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Early View

Research letter

Failure with acquired resistance of an optimised bedaquiline-based treatment regimen for pulmonary *Mycobacterium avium* complex disease

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Failure with acquired resistance of an optimized bedaquiline-based treatment regimen for pulmonary *Mycobacterium avium* complex disease

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Take Home Message: In its current dose, bedaquiline is probably not a viable treatment option for treatment-refractory MAC-PD; even with adequate exposures, treatment fails due to acquired resistance documented by increasing MICs and whole genome sequencing

Five years ago, a then 50 year old woman presented with long-standing fatigue, dyspnea and a chronic productive cough. Based on a CT scan of the thorax and multiple positive cultures she was diagnosed with nodular-bronchiectatic *Mycobacterium avium* pulmonary disease; she was also found to have a heterozygous ΔF508 *CFTR* gene mutation. She commenced therapy with rifabutin 300 mg once daily (od), ethambutol 1200 mg od and azithromycin 500 mg od. After 15 months of ongoing symptoms, radiographic deterioration and persistent culture positivity, clofazimine 100 mg od and thrice weekly intravenous amikacin 15mg/kg were added to the regimen. Amikacin was halted after 4 months; the remaining four drugs were continued. The dose of azithromycin was lowered to 250mg od after 9 months because of hearing loss.

After two years of stable symptoms and radiology, but persistent culture positivity, rifabutin was replaced by bedaquiline, in the 400 mg od for 2 weeks and then 200 mg thrice weekly dosing regimen used for tuberculosis (TB). Azithromycin was switched to clarithromycin 500 mg twice daily and ethambutol and clofazimine were maintained. Therapeutic drug monitoring on the last day of the daily bedaquiline dosing (i.e. at the end of the loading phase, thus at steady state) showed peak and trough bedaquiline plasma concentrations of 3.4 mg/L and 1.2 mg/L and a total exposure (AUC_{0-24h}) of circa 43 h*mg/L. The ethambutol peak serum concentration was 3.0 mg/L with an AUC of 28.5 h*mg/L and the clarithromycin peak plasma concentration was 1.4 mg/L.

This bedaquiline-containing regimen, too, did not lead to culture conversion. Moreover, the *M. avium* isolates obtained during and after bedaquiline treatment showed an increase in bedaquiline and clofazimine minimum inhibitory concentration (MIC) and, by whole-genome sequencing, a new mutation in the regulator of the efflux pump involved in bedaquiline and clofazimine resistance (see Figure).

After 12 months and with persistent symptoms and culture positivity, bedaquiline was stopped and a bilobectomy of the right upper and middle lobe was performed. Post-operatively, rifabutin 300mg od and amikacin liposomal inhalation solution were added to the clofazimine-ethambutol-clarithromycin regimen. Although surgical samples were culture positive, sputum culture conversion

has been attained after surgery and has lasted for six months now.

Throughout the course of treatment, all *M. avium* isolates remained macrolide- and amikacin-susceptible and no QTc interval prolongation was observed by ECG monitoring.

Bedaquiline was developed for treatment of multidrug-resistant tuberculosis (MDR-TB), but has also been explored for use in disease caused by nontuberculous mycobacteria [1]. To date, bedaquiline treatment and outcome data of 13 patients with *Mycobacterium avium* complex pulmonary disease (MAC-PD) have been reported and these were not favorable [1]. After an initial clinical, radiological and microbiological response [1], most patients' treatment failed with the emergence of mutational resistance to bedaquiline [2]. Most of these patients used a rifamycin (rifampicin or rifabutin) and bedaquiline as parts of their treatment regimen and rifampicin is known to decrease the systemic exposure to bedaquiline by 75% through induction of CYP3A4 [3]; thus, the emergence of bedaquiline resistance may have resulted from suboptimal exposure.

Clofazimine has been shown to be as active as rifampicin in three-drug regimens that also feature ethambutol and a macrolide [4], it does not affect pharmacokinetics of bedaquiline [5] and is synergistic with bedaquiline against *M. avium* complex bacteria [6].

Hence, upon introducing bedaquiline we aimed to optimize its efficacy in the regimen by stopping rifabutin and continuing clofazimine. Furthermore the change of azithromycin to clarithromycin (an inhibitor of CYP3A4), might cause an increased exposure to bedaquiline [7]. The bedaquiline serum concentrations at the end of week 2 (peak 3.4 mg/L and trough 1.2 mg/L) were at least similar to the peak (2.76 mg/L) and trough concentrations (0.73 mg/L) reported by van Heeswijk *et al*. [7], showing that drug-drug interactions were successfully avoided. This is important, as the bacteriostatic effect of bedaquiline against *M. avium* is known to be exposure-dependent [6]. Nonetheless, the bedaquiline-clofazimine-ethambutol-clarithromycin failed to achieve culture conversion. Exposure to ethambutol was adequate, but exposure to clarithromycin, as reflected in its peak concentration of

1.4 mg/L, was lower than the mean of 3.91 mg/L in MAC-PD patients treated without rifamycins [8]. The low clarithromycin exposure may have contributed to the poor response to therapy. The fact that bedaquiline was a single drug added to an already failing regimen, may also have contributed to the poor outcome of the bedaquiline-containing regimen.

