereas there were 66 in the placebo group. The average number of Infections per Subject was lower in the BCG than placebo groups, with Poisson statistics indicating a significant difference at $p < 0.0001$. A violin plot demonstrates more subjects in the placebo group had many more infectious events as compared to the subjects in the BCG group (Figure 4B). Taking into account the difference in number of subjects in the BCG ($n = 93$) and placebo ($n = 48$) groups with the 2:1 randomization, the heatmap shows a breakdown of the various infectious events per subject (Figure 4C). The darker shades of red for many of the infections indicates a higher infection rate in the placebo group than in the BCG group. Taken together, these results indicate that BCG-treated subjects have fewer infectious events compared to placebo-treated subjects.

We analyzed the accumulation of infectious events over time for the full 34 months of the Phase II and Phase III randomized double blinded clinical trials, a period covering almost the entire COVID-19 pandemic in the United States. The Consort Diagram places the previous Phase II trial and current Phase III trial on a timeline of dominant SARS-CoV-2 variants (Figure 5A). To take account of the different sizes of BCG and placebo groups (BCG $n = 93$; Placebo $n = 48$), we studied the cumulative rate of Infectious Events per Subject. For the current Phase III clinical trial, the data demonstrates that subjects in the placebo group accumulate infectious diseases at a faster rate compared to the BCG group (Figure 5B). The combined total infectious disease events in the Phase II and Phase III clinical trials (34 months) shows strongly that BCG-treated subjects have a lower rate of infection across the whole period as compared to the placebo-treated subjects (Figure 5C; Poisson statistics $p < 0.0001$).

We also performed Andersen-Gill Cox Proportional Hazard Statistics to evaluate whether the length of time that subjects were vaccinated with COVID-19 vaccines had an effect (beneficial or detrimental) on overall infectious disease rates. This was done by including the number of calendar days for COVID-19 vaccination as a parameter. Using infectious disease data for both the Phase II and the Phase III clinical trials, the resulting p-value was nonsignificant ($p = 0.154$). The Hazards ratio was 0.998 (95% CI 0.995–1.001). We conclude that the amount of time that a subject is treated with COVID-19 vaccines does not play a beneficial or harmful role in the prevention of infections afforded by BCG.

Commercial COVID-19 vaccines offer no protection of type 1 diabetics from COVID-19 disease

Although not a primary outcome of this Phase III clinical trial, it was important to evaluate the impact of the commercially available COVID-19 vaccines for type 1 diabetics in the absence or presence of BCG vaccination. During this trial, rollout of the commercial, predominantly mRNA vaccines, occurred. Receipt of Pfizer, Moderna and Johnson & Johnson commercial vaccines in our study population is recorded in Table 1.

First, we quantified the efficacy of commercial COVID-19 vaccines in the absence of BCG vaccination as an observational outcome (Figure 6). Placebo recipients (non-BCG vaccinated) were divided into COVID-19-vaccinated and never-COVID-19-vaccinated cohorts. The number of subjects in each group with COVID-19 disease was tabulated. As was the case for the overall study, a subject was considered to have COVID-19 disease when they had one or more COVID-19 symptoms as defined by the FDA,[39] with a length of illness of at least one day, as well as a positive test for COVID-19 at Point of Care via PCR or Rapid Antigen testing.

Out of a total of 48 placebo subjects, 39 received commercial COVID-19 vaccines and 9 never received COVID-19 vaccines. In the COVID-19 vaccine-treated cohort, 17 (43.6%) had COVID-19, whereas in the never-COVID-19-vaccinated cohort 3 (33.3%) had COVID-19 (Figure 6A).

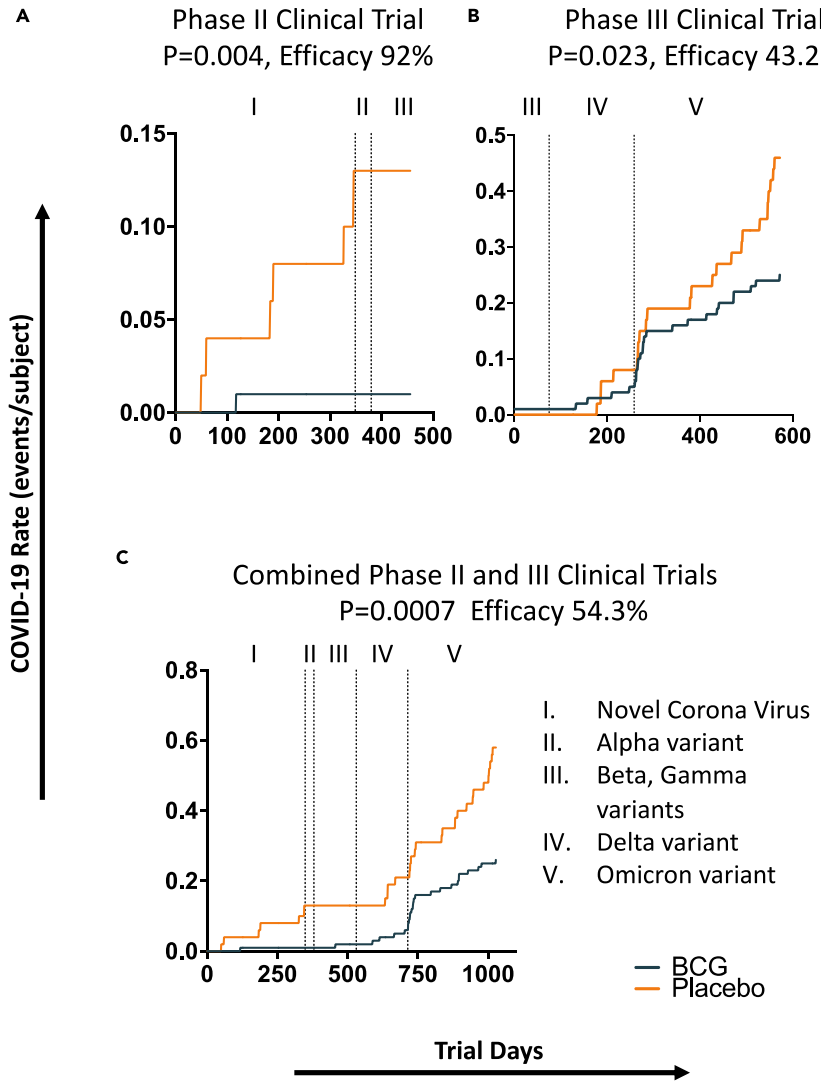

Figure 3. BCG vaccines protect type 1 diabetics from COVID-19 disease events over nearly the entire US pandemic

Based on the start date of each COVID-19 event, the number of days since the start of either the Phase II trial or the current Phase III clinical trial were calculated for each COVID-19 event in the BCG and placebo groups separately. The number of subjects in the BCG and placebo groups were different (Phase II, BCG $n = 96$ and placebo $n = 48$; current Phase III, BCG $n = 93$ and placebo $n = 48$). The data were normalized relative to group size by expressing the cumulative number of COVID-19 events as a rate per subject. The cumulative event rate per subject was then plotted in a step graph against elapsed time in days. (A) Cumulative COVID-19 event rate per subject during Phase II trial (1/1/2020 to 4/1/2021). The placebo group experienced a much higher rate of COVID-19 events per subject compared to the BCG group.[13]

(B) Cumulative COVID-19 event rate per subject during Phase III trial (4/1/2021 to 11/3/2022). The placebo group experienced a much higher rate of COVID-19 events per subject as compared to the BCG group. The dotted vertical lines delineate the approximate periods during which various SARS-CoV-2 variants were dominant in the United States. Note the sudden increase in COVID-19 event rates once the Omicron variant supplanted earlier ones.

(C) Phase II and Phase III trials combined. The dominant variants are delineated by the dotted vertical lines. Regardless of time period in the US pandemic, BCG vaccines continuously protect from COVID-19 disease in the BCG-vaccinated vs. placebo type 1 diabetic population. The number at risk data for these figures is shown in Figure S2B.

