Drug-resistance mechanisms and tuberculosis drugs

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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.

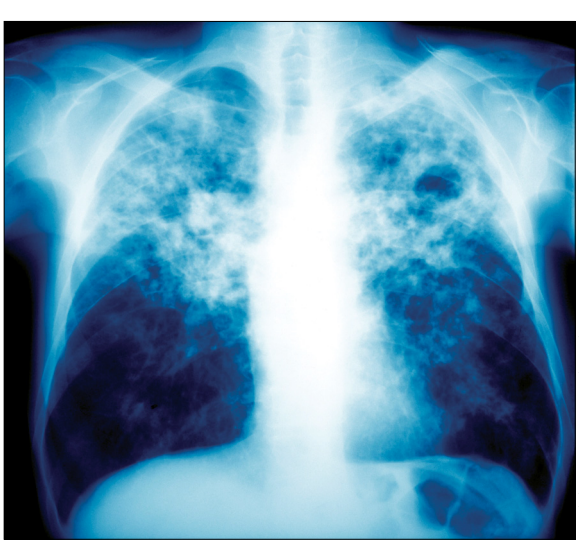
A potential solution is to link the elucidation of resistance mechanisms to the approval process for new antibiotics, as is already the case for resistance to antivirals.[4-6] Where appropriate, this approach should also include the resistance mechanisms of older antibiotics that will be included in 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]

See Online for appendix

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



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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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Familial hypercholesterolaemia: PCSK9 inhibitors are coming

Familial hypercholesterolaemia is an inherited disorder that substantially increases risk of coronary artery disease.[1,2] Recent population data show that the heterozygous and homozygous forms of familial hypercholesterolaemia affect one in 200[1] and one in 160 000–300 000 people,[3] respectively, worldwide. Plasma concentrations of LDL cholesterol (LDL-C) are increased in the disorder because of hypocatabolism of LDL particles by the liver, which is related to the severity of underlying genetic mutations mostly affecting the LDL receptor gene. The clinical sequelae are tendon xanthomas, premature coronary artery disease, and, in homozygous familial hypercholesterolaemia, valvular or supravalvular aortic stenosis.[4] The raised risk of coronary artery disease in familial hypercholesterolaemia is caused by lifelong exposure to high LDL-C from birth.[1] Recent evidence has also reaffirmed a proatherogenic effect of raised concentrations of the LDL-like particle plasma lipoprotein(a).[5]

Statins lower plasma LDL-C concentrations and improve coronary artery disease outcomes and survival in familial hypercholesterolaemia[6] by increasing hepatic expression of the LDL receptor and plasma clearance of LDL. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secretory serine protease that homoeostatically regulates the amount of plasma LDL-C by interacting with the LDL receptor.[7] After binding and internalisation, PCSK9 directs the LDL receptor to lysosomal degradation, inhibiting its recycling to the hepatocyte surface and thus catabolism of plasma LDL. Therapeutic inhibition of HMGCoA reductase by statins upregulates PCSK9,[7] limiting the effectiveness of statins in lowering plasma LDL-C. Gain-of-function mutations in the *PCSK9* gene cause a phenotype that mimics familial hypercholesterolaemia.[1,7]

Despite the effectiveness of cholesterol-lowering therapies, most patients with familial hypercholesterolaemia cannot achieve plasma LDL cholesterol targets recommended to prevent cardiovascular events.[1,8] This challenge is virtually universal in patients with homozygous familial hypercholesterolaemia, for whom an apoB100 antisense oligonucleotide (mipomersen)[9] and a microsomal triglyceride transfer protein inhibitor (lomitapide)[10] were recently approved as adjunctive treatments that can reduce LDL-C by a further 25–40%.[9,10] However, this reduction is achieved at substantially increased monetary costs and clinical side-effects.[9–11]

The recent development of monoclonal antibodies against PCSK9 is a revolution in the treatment of familial and other types of hypercholesterolaemia. These drugs, injected subcutaneously every 15 or 30 days, bind

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