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Preclinical Evidence for Implementing a Prime-boost Vaccine Strategy for Tuberculosis

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Abstract

In this review, published peer-reviewed preclinical studies using prime-boost tuberculosis (TB) vaccine regimens in animal challenge models for tuberculosis have been evaluated. These studies have been divided into groups that describe prime-boost vaccine combinations that performed better than, equivalent to, or worse than the currently used BCG vaccine. Review of the data have revealed interesting findings, including that more than half of the published studies using BCG as a prime combined with a novel boost vaccine give better efficacy than BCG alone and that the greatest reduction in *Mycobacterium tuberculosis* (*M.tb.*) colonization of animal tissues is provided by viral vectored vaccines delivered intranasally. Careful evaluation of these data should assist in defining the value of prime-boost regimens for advancement into human TB vaccine trials and stimulate the development of criteria for choosing which vaccine candidates should be studied further.

Keywords

Tuberculosis; Vaccines; Animal Models

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Introduction

Tuberculosis remains a significant cause of mortality despite global efforts to impede its impact. Recent estimates suggest that 1.4 million people die from the disease each year, with the majority of cases occurring in Africa and South East Asia [1]. As recommended by WHO, *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) is widely administered to newborns or young infants in most of the world where tuberculosis is endemic, and it is the only vaccine available to protect against tuberculosis. A live attenuated strain of *M. bovis* developed nearly a century ago, BCG has been shown to be at least partially effective in children for the prevention of more serious forms of tuberculosis, such as tuberculous meningitis and miliary tuberculosis [2]. However, the protective efficacy of the vaccine is highly variable in adults, ranging from zero to eighty percent in a systematic review of data from clinical trials [3]. These findings highlight the need for a new TB vaccine, ideally one that effectively prevents both TB disease and transmission, to aid in the control of the global TB epidemic.

A prime-boost strategy that would combine the use of BCG or a BCG replacement vaccine with a subsequently administered novel vaccine candidate is currently a favored approach for introducing new TB vaccines into human populations. It is suspected that declining immunological memory after BCG vaccination is one cause of the vaccine's highly variable efficacy [4]. Consequently, there has been growing interest in the development of a novel vaccine strategy that introduces a new booster vaccine to enhance the immunity induced by initial BCG vaccination. Such a prime-boost regimen is a pragmatic approach since it is estimated that nearly eighty percent of newborns worldwide are or have been inoculated with BCG. Thus, an effective booster vaccine would benefit a significant proportion of the global population that has already received BCG vaccination [5]. Furthermore, BCG administration has been estimated to be a highly cost-effective public health intervention, even though BCG does not protect against adult pulmonary tuberculosis [6]. Overall, the development of a vaccine to boost BCG immunity may be the most pragmatic and effective strategy for testing and introducing new TB vaccines particularly for children and adolescents.

To ascertain which new TB vaccine candidates protect against *M.tb.* infection and progression of disease, many different prime-boost combinations have been studied in animal models. This research includes studies to test the preclinical efficacy of immunization strategies giving a BCG prime followed by a boost with a viral vector, recombinant protein with adjuvant or DNA vaccine. Alternatively, non-BCG vaccine candidates have been used as the priming vaccine followed by boosting with BCG or other heterologous platforms. In this article, we have reviewed the literature on prime-boost studies performed in animal models for TB vaccines – specifically those studies reporting on a heterologous prime-boost regimen, using the common search engines for published medical studies PubMed, [http://www.ncbi.nlm.nih.gov/pubmed/]ScienceDirect, [http://www.sciencedirect.com/] and Google Scholar, [http://scholar.google.com/]. In so doing, our major objective was to provide a summary report on the effectiveness of different preclinical prime-boost combinations to help interested investigators evaluate the success or failure of specific prime-boost vaccine combinations and strategies. We hope this report will be useful for those interested in assessing the overall value of the heterologous prime-boost strategy for TB vaccine development in human trials and stimulate the development of new strategies that advance the introduction of safe and effective new TB vaccines.

Comparing preclinical studies that evaluate combinations of TB vaccine candidates in animal challenge models for tuberculosis is a difficult task. In general, while the dataset evaluated in this analysis contains similarities within the general experimental models (such

as specific timepoints for determining *M.tb.* bacterial burden in the lungs and spleen following challenge), there are many details that lack standardization amongst the reviewed studies, including: BCG immunization strains, *M.tb.* challenge strains, prime-boost vaccination schedules, challenge dose and the time intervals between immunization and challenge. The need for standardization of preclinical experiments is difficult to achieve since, even when standardized challenge and vaccine strains are made available, investigators often prefer to use the strain(s) with which they are most familiar. Therefore, in summarizing the experiments we have focused our attention on the data that demonstrate protection as revealed by bacterial load (colony counts) in lung and spleen tissues and/or survival studies. Histopathology is noted but, in most cases, was confirmatory to the primary read-out (bacterial load or survival). Gross pathology scoring is a key read-out in large animal models (cattle, macaques) and was therefore also used in certain analyses as the primary measure of protective efficacy. In order to simplify our analyses we have discounted the differences among the experimental protocols and focused on the main outcome(s) of the studies. Since the immune response that correlates with vaccine protection has not been well defined, we have not compared the interesting immunological data provided in many of these studies. In addition, the data presented here are limited to that which has been published. As negative results are seldom published, these analyses are therefore limited by this selection bias. Nevertheless, interesting observations can be made that we hope will further the development of new TB vaccine candidates, establish the value of prime-boost immunization strategies and help identify specific vaccine combinations to carry forward into human clinical studies.

Results/Discussion

The primary purpose of this report is to serve as a useful tool to review the preclinical prime-boost studies that are of most interest to TB vaccine investigators. In the Tables, we have attempted to provide the critical information found in the preclinical studies, including: vaccines used, challenge strain and a summary of the protection results. Protection data that reflect the major outcomes of the publication are provided, and we have not attempted to include all results found in each paper or accompanying immunogenicity data. Interested readers are directed to the citation for a more detailed reading of the research. Although we have provided some numbers for protection in Tables 1 and 2 that are extracted from the publications, in some cases, where the actual data was not provided, these are our interpretation of the graphic data provided in the publication. Since in a number of cases it was difficult to evaluate the significance of the results because of a lack of raw data, we have limited our general identification of these to “better,” “equivalent,” or “worse” than the prime. Lastly, if a particular prime-boost preclinical publication was omitted, we apologize in advance.

Comparative analyses

To compare the published prime-boost studies that have been performed in animal models of tuberculosis we divided our analyses into BCG prime-TB vaccine boost studies that performed better than BCG (Table 1A), equivalent to BCG (Table 1B) or worse than BCG (Table 1C) and non-BCG prime-TB vaccine boost studies that performed better than BCG (Table 2A) or equivalent to BCG (Table 2B). All studies were grouped by animal species as well. The identifying name of the vaccines provided in the original citation is used in the comparative tables. For those vaccines where the antigen composition is not obvious, we have provided a companion table describing the primary content of the vaccines (Table 3).

