



# Extremely Low Hit Rate in a Diverse Chemical Drug Screen Targeting *Mycobacterium abscessus*

Jakob J. Malin,<sup>a,b</sup> Sandra Winter,<sup>a,b</sup> Edeltraud van Gumpel,<sup>a,b</sup> Georg Plum,<sup>c</sup> Jan Rybniker<sup>a,b,d</sup>

<sup>a</sup>Department I of Internal Medicine, Division of Infectious Diseases, University of Cologne, Cologne, Germany

<sup>b</sup>Faculty of Medicine, Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany

<sup>c</sup>Faculty of Medicine and University Hospital of Cologne, Institute for Medical Microbiology, Immunology, and Hygiene, University of Cologne, Cologne, Germany

<sup>d</sup>German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, Cologne, Germany

**KEYWORDS** *Mycobacterium abscessus*, NTM, drug discovery, drug screening, emerging pathogens, hit rate

*Mycobacterium abscessus*, a rapidly growing nontuberculous mycobacterium (NTM), is increasingly recognized as an important pathogen causing soft tissue and lung infections as well as severe disseminated infections in immunocompromised patients (1, 2). Due to the intrinsic resistance to most of the classic antituberculous drugs and many other antibiotics, infections with *M. abscessus* are extremely difficult to manage (3, 4). The spread of strains which acquired additional resistance-causing mutations and the requirement for extended treatment periods, often leading to severe adverse events, stress the need for identification of new compounds active against this pathogen (2). Recent drug screening efforts have focused on testing of preselected compounds and compound libraries containing substances with known antimycobacterial or antibacterial activity which led to identification of some compounds inhibiting growth of *M. abscessus* (5–9). Given the high attrition rate of hit and lead compounds during preclinical and clinical development, additional efforts may be needed to identify promising clinical candidates against *M. abscessus*. Phenotypic whole-cell screening of large, diverse synthetic small-molecule libraries led to the identification of potent and selective antituberculous drugs, some of which have been tested successfully in clinical trials (8, 10, 11). While the expected hit rate for these classic *M. tuberculosis* whole-cell screens is between 0.3 and 1%, this important information is missing for *M. abscessus* strains (12).

Here, we report on a head-to-head comparison of *M. abscessus* and *Mycobacterium tuberculosis* in a whole-cell phenotypic screen using a diverse chemical library of 10,000 synthetic small molecules (World Diversity Set III, SPECS, Netherlands) (13). We established a resazurin microtiter assay (REMA) for *M. abscessus* in analogy to the *M. tuberculosis* REMA which was previously described (14, 15). Log phase *M. abscessus* ATCC 19977 with an optical density at 600 nm (OD<sub>600</sub>) of 0.0001 was incubated in 96-well plates at 37°C in Middlebrook 7H9 broth supplemented with 10% ADC (albumin, dextrose, catalase), 0.5% glycerol, and 0.05% Tween 80. After 72 hours, resazurin was added (0.025% wt/vol), and fluorescence was measured after 2 hours of incubation (excitation at 560 nm and emission at 590 nm). When using dimethyl sulfoxide (DMSO) and clarithromycin (20 μM) as controls, the assay had a Z-factor of 0.78 in a 96-well microplate format. Molecules were tested in parallel REMA against *M. abscessus* or *M. tuberculosis* Erdman in duplicates at concentrations of 20 μM and 10 μM, respectively.

In the *M. tuberculosis* primary screen, we observed the expected hit rate of 0.7% (72 hits). In contrast to that, the *M. abscessus* screen revealed only 7 substances causing growth inhibition of 50% or higher (hit rate of 0.07%) (Table 1). *M. abscessus* hits were retested, followed by MIC determination in *M. abscessus* and *M. tuberculosis* using 2-fold serial dilutions and a starting concentration of 100 μM. To estimate the cellular toxicity

**Citation** Malin JJ, Winter S, van Gumpel E, Plum G, Rybniker J. 2019. Extremely low hit rate in a diverse chemical drug screen targeting *Mycobacterium abscessus*. *Antimicrob Agents Chemother* 63:e01008-19. <https://doi.org/10.1128/AAC.01008-19>.

