

## Colworth prize lecture 2016:

Moynihan, Patrick; Besra, Gurdyal

DOI:

[10.1099/mic.0.000522](https://doi.org/10.1099/mic.0.000522)

License:

Other (please specify with Rights Statement)

*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Moynihan, P & Besra, G 2017, 'Colworth prize lecture 2016: exploiting new biological targets from a whole-cell phenotypic screening campaign for TB drug discovery', *Microbiology*, vol. 163, no. 10. <https://doi.org/10.1099/mic.0.000522>

[Link to publication on Research at Birmingham portal](#)

### **Publisher Rights Statement:**

Article originally published in *Microbiology* on 12/09/2017. <http://mic.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.000522>

### **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

1 **Colworth Prize Lecture 2016: Exploiting new biological targets**  
2 **from a whole-cell phenotypic screening campaign for TB drug**  
3 **discovery**

4  
5  
6 Patrick J. Moynihan<sup>1</sup> & Gurdyal S. Besra<sup>1,\*</sup>

7  
8 <sup>1</sup>Institute of Microbiology and Infection, School of Biosciences, University of Birmingham,  
9 Edgbaston, Birmingham, UK.

10  
11 \*Corresponding author: Telephone: +44 (0) 121 41 58125; Email: [g.besra@bham.ac.uk](mailto:g.besra@bham.ac.uk)

12  
13  
14 **Abstract**

15  
16 *Mycobacterium tuberculosis* is the etiological agent of tuberculosis (TB) and is the leading  
17 bacterial cause of mortality and morbidity in the world. One third of the world's population is  
18 infected with TB, and in conjunction with HIV represents a serious problem that urgently needs  
19 addressing. TB is a disease of poverty and mostly affects young adults in their productive years,  
20 primarily in the developing world. The most recent report from the World Health Organisation  
21 states that 8 million new cases of TB were reported and that ~1.5 million people died from TB.  
22 The efficacy of treatment is threatened by the emergence of multi-drug and extensively-drug  
23 resistant strains of *M. tuberculosis*. It can be argued that, globally, *M. tuberculosis* is the single  
24 most important infectious agent affecting mankind. Our research aims to establish an academic-  
25 industrial partnership with the goal of discovering new drug targets and hit-to-lead new  
26 chemical entities for TB drug discovery.

27  
28 **Introduction**

29 In 2015 seventeen new Sustainable Development Goals (SDGs) were adopted by the United  
30 Nations [1]. Included amongst these goals was the ambitious aim to 'end the epidemics of  
31 AIDS, tuberculosis, malaria and neglected tropical diseases' [1]. Despite progress towards  
32 reaching this aim, the frequency of multi-drug resistant (MDR) and extensively-drug resistant

1 (XDR) forms of TB is rising and threatening to undermine global TB containment efforts as  
2 current treatment regimens lose their efficacy [2]. The continued high prevalence of TB can, at  
3 least in part, be attributed to problems with current anti-TB drugs. The nature of the treatment,  
4 which involves a combination of up to four drugs taken for a minimum of six months, and  
5 associated side-effects, often causes patients to discontinue therapy prematurely, leading to  
6 infection relapse and exacerbation of the problem of drug resistance, which is already  
7 beginning to emerge for the recently approved TB drugs, Sirturo™ (TMC207) and Deltyba™  
8 (Delamanid) [3,4]. Whilst poverty remains one of the main drivers of the TB pandemic, the  
9 development of new drugs remains a critical component of any plan to tackle TB. It is therefore  
10 vital that we replenish the drug pipeline with new targets and leads to establish more robust  
11 combination regimens for treating MDR/XDR-TB in order to ease the economic and health  
12 burden of this disease on Society (Figure 1).

13 Many organisations, continue to employ a traditional target-based approach to antibiotic drug  
14 discovery, even in the knowledge that it is blighted by high attrition rates [5,6]. Applied to TB  
15 drug discovery, the problems of this approach are compounded by the limited number of  
16 validated targets. To address these challenges, we [7, reviewed in 8], and others [9-11], are  
17 increasingly turning to whole-cell screens to identify hits as well as new targets. Having  
18 demonstrated access to the target, hits are selected based upon their antibacterial activity and  
19 provide privileged starting points for target-focused medicinal chemistry programmes.