While the percent positive for COVID-19 in the never-vaccinated group trended lower, the difference was not statistically significant (one-tailed Fisher's exact test $p = 0.43$).

Since different SARS-CoV-2 variants were dominant during 2021 (Beta, Gamma and Delta) as compared to 2022 (mainly Omicron) and commercial COVID-19 vaccines have short duration of efficacy and different efficacy depending on the variant, we also analyzed the approximate two-year time period as separate years (Figure 6B). No statistical significance was reached in either year. In 2021, 12.8% of the COVID-19 vaccinated subjects were COVID-19-positive versus 22.2% of the never-COVID-19-vaccinated subjects. The one-tailed Fisher's exact test

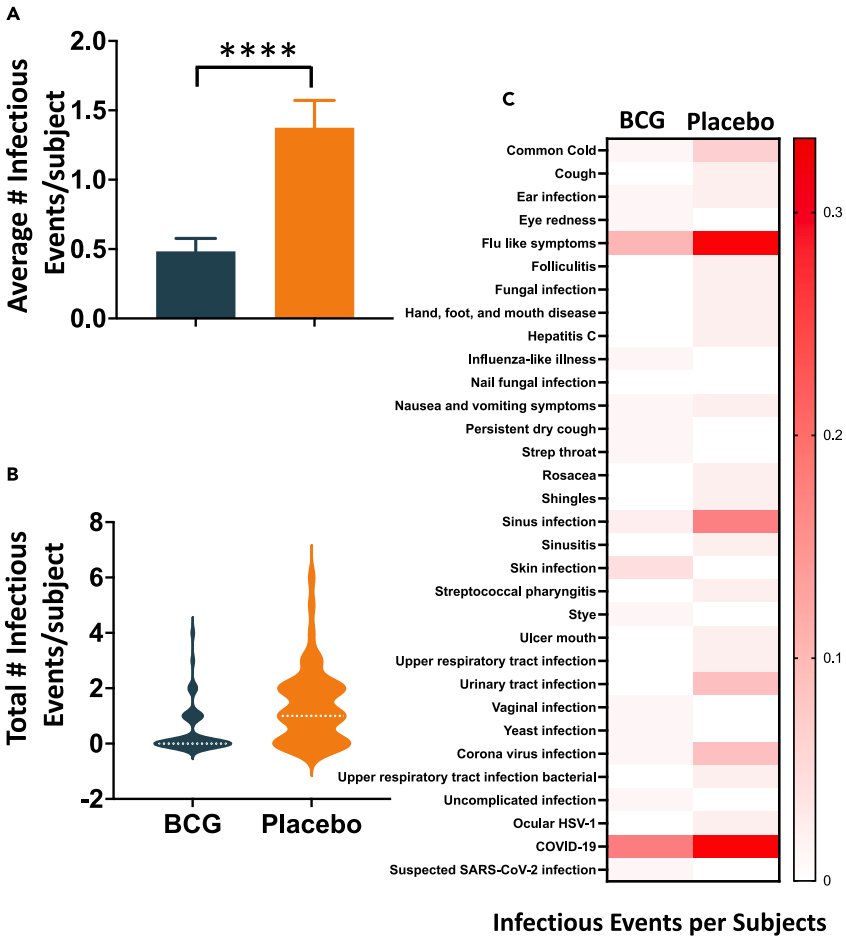

Figure 4. BCG vaccines protect type 1 diabetic subjects against infectious diseases

(A) The bar plot shows the average rate and SEM across all infectious disease events in BCG (blue) versus placebo (orange) subjects. Averages were calculated and then expressed as a rate (average events/subject) to take account of the different numbers of participants in each group. Patient numbers per group: BCG (n = 93) vs. placebo (n = 48). Total number of infectious events in the BCG group is 44 vs. placebo group is 66. Average rate/subject: BCG, 0.47; Placebo, 1.38. Poisson statistics indicate that the difference between BCG and placebo groups is significant at $p < 0.0001$. Data are represented as mean ± SEM.

(B) The Violin plot shows the density of the number of Infectious Events per Subject during the entire 19-month trial period. The distribution in the orange plot (placebo) shows greater number compared to the blue plot (BCG). This is also evident from the higher median in the orange plot (white dotted lines indicate the median).

(C) Heatmap of BCG versus placebo groups shows the number of various types of infectious events per patient over the entire Phase III trial. The darker red colors in the placebo group indicate that this group tended to have a higher number of infection events as compared to the BCG group. A detailed listing of the data in this heatmap is shown in Figure S3. ****$p < 0.0001$.

showed no significant difference, $p = 0.39$. In 2022, 30.8% of the COVID-19 vaccinated subjects were COVID-19-positive versus 22.2% of never-COVID-19-vaccinated subjects. The one-tailed Fisher's exact test was also not significant, $p = 0.47$. These observational results indicate that the commercially available COVID-19 vaccines do not appear to protect type 1 diabetes against COVID-19 disease, at least during the time period under study.

BCG affords broad protection of type 1 diabetic subjects against infectious diseases irrespective of COVID-19 vaccine

Another way to study the impact of commercial COVID-19 vaccines is to analyze infectious disease outcomes (which include COVID-19). This analysis is by treatment group (BCG vs. placebo) and by time periods within this Phase III trial: pre-COVID-19 vaccines, post-COVID-19 vaccines, and pre- and post-COVID-19 vaccines (Figure 7). Since the commercial COVID-19 vaccines were received at random or at individualized times, we calculated the number of months that each subject was pre-COVID-19 vaccine (i.e., time before receiving the COVID vaccine) and post-COVID-19 vaccine (i.e., time after receiving the vaccine) and then expressed the data as the rate of Infectious Events per Subject and per Month. We performed Poisson statistics for Figure 7C (Pre- + Post-COVID-vaccines; Poisson distribution) since this encompassed a constant time period for each subject (581 days). In the three time periods of analysis per Subject, the rate of COVID-19 disease in the BCG group was

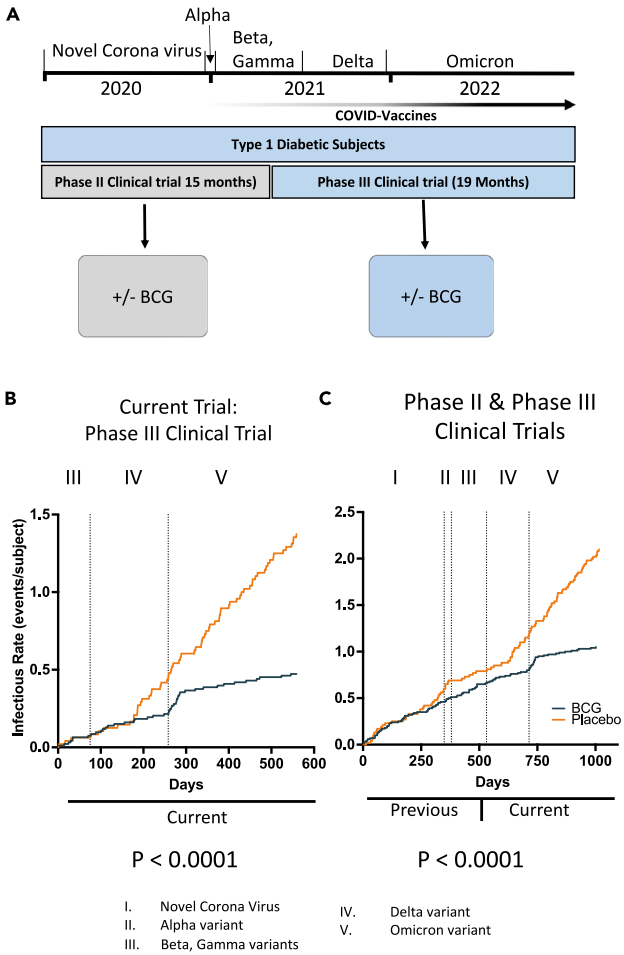

Figure 5. Regardless of the time frame of the US pandemic, BCG vaccines continuously protect type 1 diabetics from all infectious diseases

Based on the start date of each event, the number of days since the start of the Phase II or the current Phase III trial was calculated for each Infectious Event in the BCG vs. placebo groups separately. The number of subjects in the BCG group and the placebo group were different (Phase II, BCG $n = 96$ and Placebo $n = 48$; Phase III, BCG $n = 93$; Placebo $n = 48$). Therefore, the data were normalized relative to the group size by expressing the cumulative number of infectious events as a rate per subject. The Cumulative Event Rate per Subject was then plotted in a step graph against elapsed time in days. For a list of types of Infectious Events observed, and the total number of infections per subject please see Figure 4B. The names of infectious disease events are listed in Figure S3. Note that this includes, but is not limited to, COVID-19 events. (A) Consort diagram shows the timeline of the Phase II and Phase III BCG trials in type 1 diabetic subjects, in relation to the dominant SARS-CoV-2 variant.

