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New Perspectives in Tuberculosis Prevention and Control

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SUMMARY

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Aim of study. The aim of the study was to assess the current approach to novel anti TB vaccines as the new tools for TB prevention and control.

Objectives. 1. To describe the current epidemiological situation of TB in the world. 2. To evaluate BCG as a current strategy for TB prevention and control. 3. To find out the main types of the new vaccines against TB and to describe their role in today’s medicine.

Methods. Data discussing TB disease, BCG and novel anti- TB vaccines under development, mainly in the years 2006-2016 was collected from databases ScienceDirect, UpToDate, EMBASE, PubMed, PLOS ONE, CSH perspectives in Medicine, The Lancet, WHO and CDC data. Key terms included tuberculosis, BCG and new anti- TB vaccines.

Results. TB is an infectious disease caused by Mycobacterium tuberculosis. The prevalence of TB is decreasing all over the world but it is still one of the most common causes of death, causing about 1.8 million deaths per year worldwide. BCG is the only vaccine approved against TB. It has protective effect against military and meningeal TB but only minor benefit against adult pulmonary TB and latent TB. Research for novel anti TB vaccines increased largely. Vaccines under development were presented in this paper.

Conclusions. 1. TB is one of the most lethal infectious diseases and is poorly controlled worldwide. 2. BCG is the only vaccine currently approved against TB but it is unable fully prevent or control TB. This emphasizes the urgent need for new more effective strategies. 3. New vaccines are the key component in achieving the end of TB epidemic and some of them are under development. Most of the new vaccines are devoted to replace BCG but post- exposure and therapeutic vaccines are investigated as well. All of them are classified by different delivery systems and divided into subunit vaccines, which include viral vector vaccines and adjuvant protein vaccines, live vaccines and Mycobacterium cell or extract vaccines. The most promising vaccine is MTBVAC, but until now none of the new vaccines is able to replace BCG.
CONFLICT OF INTEREST

The author reports no conflicts of interest.
ETHICS COMMITTEE CLEARANCE

Ethics committee clearance was not required.
ABBREVIATIONS LIST

TB- Tuberculosis
BCG- Bacillus Calmette- Guérin
WHO- World Health Organization
HIV- Human immunodeficiency virus
DOTS- Directly Observed Therapy, short course
PPD- Purified protein derivative
MDR-TB- Multidrug-resistant tuberculosis
XDR-TB- Extensively drug-resistant tuberculosis
rBCG- recombinant Bacillus Calmette- Guérin
MVA85A- Modified Vaccinia virus Ankara expressing *Mycobacterial* antigen 85A
QFT- GIT - Quantiferon Gold in Tube
UK- United Kingdom
Ag- Antigen
IFNγ - Interferon gamma
ELISpot- Enzyme linked immunosorbent spot
ICS- Intracellular cytokine staining
TNFα - Tumor necrosis factor alpha
IL- Interleukin
ESAT-6- Early secretary antigenic target
Th1- T helper-1 cell
hly- Listeriolysin O

PEST- proline, glutamate, serine and threonine

MHC- Major histocompatibility complex
INTRODUCTION

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. According to the World Health Organization (WHO), TB is one of the ten most common causes of death even more common than malaria and human immunodeficiency virus (HIV) [1]. In 2015, approximately 10.4 million new TB cases were diagnosed around the world, leading to around 1.8 million deaths [1]. The epidemiology of TB is different among different countries, with most cases occurring in developing countries [2]. This strongly emphasizes the need for better control of TB which has cost many lives in the past as well as nowadays.

BCG vaccine was introduced in 1921, more than 90 years ago, and until now it is the only vaccine approved against TB. BCG became part of the expanded program of immunization in 1974 [4]. It was found effective when administered in infants, with protective effects mainly against the disseminated form of TB, but showed only minor effect in prevention of pulmonary TB, which is the most prevalent form of TB [4].

Nowadays, with the development of modern technologies, new approaches in the development of an effective and safe vaccine against TB are the goal of many researchers. In this work, novel approaches in TB vaccines, aimed at boosting current BCG as well as the novel vaccines aiming to replace BCG, will be presented.
AIM AND OBJECTIVES

The aim of the study was to assess the current approach to novel anti TB vaccines as the new tools for TB prevention and control.

