THE LANCET
Microbe

Supplementary appendix 1

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Derendinger B, Dippenaar A, de Vos M, et al. Bedaquiline resistance in patients with drug-resistant tuberculosis in Cape Town, South Africa: a retrospective longitudinal cohort study. *Lancet Microbe* 2023; published online Nov 3. https://doi.org/10.1016/S2666-5247(23)00172-6.

Appendix 1

Table of Contents

Methods

Next-generation sequencing analyses

WGS analysis (alignment, variant calling, drug resistance prediction)

Raw WGS data were analysed as described (1). Briefly, reads were trimmed and filtered using Trimmomatic (2) using a sliding-window, and mapped to *M. tuberculosis* H37Rv (Genbank: AL123456) using BWA (3), NovoAlign (Novocraft) and SMALT(4). Variants were identified using SAMtools (5) and GATK (6). TB profiler (version 3.0.4) was used to determine genotypic drug susceptibility profiles from raw WGS data using default parameters using the default reporting allele frequency of 10%.

Pairwise comparisons

Variants identified at a heterogeneity frequency of >70% were used to calculate the distance between the baseline and follow-up isolate of each patient to determine the intrapatient variant distance.

Clustering and phylogenetic analyses

Phylogenetic analyses included concatenated sequences of 101 *Mycobacterium tuberculosis* complex isolates (76 from the study, 25 representative of the complex (7-10) (**Supplementary data file, Sheet 2**). High confidence single nucleotide polymorphisms (SNPs) with ≥95% frequency (excluding *pe/ppe*, repeat, insertion sequences, and bacteriophages regions) (n=27,251) were used to construct a maximum likelihood phylogeny tree with IQ-TREE (version 2.0.6; 1,000 bootstrap pseudo-replicates, ultrafast automatic model selection) (11) and visualised and annotated using interactive Tree of Life (iTOL) (v6) (12). Transmission clusters were evaluated using *ape (13)* and *adegenet (14)* under SNPs thresholds of 5 and 12, with 12 (previously defined as the upper threshold of genomic relatedness noted within patient and

between epidemiologically linked cases (15)) used to define a cluster. Pairwise comparisons of SNPs and indels (≤50 bp, >70% heterogeneity frequency (16)) were done between baseline and follow-up isolates of each patient to determine intrapatient variant distance.

Targeted deep sequencing analyses

The single-molecule overlapping-read (SMOR) analysis tool was used with TGen's Amplicon Sequencing Analysis Pipeline (ASAP) as described (17) to examine rare mutations and low-level variation in sequence data. bbduk was used for adapter removal, after which reads were mapped against amplicon or gene-specific reference sequences [*M. tuberculosis* H37Rv (Genbank: AL123456)] using bowtie2. Bowtie2's alignment parameters were adjusted to facilitate local alignment, and the reference gap open and extension penalties reduced to 3 and 1, respectively; allowing insertions near read end to be called rather than soft-clipped. The minimum breadth-of-coverage needed for alignment was set to zero, because the reference used was the whole gene not just amplicon sequences. ASAP, using SMOR analysis, automates the acquisition of counts at a position of interest. For each read pair collected, the frequency at which each nucleotide appears at a given position of interest is tallied on both reads. Paired reads that disagree are considered sequencing error and excluded. This allows for low-level subpopulation detection, as it reduces the likelihood that a SNP call will be made erroneously through Illumina sequencing-by-synthesis errors. A Next Gen genotypic call of resistance was made if a resistant subpopulation was detected at ≥1%, while lower proportions were recorded. For this analysis, in addition to excluding reads that were not paired, SNPs were not called at locations were the read depth was <500, as this would have required fewer than five paired reads to facilitate a 1% SNP call.

Haplotype analyses

For the haplotype calling, "region of interest" (ROI) assays were created for the *rv0678* gene corresponding to the length of each amplicon used to amplify the gene for sequencing (18). This produced ROIs at positions 778990-779114 for amplicon 1, 779085-779224 for amplicon 2, 779130-779303 for amplicon 3, and 779305-779488 for amplicon 4 of the H37Rv reference (GenBank accession number AL123456.3) used in the BAM files. ASAP inspects each read aligning to the ROI and extracts the nucleotide sequence. Reads that do not have complete coverage of the ROI are discarded. Additionally, SMOR analysis discards any reads where the forward and reverse reads do not contain identical sequences across the ROI. Remaining nucleotide sequences are added to a counter variable which tracks the number of occurrences for each unique sequence. Each unique sequence occurring in at least 0.1% of the total read depth at the ROI are output in the final report for each isolate, provided sufficient sequencing depth to call to 0.1%. Counts of the number of read pairs matching each sequence are included, and nucleotide variants are underlined to make it easy to see the differences between each sequence.

Dosing of BDQ

Recommended BDQ dosing was 400mg daily for two weeks, then 200mg daily on Mondays, Wednesdays, and Fridays for a total of six months (19).

Results

Comparison of variant gain/loss between timepoints of background drugs (based on WGS)

Most drug-resistance conferring mutations were fixed in baseline and follow-up isolates 79% (30/38). This was not the case in the below isolates, grouped according to their BDQ-susceptibility status:

Baseline and follow-up BDQ susceptible

- 30-B01 gained a fluoroquinolone (FQ)-resistance conferring mutation: gyrA Ala90Val (35%) in follow-up isolate (WT in baseline)
- 32-B03 lost two heterogeneous isoniazid (INH)-resistance conferring fabG1 mutations that were present at baseline [-15C>T (64%); -8T>C (36%)]. In this case, a katG 315 INH resistance-conferring mutation was present at 100% in the baseline and follow-up isolates.

