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Immediate Bacille Calmette-Guérin vaccination to neonates requiring perinatal treatment at the maternity ward in Guinea-Bissau: A randomized controlled trial

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Running title: RCT of BCG to hospital-admitted neonates

Brief 40-word summary: In a randomized controlled trial from Guinea-Bissau, providing BCG immediately compared with BCG-at-discharge to neonates admitted to the neonatal intensive care

unit did not affect overall in-hospital mortality significantly, but tended to reduce deaths from infectious diseases.

Ethical approval: The study protocol was approved by the Guinea-Bissau Health Ministry's Research Coordination Committee (Reference number: CNES-2013-0054) and given consultative approval by the Central Danish Ethical Committee (Case No: 1303771-1). A subsequent protocol revision was equally approved by both committees (CNES-2014-001, 1303771-2).

The trial was conducted in accordance with the Helsinki Declaration ethical standards, and a Data and Safety Monitoring Board (DSMB) oversaw the trial. Free healthcare consultations and essential drugs were provided to all infants invited to participate in the study, which was registered at **ClinicalTrials.gov** with registration number NCT01989026 on November 20, 2013.

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ABSTRACT (200)

Background: Randomized controlled trials (RCTs) indicate that Bacille Calmette-Guérin (BCG) vaccination provides broad beneficial *non-specific* protection against infections. We investigated the effect on in-hospital mortality of providing BCG immediately upon admission to a neonatal intensive care unit (NICU), rather than BCG-at-discharge. The pre-trial NICU mortality was 13% and we hypothesized that BCG would reduce mortality by 40%.

Methods: Parallel-group, open-label RCT initiated in 2013 in Guinea-Bissau. NICU-admitted neonates were randomized 1:1 to BCG+Oral Polio Vaccine (OPV) immediately (intervention) versus BCG+OPV at hospital discharge (control; usual practice). The trial was discontinued due to decreasing in-hospital mortality and major NICU restructuring. We assessed overall and disease-specific mortality by randomization allocation in Cox Proportional Hazards models providing Mortality Rate Ratios (MRRs).

Results: We recruited 3,353 neonates and the overall mortality was 3.1% (52/1676) for BCG-vaccinated neonates versus 3.3% (55/1677) for controls, MRR=0.94 (0.64-1.36). For non-infectious causes of death the MRR was 1.20 (0.70-2.07) and there tended to be fewer deaths from infections in the BCG group (N=14) than among controls (N=21), MRR=0.65 (0.33-1.28).

Conclusions: Providing BCG+OPV to frail neonates was safe and might protect against fatal infection in the immediate newborn period. Deaths due to prematurity and perinatal complications were unaffected by BCG.

Keywords: Bacille Calmette-Guérin vaccine; vaccination at birth; neonatal mortality; non-specific effects of vaccines.

BACKGROUND (279)

In tuberculosis (TB) endemic areas, the Bacille Calmette-Guérin (BCG) vaccine is recommended at birth and is one of the most widely used vaccines in the world.[1] While BCG provides variable protection against pulmonary TB and good protection against disseminated TB, evidence from observational studies and randomized controlled trials (RCTs) show that BCG also has beneficial *non-specific effects* (NSEs), leading to a reduction in all-cause mortality and morbidity of 30-50%.[2–8] In 2014, a review of NSEs conducted by the World Health Organization concluded that BCG vaccination reduces child mortality much more than explained by prevention of TB, and recommended further research.[9]

Immunological studies suggest that possible immunological pathways of BCG's NSEs are "*trained innate immunity*", induction of emergency granulopoiesis and/or induction of heterologous T-cell immunity.[10–12]

In Guinea-Bissau, the Bandim Health Project (BHP, www.bandim.org) has conducted a series of large-scale epidemiological studies and RCTs evaluating the overall effects of BCG and other childhood vaccines.[2–7,13–17] We have previously shown in RCTs that providing BCG to healthy low-weight neonates at hospital discharge reduces neonatal mortality by 38% (95% Confidence Interval (CI): 0.46-0.83) due to protection against death from infection, specifically from sepsis and pneumonia.[2–5] Given the strongly beneficial non-specific effects of BCG we speculated that BCG might help reduce the high mortality observed in frail newborns admitted for intensive care. We therefore conducted the present RCT to evaluate whether providing BCG early, i.e. immediately on admission to the Neonatal Intensive Care Unit (NICU), rather than at discharge, could protect this highly vulnerable group against severe infections and help reduce the high NICU mortality.[18]

We hypothesized that receiving BCG at birth would reduce the overall NICU mortality by 40%.

METHODS (1,216)

Setting

The trial was conducted at Hospital Nacional Simão Mendes (HNSM)'s maternity ward, which is Guinea-Bissau's principal birthplace with ~7,000 deliveries/year and is located in the capital Bissau. BHP maintains a routine data collection system to register births, vaccinations, admissions and outcomes at the HNSM maternity and pediatric wards.[5,17–20] When the trial was planned, the obstetrical facilities were limited and the stillbirth rate 9.9%.[19] Of the live-born, approximately 5% were admitted to the NICU, where the pretrial mortality risk was 13%.[18] A birth weight <1500g, Apgar score ≤3, single motherhood and birth by C-section are known risk factors for admission to the NICU.[18]. Further details of the NICU are provided in the Appendix. At the adjacent pediatric ward, children aged 0-18 years are treated and there are approximately 6,000 admissions/year with previously reported case-fatality rates of 5%-12%.[5,17,20,21] Approximately 7% of our NICU-enrolments were transferred to the pediatric ward for treatment.

Study Design

This hospital-based, open-label, parallel-group RCT was initiated in October 2013. The recommended vaccination schedule at birth in Guinea-Bissau is co-administered BCG and Oral Polio Vaccine (OPV), and the usual practice is to give both at discharge. Thus, NICU-admitted neonates were randomized 1:1 to BCG+Oral Polio Vaccine (OPV) immediately (intervention; vaccines provided in the morning following admission) versus BCG+OPV at hospital discharge (control; usual practice).

Inclusion and exclusion criteria

Initially, all neonates admitted to the NICU were eligible. From February 2014, on advice from the Data Safety and Monitoring Board that intended to exclude the most vulnerable and likely moribund

neonates, the following exclusion criteria were added, for assessment by local neonatal nurses: weight at admission<1250g and Apgar score<2.

Enrollment and Informed Consent

Eligible neonates were invited to participate the morning following admission to the NICU. Mothers/guardians were provided written study information in Portuguese and a verbal explanation of the study in the local language Creole and were invited to ask questions. At enrolment, we collected socio-economic data and recorded the maternal mid-upper-arm circumference along with weight and twinning status.

