Drug-resistance mechanisms and tuberculosis drugs

Claudio U Köser,* Babak Javid, Kathleen Liddell, Matthew J Ellington, Silke Feuerriegel, Stefan Niemann, Nicholas M Brown, William J Burman, Ibrahim Abubakar, Nazir A Ismail, David Moore, Sharon J Peacock, and M Estée Török

Department of Medicine, Addenbrooke's Hospital, University of Cambridge, Cambridge CB2 0QW, UK (CUK, BJ, SJP, MET);
School of Medicine, Tsinghua University, Beijing, China (BJ);
Faculty of Law, University of Cambridge, Cambridge, UK (KL);
Clinical Microbiology and Public Health Laboratory, Public Health England, Cambridge, UK (MJE, NMB, SJP, MET);
Molecular Mycobacteriology, Research Center Borstel, Borstel, Germany (SF, SN);
German Centre for Infection Research, Borstel, Germany (SF);
Denver Public Health, Denver, USA (WJB);
Tuberculosis Section, Centre for Infectious Disease Surveillance and Control, Public Health England, London, UK (IA);
Research Department of Infection and Population Health, University College London, London, UK (IA);
Centre for Tuberculosis, National Institute for Communicable Diseases, Johannesburg, South Africa (NAI);
TB Centre, London School of Hygiene and Tropical Medicine, London, UK (DM);
Laboratorio de Investigación de Enfermedades Infecciosas, Universidad Peruana Cayetano Heredia, Lima, Peru (DM);
Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK (SJP, MET);
and Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK (SJP)
cuk21@cam.ac.uk

Bedaquiline and delamanid, novel classes of anti-tuberculosis drugs, have been recently approved for the treatment of multidrug-resistant tuberculosis.[1] Antimicrobial resistance invariably follows the introduction of new drugs, and appropriate drug-susceptibility testing assays are needed to detect resistance and tailor treatment regimens that contain new agents.[2,3] Given that phenotypic drug-susceptibility testing is slow, technically demanding, and, in some cases, unreliable, future assays are likely to be based on rapid molecular techniques. To design such assays, research to unravel the genetic basis of resistance is urgently required (appendix).[2] The question is how to ensure that this research occurs in a timely way, before the emergence and spread of resistance.

A potential solution is to link the elucidation of resistance mechanisms to the approval process for new antibiotics, as is already the case for resistance to antivirals.[4-6] Where appropriate, this approach should also include the resistance mechanisms of older antibiotics that will be included in new regimens. For many bacteria and antibiotics it is not feasible to identify resistance before market release because of horizontal transfer of resistance genes between bacteria. By contrast, resistance in the *Mycobacterium tuberculosis* complex (MTBC) arises exclusively by chromosomal changes.[7] Therefore, mechanisms of resistance can be studied by multiple methods, including the selection of drug-resistant mutants in vitro and in-vivo animal infection models, and by examining drug-resistant mutants from clinical trials.[8]

Next-generation sequencing showed that bedaquiline resistance arises through mutations in the ATP synthase.[9,10] Yet it was only after regulatory approval of bedaquiline—and more than 8 years after the identification of the target of bedaquiline—that it was shown that resistance can also arise through the mutational upregulation of an efflux pump.[8,10,11] Importantly, this mechanism also confers cross-resistance to clofazimine.[3,8,11] As a result, regimens that contain both drugs might have to be reconsidered if these mutations are found to be common and to increase the minimum inhibitory concentrations significantly to reduce treatment success.[12,13] It is questionable whether these regimens would have been evaluated at all, had the bedaquiline resistance mechanisms been elucidated comprehensively in the early stages

of drug development. Moreover, had this genetic information been available at the time of approval of bedaquiline, regulators might have required for this cross-resistance to be formally labelled.[3,8,11]

The early identification of resistance mechanisms would also minimise the chance of developing antibiotics that are not effective across the world.[7] Clinical trials only include patients infected with a limited number of MTBC genotypes, which raises the possibility that intrinsic antibiotic resistance could be missed.[7] By contrast, intrinsically resistant strains could be screened for by assessing the conservation of resistance genes in the genomes of the thousands of phylogenetically diverse MTBC isolates that have been sequenced to date.[7] This approach has already raised the possibility that *Mycobacterium canettii*, which causes tuberculosis in the Horn of Africa and is intrinsically resistant to pyrazinamide, might also be intrinsically resistant to PA-824.[7,14] Consequently, the regimen of PA-824/pyrazinamide/moxifloxacin, which is about to be assessed in phase 3 clinical trials, might lead to monotherapy of patients with *M canettii* infection.[12]

The development and periodic revision of guidelines to determine resistance mechanisms as part of drug development would benefit from close cooperation between academic experts, funding agencies, pharmaceutical companies, and regulatory authorities, as has occurred for antivirals in the past.[3-6,15] Such work would require a flexible approach, depending on the properties of the particular antibiotic. For example, it might not be readily possible to select for in-vitro resistance to some agents.[16] An analysis of the detailed mechanism of resistance would be desirable but not essential for the approval of new agents.

