Tuberculosis Vaccine Regulatory Workshop

Co-Sponsored by:
National Institute of Allergy and Infectious Diseases (NIAID)/
National Institutes of Health (NIH)
and
Center for Biologics and Evaluation and Research (CBER)/
U.S. Food and Drug Administration (FDA)

Held at the:
NIAID Suite 250
Conference Room 2C13
The Fernwood Building
Bethesda, Maryland

Tuesday, December 9, 2003

Meeting Summary

WELCOME, INTRODUCTIONS, MEETING GOALS *Christine Sizemore – NIH*
Michael Brennan – FDA

Dr. Christine Sizemore, Program Officer for Tuberculosis, Leprosy, and Other Mycobacterial Diseases, NIAID, NIH, and Dr. Michael Brennan, Senior Investigator, Laboratory of Mycobacterial Diseases and Cellular Immunology, CBER, FDA, welcomed participants and attendees to the day-long meeting.

In opening the conference, Dr. Sizemore explained that as part of NIH's mission to support and conduct research to strengthen the foundation of scientific knowledge and its translation into new interventions, NIAID serves as the lead institution for research on tuberculosis through its Division of Microbiology and Infections Diseases (DMID) (www.niaid.nih.gov/dmid/tuberculosis/). In this role, NIAID provides contract resources to support basic TB research; the development of candidate drugs, vaccines, and diagnostics for TB; and clinical testing of new candidates for the prevention, diagnosis, and treatment of TB. NIAID encourages investigators to submit applications covering all of these aspects of tuberculosis research.

Moving research from the lab to clinical trials involves translating discoveries from basic research into candidate vaccines, which are then tested in preclinical studies and subsequently early clinical trials for safety and efficacy. Between the basic and clinical research, targets are identified and validated.

While the NIH encourages and supports TB vaccine research, the FDA, through CBER, has the primary statutory authority for regulating vaccine development and approval, including oversight of Investigational New Drug (IND) applications (CBER guidance www.fda.gov/cber/reading.htm and www.fda.gov/cber/guidelines.htm; regulations/SOPs http://www.fda.gov/cber/regsopp/regsopp.htm). In this capacity, CBER provides a framework for early opportunities for TB vaccine developers to interact with FDA; the procedures for requesting and conducting a pre-IND meeting; and the content and format of INDs, including preclinical and clinical testing.

The main goal of the meeting was to offer investigators an opportunity to learn about and discuss regulatory issues for TB vaccines. In keeping with this goal, the presentations and the comment sessions:

- Provided an overview of the regulatory process for vaccines in the United States,
- Focused on preclinical development and Phase I/II trials, and
- Identified key issues in TB vaccine research and development.

The meeting was not designed for detailed discussions of individual vaccine candidates or of the shortcomings or merits of candidate vaccines, the science of vaccine development, or phase III trials. Although the current meeting focused on U.S.-related research and regulations, the Co-Chairs acknowledged that many TB vaccines are tested (e.g., in Phase III clinical trials) and primarily used in other countries.

SESSION I: OVERVIEW OF THE U.S. FDA REGULATORY PROCESS

General IND Issues *Julienne Vaillancourt – FDA*

Julienne Vaillancourt, Regulatory Project Manager, Office of Vaccines and Related Products, CBER, explained that the FDA has regulatory authority for the development and approval of vaccines for human use, per several statutes, including the Biologics Control Act (1902); Section 351 of the Public Health Service Act (1944); and the Federal Food, Drug, and Cosmetic Act (1938). The U.S. Department of Agriculture (USDA) has regulatory authority of vaccines for animal use. Biologics are, by definition, drugs, but are regulated by CBER.

The *Code of Federal Regulations* (*CFR*) provides specific regulatory requirements for vaccine research and development, and investigators should become familiar with relevant sections of the *CFR*, including 21 *CFR* Parts 600–680 (Biologics), Part 312 (Investigational New Drugs or INDs), Part 314.126 (Adequate and Well-Controlled Studies), Part 50 (Informed Consent), Part 56 (Institutional Review Boards or IRBs), Parts 210 and 211 (current Good Manufacturing Practices or cGMPs), Part 58 (Good Laboratory Practice or GLP for Nonclinical Lab Studies), and Part 800 (*In vitro* Diagnostics). The *CFR* may be accessed online at www.gpoaccess.gov/cfr/index.html.
CBER approves vaccines that are demonstrated to be:

- Safe (21 *CFR* 600.3), defined as relative freedom from harmful effect when prudently administered,
- Pure (21 *CFR* 600.3), defined as relative freedom from extraneous matter in the finished product,
- Potent (21 CFR 600.3), defined as having the specific ability . . . to effect a given result; and
- Manufactured consistently according to current Good Manufacturing Practices (GMPs) (21 *CFR* 210–211).

The development of vaccines for human use may be divided into two major phases: the pre-IND phase and the IND phase. During the pre-IND phase the rationale for the product based on disease pathogenesis is developed, then research to identify the immunogen is conducted, the manufacturing process is developed, and preclinical studies are done. If a candidate product is successfully identified and developed in the pre-IND phase, it may then move into the IND phase, where it will be tested for the first time in humans. Initially it will be evaluated in small studies to evaluate product safety and subsequently in larger scale studies to additionally evaluate dose range, immunogenicity, and efficacy. Regardless, of the phase of study, safety is always evaluated. Additional nonclinical studies may be conducted in the IND phase.

CBER reviews new biological products, as well as new indications for already approved products. The review includes a thorough evaluation of the scientific and clinical data submitted by sponsors to determine whether the product meets CBER's standards for approval. CBER then makes a decision based on the risk-benefit for the intended population and the product's intended use.

CBER reviews and regulates biological products during their development under IND, in the context of a submitted Biologics License Application or BLA and in the post marketing period, which occurs after a biological product has been approved for licensure. Prior to the IND phase, the sponsor should design a clinical investigational plan for the product. The details of this plan may change over time as the product moves through development. During the IND stage of development the vaccine product is first tested in Phase 1 studies for safety and immunogenicity; and then tested in Phase 2 studies for dose ranging, as well as safety and immunogenicity, and finally in larger scale Phase 3 studies for safety, immunogenicity and efficacy. Each phase supports and forms the foundation for the next phase of the IND process. Once the sponsor has successfully conducted the necessary studies under IND and adequately developed the product for intended large scale production and use, the sponsor may submit a Biologics License Application or BLA, which must include data to support approval and inspection. Following BLA approval, a sponsor may submit additional information to the BLA in the form of Biologic License Supplements (BLSs), which might contain data to support identification of new indications for a product or changes in the dosing regimen, manufacturing process, or equipment and facilities used to produce the vaccine. Once submitted, a BLS undergoes a review process as well.

The scope of the IND submission is defined in 21 *CFR* 312.1 as a mechanism that allows an investigational new drug to be lawfully shipped across state lines for the purpose of conducting a clinical study of that drug. The FDA's objectives in reviewing an IND submission are outlined in 21 *CFR* 312.22 as follows: in all phases of the investigation, to assure the safety and rights of subjects; in Phase 1 investigations, to assess product safety; and in Phases 2 and 3 investigations, to assure that the

quality of the scientific evaluation of drugs is adequate to permit an evaluation of the drug's effectiveness and safety.

Early dialogue between researchers and CBER staff is encouraged. Such dialogue may begin long before a pre-IND meeting, in which case it may occur via teleconference, scientific meeting, or outreach presentation. Dialogue also may take the form of focused technical discussion similar to the current meeting in which topics such as the general design or pharmacokinetic and toxicologic studies, product assays, and product characterization are presented. CBER staff are also willing, time and resources permitting, to informally review faxed one-page concepts papers, proposals, or preliminary findings from vaccine researchers and developers. It is important to keep in mind that advice and feedback provided through such informal review and dialogue is not binding

The next opportunity for early dialogue is the pre-IND meeting, which is an official meeting that allows for an interface between pre-IND and IND phases and which provides researchers with a "dress rehearsal," so to speak, for submission of the IND. The pre-IND meeting is an opportunity to discuss and identify product safety issues, the design of animal studies needed to initiate human testing, and potential clinical hold issues. To convene a pre-IND meeting, which is a Type B meeting per the Prescription Drug User Fee Act (PDUFA) 2, a written request (via fax or mail) must be sent to the Division of Vaccines and Related Products Applications in OVRR/DVRPA at CBER. The written request the letter should contain sufficient information to allow CBER to consider whether the request for the meeting should be granted. CBER must respond to the request within 14 days. The meeting, in turn, must be scheduled to occur within 60 days of receipt of the request. Ms. Vaillancourt noted that generally only one pre-IND meeting is granted per product.

The pre-IND meeting materials ("pre-read" materials) must be received by CBER/OVRR at least 4 weeks prior to the meeting. The materials should include but are not limited to the purpose and description of the product, objectives, proposed indication, questions for CBER (focused primarily on how best to proceed with clinical trials), a list of sponsor participants, supporting data summaries (preclinical, clinical), a protocol summary or draft, and reprints of key references. The CDER/CBER Guidance for Industry, entitled "Formal Meetings with Sponsors and Applicants for PDUFA Products" (3/27/2000) provides more detail on requesting such meetings with CBER and may be found on the following website: http://www.fda.gov/cber/guidelines.htm. In order to have a successful pre-IND meeting with CBER it is recommended that a complete background package that adequately represents the data to be provided in the IND be submitted, the meeting agenda be limited to the issues and immediate questions for CBER, and the issues and questions for CBER primarily concern how best to proceed into clinical trials.

The CBER/OVRR IND review team includes a primary reviewer/regulatory project manager, a clinical reviewer, a product reviewer, a statistical reviewer, and persons with other expertise (e.g., toxicologist, clinical specialist based on indication) as needed. The Division of Vaccines and Related Products Applications (DVRPA) within OVRR at CBER is the application Division, which primarily receives INDs from CBER's document room once submitted. Primary reviewers/regulatory managers in DVRPA serve as the points of contacts for assigned vaccine INDs and manage the overall review of these INDs. Clinical reviewers of vaccine INDs are also among the staff in DVRPA. Product

reviewers who would be assigned to TB vaccine INDs are in the Division of Bacterial Parasitic and Allergenic Products (DBPAP) within OVRR. Statistical reviewers in CBER, regardless of the product assignment are in the Office of Biostatistics and Epidemiology (DBE).

The required contents and format of an IND for submission are outlined in 21 CFR 312.23. Likewise, the elements to be included in a clinical protocol are outlined in 21 CFR 312.23(a) (6)(iii). Ms. Vaillancourt provided the following helpful hints for IND Original Submissions: paginate the entire submission; contact DVRPA prior to submission of the IND to alert the Division about the planned submission, as well as to ask whether additional copies might be needed; include the consent form, case report form, and patient diary with the protocol; tabulate supportive preclinical and clinical data; provide reprints of key references; never describe safety data from previous clinical studies as "well tolerated" – instead provide details about how safety was monitored and the actual results; and provide bovine-source documentation, if available.