In April 2015, we initiated an immunological sub-study nested within the RCT aiming to identify immune profiles induced by BCG and their correlation with survival; blood samples were collected 21-24 hours after enrolment via heel-prick blood draw. Pilot data from 40 healthy sub-study participants have been reported separately.[11] To provide a benefit to participants, blood glucose levels were measured for all participants of the sub-study and hypoglycemic neonates (blood glucose≤2.5 mmol/L) were either encouraged to breastfeed, formula feed or provided oral glucose or, at the discretion of the local medical team, referred to the pediatric ward. The sub-study continued for the remainder of the trial (1,332 neonates sampled in total).

Intervention

The BCG strain was BCG-Denmark (Copenhagen strain 1331, Statens Serum Institut (SSI)) from October 2013 to June 2016 and BCG-Japan (Tokyo strain 172, Japan BCG Laboratory) from July 2016 to August 2017 due to a production halt at SSI. Two vaccinators with >15 years of experience performed all vaccinations by intradermal injection of 0.05 ml reconstituted BCG in the left deltoid region, followed by administration of OPV. The control group received BCG+OPV at discharge from the hospital in accordance with the standard of care.

Randomization

The mother/guardian drew a randomization lot from a bag of sealed, opaque envelopes. Neonates were randomized 1:1 to receive either BCG+OPV immediately or upon hospital discharge. Initially, randomization was stratified by NICU placement (incubators or ordinary cribs). Further stratification by sex and weight group (1250-1499g, 1500-1999g, 2000-2499g, >2500g) was added in February 2014. Same-sex twins were allocated to the same treatment to avoid misclassification during follow-up.

Outcomes and hypothesis

The main hypothesis was that providing immediate BCG would reduce in-hospital mortality (main outcome) by 40% compared with vaccination at discharge. Secondary outcomes were the impact of BCG on different causes of death, duration of NICU admission and weight change while admitted.

Blinding

The mother/guardian was informed whether the child was randomized to be vaccinated immediately or at the time of discharge. No placebo vaccine was used. BCG vaccination usually leaves a small white papule on the skin that disappears within 15-30 minutes. Redness can develop within a few days, and a pus-containing papule typically appears 2 to 4 weeks after vaccination.[22] To blind the clinical staff, a band aid was placed on the upper left arm in both treatment groups (for a maximum of 3 days) and the assistant dedicated to in-hospital follow-up procedures was not involved in the inclusion procedure or the vaccination of participants.

Follow-up

The weight and vital status for enrolled neonates was monitored daily and follow-up continued at the adjacent pediatric ward for neonates transferred there. At hospital discharge, we ensured that controls received the recommended BCG+OPV. We have previously observed that moribund infants are sometimes taken out of the hospital by their parents without proper medical discharge, and that

these children have a high subsequent mortality.[20] If a neonate died within 24 hours after leaving the hospital, it was therefore recorded as a study death (BCG 0, Control 2).

Evaluation of causes of death

A senior pediatrician blinded to the treatment allocation conducted reviews of the NICU clinical chart data and Pediatric ward admission data (where applicable) and categorized deaths into five broad disease categories: infection, birth complication, respiratory insufficiency/prematurity, dehydration, or unknown cause.

Sample size

Calculated by events and assuming a 12% mortality risk, the required sample size was 1,262 neonates corresponding to 122 in-hospital deaths to demonstrate a 40% mortality reduction with 80% power and a significance level of 5%.

Events that affected the trial

Several external events and changes affected the trial, such as the BCG-Denmark production halt, and changes in hospital services (**Appendix**).

In 2016, Doctors Without Borders (Médecins Sans Frontières, MSF) implemented a large intervention at the hospital's pediatric ward. With their help a new NICU was built in 2017 adjacent to the maternity ward, which resulted in substantial organizational and structural changes.[23] The improved treatment standards were likely to accelerate the trend of declining mortality, and the trial status was therefore discussed with the DSMB, who recommended to discontinue the trial in August 2017, when 107 of 122 deaths had occurred corresponding to 88% of the originally planned number. The various time periods and major events that occurred during the trial are presented in **Supplementary Figure 1**.

Statistical analyses

We assessed effects on all-cause mortality (primary outcome) and major causes of death (secondary outcome) in Cox Proportional Hazards models providing Mortality Rate Ratios (MRRs) with age as the underlying time in the analysis; age was thus inherently controlled for. We had pre-specified to account for interdependency among twin pairs (using the Stata *cluster* command) and to stratify all analyses by sex, weight group (1250-1499g, 1500-1999g, 2000-2499g, ≥2500g) and season of enrolment (rainy: June-November, dry: December-May). Cumulative mortality curves for the first 7 days following randomization (where >90% of deaths occurred) for all-cause deaths and infectious disease deaths were computed using the Kaplan-Meier estimate. Median values of duration of admission (with 25-75% centiles) were analyzed using Wilcoxon rank sum test (non-parametric). Weight changes during admission were analyzed using linear regression adjusted for duration of admission and with censoring of weight changes >150g/day due to implausibility. All analyses were conducted overall and by sex using Stata16 (Stata Corp, College Station, Texas), and all estimates are reported with 95% CIs.

RESULTS (709)

Between October 2013 to August 2017, 3,915 neonates were admitted to the NICU, of which 86% (3,364/3,915) were eligible to participate in the trial. Three families declined to participate, and we thus enrolled 3,361 neonates (**Figure 1**). Of these, six children were excluded due to inclusion errors and one twin-pair was excluded due to confusion at follow-up. We thus included 3,353 neonates in the analysis, of which 1,676 received immediate BCG and 1,677 were controls (**Figure 1**). Baseline characteristics were well-balanced between intervention and control (**Table 1**). The C-section rate was 77% (2571/3346).

Mortality

The total follow-up time for the 3,353 neonates was 19,361 person-days during which 107 in-hospital deaths occurred. The mortality among enrolled neonates declined from 5.9% (14/236 neonates) in 2013 to 1.2% (4/335 neonates) in 2017 (p=0.002). The all-cause mortality risk was 3.1% (52/1,677) for neonates that received immediate BCG vs. 3.3% (55/1,676) for controls and the corresponding BCG/Control MRR 0.94 (0.64-1.36) (**Table 2, Figure 2)**; 1.09 (0.59-2.01) for neonates delivered by C-section and 0.82 (0.50-1.32) for vaginal births**.**

By randomization strata

By sex, BCG tended to be beneficial for females, MRR 0.67 (0.35-1.30), but not for males, MRR 1.10 (0.69-1.76) (p for same effect=0.24, **Table 2**). By season, the rainy season MRR was 0.85 (0.49-1.47) and the dry season MRR=0.98 (0.59-1.62) (p for same effect=0.71, **Table 2**).

There were no differences in the effects of BCG by inclusion weight category.