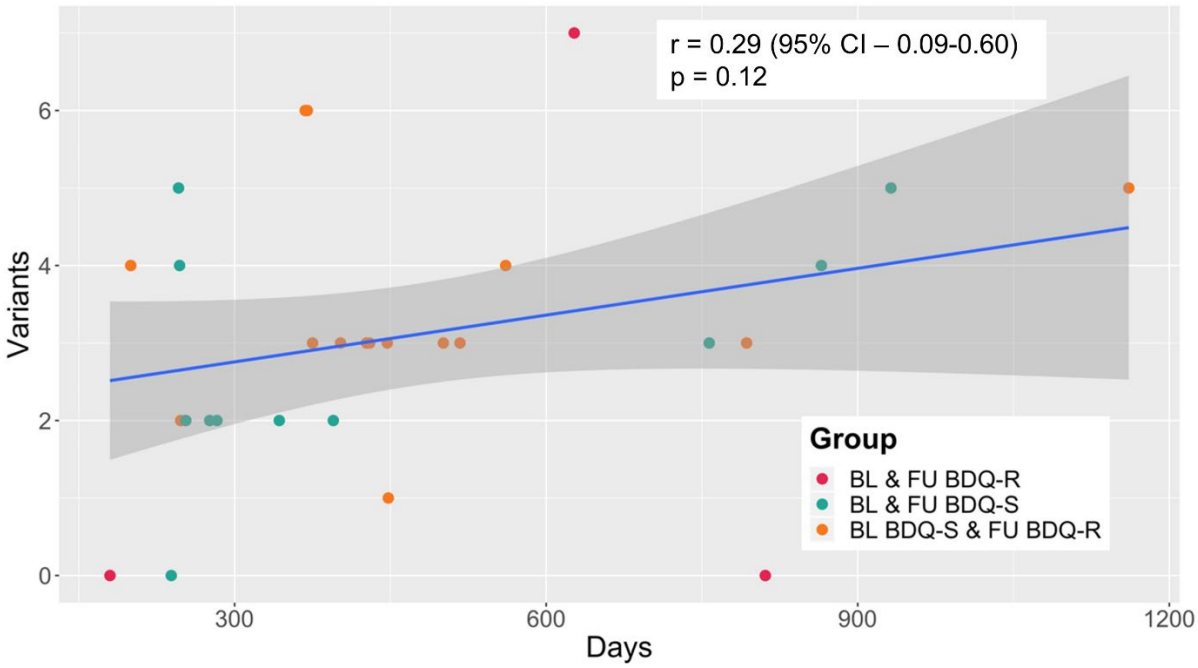
Baseline BDQ-susceptible and follow-up BDQ-resistant

- 04-A04 has a mixed infection at baseline and is heteroresisistant to various drugs due to mixed infection and not evolution
- 09-A09 at baseline loses an ethambutol-resistance conferring mutation: embA_c.-16C>T (85%) and then at follow-up, embA_c.-12C>T (fixed) is gained.
- 14-A14 (FQ) gyrB_p.Glu501Asp (18%) is lost in baseline. And two FQ-resistance conferring mutations [gyrA_p.Ala90Val (76%) and gyrA_p.Ser91Pro (25%)] are gained in follow-up. Based on the BDQ-resistance causing variant (mmpR5_p.Leu74Val (79%)) allele frequency, the BDQ-resistant population could be linked to the population with the gyrA codon 90 mutation.

- 25-A25 follow-up isolate gains a pncA_c.470_470del (87%), which is likely linked to the BDQ-resistant population [(mmpR5_c.141_142insC (87%)].
- 28-A28 is resistant to PZA at baseline due to a fixed pncA_c.517_518insG mutation, the allele frequency of which is 85% in the follow-up isolate.

Baseline and follow-up BDQ-resistant

- 26-A26: baseline PZA-susceptible isolate gains PZA resistance due to the presence of pncA_c.449_450insGG (54%).



Supplementary Figure 1.

Variant distance (SNPs, indels) from 29 patients showed a trend towards a positive linear correlation with days between baseline and follow-up sample collection. Patients (n=7) with isolates with variant distances indicative of reinfection (≥39 variants apart) were omitted. Abbreviations: BDQ-S – bedaquiline susceptible, BDQ-R – bedaquiline resistant, BL – baseline, FU – follow-up, SNP – single nucleotide polymorphism.

Supplementary Table 1.

BDQ resistance-associated genomic regions analysed by TDS. Universal tail sequences (in bold) are described previously (20). Amplicons sizes and positions are given. Abbreviations: BDQ – bedaquiline, bp – base pair, F – forward, R – reverse, TDS – targeted deep sequencing

Primer name (direction)	Sequence
atpE **(246 bp)** **-56 bp upstream of gene to 95 bp downstream of gene**	
atpEf-84 (F)	**ACCCAACTGAATGGAGC**AGCCAAGCGATGGAGCTCGAAGAGGAAC
atpEr222 (R)	**ACGCACTTGACTTGTCTTC**GAACAGCGCCATAAAMGCCAGGTTGATG
atpEf130 (F)	**ACCCAACTGAATGGAGC**AGGCGCAAGGGCGGCTGTTCACA
atpEr+96 (R)	**ACGCACTTGACTTGTCTTC**GCTGGACTCGCCGCCTTCCTCTGC
Rv0678 **(498 bp)** **-33 bp upstream of gene to 495 bp in gene**	
Rv0678f-57 (F)	**ACCCAACTGAATGGAGC**CACGCCGGTCTGGTGACGCATACC
Rv0678r124 (R)	**ACGCACTTGACTTGTCTTC**AGCCCAACAATCGACCCGCCAACC
Rv0678f115-1 (F)	**ACCCAACTGAATGGAGC**TGTTGGGCTGGCTGCTGGTGTGTGAT
Rv0678r336-1 (R)	**ACGCACTTGACTTGTCTTC**GGCCATTGCCCGGATGCGTTCA
Rv0678f115-2 (F)	**ACCCAACTGAATGGAGC**TATTGGGCTGGtTGCTGGTGTGTGAT
Rv0678r336-2 (R)	**ACGCACTTGACTTGTCTTC**GGCCATCGCCCGGATGCGCTCA
Rv0678f291-1 (F)	**ACCCAACTGAATGGAGC**AACGCTTTCGCGGCTGGCGAG
Rv0678f291-2 (F)	**ACCCAACTGAATGGAGC**AATGCTTTCGCGGCCGGCGAG
Rv0678r+24 (R)	**ACGCACTTGACTTGTCTTC**TCGGTCAGATTGCGAGGTTGCTCATCA
pepQ **(1119 bp)** **-33 bp upstream of gene to 1112 bp in gene**	
pepQf-60 (F)	**ACCCAACTGAATGGAGC**CAACCCGCGCAGCATCCAGTTAGTCAT
pepQr152 (R)	**ACGCACTTGACTTGTCTTC**CGCGCTCATCGGCGAACACCA
pepQf199 (F)	**ACCCAACTGAATGGAGC**AAGCGCCCGACCTCGAAGTGGC
pepQr421 (R)	**ACGCACTTGACTTGTCTTC**CCAGCTCGCCGGCGTCTTTAACCT
pepQf496 (F)	**ACCCAACTGAATGGAGC**GCCGAACCGAACGGCAGGTGAGC
pepQr713-1 (R)	**ACGCACTTGACTTGTCTTC**ACGAAGGTGCGGGTCATATCGGAGTGGTA
pepQr713-2 (R)	**ACGCACTTGACTTGTCTTC**ACGAAGGTGTGGGTCATATCGCAGTGGTA
pepQf899 (F)	**ACCCAACTGAATGGAGC**GCAGATACATGAAGCGCCGGGCATC
pepQr+19 (R)	**ACGCACTTGACTTGTCTTC**TGGTCGCCACGTGGGTCTCCTACAGA
pepQf74 (F)	**ACCCAACTGAATGGAGC**CGACCTGATAAACGTGCGATATCTATCAGGCTTC
pepQr324 (R)	**ACGCACTTGACTTGTCTTC**GTCCAGGCCGTCCACCGTGACCA
pepQf351 (F)	**ACCCAACTGAATGGAGC**AACACCGAGTTGGTGCGGGCATCC
pepQr535 (R)	**ACGCACTTGACTTGTCTTC**GGGCCTCCAGCTCGCGGCTCAC
pepQf640 (F)	**ACCCAACTGAATGGAGC**CGGCGATTTCGTGAAGATCGACTTCGG
pepQr968 (R)	**ACGCACTTGACTTGTCTTC**GTCACCACGGAGCCCGCCAGTAGTGTA

Supplementary Table 2.

Proportion of patients on each TB drug (other than BDQ) and the drugs' likely effectiveness based on WGS, stratified by follow-up phenotypic BDQ result. No single drug was more likely to be used in BDQ-susceptible or -resistant patients, however, in patients receiving LFX, LFX was more likely to be ineffectively used in BDQ-resistant than -susceptible patients. The same applied to PZA and CFZ. This highlights the need for DST to prevent likely ineffective treatment and alternative drugs. Data are n/N (%). Abbreviations: BDQ – bedaquiline, CFZ – clofazimine, DST – drug susceptibility testing, LFX – levofloxacin, PZA – pyrazinamide, TB – tuberculosis, WGS – whole genome sequencing, pDST – phenotypic drug susceptibility testing.

	Overall (n=38)	Follow-up BDQ pDST status	
		Susceptible (n=16)	Resistant (n=22)
High dose isoniazid[†]	21/38 (55)	9/16 (56)	12/22 (55) p=0.917
Likely effective	20/20 (100)	8/8 (100)	12/12 (100) p>0.999
Pyrazinamide	35/38 (92)	15/16 (94)	20/22 (91) p=0.749
Likely effective	11/3 (33)	8/14 (57)	3/19 (16) **p=0.013**
Ethambutol	25/38 (66)	13/16 (81)	12/22 (55) p=0.087
Likely effective	6/37 (16)	5/13 (38)	1/11 (9) p=0.098
Levofloxacin	25/38 (66)	11/16 (69)	14/22 (64) p=0.743
Likely effective	4/24 (17)	4/10[*] (40)	0/14 (0) **p=0.010**
Moxifloxacin	24/38 (63)	11/16 (69)	13/22 (59) p=0.542
Likely effective	5/23 (22)	3/11 (27)	2/12 (17) p=0.538
Capreomycin	1/38 (3)	1/16 (6)	0/22 (0) p=0.235
Likely effective	1/1 (100)	1/1 (100)	N/A
Amikacin	3/38 (8)	1/16 (6)	2/22 (9) p=0.749
Likely effective	2/3 (67)	1/1 (100)	1/2 (50) p=0.387
Kanamycin	20/38 (53)	8/16 (50)	12/22 (55) p=0.782
Likely effective	14/19 (74)	7/8 (88)	7/11 (64) p=0.244

Linezolid	25/38 (66)	10/16 (63)	15/22 (68) p=0.716
Likely effective	23/24 (96)	9/9 (100)	14/15 (93) p=0.429
Clofazimine	25/38 (66)	8/16 (50)	17/22 (77) p=0.080
Likely effective	8/36 (22)	6/15 (40)	2/21 (10) **p=0.030**
Terizidone	33/38 (87)	14/16 (88)	19/22 (86) p=0.919
Likely effective	28/31 (90)	13/13 (100)	15/18 (83) p=0.121
Delamanid	9/38 (34)	3/16 (19)	6/22 (27) p=0.542
Likely effective	9/9 (100)	3/3 (100)	6/6 (100) p>0.999
Ethionamide	26/38 (68)	11/16 (69)	15/22 (68) p=0.970
Likely effective	6/25 (24)	4/11 (36)	2/13 (15) p=0.237
4-aminosalicylic acid	21/38 (55)	7/16 (44)	14/22 (64) p=0.224
Likely effective	18/19 (95)	5/6 (83)	13/13 (100) p=0.131

Two patients excluded due to unknown treatment regimens
Four WGS results unavailable
*10 mg/kg per South African treatment guideline (21)

Supplementary Table 3.

Proportion of patients that had drug resistance (other than BDQ), based on WGS data, stratified by baseline and follow-up. The proportion of people with resistance to fluoroquinolones and clofazimine increased.

Drug	Baseline (%)	Follow-up (%)
Rifampicin	39/39 (100)	35/37 (95) p=0.14
Isoniazid	35/39 (90)	33/37 (89) p=0.94
Ethambutol	24/39 (62)	26/37 (70) p=0.42
Pyrazinamide	25/38 (64)	24/37 (65) p=0.94
Streptomycin	24/39 (62)	22/37 (59) p=0.85
Fluoroquinolones	18/39 (46)	28/37 (76) **p=0.01**
Aminoglycosides	6/39 (15)	7/37 (19) p=0.68
Kanamycin	7/39 (18)	10/37 (27) p=0.34
Amikacin	6/39 (15)	7/37 (19) p=0.68
Capreomycin	6/39 (15)	7/37 (19) p=0.68
Ethionamide	26/39 (67)	27/37 (73) p=0.54
Para-aminosalicylic acid	2/39 (5)	1/37 (3) p=0.59
Linezolid	1/39 (3)	1/37 (3) p=0.97
Delamanid	0/39 (0)	0/37 (0) p>0.99
Clofazimine	3/39 (8)	21/37 (57) **p<0.001**

Supplementary Table 4.