(B) Cumulative Infectious Event rate per Subject during the Phase III trial (4/1/2021 to 11/3/2022). The placebo group experienced a much higher rate of Infectious Events per Subject compared to the BCG group. The dotted vertical lines delineate the approximate periods of predominance of SARS-CoV-2 variants in the United States. (Poisson statistics $p < 0.0001$).

(C) Phase II and Phase III trial periods combined. The dominant variants are delineated by dotted vertical lines. (Poisson statistics $p < 0.0001$). We also performed an Andersen-Gill Cox proportional hazard statistic on these data to see the positive or negative impact of COVID-19 vaccines on BCG protective effects. With calendar days of COVID-19 vaccinations as a variable we showed that there was no significant benefit or harm of COVID-19 vaccines on the prevention of overall BCG prevention of infectious diseases ($p = 0.253$). (C) Phase II and Phase III trial periods combined. The dominant variants are delineated by dotted vertical lines. (Poisson statistics $p < 0.0001$). Andersen-Gill Cox proportional hazard statistics with calendar days of COVID-19 vaccination as a variable was also not significant ($p = 0.154$).

always low and was always lower than that in the placebo group, regardless of whether the subjects had received a COVID-19 vaccine ($p < 0.001$). This analysis is consistent with the earlier one (Figure 6) that the commercial COVID-19 vaccines did not protect type 1 diabetics from COVID-19. The data also supports the conclusion that concurrent COVID-19 vaccines did not harm BCG recipients.

DISCUSSION

This randomized, double-blinded placebo-controlled Phase III trial conducted late in the US pandemic shows that multidose BCG vaccines in a vulnerable type 1 diabetic population protect from COVID-19 disease and infectious diseases. This was a time of sequential dominance of

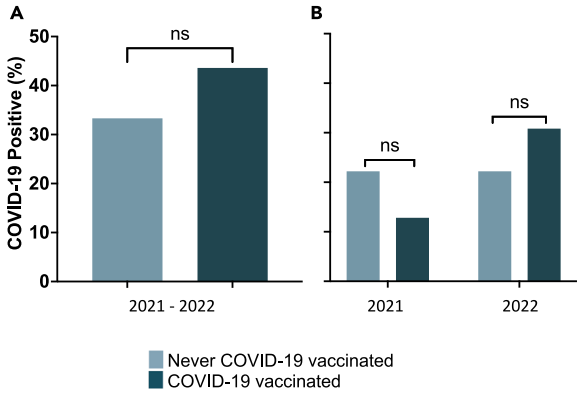

Figure 6. COVID-19 vaccines (Pfizer, Moderna, J&J) do not protect type 1 diabetic subjects from COVID-19 disease

(A) To assess the efficacy of the COVID-19 vaccines by themselves in our type 1 diabetic subjects, we excluded BCG as a potential confounding factor and analyzed only the placebo (No BCG) cohort. The placebo type 1 diabetic subjects were divided into COVID-19 vaccinated and never COVID-19 vaccinated groups. Out of a total of 48 subjects in the placebo (No BCG) arm, 39 were COVID-vaccinated and 9 were never COVID-vaccinated. We then counted the number of subjects that were diagnosed with COVID-19 disease. In the COVID-vaccine-treated cohort, 17 out of 39 subjects (43.6%) were COVID-19 positive, whereas in the never vaccinated cohort 3 out of 9 (33.3%) tested positive. While the percent positive in the never vaccinated group trended lower, the difference was not statistically significant (one-tailed Fisher's exact test $p = 0.43$).

(B) Comparison of effectiveness of COVID-19 vaccines in 2021 versus 2022. We quantified the percentage of subjects with COVID-19 disease. This observational analysis only included placebo subjects not given the BCG vaccine. No statistical significance was reached in either year (one-tailed Fisher's exact test: 2021, $p = 0.39$; 2022, $p = 0.47$), confirming that the commercially available COVID-19 vaccines did protect type 1 diabetic subjects from COVID-19.

Beta, Gamma, Delta, and Omicron SARS-CoV-2 variants, all more transmissible and some less virulent than earlier strains.[38] Based on observational data, we also found no efficacy of commercial mRNA vaccines for this infectious disease-vulnerable type 1 diabetic population. Finally, receipt of COVID-19 vaccination had no bearing on the efficacy of concurrent BCG vaccination.

This is the second successful test of the BCG vaccine in the United States. The two back-to-back trials—a Phase II and Phase III trial—share some similarities and some differences (Figure S2). Both used the Tokyo 174 BCG strain given as a multi-dose intradermal regimen, and both excluded a history of neonatal BCG and prior TB. The Phase II trial occurred at the start of the pandemic when approved diagnostics were not yet widely available. COVID-19 disease was confirmed through COVID-19 antibody profiling.[13] The current Phase III clinical trial used point of care COVID-19 diagnostics for detecting and confirming COVID-19. The Phase II trial showed near total protection of the BCG group from COVID-19 disease (92% vs. placebo; $p = 0.0036$) with the less transmissible but highly virulent SARS-CoV-2 variants early in the pandemic (the original and Alpha variant). The Phase III trial revealed somewhat lower protection of BCG vaccines against COVID-19 disease late in the pandemic with the more transmissible SARS-CoV-2 variants (43% vs. placebo; $p = 0.023$), but still with continuing statistical significance. Taken together, the two trials show that BCG vaccines provided continuous protection of type 1 diabetic subjects for nearly the entire COVID-19 pandemic in the United States. BCG protection is agnostic to the genetic variants of COVID-19 and even protected the vaccinated subjects from Omicron, the most transmissible variant.[38]

Both Phase II and Phase III RCT trials also found platform protection of the BCG vaccines (vs. placebo) against other infectious diseases ($p < 0.01$, $p < 0.0001$, respectively). All clinical trial recipients received multiple doses of BCG and in both trials the participants had received >3 BCG vaccines over a 2-year period prior to the start of monitoring for infectious disease risk in either the randomized double blinded Phase II or Phase III clinical trial.

