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Original Research Article

New Treatment Methods to Treat Tuberculosis

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Abstract: Internationally it is estimated that around 273,000 new cases of multidrug-resistant tuberculosis (MDR-TB, resistant to isoniazid and rifampicin) occurs every year. To improvise and efiiciently manage MDR-TB in the context of the DOTS strategy, WHO and partners have been piloting an expanded treatment strategy called DOTS-Plus. However, standard definitions for MDR-TB registration or Statndard Operating Procedures (SOP's) treatment outcomes do not exist. We document a set of definitions and standards to assess treatment outcomes of MDR-TB patients under the DOTS strategy.
Keywords: Multidrug resistant TB, DOTS, WHO, Isoniazid and Rifampicin

INTRODUCTION

A recent report estimates that around 273,000 new cases of multidrug-resistant tuberculosis (MDR-TB)--3.2% of all new TB cases--occurred worldwide in 2014 [1]. MDR-TB is caused by strains of *Mycobacterium tuberculosis* resistant to at least the two most efficient anti-TB drugs, isoniazid and rifampicin. As such, the treatment of MDR-TB requires the use of second-line anti-TB drugs, which are less cost effective, more toxic, and less effective than first-line drugs. In order to efficently manage the MDR-TB in the context of the DOTS strategy [1], particularly in the low- and the middle-income countries, WHO and its associated international partners are piloting or initiating an expanded TB treatment strategy called DOTS-Plus, which treats MDR-TB patients in a programmed manner [2]. The Green Light Committee is the implementing authority, several of these projects have been established across the globe, and their cumulative evidence will be critical to a framework of policy recommendations regarding MDR-TB management [3]. Till today standard definitions for MDR-TB treatment outcomes do not exist.

In 1997, WHO published a set of outcome definitions and recommended standards for conducting cohort analyses for patients treated under the DOTS strategy [4]. These definitions have been periodically revised and widely accepted and applied by national TB control programs (NTPs) as an integral part of recording and reporting under the DOTS strategy. The use of these definitions in yearly NTP publications allows comparison of treatment outcomes across countries, fostering an accepted standardized operational approaches (SOP's) to TB clinical care and achievable case management [5]. However, these set definitions are not applicable for MDR-TB as MDR-TB case management has several unique characteristics: treatment duration (up to 24 months for MDR-TB patients versus six months for drug-susceptible TB patients), enrolllment criteria (history of treatment with first or second-line drugs),and more complex monitoring mechanisms

Globally, these two factors most likely influence the TB burden. They are:

• DOTS expansion, which delivers increased TB detection and cure rates in areas of DOTS coverage. There has been a renewed vigour for DOTS expansion, with rapidly covering the diseased population in the high-incidence countries (India 45%; China 68%; Indonesia 98% and Bangladesh 95%). Results from Peru Vietnam, and from China show that DOTS can significantly reduce the TB burden, however we will need to substantiate the improvement of the case-finding tools and strategies, which currently are still a

minority in TB patients despite having 100% DOTS coverage.

• The HIV/AIDS, particularly in Sub-Saharan Africa, but increasingly in South-East Asia, India and China is pandemic. National data of various countries show that HIV-TB figure has grown from 4% of the global TB burden in 1995 to 12% in 2000, with Africa now representing 20% of TB cases. Without Innovation of new TB tools and new approaches, AIDS epidemics in other areas of the world will be catastrophic for TB control. Scaling up of joint TB/HIV activities is required.

What is the TB Control Strategy?

The fundamental strategy recommended for TB control for all epidemiological settings, is DOTS (Directly Observed Treatment, Short-course). There are four key elements of this approach :

- Strong Political commitment and Availability of resources to address TB
- Uninterrupted / Unstoppable supply of the four to six most effective anti-TB drugs in specific time frame.
- A standardised reporting and recording system to enable outcome assessment of patients and TB programmes
- To detect patients with smear-positive pulmonary TB through sputum microscopy

The DOTS Strategy: a historical perspective[6]

The goals of TB control are to reduce morbidity, mortality and transmission of the disease, while minimizing the emergence of drug resistance, until TB no longer poses a threat to the public's health. To achieve this, it is necessary to ensure the diagnosis, treatment, and cure of each patient with active TB disease, particularly those capable of transmitting the disease. This will both prevent transmission of TB to uninfected individuals and prevent the emergence of drug resistant strains of the TB bacteria.

