Rapid emergence of Mycobacterium tuberculosis bedaquiline resistance: lessons not to repeat past errors

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Rapid emergence of *Mycobacterium tuberculosis* bedaquiline resistance: lessons not to repeat past errors

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Word count : 1181

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Bedaquiline (BDQ), has demonstrated potent clinical activity against multi (MDR) and extensively (XDR) drug resistant *M. tuberculosis* complex strains [1–3]. It has now been used in more than 50 countries, and it is estimated that approximately 2500 patients have been treated by BDQ by the end of 2015. In spite of a recent clinical use, there are few reports of BDQ resistant strains [4, 5]. Mutations in the *rv0678* gene encoding the MmpL5 efflux pump repressor generate low-level BDQ resistance and clofazimine (CFZ) cross-resistance [6]. This mechanism is, to our knowledge, the sole mechanism of BDQ resistance described in clinical strains [4, 5]. Despite a recent introduction in France in 2011 for XDR and MDR-TB treatment, we report herein four BDQ-resistant cases, and discuss strategies to avoid a surge of BDQ resistance.

In France, all MDR *M. tuberculosis* complex strains are sent to the National Reference Center for Mycobacteria (NRC) for complete genotypic and phenotypic drug susceptibility testing (DST), including BDQ since 2014. BDQ minimum inhibitory concentration (MIC) was measured in 7H11 medium in polystyrene petri dishes for all strains that were resistant to a screening concentration of 64 mg/L in Lowenstein-Jensen medium. For each MIC measure, H37Rv was included as susceptible control. For all strains screened as BDQ resistant, the *atpE* and *rv0678* genes were sequenced [4]. BDQ dry powder was kindly supplied by Janssen. The clinical history of patients harbouring BDQ-resistant strains was retrospectively abstracted from medical records. Ethics approval of the study protocol was granted by Bligny Hospital Institutional Review Board.

Between January 1st 2014 and December 31st 2015, 209 MDR, including 40 XDR, *M. tuberculosis* complex strains were routinely tested at the NRC for BDQ susceptibility. Among these, 4 (2%) had elevated BDQ MICs, between 0.25 and 0.5 mg/L as

compared to 0.03 mg/L for the H37Rv reference strain. The genotypic and phenotypic characteristics of these 4 unrelated strains are summarized in the table.

Patient 1, born in Pakistan, had no past history of tuberculosis or leprosy treatment and never received BDQ nor CFZ. He was diagnosed with bone and pulmonary MDR-TB on arrival in France in January 2015. His strain isolated before any tuberculosis treatment displayed BDQ resistance.

Patient 2 was treated for MDR-TB in Romania from September 2013 to November 2014 but has never received BDQ nor CFZ. He was diagnosed in France with cavitary lung MDR-TB in October 2015. The strain isolated at that time displayed BDQ resistance.

Patient 3 has been treated in Georgia for 24 months from 2011 to 2013 for MDR-TB with ethionamide (ETH), para-aminosalycilic acid (PAS), capreomycin (CAP), cycloserine (CYC), amoxicillin-clavulanate (AMC), a regimen subsequently supplemented with pyrazinamide (PZA) and BDQ for the last 4 months. In January 2014, he was diagnosed in France with cavitary lung XDR-TB, with a BDQ-resistant strain.

Patient 4 arrived in France from Georgia in 2013 after a 10-year history of tuberculosis treatment, with extensive bilateral cavitary lung lesions. In July 2013, he started treatment with PZA, amikacin (AMK), ETH, PAS, linezolid (LNZ), ethambutol (EMB) and BDQ. The strain isolated on arrival in France was only susceptible to AMK, PAS, LNZ, and had BDQ MIC in the normal range. In August 2013, EMB and LNZ were withdrawn, due respectively to in vitro resistance and to peripheral neuropathy, and imipenem (IMP)/AMC was introduced. He became sputum culture negative in October 2013. In December 2013, IMP/AMC was stopped and CFZ was introduced. In

September 2014, AMK was stopped. In October 2014, the patient reverted to sputum culture positivity. DST of the 2014 strain showed the same susceptibility profile as the initial 2013 strain, except for appearance of BDQ resistance.

The rapid appearance of BDQ resistance is alarming. These 4 cases can be grouped into 2 profiles, which raise different questions.

The first profile is the one of patients 1 and 2 who had never been treated with BDQ. Considering that tuberculosis is transmitted from a patient to another, one can hypothesize that BDQ resistance was selected by BDQ or CFZ treatment in the transmission chain. This is unlikely for BDQ, because of its recent use. CFZ however, may have been prescribed for a non-tuberculous disease such as leprosy. Finally, other compounds could select for BDQ resistance through *rv0678* mutations, because the recognized mechanism of resistance affects an efflux pump. Altogether, these data underline the importance of including BDQ DST in national programs implementing BDQ for the treatment of MDR or XDR TB.