It is appreciated that in naive adult populations, the BCG vaccine administered as an intradermal vaccine takes at least two years to achieve full protection for a number of off-target effects.[13,16,32] The Phase II clinical trial monitored subjects for 15 months, while the second clinical trial monitored subjects for the next 18 months. The Phase III trial coincided with the US rollout of commercial anti-COVID-19 vaccines predominantly of the mRNA type. It enabled observational evaluation of mRNA efficacy as well as impact on concurrent BCG vaccine. The analysis found neither benefit nor harm of the antigen-specific vaccine technology in type 1 diabetic subjects ($p = 0.43$), as has also been reported by others.[40,41]

We attribute the success of our Phase II and III trials to several methodological features. First, the follow-up time exceeded two years. BCG vaccines in US adults take two years to realize infectious disease protection or other off-target benefits such as protection from autoimmunity in naive previously non-vaccinated adults.[16,32] Shortly after vaccination and within weeks, BCG stimulates innate immunity that certainly is related to the introduction of bacterial foreign products.[42] For off-target effects in adults, BCG causes changes in DNA methylation of genes of the innate and adaptive immune system and of metabolism pathways, and signaling pathways become rewired in a time frame that correlates with the slow but durable clinical improvements.[34,43–45] Second, we excluded individuals with a history of BCG or TB exposure. Protection from past exposure of this kind probably lasts decades, and thus would tend to obscure finding a benefit from a recent BCG vaccine. Third, we used a potent strain of BCG (Tokyo 174). Like the use of BCG for bladder cancer, various strains of BCG have very different efficacy, and the Tokyo 174 strain continues to show strong off-target effects.[46] Fourth, we used multiple BCG doses. Animal and human studies show

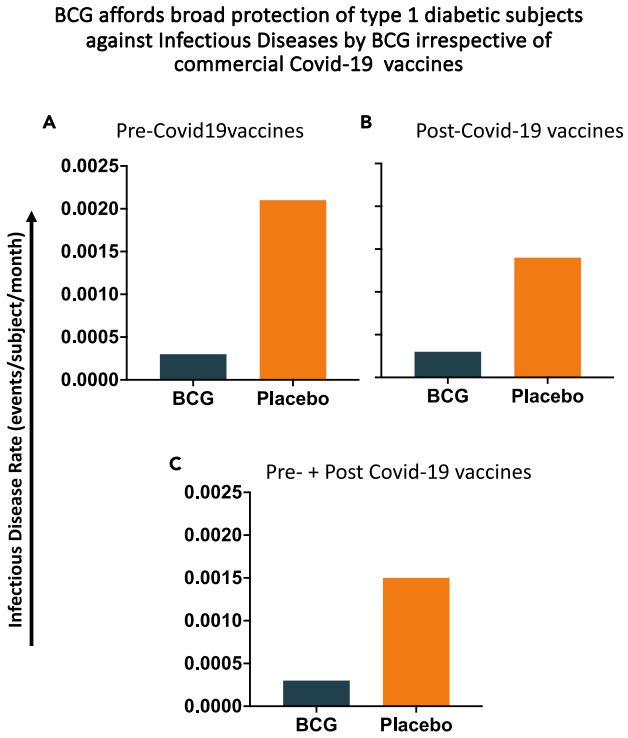

Figure 7. BCG affords broad protection of type 1 diabetic subjects against infectious diseases irrespective of COVID-19 vaccines

Since type 1 diabetic subjects were vaccinated with commercial COVID-19 vaccines at different timepoints during this current Phase III clinical trial, the number of months pre-COVID-19-vaccine and post-COVID-19 vaccine were calculated for BCG (n = 93) and placebo (n = 48) groups separately. The cumulative number of Infectious Events in BCG vs. placebo pre-COVID-19 vaccine and post-COVID-19 vaccines were then determined and the rate of Infectious Events per Subject and per Month was calculated. Never-COVID-19-vaccinated subjects were considered as pre-COVID-vaccine for the entire duration of the Phase III clinical trial. Differences between the BCG and placebo groups were determined using Poisson statistics.

(A) Infectious Events rate per Subject per Month before treatment with commercial COVID-19 vaccines is much higher in the placebo (No BCG) group as compared to the BCG group.

(B) Infectious Events rate per Subject per Month after treatment with commercial COVID-19 vaccines is also much higher in the placebo (No BCG) group as compared to the BCG group.

(C) Infectious Events rate per Subject per Month over the entire course of the Phase III trial, i.e., both pre- and post-COVID-vaccines, show the same pattern. Andersen-Gill Cox Proportional Hazard statistics showed that there was no significant effect of COVID-19 vaccines on the time on the prevention or acceleration of overall infectious diseases (p = 0.253). Overall, these results indicate that BCG broadly protects type 1 diabetes against infectious diseases, irrespective of presence or absence of commercial COVID-19 vaccines. Further, commercial COVID-19 vaccines did not interfere with platform BCG vaccine protection from COVID-19 and overall infectious disease.

that multi-dosing is best for metabolic changes and T cell changes through epigenetic reprogramming in both the adaptive and innate immune system.[34,43–45] Fifth, we studied an infectious disease-vulnerable population of type 1 diabetics,[35] which facilitated finding an effect. Similarly, other positive BCG trials for COVID-19 were in high-risk populations.[21,22] In contrast, three negative trials studied health care workers,[25,27,29] a healthy population often resistant to infections and lacking immune compromise.

We are not the first to report that diabetic subjects, including type 1 diabetic subjects, are not adequately protected against COVID-19 by the mRNA vaccines.[40,41] These studies show that diabetic subjects, as a group, are at risk of breakthrough COVID-19 infection post-COVID-vaccination. Type 1 diabetic subjects, more specifically, are likely to have an even worse risk of breakthrough infection, an inference based on research showing that type 1 diabetics vs. type 2 diabetics are at least twice as likely to die from COVID-19.[47] Given the lack of efficacy of mRNA vaccination for type 1 diabetics, there is still an unmet need for this patient population, especially as relates to new and emerging SARS CoV-2 variants. Given the high rate of infectious diseases in the vulnerable type 1 diabetic population, there is still an unmet need for this patient population for a platform infectious disease protection.

The lack of efficacy of mRNA vaccines reported here is buttressed by research showing that mRNA vaccines in type 1 diabetic subjects do not elicit any T cell responses.[48] The finding fits with other data that type 1 diabetic subjects do not have the ability to mount an appropriate immune response using T cells of the adaptive immune response. Mechanistic data from our laboratory show that BCG's benefit for COVID-19 and infectious disease may stem from its strengthening the T cell response through de-methylation of many of the key T cell receptor (TCR) genes.[34] The data show that type 1 diabetic subjects ordinarily have low expression of the TCR complex resulting from

over-methylation of the TCR genes as well as the associated CD3 protein of the TCR complexes. BCG corrects this defect over a three-year period.

In the infectious disease field, there is a pressing need for new antibiotics or vaccines to meet the pace of emerging infectious diseases. While mRNA vaccines are a leap forward for most individuals based on alacrity of vaccine production and biological response, the protection is short-term (a matter of months) in the face of viral genetic drift.[49] BCG vaccines appear to offer a platform opportunity but with caveat that benefits may not be manifest for a minimum of 2 years. Nevertheless, BCG offers the prospect of near lifelong protection.[50]

Limitations of the study

This trial was conducted in an infectious disease-vulnerable population, type 1 diabetic subjects, and therefore findings may not apply to non-diabetic patient populations or healthy populations. This clinical trial was small in size, but it had no drop-outs. Also, this long trial in a randomized double-blinded clinical trial format allowed subject exposure to many SARS-CoV-2 variants and infectious disease exposures. This longer in time clinical trial format is an alternative trial design compared to mRNA vaccine trials that enroll large numbers of patients but with shorter follow-up of months instead of years to capture the specific viral variant the mRNA vaccine was designed for.

Another limitation of this study is reliance on observational data regarding efficacy of mRNA vaccines, whether alone or with concurrent BCG vaccination. Observational data cannot be used to infer cause and effect. Further, we do not know if the lack of mRNA vaccine efficacy against COVID-19 reported here is due to the underlying disease state (i.e., type 1 diabetes) or due to exposure to new viral variants that emerged over the course of study.

Another limitation is that we did not genetically sequence the COVID-19 variants from infected cohorts but used the US-based epidemiology time frame to document exposure. Finally, this was a US-based trial, so findings may not necessarily apply to other countries.

STAR★METHODS

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

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

W.M.K. and D.L.F. conceived the study and participated in the study design. D.L.F. was the PI on the study and overall ran the clinical trial. The clinical trial management team interacted with the clinical trial subjects, performed clinical trial management, scheduled patients, performed blood draws, etc. and was composed of E.R.H., G.E.W., M.S.V., R.G., E.R.B., N.S.H., J.E.B., and D.L.F. W.M.K. prepared the blinded vaccines using the pharmacy-generated randomization codes. W.M.K. and H.Z. performed the data analysis. W.M.K. and D.L.F. wrote the manuscript. All authors read, revised, and approved the final manuscript for publication.

