Autologous mesenchymal stromal cell infusion as adjunct treatment in patients with multidrug and extensively drug-resistant tuberculosis: an open-label phase 1 safety trial

Aliaksandr Skrahin, Raija K Ahmed*, Giovanni Ferrara, Lalit Rane, Thomas Poiret, Yanina Isaikina, Alena Skrahina, Alimuddin Zumla, Markus J Maeurer*

Summary

Background Novel treatment options are urgently needed for multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis, which are associated with immune dysfunction and poor treatment outcomes. Mesenchymal stromal cells (MSCs) are immunomodulatory and adjunct autologous treatment with bone marrow-derived MSCs might improve clinical outcome by transforming chronic inflammation into productive immune responses. Our aim was to assess the safety of infusion of autologous MSCs as an adjunct treatment in patients with tuberculosis.

Methods 30 patients with microbiologically confirmed MDR or XDR tuberculosis were treated with single-dose autologous bone marrow-derived MSCs (aimed for 1×10^6 cells per kg), within 4 weeks of the start of antituberculosis-drug treatment in a specialist centre in Minsk, Belarus. Inclusion patients were those with pulmonary tuberculosis confirmed by sputum smear microscopy, culture, or both; MDR or XDR tuberculosis confirmed by drug-susceptibility testing to first-line and second-line drugs; age older than 21 years to 65 years or younger; and absence of lesion compatible with a malignant process or ongoing tuberculosis in organs other than the lungs and pleura. In addition to the inclusion criteria, patients were excluded if they were pregnant, coinfected with HIV, or infected with hepatitis B, C, or both. The primary endpoint was safety measured by MSC-infusion related events; any tuberculosis-related event within the 6 month observation period that related to a worsening of the underlying infectious disease, measured by conversion of *Mycobacterium tuberculosis* culture or microscopic examination; or any adverse event defined clinically or by changes in blood haematology and biochemistry variables, measured monthly for 6 months after MSC infusion per protocol. This study is registered with the German Clinical Trials Registry, number DRKS00000763.

Findings The most common (grade 1 or 2) adverse events were high cholesterol levels (14 of 30 patients), nausea (11 of 30 patients), and lymphopenia or diarrhoea (ten of 30 patients). There were no serious adverse events reported. We recorded two grade 3 events that were transitory—ie, increased plasma potassium ion concentrations in one patient and a transitory grade 3 γ-glutamyltransferase elevation in another patient.

Interpretation MSCs as an adjunct therapy are safe and can now be explored further for the treatment of patients with MDR or XDR tuberculosis in combination with standard drug regimens. Adjunct treatment with MSCs needs to be evaluated in controlled phase 2 trials to assess effects on immune responses and clinical and microbiological outcomes.

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Introduction

The identification and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis[1,2] poses a major threat to global control of tuberculosis. WHO estimates that about 440 000 cases were due to MDR tuberculosis in 2008 with increasing prevalence in eastern Europe and southern Africa.[3–5] Novel treatment options are urgently needed for MDR and XDR tuberculosis since they are associated with immune dysfunction and poor treatment outcomes. A robust response by T-helper-1 (Th1) cells is believed to be helpful in containing *Mycobacterium tuberculosis*; adjunct treatments have therefore often aimed to induce or augment a Th1-cell response in patients with tuberculosis.[6] However, the highest local and systemic levels of Th1-cell-type responses, including interleukin 18 production,[7] have been identified in patients with the most severe forms of tuberculosis—ie, individuals in whom *M tuberculosis* was not contained. This led to the suggestion that immunotherapeutic approaches might reduce aberrantly high inflammatory immune responses in tuberculosis;[8] for example, by using corticosteroids for the adjunct treatment of tuberculosis,[9] in part via reduction of tumour necrosis factor α (TNFα).[10]

Curbing damaging and ineffective host immune responses associated with infections has been suggested to be centrally important in tackling increased resistance to antibiotics in general;[11] this refocusing of the immune response needs new thinking and approaches to

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*Contributed equally

Republican Research and Practical Centre for Pulmonology and TB, Minsk, Belarus (A Skrahin MD, A Skrahina MD); **Department of Intensive Care, Belarussian State Medical University, Minsk, Belarus** (A Skrahin); **Swedish Institute for Infectious Disease Control (SMI), Solna, Sweden** (R K Ahmed PhD); **Section of Respiratory Diseases, Department of Internal Medicine, University of Perugia, Perugia, Italy** (G Ferrara MD); **Department of Microbiology, Tumour and Cell Biology, MTC** (L Rane MSc, Prof M J Maeurer MD), **and Therapeutic Immunology (TIM), Department of Laboratory Medicine** (T Poiret MSc, Prof M J Maeurer), **Karolinska Institutet, Stockholm, Sweden; Laboratory of Cellular Biotechnology and Cytotherapy, Belarussian Research Centre for Paediatric Oncology, Haematology and Immunology, Minsk, Belarus** (Y Isaikina PhD); **Division of Infection and Immunity, Centre for Clinical Microbiology, University College London, London, UK** (Prof A Zumla FRCP); **National Institute of Health Research-Biomedical Research Centre, University College Hospitals NHS Foundation Trust, London, UK** (Prof A Zumla); **and Centre for Allogeneic Stem Cell Transplantation, CAST, Karolinska University Hospital, Stockholm, Sweden** (Prof M J Maeurer)

Correspondence to:
Prof Markus J Maeurer, TIM, F79, Hälsovägen, Karolinska University Hospital Huddinge, SE 14186 Stockholm, Sweden
markus.maeurer@ki.se

See Online for appendix

restricting pathogen-induced damage to the host. One option to reduce inflammation-induced damage in infectious diseases is the use of bone-marrow-derived mesenchymal stromal cells (MSCs).[12]

MSCs were originally identified in the 1970s in cellular bone-marrow suspensions by their capacity to adhere to plastic—still the standard in MSC procurement. Although first identified in bone marrow,[13] MSCs have now been shown to be present in various tissues and organs.[14,15] Their function in bone marrow is to facilitate haemopoiesis,[16] and to support cell growth and cellular organisation in adult organ tissues.[14] For example, MSCs have been shown to increase the proliferative potential of bronchoalveolar stem cells[17] and to restore lung epithelium via the donation of mitochondria to other cells.[18,19] In general, MSCs seem to facilitate organ homoeostasis and to repair damaged and traumatised tissues and organs—including the lungs.[20]

In view of the immune-modulatory and tissue-saving capacity of MSCs, we hypothesised that adjunct autologous, bone-marrow-derived MSCs might aid in the clinical management of patients with drug-resistant tuberculosis by transforming chronic inflammation into productive immune responses. A worsening of tuberculosis was a major concern since recent studies suggested that allogeneic MSCs might be associated with an increased risk of infections in allogeneic stem-cell transplantation[21] or in the treatment of autoimmune diseases.[22] Most clinical MSC trials in regenerative medicine have been done with allogeneic MSCs,[23] since allogeneic MSC production is easier to streamline and quality control compared with the production of autologous MSCs. However, several clinical studies have been done with autologous MSCs, where a more extended action of MSCs is thought to be necessary—eg, for the treatment of stroke patients.[24] A recent head-to-head comparison (POSEIDON trial)[25] of allogeneic versus autologous MSCs for the treatment of patients with ischaemic cardiomyopathy reported that both sources were equivalent. We chose to use autologous MSCs in our study because of safety considerations (ie, autologous material will not introduce new infectious organisms to the patient) and to enable an extended biological activity of the cell product (ie, autologous cells allow an extended biological activity because they are from the patient and are not attacked by the patient's own immune system). We report the outcome of a phase 1 safety clinical trial in 30 individuals, started in 2009, using autologous MSCs for the adjunct treatment of MDR and XDR tuberculosis[5] in Belarus, where greater than 76% of previously treated patients present with resistant forms of tuberculosis.[26] These forms are associated with substantial lung damage, immune dysfunction, and poor treatment outcomes.[4,5,27]

Methods

Participants

Between September, 2009, and June, 2011, 30 patients admitted to the Republican Research and Practical Centre for Pulmonology and Tuberculosis (RRPCPTB; Minsk, Belarus) were recruited after written informed consent. The main inclusion criteria (for the protocol see appendix) were pulmonary tuberculosis confirmed by sputum smear microscopy, culture, or both; MDR or XDR tuberculosis confirmed by drug-susceptibility testing to first-line and second-line drugs; age older than 21 years to 65 years or younger; and absence of lesion compatible with a malignant process or ongoing tuberculosis in organs other than the lungs and pleura.[28] The decision to offer MSC treatment to new patients or patients with previous MDR or XDR tuberculosis was taken if a new course of individualised therapy, based on the drug-susceptibility pattern, was started and the bone marrow aspiration could be done within 1 month of the start of individualised treatments for MDR or XDR tuberculosis. Thus patients were already treated with antituberculosis-drug regimens before the MSC infusion.