After CBER receives an IND original submission, CBER has 30 days to review the submission and inform the sponsor as to whether the proposed study under the IND may proceed or not. If CBER does not allow the study to proceed as proposed, the study is said to be "placed on clinical hold." 21 *CFR* 312.42 defines a clinical hold as an order by FDA to the sponsor to delay a proposed clinical investigation or suspend an ongoing investigation. A clinical hold may apply to one or more of the investigations covered by an IND. For a proposed study, subjects may not receive the study vaccine. For an ongoing study, no new subjects may be recruited and given the vaccine, and participants already in the study should receive no additional doses of the vaccine. The grounds for a clinical hold on Phase 1, 2, and 3 studies are found in 21 *CFR* 312.42(b) (1) and (2). A sponsor will be notified of a clinical hold decision by phone on or before 30 days of CBER's receipt of the IND application. CBER may also issue a separate advice or information request (AI) letter with non-hold issues to the sponsor; however, CBER is not required to do and therefore, CBER is not required to issue such letters within a certain time limit. Once a sponsor submits an IND amendment containing a complete response to the issues identified in the clinical hold letter, the FDA must respond in writing within 30 days to maintain or remove the hold. A sponsor may not proceed with a study until notification from FDA that the hold has been lifted. If no clinical holds are identified and the IND study is allowed to proceed, the IND goes into effect 30 days after it is received by FDA [21 *CFR* 312.40(b)(1)].

The four basic types of IND amendments are protocol, information, safety, and annual report amendments. 21 *CFR* 312.30 provides requirements for protocol amendments, including changes in a protocol relating to subject safety, scope of investigation, scientific quality, and new investigators. 21 *CFR* 312.31 provides requirements for submitting IND information amendments. 21 *CFR* 312.32 provides requirements for submitting IND safety reports, which must be received by CBER within 7 days via phone or fax for unexpected fatal and life-threatening experiences associated with the use of the vaccine or 15 days for original written reports of serious and unexpected adverse experiences associated with the use of the vaccine or animal data that suggest a significant risk from the sponsor's initial receipt of the safety-related information. 21 *CFR* 312.33 provides requirements for submitting annual reports.

By becoming familiar with IND regulations and guidance, investigators can avoid many if not all of the common pitfalls associated with vaccine IND submissions. These pitfalls are found in every aspect of the IND application process: manufacturing, preclinical testing, and clinical protocols. Ms.Vaillancourt highlighted a few of the common preclinical and clinical pitfalls, including failure to provide data on pyrogenicity, attenuation concerning live vaccine potency, and adjuvant justification; the lack of Good Laboratory Practice (GLP) safety testing of a novel product prior to proposed Phase 1 testing; inadequate stopping rules for individuals and an entire cohort; no or inadequate safety follow-up; inadequate or no statistical analysis plan; and inconsistencies within the protocol and between the protocol and other documents. As before, researchers were strongly encouraged to refer to the regulations and guidance to ensure that what they intend to submit is complete prior to submission. Ms. Vaillancourt summarized key points of her presentation as follows:

- The regulation of vaccines is based on sound science, law, and public health impact.
- Early and open communication with CBER may facilitate vaccine development and resolution of issues.
- Pre-IND meetings are strongly recommended.
- CBER advice is based on regulatory requirements and experience.
- Many pitfalls can be avoided if sponsors use available guidance and other resources, ask questions, and consider CBER advice.

The following contact information was provided: E-mail: OCTMA@CBER.FDA.GOV; Fax: 1-888-CBER-FAX; Phone numbers: 301-827-3070 (DVRPA), 301-827-1800 (OCTMA).

Additional references and resources included:

- CBER guidance, including guidance for industry, general guidelines, points to consider Federal Register notices, ICH topics and guidelines, reviewers' guide, and CBER SOPPs: www.fda.gov/cber/reading.htm
- CBER standard operating procedures and policies (SOPPs): http://www.fda.gov/cber/regsopp/regsopp.htm
- BSE issues including estimating risk: www.fda.gov/cber/bse/bse.htm
- Goldenthal KL et al. Preventive HIV type 1 vaccine clinical trials: a regulatory perspective. *AIDS Res Hum Retroviruses* 14 (Suppl 3): S333-S340, 1998
- Baylor N, Midthun K. Regulation and Testing of Vaccines. In: Vaccines, 4th ed., WB Saunders, 2004
- Shapiro SZ. The HIV/AIDS vaccine researchers' orientation to the process of preparing a US FDA application for an investigational new drug (IND): What it is all about and how you start by preparing for your pre-IND meeting. *Vaccine* 20(9-10):1261-1280, 2002

Questions/Comments

In response to a question, Ms. Vaillancourt noted that investigators may submit a draft study report early in the IND process, with the understanding that a formal and final report subsequently will be required.

GMP Manufacturing and Preclinical Testing *Sheldon Morris – FDA*

Dr. Sheldon Morris, Chief, Laboratory of Mycobacterial Diseases and Cellular Immunology, CBER, highlighted various aspects of GMPs, as outlined in 21 *CFR* 210 and 211 (general GMP regulations), 21 *CFR* 610, and 21 *CFR* 312 (related to INDs). GMPs cover a broad range of factors and specifications associated with vaccine production, including facilities, raw materials and components, equipment, validated procedures, environmental monitoring, personnel, batch records, and documentation. Conditions for each phase or facet vary according to the process and product being developed. SOPs need to be developed for each GMP component and for the full process, and all steps and SOPs need to be documented. The entire manufacturing process also needs to be standardized and validated, which allow for consistency across all phases of production.

Facilities must meet certain space and environmental requirements and provide systems for monitoring the manufacturing environment and equipment. The purity of the air can vary from class 100 air, which is sterile (i.e., 3,520 particles, 1 microbe/m^3), to class 100,000 air (i.e., 3,520,000 particles, up to 100 microbes/m^3). Various HEPA filters and other treatments are necessary to meet the desired level of air quality. Other environmental factors, such as the water supplies and surfaces, also need to be monitored for quality assurance and quality control purposes; the specifications for water used for injection versus cleaning, for example, will vary considerably. SOPs may include tracking of frequency, time, and duration of sampling.

GMPs exist for master seed production and characterization. The primary seed lot should be produced in sufficient volume to serve study needs and should be stored in two locations. As with all seed lots, the primary seed lot should be tested for activity and contamination, and it should be free of bovine spongiform encephalopathy (BSE) agents. The secondary seed lot is the "working seed." A complete record of each batch production is to be maintained, with every step such as the sources of the raw materials and buffer and media characteristics, documented. Testing of raw materials and components involve the development of SOPs for the receipt, quarantine, storage, and release of these materials, and for testing for contamination. Product packaging systems should be defined.
A product that moves to Phase 1 preclinical testing must meet criteria for safety, purity, sterility, potency, stability, and other characteristics, as defined in 21 *CFR* 600.3. General safety studies are conducted using a small number of different mammalian species (e.g., mice, guinea pigs) that are monitored for weight gain and survival 7 days post-exposure to the product (21 *CFR* 610.11). Live mycobacteria vaccine strains and TB-derived products should undergo further testing in guinea pigs (given the equivalent of at least one maximum allowable human dose) to assess whether virulent mycobacteria are contaminating the final product. A six-week survival study and other tests for the presence of virulent TB, including postmortem examination for TB disease, are often required. Sterility is confirmed with negative 14-day fluid thioglycolate media and soybean casein digest media assays (21 *CFR* 610.12). Bioburden assessments are required for all products, even live attenuated mycobacterial strains. Potency assays are generally required only for Phase 2 and 3 studies. Potency testing often shows that a biologic agent induces an appropriate immune response, which may or may not directly correlate with product efficacy. However, potency is also used as a measure of manufacturing consistency and product stability. A broad range of *in vitro* and *in vivo* potency tests is

available (e.g., for BCG, viability testing and DTH response; mouse protection assay for typhoid and plague), and investigators may wish to consult with FDA to identify the most appropriate test(s) for their product. Stability translates into product shelf life, which preferably is at least 1 year. Dr. Morris noted that many researchers forget to determine product stability; however, he reminded the audience, some evidence of product stability may be required to initiate clinical trials. Stability is determined by testing for potency, moisture (if applicable), and other product characteristics at various time intervals. Common concerns and pitfalls associated with GMPs in the preclinical phase include not having sufficient supporting data and/or documentation, not identifying lots clearly, inadequate or incomplete product test results, inappropriate testing, not conducting stability or toxicity testing, and ensuring that the preclinical product that is tested matches the clinical vaccine formulation. Early dialogue with CBER and the pre-IND meeting should help researchers reduce or eliminate these oversights.

Questions/Comments

Regarding tests for shelf life, stability, and other characteristics, Dr. Morris commented that these tests preferably will be completed by the end of IND Phase 1. The specific requirements are set forth in the *CFR*.

Per a prior question about effectiveness versus efficacy, Dr. Norman Baylor, Director, CBER/FDA/OVRR, noted that efficacy represents a "calculated" response indicating how effective a product is against a placebo, measured in part by a number or proportion of persons giving an expected response. Effectiveness may be considered as the product inducing some response that may or may not be of clinical benefit. Another view of effectiveness involves Phase IV clinical trials, which measure the distribution and performance of the vaccine in the body. In sum, the goal is to show that the vaccine protects against a specific infection in a target population.

Toxicology/Nonclincal Testing of Adjuvants *Marion Gruber – FDA*

Dr. Marion Gruber, Division of Vaccines and Related Products Applications, CBER, noted that nonclinical testing of vaccine products is an evolving area of research that helps lay the foundation for preclinical testing in animals. Preclinical safety assessment includes product characterization, proof of concept studies, and animal safety testing; it is a prerequisite to the initiation of clinical trials. Nonclinical safety assessment may be defined as preclinical safety assessment plus further product characterization and safety assessments during various stages of clinical product development. Nonclinical safety assessments may be required if changes to the product manufacturing and/or formulation are made and evaluates potential safety concerns that may have arisen from Phase 1 and Phase 2 clinical trials. Thus, safety assessments can be conducted prior to Phase 1 clinical trials or in parallel with early-stage clinical testing to address specific safety concerns depending on clinical indications.

Vaccines are defined as a heterogeneous class of medicinal products containing antigenic substances capable of inducing specific, active, and protective host immunity against an infectious agent or pathogen. Preventive TB vaccines prime an immune response to initial infections. In contrast,

postexposure and therapeutic vaccines are designed to prevent infection to progressing to disease in those already exposed to *Mycobacterium tuberculosis*.

Key components in nonclincal safety evaluations include product characterization, standardized and documentation of all aspects of the manufacturing process (as described earlier), and a range of *in vitro* and *in vivo* animals studies to characterize as completely as possible product safety (e.g., pyrogenicity, virulence, reversion to virulence, biodistribution, integration). Dr. Gruber pointed to 21 *CFR* 312,23(a)8, which states:

> "Adequate information about pharmacological and toxicological studies of the drug involving laboratory animals or *in vitro*, on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and other tests required varies with the duration and nature of the proposed clinical investigations . . . As drug development proceeds, the sponsor is required to submit informational amendments, as appropriate, with additional information pertinent to safety."

CBER takes a case-by-case approach in identifying the safety concerns and potential toxicity of each candidate TB vaccine by assessing the inherent toxicity of the vaccine, the toxicity of impurities and contaminants, toxicity resulting from the interaction of components, toxicity linked to the immune response induced. Risk-benefit analysis takes into consideration the target population, route of administration (ROA), available clinical data, mechanism of action, and product features. The safety assessment also determines whether the proposed or completed studies are adequate to identify toxic effects, and it seeks a balance in the interpretation of all data to predict the potential value of the product.