Objectives of the thesis:
1. To describe the current epidemiological situation of TB in the world.
2. To evaluate BCG as a current strategy for TB prevention and control.
3. To find out the main types of the new vaccines against TB and to describe their role in today’s medicine.
1. LITERATURE REVIEW

1.1 Tuberculosis overview

According to WHO data, TB is one of the ten most common causes of death, more common than malaria and HIV [1]. In 2015, approximately 10.4 million new cases of TB were diagnosed around the world, leading to approximately 1.8 million deaths [1]. The incidence of TB varies among different countries around the world, a map demonstrating the various incidence rates in 2015 in Fig. 1. High incidence countries have 100/100,000 case or higher, as seen in India, Micronesia, Southeast Asia and Africa. Intermediate rates are of 26 to 100 /100,000 cases, including countries as Central and South America. And low rate counties have 25/100,000 cases or less as in Australia, Canada and Japan [2]. Majority of cases reported from Asia which account for 58% and form Africa accounting for 27% [3].

Fig.1 TB incidence rates around the world at 2015
(World Health Organization 2016, Global tuberculosis report)

TB prevalence has been decreasing greatly due to application of Directly Observed Therapy, short course (DOTS) in 1994-1995. DOTS assisted in decline of chronic untreated cases and shortened disease
duration. It is estimated that about 22 million lives were saved [3]. Unfortunately rate of TB morbidity and mortality in some regions failed to show sufficient decline, possibly due to higher rates of treatment resistant infections or HIV, as seen in Africa [3].

1.1.1 Tuberculosis discovery

Tuberculosis is an infectious disease caused by *M. tuberculosis*, an intracellular obligate aerobe. Humans are the only reservoirs of *M. tuberculosis*. The pathogen’s main route of transmission is airborne, spreading from one infectious person to another, far less common routes include transdermal and gastrointestinal. *M. tuberculosis* is thought to be one of the most deadly pathogens, responsible for more deaths than any other microbial pathogen [5]. Better apprehension of TB pathogenesis started at beginning of 19th century by Théophile Laennec, both regarding pulmonary and extrapulmonary forms of TB. The next big step towards deeper TB understanding occurred at 1882 by Hermann Heinrich Robert Koch which discovered tubercle bacillus and was able to demonstrate its presence in tissues of animals and humans suffering from TB. In 1890, Koch presented tuberculin, which he thought to be the cure of TB, although tuberculin was rapidly determined ineffective as a treatment, Koch concluded it might have a diagnostic use. In 1905 Koch was awarded the Noble Prize in Medicine and Physiology for his discovery. In 1909 Clemens Freiherr von Pirquet published the tuberculin reaction has a useful diagnostic tool for latent TB, a term he set for the first time. One year later, Charles Mantoux presented the use of cannulated needle and syringe for intracutaneous administration of tuberculin and in 1930, Florence Seibert introduced purified protein derivative (PPD) which is used until now days as screening tool for TB exposure [5].

1.1.2 Tuberculosis forms

The most common form of the disease is pulmonary TB, but extra pulmonary sites as kidneys, lymph nodes, spine and brain may be involved as well [6]. Exposure to *M.tuberculosis* can lead to either of the following: clearance of bacteria by immune system, primary TB disease, latent infection or reactivation disease [7, 8]. Approximately 5 to 10 percent of infected individuals will develop primary TB infection, most commonly during the first two years [7, 8].
Latent TB can be defined as an inactive disease, the person will have no symptoms and will not be contagious to his environment. They will show a positive skin reaction TB test [8]. Factors as HIV, malignancy, corticosteroids use and other cause which suppress the immune system may cause reactivation of latent infection into active TB disease. In the absence of immunosuppressive factors the risk of reactivation is only 5 to 10 percents [7, 8]. Reactivated TB disease is more likely to be localized when compared to primary TB [8]. Another form is disseminated TB, which occurs when the infection spreads from the lungs via blood, lymph or by mechanical erosion, into other body organs. Disseminated TB can lead to death in 80 percent of cases without appropriate treatment [8].

According to WHO in 2015 about 1 million of children were infected with TB worldwide, accounting for 5 to 15 percent of all TB cases [9, 11]. In children TB diagnosis is more complicated and some of those dying from TB are incorrectly sorted as pneumonia, meningitis, HIV or other diseases [9]. Poor diagnosis and reporting of cases prevent a more accurate estimation of the global burden of TB in children [10]. Most children develop the disease within 1 year after primary infection. The risk profile has a bimodal distribution, with youngest children and adolescents being at increased risk [10]. In children as in adults, pulmonary TB is the most common form, but compared to adults children are more likely to develop extra pulmonary TB as miliary TB and TB meningitis [11]. Although the amount of TB cases as declined, the proportion of extra pulmonary TB cases remained relatively unchanged [12]. Common extra pulmonary TB types include: TB lymphadenitis, Pleural TB, miliary TB, TB peritonitis, renal TB, genital TB, intestinal TB and TB pericarditis [12].

