

## Therapeutic Vaccination of Treatment-Failed TB Patients on “Palliative” Support Consisting of Isoniazid and Rifampicin

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### Abstract

Open label phase 2b retrospectively controlled trial was conducted in 72 treatment-failed TB patients on so-called palliative support consisting of isoniazid (H) and rifampicin (R) with or without adjunct immunotherapy. The intervention group (N=36) received once-daily pill of V-5 Immunitor (V5) and comparison control (N=36) received HR only. The subjects in V5 and control groups had military: cavitary: MDR-TB: DR-TB: TB/HIV at 10: 26: 5: 4: 1 and 14: 22: 6: 6: 2 ratios, respectively. After 3 months 69.4% of V5-treated patients experienced negative sputum smear conversion (P<0.0001) vs. 16.7% (P=0.02) in comparison group who were treated for 3.5 months on average. 9 out of 10 V5 recipients with drug-resistant TB and TB/HIV became sputum negative, whereas none of 14 patients with same diagnosis converted (P<0.0001; OR 1732; 100-30008 at 95% CI). TB-associated inflammation was downregulated by V5 as shown by normalization of leukocytosis 7.9 vs. 6.2 x10<sup>9</sup>/L (P=0.005) and decreased erythrocyte sedimentation rate 22.7 vs. 13.3 mm/h (P<0.0001), whereas among HR recipients changes were smaller, i.e., 7.2 vs. 7.5 x10<sup>9</sup>/L (P=0.49) and 26.4 vs. 22.5 mm/h (P<0.0001). The body °C temperature was reduced from 37.4 ± 0.5 to 37.1 ± 0.3 (P=0.001) and from 37.7 ± 0.5 to 37.2 ± 0.5 (P=0.0002) in V5 and control respectively. In V5 arm average body weight accrual 2.2 ± 1.7 kg (P<0.0001) was higher than 0.08 ± 1.1 kg in control. No adverse effects or reactivation of disease were seen at any time. V5 is safe and in combination with simple two-drug regimen was highly effective as an immune adjunct for management of treatment-failed and/or drug-resistant TB.

### Introduction

Tuberculosis (TB) has become global public health priority [1]. Currently available chemotherapies for the treatment of TB are not ideal. The length of therapy, coupled with side effects, often results in poor patient adherence, treatment failure, and the emergence of drug resistance [2]. Current so-called short-course chemotherapy still requires 6 months, with isoniazid, rifampicin, pyrazinamide and ethambutol during first 2 months of intensive phase and isoniazid and rifampicin for continuation phase. The global target for successful TB treatment is set at 85% or higher, however the worsening epidemic of drug resistant TB converging with HIV reduces the likelihood of attaining this goal. New approaches are needed to shorten standard short-course and increase cure rates. Although several new drugs are now in pipeline, it is unlikely that a useful therapy will emerge soon [3]. All this necessitates formulation of novel strategies to combat TB; one of them is to find simplified treatment regimens among existing drugs, which is a major priority of the Global Plan to Stop TB [3]. The idea that immunotherapy might improve treatment outcomes started gaining consensus in recent years [4].

Oral therapeutic vaccine V5 Immunitor was originally developed for the management of chronic hepatitis B and C [5-7]. During clinical trial in 20 patients with hepatitis C, who happened to have pulmonary TB with HIV-co-infection, V5 produced mycobacterial clearance in sputum smears of 94.4% of patients [8]. Due to this surprising outcome a placebo-controlled, randomized trial has been initiated. Two preliminary reports from this trial were already published, confirming that V5 was safe and effective [9,10]. During this study we had some patients who have repeatedly failed all available therapies and were placed on so-called palliative support consisting of isoniazid and rifampicin. Despite treatment failure and seemingly ineffective

two-drug chemotherapy many of these patients became sputum smear negative within one month [11]. The present study was aimed to expand this observation by recruiting 36 failure patients and following them for 3 months. As a control we have randomly selected the same number of patients who were receiving H and R only.

### Materials and Methods

#### V5 immunitor

V5 is derived from the pooled blood of donors with HBV and HCV, which after heat- and chemical inactivation was formulated into an oral pill [5-7]. It is well known that one third of people carry *M. tuberculosis* without showing symptoms of the disease. Therefore, V5 inherently has circulating *M. tuberculosis* antigens. As we had not known prior to this accidental discovery that V5 may affect TB we have no much information regarding the exact content of *M. tuberculosis* antigens.

