Summary of PZA Day at CDC

Thursday, December 15, 2011, CDC, Atlanta

Purpose

PZA is still a unique and essential agent for tuberculosis (TB) therapy in combination with both current and new drugs. Reliable and practical DST testing for PZA would greatly faciliate care, and rapid (genetic-based) DST would also facilitate clinical trials of several new PZA-based combinations for multidrug-resistant (MDR) TB. Inspired by recent meetings on diagnostics, the emerging view on the dependence of future regimens on PZA, and various challenges with PZA DST on CDC-related projects, CDC hosted a meeting to strengthen internal USG interaction among CDC, FDA, and NIH on relevant questions and actions forward, with the aim of better facilitating ongoing partner planning and efforts to advance PZA DST in both the short-term and long-term. CDC invited The TB Drug Alliance to be a partner in this first focused step forward. Importantly, these joint efforts to improve PZA DST will also be applicable to development of DST and resistance monitoring for new TB drugs.

Action items

1) A comprehensive inventory of current activities focused on molecular analysis and conventional drug susceptibility testing for PZA and the parties involved should be developed.
    a. Inventory development should begin with partners in the Diagnostics Research Forum and include follow-up with the Gates Foundation to determine the current status of the TB Drug Resistance Mutation Database Project.
    b. A draft product should be available for the Diagnostics Research Forum PZA meeting hosted by NIH and scheduled for 2012.

2) NIH and CDC will coordinate with each other in the planning of a follow-on broader PZA meeting to be held in 2012.

3) Collaborative efforts should be coordinated with interested parties identified through the research inventory to identify the essential components of a mutation database to better understand and identify PZA resistance. These efforts would also include review of literature and compilation of what is already known about *pncA* mutations.

4) Overview of meeting outcomes should be available for the next Jupiter call in January by DTBE's Laboratory Branch to make some preliminary decisions on how best to proceed.

5) NIAID to explore establishment of sequencing capability at a couple of laboratories in South Africa and elsewhere to be used for clinical research purposes, based on mutual interest among DAIDS/ACTG, DTBE/TBTC, and GTAB and will coordinate efforts to meet this objective.

6) DTBE's IRPB and LB will review progress and establish next steps around PZA DST needed to meet PETTS study objectives.

7) A plan should be made for developing clinical trial service laboratory capabilities to provide rapid turn-around on *pncA* sequencing results in one or two key regions by 2013.

Questions

1. What is agreement of the current, growth-based PZA DST with other tests? What is the range of expected variation from assay to assay, method to method, and laboratory to laboratory?

   The current capacity of available assays to accurately identify PZA resistance is insufficient and unacceptable with varying amounts of discordance. Based on the importance of PZA to new treatment regimens, progress must be made. Existing data are primarily anecdotal in nature and there is a need exists for systematic prospective approach to data collection.

   One potential approach for analyzing new methodologies is examining performance in different laboratory settings in trained hands and correlation of results with a central laboratory. In addition, proficiency panels may be needed to assess test performance and consistency. A prospective design is essential to for defining the amount of variation and determining if it is acceptable.

2. How can growth-based PZA DST be improved? What are the pros and cons of each method, and could a single type be standardized for research purposes?

   Growth-based testing could be improved by optimizing inoculum preparation, identifying the range of minimum inhibitory concentrations based on genetic mutations (primarily in *pncA)*, and examining the performance of alternative approaches, such as the use of nicotinamide instead of PZA. A standardized approach for DST development will be needed for new antituberculosis drugs as well. The STOP Partnership Working Group on New Drugs has agreed to examine approaches to this larger question that extends beyond PZA DST. The collective experience gathered as part of the optimization of testing for PZA may help to inform the larger process. Approaches need to be collaborative and to include an international perspective. In addition to MGIT, data collection for other available culture-based products should be included.

   Future meetings will be needed to learn what research is already underway. CDC has one approach that was described at this meeting, and additional input will be critical. In addition, data regarding the correlation of genetic and phenotypic data with clinical significance and outcomes are needed. In the interim, additional information might be

obtained from functional assays for pyrazinamidase activity and correlation with animal studies.