1.1.3 Tuberculosis treatment

According to WHO at least 85% of newly diagnosed TB cases should be treated, but it may be difficult to achieve in some counties, as those with higher rates of drug resistance, poor compliance of population and not well developed DOTS system. TB drug susceptibility can be classified as: Drug- resistant TB, when detected resistance to one of the four first line drugs used for treatment (Isoniazid, Rifampicin, Pyrazinamide and Ethambutol), Multidrug-resistant TB (MDR- TB) defined as a resistance to both Isoniazid and Rifampicin, and Extensively drug-resistant TB (XDR-TB) defined as a resistance to Isoniazid, Rifampicin and at least one second line drug as Capreomycin, Kanamycin and Amikacin [8, 13].

Drug susceptible active TB is treated by a regimen divided into two periods, the intensive phase including
Two months course of the following drugs: Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol, and the continuation phase which includes four months therapy with Isoniazid and Rifampicin [13].

MDR- TB and XDR- TB are of great danger to worldwide TB control programs [14]. In many cases treatment strategies of MDR- TB and XDR- TB are not clear, some may use different drug regimens or increasing dosage of the same drugs [14]. As seen in the Fig. 2, we can notice significant epidemics of MDR- TB in Eastern Europe and central Asia while other areas remain with relative stable levels.

**Fig. 2 New cases of TB with MDR-TB showed in percentage, at 2015**

(World Health Organization 2016, Global tuberculosis report)

1.1.4 The WHO „End TB Strategy”

Now days the decrease in TB incidence rates is very slow in high burden countries as Africa and Asia, an intermediate decline level of about 5 percent in China and Cambodia, and a greater decrease in TB incidence rates is observed in low- incidence countries, overall producing a global decline of only 1.5 percent a year in TB incidence rates [15]. Therefore in order to decrease significantly the burden of TB around the world WHO has presented the „End TB Strategy”. A scheme of „End TB Strategy” is presented by Fig. 3.
The „End TB Strategy” was presented in May 2014, it covers the period of 2016 to 2035, with the aim to end the global TB epidemics [16]. The strategy indicators include reduction of 35% in TB deaths by 2020, 75% by 2025, 90% by 2030 and 95% by 2035 [16]. As well as reduction in TB incidence rates of 20% by 2020, 50% by 2025, 80% by 2030 and 90% by 2035 which is about 10 TB cases per 100,000 individuals [16]. All indicators are compared to the incidence reported in 2015 [16]. Development of a new and effective TB vaccine is a key component in achieving those goals by 2035. Most experts in TB prevention can agree that mass vaccination of adolescents and young adults in countries with high TB incidence will achieve the greatest impact on disease control [17]. Latent TB infection thought to affect approximately one third of human population worldwide, therefore some scientist believe that adequate treatment of latent TB must be achieved in order to reach the „End TB Strategy” goal treatment [18].

Fig.3 WHO goals of TB incidence rate decline according to the „End TB Strategy”
(World Health Organization 2016, The End TB strategy)

1.2 The BCG vaccine

1.2.1 BCG development
BCG is a live attenuated vaccine, containing *Mycobacterium bovis* strains. Albert Calmette and Camille Guérin managed to isolated *M. bovis* from a bovine TB infected cow in 1908 at the Pasteur Institute in Lille, France. BCG was first introduced to human use in 1921 and has become part of the expanded program of immunization in 1974 [4, 19]. BCG has been used for more than 90 years and is thought to be the oldest vaccine still in use. BCG was administered more than four billion doses worldwide [19, 20]. Despite many ongoing studies, until nowadays BCG is the only vaccine approved against TB. BCG development is considered as an important milestone in the global fight against TB [19].

1.2.2 BCG sub- strains

Although all BCG vaccines produced originate from the isolate of *M. bovis*, they undergo different processing in different laboratories and conditions. Currently the most common used sub- strains include Pasteur 1173 P2, the Danish 1331, the Glaxo 1077, the Tokyo 172-1 and the Russian BCG, covering approximately 90% of all BCG vaccines produced. The Russian BCG is more commonly used in high- incidence countries. The Danish and Pasteur BCG sub- strains have showed greater immunogenicity but they did not produce higher efficacy in field trials [19, 20]. Different sub- strains induce diverse immunity and vaccines genetics is thought to be associated with difference in incidence of adverse reaction. No scientific evidence was found of variations in protective immunity between the sub- strains [21]. WHO is currently trying to organize standards for BCG production, by formulating a proper of regulations and protocols in order to equalize manufacturing process between all laboratories.