Select characteristics of patients based on follow-up BDQ resistance status after baseline BDQ resistant strains and, separately, also reinfections were excluded.
Compared to comparisons involving all patients (**Main text, Results**), done without exclusions based on baseline BDQ phenotype and reinfection, fewer variables were significantly associated with resistance at follow-up when both these exclusions were made (only ≤4 likely effective drugs). Data are n/N or median (IQR) unless otherwise stated. Abbreviations: BDQ – bedaquiline, CFZ – clofazimine, FQ – fluoroquinolone, IQR – interquartile range, OR – odds ratio, R – resistance, TB – tuberculosis, WGS – whole genome sequencing

	Baseline BDQ R omitted			**Baseline BDQ R and reinfections omitted**		
	BDQ phenotype at follow-up			**BDQ phenotype at follow-up**		
	Susceptible (n=18)	**Resistant (n=19)**	**OR (95% CI)**	**Susceptible (n=13)**	**Resistant (n=18)**	**OR (95% CI)**
Baseline FQ resistance*	4/18 (22)	12/18 (67) **p=0.01**	7 (2-31) **p=0.01**	4/13 (31)	11/17 (65) p=0.07	4 (1-19) p=0.06
Any CFZ exposure (prior or concurrent)	10/18 (56)	16/19 (84) p=0.06	4 (1-20) p=0.05	8/13 (62)	15/18 (83) p=0.17	3 (0.6-17) p=0.17
≤4 likely effective drugs (excluding BDQ)†	5/16 (31)	16/18 (89) **p<0.01**	16 (3-99) **p<0.01**	3/11 (27)	15/17 (88) **p<0.01**	20 (2-145) **p<0.01**
Unfavourable outcome	6/18 (33)	17/19 (89) **p<0.01**	Not calculable (few resistance cases with favourable outcomes)	5/13 (38)	16/18 (89) **p<0.01**	Not calculable (few resistance cases with favourable outcomes)

*Detected by WGS or programmatic line probe assay. One result unavailable
†Two patients were excluded due to unknown background TB drug regimens and two WGS sequencing results unavailable

Supplementary Table 5.

Phenotypic and genotypic DST result for three patients with phenotypic BDQ resistance at baseline (all also resistant at follow-up). TDS frequently detected variants that WGS did not, however, one patient at follow-up (37-B-08) had a variant (*pepQ* 693 A ins) exclusively detected by WGS. The last row shows summary data. As detailed in **Methods**, TDS was done on *Rv0678*, *atpE*, and *pepQ*. WGS was also done on at *Rv0676c*, *Rv0677c* and *Rv1979c*. No variants were detected in *atpE*, *Rv0677c* and *Rv1979c* and these columns are omitted. There was no evidence of reinfection. Prior CFZ exposure, days on BDQ (with programmatic treatment outcome), SNP distances, and the specific variant (proportion of reads indicated) are shown (blue indicates variant loss, red indicates gain). Variants previously described (22) are bolded. The only variants the baseline BDQ-resistant patient 26-A26 had were in *Rv0676c*, however, these also appeared in BDQ-susceptible isolates **(Supplementary Table 5)**, suggesting these are lineage markers and this isolate has an unknown resistance mechanism that, at follow-up, led to the emergence of previously described *Rv0678* variants. Abbreviations: BDQ – bedaquiline, CFZ – clofazimine, indels – insertions and deletions, R – resistant, S – susceptible, SNPs – single nucleotide polymorphisms, TDS – targeted deep sequencing, WGS – whole genome sequencing, WT – wildtype. Data are % unless otherwise stated.

Patient identifier (CFZ exposure)	Days BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS		WGS		
					Rv0678	*pepQ*	*Rv0678*	*pepQ*	*Rv0676c*
37-B08 (Yes)	208 (Cured)	Baseline	R	7 (likely intrapatient evolution)	*SNPs* 73 G/A (2) 119 T/C (9) 122 G/T (2) 194 G/T (1) 226 C/T (19) 391 C/T (2) 417 G/T (2) *Indels* **192 G ins (1)** 141 T del (2) 482 C del (10)	WT (100)	*SNPs* 226 C/T (26)	WT (100)	*SNPs* 1266 G/C (22) 2842 T/C (100)
		Follow-up	R		WT (100)	WT (100)	WT (100)	*Indels* 693 A ins (97)	*SNPs* 2842 T/C (100)
26-A26* (Yes)	201 (Treatment failed)	Baseline	R	0 (no change)	*SNPs* -11 C/A (100)	WT (100)	*SNPs* -11 C/A (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**
		Follow-up	R		*SNPs* -11 C/A (100) 394 C/T (3) 461 T/C (17) *Indels* 132 GT ins (10) 137 TGA ins (3)	WT (100)	*SNPs* -11 C/A (98) 461 T/C (21) *Indels* 132 GT ins (13) **192 G ins (50)**	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**

Patient identifier (CFZ exposure)	Days BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS				WGS								
					Rv0678			*pepQ*	*Rv0678*			*pepQ*			*Rv0676c*		
					192 G ins (57) 217 A del (2) 218 T del (2)												
29-A29 (Yes)	91 (Died)	Baseline	R	0 (no change)	*SNPs* **136 T/C (100)**			WT (100)	*SNPs* **136 T/C (100)**			WT (100)			*SNPs* **2381 G/A (100)** **2842 T/C (100)**		
		Follow-up	R		*SNPs* **136 T/C (100)**			WT (100)	*SNPs* **136 T/C (100)**			WT (100)			*SNPs* **2381 G/A (100)** **2842 T/C (100)**		
Sub-total (Yes=3)	**Median (IQR) days of BDQ treatment** 201 (91-208) Cured (n=1) Treatment failed (n=1) Died (n=1)	Baseline	R n=3	**Median (IQR) variant distance** 0 (0-7) Likely intrapatient evolution (n=1) No change (n=2)	*SNPs* -11 C/A 73 G/A 119 T/C 122 G/T **136 T/C** 194 G/T 226 C/T 391 C/T 417 G/T *Indels* **192 G ins** 141 T del 482 C del	*n* 1 1 1 1 **1** 1 1 1 1 *n* **1** 1 1	*Median (IQR)* N/A N/A N/A N/A **N/A** N/A N/A N/A N/A **N/A** N/A N/A	-	*SNPs* -11 C/A **136 T/C** 226 C/T	*n* 1 **1** 1	*Median (IQR)* N/A N/A N/A	-			*SNPs* 1266 G/C **2299 C/T** **2381 G/A** **2842 T/C**	*n* 1 **1** **2** **3**	*Median (IQR)* N/A **N/A** **100 (100-100)** **100 (100-100)**
		Follow-up	R n=3		*SNPs* -11 C/A **136 T/C** 394 C/T 461 T/C *Indels* 132 GT ins 137 TGA ins **192 G ins** 217 A del 218 T del	*n* 1 **1** 1 1 *n* 1 1 **1** 1 1	*Median (IQR)* N/A **N/A** N/A N/A N/A N/A **N/A** N/A N/A	-	*SNPs* -11 C/A **136 T/C** 461 T/C *Indels* 132 GT ins **192 G ins**	*n* 1 **1** 1 *n* 1 **1**	*Median (IQR)* N/A **N/A** N/A N/A **N/A**	*Indels* 693 A ins	*n* 1	*Median (IQR)* N/A	*SNPs* **2299 C/T** **2381 G/A** **2842 T/C**	*n* **1** **2** **3**	*Median (IQR)* **N/A** **100 (100-100)** **100 (100-100)**

Supplementary Table 6.

