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Original Research

Recombinant interleukin-7 treatment of refractory *Mycobacterium avium* complex lung disease (IMPULSE-7): a pilot phase II, single-center, randomized, clinical trial

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Abstract

Background: Nontuberculous mycobacteria disease is an emerging opportunistic infection that is often refractory to therapy. Interleukin 7 (IL-7) is a pleiotropic cytokine with broad-ranging effects that enhance immunity and augment monocyte/macrophage anti-*Mycobacterium avium* killing in vitro.

Objectives: This study evaluated IL-7 in patients with refractory *Mycobacterium avium* complex lung disease (MAC-LD).

Design: Prospective, single-center, randomized, study of IL-7 in patients with refractory MAC-LD.

Methods: Randomization (two sets of 4 weekly IL-7 injections) was stratified based on the presence of pulmonary cavities. The primary outcome was sputum culture conversion to negative within 6 months. Exploratory outcomes included investigation of potential molecular mechanisms of immunosuppression via single-cell RNA sequencing (scRNA-seq).

Results: Of the eight participants enrolled, six completed the IL-7 regimen, one completed one 4-week therapy, and one received a single dose of IL-7. All six participants who completed the regimen showed an increased absolute lymphocyte count (ALC), yet none converted their sputum culture to negative at 6 months. Similarly, there were no differences in secondary outcomes compared to baseline. IL-7 was well tolerated, and two participants showed an increase in time-positivity for MAC in their sputum culture. scRNA-seq revealed increased expression of genes involved in immunosuppressive pathways.

Conclusion: In adults with refractory MAC-LD, IL-7 did not result in sputum culture conversion. IL-7 reversed the underlying lymphopenia associated with MAC-LD and led to a sustained increase in ALC. The study was limited by a small sample size, and although a longer course of IL-7 combined with newer antimicrobials for may warrant further investigation, structural lung disease may be a stronger predictor of cure than immune dysfunction in MAC-LD.

Trial registration: The trial was registered in clinicaltrials.gov (NCT04154826).

Plain language summary

IL-7 for refractory MAC lung disease (IMPULSE-7)

We present the results of a proof-of-concept trial that investigated the use of recombinant IL-7 as an adjuvant host-directed therapy for the treatment of refractory Mycobacterium avium complex lung disease (MAC-LD). Unfortunately, this was a negative study, limited by

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the small number of patients included, as we stopped recruitment early due to the lack of a clinical signal suggesting that IL-7 would be effective in achieving the primary outcome. However, we believe it is important to publish negative studies to help researchers explore alternative pathways in the management of refractory MAC-LD, where therapies to achieve microbiological cure are greatly needed.

Keywords: IL-7, nontuberculous mycobacteria, NTM, pulmonary disease, randomized clinical trial, refractory

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Introduction

Nontuberculous mycobacteria (NTM) are ubiquitous, environmental, opportunistic microorganisms, with most infections acquired by inhalation, microaspiration, or direct inoculation.[1] Once considered rare, a recent meta-analysis found that NTM disease incidence per 100,000 persons per year has been increasing by 4.1% annually, worldwide.[2] NTM lung disease (NTMLD) accounts for >85% of all NTM infections, with two species, *Mycobacterium avium* complex (MAC) and *M. abscessus* complex, which are responsible for 70%–90% of all infections. Current NTMLD treatment guidelines recommend the use of a lengthy, multidrug antimicrobial regimen, with most participants being treated for 18–24 months.[3,4] However, overall treatment success in NTMLD is 30%–50%,[5,6] and a meta-analysis estimated a pooled treatment success rate of 60% for MAC lung disease (MAC-LD).[7] Furthermore, refractory patients (i.e., those with culture positivity after at least 6 months of guideline-based treatment) have very few treatment options, and 5-year mortality for NTMLD patients is approximately 40%, independent of the receipt of antimicrobial therapy.[4,8,9]

MAC-LD frequently infects individuals who are of advanced age, have significant comorbidities, and/or are malnourished; clinical characteristics that are associated with impaired immunity.[10] Studies of immune effector cells from participants with MAC-LD have demonstrated that there is increased expression of the programmed cell death protein 1 (PD-1), a T cell inhibitor receptor that mediates T cell exhaustion. Other immunosuppressive mechanisms that have been identified in patients with MAC-LD include an increase in T regulatory cells and myeloid-derived suppressor cells.[10] In addition to phenotypic changes in immune suppression, CD4 and CD8 T cells from patients with MAC-LD have functional impairment, as evidenced by decreased production of key cytokines, including IFN-γ and TNF-α, which are thought to be essential in controlling mycobacterial pathogens.[11] Not only is there a decrease in lymphocyte function but lymphopenia has also been associated with an increased risk of redeveloping MAC-LD after antimicrobial treatment completion.[12,13] Collectively, these findings support that there are numerous defects impairing the host response in patients with MAC-LD which may contribute to the morbidity and mortality of this disease.

