

Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



www.elsevier.com/locate/intimp



## Effect of oral immunomodulator Dzherelo in TB/HIV co-infected patients receiving anti-tuberculosis therapy under DOTS

Lyudmila G. Nikolaeva<sup>a,\*</sup>, Tatyana V. Maystat<sup>a</sup>, Volodymyr S. Pylypchuk<sup>b</sup>, Yuri L. Volyanskii<sup>c</sup>, Lilia A. Masyuk<sup>d</sup>, Galyna A. Kutsyna<sup>e</sup>

<sup>a</sup> Kharkov Regional AIDS Prophylaxis and Prevention Center, Kharkov Medical Academy of Postgraduate Education, 6 Bor'by street, Kharkov 61044, Ukraine

<sup>b</sup> Ekomed LLC., Prospect Pravdy 80-A, Kiev 04208, Ukraine

<sup>c</sup> I.I. Mechnikov Institute of Microbiology and Immunology, Kharkov 61057, Ukraine

<sup>d</sup> Jovtnevsky Correctional Colony No.17, State Department of the Penitentiary of Ukraine in Kharkov Region, Kharkov, Ukraine

<sup>e</sup> Luhansk Regional AIDS Center and Luhansk State Medical University, Luhansk 91045, Ukraine

Received 12 December 2007; received in revised form 31 January 2008; accepted 31 January 2008

### KEYWORDS

*Mycobacterium tuberculosis*;  
HIV;  
Herbal;  
Phytotherapy;  
Immunotherapy;  
Immunomodulator

### Abstract

Open-label, phase II clinical trial was conducted in 40 HIV/TB dually infected patients to evaluate the effect of oral immunomodulator Dzherelo on immune and viral parameters. The anti-retroviral therapy naïve patients were randomized into two equal groups to be given anti-tuberculosis therapy (ATT) under DOTS. The arm A, which served as a control, received Isoniazid (H); Rifampicin (R); Pyrazinamide (Z); Streptomycin (S); and Ethambutol (E), and arm B received 50 drops of Dzherelo twice per day in addition to the daily dose of HRZSE. After 2 months the total CD3<sup>+</sup> lymphocytes increased from 728 to 921 cells/ $\mu$ l ( $P=0.025$ ) in Dzherelo recipients, whereas in the control group they decreased from 651 to 585 cells ( $P=0.25$ ). The population of CD4 T-cells expanded in Dzherelo arm (174 to 283;  $P=0.00003$ ) but declined in ATT group (182 to 174;  $P=0.34$ ). The CD8 cells fluctuated slightly upward in both groups: 159 > 180 ( $P=0.17$ ) and 159 > 183 ( $P=0.13$ ). The ratio between CD4/CD8 cells deteriorated in arm A (1.213 > 0.943;  $P=0.002$ ) but improved in arm B (1.244 > 1.536;  $P=0.007$ ). The percent of CD3+HLA-DR+ activated lymphocytes had fallen in ATT group (22.6 > 20.5;  $P=0.004$ ), but rose in Dzherelo recipients (21.5 > 30.5;  $P=0.0001$ ). The changes in CD20+ B lymphocytes were insignificant in both arms (28.4% > 28.6%;  $P=0.4$ ) and (27.2% > 26.7%;  $P=0.38$ ). No difference was seen in the amount of CD3-CD16+CD56+ natural killer (NK) cells in arm A (21.3% > 22.6%;  $P=0.1$ ), while in Dzherelo recipients they declined significantly (19.9% > 14.5%;  $P=0.0026$ ). The viral load, measured by plasma RNA-PCR, decreased in Dzherelo group (2174 > 1558;  $P=0.002$ ), but increased in ATT group

\* Corresponding author.

E-mail address: [kutsyna@list.ru](mailto:kutsyna@list.ru) (G.A. Kutsyna).

(1907 > 2076 copies/ml;  $P=0.03$ ). Dzherelo has a favorable effect on the immune status and viral burden in HIV/TB patients when given as the immunomodulating adjunct to ATT.  
© 2008 Elsevier B.V. All rights reserved.

## 1. Introduction

In resource-limited countries, including Ukraine, tuberculosis (TB) is the leading cause of death among individuals infected with HIV [1,2]. The prognosis of disease among HIV/TB co-infected patients is unfavorable, especially in advanced HIV disease. The World Health Organization (WHO) estimates that a person with both HIV and TB infection is thirty times more likely to become ill with TB than a person with TB infection alone [3]. In developing countries about one-third of all AIDS-related deaths results from TB.

Ukraine has the highest prevalence of HIV/TB co-infection in Eastern Europe [4]. The effectiveness of TB therapy is significantly lower among patients with HIV/AIDS [1,2]. The rate of relapse and mortality are consistently higher even when HIV/TB patients are treated under directly observed treatment-short course (DOTS) regimen [3]. Drug resistance accompanied by HIV-associated immunodeficiency is the main cause of treatment failure. The TB drug resistance among HIV-positive individuals in countries of the former Soviet Union is in the 10–43% range [5]. The recently published survey of Nikolayevskyy et al., reported that in Ukraine the multi-drug (MDR) resistance rates were significantly higher among former prison inmates compared with non-prisoners (54.8% vs. 27.3%) [6].