The goals of preclinical safety studies for TB vaccines are to determine the safety, potency, and purity of vaccines; to support entry of candidates into clinical trials, where human safety is ultimately evaluated; maximize the benefit-to-risk ratio of vaccine development; determine a safe dose; identify rare toxicities or potential effects on subpopulations often addressable only in humans; and identify any potential or unknown toxicities in target organs. Dr. Gruber continued by noting that preclinical testing programs are product specific. Unique safety concerns need to be addressed using *in vitro* and *in vivo* methods and tests specifically tailored to the particular product category. For example, live attenuated BCG strains and live vectors should be tested in assays demonstrating sufficient attenuation and inability to revert to the wild-type strain. In contrast, DNA vaccines should undergo testing for tissue distribution, persistence, expression, and integration. Vaccines formulated with adjuvant should include safety testing of the adjuvant. The key point to keep in mind is that no one study design fits all products; investigators need to tailor safety testing programs to the type of vaccine under development.

Study designs for toxicity assessment should include dedicated, stand-alone toxicity studies *or* combination safety/activity studies; control arms should include baseline measures and comparisons to test group and address reversibility of adverse effects and delayed adverse effects. The ROA and dosages administered in toxicity studies should correspond to clinically intended ROA and dose. Dr. Gruber noted that the total number of doses should be equal to or greater than the number of doses to

be administered clinically (i.e., "N plus 1" dosing). Studies should include episodic dosing (e.g., weeks between doses) should be studied where applicable.
In general, a dose response may not need to be demonstrated, with some possible exceptions, such as adjuvants. The dose to be administered will be defined by the immune response. The volume should be the same as that administered to humans (i.e., 1x); it can be scaled using a mg/kg-dose, if the 1x dose is not feasible. It is important that investigators do not change the product formulation in conducting safety testing.

A broad range of parameters should be monitored when determining the safety of a TB vaccine, including local/systemic events, immunogenicity, clinical observations (e.g., general health, body weight and food consumption, injection site, limb use impairment), serum chemistries including liver and renal function tests (i.e., ALT, AST, creatine kinase, BUN), hematologic analysis (CBC and differential), and injection site histopathology. Postmortem examination should include necropsy, organ description and weights, and histopathology on tissues including evaluation of immune organs. These tests follow GLP regulations and guidance (21 *CFR* 58.1). Assessment of the immune response includes characterization of the immune response and changes in immune parameters; parameters to be evaluated include white blood cell count and histopathological examination of bone marrow and lymphoid tissue. If toxicity is suggested, future testing may take a tiered testing approach. In some cases, specific immune investigations and testing for hypersensitivity reactions may be necessary.

Certain facets of preclinical testing involve the study of "relevant" animal species, which can be a challenge. Such species must be susceptible and respond to the test article activity; for example, they must develop an immune response following vaccination. Ideally, the species should be sensitive to the pathogenic organism or toxin. Dr. Gruber noted that although including a variety of species is important in assessing safety, INDs will be reviewed on a case-by-case basis and one relevant animal species may be sufficient. She added that non-human primates generally are not necessary for safety testing of vaccines. Researchers need to recognize the limitations of animal models, exercise judicious use of animals, and consider the use of naïve vs. partially immune or immune animals in their studies. The choice of animal model(s) for testing of a particular product needs to be justified and valid (e.g., through historic control data such as hematological, serum chemistry parameters, pathology).

Preclinical toxicity studies should be conducted p*rior* to initiating Phase 1 clinical trials. Investigators should discuss proposed study designs and tests/assays with CBER prior to or during the pre-IND meeting; this dialogue should include adequate information on the proposed clinical plan for the product. Toxicity protocols also should be submitted to CBER for review prior to initiation of animal studies in an effort to conduct only those additional toxicity studies needed. A summary of the toxicity studies conducted should be included in the original IND, with the investigator providing full tabulation of data and a summary of the safety of the clinically intended dose/ROA. Dr. Gruber noted that additional toxicity studies may be necessary as product/clinical development continues.

In summary, nonclinical and preclinical safety assessment is a key component in vaccine development. Assessment plans are considered on a case-by-case basis, depending on the type of vaccine, product formulation, dosing route, and intended target population. Approaches to this testing continues to

evolve, and ongoing discussions are needed to reach further consensus on the models and methods used in the assessment.

Questions/Comments

In response to a question about "early" dialogue, Dr. Gruber commented that comments, suggestions, and feedback given prior to the pre-IND meeting is less formal and is not binding compared to discussions in pre-IND and IND meetings and communications.

Dr. Barton Haynes, Director, Human Vaccine Institute, Duke University School of Medicine inquired about requirements for studies using non-human primates and SIV-infected animals given TB vaccines. Dr. Morris replied that there currently are no general requirements for studies of these animals; decisions are made based on a case-by-case basis using the scientific rationale and available information on the model. Studies of live attenuated vaccine are likely to present a greater number of safety issues than other studies, however. Dr. Morris encouraged investigators interested in these models to contact CBER before proceeding with study plans.

SESSION II. VACCINE DEVELOPMENT: FROM THE LABORATORY TO FDA—TWO PERSPECTIVES

DMID Perspective for Conducting Clinical Trials *Lydia Falk – NIH*

Dr. Lydia Falk, Director, Office of Regulatory Affairs, DMID, NIAID, discussed DMID's perspective on the interaction of NIH, FDA, industry, and academia and the conduct clinical trials.

In following up on Dr. Sizemore's introduction, Dr. Falk noted that NIH's primary role in vaccine development is facilitating the translation of basic research discoveries into clinical applications and funding this research spectrum through grants, contracts, and cooperative agreements. Dr. Falk explained that the guiding principles for all phases of this work are the protection of human subjects, the conduct of studies in compliance with Good Clinical Practice (GCP) guidelines, and the generation of robust data.

The regulatory framework for clinical studies has many players. The U.S. government regulatory authority for vaccine research, development, and approval is the FDA, which oversees INDs, BLAs, and NDAs (New Drug Applications). Other regulatory elements that may come into play within and outside of the United States include the NIH TOA (Terms of Award) for grants and cooperative agreements, the ICH (International Conference on Harmonisation), and European regulatory authorities and agencies (e.g., National Competent Authority, EMEA, Medicines and Health Products Regulatory Agency). Because TB affects populations around the world, largely outside the United States, the regulatory authorities within many countries also have a clear role in TB vaccine R&D and use.

The DMID/NIH assesses a range of facets of clinical trials conducted under an IND, including human subjects risk level, vulnerable populations, the impact of the research on public health policy, and product development feasibility. An IND allows for FDA authorization to conduct clinical research using an unlicensed product or off-label use of licensed products. INDs also allow for transport of unlicensed drugs for clinical study.

The FDA may exempt a drug from an IND if it is lawfully marketed in the United States *and* if it meets all of the following criteria:

- It is not intended to be reported to the FDA as a well-controlled study in support of a label change,
- It is not intended to support change in advertising,
- The ROA, dose, or patient population does not result in increased risks, and
- The study is conducted in compliance with requirements for IRB and informed consent.

Factors considered in DMID-supported clinical vaccine research include:

- Review and approval of protocols. These steps include scientific review from a Program perspective; regulatory and product reviews; and review of the protocol, consent forms, and safety monitoring plans.
- Drafting of clinical TOAs to ensure adequate conduct and safety oversight. This step includes developing a safety monitoring plan, ensuring GCP compliance, doing a QA audit, and conducting the study under an IND.
- Ensure adequate human subjects protections are in place (e.g., Federal Wide Assurance/FWA).
- Develop clinical trials agreements for studies in the DMID-contract networks, which define each sponsor's or partner's responsibilities.

Dr. Falk then outlined the timeline for developing and submitting an IND and the steps following the IND submission when DMID is the sponsor. In the initial pre-IND phase, researchers discuss and develop a clinical protocol and a toxicity testing protocol, and provide data on chemistry and manufacturing of the product; these activities can take months to years. Required elements include filing summary chemistry/manufacturer/controls (CMC) information if the product is not licensed in the United States; developing clinical trials and investigator's brochures; providing where available data on previous nonclinical and clinical (human) experience regarding safety and efficacy at the dose, ROA, dosing regimen, and populations for individual and combination therapies; and providing nonclinical data to support proof of concept for product activity and efficacy.
Investigators then submit these materials to DMID for review and preparation of a meeting package for the FDA; these steps generally take more than 30 days, and the FDA will assign a meeting date within approximately 14 days. The pre-IND meeting will occur within 30–60 days. The investigators should respond to FDA's pre-IND comments and incorporate the responses in the IND (per 21 *CFR* 312.33). The post-IND review period is approximately 30 days. Once investigators have adequately responded to any FDA comments, the protocol undergoes IRB review and approval, the drug is shipped, and the study commences. Researchers must follow various reporting, monitoring, and documentation requirements as the study progresses.

Dr. Falk noted that investigators conducting clinical research on TB vaccines face several additional potential challenges:

- Where will the vaccine be used and what are the regulatory issues in that country or region? When conducting trials in endemic areas, investigators need to determine the role of the FDA in studies where there is no U.S. commercial interest, identify relevant clinical endpoints, evaluate product effectiveness against a complex background of therapies and concomitant diseases, and demonstrate a high level of sensitivity to changing public health policies.
- What are the sources of the products to be used? Documented GMPs must be put in place.
- Will the products be imported and exported? If so, what special considerations and regulations must be taken into account?
- For NIH-funded research, do the studies meet NIH requirements for diversity of gender, age, and ethnicity even if they are conducted outside the United States?

Questions/Comments

Regarding a question about addressing NIH diversity issues in conjunction with FDA's more "exclusionary" criteria in the early stages of clinical research, Dr. Falk noted that these two policies are not necessarily incompatible or in conflict. As the clinical research progresses, for example, the FDA does seek trials in broader populations. Investigators also can study different populations in a "tiered" or staged manner, in which subsequent studies include different or broader populations. It is important to keep in mind, however, that researchers must provide justifications for excluding certain populations. Dr. Falk encouraged investigators to raise these issues early in their conversations with NIH and FDA.

The Biologics Consulting Group *James Kenimer – TBCG*

Dr. James Kenimer, President, The Biologics Consulting Group (TBCG) reviewed common misperceptions and mistakes associated with vaccine development and identified concepts and strategies to avoid these mistakes.

The first error in judgment that many investigators have is to assume that *product development will be 'a piece of cake.'* Dr. Kenimer pointed out that product development differs in significant ways from product discovery and basic research and requires a certain set of skills and knowledge beyond the lab. A key to successful vaccine development is recognizing that the process is a team effort that requires assembling the appropriate experts early in the process. It is important to see the "big picture," which incorporates basic and clinical science, manufacturing, preclinical and clinical testing, and regulatory and quality control issues, and experts from these varied disciplines.

Investigators also tend to think that *it's too soon to worry about regulatory issues*. Dr. Kenimer emphasized, as did the other presenters, that regulatory issues and dialogue are critical at all stages of product development. Mistakes made early can have long-lasting effects on the entire development and approval process. Mistakes caught early can shorten product development time significantly.

Thus, investigators should integrate regulatory awareness into all decision making steps. They also should hire or consult with a senior regulatory affairs (RA) expert as early in the process as possible.

