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A mutation associated with clofazimine and bedaquiline cross-resistance in MDR-TB following bedaquiline treatment

To the Editor:

The world-wide increase in the incidence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) poses a major clinical challenge. The treatment outcome of MDR-TB and XDR-TB patients is often poor and unsuccessful in the absence of an optimal number of active drugs [1]. Novel antituberculous compounds are urgently required and only very few, such as bedaquiline, have recently been approved for tuberculosis treatment [2]. In a recent phase 2b clinical trial that was based on a 160 newly diagnosed MDR-TB patients, the addition of bedaquiline to a preferred background regimen for 24 weeks resulted in faster culture conversion and significantly more culture conversion at 120 weeks compared with the control group. However, there were more deaths in the bedaquiline than in the placebo group and half of these patients died due to tuberculosis. So far, it is unclear whether the death of any of these patients may have been associated with diminished susceptibility to bedaquiline [3].

Our study indicates that emergence of drug resistance to bedaquiline is already an ongoing threat, as we provide *in vivo* evidence of acquired resistance due to a mutation in an efflux pump-related gene, and its association with clofazimine and bedaquiline cross resistance in an *Mycobacterium tuberculosis* isolate from a patient with MDR-TB. In January 2011, a Tibetan refugee hospitalised with bilateral cavernous chest radiograph abnormality was diagnosed with MDR-TB at the Swiss Reference Center for Mycobacteria, Zurich, Switzerland. The *M. tuberculosis* isolate from the patient showed resistance to isoniazid, rifampicin, pyrazinamide, ethionamide, linezolid, moxifloxacin and streptomycin by quantitative drug susceptibility testing (DST) in the BACTEC MGIT 960 system (Becton-Dickinson Inc., East Rutherford, NJ, USA) (table 1) [4]. In line with the DST results, a combined and directly observed antituberculous therapy was initiated with cycloserine, capreomycin, para-aminosalicylic acid (PAS) and ethambutol. Published evidence indicates that treatment outcome of patients with MDR-TB whose isolates show resistance either to pyrazinamide or fluoroquinolones is poor in the absence of an adequate number of active drugs [5, 6]. In order to strengthen the efficacy of therapy with the less potent second-line drugs, the patient received bedaquiline on a compassionate basis between September 2011 and February 2012. Culture conversion was confirmed at the end of October 2011. The patient remained culture negative and therapy was terminated in March 2013.

In August 2013, the patient was re-admitted with fever, cough and acid-fast bacillus-positive sputum microscopy. Therapy was re-initiated with cycloserine, capreomycin, PAS, ethambutol, clofazimine and inhaled amikacin. Re-application for bedaquiline treatment was rejected by the manufacturer on the basis that the patient had already received treatment on a compassionate basis for 6 months. DST of the relapse isolate in 2013 confirmed the previous resistance pattern but, to our surprise, revealed additional resistance to clofazimine. The 2011 isolate was susceptible to clofazimine (table 1). Most notably, the patient never received clofazimine. Genotyping using 24-locus mycobacterial interspersed repetitive unit variable number tandem repeats did not identify differences between the post-relapse and the previous isolates from 2011, indicating a common clonal origin of these isolates [7]. Recently, Hartkoorn *et al.* [8] described a mechanism of cross-resistance between clofazimine and bedaquiline in *in vitro*-selected

TABLE 1 Quantitative phenotypic drug susceptibility testing results for first- and second-line antituberculous drugs[#], and DNA sequencing results of resistance-associated genes

