the radiology department. This, arguably, limits the optimization of respiratory support during neonatal intensive care with existing monitoring tools focusing on ventilation alone. A further strength of this case report is the lack of any adjunctive respiratory support during the measurements.

EIT demonstrated an almost complete lack of tidal ventilation in the left lung. EIT has been repeatedly shown to be able to differentiate single-lung ventilation in animal and human reports (5). Interestingly, EIT detected some residual tidal ventilation that clinicians would not have seen without the EIT recordings. The pulmonary artery infarct likely occurred after the pseudoglandular period and airway development. The residual ventilation likely represents bronchial tidal air movement and collateral ventilation via pores of Kohn. This suggests that EIT is able to image complex ventilation patterns within the lung at a resolution not currently possible within a bedside tool. EIT holds potential in monitoring ventilation and perfusion development in conditions characterized by unilateral lung differences and altered pulmonary blood flow, such as congenital diaphragmatic hernia and after cardiac surgery, over longer time periods.

To date, the use of EIT to measure lung perfusion has been limited to human observations in which the perfusion state was not known (2) or animal models with selective occlusion of a single pulmonary artery and injection of an electrical impedance contrast agent (3). Our data support the preclinical data demonstrating the ability of EIT to selectively measure lung perfusion. The clear differentiation between the heartbeat-related signals within the left and right lung suggests that the cardiac-domain impedance measures within the anatomical regions of the chest containing lung are not exclusively due to heart movement being transmitted through lung regions.

Conclusions. This case report describes the first report of EIT to measure perfusion and ventilation patterns in a newborn with a known loss of both in a single lung. EIT was able to demonstrate the perfusion and ventilation defects found on static radiological imaging. Importantly, EIT recordings within the heart-rate domain were independent of heart movement and at a respiratory resolution able to identify small areas of tidal ventilation. This suggests that EIT may hold promise as the first tool able to dynamically measure ventilation-perfusion matching over time in infants. ■

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Pharmacologic Exhaustion of Suppressor Cells with Tasquinimod Enhances Bacterial Clearance during Tuberculosis

To the Editor:

Suppressor cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), are important components of the immune response generated against tuberculosis (TB).

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Figure 1. Tasquinimod (TSQ) treatment decreases tuberculosis disease progression and depletes regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) in lungs and spleens of *Mycobacterium tuberculosis*-infected mice. 129S2 mice were infected with *M. tuberculosis* H37Rv by aerosol route to achieve a lung implantation of $2.4 \log_{10}$ cfu 1 day after infection. A group of mice was treated with TSQ (10 mg/kg oral) daily for 7 d/wk starting from Day 1. (A) Lungs were homogenized, diluted, and plated for cfu counts at indicated time points. (B) Single-cell suspensions from lungs and spleens of infected mice 21 days after treatment were analyzed by flow cytometry for frequency of $CD4^+CD25^+FoxP3^+$ Tregs, $CD11b^+Gr1^{HI}$ MDSCs, $CD124^+$ -expressing MDSCs, and $CD8^+$ T cells. ****** $P < 0.01$.

Tasquinimod (TSQ), a novel antitumor compound currently being developed to treat various cancer forms, is a small molecule that restricts suppressive cell frequency in the tumor microenvironment (1). We sought to determine whether TSQ could be used as host-directed immunotherapy to treat

tuberculosis by transiently depleting MDSCs and related cellular populations and thereby serve as a potential adjunctive agent to shorten TB treatment duration.

We evaluated the effect of TSQ in an acute mouse model of TB infection and analyzed the cellular composition and bacterial

Figure 2. Tasquinimod (TSQ) treatment potentiates standard tuberculosis chemotherapy. Groups of mice (five mice per time point) were infected with $2.8 \log_{10}$ units of *Mycobacterium tuberculosis* H37Rv and treated with rifampin (R; 10 mg/kg), isoniazid (H; 10 mg/kg), and pyrazinamide (Z; 150 mg/kg), with or without TSQ (10 mg/kg oral) from Day 14, daily for 5 d/wk. (A and B) The lungs (A) and spleens (B) were homogenized, diluted, and plated for cfu counts at Day 21. $^{**}P < 0.01$.

burden in organs. Briefly, 129S2 (129S2/SvPasCrl) mice (Charles River Laboratory) were infected through aerosol route using *Mycobacterium tuberculosis* H37Rv as previously described (2). Animals were treated with 10 mg/kg TSQ daily, as reported earlier (3). Mouse organs were homogenized, diluted, and plated for colony-forming units (cfus). For flow cytometry, organs were harvested on Day 21, and single-cell suspensions were stained with CD4, CD8, CD25, CD124, FoxP3, CD11b, and Gr1 fluorescently labeled antibodies. The data were analyzed using BD FACSDiva (BD Biosciences) software. All experiments were in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* (NIH). Lung cfu counts were $\log_{10}$ transformed before analysis. Data, presented as mean $\pm$ SEM, were evaluated using Student's *t* test.