Interleukin 7 (IL-7) has been termed the "maestro" of the immune system because of its stimulatory effects on various immune cells.[14] IL-7 is a pleiotropic cytokine that is a growth factor for CD4 and CD8 T cells but also indirectly increases neutrophil and monocyte function and recruitment to sites of infection. Importantly, IL-7 has shown to improve survival in animal models of bacterial, fungal, and mycobacterial infections.[15–17] Furthermore, in a multi-center, phase II, placebo-controlled trial of IL-7 in participants with septic shock, IL-7 was well-tolerated and caused a 3–4-fold increase in circulating lymphocytes with improved adaptive immunity.[18] Studies have shown that many of the genes that are associated with improved survival in participants with NTMLD are related to T cell function.[19] Tantawichien et al demonstrated that IL-7 induced anti-*M. avium* activity in human monocyte-derived macrophage with up to a 50% reduction in bacteria.[20] Similarly, Terrazzini and associates reported that IL-7 unveiled and supported re-activation of pathogen-specific T cells with possible diagnostic, prognostic, and therapeutic significance, of clinical

value especially in conditions of pathogen persistence and chronic infection like mycobacterial infections.[21]

Given the low success rates of current treatments, elevated mortality rates, and role of defective immunity in MAC-LD, we hypothesized that a strategy to improve host immunity using IL-7 may be advantageous in patients with MAC-LD. We performed a single-center, randomized clinical trial of IL-7 to ascertain the safety, efficacy, and tolerability of two dose regimens of recombinant human IL-7 (CYT-107) in patients with refractory MAC-LD. Additionally, we performed scRNA-seq on three patient samples to investigate the underlying molecular mechanisms of immune suppression in patients with MAC-LD.

Methods

Trial design and oversight

This was a prospective, single-center, randomized, phase II, single-blinded, two-dose level trial aimed at testing the anti-mycobacterial activity of IL-7 in participants with refractory MAC-LD (i.e., persistently positive sputum cultures after ⩾6 months of guideline-based treatment with a multidrug regimen containing a macrolide and at least one other antimicrobial agent with activity against MAC).

The study was performed at Washington University School of Medicine and registered at clinicaltrials.gov (NCT04154826). The study was conducted and reported in accordance with the CONSORT statement (Supplemental Material).[22] The study was approved by Washington University's Institutional Review Board and conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Council for Harmonization Guidance for Good Clinical Practice. All participants provided written informed consent prior to their inclusion in the trial.

Participants and procedures

Eligible participants were enrolled in this study between January 2020 and September 2022. Participants had to be 18–85 years of age, have refractory MAC-LD including a positive sputum culture within 2 months of enrollment, be on stable guideline-based therapy (i.e., unchanged in the last 28 days), be able to produce sputum, and/or be willing to undergo sputum induction. Refractory MAC-LD was defined as having sputum cultures persistently positive despite being on guideline-based therapy, including the use of amikacin liposomal inhalation suspension (ALIS). The estimated sample size was based on the assumption that the intervention would achieve sputum conversion at 6 months in 29% of the participants[23] and that without any intervention, culture conversion was going to be achieved in ⩽5% of participants, given that the study enrolled patients with refractory MAC-LD who had not achieved culture conversion to negative despite receiving treatment with ALIS or where not suitable to such treatment.

Eligible participants were randomized to study drug treatment and allocated in a 1:1 ratio to either 10 or 20 μg/kg/week ideal body weight for two 4-week treatment periods. IL-7 was administered intramuscularly. Randomization was stratified based on the presence of pulmonary cavities (a maximum of three participants with pulmonary cavitary disease were allocated to each dose group), and randomization was assigned by the REDCap Cloud database following eCRF data entry by the research team. The Investigative Pharmacist received a system-generated email notification from REDCap Cloud to indicate the stratified randomization assignment. The IL-7 dosing regimens were chosen based on their ability to provide good T cell recovery in various populations of lymphopenic patients, and upon the half-life of CYT-107 as determined in other clinical studies.[17,24,25]

Endpoints

The primary endpoint was the proportion of subjects with sputum culture conversion to negative at 6 months. Secondary endpoints included changes in the 6 minute walk test, forced expiratory volume (FEV1), ratio of FEV1 to forced vital capacity (FVC), changes in chest computed tomography (CT) scans using the Timika score, assessment of Health-related Quality of Life (HRQoL) using the participant-reported outcomes measurement information system (PROMIS-29), immunogenicity, and tolerance of IL-7. Additional endpoints included the effect of IL-7 on immune cell counts (absolute

lymphocyte, monocyte, and neutrophil counts). Midway through the study enrollment, we amended the protocol to perform single-cell RNA sequencing with the aim of gaining insights into the potential immune suppression mechanisms in patients with IL-7.