Patient categorisation was done in accordance with the 2008 update of the *Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis*,[29] which was in effect at the time of trial design and patient recruitment. Registration data were collected on the history of previous treatments for tuberculosis; for example, whether patients were previously treated with first-line drugs only or whether patients were previously treated with second-line drugs. These data enabled the easy differentiation of patients with MDR or XDR tuberculosis in terms of the WHO definitions—ie, data collected at trial registration were used to divide patients into "new", "previously treated with first line", and "previously treated with second line" groups. The entire range of antituberculosis drugs was recorded for transparency. The term "chronic" was used to signify patients who received at least two or more courses of treatment with second-line drugs and who did not respond to that treatment before entry into the study. Patients were excluded if they were younger than 21 years, pregnant, coinfected with HIV, or infected with hepatitis B, C, or both. To be defined as MDR tuberculosis, the infecting strain had to be resistant to at least isoniazid and rifampicin; XDR tuberculosis was similarly defined with the addition of resistance to any fluoroquinolone and at least one injectable second-line drug.[2,3,5] All patients were treated with the best personalised tailored second-line regimens on the basis of drug-susceptibility testing.

The RRPCPTB laboratory is a quality controlled microbiological laboratory and proficiency testing concerning the identification of *M tuberculosis* and drug-susceptibility testing are done regularly by the Swedish Institute for Infectious Disease Control. Diagnoses of tuberculosis were confirmed by direct microscopy after Ziehl–Neelsen staining, culture using the BACTEC MGIT 960 system (Becton Dickinson, Sparks, MD, USA) and solid Löwenstein–Jensen medium, or both. The drug susceptibility of every isolate of *M tuberculosis* was assessed

with BACTEC MGIT 960 supplemented with isoniazid (0·1 μg/mL), rifampicin (1·0 μg/mL), ethambutol (5·0 μg/mL), or streptomycin (1·0 μg/mL). The isolates identified as MDR were then tested for resistance to second-line antituberculosis drugs with BACTEC MGIT 960 supplemented with kanamycin (2·5 μg/mL), amikacin (1·0 μg/mL), capreomycin (2·5 μg/mL), ofloxacin (2·0 μg/mL), or levofloxacin (1·5 μg/mL), and using Löwenstein-Jensen medium supplemented with ethionamide (40·0 μg/mL), para-aminosalicylic acid (1·0 μg/mL), or cycloserine (30·0 μg/mL).[30,31]

Furthermore, in another 30 patients with MDR or XDR tuberculosis (not treated with autologous MSCs), samples from routine laboratory tests and radiographs (matched to the timepoints of MSC-treated patients and after 6 months—ie, the observation period) were assessed to gauge the development of the disease and laboratory variables over time without adjunct MSC therapy (appendix). The laboratory and clinical follow-up of the population with tuberculosis who did not receive treatment with MSCs was matched to the study population—ie, this cohort consisted of patients with MDR or XDR tuberculosis who met the inclusion criteria and agreed to participate in the study but did not opt for treatment with MSCs. These individuals were consecutively recruited into the study.

The clinical treatment protocol was approved by the RRPCPTB ethics committee (first approval at EC meeting of Feb 3, 2009). The jurisdiction concerning the clinical study rested with the RRPCPTB ethical committee in Belarus; the ethics committee in Sweden had been informed and provided ethical clearance before entry of patient material (derived from the clinical study) into Sweden for subsequent analysis of immune cells (approval 2011/863-31/2).

Procedures

The primary endpoint of this study was safety of autologous MSCs used as adjunct treatment in patients with MDR or XDR tuberculosis. The secondary endpoint was changes in immunological markers.

Chest radiographs were taken before, 2 months after, and 6 months after MSC infusion. Routine blood tests included differential blood counts, haemoglobin, ESR, total protein, albumin, total globulins, glucose, urea, C-reactive protein (CRP), creatinine, cholesterol, total (direct and indirect) bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase, lactate dehydrogenase, α-amylase, γ-glutamyltransferase, creatine phosphokinase, sodium, potassium, and calcium. Sputum microscopy and culture for *M tuberculosis* was done every month after MSC infusion. Chest radiographs were scored by an experienced tuberculosis physician (A Skrahin) in accordance with the work of Ralph and colleagues[32] (proportion of lung involvement, plus 40 if cavities are present) to gauge changes related to tuberculosis. The difference in the radiograph score between timepoint 0 and 6 months after MSC infusion was calculated as

$$\textit{Difference (\%)} = \frac{\textit{Score at time } 0 - \textit{Score after } 6 \textit{ months}}{\textit{Score at time } 0} \times 100$$

and classified as improved (10% or greater difference, showing a decrease of the score), worsened (–5% or greater variation, showing an increase in the score), or stable (variation of less than 5%).

Cells were processed and produced at the Laboratory of Cellular Biotechnology and Cytotherapy of Belarussian Research Centre for Paediatric Oncology, Haematology and Immunology (BRCPOHI; Minsk, Belarus), responsible for the procurement of MSCs for the treatment of corticosteroid-resistant graft-versus-host disease for patients after autologous haemopoietic stem-cell transplantation. BRCPOHI is a state organisation with the state registration certificate authorising procurement of cells for cellular therapy of patients including haemopoietic stem cells and MSCs. All requirements concerning quality assurance required by national regulatory authorities are met and MSC products are subjected to internal quality control measures.

MSC sterility was done after each passage and from the final product of MSCs (ready to be infused) using an automated system BACTEC 9120 (Becton Dickinson). The cell culture samples of MSCs were added to BACTEC Peds Plus/F culture vials (for bacteria) and to BACTEC Mycosis-IC/F culture vials (for fungal pathogens). Hence MSC cultures were tested for sterility three times (since the culture was split three times) before infusion. Sterility before infusion was done by adding MSC culture material into the BACTEC 9120 and BACTEC Peds Plus/F culture vials 3 days before MSC infusion. The infusion of MSCs into the patient was scheduled if the automated system did not show any growth and the Gram stain from these culture vessels tested negative—as well as the culture from material from the culture vessel (after the initial 2 day cultures in the automated system) streaked onto agar plates incubated both aerobically and anaerobically. Bacterial and fungal cultures in liquid media were continued and stopped after 2 weeks in the automated BACTEC system.

In addition to the internal quality control measures, external quality control was done for MSCs (in nine clinical MSC samples, shipped to Karolinska Institutet), where cell compositions, cell dose, and sterility was confirmed. Three MSC samples were further analysed with real-time PCR for CD105, CD73, and CD90 markers. The study was registered and approved by the Expert Board of the Ministry of Health on June 8, 2009 (state registration number 20091063 НИОК[Т]Р). Audits from the Expert Board of the Ministry of Health were in accordance with national regulations and were done on a regular basis (two times a year).

In detail, bone marrow aspirates of 40–80 mL were obtained from the iliac crest from recruited patients with MDR or XDR tuberculosis in accordance with standard procedures. Heparinised bone marrow was mixed with a double volume of RPMI medium and ficolled to obtain mononuclear cells, which were collected and washed twice with phosphate-buffered saline and resuspended in human MSC medium, Iscove's modified Dulbecco's medium (Invitrogen, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (mesenchymal stem-cell qualified; Invitrogen) and 2 mmol/L of L-glutamine and 10^{-4} mol/L of 2-mercaptoethanol. Cells were plated at 1×10^7 cells per mL in T75 flasks and cultured at 37°C in 5% CO_2. The starting volume was 30 mL and medium was added as needed. Floating cells were removed at days 3 and 5. When the cultures reached 70% confluence, they were detached by trypsin and EDTA treatment, counted, washed and passaged at 1×10^6 cells per mL in T75 flasks. After each passage, sterility controls were done. After two to four passages, cells were harvested and tested by flow cytometry for immune markers including CD166, CD90, CD105, CD73, CD44, CD45, CD14, and CD34 (appendix); the minimal test requirements for inclusion in the study were the flow cytometric analysis (ie, CD73, CD90, CD105 plus CD45, and CD34), microscopic examination, and MSC differentiation in accordance with guidelines in place at the time of trial design and initiation.[33] MSCs were washed three times in 0·9% NaCl solution before infusion, resuspended in 0·9% NaCl and 5% human serum albumin (Octapharma, Vienna, Austria) solution, adjusted to 1×10^6 cells per mL, and slowly intravenously infused in 5% human serum albumin.[34] The entire MSC cell dose was given as a slow (5 min) bolus injection via an intravenous line. The MSC production followed the requirements as described by Dominici and colleagues.[33] Most clinical trials used MSCs in the range of $0\cdot5–3\cdot0\times10^6$ cells per kg;[35,36] we aimed to obtain 1×10^6 cells per kg. The capacity to expand MSCs differs from donor to donor. In some cases, fewer than 1×10^6 cells per kg MSCs could be expanded for infusion and lower numbers of MSCs were not excluded from infusion.