20 However, one of the greatest challenges of scientific research is effectively transitioning good  
21 ideas and excellent science into translational outcomes. This process is rarely straightforward  
22 and often involves significant elements of serendipity. The history of drug discovery in general  
23 and antibiotic development in particular is littered with compounds for which ideal  
24 translational outcomes were not met. This high rate of attrition reflects the challenging  
25 landscape of drug discovery. Efforts to improve this hit rate have intensified, largely based on  
26 “smarter” screening strategies which take into account more parameters. This is illustrated by  
27 the high-throughput screening (HTS) campaigns of GSK’s compound repository (>2.5 million  
28 compounds) which produced what is now referred to as the TB box-set (177 compounds) [7].  
29 A selection of these hits ( $MIC_{99} \sim 1 \mu M$ ) were ranked according to their anti-TB activity,  
30 cytotoxicity and physico-chemical properties (*e.g.* cLogP, molecular weight, polar surface  
31 area). A second phenotypic HTS of GSK’s new 254,053 diversity set, the profile of which  
32 reflects the latest intelligence on how specific physico-chemical property descriptors ( $sp^3$   
33 character, lipophilicity/water solubility, molecular size) affect attrition at the various stages of

1 drug discovery after filtering by SMARTS and pIC50 data, provided 51 additional hits against  
2 *M. tuberculosis* with MIC<sub>99</sub> < 10 μM, and expanded the TB box-set to 228 compounds.

3 The process of improving a screen however still rests firmly on a strong foundation of basic  
4 biology. For example, the explosion in genomic sequencing has lead to unparalleled  
5 information about the molecular blue-print for all forms of life. The success of genomic  
6 annotation is informed by and depends upon on the availability of classical molecular and  
7 biochemical studies. The wealth of genomic sequencing would have a fraction of its usefulness  
8 if it were not for this basic functional information. It is possible that within the TB-Box-set  
9 may well be the next rifampicin or isoniazid, however without detailed knowledge of their  
10 mode of action, they remain un-useable. As a consequence, the success of phenotypic screening  
11 in (TB) drug discovery rests on there being efficient strategies for elucidating the cellular  
12 targets of identified hits. Fortunately, state-of-the-art genomic, proteomic and metabolomic  
13 tools are facilitating accelerated target identification, making whole-cell screens a viable (if not  
14 now preferred) alternative to the traditional methodologies that have been used to identify anti-  
15 TB agents and, just as importantly, new targets. Nevertheless, this approach is not without its  
16 challenges: in many instances, target identification rests on the generation of spontaneous drug-  
17 resistant mutants, with the expectation that resistance-conferring mutations, revealed by whole-  
18 genome sequencing (WGS), identifies the protein target of a given hit. *M. tuberculosis* F<sub>0</sub>F<sub>1</sub>  
19 ATP synthase, for example, was identified in this way as the target for TMC207 [3]. However,  
20 resistance can occur through other mechanisms, which means that spontaneous drug-resistant  
21 mutations may not only arise in the drug target but also in other cellular proteins that interact  
22 with the inhibitor (*e.g.* InhA/KatG in the case of isoniazid [12,13]) or indeed the target. For  
23 these reasons, a strategy involving parallel orthogonal approaches must be used to ensure  
24 definitive and robust target identification. In this way, the development of new antimicrobials  
25 must be intimately tied to basic biology and the study of both the host and the pathogen. Three  
26 recent examples typify this relationship.

27

### 28 **It's all in the details**

29

30 The nitro-benzothiazinone (BTZ) family of anti-tubercular compounds were first identified in  
31 2009 [14]. In that study, the BTZs were found to block the synthesis of the essential  
32 mycobacterial cell-wall polymer arabinan. A more precise mode of action was determined

1 through a combination of structure-activity studies, resistant mutant generation and  
2 transcriptomics, leading Makaraov and colleagues to identify DprE1 as the target of the BTZ  
3 compounds. This was a critical step in defining mode-of-action for these compounds, but was  
4 lacking molecular details. In 2012, two independent studies solved this problem by determining  
5 the first crystal structure of DprE1 in complex with BTZ [15,16]. The solved structures  
6 highlighted the critical moieties of the BTZ compound for DprE1 inhibition, information which  
7 is critical for lead-optimisation. Further studies demonstrated that the lethality of the BTZ  
8 compounds comes about not only through inhibition of DprE1 (and therefore blocking arabinan  
9 biosynthesis), but also as a result of a blockage in decaprenyl-phosphate recycling. This was  
10 demonstrated by the viability of a *Corynebacterium glutamicum* mutant lacking the enzyme  
11 acting upstream of DprE1, UbiA thereby also generating an arabinan-less mutant but one which  
12 does not generate decaprenyl-intermediates as a dead end [17]. The accumulation of  
13 decaprenyl-phosphoarabinose acts as a sink for this critical carrier molecule, which is in turn  
14 lethal. A detailed understanding of this relationship allows for a more nuanced view of how the  
15 pathway can be interfered with in order to kill these bacteria.