	TB isolate[¶]		TB relapse isolate[+]	
	MGIT 960 phenotype	**Resistance genotype**	**MGIT 960 phenotype**	**Resistance genotype**
Isoniazid mg·L^{-1}		*katG* Ser315Thr		*katG* Ser315Thr
0.1	**Resistant**	*inhA* wild type	**Resistant**	*inhA* wild type
1.0	Resistant		Resistant	
3.0	Resistant		Resistant	
10.0	Resistant		Resistant	
Rifampicin mg·L^{-1}		*rpoB* Ser531Leu		*rpoB* Ser531Leu
1.0	**Resistant**		**Resistant**	
4.0	Resistant		Resistant	
20.0	Resistant		Resistant	
Rifabutin mg·L^{-1}				
0.1	Resistant		Resistant	
0.4	**Resistant**		**Resistant**	
2.0	Not performed		Resistant	
Ethambutol mg·L^{-1}		Not applicable		Not applicable
5.0	**Susceptible**		**Susceptible**	
12.5	Susceptible		Susceptible	
50.0	Susceptible		Susceptible	
Streptomycin mg·L^{-1}		*rpsL* Lys88Arg		*rpsL* Lys88Arg
1.0	**Resistant**	*rrs* wild type (530 region)	**Resistant**	*rrs* wild type (530 region)
4.0	Susceptible		Susceptible	
20.0	Susceptible		Susceptible	
Amikacin mg·L^{-1}		*rrs* wild type (1400 region)		*rrs* wild type (1400 region)
1.0	**Susceptible**		**Susceptible**	
4.0	Susceptible		Susceptible	
20.0	Susceptible		Susceptible	
Capreomycin mg·L^{-1}		*rrs* wild type (1400 region)		*rrs* wild type (1400 region)
2.5	**Susceptible**	*tlyA* wild type	**Susceptible**	*tlyA* wild type
5.0	Susceptible		Susceptible	
25.0	Susceptible		Susceptible	
Ethionamide mg·L^{-1}		*ethA* Ser266Arg		*ethA* Ser266Arg
5.0	**Resistant**	*inhA* wild type	**Resistant**	
10.0	Resistant		Resistant	
25.0	Resistant		Resistant	
Linezolid mg·L^{-1}		*rrl* A2572C		*rrl* A2572C
1.0	**Resistant**	*rrl* G2576T	**Resistant**	*rrl* G2576T
4.0	Resistant		Resistant	
16.0	Susceptible		Susceptible	
Moxifloxacin mg·L^{-1}		*gyrA* Asp94Tyr		*gyrA* Asp94Tyr
0.25	**Resistant**		**Resistant**	
0.5	Resistant		Resistant	
2.5	Susceptible		Resistant	
7.5	Not performed		Susceptible	
Para-aminosalicylic acid mg·L^{-1}		Not applicable		Not applicable
4	Susceptible		Susceptible	
16	Susceptible		Susceptible	
64	Susceptible		Susceptible	
Cycloserine mg·L^{-1}		Not applicable		Not applicable
50	Susceptible		Susceptible	
Pyrazinamide mg·L^{-1}		*pncA* Gly107 Stop		*pncA* Gly107Stop
100	**Resistant**		**Resistant**	
Clofazimine mg·L^{-1}		Rv0678 wild type		Rv0678 fMet1Ala
0.5	Resistant		Resistant	
1.0	Susceptible		Resistant	
4.0	Susceptible		Resistant	
Bedaquiline		*atpE* wild type		*atpE* wild type
Not available	Not available	Rv0678 wild type	Not applicable	Rv0678 fMet1Ala

Critical concentrations of first- and second-line antituberculosis drugs in the MGIT 960 system are highlighted in bold. Critical concentrations are not established for para-aminosalicylic acid, cycloserine, clofazimine or bedaquiline in the BACTEC MGIT 960 system. TB: tuberculosis. [#]: using the BACTEC MGIT 960 system (Becton-Dickinson Inc., East Rutherford, NJ, USA) with the TB eXiST module (Becton-Dickinson Microbiology Systems, Sparks, MD, USA); [¶]: January 2011; [+]: August 2013.

mutants due to mutation in the Rv0678 regulatory gene resulting in upregulation of the MmpL5 efflux pump gene. In a previous study by MILANO *et al.* [9], numerous mutations in Rv0678 were found to lead to de-repression of *mmpL5*. Analysis of the Rv0678 gene of the initial isolate of the patient from 2011 showed a wild-type sequence [8]. In contrast, the Rv0678 gene sequence of the August 2013 isolate revealed a mutation at nucleotide position 2 (GTG→GCG), resulting in the loss of the start codon (replacement of *N*-formylmethionine by alanine), suggesting that overexpression of the *mmpL5* efflux pump gene due to impaired Rv0678 function is responsible for the observed resistance. Notably, the relapse isolates of the patient did not have any mutations in the *atpE* gene, which was previously suggested to be associated with bedaquiline resistance [10].

