TO THE EDITOR: Bedaquiline improves survival among persons with multidrug-resistant tuberculosis (MDR-TB).1 We report the case of a 65-year-old South African man who was negative for human immunodeficiency virus and in whom MDR-TB was diagnosed in 2013 (resistant to rifampin and isoniazid; phenotypically susceptible to a fluoroquinolone and amikacin). A baseline radiograph showed changes consistent with bilateral tuberculosis with left apex cavitation. He started standardized treatment that included moxifloxacin, pyrazinamide, kanamycin, ethionamide, isoniazid, and terizidone. After initial sputum culture conversion (at month 3) and clinical improvement, the patient again became culture-positive, and bilateral cavitation developed. After detection of phenotypic resistance to fluoroquinolones (at month 6), his treatment was revised (at month 8) to include high-dose isoniazid, ethambutol, pyrazinamide, terizidone, linezolid, paraaminosalicylic acid, and kanamycin (Fig. 1 and the Supplementary Appendix, available with the full text of this letter at NEJM.org). Bedaquiline was added 22 days later and was administered for 6 months.2 The patient remained culture-positive (treatment failure), and treatment was stopped 15 months after revision of the regimen. The patient died 7 months later.

Overall, eight Mycobacterium tuberculosis isolates (A through H) were assessed by means of whole-genome sequencing, targeted deep sequencing3 of Rv0678, and phenotypic bedaquiline resistance testing. Whole-genome sequencing of isolate A, which was obtained 4.7 months after the initiation of standard MDR-TB treatment, revealed a Beijing strain with mutations conferring resistance to rifampin, isoniazid, ethambutol, ethionamide, fluoroquinolones, pyrazinamide, and streptomycin (Fig. 1). Whole-genome sequencing of isolate C, obtained 2 months after treatment revision, suggested that there were five potentially effective drugs in the regimen the patient had been receiving at the time that bedaquiline (to which the isolate was phenotypically susceptible) was added. Targeted deep sequencing of isolate C revealed the presence of a base-pair insertion in Rv0678 at a variant frequency of 0.05% (at position 192), indicating microheteroresistance (i.e., the presence of resistance-associated alleles at a frequency of <1%). This variant was not present in isolate B, which had been obtained before bedaquiline treatment. Isolate D, obtained after bedaquiline cessation, had this insertion in more than 90% of the bacterial population. The frequency of the insertion in Rv0678 at position 192 decreased in subsequent isolates, but two different insertions in Rv0678 emerged (insertion of GA at position 138 in isolate F, and insertion of G at position 138 in isolate G). The G insertion at position 138 became fixed after all treatment was stopped (iso-
Isolates D, E, F, G, and H were phenotypically resistant to bedaquiline. This case shows the emergence of bedaquiline resistance despite the presence of five poten-

tially effective drugs and good adherence (based on clinical notes). The emergence of \textit{Rv0678} variants after completion of 6 months of bedaquiline treatment shows the risk of resistance am-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Chronology of Diagnosis and Treatment.}
\end{figure}

Shown is a summary of treatment, genotypic drug resistance (based on whole-genome sequencing), phenotypic bedaquiline drug-susceptibility testing (DST), targeted deep sequencing, and treatment monitoring during the standard treatment regimen and a subsequent individualized bedaquiline-containing regimen. Overall, eight isolates (A through H) collected between 4.7 months after the initiation of the standard regimen and 6 months after all tuberculosis treatment was stopped were analyzed by whole-genome sequencing, targeted deep sequencing of \textit{Rv0678}, and phenotypic bedaquiline DST. The patient was initially given a diagnosis of multidrug-resistant tuberculosis (MDR-TB) with low-level isoniazid (INH) resistance, determined with the use of Genotype MTBDRplus, and was treated with a standard MDR-TB regimen. However, he remained culture-positive. In accordance with guidelines, subsequent isolates were phenotypically characterized for susceptibility to ofloxacin (OFX) and amikacin (AMI). Fluoroquinolone resistance (indicated by resistance to OFX) was first noted 6 months after treatment initiation. All isolates remained susceptible to second-line injectable antibiotics. At 8.1 months, a revised regimen was initiated, with subsequent addition of bedaquiline (BDQ) (22 days after the initiation of the revised regimen) and withdrawal of pyrazinamide (Z) and ethambutol (E) (2 months after the initiation of the revised regimen). BDQ was administered for 6 months. At month 6 of the revised regimen, the patient declined treatment with kanamycin (KAN); he continued to not receive KAN for a duration of 2.4 months. Treatment with the individualized regimen was continued until the outcome of treatment failure at 15 months. Phenotypic DST showed that all isolates with a variant frequency of more than 1% in \textit{Rv0678} were found by mycobacteria growth indicator tube (MGIT) testing to be resistant to BDQ at a concentration of 1 µg per milliliter. ETH denotes ethionamide, LZD linezolid, MXF moxifloxacin, PAS paraaminosalicylic acid, R resistant, S susceptible, and TZD terizidone.
plification after cessation of treatment with a drug that has a long half-life (5.5 months for bedaquiline).5

Margaretha de Vos, Ph.D.
Stellenbosch University
Cape Town, South Africa
margab@sun.ac.za

Kristin B. Wiggins, B.S.
Translational Genomics Research Institute
Flagstaff, AZ

Brigitta Derendinger, B.Sc.
Anzaan Dippenaar, Ph.D.
Melanie Grobbelaar, Ph.D.
Stellenbosch University
Cape Town, South Africa

Tania Dolby, B.S.
National Health Laboratory Services
Cape Town, South Africa

Scott Burns, B.S.
Centers for Disease Control and Prevention
Atlanta, GA

Marco Schito, Ph.D.
Critical Path Institute
Tucson, AZ

David M. Engeltalher, Ph.D.
Translational Genomics Research Institute
Flagstaff, AZ

John Metcalfe, M.D., Ph.D.
University of California, San Francisco
San Francisco, CA

Grant Theron, Ph.D.
Stellenbosch University
Cape Town, South Africa

Annelies van Rie, M.D., Ph.D.
University of Antwerp
Antwerp, Belgium

James Posey, Ph.D.
Centers for Disease Control and Prevention
Atlanta, GA

Rob Warren, Ph.D.
South African Medical Research Council
Cape Town, South Africa

Helen Cox, Ph.D.
University of Cape Town
Cape Town, South Africa

Drs. de Vos and Ley and Drs. Warren and Cox contributed equally to this letter.

Use of trade names is for identification only and does not constitute endorsement by the Department of Health and Human Services, the Public Health Service, or the Centers for Disease Control and Prevention (CDC). The findings and conclusions in this letter are those of the authors and do not necessarily represent the views of the CDC, the National Institutes of Health (NIH), or the South African Medical Research Council.

This is the New England Journal of Medicine version of record, which includes all journal editing and enhancements. The Author Final Manuscript, which is the author's version after external peer review and before publication in the Journal, is available under a CC BY license at PMC6518951.

Supported by the National Research Foundation, the South African Medical Research Council, the Stellenbosch University Faculty of Medicine Health Sciences, and a grant from the NIH (R01AI131939). Dr. Cox is supported by a Welcome Trust fellowship (099818/Z/12/Z); Drs. de Vos, Ley, van Rie, and Warren are supported by TORCH funding through the Flemish Fund for Scientific Research (FWO G0F8316N); Dr. Ley is supported by a grant from the Swiss National Science Foundation (P2BSP3 _165379); and Dr. Theron is supported by the EDCTP2 program supported by the European Union (grant SF1401, OPTIMAL DIAGNOSIS). The sequencing work was performed at the Translational Genomics Research Institute and supported by a grant from the Bill and Melinda Gates Foundation (OPP1115887, to Dr. Schito) for the ReSeqTB sequencing platform.

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.


DOI: 10.1056/NEJMoa1815121