Avoiding FDA in the hope that the agency 'won't notice' an error or other problem is another common mistake that investigators make. At some point, however, the FDA will take notice, probably after much time has passed and perhaps millions of dollars have been spent. The current FDA meeting procedures encourages early and ongoing dialogue and stresses the importance of asking tough questions early in the process. Dr. Kenimer pointed out that the FDA is structured to respond to proposals, not to provide a roadmap. FDA is the best and most likely least expensive consultant, and researchers should make good use of that expertise. If a research team or company needs to outsource, however, many consultants are available. It also is prudent to have in-house regulatory affairs expertise involved in all aspects and phases of product development.

Many investigators mistakenly assume that *if the FDA didn't say 'no,' it must mean 'yes.'* Dr. Kenimer noted that the FDA rarely responds with a flat-out rejection or directive (i.e., "You must do the following . . ."). Rather, feedback and guidance is more likely to be in the form of "Have you considered . . .?" or "We suggest you try . . ." or "We need more information about . . ." Investigators should always respond to all FDA comments, even if it is to state that a certain suggestion was considered but not followed (i.e., and then provide the rationale for not pursuing that suggestion). Dr. Kenimer added that FDA reviewers try to provide investigators with useful information that will strengthen applications, proposals, and the research and development process.

The final error in judgment involves' researchers thinking that *clinical development is going well, and product development can catch up later.* Allowing clinical development to outrun product development could result in serious problems. Product development, which often takes longer than planned, should not be rushed. Because of all the issues that can arise during product development, investigators are advised to make this component of the research enterprise a priority from the start of the process. They also need to keep in mind that the process represents a team effort; as such, basic and clinical researchers need to work together, and solid communication and collaboration between these two disciplines need to be fostered and maintained.

In closing, Dr. Kenimer emphasized the importance of investigators taking a team approach to vaccine development and to make every effort to establish and maintain credibility with FDA. To accomplish this goal, provide the FDA with complete and easily reviewable submissions, clearly explain rationales for all requests, be prompt and thorough in response to requests to information, and be transparent.

SESSION III. CLINICAL TRIAL ISSUES

Basics of Phase 1/2 clinical trials *Steve Rosenthal – FDA*

Dr. Steve Rosenthal, Medical Officer, CBER, focused on Phase 1 and 2 trials in the development of preventive vaccine clinical trials, including encouraging sponsors to identify global development goals

early with respect to target populations, label indications, and anticipated use. The presentation also identified special considerations for vaccine development.

Because each phase of product development forms the foundation for subsequent phases, which often involve more extensive and more costly testing in humans, early clinical trials and testing of candidate vaccines undergo heavy scrutiny. The series of pre-IND, IND, and post-IND meetings with the FDA (per 21 *CFR* 312.47) are designed to facilitate the entire product development process. The pre-IND (pre-Phase 1) meeting focuses on manufacturing issues, product characterization, lot release, animal safety, immunogenicity, and comments on the proposed Phase 1 protocol(s). The next meeting takes place close to the end of Phase 2, when participants discuss efficacy trial protocol(s) and updates and status of Phase 1/2 and assay data. Investigators whose products move through Phase 3 next meet to discuss pre-BLA issues; at this meeting, data from the prior clinical trials and product assays are discussed and updated, and an outline of a BLA is formulated. Licensed products may then move to Phase 4 clinical trials, which continue to assess product safety and efficacy in larger cohorts.

General considerations for a Phase 1 study include:

- Identify study objectives and endpoints. The primary goal is to test safety and tolerability and the secondary goal to assess preliminary immunogenicity
- Monitor the study closely for safety
- Enroll only adults, at least for first Phase 1 study
- Keep the sample size to a relatively small number (i.e., 20 to 80)
- Provide special instructions for vaccinees, if needed

Features and components to consider when designing a Phase 1 study:

- Take into account vaccine-specific features (e.g., live vaccine)
- Develop inclusion and exclusion criteria. For example, participants will include healthy adult volunteers between 18 and 40 years old (recommended for a phase 1 study)
- Determine additional special criteria or characteristics, such as age, serostatus, concomitant medications allowed, and other factors
- Identify vaccinee contacts (e.g., vaccinia) as applicable

Safety monitoring of a Phase 1 study should be detailed and comprehensive. The goals at this stage are to protect subjects by monitoring local, systemic, and potential end-organ toxicity and to identify major toxicity. These goals are achieved through regular clinical visits, which include a clinical examination and review of symptoms and diary cards; laboratory studies, such as CBCs/hematology, blood chemistries/enzymes for organ function [hepatic, renal (U/A), endocrine]; and others as indicated from results of preclinical toxicology studies and prior experience with similar vaccines. Product safety and activity (e.g., as indicated through immunogenicity measures) should be assessed according to a predetermined time schedule and include post-vaccination monitoring. The monitoring tools employed during the study, such as prototype case report forms (CRFs), diary cards, scripted interviews, and other items (e.g., photographs) are to be submitted to with the protocol, regardless of the phase.

Toxicity grading scales used in Phase 1 trials define grades for specifically monitored parameters (clinical and laboratory AEs) based on healthy volunteers. The protocol also must include rules for stopping the study, identify specific criteria for stopping, and address all grade 3 (severe) or grade 4 (serious) AEs. If the stop criteria are met, researchers must stop vaccination and investigate the events; an external safety review (e.g., DSMB) should also be done. If appropriate, the study may be resumed with or without modifications. Investigators must provide details about dose escalation in Phase 1 studies, including criteria for dose escalation and a safety review of lowest dose cohort. If results show the product is safe at a low dose, the study may proceed with a higher dose.

Goals of Phase 2 studies involve assessing immunogenicity and product safety. The presence and extent of an immune response should be tested based on dose-ranging data and is used to identify preferred dose, schedule, formulation, and ROA for advancement to Phase 3. Safety monitoring should include more precise estimates of common adverse events, local reactogenicity, and systemic effects. Phase 2 trials are conducted with up to several hundred subjects from a broader population base than Phase 1 studies. Phase 2 trials are often randomized and controlled. Vaccine-elicited immune responses are assessed qualitatively and quantitatively. Pilot evaluation of efficacy endpoints may be incorporated into a Phase 2 trial where feasible.

Planning for a Phase 3 trial is often concurrent with the conduct of a Phase 2 trial; pilot evaluations can help focus such planning. Logistics and protocol features assessed and considered for future research include level of determining compliance with protocol and ability to accrue subjects, identifying target populations for licensure, identifying the best monitoring tools, and defining proper sample handling.

Phase 3 studies are designed to generate adequate safety, immunogenicity, and efficacy data to support proposed product use(s) and indication(s) in the target population(s). The key objectives of a Phase 3 study are to define product efficacy through clinical endpoints and/or immune response endpoints and to develop a prelicensure safety database based on data from thousands of humans regardless of path to licensure. The use of the "animal rule" may apply in some cases. This rule was developed in response to development of agents to protect against chemical and biological terrorism when human efficacy trials are not feasible or ethical. The criteria for the dose administered in a Phase 3 trial must consider results of animal efficacy studies and compare the immune response in animals versus humans. Phase 3 safety data are collected at the appropriate vaccine dose compared with an appropriate control group in a randomized, controlled study.

Phase 4 postmarketing studies focus on identifying rare adverse events, delayed-onset and long-term effects, subpopulations, and product efficacy. At the time of product license approval, investigators may wish to provide FDA with recent vaccine approval letters in addition to results of Phase 4 testing.

Dr. Rosenthal provided the following resources to assist investigators move through all phases of vaccine development:

- FDA Guidance Documents for Industry: http://www.fda.gov/cber/guidelines.htm, http://www.fda.gov/cder/

- International Conference on Harmonisation: E6: http://www.ich.org/

Questions/Comments

Dr. Ingrid Kromann, Head, Vaccine Development Department, Statens Serum Institute (Denmark), inquired about timing of dose escalation in a Phase 1 trial in conjunction with safety evaluations. Dr. Rosenthal stated that each case is reviewed on its own merits, adding that investigators should provide the FDA with the rationale for dose escalation and supporting data on adverse events. It is important to capture as much information on all events that occur.

Another attendee asked about whether Phase 1 therapeutic vaccine trials use healthy volunteers. Dr. Rosenthal noted that these types of studies do not use healthy subjects. Researchers still need to collect safety data and analyze the results to try to discriminate between the effects of the vaccine versus disease. Such studies also need to make sure that the vaccine is not aggravating existing disease. Phase 1 trials may not be able to evaluate all of these parameters adequately because of their small sample size; larger studies should be able to make these determinations, however. Regarding inclusion of a placebo control group in a Phase 1 study, Dr. Rosenthal stated that a placebo group is an option but most Phase 1 trials are open label and unblinded to investigators and participants. Blinded and/or placebo-controlled Phase 1 studies must be monitored very closely so that groups/individual participants can be unmasked as quickly as possible in case of serious adverse events.

Phase II feasibility trials of TB vaccines for Targeted Populations:

Manufacturers perspectives *Ripley Ballou – GSK*

Dr. W. Ripley Ballou, Vice President, Clinical R&D Emerging Disease Vaccines, GlaxoSmithKline Biologicals (Belgium), explained that vaccine feasibility trials are conducted to reduce risks in populations and individuals and to optimize efficacy, ROA, and other factors, which is done in Phase 3 studies. In brief, feasibility studies should come as close to meeting licensing requirements as possible.

Key issues to address prior to the conduct of a vaccine feasibility trial include assessing the role of access and consent in the administration of the product to the target population, and the ethics of conducting a study in those populations. Investigators must take into account the impact of other health conditions on the efficacy and safety of the vaccine in the cohort. For example, assessment of vaccine safety should take into account specific settings, such as prior exposure to the Bacillus Calmette-Guerin (BCG) vaccine, latent TB infections (LTBI) and successfully treated TB, HIV co-infection, and use of anti-retroviral therapies. Case definitions and risk also must be considered and assessed. Regarding proof of concept, the goals of the study should be achieved in as short a timeframe as possible with clear "Go/No Go" criteria to proceed in place. Dr. Ballou noted that the precision of a Phase 3 trial (e.g., tight confidence intervals) may not be necessary. Dose scheduling, product formulation, and age-specific dosing are optimized through feasibility studies. Researchers

should also conduct appropriate epidemiology studies and assessments in conjunction with feasibility trials.

The ability of a TB vaccine to prevent initial infection or re-infection may be studied at four intervention points: prior to infection, LTBI, recurrent TB, or primary TB infections (e.g., in infants and adolescents). Dr. Ballou described possible scenarios for feasibility trials. For prevention of pediatric TB, which perhaps should be the initial focus, many high-incidence populations exist that would benefit from an effective and safe vaccine. Medical and epidemiologic evidence is important in guiding such trials, which can take advantage of BCG priming. The primary disadvantages of conducting these trials are the limited ability to assess CMI readouts and the specificity of case definitions. Dr. Ballou noted that pediatric TB cases represent a largely neglected population, perhaps in part because the group poses difficult diagnostic challenges. Many children are well but have had household contact to TB through a family member. In other cases, unwell children infected with TB give a negative test. Thus, it is not certain whether standard clinical scoring systems used for diagnosis will be acceptable for vaccine trials.

For the prevention of TB re-infection, the pros of conducting feasibility studies include the existence of high-risk populations, clearly defined endpoints, and access to effective drugs through prescription programs. Despite these advantages, the available populations may be a pool of inherently poor candidates for vaccination. In addition, studies to assess re-infection must be very large and, as a result, are very costly.

