

Development of an Intracellular Screen for New Compounds Able To Inhibit *Mycobacterium tuberculosis* Growth in Human Macrophages

Flavia Sorrentino,^{a,b} Ruben Gonzalez del Rio,^b Xingji Zheng,^a Jesus Presa Matilla,^b Pedro Torres Gomez,^b Maria Martinez Hoyos,^b Maria Esther Perez Herran,^b Alfonso Mendoza Losana,^b Yossef Av-Gay^a

Department of Medicine, Division of Infectious Diseases, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada^a; Diseases of the Developing World, GSK, Tres Cantos, Madrid, Spain^b

Here we describe the development and validation of an intracellular high-throughput screening assay for finding new antituberculosis compounds active in human macrophages. The assay consists of a luciferase-based primary identification assay, followed by a green fluorescent protein-based secondary profiling assay. Standard tuberculosis drugs and 158 previously recognized active antimycobacterial compounds were used to evaluate assay robustness. Data show that the assay developed is a short and valuable tool for the discovery of new antimycobacterial compounds.

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* affects 9.0 million people annually, with 1.5 million deaths in 2013 (1). Standard TB treatment involves a regimen of four antibiotics taken daily for 6 to 9 months. However, the long treatment duration, toxicity, and interaction with antiretrovirals lead to poor patient compliance and treatment failure. Novel TB drug regimens are therefore urgently needed to treat both standard and drug-resistant forms of TB. Two new drugs, bedaquiline (2) and delamanid (3), were recently approved for the treatment of multidrug-resistant (MDR) TB, and other compounds are in the clinical development pipeline (4). Yet, the search for new TB drug candidates with different modes of action seeks to increase the chances of finding new drugs.

Screening of chemical libraries is the first crucial step in the antimicrobial discovery process. Potential antimycobacterial agents are identified by testing chemicals for the ability to inhibit *M. tuberculosis* growth under *in vitro* growth conditions in culture medium. However, *in vitro* screening results are often misleading, as the culture broth does not reflect the environment *M. tuberculosis* encounters *in vivo* during the natural course of the disease, neglecting important factors such as compound activation, membrane permeability, removal by efflux pump, and toxicity to mammalian cells (4). Furthermore, adaptive metabolic changes that *M. tuberculosis* undergoes within the host may affect compound activity (5). *Ex vivo* screening, in the macrophage, may represent physiological conditions that mimic disease and take into consideration the favorable contribution of host cells in the process of eradicating *M. tuberculosis*.

M. tuberculosis's intracellular lifestyle presents an attractive area for new drug discovery programs. A successful example is the intracellular high-content screening campaign that led to the discovery of Q203 (6). Image-based high-content screening technologies are being adopted more frequently to evaluate the activities of compounds against *M. tuberculosis* by using various cell types (7–9) or the granuloma infection model (10).

High-content screening against *M. tuberculosis* is a robust and informative assay; however, it is still lacking in terms of speed and simplicity since the endpoint assay requires multiple steps for staining, image acquisition, and cumbersome data analysis. In addition, most of the intracellular compound screening done so far was performed inside epithelial cells (11) or murine macrophages

(7–9), which are not natural hosts of *M. tuberculosis*. In this report, we describe a new, fast, and robust intracellular high-throughput screening (HTS) method well suited for extensive compound screening campaigns against intracellular *M. tuberculosis*. Using this assay, we demonstrate the successful identification of a set of highly active intracellular compounds from a previously identified set of *in vitro*-active anti-*M. tuberculosis* compounds.

Our new protocol was developed to identify compounds active against intracellular *M. tuberculosis* by using THP-1 human monocytes infected with *M. tuberculosis* strains expressing either a luciferase or a green fluorescent protein (GFP) reporter gene for primary identification and a secondary profiling assay, respectively (see Fig. S1 in the supplemental material). Large-scale production of uniformly infected THP-1 cells was achieved by performing the differentiation-infection in a single step and in bulk by using roller bottles with up to 1 liter of total volume, which is significantly different from microplate intracellular assays (7–9) and represents a key strategy to improve assay robustness by decreasing well-to-well variation, as reflected by the excellent *Z'* scores obtained (Table 1). Different infection times and multiplicities of infection (MOIs) were tested (see the supplemental material) for *M. tuberculosis* constitutively expressing either luciferase or GFP. The conditions that worked best were concomitant differentiation and infection for 4 h with 40 ng ml⁻¹ of phorbol myristate acetate at an MOI of 1:1. Intracellular bacterial loads were quantified to check the potency of the compounds by measuring either luciferase luminescence or GFP fluorescence. The

Received 8 August 2015 Returned for modification 19 August 2015

Accepted 23 October 2015

Accepted manuscript posted online 26 October 2015

Citation Sorrentino F, Gonzalez del Rio R, Zheng X, Presa Matilla J, Torres Gomez P, Martinez Hoyos M, Perez Herran ME, Mendoza Losana A, Av-Gay Y. 2016. Development of an intracellular screen for new compounds able to inhibit *Mycobacterium tuberculosis* growth in human macrophages. *Antimicrob Agents Chemother* 60:640–645. doi:10.1128/AAC.01920-15.

Address correspondence to Yossef Av-Gay, yossi@mail.ubc.ca.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/AAC.01920-15>.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

TABLE 1 Intracellular MIC₉₀ of standard TB drugs obtained by primary and secondary assay in comparison with *in vitro* MIC₉₀^a

Drug	Intracellular MIC ₉₀ (μM)		<i>In vitro</i> MIC ₉₀ (μM) in H37Rv ^b
	Primary assay ^c	Secondary assay ^d	
Rifampin	0.02	0.06	0.03
Linezolid	1.85	2.14	0.7
Moxifloxacin	0.62	0.45	0.07
Pyrazinamide	>50	>50	Inactive
Levofloxacin	1.85	0.78	1.3
Ethambutol	16.67	14.63	4.6
4-Aminosalicylic acid	50	>50	6.5
Isoniazid	0.62	0.43	0.16
Ofloxacin	5.56	8.36	2.2

^a Data are the average results of two representative experiments.

^b Reference 21.

^c Assay robustness (Z'), 0.67, 0.64.

^d Assay robustness (Z'), 0.67, 0.76.

optimal time point for measurement of growth inhibition in the primary assay was 5 days postinfection with the H37Rv strain expressing the luciferase gene. A shorter incubation time of only 4 days was required for the secondary assay with the Erdman strain expressing GFP, where intracellular mycobacterial growth was substantial and little apparent macrophage lysis was observed. These results are consistent with previous reports showing that the Erdman strain is more virulent than the H37Rv strain (12). Overall, the use of reporter genes to quantify bacterial loads along with concurrent differentiation and infection protocols and a 384-well plate format increased the assay throughput and shortened the screening time from weeks to only 5 or 6 days.

A dose-response assay was performed to determine the intracellular MICs of first- and second-line anti-TB drugs. MIC₉₀ values were interpolated from dose-response curves obtained by testing the compounds with the luciferase and GFP assays and by plotting the percentage of growth inhibition against the log concentration of the standard compounds (see Fig. S2 in the supplemental material). The intracellular MIC₉₀ values obtained with both assays are summarized in Table 1. Overall, a good correlation between the MIC₉₀ determined by the primary and secondary assays was observed. Rifampin was the most powerful intracellular compound in both assays, with MIC₉₀ values of 0.02 to 0.06 μM, followed by isoniazid and moxifloxacin, both with MIC₉₀ below 1 μM, which is consistent with previous reports (13, 14). Linezolid and levofloxacin demonstrated good intracellular activity, with a MIC₉₀ of <2.5 μM, followed by ofloxacin and ethambutol with moderate activity (MIC₉₀, <20), as reported elsewhere (13–15). Pyrazinamide did not exhibit any intracellular growth inhibition at any of the concentrations tested compared to a dimethyl sulfoxide (DMSO) negative control, consistent with previous data and in agreement with pyrazinamide's sterilizing activity against non-replicating bacteria (16–19).

Statistical analysis was performed to assess assay quality, and Z' values were calculated (20). The primary assay showed an average Z' value of 0.65, and that of the secondary assay was similar at 0.71. This is a well-defined signal window for both assays, suggesting that the assays performed well and could be used for HTS to test a larger number of compounds.

Next, the primary assay was used to screen a larger set of com-

pounds shown to be highly active (MICs of ≤10 μM) against *M. tuberculosis* H37Rv under *in vitro* growth conditions (21). One hundred fifty-eight of these compounds were first tested in duplicate at a single concentration of 50 μM. Fourteen of the initial 158 compounds did not show intracellular activity and were discarded (see Table S1 in the supplemental material). One hundred forty-four compounds showed intracellular activity, with MICs below 50 μM, and were selected for dose-response studies.

Using the primary assay, 144 hits from the single-concentration assay were tested in a dose-response study. Figure 1A shows the distribution of the intracellular MIC₉₀ of all of the compounds; for a summary of the individual MIC₉₀, see Table S1 in the supplemental material. Of the 144 compounds tested, 90.3% were confirmed as active, with intracellular MIC₉₀ of <50 μM. Of the 144 compounds tested in the dose-response study, 9.7% were found to have intracellular MIC₉₀ above 50 μM. The previously reported extracellular MIC₉₀ of each hit compound (21) was compared to the intracellular MIC₉₀ obtained.

Extracellular/intracellular MIC₉₀ ratios are illustrated in Fig. 1B. Fifty-six compounds were more active in macrophages than in culture broth, showing extracellular/intracellular MIC₉₀ ratios above 1. These compounds might be targeting pathways, either in the bacteria or in macrophages, that are essential during infection but not during *in vitro* growth (22–25). Although these compounds were identified initially as active under *in vitro* growth conditions, higher concentrations of them in macrophages, their intracellular conversion into active metabolites (26), or host cell

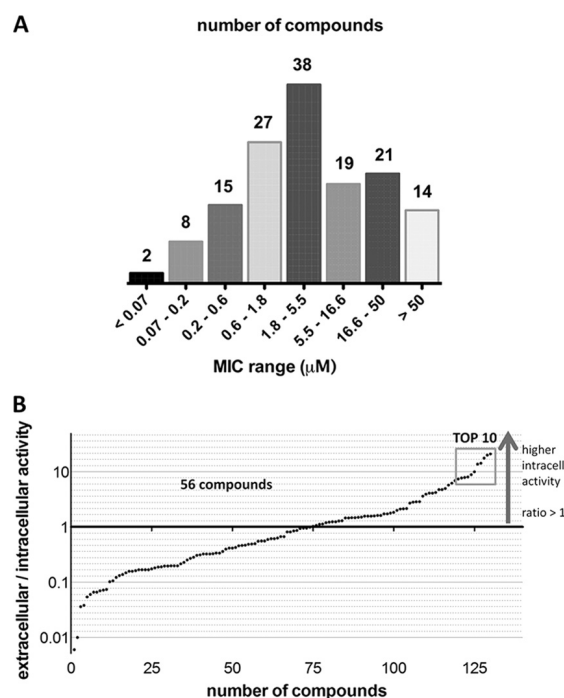
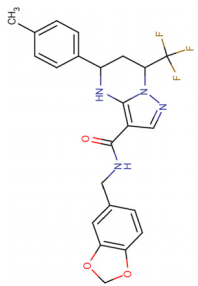
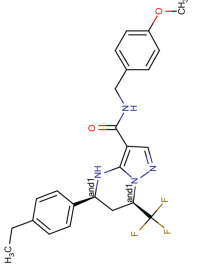
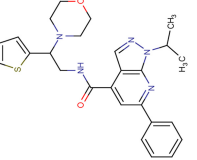
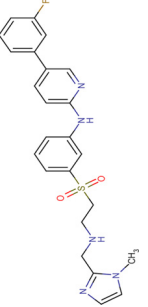


FIG 1 Results of pilot screening of the set of compounds active *in vitro*. Panel A shows the distribution of the MIC ranges of the compounds tested (144 compounds distributed into eight MIC ranges). Panel B shows a comparison of compound potency in broth (extracellular MIC₉₀) with activity in macrophages (intracellular MIC₉₀). The extracellular/intracellular MIC₉₀ ratios of all of the compounds were plotted. Compounds with ratios of >1 have higher activity in macrophages than *in vitro*, while compounds with ratios of <1 have higher *in vitro* activity. The 10 compounds with the highest intracellular versus extracellular activity are further described in Table 2.

TABLE 2 Top 10 hit compounds from intracellular HTS of *in vitro*-active TB compounds

Compound no.	Structure	<i>In vitro</i> MIC ₉₀ (μM) in H37Rv	Intracellular MIC ₉₀ (μM) in THP-1 primary assay	H37Rv/THP-1 MIC ₉₀ ratio	Intracellular MIC ₉₀ (μM) in THP-1 secondary assay	Cytotoxicity (μM) in:		Structure/target
						HepG2	THP-1	
GSK1589671A		4.4	0.2	20.9	1.9	>100	>50	THPP family, ^a possibly targeting MmpL3
GSK1985270A		4.2	0.2	20	2.4	>100	50	
GSK2043267A		3.7	0.2	17.6	0.8	>100	>50	
GW623128X		1	0.07	14.3	0.4	>100	>50	Adamantyl family, targeting MmpL3 ^b
GSK1385423A		8.4	0.6	13.8	Autofluorescent	25	>50	
GSK937213A		6.1	0.6	10	25.9	>100	>50	

TABLE 2 (Continued)

Chemical Structure	5.4	0.6	8.8	3.6	>100	>50	THPP family, ^a possibly targeting MmpL3
							
GSK937733A							
							
GSK1589673A	4.9	0.6	8	0.8	40	45	THPP family, targeting MmpL3 ^a
							
GSK1941290A	4.8	0.6	7.9	5.6	80	35	
							
GSK1826247A	4.7	0.6	7.7	>50	40	47	

^a Reference 30.^b Reference 31.

