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

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Abstract

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

Introduction

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

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

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

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

drug discovery after filtering by SMARTS and pIC50 data, provided 51 additional hits against *M. tuberculosis* with MIC_{99} < 10 μM, and expanded the TB box-set to 228 compounds.

The process of improving a screen however still rests firmly on a strong foundation of basic biology. For example, the explosion in genomic sequencing has lead to unparalleled information about the molecular blue-print for all forms of life. The success of genomic annotation is informed by and depends upon on the availability of classical molecular and biochemical studies. The wealth of genomic sequencing would have a fraction of its usefulness if it were not for this basic functional information. It is possible that within the TB-Box-set may well be the next rifampicin or isoniazid, however without detailed knowledge of their mode of action, they remain un-useable. As a consequence, the success of phenotypic screening in (TB) drug discovery rests on there being efficient strategies for elucidating the cellular targets of identified hits. Fortunately, state-of-the-art genomic, proteomic and metabolomic tools are facilitating accelerated target identification, making whole-cell screens a viable (if not now preferred) alternative to the traditional methodologies that have been used to identify anti-TB agents and, just as importantly, new targets. Nevertheless, this approach is not without its challenges: in many instances, target identification rests on the generation of spontaneous drug-resistant mutants, with the expectation that resistance-conferring mutations, revealed by whole-genome sequencing (WGS), identifies the protein target of a given hit. *M. tuberculosis* F_0F_1 ATP synthase, for example, was identified in this way as the target for TMC207 [3]. However, resistance can occur through other mechanisms, which means that spontaneous drug-resistant mutations may not only arise in the drug target but also in other cellular proteins that interact with the inhibitor (*e.g.* InhA/KatG in the case of isoniazid [12,13]) or indeed the target. For these reasons, a strategy involving parallel orthogonal approaches must be used to ensure definitive and robust target identification. In this way, the development of new antimicrobials must be intimately tied to basic biology and the study of both the host and the pathogen. Three recent examples typify this relationship.

It's all in the details

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

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

A case of mistaken identity

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

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

A new scaffold for a known target

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

Conclusion

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

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

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Figures

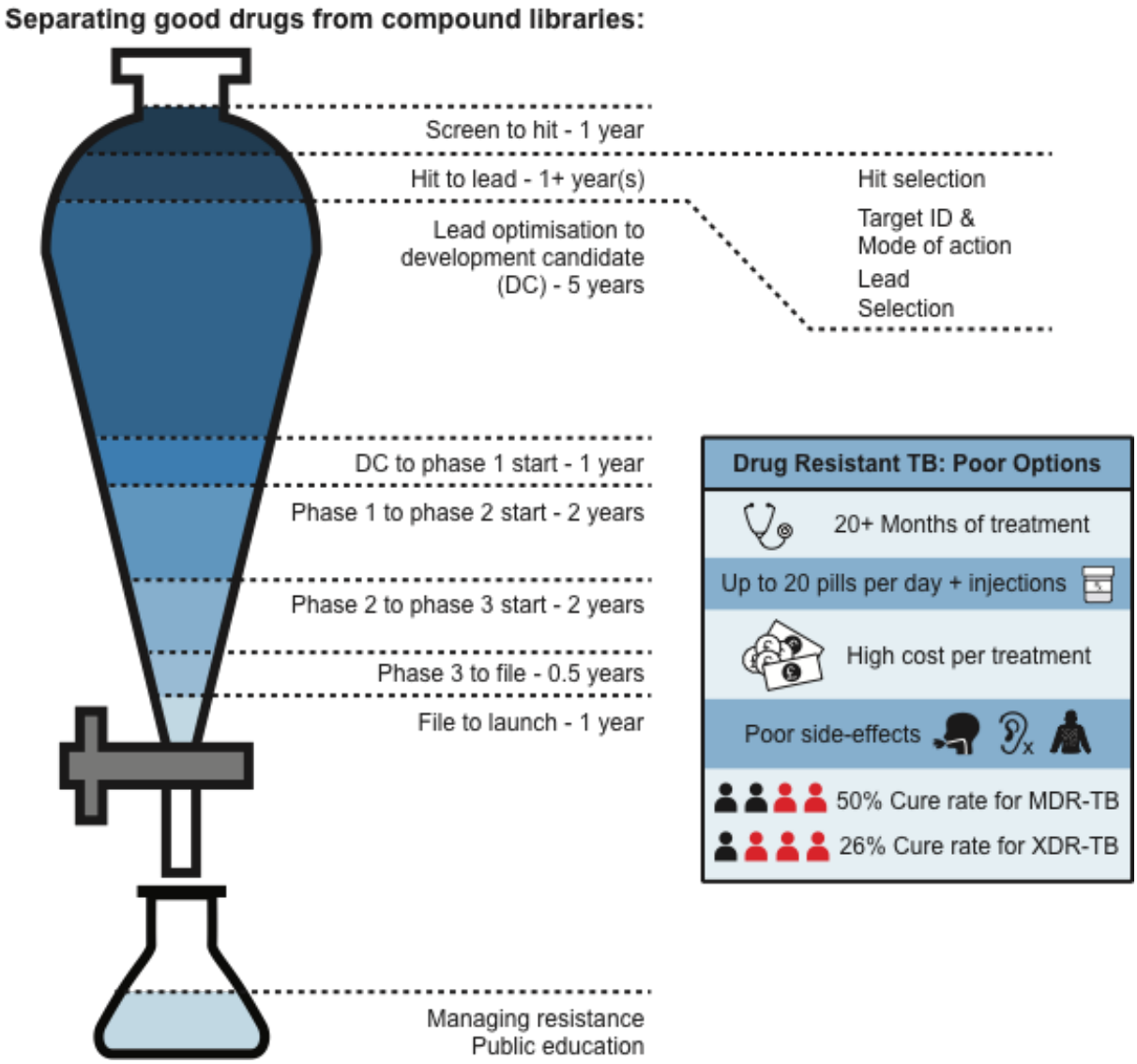

Figure 1. **Separating good drugs from compound libraries.** The rise of MDR- and XDR-TB has highlighted the poor options that populate first and second-line drug formulations. The identification of new drugs is a long and difficult process in which very few compounds make it successfully from screening libraries to useful medicines. Compounds are eliminated for a variety of reasons including poorly defined mode(s)-of-action, poor pharmacokinetic-pharmacodynamic properties and toxicity among others. The identification of good hits in tuberculosis drug-discovery has significantly benefited from industry-academic collaborations. In particular, this has recently been enhanced through more robust mode-of-action studies and whole-cell phenotypic screening.