Single-cell RNA sequencing

To gain mechanistic insights into immune suppression mechanisms occurring in MAC-LD patients, single-cell suspensions from three MAC-LD patients were collected prior to the initiation of IL-7 therapy, and the resulting dataset was compared to peripheral blood mononuclear cells (PBMC) from control subjects. Blood samples were collected from three patients prior to the initiation of IL-7 therapy. Single-cell suspensions were prepared prior to processing for scRNA-seq via red blood cell lysis (10x RBC Lysis Buffer, BioLegend, San Diego, CA, USA). The cells were sequenced using the 10X Genomics platform with chemistry version 3. The CellRanger pipeline (version 7.0.0., 10X Genomics) was used to process Chromium single-cell RNA-seq output to align reads to the GRCh38 reference genome and generate gene-cell expression matrices. The uniquely aligned reads were used to quantify gene expression levels for all *Ensembl* genes. The controls were chosen to have a similar age distribution (specific ages of controls: 49, 50, 51, 52, 53, 55, 56, 57, 58, 59, 60, 61, 65, 72, 73, 74) and a similar sex ratio (10 females:5 males); they were nonsmokers without a history of cancer, chronic inflammatory conditions (arthritis, Crohn's disease, colitis, dermatitis, fibromyalgia, or lupus), or blood-borne infections (HIV, hepatitis B, and C).

Downstream analyses were performed using Seurat R software package version 4.0 (http://satijalab.org/seurat/). Low-quality cells were filtered from the dataset if the number of genes detected was <500 or >3500, or the percentage of mitochondrion reads was $>5\%$. The control samples used in this analysis were obtained from a previously published dataset, in which TCR genes were regressed to avoid donor-specific TCR rearrangement confounders.[26] After removing unwanted cells and regressing out mitochondrial genes from the dataset, raw UMI counts in each cell were both scaled and normalized using the *SCTransform* algorithm. The control data used for this analysis were previously published.[26] Highly variable genes were identified and selected for PCA reduction of high-dimensional data. Cells in this reduced space were harmonized to adjust for batch effects coming from the two datasets and from different donors using the *Harmony* tool implemented in Seurat v4. These low-dimensional corrected Harmony embeddings were used for downstream analyses. Graph-based clustering was performed on the reduced data for clustering analysis using Seurat v4. The resolution in the *FindClusters* function in Seurat was set to 0.9 and the clustering results were shown in a UMAP plot. For different cell types, cells were annotated based on top markers and also to by comparing them to the annotation in the control samples. For each cluster, DEGs were generated relative to cells in all other clusters via the *FindMarkers* function, where genes were considered DEGs if they were present in at least 10% of cells and if they were expressed at a higher than 2.5 log Fold Change (FC) in that cluster as compared to other clusters. Heatmaps of individual cells were plotted using the *DoHeatmap* function from Seurat by selecting the top 10 DEGs in each cluster. Genes that were discarded during the *SCTransform* process were not represented in those heatmaps. Given that our prespecified an interim evaluation when data led to early termination of the study due to lack of efficacy, we only performed these analyses in participants 7–9.

Statistical analysis

Descriptive statistics for baseline characteristics and study outcomes are presented in aggregate and stratified by treatment arm and separately for the intention-to-treat (ITT) and per-protocol datasets. Continuous variables were summarized using median and interquartile range (IQR), and comparisons were made using Wilcoxon signed-rank test for related samples. Categorical variables are summarized as numbers and proportions. All patients who received $\geqslant 1$ injection were included in the safety analysis. An interim evaluation by an independent clinical medical monitor, an expert with experience in the management of NTMLD and not involved in the conduct of the trial as an investigator, was planned to assess IL-7 effect on the primary and secondary endpoints when half of the anticipated 12 participants were eligible for analysis of the primary endpoint. All statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and p values < 0.05 were considered significant.

Results

Participants

Ten subjects were screened and eight were enrolled between December 2020 and November 2022 (Table 1). All participants were Caucasian, with a median age of 60 years (interquartile range (IQR), 55–65), and 7 (87.5%) were female. The most common chest tomography findings were bronchiectasis (100%) and tree-in-bud nodularities (87.5%), and three participants (37%) had cavitary disease. Two participants with cavitary disease were randomized to low-dose IL-7. At baseline, the participant's median (IQR) duration of MAC-LD therapy prior to enrollment was 3.4 years (1.9–4). All participants were on a macrolide-based multidrug regimen at baseline; two also were on ALIS, and four had been previously treated with ALIS amikacin liposomal inhalation suspension for >6 months and failed to clear their infection. Participants who previously received amikacin liposomal inhalation suspension discontinued it due to toxicity. Two participants had amikacin resistance, and two had macrolide resistance at enrollment. Other than the antimicrobial therapy received, all participants had optimized management of their underlying lung disease by their standard of care pulmonologist. None of the participants had cancer (history or active) nor known underlying immunosuppression.