Peripheral-blood mononuclear cells (PBMCs) were obtained by venepuncture, isolated by ficoll-hypaque density gradient centrifugation from heparinised blood, with subsequent freezing in liquid nitrogen for analysis by flow cytometry. All samples from different timepoints were tested, along with appropriate controls from healthy blood donors, in the same experiment at the same day. Blood was collected at day 0 (before MSC infusion), and monthly after MSC infusion for the entire observation period. The study protocol stipulated a more detailed immunological analysis of immune responses in the first ten patients after initiation of the protocol. Immune cells from healthy donors exhibited strong cytokine production as described by Magalhaes and colleagues.[37] Haematological and biochemical analysis was done in all 30 patients. Then heparin blood samples from the first ten patients were analysed for the whole-blood assay (WBA; antigen-specific interferon-γ production) and the PBMC samples from these ten patients were analysed for intracellular cytokine staining and STAT5 phosphorylation. Datapoints that are not visible imply that the values were zero (eg, in the interferon-γ production assay).

Immune marker analysis was done on thawed PBMCs as described previously;[37,38] the antibodies are listed in the appendix. Samples obtained before and at different timepoints after infusion of MSCs from each individual were processed the same day. The markers CD45RA and CCR7 help to group T cells into precursor, memory-T-cell subsets, and terminally differentiated T cells;[39] these markers establish if T cells are long lived, contribute to immune memory, and are able to access tissues. Immune marker analysis was done in (non-expanded) PBMCs and not in the 7 day expanded immune cells from the WBA assay.

Cytokine production (interleukin 2, interferon γ, TNFα, and interleukin 17) was analysed in PBMCs,[37] as described in detail in the appendix. This 6 h assay measures the production of cytokines in response to a molecularly defined stimulus and relates to the ex-vivo production of cytokines in effector T cells. Simultaneous production of different cytokines from the same cell has been suggested to be associated with improved responses to *M tuberculosis*; interferon γ, TNFα, and interleukin 17 are believed to be instrumental in anti-*M tuberculosis* responses.

Constitutive and cytokine-induced phosphorylated STAT5 expression was assessed in PBMCs as described previously.[37,40] Immune-competent T cells respond with strong STAT5 phosphorylation in response to interleukin 7 and 2.

Assessment of cellular immune responses in whole blood (WBA) was done as described previously.[38] The WBA has been shown to gauge CD8+ and CD4+ T-cell responses and interferon-γ production to test antigens,[41] including *M tuberculosis* antigens.[42,43] Briefly, heparinised blood is diluted with cell-culture medium and immune cells are expanded for 7 days until assay readout (appendix). The 7 day WBA expansion measures CD4+ and CD8+ T-cell responses and not responses of natural killer cells. We tested a comprehensive panel of molecularly defined *M tuberculosis* antigens (appendix) that were commonly recognised in blood from patients with pulmonary tuberculosis in Belarus[44] (as well as in Honduras[45]) since the MHC diversity of the patients and the status of tuberculosis might vary and need a more comprehensive target *M tuberculosis* panel to gauge memory T-cell responses involved in *M tuberculosis* containment. The WBA measures whether immune cells from an individual are able to respond at all to a positive control (phytohaemagglutinin) and whether constitutive interferon-γ production is present. The background (medium) interferon-γ responses have been subtracted for each *M tuberculosis* antigen as described in detail

	Sex	Age, years	Case definition*	Drug-resistance status	Drug-susceptibility testing	Number of previous second-line treatments	Second-line treatment regimen at the time of MSC infusion	Microscopy/culture at the time of MSC infusion	MSC dose×10⁶ (cells per kg)	Radiology at 6 months
1	Male	31	New case	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, amikacin, kanamycin, ofloxacin, ethionamide, cycloserine	0	Moxifloxacin, PAS, protionamide, cycloserine, capreomycin, linezolid	–/+	55 (0·75)	Improved
2	Male	39	Previously treated	MDR	Isoniazid, rifampicin, streptomycin	1	Capreomycin, ofloxacin, pyrazinamide, cycloserine, ethionamide	–/+	17 (0·25)	Improved
3	Male	50	Chronic	MDR	Isoniazid, rifampicin, ethambutol, streptomycin, amikacin, kanamycin, capreomycin, protionamide	2	Levofloxacin, capreomycin, cycloserine, ethionamide, PAS, co-amoxiclav†	–/+	94 (1·30)	Worsened
4	Male	24	Chronic	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, ofloxacin, amikacin, capreomycin, kanamycin, PAS, cycloserine	3	Moxifloxacin, capreomycin, cycloserine, ethionamide, PAS, co-amoxiclav	–/+	74 (1·04)	Stable
5	Female	25	Chronic	MDR	Isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide, ofloxacin, PAS, ethionamide	2	Moxifloxacin, protionamide, kanamycin, co-amoxiclav, clarithromycin, PAS	–/+	65 (1·14)	Worsened
6	Female	29	Previously treated	MDR	Isoniazid, streptomycin, rifampicin, ethambutol	1	Pyrazinamide, kanamycin, ofloxacin, PAS, ethionamide, cycloserine	–/–	41 (0·70)	Improved
7	Male	41	Chronic	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide, amikacin, kanamycin, ofloxacin, PAS, ethionamide, capreomycin	2	Capreomycin, protionamide, PAS, cycloserine, co-amoxiclav, moxifloxacin, linezolid	–/+	43 (0·67)	Improved
8	Male	25	Chronic	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, kanamycin, amikacin, ofloxacin, ethionamide, capreomycin	3	Levofloxacin, protionamide, PAS, capreomycin, cycloserine, co-amoxiclav, clarithromycin	+/+	76 (0·95)	Stable
9	Female	29	Previously treated	MDR	Isoniazid, rifampicin, streptomycin, pyrazinamide, ethambutol, ethionamide	1	PAS, kanamycin, ofloxacin, ethionamide, cycloserine	–/+	13 (0·25)	Improved
10	Female	26	Chronic	XDR	Isoniazid, rifampicin, streptomycin, amikacin, kanamycin, ethambutol, ofloxacin	2	Levofloxacin, protionamide, PAS, capreomycin, cycloserine, co-amoxiclav	–/+	62 (0·79)	Improved
11	Female	29	Chronic	XDR	Isoniazid, streptomycin, rifampicin, ethambutol, kanamycin, amikacin, ethionamide, ofloxacin, capreomycin	2	PAS, protionamide, cycloserine, capreomycin, levofloxacin, co-amoxiclav, clarithromycin	–/+	69 (1·50)	Improved
12	Female	29	Chronic	MDR	Isoniazid, rifampicin, streptomycin, ethambutol	2	Pyrazinamide, kanamycin, ofloxacin, protionamide, cycloserine, PAS	–/+	22 (0·37)	Improved
13	Male	32	Previously treated	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide, amikacin, kanamycin, capreomycin, ofloxacin	0	PAS, levofloxacin, capreomycin, cycloserine, protionamide, co-amoxiclav	–/+	56 (0·70)	Improved
14	Female	24	Chronic	MDR	Isoniazid, rifampicin, streptomycin, ethambutol, amikacin, kanamycin	2	Ofloxacin, ethionamide, PAS, capreomycin, cycloserine	–/+	380 (6·00)	Improved
15	Female	22	Previously treated	MDR	Isoniazid, rifampicin, streptomycin, ethambutol	1	Kanamycin, ofloxacin, protionamide, PAS, pyrazinamide	–/+	20 (0·34)	Improved
16	Female	20	New case	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, kanamycin, amikacin, ofloxacin, levofloxacin, protionamide	0	Protionamide, levofloxacin, cycloserine, capreomycin, PAS, co-amoxiclav, clarithromycin	–/+	100 (1·81)	Improved
17	Male	21	New case	MDR	Isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide, ofloxacin, levofloxacin	0	Capreomycin, levofloxacin, protionamide, PAS, cycloserine, co-amoxiclav	–/+	71 (0·98)	Improved
18	Female	41	New case	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, kanamycin, ofloxacin, ethionamide, PAS	0	Moxifloxacin, amikacin, PAS, cycloserine, protionamide, co-amoxiclav‡	–/+	11 (0·20)	Improved
19	Female	35	New case	MDR	Isoniazid, rifampicin, streptomycin	0	Kanamycin, levofloxacin, protionamide, cycloserine, PAS	–/+	70 (1·06)	Improved