Results

Tasquinimod monotherapy enhances mycobacterial clearance in the mouse model. To evaluate the effect of TSQ treatment during tuberculosis, we quantified cfus at 1, 14, and 21 days after infection and TSQ treatment in lungs and spleens. Lung cfu counts in the TSQ-treated group were 0.67 and 0.76 log units lower than the untreated control at Days 7 and 21, respectively ($P < 0.001$) (Figure 1A). At Day 21, the spleen burden for untreated animals was $4.59 (\pm 0.06)$ log units, whereas TSQ-treated spleens were 0.55 log units lower than untreated controls. These data demonstrate that TSQ treatment is successful in reducing *M. tuberculosis* burden in the infected organs.

Adjunctive tasquinimod treatment decreases Treg and MDSC frequencies during TB infection. To better understand the cellular populations involved in the effects of TSQ treatment in TB-infected animals, we analyzed the number of Tregs and MDSCs in treated lungs and spleens. The percentages of $CD4^{+}CD25^{+}FoxP3^{+}$ cells (Tregs) in lungs at Day 21 were found to be 4.9% for the untreated animals and 3.2% for the TSQ-treated group ($P < 0.01$), indicating a significant decrease of Tregs after treatment (Figure 1B).

Next, we investigated the numbers of MDSCs in lungs and spleens of untreated or TSQ-treated infected mice. The

percentages of $CD11b^{+}Gr1^{Hi}$ cells of total leukocytes in lungs at Day 21 were found to be 12.0% for the untreated animals and 6.2% for the treated group ($P = 0.005$), indicating a significant decrease in MDSCs in lungs after TSQ treatment (Figure 1B). We observed similar reduction in splenic MDSC frequencies.

It has been recently demonstrated that excessive MDSC accumulation in lungs correlated with elevated surface expression of CD124 (IL-4R α) on MDSCs during TB infection (4). We determined the frequencies of MDSCs expressing surface CD124 and found that the subset frequencies were reduced from 14.2% in untreated controls to 5.7% in TSQ-treated lungs on Day 21 (Figure 1B). To determine whether the observed inhibition of bacterial growth by TSQ treatment was associated with improved immune responses, we examined $CD8^{+}$ T cells from lungs of the treated animals. We observed an increase in $CD8^{+}$ T-cell frequencies from 3.4% in untreated infected animals to 5.4% in TSQ-treated animals on Day 21 ($P = 0.07$), indicating induction of effector T cells associated with TSQ immunotherapy (Figure 1B).

Tasquinimod enhances antimycobacterial activity of standard TB treatment. To determine the potential of TSQ as an adjunctive therapeutic when given with standard TB treatment, we administered the drugs rifampin (R), isoniazid (H), and pyrazinamide (Z) in the presence and absence of TSQ treatment. An additional group of mice received TSQ monotherapy. Mice were infected with *M. tuberculosis* H37Rv and treated daily with RHZ with or without TSQ, starting on Day 14 after infection. In mouse lungs (Figure 2A), standard RHZ treatment yielded a $1.91 \log_{10}$ unit cfu decrease at day 21 compared with the untreated controls, whereas the RHZ-plus-TSQ group gave quantitative cfu counts that were $2.24 \log_{10}$ units lower ($P = 0.003$). We observed comparable effects of TSQ addition on spleen burden (Figure 2B, $P = 0.002$). Taken together, these results demonstrate that TSQ therapy significantly potentiates the bactericidal effectiveness of standard RHZ treatment.

Discussion

TSQ is a quinoline-3-carboxamide analog that suppresses the local recruitment and function of MDSCs, Tregs, and their

soluble mediators, thereby disrupting the immunosuppressive tumor microenvironment (4–6). Moreover, it was recently shown that granuloma formation was markedly impaired by TSQ treatment in a guinea pig model (7). We recently demonstrated that denileukin diftotox, a recombinant protein toxin targeting CD25⁺ cells, restricted bacterial replication and depleted suppressor cells in TB-infected organs (8). Our data here reveal that TSQ mono-treatment reduced the bacterial burden in lungs and spleens compared with the untreated animals and was found to be associated with a significant depletion of Tregs and MDSCs in relevant organs. We were unable to observe a direct antimicrobial effect of TSQ against *M. tuberculosis* H37Rv using *in vitro* plate assay. Taken together, these observations suggest that the observed effects are likely due to modulation of immune responses during TSQ treatment of TB-infected animals.

Immune system failure during TB dictates the need of immunomodulatory strategies to enhance immune responses. Several strategies targeting different effector mechanisms to modify lung inflammation have shown promise toward shortening TB treatment duration and preventing the development of microbial resistance (9). It has been recently demonstrated that highly susceptible mouse strain 129S2 closely mimics active TB in patients compared with a highly resistant strain, C57BL/6 (10). Using *in vivo* studies in the TB mouse model, our study demonstrates that TSQ depletes immunosuppressive cells during therapy, leading to reduced bacterial proliferation, and that it potentiates standard antimicrobial therapy for TB treatment. Our results indicate that TSQ offers considerable promise as a novel adjunctive, host-directed therapy for tuberculosis. ■

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Different Background, Short Duration, and Inappropriate Participants May Harm Your Conclusion

To the Editor:

In their randomized, double-blind, triple-dummy study, Chapman and colleagues suggested that in patients receiving long-term triple therapy and with no more than one exacerbation in the previous year, the direct change from triple therapy (tiotropium plus salmeterol/fluticasone) to indacaterol/glycopyrronium led to a small decrease in lung function, but with no significant difference in the rates of chronic obstructive pulmonary disease (COPD)

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