The effectiveness of vaccines to prevent recurrent disease (relapse) may be assessed through readily identifiable target populations. Such feasibility studies build on the DOTS (directly observed treatment, short-course) infrastructure and have predictable clinical failure rates. Investigators need to recognize, however, that clinical feasibility data on relapse are limited and that the relevance of recurrent disease to reactivation of LTBI is not clear.

Post-proof of concept efficacy studies are designed to test vaccines to prevent primary infection in children and adults. In studies to prevent reactivation of LTBI, there are clearly defined endpoints and a high public impact with successful intervention. These large-scale feasibility studies require a relatively long follow-up period and may require testing of age-specific regimens.

Other issues to consider before proceeding with a vaccine feasibility study include regulatory strategies and market preparedness. For example, should the product be developed under and IND, and will it be registered in countries with a weak regulatory authority? Regarding marketing, investigators should be involved in continued efforts to understand the public health impact of their product and maintain informed and accurate demand forecasts.

Questions/Comments

Feasibility studies in high-incidence groups preferably are conduced with less than 5,000 participants. Approximately 80 percent of the residents in the Cape Town, South Africa, are exposed to TB, which could provide true cases of pediatric TB to consider for vaccine testing. However, the specificity of

diagnosis in pediatric TB cases remains a hurdle. Many but not all children suspected as being infected present with pulmonary disease, failure to thrive, and have an abnormal chest x-ray; whether these factors can be used as endpoints is not clear. Results of culture studies may indicate definite cases, probable cases, and possible cases. Dr. Greg Hussey has been working with the Cape Town community and has been studying intradermal versus subcutaneous BCG. The study has a fairly low eligibility threshold for recruitment: household exposure. About two-thirds (70 percent) have bacterial confirmation, and about 17 percent of children with positive cultures have clean x-rays and no symptoms.

Dr. Brennan commented on resources and agencies to consider regarding licensing a vaccine through the FDA for use in another country. CBER has a global vaccine group in the Director's Office to discuss issues related to this licensing strategy. WHO has a Regulatory Working Group that will meet to address international/global licensing issues. The FDA will be meeting with EMEA and Japanese and other national regulatory authorities in March 2004 to address these issues.

FDA perspective Rosemary Tiernan – FDA

Dr. Rosemary Tiernan, Division of Vaccines and Related Products Applications, CBER, described FDA's perspective on TB vaccine development addressing issues related to Phase 2 clinical trials. She noted that the current standard of care in the United States does not include BCG administration, except in select circumstances. Nevertheless, a tuberculosis vaccine could prove useful for administration to a range of higher risk U.S. patient populations such as the homeless and health care workers. FDA appreciates the global concern regarding the need to develop a vaccine for TB. However, at this time, we believe that the population for whom this vaccine would be indicated is mainly in the developing world. Consequently, FDA faces the challenge of regulating the development of a product whose use may be primarily in a non-U.S. population. Investigators planning Phase 2 clinical trials as part of a TB vaccine development program should consider the non-U.S. population(s) that would be expected to benefit the most from a TB vaccine.

The primary goal of Phase 2 clinical trials includes safety assessments, immunogenicity testing (e.g., validate assays), dose finding (i.e., identify the optimal formulation, route of administration, dosing schedule), and adjuvant testing (i.e., identify the added value of the adjuvant in terms of immunogenicity and/or stability).

Design issues to address in planning a Phase 2 study include logistics, target population(s), BCG and live vaccine issues, immunogenicity and safety endpoints, and product safety and efficacy. Investigators also must take into consideration the primary location(s) or region(s) where the vaccine will be administered based on epidemiology and incidence data and the infrastructure for access and delivery of the test product.

A Phase 2 vaccine study should include 50 to 500 participants and include at least 6 months of safety follow-up after administration of the last dose of study vaccine. A key challenge in designing a Phase 2 study is determining the appropriate endpoints and diagnostic tests to include in the trial. Case

definitions for latent TB infection and active TB disease in the study cohort(s) also must be clearly identified.

Study populations may include immunocompetent adults, who have no evidence of latent or active TB (negative PPD test v. QFT) and who have not received BCG, and sensitized immunocompetent adults, including persons who have received BCG or who are PPD-positive but not a recent converter requiring therapy or having active disease. If the ultimate goal of the trial is to evaluate the vaccine in a highly TB-endemic area in an un-sensitized population such as neonates, particular attention must be made to ensure the safety and protection of the study participants. As such, investigators need to consider whether the vaccine will be used in an area where HIV is highly prevalent in addition to TB, and whether the vaccine will be used as a preventive or therapeutic tool. In addition, researchers must discuss the type of safety and efficacy data that will be required prior to enrolling infants and prior to studying a new vaccine in an HIV-positive population.

Issues to consider regarding BCG include its efficacy, which ranges from 50 to 80 percent; its long-term effectiveness, which may persist for 10 to 20 years; how the different BCG strains used in various formulations may affect results and impact the trial design and analyses; different routes of administration for BCG; the ethics of withholding BCG; and the infrastructure for mass administration of BCG, which generally is very good.

Factors and risks associated with administration of live TB vaccine include risks associated with dissemination of the vaccine in HIV-infected populations, the potential for INH resistance (live vaccine strains may have a higher MIC for INH) and antibiotic resistance (vaccines may carry plasmid markers with resistance to antibiotics that may need to be used to treat TB), and the possible loss of PPD reactivity as a surveillance tool.

If a prime boost strategy is used in clinical trials (persons inoculated with BCG as infants are considered to be “primed” and they later receive a “boost” with study vaccine), it will be important to describe the rationale for using this approach including the reasons to support the decision to “boost” at a specific time.

Immunogenicity endpoints include parameters to validate assays used to assess cell mediated immunity and humoral immunity, and response to PPD. Regarding PPD response, the study should determine whether this response shows a significant correlation with other immune response measures in conjunction with product efficacy.

Safety endpoints should address product-specific concerns, local and systemic reactions assessed in part through a grading system, and appropriate lab tests. Investigators must develop a plan to monitor for dissemination of the vaccine strain versus acquisition of TB infection or disease; as part of this monitoring plan, investigators need to track and document all adverse events, such as fever, weight loss, headache, and cough, and this may be particularly challenging in countries endemic for diseases such as malaria, dengue, and others. A data safety and monitoring board may be established to oversee these efforts.

Efficacy endpoints may include tuberculin conversion, decrease in incidence of TB infection, or decreased incidence of active TB disease. Field trials will be necessary to evaluate the impact of vaccination on the development of TB infection and disease in the study population.

Special considerations and protections should be taken into account for vulnerable populations. These include but are not limited to ensuring that fully informed consent is obtained that outlines the risks of administering a live vaccine. In addition, when discussing clinical studies in children, withholding BCG even in countries with BCG failures may not be ethical. It will be important to develop a plan outlining how one will progress from the clinical testing of the candidate vaccine in healthy adults to children and/or other vulnerable populations.

In summary, the early phase studies must support product efficacy and safety. Investigators need to keep in mind that the path to licensure can vary considerably depending on the epidemiology of TB in the country of interest Field efficacy trials will be required and if necessary, questions may be brought to the Advisory Committee for consideration.

Regulatory issues in developing countries *Christopher Whalen – CWRU*

Dr Christopher Whalen, Department of Epidemiology and Biostatistics, Case Western Reserve University, School of Medicine, described vaccine studies conducted in Uganda. He discussed various regulatory issues, study concerns and challenges associated with planning and conducting these studies.

Once Phase 1 and 2 trials are completed, a new TB vaccine may be tested in populations endemic or epidemic for TB. As noted by previous speakers, other conditions, such as HIV and malaria, are often present and confound ongoing studies and potentially patient safety. A non-zero sum outcome is the goal. Investigators must gather as much epidemiologic and demographic information about the region and its people as possible before proceeding with clinical testing.

Four IND-based clinical vaccine studies have been completed or are underway in Uganda with support from NIH and the CDC. These include a Phase 1 ALVAC HIV vaccine study, a Phase 2 *M. vaccae* trial, a Phase 2 trial that is assessing IL-2 immune-modulating treatment in TB, and a Phase 3 study examining treatment-shortening interventions for TB. Epidemiology studies are assessing genetic susceptibilities, access for children, and community-based participation.

Key factors that must be taken into consideration in conducting clinical trials, particularly outside the United States, have been described in the Belmont Report and include ensuring that participants are fully informed of the study purpose, design, and risks; ensuring that adequate protections are in place, with special protections for vulnerable populations; conducting and taking into account the results of a risk-benefit analysis; and ensuring fairness and justice for participants in the trial.

Dr. Whalen outlined some of the obstacles to regulatory compliance in developing countries and offered ideas on how investigators may overcome those obstacles. He noted that these steps will

involve negotiations with the investigators, the regulatory authorities in the United States and in the study country, and the participating community.
Regarding *informed consent* issues, investigators need to ensure that participants understand key aspects of the study design, such as randomization and placebo, as well as potential risks. Approaches to convey these concepts, particularly to low-literate and uneducated populations, must be developed and tested. Researchers can *fill information gaps and communicate information about the study* by engaging the community, including health educators, health counselors, and translators, to recruit, enroll, and retain participants into clinical trials. *Identifying the risks and benefits* of the study and of vaccine use in communities and countries such as Uganda, where the threat of TB is great, can help strengthen arguments for conducting clinical trials. Individuals and communities that understand the potential benefits of an effective vaccine versus the risks of TB (or other disease) may be willing to accept some additional risk associated with participating in a trial of a candidate vaccine. As with other factors, the risks and benefits must be defined and explained clearly and fully. Addressing the issue of *voluntary participation* may present another challenge, given the social norms and paternalistic or authoritarian nature of the medical community in some countries. In addition to addressing the above issues of clear communication and informed consent, offering participants incentives such as transportation costs and follow-up healthcare may encourage individuals to participate without being coercive. Regarding studying different *age* groups, particularly children, it is important to determine cultural norms for defining adulthood and adolescence, for example. Investigators also should determine the cultural meaning of other features, such as signatures.

Investigators should plan to have several approaches to monitoring a trial, interim findings, and study results. For example, a "Documenting Council" may oversee consents and medical records. IRBs review initial protocols, protocol amendments, and reports of adverse events and make recommendations based on those submissions and reviews. The number of IRBs reviewing each protocol will vary considerably, depending on the number of institutions and agencies overseeing a study. Reviews and reports to be submitted by investigators to the regulatory authorities and review boards include initial reviews, annual reports, meeting manuals where indicated, review boards members' qualifications, and reports of how the study meets all regulatory requirements.

Source documentation is critical to licensing a vaccine. However, many differences exist between the United States and many other countries regarding how and what information is documented. For example, in many African countries, information included in an individual's medical records often is minimal, compared to American standards; characteristics common to our medical records system, such as dictation, medical consults, and nursing notes, are uncommon in Africa. In addition, medical records belong to the patients, and identifiers, such as hospital and national ID numbers, usually are not used. Some documentation can be used to generate case report forms (CRFs); whether a medical record can be used as a CRF is not clear in all cases, however. Data entry may be done on paper or by electronic means.