1.2.3 BCG advantage and disadvantage

In meta- analyses, BCG shows an average of 86% effectiveness in the prevention of TB [21], but the main protection given by BCG is against military and meningeal forms of TB for infant and children populations, showing only minor protective benefit against adult pulmonary TB, which is the most common form of the disease [21,22]. BCG did not show a protective benefit in reactivated TB disease. According to most trials the protective benefits of BCG last only for 10 to 15 years. However an exception was found in a study of American Indians and Alaskan natives communities showing about 50% efficacy even after 50 years [20, 21].
In most cases of BCG vaccination were reported only mild adverse events, including injection site reactions described as skin papule, erythema and tenderness [21]. The papule may progress to ulceration and result in a small scar. Another common adverse event is mild regional lymph node enlargement, mostly in axillary area [21, 22]. A major disadvantage of BCG is its high potential for severe complications when administered to immunocompromised individuals, as those with HIV [21, 23]. Complications can include disseminated BCG disease, lymphadenitis, immune reconstitution inflammatory syndrome and others.

1.2.4 WHO recommendations for BCG vaccination

WHO recommends intradermal BCG administration for infants living in high burden TB countries soon after birth, and for children at high risk who are living in low burden TB countries [21, 23, 24]. The ideal age of BCG vaccination remains in dispute, as evident by different ages of vaccination used in various counties [25]. As mentioned before severe adverse events may occur when vaccinating HIV infected individuals, therefore, WHO states that children known to be HIV positive, or those with unknown HIV status and HIV associated symptoms, should not be vaccinated [21, 23, 24]. According to WHO currently there is no proven benefit of BCG revaccination [21].

1.3 New vaccine designs and approaches

In the last decade development of new anti TB vaccines has increased significantly, nevertheless the discovery of an effective and safe anti TB vaccine is still a continuous challenge [26, 27]. In order to achieve „End TB Strategy” goals, an advanced research of relevant biomarkers and profound understanding of immunity processes of both active and latent TB should be reached [26].

New vaccines designed for TB control can be classified according to their delivery systems. They can be subunit vaccines which are delivered by a viral vector or adjuvant recombinant proteins, live vaccines including recombinant Bacillus Calmette- Guérin (rBCG) or live attenuated M. tuberculosis vaccines, or they can be vaccines delivered by Mycobacterium extract or whole cell. A summary of the new anti TB vaccines will be presented in Table.1 and Table.2 in results section. Detection of protective effect against M. tuberculosis in animal challenge models will be one of the criteria
for continuation towards clinical trials, those can involve mice, guinea pigs and cattle [27]. Another challenge for the investigators is their incomplete understanding of the level and quality of immune response which will be needed in order to produce an effective vaccine for humans in the future [27].
2. RESEARCH METHODOLOGY AND METHODS

2.1 Research planning

Research work organization was started by collecting studies which discuss TB disease and its burden, evaluating BCG and new anti-TB vaccines under development, mainly in the years 2006-2016. The studies were collected from databases ScienceDirect, UpToDate, EMBASE, PubMed, PLOS ONE, CSH perspectives in Medicine, The Lancet, WHO and CDC data. Key terms included tuberculosis, BCG and new anti-TB vaccines.

2.2 Object of study

In this work I have collected and analyze data about the new perspectives in TB prevention and control. The studied vaccines include the old BCG and novel vaccines including (a) subunit vaccines, (b) live vaccines and (c) *Mycobactrium* cell or extract vaccines.

2.3 Selection of data

After gathering data from databases, journals and websites, the abstract section was read to evaluate study relevance. Inclusion criteria were (a) animal and human studies evaluating BCG effects (b) new anti-TB vaccines effects, (c) comparison between the new vaccines and BCG and (d) those assessing TB burden now days and the need for new effective vaccine. All relevant studies were divided into topics as general TB review, BCG, new TB vaccines and subtopics as viral vector vaccines, protein adjuvant vaccines, live vaccines and mycobacterium cell or extract vaccines. Then detailed reading of all studies was done with highlighting the key features of each study. Exclusion criteria included studies which (a) were not about TB vaccines (BCG or new anti-TB vaccines), (b) studies in which vaccine effect was difficult to interpret and (c) repeatedly published literatures or similar literatures.
2.4 Research method

Systematic literature review of published studies is a theoretical type of research, data collection, analysis and summary of results. Assessment of safety and immunogenicity of those new vaccines under development nowadays was according to the results presented in published studies collected. This methodology also allows other researchers to update the review at a later time in order to integrate new findings when the next stages of clinical trial will be published and assessing if the results are of enhanced beneficial effect with vaccine progression towards next clinical trial phase or are if they are of no clinical significance.