The threat of emerging bedaquiline resistance associated with this cross-resistance mechanism is underlined by the compassionate use of bedaquiline in patients with XDR-TB and pre-XDR-TB who receive clofazimine as part of their combination therapy [11]. During the preparation of our manuscript, similar findings have been reported by ANDRIES *et al.* [12] from Janssen Pharmaceutica (Beerse, Belgium), the manufacturer of bedaquiline. Unfortunately, our requests to obtain bedaquiline to establish DST were declined by the manufacturer and TB Alliance (Brussels, Belgium), as the drug is not intended to be made available for *in vitro* DST before a manufacturer-sponsored DST validation study may be published in the first half of 2015. The lack of access to a high-standard reference laboratory, with vast experience in developing and standardising novel DST methods to newer antituberculous compounds already used for non-trial treatment, poses a serious drawback to the surveillance of emerging drug resistance and determination of the remaining therapeutic options in MDR-TB.

In order to avoid further development and spread of this newly identified mechanism of cross-resistance between clofazimine and bedaquiline, and to be able to rapidly and adequately identify and confirm treatment failure to these drugs in the clinical practice, we believe that clinicians need to be aware of this threat, and we urge that routine phenotypic DST should be available for all patients receiving clofazimine or bedaquiline therapy. In addition, detection of mutations in the Rv0678 gene may indicate that revisions in the therapeutic regimen are necessary. Not least, our findings reiterate that pharmaceutical companies need to coordinate their priorities with tuberculosis control activities.

After the acceptance of this manuscript, bedaquiline quantitative DST was established in the BACTEC MGIT 960 system using a commercially available bedaquiline tablet. Suspicion of bedaquiline resistance in the relapse isolate (August 2013), based on the mutation identified in Rv0678, was corroborated by a 10-fold minimal inhibitory concentration increase compared with the initial isolate (January 2011) from the patient.

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Acquired clofazimine and bedaquiline cross-resistance in a patient due to mutation in the *M. tuberculosis* Rv0678 gene http://ow.ly/Cnkk7

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Impaired lung function is associated with systemic inflammation and macrophage activation

To the Editor:

Lung function impairment, as assessed by a reduction in the forced expiratory volume measured in the first second of exhalation (FEV_1) and forced vital capacity (FVC), contributes significantly to several major health issues, such as all-cause mortality [1, 2], chronic lung disease prevalence (chronic obstructive pulmonary disease (COPD) and asthma) [3, 4], and death from cardiovascular disease [5]. Preservation of lung health during ageing [6] through identification of modifiable risk factors is a key research priority.

Low-grade chronic systemic inflammation, determined by elevation of C-reactive protein (CRP) and interleukin (IL)-6 is a common mechanistic pathway contributing to reduced lung function [4, 5, 7] and increased cardiovascular events [8, 9]. Systemic inflammation is also a feature of chronic respiratory conditions including asthma and COPD [10]. However, the influence of systemic inflammation on lung function across different populations needs further investigation if targeting this inflammation is to be developed as a strategy in preventing lung function decline. Circulating soluble (s) CD163 is a biomarker of macrophage activation and is elevated in many inflammatory and infectious disease states [11]. While macrophage activation has been associated with chronic lung disease, it has not previously been assessed in relation to lung health at a population level.

The Inuit indigenous population of Greenland provides a unique and important opportunity to assess the relationships between systemic inflammation and macrophage activation on lung function impairment in a genetically homogenous population. We hypothesised that lung function impairment would be associated with systemic inflammation and macrophage activation. This study investigates systemic inflammation and macrophage activation as predictors of lung function in an Inuit indigenous population residing in the Arctic (Greenland) or Western Europe (Denmark).

TABLE 1 Association between inflammatory markers and lung function adjusted for age, sex, smoking and height

	FEV_1 n=1068		FVC n=1048	
	β	p-value	β	p-value
lnIL6	−0.032±0.022	0.144		>0.2
lnCRP	−0.060±0.013	**<0.0001**	−0.078±0.013	**<0.0001**
lnCD163	−0.057±0.027	**0.034**	−0.080±0.031	**0.011**

Data are presented as β±SE. Bold indicates significance. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity.