Beyond ORBIT: Mapping the Constellation of Pseudomonas Endotypes in Bronchiectasis Clinical Trials

Bronchiectasis antimicrobial trials are marked by paradoxes. Macrolides, lacking *in vivo* activity against *Pseudomonas aeruginosa*, show clear benefit, whereas *Pseudomonas*-active agents often fail, raising concerns of resistance, especially in chronic *P. aeruginosa* infection associated with worse outcomes (1–3). Several large multicenter studies of inhaled antimicrobials, including AIR-BX, RESPIRE, and ORBIT, have reported inconsistent results despite rigorous phase 3 designs evaluating inhaled formulations of *Pseudomonas*-active antibiotic agents (4–6). Among these, ORBIT (Once daily Respiratory Bronchiectasis Inhalation Treatment) stands out, encompassing two identically designed trials of inhaled liposomal ciprofloxacin (ORBIT-3 and ORBIT-4) in patients with chronic *P. aeruginosa* infection. Unlike AIR-BX and RESPIRE, ORBIT targeted a microbiologically defined phenotype: a focused strategy that should, in theory, reduce heterogeneity (6). This fact makes the failure of ORBIT-3 at once disappointing and all the more intriguing.

How did such disparate outcomes emerge from a targeted trial of a plausible therapy? The default explanation is often statistical noise, or the assumption that inhaled antibiotic agents are inconsistently effective (7). Why does the statistical variance that ORBIT hoped to constrain remain inflated? What biological heterogeneity—microbial, immunological, or otherwise—continues to elude trial design? How does σ remain intractably large, even while constraining for a specific microbial etiology? The ongoing bronchiectasis "renaissance" and the growing recognition of microbiome and immune correlates of disease are beginning to offer clues (8). Informative insights are emerging that point to novel approaches for improved stratification, covariate adjustment, and confounder resolution (9). Despite targeting a defined bronchiectasis phenotype, could the ORBIT trials have been interpreting therapeutic responses using an incomplete biological map?

In this issue of the *Journal*, Hull and colleagues (pp. 1397–1408) put multi-omic bronchiectasis insights into practice. They offer the deeper, data-driven explanation that these trials may have lacked important biological resolution (10). Using a large subset of sputum samples prospectively collected from ORBIT-3 and ORBIT-4 participants, targeted (16S rRNA) microbiome sequencing and proteomic profiling was performed. The central hypothesis explored was that patients with chronic *P. aeruginosa* infection exhibit biological heterogeneity underlying the discrepant treatment effects observed between ORBIT-3 and ORBIT-4. To address this, the study captured and integrated microbiome, proteome, and host immune response profiles. The findings were striking. Even within *P. aeruginosa*–positive patients, there existed profound variability in microbial community structure and host response. This suggests the general categorization of "chronic *Pseudomonas* infection" lacks specificity and masks *Pseudomonas* endotypes in these patients. Whereas some patients had airway communities dominated by *Pseudomonas*, others maintained higher microbiome diversity and significant proportions of commensal genera such as *Rothia* and *Veillonella* while also meeting the trial definition of chronic *P. aeruginosa* infection (Figure 1). The latter observation is intriguing given the emerging role of commensals, pathobionts, and the broader microbial interactome in bronchiectasis and chronic respiratory disease research (11). In particular, the ecological interplay between *P. aeruginosa* and cocolonizing airway taxa may reflect or even shape distinct exacerbation risk profiles (12, 13). Notably, distinct inflammatory endotypes with a low-diversity, neutrophil-dominant profile (cluster 1), marked by high *Pseudomonas* and *Neisseria* abundance, contrasted with a more treatment-responsive endotype (cluster 2) enriched for *Rothia*, *Gemella*, and *Veillonella*, exhibiting immunoglobulin-related proteins and regulatory immune markers. This potentially points to the opposing roles of these taxa in modulating airway inflammation and antibiotic response in *P. aeruginosa*, which, through targeted early intervention, might prevent the establishment of recalcitrant, low-diversity *Pseudomonas*-dominated microbiotypes that are less amenable to treatment.