2.5 Method of data analysis

Collected studies were classified according to their stage, either of preclinical or clinical trials. Then the studies were classified according to the vaccine properties. In all studies safety and immunogenicity data were highlighted. All data was described in a table, from which a representative of each vaccine type, of the most advanced clinical trial is noticed in the result section.
3. RESULTS

3.1 Data collection

I found 42 articles representing the aim and objectives of my work. Data discussing novel anti-TB vaccines was found in 17 studies, out of them 11 studies focused on subunit vaccines (6 on viral vector vaccines and 5 on adjuvant protein vaccines), 4 studies on live vaccines and 2 focused on *Mycobacterium* cell or extract vaccines. The summary of the data is presented in the Table 1 and Table 2.

3.2 The reasons of urgent need for new anti TB vaccines

Although the prevalence of TB is decreasing worldwide, the rate of TB morbidity and mortality in some regions failed to show sufficient decline and the prevalence of the disease is still high, it is especially seen in low-income countries [3]. Clearly BCG vaccination cannot totally prevent and control TB infection [20, 21, 22].

The WHO „End TB Strategy” was presented in May 2014 aiming to end the global TB epidemics [16]. Development of a new and effective TB vaccine is a key component in achieving the goals of the „End TB Strategy” by 2035.

In the last decade development of new anti TB vaccines has increased significantly, but nevertheless the discovery of an effective and safe anti TB vaccine continues to be a great challenge [26, 27].

3.3 Subunit vaccines

Subunit vaccines are designed to enhance BCG effect. It was thought that BCG limited effect against adult pulmonary TB is due to decreasing vaccine induced T cell immunity over the years, this assumption together with the beneficial effect of BCG against the disseminated form of TB in children are basis of production of a vaccine used to boost BCG effect rather than formation of fully new vaccine.

3.3.1 Viral vector vaccines
Modified Vaccinia virus Ankara expressing *Mycobacterial* antigen 85A (MVA85A) is one of the most famous representatives of this category. MVA85A has showed protective effect against TB in animal models, as well as immunogenicity [28, 29] which supported the progression towards clinical trials. Phase I and IIa clinical trials of MVA85A were managed in United Kingdom (UK), Gambia, South Africa and Senegal. Tested populations included healthy children, adolescents and adults which showed good safety profile. More importantly MVA85A was also tested in HIV positive adults, which also showed good vaccine tolerability [21, 29]. Human challenge models were used for development of some vaccines as influenza, dengue fever and typhoid. Since intentional induction of TB infection considered unethical in humans, a model using BCG as a surrogate for *M. tuberculosis* infection can show variations in antimicrobial immunity caused due to past BCG vaccination [30]. When comparing this human BCG challenge model with the animal *M. tuberculosis*, MVA85A demonstrated improved efficacy when administered as BCG boost than administration of BCG alone. However, such improvement was not shown in human BCG challenge model [30]. Phase IIb of clinical trial was conducted in infants showed that MVA85A managed to induce an antigen-85 (Ag-85) specific T cell response, which was measured by *ex-vivo* interferon gamma (IFNγ) enzyme linked immunosorbent spot (ELISpot). Blood intracellular cytokine staining (ICS) assay demonstrated CD4 positive T cells mainly noting IFNγ, tumor necrosis factor alpha (TNFα) and interleukin-2 (IL-2), compared to BCG-specific CD4 positive T cells reaction which mainly shows IFNγ alone [29, 31]. This variation of reaction can be the basis of concluding MVA85A vaccine as ineffective against TB, showing no additional benefit to BCG vaccination alone [29, 31]. During phase IIb MVA85A was well tolerated in humans, causing no significantly severe adverse reactions [31]. Interestingly, after MVA85A phase IIb trial was conducted, a new meta-analysis study claims that there was no MVA85A vaccine efficacy detected in tested animals, raising the questionable decision of proceeding to infant testing [32], which as mentioned above showed no vaccine efficacy.