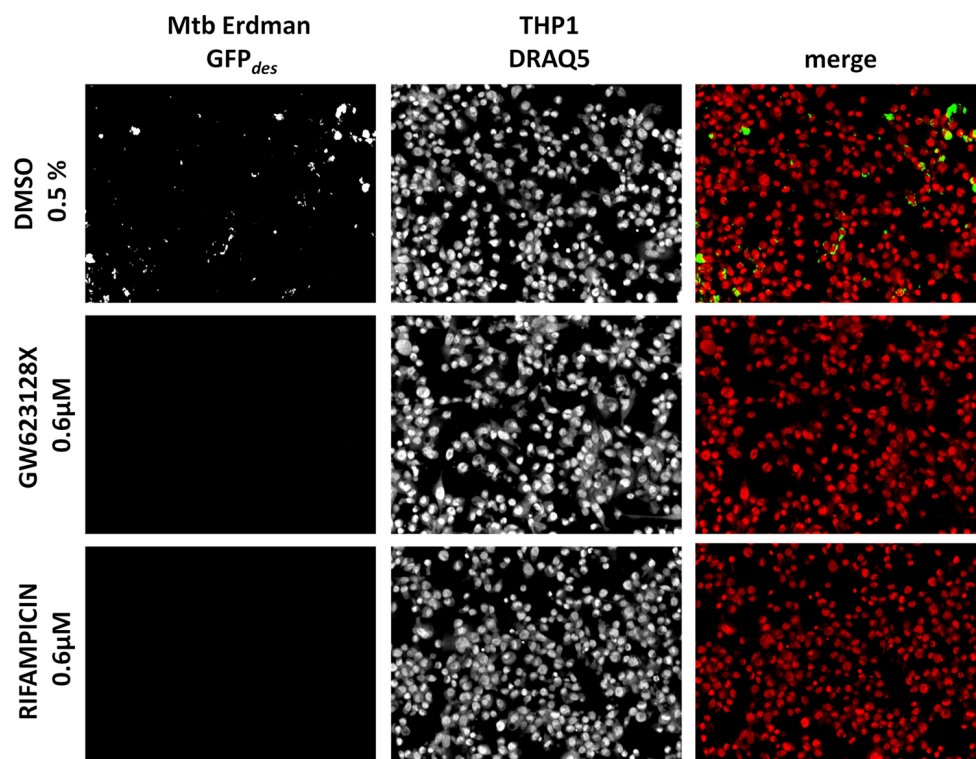


FIG 2 Secondary intracellular assay of the top 10 compounds from the set of *in vitro*-active compounds. Pictures of *M. tuberculosis* (Mtb) Erdman-GFP_{des}-infected THP-1 cells at day 4 in the presence of DMSO at 0.5%, compound GW623128X at 0.6 μ M, and rifampin at 0.6 μ M are shown.

interaction such as that of clofazimine (27, 28) could better explain our findings.

Seventy-four compounds had extracellular/intracellular MIC₉₀ ratios below 1, indicating higher activity *in vitro*. This could be due to poor cell membrane permeability for the compound, activation of bacterial efflux pumps by macrophages (29), or even inactivation of the compound by host cell-derived metabolites (e.g., reactive oxygen or nitrogen species) or an acidic pH.

The 10 compounds with the highest extracellular/intracellular MIC₉₀ ratios, as well as their chemical properties, are shown in Table 2. Among them are GSK1589671A (20.8-fold higher intracellular activity), GSK1985270A (20-fold higher intracellular activity), and GSK2043267A (17.6-fold higher intracellular activity).

The intracellular activities of all 158 compounds at 50 μ M were also tested by the secondary assay with the Opera High Content Screening System. The top 10 hit compounds from the primary screening were tested in a dose-response assay. On day 4, the percentage of infected cells reached an average of 34.3% in the DMSO control and 4.8% in the rifampin control (Fig. 2). Dose-dependent decreases in the bacterial loads and percentages of infection were obtained with all of the compounds tested (see Fig. S3 in the supplemental material). The MIC₉₀s obtained were consistent and in agreement with those obtained in the primary assay. However, high MIC₉₀s of two compounds, GSK937213A and GSK1826247A, were obtained in the secondary assay. High lipophilicity (property forecast indexes of 9.8 and 8.4, respectively) and poor solubility could explain the difference between the data obtained in the primary and secondary assays with these two compounds. GSK1385423A appeared to be autofluorescent, interfering and thus giving false-negative results at the highest concentra-

tions in the secondary assay (see Fig. S4 in the supplemental material).

In conclusion, we have completed a pilot intracellular HTS assay with the differentiated human monocytic cell line THP-1. Robust assays were developed that enable the identification of compounds able to inhibit *M. tuberculosis* growth intracellularly. We believe that these assays will assist TB drug discovery by improving the ability to identify compounds that target mycobacterial and/or host cell proteins required for bacterial entry, replication, and survival in macrophages.

ACKNOWLEDGMENTS

We thank Neeraj Dahr for the kind gift of the *M. tuberculosis* Erdman GFP strain. We are grateful to Jeffrey Helm for help reviewing the manuscript. We also thank GSK Sample Management Technologies facilities for the preparation of compound-predispensed 384-well plates.

FUNDING INFORMATION

This work was supported by the Tres Cantos Open Lab Foundation. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

REFERENCES

1. World Health Organization. 2014. Global tuberculosis report 2014. World Health Organization, Geneva, Switzerland. www.who.int/tb/publications/global_report/en/.
2. Diacon AH, Pym A, Grobusch M, Patientia R, Rustomjee R, Page-Shipp L, Pistorius C, Krause R, Bogoshi M, Churchyard G, Venter A, Allen J, Palomino JC, De Marez T, van Heeswijk RP, Lounis N, Meyvisch P, Verbeeck J, Parys W, de Beule K, Andries K, McNeeley DF. 2009. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. *N Engl J Med* 360:2397–2405. <http://dx.doi.org/10.1056/NEJMoa0808427>.

3. Gler MT, Skripconoka V, Sanchez-Garavito E, Xiao H, Cabrera-Rivero JL, Vargas-Vasquez DE, Gao M, Awad M, Park SK, Shim TS, Suh GY, Danilovits M, Ogata H, Kurve A, Chang J, Suzuki K, Tupasi T, Koh WJ, Seaworth B, Geiter LJ, Wells CD. 2012. Delamanid for multidrug-resistant pulmonary tuberculosis. *N Engl J Med* 366:2151–2160. <http://dx.doi.org/10.1056/NEJMoa1112433>.
4. Mdluli K, Kaneko T, Upton A. 2015. The tuberculosis drug discovery and development pipeline and emerging drug targets. *Cold Spring Harb Perspect Med* 5:a021154. <http://dx.doi.org/10.1101/cshperspect.a021154>.
5. Galagan JE, Minch K, Peterson M, Lyubetskaya A, Azizi E, Sweet L, Gomes A, Rustad T, Dolganov G, Glotova I, Abeel T, Mahwinney C, Kennedy AD, Allard R, Brabant W, Krueger A, Jaini S, Honda B, Yu WH, Hickey MJ, Zucker J, Garay C, Weiner B, Sisk P, Stolte C, Winkler J K, Van de Peer Y, Iazzetti P, Camacho D, Dreyfuss J, Liu Y, Dorhoi A, Mollenkopf HJ, Drogaris P, Lamontagne J, Zhou Y, Piquenot J, Park ST, Raman S, Kaufmann SH, Mohney RP, Chelsky D, Moody DB, Sherman DR, Schoolnik GK. 2013. The *Mycobacterium tuberculosis* regulatory network and hypoxia. *Nature* 499:178–183. <http://dx.doi.org/10.1038/nature12337>.
6. Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, Jiricek J, Jung J, Jeon HK, Cechetto J, Christophe T, Lee H, Kempf M, Jackson M, Lenaerts AJ, Pham H, Jones V, Seo MJ, Kim YM, Seo M, Seo JJ, Park D, Ko Y, Choi I, Kim R, Kim SY, Lim S, Yim SA, Nam J, Kang H, Kwon H, Oh CT, Cho Y, Jang Y, Kim J, Chua A, Tan BH, Nanjundappa MB, Rao SP, Barnes WS, Wintjens R, Walker JR, Alonso S, Lee S, Kim J, Oh S, Oh T, Nehrass U, Han SJ, No Z, Lee J, Brodin P, Cho SN, Nam K, Kim J. 2013. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. *Nat Med* 19:1157–1160. <http://dx.doi.org/10.1038/nm.3262>.
7. Queval CJ, Song OR, Delorme V, Iantomasi R, Veyron-Churlet R, Deboosere N, Landry V, Baulard A, Brodin P. 2014. A microscopic phenotypic assay for the quantification of intracellular mycobacteria adapted for high-throughput/high-content screening. *J Vis Exp* 83:e51114. <http://dx.doi.org/10.3791/51114>.
8. Stanley SA, Barczak AK, Silvis MR, Luo SS, Sogi K, Vokes M, Bray MA, Carpenter AE, Moore CB, Siddiqi N, Rubin EJ, Hung DT. 2014. Identification of host-targeted small molecules that restrict intracellular *Mycobacterium tuberculosis* growth. *PLoS Pathog* 10:e1003946. <http://dx.doi.org/10.1371/journal.ppat.1003946>.
9. VanderVen BC, Fahey RJ, Lee W, Liu Y, Abramovitch RB, Memmott C, Crowe AM, Eltis LD, Perola E, Deininger DD, Wang T, Locher CP, Russell DG. 2015. Novel inhibitors of cholesterol degradation in *Mycobacterium tuberculosis* reveal how the bacterium's metabolism is constrained by the intracellular environment. *PLoS Pathog* 11:e1004679. <http://dx.doi.org/10.1371/journal.ppat.1004679>.
10. Silva-Miranda M, Ekaza E, Breiman A, Asehnoune K, Barros-Aguirre D, Pethe K, Ewann F, Brodin P, Ballell-Pages L, Altare F. 2015. High-content screening technology combined with a human granuloma model as a new approach to evaluate the activities of drugs against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 59:693–697. <http://dx.doi.org/10.1128/AAC.03705-14>.
11. Rybniker J, Chen JM, Sala C, Hartkoorn RC, Vocat A, Benjak A, Boy-Rottger S, Zhang M, Szekely R, Greff Z, Orfi L, Szabadkai I, Pato J, Keri G, Cole ST. 2014. Anticytolytic screen identifies inhibitors of mycobacterial virulence protein secretion. *Cell Host Microbe* 16:538–548. <http://dx.doi.org/10.1016/j.chom.2014.09.008>.
12. North RJ, Izzo AA. 1993. Mycobacterial virulence. Virulent strains of *Mycobacteria tuberculosis* have faster in vivo doubling times and are better equipped to resist growth-inhibiting functions of macrophages in the presence and absence of specific immunity. *J Exp Med* 177:1723–1733.
13. Chanwong S, Maneekarn N, Makonkawkeyoon L, Makonkawkeyoon S. 2007. Intracellular growth and drug susceptibility of *Mycobacterium tuberculosis* in macrophages. *Tuberculosis (Edinb)* 87:130–133. <http://dx.doi.org/10.1016/j.tube.2006.06.001>.
14. Hartkoorn RC, Chandler B, Owen A, Ward SA, Bertel SS, Back DJ, Khoo SH. 2007. Differential drug susceptibility of intracellular and extracellular tuberculosis, and the impact of P-glycoprotein. *Tuberculosis (Edinb)* 87:248–255. <http://dx.doi.org/10.1016/j.tube.2006.12.001>.
15. Rey-Jurado E, Tudo G, Soy D, Gonzalez-Martin J. 2013. Activity and interactions of levofloxacin, linezolid, ethambutol and amikacin in three-drug combinations against *Mycobacterium tuberculosis* isolates in a human macrophage model. *Int J Antimicrob Agents* 42:524–530. <http://dx.doi.org/10.1016/j.ijantimicag.2013.07.014>.
16. Zhang Y, Scorpio A, Nikaido H, Sun Z. 1999. Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of *Mycobacterium tuberculosis* to pyrazinamide. *J Bacteriol* 181:2044–2049.
17. Shi W, Zhang X, Jiang X, Yuan H, Lee J S, Barry CE, III, Wang H, Zhang W, Zhang Y. 2011. Pyrazinamide inhibits trans-translation in *Mycobacterium tuberculosis*. *Science* 333:1630–1632. <http://dx.doi.org/10.1126/science.1208813>.
18. Grosset J, Almeida D, Converse PJ, Tyagi S, Li SY, Ammerman NC, Pym AS, Wallengren K, Hafner R, Lalloo U, Swindells S, Bishai WR. 2012. Modeling early bactericidal activity in murine tuberculosis provides insights into the activity of isoniazid and pyrazinamide. *Proc Natl Acad Sci U S A* 109:15001–15005. <http://dx.doi.org/10.1073/pnas.1203636109>.
19. Heifets L, Higgins M, Simon B. 2000. Pyrazinamide is not active against *Mycobacterium tuberculosis* residing in cultured human monocyte-derived macrophages. *Int J Tuberc Lung Dis* 4:491–495.
20. Zhang JH, Chung TD, Oldenburg KR. 1999. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen* 4:67–73. <http://dx.doi.org/10.1177/108705719900400206>.
21. Ballell L, Bates RH, Young RJ, Alvarez-Gomez D, Alvarez-Ruiz E, Barros V, Blanco D, Crespo B, Escribano J, Gonzalez R, Lozano S, Huss S, Santos-Villarejo A, Martin-Plaza JJ, Mendoza A, Rebollo-Lopez MJ, Remuñán-Blanco M, Lavandera JL, Perez-Herran E, Gamo-Benito FJ, Garcia-Bustos JF, Barros D, Castro JP, Cammack N. 2013. Fueling open-source drug discovery: 177 small-molecule leads against tuberculosis. *ChemMedChem* 8:313–321. <http://dx.doi.org/10.1002/cmcd.201200428>.
