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BIOGRAPHICAL SKETCH

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NAME: Wassihun Wedajo Aragaw	eRA COMMONS USERNAME:
POSITION TITLE: Research Assistant Member	
ORGANIZATION: Hackensack Meridian Health, Center for Discovery and Innovation, Nutley, NJ, USA	

EDUCATION/TRAINING

(Begin with baccalaureate or other initial professional education, include postdoctoral training if applicable.)

INSTITUTION AND LOCATION	DEGREE	Completion Date MM/YYYY	FIELD OF STUDY
Jimma University, College of Natural Sciences, Ethiopia	B.Ed.	06/2005	Biology (Minor in Chemistry)
Addis Ababa University, College of Natural Sciences, Ethiopia	M.Sc.	04/2011	Biology (Applied Genetics)
National University of Singapore, School of Medicine, Singapore	Ph.D.	12/2018	Biomedical Sciences
Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ	Postdoctoral	03/2025	Drug Discovery, Mycobacteriology

A. PERSONAL STATEMENT

I am a biomedical scientist specializing in antimicrobial drug discovery, with a focus on the identification and pharmacological characterization of novel anti-infective agents against nontuberculous mycobacteria (NTM) and tuberculosis. Since joining the Center for Discovery and Innovation (CDI) in 2019 as a Postdoctoral Research Fellow, and in my current role as Research Assistant Member, I have led multiple early-stage drug discovery programs in the laboratory of Professor Thomas Dick. My research sits at the intersection of microbiology, chemical biology, and pharmacology, spanning the full early drug discovery pipeline from whole-cell screening and target identification to mechanism-of-action studies, in vitro resistance profiling, and in vivo efficacy evaluation.

My doctoral research at the National University of Singapore, in the laboratory of Professor Thomas Dick, centered on triazaspiroalkene-based inhibitors of *Mycobacterium tuberculosis* (Mtb) dihydrofolate reductase (DHFR). This work yielded potent and selective Mtb DHFR inhibitors. Subsequent investigation on the mechanism of action revealed intrabacterial metabolism as a modulator of drug potency, a concept with broad implications for antimycobacterial drug design.

My current research interests pursue two complementary strategies to advance the *Mycobacterium abscessus* (Mab) drug discovery pipeline. The first is de novo target-lead discovery: high-throughput whole-cell screening of diverse chemical libraries against Mab to identify novel active scaffolds, followed by target deconvolution to establish new target-lead couples for medicinal chemistry optimization. The second is Mab-specific chemical optimization of known antibiotic classes, including fluoroquinolones, against pharmacologically validated targets, with the goal of overcoming the intrinsic potency gaps that limit their clinical utility in NTM lung disease. Supporting both strategies is an ongoing interest in developing robust preclinical infection models for sustained Mab pulmonary infection in immunocompetent mice as platforms for in vivo drug efficacy testing.

My expertise, spanning mycobacteriology, microbial genetics, chemical biology, and biochemistry, positions me well to drive programs across the full early drug discovery spectrum: from target identification and in vitro characterization (whole-cell screening, target deconvolution, mechanism-of-action studies, resistance and tolerance profiling) through to in vivo efficacy evaluation (PK/PD profiling and preclinical candidate selection in animal models of pulmonary infection).

B. POSITIONS, SCIENTIFIC APPOINTMENTS, AND HONORS

Positions and Scientific Appointments

2025 – Present	Research Assistant Member, Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ
2022 – 2025	Senior Research Associate, Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ
2021 – 2022	Research Associate, Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ
2019 – 2021	Postdoctoral Research Fellow, Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, NJ
2018 – 2019	Visiting Researcher, Rutgers University, Newark, NJ
2014 – 2018	Graduate Teaching Assistant, Dept. of Microbiology and Immunology, National University of Singapore
2011 – 2014	Lecturer (Stream Leader, Genetics & Molecular Biology), Dept. of Biology, Jimma University, Ethiopia
2009 – 2011	MSc Research Fellow, Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia

Honors and Awards

2017	SPRINT-TB Young Investigator Award, 3rd SPRINT-TB Annual Conference, National University of Singapore
2014 – 2018	Singapore International Graduate Award (SINGA) PhD Scholarship, Singapore
2012	Tore Godal Prize Award: annual prize for young Ethiopian researchers in infectious diseases, Addis Ababa, Ethiopia
2011	Best Tuberculosis Research Award, 6th National Tuberculosis Conference, University of Gondar & TB Research Advisory Committee, Ethiopia
2009 – 2011	M.Sc. Research Fellowship, Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia

C. CONTRIBUTIONS TO SCIENCE

1. Revealing a Novel Mechanism Driving Potency of a DHFR Inhibitor in *Mycobacterium tuberculosis*

Dihydrofolate reductase (DHFR) is an essential enzyme in the folate biosynthetic pathway and a well-validated antibacterial drug target. In *Mycobacterium tuberculosis* (Mtb), DHFR remains an underexploited target with no clinically approved inhibitor. During my doctoral training, I characterized triazaspiroalkene compounds as potent and selective inhibitors of Mtb DHFR. This work revealed an unexpected and mechanistically novel phenomenon: the intrabacterial potency of triaza-coumarin (TA-C), a Mtb DHFR inhibitor with moderate biochemical activity, is amplified by approximately two orders of magnitude through sequential metabolic activation by multiple $F_{420}H_2$-dependent oxidoreductases (FDORs).