DECLARATION OF INTERESTS

No author or author family member owns the study drug nor does any author or family member consult for the drug producer or have ownership interests in BCG Japan Laboratories. None of this clinical trial work was financially supported by the maker/manufacturer/owner of this drug, BCG Japan Laboratories. No Author has any ownership rights to the study drug. No authors receive consulting or research support from BCG Japan Laboratories.

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REFERENCES

1. Aaby, P., Roth, A., Ravn, H., Napirna, B.M., Rodrigues, A., Lisse, I.M., Stensballe, L., Diness, B.R., Lausch, K.R., Lund, N., et al. (2011). Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? J. Infect. Dis. *204*, 245–252. https://doi.org/10.1093/infdis/jir240.
2. Biering-Sorensen, S., Aaby, P., Lund, N., Monteiro, I., Jensen, K.J., Eriksen, H.B., Schaltz-Buchholzer, F., Jorgensen, A.S.P., Rodrigues, A., Fisker, A.B., and Benn, C.S. (2017). Early BCG-Denmark and Neonatal Mortality Among Infants Weighing <2500 g: A Randomized Controlled Trial. Clin. Infect. Dis. *65*, 1183–1190. https://doi.org/10.1093/cid/cix525.
3. Prentice, S., Nassanga, B., Webb, E.L., Akello, F., Kiwudhu, F., Akurut, H., Elliott, A.M., Arts, R.J.W., Netea, M.G., Dockrell, H.M., et al. (2021). BCG-induced non-specific effects on heterologous infectious disease in Ugandan neonates: an investigator-blind randomised controlled trial. Lancet Infect. Dis. *21*, 993–1003. https://doi.org/10.1016/S1473-3099(20)30653-8.
4. Biering-Sorensen, S., Aaby, P., Napirna, B.M., Roth, A., Ravn, H., Rodrigues, A., Whittle, H., and Benn, C.S. (2012). Small randomized trial among low-birth-weight children receiving bacillus Calmette-Guerin vaccination at first health center contact. Pediatr. Infect. Dis. J. *31*, 306–308. https://doi.org/10.1097/INF.0b013e3182458289.
5. de Castro, M.J., Pardo-Seco, J., and Martinón-Torres, F. (2015). Nonspecific (Heterologous) Protection of Neonatal BCG Vaccination Against Hospitalization Due to Respiratory Infection and Sepsis. Clin. Infect. Dis. *60*, 1611–1619. https://doi.org/10.1093/cid/civ144.
6. Garly, M.L., Martins, C.L., Balé, C., Baldé, M.A., Hedegaard, K.L., Gustafson, P., Lisse, I.M., Whittle, H.C., and Aaby, P. (2003). BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa. A non-specific beneficial effect of BCG? Vaccine *21*, 2782–2790. https://doi.org/10.1016/s0264-410x(03)00181-6.
7. Kristensen, I., Aaby, P., and Jensen, H. (2000). Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. BMJ *321*, 1435–1438. https://doi.org/10.1136/bmj.321.7274.1435.
8. Ponnighaus, J.M., Fine, P.E., Sterne, J.A., Wilson, R.J., Msosa, E., Gruer, P.J., Jenkins, P.A., Lucas, S.B., Liomba, N.G., and Bliss, L. (1992). Efficacy of BCG vaccine against leprosy and tuberculosis in northern Malawi. Lancet *339*, 636–639. https://doi.org/10.1016/0140-6736(92)90794-4.
9. Roth, A., Gustafson, P., Nhaga, A., Djana, Q., Poulsen, A., Garly, M.L., Jensen, H., Sodemann, M., Rodriques, A., and Aaby, P. (2005). BCG vaccination scar associated with better childhood survival in Guinea-Bissau. Int. J. Epidemiol. *34*, 540–547. https://doi.org/10.1093/ije/dyh392.
10. Stensballe, L.G., Nante, E., Jensen, I.P., Kofoed, P.E., Poulsen, A., Jensen, H., Newport, M., Marchant, A., and Aaby, P. (2005). Acute lower respiratory tract infections and respiratory syncytial virus in infants in Guinea-Bissau: a beneficial effect of BCG vaccination for girls community based case-control study. Vaccine *23*, 1251–1257. https://doi.org/10.1016/j.vaccine.2004.09.006.
11. Walk, J., de Bree, L.C.J., Graumans, W., Stoter, R., van Gemert, G.J., van de Vegte-Bolmer, M., Teelen, K., Hermsen, C.C., Arts, R.J.W., Behet, M.C., et al. (2019). Outcomes of controlled human malaria infection after BCG vaccination. Nat. Commun. *10*, 874. https://doi.org/10.1038/s41467-019-08659-3.
12. Wardhana, Datau, E.A., Datau, E.A., Sultana, A., Mandang, V.V.V., and Jim, E. (2011). The efficacy of Bacillus Calmette-Guerin vaccinations for the prevention of acute upper respiratory tract infection in the elderly. Acta Med. Indones. *43*, 185–190.
13. Faustman, D.L., Lee, A., Hostetter, E.R., Aristarkhova, A., Ng, N.C., Shpilsky, G.F., Tran, L., Wolfe, G., Takahashi, H., Dias, H.F., et al. (2022). Multiple BCG vaccinations for the prevention of COVID-19 and other infectious diseases in type 1 diabetes. Cell Rep. Med. *3*, 100728. https://doi.org/10.1016/j.xcrm.2022.100728.
14. Nemes, E., Geldenhuys, H., Rozot, V., Rutkowski, K.T., Ratangee, F., Bilek, N., Mabwe, S., Makhethe, L., Erasmus, M., Toefy, A., et al. (2018). Prevention of M. tuberculosis Infection with H4:IC31 Vaccine or BCG Revaccination. N. Engl. J. Med. *379*, 138–149. https://doi.org/10.1056/NEJMoa1714021.
15. Nguipdop-Djomo, P., Heldal, E., Rodrigues, L.C., Abubakar, I., and Mangtani, P. (2016). Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: a retrospective population-based cohort study. Lancet Infect. Dis. *16*, 219–226. https://doi.org/10.1016/S1473-3099(15)00400-4.
16. Shpilsky, G.F., Takahashi, H., Aristarkhova, A., Weil, M., Ng, N., Nelson, K.J., Lee, A., Zheng, H., Kühtreiber, W.M., and Faustman, D.L. (2021). Bacillus Calmette-Guerin 's beneficial impact on glucose metabolism: evidence for broad based applications. iScience *24*, 103150. https://doi.org/10.1016/j.isci.2021.103150.
17. WHO (2018). BCG vaccines: WHO position paper - February 2018. Wkly. Epidemiol. Rec. *93*, 73–96.
18. Feikin, D.R., Higdon, M.M., Andrews, N., Collie, S., Deloria Knoll, M., Kwong, J.C., Link-Gelles, R., Pilishvili, T., and Patel, M.K. (2023). Assessing COVID-19 vaccine effectiveness against Omicron subvariants: Report from a meeting of the World Health Organization. Vaccine *41*, 2329–2338. https://doi.org/10.1016/j.vaccine.2023.02.020.
19. Dos Anjos, L.R.B., da Costa, A.C., Cardoso, A.d.R.O., Guimarães, R.A., Rodrigues, R.L., Ribeiro, K.M., Borges, K.C.M., Carvalho, A.C.d.O., Dias, C.I.S., Rezende, A.d.O., et al. (2022). Efficacy and Safety of BCG Revaccination With M. bovis BCG Moscow to Prevent COVID-19 Infection in Health Care Workers: A Randomized Phase II Clinical Trial. Front. Immunol. *13*, 841868. https://doi.org/10.3389/fimmu.2022.841868.
20. Moorlag, S.J.C.F.M., Taks, E., Ten Doesschate, T., van der Vaart, T.W., Janssen, A.B., Müller, L., Ostermann, P., Dijkstra, H., Lemmers, H., Simonetti, E., et al. (2022). Efficacy of Bacillus Calmette-Guerin vaccination against respiratory tract infections in the elderly during the Covid-19 pandemic. Clin. Infect. Dis. *75*, e938–e946. https://doi.org/10.1093/cid/ciac182.
21. Sinha, S., Ajayababu, A., Thukral, H., Gupta, S., Guha, S.K., Basu, A., Gupta, G., Thakur, P., Lingaiah, R., Das, B.K., et al. (2022). Efficacy of Bacillus Calmette-Guerin (BCG) Vaccination in Reducing the Incidence and Severity of COVID-19 in High-Risk Population (BRIC): a Phase III, Multi-centre, Quadruple-Blind Randomised Control Trial. Infect. Dis. Ther. *11*, 2205–2217. https://doi.org/10.1007/s40121-022-00703-y.
22. Tsilika, M., Taks, E., Dolianitis, K., Kotsaki, A., Leventogiannis, K., Damoulari, C., Kostoula, M., Paneta, M., Adamis, G., Papanikolaou, I., et al. (2022). ACTIVATE-2: A Double-Blind Randomized Trial of BCG Vaccination Against COVID-19 in Individuals at Risk. Front. Immunol. *13*, 873067. https://doi.org/10.3389/fimmu.2022.873067.
23. Santos, A.P., Werneck, G.L., Dalvi, A.P.R., Dos Santos, C.C., Tierno, P.F.G.M.M., Condelo, H.S., Macedo, B., de Medeiros Leung, J.A., de Souza Nogueira, J., Malvão, L., et al. (2023). The effect of BCG vaccination on infection and antibody levels against SARS-CoV-2-The results of ProBCG: a multicenter randomized clinical trial in Brazil. Int. J. Infect. Dis. *130*, 8–16. https://doi.org/10.1016/j.ijid.2023.02.014.
24. Czajka, H., Zapolnik, P., Krzych, Ł., Kmiecik, W., Stopyra, L., Nowakowska, A., Jackowska, T., Darmochwał-Kolarz, D., Szymański, H., Radziewicz-Winnicki, I., and Mazur, A. (2022). A Multi-Center, Randomised, Double-Blind, Placebo-Controlled Phase III Clinical Trial Evaluating the Impact of BCG Re-Vaccination on the Incidence and Severity of SARS-CoV-2 Infections among Symptomatic Healthcare Professionals during the COVID-19 Pandemic in Poland-First Results. Vaccines (Basel) *10*, 314. https://doi.org/10.3390/vaccines10020314.
25. Ten Doesschate, T., van der Vaart, T.W., Debisarun, P.A., Taks, E., Moorlag, S.J.C.F.M., Paternotte, N., Boersma, W.G., Kuiper, V.P., Roukens, A.H.E., Rijnders, B.J.A., et al. (2022). Bacillus Calmette-Guerin vaccine to reduce healthcare worker absenteeism in COVID-19 pandemic, a randomized controlled trial. Clin. Microbiol. Infect. *28*, 1278–1285. https://doi.org/10.1016/j.cmi.2022.04.009.
26. Upton, C.M., van Wijk, R.C., Mockeliunas, L., Simonsson, U.S.H., McHarry, K., van den Hoogen, G., Muller, C., von Delft, A., van der Westhuizen, H.M., van Crevel, R., et al. (2022).