Individual patient information [BDQ phenotypic DST (1µg/ml), TDS, WGS] in those with phenotypic BDQ resistance at follow-up but not baseline. CFZ exposure, BDQ treatment duration and outcome, whether intrapatient evolution or reinfection were likely, together with the specific variants and the percentage of reads are shown. The last row shows summary data. TDS frequently detected additional variants that WGS did not but all genes with TDS-detected variants had WGS-detected variants. *rv0678*, *rv0676c* and *rv1979c* variants were detected and no *atpE*, *pepQ* or *Rv0677c* variants were detected. Prior CFZ exposure, days on BDQ (with programmatic treatment outcome), SNP distances, and the specific variant (proportion of reads indicated) are shown (blue indicates variant loss, red indicates gain). Variants previously described(22) are bolded. In baseline susceptible isolates, most had no *rv0678* variants [33% (6/18) had -11C/A variants]. When comparing baseline and follow-up isolates, 94% (16/17) with newly gained resistance appeared to be due to intrapatient evolution, however, 6% (1/17) patients had evidence of reinfection. Abbreviations: BDQ – bedaquiline, CFZ – clofazimine, indels – insertions and deletions, R – resistant, S – susceptible, SNPs – single nucleotide polymorphisms, TDS – targeted deep sequencing, WGS – whole genome sequencing, WT – wildtype. Data are % unless otherwise stated.

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS	WGS		
					rv0678	*rv0678*	*rv0676c*	*rv1979c*
19-A19 (Yes)	168 (Treatment failed)	Baseline	S	3 (likely intrapatient evolution)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs* **5 G/T (5)** 77 A/T (3) 265 C/T (2) *Indels* 124 G ins (21) **16 G del (3)**	*Indels* 124 G ins (19)	*SNPs* **2842 T/C (100)**	WT (100)
22-A22 (Yes)	168 (Treatment failed)	Baseline	S	5 (likely intrapatient evolution)	*SNPs* -11 C/A (100)	*SNPs* -11 C/A (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs* - 11 C/A (100) *Indels* **138 G ins (99)**	*SNPs* - 11 C/A (100) *Indels* **138 G ins (99)**	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
27-A27 (Yes)	168 (Treatment failed)	Baseline	S	39 (likely reinfection)	*SNPs* -11 C/A (100)	*SNPs* -11 C/A (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs* -11 C/A (100) 137 G/A (77)	*SNPs* -11 C/A (100) 137 G/A (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)**	WT (100)

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS	WGS		
					rv0678	*rv0678*	*rv0676c*	*rv1979c*
							2842 T/C (100)	
38-B09 (Yes)	265 (Treatment failed)	Baseline	S	4 (likely intrapatient evolution)	WT (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)** **2889 C/T (100)**	WT (100)
		Follow-up	R		*SNPs* **107 C/T (5)** *Indels* **141 C ins (93)**	*Indels* **141 C ins (98)**	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)** **2889 C/T (100)**	WT (100)
04-A04 (Yes)	192 (Died)	Baseline	S	221 (likely reinfection, two strains present at baseline supplanted by a new strain at follow-up)	WT (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 151 A/T (72)
		Follow-up	R		*SNPs* 278 T/C (100)	*SNPs* 278 T/C (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)** **2889 C/T (100)**	WT (100)
09-A09 (Yes)	168 (Died)	Baseline	S	2 (likely intrapatient evolution)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
		Follow-up	R		WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
10-A10 (Yes)	356 (Died)	Baseline	S	Noncalculable	WT (100)	No WGS available		
		Follow-up	R		*Indels* 140 TC ins (97) **141 C ins (1)**	*Indels* 140 TC ins (91)	*SNPs* **2842 T/C (100)**	WT (100)
12-A12 (Yes)	165 (Died)	Baseline	S	Noncalculable	WT (100.00)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs* 461 T/G (11) *Indels* 140 TC ins (15) **141 C ins (74)**	No WGS available		
14-A14 (Yes)	168 (Died)	Baseline	S	3 (likely intrapatient evolution)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs* **107 C/T (33)** 167 T/C (2) **220 C/G (48)** *Indels* 136 G ins (2) 390 C ins (4) **16 G del (5)**	*SNPs* **220 C/G (79)**	*SNPs* **2842 T/C (100)**	WT (100)
18-A18 (Yes)	368 (Died)	Baseline	S	6 (likely intrapatient evolution)	*SNPs* -11 C/A (100)	*SNPs* -11 C/A (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs*	*SNPs*	*SNPs*	WT (100)

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS	WGS		
					rv0678	*rv0678*	*rv0676c*	*rv1979c*
					-11 C/A (100) 374 T/C (12) *Indels* **192 G ins (16)** 268 G ins (46) **193 G del (2)** 419 GGGATCTGTT del (2)	-11 C/A (100) *Indels* 268 G ins (17)	**2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	
21-A21 (Yes)	154 (Died)	Baseline	S	3 (likely intrapatient evolution)	*SNPs* -11 C/A (100)	*SNPs* -11 C/A (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs* -8 T/G (10) -11 C/A (100) 343 C/T (1) *Indels* 289 C del (85) 292 A del (2) 383 C del (1)	*SNPs* -8 T/G (13) -11 C/A (100) *Indels* 289 C del (88)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
25-A25 (Yes)	365 (Died)	Baseline	S	3 (likely intrapatient evolution)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
		Follow-up	R		*Indels* **141 C ins (92)** **192 G ins (4)**	*Indels* **141 C ins (87)**	*SNPs* **2842 T/C (100)**	WT (100)
36-B07 (Yes)	290 (Died)	Baseline	S	3 (likely intrapatient evolution)	WT (100)	WT (100)	*SNPs* **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs* 343 C/T (4) *Indels* 138 GA ins (3) **141 C ins (85)** 432 A del (7) 433 T del (7) 434 A del (7)	*Indels* **141 C ins (85)**	*SNPs* **2381 G/A (100)** **2842 T/C (100)**	WT (100)
39-B10 (Yes)	393 (Died)	Baseline	S	3 (likely intrapatient evolution)	WT (100.00)	WT (100)	*SNPs* **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 1266 C/T (100)
		Follow-up	R		*Indels* **141 C ins (99)**	*Indels* **141 C ins (95)**	*SNPs* **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 1266 C/T (100)
28-A28 (Yes)	177 (Loss to follow-up)	Baseline	No result	4 (likely intrapatient evolution)	WT (100)	WT (100)	*SNPs* **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 1266 C/T (100)
		Follow-up	R		*SNPs* 122 G/A (4) 128 T/G (2)	*Indels* **132 GT ins (54)** **138 G ins (38)**	*SNPs* **2381 G/A (100)** 2842 T/C (100)	*SNPs* 1266 C/T (100)

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS	WGS		
					rv0678	*rv0678*	*rv0676c*	*rv1979c*
					437 T/G (4) *Indels* **132 GT ins (29)** 136 G ins (18) **138 G ins (16)** **141 C ins (9)** **192 G ins (2)** 210 G ins (3) 269 C ins (1) 318 CG ins (2) 334 CC ins (1) 423 C ins (1) 426 T ins (4)			
40-B11 (Yes)	265 (Not evaluated)	Baseline	S	6 (likely intrapatient evolution)	*SNPs* -11 C/A (100)	*SNPs* -11 C/A (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs* -11 C/A (100) *Indels* 292 A del (100)	*SNPs* -11 C/A (100) *Indels* 292 A del (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
03-A03 (No)	375 (Died)	Baseline	S	3 (likely intrapatient evolution)	WT (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 151 A/T (100)
		Follow-up	R		*SNPs* 304 G/C (4) *Indels* **132 GT ins (2)** **138 G ins (19)** 138 GA ins (43) **93 G del (12)**	*Indels* Gene disruption*	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 151 A/T (100)
33-B04 (No)	430 (Died)	Baseline	S	3 (likely intrapatient evolution)	*SNPs* -11 C/A (100)	*SNPs* -11 C/A (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	R		*SNPs* -11 C/A (100) **136 T/C (12)** 263 A/G (83) *Indels* **16 G del (6)**	*SNPs* -11 C/A (100) **136 T/C (16)** 263 A/G (85)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
35-B06 (No)	163 (Died)	Baseline	S	1 (likely intrapatient evolution)	WT (100.00)	WT (100.00)	*SNPs* **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 1266 C/T (100)
		Follow-up	R		WT (100.00)	WT (100.00)	*SNPs* **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 1266 C/T (100)
	Median (IQR) days of BDQ treatment	Baseline	S n=18	**Median (IQR) variant distance**	*SNPs* | *n* | *Median (IQR)*	*SNPs* | *n* | *Median (IQR)*	*SNPs* | *n* | *Median (IQR)*	*SNPs* | *n* | *Median (IQR)*

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS			WGS								
					rv0678			*rv0678*	*rv0676c*					*rv1979c*		
Sub-total (Yes=16 No=3)	192 (168-267) Treatment failed (n=4) Died (n=13) Loss to follow-up (n=1) Not evaluated (n=1)			3 (3-6) Likely intrapatient evolution (n=15) Likely reinfection (n=2) Noncalculable (n=2)	-11 C/A	6	100 (100-100)	-11 C/A	6	100 (100-100)	**2299 C/T** **2381 G/A** **2842 T/C** **2889 C/T**	9 13 18 1	100 (100-100) 100 (100-100) 100 (100-100) N/A	151 A/T 1266 C/T	2 3	86 (72-100) 100 (100-100)
		Follow-up	R n=19		*SNPs*	*n*	*Median (IQR)*	*SNPs*	*n*	*Median (IQR)*	*SNPs*	*n*	*Median (IQR)*	*SNPs*	*n*	*Median (IQR)*
					-8 T/G	1	N/A	-8 T/G	1	N/A	**2299 C/T**	9	100 (100-100)	151 A/T	1	N/A
					-11 C/A	6	100 (100-100)	-11 C/A	6	100 (100-100)	**2381 G/A**	13	100 (100-100)	1266 C/T	3	100 (100-100)
					5 G/T	**1**	**N/A**	**5 G/T**	**1**	**N/A**	**2842 T/C**	17	100 (100-100)			
					77 A/T	1	N/A	**136 T/C**	**1**	**N/A**	**2889 C/T**	2	100 (100-100)			
					107 C/T	**2**	**19 (5-33)**	137 G/A	1	N/A						
					122 G/A	1	N/A	**220 C/G**	**1**	**N/A**						
					128 T/G	1	N/A	263 A/G	1	N/A						
					136 T/C	**1**	**N/A**	278 T/C	1	N/A						
					167 T/C	1	N/A	*Indels*	*n*	*Median (IQR)*						
					220 C/G	**1**	**N/A**	124 G ins	1	N/A						
					263 A/G	1	N/A	**132 GT ins**	**1**	**N/A**						
					265 C/T	1	N/A	136 G ins	1	N/A						
					278 T/C	1	N/A	**138 G ins**	**2**	**69 (40-98)**						
					304 G/C	1	N/A	140 TC ins	1	N/A						
					343 C/T	2	3 (1-4)	**141 C ins**	**4**	**91 (80-95)**						
					374 T/C	1	N/A	**192 G ins**	**1**	**N/A**						
					437 T/G	1	N/A	268 G ins	1	N/A						
					461 T/G	1	N/A	289 C del	1	N/A						
					Indels	*n*	*Median (IQR)*	292 A del	1	N/A						
					124 G ins	1	N/A									
					132 GT ins	**1**	**N/A**									
					136 G ins	2	10 (2-17)									
					138 G ins	**3**	**19 (16-99)**									
					138 GA ins	2	23 (19-26)									
					140 TC ins	2	56 (15-97)									
					141 C ins	**7**	**85 (9-93)**									
					192 G ins	**3**	**4 (2-16)**									
					210 G ins	1	N/A									
					268 G ins	1	N/A									
					269 C ins	1	N/A									

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS			WGS								
					rv0678			*rv0678*	*rv0676c*						*rv1979c*	
					318 CG ins	1	N/A									
					334 CC ins	1	N/A									
					390 C ins	1	N/A									
					423 C ins	1	N/A									
					426 T ins	1	N/A									
					16 G del	**3**	**5 (3-6)**									
					93 G del	**1**	**N/A**									
					193 G del	**1**	**N/A**									
					289 C del	1	N/A									
					292 A del	2	51 (2-100)									
					383 C del	1	N/A									
					419 GGGATCTGTT del	1	N/A									
					432 A del	1	N/A									
					433 T del	1	N/A									
					434 A del	1	N/A									

Supplementary Table 7.

Phenotypic and genotypic DST result for patients with no phenotypic BDQ resistance and at baseline and follow-up. The last row shows summary data. As detailed in **Methods**, TDS was done on *Rv0678*, *atpE*, and *pepQ*. WGS was also done on at *Rv0676c*, *Rv0677c* and *Rv1979c*. No variants were detected in *atpE*, and *Rv0677c* and these columns are omitted. When comparing baseline and follow-up isolates, 63% (10/16) with newly gained resistance appeared to be due to intrapatient evolution, however, 31% (5/16) patients had evidence of reinfection. Prior CFZ exposure, days on BDQ (with programmatic treatment outcome), SNP distances, and the specific variant (proportion of reads indicated) are shown (blue indicates variant loss, red indicates gain). Variants previously described (22) are bolded. In baseline isolates, 35% (6/17) compared to 20% (3/15) follow up isolates had *Rv0678* -11 C/A variants detected. Two phenotypically susceptible isolates have *pepQ* variants detected (32-B03, 13-A13). Abbreviations: BDQ – bedaquiline, CFZ – clofazimine, indels – insertions and deletions, R – resistant, S – susceptible, SNPs – single nucleotide polymorphisms, TDS – targeted deep sequencing, WGS – whole genome sequencing, WT – wildtype. Data are % unless otherwise stated.

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS		WGS			
					Rv0678	*pepQ*	*Rv0678*	*pepQ*	*Rv0676c*	*Rv1979c*
31-B02 (Yes)	862 (Cured)	Baseline	S	3 (likely intrapatient evolution)	WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
		Follow-up	S		WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
06-A06 (Yes)	178 (Treatment completed)	Baseline	S	2 (likely intrapatient evolution)	WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)** 2889 C/T (100)	WT (100)
		Follow-up	S		WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)** 2889 C/T (100)	WT (100)
15-A15 (Yes)	168 (Treatment completed)	Baseline	S	838 (likely reinfection)	WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* 1792 A/T (100) **2842 T/C (100)**	WT (100)
		Follow-up	S		WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
02-A02 (Yes)	186 (Died)	Baseline	S	4 (likely intrapatient evolution)	*SNPs* -11 C/A (100)	WT (100)	*SNPs* -11 C/A (100)	WT (100)	*SNPs* **2381 G/A (100)** **2842 T/C (100)** **2889 C/T (100)**	WT (100)
		Follow-up	S		*SNPs* -11 C/A (100)	WT (100)	*SNPs* -11 C/A (100)	WT (100)	*SNPs* **2381 G/A (100)** **2842 T/C (100)** **2889 C/T (100)**	WT (100)
05-A05 (Yes)	343 (Died)	Baseline	S	2 (likely intrapatient evolution)	**SNPs** -11 C/A (100)	WT (100)	*SNPs* -11 C/A (99)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)**	WT (100)

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS		WGS			
					Rv0678	*pepQ*	*Rv0678*	*pepQ*	*Rv0676c*	*Rv1979c*
									2842 T/C (100)	
		Follow-up	S		**SNPs** -11 C/A (100)	WT (100)	*SNPs* -11 C/A (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
32-B03 (Yes)	126 (Died)	Baseline	S	5 (likely intrapatient evolution)	WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2381 G/A (100)** **2842 T/C (100)**	*SNPs* **1266 C/T (100)**
		Follow-up	S		WT (100)	*Indels* 241 G ins (100) 219 G del (100)	WT (100)	*Indels* 241 G in (98) 219 G del (100)	*SNPs* **2381 G/A (100)** **2842 T/C (100)**	*SNPs* **1266 C/T (100)**
11-A11 (Yes)	218 (Loss to follow-up)	Baseline	S	77 (likely reinfection)	WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
		Follow-up	S		WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
24-A24 (Yes)	168 (Loss to follow-up)	Baseline	S	Noncalculable	*SNPs* -11 C/A (100)	WT (100)	*SNPs* -11 C/A (99)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	S		*SNPs* -11 C/A (100)	WT (100)	No WGS available			
17-A17 (Yes)	283 (Not evaluated)	Baseline	S	2 (likely intrapatient evolution)	WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 151 A/T (100)
		Follow-up	S		WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	*SNPs* 151 A/T (100)
20-A20 (No)	168 (Cured)	Baseline	S	240 (likely reinfection)	*SNPs* -11 C/A (100)	WT (100)	*SNPs* - 11 C/A (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	S		WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
30-B01 (No)	843 (Cured)	Baseline	S	0 (no change)	WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
		Follow-up	S		WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
08-A08 (No)	168 (Treatment completed)	Baseline	S	Noncalculable	*SNPs* -11 C/A (100)	WT (100)	*SNPs* -11 C/A (99)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**	WT (100)
		Follow-up	S		WT (100)	WT (100)	No WGS available			
16-A16 (No)	170 (Treatment completed)	Baseline	S	1245 (likely reinfection)	WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2842 T/C (100)**	WT (100)
		Follow-up	S		WT (100)	WT (100)	WT (100)	WT (100)	*SNPs* **2299 C/T (100)** **2381 G/A (100)**	*SNPs* 151 A/T (100)

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS						WGS											
					Rv0678			*pepQ*			*Rv0678*			*pepQ*			*Rv0676c*			*Rv1979c*		
																	2842 T/C (100)					
01-A01 (No)	234 (Died)	Baseline	S	2 (likely intrapatient evolution)	*SNPs* -11 C/A (100)			WT (100)			*SNPs* -11 C/A (100)			WT (100)			*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**			WT (100)		
		Follow-up	S		*SNPs* -11 C/A (100)			WT (100)			*SNPs* -11 C/A (100)			WT (100)			*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**			WT (100)		
07-A07 (No)	168 (Died)	Baseline	S	1271 (likely reinfection)	WT (100)			WT (100)			WT (100)			WT (100)			*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)** 2889 C/T (100)			WT (100)		
		Follow-up	S		WT (100)			WT (100)			WT (100)			WT (100)			*SNPs* **2842 T/C (100)**			WT (100)		
23-A23 (No)	168 (Died)	Baseline	S	4 (likely intrapatient evolution)	WT (100)			WT (100)			WT (100)			WT (100)			*SNPs* **2381 G/A (100)** **2842 T/C (100)**			*SNPs* **1266 C/T (100)**		
		Follow-up	S		WT (100)			WT (100)			WT (100)			WT (100)			*SNPs* **2381 G/A (100)** **2842 T/C (100)**			*SNPs* **1266 C/T (100)**		
34-B05 (No)	276 (Loss to follow-up)	Baseline	S	2 (likely intrapatient evolution)	WT (100)			WT (100)			WT (100)			WT (100)			*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**			*SNPs* 151 A/T (100)		
		Follow-up	S		WT (100)			WT (100)			WT (100)			WT (100)			*SNPs* **2299 C/T (100)** **2381 G/A (100)** **2842 T/C (100)**			*SNPs* 151 A/T (100)		
13-A13 (No)	675 (Not evaluated)	Baseline	No Result	5 (likely intrapatient evolution)	WT (100)			WT (100)			WT (100)			WT (100)			*SNPs* **2842 T/C (100)**			WT (100)		
		Follow-up	S		WT (100)			WT (100)			WT (100)			*SNPs* 260 C/G			*SNPs* **2842 T/C (100)**			WT (100)		
(Sub-total (Yes=9 No=9)	**Median (IQR) days of BDQ treatment** 181 (168-267) Cured (n=3) Treatment completed (n=4) Died (n=6) Loss to follow-up (n=3) Not evaluated (n=2)	Baseline	S n=17	**Median (IQR) variant distance** 4 (2-148) No change (n=1) Likely intrapatient evolution (n=10) Likely reinfection (n=5) Noncalculable (n=2)	*SNPs* -11 C/A	*n* 6	*Median (IQR)* 100 (100-100)	-			*SNPs* -11 C/A	*n* 6	*Median (IQR)* 100 (99-100)	-			*SNPs* 1792 A/T **2299 C/T** **2381 G/A** **2842 T/C** 2889 C/T	*n* 1 **8** **12** **18** 3	*Median (IQR)* N/A **100 (100-100)** **100 (100-100)** **100 (100-100)** 100 (100-100)	*SNPs* 151 A/T **1266 C/T**	*n* 2 **2**	*Median (IQR)* 100 (100-100) **100 (100-100)**
		Follow-up	S n=18		*SNPs*	*n*	*Median (IQR)*	*Indels*	*n*	*Median (IQR)*	*SNPs*	*n*	*Median (IQR)*	*SNPs*	*n*	*Median (IQR)*	*SNPs*	*n*	*Median (IQR)*	*SNPs*	*n*	*Median (IQR)*

