Department of Immunology

Director: Stefan H. E. Kaufmann

3. Rational Vaccine Development

3. Rational Vaccine Development

The recombinant vaccine BCG Δ*ureC*::*hly*, which was developed by our team over the last 2 decades, has now entered an exciting stage [1]. The vaccine has been licensed to Vakzine Projekt Management (VPM) GmbH in Hannover, who termed it VPM1002. Later, it was sublicensed to the largest vaccine producer in the world, Serum Institute India (Pune, India). The vaccine has now successfully completed two phase I clinical trials in adults: the first in Germany (NCT00749034) [2] and the second in South Africa (NCT01113281). Moreover, a phase IIa clinical study in infants in South Africa (NCT01479972) has been successfully completed. Thus, VPM1002 is safe and immunogenic in adults and infants. Currently a phase II trial in HIV-exposed newborns is ongoing in South Africa (NCT02391415), which will accumulate sufficient safety and immunogenicity data to register for a phase IIb efficacy trial in infants in South Africa in the near future. Most recently, the protocol for a phase III trial with VPM1002 has been submitted to the responsible Indian regulatory agency. The goal of this study is to assess vaccine efficacy in a post-exposure setting in adults. Study participants are former TB patients who have successfully completed conventional drug therapy (multiresistant TB excluded). In this setting, recurrence rate, i.e., TB as a result of relapse or of reinfection, is in the order of 10%. Hence, this study group allows assessment of protective efficacy in ca. 2000 individuals per study group. Moreover, since BCG vaccination is not considered as treatment of choice, the trial comprises two study arms only: vaccination with VPM1002 and no vaccination. Recruitment is expected to last for ca. 12 months and the study itself is planned to last another 12 months, since the majority of TB cases in this setting develop within this time period.

Concomitant studies in the wet lab are aimed at shedding more light on immunologic mechanisms underlying the improved protective efficacy of VPM1002 over canonical BCG. These studies have revealed a major role for central memory $CD4^+$ T cells in the improved vaccine efficacy of VPM1002 [3], and generated compelling evidence for a major contribution of inflammasome activation and autophagy [4]. Finally, recent experiments revealed profound protection induced by rBCG Δ*ureC::hly* given post-exposure Mtb infection [5]. This finding was highly supportive for the move forward to a phase III trial in India. In parallel, we aim at further improving the BCG Δ*ureC::hly* candidate with respect to safety and efficacy. A safer vaccine candidate has been created by removing vitamin B6 synthesis in the live vaccine strain [6]. In fact this vaccine strain is extremely safe both in immunocompetent and immune-deficient mice. However, protection is reduced to the level of that stimulated by parental BCG.

In an attempt to further improve the already profound protection afforded by VPM1002, we integrated relevant cytokine genes into VPM1002, but without effect [7]. More successful was an approach in which we deleted the anti-apoptotic nuoG gene [17]. Our work not only identified a role for nuoG in xenophagic pathways but also demonstrated further improvement of vaccine efficacy while sustaining the excellent safety profile. As part of the ADITEC vaccine consortium, we have generated canonical BCG and VPM1002 expressing the H56 protein, which is a fusion protein containing epitopes from

Department of Immunology

Director: Stefan H. E. Kaufmann

3. Rational Vaccine Development

three Mtb antigens, ESAT6, Ag85B and Rv2660 [8]. These recombinant strains are currently being tested in homologous and heterologous prime–boost studies together with intradermal DNA vaccination with H56 DNA. In addition to creating new vaccines, we are also interested in the impact of the route of administration on the efficacy of BCG and in novel vaccine candidates, including those generated by our group. To this end, we have already found improved vaccine efficacy of BCG administered by the mucosal route over the parenteral route. Intriguingly, mucosal BCG vaccination strongly induced airway resident memory T cells of both CD4 and CD8 subpopulations.