Safety and efficacy of BCG re-vaccination in relation to COVID-19 morbidity in healthcare workers: A double-blind, randomised, controlled, phase 3 trial. EClinicalMedicine *48*, 101414. https://doi.org/10.1016/j.eclinm.2022.101414.

27. Pittet, L.F., Messina, N.L., Orsini, F., Moore, C.L., Abruzzo, V., Barry, S., Bonnici, R., Bonten, M., Campbell, J., Croda, J., et al. (2023). Randomized Trial of BCG Vaccine to Protect against Covid-19 in Health Care Workers. N. Engl. J. Med. *388*, 1582–1596. https://doi.org/10.1056/NEJMoa2212616.
28. Koekenbier, E.L., Fohse, K., van de Maat, J.S., Oosterheert, J.J., van Nieuwkoop, C., Hoogerwerf, J.J., Grobusch, M.P., van den Bosch, M.A.A.J., van de Wijgert, J.H.H., Netea, M.G., et al. (2023). Bacillus Calmette-Guerin vaccine for prevention of COVID-19 and other respiratory tract infections in older adults with comorbidities: a randomized controlled trial. Clin. Microbiol. Infect. *29*, 781–788. https://doi.org/10.1016/j.cmi.2023.01.019.
29. Madsen, A.M.R., Schaltz-Buchholzer, F., Nielsen, S., Benfield, T., Bjerregaard-Andersen, M., Dalgaard, L.S., Dam, C., Ditlev, S.B., Faizi, G., Azizi, M., et al. (2024). Using BCG vaccine to enhance non-specific protection of health care workers during the COVID-19 pandemic: a randomized controlled trial. J. Infect. Dis. *229*, 384–393. https://doi.org/10.1093/infdis/jiad422.
30. Pittet, L.F., Messina, N.L., and Curtis, N. (2023). BCG to Protect against Covid-19 in Health Care Workers. Reply. N. Engl. J. Med. *389*, 192. https://doi.org/10.1056/NEJMc2306483.
31. Noble, C.C.A., Messina, N.L., Pittet, L.F., and Curtis, N. (2023). Interpreting the results of trials of BCG vaccination for protection against COVID-19. J. Infect. Dis. *228*, 1467–1478. https://doi.org/10.1093/infdis/jiad316.
32. Kuhtreiber, W.M., Tran, L., Kim, T., Dybala, M., Nguyen, B., Plager, S., Huang, D., Janes, S., Defusco, A., Baum, D., et al. (2018). Long-term reduction in hyperglycemia in advanced type 1 diabetes: the value of induced aerobic glycolysis with BCG vaccinations. NPJ Vaccines *3*, 23. https://doi.org/10.1038/s41541-018-0062-8.
33. Ristori, G., Romano, S., Cannoni, S., Visconti, A., Tinelli, E., Mendozzi, L., Cecconi, P., Lanzillo, R., Quarantelli, M., Buttinelli, C., et al. (2014). Effects of Bacille Calmette-Guerin after the first demyelinating event in the CNS. Neurology *82*, 41–48. https://doi.org/10.1212/01.wnl.0000438216.93319.ab.
34. Takahashi, H., Kühtreiber, W.M., Keefe, R.C., Lee, A.H., Aristarkhova, A., Dias, H.F., Ng, N., Nelson, K.J., Bien, S., Scheffey, D., and Faustman, D.L. (2022). BCG vaccinations drive epigenetic changes to the human T cell receptor: Restored expression in type 1 diabetes. Sci. Adv. *8*, eabq7240. https://doi.org/10.1126/sciadv.abq7240.
35. Carey, I.M., Critchley, J.A., DeWilde, S., Harris, T., Hosking, F.J., and Cook, D.G. (2018). Risk of Infection in Type 1 and Type 2 Diabetes Compared With the General Population: A Matched Cohort Study. Diabetes Care *41*, 513–521. https://doi.org/10.2337/dc17-2131.
36. Mathieu, E., Ritchie, H., Rodés-Guirao, L., Appel, C., Giattino, C., Hasell, J., Macdonald, B., Dattani, S., Beltekian, D., Ortiz-Ospina, E., and Roser, M. (2020). Coronavirus Pandemic (COVID-19). Our World in Data.
37. John Hopkins Coronavirus Resource Center, T.C.f.S.S.a.E. (2020). Coronavirus COVID-19 Global Cases. https://coronavirus.jhu.edu/map.html.
38. Telenti, A., Hodcroft, E.B., and Robertson, D.L. (2022). The Evolution and Biology of SARS-CoV-2 Variants. Cold Spring Harb. Perspect. Med. *12*, a041390. https://doi.org/10.1101/cshperspect.a041390.
39. FDA, U. (2019). Process for Making Available Guidance Documents Related to Coronavirus Disease 2019. https://www.federalregister.gov/documents/2020/03/25/2020-06222/process-for-making-available-guidance-documents-related-to-coronavirus-disease-2019.
40. Basso, P., Negro, C., Cegolon, L., and Larese Filon, F. (2022). Risk of Vaccine Breakthrough SARS-CoV-2 Infection and Associated Factors in Healthcare Workers of Trieste Teaching Hospitals (North-Eastern Italy). Viruses *14*, 336. https://doi.org/10.3390/v14020336.
41. Brosh-Nissimov, T., Orenbuch-Harroch, E., Chowers, M., Elbaz, M., Nesher, L., Stein, M., Maor, Y., Cohen, R., Hussein, K., Weinberger, M., et al. (2021). BNT162b2 vaccine breakthrough: clinical characteristics of 152 fully vaccinated hospitalized COVID-19 patients in Israel. Clin. Microbiol. Infect. *27*, 1652–1657. https://doi.org/10.1016/j.cmi.2021.06.036.
42. Netea, M.G., Domínguez-Andrés, J., Barreiro, L.B., Chavakis, T., Divangahi, M., Fuchs, E., Joosten, L.A.B., van der Meer, J.W.M., Mhlanga, M.M., Mulder, W.J.M., et al. (2020). Defining trained immunity and its role in health and disease. Nat. Rev. Immunol. *20*, 375–388. https://doi.org/10.1038/s41577-020-0285-6.
43. Keefe, R.C., Takahashi, H., Tran, L., Nelson, K., Ng, N., Kühtreiber, W.M., and Faustman, D.L. (2021). BCG therapy is associated with long-term, durable induction of Treg signature genes by epigenetic modulation. Sci. Rep. *11*, 14933. https://doi.org/10.1038/s41598-021-94529-2.
44. Dias, H.F., Kuhtreiber, W.M., Nelson, K.J., Ng, N.C., Zheng, H., and Faustman, D.L. (2022). Epigenetic changes related to glucose metabolism in type 1 diabetes after BCG vaccinations: A vital role for KDM2B. Vaccine *40*, 1540–1554. https://doi.org/10.1016/j.vaccine.2021.04.011.