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	Sex	Age, years	Case definition*	Drug-resistance status	Drug-susceptibility testing	Number of previous second-line treatments	Second-line treatment regimen at the time of MSC infusion	Microscopy/ culture at the time of MSC infusion	MSC dose×10⁶ (cells per kg)	Radiology at 6 months
(Continued from previous page)										
20	Male	22	New case	XDR	Isoniazid, streptomycin, rifampicin, ethambutol, kanamycin, ofloxacin, PAS, capreomycin, cycloserine	0	Amikacin, levofloxacin, protionamide, PAS, cycloserine, co-amoxiclav	–/+	94 (1·20)	Stable
21	Male	21	Chronic	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, kanamycin, amikacin, ofloxacin, protionamide	1	Capreomycin, levofloxacin, protionamide, PAS, cycloserine, co-amoxiclav	–/+	67 (1·05)	Improved
22	Female	24	Previously treated	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, amikacin, kanamycin, ofloxacin	0	Pyrazinamide, capreomycin, levofloxacin, protionamide, PAS, cycloserine, co-amoxiclav	–/+	63 (0·95)	Improved
23	Female	21	New case	MDR	Isoniazid, rifampicin, streptomycin, pyrazinamide	0	Ethambutol, capreomycin, ofloxacin, protionamide, cycloserine, PAS	–/+	65 (0·80)	Improved
24	Female	43	Chronic	MDR	Isoniazid, rifampicin, ethambutol, streptomycin, pyrazinamide, kanamycin, amikacin, ethionamide	2	Capreomycin, pyrazinamide, levofloxacin, protionamide, cycloserine, PAS	–/+	60 (1·06)	Worsened
25	Male	45	Previously treated	MDR	Isoniazid, rifampicin, streptomycin, ofloxacin	0	Pyrazinamide, ethambutol, capreomycin, moxifloxacin, ethionamide, cycloserine	–/+	53 (0·62)	Improved
26	Female	33	New case	MDR	Isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide, kanamycin, PAS	0	Pyrazinamide, capreomycin, levofloxacin, protionamide, cycloserine, PAS	–/+	58 (1·20)	Improved
27	Female	19	Chronic	XDR	Isoniazid, rifampicin, streptomycin, ethambutol, amikacin, kanamycin, ofloxacin, capreomycin	2	Pyrazinamide, capreomycin, moxifloxacin, ethionamide, PAS, cycloserine, co-amoxiclav	+/+	51 (1·12)	Worsened
28	Male	39	New case	MDR	Isoniazid, rifampicin, streptomycin, ethambutol, amikacin, kanamycin, capreomycin	0	Pyrazinamide, capreomycin, levofloxacin, protionamide, cycloserine, PAS	–/+	110 (1·84)	Improved
29	Male	34	Previously treated	MDR	Isoniazid, rifampicin, streptomycin, pyrazinamide	0	Pyrazinamide, ethambutol, capreomycin, levofloxacin, protionamide, cycloserine, PAS	–/+	98 (1·14)	Stable
30	Male	46	Previously treated	MDR	Isoniazid, rifampicin, streptomycin, ethambutol, kanamycin, capreomycin	0	Pyrazinamide, amikacin, levofloxacin, protionamide, cycloserine, PAS	–/+	41 (0·41)	Stable

MSCs=mesenchymal stromal cells. MDR=multidrug resistant. XDR=extensively drug resistant. PAS=para-aminosalicylic acid. *Chronic case means more than two unsuccessful rounds of therapy. †Seven-rib thoracoplasty. ‡Lobectomy right-upper lobe during therapy.

Table 1: **Patient characteristics by identification number**

previously[44,46]—ie, the interferon-γ values reported for each *M tuberculosis* antigen represent antigen-specific T-cell responses. interferon-γ production of T cells from patients with tuberculosis can be inherently low or high in response to certain *M tuberculosis* target antigens, associated with the nature and biology of the target—eg, antigens associated with *M tuberculosis* latency.[47]

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Statistical analysis

Differences between the proportions of immune responses in blood from patients, defined by interferon-γ production (WBA) before and after treatment were analysed with the Wilcoxon test and the Fisher exact test was used to analyse differences in the tuberculosis diagnostics (microscopy and culture) using the GraphPad version 4.

We recorded three categories of events within the 6 month observation period: worsening or exacerbation of tuberculosis, defined as conversion from smear negative to smear positive; conversion from *M tuberculosis* culture negative to culture positive, or both; and adverse events associated with the intravenous administration of MSCs measured by standard means in accordance with the Common Terminology Criteria for Adverse Events (CTCAE). Terminology and classification for adverse events was done in accordance with CTCAE version 3. In addition to standard means (eg, blood pressure, temperature, heart rate), SpO_2 (as a sign of hypoxia) was measured at the time of MSC infusion and 1 h after infusion—the values were within the normal range before study entry. Other pulmonary variables (eg, vital capacity, FEV_1, carbon monoxide diffusion capacity [also recommended by CTCAE]) were not done for patients with MDR or XDR tuberculosis in accordance with institutional infection control measures; any adverse effect according to CTCAE within the 6 month

observation period including laboratory parameters and clinical performance was noted. Chest radiographic assessment was done at the time of MSC infusion and after 6 months—ie, at completion of the observation period. The data are part of the adverse event report, since a more detailed examination of pulmonary alterations (ie, measured by high-resolution CT) would be needed to gauge objectively subtle changes in pulmonary structures.

Tuberculosis treatment outcome was reported 18 months after initiation of therapy (and is therefore outside the 6 month observation period of the MSC safety trial). The terms "cure", "treatment completed", "treatment failure", and "default" relate to the nomenclature of the tuberculosis treatment in accordance with those published in the guidelines.[29] This study is registered with the German Clinical Trials Registry, number DRKS00000763.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Table 1 details the characteristics of the 30 patients with drug-resistant pulmonary tuberculosis. Of these 30 patients, 17 had MDR tuberculosis and 13 XDR tuberculosis. All patients were *M tuberculosis* culture positive and received infusions of autologous MSCs. Flow cytometric analysis of MSCs showed that they exhibited typical markers including CD90, CD105, CD44, and CD166; they stained negative for CD45, CD34, and CD14 (figure 1). Nine patients represented new cases, nine had been treated previously for tuberculosis, and twelve had chronic cases of tuberculosis.

No severe adverse events were recorded in the 6 months of the study. Because of underlying disease, many patients experienced pulmonary symptoms (mostly cough) before MSC infusion (at baseline) due to pulmonary tuberculosis. With regard to effects on the underlying disease, a major concern was the potential immunosuppressive effect of MSC infusion that could lead to deterioration of tuberculosis. Therefore, a systematic and careful screening of the sputum samples (microscopy and culture) was adopted. The term "exacerbation" refers to a rapid deterioration of the disease within the 6 month observation period defined by *M tuberculosis* positive direct sputum or culture examination (appendix). This event was not recorded for any patients. No patients experienced even mild (grade 1) events associated with intravenous MSC infusion. This lack of adverse events was also true for pulmonary symptoms (eg, bronchospasm, dyspnoea) during MSC infusion or during the 6 month observation period. SpO_2 (as a sign of hypoxia) was measured at the time of MSC infusion and 1 h after infusion; the value was within normal range and none of the patients exhibited a decrease in SpO_2.

Table 2 lists the adverse events according to standard International Conference on Harmonisation good clinical practice definitions covering the first 6 months after MSC infusion. The most common adverse events (grade 1 and 2) were increased cholesterol, nausea, diarrhoea, and lymphopenia. Most of the adverse events existed before MSC infusion (eg, lymphopenia); other adverse events (eg, high cholesterol) were attributed to the liver-related toxic effects of antituberculosis drugs. We recorded two grade 3 events that were transitory—ie, increased plasma potassium ion concentrations in one patient and a transitory grade 3 γ-glutamyltransferase elevation in another patient. There were some changes from baseline values of standard haematology and biochemistry, but they did not persist and no values were outside of the normal range (figure 2, appendix).