### 3.3.2 Adjuvant protein vaccines

Several adjuvant protein based vaccines are under development currently. H56: IC31 was designed as a post-exposure vaccine. H56 is a fusion protein composed of Ag85B, early secretary antigenic target (ESAT-6), which also make H1 fusion protein, and Rv2660c. Ag85B and ESAT-6 are found to be secreted during acute infection phase and Rv2660c was detected in high level under nutrient
stress, therefore suggesting their importance for intracellular survival. H56 protein is formulated in IC31 adjuvant which has demonstrated strong T helper-1 cell (Th1) response [33, 34]. During animal studies boosting BCG with H56: IC31 showed to be effective in latent TB infection containment demonstrated by limited disease spread and pathology at necropsy, prevention of disease reactivation after administration of anti-TNF antibody, and increased immune response to all H56: IC31 was seen by IFNγ and IL-2 ELISpot assay [33]. The positive results provided basis for human trials. Phase I human trial was conducted comparing H56: IC31 effects in TB infected and non-infected healthy adults. The vaccine showed acceptable tolerability and safety profile. Most common adverse events included injection site reactions and 36% of individual had experienced transient bradycardia, no severe adverse events were reported [34]. Vaccine induced CD4 positive T cells were mainly ESAT-6 and Ag85B specific with only low amounts of Rv2660c specific cell reaction. Surprisingly, lower vaccine doses had higher levels of IFNγ, TNFα and IL-2 CD4 positive T cells, illustrating positive immunogenic effect of H56: IC31 when given as BCG boosting vaccine [34].

H4: IC31 was designed as a preventive vaccine. H4 is a fusion protein of TB10.4 and Ag85B, two immunogenic Mycobacterial Ag’s, formulated in IC31 adjuvant [35, 36]. During animal studies H4: IC31 showed to be effective as BCG boosting vaccine, leading to increased and more durable immune response by multifunctional T cells [35]. Proceeding to phase I clinical trial, H4: IC31 had demonstrated an acceptable safety profile, most common adverse events were local injection site reactions and mild fatigue. Immunogenicity was assessed by ELISpot and ICS assays, showing durable and increased multifunctional H4- specific T cell response, thought to be associated with long memory response [36]. Interestingly, H4: IC31 showed to be most effective with lower vaccine doses, similar to result found in H56: IC31 trials [34, 36].

M72/AS01 designed as a preventive vaccine. M72 is a fusion protein of Mycobacterial Ag’s Rv1196 and Rv0125, formulated in AS01 adjuvant. Phase I/II clinical trial showed acceptable safety profile with no severe adverse events reported. M72/AS01 was immunogenic, enhancing multifunctional specific CD4 positive T cells as well as showing humoral immune responses, lasting for up to three years [37].

3.4 Live vaccines
MTBVAC is a live attenuated vaccine, designed as preventive vaccine aiming to replace BCG. MTBVAC is a derivative of *M. tuberculosis* isolate MT103. MTBVAC consists of two deletion mutations of virulent genes without antibiotic resistance markers, phoP gene which codes for transcription factor and fadD26 gene, his deletion stops the synthesis of surface virulent lipids. An experiment in newborn mice showed the vaccine to be safe with no evidence of pathological changes according to histopathological findings, the tested mice had no developmental or structural changes [38]. MTBVAC was found to be more immunogenic and with enhanced protection efficacy when compared to BCG [38]. Evidence of the vaccine improved longer protection can be concluded in guinea pigs, non-human primates and mice, by induced higher levels of Ag- specific CD4 positive T cells differentiation to central memory T cells [39]. MTBVAC is the first *M. tuberculosis* based live vaccine which completes the requirements to proceed into clinical trials [39].

VPM1002 was designed as a preventive vaccine, it is an rBCG expresses listeriolysin O (hly) which originates from *Listeria monocytogenes* and deleted urease C (ureC) gene that enables optimal acidic environment for hly action [40]. Hly forms pres in acidic environment and has amino acid sequence of proline, glutamate, serine and threonine (PEST) causing rapid phagosomal degradation once protein appears in cytosome of human cells. Hly activity allows potentiation of major histocompatibility complex (MHC) I and CD8 positive T cells [40], also seen CD4 positive T cells inducing IFNγ, TNFα, IL-17 [41]. Ten preclinical animal studies were done demonstrating improved protection of MVP1002 when compared to BCG control groups. Safety profile in preclinical studies was acceptable, local injection site reactions were the most common adverse events [40]. At phase II trial in South African newborns the vaccine was showing both immunogenic and safe [41].