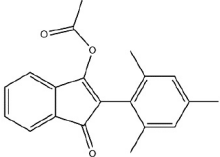
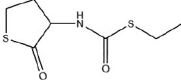
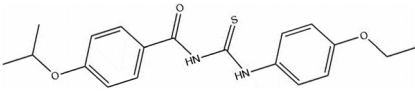
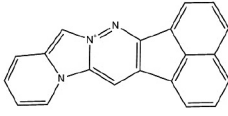
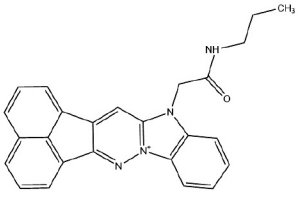
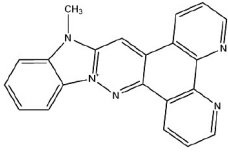
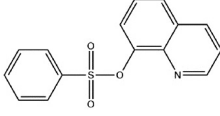
**Copyright** © 2019 American Society for Microbiology. All Rights Reserved.

Address correspondence to Jan Rybniker, [jan.rybniker@uk-koeln.de](mailto:jan.rybniker@uk-koeln.de).

**Accepted manuscript posted online** 19 August 2019

**Published** 22 October 2019

**TABLE 1** Properties of *M. abscessus* REMA hit compounds<sup>a</sup>

Substance	Molecular structure	MIC ( $\mu\text{M}$ ) <i>M. abscessus</i>	MIC ( $\mu\text{M}$ ) <i>M. tuberculosis</i>	IC <sub>50</sub> ( $\mu\text{M}$ ) <sup>b</sup>	SI ( <i>M. abscessus</i> ) <sup>c</sup>
1		6.1	6.3	54.2	8.9
2		22	>100	>100	>4.5
3		23.7	25.4	>100	>4.2
4		>100	3.5	2.1	<0.02
5		40.8	6.9	39.7	0.97
6		>100	45.4	17.2	<0.17
7		>100	>100	>100	ND
Positive control	Clarithromycin	0.7	ND	ND	ND
Positive control	Isoniazid	ND	1.3	ND	ND

<sup>a</sup>ND, not determined.<sup>b</sup>IC<sub>50</sub>, half maximal inhibitory concentration, measured in human hepatoma HepG2 cells.<sup>c</sup>SI, selectivity index (MIC against *M. abscessus*/IC<sub>50</sub>).

(50% inhibitory concentration [IC<sub>50</sub>]) of the seven hit compounds, we used standard human hepatoma HepG2 cell cytotoxicity assays and calculated the corresponding selectivity indices (SI) (IC<sub>50</sub>/MIC). Growth inhibition of *M. abscessus* was confirmed for all 7 hit compounds. However, only compound 1 had a MIC below 20  $\mu\text{M}$  (6.1  $\mu\text{M}$ ). The selectivity index for this substance was 8.9 (Table 1). Hit compound 2, a carbamothioate, and hit compound 3, a thiourea, had MICs of 22  $\mu\text{M}$  and 23.7  $\mu\text{M}$ , respectively. IC<sub>50</sub> cytotoxicity values were >100  $\mu\text{M}$ . A database search revealed that both substances display known antituberculous activity (Collaborative Drug Discovery Vault) (16). This also holds true for the imidazo-pyridazine compounds 4 to 6, all which displayed an SI of <1 due to pronounced cytotoxicity. Hit compound 7, a quinoline, did not reach a MIC of  $\leq 100$   $\mu\text{M}$ .