In the TB vaccine candidate VPM1002 rBCG Δ*ureC::hly*, the gene encoding urease C was exchanged by the listeriolysin-encoding gene. Listeriolysin perforates the phagosomal membrane at an acidic pH, which is achieved by urease C deletion. The vaccine candidate has been licensed to Vakzine Projekt Management (VPM) and sublicensed to Serum Institute India (SSI, Pune, India). It had completed a phase I trial in Germany (NCT 00749034) successfully. In this trial, three escalating doses were given to young adults (10^4, 10^5, 10^6 CFUs VPM1002) and compared to 10^6 CFUs BCG. The vaccine study included both BCG pre-exposed and unexposed adult volunteers and the vaccine trial ranged over 6 months with a 6-month follow-up period [2]. Subsequently a similar phase I trial was performed in South Africa (NCT 01113281) which was completed successfully in 2011. The same regimen (three escalating doses of VPM1002 and 10^6 CFUs BCG as comparator) was performed however only in BCG pre-exposed adult volunteers. Both phase I studies demonstrated immunogenicity and safety in adults of VPM1002. A phase IIa trial in South Africa (NCT 01479972) was completed successfully in 2013. In this trial study, three escalating doses of 10^4, 10^5, 10^6 CFUs VPM1002 were compared to 10^6 CFUs BCG in newborns. This study demonstrated safety and immunogenicity of VPM1002 in newborns. Currently, a phase II trial is being performed in South Africa (NCT 02391415) including 416 newborn from HIV^+ and HIV^- mothers, with the first baby vaccinated in June 2015. This clinical study primarily aims at defining safety of VPM1002 in HIV-exposed newborns. Currently, VPM1002 is under negotiation with regulatory agencies in India, for a multicentric phase III trial in adults. The goal of this study is to determine the capacity of VPM1002 to protect against relapse when given after successfully completed drug treatment of TB patients. It is therefore a post-exposure study of healthy individuals after cure of TB. It is expected that ca. 10% drug-treated TB patients in this setting will relapse and the goal is to determine the efficacy of VPM1002 in preventing TB recurrence. In parallel, a multicentric phase I/II therapy trial for bladder cancer (NCT 02371447) is being performed in Switzerland. It has been approved since 2015, with the first instillation in September 2015. BCG is the standard of care for nonmuscle invasive bladder cancer, and the study aims to determine the therapeutic effects of VPM1002 in bladder cancer patients with previous failure of canonical BCG treatment.

BCG has been used for vaccination against TB for nearly a century. Here, we analyzed immunity induced by a live TB vaccine candidate, recombinant BCG Δ*ureC::hly* vaccine (rBCG), with proven preclinical and clinical safety and immunogenicity [2;9]. We pursued in-depth analysis of the endogenous mycobacteria-specific $CD4^+$ T-cell population, comparing the more efficacious rBCG with canonical BCG to determine which T-cell memory responses are prerequisites for superior protection against TB. rBCG induced higher numbers and proportions of antigen-specific memory $CD4^+$ T cells

than BCG, with a $CXCR5^{+}CCR7^{+}$ phenotype and low expression of the effector transcription factors T-bet and Bcl-6. We found that the superior protection of rBCG, compared with BCG, correlated with higher proportions and numbers of these central memory T cells and of T follicular helper cells associated with specific antibody responses. Adoptive transfer of mycobacteria-specific central memory T cells validated their critical role in protection against pulmonary TB.