Using spontaneous resistance mutagenesis, I mapped two distinct resistance mechanisms: mutations in thymidylate synthase *thyA* (confirming folate pathway interference) and in F_{420} biosynthetic genes (*fbiA*, *fbiC*, *fgd1*), demonstrating that $F_{420}H_2$-dependent reduction is essential for full potency. By chemically blocking the putative site of FDOR-mediated reduction, we reproduced the F_{420}-dependent resistance phenotype in a resistant analog, providing chemical genetic confirmation. Biochemical screening of recombinant Mtb FDORs identified Ddn (Rv3547) and Rv1558 as the principal activating enzymes. The reduced product TA-C-Red undergoes spontaneous hydrolysis to TA-C-Acid, an extremely potent DHFR inhibitor, revealing a multienzyme cascade amplification mechanism. Crucially, unlike classical prodrugs that are completely inert before activation, TA-C retains baseline whole-cell activity in the absence of intracellular bioactivation making it the first compound of its kind and defining a new class of DHFR inhibitors. The work uncovered a fundamentally new tuberculosis treatment pathway: the TB bacterium itself takes up and metabolizes TA-C in a "Trojan horse" fashion, generating intracellular byproducts that inhibit the pathogen from within with two orders of magnitude greater potency than

the parent compound. This new antibacterial drug discovery concept, exploiting the bacterium's own enzymatic machinery to amplify on-target drug activity, has broad implications for the rational design of next-generation antimycobacterials.

i. **Aragaw WW**, Lee BM, Yang X, Zimmerman MD, Gengenbacher M, Dartois V, Chui WK, Jackson CJ, Dick T. Potency boost of a *Mycobacterium tuberculosis* dihydrofolate reductase inhibitor by multienzyme F420H2-dependent reduction. Proc Natl Acad Sci USA. 2021;118(25):e2025172118. PMID: 34161270

ii. Yang X*, **Aragaw WW***, Yamada Y, Sue-Li D, Neo JJL, Dick T, Chui WK. 1,3,5-Triazaspiro[5.5]undeca-2,4-dienes as selective *Mycobacterium tuberculosis* dihydrofolate reductase inhibitors with potent whole-cell activity. Eur J Med Chem. 2018;144:262–276. PMID: 29227810 (*co-first author)

2. Characterizing In Vitro Resistance of Novel NTM Drug Candidate SPR720 to Inform Clinical Development

The development of new antibiotics requires an understanding of the resistance mechanisms. In collaboration with Spero Therapeutics, which have patented this work for further development, I led the first systematic characterization of resistance to SPR719 (fobrepodacin), a novel aminobenzimidazole inhibitor of the DNA gyrase B ATPase. This work directly supported the FDA IND application for SPR720, the oral prodrug of SPR719. Through comprehensive phenotypic and genomic analyses in *Mycobacterium avium* and *Mycobacterium abscessus*, I characterized both target-based and indirect resistance mechanisms, establishing DNA gyrase as the primary mechanism of action while also identifying pathways that could confer reduced drug susceptibility. Such characterization is particularly critical for drug candidates entering the clinic, as it provides a framework to proactively anticipate and monitor the potential emergence of resistance during treatment. Collectively, this work informs clinical strategy, supports regulatory decision-making, and advances the development of more effective and reliable therapies for NTM lung disease.

i. **Aragaw WW**, Cotroneo N, Stokes S, Pucci M, Critchley I, Gengenbacher M, Dick T. In vitro resistance against DNA gyrase inhibitor SPR719 in *Mycobacterium avium* and *Mycobacterium abscessus*. Microbiol Spectrum. 2022;10(1):e01321-21. PMID: 35019671

3. Pharmacological Target Validation DHFR for *Mycobacterium abscessus* lung disease

The *Mycobacterium abscessus* (Mab) drug discovery pipeline is critically underpopulated. A particular deficiency is the lack of pharmacologically validated target-lead couples to initiate de novo drug discovery campaigns. Despite being genetically essential in Mab, DHFR had never been pharmacologically validated as a drug target in this pathogen. Trimethoprim, the canonical DHFR inhibitor, is not active against Mab. I addressed this gap by demonstrating that PQD-1, a pyrrolo-quinazoline previously identified as a Mtb DHFR inhibitor, exerts potent whole-cell activity against Mab.

Through a combination of biochemical, genetic, in silico, and structure-activity relationship (SAR) studies, I showed that: (i) PQD-1 is a potent inhibitor of recombinant Mab DHFR, while trimethoprim is inactive; (ii) overexpression of *dfrA* in Mab confers reduced susceptibility to PQD-1, providing genetic confirmation that DHFR is the antibacterial target; (iii) resistance mutations map to *thyA* (thymidylate synthase), consistent with the folate pathway mechanism characterized in Mtb; and (iv) PQD-1 synergizes with the DHPS inhibitor sulfamethoxazole, recapitulating the successful cotrimoxazole combination strategy used against Gram-negative bacteria. SAR studies with a panel of PQD-1 analogs demonstrated a dynamic structure-activity relationship, identifying chemical features essential for potency and providing a basis for medicinal chemistry optimization.

i. **Aragaw WW**, Negatu DA, Bungard CJ, Dartois VA, El Marrouni A, Nickbarg EB, Olsen DB, Warrass R, Dick T. Pharmacological validation of dihydrofolate reductase as a drug target in *Mycobacterium abscessus*. Antimicrob Agents Chemother. 2024;68(1):e00717-23. PMID: 38018963

Complete List of Published Work

Full list of publications available via Google Scholar or PubMed