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New tuberculosis vaccines

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A B S T R A C T

The current tuberculosis (TB) vaccine, bacille Calmette-Guerin (BCG), is a live vaccine used worldwide, as it protects against severe forms of the disease, saving thousands of lives every year, but its efficacy against pulmonary forms of TB, responsible for transmission of the diseases, is variable.

For more than 80 years now no new TB vaccines have been successfully developed. Over the last decade the effort of the scientific community has resulted in the design and construction of promising vaccine candidates. The goal is to develop a new generation of vaccines effective against respiratory forms of the disease. We will focus this review on new prophylactic vaccine candidates that aim to prevent TB diseases. Two are the main strategies used to improve the immunity conferred by the current BCG vaccine, by boosting it with new subunit vaccines, and a second strategy is focused on the construction of new more effective live vaccines, capable to replace the current BCG and to be used as prime vaccines.

After rigorous preclinical studies in different animal models new TB vaccine candidates enter in clinical trials in humans. First, a small Phase I for safety followed by immunological evaluation in Phase II trials and finally evaluated in large population Phase III efficacy trials in endemic countries. At present BCG prime and boost with different subunit vaccine candidates are the more advanced assessed in Phase II. Two prime vaccines (based on recombinant BCG) have been successfully evaluated for safety in Phase I trials. A short number of live attenuated vaccines are in advance preclinical studies and the candidates ready to enter Phase I safety trials are produced under current good manufacturing practices.

Nuevas vacunas contra la tuberculosis

Palabras clave:
Bacilo de Calmette-Guérin (BCG)
Tuberculosis
Vacunas subunidades
Vacunas vivas atenuadas

R E S U M E N

La actual vacuna contra la tuberculosis, bacilo de Calmette-Guerin (BCG), en uso desde 1921, protege contra las formas más graves de la enfermedad, pero su eficacia es muy variable contra las formas de tuberculosis pulmonar, por lo que la investigación y desarrollo de nuevas vacunas es un reto importante para la comunidad científica.

En la última década, importantes esfuerzos en investigación han dado como resultado el diseño y construcción de nuevos candidatos a vacunas contra la tuberculosis. El objetivo es desarrollar una nueva generación de vacunas eficaces contra las formas respiratorias de la enfermedad, responsables de la transmisión de la tuberculosis.

Esta revisión se centra en el progreso preclínico y clínico de nuevas vacunas profilácticas, dirigidas a prevenir la enfermedad. Dos son las principales estrategias: la primera busca mejorar la inmunidad conferida por BCG, revacunando con vacunas subunidades, y la segunda estrategia se centra en la construcción de nuevas vacunas vivas más eficaces, capaces de sustituir la actual BCG.

Tras rigurosos estudios preclínicos en modelos animales, algunos candidatos a la vacuna contra la tuberculosis han entrado en ensayos clínicos en humanos. Actualmente, las vacunas subunidades son las más avanzadas en estos ensayos y están siendo evaluadas en fase II en individuos previamente vacunados con BCG. Vacunas vivas basadas en BCG recombinante se están evaluado en fase I, y nuevas vacunas vivas atenuadas han mostrado buenos resultados de atenuación y protección en estudios preclínicos, y están listas para entrar en ensayos de fase I en humanos.

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Introduction

Prophylactic vaccines are one of the most useful and cost-effective tools for reducing the morbidity and mortality associated with infectious diseases.[1] There is no other vaccine so widely used and as controversial in terms of efficacy as the current bacille Calmette-Guerin (BCG). Its protective effects in randomized controlled trials and case-control studies have provided mixed results ranging from excellent protection to no protection against tuberculosis.[2] The majority of studies have shown that BCG vaccine produces a higher degree of protection against severe forms of TB such as TB meningitis and TB disseminated, than against moderate forms of the disease.[3,4]