Follow-up cultures during bedaquiline therapy revealed an increase in bedaquiline and clofazimine MIC of the causative *M. avium* strain (Figure), determined by broth microdilution following CLSI guidelines [6]. This finding prompted us to perform whole-genome sequencing of isolates obtained over the entire course of the patient's disease (Figure). Whole-genome sequencing revealed a 173W>173R|2544950A>G mutation in the MAV_2512 locus (reference genome *M. avium* 104; GenBank accession number CP000479), no mutations associated with macrolide or aminoglycoside resistance and it showed that all isolates were <6 SNPs different, suggesting a persistent monoclonal infection; the MAV_2512 173W>173R|2544950A>G mutation was already present in 56% of the sequence reads obtained from the *M. avium* isolates after 7 months, but only passed the >80% threshold in the isolate obtained after 12 months of bedaquiline therapy (Figure). Of note, this mutation only emerged after bedaquiline was added to clofazimine in the regimen. The affected gene encodes the *tetR* family transcriptional regulator of the MmpL5/MmpS5 drug transporter, shown to be involved in acquired low-level bedaquiline resistance in *M. intracellulare* as well [2]. Its functional counterpart in *M. tuberculosis* is *Rv0678*, a *MarR* family transcriptional regulator; mutations in this gene also lead to low-level bedaquiline and clofazimine resistance in *M. tuberculosis* through increased MmpS5/MmpL5 expression [9].

The acquired mutation in the *tetR* family transcriptional regulator of the MmpL5/MmpS5 drug transporter is likely the mechanism responsible for the increase in the bedaquiline and clofazimine MIC of the causative *M. avium* strain and is apparently not prevented by bedaquiline exposures considered adequate in MDR-TB treatment. The current bedaquiline dose was the highest dose in the only published dose finding study [10], yet the exposures reached with this dose seem too low to

exert a microbiological effect and do not prevent acquired drug resistance in MAC-PD. Increased doses may be required for a therapeutic effect, but their safety remains to be established.

In summary, the patient presented here experienced failure, with acquired bedaquiline resistance, of an optimized bedaquiline-based treatment regimen, designed to minimize drug-drug interactions, maximize bedaquiline exposure and exploit bedaquiline-clofazimine synergy. In its current dose, bedaquiline is probably not a viable treatment option for treatment-refractory MAC-PD.

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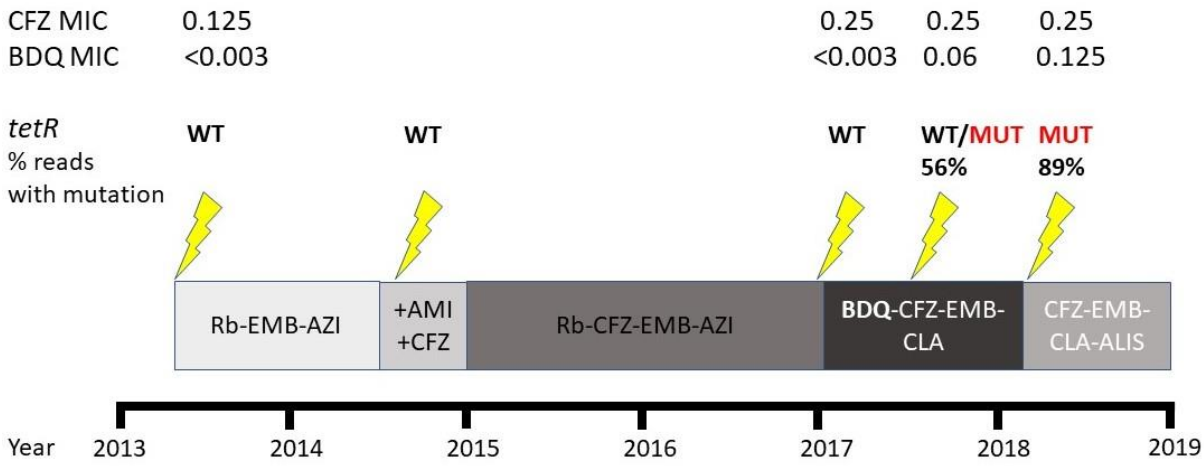
Figure

Note: CFZ, clofazimine; BDQ, bedaquiline; MIC, minimum inhibitory concentration; WT, wild type sequence; MUT, mutant; Rb, rifabutin; EMB, ethambutol; AZI, azithromycin; AMI, amikacin; CLA, clarithromycin; ALIS, amikacin liposomal inhalation solution


CFZ MIC
0.125
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0.25
0.25
BDQ MIC
<0.003
<0.003
0.06
0.125
tetR
% reads
with mutation
WT
WT
WT
WT/MUT
56%
MUT
89%
Rb-EMB-AZI
+AMI
+CFZ
Rb-CFZ-EMB-AZI
BDQ-CFZ-EMB-
CLA
CFZ-EMB-
CLA-ALIS
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