Cause of death

Infections represented 33% (35/107) of the trial deaths while non-infectious causes represented 48% (51/108) and consisted of: perinatal complications 15% (16/107), respiratory insufficiency/prematurity 31% (33/107) and dehydration 2% (2/107). The remaining 20% (21/107) of deaths were of unknown cause. There were no significant differences in the mortality risk by randomization allocation for the different causes of death (**Table 3, Supplementary Table 1**). The risk of death from infection was 0.8% (14/1,676) in the immediate BCG group versus 1.3% (21/1,677) for controls, the MRR being 0.65 (0.33-1.28) (**Figure 3**). The MRR was 1.06 (0.37-3.05) for neonates delivered by C-section and 0.42 (0.17-1.06) for vaginal births (p for same effect=0.19) (**Supplementary Table 2**). The MRR for non-infectious diseases (perinatal complications, respiratory insufficiency/prematurity, and dehydration) was 1.20 (0.70-2.07) (p for same effect=0.16, **Table 3**). By birth route, the MRR for non-infectious diseases was 1.03 (0.41-2.59) for neonates born by C-section and 1.29 (0.66-2.52) for normal births (**Supplementary Table 2**).

By BCG strain

For the period utilizing BCG-Denmark (96 deaths: 46 BCG, 50 Control), the BCG/Control MRR was 0.90 (0.61-1.33), while the MRR for the period using BCG-Japan (11 deaths: 6 BCG, 5 Control) was 1.24 (0.35-4.37) (p for same effect=0.64, **Supplementary Figure 1**).

Duration of admission

For incubator infants, the median admission length was 5 days (25-75% centile: 3-7 days) for 1014 BCG recipients vs. 4 days (3-6 days) for 1013 controls (p=0.17) and for crib admissions, it was 6 (4-7 days) for 662 BCG recipients vs. 5 (4-7 days) for 664 controls (p=0.29).

Weight change during admission

Excluding 4.1% (136/3343) of cohort neonates that had unlikely weight changes of >150g/day, the overall median weight change during admission was 0g (-140g to 100g) in the immediate BCG group vs. -10g (-120g to 100g) among controls (p=0.55).

Referrals to the pediatric ward

Referrals increased from 2% (4/236) in 2013 to 10% (34/335) in 2017 (p<0.001) and a total of 7% (223/3343) of the neonates (116 BCG, 107 control) were transferred to the pediatric ward (BCG/Control Relative Risk=1.09 (0.84-1.40), **Supplementary Table 3**). The mortality was 19% (43/223) for transferred neonates; 20% (23/116) for BCG-vaccinated and 19% (20/107) for controls, MRR=0.93 (0.47-1.83).

OPV vaccination campaigns

Since we have found OPV campaigns to interfere with other health interventions with a major beneficial impact on subsequent child survival[24], in the protocol we had prespecified that we would conduct a sensitivity analysis censoring children at the time of the intervention, if such

campaigns were implemented for children at the NICU. Four nationwide OPV campaigns occurred during the study, during which 3.4% (114/3343) of participants were admitted at the NICU. Of these neonates, 2 died (2 BCG, 0 Control). After censoring the neonates present at the NICU during OPV campaigns, the MRR was 0.89 (0.61-1.30).

Adverse events

During follow-up at the NICU, we did not observe any adverse events related to BCG.

DISCUSSION (1,218)

Main findings

There was no benefit on overall mortality of providing BCG immediately to neonates admitted to the NICU in Guinea-Bissau. Most trial deaths were caused by perinatal complications and prematurity and only a third of deaths were due to infections. Though not significant, there was a tendency for fewer deaths from infectious diseases among immediate BCG recipients.

Strengths and weaknesses

To our knowledge, this is the first trial providing BCG to neonates at admission to an NICU setting in Sub-Saharan Africa. The trial was carried out by a highly experienced team of hospital-based staff.

Our work has several limitations. The overall in-hospital mortality was substantially lower (3%) than anticipated (12%). The reduced mortality might partly have been due to improved treatment standards that were further accelerated by MSF interventions occurring during the trial. While this decline in NICU mortality was very welcome, it reduced study power and prolonged the trial until the DSMB advised to discontinue the trial. It is possible that provision of improved care to both groups at the pediatric ward and within the immunological study (from April 2015) may have reduced the effect of the intervention. The immunological sub-study involved measurements of blood glucose

levels. In cases of hypoglycemia, the neonatal nurses intervened, which may have impacted the clinical course and increased referrals to the pediatric ward.

Our data regarding causes of deaths was limited by the availability of diagnostic tools, e.g., no laboratory results or blood-cultures to establish a sepsis diagnosis, and sometimes insufficient clinical data. Hence, caution is warranted in the interpretation of the cause-of-death data.

To exclude the most vulnerable neonates, a protocol revision was undertaken in February 2014 (i.e. soon after study initiation) and the randomization and enrolment criteria were thus not uniform throughout the entire trial. Furthermore, an unanticipated production halt at SSI occurred in July 2016 which resulted in a world-wide shortage of BCG-Denmark. We thus finished the trial using BCG-Japan. We note that both strains are held in high regard for their immunogenicity.[17,25]

The trial furthermore did not provide a placebo injection as this was deemed unethical, and though a concealment band aid was used, preferential care could potentially have been given to one group which would affect outcomes. Based on our clinical observations, we believe this was not the case.

There was a tendency for a greater protective effect of BCG in females than in males, but both sexes appeared to benefit from BCG when focusing on infectious deaths.

Consistency with previous findings

We did not detect a beneficial effect of BCG on overall mortality in the present trial. Several aspects may have contributed to this. Enrolment in the present trial had occurred 1-2 days earlier than in the previous trials where BCG was administered at discharge from the hospital. The previous trials had enrolled healthy LBW newborns [4], not frail newborns admitted to the NICU. This likely increased the relative importance of disease etiologies such as perinatal complications, respiratory insufficiency, and prematurity. Furthermore, BCG may not have the same beneficial effects when

given to frail, moribund children. It is also noteworthy that in the most recent trial of BCG to healthy LBW newborns, less than 10% were delivered by C-section [4] and for all HNSM births between 2007-13, <15% were by C-section.[19] In the present trial, 77% were delivered by C-section and there was no effect of BCG in neonates born by C-section. The disease etiologies affecting these newborns might have been affected by the administration of antibiotics following the C-section. A previous trial in Danish children, however, reported a borderline significant interaction, with a trend for a more protective effect of BCG against hospitalizations for infections in children born by C-section.[26] Future BCG trials should be encouraged to present data stratified by mode of delivery.

A previous analysis has revealed a marked effect on all-cause mortality already by three days after randomization to BCG provided at hospital discharge vs. no-BCG[27] and BCG-induced emergency granulopoiesis has been shown to result in a significant increase in neutrophil numbers by 72 hours post-vaccination.[11] This was directly and quantitatively responsible for protection from sepsis in a murine model of neonatal sepsis[11], and we note that the possible protection against death from infection induced by BCG in our data appears to occur also by three to four days after randomization.