45. Kuhtreiber, W.M., Takahashi, H., Keefe, R.C., Song, Y., Tran, L., Luck, T.G., Shpilsky, G., Moore, L., Sinton, S.M., Graham, J.C., and Faustman, D.L. (2020). BCG Vaccinations Upregulate Myc, a Central Switch for Improved Glucose Metabolism in Diabetes. iScience *23*, 101085. https://doi.org/10.1016/j.isci.2020.101085.
46. Angelidou, A., Conti, M.G., Diray-Arce, J., Benn, C.S., Shann, F., Netea, M.G., Liu, M., Potluri, L.P., Sanchez-Schmitz, G., Husson, R., et al. (2020). Licensed Bacille Calmette-Guerin (BCG) formulations differ markedly in bacterial viability, RNA content and innate immune activation. Vaccine *38*, 2229–2240. https://doi.org/10.1016/j.vaccine.2019.11.060.
47. Demirci, I., Haymana, C., Tasci, I., Satman, I., Atmaca, A., Sahin, M., Ata, N., Unluturk, U., Cakal, E., Dagdelen, S., et al. (2022). Higher rate of COVID-19 mortality in patients with type 1 than type 2 diabetes: a nationwide study. Endokrynol. Pol. *73*, 87–95. https://doi.org/10.5603/EP.a2022.0008.
48. D'Addio, F., Sabiu, G., Usuelli, V., Assi, E., Abdelsalam, A., Maestroni, A., Seelam, A.J., Ben Nasr, M., Loretelli, C., Mileto, D., et al. (2022). Immunogenicity and Safety of SARS-CoV-2 mRNA Vaccines in a Cohort of Patients With Type 1 Diabetes. Diabetes *71*, 1800–1806. https://doi.org/10.2337/db22-0053.
49. Chemaitelly, H., Ayoub, H.H., AlMukdad, S., Coyle, P., Tang, P., Yassine, H.M., Al-Khatib, H.A., Smatti, M.K., Hasan, M.R., Al-Kanaani, Z., et al. (2022). Duration of mRNA vaccine protection against SARS-CoV-2 Omicron BA.1 and BA.2 subvariants in Qatar. Nat. Commun. *13*, 3082. https://doi.org/10.1038/s41467-022-30895-3.
50. Aronson, N.E., Santosham, M., Comstock, G.W., Howard, R.S., Moulton, L.H., Rhoades, E.R., and Harrison, L.H. (2004). Long-term efficacy of BCG vaccine in American Indians and Alaska Natives: A 60-year follow-up study. JAMA *291*, 2086–2091. https://doi.org/10.1001/jama.291.17.2086.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
BCG vaccine (Bacillus Calmette-Guérin)	Japan BCG Laboratory, Tokyo, Japan	Japan 174 strain
Critical commercial assays		
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

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, MD, PhD (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

Late in the COVID-19 pandemic we conducted this Phase III US based clinical trial to determine the safety and efficacy of up to 6 doses of BCG vaccines versus placebo for the prevention of COVID-19 and other infectious diseases. A total of 141 participants were enrolled in this Phase III clinical trial evaluating the efficacy of the BCG vaccine in protecting infectious disease-vulnerable, type 1 diabetic subjects from April 2021 to November 2022 (Figure 1). A total of 141 participants also finished the trial; there was no patient attrition. This was a randomized, double-blinded clinical trial with a 2:1 enrollment. During this 19-month course, the US population was no longer exposed to the early original SARS-CoV-2 virus and Alpha variant but instead was predominantly exposed to the Beta, Gamma, Delta and Omicron variants (Figure 1). Both the incidence of COVID-19 disease and other infectious diseases was determined using the methods described below. All participants at the time of enrollment met the criteria of having no previous or current tuberculosis, no history of BCG vaccinations, even during childhood, and being born in the US. Throughout the trial, subjects were monitored for any infectious diseases (viral, bacterial, parasitic, etc.) using a direct MedDRA documentation system. It is important to note that newborn or childhood BCG vaccinations have never been offered in the US. Starting in early 2021, gradually mRNA vaccines became available in the US and although not a primary outcome of this trial, it was possible in the placebo group to see if the combined use of either the two mRNA-based vaccines from Pfizer-BioNTech and Moderna, as well as a Viral Vector vaccine from J&J/Janssen had any efficacy or harm in this infectious disease-vulnerable population. The use of these commercial vaccines in male and female placebo subjects is shown in Table 1.

At the start of this current infectious disease related clinical trial, subjects were volunteers with a mean age of 44 $\pm$ 1.1 years with co-morbid type 1 diabetes of long-standing origins, mean duration 17.9 $\pm$ 0.06 (Table 1). The current Phase III clinical trial was a US-based randomized clinical trial using already enrolled double-blinded subjects in an ongoing double blinded trial looking at the impact of the multi-dose BCG vaccines on metabolic outcomes (US FDA IND #16434; MGH protocol #2003P002633). This trial is registered in the clinicaltrials.gov NCT site as: NCT02081326. The randomized type 1 diabetic subjects receiving BCG or placebo vaccines had also been used early in the pandemic, prior to mRNA vaccines for COVID-19 disease and infectious disease incidences study and this data is published (Figure 1).[13] This earlier study was terminated when the two way comparisons of the written protocol comparing BCG to placebo were no longer a valid trial with the roll out of the commerical mRNA vaccines.

