Journal of Infectious Diseases Advance Access published May 30, 2016

Vaccination against tuberculosis with whole cell mycobacterial vaccines

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Summary

Live attenuated and killed whole cell vaccines offer a promising vaccination strategy against tuberculosis. A number of whole cell vaccine candidates, based on recombinant BCG, attenuated *Mycobacterium tuberculosis,* or related mycobacterial species are in various stages of preclinical or clinical development. In this review, we discuss the vaccine candidates and key factors shaping the development pathway for live and killed whole cell vaccines, and provide an update on progress.

Development of vaccines against many human pathogens was traditionally based on attenuation or inactivation of the pathogenic organism. This approach has been very successful and many live attenuated vaccines confer highly durable immune responses that provide protective immunity for decades [1].

Live attenuated or killed whole cell vaccines (WCV) against mycobacteria also have potential advantages over protein–adjuvant formulations and recombinant viral-vectored constructs for vaccination against tuberculosis (TB). This is supported by a plethora of evidence of vaccination against TB with Bacille Calmette Guerin (BCG), an attenuated strain derived from *Mycobacterium bovis* originally isolated from cows. BCG is typically the most protective vaccine against *Mycobacterium tuberculosis* in experimental animal models. A number of human trials of BCG also show partial vaccine efficacy against TB [2, 3]. The advantages of WCV over protein-adjuvant formulations and viral-vectored constructs are hypothesized to be due to their broad antigen composition, which includes the (almost) complete protein repertoire, lipids, carbohydrates and other moieties that may be antigenic and induce donor unrestricted T cell responses, B cell responses and possibly also NK and ILC responses. Live WCV also possess an ability to induce long-lasting memory immune responses, probably related to their restricted persistence or replication *in vivo*, as typified by other whole cell vaccines (e.g. measles, yellow fever, polio). In addition, BCG vaccination may have a general positive effect on mortality due to other diseases, at least in resource-limited countries [4]. This effect may rely, at least in part, on epigenetic imprinting of immune

cells after BCG vaccination, a process referred to as “trained immunity” [5]. It is most likely that these positive effects are also induced by live WCV.

Live attenuated TB vaccines thus offer a promising vaccination strategy against TB, and a number of vaccine candidates based on recombinant BCG and attenuated *M. tuberculosis* are in preclinical or clinical development. To be considered for licensing the WHO recommends that these vaccine candidates will need to be either on their own or as heterologous boosts of BCG, i/ safer than BCG or ii/ at least as safe as BCG and more efficacious than BCG in a prophylactic setting. Therefore, it is expected that various new WCV candidates will target different indications: (1) WCV safer than BCG will primarily target neonates, since BCG is already (partially) protective in this population; (2) a WCV developed as a booster of BCG and to be used in adolescents will have to fill particular booster conditions, e.g. inducing tolerable and/or acceptable adverse events after secondary or tertiary booster administration; (3) a WCV expressing latency antigens is needed for adolescents and young adults with latent *M. tuberculosis* infection (LTBI); and (4) a highly attenuated or even killed WCV may be particularly interesting for therapy in adjunct to chemotherapy, especially for immunocompromised individuals such as those with AIDS.

Here we review recent progress in clinical and preclinical development of WCV for TB (Tables 1 and 2, listing WCV in clinical or preclinical development, respectively).

Lessons from VPM1002 and MTBVAC, the most clinically advanced WCV

The recombinant BCG, VPM1002, is at the most advanced stage of clinical development. VPM1002 expresses listeriolysin (LLO, encoded by the gene *hly* in *Listeria monocytogenes*), which is known to perforate the phagosomal membrane and is biologically active at a pH of 5.5 [6]. Optimal biological activity of LLO in the phagosome containing VPM1002 is achieved by deletion of the urease C subunit-encoding gene (*ureC*), which functions to reduce acidification of the phagosomal compartment. VPM1002 is therefore designed to allow enhanced release of BCG-derived antigens into the cytosol and increased apoptosis and xenophagy of host cells *in vitro* [7, 8]. After extensive preclinical development VPM1002 successfully completed two phase I trials (NCT 00749034 [9, 10] and NCT 01113281) and one phase IIa trial in infants (NCT 01479972), which show that it is safe and immunogenic in adolescents/adults and infants. It is currently being assessed in large cohorts of newborns from HIV^+ and HIV^- mothers (NCT 02391415). VPM1002 has been found to be highly efficacious, with an excellent safety record in preclinical models as compared to BCG [7]. In a mouse model, VPM1002 induced central memory T cells to a greater degree than BCG and when adoptively transferred, this central memory cell population provided protection with high efficiency [11]. *In vitro* studies indicate that VPM1002 induces increased apoptotic and xenophagic events, which may underlie the promising safety and efficacy record to date [8]. More recently, it has also been shown to be highly effective as post-exposure vaccine in an experimental mouse model of LTBI (M. Gengenbacher et al. Submitted). Currently it is being prepared for a phase III trial in India comprising a group of

TB patients who had completed drug treatment, expected with a risk of relapse in the order of 10%. Hence, this is a high-risk group allowing assessment in study groups of 1,000-2,000 individuals.

MTBVAC is a live rationally attenuated derivative of the *M. tuberculosis* isolate MT103, which belongs to lineage 4 (Euro-American), one of the most widespread lineages of *M. tuberculosis*. MTBVAC contains all the genes present in *M. tuberculosis* strains commonly transmitted between humans by the aerosol route, including the genes that are deleted in *M. bovis* and BCG. MTBVAC contains two independent stable deletion mutations in the virulence genes *phoP* and *fadD26.* These deletions were generated in the absence of antibiotic resistance markers, fulfilling the Geneva consensus requirements for progressing live mycobacterial vaccines to clinical trials [12]. PhoP is a transcription factor that controls expression of 2% of the *M. tuberculosis* genome, including production of immunomodulatory cell-wall lipids and early secretory antigenic target (ESAT)-6 secretion [13]. Deletion of *fadD26* leads to complete abrogation of synthesis of the virulence surface lipids phtiocerol dimycocerosates (PDIMs) [14]. Extensive preclinical studies demonstrated adequate attenuation and safety of MTBVAC comparable to BCG, with superior immunogenicity and efficacy against *M. tuberculosis* [12, 15]. A first-in-human MTBVAC clinical trial was recently completed successfully in healthy adults in Lausanne, Switzerland (NCT02013245) [16]. In this trial, when MTBVAC was given at the same dose as BCG ($5x10^5$ CFU), there were more responders in the MTBVAC group than in the BCG group, with a greater frequency of polyfunctional $CD4^+$ central memory T cells. However, this study

has the limitation, as a phase I first in-human trial, that the secondary objective (immunogenicity) was not powered for statistical analysis. Nevertheless, MTBVAC is the first live-attenuated *M. tuberculosis* vaccine to enter clinical trials and to date has shown a comparable safety profile to BCG [16]. A notable finding in the first trial was the absence of ESAT-6 and CFP-10-specific T cell responses at the end of the study [16], suggesting that interferon-γ release assays (IGRAs) could be utilized as study endpoints in future efficacy trials to test efficacy against *M. tuberculosis* infection. The immunogenicity data show that MTBVAC is at least as immunogenic as BCG. Altogether these data supported the advanced clinical development in high-burden countries where TB is endemic. A dose-escalation safety and immunogenicity study to compare MTBVAC to BCG in newborns with a safety arm in adults is currently ongoing in South Africa (NCT02729571).

Other WCV candidates

Other WCV candidates have completed preclinical development and entered or are about to enter clinical trials in humans. They include candidates to be used either as therapeutic or preventive vaccines.

Therapeutic vaccine candidates

RUTI is a polyantigenic liposomal vaccine made of detoxified, fragmented *M. tuberculosis* cells. It is targeted for the prevention of active TB in subjects with LTBI. A phase IIa clinical trial was completed in 2014 in South Africa [17]. Three different doses of RUTI were compared to placebo for safety,

tolerability and immunogenicity in HIV-infected and -uninfected subjects with latent *M. tuberculosis* infection after completion of one-month isoniazid treatment before vaccination. RUTI was well tolerated and its immunogenicity profile suggested a single injection of one of the highest doses might be optimal and sufficient, which will be tested in future trials. New trials are being planned, including evaluation of the efficacy of RUTI in specific populations such as patients with MDR-TB (C. Villaplana and P.J. Cardona, Personal communication).

M. indicus pranii preparation was found to have potential effects against TB when used as an aerosol-delivered adjunct to chemotherapy in animal models, including guinea pigs [18]. However, in a recent phase III clinical trial in patients with TB pericarditis *M. indicus pranii* vaccination had no immunotherapeutic effect either alone or adjunctive to prednisolone [19]. Based on clinical evidence that this vaccine candidate can modulate immunopathology in sepsis [20], it is currently being assessed as an immunotherapeutic agent in a phase IIb trial in patients with severe sepsis (ClinicalTrials.gov reference, NCT02330432).

Vaccine preparations of the non-tuberculous mycobacteria, *M. vaccae* and *M. obuense,* have also been extensively developed in preclinical studies and clinical trials. Killed *M. vaccae* was studied for use as an immunotherapeutic agent against leprosy and TB [21]. Killed *M. vaccae* as well as a *M. vaccae* lysate, made by a press method, have been assessed as adjunct therapy to anti-TB treatment in many trials in different countries, including in HIV-infected

persons. Two meta-analyses by Yang et al. [22, 23] suggested that *M. vaccae* therapy led to moderate improvements in sputum conversion and radiographical appearances. A phase III trial of the lysate preparation of *M. vaccae* is currently underway in China (personal communication, Ann Ginsberg, Aeras).

Heat-killed preparations referred to as SRL172 and DAR-901, initially thought to be *M. vaccae* but recently identified as *M. obuense,* have also been tested in numerous trials [24, 25]. One such a *M. obuense* preparation was tested as a booster in a phase III trial known as the Dar-Dar trial in BCG-vaccinated and HIV-infected adults in Tanzania [26]. The data suggest that multiple-dose administration of inactivated, whole cell *M. obuense* may prevent HIV-associated TB. The conclusions of this trial need to be confirmed in further trials; a phase I of the DAR-901 candidate is currently underway (personal communication, Ann Ginsberg, Aeras).

Preventive live vaccine candidates

Many other live WCV candidates are in the preclinical development pipeline, some with very promising results (**Table 2**). For instance, a BCG mutant inactivated in *zmp1*, a gene involved in inflammasome inhibition, appeared more immunogenic and safer than BCG in mice and is more protective than BCG in mice and guinea pigs [27]. This candidate is poised to enter phase I clinical trial soon (P. Sander, Personal communication). Other attenuated *M. tuberculosis* strains, inactivated in the transcriptional regulator SigH [28] or in metabolic genes such as *panCD* or *lysA*, involved in pantothenate and lysine

biosynthesis respectively, amongst others [29, 30] are also in preclinical development and may enter phase I trials in the future.

Safety of WCV

Safety of live WCV is a critically important consideration. The safety concerns are exemplified by the observation that BCG, a highly attenuated live vaccine that has been given to ca. 4 billion people, can cause disseminated disease in immunocompromised persons. The inherently difficult question for WCV is the optimal degree of attenuation. According to WHO recommendations two independent genetic inactivations must be made in an *M. tuberculosis*-based vaccine candidate before it can enter clinical trials [31]. Highly attenuated strains, completely incapable of *in vivo* replication, are most likely very safe, but might fail to induce sufficient immunity for long-term protection. For example, a *M. tuberculosis* pantothenate auxotrophic mutant was found much safer and as protective as BCG in mice [30]; whether strains of this nature confer long-term protection against TB remains to be evaluated in appropriate experimental settings. The BCG-Δ*zmp1* mutant [27] and VPM1002 (see above) are good examples of WCV that appear safer and more immunogenic and protective than BCG, at least in animal models.

On the other hand, less attenuated strains may persist for longer periods of time *in vivo* and therefore be highly immunogenic, but may also be associated with unacceptable adverse events. For example, a phase I clinical safety trial of a recombinant BCG vaccine, which expressed perfringolysin, Ag85A, Ag85B, and Rv3407, had to be terminated because two vaccine recipients presented with shingles reactivation [32].

Given the rich literature on safety and efficacy of BCG, it is ideal that studies of WCV candidates be performed with BCG as a comparator, to allow comparison of adverse events between the WCV candidates with BCG.

Which animal models are preferred for preclinical WCV studies?

A remarkable range of animal species have been utilized in TB vaccine research, including mice, guinea pigs, rabbits, mini pigs, badgers, cattle, and rhesus or cynomolgus macaques. A recent development in the TB vaccine community is a renewed emphasis on more stringent preclinical data about vaccine efficacy. Further, the Bill and Melinda Gates Foundation has communicated that significant vaccine efficacy in non-human primates is a prerequisite for their support of late-phase human trials. In light of this background, selection of an appropriate animal model and study design of animal experiments is important. Different approaches and species may be required for efficacy testing and in-depth immunological studies of WCV in animal models. For example, guinea pigs may be suitable for the former, whereas the virtually unlimited immunological reagents and genetic approaches for murine experimentation may encourage mechanistic immunological studies in mice. In light of the clear differences in BCG efficacy between unsensitized and sensitized persons [33], a good animal model for latent *M. tuberculosis* infection would be highly desirable.

Can WCV be used as heterologous boosts of childhood BCG vaccination?

A large number of human studies suggest that homologous boosting of childhood BCG vaccination with BCG does not provide additional benefit [34]. Although it must be acknowledged that none of these studies systematically optimized the interval between administrations of BCG, or any other parameters of the homologous prime-boost protocol, it is generally believed that revaccination with BCG is inefficient. It remains possibe that BCG revaccination in humans can be optimised to achieve greater efficacy, as supported by a number of studies of homologous BCG revaccination in cattle, deer and wild pigs, which have shown enhanced efficacy over single BCG [35-37]. Importantly though, BCG revaccination is not associated with major safety concerns in these studies. A recent study in tuberculin skin test (TST)+ adults also reported that BCG-revaccination was safe and well tolerated and that injection site reactogenicity was similar to that of primary BCG vaccination [38].

If BCG revaccination itself does not confer better protection than a BCG prime, how can a single heterologous WCV administration before or during adolescence be expected to boost BCG-induced immunity? Although speculative at this stage, we propose that a WCV that is better than BCG as a prime may also be better than BCG as a boost. This speculation might also hold true regarding the ability of such a better WCV to overcome the limitations of BCG-induced protection, thought to be due to exposure to environmental non-tuberculous mycobacteria or to helminthic infection, for example [39]. In addition, recent modelling results suggest targeting of

adolescents with TB vaccines will be more cost effective and have greater impact on transmission of *M. tuberculosis* [40]. Since BCG is the only currently licenced vaccine, it is likely that advancing such heterologous strategies to efficacy trials will require strong evidence in preclinical models. Selection of animal models ideal for testing such approaches is therefore an important issue.

Important further considerations include selection of revaccination intervals (for example, short or long intervals between prime and boost), environmental exposure of animals, and how to define protective efficacy in animal models.

What is the optimal administration route for WCV?

BCG is given as an intradermal vaccination in most countries. Can efficacy of WCV be improved by changing the route of immunization to percutaneous, intramuscular, or mucosal routes (although the latter raises safety concerns that will need to be addressed before proceeding for clinical trials)? Experiments have already started in order to test the efficacy of WCV, such as MTBVAC and VPM1002, when delivered into the lungs via alternative routes, such as the intratracheal, intranasal, oral and aerosol routes. A recent murine study showed that BCG administered intranasally, but not subcutaneously, confers robust protection against pulmonary *M tuberculosis* challenge, indicating that pulmonary vaccination triggers a specific mucosal immune response [41]. These results demonstrate that airway delivery of BCG can overcome the lack of protection observed when BCG is given parenterally. Respiratory administration could therefore be advantageous in TB-endemic countries, where intradermally administered BCG has inefficient effectiveness against pulmonary TB.

Conclusion

Many questions in the development of TB WCV remain. Much will be learnt from the many preclinical studies and clinical studies currently underway. It is critical that rare and expensive efficacy trials in humans are appropriately leveraged to perform exploratory studies that maximise the knowledge gained.

Acknowledgments

We acknowledge funding from EC HORIZON 2020 (contract TBVAC2020 no. 643381 to S.H.E.K., C.M. and O.N.), FP7 (contract NEWTBVAC no. 241745 to S.H.E.K., C.M. and O.N.), the Bundesministeriums für Bildung und Forschung (BMBF, contract inVAC no. 03ZZ0806A to S.H.E.K.) and the Spanish Ministry of Economy and Competitiveness [contract BIO2014-5258P to C.M.].

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Table 1. WCV candidates in clinical development

Vaccine candidate	**Backbone**	**Live/ dead**	**Modifications**	**Purpose[a]**	**Safe in HIV+ individuals**	**Clinical trial stage**	**References**
VPM1002	Recombinant BCG	Live	LLO inserted, *ureC* deletion	P	Yes	Phase II	[7, 9]
MTBVAC	Attenuated *M. tuberculosis*	Live	*phoP & fadD26* deletions	P	Not tested in human. Safer than BCG in SCID mice	Phase I	[12, 16]
RUTI	Detoxified & fragmented cells *M. tuberculosis*	Dead	*Polyantigenic liposomal preparation*	T	Yes	Phase II	[17]
M. indicus pranii	*M. indicus pranii* (formerly *Mycobacterium* w)	Dead	Heat-killed	T	Yes	Phase II	[18, 19]
M. vaccae or SRL172 or DAR-901	*M. vaccae* or *M. obuense* (formerly thought to be *M. vaccae*)	Dead	Heat-killed or irradiated	T	Yes	Phase III, Phase IIb (sepsis)	[22-24, 26, 42]

a. P, prophylactic; T, therapeutic

Table 2. WCV candidates in preclinical development

Vaccine candidate	Backbone	Live/dead	Modifications	Purpose[a]	Safe in HIV+ individuals	References
BCGΔ*zmp1*	Recombinant BCG	Live	*zmp1* deletion	P	Yes (safer than BCG in SCID mice)	[27]
MtbΔ*sigH*	Recombinant *M. tuberculosis*	Live	*sigH* deletion	P	Possibly, not stipulated	[28]
sigE mutant	Recombinant *M. tuberculosis*	Live	*sigE* deletion	P	Yes (safer than BCG in nude mice)	[43]
Δ*leuD* Δ*panCD*	Recombinant *M. tuberculosis*	Live	*leuD* & *panCD* deletions	P	Yes (safer than BCG in SCID mice)	[44]
$mc^2$6020	Recombinant *M. tuberculosis*	Live	*lysA* & *panCD* deletions	P	Yes (safer than H37Rv in SCID and than BCG IFNγ knock-out mice)	[29, 45]
Δ*secA2*	Recombinant *M. tuberculosis*	Live	*secA2* deletion	P	Possibly, not stipulated	[46]
Δ*lysA* Δ*secA2*	Recombinant *M. tuberculosis*	Live	*lysA* & *secA2* deletion	P	Yes (safer than BCG in SCID mice)	[47]
$mc^2$6030	Recombinant *M. tuberculosis*	Live	RD1 & *panCD* deletions	P	Yes (safer than H37Rv in SCID and than BCG IFNγ knock-out mice)	[45, 48]
BCG::ESAT6-L28A/L29S	Recombinant BCG	Live	Reconstituted with ESX-1, ESAT-6 mutated	P	Yes (safer than BCG in SCID mice)	[49]

			L28A/L29S			
BCGΔ*sapM*	Recombinant BCG	Live	*sapM* delection	P	Not definitive, Persistence in immunocompetent mice equivalent to BCG	[50]