mortality in a clinical cohort, thereby lending support to the utility of the novel HB measure in both the clinical and population health settings. These data underscore the importance of the role of nocturnal hypoxia specifically linked to OSA in portending cardiovascular risk. Future opportunities include clarifying explanatory sleep apnea–specific hypoxic mechanistic pathways contributing to cardiovascular risk. Given intermittent hypoxia in OSA has been implicated in impaired function of orexin (alerting) neurons (11), an enhanced understanding of the intersection of HB and symptom-based phenotypes will be useful to inform OSA risk stratification and potentially treatment responsiveness. ■

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References

1. Azarbarzin A, Sands SA, Stone KL, Taranto-Montemurro L, Messineo L, Terrill PI, *et al.* The hypoxic burden of sleep apnoea predicts cardiovascular disease-related mortality: the Osteoporotic Fractures in Men Study and the Sleep Heart Health Study. *Eur Heart J* 2019;40:1149–1157.
2. Trzepizur W, Blanchard M, Ganem T, Balusson F, Feuilloy M, Girault J-M, *et al.*; ERMES study group. Sleep apnea–specific hypoxic burden, symptom subtypes, and risk of cardiovascular events and all-cause mortality. *Am J Respir Crit Care Med* 2022;205:108–117.
3. Azarbarzin A, Sands SA, Taranto-Montemurro L, Vena D, Sofer T, Kim SW, *et al.* The sleep apnea-specific hypoxic burden predicts incident heart failure. *Chest* 2020;158:739–750.
4. Jackson CL, Umesi C, Gaston SA, Azarbarzin A, Lunyera J, McGrath JA, *et al.* Multiple, objectively measured sleep dimensions including hypoxic burden and chronic kidney disease: findings from the Multi-Ethnic Study of Atherosclerosis. *Thorax* 2021; 76:704–713.
5. Kim JS, Azarbarzin A, Wang R, Djonlagic IE, Punjabi NM, Zee PC, *et al.* Association of novel measures of sleep disturbances with blood pressure: the Multi-Ethnic Study of Atherosclerosis. *Thorax* 2020;75: 57–63.
6. Terrill PI, Landry S, Edwards BA, Joosten S, Azarbarzin A, Mann D, *et al.* Nocturnal hypoxaemia is a risk factor for mortality in middle aged women. 30th Annual Scientific Meeting (ASM) of Australasian-Sleep-Association and the Australasian-Sleep-Technologists-Association (Sleep DownUnder), Brisbane, Australia, 17–20 October 2018. Chichester, West Sussex, UK: Wiley-Blackwell Publishing; 2018.
7. Mazzotti DR, Keenan BT, Lim DC, Gottlieb DJ, Kim J, Pack AI. Symptom subtypes of obstructive sleep apnea predict incidence of cardiovascular outcomes. *Am J Respir Crit Care Med* 2019;200: 493–506.
8. Bratton DJ, Stradling JR, Barbé F, Kohler M. Effect of CPAP on blood pressure in patients with minimally symptomatic obstructive sleep apnoea: a meta-analysis using individual patient data from four randomised controlled trials. *Thorax* 2014;69:1128–1135.
9. Sánchez-de-la-Torre M, Sánchez-de-la-Torre A, Bertran S, Abad J, Duran-Cantolla J, Cabriada V, *et al.*; Spanish Sleep Network. Effect of obstructive sleep apnoea and its treatment with continuous positive airway pressure on the prevalence of cardiovascular events in patients with acute coronary syndrome (ISAACC study): a randomised controlled trial. *Lancet Respir Med* 2020;8:359–367.
10. Weller BE, Bowen NK, Faubert SJ. Latent class analysis: a guide to best practice. *J Black Psychol* 2020;46:287–311.
11. Dergacheva O, Yamanaka A, Schwartz AR, Polotsky VY, Mendelowitz D. Hypoxia and hypercapnia inhibit hypothalamic orexin neurons in rats. *J Neurophysiol* 2016;116:2250–2259.

2021 American Thoracic Society BEAR Cage Winning Proposal: Microbiome Transplant in Pulmonary Arterial Hypertension

What Is the Evidence that the Gut Microbiome Plays a Role in Pulmonary and Cardiovascular Disease?

The gut microbiome is integral to host physiology, including metabolism and immunity (1, 2). Interest in how the microbiome impacts chronic diseases, including chronic obstructive pulmonary disease, asthma, heart failure, idiopathic pulmonary fibrosis (3–5), and others, has been growing. Fecal microbiomes in patients with chronic obstructive pulmonary disease differ in relative abundances of several bacterial species and microbial metabolites compared with healthy control subjects (6). Lower fecal microbiota diversity, assessed by 16S rRNA gene sequencing within the first year of life, correlates with asthma development by age 7 years (7), and infants deemed to be at risk for asthma development have lower levels of the bacterial-produced, antiinflammatory, short-chain fatty acid (SCFA) acetate in their feces (8). Mice supplemented with SCFAs by including acetate in their drinking water develop significantly reduced lung inflammatory cellular infiltrates, whereas mice fed a low-fiber diet

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have reduced levels of SCFAs in their circulation and increased airway hyperreactivity (9). Trimethylamine N-oxide (TMAO), a bacterial-derived metabolite produced during red meat digestion, has been linked to stroke and myocardial infarction, and *in vitro* studies have shown that TMAO increases platelet activation and aggregation (10). Mice given chow supplemented with TMAO have significantly reduced cardiomyocyte transverse tubule power and left ventricular ejection fraction compared with control mice fed normal chow (11). These studies make a strong argument for the characterization of the gut microbiome to influence the development of novel treatments.

Is There Evidence in Pulmonary Arterial Hypertension for a Gut–Lung Axis?

Pulmonary arterial hypertension (PAH) is characterized by perivascular lung inflammation and pulmonary vascular remodeling, resulting in increased pulmonary vascular resistance. An increase in right ventricular afterload leads to right-sided heart failure and, ultimately, death. Despite the development of pharmacologic therapies, PAH mortality has not significantly improved (12–14). Although inflammation plays a mechanistic role in PAH, the underlying factors causing it remain unclear. Inflammation in PAH could be driven by an imbalance of pro- and antiinflammatory intestinal microbial metabolites, cytokines, and other mediators and/or direct effects of circulating bacteria all stemming from dysbiosis, gut-barrier dysfunction leading to increased permeability of metabolites and/or bacteria, and, possibly, decreased hepatic filtration of inflammatory gut microbial metabolites. There is evidence that PAH development is, in part, linked to the gut microbiome. One possible etiologic mechanism is the direct translocation of bacteria or bacterial components from the gut into the circulation (15). Supporting this is the finding that mice heterozygous deficient for the *BMPR2* (bone morphogenetic protein receptor type II) allele develop PAH after acute exposure to LPS, whereas wild-type mice do not (16). *BMPR2* germline mutations are found in 20% of idiopathic and 80% of heritable PAH cases (17). Exposure to LPS was associated with increased cytokine secretion (both in the murine model and *in vitro* with cultured pulmonary artery smooth muscle cells from humans and mice with PAH) (18). Therefore, TLR4 (Toll-like receptor 4), the receptor for LPS, is thought to play a pivotal role in the development of PAH. Supporting this proposal, it was found that TLR4-deficient mice are resistant to developing PAH in a hypoxia model (16). Supporting a role for gut permeability in PAH, rats treated with monocrotaline that develop PAH have increased levels of systemic intestinal fatty acid binding protein (a marker of gut permeability) (19), small intestinal fibrosis (19), and increased Firmicutes-to-Bacteroidetes ratio compared with control rats (19). The sugen-hypoxia rat model of PAH also replicates an increased Firmicutes-to-Bacteroidetes ratio (20, 21). Further strengthening the association of how the gut microbiota may augment the development of PAH, rats given antibiotics before and after sugen injection and during hypoxia develop significantly reduced right-ventricular systolic pressure and reduced vascular remodeling of pulmonary arteries compared with rats only treated with sugen and hypoxia (21). It is unknown if these effects are causal in promoting the development of PAH or are a bystander effect of gut hypoxia or hypoperfusion, and further research is needed to answer this question. However, these studies suggest that the microbiome does play a role in either the development or advancement of PAH. Thus, we hypothesized that PAH is characterized by gut dysbiosis, leading to altered intestinal permeability and an altered burden of circulating microbial metabolic products promoting perivascular inflammation and PAH.

How Is the Human Gut Microbiome in PAH Different from that of Healthy Control Subjects?

An analysis of fecal microbiome compositions of patients with PAH versus a reference cohort using shotgun metagenomics sequencing has shown distinct differences when ecological metrics are measured, including decreases in both richness and diversity as measured by the Shannon and Simpson indices, decreases in evenness (less equal distribution in relative abundances of all species), and distinction in relative abundances of the specific species present (22). Providing further evidence that the microbiome influences disease in PAH, this study found that the PAH cohort had increases in species that are positively correlated with TMAO production and decreases in species that are positively correlated with SCFA secretion compared with the reference cohort (22).

How Can This Knowledge Be Applied to Treat PAH?

We plan to determine if microbiota transplant therapy (MTT), also known as fecal microbiota transplant, can treat PAH. This therapy uses purified bacteria from fecal samples from rigorously screened healthy donors that are stringently tested for infection-causing pathogens and then lyophilized and placed in capsule form to be taken by mouth. MTT is a safe treatment for *Clostridioides difficile* colitis (23) and is safe in immunocompromised patients (24). The oral route of administration will allow for repeated dosing, which we anticipate will be important in the absence of antibiotic conditioning that creates an ecological space for engraftment.

To test MTT, we aim to determine its safety and feasibility in PAH. Our group has a phase I safety and feasibility trial for MTT in PAH approved by the U.S. Food and Drug Administration. In this open-label trial, 12 patients diagnosed with PAH will receive once-daily MTT capsules for 7 days. Our primary endpoints will be safety and feasibility. To address these endpoints, we will be monitoring patients for success in taking capsules and occurrence of adverse events. Patients will be monitored daily for 2 weeks and then monthly for 6 months. A timeline of this study is shown in Figure 1. Although we will not be selecting patients for the study based on baseline microbiome characteristics or on specific PAH treatments, if safe and feasible, future randomized placebo-controlled trials can help determine how MTT impacts disease based on baseline microbiome characteristics and how various pharmacologic therapies may be impacted or may impact the efficacy of MTT (as bacterial metabolism can affect metabolism of pharmacologic therapies [25]). Future, randomized placebo-controlled trials can also determine how MTT can impact concurrent pharmacologic therapeutic effects on secondary hemodynamic endpoints.

What Would Success Look Like?

We aim to show that MTT is both safe and feasible in PAH. If achieved, future clinical studies can test the efficacy of MTT in PAH,