A meta-analysis of three RCTs from Guinea-Bissau that provided BCG-Denmark vs. no-BCG to healthy low-weight newborns at hospital discharge reported a 38% reduction in neonatal mortality associated with BCG, primarily due to fewer deaths from infections.[4] Likewise, an analysis of post-discharge pediatric ward admissions within the same cohort revealed that BCG markedly reduced the risk of in-hospital deaths caused by infections.[5] A recent RCT from Uganda reported that BCG-Denmark provided at discharge was associated with a 29% lower incidence of non-TB infections between birth and 6 weeks of age, when compared to no-BCG.[8]

BCG is thus thought to mainly affect morbidity and mortality from infection, but only 33% of the deaths in the present trial were known to be due to infection. While there tended to be fewer

infectious deaths among BCG recipients versus controls, the study lacked power to conclusively demonstrate an effect. It is noteworthy, however, that the effect of BCG on non-infectious causes trended in the opposite direction. In the previous Guinea-Bissau RCTs, 69% (69/100) of the neonatal deaths were caused by infection, and essentially similar effects were reported, the MRR of BCG vs. no BCG being 0.57 (0.35-0.93) for infectious diseases and 1.20 (0.58-2.49) for non-infectious diseases (**Table 4**).[4] The tendency we report of fewer deaths from infections associated with immediate BCG is thus consistent with the previous findings from Guinea-Bissau and Uganda. Based on the data, it cannot be excluded that BCG may have a negative impact on non-infectious deaths, but we do not have any immediate explanation why that should be the case; however, if possible, it should be evaluated in future trials.

The importance of intervention timing, the disease-spectrum affecting the children, and their state at the time of vaccination is further supported by two large-scale trials from India, where the less immunogenic BCG-Russia administered at birth to frail NICU-admitted newborns weighing <2000g had no effect on the NICU mortality.[28] Across the two trials, 40% (345/872) of deaths were due to infection, while clinical conditions associated with prematurity (e.g., hyaline membrane disease, intravascular hemorrhages and other causes) represented >50% of deaths.

Hence, the results from ours as well as the two India BCG trials suggest that the effect of providing early BCG to frail NICU-admitted newborns might be less marked, compared to BCG-Denmark provided to healthy LBW neonates at hospital discharge.

Implications

While not associated with detectable benefits on overall in-hospital mortality in this trial, BCG appeared to reduce the risk of severe infection. Since BCG is associated with long-term survival benefits,[6,29,30] and that providing BCG at the first given opportunity is logistically the simplest

approach to achieve a higher overall vaccination coverage, then administration of BCG to all newborns, irrespective of frailty, would have the greatest effect and probably be good policy.

CONCLUSION

Providing BCG to frail neonates at admission to the NICU was safe. We detected no effect of immediate BCG on overall mortality but found a tendency for fewer deaths from infections among BCG recipients, consistent with previous findings.

Foot note page

Conflict of interest statement

Several of the authors have been affiliated with the Statens Serum Institute (SSI), Copenhagen, Denmark. SSI was the producer of BCG-Denmark when this trial was conducted. SSI did, however, not fund the study or the researchers involved, nor did it have any influence on the study design, data collection, analysis, interpretation, writing or decision to submit the present paper for publication. None of the authors have any other associations that might pose a conflict of interest.

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Data sharing statement

Deidentified participant data with a data dictionary can be shared after approval of a data-sharing proposal sent to Professor Christine Stabell Benn (cbenn@health.sdu.dk).

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Authors' contributions

CSB and MBA were the principal investigators and guarantors of the study. CSB, PAA, MBA, FSB and TRK designed the study. CNG, FSB, HNF, IM, KLL, MBA, NA and SS supervised the data collection and data entry. LC attended the children at the NICU and provided important clinical input. FSB and MBA conducted the statistical analyses. FSB and MBA wrote the first draft of the paper and all authors approved the final manuscript.

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FIGURE TITLES AND LEGENDS

Figure 1. *Study flowchart.*

Abbreviation: BCG, Bacille Calmette-Guérin.

Figure 1. *Kaplan-Meier cumulative in-hospital mortality incidence up to 7 days after randomization. Neonates were at risk until they were discharged, had died, or were lost to follow-up.*

The statistical analysis is a Cox Proportional Hazards cluster-analysis stratified by season, sex, and weight group.

Abbreviations: BCG, Bacille Calmette-Guérin; MRR, Mortality Rate Ratio.

Figure 3. *Kaplan-Meier cumulative in-hospital mortality incidence from infections up to 7 days after randomization. Neonates were at risk until they were discharged, had died, or were lost to follow-up.*

The statistical analysis is a Cox Proportional Hazards cluster-analysis stratified by season, sex, and weight group.

Abbreviations: BCG, Bacille Calmette-Guérin; MRR, Mortality Rate Ratio.

Supplementary Figure 1. Major events occurring during the trial.

The statistical analysis is a Cox Proportional Hazards cluster-analysis stratified by season, sex, and weight group.

Abbreviations: MRR, Mortality Rate Ratio; BCG, Bacillus Calmette-Guérin.

426 ***Table 1.*** *Baseline Characteristics for Intervention and Control Children.*

	Immediate BCG	**Controls**
	N=1676	N=1677
Infant characteristics		
Male sex	54.7% (917/1676)	54.0% (906/1677)
Weight at inclusion (g)[a]	2815 (N=1676)	2816 (N=1677)
1250-1499 g	1385 (N=64)	1388 (N=57)
1500-1999 g	1755 (N=186)	1766 (N=200)
2000-2499 g	2252 (N=233)	2245 (N=228)
>2500 g	3178 (N=1187)	3174 (N=1189)
Mean Apgar score (one minute) [SD]	6.5 (N=1667) [2.1]	6.5 (N=1664) [2.1]
Twin or triplet	15.2% (254/1676)	15.2% (254/1677)
Randomized>24h after birth	7.0% (117/1676)	7.9% (132/1676)
Born before 33 weeks of gestation[b]	10.3% (48/466)	9.1% (44/484)
Born by caesarean section	76.9% (1287/1674)	76.8% (1284/1672)
Maternal characteristics		
Age in years (25-75% centiles)[c]	26 (21-31, N=1673)	26 (22-30, N=1672)
From BHP study area	14.6% (245/1676)	14.5% (243/1677)
Mother dead before inclusion	0.7% (11/1676)	0.7% (11/1677)

Mother is literate	71.7% (1188/1657)	75.1% (1234/1643)
No maternal schooling	27.0% (449/1661)	24.5% (407/1663)
Maternal scar prevalence[d]	54.9% (441/804)	58.1% (462/795)
Mother severely ill during pregnancy	14.1% (234/1666)	15.1% (250/1660)
Pregnancy card available	88.9% (1488/1674)	89.6% (1499/1674)
High risk pregnancy[e]	9.5% (63/661)	12.2% (86/705)
High blood pressure during pregnancy[f]	3.9% (58/1472)	4.6% (68/1476)

Cells are percent (n/N) or mean. For continuous data, we have added the total number [N] of observations.

[a] Including neonates from the beginning of the study (before new enrolment criteria) with weight<1250 g (9 neonates in total).

[b] Very preterm, based on the last date of menstruation indicated on public health system pregnancy cards (if available).