Commendably, Hull and colleagues take their analysis beyond descriptive "-omics." They show that, when trial results are adjusted for baseline microbiota and geography, two features that differ meaningfully between ORBIT-3 and ORBIT-4, the treatment effect estimates converge, with both trials yielding a similar reduction in exacerbation frequency of approximately 20% with ciprofloxacin (Figure 1). The convergence is noteworthy: reframing interpretation and illustrating the importance of the microbiome and its host immunological corollary in the setting of antiinfective clinical trials. The microbiome is rarely accounted for in stratification or randomization, yet emerges here as a key contributor to the clinical effect. Despite identical design, ORBIT-3 and ORBIT-4 recruited from different regions—ORBIT-3 primarily from Eastern Europe and

Artificial Intelligence Disclaimer: Large language models (ChatGPT, OpenAI GPT-4o, 2024–2025) were used to support code generation for illustrative Gaussian treatment effect models and to assist with editorial phrasing and structural refinement. Generative tools were also employed in the conceptual design of vector-based visual elements, with downstream conversion to publication-ready formats (PDF, SVG) facilitated via vector.ai. At no point were manuscript data, confidential content, or unpublished materials from the primary article under review shared with or uploaded to any generative platform. All AI-assisted outputs were critically reviewed, validated, and finalized by the author, who maintains full responsibility for the integrity and interpretation of the editorial content.

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ORBIT-3 and ORBIT-4 trials revisited