The effectiveness of BCG vaccine also appears to vary with latitude - the greater the distance from Ecuador, the greater the effectiveness of the vaccine. Probably exposure to nonpathogenic mycobacteria, which is more intense in warm climates, induces a degree of protective immunity in exposed populations, masking the potential BCG protection against TB.[5] Most evidence suggests that BCG efficacy is maintained after 10 years of vaccination have been described as 60 years.[6] Since today is possible to differentiate between vaccinated individual and infected with *Mycobacterium tuberculosis* (MTB) by using Interferon gamma based assays,[7] a recent study showed that BCG could protect not only TB disease but also MTB infection.[8]

Since one third of the world population is infected by MTB, new strategies that could avoid the progression of latent infection to active disease, such as immunotherapeutic vaccines designed for use post-infection in adults, could have tremendous impact on TB control. Today some therapeutic vaccines are in Phase I clinical trials for safety.[9]

Considering the natural history of tuberculosis and its wide distribution by age, there is a need for vaccines that can prevent disease having a greater efficacy in preventing respiratory forms of TB in adults and help avoiding transmission of TB. That is why research on new TB vaccine strategies, which improve the efficacy of the present BCG to prevent respiratory forms of the disease or the development of new TB vaccines more effective than the current BCG is a priority. We will focus this review on prophylactic vaccines that can prevent TB disease, with special emphasis on pulmonary forms of TB responsible for transmission of the disease.

Bacille Calmette-Guerin the present vaccine in use against tuberculosis

BCG is the current vaccine for tuberculosis. It is a live vaccine obtained from a strain of *Mycobacterium bovis* isolated from an infected cow. Calmette and Guérin needed thirteen years and over 200 subcultures to obtain the attenuated version of the original virulent strain of *M. bovis* isolated.

BCG was used in 1921 for the first time in children, orally. Later other methods were introduced for administration, including the currently in use, intradermal injection. It is estimated that the systematic use of BCG is saving thousands of lives each year but its benefit seems to be linked to the prevention of severe childhood forms of disease, including extra pulmonary TB and the often fatal TB meningitis.[3,4] For this reason, BCG vaccination is recommended and include in the calendar of vaccination by WHO in countries with high incidence of TB. The 100 million BCG vaccinations given to infants in 2002 will have prevented 30,000 TB meningitis cases in children during their first 5 years of life, and about 11,000 cases of disseminated or miliary TB.[10] In Spain, BCG is not applied systematically in the pediatric population and is not included in the immunization schedules of the various autonomous communities, although it is present in the immunization schedule of the Basque Country.

Despite BCG being the most widely used vaccine in human history, the mechanisms of attenuation are just starting to be understood. Recent genetic studies have revealed that that the current vaccine has lost more than 100 genes in Regions of Difference (RD) from its original genome, with some regions involved in virulence such as RD1, which codes for ESAT-6 secreted protein implicated in MTB virulence.[11] BCG is a highly immunogenic complex, immunogen and which induces a cell type immunity. Originally each BCG was known by the name of the place of production, e.g., BCG (Russia), BCG (Brazil) and BCG (Japan), among others, or after the laboratories where they were produced, such as BCG Pasteur, BCG Meriéux or BCG Glaxo. The name could be followed by the number of the last passage from which it was obtained, as BCG Pasteur 1173, for example. Interestingly from 1921 BCG was generosity distributed worldwide between microbiologists, resulting in the evolution of a number of daughter substrains, as laboratories have used various procedures in the preparation of BCG changing its residual virulence and immunogenic characteristics. This lasted until in the early 1960's when batch 1331 of BCG Danish was freeze-dried and was adopted as the primary seed lot in 1966.[11] During the subcultivation process that led to attenuation, BCG has lost so many genes with respect to MTB and has been considered that BCG could be attenuated to the impotence.[12] Subculture of the original BCG strain in different laboratories and this makes that BCG is not a single organism but comprise a number of substrains that differ in genotype and phenotype with different immunological properties.[13]

The publication in 2007 of the complete sequence of the first BCG strain (Pasteur) and in 2009 BCG Tokyo, has revealed differences in genetic and molecular characteristics.[14,15] These would explain the differences found in the substrains currently used as a BCG vaccine. BCG Tokyo and BCG Pasteur are "more immunogenic" and probably more capable than other strains "less immunogenic" (BCG Glaxo, BCG Danish). Between the molecular mechanisms that contribute to attenuation, BCG substrains comprise natural mutants of major virulence factors of MTB including ESAT6, PDIM/PGL and PhoP, and differ markedly in virulence level that could be responsible for the differences in the protective efficacy and tuberculin reactivity or adverse reactions between different substrains.[11,16] The study and understanding of these new insights have extremely important implications for the development of future vaccines.