[c] Expressed as median (with 25-75% centiles).

[d] Data collection initiated on July 10, 2015.

[e] Indicated on pregnancy card (if available).

[f] Above 140/90 mmHg as indicated on pregnancy card (if available).

Abbreviations: BCG, Bacille Calmette-Guérin; BHP, Bandim Health Project.

438 ***Table 2.*** *Mortality risk by randomization allocation and strata*

	Immediate BCG	**Control**	**MRR[a]** (95% CI)
Overall	3.1% (52/1676)	3.3% (55/1677)	0.94 (0.64-1.36)
Male	3.9% (36/917)	3.7% (33/906)	1.10 (0.69-1.76)[b]
Female	2.1% (16/759)	2.9% (22/771)	0.67 (0.35-1.30)[b]
By weight group at inclusion[c]			
<1500 g	21.4% (15/70)	13.3% (8/60)	1.69 (0.73-3.93)
1500-1999 g	7.0% (13/186)	10.5% (21/200)	0.68 (0.34-1.37)
2000-2499 g	2.6% (6/233)	3.1% (7/228)	0.79 (0.26-2.37)
>2500 g	1.5% (18/1187)	1.6% (19/1189)	0.93 (0.48-1.79)
By season of inclusion[d]			
Rainy season	2.9% (25/875)	3.1% (27/861)	0.85 (0.49-1.47)

Dry season	3.4% (27/801)	3.4% (28/816)	0.98 (0.59-1.62)

[a] Cox Proportional Hazards cluster-analysis stratified by season, sex, and weight group. Includes two deaths (0 BCG, 2 Control) that occurred within 24 hours after discharge.

[b] Analysis stratified by weight group and season of inclusion, p for same effect for males and females=0.24.

[c] Analysis stratified by sex and season of inclusion.

[d] Rainy season: June-November, dry season: December-May. Analysis stratified by weight group and sex, p for same effect in rainy and dry season=0.71.

Abbreviations: BCG, Bacillus Calmette-Guérin; CI, Confidence Interval; MRR, Mortality Rate Ratio.

Table 3*. Infectious, non-infectious and unknown causes of death by randomization allocation, overall and by sex.*

	Immediate BCG	Control	MRR[a]
	Deaths in percent (n/N)	Deaths in percent (n/N)	(95% CI)
Infectious deaths			
Overall	0.8% (14/1676)	1.3% (21/1677)	0.65 (0.33-1.28)
Male	1.0% (9/917)	1.3% (12/906)	0.72 (0.30-1.73)
Female	0.7% (5/759)	1.2% (9/771)	0.53 (0.18-1.58)
Non-infectious deaths[b]			
Overall	1.7% (28/1676)	1.4% (23/1677)	1.20 (0.70-2.07)[c]
Male	2.2% (20/917)	1.7% (15/906)	1.38 (0.71-2.66)
Female	1.1% (8/759)	1.0% (8/771)	0.88 (0.32-2.42)
Unknown cause of death			
Overall	0.6% (10/1676)	0.7% (11/1677)	0.93 (0.39-2.21)
Male	0.8% (7/917)	0.7% (6/906)	1.21 (0.40-3.65)
Female	0.4% (3/759)	0.7% (5/771)	0.59 (0.14-2.45)

[a] Cox Proportional Hazards cluster-analysis stratified by season, sex (overall estimate), and weight group.

[b] Encompasses neonates that died from perinatal complications, respiratory insufficiency/prematurity, and dehydration.

[c] p for same effect (infection and non-infectious causes of deaths)=0.16.

Abbreviations: BCG, Bacillus Calmette-Guérin; CI, Confidence Interval; MRR, Mortality Rate Ratio.

458 ***Table 4.*** *Overview of trials that has evaluated the non-specific effects of providing BCG within the first days after birth.*

Trial [ref]	Country	Inclusion criteria	BCG strain	Outcome	% of events caused by infection	HR infectious diseases	HR non-infectious diseases	Combined HR
Prentice [8]	Uganda	Healthy neonates discharged from the hospital	BCG-Denmark+OPV	Non-TB infectious disease incidence	NA	0.71 (0.53-0.95)	NA	NA
Biering-Sørensen (3 RCTs) [4]	Guinea-Bissau	Healthy LBW neonates discharged from the hospital	BCG-Denmark+OPV	Neonatal all-cause mortality	69% (69/100)	0.57 (0.35-0.93)[1]	1.20 (0.58-2.49)[2]	0.62 (0.46-0.83)
Present study	Guinea-Bissau	NICU-admitted neonates	BCG-Denmark (July 2016: BCG-Japan)+OPV	All-cause mortality during NICU admission	33% (35/107)	0.65 (0.33-1.28)[1]	1.20 (0.70-2.07)[2]	0.94 (0.64-1.36)
Jayaraman (2 RCTs) [28]	India	NICU-admitted neonates weighing <2000g	BCG-Russia (+/- OPV)	All-cause mortality during NICU admission	40% (345/872)	NA	NA	0.98 (0.85-1.11)