There would be many advantages in sharing the resulting strain collections, sequence data, markers of resistance, and drug-susceptibility testing results, as is standard practice in HIV research.[17] We, therefore, have serious concerns about the patenting of resistance mechanisms, which has already occurred for several tuberculosis resistance mechanisms. For example, a university patented the "isolated" nucleic acid sequence of *pncA* (patent number US5846718), mutations in which confer resistance to pyrazinamide. This claim was probably invalidated by the US Supreme Court ruling in Association for Molecular Pathology *v.*Myriad Genetics in 2013, which found that a "naturally occurring DNA segment is a product of nature and not patent-eligible merely because it has been isolated".[18] This ruling has no direct bearing on the equivalent *pncA* patents granted in Canada (CA2254828) and Europe (EP0904410), all of which have lapsed for other reasons.

The patenting of isolated genes remains legal in many countries, as affirmed most recently by the Federal Court of Australia.[19,20] More recently, the same university filed a patent for the detection of *rpsA*mutations as a marker for pyrazinamide resistance that could potentially cover any molecular method to detect mutations in this gene.[21] In light of the ruling by the US Supreme Court in Mayo *v.* Prometheus, however, a biomarker patent of this kind is unlikely to be valid in the USA because the correlation between*rpsA* mutations and pyrazinamide resistance would be regarded as a law of nature.[18] Whether similar biomarker patents could be refused or invalidated in other jurisdictions is less clear.

Irrespective of the legality of such patents, we are concerned by attempts to monopolise knowledge about resistance mechanisms, including through the use of trade secrets in relation to clinical data.[22]Understanding resistance mechanisms is vital for the safe and effective treatment of patients, as well as for long-term antibiotic stewardship. The early and comprehensive elucidation of resistance mechanisms to drugs for tuberculosis during drug development is in the common interest of patients, clinicians, academics, and pharmaceutical companies. Moreover, the resulting knowledge should be made publicly available at no cost.

This needs appropriate regulatory and business models for antibiotic drug development that promote or mandate public sharing of knowledge about resistance and its mechanisms,[17] as well as addressing the many other tensions in antibiotic innovation.[23]

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Footnotes

KL, IA, NAI, and KL declare no competing interests.

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Supplementary Appendix

Depending on the nature of the resistance mechanism(s) to a novel antibiotic, not all technologies may be appropriate to detect them. Rapid molecular assays, such as the Cepheid GeneXpert system, can be used directly on clinical samples but only interrogate short stretches of bacterial DNA.[1] In the case of PA-824 (pretomanid) resistance can arise through mutations in five non-essential genes (*fgd1*, *fbiC*, *fbiA*, *fbiB* and *ddn*) with a total length of 6.4 kilobase pairs.[2-4] Therefore phenotypic drug-susceptibility testing or rapid whole-genome sequencing might be the only diagnostic option to detect resistance to this agent,[5, 6] unless one particular set(s) of mutations are found to be dominant in clinical isolates, as is the case with *katG* mutations and high-level isoniazid resistance.[7, 8] Irrespective of the technology used, a comprehensive understanding of the natural diversity in the resistance gene(s) for each antibiotic is crucial to avoid systematic false-positive results due to polymorphisms that do not cause resistance.[9-12]

The study of large collections of *in vitro* and *in vivo* mutants to correlate different mutations with minimum inhibitory concentrations (MICs) might inform the design of pre-clinical and clinical trials and, subsequently, improved diagnostic and screening tests.[12] If certain mechanisms or mutations led to only marginally elevated MICs (as is the case with *inhA* mutations and isoniazid[9] or with certain *rpoB* mutations and rifampicin[13]), higher doses or more frequent dosing of the novel therapeutic agent to overcome this low level of resistance could be evaluated.[14] This would benefit patients, clinicians and pharmaceutical companies by increasing the number of TB cases that could be treated with that particular drug. The resulting knowledge could also be used to determine appropriate breakpoints for phenotypic assays – which is not straightforward for MTBC[15, 16] – and to validate the reliability of these assays.[17] Finally, individual mutants could be used to quantify the risk of developing further resistance, as certain low-level resistance mechanisms have been found to increase the chance of developing high-level resistance.[11, 18]

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