The need for new tools for tuberculosis control, from preclinical to clinical trials

The scientific community effort has resulted in the construction of numerous vaccine candidates over the last decade. In search of candidate vaccines to enhance the effectiveness of the current BCG, there are various teams that have used different approaches. Two main strategies used to improve the immunity are subunit vaccines to "boost" the current BCG vaccine and new more efficient "prime" strategies, able to replace the current BCG, such as novel attenuated live vaccines.

Subunit vaccines mainly use antigens of the bacillus of tuberculosis to improve the immunogenicity of BCG. These MTB antigens consist generally in protein selected for their ability to be recognized by the immune system after infection in humans. These proteins can be administered directly with potent adjuvans or by inserting their genes in different viruses genetically modified such as non replicative vaccinia virus.

Live vaccines mainly consist in nonvirulent mycobacterial strains such as *Mycobacterium vaccae*[17] or the most promising use of recombinant BCG including MTB antigens or other genes from a different microorganism than MTB. Finally another approach is based in the construction from the scratch of rationally attenuated MTB strains which are based on the removal of virulence genes and immunomodulatory lipids which rending safe and more effective TB vaccines.

All vaccine candidates are evaluated to know their safety and effectiveness as degree of protection against MTB disease. In the

evaluation of new vaccines, BCG remains the "gold standard." Studies have been conducted in various animal models and the most commonly used animal model is the mouse, followed by the guinea pig. Primate models have been developed and are being used as a final testing prior to entry in clinical trials.

The advantage of mouse model is based on the amount of reagents and genetic information available, and because of its logistical and economical advantages in comparison with other models like guinea pig. Mice have certain tolerance to infection with MTB, whereas it triggers a moderate inflammatory reaction that allows the control of the bacillary concentration to a low level but without ending up eradicating it. The commonest route of infection is the intravenous route, because switches on acquired immunity very rapidly. The experimental model induced by aerosol is the most physiologic infection route and at the same time is more aggressive for the host than the intravenous one. This is because the induction of immunity is quicker after the intravenous inoculation than in the aerosol one. In this model has been demonstrated that the immunity against this infection is based essentially on the stimulus of a Th1 type response, that is to say, in the stimulation of T cells CD4+ able to produce gamma interferon and to activate the infected macrophages.[18]

Protection in new vaccines using the guinea pig model has become a compulsory experiment since the extreme sensibility that has demonstrated this animal against MTB, and the toxic response generated allowing the comparison between different TB vaccine candidates.[19] On the other hand, the necessity to evaluate the protection of any new vaccine before to carry on human clinical trials in an experimental model closer physiologically to humans has lead to the development of the primate model. In this model the protection mechanisms against the MTB infection are established.[20]

Once the candidate is proven safe and effective, is proposed for study in clinical trials in humans. Clinical trials are divided into 3 consecutive phases, phases I-III, which correspond to the actual development stage, and a phase IV or post-marketing pharmaco vigilance and be released. The first phase of the trial, Phase I, aims to determine the safety and biological effects, including immunogenicity and takes place in a small group of healthy volunteers. Phase II focuses on the immunogenicity and to determine the effectiveness of the vaccine in a limited number of volunteers. Since there is not a clear correlation of protection in humans, the only way to demonstrate the efficacy of a new vaccine candidate is to test in a population with a high incidence of tuberculosis and compare unvaccinated, with gold standard BCG vaccinated. Phase III aims to assess the safety and effectiveness in vaccinated and unvaccinated volunteers by large double-blind trials. Phase III of the vaccine against tuberculosis is predicted long (3-4 years) in the absence of biological markers of protection and require the study of their effectiveness in reducing disease in immunized individuals, the participation of thousands of individuals. According to the Global Plan to Stop TB to have a new licensed vaccine available for 2015, it is estimated that at least 20 vaccine candidates should enter phase I safety trials, with about half going forward for immunological evaluation in phase II trials and three / four being evaluated in phase III efficacy trials.[10]