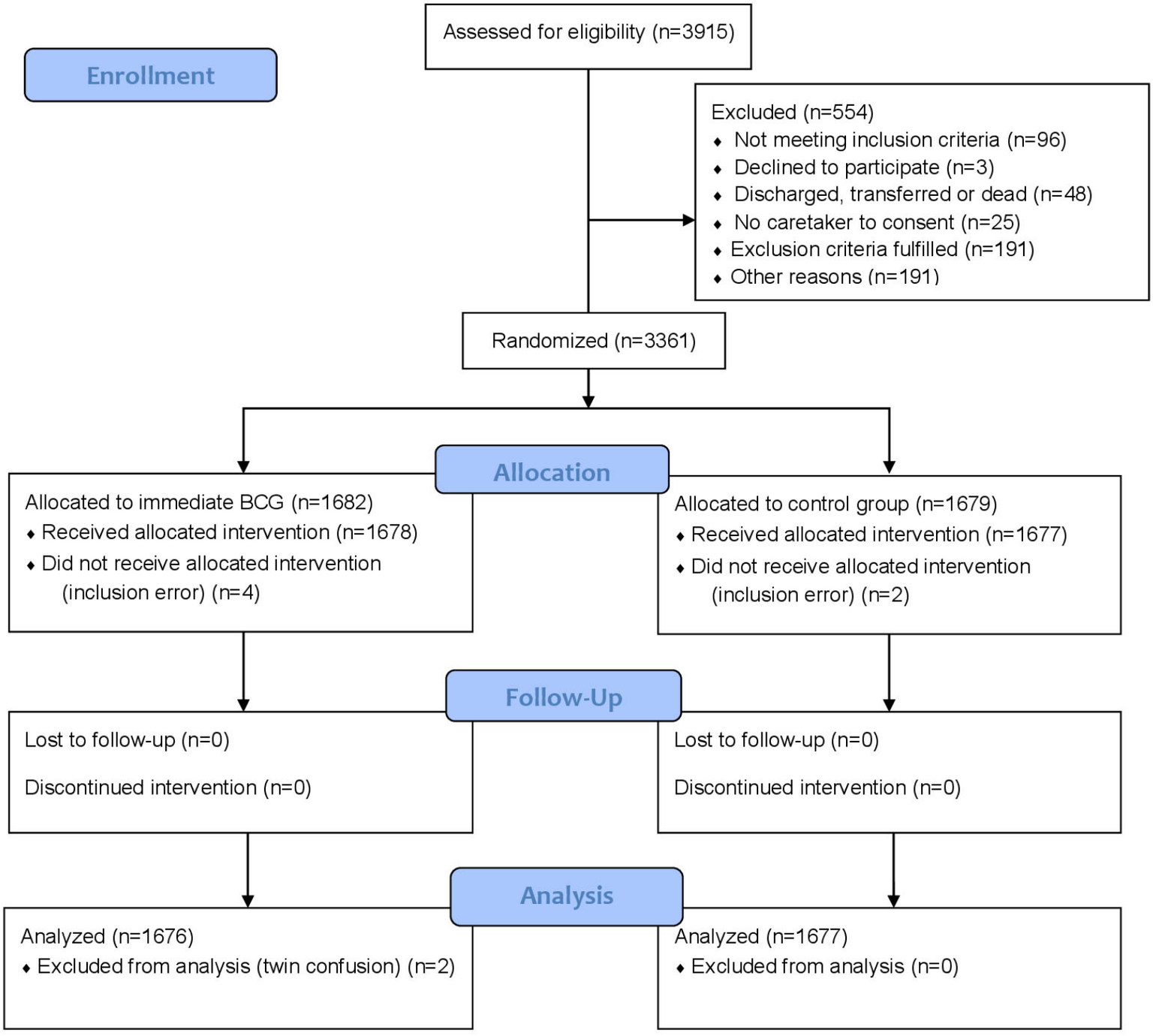
459 Abbreviations: BCG, Bacille Calmette-Guérin; HR, Hazard Ratio; LBW, low birth weight; NA, not available; OPV, Oral Polio Vaccine; TB, Tuberculosis.

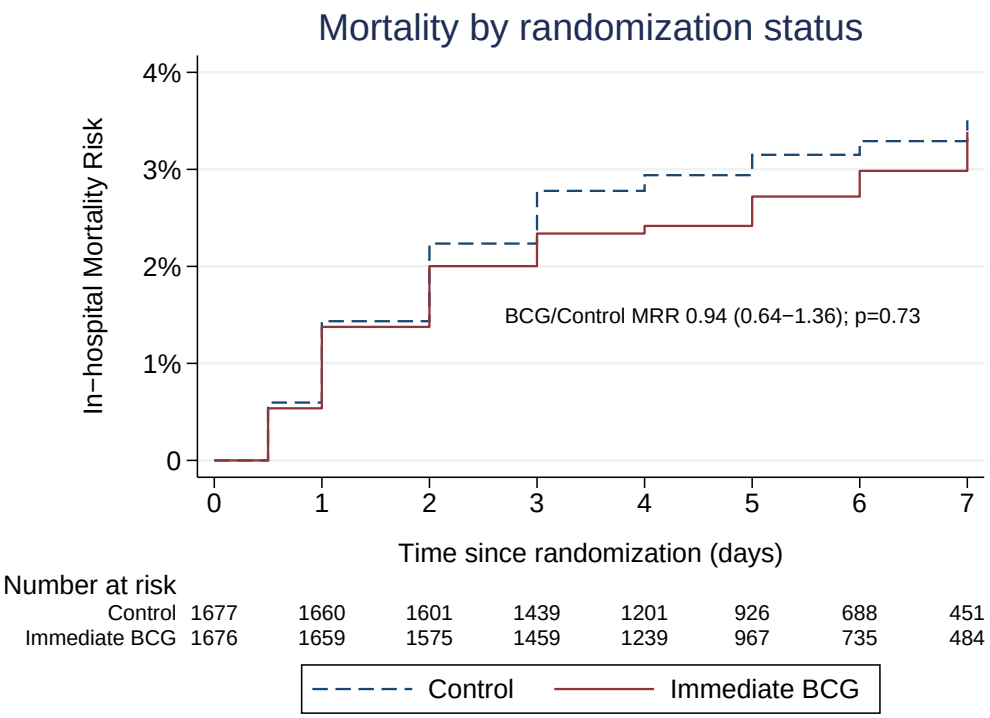
460 [1] Combined infectious disease death HR for Bissau trials: 0.60 (0.40-0.89), p=0.01. [2] Combined non-infectious disease death HR for Bissau trials: 1.20

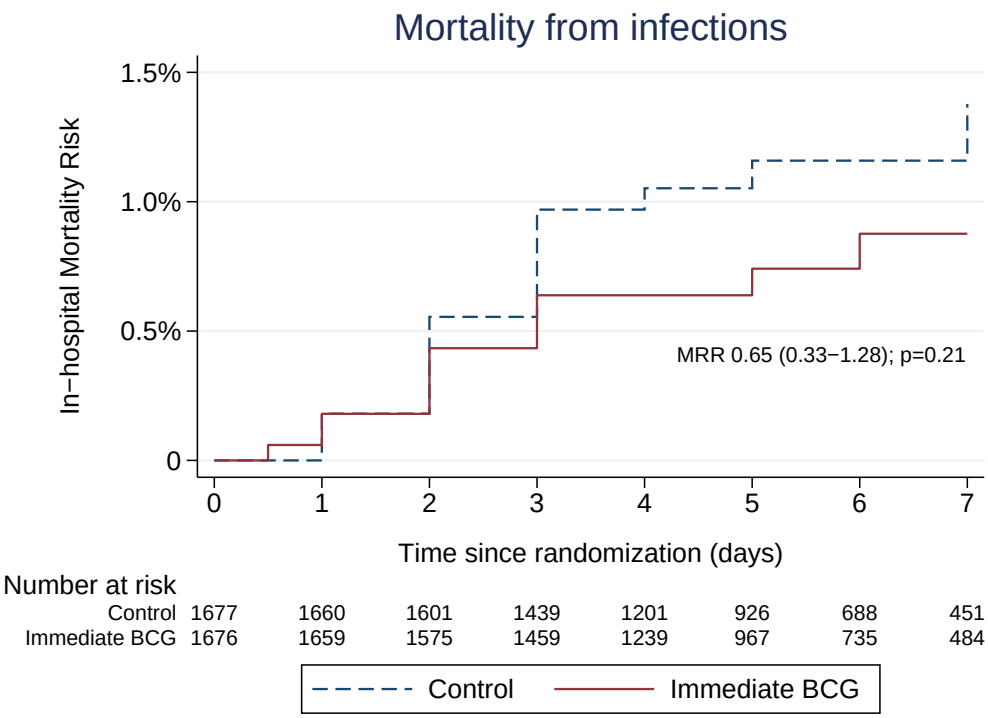
461 (0.78-1.85), p=0.41 (p for same effect=0.02).