In conclusion, we show that high-throughput screening of randomly selected molecules yields extremely low hit rates for compounds targeting *M. abscessus*. Despite using a two-times-higher compound concentration in the *M. abscessus* assay, the hit rate was 10 times lower than results obtained for *M. tuberculosis*. From a total of seven hits, only one showed a MIC below 10  $\mu$ M and moderate cytotoxicity. Five of the seven *M. abscessus* hits were either positive in our *M. tuberculosis* REMA or had been described previously as hits in other *M. tuberculosis* screening campaigns (16). Upon testing of *M. abscessus* hits against *M. tuberculosis*, we identified only one substance (number 2) which seems to possess selective activity against *M. abscessus* (Table 1). The disappointing results observed in this *M. abscessus* small-molecule screening study can be explained by the high intrinsic drug resistance of NTM that might reflect an evolutionary adaptation to hostile environments (soil and water) (4, 17). NTM have a highly flexible system of gene regulation altering growth rate, metabolism, and inducible expression of genes directly facilitating drug resistance (e.g., genes encoding efflux pumps). One example is the transcriptional regulator WhiB7, which is involved in various mechanisms of inducible drug resistance in mycobacteria (18–22). Recently, it was shown that WhiB7 confers species-specific patterns of gene induction that might explain differences in drug susceptibilities among different mycobacterial species. For *M. abscessus*, it was shown that resistance to amikacin is induced by the WhiB7-regulated gene *eis2*, which seems to be unique for this species (23).

Based on our findings, we suggest retesting and repurposing substances that have already been tested positive in screens targeting *M. tuberculosis* and Gram-positive bacteria, rather than performing large-scale phenotypic screening against *M. abscessus* (5–7, 24, 25). In addition, known antimycobacterial drugs may exhibit synergistic effects as shown for combinations of various drug classes acting against *M. abscessus* (26–28). Changing assay conditions and using different media may also increase hit rates, as recently shown for experiments performed using Mueller-Hinton broth (6). Whether this will translate into compounds with good *in vivo* activity against *M. abscessus* requires further investigation.

## ACKNOWLEDGMENTS

J.R. receives funding from the Thematic Translational Unit Tuberculosis (TTU TB, grant numbers TTU 02.806 and 02.905) of the German Center of Infection Research (DZIF). Financial support was also received from the German Research Foundation (DFG RY 159) and the Center for Molecular Medicine Cologne (ZMMK—CAP8). J.J.M. receives funding from DZIF (stipend TI 07.001\_Malin\_00 [TTU TB]).

## REFERENCES

- Petrini B. 2006. Mycobacterium abscessus: an emerging rapid-growing potential pathogen. *APMIS* 114:319–328. [https://doi.org/10.1111/j.1600-0463.2006.apm\\_390.x](https://doi.org/10.1111/j.1600-0463.2006.apm_390.x).
- Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P, Verma D, Hill E, Drijkoningen J, Gilligan P, Esther CR, Noone PG, Giddings O, Bell SC, Thomson R, Wainwright CE, Coulter C, Pandey S, Wood ME, Stockwell RE, Ramsay KA, Sherrard LJ, Kidd TJ, Jabbour N, Johnson GR, Knibbs LD, Morawska L, Sly PD, Jones A, Bilton D, Laurenson I, Ruddy M, Bourke S, Bowler IC, Chapman SJ, Clayton A, Cullen M, Daniels T, Dempsey O, Denton M, Desai M, Drew RJ, Edenborough F, Evans J, Folb J, Humphrey H, Isalska B, Jensen-Fangel S, Jönsson B, Jones AM, et al. 2016. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. *Science* 354:751–757. <https://doi.org/10.1126/science.aaf8156>.
- Luthra S, Rominski A, Sander P. 2018. The role of antibiotic-target-modifying and antibiotic-modifying enzymes in Mycobacterium abscessus drug resistance. *Front Microbiol* 9:2179. <https://doi.org/10.3389/fmicb.2018.02179>.
- Nessar R, Cambau E, Reyat JM, Murray A, Gicquel B. 2012. Mycobacterium abscessus: a new antibiotic nightmare. *J Antimicrob Chemother* 67:810–818. <https://doi.org/10.1093/jac/dkr578>.
- Low JL, Wu ML, Aziz DB, Laleu B, Dick T. 2017. Screening of TB actives for activity against nontuberculous mycobacteria delivers high hit rates. *Front Microbiol* 8:1539. <https://doi.org/10.3389/fmicb.2017.01539>.
- Richter A, Strauch A, Chao J, Ko M, Av-Gay Y. 2018. Screening of preselected libraries targeting Mycobacterium abscessus for drug discovery. *Antimicrob Agents Chemother* 62:e00828-18. <https://doi.org/10.1128/AAC.00828-18>.
- Aziz DB, Low JL, Wu ML, Gengenbacher M, Teo JWP, Dartois V, Dick T. 2017. Rifabutin is active against Mycobacterium abscessus complex. *Antimicrob Agents Chemother* 61:e00155-17. <https://doi.org/10.1128/AAC.00155-17>.
- Wu ML, Aziz DB, Dartois V, Dick T. 2018. NTM drug discovery: status, gaps and the way forward. *Drug Discov Today* 23:1502–1519. <https://doi.org/10.1016/j.drudis.2018.04.001>.
- Kaushik A, Ammerman NC, Martins O, Parrish NM, Nuernberger EL. 2019. In vitro activity of new tetracycline analogs omadacycline and eravacycline against drug-resistant clinical isolates of Mycobacterium abscessus. *Antimicrob Agents Chemother* 63. <https://doi.org/10.1128/AAC.00470-19>.
- Lechartier B, Rybniker J, Zumla A, Cole ST. 2014. Tuberculosis drug discovery in the post-post-genomic era. *EMBO Mol Med* 6:158–168. <https://doi.org/10.1002/emmm.201201772>.
- Herrmann J, Rybniker J, Muller R. 2017. Novel and revisited approaches

