TUBERCULOSIS TIMEBOMB A GLOBAL EMERGENCY: NEED FOR ALTERNATIVE VACCINES

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Tuberculosis (TB) also known as white plague is a major infectious disease and a global emergency. It is one of the oldest recorded human afflictions and still continues to cause widespread morbidity and mortality in children and adults worldwide, despite the extensive use of a live attenuated vaccine and several antibiotics. One third of the world's population is already infected and about 3 million people die and 8 million people develop the active disease each year[1-2]. In the last decade there is a sharp rise in the cases of TB mainly due to emergence of multi-drug resistant (MTD), extensively drug-resistant (XDR) and totally drug-resistant (TDR) strains of Mycobacterium *tuberculosis*, which are virtually untreatable[3-7].

In the beginning of the last quarter of the 20th century, there was a glimmer of hope that TB could be brought under control; however, this was dashed with the emergence of HIV/AIDS TB co-infection[8]. HIV compromises the immune system, which needs to be competent for control of latent *M. tuberculosis* infection, and hence increases risk of active TB manifold, from 10% risk over a lifetime to 10% risk within of TB is becoming worse day by day. Use of BCG is effective in preventing TB in efficacies. The only approved and currently available vaccine against TB i.e. BCG has young children that too with varied the year of co-infection[9]. Despite the fact that highest number of people on the earth has been vaccinated with BCG still scenario been given 4 billion times over the last 100 years since its development; we don't even understand the precise immune mechanisms that protect BCG vaccinated infants and lacks protection in adults probably due to absence of generating long lasting memory cells. Thus, an effective vaccine for the prevention of pulmonary TB in adolescents and adults, many of whom are latently infected with M. tuberculosis in countries in which TB is endemic, is urgently needed to control the TB time bomb.

Immunity to Mtb is a two-edged sword: it protects the human host against disseminating infection, but also facilitates transmission of TB to contacts. Therefore, TB vaccines not only need to induce optimal immunity to Mtb, but also a balanced response that favors protective and avoids pathogenic mechanisms[10]. But the most successful vaccines today target pathogens against which humoral immunity suffices to achieve protection and often sterile eradication but TB vaccines need to drive primarily the cellular arm of the immune system.

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+Table 1: Next Generation vaccine candidates against Tuberculosis

Type of Vaccine	Product	Description	Indication	Sponsors
Recombinant Protein	r30	30KDa mtb Ag85B protein purified from rM. Smegmatis	B and PI	UCLA, NIH, NIAD
	R32KDA (Recombinant 85A)	Purified recombinant Ag85A protein from BCG	B, PI and IT	LEPRA Society, Blue Peter Research Centre, and BMMRC
	Latency fusion protein	recombinant fusion protein composed of antigens 85A- 85B-Rv3407, Rv3407-Rv1733c-Rv2626c, Rv0867c-Rv-1884-Rv2389c	B	Aeras
Recombinant Live	rBCG(mtbB)30	rBCG with limited replication overexpressing the 30kDa Mtb Ag85B	P	UCLA, NIH, NIAD
	HG856-BCG	rBCG overexpressing chimeric ESAT-6/Ag85A DNA fusion protein	B and PI	Shanghai Public Clinical Health Centre
	BCG zmp1	BCG zmp 1 deletion mutant	P	University of Zurich, TBVI
	rBCG38	rBCG Tice strain overexpressing the38KDa protein	P and B	Universidad Nacional Autonoma de Mexico
	Disruption of the SapM locus	Recombinant M. bovis BCG in which the SapM locus has been disrupted	P	FWO-Ghent University-VIB
	rBCG85C	rBCG overexpressing antigen 85C of M tb	P	University of Delhi; DBT, Govt. of India
	Streptomyces live vector	Recombinant Streptomyces expressing multiple T and B epitopes of Mtb.	P, B, PI and IT	Finlay Institute; Institute of Pharmacy and Food Cuba
	rBCG T+B rM. Smegmatis T+B	rBCG and rM. Smegmatis expressing multiple T and B epitopes of Mtb	P, B and PI	Finlay Institute; Universiti Sains Malaysia
Viral Vectored	rhPIV2-Ag85B	Replication deficient human parainfluenza type-2 virus expressing Ag85B	P and B	NIBI Japan; Japan BCG Laboratory
	Recombinant LCMV	r-LCMV expressing Ag85A, Ag85B or Ag85B-ESAT-6	P, B, PI and IT	University of Geneva, TBVI
DNA	HG856A	Chimeric DNA vaccines-ESAT-6/Ag85A; Ag85A/Ag85B	B and IT	Shanghai H&G Biotech
	DNAacr	DNA vaccine expressing alpha crystalline, a key latency associated antigen of Mtb	B	University of Delhi; DBT, Govt. of India
	HVJ-Envelope/HSP65 DNA+IL-12 DNA	Combination of DNA vaccines expressing Mtb HSP-65 & IL-12	B, PI and IT	Osaka University
	pUMVC6/7 DNA	DNA vaccine plasmid vector pUMVC6 or pUMVC7 expressing Rv 3872, Rv 3873, Rv3874, Rv3875 or Rv3619c	P	Kuwait University
	HG85A/B	Chimeric DNA vaccines-Ag85A/B	B and IT	Shanghai H&G Biotech