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram




Mortality by randomization status
In−hospital Mortality Risk
4%
3%
2%
1%
0
BCG/Control MRR 0.94 (0.64−1.36); p=0.73
0
1
2
3
4
5
6
7
Time since randomization (days)
Number at risk
Control 1677 1660 1601 1439 1201 926 688 451
Immediate BCG 1676 1659 1575 1459 1239 967 735 484
Control
Immediate BCG


Mortality from infections
In−hospital Mortality Risk
1.5%
1.0%
0.5%
0
MRR 0.65 (0.33−1.28); p=0.21
0
1
2
3
4
5
6
7
Time since randomization (days)
Number at risk
Control 1677 1660 1601 1439 1201 926 688 451
Immediate BCG 1676 1659 1575 1459 1239 967 735 484
Control
Immediate BCG

Appendix

Description of the NICU facility

During the trial period, the NICU facilities were modest and subject to frequent interruptions in water and electricity, as well as a lack of equipment and medications. It is thus not comparable to NICUs in high-income settings, but we use the term "NICU" as this was the unit's official designation. The staff consisted of trained nurses that were mainly present during daytime. Newborns requiring additional care were placed in incubators, sometimes for weeks and even months in rarer cases. Treatment options were limited, and consisted mainly of registration of vital signs, treatment of hypoglycaemia/hypothermia and administration of medications (e.g., antibiotics). Nasal oxygen and nasogastric feeding tubes were available. The NICU also had smaller cribs, which were normally reserved for neonates born by C-section. The most common antibiotic given prior to C-section at the Maternity Ward was intravenous Ceftriaxone. Neonates at the smaller cribs of the NICU were often not overtly ill, and their length of stay usually depended mainly on maternal recovery.

Factors that affected the trial

Discontinuation of crib inclusions

During the trial, it became evident that the vast majority of deaths (~90%) occurred among incubator neonates. Hence, the relatively larger number of children placed in the small NICU cribs made only a limited contribution to addressing the research question. After elaboration with the DSMB, inclusions of crib neonates were therefore halted by March 31, 2016.

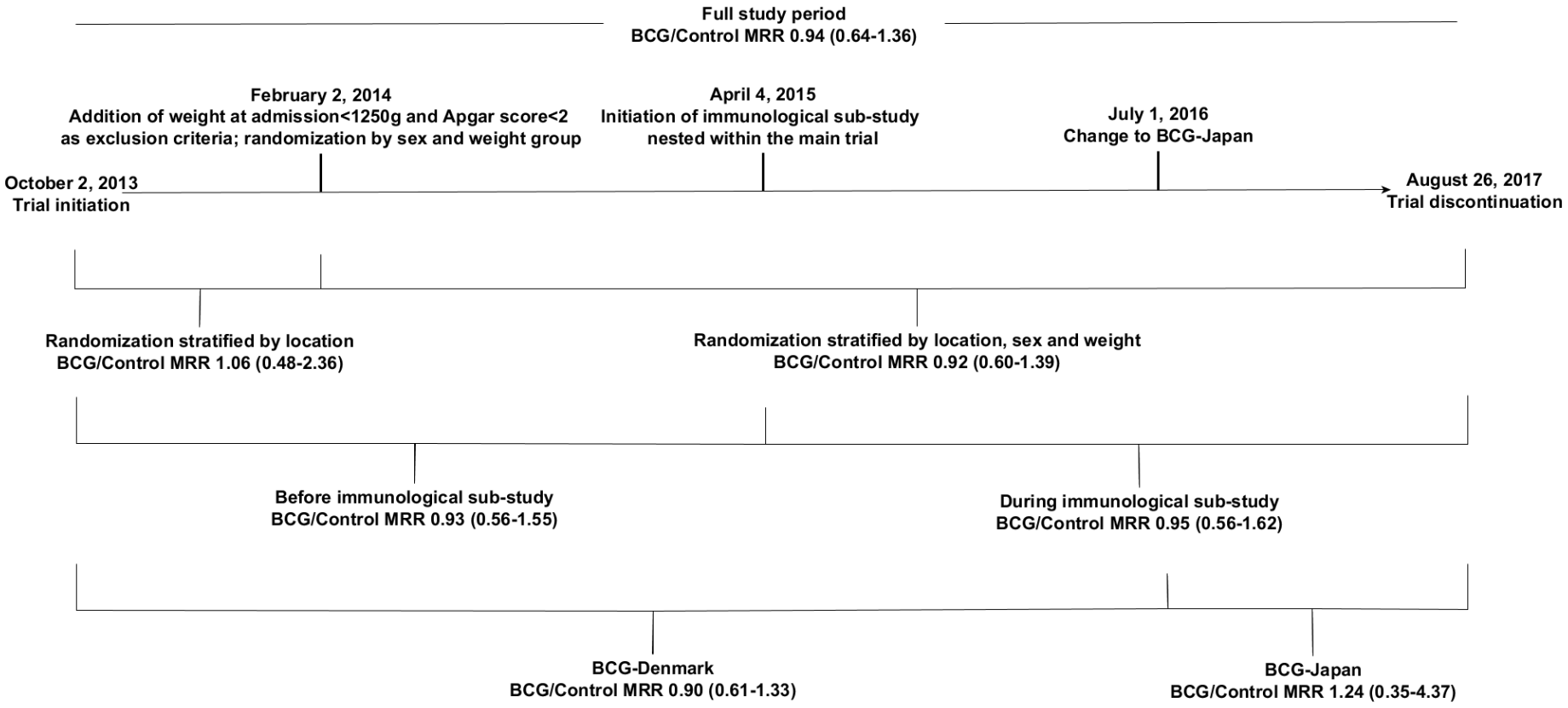
Change in BCG strain

Due to an unanticipated production halt of the BCG-Denmark strain, our last stock expired by July 1, 2016. We obtained permission by the Ethical Committees in Denmark and Guinea-Bissau to continue the trial using the BCG-Japan strain.

Interruptions in hospital services

We had pre-specified censoring of neonates born during prolonged disruptions of hospital services, i.e. strikes or electricity failures. However, after review of the study log, we did not identify events to merit censoring of neonates for this reason.

Supplementary Figure 1. Major events occurring during the trial.



The statistical analysis is a Cox Proportional Hazards cluster-analysis stratified by season, sex, and weight group.

Abbreviations: MRR, Mortality Rate Ratio; BCG, Bacillus Calmette-Guérin.