16

### 17 **A case of mistaken identity**

18

19 In 2013, the tetrahydropyrazolo[1,5-*a*]pyrimidine-3-carboxamide (THPP) family of  
20 compounds were demonstrated to have remarkable anti-tubercular effects. This compound  
21 series was first described by Remuiñán *et al* in 2013 [18]. Using a combination of resistant  
22 mutant generation and lipid profiling a putative trehalose-monomycolate transporter named  
23 MmpL3, was concluded to be the likely target for these compounds. Surprisingly, MmpL3 had  
24 also been determined to be the likely target of several distinct classes of molecules including  
25 SQ109, adamantyl ureas, BM212, N-benzyl- 6',7'-dihydrospiro[piperidine-4,4'-thieno[3,2-  
26 c]pyran] and indolcarboxamides [19-24]. By combining traditional biochemical and lipid  
27 profiling methods with cutting edge chemical proteomics and genetic screens, Cox and  
28 colleagues were able to show that the target of the THPPs is actually a crotonase-like protein  
29 called EchA6 and not MmpL3 [25]. This protein appears to play a role in a shunt pathway  
30 between the FAS-I and -II fatty acid synthesis pathways and so loss of EchA6 phenocopies the  
31 mycolate-lipid profile associated with MmpL3 depletion. The role of MmpL3 in this pathway  
32 is still somewhat murky. The simplest explanation is that it is moon-lighting as a drug importer,  
33 a feature which could have profound consequences in drug discovery. This re-assignment  
34 highlights the need for better MmpL3 functional assays and the importance of unbiased

1 approaches, such as chemical proteomics to determine mode-of-action for hit compounds.

## 2 **A new scaffold for a known target**

3 The biosynthesis of mycolic acids is one of the best developed targets for mycobacterial-  
4 specific antibiotics. These cell-wall polymers form the outer membrane of the bacterium and  
5 are essential for their viability [26]. The key anti-mycobacterial drug isoniazid exerts its effect  
6 by blocking the enoyl-acyl carrier protein InhA [13]. A wealth of basic biochemistry and  
7 biology has gone into understanding the mechanism of this pro-drug and has primed the field  
8 for study of this pathway. A new family of indazole sulfonamides were found to possess anti-  
9 tubercular activity against KasA [27]. KasA is a condensing enzyme in the FAS-II fatty acid  
10 synthesis pathway and is essential for viability in mycobacteria [28]. Past studies have  
11 identified KasA inhibitors, including thiolactomycin which inhibits a broad array of Kas-like  
12 enzymes [29]. Critical to the success of the key hit indazole sulfonamide is that it has excellent  
13 pharmacokinetic properties, allowing for *in vivo* studies supporting its development as an anti-  
14 tubercular compound. While resistant-mutant generation suggested that KasA was indeed the  
15 target of this compound, prior experience with the THPPs highlighted the need for more robust  
16 target identification. In this case that included structural biology, chemical proteomics and *in*  
17 *vitro* biochemical assays. The co-crystal structure of KasA and hit molecule from the indazole  
18 sulfonamide series identified a distinct inhibition mechanism from existing inhibitors  
19 explaining the remarkable specificity of this series.

## 20 **Conclusion**

21 The field of mycobacterial drug discovery is littered with compounds that are either potent  
22 against target enzymes but have poor anti-bacterial power or have good anti-bacterial power  
23 but unknown mechanism(s). This high rate of attrition is made worse by the large number of  
24 compounds that prove to be cytotoxic or have otherwise poor pharmacokinetic properties.  
25 Central to this is the need for robust mode-of-action pipelines using a combination of traditional  
26 and modern tools. The three studies described above further highlight the absolute requirement  
27 of a strong basic science background in the field to enable drug discovery. This is where the  
28 interface between academic and industrial science is made very important. The open drug-  
29 discovery initiative spear-headed by GSK is an excellent example of the promise of this type  
30 of collaboration. Through their efforts in identifying anti-mycobacterial compounds with good  
31 pharmacokinetic properties the wealth of knowledge about the tubercule bacilli generated in

1 academia and elsewhere can be leveraged to develop new drugs aimed at tackling one of the  
2 biggest challenges in human-health today.