- in antituberculosis drug discovery. *Curr Opin Biotechnol* 48:94–101. <https://doi.org/10.1016/j.copbio.2017.03.023>.
12. Manjunatha UH, Smith PW. 2015. Perspective: challenges and opportunities in TB drug discovery from phenotypic screening. *Bioorg Med Chem* 23:5087–5097. <https://doi.org/10.1016/j.bmc.2014.12.031>.
  13. SPECS. 2019. World Diversity Set 3 factsheet. <https://www.specs.net/transfer.php?code=SPECSPH9TJQygpSymZUO1Lv9TLJAOp2uyMKEsl0EGZl5jMTM8H3OyL3AIESyEjWuJGW4>. Accessed 3 July 2019.
  14. Rybniker J, Chen JM, Sala C, Hartkoorn RC, Vocat A, Benjak A, Boy-Röttger S, Zhang M, Székely R, Greff Z, Orfi L, Szabadkai I, Pató J, Kéri G, Cole ST. 2014. Anticytolytic screen identifies inhibitors of mycobacterial virulence protein secretion. *Cell Host Microbe* 16:538–548. <https://doi.org/10.1016/j.chom.2014.09.008>.
  15. Rybniker J, Vocat A, Sala C, Busso P, Pojer F, Benjak A, Cole ST. 2015. Lansoprazole is an antituberculous prodrug targeting cytochrome bc1. *Nat Commun* 6:7659. <https://doi.org/10.1038/ncomms8659>.
  16. Ekins S, Clark AM, Dole K, Gregory K, McNutt AM, Spektor AC, Weatherall C, Litterman NK, Bunin BA. 2018. Data mining and computational modeling of high-throughput screening datasets. *Methods Mol Biol* 1755:197–221. [https://doi.org/10.1007/978-1-4939-7724-6\\_14](https://doi.org/10.1007/978-1-4939-7724-6_14).
  17. van Ingen J, Boeree MJ, van Soolingen D, Mouton JW. 2012. Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. *Drug Resist Updat* 15:149–161. <https://doi.org/10.1016/j.drug.2012.04.001>.
  18. Burian J, Ramon-Garcia S, Howes CG, Thompson CJ. 2012. WhiB7, a transcriptional activator that coordinates physiology with intrinsic drug resistance in *Mycobacterium tuberculosis*. *Expert Rev Anti Infect Ther* 10:1037–1047. <https://doi.org/10.1586/eri.12.90>.
  19. Ramon-Garcia S, Ng C, Jensen PR, Dosanjh M, Burian J, Morris RP, Folcher M, Eltis LD, Grzesiek S, Nguyen L, Thompson CJ. 2013. WhiB7, an Fe-S-dependent transcription factor that activates species-specific repertoires of drug resistance determinants in actinobacteria. *J Biol Chem* 288:34514–34528. <https://doi.org/10.1074/jbc.M113.516385>.
  20. Geiman DE, Raghunand TR, Agarwal N, Bishai WR. 2006. Differential gene expression in response to exposure to antimycobacterial agents and other stress conditions among seven *Mycobacterium tuberculosis* whiB-like genes. *Antimicrob Agents Chemother* 50:2836–2841. <https://doi.org/10.1128/AAC.00295-06>.