Improving bacille Calmette-Guerin: subunit vaccine candidates

Numerous subunit vaccines have been developed using different experimental approaches. The justification for these vaccines is that a few antigens can achieve the same protection that comes with the complete bacteria and their use would provide the vaccine safe, reproducible and without posing problems for application to immunocompromised individuals. To date subunit vaccines alone have not demonstrated a better protection than BCG in different animal model tested and is for that that the new strategy fort subunit vaccines is to be used as boost Improving the effectiveness of BCG.[21] These approaches are aimed at enhancing the protection of BCG. The experiments consist of priming revaccinated animals with BCG and boosting with subunit vaccine candidates.

Subunit antigens have been chosen by different approaches. The study of the immune response in healthy individuals, who have been in contact with MTB, has identified several antigens that would be key to containing tuberculosis infection. The immunization consists of a recombinant fusion protein Mtb72 and adjuvant that provokes a good cellular immune response. This candidate vaccine of GlaxoSmithKline Biologicals (Rixensart, Belgium), Mtb72 has passed Phase I in humans and in collaboration with Aeras Global TB Vaccine Foundation is allowing its progress in clinical trials for safety and immunity, involving adults previously infected with TB or vaccinated with BCG.[22,23]

Another promising approach is the use of ESAT 6 that has been developed from antigens of the bacillus of tuberculosis. It is a major secreted protein. In experimental animals infected, treated and reinfected with MTB, it was noted that at the time of reinfection animals developed a strong T cell response to ESAT-6 making this protein relevant as a source of protective immunity. By merging with another major antigen Ag85B, derivatives have been built of this protein. This subunit vaccine administered with different adjuvants causes an immune response in mouse and primate models inducing protection against infection with MTB. Currently this candidate is in Phase I within the European TB Vaccine Initiative TBVI.[24,25]

Adrian Hill's group at the University of Oxford has used the non replicative modified vaccinia virus Ankara (MVA) to introduce an antigen of the bacillus of tuberculosis (Ag85A). An experiment involving prime vaccination of guinea pigs with BCG, boost with the virus carrying Ag85A and boost with Ag85A protein with an adjuvant, shows greater protection than BCG alone in this animal models.[19] Phase I studies with this candidate have been published[26] and currently is in Phase II clinical evaluation for efficacy and safety in previously BCG vaccinated individuals.[27]

Development of prime live vaccines based on recombinant bacille Calmette-Guerin

BCG-based vaccines consist of recombinant BCG derived from genetically modified strains of BCG aiming to increase their immunity. Different groups have chosen this strategy to build their candidates. The group of Marcus Horwitz of UCLA (University of California, Los Angeles, USA) developed through genetic engineering techniques rBCG30 strain. It is based on the Tice BCG strain, enhanced with MTB antigen Ag85B, and its overexpression is achieved by increasing the stimulation of the immune system.[28,29] rBCG30 has been noted that the first live vaccine candidate has passed Phase I clinical evaluation. However, as a consequence of less than expected immunogenicity, rBCG30 is no longer in clinical development.

Different strategy is used in the construction of rBCG: RD1 based on the introduction of MTB genes that are absent in BCG coding for ESAT6.[30,31] Such as approach has shown higher protection in animal models but rigorous residual virulence studies are ongoing for regulatory approval to enter in clinical trials.