3. What is the expected accuracy of genetic-based testing for PZA resistance?

   This question might be revised to read: What is an acceptable level of association of genetic tests for PZA resistance with clinically relevant outcomes? Currently the correlation of genetic-based tests to detect *pncA* mutations with phenotypic tests for PZA resistance may be in the 80-90% range depending on confidence in the phenotypic test used for comparison. The benchmark for performance has been set by the high sensitivity and specificity of molecular assays such as the Cepheid GeneXpert MTB/RIF assay. Differences in enzymatic activity of the pyrazinamidase could exist based on the *pncA* allele that is present, thereby contributing to variations in the level of resistance. One approach to understanding this variation is an analysis of the activity of the enzyme measured after mutations of mutated *pncA* alleles in a whole cell assay. The usefulness of genetic or enzymatic-based assays is tied to the ability to use the drug clinically based on expected patient outcomes. Understanding the clinical significance is important for determining which mutations to target for assay development. With growing interest in using PZA, and growing recognition of its synergy with new drugs (e.g., bedaquiline) and in new regimens (e.g., PaMZ), the problem takes on increased urgency.

   For the purposes of assay development, and as a clinical trial inclusion criterion for a drug that depends on PZA synergy, one might assume that all promoter and nonsynonymous pncA mutations have the potential to confer PZA resistance. Perhaps an initial assay need only differentiate between (a non-silent) mutant and wild-type *pncA.* Based on this assumption, a "good enough" test might be developed quickly to use in clinical trials. Assays of this nature might be useful for rapidly excluding patients from trials involving PZA as part of a new treatment regimen based on the "likely" potential for PZA resistance until sufficiently robust correlation databases have been developed. It was noted that about 10% of apparent PZA resistance cannot be attributed to *pncA* mutations.

4. How can we move forward with *pncA* sequencing of isolates to develop a database for the development of genetic-based testing?

   An inventory of mutations must be developed. In addition, other data that might usefully be collected could include the phenotypic result, PZase activity, PZA MIC, methods of genotypic and phenotypic testing, and animal assay results and clinical outcomes when available. An inventory of current research activities is needed to determine which data are currently available, who holds these data, is information, and what additional data are needed. This process for data inventory should be international in scope and include compilation of the literature. After the meeting, two recent

publications were cited as relevant examples: INT J TUBERC LUNG DIS 16(2):221–223 (Cambodia) and INT J TUBERC LUNG DIS 16(1):98–103 (South Korea).

5. How might we develop a rapid *pncA* sequencing capability to support PZA combination trials before 2013?

   The development of a rapid genetic assay for timely detection of mutations associated with PZA resistance is of the highest priority. Ideally results would be available within 2 days of specimen collection. There are a number of technical and logistical issues that must be considered. These include selection of a standard platform and methodology, identification of laboratories with the capacity to participate, development of a proficiency testing panel for assessing performance, and commitment from parties conducting internationally-based trials to support the initiative. Potential barriers will need to be identified. Establishing a few regional laboratories to provide *pncA* mutation information within two days to identify resistance to PZA for determination of trial eligibility is a priority.

6. What technical requirements (for example, laboratory capacity, isolate banks, validation and EQA processes) are needed to get a molecular PZA DST program up and running?

   A number of partners including FIND and the Global Laboratory Initiative have implementation experience and could provide logistical consultation including cost estimates. The initial focus would be establishment of a program for use in enrolling patients in clinical trials of new treatment regimens. At the same time a simpler method for determination of *pncA* mutations could be developed for commercialization. Accordingly, engagement of industry is an important next step. Piloting in a limited number of locations would help to establish a realistic budget and identify systems issues (e.g., shipping, reporting, communication, bioinformatics needs). The program might progress in multiple phases. A first step that could be implemented soon would be use of a rapid genetic sequencing-based assay for enrollment of patients in clinical trials. A parallel process for using this approach for clinical care will require collaboration with multiple groups to make it a reality. Continual data collection for quality improvement would contribute to development of commercial genotypic and phenotypic assays. The resultant model could then be used to develop molecular resistance assays for new anti-tuberculosis drugs in the pipeline.

7. How can less demanding and more affordable genetic-based rapid PZA susceptibility testing methods be developed? What are the barriers that need to be overcome?

   PZA resistance-associated mutations in the *pncA* gene can be located within its regulatory region or throughout the length of the open reading frame (ORF). Because *pncA* lacks a short resistance-determining region similar to other genes implicated in drug resistance (e.g., *rpoB* and *gyrA),* development of a simple genetic assay may prove

more complicated and costly. In addition, the clinical significance of many *pncA* mutations is still unknown. Therefore, consideration should be given to development of phenotypic-based assays as well. For example, pyrazinamidase activity could be examined. Our thinking should extend beyond *M. tuberculosis* to other respiratory pathogens, and to how this process might inform those research efforts. Other technologies, for example MODS and the Hain platform, might be adopted to address PZA DST. After the meeting, a relevant publication ahead of print was noted on a line-probe assay: J. Clin. Microbiol. doi:10.1128/JCM.05638-11, 28 December 2011.

8. What is the R&D time frame for such development?

   We believe that a phased approach to program development for rapid analysis of mutations in *pncA* for use in clinical trials, using currently available platforms and genetic data, can produce a usable product by 4th Quarter 2013.

9. What is the role and interest of USG in improving PZA DST, given a key next step is to coordinate with partners: TBTC, ACTG, PETTS, and TB Alliance, PHRI, academic investigators, CDRC, industry, and the three relevant STOP TB Partnership Working Groups?

   There is strong interest in improving PZA DST among several stakeholders. The process must be inclusive and well-coordinated, while sustaining the current momentum for continued progress.

10. What are the major steps to be taken by CDC and NIH to move forward?

    The major steps CDC and NIH face include the selection of active partners, identification of the appropriate, accessible populations for assessment, recruitment of the right mix of expertise, and development of a process for partner engagement and collaboration. A primary question is the cost of this effort and whether sources of funding can be identified. Coordination and awareness among all partners is critical to success.

11. What are the resources available and organizational advantages and challenges to making progress?

    The Diagnostic Research Forum being developed by NIAID and its multiple partners is already focused on advancing diagnostics. Lessons may be learned from the history of diagnostic and drug development, as well as the development of DST, as an efficient way of making decisions and moving forward. A project focused on PZA will serve as an initial centerpiece for better DST development that can then be applied to new anti-TB drugs.

PZA Day at CDC Agenda

Thursday, December 15, 2011, CDC, Atlanta
1600 Clifton Road, Atlanta Georgia, 30333; Building 19, Room 245; Time: 10:00 to 17:00

We will try to stick to questions of clarification following each presentation, saving time for broader discussion at the end of the meeting.

09:30-10:00 Arrival

10:00-10:05 Welcome (*Dr. Castro*)

10:05-10:15 Introduction and Statement of Purpose by *Michael Iademarco* (DTBE) and *Richard Hafner* (Division of AIDS and *Rapporteur*); *Angela Starks* (Recorder)

10:15-10:45 Introduction of Guests and Importance of pyrazinamide (PZA) and its DST in Clinical Trials

-Division of Microbiology and Infectious Diseases (*Christine Sizemore*)
-TB Alliance (*Khisi Mdluli*)
-FDA (*Eileen Navarro-Almario and Frederic Marsik*)

10:45-11:15 PZA: The Fundamentals (*Glenn Morlock,* ART)

11:15-11:30 Epidemiology of PZA-resistant TB based on the National TB Surveillance System Data (*Ekaterina Kurbatova*, IRPB)

11:30-12:00 CDC Reference Laboratory and Technical Experience with PZA DST (*Beverly Metchock*, RLT)

12:00-13:30 Working lunch with PIZZA and informal discussion on-site

13:30-14:00 Overview of the PETTS study, with a Focus on PZA DST and Outcomes (*Peter Cegielski,* IRPB)

14:00-14:45 CDC Laboratory Research on PZA (*Jamie Posey*, ART)

14:45-15:00 Break

15:00-16:30 Facilitated Discussion of Coordinated and Collaborative Way Forward

16:30-17:00 *Rapporteur* Summation (*Richard Hafner*, DAIDS)

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Attendee List

Ades, Eddie
Castro, Kenneth G.
Cavanaugh, Joseph S.
Cegielski, Peter
Chorba, Terence L.
Dalton, Tracy
Degruy, Kyle
Driscoll, Jeffrey
Franklin, Jameelah
Goldberg, Stefan
Hafner, Richard E.
Hartline, Denise
Iademarco, Michael
Jaffe, Harold W.
Kim, Peter S.
Kurbatova, Ekaterina
Laughon, Barbara E.
MacKenzie, William R.
Makhene, Mamodikoe (phone)
Marsik, Fredrick J.
Mase, Sundari
Mdluli, Khisimuzi
Metchock, Beverly
Morlock, Glenn
Navarro Almario, Eileen E.
Plikaytis, Bonnie
Poole, Freddie (phone)
Posey, James
Poynter, Tameka
Schito, Marco L.
Shawar, Ribhi (phone)
Shinnick, Thomas (phone)
Sikes, David
Sizemore, Christine F. (phone)
Starks, Angela
Temporado, David
Vernon, Andrew
Willby, Melisa
Williams, Sharon D. (phone)