Manas et al., indicated that *Mycobacterium tuberculosis* and HIV infection were associated with pronounced deterioration of the immune system as evidenced by higher depletion rate of helper CD4+ lymphocytes [7]. Similarly, advanced HIV infection is also characterized by low number of helper lymphocytes. Several studies have shown that immune control of *M. tuberculosis*, similarly to HIV, is mediated by cellular rather than humoral immune response [8,9]. Hence, the immunotherapeutic approaches toward TB began receiving more attention recently [10,11]. There are many types of immune modulators that have been used clinically for viral infections [12], but for TB the choice of immune interventions is limited [10,11]. The T-cell responses during TB may help contain *M. tuberculosis* but may also cause inflammatory damage to the host [8–10].

Oral immunomodulator Dzherelo (Immunoxel) is used in Ukraine for the management of HIV infections, including patients co-infected with TB [13–15]. Recent studies have indicated that Dzherelo can significantly shorten the duration of treatment and helps to achieve higher response rate even in those with MDR and XDR forms of TB [14]. Dzherelo has also been found to decrease the hepatotoxicity associated with ATT [16]. Dzherelo contains concentrated aqueous-alcohol extract from medicinal plants such as Aloe, Common knotgrass, Yarrow, Purple coneflower, St. John's Wort, Centaury, Nettle, Snowball tree berries, Dandelion, Sweet-sedge, Oregano, Marigold, Seabuckthorn fruit, Elecampane, Tormentil, Greater plantain, Wormwood, Siberian golden root, Licorice, Cottonweed, Fennel, Birch tree fungus, Thyme, Three-lobe Beggarticks, Sage, Dog rose fruit, and Juniper fruit.

Dzherelo has been approved in 1997 by the Ministry of Health of Ukraine as an immunomodulating supplement, which so far has been used by over 150,000 individuals for various indications including chronic bacterial and viral infections such as TB and HIV, autoimmune diseases, and malignancy [13–15]. Our study was aimed at evaluating the effect of Dzherelo on lymphocyte subsets and viral load among HIV/TB patients in comparison to control population which received DOTS alone.

## 2. Materials and methods

### 2.1. Patients

The patients, aged 20–49 years, have been selected and randomly divided into arms A and B, each consisting of 20 patients. The randomization was done according to allocation sequence generated with a random number table. The patients' heterogeneity was minimized by exclusion/inclusion criteria such as age bracket, gender, TB co-infection, alcohol/drug abuse, prior treatment, and disease stage. Another inclusion criterion was the lack of any form of anti-retroviral therapy prior to and during the trial. The majority of patients in our study were correctional facility inmates in advanced clinical stage III of HIV infection with average baseline CD4+ T-cell count below 200 cells/ $\mu$ l. The diagnosis of HIV infection was established by standard ELISA test further confirmed by Western blot analysis. Active pulmonary tuberculosis was certified by a medical history and clinical findings compatible with pulmonary tuberculosis, a chest X-ray showing lung involvement, and positive sputum smear for acid-fast bacilli or the culture of *M. tuberculosis*. The participation in this trial was voluntary and patients were enrolled only after signing the written consent indicating that they were free to withdraw from the study at any time. The conduct of the trial was approved by the State Department of the Penitentiary of Kharkov region, Ukraine.

### 2.2. Treatment regimens

None of the patients received anti-retroviral therapy during 2-months of follow-up. All patients received standard anti-tuberculosis therapy (ATT) administered under DOTS schedule which consisted of one daily dose of Isoniazid (H) 300 mg; Rifampicin (R) 600 mg; Pyrazinamide (Z) 2000 mg; Streptomycin (S) 1000 mg; and Ethambutol (E) 1200 mg. All anti-TB drugs were procured through the centralized national supply system of Ukraine. The arm B received, in addition to HRZSE, twice per day dose of Dzherelo which was given as 50 drops diluted in 100 ml of water. The over-the-counter phytoconcentrate Dzherelo was generously supplied by Ekomed company. Dzherelo contains concentrated aqueous-alcohol extract from medicinal plants such as Aloe (*Aloe arborescens*), Common knotgrass (*Polygonum aviculare*), Yarrow (*Achillea millefolium*), Purple coneflower (*Echinacea purpurea*), St. John's Wort (*Hypericum perforatum*), Centaury (*Centaureum erythraea*), Snowball tree berries (*Viburnum opulus*), Nettle (*Urtica dioica*), Dandelion (*Taraxacum officinale*), Sweet-sedge (*Acorus calamus*), Oregano (*Oreganum majorana*), Marigold (*Calendula officinalis*), Seabuckthorn berries (*Hippophae rhamnoides*), Elecampane (*Inula helenium*), Tormentil (*Potentilla erecta*), Greater plantain (*Plantago major*), Wormwood (*Artemisia* sp.), Siberian golden root (*Rhodiola rosea*), Cudweed (*Gnaphalium uliginosum*), Licorice (*Glycyrrhiza glabra*), Fennel (*Foeniculum vulgare*), Chaga (*Inonotus*

*obliquus*), Thyme (*Thymus vulgaris*), Three-lobe Beggarticks (*Bidens tripartite*), Sage (*Salvia officinalis*), Dog rose (*Rosa canina*), and Juniper berries (*Juniperus communis*). Dzherelo was approved in 1997 by the Ministry of Health of Ukraine as a dietary herbal supplement. In 1999 Dzherelo was recommended by the Ministry of Health as an immune adjunct to the therapy of pulmonary tuberculosis [15]. In 2006 it has received the status of a functional food—special category of supplements which can carry medical claims substantiated by clinical evidence.

### 2.3. Immunophenotyping of lymphocyte subpopulations

The samples of peripheral blood of patients with HIV/TB were analyzed using commercially available Clonospctr panel of monoclonal antibodies against surface antigens of lymphocytes (MedBioSpectr, Moscow, Russia). Assays were carried out at study entry and after 1 and 2 months on the therapy. The absolute and percent values of the following subpopulations were assessed in a blinded fashion by fluorescent microscopy: total T lymphocytes (CD3+); helper T lymphocytes (CD3+CD4+); cytotoxic T lymphocytes (CD3+CD8+); B lymphocytes (CD20+); natural killer or NK cells (CD3–CD16+CD56+); and activated T lymphocytes (CD3+HLA-DR+). In addition the changes in the ratio between CD4 and CD8 cells were evaluated as part of the assessment of the immune status of patients. The samples of the blood from 19 healthy blood donors were analyzed as a reference for normal values.

### 2.4. PCR analysis

Stored frozen samples of plasma were processed in bulk by using commercially available PCR kit (AmpliSense HIV-1, Central Research Institute of Epidemiology, Moscow, Russia) designed for quantitative analysis of HIV-RNA copies. Tests were carried on plasma samples collected at baseline and after 2 months of the therapy.

### 2.5. Statistical analysis

The obtained results were analyzed with the aid of statistical software STATMOST (Datamost, South Sandy, UT). The baseline cell numbers relative to the 1st and 2nd months of follow-up were evaluated by paired Student *t*-test. The non-parametric values of viral load were analyzed by Wilcoxon signed rank test. The resulting probability values were considered as significant at the cut-off levels of  $P \leq 0.05$ .

## 3. Results

After 1 month on the therapy there was a clear distinction between recipients of ATT alone and those who received ATT

along with the daily dose of Dzherelo. This disparity became even more evident at the end of 2nd month of therapy. Some but not all immune markers of lymphocyte subsets have been impacted in a statistically significant manner. The changes in viral load among HIV/TB patients of both groups have also reached statistical significance even though none of the patients have ever received the anti-retroviral therapy. These findings are summarized below.

### 3.1. CD3+ total T lymphocytes

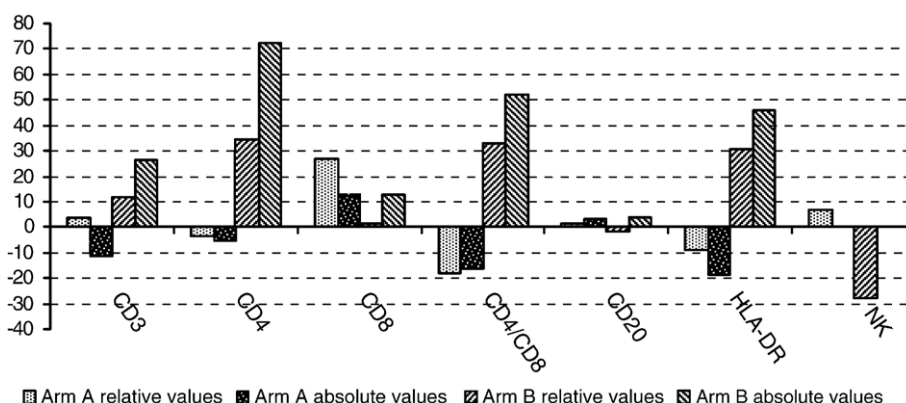
After 1 month on ATT alone the absolute and percent (%) values of total CD3+ lymphocytes per microliter of blood have not changed appreciably, i.e., 650(36.8%) vs. 634(37.2%);  $P=0.38$  ( $P=0.23$ ), as analyzed hereinafter by paired Student *t*-test. However, in the HRZSE group receiving Dzherelo there was a significant difference in total CD3+ values as early as 1month post-therapy: 728 (37.5%) vs. 902(40.4%);  $P=0.015$  ( $P=0.04$ ). After 2 months the number of total CD3+ lymphocytes increased further to 921 in arm B ( $P=0.025$ ) whereas in the control group it decreased to 585 cells ( $P=0.25$ ). The difference in treatment outcomes, i.e., 921 vs. 585 was significant ( $P=0.0036$ ), while baseline values between two groups were not statistically different ( $P=0.15$ ).