A third strategy is to increase cellular immunity conferred by BCG by inserting the gene that encodes for a protein of *Listeria monocytogenes*. This gene inside the infected macrophage increased presentation of BCG antigens to other immune system cells. This line of research is led by the group of Stefan Kaufmann, director of Max Planck Institute in Berlin and today this candidate is successfully exiting Phase I safety trials, with plans to enter Phase II in TB endemic countries for safety and immunogenicity.[32,33]

Prime vaccines based in new live attenuated vaccines

Classical vaccine candidates have to mimic natural infection as closely as possible without causing disease.[10] Provided that immune

response evolved to provide protection against infectious diseases, the optimal development of a protective immune response by a vaccine should reproduce the steps and processes elicited during the establishment of natural immunity.[34] Epidemiological and animal studies indicate that previous infection with tuberculosis confers relative protection against subsequent disease due to post exposure.[35,36]

The improvement of live attenuated vaccines has often been limited by a lack of tools for genetic manipulation of mycobacteria. Pioneering studies performed in Europe by the group of Brigitte Gicquel[37,38] and USA by the group of William Jacobs[39] were able to develop the mycobacterial genetic tools today in use. This strategy is more laborious, meaning starting from scratch and demonstrating in a first stage the attenuation of the vaccine candidate constructed and, secondly, a higher immunity to TB.

Pantothenic acid (vitamin B5) is an essential molecule for the synthesis of coenzyme A and acyl-carrier proteins, two important molecules in fatty acid metabolism and biosynthesis of polyketides among other metabolic reactions. A double-deletion mutant of MTB in the *panC* and *panD* genes involved in the pantothenate synthesis has been constructed.[40] This auxotrophic mutant is attenuated when tested in Balb/c and SCID mice, and conferred protection when used as subcutaneous vaccine in mice challenged with low aerosol doses of virulent H37Rv. In order to increase attenuation and stability of attenuated phenotype a deletion of virulence region RD1 was performed.[41] A representative of this strategy is the mc²6020 strain constructed by inactivation of the *panCD* and *lysA* genes involved in pantothenate and lysine metabolism respectively.[40]). Protection levels equivalent to BCG were generated in the lungs and spleen of vaccinated guinea pigs, with reduced dissemination of infection to the spleen at five weeks after aerosol challenge with MTB.[42]

Another strategy to rationally attenuate MTB consists on inactivation of *secA*. This gene encodes a component of a mycobacterial protein secretion system involved in inhibiting the host immune system and consequently promoting MTB survival within the host. Conversely, inactivation of *secA* results in increased host cell apoptosis and increased priming of antigen-specific $CD8^+$ T cells *in vivo*. These results pave the way for a new approach to improving live vaccine candidates; the *secA* mutant is currently in preclinical development.[43]

In order to build a new vaccine from a MTB a rational inactivation of major its virulence genes, was performed by the by the Group of the University of Zaragoza, in collaboration with the Pasteur Institute. The hypothesis to build a new live vaccine arose from studies on molecular epidemiology of multidrug-resistant MTB strains, assuming that if only a few MTB complex strains are transmitted in a different way than the rest, they have some characteristic that makes them increase their virulence. Therefore, if we decipher the role of the gene in MTB know how to increase their virulence, is possible to inactivate these genes to decrease its virulence. In the late 1990´s the group focus their studies on the strain which caused the largest outbreak of multidrug-resistant TB described in Europe,[44] later described as an *M. bovis* XDR strain.[45] It was found that *phoP* gene, annotated as a possible regulator of virulence genes in the genome of MTB, was highly expressed in this strain, so it was hypothesised that its inactivation may reduce the virulence of MTB. Using genetic engineering techniques *phoP* gene was inactivated. The inactivation of this single gene led to an important attenuation both the cellular model as in the mouse model. Subsequent studies have shown attenuation in the mouse model with reduced immune system (SCID mice, severe combined immunodeficiency) and higher attenuation than BCG Pasteur.[46]

Today we know that the *phoP* gene regulates 2% of the genes of MTB most involved with virulence, including complex lipids, which could be the basis of its high attenuation. Trials of protection against infection, conducted in collaboration with national and international teams from Spain, England, France and Mexico, have shown promising results in the mouse model and superior protection and greater immunity to BCG in the guinea pig model.[20,46] Immunity conferred by the *phoP* mutant is under study. Experiments with primates performed in the PBRC in the Netherlands, were very encouraging.[20]

Another important question that arises is whether after MTB attenuation the sensitivity profile to antituberculous drugs could be changed. The *phoP* mutant strain was found to be fully sensitive to ethambutol, isoniazid, rifampicin and streptomycin. And the mutant was more sensitive to isoniazid than MTB wild type and this could be due to changes in the cell envelope of the *phoP* mutant.[47,48] All these results indicate that MTB *phoP* mutant is sensitive to major antituberculous drugs and, in case of hypothetical infection with the vaccine strain, it would be possible to be treated.