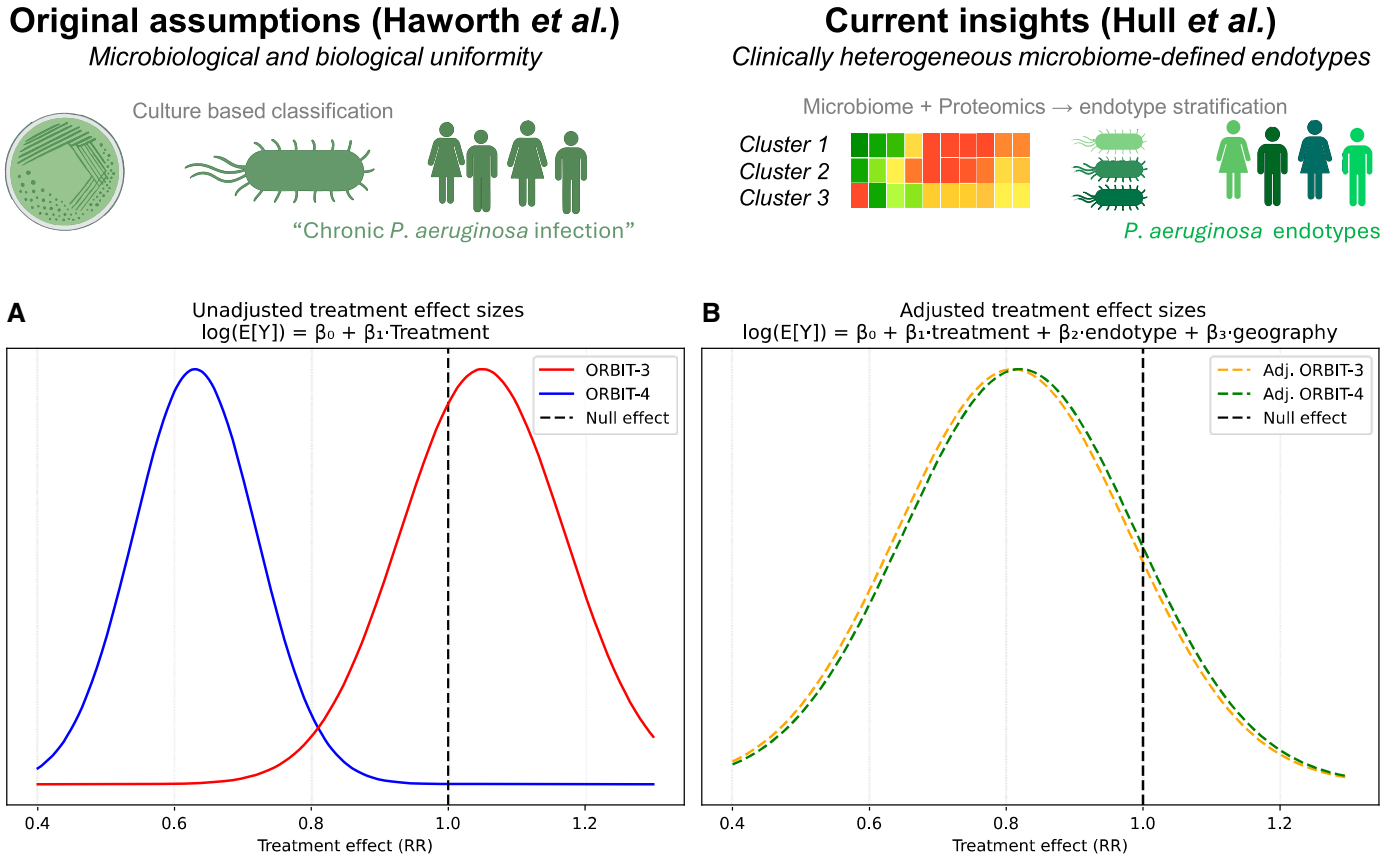

Figure 1. Reconciling divergent outcomes in the ORBIT (Once daily Respiratory Bronchiectasis Inhalation Treatment) trials through stratification of *Pseudomonas* endotypes. Upper: Both trials enrolled patients with clinically defined chronic *Pseudomonas aeruginosa* infection, with analysis assuming microbiological and biological uniformity. Reanalysis by Hull and colleagues demonstrates that this masks geographically structured endotypes that are identifiable through integrated microbiome and proteomic profiling. Lower: (*A*) unadjusted treatment effect estimates, with ORBIT-3 (red) and ORBIT-4 (blue) producing divergent outcomes. (*B*) Adjusted estimates after stratification by microbiome cluster and geography revealing convergence. Effect estimates and their uncertainties, derived from Poisson regression, are visualized on the log(E[Y]) scale. Curves reflect the approximate distribution of log-linear treatment effects, allowing visual comparison of residual variance (σ). Curves are scaled to equal peak height for clarity; the dashed vertical line represents the null effect (relative risk, 1.0).

ORBIT-4 from Western Europe and North America—further supporting associations between geography, microbiome, and clinical outcomes in bronchiectasis (14, 15). The study calls for microbiome-directed stratification as a means of improving the resolution, discriminatory power, and ability to accurately detect therapeutic effects in bronchiectasis as a solution to past failures (16). The work points to candidate biomarkers such as neutrophil elastase, LSP1, and several immunoglobulin chains as promising leads for future applied stratification. Ultimately, without endotypic stratification, ORBIT-3 likely underestimated and ORBIT-4 overestimated treatment efficacy, leading to potential type II and type I errors, respectively.

Although this work has profound implications for bronchiectasis clinical trials, it is not without limitation. The analyses are *post hoc* and exploratory, with limited -omic data, geographically limited proteomics, absence of fungal/viral profiling, and no metagenomic or functional resistome characterization. Deeper analysis of *P. aeruginosa* phenotypic variation (biofilm formation, virulence, quorum sensing, mucoid conversion) may too have offered further mechanistic insights into divergent trial outcomes. Different proteomic platforms were employed (Olink in ORBIT-3, mass spectrometry in ORBIT-4), and clustering was performed separately in each trial. Despite this, both analyses converged on a three-cluster structure with analogous immunomicrobial features. The authors are transparent about these caveats. They offer a carefully argued, biologically grounded rationale for inconsistency across past trials and a framework for interpreting inhaled antibiotic effects going forward. Indeed, this work challenges the "one-size-fits-all" assumption that has underpinned bronchiectasis drug development, even in ORBIT, a very rational and focused trial. It suggests that future studies must be larger, powered for approximately 20% effects, and stratified, explicitly or analytically, by microbiome-informed endotypes. As with any advance, replication and validation will be key. Yet, this work offers a coherent, mechanistically supported explanation for past inconsistency, and a credible path forward.

Microbiome profiles and inflammatory endotypes, though measurable, are almost always excluded from trial design. Their effects are absorbed into the error term and misclassified as noise, masking true treatment signals. Hull and colleagues boldly go where no bronchiectasis trial has gone before, taking ORBIT down from the stratosphere and into the microbial ground truth of the airway. When accounted for, the divergent results of ORBIT-3 and ORBIT-4 converge and inflated error. (σ) is recast as unmeasured structure. This reveals distinct microbial communities, divergent host responses, and fundamentally different contexts for antibiotic efficacy. ■

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Micheál Mac Aogáin, Ph.D.
Department of Biochemistry
St. James's Hospital
Dublin, Ireland
and
School of Medicine
Trinity College
Dublin, Ireland

ORCID ID: 0000-0002-1726-7700 (M.M.A.).

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