### 3 **Acknowledgements**

4 GSB acknowledges support in the form of a Personal Research Chair from Mr. James Bardrick  
5 and the UK Medical Research Council (grant MR/K012118/1) and The Wellcome Trust (grant  
6 081569/Z/06/Z). PJM acknowledges support in the form of a Future Leaders Fellowship from  
7 the UK Biotechnology and Biological Sciences Research Council (grant BB/N011945/1).

### 8 **Conflict of Interest**

9 The authors have no conflicts of interest to declare.

### 10 **References**

- 11 1. **United Nations**. Draft Outcome Document of the United Nations Summit for the  
12 Adoption of the Post-2015 Development Agenda. New York: Sixty-ninth session of  
13 the General Assembly of the United Nations; 2015.
- 14 2. **World Health Organisation**. TB Fast Sheet No. 104. Geneva: World Health  
15 Organization; 2014.
- 16 3. **Andries K, Verhasselt P, Guillemont J, Göhlmann HWH, Neefs J-M, et al**. A  
17 diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*.  
18 *Science* 2005;307:223–227.
- 19 4. **Bloemberg GV, Keller PM, Stucki D, Stuckia D, Trauner A, et al**. Acquired  
20 Resistance to Bedaquiline and Delamanid in Therapy for Tuberculosis. *N. Engl. J.*  
21 *Med.* 2015;373:1986–1988.
- 22 5. **Palomino JC, Martin A**. TMC207 becomes bedaquiline, a new anti-TB drug. *Future*  
23 *Microbiol* 2013;8:1071–1080.
- 24 6. **Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL**. Drugs for bad bugs:  
25 confronting the challenges of antibacterial discovery. *Nature Reviews Drug Discovery*  
26 2007;6:29–40.
- 27 7. **Ballell L, Bates RH, Young RJ, Alvarez-Gomez D, Alvarez-Ruiz E, et al**. Fueling  
28 open-source drug discovery: 177 small-molecule leads against tuberculosis.  
29 *ChemMedChem* 2013;8:313–321.
- 30 8. **Cooper CB**. Development of *Mycobacterium tuberculosis* whole cell screening hits as  
31 potential antituberculosis agents. *J Med Chem* 2013;56:7755–7760.
- 32 9. **Grant SS, Kawate T, Nag PP, Silvis MR, Gordon K, et al**. Identification of novel  
33 inhibitors of nonreplicating *Mycobacterium tuberculosis* using a carbon starvation