Investigators must also pay close attention to the logistics and requirements involving drug production, handling, importation, and storage. They should contact the local authority early in the drug development process and remain in contact as clinical testing approaches. Ensuring that proper storage equipment—particularly the ability to keep samples frozen and to respond immediately to power

failures—and monitoring are in place is also essential, as are transportation and documentation. Regarding laboratory certification, in the United States, CLIA (Clinical Laboratory Improvement Amendment) and certain organizations [(e.g., the College of American Pathology (CAP)] certify labs. In contrast, labs in developing countries generally are not certified; further, most would not meet CLIA standards. As a result, acceptable laboratory practices and standards often need to be negotiated.

Tracking and reporting adverse events can present additional challenges, and investigators should ensure that an appropriate safety net is in place and reporting of AEs and other events. They should be aware of cultural norms and attitudes that could affect timely reporting and treatment. A QC/QA management program should be in place, and arrangements for financial disclosures should be made. Informed consent for storage and future use of specimens should be obtained.

Dr. Whalen closed his presentation by quoting an African proverb: "Take only what you need. Leave more than you take."

Questions/Comments

Regarding a question about the length of the consent form for the Uganda studies, Dr. Whalen noted that the original consent form was 54 pages long to meet all regulatory requirements; a final 15-page version eventually was drafted. In response to a follow-up query about acceptance of even the shorter consent, Dr. Whalen stated that the local ethics committee has approved the form but questioned its length as well as the details and process of informed consent. Time in individual counseling and in a group setting has helped participants and others to understand the process and to ask specific and general questions about the study and informed consent.

In discussing testing of vaccines, Dr. Leonard Sacks, CDER, FDA, noted that vaccines for a range of conditions, including malaria, HIV, and TB, have been tested outside the United States. One goal of these studies is to develop a hierarchy of endpoints and to generate comparative data that can be used to identify disease-modifying events or effects following vaccine administration.

Immunological assays in TB vaccine studies *Daniel Hoft – SLU*

Dr. Daniel Hoft, Professor, St. Louis University Health Sciences Center, described various immunological assays used primarily in Phase 1 clinical trials. Although safety is critical in Phase 1 and Phase 2 trials in particular, immunogenicity evaluations are important throughout TB vaccine development.

Immunogenicity may be defined as the induction of an immune response that can be measured. Efficacy may be defined as allowing for protection against a disease process, whereas effectiveness refers to "real-world" scenarios including mitigating factors that, in turn, can modify efficacy. For example, BCG is highly immunogenic, and it can be efficacious (i.e., produce the desired immune response) in some populations. However, in "real-world" terms, BCG is not highly effective in that it has not altered disease incidence. A new TB vaccine must meet all three criteria; that is, it must be

immunogenic, efficacious, and effective. Phase 1 and Phase 2 trials need to identify sound immunological correlates that are predictive of efficacy and effectiveness.

In selecting vaccines to include in Phase 1 studies, investigators should look for animal data demonstrating induction of type 1 immunity, animal data demonstrating greater efficacy than BCG or improved safety, and lack of PPD cross-reactivity. Development of both mucosal and systemic vaccines should be considered, and long-term industrial support should be secured. Initial study populations should include healthy young adults who are PPD-negative and HIV-negative without known exposure to TB and have minimal contact with infected persons. Subsequent trial populations may include larger groups of persons who are PPD-positive without active disease.

Phase 1 immunoassay study designs should address:

- Sample size (small initially to identify large effects mediated by highly potent vaccines).
- Vaccine routes of administration and vaccine schedules.
- Kinetics to track peak vaccine-activated effector responses and immune memory.
- Controls: For adults, control groups should include pre-vaccination and concurrent placebo groups. For children, the study groups should include a test product group, and a standard BCG vaccinated group. Studies of children and infants should not have a placebo group.
- Comparisons with BCG immunogenicity.
- Randomization and double-blinding.

Assays for basic immune responses in Phase 1 studies should test for:

- Type 1 immunity [e.g., Th1/CTL (cytotoxic T lymphocytes) responses].
- Expression of Th1 memory precursor markers, such as IFN-γ, IL-12R &/or Tbet.
- IFN-γ/perforin/proliferative responses mediated by CD8+ CTL.
- Immune responses capable of inhibiting mycobacterial growth.

Focus of immune endpoints in Phase 1 studies:

- Antigen-specific cellular immunity.
- Responses to live mycobacterial stimulation.
- Responses specific for relevant purified mycobacterial antigens.
- DTH responses are not a good Type 1 immune endpoint because they do not always correlate with disease protection or with other surrogate markers associated with protection.

Specific immune assays to consider include antigen-specific lymphoproliferation assays, and IFN-γ measurements by analyses of secretion, single cell ELISPOT and intracellular flow cytometry methods. Dr. Hoft identified the pros and cons of these assays:

- All types of tests can be done on fresh whole blood or frozen PBMCs. IFN-γ responses and proliferation assays in studies with small sample sizes can be highly variable. Both assays are less reliable with frozen specimens.
- Proliferation assays measure functional expansion capacity, whereas IFN-γ-based assays measure the type 1 immune phenotype. Proliferation responses are not specific to type 1 immune cells, and not all T cells capable of making IFN-γ responses can expand in response to antigenic stimulation.
- Both types of assays have been validated in BCG trials.
- Neither type of assay is known to highly correlate with inhibitory T cell responses.

ELISPOT assays:

- Are minimally labor intensive & expensive compared with many other assays.
- Easily quantify only total IFN-γ producing cells (Th1 versus Tcl versus innate cellular responses may be detected).
- Appear to allow for enhanced sensitivity and specificity.
- Identify peak effector responses.
- Do not measure proliferative capacity.
- Are not validated in BCG trials.
- Are less useful using frozen cells, especially with small sample sizes.
- Not known whether ELISPOT responses correlate with T cell inhibition.

Intracellular flow cytometric detection of IFN-γ responses:

- Can be used with fresh or frozen samples.
- Can enumerate and distinguish Th1 and Tcl cell responses.
- May identify peak effector response.
- Have not included measurements of proliferative capacity although this could be done using CFSE dilution.
- Not known whether these responses directly correlate with T cell inhibitory capacity.
- Have not been validated in BCG trials.
- Are relatively expensive because of flow cytometry.

Mycobacterial growth inhibition assays:

- Whole blood inhibition assays are most amenable for use in large-scale field trials, but can only be done with fresh samples.
- More complicated inhibition assays using antigen expanded T cells and infected macrophages purified from total PBMC measure the most relevant immune responses effective against intracellular mycobacteria, and can be completed with either fresh or frozen samples (but work best when fresh samples are used).
- Both whole blood inhibition assays and more complicated assays of intracellular inhibitory activities have been validated in BCG trials.

- Whether these assays are better than other assays at predicting *in vivo* protection is not known. In addition, these assays are labor intensive and costly.

A primary motivation for developing an intradermal BCG challenge model is that such a model could measure *in vivo* protection. Such a model may be used to validate a variety of assays and to assess efficacy in a small number of subjects.

To be interested in moving beyond Phase 1 trials, the following should apply:

- There should be no initial safety concerns, or safety should be enhanced.
- Demonstrated immunogenicity should be greater than that shown for BCG.
- The product should not inhibit T cell responses.
- Phase 1 trials should be repeated in PPD-positive populations.
- The dose, dosing schedule, ROA, and product formulation must be determined.

Questions/Comments

An attendee inquired how protection against infection could be affected by different ROA and revaccination. Dr. Hoft noted that both mucosal and systemic BCG vaccinations have induced protective immune responses. In addition, large revaccination studies have shown no increased risks with secondary BCG booster vaccinations/challenges, but have failed to date to document the induction of enhanced protection.

In response to a question about the endpoints for use in BCG challenge studies, Dr. Hoft stated that investigators focusing on other infectious disease models have been successful by quantifying recoverable levels of viable model pathogens over time with standard culture techniques, in conjunction with measurements of relative changes in molecular pathogen equivalents using real-time PCR methods. A follow-up question focused on whether any BCG studies have made quantitative comparisons in large-scale genetically unrelated populations? Dr. Hoft replied that differences in genetically unrelated populations have been shown to have some effects, although usually only to a minor degree. It is important to remember that if a new vaccine induced only a 10 % increase in responses, and even if that increase is statistically significant, the protection may not be sufficient to reduce overall prevalence of TB infection and disease. A minimum of at least a two-fold increase in detectable responses might be necessary to see a population effect.

Another question focused on validation of assays per FDA regulations and how to show a relationship between vaccine administration and immune responses to fulfill FDA criteria. Initial studies show that using the BCG model, various TB vaccines can induce significantly increased relevant immune responses. Dr. Hoft recommended that an advisory committee, in conjunction with consultation with FDA, would likely prove helpful in determining whether a certain product meets FDA requirements.

LUNCHEON PRESENTATION:
A TB VACCINE STUDY IN THE UK *Adrian Hill – Oxford U*

Dr. Adrian Hill, Professor of Human Genetics, Wellcome Trust Principal Research Fellow, Oxford University, described the development and Phase 1 clinical testing of a new TB vaccine. The vaccine uses a two-part "prime-boost" strategy in which the priming vaccine, BCG, is followed by a boosting vaccine, a modified vaccinia virus Ankara (MVA) into which one gene from *M. tuberculosis* has been inserted. The new vaccine, MVA85A, is engineered from attenuated vaccinia virus that expresses the 85A antigen. This work builds on prior prime-boost studies that showed promise in the development of a malaria vaccine.

MVA85A is the first TB subunit vaccine candidate to enter clinical evaluation. It is being tested in the United Kingdom and The Gambia. The rationale for a boosting TB vaccine focuses on BCG efficacy and initial immune response. As Dr. Hill noted, BCG has low efficacy in teens and adults in Asia and Africa, where many regions are endemic to TB and where environmental bacteria often mask and inhibit BCG's effects. A boosting vaccine that can build on pre-existing anti-mycobacterial T cell responses should benefit from these responses rather than be inhibited by them. Possible aims of a new TB vaccine include prevention of primary infection, improved disease treatment, and particularly for the BCG-MVA85A prime-boost strategy undergoing testing, preventing disease in persons already infected by boosting immunity.

Heterologous prime-boost immunization involves two different vaccines, each encoding the same antigen, given several weeks apart. This strategy induces high levels of CD4+ and CD8+ T cells, with poxviruses and adenoviruses producing the strongest response. Several regimens have been tested in humans for various diseases thus far: DNA-MVA, FP9-MVA, and DNA-adenovirus. Features of MVA include:

- It is a non-replicating vaccinia strain that produces deletions in host range genes and cytokine receptor genes.
- It has been safety tested in 120,000 people immunized against smallpox and more than 500 persons immunized with recombinants.
- It has demonstrated strong boosting of CD4+ and CD8+ T cells in humans (McConkey et al., *Nature Medicine*, 2003).
- It has been studied in clinical trials for malaria, HIV, melanoma, hepatitis, and HPV.

Rationales for using BCG in prime-boost regimes include:

- The need for a common antigen to prime and boost.
- Antigen 85A is immunodominant and conserved across mycobacterial species and in all BCG strains.
- Enhanced efficacy of BCG-MVAAg85A has been demonstrated in BALB/c mice (Goonetilleke et al., *J Immunol*. 171:1602-1609, 2003).
- BCG-MVA-fowlpox induced a stronger protective effect than BCG in guinea pigs (Rawkins et al.).