### 3.5 *Mycobacterium* cell or extract vaccines

As a representative of this group we will assess RUTI which was designed as a therapeutic vaccine. RUTI is a polyantigenic liposomal vaccine consists of detoxified fragmented *M. tuberculosis* cells [23]. The goal of this vaccine was to enhance the existing dormant immunological response of the body against *M. tuberculosis*, as well as to trigger new immunological reaction against the latent infection [42]. During phase II clinical trial conducted in South Africa vaccine was tested with latent TB both in HIV- negative and HIV- positive individuals. Results showed an acceptable tolerability and safety profile with mainly mild local adverse events as erythema, pain, swelling and local nodules which were more
common among HIV-positive patients. RUTI had no adverse influence on HIV-positive patients when assessing changes in viral loads or CD4 positive T cells counts. Lower doses demonstrated better immunogenicity and in 50µg RUTI has lost its polyantigenic effect. RUTI demonstrated cellular polyantigenic reaction, with no humoral immune response [42].

Table 1: Overview of new anti-TB vaccines in clinical trials

<table>
<thead>
<tr>
<th>No.</th>
<th>Vaccine name</th>
<th>Vaccine aim</th>
<th>Delivery system</th>
<th>Antigen or Rv</th>
<th>Trial phase</th>
<th>Reference</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>MVA85A</td>
<td>Preventive</td>
<td>Modified vaccinia Ankara</td>
<td>Ag85A</td>
<td>IIb</td>
<td>Michele D. Tameris et al. 2013 [31]</td>
<td>Viral vector vaccine</td>
</tr>
<tr>
<td>2.</td>
<td>H56: IC31</td>
<td>Post-exposure</td>
<td>IC31</td>
<td>Ag85B ESAT-6 Rv2660c</td>
<td>I</td>
<td>Angelique Kany Kany Luabeya et al. 2015 [34]</td>
<td>Adjuvant protein vaccine</td>
</tr>
<tr>
<td>5.</td>
<td>MTBVAC</td>
<td>Preventive</td>
<td>Live <em>mycobacteria</em></td>
<td>-</td>
<td>I</td>
<td>Ainhoa Arbues et al. 2013 [39]</td>
<td>Live attenuated vaccine</td>
</tr>
<tr>
<td>6.</td>
<td>VPM1002</td>
<td>Preventive</td>
<td>Live Rbcg with hly and ureC</td>
<td>-</td>
<td>II</td>
<td>André G. Loxton et al. 2017 [41]</td>
<td>Live vaccines</td>
</tr>
<tr>
<td>7.</td>
<td>RUTI</td>
<td>Therapeutic</td>
<td>Whole fragmented <em>M.</em></td>
<td>-</td>
<td>II</td>
<td>Andre S. Nell et al. 2014 [42]</td>
<td>Detoxified fragmented <em>M.</em></td>
</tr>
</tbody>
</table>
### Table 2: Comparison between immunogenicity and safety of new anti-TB vaccines in clinical trials

<table>
<thead>
<tr>
<th>No.</th>
<th>Vaccine name</th>
<th>Immunogenicity</th>
<th>Safety</th>
</tr>
</thead>
</table>
| 1.  | MVA85A [31]  | CD4 positive T cells inducing IFNγ, TNFα, IL-2 and IL-17.                      | At least one local adverse event in 89% of participant (45% in placebo group).  
At least one systemic adverse event in 80% of participant (76% in placebo group). 
At least one serious adverse event mostly of acute lower respiratory tract or gastroenteritis in 18% of participants (18% in placebo group). |
| 2.  | H56: IC31 [34] | Ag –specific IgG and CD4 positive T cells inducing IFNγ, TNFα, IL-2. Especially in lower H56 doses. | At least one local adverse event seen in 36% of participants.  
Bradycardia detected in 20% of cases.  
No serious adverse events reported. |
| 3.  | H4: IC31 [34, 35] | CD4 positive T cells inducing IFNγ, TNFα, IL-2. And an increase in IL-2/ TNFα-expressing cells, associated with effector and central memory like cells. Lower doses showed higher immunogenicity. | 100% of participants had at least one adverse event.  
Local injection area reactions were more common in vaccine group compared to placebo group.  
No serious adverse events reported. |
<p>| 4.  | M72 [37]     | CD4 positive T cells inducing IFNγ, TNFα, IL-2. Higher immunogenicity seen     | AS01 showed local adverse events in 100% of cases, most commonly with injection site pain. |</p>
<table>
<thead>
<tr>
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<tr>
<td>5.</td>
<td>MTBVAC</td>
<td>CD4 positive T cells inducing IFNγ.</td>
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<td></td>
<td>[39]</td>
<td>Proved to be safe in guinea pigs.</td>
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<td>6.</td>
<td>VPM1002</td>
<td>CD4 positive T cells inducing IFNγ, TNFα, IL-17 and CD8 positive T cells.</td>
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<tr>
<td></td>
<td>[41]</td>
<td>Predominantly local injection site reactions, occurring in similar rate in both VPM1002 and BCG groups.</td>
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<td></td>
<td></td>
<td>No serious adverse events reported.</td>
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<td>7.</td>
<td>RUTI</td>
<td>CD4 positive T cells inducing IFNγ.</td>
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<td></td>
<td>[42]</td>
<td>Lower doses demonstrated better immunogenicity and 50µg RUTI lost its polyantigenic effect.</td>
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<td>100% of participants had at least one adverse event, mostly local injection site reactions (79% in placebo group).</td>
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<td>Adverse events were more common in higher RUTI doses.</td>
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<td>Only two participants had severe adverse events (retinal detachment and abscess in injection site).</td>
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4. DISCUSSION