  21. Burian J, Yim G, Hsing M, Axerio-Cilies P, Cherkasov A, Spiegelman GB, Thompson CJ. 2013. The mycobacterial antibiotic resistance determinant WhiB7 acts as a transcriptional activator by binding the primary sigma factor SigA (RpoV). *Nucleic Acids Res* 41:10062–10076. <https://doi.org/10.1093/nar/gkt751>.
  22. Morris RP, Nguyen L, Gatfield J, Visconti K, Nguyen K, Schnappinger D, Ehrh S, Liu Y, Heifets L, Pieters J, Schoolnik G, Thompson CJ. 2005. Ancestral antibiotic resistance in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* 102:12200–12205. <https://doi.org/10.1073/pnas.0505446102>.
  23. Hurst-Hess K, Rudra P, Ghosh P. 2017. *Mycobacterium abscessus* WhiB7 regulates a species-specific repertoire of genes to confer extreme antibiotic resistance. *Antimicrob Agents Chemother* 61:e01347-17. <https://doi.org/10.1128/AAC.01347-17>.
  24. Le Run E, Arthur M, Mainardi JL. 2018. In vitro and intracellular activity of imipenem combined with rifabutin and avibactam against *Mycobacterium abscessus*. *Antimicrob Agents Chemother* 62:e00623-18. <https://doi.org/10.1128/AAC.00623-18>.
  25. Griffith DE, Eagle G, Thomson R, Aksamit TR, Hasegawa N, Morimoto K, Addrizzo-Harris DJ, O'Donnell AE, Marras TK, Flume PA, Loebinger MR, Morgan L, Codecasa LR, Hill AT, Ruoss SJ, Yim JJ, Ringshausen FC, Field SK, Philley JV, Wallace RJ, Jr, van Ingen J, Coulter C, Nezamis J, Winthrop KL, CONVERT Study Group. 2018. Amikacin liposome inhalation suspension for treatment-refractory lung disease caused by *Mycobacterium avium* complex (CONVERT): a prospective, open-label, randomized study. *Am J Respir Crit Care Med* 198:1559. <https://doi.org/10.1164/rccm.201807-1318OC>.
  26. Story-Roller E, Maggioncalda EC, Lamichhane G. 2019. Select beta-lactam combinations exhibit synergy against *Mycobacterium abscessus* in vitro. *Antimicrob Agents Chemother* 63:e02613-18. <https://doi.org/10.1128/AAC.02613-18>.
  27. Pryjma M, Burian J, Thompson CJ. 2018. Rifabutin acts in synergy and is bactericidal with frontline *Mycobacterium abscessus* antibiotics clarithromycin and tigecycline, suggesting a potent treatment combination. *Antimicrob Agents Chemother* 62:e00283-18. <https://doi.org/10.1128/AAC.00283-18>.
  28. van Ingen J, Totten SE, Helstrom NK, Heifets LB, Boeree MJ, Daley CL. 2012. In vitro synergy between clofazimine and amikacin in treatment of nontuberculous mycobacterial disease. *Antimicrob Agents Chemother* 56:6324–6327. <https://doi.org/10.1128/AAC.01505-12>.