Patient identifier (CFZ exposure)	Days of BDQ treatment duration (treatment outcome)	Timepoint	BDQ phenotypic DST (1µg/ml)	SNP distance (interpretation)	TDS						WGS											
					Rv0678			*pepQ*			*Rv0678*			*pepQ*			*Rv0676c*			*Rv1979c*		
					-11 C/A	4	100 (100-100)	241 G ins	1	N/A	-11 C/A	3	100 (100-100)	260 C/G	1	N/A	**2299 C/T**	**6**	**100 (100-100)**	151 A/T	3	100 (100-100)
								219 G del	1	N/A				*Indels*	*n*	*Median (IQR)*	**2381 G/A**	**10**	**100 (100-100)**	**1266 C/T**	**2**	**100 (100-100)**
														241 G ins	1	N/A	**2842 T/C**	**16**	**100 (100-100)**			
														219 G del	1	N/A						
																	2889 C/T	2	100 (100-100)			

References

1. Dippenaar A, De Vos M, Marx FM, Adroub SA, van Helden PD, Pain A, et al. Whole genome sequencing provides additional insights into recurrent tuberculosis classified as endogenous reactivation by IS6110 DNA fingerprinting. Infection, Genetics Evolution. 2019;75:103948.
2. Bolger AM, Lohse M, Usadel BJB. Trimmomatic: a flexible trimmer for Illumina sequence data. 2014;30(15):2114-20.
3. Li H, Durbin RJb. Fast and accurate short read alignment with Burrows–Wheeler transform. 2009;25(14):1754-60.
4. Ponstingl H NZ, editor. Intelligent Systems for Molecular Biology 2010 meeting; 2010.
5. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. 2009;25(16):2078-9.
6. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. 2010;20(9):1297-303.
7. Comas I, Chakravartti J, Small PM, Galagan J, Niemann S, Kremer K, et al. Human T cell epitopes of Mycobacterium tuberculosis are evolutionarily hyperconserved. Nat Genet. 2010;42(6):498-503.
8. Blouin Y, Hauck Y, Soler C, Fabre M, Vong R, Dehan C, et al. Significance of the identification in the Horn of Africa of an exceptionally deep branching Mycobacterium tuberculosis clade. PLoS One. 2012;7(12):e52841.
9. Coscolla M, Lewin A, Metzger S, Maetz-Rennsing K, Calvignac-Spencer S, Nitsche A, et al. Novel Mycobacterium tuberculosis complex isolate from a wild chimpanzee. Emerg Infect Dis. 2013;19(6):969-76.
10. Bos KI, Harkins KM, Herbig A, Coscolla M, Weber N, Comas I, et al. Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. Nature. 2014;514(7523):494-7.
11. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32(1):268-74.
12. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. Nucleic Acids Res. 2019;47(W1):W256-W9.
13. Paradis E, Schliep KJB. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. 2019;35(3):526-8.
14. Jombart T, Ahmed IJB. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. 2011;27(21):3070-1.
15. Walker TM, Lalor MK, Broda A, Ortega LS, Morgan M, Parker L, et al. Assessment of Mycobacterium tuberculosis transmission in Oxfordshire, UK, 2007-12, with whole pathogen genome sequences: an observational study. Lancet Respir Med. 2014;2(4):285-92.
16. Black P, De Vos M, Louw G, Van der Merwe R, Dippenaar A, Streicher E, et al. Whole genome sequencing reveals genomic heterogeneity and antibiotic purification in Mycobacterium tuberculosis isolates. BMC genomics. 2015;16(1):857.
17. Colman RE, Anderson J, Lemmer D, Lehmkuhl E, Georghiou SB, Heaton H, et al. Rapid drug susceptibility testing of drug resistant Mycobacterium tuberculosis directly from clinical samples using amplicon sequencing: A proof of concept study. Journal of clinical microbiology. 2016:JCM. 00535-16.
18. Willby M, Chopra P, Lemmer D, Klein K, Dalton TL, Engelthaler DM, et al. Molecular evaluation of fluoroquinolone resistance in serial Mycobacterium tuberculosis isolates from individuals diagnosed with multidrug-resistant tuberculosis. Antimicrobial Agents Chemotherapy. 2020;65(1):e01663-20.
19. TB DD-R. Introduction of new drugs, drug regimens and management for drug-resistant TB in South Africa: policy framework. National Department of Health. 2015.
20. Colman RE, Schupp JM, Hicks ND, Smith DE, Buchhagen JL, Valafar F, et al. Detection of low-level mixed-population drug resistance in Mycobacterium tuberculosis using high fidelity amplicon sequencing. 2015;10(5):e0126626.

21. Mudaly V, Voget J. Clinical Guidelines & Standard Operating Procedure for the Implementation of the Short & Long DR-TB regimens for Adults, Adolescents and Children.
22. Ismail N, Rivière E, Limberis J, Huo S, Metcalfe JZ, Warren RM, et al. Genetic variants and their association with phenotypic resistance to bedaquiline in Mycobacterium tuberculosis: a systematic review and individual isolate data analysis. The Lancet Microbe. 2021;2(11):e604-e16.