The protocol, outcomes, data analysis methods, methods for COVID-19 diagnosis, non-overlapping trial length, COVID-19 genetic variant exposures and consents for the current COVID-19 prevention trial were approved as a new study by MGH and the FDA and filed as an amendment to the original FDA IND and MGH protocols. This trial was labeled a Phase III clinical trial. A study synopsis is provided (Figure S1). The

subjects in this Phase III clinical trial and the earlier Phase II clinical trial were overlapping and were all type 1 diabetic subjects. Figure S2 shows the differences in the two clinical trials. This trial was performed in accordance with ethical principles in the Declaration of Helsinki and are consistent with the International Conference on Harmonization/Good Clinical Practice. At the start of this Phase III clinical trial 30% of subjects (BCG and placebo) had received 5 BCG/placebo vaccines and 70% of subjects had received 6 BCG/placebo vaccines. For the subjects with only 5 vaccines at the time of consent, the average number of days until that sixth vaccine was 124 $\pm$ 16 days. For the subjects with only 5 vaccines at the time of consent, the average number of days since the last BCG vaccine was 241 days. This data is calculated from the protocol approval date of April 2021.

The initial exclusion criteria for the trial included a positive purified protein derivative (PPD) test, a positive T-spot test for tuberculosis performed at screening; or being born in a foreign country with mandatory BCG vaccinations. These criteria were implemented to ensure that participants did not have existing long-term protection against *Mycobacterium bovis* (the source of the BCG vaccine) or *Mycobacterium tuberculosis* (TB) due to previous exposures. Additionally, individuals actively undergoing high dose glucocorticoid treatment; taking chronic immunosuppressive medications; or living with an immunosuppressed person were also excluded to prevent adverse events from the administration of the live vaccine. All participants resided in the United States, with the largest number (36) coming from Massachusetts, followed by 16 from New York and 11 from Texas.

Both MGH and FDA approved COVID-19 survey were sent through email at two month intervals and the patients were also seen in the clinic every 6 months. The COVID-19 survey was under the FDA Guidance recommendations. There was good compliance. Also these subjects would also sometimes email or phone the clinic with any illness updates and reporting at non-clinic appointment times. For this trial, PCR was approved so this method was used to confirm COVID-19 disease.

This trial had three levels of oversight. At 6-month to yearly intervals, audits were conducted either by the Massachusetts General Brigham (MGB) Division of Quality Management or by external auditors (Advanced Clinical Trials, Deerfield, IL). Unblinded statisticians (H.Z.) processed all trial data, and an independent DSMB met every six months to a year to ensure subject safety and compliance with reporting.

The randomization code for administering either the BCG vaccine or placebo saline to study participants was generated by the MGH Research Pharmacy and securely held by them. Both the BCG and Placebo preparations appeared identical in the syringe, making it easier to blind the study staff and participants. Unlike antigen-specific vaccines that often cause multi-day systemic reactions, site soreness and missed workdays, the BCG vaccine did not induce any systemic or local symptoms immediately after administration. The BCG vaccine in adults administered as an intradermal vaccine after 4 weeks can induce a skin reaction that was blinded to study staff. Throughout the study, investigators and study staff remained blinded to both the primary study outcomes and the parallel study outcomes related to COVID-19 and infectious diseases. The statistical analysis team after November 2022 was unblinded to tabulate the data presented in this paper.

METHOD DETAILS

Procedures

We assessed late in the US COVID-19 pandemic, the safety and efficacy of 6 doses of BCG vaccines of the Tokyo-172 strain for COVID-19 and other infectious diseases. This was a community-based, but broadly US recruited randomized double blinded placebo controlled clinical trial. The BCG vaccine or saline placebo (both 0.1 mL volume per dose) was administered intradermally starting 2.5–3 years prior to this phase of the COVID-19 pandemic (Figure 1). The first two BCG or Placebo doses were given 4 weeks apart and subsequently at yearly intervals additional booster doses of BCG or Placebo were administered, for a total of up to 6 doses over a period of 60 months (5 years) (Figure 1). Site staff were responsible for reporting all drug- and non-drug-related safety information and were blinded to group assignments.

In our published Phase II clinical trial, we used antibody profiling, a technology called the COVID Scan assay (CDI Labs, Baltimore, MD) to identify COVID-19 infected subjects based on the presence of arrays of anti-SARS-CoV-2 antibodies.[13] This method had been used because there were no COVID-19 commercial diagnostic methods. With this Phase III clinical trial commercial COVID-19 diagnostics were used to diagnose COVID-19 disease. We therefore relied on Point of Care based PCR and/or Rapid Antigen testing to confirm COVID-19 in subjects that presented with one or more COVID-19 symptoms as defined by the FDA Guidance Document[39] and at least one day of illness (see below). It is important to additionally note that antibody scanning diagnostic methods were also no longer feasible with the rollout of COVID-19 vaccines; indeed the vaccines can mimic the actual disease (see Figure S4).

COVID-19 is defined according to FDA by the presence of at least one of 12 symptoms (ref. 1) including headache, chills/shivering, diarrhea, nausea/vomiting, fatigue, shortness of breath, loss of smell or taste, muscle aches, nasal congestion, cough, sore throat and fever). 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 an Average Symptom Score were tabulated. Then, multiplying by the length of illness, the Average Infectious Symptom Index was assessed.

During the trial period the launch of the mRNA vaccines happened, so an analysis of the efficacy of these licensed vaccines products in the absence of BCG vaccination was also conducted.

Outcomes

We determined if multi-dosing with BCG-Japan (Tokyo-172) vaccine can protect high-risk T1D subjects from COVID-19, both in the presence and in the absence of COVID-19 commerical vaccines. We also determined if BCG-Japan can protect from other infectious diseases in the presence of COVID-19 vaccines. Finally, we also looked at the efficacy of the COVID-19 vaccines to protect the high-risk T1D subjects from

COVID-19 disease in the absence of BCG. We did not study severe COVID-19 disease as measured by hospitalizations or deaths because the period under study saw to some extent less lethal viral variants.

The study synopsis is provided (Figure S1). These outcomes conformed to the primary US based trials for the commercial COVID-19 vaccines (Pfizer-BioNTech and the Moderna clinical trials). For this trial we reported adverse events as they relate to infectious diseases. There were no moderate or severe adverse events related to the BCG vaccines. In contrast to the commerical COVID-19 vaccines, the administration of the BCG vaccines, even with multi-dosing, does not induce arm sourness, fever, fatigue, or other flu like symptoms in the 48 h after administration thus assisting in the blinding of the clinical trial. For this report, the full skin reaction logs belong to a time period not overlapping with this clinical trial since most had received their full series. For vaccine safety for this clinical trial, no reports of a vaccine site composed of redness, swelling and/or a scar greater that 3 cms was reported.

QUANTIFICATION AND STATISTICAL ANALYSIS

Vaccine efficacy is defined by (p1 – p2)/p1 × 100, where p1 is the % COVID-19 positive in the placebo group and p2 is the % COVID-19 positive in the BCG group. The number of COVID-19 positive patients in the BCG versus the placebo cohorts were compared using Fisher's exact test (available online at https://www.graphpad.com/quickcalcs/contingency1/). To satisfy the requirements for this test, each subject was only counted once, even if the subject experienced more than one COVID-19 event. To be able to include multiple events per subject we created charts of rate of events per subject and used Poisson distribution statistics for counting scenarios that included the whole current trial period. A similar approach was also used to compare all infectious events (both COVID-19 and non-COVID-19) between the cohorts. Statistics were considered significant at $p < 0.05$.

OVERSIGHT OF THIS CLINICAL TRIAL

This trial was approved by the MGH human studies and by the US Food and Drug Administration with external audits at yearly intervals conducted by Advanced Clinical. The safety of BCG was monitored by an independent Data and Safety Monitoring Board (DSMB). The investigators designed the trial. The funders and the drug supplier Japan Laboratories had no role in the collection, analysis or interpretation of the data or in the preparation, review or approval of the manuscript.

ADDITIONAL RESOURCES

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