Inoculation of a high dose of vaccine candidates in guinea pigs is a study inspired by the BCG validation standards for toxicity. In one such study, animals were inoculated with 2.5×10^6 CFU (colony-forming units) of attenuated vaccine (50 times the standard vaccination dose).[49] Data indicate the lack of toxicity of *phoP* mutant strain since the health status of the animals was satisfactory, as evidenced by the constant increase of their weight and lack of pathology after the end of a 6 month follow up. Determination of DTH in guinea pigs at the end of this study reflected similar values to the ones obtained 4 or 6 weeks after immunization with BCG, thus reflecting the conservation of the immune response.

The use of post-exposure infection models for checking toxicity when vaccines are administered in a therapeutic way is based on previous data showing that this administration can be dangerous because of potential induction of the "Koch phenomenon". A number of vaccine candidates have recently been tested to assess the effectiveness and lack of toxicity after post exposure vaccination. The results suggest that although most vaccine candidates are unlikely to evoke the "Koch phenomenon", extreme caution should be taken to avoid serious reactions in previously infected individuals in clinical trials. By using previous validated models of post-exposure infection in guinea pigs and mice to address this question, and we can conclude that no toxic effects have been developed in any of the cases, as it has been demonstrated after examining the bacillary concentration and histology of the tissues.[49] In fact, in the guinea pig post-exposure model, the administration of *phoP* mutant vaccine decreased the pathology, which could be related to a kind of protective effect, although this was not confirmed by a reduction in the bacillary counts. In any case, the lack of toxicity in these models gives an idea about how safe this vaccine is, including the potential secure profile to be used in subjects with latent TB infection. All together extended safety studies encourage the use of *phoP* mutant strain as a starting point for the construction of a next generation attenuated live vaccines.[49]

For live vaccines based on attenuated MTB, a consensus document was developed at the Geneva conference and the presence of at least two non-reverting independent mutations in the mycobacterial genome was recommended in order to avoid reversion and elimination of antibiotic resistance markers.[50] Regulatory issues are fundamental for the development of new tuberculosis vaccines[51] and a second Geneva Consensus include recommendations for novel live TB vaccines prior to entry in phase I safety trials, criteria through to Phase III, review of manufacturing considerations and considering requirements and associated issues related to the use of these new vaccines within an existing BCG vaccination programme.[52]

Future challenges

Vaccination against tuberculosis poses great challenges. One third of the world population is infected with TB bacilli, for this whom investigation in so-called "therapeutic vaccines" aimed at reducing the time of treatment with conventional drugs is ongoing. Another challenge in vaccination against TB is to protect the population

Table 1
Tuberculosis vaccine candidates using a heterologous "prime-boost strategy" to complement the immune response induced by current bacille Calmette-Guerin (BCG)

Type of vaccine	Product	Sponsor	Status 2010	Product description
Viral vector	MVA-85A Aeras-485	Oxford/Aeras	Phase I, Phase II	Mofdified vaccinia Ankara vector expressing MTB antigen 85A
Viral vector	Aeras-402 Crucell Ad35	Crucell/Aeras	Phase I, Phase II	Replication-deficient adenovirus 35 vector expressing MTB antigens 85A, 85B, TB10.4
Recombinant protein	Mtb72f	GSK/Aeras	Phase I, Phase II	Recombinant protein composed of a fusion of MTB antigens Rv1196 and Rv0125 and adjuvant
Recombinant protein	Hyrbid +IC-311	SSI/Intercell/TBVI	Phase I, Phase II	Adjuvanted recombinant protein composed of MTB antigens 85B and ESAT-6