Historically, efforts to treat TB were highly ineffective and could take several years, but in the 1950s, the development of new drugs given in combination cured TB and revolutionized treatment by eliminating the need for lengthy hospitalizations. However, despite the availability of these effective drugs, TB continued to be a persistent problem. TB treatment is complicated and requires a minimum of 6 months of multi-drug therapy. Years after the development of effective anti-TB drugs, it became apparent how difficult it is for patients to complete their full course of therapy without some support from the health system. The practice of "Directly Observed Therapy" (DOT), in which a trained individual watches a patient take each dose of their medicines, was developed to address this problem. While DOT was initially considered an absolute requirement for TB treatment, randomized controlled trials have not clearly demonstrated the effectiveness of DOT compared to self-administered therapy under good program conditions with close monitoring. Though the debate continues, DOT remains the national policy and preferred treatment approach in countries that are implementing the DOTS strategy. Many countries are exploring options of making DOT less burdensome on the health care system by making arrangements for laypeople to provide DOT in the patient's community.

Around the same time as the introduction of DOT, the discovery of highly effective drugs such as rifampin made it possible to cure the majority of TB cases in six to eight months. The new rifampin containing regimen became known as Short Course Therapy. These two dramatic breakthroughs in TB treatment formed the basis of the DOTS approach—**D**irectly **O**bserved **T**herapy, **S**hort Course.

The short course therapy administered as DOT was not efficacious in controlling TB, an even more comprehensive approach to TB control was required. Developed by the International Union Against Tuberculosis and Lung Diseases (IUATLD) through their work in Africa, the DOTS strategy is a five-pronged comprehensive approach that builds upon the administration of therapy under direct observation. This strategy has been endorsed by the WHO[7].

The five components of the DOTS Strategy are:

- Sustained political commitment
- Case detection by quality-assured sputum smear microscopy
- Treatment of TB cases with standard short-course chemotherapy regimens under proper case-management conditions including direct observation of treatment
- Uninterrupted supply of quality-assured anti-TB drugs
- Recording and reporting system enabling outcome assessment and management of program effectiveness

In order for a National TB Program (NTP) to function optimally, all elements of the DOTS strategy need to be in place. However, it is a mandatory responsibility of the authority engaging in one element of TB control programming to ensure that 1) the essential complementary components are being addressed by the government or other partner(s), 2) the timing of the proposed component(s) is appropriate, and 3) that all the activities are coordinated and integrated into the host government's NTP, as well as coordinated with other partners working to strengthen the NTP.

It is important to emphasize that inadequate TB treatment is worse than no treatment. The rapidly rising rates of drug resistant TB in many countries (e.g., the former Soviet Union countries) are due to previous

poor treatment—specifically, a failure of patients to take drugs consistently and/or to drop out of treatment prior to completion of the prescribed course. With the treatment of drug resistant cases being much longer, more difficult, and vastly more expensive than that of drug sensitive cases, the very ability of public health measures to control tuberculosis is threatened by rising rates of drug resistant cases. For this reason, organizations and governments should not embark on TB treatment projects unless they are willing to commit the resources and management effort and skill needed to ensure treatment success rates above 85%, the level at which emergence of resistance is controlled.

In long term, funding would play a major role to manufacture vaccine lots for larger-scale clinical trials. Manufacture will almost certainly need to be done by organizations or in association with industry, who have the technical expertise to scale up production and to handle Large volumes of potentially infectious biological material. At this crtical stage incentives would be needed to encourage and reward the industries who participate in this commercially less competitive area.