Key: P= Prime, B= Boost, PI= Post Immunization and IT= Immunotherapy

So the new vaccines candidates are needed which are better than BCG and must protect not only toddlers but also adults; be effective when administered pre-exposure and post-exposure with M. tuberculosis; and ultimately the next-generation candidates should be capable of eradicating or preventing infection with M. tuberculosis, while current vaccine candidates are aimed at preventing active TB disease by controlling latent M. tuberculosis infection[5]. Currently extensive efforts are being put into the development of a better vaccine. Some of the live vaccine alternatives are quite promising for prevention of primary disease. The development of new TB vaccines should follows two basic

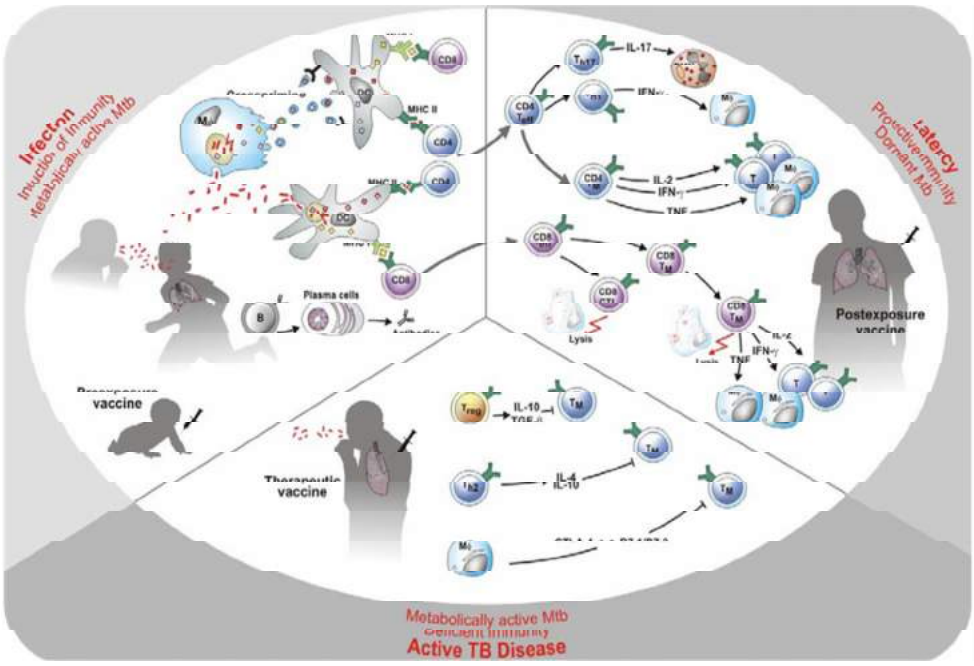


Figure*: *The three stages of tuberculosiss Disease. Stage *1: Infection of Mycobacterium tuberculosis (Mtb) frequently occurs at a young age. Metabolically active Mtb are inhaled and subsequently T-cells are stimulated which carry the major burden of acquired immunity. These include major histocompatability complex class II (MHC II)-restricted CD4 T-cells and MHC I-restricted CD8 T-cells. B cells are also activated but their protective role in TB remains elusive. Pre-exposure vaccines are given at this early stage. Novel pre-exposure vaccine candidates are given very soon after birth and thus generally before infection with Mtb. They either substitute for Bacille Calmette Gue´ rin (BCG) or boost immunity induced by BCG.* ***Stage 2:*** *Acquired immunity comprising CD4 and CD8 T-cells contains Mtb in a dormant stage within solid granulomas. T-cells produce type I cytokines and cytolytic effector molecules. They become memory T-cells which concomitantly produce multiple cytokines. Individuals remain latently infected without clinical signs of active tuberculosis (TB). Post-exposure vaccines are given to adolescents or adults who are latently infected but healthy.