Dr. Hill highlighted findings of three small-scale Phase 1 trials of BCG alone, MVA85A alone, and the BCG prime-MVA85A boost. The studies were conducted in the U.K. by Helen McShane and colleagues at Oxford, and study population included 11 mycobacteria-naïve healthy adults between 18 and 45 years old. Immunogenicity tests and measures included *ex vivo* IFN-gamma ELISPOT assay using antigens [85A (purified), 85B (recombinant), and PPD) and peptides (85A, ESAT6/CFP10)

MVA85A is manufactured under cGMP conditions at IDT in Germany using a seed stock. The working seed virus (WSV) is obtained from MSV cultured on chick embryo fibroblasts following sucrose cushion purification. The test product is titered and filled at IDT at a relatively low cost. GLP toxicology studies conducted on the clinical test lot include two-dose assessments in a single species; tissue distribution studies including culturing and PCR; potency and stability testing including virus stability with repeated passage and ELISPOT; and sequencing of the insert. The insert design is an Ag85A conserved sequence with a tPA leader sequence and a PK antibody tag at the C-terminus. It is not codon optimized. Murine T cell epitopes in BALB/c mice are used for potency testing.

The protocols and candidate product are subject to several regulatory reviews and approvals by local ethics committee; the Medicines Control Agency (MHRA); the Department of Environment for contained use of MVA as a Category 1 agent; and the Health and Safety Executive Committee, which oversees GMO issues in the U.K. Some general issues regarding regulatory criteria and approvals include:

- What is required and what is recommended? Various consultants advise researchers and companies on these questions.
- What are the requirements for Phase 1 assessments versus licensure?
- Separate regulatory filings may be used for multiple vaccines in prime-boost studies. "Combined" product toxicology for prime-boost vaccines may not be required.

Dr. Hill pointed out that nearly all malaria, HIV, and TB candidate vaccines in Phase 1 trials will never reach the licensing stage.

In one vaccination trial of 14 healthy adult volunteers in the United Kingdom, participants were immunized with $5X10^7$ pfu i.d.; 11 subjects were immunized twice with MVA85A, and 3 were immunized once. The first immunization occurred at 1 week after screening, and the second immunization occurred at week 4. Follow-up continued for 24 weeks after the last immunization. MVA85A induced strong immune responses in the volunteers. T cell responses to Ag85A were very strong. These initial findings suggest that the subjects may have pre-existing T cell memory to a cross-reactive antigen. BCG-MVA85A immunogenicity responses were stronger than for MVA85A alone when the prime and boost vaccines were given 1 month apart. Safety data overall have been good in clinical studies of MVA85A. Local reactions of redness, inflammation, and itching lasted for up to 3 days and were mild in 11 of 14 participants and moderate in the remaining 3 subjects. Four participants reported mild systemic reactions.

The clinical studies underway in The Gambia undertaken by the MRC unit (H. Ibanga, P. Hill, R Brookes) are designed to replicate the studies conducted in the U.K. Dr. Hill reported that a small number of healthy adult volunteers had completed the study in The Gambia thus far. Next steps include:

- Completing the Phase 1 studies in the U.K. and The Gambia.
- Further evaluating immune responses using cultured ELISPOT and FACS.
- Conducting Phase 1 trials in *M. tuberculosis*-infected healthy individuals, which have been approved.
- Evaluating fowlpox-Ag85A immunization
- Conducting studies in HIV-positive individuals.
- Conducting "BCG challenge" studies as a possible efficacy model.

Another safety and regulatory issue of concern is what is known as the "Koch phenomenon," in which a hyper-responsiveness to mycobacterial antigens develops in vaccinated subjects with TB disease. Dr. Hill noted that a similar reactivity has been observed with some therapeutic vaccines in some animal models. Whether the same type of reaction could occur in otherwise healthy, non-diseased vacinees administered a new generation of TB vaccines is not clear. Dr. Hill suggested the following approach to avoid the Koch phenomenon:

- Vaccinate sequentially. Test first for mycobacterially-naïve individuals via skin testing and ELISPOT. Then test for BCG-positive, *M. tuberculosis*-negative persons. *M. tuberculosis*-infected healthy individuals may be considered next and post-treatment TB patients last.
- Monitor participants for "mild" Koch reactions (e.g., via CRP, chest x-rays, serial CT scans).

Future research investigations should focus on live vectors in HIV-positive populations in studies that:

- Distinguish between replicating BCG and nonreplicating viral vectors (e.g., MVA, FP9)
- Determine the effects of MVA in conjunction with HIV immunotherapy.

Efficacy trials of new TB vaccines require sample sizes in the thousands with many years of follow-up even at moderate incidence rates. Studies of high-risk populations (e.g., household contact, HIV-positives) allow for smaller, more rapid assessment of safety and efficacy. However, ethical considerations must be made in balancing risk with possible need for prophylactic treatment (e.g., chemoprophylaxis).

In closing, Dr. Hill commented briefly on post-exposure vaccination, which represents a new concept in TB vaccine development. Issues to consider in this area of investigation include assessing the risk of immunopathology and identifying tests to confirm that risk; determining the incidence at which recent skin-test converters should receive chemoprophylaxis; and deciding whether careful follow-up is an ethical alternative to prophylaxis or another intervention.

Questions/Comments

Dr. Peter Small, Senior Program Officer, Tuberculosis, Bill and Melinda Gates Foundation, inquired about a rationale for choosing a specific BCG strain for clinical trials and licensing. Dr. Hill replied that because these investigations are still on new ground, this issue is still under exploration. He noted that the first strain used was pulled off the market within 6 months, and it was replaced with an SSI strain; few notable differences are expected with the newer strain.

Dr. Zheng Chen, Associate Professor of Medicine, Harvard Medical School, asked about separating out the CD4 versus the CD8 responses. Dr. Hill noted that the response appears to depend on the priming agent.

In response to a question from Dr. Ronald Mayner, Aeras Global Tuberculosis Vaccine Foundation, about dosing (given that BCG is a replicating agent and MVA85A is not), Dr. Hill explained that because of the very strong responses seen with the initial, single dose, the team did not see a need to increase the dose at least in the short term. In addition, other studies of these vectors supported the chosen dose as a reasonable starting point.

Dr. Lewellys Barker, Aeras Global Tuberculosis Vaccine Foundation, asked about the history of BCG exposure in the volunteers from The Gambia. Dr. Hill noted that the research team initially sought to recruit only BCG-naïve participants; however, that approach, although possible, was associated with slow enrollment. The eligibility requirements had recently been revised following further safety data from initial volunteers to allow BCG-vaccinated individuals to participate in the study, but only if they also meet very strict T cell response requirements.

SESSION IV. SPECIFIC VACCINE ISSUES

DNA vaccines

Dennis Klinman – FDA

Dr. Dennis Klinman, Division of Viral Products, CBER, opened his presentation by explaining that DNA plasmids are designed so that a strong promoter drives the expression of one or more genes encoding the protein(s) of interest. The immunogenicity of DNA plasmids promised to revolutionize vaccine development by eliminating roadblocks to vaccine development, such as pathogen isolation, growth, purification, and attenuation; and protein identification, production, and purification. The tools of molecular biology could advance these research efforts further through the isolation and cloning relevant genes.

In addition, animal studies indicated that DNA vaccines could induce protective antibody and CTL responses *in vivo*. For example, IgM and IgG responses were seen at 1 week and at 2–3 weeks, respectively. A memory response was observed after boosting, and the duration of the response was influenced by the strength of the immunogen. The cytokine response was dominated by Th1 cytokine production (IFNγ), with modest Th2 immunity (IL-4) also observed. These responses were influenced by the site of injection and the nature of the encoded antigen. The CTL response generally arises only after boosting, is associated with strong Th1 response, and persists over time.

Quality control is fundamental to DNA vaccine production. Principles common to all vaccine manufacturing include:

- Detailed manufacturing procedures must be in place to ensure consistency across all phases of production.
- Compatible components must be defined.
- Product characterization specs must be defined.
- Batches and formulations must be tested for the presence of extraneous materials/contamination.
- The product must undergo stability testing, including genetic stability.
- Recommendations for lot release testing must be in place.

Features of lot release testing include:

- Sterility testing to detect bacterial or fungal contamination.
- General safety testing in guinea pigs and mice to detect extraneous toxic contaminants.
- Identity testing to check for particle size, restriction endonuclease digestion pattern, and the percent of the plasmid that is circular or supercoiled.
- Purity testing to ensure freedom from protein, RNA, endotoxin, and bacterial DNA contamination.
- *In vivo* or *in vitro* potency testing to assess immunogencity or transfection/translation efficiency.
- Testing for removal of process contamination.

Several safety issues are associated with DNA vaccines. With respect to induction of autoimmunity, investigators should monitor local inflammatory responses (myositis) and organ-specific autoimmunity. The persistence and integration of plasmid DNA can be detected through sites of uptake and expression, persistence of plasmid and protein product, and integration into the host genome. General toxicity studies also should be conducted.

To date, no systemic or organ-specific autoimmunity has been observed in DNA-vaccinated volunteers. As such, CBER no longer requires preclinical studies to examine whether DNA vaccines induce autoimmune disease. However, if the formulation or content of a specific DNA vaccine raises concern that immunization may induce autoimmunity, specific pre-clinical and Phase 1 clinical assessments will be requested on a case-by-case basis.

Integration of plasmid DNA may cause genetic toxicity through several mechanisms, including alteration by vaccine-derived promoters/enhancers of expression of host genes (including oncogenes), genomic instability (breaks or rearrangements), inactivation of tumor suppressors, and germline alteration following integration into reproductive tissue. Studies assessing the persistence of DNA vaccines *in vivo* indicate:

- Initial vaccine uptake is influenced by transfection efficiency and the method and dose of plasmid delivery.

- Plasmid persists long-term primarily at the site of vaccine injection/administration.
- Plasmid levels fall by several orders of magnitude within 2 months of administration.
- Fewer than 30 copies of plasmid/million host cell genomes persist long-term, corresponding to calculated integration rates that are 1,000-fold lower than the natural mutation rates.
- No long-term persistence has been reported for reproductive organs.

On the basis of these findings, revisions to the CBER guidelines for plasmid vaccine development have been proposed as follows:

- Integration studies will be required only when persistence studies indicate that plasmid is present for prolonged periods at high copy number (>300 copies/10^6 host genomes) *in vivo*, and
- Biodistribution/persistence studies will be waived for DNA vaccines demonstrably similar to those already approved for clinical trial.

Dr. Klinman noted, however, that with respect to plasmid vaccine testing, sponsors should contact the FDA for advice when:

- New or significantly modified plasmids are proposed for clinical use,
- The formulation of the DNA vaccine and/or its method or route of delivery may significantly increase cellular uptake or alter plasmid distribution, and/or
- If differences in the behavior of "approved" plasmids are observed.

Preclinical toxicity testing should include the full range of serum chemistries including liver and renal function tests, hematologic tests (CBC, differential), clinical assessments (general health, injection site observation), and necropsy (acute: 2-3 days after immunization; chronic: 2-3 weeks). Preclinical safety assessment should be conducted using animals that have been immunized twice/month for 5 months. Investigators should look for lasting changes in the immune milieu; no deaths; normal serology and urinalysis; and no macroscopic or microscopic changes in spleen, lymph nodes, liver, kidney, intestine, heart, lungs, or adrenals.