Studies using BCG as the prime vaccine

Of the forty-five individual animal studies evaluated that used a BCG prime with a novel vaccine boost, twenty-six (58%) demonstrated better efficacy than BCG alone (Table 1A). Of these twenty-six, fourteen used a mouse model, seven were performed in guinea pigs, three in non-human primates and two in cattle. Fourteen of these successful booster vaccines contained Ag85 A or B (54%) as part of the booster vaccine. For comparison, of the seventeen BCG prime-boost studies that demonstrated equivalent protection to BCG (Table 1B) twelve were performed in mice and five in guinea pigs, with twelve of the boosters containing Ag85. Nine booster vaccines that showed better or equivalent protection following a BCG prime contained the ESAT-6 antigen not present in BCG. Strictly speaking, the lack of an immunological prime precludes reference to this vaccination as a "boost." However, ESAT-6 is often combined with the immunodominant antigen Ag85B as a fusion protein. Pre-existing responses against Ag85B may provide cognate help for responses to the fusion protein components. Three of the booster vaccines that were better than BCG and five of those that were equivalent to BCG were Ag85B-ESAT-6 heterodimer vaccines. Six of the vaccines tested in Table 1A demonstrated a total protection of the prime-boost immunization compared with naïve controls of ≥ 1.75 log CFU (highlighted boxes).

Two prime-boost studies gave results that showed that the protection provided by the BCG prime was lost following the booster vaccine (Table 1C). In one case the boost vaccine expressed a heat shock protein (hsp65) [7], and in the second case a recombinant BCG expressing ESAT-6 was followed by a DNA vaccine boost also expressing ESAT-6 [8]. Since in both cases a good immune response to the booster antigen is induced, it has been proposed that an "exaggerated immunity" against the DNA vaccine components could result in a worsening of the inflammation and disease process [8].

One vaccine, the vaccinia vectored MVA85A, was successful in boosting BCG protection in four different animal models: a mouse, guinea pig and rhesus model of tuberculosis and an *M. bovis* infection model in cattle, as indicated by the relevant protection data. Also, the M72F vaccine which contains a fusion protein of Mtb39a and Mtb32 together with an adjuvant, gave better protection than BCG when used as a booster vaccine for BCG in cynomologous monkeys and produced equivalent protection in mouse and guinea pig models. Both boosting BCG with Hybrid 1 (a recombinant Ag85B-ESAT-6 subunit vaccine delivered with IC31 adjuvant) and Hyvac4 (a recombinant Ag85B-TB10.4 subunit vaccine delivered with IC31 adjuvant) gave better protection than BCG alone in mice and guinea pigs, respectively. Having successfully completed preclinical testing in at least one animal model, all three of these vaccines are currently progressing in human clinical trials. In fact, MVA85A is presently being studied as a booster vaccine in infants previously immunized with BCG in South Africa. It is important to point out, however, that in some individual studies these same vaccines have not shown superiority as a booster for a BCG prime [9] and that this has not precluded entry of the vaccines into human clinical studies. This indicates that the overall portfolio of pre-clinical efficacy has been used to support the progression to clinical trials rather than stand-alone studies, which illustrates the importance of conducting pre-clinical studies in multiple animal species.

In studies using plasmid DNA as a boost for BCG, three have shown superior protection and two equivalent protection, suggesting that nucleic acid-based vaccines expressing protective antigens may be of value. Two of the DNA boosts providing superior protection over BCG alone express heat shock proteins that have also been shown to give protection in animals infected with *M.tb.* [10]. It should be noted that safety issues with the use of heat shock antigens in humans have been the subject of some discussion [11,12].

Figure 1 is a schematic showing the log reduction of CFU compared with BCG for those booster vaccines that have demonstrated superior protection in preclinical studies and have used bacterial load as a measure of protection (limited to those studies shown in Table 1A). Protection data are provided for the lung (Fig. 1A) and spleen (Fig. 1B) where available. Most data show an enhanced reduction in *M.tb.* colony counts of approximately 0.5 log CFUs in boosted animals compared with the BCG control in the relevant animal model. The greatest reduction seen is provided by the two viral vectored vaccines, MVA85A and AdAg85A. However, these data reflect intranasal administration which is not directly comparable to most other studies, including current human studies [13]. The results do, however, point to the potential enhancement of the prime-boost regimen when combined with the induction of mucosal immunity by intranasal delivery. A caveat is that the data shown in Figure 1 and Table 1A need to be considered within the context of the extent of protection provided by BCG compared to naïve controls. For example, if ≤ 0.3 log CFU reduction by BCG immunization alone is used as a cutoff for "weak BCG protection" (shown by an asterisk in Fig. 1) then four of the boost vaccines shown in Figure 1 may have an advantage in demonstrating enhanced protection beyond BCG alone. Only a few studies have demonstrated some ability of BCG to boost BCG (see H-kBCG results in Table 1A and Fig. 1). Most preclinical studies, however, suggest that revaccination with BCG does not enhance protection [14-16], and this approach has been questioned by the lack of improved efficacy observed in the REVAC human studies trial in Brazil [17].

Studies using prime vaccines other than BCG

A number of prime-boost preclinical studies not using BCG for the initial immunization have been published. Table 2 summarizes those studies demonstrating better protection than BCG (Table 2A) and those showing protection equivalent to BCG (Table 2B). Except for the studies by Kolibab et al. 2010 [18] and Elvang et al. 2009 [19], most of the studies used a DNA vaccine for the primary immunization. In eight prime-boost regimens protecting better than BCG alone (Table 2A), four of the DNA primes expressed Ag85 (A or B), three expressed heat shock proteins and one expressed a ribosomal protein. In general, surprisingly few *M.tb.* antigens have been tested as DNA vaccine constructs in prime-boost TB vaccine studies, which may reflect the lack of success of DNA vaccines in general against human infectious disease. Nearly all regimens used a dose of BCG vaccine as the secondary immunization while one used an adenovirus expressing Ag85A matched to the DNA prime [20]. Of interest is the fact that four different BCG strains (Glaxo, Pasteur, GL2 [21], and Tokyo) were used in the DNA-prime/BCG boost studies, suggesting that BCGs with different genotypes give similar results in this combination. Therefore, taken together these studies provide a foundation for pursuing DNA vaccines as a part of a "reverse" prime-boost strategy for tuberculosis using live BCG vaccines for the second immunization. Three of the vaccines tested in Table 2A demonstrated a total protection of the prime-boost immunization compared with naïve controls of ≥ 1.75 log CFU (highlighted boxes).

However, not all prime-boost regimens using DNA vaccines were as successful as those shown in Table 2A. Eight prime-boost preclinical studies using primes other than BCG demonstrated no increased protection compared to BCG alone (Table 2B). Five used DNA vaccines as the primary immunization, one used the adjuvanted fusion protein rH4 (Ag85B fused with TB10.4 – an ESAT 6-like antigen), another used the fusion protein E6-85 and another used an MVA vectored vaccine expressing five *M.tb.* antigens. Various vaccine constructs were used as boosts including viral and bacterial vectors, protein and live BCG. In general, all these prime-boost regimens showed a slight but not statistically significant improvement compared with BCG, which was used as the positive control. Although results are limited to date, prime-boost regimens that do not include BCG are of interest since they

could be considered novel approaches for human immunization programs, particularly in populations where live vaccines are not recommended.

Using BCG as a primary vaccine in prime-boost strategies

Comparative analyses of BCG experiments in animal models of tuberculosis suffer from some of the same issues that have troubled the interpretation of human clinical trials with BCG vaccines for a number of years. Primary among these are the genotypic and phenotypic differences among BCG strains and the subsequent potential for variable immunogenicity and efficacy. In addition, the use of different culture conditions to propagate BCG and, in the case of commercial BCG products, differences in manufacturing methods can result in variable BCG vaccines. Indeed, recent evidence confirms older observations, indicating that commercial BCG contains two genotypes based on specific frequently-occurring genetic deletions [22]. The variable characteristics of BCG vaccines need to be considered during the interpretation of experimental results. However, as observed in the experiments evaluated in this report, protection with BCG is a highly consistent finding in animal models of TB. In fact, a key issue in using TB animal models for studying the ability of vaccine candidates to boost primary BCG immunization is that BCG commonly provides a 1 to 1.5 $\log_{10}$ reduction in CFU in the lungs and spleen of mice as observed in a number of studies summarized in this analysis. This consistent reduction in *M.tb.* colonies recovered from animal tissues may reflect the ‘maximum’ protective effect of vaccines in these models. This allows only a minimal “window” to detect additive or synergistic effects due to a booster vaccine, which would be difficult to demonstrate in a statistically significant manner. Using a “weak” BCG has been offered as a solution to this problem and is worth studying since this may mimic the poor estimates of efficacy observed in some human BCG vaccine studies. However, possible differences resulting from boosting a potent compared with a weak BCG would need to be considered, as would the differences in sensitivity of various animals to BCG. For example, these differences may account for the more limited ability to show BCG boosting in guinea pigs and non-human primates compared with mice.

A better approach may be to use a “hypervirulent” *M.tb.* clinical strain as the challenge strain in preclinical vaccine studies, which have demonstrated reduced protection by BCG compared with other challenge strains in mouse studies [23]. This strategy may provide a better model for finding vaccines that are better than BCG alone. Alterations in study design, such as lengthening the time between prime and boost and evaluating efficacy at later time points [24,25] have been shown to increase the discriminative power to show an improvement upon BCG. Indeed in reviewing the data for this report, we have found a trend towards better protection linked to increasing the time interval between boost and *M.tb.* challenge in the animal models. However, there is a wide variation in the time intervals used between challenge and sacrifice among investigators. All of these approaches are aimed at reducing the efficacy of BCG in an experimental setting to improve the ability to screen candidates and allow vaccine developers to prioritize the most promising boost candidate for further development.

The last decade has seen a surge of activity in evaluating new TB vaccine candidates as boosters for BCG as well as modified BCGs in human clinical testing. At least 3 modified BCGs for better priming and 8 boosting candidates have entered into clinical testing during this period [13]. This approach is based on the hypothesis that a stronger and more durable immune response to *M.tb.* antigens achieved with prime-boost regimens will produce better and more durable protection than is currently provided by newborn or early infant immunization with BCG alone. The case for this approach has been somewhat bolstered by many of the prime-boost studies utilizing an *M.tb.* challenge summarized herein. When measured in animals, it has not been difficult to show more robust cell-mediated immune

responses following many of the prime-boost regimens tested compared to the responses to BCG alone, which are commonly quite modest. It must be recognized, however, that the increased protection provided by the investigational vaccine prime-boost combinations studied in the animal models do not for the most part provide very large increases (e.g., multiple logs of CFUs or sterilization) in protection compared with that provided by BCG alone. We are left then with a quandary: Is BCG too protective in animal models to see differences in challenge tests, or are there instead shortcomings in the animal models used or in the new vaccine candidates, as reflected by the current data? The answer may only be ascertainable by evaluation of the most promising prime-boost combinations in human efficacy trials; such evaluation is already at an early stage with one candidate, MVA85A, which is well advanced in a proof-of-concept study in infants in South Africa.

The studies described in this review reflect the intrinsic difficulties in providing reproducible results in animal testing that is complicated by a number of variables including: 1) the potency of the vaccines, 2) the *M.tb.* challenge strain used, 3) differences in immunization and challenge regimens, and 4) the condition of the animals themselves. As a result of these confounders in the experiments reviewed, the significance of this comparison is open to criticism. This issue could be alleviated, in part, by head-to-head animal experiments comparing different vaccines in the same study. However, it seems clear that some booster vaccines fail to show synergistic efficacy compared with BCG alone in some experiments, while in other experiments and with other vaccine combinations a reasonable difference can be found when using a measure of bacterial load in tissues, survival or histopathological findings as measures of protection. So, can these experiments in animals be used to support a decision for entering a new vaccine candidate into a prime-boost immunization program in human subjects?

While it is commonly accepted that no animal model very closely resembles human tuberculosis disease, animal cells and tissues, including the lung, are infected with *M.tb.* and demonstrate histopathological changes including influxes of inflammatory cells. This and the fact that some vaccine preparations can reduce the bacterial load in tissues and extend survival of diseased animals while others do not indicate that models are useful as a first step in identifying promising vaccine candidates. Since BCG is given to infants at birth in a number of TB-endemic countries, a BCG prime immunization followed by a booster vaccine candidate is a pragmatic strategy for the introduction of new TB vaccines in target countries. As observed in Tables 1A and 2A, in a number of cases, vaccines tested in BCG prime-boost strategies in animal models can improve upon BCG. These data can be seen as either a starting point for further preclinical studies or as evidence for submission to a regulatory authority for approval of human clinical trials. One difficulty is how to interpret the data from vaccines in prime-boost studies that contain candidate antigens that both succeed and fail in animal models. Here, reproducibility of multiple experiments, modification of animal models, head-to-head comparability studies and the use of strict go, no-go criteria for selection of the best vaccines will be required.

In summary, these analyses indicate: 1) that animal models provide evidence that certain new TB vaccines can boost the effectiveness of BCG, which is commonly used to immunize infants in countries endemic for tuberculosis, 2) that delivery of booster vaccines that elicit mucosal immunity, particularly viral vectored TB vaccines, are most efficient in eliciting protective immunity in animals previously immunized with BCG, 3) that there should be further analyses of DNA vaccines as part of a prime-boost regimen, particularly in providing a proof of concept that they can elicit effective immunity in humans and (4) that there is a need for including novel antigens in prime boost studies since more than half of the vaccine candidates that successfully boosted BCG in the studies reviewed contained the Ag85 antigen. Further, these comparative analyses suggest that in the future, standardization of

animal models for tuberculosis and protocols for measuring vaccine effects would be a useful tool for comparing different products. Also, since it can be difficult to persuade vaccine sponsors to add their vaccine into head-to-head comparative studies, particularly if the vaccines have entered the clinic, performing independent pre-clinical experiments in a standardized model may be the most pragmatic approach to obtain comparative data. In addition, this report highlights the value of sharing data via publication in peer-reviewed journals (including publication of negative results) for comparing different vaccines and vaccine regimens and for determining which vaccines and immunization profiles should move into human clinical studies. In the future, animal models for tuberculosis will continue to be valuable as they are refined to address new questions raised by the outcomes of the TB vaccine trials in human populations.

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Highlights of Brennan et al. *Preclinical Evidence for Implementing a Prime-boost Vaccine Strategy for Tuberculosis*

- Over half of the BCG prime-boost studies provided better protection than BCG alone.
- Ag85A and B are the most studied *M.tb.* antigens in prime-boost animal models.
- Intranasally delivered viral vectored vaccines gave the most enhanced protection.
- A non-BCG prime with a BCG boost regimen may be a safer option for infants.
- Animal models of TB should be refined pending efficacy data in humans.

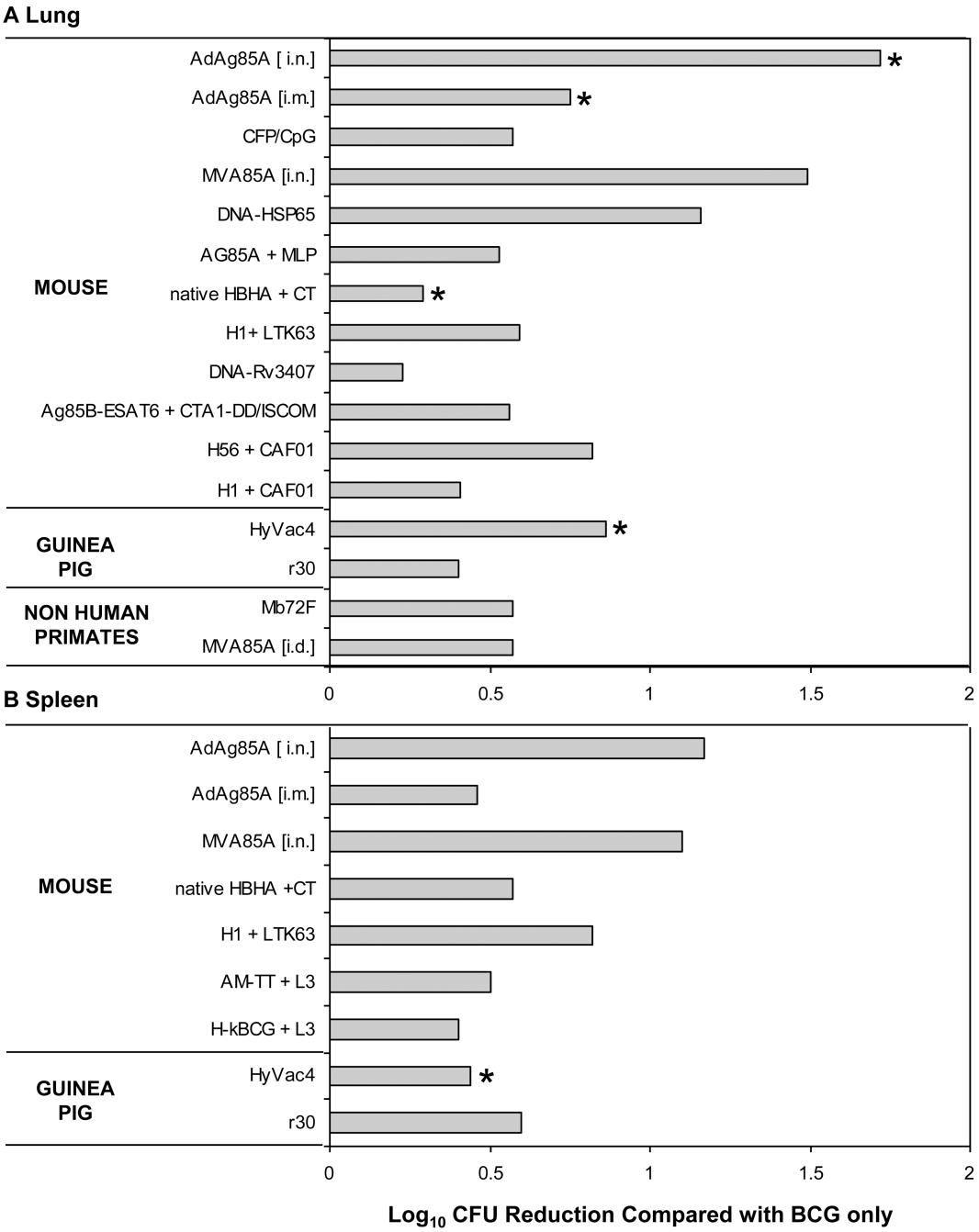

Figure 1. Log reduction of CFU provided by prime-boost vaccine compared with BCG alone. (See Table 1A vaccines.)

Prime-boost combinations that protect better than BCG alone. Certain investigational TB vaccines reduce the colonization of lungs (A) and spleens (B) when used to immunize animals previously primed with BCG vaccines and then challenged with virulent strains of *M.tb*. Data is taken from the studies described in Table 1A that include CFU data from lungs and spleen compared with a BCG vaccine control. Experiments where protection by BCG alone was ≤ 0.3 log10 CFU reduction are indicated by an asterisk (*). Statistical analyses were not performed for this set of data due to the lack of access to raw data in certain studies.

* Indicates experiments where protection from BCG alone was ≤ 0.3 log10 CFU reduction.

Table 1

Table 1A: BCG Prime - TB Vaccine Boost Studies Performing Better than BCG.

Model	Vaccines		Challenge	Boost/ChallengInterval	Challenge/ Sacrifice Interval	Protection				Citation
						Bacterial Load(Log_{10}CFU)[a]		Histopathology/ Gross pathology	Survival[b]	
	Prime	Boost				vs. BCG	vs. Naïve			
Mouse BALB/c	BCG Connaught [s.c.]	AdAg85A [i.n.]	H37Rv [i.n, 10^5 cfu]	4 weeks	5 days	L: -1.72 * S: -1.17 *	L: -2 S: -1.50 *	N/A	N/A	[26]
Mouse BALB/c	BCG Connaught [s.c.]	AdAg85A [i.m.]	H37Rv [i.n, 10^5 cfu]	4 weeks	5 days	L: -0.75 * S: -0.46 *	L: -1.02 * S: -0.79 *	N/A	N/A	[26]
Mouse BALB/c	BCG Moreau [s.c.]	CFP/CpG protein [s.c.]	H37Rv [i.t., 10^5 bacilli]	60 days	30 days	L: -0.57 * S: N/A	L: -1.75 * S: N/A	Improvement over BCG	N/A	[27]
Mouse BALB/c	BCG Pasteur [i.n.]	MVA85A [i.n.]	H 3 7 R v [aerosol, 250 cfu]	4 weeks	6 weeks	L: -1.49 * S: -1.10 *	L: -2.5 S: -1.5	N/A	N/A	[28]
Mouse BALB/c	BCG Pasteur [i.n]	DNA-HSP65 [i.m.]	H 3 7 R v [i.t., 10^5 bacilli]	15 days	30 days	L: -1.16 S: N/A	L: -3.1 S: N/A	Improvement over BCG	N/A	[29]
Mouse, C57BL/6J	BCG Pasteur [s.c.]	Ag85A + MPL [s.c.]	H37Rv [aerosol, 50 cfu]	5 months	30 days	L: -0.53 S: N/A	L: -1.00 S: N/A	Improvement over BCG	N/A	[30]
Mouse BALB/c	BCG Pasteur [s.c.]	native HBHA + CT [s.c.]	*M. bovis* [i.n, 10^7 cfu]	4 weeks	3 weeks	L: -0.29 * S: -0.57 *	L: -0.48 * S: -1.12 *	N/A	N/A	[31]
Mouse BALB/c × C57BL/6 F1	BCG SSI [s.c.]	H1 + LTK63 [i.n.]	*M. t b.* E r d m a n [aerosol, ~100 cfu]	8 weeks	6 weeks	L: -0.59 S: -0.82	L: -0.96 * S: -1.15 *	N/A	N/A	[32]
Mouse C57BL/6J	BCG SSI [s.c.]	AM-TT + L3 [i.n.]	H 3 7 R v [i.v.,3x10^5 cfu]	10 weeks	8 weeks	L: NS S: -0.5	L: -1.23 * S: -1.04 *	Improvement over Naïve	N/A	[33]
Mouse C57BL/6J	BCG SSI [s.c.]	H-kBCG + L3 [i.n.]	H 3 7 R v [i.v.,3x10^5 cfu]	10 weeks	8 weeks	L: NS S: -0.4	L: -1.08 * S: -0.94 *	No significant improvement over Naïve	N/A	[33]
Mouse BALB/c	BCG SSI 1331 [i.v.]	DNA-*Rv3407* [i.m.]	H 37Rv [aerosol, ~200 bacilli]	21 days	30 days	L: -0.23 S: N/A	L: -0.72 * S:N/A	N/A	N/A	[34]
Mouse C57BL/6J	BCG SSI 1331 [s.c.]	Ag85B-ESAT6 + CTA1-DD/ISCOM [i.n.]	*M.tb.* Erdman [aerosol, ~100 cfu]	6 weeks	6 weeks	L: -0.56 S: NS	L: -0.99 * S: -0.85 *	N/A	N/A	[35]
Mouse CB6F1	BCG SSI 1331 [s.c.]	H56 + CAF01	*M.tb.* Erdman [aerosol, ~100 cfu]	6 weeks	24 weeks	L: -0.82 S: NA	L: -1.87 S: NA	N/A	N/A	[36]
Mouse CB6F1	BCG SSI 1331 [s.c.]	H1 + CAF01	*M.tb.* Erdman [aerosol, ~100 cfu]	6 weeks	24 weeks	L: -0.41 S: NA	L: -1.46 S: NA	N/A	N/A	[36]
Guinea Pig Hartley (female)	BCG Pasteur [i.d.]	ID93 + GLA-SE [s.c.]	H 3 7 R v [aerosol, 20-50 bacilli]	4 weeks	N/A	L: N/A S: N/A	L:N/A S: N/A	Improvement over BCG	Naïve: 0% BCG: 33% P/B: 87.5%[61 wks p.c.]	[37]

Table 1A: BCG Prime - TB Vaccine Boost Studies Performing Better than BCG.

Model	Vaccines		Challenge	Boost/ChallengInterval	Challenge/ Sacrifice Interval	Protection				Citation
						Bacterial Load(Log_{10}CFU)[a]		Histopathology/ Gross pathology	Survival[b]	
	Prime	Boost				vs. BCG	vs. Naïve			
Guinea Pig Dunkin-Hartley	BCG [s.c.]	1.MVA85A [i.d.]2. FP9.85A [i.d.]	H37Rv [aerosol, 500 cfu]	6 weeks	26 weeks	L: N/A S: N/A	L: N/A S: N/A	N/A	Naïve: 0% BCG: 33% P/B: 100%[26 wks p.c.]	[38]
Guinea Pig Hartley	BCG SSI 1331 [i.d.]	AdAg85A [i.m.]	H37 R v [aerosol, 10-15 cfu]	6 weeks	40 days for CFU counts	L: NS S: NS	L: NS S: -2.65 *	N/A	Naïve: 177 days BCG: 308 days P/B: 412 days	[39]
Guinea Pig Hartley	BCG SSI 1331 [i.d.]	AdAg85A [i.n.]	H37Rv [aerosol, 10-15 cfu]	6 weeks	40 days for CFU counts	L: NS S: NS	L: -0.81 * S: -2.57 *	N/A	Naïve: 177 days BCG: 308 days P/B: 467 days	[39]
Guinea Pig Hartley	BCG SSI 1331 [i.d.]	HyVac4 [i.m.]	*M.tb.* Erdman [aerosol, 10-50 cfu]	8 weeks	56 weeks (or death)	L: -0.86 S: -0.44	L: -1.01 S: -0.61	Improvement over BCG	Naïve: 0% BCG: 7% P/B: 17%[56 wks p.c.]	[40]
Guinea Pig Hartley (male)	BCG Tice [i.d.]	r30 [i.d.]	*M. tb.* E r d m a n [aerosol, ~100 cfu]	3-4 weeks	10 weeks	L: -0.4 S: -0.6	L: -1.8 S: -2.7	N/A	N/A	[41]
Guinea Pig Dunkin-Hartley	BCG Tice [s.c.]	P-rAg85B [aerosol]	H 3 7 R v [aerosol, ~10-15 bacilli]	6 weeks	4 weeks	L: NS S: NS	L: -1.46 S: -2.85	Improvement over BCG	N/A	[42]
Cynomolgus	BCG Pasteur [i.d.]	Mtb72F + AS02A [i.m.]	*M.tb.*Erdman [i.t., 500 cfu]	4 weeks	76 weeks(or death)	L: -0.57 * S: N/A	L: -1.29 * S: N/A	Improvement over BCG in gross pathology score	Naïve: 33% BCG: 50% P/B: 67%[76 wks p.c.]	[43]
Rhesus	BCG SSI [i.d.]	MVA85A [i.d.]	*M tb.*Erdman [i.t., 103 cfu]	9 weeks	16/17 wks (orhumaneendpoint)	L: -0.57 S: N/A	L: -1.0 S: N/A	Improvement over BCG in gross pathology score	N/A	[44]
Cynomolgus	BCG Tokyo [i.d.]	HJV liposome/HSP65 DNA + IL-12 DNA [i.m.]	*M.tb.* Erdman [i.t., 500 cfu]	4 weeks	N/A	L: N/A S: N/A	L:N/A S:N/A	N/A	Naïve: 50% BCG: 33% P/B: 100%[12 mos p.c.]	[45]
Cattle Friesian	BCG SSI [s.c.]	MVA85A [i.d]	*M. bovis* (strain AF 2122/97) [i.t.,2x10^3 cfu]	6 weeks	14 weeks	LN: NS	LN: NS	Improved multi-parameter pathology score over BCG	N/A	[46]
Cattle Friesian	BCG SSI [s.c.]	Ad85A [i.d.]	*M. bovis* (strain AF 2122/97) [i.t.,2x10^3 cfu]	6 weeks	14 weeks	LN: NS	L: -1.729	Improved multi-parameter pathology score over BCG	N/A	[46]