- 1 model. *ACS Chem. Biol.* 2013;8:2224–2234.
- 2 10. **Reynolds RC, Ananthan S, Faaleolea E, Hobrath JV, Kwong CD, et al.** High  
3 throughput screening of a library based on kinase inhibitor scaffolds against  
4 *Mycobacterium tuberculosis* H37Rv. *Tuberculosis* 2012;92:72–83.
- 5 11. **Manjunatha UH, Smith PW.** Perspective: Challenges and opportunities in TB drug  
6 discovery from phenotypic screening. *Bioorg. Med. Chem.* 2015;23:5087–5097.
- 7 12. **Zhang Y, Heym B, Allen B, Young D, Cole S.** The catalase-peroxidase gene and  
8 isoniazid resistance of *Mycobacterium tuberculosis*. *Nature* 1992;358:591–593.
- 9 13. **Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, et al.** *inhA*, a  
10 gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*.  
11 *Science* 1994;263:227–230.
- 12 14. **Makarov V, Manina G, Mikušová K, Möllmann U, Ryabova O, et al.**  
13 Benzothiazinones kill *Mycobacterium tuberculosis* by blocking arabinan synthesis.  
14 *Science* 2009;324:801–804.
- 15 15. **Batt SM, Jabeen T, Bhowruth V, Quill L, Lund PA, et al.** Structural basis of  
16 inhibition of *Mycobacterium tuberculosis* DprE1 by benzothiazinone inhibitors. *Proc*  
17 *Natl Acad Sci USA* 2012;109:11354–11359.
- 18 16. **Neres J, Pojer F, Molteni E, Chiarelli LR, Dhar N, et al.** Structural basis for  
19 benzothiazinone-mediated killing of *Mycobacterium tuberculosis*. *Science*  
20 *Translational Medicine* 2012;4:150ra121.
- 21 17. **Grover S, Alderwick LJ, Mishra AK, Krumbach K, Marienhagen J, et al.**  
22 Benzothiazinones Mediate Killing of Corynebacterineae by Blocking Decaprenyl  
23 Phosphate Recycling Involved in Cell Wall Biosynthesis. *J Biol Chem*  
24 2014;289:6177–6187.
- 25 18. **Remuiñán MJ, Pérez-Herrán E, Rullas J, Alemparte C, Martínez-Hoyos M, et al.**  
26 Tetrahydropyrazolo[1,5-a]pyrimidine-3-carboxamide and N-benzyl-6',7'-  
27 dihydrospiro[piperidine-4,4'-thieno[3,2-c]pyran] analogues with bactericidal efficacy  
28 against *Mycobacterium tuberculosis* targeting MmpL3. *PLoS ONE* 2013;8:e60933.
- 29 19. **Grzegorzewicz AE, Pham H, Gundi VAKB, Scherman MS, North EJ, et al.**  
30 Inhibition of mycolic acid transport across the *Mycobacterium tuberculosis* plasma  
31 membrane. *Nat Chem Biol* 2012;8:334–341.
- 32 20. **La Rosa V, Poce G, Canseco JO, Buroni S, Pasca MR, et al.** MmpL3 is the cellular  
33 target of the antitubercular pyrrole derivative BM212. *Antimicrob Agents Chemother*  
34 2012;56:324–331.
- 35 21. **Li K, Schurig-Briccio LA, Feng X, Upadhyay A, Pujari V, et al.** Multitarget drug  
36 discovery for tuberculosis and other infectious diseases. *J Med Chem* 2014;57:3126–  
37 139.
- 38 22. **Li W, Upadhyay A, Fontes FL, North EJ, Wang Y, et al.** Novel insights into the  
39 mechanism of inhibition of MmpL3, a target of multiple pharmacophores in