The recombinant BCG Δ*ureC::hly* (rBCG) vaccine candidate induces improved protection against TB over parental BCG (pBCG) in preclinical studies and has successfully completed a phase IIa clinical trial [2;3;9]. However, the mechanisms responsible for superior vaccine efficacy of rBCG are still incompletely understood. Here, we investigated the underlying biological mechanisms elicited by the rBCG vaccine candidate relevant to its protective efficacy. THP-1 macrophages were infected with pBCG or rBCG, and inflammasome activation and autophagy were evaluated. In addition, mice were vaccinated with pBCG or rBCG and gene expression in the draining lymph nodes was analyzed by microarray at day 1 post-vaccination. BCG-derived DNA was detected in the cytosol of rBCG-infected macrophages. rBCG infection was associated with enhanced absent in melanoma 2 (AIM2) inflammasome activation, increased activation of caspases and production of IL-1β and IL-18, as well as induction of AIM2- and stimulator of IFN genes (STING)-dependent autophagy. Similarly, mice vaccinated with rBCG showed early increased expression of *Il-1b* and *Il-18*, as well as transmembrane protein 173 (*Tmem173*, the gene encoding STING). In conclusion, rBCG stimulates AIM2 inflammasome activation and autophagy, suggesting that these cell-autonomous functions should be exploited for improved vaccine design.

BCG, a live vaccine derived from virulent *M. bovis*, was introduced almost a century ago to fight the global TB pandemic. Until now, BCG remains the only TB vaccine in clinical use [10]. It is relatively safe in immunocompetent individuals and significantly reduces mortality and extrapulmonary TB in infants. However, it fails to protect against pulmonary TB, the most prevalent form of the disease and the source of spread. In high endemic areas, a large proportion of adolescents and adults carry latent TB infection (LTBI). Hence, a TB vaccine for adolescents and adults frequently needs to be administered post-exposure with Mtb. We have developed recombinant BCG Δ*ureC*::*hly*, which secrets pore-forming listeriolysin O (LLO) of *Listeria monocytogenes* to achieve superior pre-clinical protection and safety. This is clinically the most advanced viable TB vaccine candidate, which has successfully demonstrated safety and immunogenicity in adults and infants. Here we assessed the protective capacity of BCG Δ*ureC*::*hly* as post-exposure vaccine. Mice with LTBI were better protected when immunized with BCG Δ*ureC*::*hly* as compared to vaccination with canonical BCG. The profound protection achieved in our preclinical model strongly suggests that BCG Δ*ureC*::*hly* should be evaluated as a post-exposure vaccine in adolescents/adults with LTBI.

The only TB vaccine in use today, BCG, provides insufficient protection and can cause adverse events in immunocompromised individuals, such as BCGosis in HIV^{+} newborns. We previously reported improved preclinical efficacy and safety of the recombinant vaccine candidate BCG

Department of Immunology

Director: Stefan H. E. Kaufmann

3. Rational Vaccine Development

ΔureC::hly, which secretes the pore-forming LLO of *Listeria monocytogenes*. Here, we evaluate a second-generation construct, BCG *ΔureC::hly Δpdx1*, which is deficient in pyridoxine synthase, an enzyme that is required for biosynthesis of the essential cofactor vitamin B6 [11]. This candidate was auxotrophic for vitamin B6 in a concentration-dependent manner, as was its survival *in vivo*. BCG *ΔureC::hly Δpdx1* showed markedly restricted dissemination in subcutaneously vaccinated mice, which was ameliorated by dietary supplementation with vitamin B6. The construct was safer in severe combined immunodeficiency mice than the parental BCG *ΔureC::hly*. A prompt innate immune response to vaccination, measured by secretion of IL-6, granulocyte colony-stimulating factor, keratinocyte cytokine, and macrophage inflammatory protein-1α, remained independent of vitamin B6 administration, while acquired immunity, notably stimulation of antigen-specific CD4 T cells, B cells, and memory T cells, was contingent on vitamin B6 administration. The early protection provided by BCG *ΔureC::hly Δpdx1* in a murine Mtb aerosol challenge model consistently depended on vitamin B6 supplementation. Prime-boost vaccination increased protection against the canonical Mtb H37Rv laboratory strain and a clinical isolate of the Beijing/W lineage. We demonstrate that the efficacy of a profoundly attenuated recombinant BCG vaccine construct can be modulated by external administration of a small molecule. This principle fosters the development of safer vaccines required for immunocompromised individuals, notably HIV^+ infants.