* ***Stage 3:*** *Mechanisms leading to deficient immunity and disease reactivation are numerous and include production of suppressive cytokines such as interleukin (IL)-10 and transforming growth factor-beta (TGFb) by T helper 2 (Th2) cells and regulatory T(reg) cells as well as T-cell exhaustion mediated by inhibitory receptor-coreceptor interactions on antigen presenting cells (APCs) and T-cells. Mtb becomes metabolically active and granulomas become caseous. Mtb can be spread to other organs and to other individuals. Therapeutic vaccines are given to TB patients in adjunct to chemotherapy. (adopted from Plos pathogen Kaufman S. H. E)*

avenues; the first aims at replacing BCG either by genetically attenuated *Mtb* or by improved recombinant (r)BCG. And in both the cases the vaccine should be: more immunogenic, capable of inducing long lasting protection; safer for human use and should also induce protection against highly virulent clinical isolates such as MDR, XDR, *TDR and Mtb* Beijing strains.

The promising alternatives are recombinant BCG expressing listeriolysin from Listeria monocytogenes, over-expression of antigen 85B in BCG or recombinant BCG expressing cytokines like IL-18 and IFN-g for more profound

Tuberculosis in different phases of clinical trails

Type of Vaccine	Candidate	Description	Clinical trial status
Fusion protein in adjuvant for pre-exposure booster vaccination	Hybrid 1+IC31	Fusion of Ag85B and ESAT-6 in adjuvant IC31	Phase I completed
	Aeras-404:Hyvac4+IC31	Fusion of Ag85B and TB10.4 in adjuvant IC31	Phase I ongoing
	M72AS01 or AS02	Fusion of Rv1196 and Rv0125 in Adjuvant AS01 or AS02	Phase II ongoing
	Hybrid 1+CAF01	Fusion of Ag85B and ESAT-6 in adjuvant CAF01	Phase I ongoing
	Hybrid 56+IC31	Fusion of Ag85B, ESAT-6 and Rv2660c in adjuvant IC31	Phase I ongoing
Recombinant BCG for pre-exposure prime vaccination	rBCG30	rBCG-expressing Ag85B	Phase I completed
	VPM 1002	rBCG-expressing listeriolysin and urease deletion	Phase II ongoing
	Aeras-422	rBCG-expressing listeriolysin	Phase I terminated
Viral-vectors for pre-exposure booster vaccination	AdAg85A	Replication deficient adenovirus 5 expressing Ag85A	Phase I
	Crucell Ad35/Aeras-402	Replication deficient adenovirus 35 expressing Ag85A, Ag85B, TB10.4	Phase II ongoing
	Oxford MVA85A/Aeras-485	Modified vaccinia Ankara expressing Ag85A	Phase II ongoing
Whole bacterial cell vaccine for therapeutic vaccination	M. vacca	Inactivated M. vacca	Phase Ii completed
	RUTI	Detoxified M. tuberculosis in liposomes	Phase II ongoing