Clinical outcomes

Four participants (50%) were randomized to receive high-dose IL-7. Six (75%) participants completed the full treatment cycle, and two participants (25%) received partial treatment cycles once the results of the interim evaluation became available. Of the six participants who completed the full treatment, all had an increase in the absolute lymphocyte count (ALC), but none converted their sputum culture to negative at month 6. Although none of the participants cleared their sputum culture, two participants showed an increase in the number of days it took for the sputum culture to become positive for MAC during the IL-7 treatment cycles, one in the high-dose and one in the low-dose arm (Figure 1; IMP3 and IMP6, respectively). One participant in the low-dose arm reported a decrease in daily sputum production. The study was terminated prematurely because of the lack of efficacy observed in the predetermined interim analysis.

Compared to baseline, there was no improvement in the median 6-minute walk test (364 vs 433 m; $p = 0.14$), median FEV1 (1.68 vs 1.48 L, $p = 0.49$), and the proportion of change of the FEV1/FVC (76 vs 68%; $p = 0.34$). The median score on any of the PROMIS-29 domains (depression, anxiety, physical function, pain Interference, fatigue, sleep disturbance, and ability to participate in social roles and activities) at month 6 was also not significantly different compared to baseline. The most common chest tomography findings were bronchiectasis (100%) and tree-in-bud nodularities (87.5%), and in the five participants with chest CT available at 6 months, the median (IQR) Timika score at baseline (45, 40–61.25) was not significantly different at 6-months (55, 40–55; $p = 1.0$) (Table 2).

IL-7 was well tolerated, and none of the participants discontinued the study drug because of adverse events. Injection-site reaction (grade 1–2) occurred in all patients and manifested as a non-tender, non-pruritic, erythematous patch that resolved within 72–96 h without any specific intervention. One patient developed myalgias and low-grade fever approximately 6–8 h after each injection of IL-7. These symptoms were ameliorated by pretreatment with acetaminophen and/or ibuprofen. Five patients developed low-titer binding antibodies that were non-neutralizing.

Results of single-cell RNA sequencing

The control subjects had similar age and sex ratios to those of MAC-LD patients. Large-scale analyses revealed that the largest contributors to the MAC-LD phenotype were $CD4^+$ T cells and monocytes. To understand the drivers of these differences, we extracted $CD4^+$ T cells from the combined dataset (Figure 2(a)). CD4 T cells were defined in the independent datasets first and then as high-quality cells expressing CD3E and CD4 and lacking expression of CD8A. We observed that MAC-LD patients had enrichment of the following CD4 T cell clusters: FOXP3+ regulatory T cells (Tregs), BACH2+ CD4 T cells, KLRB1+MAF+ CD4 T cells, a subset of KCNJ15-expressing CD4 T cells, and KLRB1+RORA+ T cells (Figure 2(b) and (c)). They had a significant reduction in subsets of

Table 1. Clinical characteristics of participants with refractory* *Mycobacterium avium* complex pulmonary disease at the time of enrollment.

Age in years and sex	Years living with MAC-PD prior to enrollment	Prior antimicrobials received	Duration of prior therapy in months	Number of AFB sputum cultures in the prior year		MAC drug susceptibility (MIC in μg/ml, interpretation)	Companion antimicrobials with IL-7	Body mass index (kg/m²)	Radiographic features
				+	–				
69 F	11	Azithromycin Ethambutol Rifampin Clofazimine	20	2	0	CLR: 4, S AMK >64, R ALIS >64, R	Azithromycin Ethambutol Rifampin	21.1	Cavitary
71 F	3	Azithromycin Ethambutol Rifampin ALIS	36	8	1	CLR: 1, S AMK >256, R ALIS >256, R	ALIS Azithromycin Ethambutol Rifampin	18.4	Nodular
70 F	5	Clarithromycin Azithromycin Rifampin Ethambutol ALIS	44	9	0	CLR: 4, S AMK: 32, I ALIS: 32, S	Azithromycin Rifampin	21.6	Nodular
65 F	4	Azithromycin Ethambutol Rifampin	44	5	4	CLR: 0.5, S AMK: 4, S ALIS: 4, S	Azithromycin Ethambutol Rifampin	20.5	Nodular
81 F	3	Azithromycin Amikacin IV Ethambutol Rifampin	18	5	0	CLR: 2, S AMK: 32, I ALIS: 32, S	Azithromycin Ethambutol Rifampin	23.1	Cavitary
45 M	1	Azithromycin Ethambutol Rifampin ALIS Omadacycline	12	4	1	CLR >64, R AMK >64, R ALIS >64, R	Azithromycin Ethambutol Omadacycline Rifampin	26.3	Nodular
71 F	1	Azithromycin Clofazimine Ethambutol Rifabutin	14	5	0	CLR >64, R AMK >64, R ALIS >64, R	Azithromycin Ethambutol Rifabutin ALIS	22.2	Nodular
50 F	5	Azithromycin Ethambutol Rifampin	14	5	0	CLR >64, R AMK >64, R ALIS >64, R	Azithromycin Ethambutol Rifampin	24	Cavitary

