SUPPLEMENTAL MATERIALS

Strongly bactericidal all-oral β-lactam combinations for the treatment of *Mycobacterium abscessus* lung disease

Dereje A. Negatu, Matthew D. Zimmerman, Véronique Dartois, Thomas Dick

a Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, New Jersey, USA

b Center for Innovative Drug Development and Therapeutic Trials for Africa (CDT-Africa), Addis Ababa University, Addis Ababa, Ethiopia

c Department of Medical Sciences, Hackensack Meridian School of Medicine, Nutley, New Jersey, USA

d Department of Microbiology and Immunology, Georgetown University, Washington, DC, USA

Running Title: Combination of oral β lactams against *M. abscessus*

Keywords: Non-tuberculous mycobacteria, NTM, synergy, sulopenem, tebipenem, cefuroxime, amoxicillin, avibactam

#Address correspondence to Thomas Dick: thomas.dick.cdi@gmail.com
### Table S1. Drugs used in the study: oral prodrug form, source, solvent and clinical status

| No. | Drug               | Prodrug form        | Class            | Catalog # | Source          | Solvent | FDA status    | Clinical development |
|-----|--------------------|---------------------|------------------|-----------|-----------------|---------|--------------|----------------------|
| 1   | Clarithromycin (CLR) | N/A                 | Macrolide        | C9742     | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 2   | Avibactam (AVI)     | ARX-1796            | Diazabicyclooctane | HY-14879A | MedChemExpress | DMSO    | Approved<sup>a</sup> | Phase 1 (NCT03931876) |
| 3   | Sulopenem (SUP)     | Sulopenem etzadroxil | Penem            | PZ0042    | Sigma-Aldrich   | DMSO    | Not approved | Phase 3 (NCT03357614) |
| 4   | Faropenem (FPM)     | Faropenem medoxomil | Penem            | F8182     | Sigma-Aldrich   | DMSO    | Not approved | Phase 2 (NCT02381470) |
| 5   | Tebibenem (TBP)     | Tebibenem pivoxil   | Carbapenem       | 161715-21-5 | MuseChem     | DMSO    | Not approved | Phase 4 (NCT04664803) |
| 6   | Imipenem (IPM)      | N/A                 | Carbapenem       | PHR1796   | Sigma-Aldrich   | Water   | Approved     |                      |
| 7   | Cephalexin (LEX)    | N/A                 | Cephalosporin    | PHR1848   | Sigma-Aldrich   | Water   | Approved     |                      |
| 8   | Cefaclor (CEC)      | N/A                 | Cephalosporin    | PHR1283   | Sigma-Aldrich   | Water   | Approved     |                      |
| 9   | Cefradine (CED)     | N/A                 | Cephalosporin    | C0690000  | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 10  | Ceftibuten (CTB)    | N/A                 | Cephalosporin    | SML0037   | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 11  | Cefprozil (CPR)     | N/A                 | Cephalosporin    | Y0001371  | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 12  | Cefpodoxime (CPD)   | Cefpodoxime proxetil | Cephalosporin  | 32344     | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 13  | Cefixime (CFM)      | N/A                 | Cephalosporin    | CDS021590 | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 14  | Cefdinir (CDR)      | N/A                 | Cephalosporin    | C7118     | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 15  | Cefadroxil (CFR)    | N/A                 | Cephalosporin    | C0650000  | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 16  | Cefuroxime (CXM)    | Cefuroxime axetil   | Cephalosporin    | C4417     | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 17  | Cefditoren (CDN)    | Cefditoren pivoxil  | Cephalosporin    | HY-17452  | MedChemExpress | DMSO    | Approved     |                      |
| 18  | Cefetamet (FET)     | Cefetamet pivoxil   | Cephalosporin    | HY-B1894A | MedChemExpress | DMSO    | Not approved | Phase 4 (NCT04664803) |
| 19  | Cefoxitin (FOX)     | N/A                 | Cephalosporin    | C4786     | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 20  | Penicillin V (PCV)  | N/A                 | Penicillin       | PVR2644   | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 21  | Amdinocillin (AMD)  | Pivmecillinam       | Penicillin       | 32887-01-7 | MuseChem     | DMSO    | Approved     |                      |
| 22  | Flucloxacillin (FLX)| N/A                 | Penicillin       | SML1023   | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 23  | Dicloxacillin (DCX) | N/A                 | Penicillin       | 46182     | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 24  | Cloxacillin (CLX)   | N/A                 | Penicillin       | PVR1922   | Sigma-Aldrich   | DMSO    | Approved     |                      |
| 25  | Ampicillin (AMP)    | Bacampicillin       | Penicillin       | HY-B0522  | MedChemExpress | Water   | Approved     |                      |
| 26  | Amoxicillin (AMX)   | N/A                 | Penicillin       | 1031503   | Sigma-Aldrich   | DMSO    | Approved     |Phase 2 (NCT02381470) |
Only the injectable form of AVI is approved. The oral AVI prodrug ARX-1796 is in Phase 1 clinical development. Sulopenem etzadroxil, in clinical development for complicated urinary tract infections. Faropenem, in clinical development for tuberculosis. Tebipenem pivoxil hydrobromide, in clinical development for complicated urinary tract infections and acute pyelonephritis. Cefetamet pivoxil, in clinical development for sinusitis. Amoxicillin, in clinical development for tuberculosis.
Fig. S1. Structures of β lactams SUP, TBP, CXM, AMX and AMP, and β-lactamase inhibitor AVI. Structures were derived from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) using the IUPAC name of the compounds.
**Fig. S2.** Growth inhibition dose-response curves for SUP, TBP, CXM and AMX with and without 4 μg/mL AVI against three *M. abscessus* subspecies reference strains. Mab absc, *M. abscessus* subsp. *abscessus* ATCC19977; Mab boll, *M. abscessus* subsp. *bolletii* CCUG50184T; Mab mass, *M. abscessus* subsp. *massiliense* CCUG48898T). ‘+’, activity of β-lactam in the presence of 4 μg/mL AVI. CLR was included as assay control. IMP and FOX were included as clinically used parenteral comparators. The inserted table shows MIC$_{50}$ values (concentrations inhibiting 50% of growth) derived from the dose response curves. MIC values (concentrations inhibiting 90% of growth) derived from the curves are presented in Table 1. The experiments were carried out three time independently and means with standard deviations are shown.
Fig. S3. Agar MIC of SUP, TBP+AVI, CXM+AVI and AMX+AVI for *M. abscessus* ATCC19977. 

10^4 CFU *M. abscessus* ATCC19977 culture were spotted on agar containing increasing β-lactam concentrations as indicated. ‘+AVI’, 4 μg/mL AVI was included in the agar. The agar MIC (first concentration preventing visible growth), indicated by red circles, were SUP, 6 μM (2.5 μM); TBP+AVI, 3 μM (4.0 μM); CXM+AVI, 12 μM (5 μM); AMX+AVI, 6 μM (25 μM). Agar MIC for IPM and FOX, included as comparators, were 6 μM (20 μM) and 48 μM (30 μM). Agar MIC for CLR, included as assay control, was 6 μM (1.6 μM). Numbers in parentheses show MICs determined in liquid cultures (Table 1). The experiment was carried out twice, yielding similar results.