Supplementary Table 1. *Causes of death by randomization allocation and inclusion weight class.*

	Immediate BCG	**Control**	**MRR[a]**
	Deaths in percent (n/N)	Deaths in percent (n/N)	(95% CI)
By cause of death			
Infection	0.8% (14/1676)	1.3% (21/1677)	0.65 (0.33-1.28)
<1500 g	2.9% (2/70)	5.0% (3/60)	0.67 (0.12-3.85)
1500-1999 g	1.1% (2/186)	3.0% (6/200)	0.36 (0.08-1.72)
2000-2499 g	1.3% (3/233)	1.8% (4/228)	0.70 (0.15-3.25)
>2500 g	0.6% (7/1187)	0.7% (8/1189)	0.83 (0.29-2.33)
Non-infectious[c]	1.7% (28/1676)	1.4% (23/1677)	1.20 (0.70-2.07)
<1500 g	17.1% (12/70)	8.3% (5/60)	2.08 (0.72-5.97)
1500-1999 g	4.3% (8/186)	6.5% (13/200)	0.70 (0.28-1.71)
2000-2499 g	0.4% (1/233)	0.9% (2/228)	0.46 (0.04-5.05)
>2500 g	0.6% (7/1187)	0.3% (3/1189)	2.35 (0.61-9.12)
Further specification of the non-infectious causes of death			
Perinatal complications	0.5% (9/1676)	0.4% (7/1677)	1.46 (0.56-3.81)
<1500 g	1.4% (1/70)	1.7% (1/60)	0.95 (0.06-14.4)
1500-1999 g	0.5% (1/186)	1.0% (2/200)	0.62 (0.06-6.58)
2000-2499 g	0% (0/233)	0.4% (1/228)	-
>2500 g	0.6% (7/1187)	0.3% (3/1189)	2.41 (0.62-9.37)
Respiratory insufficiency and prematurity	1.1% (18/1676)	0.9% (15/1677)	1.11 (0.56-2.19)
<1500 g	15.7% (11/70)	5.0% (3/60)	3.08 (0.83-11.4)
1500-1999 g	3.2% (6/186)	5.5% (11/200)	0.60 (0.21-1.71)
2000-2499 g	0.4% (1/233)	0.4% (1/228)	0.90 (0.06-14.3)
>2500 g	0% (0/1187)	0% (0/1189)	1.02 (0.92-1.13)

Other/unknown cause[d]	0.7% (11/1676)	0.7% (12/1677)	0.93 (0.41-2.13)
<1500 g	1.4% (1/70)	1.7% (1/60)	0.87 (0.06-13.8)
1500-1999 g	2.2% (4/186)	1.0% (2/200)	2.21 (0.40-12.2)
2000-2499 g	0.9% (2/233)	0.4% (1/228)	1.89 (0.17-20.9)
>2500 g	0.3% (4/1187)	0.7% (8/1189)	0.50 (0.15-1.66)

[a] Cox Proportional Hazards cluster-analysis stratified by season, sex, and weight group.

[b] p for same effect between neonates weighing <1,500g and ≥1,500g = 0.12.

[c] Encompasses neonates that died from perinatal complications, respiratory insufficiency/prematurity and dehydration.

[d] Cause unknown or dehydration (1 BCG (1500-1999 g), 1 Control (<1500 g)).

Abbreviations: BCG, Bacillus Calmette-Guérin; CI, Confidence Interval; MRR, Mortality Rate Ratio.

Supplementary Table 2. *Causes of death by birth route.* [a]

	Immediate BCG	Control	MRR [b]
	Deaths in percent (n/N)	Deaths in percent (n/N)	(95% CI)
All causes of death			
Overall	3.1% (52/1676)	3.3% (55/1677)	0.94 (0.64-1.36)
Born by C-section	1.6% (21/1287)	1.5% (19/1284)	1.09 (0.59-2.01)
Normal birth	7.8% (30/386)	9.3% (36/388)	0.82 (0.50-1.32)
By cause of death			
Infection			
Overall	0.8% (14/1676)	1.3% (21/1677)	0.65 (0.33-1.28)
Born by C-section	0.6% (8/1287)	0.6% (7/1284)	1.06 (0.37-3.05)
Normal birth	1.6% (6/386)	3.6% (14/388)	0.42 (0.17-1.06)
Non-infectious [c]			
Overall	1.7% (28/1676)	1.4% (23/1677)	1.20 (0.70-2.07)
Born by C-section	0.6% (8/1287)	0.6% (8/1284)	1.03 (0.41-2.59)
Normal birth	5.2% (20/386)	3.9% (15/388)	1.29 (0.66-2.52)
Unknown cause			
Overall	0.6% (10/1676)	0.7% (11/1677)	0.93 (0.39-2.21)
Born by C-section	0.4% (5/1287)	0.3% (4/1284)	1.26 (0.34-4.70)
Normal birth	1.3% (5/386)	1.8% (7/388)	0.77 (0.25-2.35)

[a] Seven missing datapoints (0 deaths).

[b] Cox Proportional Hazards cluster-analysis stratified by season, sex, and weight group.

[c] Encompasses neonates that died from perinatal complications, respiratory insufficiency/prematurity and dehydration.

Abbreviations: BCG, Bacillus Calmette-Guérin; CI, Confidence Interval; MRR, Mortality Rate Ratio.

Supplementary Table 3. Deaths and *referrals to the pediatric ward from the NICU by year of inclusion.*

		Year					
		2013	**2014**	**2015**	**2016**	**2017**	**Total (2013-17)**
Included (BCG, control)		236 (119, 117)	961 (478, 483)	1117 (557, 560)	704 (361, 343)	335 (161, 174)	3353 (1676, 1677)
Infectious disease deaths n/N (%)	BCG	1.7% (2/119)	0.8% (4/478)	0.7% (4/557)	1.1% (4/361)	0% (0/161)	0.8% (14/1676)
	Control	0% (0/117)	1.7% (8/483)	2.0% (11/560)	0.6% (2/343)	0% (0/174)	1.3% (21/1677)
Non-infectious disease deaths n/N (%)	BCG	5.0% (6/119)	1.1% (5/478)	2.0% (11/557)	1.4% (5/361)	0.6% (1/161)	1.7% (28/1676)
	Control	4.3% (5/117)	1.0% (5/483)	1.4% (8/560)	1.2% (4/343)	0.6% (1/174)	1.4% (23/1677)
Deaths from unknown cause n/N (%)	BCG	0.8% (1/119)	0.2% (1/578)	0.9% (5/557)	0.3% (1/361)	1.2% (2/161)	0.6% (10/1676)
	Control	0% (0/117)	0.6% (3/483)	1.1% (6/560)	0.6% (2/343)	0% (0/174)	0.7% (11/1677)
Referred (BCG, control)		4 (1, 3)	18 (11, 7)	93 (49, 44)	74 (39, 35)	34 (16, 18)	223 (116, 107)
% of inclusions		1.7%	1.9%	8.3%	11%	10%	6.7%

Deaths at the pediatric ward (BCG, control)	2 (0, 2)	8 (6, 2)	23 (10, 13)	9 (6, 3)	1 (1, 0)	43 (23, 20)
% of referred	50%	44%	25%	12%	3%	19%

Immediate BCG and control numbers are given in brackets behind each number (BCG, control).