#### Patients

The conduct of the trial was approved by the internal review board

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of the Lisichansk TB Dispensary with ClinicalTrials.gov identifier: NCT01222338. The study involved the total of 72 patients all of whom were treatment failures and were on so-called palliative support consisting of daily isoniazid (H; 300 mg) and rifampicin (R; 600 mg) (Table 1). This regimen is granted to a limited number of patients by regional health authorities as a result of case-by-case evaluation of medical and compliance history. V5 group comprised patients who repeatedly failed to convert after multiple treatment options have been tried unsuccessfully for an average/median  $6 \pm 3.6/5$  years (range 2-19 years). The participation in this study was voluntary and patients were eligible to enroll after signing the written consent. Thirty six patients have consented to receive daily dose of V5 Immunitor along with concomitant isoniazid and rifampicin for 3 months with monthly sputum microscopy. V5 group had 4 females and 32 males between ages 23-68 with mean  $\pm$  SD (median) age  $37.3 \pm 10.8$  (34) years. The baseline body weight ranged between 38-80 kg with mean  $\pm$  SD (median)  $57 \pm 10.7$  kg (56). One and five patients in this group had confirmed HIV and MDR-TB respectively. Four additional patients had confirmed drug resistance other than HR resistance. Ten patients had disseminated or miliary form of TB (27.8%) and the rest had cavitory involvement (72.2%). Most patients, 22 out of 36 (61.1%), had normal body temperature with overall mean  $\pm$  SD/median  $37.4 \pm 0.5/38^\circ\text{C}$ .

The control group of patients of same size was randomly selected among those who were receiving HR chemotherapy during the study period. At study conclusion, when V5 patients have completed their treatment, they were on HR combination between 1 and 7 months with an average  $\pm$  SD/median  $3.5 \pm 1.6/3.5$  months (lower 25% - upper 75% 2-4.8 months). These patients had treatment failure history for average/median  $6 \pm 3.6/5$  years (range 2-15 years). The group had female/male ratio 7/29 between 24-66 years and mean  $\pm$  SD (median)  $42.9 \pm 11.8$  (43.5) years. The baseline body weight was 41-87 kg with mean  $\pm$  SD (median)  $61.1 \pm 12.7$  (57) kg. Two and six patients in this group had confirmed HIV and MDR-TB. Additional six patients had resistance to drugs other than MDR-TB. In remaining 24 patients drug resistance status was unknown. The control group included 14 cases of disseminated TB (38.9%) and 22 fibrocaceous cavitory TB (61.1%). At study initiation 26 out of 36 (72.2%), had low grade fever with mean  $\pm$  SD/median  $37.7 \pm 0.5/38^\circ\text{C}$ .

## Laboratory Evaluation

The sputum microscopy on acid-fast bacilli (AFB) smears was conducted at baseline and at monthly intervals. TB drug resistance was determined by commercially available kit (Tulip Diagnostics, Goa, India) in some but not all patients. The failure to test every patient for drug resistance was due to lack of funds for laboratory services. MDR-TB was diagnosed when resistance to both isoniazid and rifampicin, with or without resistance to other drugs, was present. DR-TB was assigned when resistance other than MDR-TB was found. The hematology parameters were evaluated by standard routine techniques.

## Statistical Analysis

The obtained results were analyzed with commercially available statistical software (GraphPad Software Inc, La Jolla, CA, USA). The paired or unpaired Student t-tests were used as necessary for the analysis of inter- or intra-group means. The Wilcoxon test was used to compare paired before-after nonparametric values in "yes" or "no" manner. The Mann-Whitney nonparametric test was selected to evaluate the distribution of two unmatched arms. Fisher's exact two-tailed test was employed for analysis of data in a contingency table. All statistical analyses were done on intent-to-treat basis. The resulting probability values were considered as significant at  $P \leq 0.05$ .

## Results

### Homogeneity of studied populations

The selection process for the control HR group was random and did not appear to cause any significant distribution bias. The immune treatment (N=36) and control (N=36) groups matched by gender, baseline body weight, hematology biomarkers, diagnosis, clinical manifestations and time on therapy (Table 1, Figure 1). Except age, hemoglobin and baseline body temperature all other parameters in both groups were statistically indistinguishable, indicating that observed differences in outcome were not affected by baseline sample heterogeneity.