In patients 3 and 4, the background regimen failed to prevent the selection of drug resistance. Reviewing DST results and treatment histories, it appears that, except for BDQ, the only likely active drugs were PAS (for both patients) and AMK (for patient 4). We previously described a similar case in which fluoroquinolone resistance was selected despite PAS and AMK co-administration [7]. Recently, a case of BDQ resistance was reported despite EMB, PAS, CAP, and CYC combined therapy [8]. Of note, when BDQ was switched for delamanid (DLM), DLM resistance was subsequently selected [8]. The 2013 WHO recommendations on BDQ use do not address the effectiveness of BDQ companion drugs. Regimens in which BDQ is only combined with drugs with poor bactericidal activity or poor tissue diffusion such as PZA, PAS, CYC, EMB, CFZ or aminoglycosides, may not prevent the selection of BDQ

resistance. We suggest that BDQ should be always associated with at least one drug with both bactericidal and sterilizing activity for the full treatment duration in order to avoid selection of BDQ resistance. Fluoroquinolones, LNZ, DLM, and maybe ETH, may be considered in this category since they have proved in vivo activity [3, 9]. Furthermore, recent data suggest that the combination of DLM and BDQ is safe [10, 11]. If the number of remaining bactericidal drugs is low and there are important destructive lung lesions, surgery should be considered [12].

The definition of BDQ resistance is currently not completely established. EUCAST adopted 0.25 mg/L as susceptibility breakpoint in 7H11 [13] which is above the MIC 90 (0.12 mg/L) [14]. However, with such breakpoint, the strain responsible for clinical failure in patient-4 (MIC 0.25 mg/L, 16-fold higher than before BDQ treatment) would have been categorized as susceptible. The clinical impact of *rv0678* mutations conferring elevated MICs in the 0.25-0.5 mg/L range, i.e. below the usual BDQ serum level (3-4 mg/L) could be a matter of discussion. A murine experiment has shown that *rv0678* mutations increasing BDQ MICs by 4- to 8-fold reduce the 1-month BDQ bactericidal activity by 1 log10 CFU [4]. Conversely, a clinical study suggested that there was no impact on treatment efficacy of BDQ containing combined regimen [3]. Nevertheless, the case of patient 4, who became initially sputum culture negative but relapsed with positive cultures after 16 months of a BDQ-containing regimen, suggests that the clinical impact of such mutations could be meaningful. Finally, it seems that there is an overlap between MICs of wild-type and mutant strains which may require a 2 breakpoint classification with an intermediate category as for other antituberculous drugs.

Acknowledgements

CNR MyRMA is supported by an annual grant of Institut de Veille Sanitaire.

Nicolas Veziris, Christine Bernard, Lorenzo Guglielmetti, Florence Brossier, Vincent Jarlier, Jérôme Robert, Wladimir Sougakoff and Alexandra Aubry work in a laboratory that has been financed by Janssen for preclinical work on bedaquiline.

147 Table: characteristics of the 4 bedaquiline resistant *M. tuberculosis* strains

Patient	Previous treatments	*M. tuberculosis* strain				
		Lineage	BDQ MIC (mg/L)	*atpE*	*rv0678*	MIRU-VNTR
H37Rv			0.03	WT	WT	
1	No	Delhi/CAS	0.5	WT	del gg 18-19	242 235 442 244 425 173 344 742
2	INH, RFB, EMB, PZA, CAP, MXF, LNZ, TER	S	0.5	WT	WT*	233 353 212 434 215 133 336 A22
3	BDQ combined with PZA, ETH, CAP, CYC, PAS and AMC but only PAS susceptible on DST	Beijing	0.25	WT	ins g140	244 233 352 644 425 153 353 823
4	INH, RIF, EMB, PZA, SM then KAN, MXF, ETH, CYC, PAS BDQ combined with EMB, PZA, AMK, ETH, LNZ, PAS, CFZ, PAS, IMP/AMC but only PAS and AMK susceptible on DST	Beijing	0.015	WT	WT	244 233 352 644 425 173 353 623
			0.25	WT	M139T[a]	244 233 352 644 425 173 353 623

148 a : amino acid substitution in the Rv0678 protein

149 **pepQ* was sequenced and was also wild-type[16]

Abbreviations: bedaquiline (BDQ), minimal inhibitory concentration (MIC), drug susceptibility testing (DST), Mycobacterial Interspersed Repetitive Units -Variable Number of Tandem Repeats (MIRU-VNTR)

INH : isoniazid, RIF : rifampin, EMB : ethambutol, PZA : pyrazinamide, SM : streptomycin, KAN : kanamycin, AMK : amikacin, CAP : capreomycin, MXF : moxifloxacin, CYC : cycloserine, PAS : para-amino-salicylic acid, ETH : ethionamide, LNZ : linezolid, CFZ : clofazimine, RFB : rifabutin ; AMC amoxicillin-clavulanate, IMP imipenem, TER: terizidone, WT : wild-type

References

1. Diacon AH, Pym A, Grobusch MP, de los Rios JM, Gotuzzo E, Vasilyeva I, Leimane V, Andries K, Bakare N, De Marez T, Haxaire-Theeuwes M, Lounis N, Meyvisch P, De Paepe E, van Heeswijk RPG, Dannemann B, TMC207-C208 Study Group. Multidrug-resistant tuberculosis and culture conversion with bedaquiline. *N. Engl. J. Med.* 2014; 371: 723–732.