Table 1B: BCG Prime - TB Vaccine Boost Studies Performing Equivalent to BCG.

Model	Vaccine		Challenge	Boost Challenge Interval	Protection				Citation
					Bacterial Load(Log_{10}CFU)[a]		Histopatholog Gross patholoy	Survival[b]	
	Prime	Boost			vs. BCG	vs. Naïve			
Mouse C57BL/6J	BCG Moreau [oral]	85B-ESAT6 + LTK63 [i.n.]	H37Rv [aerosol, ~100 cfu]	6-7 weeks	L: NS S: NS	L: -1.14 * S: -1.20 *	N/A	N/A	[47]
Mouse C57BL/6J	BCG Moreau [s.c.]	85B-ESAT6 + DDA + MPL[s.c.]	H37Rv [aerosol, ~100 cfu]	6-7 weeks	L: NS S: NS	L: -1.14 * S: -1.05 *	N/A	N/A	[47]
Mouse C57BL/6J	BCG Pasteur [s.c.]	rMtb72F + AS02A [i.m.]	H37Rv [aerosol, 100 bacilli]	6 weeks	L: NS S: N/A	L: -0.60 * S: N/A	N/A	N/A	[48]

Table 1B: BCG Prime - TB Vaccine Boost Studies Performing Equivalent to BCG.

Model	Vaccine		Challenge	Boost Challenge Interval	Protection				Citation
					Bacterial Load(Log_{10}CFU)[a]		Histopatholog Gross patholoy	Survival[b]	
	Prime	Boost			vs. BCG	vs. Naïve			
Mouse BALB/c	BCG SSI 1331 [s.c.]	MVA85A [i.d.]	*M.tb.* Erdman [aerosol, 200 cfu]	4 weeks	L: NS S: N/A	L: -0.5 * S: N/A	N/A	N/A	[9]
Mouse BALB/c	BCG SSI 1331 [s.c.]	MVA85A [i.d.]	H37Rv [aerosol, 200 cfu]	4 weeks	L: NS S: N/A	L: -1.0 * S: N/A	N/A	N/A	[9]
Mouse C57BL/6J	BCG::RD1 [s.c]	CyaA-ESAT6 [s.c.]	H 3 7 Rv[aerosol, ~100 cfu]	15 days	L: NS S: NS	L: -1.91 * S: -2.93 *	N/A	N/A	[49]
Mouse C57BL/6J	BCG::RD1 [s.c.]	CyaA-85A [i.v.]	H37Rv [aerosol, ~100 cfu]	15 days	L: NS S: NS	L: -2.06 * S: -3.01 *	N/A	N/A	[49]
Mouse BALB/c	*DureC hly*+ BCG [s.c.]	MVA85A [i.d.]	*M.tb.* Erdman [aerosol, 200 cfu]	4 weeks	L: NS S: N/A	L: -1.85 * S: N/A	N/A	N/A	[9]
Mouse BALB/c	*DureC hly*+ BCG [s.c.]	MVA85A [i.d.]	H37Rv [aerosol, 200 cfu]	4 weeks	L: NS S: N/A	L: -1.9 * S: N/A	N/A	N/A	[9]
Mouse BALB/c	*M. Bovis* BCG (strain GL2) [i.v.]	Ag85A DNA [i.m.]	H37Rv [i.v, $2x10^5$ cfu]	100 days	L: N/A S: N/A	L: N/A S: N/A	N/A	Naïve: 11.5 wks BCG: 19 wks P/B: 21 wks	[21]
Mouse C57BL/6J	Myc3504 [oral]	8 5 B - E S AT6 + LTK63 [i.n.]	H37Rv [aerosol, ~100 cfu]	6-7 weeks	L: NS S: NS	L: -1.54 * S: -1.82l *	N/A	N/A	[47]
Mouse C57BL/6J	Myc3504 [s.c.]	85B-ESAT6 + DDA + MPL [s.c.]	H37Rv [aerosol, ~100 cfu]	6-7 weeks	L: NS S: NS	L:- 0.94 * S:- 1. 1 7 *	N/A	N/A	[47]
Guinea Pig Dunkin-Hartl	Myc3504 [oral]	85B-ESAT6 + LTK63 [i.n]	H37Rv [aerosol, 500 cfu]	10 weeks	L: N/A S: N/A	L: N/A S: N/A	N/A	Naïve: 92 days BCG: 166 days P/B: 179 days	[47]
Guinea Pig Hartley	B CG Pasteur [i.d.]	Mtb72F DNA [i.m.]	H37Rv [aerosol, 20 bacilli]	6 weeks	L: N/A S: N/A	L: N/A S: N/A	Improvement over BCG	Naïve: 0% BCG: 40% P/B: 60%[108 wks p.c.]	[48]
Guinea Pig Hartley	B CG Pasteur [i.d.]	rMtb72F + AS02A [i.d.]	H 3 7Rv [aerosol, 20 bacilli]	6 weeks	L: N/A S: N/A	L: N/A S: N/A	Improvement over BCG	Naïve: 0% BCG: 20% P/B: 60%[113 wks p.c.]	[48]
Guinea Pig Dunkin-Hartley	B CG SSI 1331 [s.c]	MVA85A [i.d.]	H37Rv [aerosol, 1000 cfu]	8 weeks	L: NS S: NS	L: -2.36 S: -1.95	N/A	Naïve: 0% BCG: 100% PB: 100%[17 weeks p.c.]	[50]
Guinea Pig Dunkin-Hartley	B CG SSI 1331 [s.c]	BCG SSI 1331 [s.c]	H 3 7 R v [aerosol, 1000 cfu]	8 weeks	L:NS S: NS	L: -2.45 S: -2.31	N/A	Naïve: 0% BCG: 100% PB: 100%[17 weeks p.c.]	[50]

Table 1C: BCG Prime - TB Vaccine Boost Studies Performing Worse than BCG.