### Lack of adverse reactions

During the entire duration of follow-up no adverse reactions or reactivation of TB attributable to V5 were identified. Quite contrary

	No.	Sex F/M	Age	Months on Rx	Dx	Body temperature ( $^\circ\text{C}$ )	Hemoglobin (g/L)		ESR (mm/h)		Leukocytes ( $\times 10^9/\text{L}$ )		Smear (+/-)		Body weight (kg)	
							before	after	before	after	before	after	before	after	before	after
V5	36	4/32	Mean $37.3 \pm 10.8$ Median 34	Mean 3 Median 3	Miliary=10 Cavitory=26 MDR=5 DR=4 TB/HIV=1	Mean $37.2 \pm 0.6$ Median 38	117.7 $\pm 16.4$	119.4 $\pm 15.4$	22.7 $\pm 15.4$	13.3 $\pm 9.9$	7.9 $\pm 3.1$	6.2 $\pm 2.4$	36/0	11/25	57 $\pm 10.7$	59.2 $\pm 10.9$
							P=0.21		P<0.0001		P=0.005		P<0.0001		P<0.0001	
Control	36	7/29	Mean $42.9 \pm 11.8$ Median 43.5	Mean $3.5 \pm 1.6$ Median 3.5	Miliary=14 Cavitory=22 MDR=6 DR=6 TB/HIV=2	Mean $37.4 \pm 0.5$ Median 37	98.8 $\pm 15.7$	98.1 $\pm 15.4$	26.4 $\pm 11.3$	22.5 $\pm 9.9$	7.2 $\pm 2.7$	7.5 $\pm 3.6$	36/0	30/6	61.1 $\pm 12.6$	61.1 $\pm 12.7$
							P=0.34		P<0.0001		P=0.49		P=0.02		P=1	
Differences between two groups		P=0.16	P=0.04	P=0.02	P=0.8	P=0.001	P<0.0001	P<0.0001	P=0.31	P<0.0001	P=0.87	P<0.0001	P=1.0	P<0.0001	P=0.14	P=0.46

**Table 1:** Summary of baseline data and treatment outcome in 72 treatment-failed TB patients treated with isoniazid and rifampicin alone (N=36) or in combination with V5 (N=36).

patients who were receiving chemotherapy along with V5 fared much better than controls. Due to alcohol and narcotic drugs' overdose two patients in V5 group have died after the first month of treatment but they were not excluded from the statistical analysis of data, so that the intent-to-treat design was not compromised. Control group was randomly selected in a retrospective manner and consisted of patients who were alive at the time of study conclusion. Nevertheless the quantitative endpoints detailed below indicate that the addition of V5 to anti-tuberculosis therapy (ATT) has resulted in better clinical outcome.

### Effect on Axillary Body Temperature

Baseline axillary body temperatures differed between two groups of patients. The average (median) °C values were lower in V5 than in the control group, i.e.,  $37.4 \pm 0.5$  (37) vs.  $37.7 \pm 0.5$  (38) ( $P=0.001$  by unpaired t-test). The proportion of febrile patients was also lower; 39% vs. 75% in control (Figure 2). At the end of treatment period the proportion of control patients who attained normal temperature increased to 50%; 3 (8.3%) reverted to low grade fever, and in remaining 41.7% patients the temperature remained normal. The mean temperature drop was  $0.5^\circ\text{C}$  ( $P=0.0002$ ). In V5 group, febrile temperature was normalized in additional 16 patients (44.4%) and in remaining 61.1% no changes were observed. The mean temperature reduction in V5 patients was smaller than in the control, i.e.,  $0.3^\circ\text{C}$  from  $37.4^\circ\text{C}$  to  $37.1^\circ\text{C}$  ( $P=0.001$ ; paired t-test). Unpaired t-test comparing the difference in outcome between