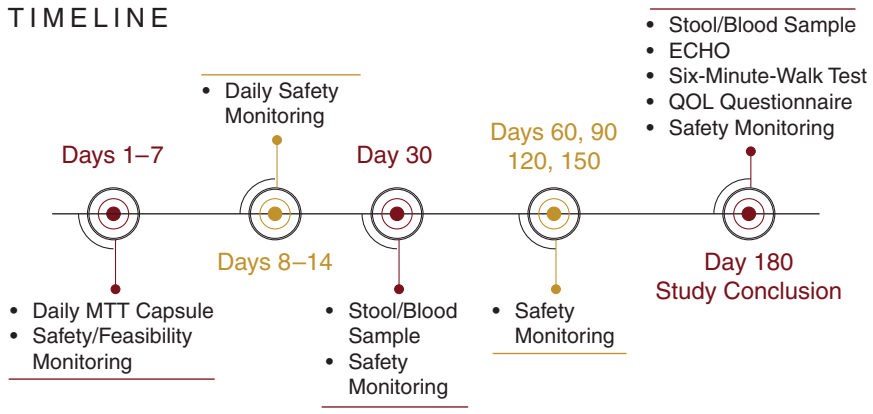

Figure 1. Timeline of open-label phase I clinical trial of microbiota transplant therapy in pulmonary arterial hypertension. ECHO = echocardiogram; MTT = microbiota transplant therapy; QOL = quality of life.

using a randomized, placebo-controlled, double-blind trial. Our initial safety and feasibility trial will allow for power calculations for our randomization through the collection of exploratory secondary endpoints. These include changes in PAH disease markers, such as right-ventricular function and pulmonary artery systolic pressure measured by echocardiography, exercise tolerance measured by a 6-minute-walk test, and a quality-of-life survey (validated emPHasis-10) (26) before and 6 months after MTT.

Stool samples from patients with PAH undergoing MTT in our phase I safety and feasibility trial will be collected before MTT and at Months 1 and 6 after MTT to study engraftment of donor microbiota. Engraftment measures how much of the donor bacteria remain in the microbiomes of patients with PAH to inform pharmacokinetics and dosing for our efficacy trial.

Conclusions

Though strides have been made in understanding the pathogenesis of PAH, and pharmacologic therapies have been developed that improve morbidity and mortality, PAH remains incurable. Therefore, novel approaches to characterize and treat this disease are needed. Our study is the first step to investigating MTT as a treatment for PAH. Additionally, future studies may also allow for the identification of a specific microbiome signature that can be used as a predictive biomarker or outcome biomarker for PAH. In future studies, if MTT succeeds or improves the efficacy of established pharmacologic therapies, MTT may become a cornerstone for an easy, nontoxic therapy to improve outcomes in PAH.

The BEAR Cage Innovation Award

Currently in its seventh year, the BEAR Cage (Building Education to Advance Research) Competition, sponsored by the American Thoracic Society Drug Device Discovery and Development Committee, provides early career investigators with the opportunity to pitch a new technology as an innovative solution to a pressing human health need. Finalists receive feedback from members of academia and industry, opening doors for further project development and collaborations. The winner of the BEAR Cage Innovation Award benefits from dedicated mentorship with the goal of accelerating clinical translation and, ultimately, impacting patient care. ■

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References

1. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res* 2020;30:492–506.
2. Visconti A, Le Roy CI, Rosa F, Rossi N, Martin TC, Mohney RP, *et al.* Interplay between the human gut microbiome and host metabolism. *Nat Commun* 2019;10:4505.
3. O'Dwyer DN, Ashley SL, Gurczynski SJ, Xia M, Wilke C, Falkowski NR, *et al.* Lung microbiota contribute to pulmonary inflammation and disease progression in pulmonary fibrosis. *Am J Respir Crit Care Med* 2019;199: 1127–1138.
4. Invernizzi R, Wu BG, Barnett J, Ghai P, Kingston S, Hewitt RJ, *et al.* The respiratory microbiome in chronic hypersensitivity pneumonitis is distinct from that of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2021;203:339–347.
5. Molyneaux PL, Willis-Owen SAG, Cox MJ, James P, Cowman S, Loebinger M, *et al.* Host-microbial interactions in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2017;195:1640–1650.
6. Bowerman KL, Rehman SF, Vaughan A, Lachner N, Budden KF, Kim RY, *et al.* Disease-associated gut microbiome and metabolome changes in patients with chronic obstructive pulmonary disease. *Nat Commun* 2020; 11:5886.

7. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clin Exp Allergy* 2014;44:842–850.
8. Arrieta MC, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S, *et al.*; CHILD Study Investigators. Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci Transl Med* 2015;7:307ra152.
9. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, *et al.* Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014;20:159–166.
10. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, *et al.* Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell* 2016;165:111–124.
11. Jin B, Ji F, Zuo A, Liu H, Qi L, He Y, *et al.* Destructive role of TMAO in T-tubule and excitation-contraction coupling in the adult cardiomyocytes. *Int Heart J* 2020;61:355–363.
12. Benza RL, Miller DP, Barst RJ, Badesch DB, Frost AE, McGoon MD. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL Registry. *Chest* 2012;142: 448–456.
13. Ling Y, Johnson MK, Kiely DG, Condliffe R, Elliot CA, Gibbs JS, *et al.* Changing demographics, epidemiology, and survival of incident pulmonary arterial hypertension: results from the pulmonary hypertension registry of the United Kingdom and Ireland. *Am J Respir Crit Care Med* 2012;186:790–796.
14. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, *et al.* Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation* 2010;122:156–163.
15. Thenappan T, Khoruts A, Chen Y, Weir EK. Can intestinal microbiota and circulating microbial products contribute to pulmonary arterial hypertension? *Am J Physiol Heart Circ Physiol* 2019;317:H1093–H1101.
16. Soon E, Crosby A, Southwood M, Yang P, Tajsic T, Toshner M, *et al.* Bone morphogenetic protein receptor type II deficiency and increased inflammatory cytokine production: a gateway to pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2015;192: 859–872.
17. McLaughlin VV, Shah SJ, Souza R, Humbert M. Management of pulmonary arterial hypertension. *J Am Coll Cardiol* 2015;65: 1976–1997.
18. Perros F, Dorfmüller P, Montani D, Hammad H, Waelput W, Girerd B, *et al.* Pulmonary lymphoid neogenesis in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2012;185:311–321.
19. Sharma RK, Oliveira AC, Yang T, Kim S, Zubcevic J, Aquino V, *et al.* Pulmonary arterial hypertension-associated changes in gut pathology and microbiota. *ERJ Open Res* 2020;6:00253-2019.
20. Callejo M, Mondejar-Parreño G, Barreira B, Izquierdo-Garcia JL, Morales-Cano D, Esquivel-Ruiz S, *et al.* Pulmonary arterial hypertension affects the rat gut microbiome. *Sci Rep* 2018;8:9681.
21. Sanada TJ, Hosomi K, Shoji H, Park J, Naito A, Ikubo Y, *et al.* Gut microbiota modification suppresses the development of pulmonary arterial hypertension in an SU5416/hypoxia rat model. *Pulm Circ* 2020; 10:2045894020929147.
22. Kim S, Rigatto K, Gazzana MB, Knorst MM, Richards EM, Pepine CJ, *et al.* Altered gut microbiome profile in patients with pulmonary arterial hypertension. *Hypertension* 2020;75:1063–1071.
23. Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, *et al.*; Fecal Microbiota Transplantation Workgroup. Treating *Clostridium difficile* infection with fecal microbiota transplantation. *Clin Gastroenterol Hepatol* 2011;9:1044–1049.
24. Kelly CR, Ihunnah C, Fischer M, Khoruts A, Surawicz C, Afzali A, *et al.* Fecal microbiota transplant for treatment of *Clostridium difficile* infection in immunocompromised patients. *Am J Gastroenterol* 2014; 109:1065–1071.
25. Tuteja S, Ferguson JF. Gut microbiome and response to cardiovascular drugs. *Circ Genom Precis Med* 2019;12:421–429.
26. Yorke J, Corris P, Gaine S, Gibbs JS, Kiely DG, Harries C, *et al.* emPHasis-10: development of a health-related quality of life measure in pulmonary hypertension. *Eur Respir J* 2014;43:1106–1113.