MATERIALS AND METHODS

Animals

Pathogen-free female out bred Hartley guinea pigs were purchased from Sanzyme Ltd., Gaganpahad, Hyderabad and held under barrier conditions in an ABL- 3 biohazard laboratory at temperature of 25±1◦C with relative humidity of 50-55% with 12hr/12hr light-dark cycle and free access to food and water *ad libitum*. The animals were acclimatized for one week before the start of the experiment. Guinea pigs weighed approximately 500 to 600 g at the beginning of the experiment and were housed two to a cage. Guinea pigs in the first study were sacrificed 30days after aerogenic infection with *M. tuberculosis* H37Rv. A second set of vaccinated guinea pigs was monitored for several months after aerogenic infection with *M. tuberculosis*H37Rv. To be cost efficient, we limited to four animals per group for these studies. The experimental protocols were approved and authenticated by the Institutional animal ethics committee (IAEC) with Reg. No.1374/ac/10/CPCSEA dated 14 sep-2010 and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) with the approval No.GNP(TRK)/CPCSEA/IAEC/2016/02

Bacterial infections

M. tuberculosis Erdman and H37Rv were previously grown to early mid-log phase in Proskauer Beck medium containing 0.05% Tween80. Cultures were then aliquoted into 1-ml tubes and stored at -70°C till their usage. Thawed aliquots were then diluted in double distilled sterile water to get the desired inoculum concentrations. An aerosol generation device (Glas-Col, Terre Haute, Ind.) was used to expose animals to an aerosol of *M. tuberculosis* and was calibrated to deliver approximately 20 to 40 bacilli into each guinea pig lung.

Vaccinations

Guinea pigs were immunized with 150 µg of CFP in MPL-TeoA. In addition, some vaccines contained rIL-12 (1µg) and /or rIL-2 (20µg). Vaccines are injected subcutaneously three times at 3-week intervals duration. The vaccine posessing CFP and IL-2 was given only twice due to as light hypersensitivity reaction occured in some animals. BCG (10^3 bacilli / guinea pig) was injected intra-dermally (i.d.) once at the same time as the third immunizations. The animals were aerogenically challenged with approximately 50 M. tuberculosis bacilli six weeks later.

DNA vaccines, consisted of control plasmid vector V1Jns(DNA-vector) and V1Jns contained the genes encoding the secreted and non-secreted forms of *M. tuberculosis* Ag85Aprotein(DNA-Ag85A), were procured from the Merck Research Laboratories. Vaccines were given intramuscularly three times at 3-week intervals. Each guinea pig was given 200 µg of plasmid DNA in saline per quadricep muscle (400 µg total per immunization). The animals were then infected aerogenically as above

RESULTS

Protection responses in guinea pigs

Experiments were performed with guinea pigs to evaluate if similar conditions of cytokine enhancement were necessary for protection in the susceptible animal model. Vaccines were given three times, 3weeks apart, to groups of outbred guinea pigs, which were then challenged via aerosol infection with around 50 viable *M. tuberculosis* H37Rv bacilli six weeks following the last injection.

The results of this study (Table1) showed a 1.35-logreduc-tion in the lungs of guinea pigs that had received intra-dermal (i.d.) BCG vaccination. A marginal but statistically significant (P = 0.048) reduction in bacterial counts with in the lungs was also seen in guinea pigs given the MPL-CFP vaccine which had been supplemented with IL-12 and IL-2 .None of the other vaccine groups exhibited any statistically significant reduction in lung bacterial counts.

Table 1: Protection responses in guinea pigs

Immunization group	Log 10 mean viable bacteria (right lung lobe) ± SE	Log 10 protection (lungs)
PBS	5.75 ± 0.16	0
BCG	4.40 ± 0.10*	1.35
MPL	5.49 ± 0.22	0.26
MPL/CFP	5.79 ± 0.25	-0.03
MPL/CFP/IL-12	6.14 ± 0.19	-0.39
MPL/CFP/IL-2	6.06 ± 0.17	-0.31
MPL/CFP/IL-12/IL-2	5.21 ± 0.05*	0.54
DNA-vector	5.82 ± 0.07	-0.07
DNA-Ag85A	5.68 ± 0.20	0.07

The calculation of the Bacterial counts in the right lower-lung lobe of guinea pigs immunized with the vaccines as shown above and aerogenically infected with M. tuberculosis 6 weeks after vaccination. Data is represented as the mean CFP/right lung lobe ± standard error (n = 4). Asterisks represent statistical significance (P< 0.05) based on the unpaired, two-tailed Student t test. Turning to practicality, how can these potential new vaccines be used clinically. The majority of the worldwide population has been immunized by BCG or is generally exposed to environmental mycobacteria. As we have de-sensitized, perhaps a more realistic use of these new vaccines (given the fact that their capacity to confer survival was less than that conferred by BCG) is to boost individuals already previously vaccinated or those who may be at risk of reactivation of the disease due to latent tuberculosis or drug-resistant tuberculosis.

The results of this study demonstrate the first evidence that two completely separate vaccines, Firstly based on a subunit vaccine consisting of a mild adjuvant admixed with purified culture filtrate proteins and enhanced by the cytokine interleukin-2 and the secondary based on immunization with DNA encoding the Ag85A protein secreted by Mycobacterium tuberculosis, may both prevent the onset of disease, which is the hallmark of the guinea pig aerogenic infection model. In both cases, the survival of vaccinated guinea pigs was smaller or shorter than the Mycobacterium bovis BCG, with mortality observed of these animals probably due to development of lymphocytic granulomas within lung tissues. A Unique feature of this approach is that it neither induced skin test reactivity to commercial tuberculin. This data thus provides optimism that development of non-living vaccines which can generate long-lived immunity which is conferred by the BCG vaccine is achievable.

DISCUSSION

The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF)[8]:

The TAACF was established by the (National Institute of Allergy and Infectious Diseases) NIAID in the year 1994 to facilitate encouragement and support academic researchers and pharmaceutical companies to re-enter the area of TB drug development. TAACF services are provided absolutely devoid of cost to researchers anywhere in the world, including Public-Private Partenerships, industrial and academic researchers and data is maintained in privacy in order to protect the user's intellectual property (IP). This service is maintained by the Southern Research Institute (SRI), along with individual services provided at five US-based research centres.

High-throughput screening (HTS) of large compound is documented in volumes in libraries – in particular industrial compound libraries with validated TB targets[9]. HTS has been provided by the SRI since 2001-02. *In vitro* screening of promising individual compounds or Novel compound groups, performed by the National Hansen's Disease Program in Baton Rouge, Louisiana. These steps are available for researchers, who have the capacity to synthesize the micro amounts of compound needed (generally in the range of 1-7mg). *In vitro* screening is slower and involves more efforts intensive than HTS, although this can be improved by automation of some steps. Compounds that successfully pass all screens are then referred for *in vivo* testing. TAACF data shows more than compounds 50,000 were screened, around 500 were potent and around 200 had promising in vitro potency and selectivity to warrant further testing as compounds with potential anti-TB activity[10].

Currently, the lack of international consensus and data on effective and economical management strategies for patients with MDR-TB leaves clinicians and NTPs with difficult choices on how to best treat patients, particularly in settings of limited compounds availability [11]. Given the grim scenario of MDR-TB and the limited number of second-line anti-TB drugs available, presently it is not possible to assess treatment efficacy under traditional controlled clinical trials[12]. Currently DOTS-Plus projects, have been framed according to country-specific rates of drug resistance, financial condiditons and available technical expertise. Collating data in a systematic manner using standard core variables and outcome definitions will allow for evidence-based decisions to be made regarding MDR-TB management under a variety of program conditions.

DOTS-Plus is for the establishment of strong basic TB control measures prior to the programmatic treatment of MDR-TB, in order to ensure that careful and Micromanagement of drug-susceptible TB patients prevents the further development of MDR-TB[13].

CONCLUTION

The set of definitions described here are designed to be practical for NTPs, stressing the importance of patient case management, and relying on a minimal set of laboratory testing requirements, as not all NTPs routinely perform drug susceptibility testing. In order to confirm their applicability and decide depending on scenario-based modifications, these definitions must be pilot or mock tested in multiple DOTS-Plus settings. Data obtained from the use of these definitions of pilot study will allow for the comparison of MDR-TB outcomes among various countries and facilitate the evaluation of MDR-TB treatment effectiveness in programmatic settings.

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