We have also analysed in greater detail haematological and biochemical parameters in patients with MDR or XDR tuberculosis and grouped them according to age, MDR or XDR tuberculosis, new versus previously treated cases, and the resistance patterns of the isolated *M tuberculosis* strains. We did not identify any differences in the safety parameters measured in these patient subsets (appendix).

Chest radiographs, obtained at the time of MSC infusion and after the observation period (6 months),

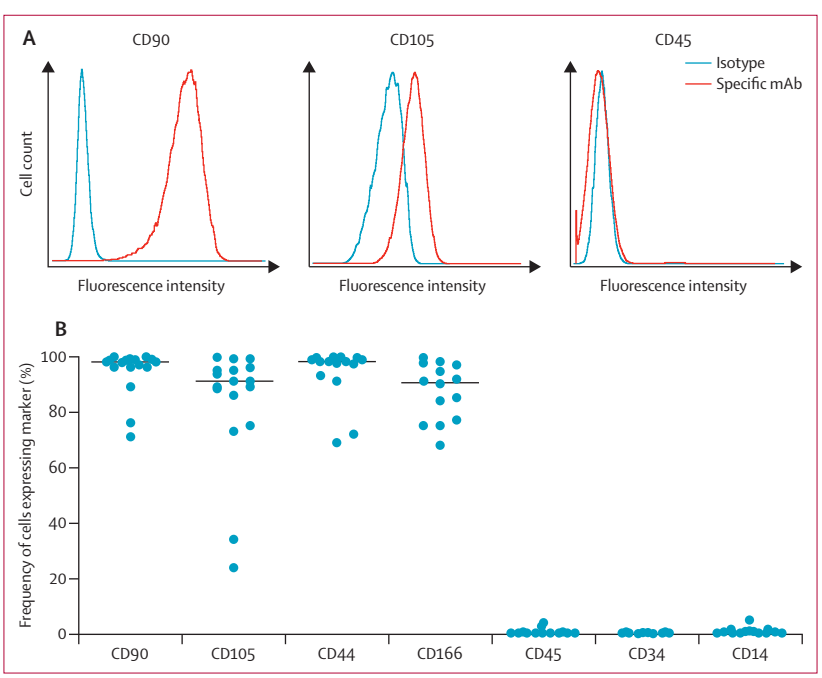

Figure 1: **MSC phenotype and quality control**
MSCs were tested for marker expression by flow cytometry in accordance with guidelines effective at the time of trial design and initiation.[33] Typical CD90 and CD105 expression and absence of CD45 in MSCs generated from patients with tuberculosis (A). Compilation of marker analysis (B) with MSC marker expression (ie, CD90, CD105, CD44 and CD166 in MSC; absence of CD45 [lymphocytes], CD34 [haemopoietic stem cells], or CD14 [monocytes] markers in MSC preparations). MSCs tested positive for CD73 (data not shown). MSC=mesenchymal stromal cell.

were compared; which was not part of the adverse event report. 21 individuals showed improvement of the chest radiograph, five showed stabilisation, and four showed worsening of the radiograph during the observation period (table 1). The detailed scores of the chest radiographs at the time of MSC infusion and after 6 months are provided in the appendix.

With regard to treatment outcomes for MDR tuberculosis (reported 18 months after the start of tuberculosis therapy, not part of the observation safety trial),[48,49] 16 individuals were deemed to be cured; two patients completed the treatment, eight were still on treatment, three showed treatment failure, one was classified as a default, and no deaths were reported (appendix). The data for age-matched and sex-matched patients with MDR or XDR tuberculosis, without MSC treatment, are also provided in the appendix.

Detection of ex-vivo intracellular cytokine production in response to the positive control stimulus (phorbol 12-myristate 13-acetate) and to the TB10.4 protein, as a marker of an anti-*M tuberculosis* response, was low in PBMCs from ten of ten individuals (<1% of CD8 or CD4+ T cells for interleukin 2, interferon γ, TNFα, or interleukin 17) at any timepoint after MSC infusion. Some patients exhibited interleukin 2 and interleukin 17 or TNFα production to the *M tuberculosis* antigen TB10.4 at day 28 after MSC infusion in CD4+ T cells (appendix); this was not statistically significant. Peripheral CD4+ and CD8+ T cells, as well as CD3+, CD4–, and CD8– T cells exhibited the phenotype of central-memory (CD45RA–CCR7+) T cells and this pattern did not change significantly after MSC infusion (appendix). So-called immune exhaustion was also evident by decreased expression of the T-cell receptor ζ chain, responsible for T-cell signalling[50] in T cells from patients with tuberculosis (appendix). Some individuals regained expression of the T-cell receptor ζ chain after MSC infusion; however, the small sample size (immune cells from five individuals) did not allow for statistical analysis.

Since we were unable to identify intracellular cytokine production in a standard 6 h ex-vivo assay, we analysed memory *M tuberculosis*-reactive T cells with a T-cell expansion assay that measures antigen-specific cytokine production in response to antigenic stimulation (figure 3). This assay measures whether T cells have the capacity to respond to any antigenic stimulation defined by interferon-γ production in response to a positive control (phytohaemagglutinin). This was the case for T cells from all ten patients with tuberculosis (figure 3) before and after MSC infusion. Baseline production of interferon γ from T cells (without antigenic stimulation) was also measured to gauge constitutive cytokine production, which was subtracted from interferon-γ production values obtained with antigen-specific stimulation. Several molecularly defined *M tuberculosis* targets were tested for differences before and after MSC infusion concerning interferon-γ production (appendix). Two *M tuberculosis* targets revealed statistically relevant differences eliciting interferon-γ production in immune cells from patients with tuberculosis before and after MSC infusion. We identified an increase of interferon-γ production in response to antigen Ag85B (Rv1886) and a decreased interferon-γ production in response to mycocerosic synthase (Rv2940c) after the observation period (figure 3).

T cells from patients with tuberculosis did not respond before MSC treatment with STAT5 phosphorylation to stimulation with interleukins 2 or 7,[37] a sign of reduced immune effector function.[40] We tested also the capacity of CD4+ and CD8+ T cells from patients to respond to cytokine stimulation (interleukin 2 and interleukin 7) defined by phosphorylation of STAT5. Immune competent T cells respond with swift phosphorylation of STAT5 upon stimulation with interleukins 2 or 7.[37] T cells from some individuals with tuberculosis showed improvement of STAT5 phosphorylation in CD4+ and CD8+ T cells in response to cytokine stimulation

	Number of patients (n=30)	Number of recorded adverse events (n=217)	Grade 1 events	Grade 2 events	Grade 3 events
Anaemia	1 (3%)	5	4 (80%)	1 (20%)	0
Neutropenia	2 (7%)	4	4 (100%)	0	0
Lymphopenia	10 (33%)	27	27 (100%)	0	0
Nausea	11 (37%)	19	19 (100%)	0	0
Vomiting	5 (17%)	6	6 (100%)	0	0
Diarrhoea	10 (33%)	14	14 (100%)	0	0
Arthralgia	5 (17%)	11	8 (73%)	3 (27%)	0
Hearing Loss	4 (13%)	4	4 (100%)	0	0
Headache	3 (10%)	3	3 (100%)	0	0
Insomnia	8 (27%)	16	16 (100%)	0	0
Seizures	2 (7%)	2	0	2 (100%)	0
Hyperthyroidism	1 (3%)	1	1 (100%)	0	0
Hypoalbuminaemia	1 (3%)	9	2 (22%)	7 (78%)	0
Hyperglycaemia	3 (10%)	7	7 (100%)	0	0
Increased alanine transaminase	4 (13%)	9	7 (78%)	2 (22%)	0
Increased aspartate transaminase	7 (23%)	12	10 (83%)	2 (17%)	0
Increased bilirubin	2 (7%)	2	2 (100%)	0	0
Increased amylase	1 (3%)	3	3 (100%)	0	0
Increased γ-glutamyltransferase	1 (3%)	1	0	0	1 (100%; transitory)
Hypercholesterolaemia	14 (47%)	35	2 (6%)	33 (94%)	0
Hypercreatinaemia	1 (3%)	1	1 (100%)	0	0
Increased creatine phosphokinase	8 (27%)	13	13 (100%)	0	0
Hypocalcaemia (total Ca)	1 (3%)	1	0	1 (100%)	0
Hyperkalaemia	4 (13%)	6	5 (83%)	0	1 (17%; transitory)
Hypernatraemia	4 (13%)	6	6 (100%)	0	0

Data are n (%). All adverse events (blood, gastrointestinal, constitutional symptoms, pain, auditory, neurology, endocrine, metabolic, laboratory) were listed in accordance with the Common Terminology Criteria for Adverse Events version 3.0 at the time of MSC infusion and during the 6 month observation period. No fever episodes were reported. Increased values for cholesterol were present before the MSC infusion; the same situation was true for vomiting or low lymphocyte counts, which were present before MSC infusion. MSC=mesenchymal stromal cell.