### 3.2. CD4+ T lymphocytes

The trends very similar to those of total CD3+ lymphocytes were observed when CD3+CD4+ lymphocyte subsets were analyzed. No significant changes were seen in ATT alone arm, i.e., 182 (24.2%) to 203(24.5%);  $P=0.063$  ( $P=0.28$ ), whereas in Dzherelo arm the helper cell counts have risen significantly from 174 (23.3%) to 257(27.3%) cells;  $P=0.00003$  ( $P=0.0004$ ). At the end of the 2nd month lymphocyte subsets have risen to 174(25.3%) and 283(31%) with probability values  $P=0.13$  and  $P=0.0000004$  for arms A and B respectively. When study completion results of Dzherelo recipients are calculated in terms of accrual in CD4+ lymphocytes relative to entry levels there was an increase of 38.5% and 24.8% in absolute and relative values (Fig. 1).

### 3.3. CD8+ T lymphocytes

The changes observed in CD3+CD8+ cytotoxic T-cell population were opposite from those seen with helper cells. In ATT alone group CD8+ cells increased in a significant manner from 159(21.7%)



**Figure 1** Treatment-induced changes in peripheral blood cells' relative (%) and absolute numbers at 2 months post-therapy as compared to their respective baseline levels for Arms A and B respectively.

**Table 1** CD4/CD8 ratios among normal donors and in HIV/TB patients during therapy

Normal blood donors (N=19)	Arm A (N=20) HRZSE			Arm B (N=20) HRZSE + Dzherelo		
	Baseline	1st month	2nd month	Baseline	1st month	2nd month
1.7±0.04	1.21±0.12	1.06±0.08	0.94±0.06	1.24±0.13	1.42±0.08	1.54±0.08
<i>P</i> values relative to baseline as analyzed by paired Student <i>t</i> -test		<i>P</i> =0.009	<i>P</i> =0.002		<i>P</i> =0.06	<i>P</i> =0.007

to 199(24.3%); *P*=0.011(*P*=0.016), while in the Dzherelo group the changes were insignificant, i.e., from 159(20.8%) to 190.2(19.8%); *P*=0.21 (*P*=0.045). At the end of the 2nd month the CTL population in ATT alone group was still above baseline, i.e., 180 cells (27.7%), an accrual that was statistically significant in percent terms (*P*=0.000009) but not significant (*P*=0.17) when calculated in absolute numbers (Fig. 1). Contrary, the 2nd month numbers of CD8 cells among Dzherelo recipients have not increased in any significant manner, from baseline levels 159(20.8%) to 183(20.7%), with *P* values being 0.13 and 0.47 for absolute and percent figures respectively.

### 3.4. CD4/CD8 ratio

The differential changes in CD4 and CD8 lymphocyte numbers had affected the CD4/CD8 ratio in patients on HRZSE alone

regimen as early as 1month after treatment initiation (Table 1). Their ratio had declined from baseline value 1.213 to 1.06 (*P*=0.009). In contrast the CD4/CD8 ratio among Dzherelo recipients had increased from 1.244 to 1.416, which was, however, slightly above the cut-off value (*P*=0.06). The disparity between CD4 and CD8 lymphocytes had progressed even further by the end of the 2nd month. Among HRZSE alone patients the ratio had declined to 0.943 (*P*=0.002), while in Dzherelo group the ratio had risen back to a level that is commonly considered as normal, i.e., 1.536 (*P*=0.007).

### 3.5. CD3+HLA-DR+ activated lymphocytes

The percent of activated CD3+HLA-DR+ T-cells decreased in ATT group (from 22.6% to 21.8%, and then to 20.5%; *P*=0.004) at the end of study, whereas in Dzherelo recipients this subpopulation

**Table 2** Effect of 2-month ATT without or with Dzherelo on HIV-RNA plasma levels

Patient no.	Arm A HIV/TB patients on HRZSE alone (N=20)			Arm B HIV/TB patients on HRZSE + Dzherelo (N=20)		
	HIV-RNA copies/ml at baseline	HIV-RNA copies/ml at 2nd month	Difference compared to baseline	HIV-RNA copies/ml at baseline	HIV-RNA copies/ml at 2nd month	Difference compared to baseline
1	227.0	324.4	+97.4	1911.2	715.0	-1196.2
2	1611.8	1754.0	+142.2	489.0	286.9	-202.1
3	571.4	511.5	-59.9	1057.9	728.5	-329.4
4	504.0	623.2	+119.2	736.0	436.6	-299.4
5	249.5	376.7	+127.2	953.1	713.6	-239.5
6	24,388.0	25,331.8	+943.8	1866.3	1042.9	-823.4
7	212.1	743.5	+531.4	848.3	339.3	-509
8	354.3	301.9	-52.4	698.6	758.5	+59.9
9	99.8	309.4	+209.6	593.8	264.5	-329.3
10	114.8	586.3	+471.5	339.3	511.5	+172.2
11	391.7	691.1	+299.4	29,096.0	21,611.8	-7484.2
12	249.5	77.3	-172.2	429.1	451.6	+22.5
13	369.3	429.1	+59.8	189.6	69.9	-119.7
14	204.6	279.4	+74.8	810.9	504.0	-306.9
15	331.8	189.9	-141.9	953.1	376.7	-576.4
16	264.5	257.0	-7.5	563.9	623.8	+59.9
17	4141.7	4912.7	+771	257.0	354.3	+97.3
18	219.6	264.5	+44.9	324.4	459.1	+134.7
19	391.7	301.9	-89.8	698.6	421.7	-276.9
20	3236.0	3258.5	+22.5	661.2	481.5	-179.7
	Mean ± SD = 1907 ± 5400 Median = 343	Mean ± SD = 2076 ± 5602 Median = 403	Mean ± SD = +170 ± 298 Median = +86 Wilcoxon signed rank test; <i>P</i> =0.03	Mean ± SD = 2174 ± 6353 Median = 699	Mean ± SD = 1558 ± 4725 Median = 470	Mean ± SD = -616 ± 1651 Median = -258 Wilcoxon signed rank test; <i>P</i> =0.002

increased by 29.5% (21.2% to 27.6% to 30.5%;  $P=0.0001$ ). The 45.5% accrual in absolute numbers was also highly significant reaching from baseline 164 cells/ $\mu$ l to 275, and then to 301, with  $P$  values being 0.00005 and 0.0003 at end of 1st and 2nd months respectively. Conversely, patients who received HRZSE only, have experienced 18% decline in absolute numbers at end of the 2nd month (from 159 to 131;  $P=0.03$ ) but there was no significant difference at one-month intermediate period (159 to 169;  $P=0.13$ ).

### 3.6. CD20+ B lymphocytes

The changes were not seen, throughout the study, neither in absolute nor percent values of CD20+ B lymphocytes. In patients who received Dzherelo B-cell counts fluctuated slightly but differences were not statistically significant either at the first month or at second month post-therapy. At the end of the first month they rose slightly from 526 (27.2%) to 612 (28.4%) cells  $P=0.093$  ( $P=0.12$ ) and at study conclusion they came down near the entry levels without any noticeable significance 552 (26.7%);  $P=0.38$ . Patients who received HRZSE alone had also displayed the unremarkable variation in CD20+ lymphocyte numbers. The entry values of 613 (28.4%) changed slightly to 592 (28.9%) at the 1st month and then remained at 633 (28.6%) toward the end of the study. None of these changes were statistically significant at any time for both arms.

### 3.7. CD3-CD16++CD56+ NK cells

The relative numbers of NK cells identified as CD3-negative but CD16+CD56+ population were not significantly affected by HRZSE alone therapy: from baseline level of 21.3% they rose to 21.9% ( $P=0.22$ ), and then to 22.6% at study conclusion ( $P=0.1$ ). The addition of daily dose of Dzherelo, however, had a strong effect on reduction of NK cells. The patients who started with an average 20% NK cells had their numbers declined to 18% ( $P=0.029$ ), which further declined to 14.5% ( $P=0.0026$ ). Even differences between the 1st and 2nd months were statistically significant ( $P=0.016$ ) indicating very rapid shift in population dynamics. Thus, at the end of 2-months therapy, the patients on Dzherelo had experienced 27.5% decline in NK cells as compared to baseline levels.

### 3.8. Viral load

The viral load, as measured by plasma RNA-PCR at baseline and at the end of the 2nd month, increased in ATT group (1907 to 2076 copies/ml;  $P=0.025$ , by Wilcoxon signed rank test) but decreased in Dzherelo group (2174 to 1558 copies;  $P=0.002$ ). About two-thirds of the patients (14/20) on HRZSE alone had shown the increase in viral load while the same number of patients on Dzherelo had a reduction in their number of viral copies (Table 2).

## 4. Discussion

Treating TB in HIV-infected individuals is a daunting task when compared to TB in HIV-negative persons [1–3,5]. This task is particularly challenging when one has to treat inmate populations which as a rule have much higher prevalence of drug-resistant TB and more advanced HIV disease [6]. Due to high failure and relapse rate of TB therapy in this particular group of patients the immune interventions are now being sought to overcome this shortcoming [10,11].

In the prior studies Dzherelo has been shown to double the success rate of ATT and shorten by about 2 months the duration of treatment even among those who had MDR-TB and XDR-TB [14–16]. It had also reduced significantly the toxic side effects of ATT, the hepatotoxicity in particular. Elevated liver aminopeptidase ALT and AST levels caused by ATT have been shown to return back to normal levels [16]. However, these studies have not dealt with the effect of Dzherelo on the immune status and viral load among HIV/TB patients. Our 2-month DOTS study conducted in a population consisting mostly of incarcerated individuals reveals that when Dzherelo is added to ATT there are significant benefits associated with this immunotherapy.

Our results indicate that as little as one-month administration of Dzherelo can produce significant increase in total CD3+ lymphocytes, CD4+ helper cells, better CD4/CD8 ratio, higher number of CD3+HLA-DR+ activated lymphocytes, and reduced number of NK cells. Dzherelo does not seem to affect CD8+ T lymphocytes nor CD20+ B lymphocyte subpopulations. Furthermore, Dzherelo appears to display inhibitory effect on viral replication resulting in statistically significant lower viral load.

We have observed highly significant increase in CD3+ total lymphocytes, CD4+ T lymphocytes, and better CD4/CD8 ratio in HIV and HIV/TB patients treated with Dzherelo (Fig. 1). In our hands Dzherelo has proven to display the same effect as reported by independent investigators [14–16]. The extent of improvement in terms of accrual in absolute and relative numbers of T-cells appears to be very similar. It is well established that elevated CD3 and CD4 counts and higher CD4/CD8 ratio are associated with better prognosis in patients with HIV as well as TB [17,18]. For this reason Dzherelo is likely to influence positively the outcome of treatment and disease progression in our study population.

The viral load is a predictor of HIV disease progression, its persistent elevation in HIV/TB co-infected patients is indicative of poor prognosis [19]. While there were earlier indications that Dzherelo may reduce the viral burden [13], our study is the first to report this phenomenon in a systemic fashion. Despite the fact that the HIV-RNA levels had decreased by less than a log the difference between baseline and outcome levels was highly significant (Table 2). This observation is encouraging since successful ATT regimens, which reported to restore immunity in HIV/TB patients, were not affecting the viral load [19,20]. What we have observed is quite unique. We are not aware of any other immunity-restoring preparation that has a dual effect on both TB and HIV. It is likely that the observed effect on viral load is mediated by immune cells since Dzherelo does not have the direct effect on HIV replication [13].

Our study reveals that at the end of study the patients on Dzherelo had 30% and 45.5% more of relative and absolute numbers of CD3+HLA-DR+ cells, whereas patients on HRZSE had their numbers declined to 18% below baseline levels. It has been shown that TB patients have significantly lower HLA-DR expressing cells, which may negatively affect T-cell immune responses and thus facilitate the disease progression [21]. For this reason Dzherelo appears to have a positive effect on a subset of activated T lymphocytes which may favorably influence the treatment outcome. We do not know to which subpopulation of T lymphocytes the CD3+HLA-DR+ activated cells belong. Judging from relative changes in CD4

and CD8 subsets it is likely that the activation marker is expressed on CD4 cells. However, due to limitations of the commercially available panel of antibodies preventing simultaneous staining with multiple fluorescent markers, we have not been able to verify this possibility. This needs to be ascertained in future studies.

Although a great deal of information is available on the role of T lymphocytes in the immune response against *M. tuberculosis*, comparatively little is understood regarding the involvement of B lymphocytes. Our results showing very little variation in CD20+ B-cell numbers do not shed any insightful information that can be interpreted in one way or other. This conclusion is perhaps reflective of the general consensus that while B-cells play a certain role in regulation of TB infection, their overall contribution is smaller than that of T lymphocytes [22]. Compared to normal blood donors the absolute and relative numbers of B-cells are almost double in both treatment groups. However, the significance of this phenomenon is unclear. According to Simonney et al., no correlation was found between elevated B-cell immune responses and progression to active disease in HIV-positive patients with TB [23].

We have observed a drastic difference between two arms in relative number of NK cells after 2 months of therapy. When compared to the entry levels the patients on Dzherelo had 27.5% less of NK cells whereas the population of these cells has not changed appreciably in HRZSE alone group. The role of NK cells in tuberculosis remains unclear. While some indicated that higher number of NK cells is critical for controlling *M. tuberculosis*, others indicated that they do not play any role in defense of the host against TB infection [24,25]. This confusion probably stems from differences in the design of studies some of which were based on measurement of functional activity but others were based on the enumeration of cells at various stages of the disease. As stated by Nirmala et al., the most likely cause for such a discrepancy is the failure to recognize that lowered NK activity during tuberculosis infection is the 'effect' and not the 'cause' for the disease [26]. Since we have not measured the functional activity of NK cells we do not know what is the significance of this phenomenon relative to the immunopathogenesis of TB. Based on the evidence that Dzherelo is highly effective as an immune adjuvant to ATT we can only speculate that in our situation the reduction in NK numbers is beneficial to the host. This explanation seems to be supported by a recent report of Barcelos et al., who indicated that symptomatic patients who failed TB chemotherapy had higher levels of NK cells [27]. Similarly, if we interpret, from our viewpoint, the study of Deveci et al., then their data indicating lower CD4 lymphocytes and higher number of NK cells in patients with active TB than in normal controls makes a sense, but is contrary to the conclusion of authors [28].

Many studies have been conducted aimed at determining the phenotype of immune cells in TB and HIV/TB co-infections. While there is a consensus that the cellular immune response plays a critical role in determining the clinical outcome after infection with *M. tuberculosis* much more has to be learned in order to have a clear picture of cellular events during the course of disease. The understanding of the immune mechanism controlling *M. tuberculosis* may result in the design of better vaccines and immunotherapies [10,11]. Opportunistic

infections such as TB cause considerable morbidity and mortality in immunocompromised AIDS patients. Therefore, high priority should be given to the efforts of prevention and treatment of infectious complications in these patients.

Currently available chemotherapy for the treatment of TB is far from ideal, requiring multiple tuberculous drugs to be taken in combination for long periods of time [1]. The extended duration of therapy, coupled with the side effects, often results in poor patient adherence, treatment failure, and the emergence of drug resistance with major social and economic implications [3,5]. The development of novel immune-based therapies is an urgent objective tuberculosis drugs' discovery. We believe that the immunotherapy is the indispensable part of therapeutic strategies against TB [29,30]. Many immune interventions are available against bacteria, protozoa, fungi and viruses. While often effective the mechanism of many immunomodulators is poorly understood [12]. This downside should be balanced against clinically confirmed benefits. Our study provides an early glimpse into the putative immune mechanism of Dzherelo, which has been successfully used as an immune adjunct to TB therapy in Ukraine [14–16]. Additional studies need to be conducted to develop better understanding of Dzherelo's properties and to enlarge the current arsenal of TB and HIV drugs.

## Acknowledgments

We thank all participants who volunteered in this study. The generosity of Ekomed in supplying Dzherelo is appreciated very much. The tireless support of clinical staff and technicians who contributed to this study has been of tremendous help to bring this study to fruition. The discussion with other investigators of Dzherelo who shared their insight and provided helpful suggestions has guided our study and we are thankful to all of them.

## References

- [1] Karachunskii MA. Tuberculosis in HIV infection. *Probl Tuberk* 2000;1:47–52.
- [2] Pokrovskii VV. Treatment of HIV-infections: success or crisis? *Ter Arkh* 2001;73:52–4.
- [3] Reid A, Scano F, Getahun H, Williams B, Dye C, Nunn P, et al. Towards universal access to HIV prevention, treatment, care, and support: the role of tuberculosis/HIV collaboration. *Lancet Infect Dis* 2006;6:483–95.
- [4] van der Werf MJ, Yegorova OB, Chentsova N, Chechulin Y, Hasker E, Petrenko VI, et al. Tuberculosis-HIV co-infection in Kiev City, Ukraine. *Emerg Infect Dis* 2006;12:766–8.
- [5] Khuadamova GT, Aruinova BK, Bidaibaev NSH, Azhmukhanbetov KA, Bairstanova KA, Bekembaeva GS. Special features of the course of tuberculosis in HIV-infected patients. *Probl Tuberk* 2001;5:34–6.
- [6] Nikolayevskyy VV, Brown TJ, Bazhora YI, Asmolov AA, Balabanova YM, Drobniewski FA. Molecular epidemiology and prevalence of mutations conferring rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* strains from the southern Ukraine. *Clin Microbiol Infect* 2007;13:129–38.
- [7] Manas E, Pulido F, Pena JM, Rubio R, Gonzalez-Garcia J, Costa R, et al. Impact of tuberculosis on the course of HIV-infected patients with a high initial CD4 lymphocyte count. *Int J Tuberc Lung Dis* 2004;8:451–7.