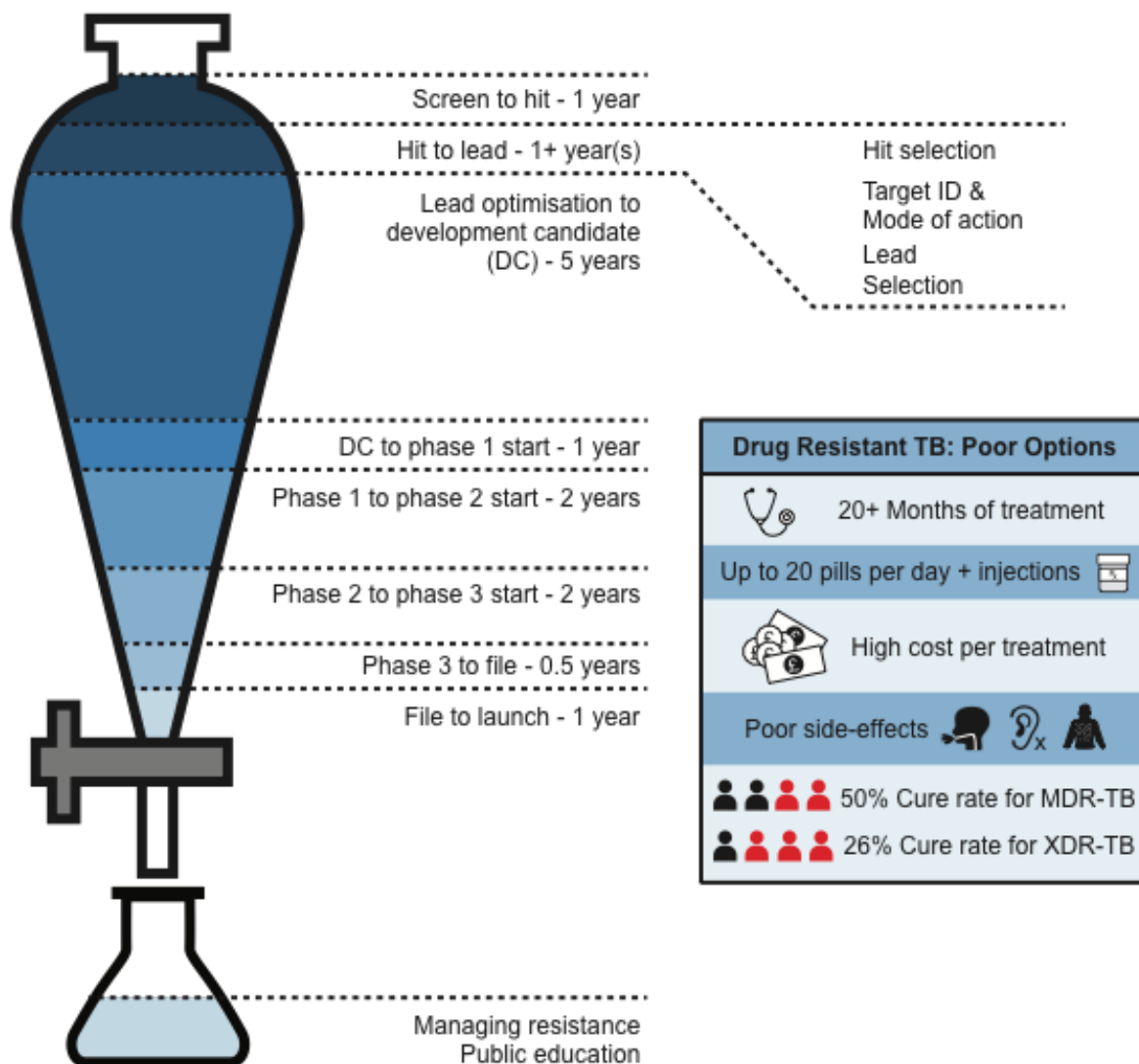
- 1 *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2014;58:6413–6423.
- 2 23. **Rao SPS, Lakshminarayana SB, Kondreddi RR, Herve M, Camacho LR, et al.**  
3 Indolcarboxamide is a preclinical candidate for treating multidrug-resistant  
4 tuberculosis. *Science Translational Medicine* 2013;5:214ra168.
- 5 24. **Tahlan K, Wilson R, Kastrinsky DB, Arora K, Nair V, et al.** SQ109 targets  
6 MmpL3, a membrane transporter of trehalose monomycolate involved in mycolic acid  
7 donation to the cell wall core of *Mycobacterium tuberculosis*. *Antimicrob Agents*  
8 *Chemother* 2012;56:1797–1809.
- 9 25. **Cox JAG, Abrahams KA, Alemparte C, Ghidelli-Disse S, Rullas J, et al.** THPP  
10 target assignment reveals EchA6 as an essential fatty acid shuttle in mycobacteria.  
11 *Nature Microbiology* 2016;1:15006.
- 12 26. **Jankute M, Cox JAG, Harrison J, Besra GS.** Assembly of the Mycobacterial Cell  
13 Wall. *Annu Rev Microbiol* 2015;69:405–423.
- 14 27. **Abrahams KA, Chung C-W, Ghidelli-Disse S, Rullas J, Rebollo-López MJ, et al.**  
15 Identification of KasA as the cellular target of an anti-tubercular scaffold. *Nat*  
16 *Commun* 2016;7:12581.
- 17 28. **Bhatt A, Kremer L, Dai AZ, Sacchetti JC, Jacobs WR.** Conditional depletion of  
18 KasA, a key enzyme of mycolic acid biosynthesis, leads to mycobacterial cell lysis. *J*  
19 *Bacteriol* 2005;187:7596–7606.
- 20 29. **Kremer L, Douglas JD, Baulard AR, Morehouse C, Guy MR, et al.**  
21 Thiolactomycin and related analogues as novel anti-mycobacterial agents targeting  
22 KasA and KasB condensing enzymes in *Mycobacterium tuberculosis*. *J Biol Chem*  
23 2000;275:16857–16864.

24

25

# 1 Figures

## Separating good drugs from compound libraries:



2

3 **Figure 1. Separating good drugs from compound libraries.** The rise of MDR- and XDR-TB has

4 highlighted the poor options that populate first and second-line drug formulations. The identification of new

5 drugs is a long and difficult process in which very few compounds make it successfully from screening libraries

6 to useful medicines. Compounds are eliminated for a variety of reasons including poorly defined mode(s)-of-

7 action, poor pharmacokinetic-pharmacodynamic properties and toxicity among others. The identification of

8 good hits in tuberculosis drug-discovery has significantly benefited from industry-academic collaborations. In

9 particular, this has recently been enhanced through more robust mode-of-action studies and whole-cell

10 phenotypic screening.