Table 2: **Adverse events 6 months after MSC adjunct therapy**

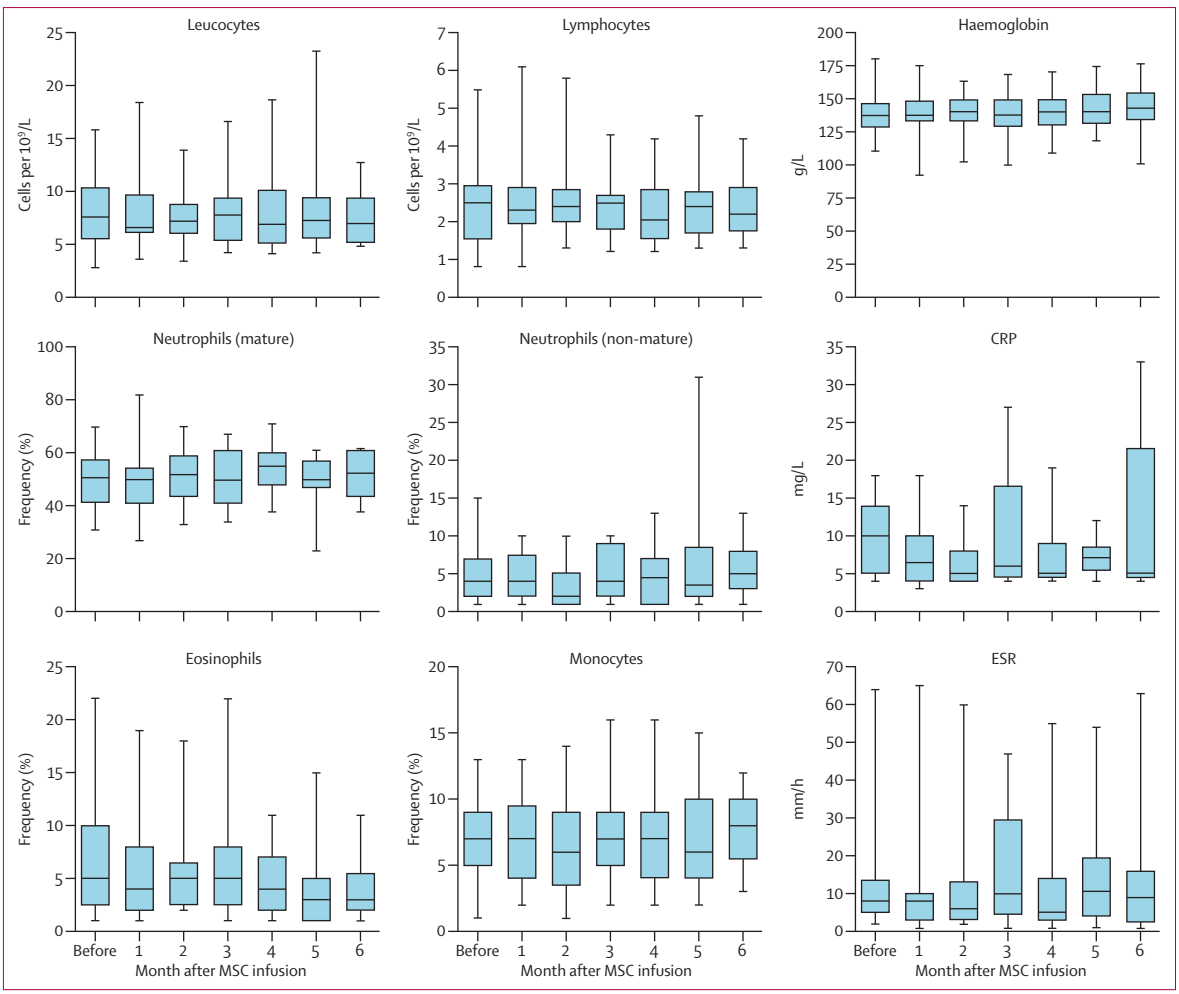

Figure 2: **Effect of MSC infusion on routine blood analyses**
The numbers show months after MSC infusion (1–6) during the 6 month observation period; patient samples were also analysed before the MSC infusion to obtain baseline values. MSC=mesenchymal stromal cell. CRP=C-reactive protein. ESR=erythrocyte sedimentation rate.

(interleukin 2 and interleukin 7) after MSC infusion (figure 4).

Discussion

This study is, to our knowledge, the first to describe the use of bone-marrow-derived autologous MSCs as an adjunct treatment for patients with MDR or XDR tuberculosis (panel). The MSCs harvested from patients with tuberculosis (who were under treatment at the time of MSC harvest) did not exhibit aberrant cell-surface-marker expression compared with MSCs harvested from healthy donors.[33] Treatment with antituberculosis drugs did not negatively affect MSC harvest; yet future studies will need to assess in greater detail whether certain anti-*M tuberculosis* drug combinations or concomitant *M tuberculosis* infection affects expansion and biology of MSCs in vitro, since data from a more recent study suggested that several factors, including donor age and sex, affect MSC function and possibly clinical efficacy.[51]

Adjunct therapy with MSCs was not associated with any severe adverse clinical events or disturbances in haematological and biochemical variables. 13% of patients with MDR or XDR tuberculosis exhibited worsening of chest radiographs, an outcome which is expected in this patient cohort on the basis of earlier studies.[4,5,26] Other adjunct immune-based therapies such as intradermal interleukin 2[52] or interferon γ via aerosol have shown limited results in improving tuberculosis-treatment outcomes or shortening the duration of therapy.[53]

Our findings show that MSC infusion was safe and well tolerated in all included patients. A recent meta-analysis assessing autologous and allogeneic MSC therapy in patients, searching Medline, Embase, and the Cochrane Central Register of Controlled Trials (to June, 2011) reported no major side-effects of MSC treatment[35] (36 randomised controlled and uncontrolled clinical studies were analysed). 27 of these studies used fetal

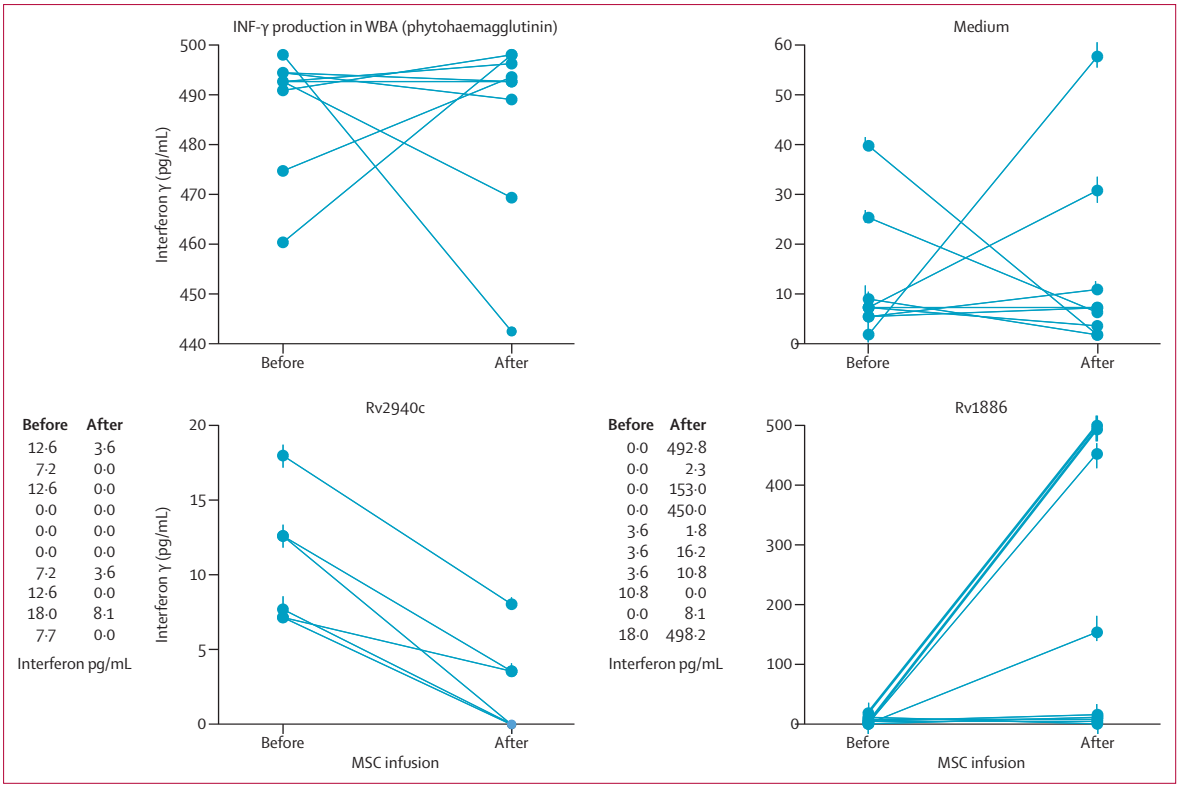

Figure 3: **Interferon-γ responses in response to Ag85B (Rv1886) after MSC infusion**
Whole blood was cultured with a positive control (phytohaemagglutinin) or with medium (negative control) and interferon γ was tested in cell-culture supernatants from PBMCs. No differences concerning spontaneous interferon-γ production and interferon-γ production in response to maximal stimulation. Note the increased interferon-γ response to Ag 85B (Rv1886) and decreased interferon-γ production in response to Ag Rv2940c after MSC infusion. A comparison of cellular immune responses before and 6 months after MSC infusion was done and background (medium) values were subtracted from *Mycobacterium tuberculosis* antigen-specific immune responses. The mean values (responses to antigenic stimulation; ie, after medium substraction) are included for the ten patients before and after MSC treatment. In total, 12 different *M tuberculosis* targets were tested for immune recognition before and after MSC infusion (appendix). Only Rv1886 and Rv2940 elicited significant differences in interferon-γ production before or after MSC infusion in PBMCs from patients with tuberculosis. MSC=mesenchymal stromal cell. PBMC=peripheral-blood mononuclear cell.

bovine serum (FBS), human serum was used in five, and four did not report the source of MSC culture supplements. The use of FBS in MSC culture medium represents several challenges (ie, batch-to-batch consistency[54]), the risk of immunogenicity (due to the transfer of xenomaterial, particularly in repeated MSC infusions[55,56]), and the risk of transfer of infectious agents (eg, prions). Of interest is the detailed comparison of infusion-related toxic effects between MSCs, cultured with different culture conditions, with or without FBS. No significant relation between MSC administration and acute toxic effects was recorded; the only toxic effect reported was transient fever. Several alternate MSC culture additives are being explored using allogeneic and autologous human serum for MSC expansion; autologous serum has been shown to be superior to allogeneic serum or FBS.[55] Serum-free medium with growth supplements would be the ideal solution to ensure batch-to-batch consistency[57] and to avoid xenoreactivity and alloreactivity associated with non-autologous serum sources.[58,59] A step in this direction is the use of platelet lysate, which has been suggested as a viable FCS substitute because of high levels of natural growth factors.[60,61]

A major initial concern in our phase 1 study was the risk of suppressing anti-*M tuberculosis* immune responses and subsequent worsening of tuberculosis, yet this has not been noted. We could also not identify decreased interferon-γ production in the WBA assay (comparing blood samples from the periods before and after MSC infusion) that would suggest immune-suppression in peripheral T-cell responses. Recovery of immune functions was shown by increased cytokine production in PBMCs (appendix) after MSC infusion and improved responses to stimulation by interleukins 2 and 7 in some patients measured by STAT5 phosphorylation (figure 4). CD4+ and CD8+ T cells from healthy individuals show low (ie, <2%) constitutively phosphorylated STAT5, which is rapidly phosphorylated upon stimulation with interleukins 2 or 7 in greater than 50% of T cells.[37] The reduced capacity of T cells to appropriately respond to antigen and cytokine stimu-

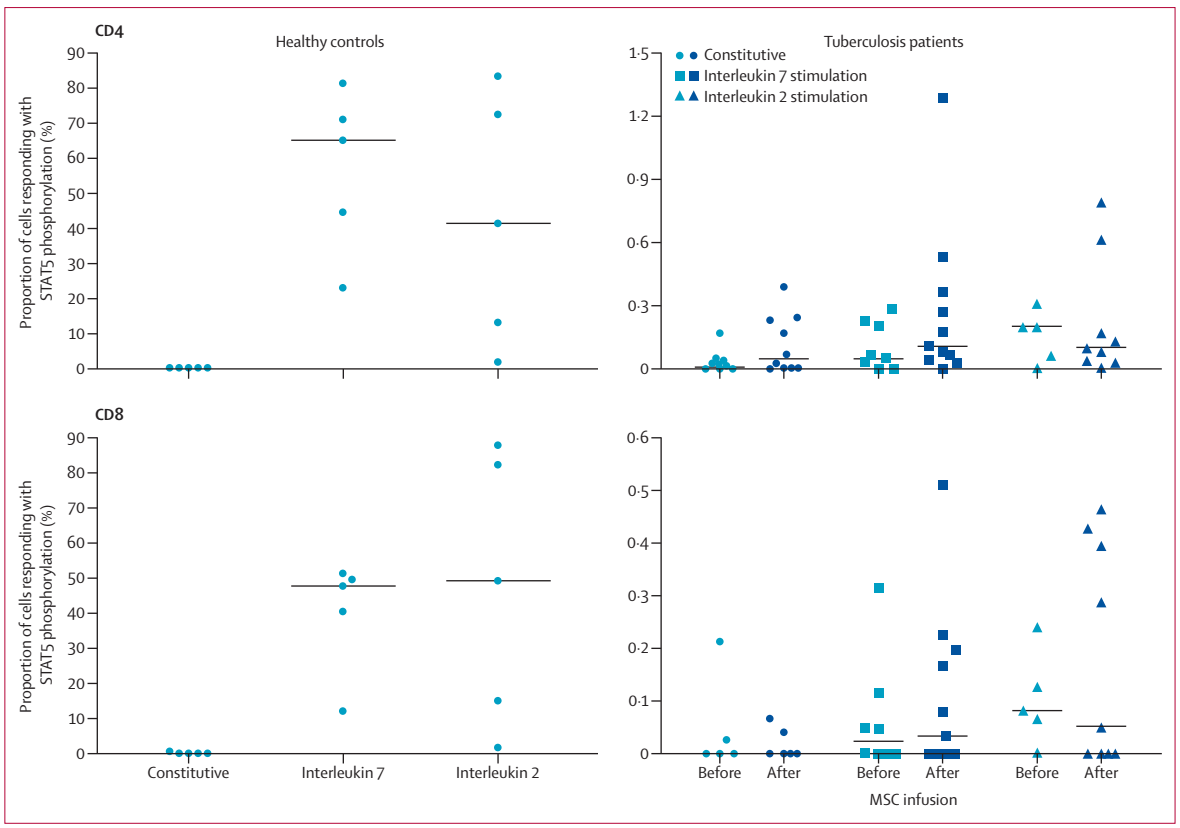

Figure 4: **STAT5-phosphorylation assay**

Mean CD4+ T-cell and CD8+ T-cell response to interleukin 2 or interleukin 7 stimulation defined by STAT5 phosphorylation. More than 50% of CD4+ and CD8+ T cells respond to stimulation with interleukin 7 or 2 defined by STAT5 phosphorylation in T cells from most healthy individuals, which served as positive controls. STAT5 phosphorylation in T cells from patients with tuberculosis before and after MSC infusion. Recovery of STAT5 phosphorylation in T cells from some patients after MSC adjunct treatment could be observed. MSC=mesenchymal stromal cell.

lation has been associated with the decreased capacity of T cells to phosphorylate STAT5 in mice.[62] Decreased STAT5 phosphorylation has also been identified in T cells from patients with chronic inflammatory diseases—eg, in individuals with Epstein–Barr virus-positive post-transplant lymphoproliferative disease,[63] in patients with cancer,[40] as well as in patients with chronic viral infections.[64] Reduction of a proinflammatory milieu, via MSC infusion, might therefore help to regain immune effector functions shown by restoration of STAT5 phosphorylation and subsequent cytokine production.

We examined immunological endpoints as the secondary study objectives. CRP decreased after MSC infusion (figure 2) at an early timepoint after MSC infusion, yet this needs to be confirmed in subsequent studies with a larger patient cohort. The ESR did not follow CRP; this observation is not unexpected, since CRP represents only a single acute-phase protein, whereas the ESR is affected by many haematological and biochemical factors, as well as sex and age.[65] At this point, no sufficiently assessed biomarker has been identified (easily accessible in peripheral blood) to gauge protective immune responses in tuberculosis.[66] We therefore addressed cellular immune responses that had been reported to be clinically and biologically meaningful—ie, *M tuberculosis*-reactive T-cell memory responses (defined by interferon-γ production in the WBA measuring memory T-cell responses),[67] T-cell differentiation and maturation markers,[37,68] intracellular cytokine production in response to *M tuberculosis* antigens (dominant TNFα, yet not interleukin 2 or interferon-γ production),[69] as well as defective STAT5 function in memory T-cell responses directed against pathogens.[63,70] Recovery was noted of *M tuberculosis*-specific interferon-γ production for the *M tuberculosis* antigen 85B (Rv1886), a component of the *M tuberculosis* cell wall, which serves also as a novel *M tuberculosis* vaccine candidate.[71,72] By contrast, immune responses directed against mycocerosic synthase were decreased (figure 3). Mycocerosic synthase, an *M tuberculosis* pathogenicity factor catalysing the synthesis of long-chain branched fatty acids (mycocerosic acids), is preferentially identified in pathogenic, slow growing bacilli.[73]

The timing of MSC infusion and subsequent immune-modulation might also represent an important factor. The immune system of the 30 patients with tuberculosis

might have been exposed for an extended period of time to antigenic *M tuberculosis* stimulation and inflammatory responses before MSC treatment. Clinical tuberculosis represents a broad disease spectrum, associated either with previously latent tuberculosis (which might have been clinically silent for many years), a new infection with *M tuberculosis* or a reinfection in individuals who had previously experienced an infection with *M tuberculosis*.[74] Two clinical examples show the importance of the right timing of biological therapy and immune-modulation: interferon-γ treatment of patients with sepsis syndrome is deleterious; it might worsen the so-called cytokine storm. However, patients with sepsis show increased survival, if interferon g is applied in a specific window of immune paralysis during the clinical course of sepsis.[75] Furthermore, immune-suppressive treatment of life-threatening influenza has been reported to be life-saving in cases of severe influenza.[76] A similar situation might be true for MSC treatment—ie, MSC-induced focused immune-suppression might be beneficial in patients with infection with tuberculosis experiencing aberrant high Th1-type immune responses associated with pulmonary tissue destruction and immune paralysis, which is, in part, shown by down-regulation of the T-cell receptor ζ chain in patients with tuberculosis,[77] which has also been noted in the present study (appendix).

Not only timing of MSC adjunct treatment, but also coinfections might modulate treatment outcomes. Note that we excluded individuals who were positive for HIV, as well as those positive for hepatitis B and C, for safety considerations in the phase 1 trial. *M tuberculosis*–HIV coinfection represents a different immunological scenario[78] and might lead to a different response pattern to MSC treatments.

Future studies are needed to define robust and clinically relevant surrogate markers to identify patients who would benefit from MSC therapy, or to gauge clinical response to MSC-based therapies in patients with infectious diseases. It would be desirable that these markers are measured easily in peripheral blood. However, it could very well be that local factors, elaborated by MSCs in pulmonary tissue, might not be easily traceable in clinical studies. The motivation to offer MSC treatment to patients with tuberculosis was the hope that MSC infusion might lead to enrichment of MSCs in destructive lung tissue curbing unproductive inflammation associated with *M tuberculosis* infection in line with more recent observations that MSCs guard against excessive inflammation and tissue destruction.[79] We have not been able to obtain transbronchial biopsies to study the in-situ situation after MSC infusion. Future studies might address the local in-situ milieu by examining the interaction of MSCs with resident cells.

Although several studies showed that the clinical efficacy of MSCs is not only related to successful expansion and engraftment, clinically relevant factors might rather be local interactions (eg, stimulation of Toll-like receptors might polarise MSCs to immune-suppression or immune-stimulation[80] in situ). The study of MSC-cellular interactions in pulmonary tuberculosis lesions will also allow the investigation of whether MSCs modulate anti-*M tuberculosis* directed immune responses, or whether MSCs also have a role in pulmonary reconstruction—MSCs exhibited therapeutic potential in preclinical models of acute lung injury,[81] as well as endotoxin-induced[82] or bleomycin-induced lung injury,[83] associated with decreased expression of transforming growth factor β1 (responsible for pulmonary fibrosis[84]). During the recruitment phase of the present study, other reports underlined the beneficial effects of MSCs in preclinical infection settings. Stimulation of human MSCs with inflammatory cytokines induced a broad range of antimicrobial effector functions mediated by the tryptophan catabolising enzyme indoleamine 2,3-dioxygenase.[85] MSCs act by reduction of oxidative stress, which was shown to be operational in a murine model of acute coxsackievirus B3-induced myocarditis.[86] Enhanced phagocytosis was, in part, responsible for increased bacterial clearance and improved survival in a murine sepsis model.[87,88] This underlines the need for detailed immunological and molecular in-situ analysis (using biopsies) upon MSC treatment of patients with tuberculosis to formulate a model of (MSC) action in pulmonary tuberculosis.

To conclude, adjunct autologous MSC therapy seems to be safe for patients with MDR or XDR tuberculosis.

***Panel:* Research in context**

Systematic review

We searched PubMed and ProMed websites both on July 15, 2008, and on July 15, 2013, for all relevant English language scientific literature with the terms "adjunct therapy", "immunotherapy", "stem cells", "stromal cells", "drug resistant tuberculosis", and "MDR/XDR-tuberculosis". This included all publications on safety and immune responses to autologous, bone-marrow-derived stromal or stem-cell infusions. Data on poor outcomes of MDR or XDR tuberculosis treatment from eastern Europe and Belarus, and WHO TB Department annual reports and PubMed publications, were also reviewed. There is scant information on adjunct immunotherapies for the treatment of MDR or XDR tuberculosis. Reports in 2008 supported the notion that anti-inflammatory treatment (with corticosteroids) might be helpful in the treatment of tuberculosis; the literature search in 2013 consolidated the notion that anti-inflammatory treatment might improve tuberculosis treatment outcomes. The literature search in 2013 also consolidated the notion that mesenchymal stromal cells (MSCs) might have organ-protective effects in preclinical infectious disease models and MSCs might also show antibacterial properties.

Interpretation

Our findings show that adjunct therapy with autologous bone-marrow-derived MSC transfusion is worth exploring in view of the poor treatment outcomes of MDR and XDR tuberculosis. Adjunct therapy with MSCs was not associated with any serious adverse events or disturbances in haematological and biochemical variables. The data suggest that adjunct cellular therapy is not only safe, it might also help to refocus cellular immune responses facilitating recovery from infection with *Mycobacterium tuberculosis* along with standard drug therapies. Since MSC adjunct therapy is safe, it needs further assessment in controlled phase 2 trials to ascertain the effects on immune responses and clinical and microbiological outcomes in patients with MDR or XDR tuberculosis.

These data are preliminary and a long-term follow-up of patients might be warranted to reveal any negative effects of MSC infusion in patients with tuberculosis. Repeated cycles with autologous MSCs are possible and can be incorporated into future trials. The harvest of bone marrow can easily be achieved and portable MSC expansion units could be employed for MSC procurement and expansion. The procedures for procuring autologous MSCs are relatively simple, and if successful in phase 2, will provide a viable adjunctive therapy for patients who have suffered from longstanding tuberculosis. Future safety trials are also needed at different clinical sites, since the exposure to *M tuberculosis*, the genetic background of the population, as well as the biology of the circulating strain (from the Belarus cohort) might affect the results in the present report. We also report in this study the outcome of individuals who did not receive MSC treatment to gauge the natural course of the disease and to learn how haematological and biochemical variables develop over time after initiation of drug therapy. This cohort cannot be used to compare results since the choice to refuse MSC adjunct therapy might relate to a milder clinical presentation of tuberculosis at the time of study entry. After addressing safety at different clinical sites, a phase 2 clinical trial is warranted, with larger patient numbers and a randomised control arm to assess the beneficial effects of adjunct autologous MSC transfusion therapy on quality and quantity of anti-*M tuberculosis* immune responses, lung-tissue regeneration, *M tuberculosis* clearance rates, and mortality rates in patients with difficult-to-treat MDR or XDR tuberculosis.

Contributors

MJM was lead principal investigator and obtained funding for the study. ASkrahin, RKA, GF, LR, AZ, and MJM designed the study, interpreted the data, and wrote the report. ASkrahina treated the patients, RKA did the ex-vivo immunological analysis, LR did the gene-expression analysis, TP did the statistical analysis, and YI was responsible for MSC procurement. ASkrahin, AZ, and MJM finalised the report. ASkrahin, GF, and MJM had full access to all data in the study and take responsibility for the integrity of the data and accuracy of the data analysis.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

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