Executive summary

Fluoroquinolones (FQs) have been a cornerstone of the treatment of multidrug-resistant (MDR) tuberculosis, and they are gaining increasing attention as possible components of new first-line chemotherapeutic regimens. A variety of FQs have been used clinically, including gatifloxacin, ciprofloxacin, ofloxacin (OFX), and moxifloxacin, which is currently being evaluated as a first-line therapeutic. Resistance to FQs in Mycobacterium tuberculosis (MtB) is most frequently due to mutations in the drug target, DNA gyrase. These occur in the quinolone resistance determining regions (QRDRs) of the gyrA and gyrB genes, and the majority of genetically characterized FQR MtB strains have mutations in these regions, most often in several highly conserved codons. However, in a recent survey of FQ resistant MtB isolates collected in Taiwan in the years 2004 and 2005, it was observed that while FQ resistance rates were generally low (3%), a sizeable fraction of FQ resistant isolates (9 of 14) had no detectable changes in the QRDR of either gyrA or gyrB. Of the 14 FQ resistant samples, 6 were resistant to another drug, and 4 of these were MDR (Wang, 2007). We now have 17 well characterized FQ resistant MtB strains isolated from patients in Taiwan, none of which have mutations in the QRDR of either gyrA or gyrB. We propose to sequence the genomes of these 17 strains in an effort to reveal the genetic basis for the FQ resistance of these organisms.

This project is critical for several reasons:

1. FQs are an important drug in the treatment of MDR TB, and it is critical to understand the full set of ways in which resistance to this class of drugs develops
2. Proper epidemiological tracking of FQ resistance requires a complete understanding of the genetic basis for resistance
3. The development of new molecular diagnostics hinges on a complete understanding of resistance-associated mutations
4. These sequencing of these strains will ultimately contribute to the larger effort to define the genetic determinants of multiple drug resistance.

We anticipate that sequencing the genomes of these 17 FQ resistant isolates will yield correlations between FQ resistance and polymorphisms in genes other than gyrA and gyrB, the two canonical loci associated with FQ resistance. While these Taiwanese strains will likely contain a large number of polymorphisms relative to any strain used as a reference, we hope to use the large number of previously sequenced genomes from FQ-sensitive MtB strains as a comparator to identify candidate FQ resistance-conferring mutations. Our belief is that these 17 strains will have in common one or more loci that contain polymorphisms, and that previously sequenced FQ sensitive strains will not contain polymorphisms in these genetic features.

These candidate FQ resistance-conferring mutations will be evaluated by engineering the candidate causative lesions into the genome of a FQ-sensitive laboratory strain. In this recombineering-based approach, long oligonucleotides carrying the polymorphisms are transformed into a drug-sensitive laboratory strain expressing an phage-derived enzyme, Che9c gene 61 (van Kessel, 2008), that promotes the replacement of the wild-type sequence with the homologous, mutation containing sequence on the
oligonucleotide. Once introduced into the genome, the contribution of these mutations to FQ resistance will be assessed using standard FQ susceptibility assays

Rationale for strain selection and the number of strains proposed in the study.

In a recent survey of FQ resistant Mtb isolates collected in Taiwan in the years 2004 and 2005, it was observed that while FQ resistance rates were generally low (3%), a sizeable fraction of FQ resistant isolates (9 of 14) had no detectable changes in the QRDR of either gyrA or gyrB. Of the 14 FQ resistant samples, 6 were resistant to another drug, and 4 of these were MDR. We now have 17 well characterized FQ resistant Mtb strains isolated from patients in Taiwan, none of which have mutations in the QRDR of either gyrA or gyrB. Comparison to existing Mtb genomes sequenced as part of this and related studies should enable the elucidation of novel FQ resistance determinants.

Mtb strains were isolated from TB patients in northern Taiwan. Strains were identified with standard biochemical tests. Drug susceptibility was assessed by serial dilution on agar plates as described in National Committee for Clinical Laboratory Standards. Susceptibility Testing of Mycobacteria, Nocardiae, and other Aerobic Actinomycetes: Approved Standard. NCCLS, Wayne, PA, USA, 2003.

REFERENCES