4.1 New types of anti TB vaccines

Currently many clinical trials are attempting to find an effective and safe vaccine for TB management and which will be able to replace BCG vaccine. The vaccine can be designed as either preventive, post-exposure or therapeutic. Different vaccines use different delivery systems and their effectiveness and safety should be assessed. New anti TB vaccines can be divided into (a) subunit vaccines, which include viral vector vaccines as MVA85A and adjuvant protein vaccines as H56: IC31, H4: IC31 and M72, (b) live vaccines as VPM1002 and MTBVAC, and (c) *Mycobactrium* cell or extract vaccines as RUTI.

4.2 Safety profile

The safety profile of most vaccines evaluated showed similar results. In all studies the most common adverse events were local injection site reactions as pain, erythema, swelling and tenderness. Severe local reactions as abscesses were less frequent. Systemic adverse events of significant effect as bradycardia which was detected in 20% of participants in H56: IC31 phase I clinical trial showed be investigated more thoroughly. Other systemic adverse events included acute lower respiratory tract or gastroenteritis in 18% of participants in MVA85A phase IIb clinical trial. Events of headache, myalgia and arthralgia of mild to moderate severity were also seen in lower extent in many vaccine trials.

4.3 Immunogenicity

Vaccines effectiveness was analyzed by measuring the levels of vaccine induced cellular and humoral immune responses. Increase in levels of CD4 positive T cells inducing IFNγ, TNFα, IL-2, IL-17, and CD8 positive cells was done by QFT- GIT, ELISpot and ICS. Most vaccines evaluated showed similar immunogenicity with increase in CD4 positive T cells inducing IFNγ, TNFα and IL-2, as demonstrated in table 2. We can see that all vaccines showed an increase in CD4 positive T cells inducing IFNγ. Increase in TNFα was in all except MTBVAC and RUTI. Increase in IL-2 was in all except MTBVAC, VPM1002 and RUTI. Increase in IL-17 was seen in clinical trial IIb of MVA85A and in
VPM1002 phase II trial vaccines. CD8 positive T cells were seen only in VPM1002 phase II trial vaccines.

Interestingly vaccines as H56: IC31, H4: IC31 and RUTI demonstrated increased immunogenicity with vaccines of lower doses when comparing to higher doses, highlighting the need to investigate the most appropriate dosage of vaccine for maximal effectiveness. However, it is not yet clear which immune response will correlate best with an effective vaccine providing immunity against TB, therefore profound understanding and research are still needed.

4.4 The perspectives of new anti TB vaccines

Until now days no vaccine from those evaluated is able to replace BCG as an effective anti TB vaccine. Further investigation and more fundamental understanding of TB and BCG immunity mechanisms is required in order to produce safe and effective vaccine.
5. CONCLUSION

1. TB is one of the most lethal infectious diseases and is poorly controlled worldwide.

2. BCG is the only vaccine currently approved against TB but it is unable fully prevent or control TB. This emphasizes the urgent need for new more effective strategies.

3. New vaccines are the key component in achieving the end of TB epidemic, some of them are under development. Most of the new vaccines are devoted to replace BCG but post- exposure and therapeutic vaccines are investigated as well. All of them are classified by different delivery systems and divided into subunit vaccines, which include viral vector vaccines (MVA85A) and adjuvant protein vaccines (H56: IC31; H4: IC31 and M72); live vaccines (VPM1002 and MTBVAC) and Mycobacterium cell or extract vaccines (RUTI). The most promising vaccine is MTBVAC, but until now none of the new vaccines is able to replace BCG. Further investigations and more fundamental understanding of TB and BCG immunity mechanisms are required.
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