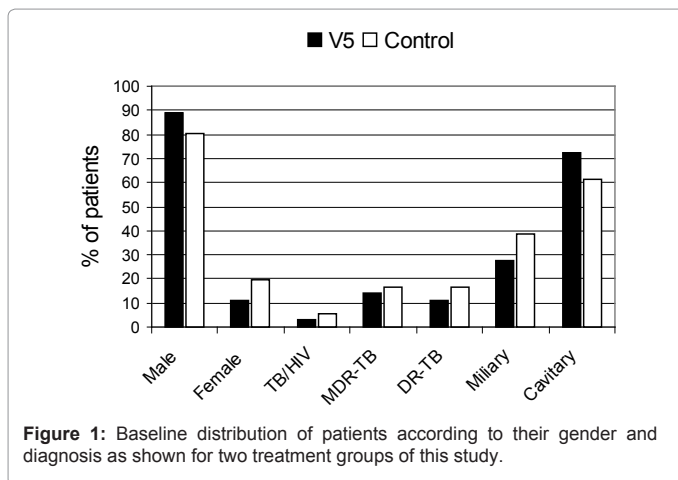


Figure 1: Baseline distribution of patients according to their gender and diagnosis as shown for two treatment groups of this study.

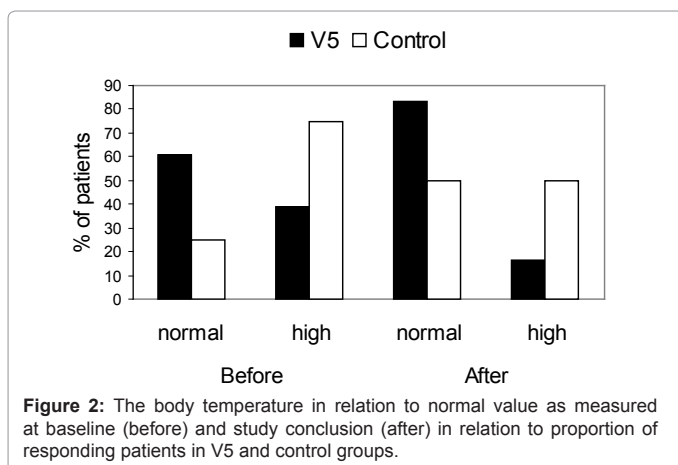


Figure 2: The body temperature in relation to normal value as measured at baseline (before) and study conclusion (after) in relation to proportion of responding patients in V5 and control groups.

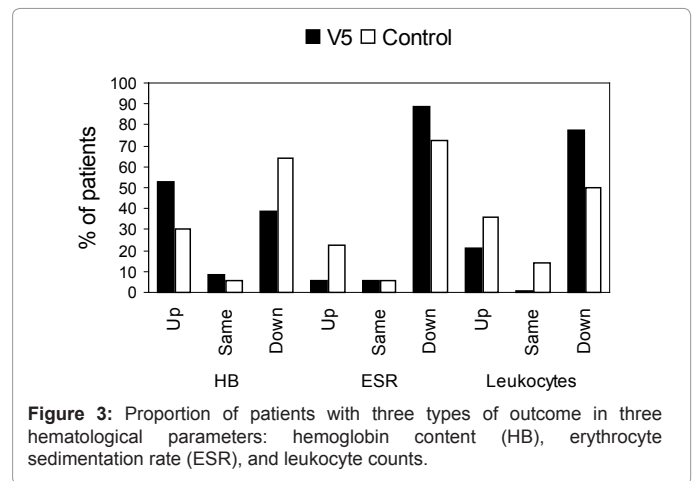


Figure 3: Proportion of patients with three types of outcome in three hematological parameters: hemoglobin content (HB), erythrocyte sedimentation rate (ESR), and leukocyte counts.

control and V5 groups has revealed that the statistical discrepancy was marginal ( $P=0.049$ ) suggesting that both treatments displayed similar defervescence trend.

### Effect on Hematology Parameters

The effect of ATT and V5 on white blood cells and hematology parameters are shown in (Table 1) and (Figure 3). Patients in V5 arm displayed positive changes that appeared to be specific to V5 intervention as opposed to the smaller effect of ATT in control group. Elevated leukocyte counts were reduced in V5-treated group from  $7.9$  to  $6.2 \times 10^9/\text{L}$  ( $P=0.005$ ), but not in the control group i.e.,  $7.2$  vs.  $7.5 \times 10^9/\text{L}$  ( $P=0.49$ ). Another marker of inflammation, the erythrocyte sedimentation rate (ESR), declined significantly in V5 group from  $22.7$  to  $13.3$  mm/h ( $P<0.0001$ ), whereas among those on HR the reduction was smaller but statistically significant, i.e.,  $26.4$  vs.  $22.5$  mm/h ( $P<0.0001$ ). The content of hemoglobin remained essentially at the same level in V5 and control patients ( $117.7 \pm 16.4$  vs.  $119.4 \pm 15.4$  g/L;  $P=0.21$ ) and ( $98.8 \pm 15.6$  vs.  $98.1 \pm 15.4$  g/L;  $P=0.33$ ) respectively. In general, V5 appeared to have more pronounced benefit in normalizing abnormal hematology picture than H and R combination (Figure 3).