The current TB vaccine, BCG, provides insufficient protection against pulmonary TB, which is the most common form of the disease accounting for an estimated 9.6 million clinical cases in 2014. We deleted the NADH dehydrogenase 1 subunit G (*nuoG*) gene [12;13] from BCG Δ*ureC*::*hly*, the most advanced live vaccine candidate in clinical development [2;3;9]. *In vitro*, deletion of *nuoG* unexpectedly led to strongly increased recruitment of the autophagosome marker LC3 to the engulfed vaccine, suggesting that *nuoG* may also inhibit xenophagic pathways. In mice, BCG Δ*ureC*::*hly* Δ*nuoG* vaccination was safer than BCG and improved protection over parental BCG Δ*ureC*::*hly*, significantly reducing TB load in murine lungs, ameliorating pulmonary pathology and enhancing immune responses. Transcriptome analysis of draining lymph nodes after vaccination with either BCG Δ*ureC*::*hly* or BCG Δ*ureC*::*hly* Δ*nuoG* demonstrated earlier and stronger induction of immune responses compared to BCG SSI, and suggested upregulation of inflammasome activation and IFN-induced GTPases. In summary, BCG Δ*ureC*::*hly* Δ*nuoG* is a promising next-generation vaccine candidate with excellent efficacy and safety.

CG, the only approved TB vaccine, provides only limited protection. Previously, we generated a recombinant derivative (BCG *ΔureC::hly*), which secretes the pore-forming LLO of *Listeria monocytogenes* [86]. This vaccine shows superior protection against TB in preclinical models and is safe in humans. Here we describe two new vaccine strains which express human IL-7 (hIL-7) or hIL-18 in the genetic background of BCG *ΔureC::hly* to modulate specific T cell immunity.

Department of Immunology

Director: Stefan H. E. Kaufmann

3. Rational Vaccine Development

Both strains exhibited an uncompromised *in vitro* growth pattern, while inducing a proinflammatory cytokine profile in human DCs. Human DCs harbouring either strain efficiently promoted secretion of IL-2 by autologous T cells in a coculture system, suggesting superior immunogenicity. BALB/c mice vaccinated with BCG *ΔureC::hly*, BCG *ΔureC::hly_hIL7* or BCG *ΔureC::hly_hIL18* developed a more robust Th1 response than after vaccination with parental BCG. Both strains provided significantly better protection than BCG in a murine Mtb challenge model but efficacy remained comparable to that afforded by BCG *ΔureC::hly*. We conclude that expression of hIL-7 or hIL-18 enhanced specific T cell responses but failed to improve protection over BCG *ΔureC::hly* in mice.

Currently, BCG is the only licensed vaccine against TB. Yet, its moderate efficacy against pulmonary TB in all age groups combined with a sharp increase in drug resistance urgently calls for improved TB vaccination strategies. Besides modifications of the vaccine vector, alternative administration routes can also improve local protection and vaccine efficacy [14]. Here, we dissected pulmonary immune responses following mucosal BCG vaccination, which conferred superior protection in an experimental mouse model of TB. In comparison to subcutaneous vaccination, mucosal vaccination by intratracheal and intranasal routes induced two spatially distinct waves of protective $CD4^+$ and $CD8^+$ T effector memory (T_{EM}) and tissue resident memory (T_{RM}) cells. Early after mucosal BCG vaccination, T cells accumulated within the lung parenchyma, whereas mycobacteria-specific T cells resided almost exclusively within the airway lumen after clearance of BCG. The airway-resident $CD8^+$ T cells displayed prototypical T_{RM} features and secreted IFN-γ and tumor necrosis factor-alpha (TNF-α) [15]. In contrast, airway-resident $CD4^+$ T cells comprised a mixture of T-bet^+ effector and $Foxp3^+$ regulatory T cells producing IL-10. Adoptive mucosal transfer experiments demonstrated an important contribution of both $CD8^+$ and $CD4^+$ T cell subsets for protection against TB. Collectively, we unraveled the mechanistic basis of superior immune protection following mucosal BCG administration by demonstrating a key role for vaccine-induced airway-resident T cells in host defense against pulmonary TB. These results have direct implications for the design of refined vaccination strategies against pulmonary TB [16].