Model	Vaccine		Challenge	Challenge Interval	Protection				Citation
					Bacterial Load ($Log_{10}CFU$)[a]		Histopathology/Gross pathology	Survival[b]	
	Prime	Boost			vs.BCG	vs. Naïve			
Mouse BALB/c	BCG Moreau [i.d.]	pVAXhsp65 [i.m.]	H37Rv [i.t., 10^5 cfu]	2 weeks	Trend toward lower cfu reduction in lungs compared with BCG	L: NS S: N/A	No significant improvement over Naïve	N/A	[7]
Guinea Pig	rBCGE6 [i.d.]	DNAE6 [i.m.]	H 3 7 R v [aerosol, 50-100 bacilli]	6 weeks	Trend toward lower cfu reduction in lungs & spleen compared with BCG	L: NS S: NS	No significant improvement over Naïve	N/A	[8]

[a]Bacterial load is measured as reduction (-) or increase (+) in log10 cfu counts in lung (L), spleen (S), or lymph node (LN) tissue of experimental prime-boost group as compared to BCG-vaccinated only (BCG) or naive groups.

[b]Survial is measured as percentage of surving animals in naive, BCG-vaccinated only (BCG), and experimental prime-boost (P/B) groups at given timepoint or as mean survival time for these groups.

NS (not statistically signifcant); N/A (data not reported);

*(value estimated based on graphic data); s.c. (subcutaneous); i.m. (intramuscular); i.d. (intradermal); i.n. (intranasal); i.t. (intratracheal); p.c. (post challenge)

Highlighted fields indicate experiments where the *M.tb.* load reduction for prime-boost versus naïve controls was ≥ 1.75 log total CFU in the lungs.

Table 2

Table 2A: Non-BCG Prime - TB Vaccine Boost Studies Performing Better than BCG.

Model	Vaccine		Challenge	Boost/Challenge Interval	Protection				Citation
					Bacterial Load(Log_{10}CFU)[a]		Histopathology/ Gross pathology	Survival[b]	
	Prime	Boost			vs. BCG	vs. Naïve			
Mouse BALB/c	Ag85A DNA [i.m.]	BCG (strain GL2) [i.v.]	H37Rv [i.v.,2x10^5 cfu]	100 days	L: NS S: NS	L: -1.63 S: NS	N/A	Naïve: 17 wks BCG: 23 wks P/B: 32 wks	[21]
Mouse BALB/c	Apa/65/70 (DNA) [i.m.]	BCG GSK [i.d.]	H37Rv [i.v., 10^5 cfu]	12-15 weeks	L: -0.65 * S: N/A	L: -3.01 * S: N/A	N/A	N/A	[51]
Mouse C57BL/6	Apa/65/70 (DNA) [i.m.]	BCG GSK [i.d.]	H37Rv [i.v., 10^5 cfu]	12-15 weeks	L: -0.21 * S: N/A	L: 1.02 * S: N/A	N/A	N/A	[51]
Mouse BALB/c	Apa/65/70 (DNA) [i.m.]	BCG Pasteur 1173 [i. d.]	*M. bovis* [i.v., 10^3 cfu]	6 weeks	L: NS S: -0.67	L: -1.29 S: -1.43	N/A	N/A	[52]
Mouse BALB/c	DNA Ag85A [i.m.]	AdAg85A [i.n.]	H37Rv [i.n., 10^4 cfu]	10 weeks	L: -0.96 * S: NS	L: -2.0 * S: -1.63 *	N/A	N/A	[20]
Mouse BALB/c	PstS3-Ag85A DNA [i.m.]	BCG (strain GL2) [i.v.]	H37Rv [i.v.,2x10^5 cfu]	100 days	N/A	N/A	N/A	Naïve: 17 wks BCG: 26.5 wks P/ B: 33 wks	[21]
Guinea Pig Dunkin-Hartley	P stS3-Ag85A DNA [i.m.]	BCG SSI 1331 [s.c]	H37Rv [aerosol, 1000 cfu]	10 weeks	L: NS S: NS	L: -1.28 S: -0.45	N/A	Naïve: 0% BCG: 33% P/B: 67% [17 weeks p.c.]	[50]
Calves	DNA Ag85B, 64, MPT-83 [i.m.]	MPT-BCG Tokyo [i.m.]	*M bovis* [i.t., 10^7 cfu]	14 weeks	L: -2.59 S: N/A	L: -4.13 S:N/A	Improvement over Naïve	N/A	[53]

Table 2B: Non-BCG Prime - TB Vaccine Boost Studies Performing Equivalent to BCG.

Model	Vaccine		Challenge	Boost/Challenge Interval	Protection				Citation
					Bactorial Load(Log_{10}CFU)[a]		Histopathology Gross patholog	Survival[b]	
	Prime	Boost			vs. BCG	vs. Naïve			
Mouse C57BL/6	E6-85 [s.c.]	M V A/IL-15/5Mtb [s.c.]	*M. t b.* Erdman [aerosol, 200 cfu]	1 month	L: NS S: NS	L: -1.15 S: NS	N/A	N/A	[18]
Mouse C57BL/6	MVA/IL-15/5Mtb [s.c.]	E 6 - 8 5 [s.c.]	*M.tb.* Erdman [aerosol, 200 cfu]	1 month	L: NS S: NS	L: -0.86 S: NS	N/A	N/A	[18]
Mouse C57BL/6	DNA expressing ESAT-6+MPT63 [i.m.]	MVA expressing E S A T- 6 +MPT63 [10^6 pfu,i.d]	H37Rv [i.p.,5x10^6 cfu]	2 weeks	L: N S S: NS	L: -0.54 * S: NS	N/A	N/A	[54]

Table 2B: Non-BCG Prime - TB Vaccine Boost Studies Performing Equivalent to BCG.

Model	Vaccine		Challenge	Boost/Challenge Interval	Protection				Citation
					Bactorial Load(Log_{10}CFU)[a]		Histopathology Gross patholog	Survival[b]	
	Prime	Boost			vs. BCG	vs. Naïve			
Mouse CB6F1 [C57BL × BALB/c]	rH4 [s.c.]	Ad-H4 [s.c.]	*M.tb.* Erdman [aerosol, 50 cfu]	6 weeks	L: NS S: +0.69 *	L: -1.2 S: -0.79 *	N/A	N/A	[19]
Mouse C57BL/6	DNA expressing ESAT-6 + Ag85A [i.m.]	BCG Pasteur 1173 [s.c.]	*M. bovis* [aerosol, 10 bacilli]	6 weeks	L: NS S: NS	L: NS S: -1.11	N/A	N/A	[55]
Mouse C57BL/6	DNA expressing ESAT-6 + Ag85A [i.m.]	WAg520 [s.c.]	*M. bovis* [aerosol, 10 bacilli]	6 weeks	L: NS S: NS	L: NS S: - 1. 7 6	N/A	N/A	[55]
Guinea Pig Dunkin-Hartley	D NA expressing Ag85A [i.m.]	MVA expressing Ag85A [i.d.]	H37Rv [aerosol, 10-50 cfu]	6 weeks	L: NS S: NS	L: NS S: -2.19	N/A	Naïve: 0% BCG: 100% P/B: 80%[17 weeks p.c.]	[50]
Fresian Calves	Apa/65/70 (DNA) [i.d./i.m.]	BCG Pasteur 1173P2 [s.c.]	*M.bovis* [i.t.,1.5x10^3 cfu]	7 weeks;8-11 weeks	LN: NS	LN: -0.53	Improvement over Naïve	N/A	[56]

[a] Bacterial load is measured as reduction (-) or increase (+) in log10 cfu counts in lung (L), spleen (S), or lymph node (LN) tissue of experimental prime-boost group as compared to BCG-vaccinated only (BCG) or naive groups.

[b] Survial is measured as percentage of surving animals in naive, BCG-vaccinated only (BCG), and experimental prime-boost (P/B) groups at given timepoint or as mean survival time for these groups.

NS (not statistically signifcant); N/A (data not reported);

* (value estimated based on graphic data); s.c. (subcutaneous); i.m. (intramuscular); i.d. (intradermal); i.n. (intranasal); i.t. (intratracheal); p.c. (post challenge)

Highlighted fields indicate experiments where the *M.tb.* load reduction for prime-boost versus naïve controls was ≥ 1.75 log total CFU in the lungs.

Table 3

Vaccine Name	Vaccine Composition or Component	Citation
Table 1A:		
AdAg85A	Adeno-5 vector expressing early secreted antigen Ag85A	[26]
CFP/CpG protein	Culture filtrate protein (CFP) plus CpG oligodeoxynucleotide adjuvant	[27]
MVA85A	Recombinant modified vaccinia virus Ankara (MVA) expressing early secreted antigen Ag85A	[28]
Ag85A + MPL	Early secreted antigen Ag85A in monophosphoryl lipid (MPL) A	[31]
native HBHA + CT	Native form of mycobacterial heparin-binding hemagglutinin plus cholera toxin (CT) adjuvant	[32]
H1 + LTK63	Fusion protein of the two early secreted antigens, Ag85B and early secretory antigenic target (ESAT-6), plus non-toxic mucosal adjuvant derived from *E. coli* heat-labile enterotoxin	[33]
AM-TT+ L3	Arabinomannan–tetanus toxoid conjugate (AM–TT) plus lipid-based suspension adjuvant	[33]
H-kBCG + L3	Heat-killed BCG conjugate (H-kBCG) plus lipid-based suspension adjuvant	[35]
CTA1-DD/ISCOM	Mucosal combined adjuvant vector composed of immune-stimulating complexes (ISCOMs) and the cholera toxin-derived fusion protein CTA1-DD	[35]
H56 + CAF01	New multistage vaccine that combines the early antigens Ag85B and 6-kDa early secretory antigenic target (ESAT-6) and the latency-associated protein Rv2660c in cationic liposomes	[36]
ID93 + GLA-SE	Recombinant fusion protein ID93 composed of the four *M.tb.* antigens Rv3619, Rv1813, Rv3620, and Rv2608 plus adjuvant of lipid formulated in a stable oil-in-water emulsion (GLA-SE)	[37]
FP9.85A	Recombinant fowlpox virus, FP9, expressing the major *M.tb.* secreted antigen antigen 85A	[38]
HyVac4	Heterologous booster vaccine, using the antigensAg85B and TB10.4 (HyVac4) delivered as a fusion molecule and formulated in the adjuvant IC31	[40]
r30	Live recombinant BCG expressing the *M.tb.* 30-kDa major secretory protein (r30), also known as antigen 85B, α-antigen, or FbpB	[41]
P-rAg85B	Recombinant antigen 85B expressed in *E. coli* and encapsulated in poly(lactic-*co*-glycolic acid) particles	[42]
Mtb72F + AS02A	Fusion protein vaccine derived from the *M.tb.* proteins Mtb32 and Mtb39 formulated in the adjuvant AS02A	[43]
HJV liposome/HSP65 DNA + IL-12 DNA	DNA vaccine expressing mycobacterial heat shock protein 65 (HSP65) and interleukin 12 (IL-12) delivered by the hemagglutinating virus of Japan (HVJ)-envelope and -liposome	[45]
Table 1B:		
85B-ESAT6 + DDA + MPL	Fusion antigen Ag85B-ESAT-6 adjuvanted in dimethyl dioctadecylammonium (DDA) bromide and monophosphoryl lipid (MPL) A	[47]
CyaA-ESAT6	*Bordetella pertussis* adenylate cyclase (CyaA) toxoid plus early secreted antigenic target (ESAT-6) protein	[49]
CyaA-85A	*Bordetella pertussis* adenylate cyclase (CyaA) toxoid plus immunodominant regions of antigen 85A	[49]
Table 1C:		
pVAXhsp65	DNA vaccine containing the hsp65 heat shock protein gene from *Mycobacterium leprae*	[7]
DNAE6	DNA vaccine expressing mycobacterial early secreted antigenic target (ESAT-6) protein	[8]
Table 2A:		
Apa/65/70	Cocktail of 3 DNA vaccines encoding Apa (Rv1860) [the alanine proline rich antigen] and heat shock proteins Hsp65 (Rv0440) and Hsp70 (Rv0350)	[52]
PstS3-Ag85A	DNA encoding PstS-3, *M.tb.* phosphate-binding transporter lipoprotein, and Ag85A, *M.tb.* early antigen	[21]
DNA Ag85B, MPT-64, MPT-83	DNA encoding Ag85B, *M.tb.*early antigen, and immunogenic, major secreted proteins MPT-64 and MPT-83 (a surface lipoprotein)	[53]
Table 2B:		
rH4	Vaccine fusion molecule rH4 (Ag85B-TB10.4) in cationic liposomes (CAF01)	[19]
E6-85	Early secreted antigenic target (ESAT-6) protein-antigen 85B fusion protein	[18]

Vaccine Name	Vaccine Composition or Component	Citation
MVA/IL-15/5Mtb	Recombinant modified vaccinia virus Ankara (MVA) overexpressing five *M.tb.* antigens 85A, 85B, ESAT6, HSP60 and Mtb39, as well as the molecular adjuvant IL-15	[18]
Ad-H4	Vaccine fusion molecule H4 (Ag85B-TB10.4) expressed in an adeno5 vector	[19]
WAg520	Live attenuated *M. bovis* strain that has a kanamycin resistence gene in a coding region of Rv2136c	[55]

This Table contains a more complete description of certain TB vaccine candidates identified in Tables 1 and 2 or of components that are part of the vaccine formulation. Vaccine names may occur in more than one table but are described only one time.