### Effect on Body Weight

Patients in both groups had similar underweight problem. The mean baseline body mass in V5 and control groups was  $57 \pm 10.7$  and  $61.1 \pm 11.2$  with median  $56$  and  $57$  kg respectively, which were statistically indistinguishable ( $P=0.14$  by unpaired t-test). In V5 arm  $7$  (19.4%) patients retained the same weight, in remaining  $29$  patients (80.6%) the increase in body mass ranged between  $1-6$  kg, with average (median) body weight accrual after 3 months equal to  $2.2 \pm 1.7$  kg (2 kg) ( $P<0.0001$ ). Weight gain observed after a 1<sup>st</sup> and 2<sup>nd</sup> month was also highly significant,  $0.86$  kg and  $1.54$  kg, respectively (both P values were less than  $0.0001$  by paired t-test). In the control group the average (median) weight accrual was only  $0.08 \pm 1.1$  kg (0 kg) and statistically not significant ( $P=1$ ). In this group  $6$  patients (16.7%) gained between  $1$  and  $4$  kg;  $4$  (11.1%) lost between  $1$  and  $4$  kg; and in remaining  $26$  (72.2%) patients no changes were observed. The treatment outcomes in mean weight accrual differed by 27.5-fold ( $2.2$  vs.  $0.08$  kg) and proportion of patients who gained weight was  $80.6\%$  vs.  $16.7\%$ ; (Fisher's test;  $P<0.0001$ ; OR 20.8; 10.1-42.9 at 95% CI (Table 1 and Figure 4).

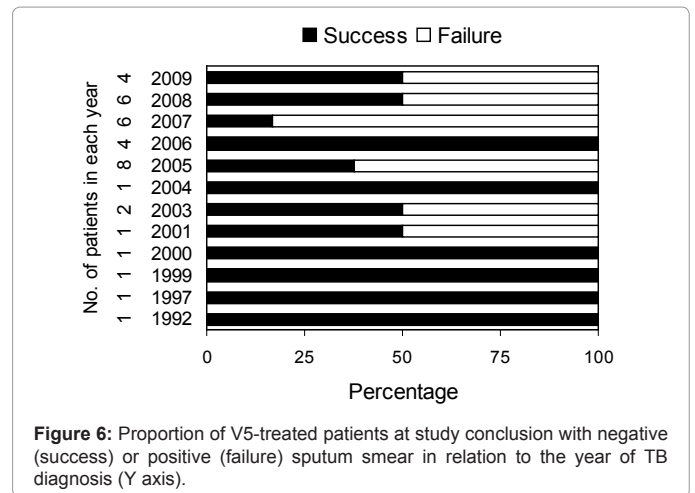
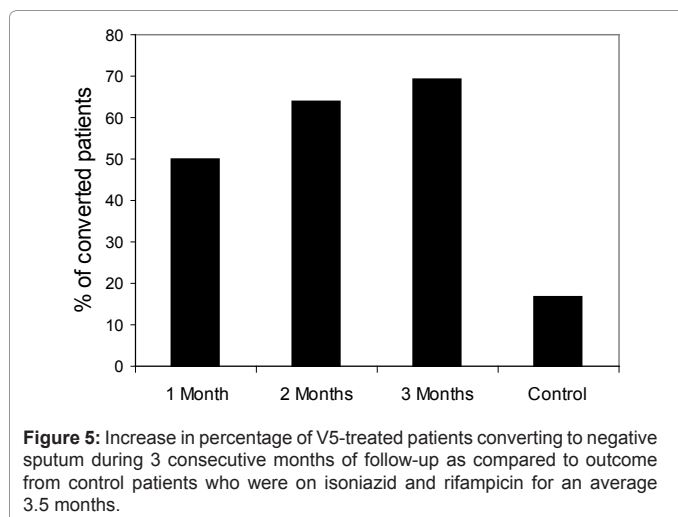
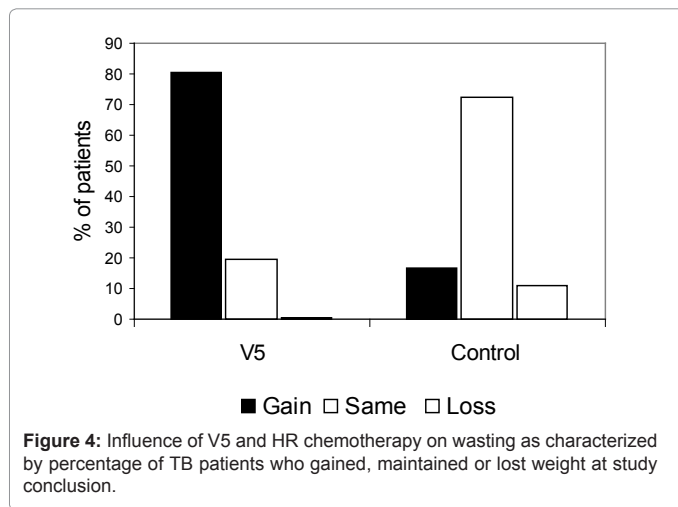
### Effect on Mycobacterial Clearance

Bacterial clearance was scored in a blinded fashion at monthly

intervals. At the end of first month half of V5-treated patients (50%) became sputum negative, which was highly significant by Wilcoxon ranking test ( $P < 0.0001$ ) (Figure 5). After 2<sup>nd</sup> and 3<sup>rd</sup> months the proportion of converted patients gradually increased to 63.9% and 69.4% respectively. Among control patients treated for on average of 3.5 months, 6 out of 36 (16.7%) converted ( $P = 0.02$ ). These patients had uncomplicated cavitary TB without HIV or MDR-TB. In V5 group 17 out of 25 (68%) patients had cavitary TB and remaining had miliary TB. Among these 25 patients one was had HIV and four MDR-TB. At baseline we had 5 and 6 MDR-TB cases in V5 and control groups respectively, but outcomes were different, i.e., none of 6 cases in the control converted while in V5 group 4 out of 5 converted. The odds ratio analysis produced OR 789.3; 47-13260 at 95% CI ( $P < 0.0001$ ). Similarly, none of 2 TB/HIV patients in the control had converted, but a single TB/HIV patient in V5 group became sputum negative. Finally, it is of interest that when treatment-failure patients on V5 have been stratified according to the length of their disease, those who had longer TB history were converted more readily (Figure 6).

## Discussion

The results of this 3-month study in treatment-failed TB patients on palliative support consisting of isoniazid and rifampicin, revealed



higher sputum conversion rate; 69.4% vs. 16.7% ( $P < 0.0001$  with OR 10.9; 5.5-21.3 at 95% CI). Even after the first month on V5, the sputum conversion was statistically more significant in comparison to control patients who were treated for 3.5 months ( $P < 0.0001$ ; OR 4.9; 2.5-9.4 at 95% CI). These findings combined with additional benefit of enhanced body mass gain, fever normalization, and potent anti-inflammatory effect support earlier studies of V5 in TB [8-11]. The fact that we have seen four-fold higher negative conversion in patients who failed every tried therapy is perhaps most impressive outcome of this study.

It is agreed that new anti-TB drugs are needed to shorten and/or simplify treatment of TB [1-3]. One of priorities is to find optimal combination among existing drugs. Isoniazid and rifampicin double regimen introduced in 1970's is regarded as effective and least toxic simple combination. When resistance to both drugs arises, treatment is complicated, prolonged, expensive, and results in poorer outcome with success rates rarely better than 65% even when 1<sup>st</sup> and 2<sup>nd</sup> line TB drugs are employed for 12-24 months [12]. In V5 and control groups we had 5 and 6 culture-confirmed MDR-TB respectively; none of those in the control had converted while in immunotherapy group 4 out of 5 became negative ( $P < 0.0001$ ); OR 789; 47-13260 at 95% CI). As we had a small number of MDR-TB cases we cannot, of course, exclude the distribution bias. However, if we add other lab-confirmed non-HR resistant cases we have total 9 and 12 cases in V5 and control respectively. Among these only one patient in V5 group remained positive, while none in the control group had converted ( $P < 0.0001$ ; OR 1564; 91-26947 at 95% CI). This implies that immune-based therapy can overcome multi-drug resistance even when the least-potent combination of TB drugs is employed.

The weight accrual during TB therapy has been identified as a reliable biomarker of treatment outcome that could be readily deployed in resource-limited settings [13]. Bernabe-Ortiz et al. [14] have demonstrated that patients with good outcome gained, after one month, mean 0.93 kg ( $P < 0.001$ ), whereas those with poor outcome lost 1.9 kg ( $P = 0.003$ ). After 3 months, those who responded favorably gained 2.4 kg ( $P < 0.001$ ) but non-responders lost 2.05 kg ( $P = 0.01$ ). Our monthly follow-up results of V5 closely match findings from Peru suggesting that variations in bodyweight can predict success of tuberculosis therapy - a concept proven to be valid even in a mouse model [15].

Other biomarkers of tuberculosis are those that appraise the intensity of inflammatory responses [16]. Leukocytosis and high erythrocyte sedimentation rate are common during active pulmonary TB. In our patient population favorable responses were seen with both markers. These results are further supported by the effect of V5 in fever control. We have worked on immunotherapy of TB over past 10 years, which resulted in a dozen published clinical trials involving close to 1,200 individuals [17-20]. In these studies we have used oral botanical immunomodulator Dzherelo or Immunoxel. What is remarkable is that clinical manifestations, inflammation, fever, and wasting improved in a manner strikingly similar to V5. This suggests to us that effective immunotherapy of any kind must produce clinical response that displays similar, if not identical trend, with these simple biomarkers.

How our results compare to previously tried immune-based interventions? Robert Koch himself was first to propose this approach back in 1890 [21]. The BCG, introduced as a prophylactic vaccine in 1921, has been occasionally used as an immune adjunct to TB therapy. In a recent large trial negative sputum conversion occurred in 98.3% of BCG recipients, which was similar to 97.2% in chemotherapy control [22]. More promising results were seen with heat-killed *M. vaccae* preparation [23]. This immune intervention usually resulted in better outcome than TB therapy alone [24]. *M. vaccae* appeared to produce favorable response in some geographical locations, but had no effect in others [23]. There are two other mycobacterium-based immune preparations, *M. phlei* and *M. w* [25-27]. Finally, an experimental vaccine RUTI, containing fragments of *M. tuberculosis*, has been developed in Spain [28]. Other immune intervention such as inhaled IFN-gamma, corticosteroids, and various cytokine or anti-cytokine regimens have produced inconsistent results and these were largely responsible for guarded attitude toward immunotherapy [29-31].

What is the mechanism of V5 action? TB is a disease whereby pulmonary or other tissues harboring mycobacteria are constantly assaulted by the host's immune system, creating chronic inflammation and ensuing tissue damage [32,33]. Yet, the prophylactic TB vaccines are designed to boost immune reaction against *M. tuberculosis* [34,35]. This approach is not likely to succeed with therapeutic vaccines. In order to treat TB one needs to induce immune tolerance to mycobacterial antigens instead of provoking immune reaction - a phenomenon first observed by Koch [21,36]. V5 originates from an allogeneic source - pooled blood of HCV+ and HBV+ donors. As one-third of people harbor latent *M. tuberculosis* this means that V5 inherently contains TB bacterium present in the donors' blood. Therefore, the administration of V5 triggers the immune response not only to hepatitis viruses (as intended originally) but also to tubercle bacilli. It is well known that when a low dose of an antigen is given orally it produces mucosal tolerance rather than immune activation [37]. Oral delivery of vaccines thus makes sense as has been demonstrated by Dlugovitzky et al. [38] in their trial of orally administered *M. vaccae*. TB is just another chronic inflammatory disease, which can be managed by restoration of self-tolerance [39]. This needs to be addressed in studies of immune mechanism of V5 action, which will contribute to the understanding of immune correlates of protection and may result in development of effective and safe immunotherapeutics and vaccines.

Despite many limitations of this study we believe that V5 has a potential to improve treatment outcome in treatment-failed TB [8-11]. V5 is safe and produces better compliance in treated patients. No

reactivation of TB was seen at any time, which is perhaps due to anti-inflammatory activity [40]. In conclusion, the combination of V5 with isoniazid and rifampicin regimen can significantly shorten the duration of treatment and produces higher response rate than chemotherapy alone.

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