Th1 cell polarization[10,11] and deletion of antiapoptotic genes to facilitate cross-priming, introduction of genes encoding dormancy antigens such as the DosR-regulated gene products for postexposure vaccination of latently infected individuals[12], subunit vaccines of fused MTB proteins with novel adjuvants, heterologous vectors such as modified vaccinia Ankara or adenovirus expressing MTB proteins; attenuating MTB by removing virulence genes such as secA. One more way to improve BCG is either by introducing immunodominant *Mtb*-specific antigens that are absent from BCG, such as RD1 locus-encoded antigens (ESAT6, CFP10)[13-17]; or by over-expressing antigens that BCG already expresses by itself (cognates of Ag85 complex), but probably not sufficiently high throughout all phases of infection. Another way to improve BCG is by introducing genetic modifications for superior targeting of essential immune pathways, for example, by enhancing or facilitating cross-priming; and to inhibit its ability to neutralize phagosomal maturation. The second major avenue to develop better TB vaccines relies on the development of subunit vaccines which are non-live, or in the case of viral vectors, non-replicating vaccines, which can be delivered safely into the human host regardless of immune-competence. Researchers are now able to exploit cutting edge technologies in designing the most potent vaccine combination by using the prime BCG vaccine with super BCG (a rBCG vaccine that expresses listeriolysin) followed by booster doses with a super subunit vaccine[18]. This logical approach of boost vaccination by introducing a second vaccine different from BCG at a later time point to use a heterologous prime–boost strategy for prevention of TB is also of high importance.

A booster dose may be used for two purposes, to strengthen the immunity of the BCG to prevent primary disease and to strengthen the immunity in individuals with latent infection with M. tuberculosis[19]. These vaccines would contain antigens secreted by metabolically active Mtb as well as dormancy antigens. However the first attempt might be to combine current vaccine candidates to

achieve sterile eradication of the pathogen, i.e., prime with the best BCG replacement vaccine followed by a selection of subunit boosters. Further the tremendous progress in standardizing animal models of MTB infection has allowed complete comparison of genetically manipulated mycobacteria and new TB vaccines across the research centres around the world[20-23].

In the near future with little bit of focus, we may be ready to reap the fruits of the efforts of decades of research in developing one or two vaccines with proven protective efficacy and safety but this by no means signals the end of scientific efforts. In fact, an emerging bottleneck may not be the number of pre-clinical TB vaccine candidates that TB researchers can produce, but rather the number of vaccines that can be Table 2: Most Advanced vaccine candidates against tested clinically in efficacy trials, given the limited clinical trial capacity worldwide, i.e., a shortage that exists not only in Africa but also in Asia. Finally, the identification of TB surrogate end-point biomarkers or "correlates of protection" may drastically reduce the need for the current long-term large-scale clinical trials, and thus will speed up TB vaccine discovery and clinical testing. But this all needs a special focus on the TB research funding and fortunately the European Commission, the National Institutes of Health, and private foundations like the Bill and Melinda Gates Foundation, have instigated several mechanisms to support TB-oriented research and clinical trials [24-25]. Hopefully, the sudden political interest in vaccine development will provide a unique opportunity for the TB research community to come up with a better vaccine candidate against TB for human use in the near future.

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REFERENCES

1. World Health Organization. Multidrug and extensively drug –resistant TB (M/XDR-TB): Global Report on Surveillance and Response, Geneva, Switzerland, 2010.
2. Stop TB Partnership. The Global Plan to Stop TB 2006–2015. WHO Press, Geneva, Switzerland 2006.
3. Announcement of the national tuberculosis prevention and control plan (2001–2010). State Council of the People's Republic of China, General Office: Beijing, China,. Document No. 75, 2001.
4. Ali Akbar Velayati, Parissa Farnia, Mohammad Reza Masjedi Recurrence after treatment success in pulmonary multidrug-resistant tuberculosis: predication by continual PCR positivity Int J Clin Exp Med 2012; 5(3):271-272.
5. Gandhi NR, Nunn P, Dheda K, Multidrug resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet 2010; 375: 1830–1843.
6. Velayati AA, Masjedi MR, Farnia P, Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. Chest 2009;136:420-425.
7. Velayati AA, Farnia P, Merza MA, Zhavnerko GK, New insight into extremely drug-resistant tuberculosis: using atomic force microscopy. Eur Respir J 2010; 36:1490-1493.
8. Ottenhoff TH Overcoming the global crisis: ''yes, we can'', but also for TB? Eur J Immunol 2009; 39: 2014–2020.
9. Kaufmann SH, Hussey G, Lambert PH New vaccines for tuberculosis. Lancet 2010; 375: 2110–2119.

10. Cooper AM Cell-mediated immune responses in tuberculosis. Annu Rev Immunol 2009; 27: 393–422.
11. Sutherland JS, Adetifa IM, Hill PC, Pattern and diversity of cytokine production differentiates between Mycobacterium tuberculosis infection and disease. Eur J Immunol 2009; 39: 723–729.
12. Kursar M, Koch M, Mittrucker HW, Cutting Edge: Regulatory T cells prevent efficient clearance of Mycobacterium tuberculosis. J Immunol 2007; 178: 2661–2665.
13. Pym AS, Brodin P, Majlessi L, Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis. Nat Med 2003;9: 533–539.
14. Ottenhoff TH, Doherty TM, van Dissel JT, First in humans: a new molecularly defined vaccine shows excellent safety and strong induction of long-lived Mycobacterium tuberculosis-specific Th1-cell like responses. Hum Vaccin 2010; 6: 1007–1015.
15. Shen HB, Wang C, Yang EZ , Novel recombinant BCG coexpressing Ag85B, ESAT-6 and mouse TNF-alpha induces significantly enhanced cellular immune and antibody responses in C57BL/6 mice. Microbiol Immunol 2010 ; 54: 435–441.
16. Ottenhoff TH, Doherty TM, van Dissel JT, First in humans: a new molecularly defined vaccine shows excellent safety and strong induction of long-lived Mycobacterium tuberculosis-specific Th1-cell like responses. Hum Vaccin 2010; 6: 1007–1015.
17. Shi CH, Wang XW, Zhang H. Immune responses and protective efficacy of the gene vaccine expressing Ag85B and ESAT6 fusion protein from Mycobacterium tuberculosis. DNA Cell Biol 2008; 27: 199–207.
18. Wu J, Ma H, Qu Q Incorporation of immunostimulatory motifs in the transcribed region of a plasmid DNA vaccine enhances Th1 immune responses and therapeutic effect against Mycobacterium tuberculosis in mice. Vaccine 2011; 29: 7624–7630
19. Tchilian EZ, Ronan EO, de Lara C, Simultaneous immunization against tuberculosis. PLoS ONE 2011; 6: e27477.
20. Skeiky, Y.A. Differential immune responses and protective efficacy induced by components of a tuberculosis polyprotein vaccine, Mtb72F, delivered as naked DNA or recombinant protein. J. Immunol. 2004; 172:7618–7628.
21. Olsen, A.W Protective effect of a tuberculosis subunit vaccine based on a fusion of antigen 85B and ESAT-6 in the aerosol guinea pig model. Infect. Immun 2004; 72:6148–6150.
22. Kaufmann, S.H., Baumann, S., and Nasser Eddine. A Exploiting immunology and molecular genetics for rational vaccine design against tuberculosis. Int. J. Tuberc. Lung Dis 2006;10:1068–1079, 2006
23. Ottenhoff T. H. M and Kaufman S. H. E Vaccines against tuberculosis; where are we and where do we need to go? PLoS Pathog 8(5): e1002607. doi:10.1371/journal/ppat.1002607, 2012.
24. Coakley C, Bhroin E, Buonadonna P. MEPs urge EU to fight tuberculosis worldwide. European Parliament February 3 2011.
25. Kupferschmidt K Infectious disease. Taking a new shot at a TB vaccine. Science 2011; 334: 1488–1490. doi:10.1371/journal.pone.0027477.