BCG is the only vaccine against TB currently in use, but while it protects against childhood TB and disseminated forms of disease, it is ineffective against pulmonary TB, the most common form of disease. The H56 fusion protein, containing epitopes from three Mtb antigens, ESAT6, Ag85B and the latency antigen Rv2660c, has been shown to confer protective immunity in pre-exposure mouse models [85]. Previously, we generated a recombinant derivative (BCG *ΔureC::hly*), which secretes the pore-forming LLO of *Listeria monocytogenes* [9]. As this vaccine has improved efficacy, we generated BCG *ΔureC::hly* expressing H56 to enhance efficacy further. Antigen-specific T cell responses to Ag85B were increased after vaccination with BCG *ΔureC::hly*::H56, but only a small number of mice showed responses to ESAT6. BCG *ΔureC::hly::H56* did not increase efficacy of either pre-exposure or post-exposure vaccination strategies. However, as BCG *ΔureC::hly* is already very effective, and the recombinant strain may not express additional inserted antigens optimally due to

Department of Immunology

Director: Stefan H. E. Kaufmann

3. Rational Vaccine Development

already expressing listeriolysin, we also generated recombinant BCG SSI expressing H56. BCG::H56 vaccination led to increased IFN-γ-producing cells specific for Ag85B, ESAT6 and Rv2660c. The efficacy of this vaccine in a challenge model is currently being tested. In addition, we are testing BCG::H56 in homologous and heterologous prime–boost models, together with intradermal DNA vaccination with optimized H56 DNA sequence. Preliminary data indicates that intradermal DNA vaccination induces increased numbers of antigen-specific T cells. Currently, we await the results of prime–boost and efficacy studies.

References

1. Kaufmann SH, Cotton MF, Eisele B, Gengenbacher M, Grode L, Hesseling AC, Walzl G. The BCG replacement vaccine VPM1002: from drawing board to clinical trial. Expert Rev Vaccines 2014 May;13(5):619-30.

2. Grode L, Ganoza CA, Brohm C, Weiner J, III, Eisele B, Kaufmann SH. Safety and immunogenicity of the recombinant BCG vaccine VPM1002 in a phase 1 open-label randomized clinical trial. Vaccine 2013 Jan 2;31:1340-8.

3. Vogelzang A, Perdomo C, Zedler U, Kuhlmann S, Hurwitz R, Gengenbacher M, Kaufmann SH. Central memory CD4+ T cells are responsible for the recombinant bacillus Calmette-Guerin deltaureC::hly vaccine's superior protection against tuberculosis. J Infect Dis 2014 Dec 15;210(12):1928-37.

4. Saiga H, Nieuwenhuizen N, Gengenbacher M, Koehler AB, Schuerer S, Moura-Alves P, Wagner I, Mollenkopf HJ, Dorhoi A, Kaufmann SH. The recombinant BCG deltaureC::hly vaccine targets the AIM2 inflammasome to induce autophagy and inflammation. J Infect Dis 2015 Jun 1;211(11):1831-41.

5. Gengenbacher M, Kaiser P, Schuerer S, Lazar D, Kaufmann SHE. Post-exposure vaccination with the vaccine candidate Bacillus Camette-Guerin *deltaureC::hly* induces superior protection in a mouse model of latent tuberculosis infection. Microbes & Infect. 18(5):364-8 (2016)

6. Gengenbacher M, Vogelzang A, Schuerer S, Lazar D, Kaiser P, Kaufmann SH. Dietary pyridoxine controls efficacy of vitamin B6-auxotrophic tuberculosis vaccine bacillus Calmette-Guerin DeltaureC::hly Deltapdx1 in mice. MBio 2014;5(3):e01262-14.