- [8] Boom WH, Canaday DH, Fulton SA, Gehring AJ, Rojas RE, Torres M. Human immunity to *M. tuberculosis*: T cell subsets and antigen processing. *Tuberculosis (Edinb)* 2003;83:98–106.
- [9] Serbina NV, Lazarevic V, Flynn JL. CD4(+) T cells are required for the development of cytotoxic CD8(+) T cells during *Mycobacterium tuberculosis* infection. *J Immunol* 2001;167:6991–7000.
- [10] Roy E, Lowrie DB, Jolles SR. Current strategies in TB immunotherapy. *Curr Mol Med* 2007;7:373–86.
- [11] Kaufmann SH. Tuberculosis: back on the immunologists' agenda. *Immunity* 2006;24:351–7.
- [12] Ershov FI. Use of immunomodulators in viral infections. *Antibiot Khimioter* 2003;48:27–32.
- [13] Chkhetian R, Pylypchuk V, Argzanova O, Prihoda, Vichrova L, Zagaydanova E, et al. Comparative effect of an immunomodulator Immunoxel (Dzherelo) when used alone or in combination with antiretroviral therapy in drug-naïve HIV infected individuals. *Int J Biotechnol* 2007;9:267–76.
- [14] Prihoda ND, Arjanova OV, Yurchenko LV, Sokolenko NI, Vihrova LA, Pylypchuk VS, et al. Open label trial of adjuvant immunotherapy with Dzherelo, Svitanok and Lizorm, in MDR-TB, XDR-TB and TB/HIV co-infected patients receiving anti-tuberculosis therapy under DOT. *J Med Plant Res* 2007;1:117–22.
- [15] Melnik VP, Panasyuk OV, Pylypchuk VS, Moshich OP, Prochenko NM, Leonenko OM. Deployment of herbal preparations Dzherelo and Svitanok for combination therapy of pulmonary tuberculosis. *Medical Institute of Ukrainian Association of People's Medicine. Information Bulletin of the Ministry of Health. UDK:616.24-002.5-085-038:615.017*. 1999, Kiev, Ukraine.
- [16] Zaitzeva SI. Clinical efficacy of phytopreparation Dzherelo and its influence on the functional status of liver in patients with destructive forms of tuberculosis. *Probl Ecol Med Gen Clin Immunol* 2006;71–72:132–40.
- [17] Rodrigues DS, Medeiros EA, Weckx LY, Bonnez W, Salomao R, Kallas EG. Immunophenotypic characterization of peripheral T lymphocytes in *Mycobacterium tuberculosis* infection and disease. *Clin Exp Immunol* 2002;128:149–54.
- [18] Uppal SS, Tewari SC, Verma S, Dhot PS. Comparison of CD4 and CD8 lymphocyte counts in HIV-negative pulmonary TB patients with those in normal blood donors and the effect of anti-tubercular treatment: hospital-based flow cytometric study. *Cytometry B Clin Cytom* 2004;61:20–6.
- [19] Kalou M, Sassan-Morokro M, Abouya L, Bile C, Maurice C, Maran M, et al. Changes in HIV RNA viral load, CD4+ T-cell counts, and levels of immune activation markers associated with anti-tuberculosis therapy and cotrimoxazole prophylaxis among HIV-infected tuberculosis patients in Abidjan, Cote d'Ivoire. *J Med Virol* 2005;75:202–8.
- [20] Kizza HM, Rodriguez B, Quinones-Mateu M, Mirza M, Aung H, Yen-Lieberman B, et al. Persistent replication of human immunodeficiency virus type 1 despite treatment of pulmonary tuberculosis in dually infected subjects. *Clin Diagn Lab Immunol* 2005;12:1298–304.
- [21] Flores-Batista VC, Boechat N, Lago PM, Lazzarini LC, Pessanha LR, Almeida AS, et al. Low expression of antigen-presenting and costimulatory molecules by lung cells from tuberculosis patients. *Braz J Med Biol Res* 2007;40:1671–9.
- [22] Bosio CM, Gardner D, Elkins KL. Infection of B cell-deficient mice with CDC 1551, a clinical isolate of *Mycobacterium tuberculosis*: delay in dissemination and development of lung pathology. *J Immunol* 2000;164:6417–25.
- [23] Simonney N, Chavanet P, Perronne C, Lepointier M, Revol F, Herrmann JL, et al. B-cell immune responses in HIV positive and HIV negative patients with tuberculosis evaluated with an ELISA using a glycolipid antigen. *Tuberculosis (Edinb)* 2007;87:109–22.
- [24] Onwubalili JK, Scott GM. Natural killer cell activity in tuberculosis. *Br J Dis Chest* 1985;79:67–76.
- [25] Ratcliffe LT, Lukey PT, MacKenzie CR, Ress SR. Reduced NK activity correlates with active disease in HIV-patients with multidrug-resistant pulmonary tuberculosis. *Clin Exp Immunol* 1994;97:373–9.
- [26] Nirmala R, Narayanan PR, Mathew R, Maran M, Deivanayagam CN. Reduced NK activity in pulmonary tuberculosis patients with/without HIV infection: identifying the defective stage and studying the effect of interleukins on NK activity. *Tuberculosis (Edinb)* 2001;81:343–52.
- [27] Barcelos W, Martins-Filho OA, Guimarães TM, Oliveira MH, Spíndola-de-Miranda S, Carvalho BN, et al. Peripheral blood mononuclear cells immunophenotyping in pulmonary tuberculosis patients before and after treatment. *Microbiol Immunol* 2006;50:597–605.
- [28] Deveci F, Akbulut HH, Celik I, Muz MH, Ilhan F. Lymphocyte subpopulations in pulmonary tuberculosis patients. *Mediat Inflamm* 2006;2006(2):89070.
- [29] Tsuyuguchi I. Immunotherapy for MDR-TB (multi-drug resistant tuberculosis)—its feasibility. *Kekkaku* 1999;74:479–91.
- [30] Pylypchuk VS. Clinical and experimental aspects rationalizing the need for immunotherapy in the treatment of patients with tuberculosis. *Probl Ecol Med Gen Clin Immunol* 2003;70:75–84.