*As defined by the Non-tuberculous Mycobacteria Network European Trials group (NTM-NET) consensus statement.[9]
AFB, acid-fast bacilli; ALIS, Amikacin liposomal inhaled suspension; AMK, Amikacin; CLR, Clarithromycin; F, female; I, intermediate; IL-7, interleukin 7; M, male; MAC, *Mycobacterium avium* complex; MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

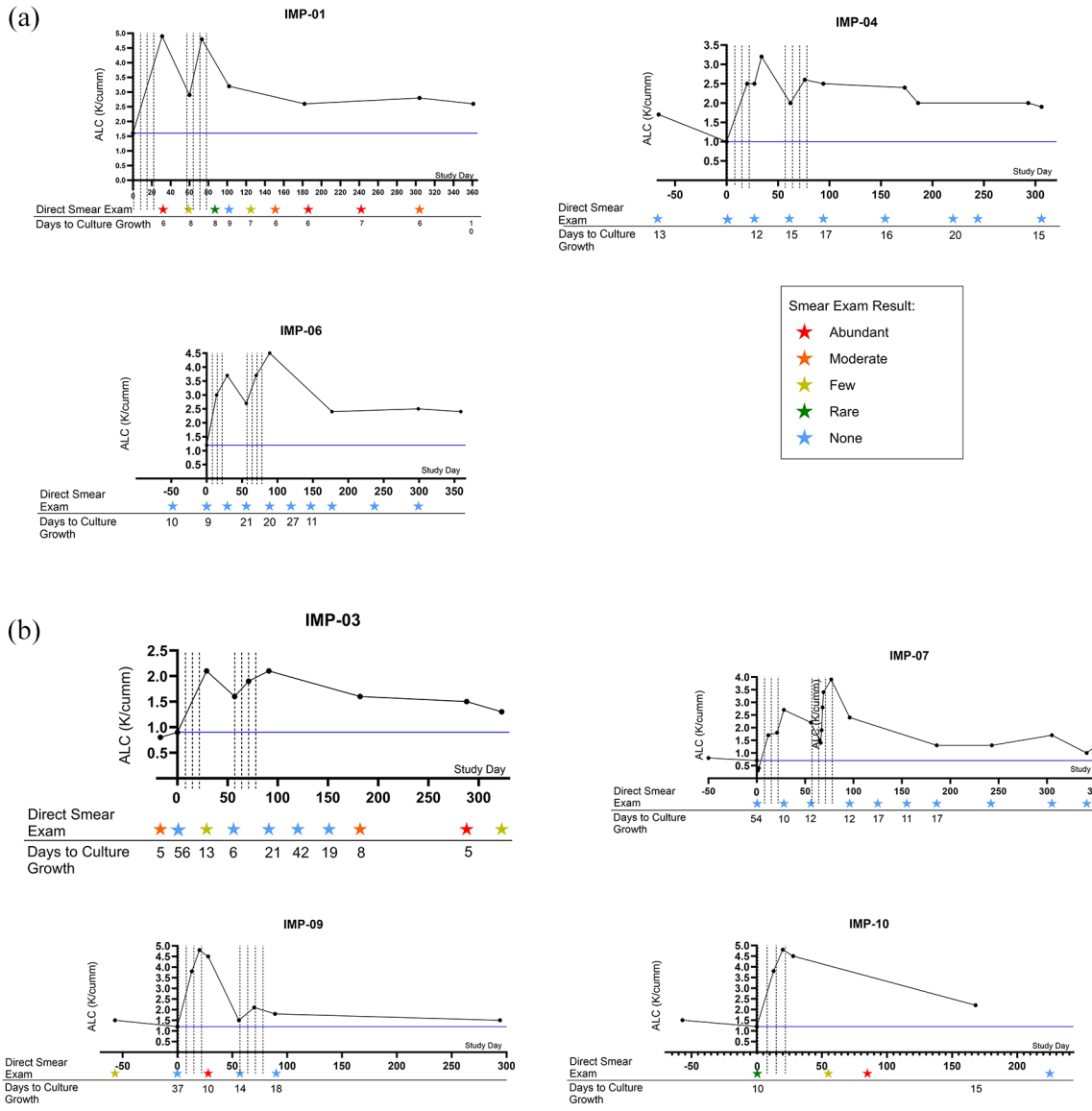

Figure 1. Effect of IL-7 on sputum culture, sputum immunohistochemical staining, and ALCs in participants with refractory *Mycobacterium avium* complex lung infection. ALC counts are depicted for each patient over the course of the trial plus prior to trial initiation as available. IL-7 injections are denoted by vertical hashed lines and occurred at days 1, 8, 15, 21, 57, 64, 71, and 78. Blue lines indicate day 0, the day of the first IL-7 infection. Absolute lymphocyte counts were obtained from blood drawn within 1–3 h prior to the first injection of IL-7. Stars (*) indicate direct smear exam results, and placement corresponds to the numerical axis (study day). Days to culture growth indicated the number of days required before the culture became positive for MAC, and numbers corresponded to the dates when the samples for the cultures were collected from the participants. Participants under panel a were randomized to low-dose IL-7 (10 μg/kg/week) and those on panel b received high-dose IL-7 (20 μg/kg/week).
ALC, absolute lymphocyte count; MAC, *Mycobacterium avium* complex.

CD4 T cells marked by productive rearrangement of the β chain TRBV20-1 and TRBV5-1 and in Th1 cells, particularly in EOMES+ Th1 cells (Figure 2(b) and (c)). The differentially expressed genes (DEGs) between MAC-LD and healthy CD4+ T cells included BCL2, a pro-survival molecule, and B2M, which is a feature of activation (Figure 2(d)). Signature genes for Treg cells, such as FOXP3 and CTLA4, were increased in MAC-LD patients, suggesting an immunosuppressive phenotype. An EOMES+ Th1 subset was specifically

Table 2. Clinical outcomes of individuals with refractory *Mycobacterial avium* complex lung disease at baseline compared to 6 months after treatment with IL-7.

Participant age and sex	IL-7 low versus high dose	Culture status at 6 mo	Adverse events (Grade*)	Discontinuation of IL-7 due to toxicity	6MWT (m)		FEV1 in L		FEV1/FVC (%)		Timika score	
					Baseline	6 mo	Baseline	6 mo	Baseline	6 mo	Baseline	6 mo
69 F	Low	Positive	Site injection rash (2)	No	457	472	2.03	1.85	75	74	55	55
71 F	High	Positive	Site injection rash (2)	No	387	433	1.62	1.66	77	83	40	55
70 F	Low	Positive	Site injection rash (2)	No	433	451	0.98	0.99	80	76	40	40
65 F	Low	Positive	Site injection rash (3)	No	320	380	1.74	1.78	69	71	15	15
81 F	High	Positive	Site injection rash (2)	No	341	308	1.15	1.39	56	60	80	65
45 M	High	NA	Site injection rash (2)	No								
71 F	High	NA	Site injection rash (2)	No								
50 F	Low	NA	Site injection rash (2)	No								

*Adverse events are graded on a scale from 1 to 5 per Common Toxicity Criteria for Adverse Events (CTCAE).
6MWT, 6 minute walking test; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; mo, months.

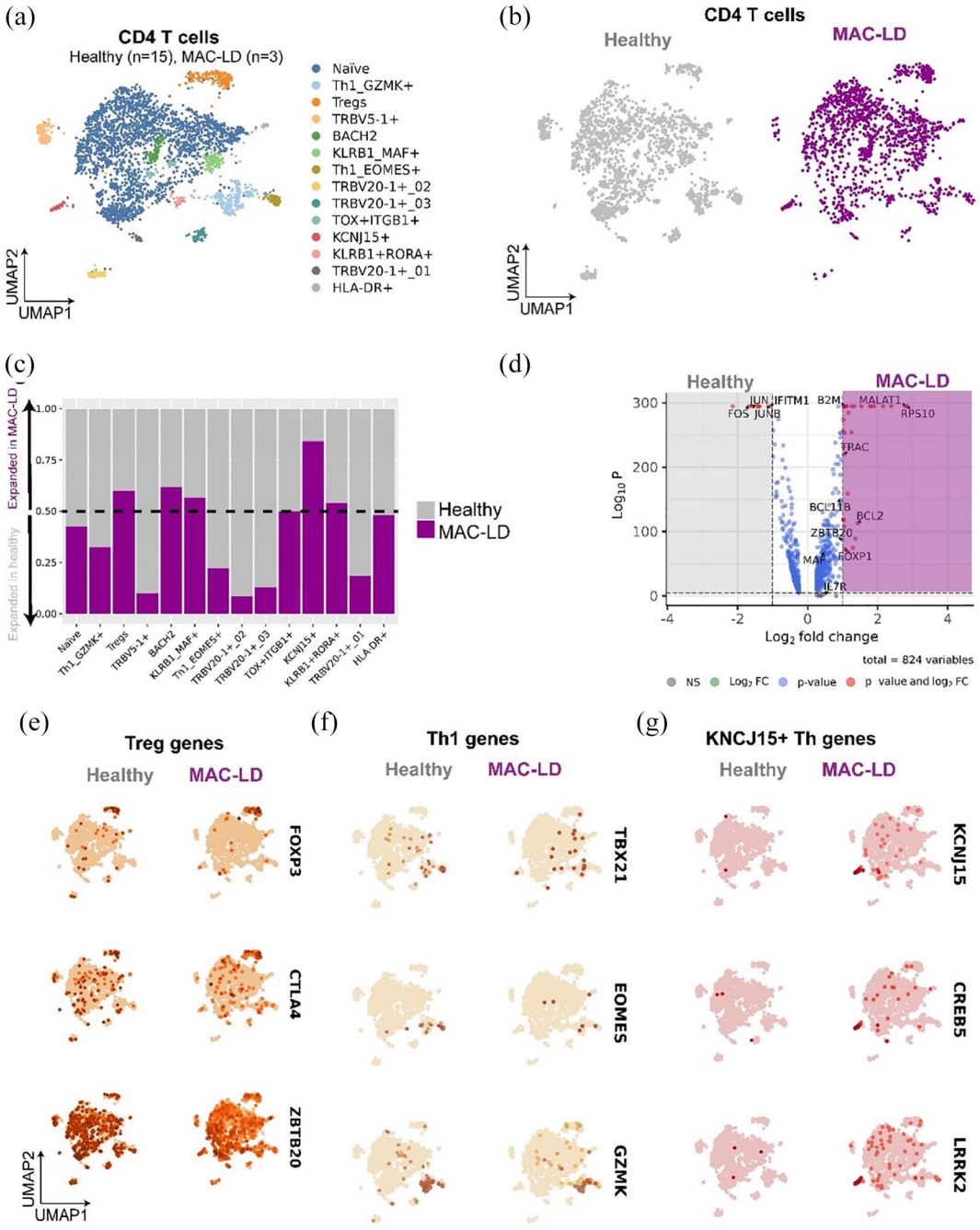

Figure 2. CD4+ Th cells from patients with MAC-LD have immunosuppressive signatures. (a) Uniform manifold approximation and projection (UMAP) plot of CD4+ T cells from MAC-LD patients at baseline ($n = 3$) and healthy controls ($n = 15$). The healthy control group consisted of 10 females and 5 males aged 49–74 years.[26] Cells are colored according to the Seurat cluster to which they belong. Clusters were annotated manually based on marker features that passed statistical significance. Finally, inter-individual variation was minimized by running the "Harmony" algorithm, regressing donor differences as a variable. (b) Cells from (a) split by health status. (c) The bar graph represents the distribution of each cell type from (a) across health statuses. The discontinuous line crosses 50%, representing a hypothetical 50%–50% contribution. (d) Volcano plot of genes that were differentially expressed in MAC-LD CD4+ T cells versus in healthy. (e) Gene expression plots of marker genes of Treg cells in healthy (left) versus MAC-LD (right) cells. (f) Representative genes of Th1 cells in healthy (left) versus MAC-LD (right) cells. (g) Representative genes of KNCJ15 cells in healthy (left) versus MAC-LD (right) cells. In (e) to (g), gene names are represented on the right of the gene expression plot.
MAC-LD, *Mycobacterium avium* complex lung disease.

depleted in MAC-LD patients (Figure 2(f)). Finally, MAC-LD patients had an enrichment in KCNJ15+ T cells, whose signature included molecules like CREB5 and LRRK2 (Figure 2(g)).

Discussion

In patients with refractory MAC-LD, the addition of IL-7 to background antimicrobial therapy did not result in sputum conversion to negative at month 6. The secondary outcomes of pulmonary function and radiological changes were also unchanged with IL-7 therapy. IL-7 did not have an impact on the participants' quality of life, although at least one participant reported a decrease in daily sputum production. Although the observed longer time to growth on mycobacterial cultures in two participants after IL-7 therapy suggests that IL-7 may have decreased the MAC burden in the lungs, it did not eradicate the infection. Furthermore, this change was not sustained over time, and the findings might have been due to interpersonal variability in sample collection. IL-7 immunotherapy was safe and well tolerated, without any major adverse effects. IL-7 was effective in increasing ALC. Intriguingly, four participants had baseline absolute lymphocyte counts (ALC) (prior to initiation of IL-7) at or below the lower limit of normal reported for most clinical laboratories (i.e., 1.0×10^3 lymphocytes/μL). This finding of a low baseline ALC in patients with NTM-LD is consistent with previous studies of patients with NTM-LD.[13]

As reported in other clinical trials using IL-7, the increase in the ALC in participants with MAC-LD persisted for many months after cessation of IL-7 administration.[27,28] IL-7 increases memory CD4 and CD8 T cells, which are long-lived and thus likely explain the sustained elevation.[13] Interestingly, a growing number of studies have reported a correlation between ALC and mortality due to infectious diseases.[29,30] Zidar et al. examined the association between low lymphocyte levels and survival in 31,178 participants in the general population who were tracked for 11 years. The results showed that individuals who had an ALC $\leqslant$ 1000/μL had a seven-fold increased risk of death due to pneumonia or influenza compared to individuals without lymphopenia. Warny et al. prospectively followed 98,344 individuals, and those who had an ALC less than 1100/μL had a 1.70 hazard ratio of dying from infection compared to individuals with normal ALC. In future studies, it would be interesting to determine the impact on incident infections in patients treated with IL-7 who have a sustained increase in their ALC.

ScRNA-seq analysis showed several potential mechanisms of T cell immune suppression. First, there was a relative increase in the number of T regulatory cells (Tregs) in patients with MAC-LD compared to age-matched control patients (Figure 2). Tregs are potent suppressors of immunity by inhibiting activation and expansion of CD4 helper T cells and of cytotoxic CD8 T cells. A second potential mechanism involves BACH2+ CD4 T cells; BACH2 is a transcriptional regulator that seems to inhibit T cell effector functions, and its mutations are associated with several diseases such as multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and others.[31] Additionally, there was an increase in Eomes, a T-box transcription factor that is thought to induce CD8 T cell exhaustion. The EOMES+ cluster also expressed granzyme K (GZMK) which is also associated with aging and exhaustion.[32]

KLRB1 is a marker of memory, and the KLRB1+ clusters are increased only mildly compared to the robust increase in other CD4+ subsets. This may indicate a memory-like response in patients under chronic stimulation. Thus, the clusters marked by TRBV20 are likely donor-specific and likely do not represent biological contribution. Additionally, because KCNJ15 has not been previously reported to be associated with T cells, there is a possibility that the KCNJ15+ population of 'T cells' represents cells that have been engulfed by phagocytes and display signatures of both T cells and monocytes.

Attempts were also made to perform scRNA-seq analysis on samples acquired within 3 h of IL-7 injection to investigate the cellular impact of treatment on T cells in the periphery; however, the number of T cells in the periphery drops precipitously following IL-7 treatment. This drop has been described in prior interventional trials, but it was not clear how quickly this happened.

This study has several limitations. First, the early termination and small number of participants

available for analysis might have underpowered the planned analyses. Furthermore, the assumptions made to justify the sample size calculation might have been fallacious. However, the early termination due to futility was in line with the pre-specified stopping criteria as determined by a data safety monitoring board. Second, the lack of a priori complete immunological assessment in all participants might have resulted in a population in which immunomodulatory therapy could have had limited efficacy. Although it is not possible to know exactly why IL-7 failed to lead to the clearance of MAC, there are several possible explanations. Refractory MAC-LD is a particularly challenging disease, as patients are already receiving a failing antimicrobial regimen, with limited availability of effective alternative treatment options. In our study, some patients had macrolide or amikacin resistance at the time of enrollment, which are the two most active antimicrobials currently available to treat MAC-LD. *M. avium* complex is also thought to inactivate the macrophage phagolysosome, the organelle that is directly responsible for their killing, by inhibiting the fusion between phagosomes and endosomal/lysosomal organelles. Although IL-7 induces CD4 and CD8 lymphocytes to produce IFN-γ, a potent activator of macrophages, it is possible that despite IL-7-induced macrophage activation, mycobacteria were still able to inhibit phagolysosome fusion and avoid their elimination by macrophages. Furthermore, the underlying structural lung disease and ongoing environmental exposure might contribute to the inability to achieve microbiological eradication in these patients.

Conclusion

The addition of IL-7 to standard background antimicrobial therapy in adults with refractory MAC-LD failed to lead to clearance of sputum at 6 months. IL-7 was effective in reversing the low baseline lymphocyte counts in most study participants, and this ALC increase persisted for months after drug cessation. However, the study was limited by the small sample size. At least one trial has been performed in NTM-LD patients using another immune-modulatory agent, that is, inhaled granulocyte monocyte-colony stimulating factor (GM-CSF).[33] In that study, full conversion was achieved in 25% of patients with treatment-refractory NTM-LD (seven participants with MAC, and one with *Mycobacterium* abscessus). Additionally, new candidate therapies such as epetraborole and bedaquiline are currently undergoing clinical trials in patients with refractory MAC-LD (NCT05327803, NCT04630145),[34,35] and it is possible that the addition of IL-7 may augment their efficacy.

Declarations

Ethics approval and consent to participate

The study received ethical approval from Washington University in St Louis IRB (approval ID #202001079). Written informed consent for inclusion in this trial was obtained from the participants prior to randomization.

Consent for publication

Written informed consent was obtained from the participants at the time of enrollment.

Author contributions

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Competing interests
The authors declare no conflicts of interest. M. Morre works for Revimmune.

Availability of data and materials

The data that support the findings of this study are not publicly available due to restrictions outlined in consent agreements with participants and the identifying nature of the data. Data can be made available upon reasonable request and in line with the consent agreed with participants, by contacting the authors.

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Supplemental material
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