7. Rao M, Vogelzang A, Kaiser P, Schuerer S, Kaufmann SH, Gengenbacher M. The tuberculosis vaccine candidate bacillus Calmette-Guerin DeltaureC::hly coexpressing human interleukin-7 or -18 enhances antigen-specific T cell responses in mice. PLoS ONE 2013;8(11):e78966.

Department of Immunology

Director: Stefan H. E. Kaufmann

3. Rational Vaccine Development

8. Aagaard C, Hoang T, Dietrich J, Cardona PJ, Izzo A, Dolganov G, Schoolnik GK, Cassidy JP, Billeskov R, Andersen P. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. Nat Med 2011 Feb;17(2):189-94.

9. Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Nasser EA, Mann P, Goosmann C, Bandermann S, Smith D, Bancroft GJ, Reyrat JM, van SD, Raupach B, Kaufmann SH. Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette-Guerin mutants that secrete listeriolysin. J Clin Invest 2005 Sep;115(9):2472-9.

10. Kaufmann SH, Gengenbacher M. Recombinant live vaccine candidates against tuberculosis. Curr Opin Biotechnol 2012 Dec;23(6):900-7.

11. Dick T, Manjunatha U, Kappes B, Gengenbacher M. Vitamin B6 biosynthesis is essential for survival and virulence of *Mycobacterium tuberculosis*. Mol Microbiol 2010 Nov;78(4):980-8.

12. Miller JL, Velmurugan K, Cowan MJ, Briken V. The type I NADH dehydrogenase of *Mycobacterium tuberculosis* counters phagosomal NOX2 activity to inhibit TNF-alpha-mediated host cell apoptosis. PLoS Pathog 2010 Apr;6(4):e1000864.

13. Velmurugan K, Chen B, Miller JL, Azogue S, Gurses S, Hsu T, Glickman M, Jacobs WR, Jr., Porcelli SA, Briken V. *Mycobacterium tuberculosis* nuoG is a virulence gene that inhibits apoptosis of infected host cells. PLoS Pathog 2007 Jul;3(7):e110.

14. Stary G, Olive A, Radovic-Moreno AF, Gondek D, Alvarez D, Basto PA, Perro M, Vrbanac VD, Tager AM, Shi J, Yethon JA, Farokhzad OC, Langer R, Starnbach MN, von Andrian UH. VACCINES. A mucosal vaccine against *Chlamydia trachomatis* generates two waves of protective memory T cells. Science 2015 Jun 19;348(6241):aaa8205.

15. Laidlaw BJ, Zhang N, Marshall HD, Staron MM, Guan T, Hu Y, Cauley LS, Craft J, Kaech SM. CD4+ T cell help guides formation of CD103+ lung-resident memory CD8+ T cells during influenza viral infection. Immunity 2014 Oct 16;41(4):633-45.

16. Kaushal D, Foreman TW, Gautam US, Alvarez X, Adekambi T, Rangel-Moreno J, Golden NA, Johnson AM, Phillips BL, Ahsan MH, Russell-Lodrigue KE, Doyle LA, Roy CJ, Didier PJ, Blanchard JL, Rengarajan J, Lackner AA, Khader SA, Mehra S. Mucosal vaccination with attenuated *Mycobacterium tuberculosis* induces strong central memory responses and protects against tuberculosis. Nat Commun 2015;6:8533.

17. Gengenbacher M, Nieuwenhuizen N, Vogelzang A, Liu H, Kaiser P, Schuerer S, Lazar D, Wagner I, Mollenkopf H-J, Kaufmann SHE. Deletion of *nuoG* from the vaccine candidate *Mycobacterium bovis* BCG Δ*ureC::hly* improves protection against tuberculosis. mBio, 7: doi:10.1128/mBio.00679-16 (2016).