Table 2
New recombinant bacille Calmette-Guerin (BCG) replacement BCG vaccine as "prime" in clinical trials

Type of vaccine	Product	Sponsor	Status 2010	Product description
Recombinant BCG Live	rBCG 30	UCLA/INH/Aeras	Phase I	rBCG Tice strain expressing 30Kda MTB Antigen 85B
Recombinant BCG Live	VPM 1002	Max Planck TBVI	Phase I	rBCG Prague strain expressing listeriolysin and carries a urease deletion mutation

Table 3
New live vaccines to replacement bacille Calmette-Guerin (BCG) vaccine as "prime" in pre clinical and good manufacturing practices (GMP)

Type of vaccine	Product	Sponsor	Status 2010	Product description
Recombinant Live	*MTBVAC01* Δ *phoP* Δ *fad26 (DIM)*	Universidad de Zaragoza Institut Pasteur BIOFABRI TBVI	GMP	Live vaccine based on attenuation of MTB by inactivation of *phoP* and *fadD26* genes
Recombinant Live	*MTB* (Δ *lysA*, Δ *panCD*, Δ *secA2*)	Albert Einstein College of Medicine	Preclinical studies	Non-replicating, MTB satin auxotrophic for lysine and pantothenate, attenuated for *secA2*

currently vaccinated with BCG. Recent trials using protein subunit vaccines in animals previously vaccinated with BCG are giving good results. Live rationally constructed vaccines derived from a *phoP* based mutant strain of tuberculosis, are in advanced preclinical stage. Live vaccines rationally attenuated by the inactivation of genes that regulate virulence, are promising vaccine candidate because of low cost of production, and by the tremendous experience, both in the use, production and distribution of BCG. In addition live vaccines are easily produced and affordable.

Today, after almost 100 years since the development of BCG, there is more than a dozen of developing preventive vaccine candidates in human trials or in advanced preclinical stages with excellent results. Due to the lake of correlation of protection the real value of their efficacy could be assed in a large scale phase trials in countries with a high incidence of TB. The development of a new vaccine that confers higher level of protection is a challenge for the scientific community that would, with a low cost, consider the eradication of tuberculosis in the medium term and replace the current BCG in the long term.[53]

For the future panorama on TB vaccines against TB there are 3 scenarios that could help to the Millennium goal to eradicate TB as a public health problem in 2050 (http://www.stoptb.org/global/plan/): *a)* a subunit vaccine to increase the immunity of individuals vaccinated with BCG administered after vaccination with BCG (boost); *b)* a new vaccine to replace BCG with a birth dose (prime); and *c)* a new vaccine to replace BCG with a birth dose plus a subunit vaccine (Tables 1-3).

Developing new vaccines against TB. Currently there are 12 vaccines in a research designed to improve the immunity of BCG and others to replace BCG.

Aeras: Foundation in the United States for the development of new vaccines against tuberculosis sponsored by the Bill Gates Foundations. http://www.aeras.org/

Tuberculosis Vaccine Initiative (TBVI). European foundation for the development of new vaccines against tuberculosis (http://www.tbvi.eu/).

In 2006, a new initiative called Stop TB was launched according to the Millennium Development Goals, it aims to reduce the global burden of tuberculosis in 2015 and posing as a goal to eliminate TB as a public health problem in 2050 (http://www.stoptb.org/globalplan/).

For a complete list of vaccine candidates including post infection and immunotherapy consult the Stop TB partnership working group on new TB vaccines (www.stoptb.org/wg/new_vaccines/).The document "vaccine pipeline" include a complete list of TB vaccine candidates in 3 sections: *a)* in clinical trials; *b)* in advanced preclinical studies and good manufacturing practices, and *c)* next generation.

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Conflict of interest

C. Martín and B. Gicquel are named inventors on a composition of matter patent for *Mycobacterium tuberculosis phoP* attenuated mutant filed by the University of Zaragoza. There are no other conflicts of interest.

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