2. Guglielmetti L, Le Dû D, Jachym M, Henry B, Martin D, Caumes E, Veziris N, Métivier N, Robert J, MDR-TB Management Group of the French National Reference Center for Mycobacteria and the Physicians of the French MDR-TB Cohort. Compassionate use of bedaquiline for the treatment of multidrug-resistant and extensively drug-resistant tuberculosis: interim analysis of a French cohort. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* 2015; 60: 188–194.

3. Pym AS, Diacon AH, Tang S-J, Conradie F, Danilovits M, Chuchottaworn C, Vasilyeva I, Andries K, Bakare N, De Marez T, Haxaire-Theeuwes M, Lounis N, Meyvisch P, Van Baelen B, van Heeswijk RPG, Dannemann B, TMC207-C209 Study Group. Bedaquiline in the treatment of multidrug- and extensively drug-resistant tuberculosis. *Eur. Respir. J.* 2016; 47: 564–574.

4. Andries K, Villellas C, Coeck N, Thys K, Gevers T, Vranckx L, Lounis N, de Jong BC, Koul A. Acquired resistance of Mycobacterium tuberculosis to bedaquiline. *PloS One* 2014; 9: e102135.

5. Somoskovi A, Bruderer V, Hömke R, Bloemberg GV, Böttger EC. A mutation associated with clofazimine and bedaquiline cross-resistance in MDR-TB following bedaquiline treatment. *Eur. Respir. J.* 2015; 45: 554–557.

6. Hartkoorn RC, Uplekar S, Cole ST. Cross-resistance between clofazimine and bedaquiline through upregulation of MmpL5 in Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* 2014; 58: 2979–2981.

7. Veziris N, Martin C, Brossier F, Bonnaud F, Denis F, Aubry A. Treatment failure in a case of extensively drug-resistant tuberculosis associated with selection of a GyrB mutant causing fluoroquinolone resistance. *Eur. J. Clin. Microbiol. Infect. Dis. Off. Publ. Eur. Soc. Clin. Microbiol.* 2007; 26: 423–425.

8. Bloemberg GV, Keller PM, Stucki D, Stuckia D, Trauner A, Borrell S, Latshang T, Coscolla M, Rothe T, Hömke R, Ritter C, Feldmann J, Schulthess B, Gagneux S, Böttger EC. Acquired Resistance to Bedaquiline and Delamanid in Therapy for Tuberculosis. *N. Engl. J. Med.* 2015; 373: 1986–1988.

9. Scardigli A, Caminero JA, Sotgiu G, Centis R, D'Ambrosio L, Migliori GB. Efficacy and tolerability of ethionamide versus prothionamide: a systematic review. *Eur. Respir. J.* 2016; 48: 946–952.

10. Lachâtre M, Rioux C, Dû DL, Fréchet-Jachym M, Veziris N, Bouvet E, Yazdanpanah Y. Bedaquiline plus delamanid for XDR tuberculosis. *Lancet Infect. Dis.* 2016; 16: 294.

11. Tadolini M, Lingtsang RD, Tiberi S, Enwerem M, D'Ambrosio L, Sadutshang TD, Centis R, Migliori GB. First case of extensively drug-resistant tuberculosis treated with both delamanid and bedaquiline. *Eur. Respir. J.* 2016; .

12. Mordant P, Henry B, Morel S, Robert J, Veziris N, Le Dû D, Frechet-Jachym M, Similowski T, Caumes É, Riquet M, Le Pimpec-Barthes F. [Adjuvant surgical resection for multidrug-resistant tuberculosis: A review]. *Rev. Mal. Respir.* 2014; 31: 511–524.

13. EUCAST: Clinical breakpoints [Internet]. [cited 2016 Apr 5].Available from: http://www.eucast.org/clinical_breakpoints/.

14. Infective Drugs Advisory Committee Meeting, Briefing Document : TMC207 (bedaquiline), Treatment of Patients with MDR-TB. Food and Drug Administration; 2012.

15. Almeida D, Ioerger T, Tyagi S, Li S-Y, Mdluli K, Andries K, Grosset J, Sacchettini J, Nuermberger E. Mutations in pepQ Confer Low-level Resistance to Bedaquiline and Clofazimine in Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* 2016; .