

Live attenuated vaccines for tuberculosis

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Tuberculosis (TB) is a leading cause of infectious death worldwide. TB is caused by the bacterium *Mycobacterium tuberculosis* (Mtb) and transmission happens via inhalation of droplets and aerosols coughed by an individual with active disease. There are about 10 million cases of active TB annually, and it is estimated that approximately 2 billion people are latently infected. Although most latently infected individuals are not sick and do not transmit the disease, in about 5%–10% of these people the disease reactivates. TB kills about 1.5 million people each year and resistance to current treatments increases steadily. Prevention of TB via a vaccine is considered the optimal solution by the World Health Organization. Presently, the only licensed vaccine against TB, Bacillus Calmette-Guérin (BCG), prevents childhood versions of TB but affords low efficiency in the prevention and transmission of the disease in adults. Hence, an improved vaccine is urgently required to combat TB.

Introduction

In May 1796, the English doctor Edward Jenner intentionally transferred cowpox material from the hand of a milkmaid to an 8-year-old boy. Jenner, who reasoned that milkmaids' frequent exposure to cowpox protected them from smallpox, wanted to prove his theory and successfully prevented the boy from developing smallpox disease following a deliberate infection. This moment commonly marks the discovery of vaccination.

However, the concept of cross-protection between cowpox and smallpox was already established in many farming communities before this pivotal moment. The farmer Benjamin Jesty had protected his wife and two sons from a smallpox epidemic in 1774 by using cowpox lesions from the udder of an infected cow and similar practices were known in India for centuries. Although it was still unknown at that time that infectious diseases are caused by microorganisms, Jenner's and Jesty's experiments provided the foundations for the development of live vaccines.

The germ theory (that pathogens cause disease) was proven towards the end of the 19th century. From this it became increasingly clear that some pathogens only cause disease in animals and are non-virulent or only cause a mild disease in humans (such as the virus causing cowpox). It also emerged that pathogens can be artificially weakened (attenuated) to no longer cause disease in humans, but to still induce a protective immune response. Based on these discoveries, in the early 20th century, the French scientists Albert Calmette and Camille Guérin started a series of experiments that would culminate in the development of a live attenuated vaccine against tuberculosis (TB).

Tuberculosis

Similar to smallpox, TB has plagued mankind for millennia and over the last 200 years, more than 1 billion people have died from TB, far more than from any other infectious disease. In 1882, Robert Koch succeeded in identifying the bacterium *Mycobacterium tuberculosis* (Mtb) as the causative agent for TB and in 1901 he showed that TB in cattle is caused by *Mycobacterium bovis*, a related but different species of mycobacterium. Inspired by Jenner's experiments with cowpox, Calmette and Guérin started their experiments with *M. bovis* in 1908. Because *M. bovis* can cause TB-like disease in humans, they passaged the bacteria more than 200 times and tested each generation on animals including guinea pigs, rabbits and cows to monitor virulence. Eleven years later, their cultures contained *M. bovis* bacteria that no longer caused disease in many animal models, including cows. Two years after that, the experimental vaccine, termed Bacillus Calmette-Guérin (BCG), was deemed ready for humans. On July 18, 1921, doctors in Paris gave BCG to an infant whose mother had died of TB only hours after giving birth. Over the following years the safety of BCG was further confirmed and it was rapidly adopted in many countries as a vaccine for infants.

Bacillus Calmette-Guérin

To date, BCG remains the only licensed and widely used vaccine against TB, and 2021 marks its 100th anniversary. In most TB-endemic regions of the world, BCG is still given to infants shortly after

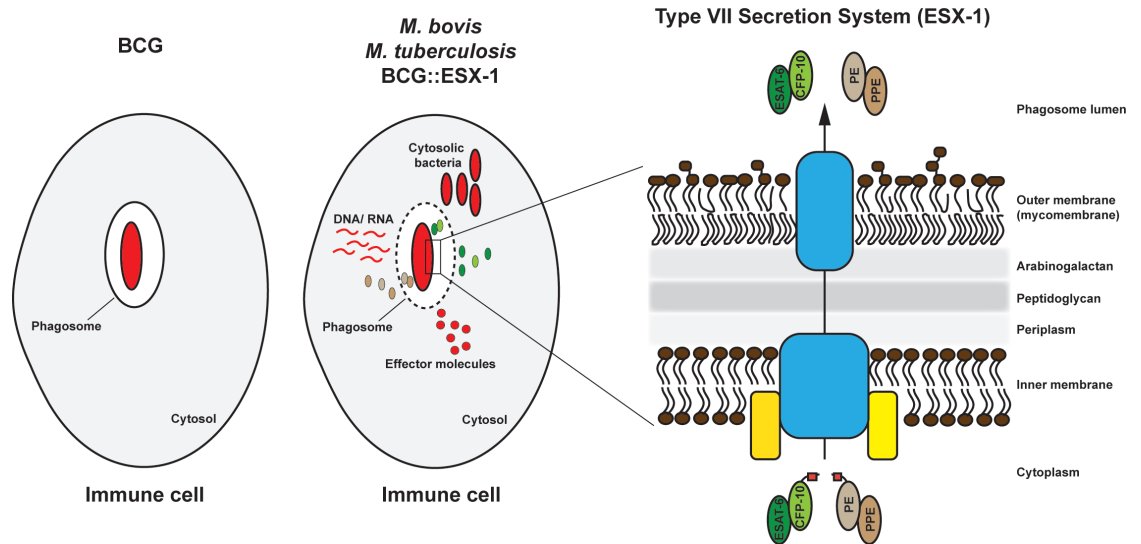


Figure 1. The loss of ESX-1 in BCG is associated with reduced virulence but also with reduced immunogenicity relative to *M. bovis*, *Mtb* and the experimental vaccine BCG::ESX-1

birth. The vaccination prevents childhood versions of TB and, hence, saves thousands of children's lives annually. However, the efficacy of BCG wanes over time, and protection against TB is often lost by young adulthood.

Because of the rapid decline of TB in many western countries after BCG was introduced and a general improvement of hygiene and living conditions, research to find a more effective TB vaccine largely stopped for most of the 20th century. As such, TB is now largely confined to low- and middle-income countries.

Towards the end of the 20th century it became clear that the development of an improved TB vaccine is critical in order to reach the World Health Organization goal of TB elimination. Renewed research efforts and better technology, including genomic sequencing and gene technology, identified that BCG lacks multiple genomic regions compared to the ancestral virulent *M. bovis* and to *Mtb*. Among others, the loss of the genetic region of difference 1 (RD1) was identified as a major attenuation event. RD1 encodes a unique mycobacterial type VII secretion system (ESX-1), a type of molecular syringe, required to inject effector molecules into the host cell and to allow escape from the phagosome. Due to the lack of ESX-1, BCG cannot escape to the cytosol and remains restricted to the phagosome (Figure 1). While these scientific breakthroughs have shed light on the limitations of BCG that occurred during the sequential passaging of *M. bovis* by Calmette and Guérin, they also opened up new avenues for improvement of BCG and the development of other live attenuated vaccines for TB.

Live attenuated vaccine candidates for TB

Over the last 20 years, approximately only 15 new TB vaccine candidates have entered clinical trials. The majority of those are subunit or viral-vectored vaccines designed to replace BCG or to boost the immune response after a primary BCG vaccination (Figure 2). Unfortunately, many of the most advanced subunit and viral-vectored TB vaccine candidates have not shown superiority over BCG. In fact, some of these clinical trials have identified that BCG re-vaccination provides better outcomes than the newly developed vaccine candidates. Consequently, there is renewed interest in live attenuated vaccines for TB and several new live vaccine candidates have been developed over the last decade. However, the enormous costs of bringing a vaccine to market (often more than US \$1bn) and the limited interest from pharmaceutical companies to invest into a disease that is largely confined to low-income countries meant that very few of those candidates have progressed to clinical trials. Live attenuated TB vaccine candidates can broadly be classified into recombinant BCG (rBCG) strains, attenuated *Mtb* strains and other bacterial species (Figure 3).

Recombinant BCG strains

BCG improvement is based on the idea that inclusion of additional genetic elements and/or deletion of particular pathways will increase immunogenicity and efficacy of the rBCG strain without increasing virulence. Currently, only one rBCG strain is in advanced clinical trials. VPM1002, also known as BCG Δ ureC::hly, expresses listeriolysin

	Preclinical	Phase 1	Phase 2a	Phase 2b	Phase 3
Infants & neonates	BCG-ZMP1		MTBVAC		VPM1002
Adolescents & adults	BCG-ZMP1	Ad5 Ag85A	MTBVAC	M72 + AS01	VPM1002
	H107	AEC/BC02	TB/Flu04L	DAR-901	MIP
	BCG, ChadOx/MVA PPE15-85A	ChadOx1.85A MVA 85A	ID93/GLA-SE	H56:IC31	
	CMV-6Ag		GamTBVac	BCG revaccination	
	CysVac2/Ad				
Therapeutic	MVA Multiphasic vac. H107	ID93/GLA-SE H56:IC31	RUTI TB/Flu04L		VPM1002

■ Subunit ■ Whole cell ■ Vector ■ Live attenuated

Figure 2. Summary of TB vaccine candidates currently undergoing clinical trials. Modified from TuBerculosis Vaccine Initiative (<https://www.tbvi.eu/what-we-do/pipeline-of-vaccines/>).

O, a pore-forming and haemolytic protein from the bacterium *Listeria monocytogenes*. To facilitate phagosome acidification and optimal function of listeriolysin O in BCG the *ureC* gene encoding urease C has been deleted. The combined deletion of *ureC* and the insertion of listeriolysin O allows access of bacterial DNA and proteins to the cytosol of immune cells, which in turn is associated with increased activation of cytosolic immune responses. VPM1002 is more effective than BCG in pre-clinical animal models, has passed phase I and II safety and immunogenicity trials in adults and infants and is currently undergoing phase III clinical trials.

A number of next-generation derivatives of VPM1002 have also been generated, including strains that (i) are auxotroph for vitamin B6 (BCG $\Delta ureC::hly \Delta pdx1$); (ii) show increased autophagy and apoptosis (BCG $\Delta ureC::hly$

$\Delta nuoG$); or (iii) secrete human cytokines (BCG $\Delta ureC::hly_{hIL7/18}$). While some of these strains have shown further increased efficacy in animal models, none of them have entered clinical trials.

Another promising rBCG strain is BCG $\Delta zmp1$. *Zmp1* encodes the zinc metalloprotease 1, a protease utilized by virulent mycobacteria to arrest phagosome maturation. Deletion of *zmp1* is associated with increased phagolysosome delivery and enhanced immunogenicity, safety and protection in various animal models, and BCG $\Delta zmp1$ is undergoing early trials.

A further concept of BCG improvement is based on the partial reversion of attenuation. When the loss of the ESX-1 secretion system of *M. bovis* was identified as a major reason for the attenuation of BCG, it was hypothesized that the incorporation of the ESX-1 secretion system of

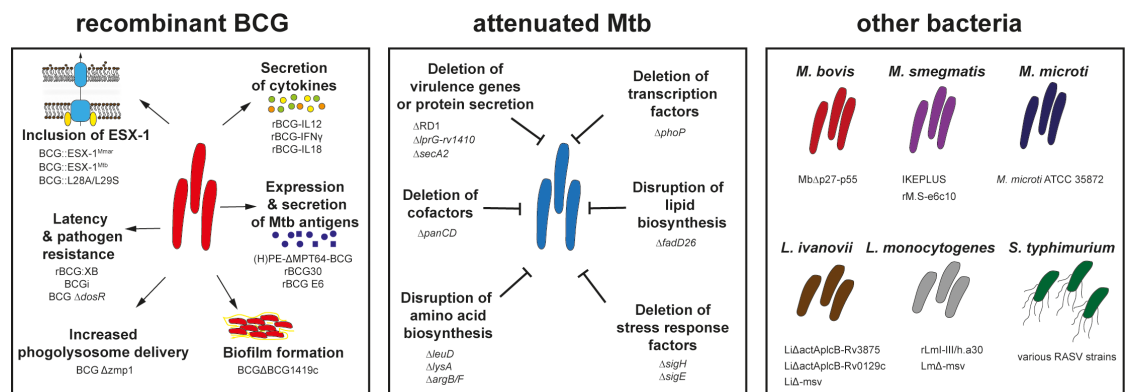


Figure 3. Examples of recombinant BCG strains, attenuated Mtb strains and other bacteria proposed as live attenuated vaccine candidates for TB

Mtb could increase immunogenicity and efficacy of BCG. The resulting BCG::ESX-1^{Mtb} strain, indeed, provided superior immunity and protection against *Mtb* challenge in mice and guinea pigs but has been deemed unsafe as a human vaccine, due to increased persistence and virulence in immunocompromised hosts. More recently, a rBCG strain expressing the ESX-1 system of *Mycobacterium marinum* (BCG::ESX-1^{Mmar}) has been developed as a potential alternative. In addition, my own research focuses on guiding ESX-1-dependent *Mtb* proteins, such as ESAT-6, to the related ESX-5 secretion system of BCG to overcome the detrimental effects of ESX-1^{Mtb} inclusion. This work has culminated in the development of the rBCG strain BCG::ESAT6-PE25SS. Both BCG::ESX-1^{Mmar} and BCG::ESAT6-PE25SS combine improved protection and immunogenicity with low virulence in animal models and await further evaluation.

In addition, several other rBCG strains have shown some advantage over BCG in small animal models, but have not reached clinical trials (Figure 3). To name a few, these include strains with increased biofilm formation (BCGΔBCG1419c), secretion of cytokines (e.g., rBCG-IL2, rBCG-IFN γ , rBCG-IL18) or *Mtb*-derived proteins (e.g., (H)PE-ΔMPT64-BCG, rBCG E6, rBCG30), expression of latency genes (e.g., rBCG:XB) or genetic pathogen resistance loci (e.g., BCGi) or a combination of the above (e.g., AERAS-422, rBCG-Ag85B-IL15).

Attenuated *Mtb* strains

Vaccine candidates that are based on *Mtb* require at least two attenuating mutations to guarantee safety and to prevent reversion to the virulent ancestral phenotype. In most cases this is achieved by deletion of genes in distinct metabolic pathways, such as amino acid synthesis, cofactors and/or transcription factors. Attenuated *Mtb* strains usually are non-virulent in animal models but provide increased immunogenicity and protection compared to BCG due to the larger antigenic repertoire.

Only one attenuated *Mtb* strain is currently in advanced clinical trials. MTBVAC (*Mtb* Δ*phoP* Δ*fadD26*) carries deletions in the transcription factor *phoP* and the lipid biosynthesis gene *fadD26*. While *phoP* is involved in intracellular survival and ESAT-6 secretion, *fadD26* plays an important role in the synthesis of virulence-associated cell wall lipids. MTBVAC is as safe as BCG and has successfully passed several clinical trial stages. Other attenuated *Mtb* strains that have shown superior protection in pre-clinical animal studies include *Mtb* ΔRD1 Δ*panCD*, *Mtb* Δ*leuD* Δ*panCD*, *Mtb* Δ*lysA* Δ*panCD*, *Mtb* Δ*lysA* Δ*secA2*, *Mtb* Δ*argB/F*, *Mtb* Δ*LprG*, *Mtb* Δ*sigH* and *Mtb* Δ*sigE* Δ*fadD26*, to name just a few (Figure 3).

However, similar to rBCG strains containing ESX-1 or its secreted proteins, attenuated *Mtb* strains suffer from an obstacle – that is cross-reactivity with the gold-standard diagnostic tool for TB, the Interferon Gamma

Release Assay (IGRA). IGRAs detect specific immune responses to proteins that are found in *Mtb* but not in BCG, including ESX-1-dependent proteins (Figure 1). Because attenuated *Mtb* strains, in most cases, still contain these proteins, vaccination with attenuated *Mtb* strains can lead to positive IGRA results and hence difficulty to distinguish vaccine-mediated immune responses from immune responses directed against *Mtb* infection. These challenges are currently addressed by the development of new diagnostic tools.

Other bacteria

Given that BCG was derived from *M. bovis*, targeted attenuation of *M. bovis* has also been trialed, but the resulting strain, MbΔp27-p55, is more virulent than BCG. Using non-pathogenic mycobacterial species and other bacteria as a live attenuated vaccine for TB is also being considered. For example, the fast growing mycobacterium *M. smegmatis* has been used to generate the TB vaccine candidate IKEPLUS. Based on the replacement of the *M. smegmatis* *esx3* locus with the *esx3* ortholog of *Mtb*, IKEPLUS provides similar protection to BCG in mice. Furthermore, *M. microti*, an animal-adapted mycobacterium, showed strong attenuation in immunocompromised mice and demonstrated similar protective efficacy as BCG. Modified strains of *Listeria ivanovii*, *Listeria monocytogenes* and recombinant attenuated *Salmonella typhimurium* vaccines (RASV) have also been used to deliver *Mtb*-derived antigens (Figure 3). In most cases improved or equivalent protection to BCG was observed in animal models, but none of these vaccine candidates has progressed to clinical trials.

Conclusions

The year 2021 marks a special occasion in the history of live attenuated TB vaccines. Hundred years after its first use, BCG remains the gold standard for TB prevention in children. While this anniversary is an important milestone, TB remains a global threat and the development of a new and effective vaccine is crucial if TB is to be significantly reduced, let alone eradicated, within the coming decades. With the largely disappointing results of subunit vaccine candidates in recent clinical trials, the various live attenuated vaccine candidates described earlier provide a glimmer of hope that an improved TB vaccine will be found. Importantly, despite being 100 years old, the mechanism by which BCG actually works is still largely unknown, and it is unclear why BCG only confers protection against childhood versions of TB and why protection often wanes in adolescence. If further research leads to a better understanding of the correlates of BCG-mediated protection, a more targeted improvement of BCG will likely be possible. ■

Further Reading

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