22. Griffin JE, Gawronski JD, Dejesus MA, Ioerger TR, Akerley BJ, Sassetti CM. 2011. High-resolution phenotypic profiling defines genes essential for mycobacterial growth and cholesterol catabolism. *PLoS Pathog* 7:e1002251. <http://dx.doi.org/10.1371/journal.ppat.1002251>.
23. Zhang YJ, Reddy MC, Ioerger TR, Rothchild AC, Dartois V, Schuster BM, Trauner A, Wallis D, Galaviz S, Huttenhower C, Sacchetti JC, Behar SM, Rubin EJ. 2013. Tryptophan biosynthesis protects mycobacteria from CD4 T-cell-mediated killing. *Cell* 155:1296–1308. <http://dx.doi.org/10.1016/j.cell.2013.10.045>.
24. Ehrst S, Rhee K, Schnappinger D. 2015. Mycobacterial genes essential for the pathogen's survival in the host. *Immunol Rev* 264:319–326. <http://dx.doi.org/10.1111/immr.12256>.
25. Weiss G, Schaible UE. 2015. Macrophage defense mechanisms against intracellular bacteria. *Immunol Rev* 264:182–203. <http://dx.doi.org/10.1111/immr.12266>.
26. Wickramasinghe SN. 1987. Evidence of drug metabolism by macrophages: possible role of macrophages in the pathogenesis of drug-induced tissue damage and in the activation of environmental procarcinogens. *Clin Lab Haematol* 9:271–280. <http://dx.doi.org/10.1111/j.1365-2257.1987.tb00091.x>.
27. Baik J, Rosania GR. 2011. Molecular imaging of intracellular drug-membrane aggregate formation. *Mol Pharm* 8:1742–1749. <http://dx.doi.org/10.1021/mp200101b>.
28. Baik J, Rosania GR. 2012. Macrophages sequester clofazimine in an intracellular liquid crystal-like supramolecular organization. *PLoS One* 7:e47494. <http://dx.doi.org/10.1371/journal.pone.0047494>.
29. Adams KN, Takaki K, Connolly LE, Wiedenhoft H, Winglee K, Humbert O, Edelstein PH, Cosma CL, Ramakrishnan L. 2011. Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. *Cell* 145:39–53. <http://dx.doi.org/10.1016/j.cell.2011.02.022>.
30. Remuñán MJ, Perez-Herran E, Rullas J, Alemparte C, Martinez-Hoyos M, Dow DJ, Afari J, Mehta N, Esquivias J, Jimenez E, Ortega-Muro F, Fraile-Gabaldon MT, Spivey VL, Loman NJ, Pallen MJ, Constantinidou C, Minick DJ, Cacho M, Rebollo-Lopez MJ, Gonzalez C, Sousa V, Angulo-Barturen I, Mendoza-Losana A, Barros D, Besra GS, Ballell L, Cammack N. 2013. Tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide and *N*-benzyl-6',7'-dihydrospiro[piperidine-4,4'-thieno[3,2-*c*]pyran] analogues with bactericidal efficacy against *Mycobacterium tuberculosis* targeting MmpL3. *PLoS One* 8:e60933. <http://dx.doi.org/10.1371/journal.pone.0060933>.
31. Grzegorzewicz AE, Pham H, Gundi VA, Scherman MS, North EJ, Hess T, Jones V, Gruppo V, Born SE, Kordulakova J, Chavadi SS, Morisseau C, Lenaerts AJ, Lee RE, McNeil MR, Jackson M. 2012. Inhibition of mycolic acid transport across the *Mycobacterium tuberculosis* plasma membrane. *Nat Chem Biol* 8:334–341. <http://dx.doi.org/10.1038/nchembio.794>.