Proposed revisions to CBER guidelines include:

- Preclinical safety studies should be performed on every novel DNA vaccine or vaccine/adjuvant combination;
- Toxicity studies should use the highest dose of vaccine planned for clinical administration; and
- Vaccine can be delivered on an accelerated schedule. For example, vaccination intervals may be shortened to every 3-4 weeks, and immunization may follow an "N + 1" vaccine dosage schedule.

CBER may modify the requirements for preclinical safety evaluation in select situations, such as when multiple variants of a specific gene (e.g., HIV-1 e*nv*) are cloned into a common plasmid vector, or when a complete safety evaluation has already been performed on a similar plasmid construct.

Dr. Klinman noted that several dozen Phase 1 clinical trials of prophylactic DNA vaccines have been conducted. Through these studies, many hundreds of normal volunteers have been vaccinated, with multi-milligram doses administered repeatedly to the same subjects. Dose escalation studies of 20 µg to 7,500 µg/subject have produced no major AEs, and local reactogenicity has been mild. Multiple dosing is required to elicit even modest immune responses in humans, however, and ongoing efforts are directed toward improving immunogenicity in humans.

Future directions and concerns include improvements in vaccine formulation and delivery to increase plasmid dissemination, cellular uptake, and persistence while decreasing the risk of integration or toxicity. Intranasal, oral, and intravenous routes may more efficiently disperse plasmid throughout the body. Liposome encapsulation or electroporation may increase plasmid uptake and the range of cells being transfected. Changes in either the vector or the gene may increase the risk of integration, whereas changes in CpG content may alter toxicity.

In closing, Dr. Klinman stated that as CBER accumulates experience with novel types of DNA vaccine, novel vaccine/adjuvant formulations, and novel vaccination strategies, FDA's science-based review of these products will continue to evolve.

Questions/Comments

Dr. Morris inquired about a robust immunotherapeutic vaccine that expresses an *Mycobacterium tuberculosis* (mtb) heat shock protein. Dr. Klinman noted that one group of investigators is testing hsp16 and hsp65 in malaria to boost immune response. The reaction appears to depend on whether the hsp is from the organism of interest; a cross reaction or a reaction against the hsp can develop. A prime-boost-boost study may be conducted under certain circumstances, for example, if prior testing showed no cross reaction and participants are fully informed of possible risks and side effects.

Another attendee asked about use of DNA vaccines in persons with autoimmune diseases. Dr. Klinman replied that persons with such conditions (e.g., with lupus or rheumatoid arthritis) are excluded from clinical trials because of compromised immune systems; however, AEs have not been observed in the few participants who have been enrolled in studies thus far.

Peptide vaccines *Hana Golding – FDA*

Dr. Hana Golding, Chief, Lab of Retrovial Research, Division of Viral Products, CBER, described several types of peptide vaccines and assays to test product efficacy and safety. Multi-epitope vaccines of interest include B cell, T helper cell (Class II restricted), CTL (Class I), and mixed-type vaccines. Delivery of peptide vaccines is associated in part on the structural form of the peptide. For example, peptides may be simple (linear) or branched or complexed with lipids to form lipopeptides. Other vaccine delivery systems may be developed using fusion peptides, plasmid DNA, viral vectors, or bacterial vectors.

In testing peptide vaccines, investigators should run appropriate physical potency assays. These include RP-HPLC and amino acid sequencing for peptides; determining the structural conformation of

plasmids (i.e., supercoiled plasmids have the greatest activity, followed by linear and then circular); and density and particle number of viral or bacterial vectors. *In vitro* gene expression assays also may be used to test potency, and immunogenicity should be tested *in vitro* and *in vivo*.
The inclusion of B-cell epitopes may be adventitious for both *in vitro* and *in vivo* assays, for example, for Western blots of infected or transfected cells and for immune response in small animals. Results of antibody binding assays can be used to test stability and immunogenicity in the development of dose range estimates; serial dilutions of immune sera are recommended. Antibody functional assays should be incorporated into advanced trials and will help establish correlates with neutralizing antibodies.

Challenges for peptide vaccine formulations include demonstrating:

- Cross-presentation of HLA Class I- or II-restricted epitopes following uptake of exogenous peptides.
- Uptake of the peptide vaccine into cytoplasm.
- Intracellular processing and presentation of multi-epitope cassettes or peptides that is clearly distinct from that of intact proteins expressed by bacterially or virally infected cells.

In addition, Western blots cannot be done on transfected or infected cells owing to the lack of antibody recognition. Further, most epitopes recognized by human HLA Class I and II molecules are not presented by murine MHC molecules. However, several transgenic mouse models expressing human MHC I and II epitopes are available.

Product development guidelines note that potency assays vary by product and evolve as a product moves through clinical trials and licensing. The ultimate assays for products going through licensing need to be validated, quantitative, and predictive of the vaccine protective activity in humans. Potential *in vitro* assays for Phase 1 and Phase 2 trials of plasmid vaccines should focus on transcription of foreign gene-containing epitopes (e.g., using quantitative PCR), Western blots of transfected or infected cells using antibodies against the foreign gene product, and assays that recognize Class I or II epitopes by antigen-specific cells established from infected individuals and vaccinated animals.

In vivo immunogenicity studies in animals may parallel the conduct of Phase 2 and Phase 3 trials and licensing application. These studies should establish positive and negative response criteria as early as possible; the number of animals tested should be large enough to accommodate the anticipated response rate. Another important feature of animal immunogenicity studies is that the epitope(s) recognized by the transgenic mice are located in the C-terminus of the multi-epitope peptide or gene cluster. Studies should include positive and negative controls for antigen presentation.

Questions/Comments

Western blots are qualitatively sound but as a quantitative tool, they are difficult to validate as a release assay. RT-PCR may be preferable. Dr. Golding agreed, adding that investigators should try to move to quantitative assays as soon as possible in the product development and testing process. Qualitative

assays are appropriate for Phase 1 trials, but more quantitative testing should begin as the product moves to the next phase.

Another attendee inquired about alternatives to animal potency models, such as the *in vitro* antigen potency assay used in testing vaccines for Hepatitis B. Dr. Golding recognized limitations of some animal models and noted that investigators and FDA staff should remain open to various options. Assessments about which assays would be most predictive of the *in vivo* immune response should be based in part on data from the manufacturer, rather than automatically requiring a certain set of assays for all products. Regarding use of microarrays (e.g., RNA expression) for viral or bacterial vectors, Dr. Golding noted that the FDA is starting to explore this option and its application in vaccine development.

Dr. Annie Mo, Associate Director, Host Defense, Antigenics, Inc., asked about the selection of epitopes based on cross-presentation. Dr. Golding replied that only some candidate epitopes may be eligible for use, and it is the researcher's responsibility to ensure that the epitopes are not clustered at the N-terminus.

Live vaccines *Karen Elkins – FDA*

Dr. Karen Elkins, Senior Investigator, Laboratory of Mycobacteria, CBER, noted that many live attenuated vaccines, particularly for organisms such as viruses with a relatively simple genome, have been approved and licensed by FDA. Fewer live attenuated vaccines exist for organisms with a more complex genomic make-up (e.g., *M. bovis* BCG). Despite many challenges, the agency will continue to consider licensing this category of vaccine for conditions and infectious agents including cholera, *Shigella*, and *Salmonella*, all of which have IND vaccine submissions.

The development of safe, efficacious live attenuated vaccines for intracellular pathogens historically has excluded subunit vaccines because only live attenuated products have been effective. To advance this research area, a much broader and more definitive understanding of efficacy, mechanisms of attenuation, and potency of live attenuated vaccines needs to be developed. Information from manufacturers as well as from clinical and basic researchers will help build this understanding. These contributions, in turn, hold potential for products with superior immunogenicity, especially regarding cellular immune responses, and improved efficacy.

Safety is an important overarching issue with respect to live attenuated bacterial vaccines. Quality control, in turn, is integral to vaccine manufacturing processes and consistency in those processes. Regarding mechanisms of attenuation, investigation is needed of the potential for reversion of naturally attenuated versus "rationally" attenuated strains to wild-type strains, including assessments of recombination in nature.

Age-related differences in safety outcomes of live attenuated vaccines must be determined in special populations, with children and the elderly being at increased risk. Other vulnerable groups include persons with a compromised immune system, pregnant women, and women of childbearing age. Another important consideration for live vaccines involves the potential for "escape" or dissemination

("shedding") of the virus, how well the pathogen can survive, once shed, and the possible impact of the disseminated virus on the health of persons unintentionally exposed to the vaccine product.

Strict quality control, in-process testing, and monitoring by the vaccine manufacturer is important to minimize the potential for exogenous contamination. Understanding the key features of gene regulation in the strain of interest (e.g., stationary phase, phase variation) can facilitate consistency in manufacturing processes. Lot release testing involves a range of assays to determine product sterility, microbial contamination, virulence, potency, and stability. Sterility testing includes modifications to traditional tests due to the live nature attenuated strains. Microbial limits tests may follow guidance from the U.S. Pharmacopoeia (USP). Potency tests assess the capacity to induce a given result. Stability testing determine product viability, the impact of moisture on the product, potency testing that includes developing a reference standard, and identifying the impact of storage conditions.

All of these tests should be developed by Phase 3 of product development. The total package of product and clinical data should support an appropriate risk-benefit relationship.

Questions/Comments

Dr. Whalen noted that under some conditions, virus shedding can be positive. Dr. Elkins commented that a distinction should be made between an IND / licensed biological product used by individuals (which is the purview of the FDA) and recommendations for use by populations for public health (which is the purview of the CDC), where an argument can be made for shedding to benefit the population. Dr. Falk added that once shedding has been identified in inpatient protocols, outpatient protocols are conducted. She asked about guidelines for quantification or acceptable limits of shedding during vaccine development. Dr. Elkins noted that each case is considered separately. At this time, there is not enough meaningful guidance to applicants. CDC would be more likely to consider societal impact and issues than FDA, whose reviews and decisions are based on benefit to individuals.

Subunit/Recombinant vaccines *Sheldon Morris – FDA*

Dr. Morris opened his second talk by pointing out the major considerations and challenges in developing efficacious and safe subunit/adjuvant vaccines, including:

- The adjuvant/antigen formulations, not just the adjuvants alone, are licensed.
- Augmentation of immune responses should be demonstrated in preclinical studies.
- Studies should address potential toxicities from adjuvant use.
- Clinical formulations should be tested in toxicity studies.
- Novel adjuvants should be tested alone in toxicity studies.
- Testing for potential contaminants, such as endotoxin, residual antibiotic, and residual Ni in his-tagged proteins. Investigators need to keep in mind difficulties in distinguishing between protein products and contaminants.

As with all vaccines, subunit vaccine products need to undergo several assays to test for potency, purity, and stability. Other concerns include consistent manufacturing practices, immune cross-

reactivity to self-antigens, consistent secondary and tertiary protein structure, and validation of liquid versus lyophilized formulations.

Dr. Morris closed the presentation by identifying concerns about developing and testing inactivated vaccines, including validating the method of virus or bacterial inactivation, ensuring consistent manufacturing processes, and using standardized potency assays to assess product characterization.

Questions/Comments

In response to a question about whether toxicity studies on clinical lots, which can be very time consuming, can be run in parallel with release studies, Dr. Morris advised investigators to not delay starting these studies. This is to ensure that cGMPs and other regulatory guidelines are met in a timely fashion as the product moves through the development and testing process.

Another attendee asked under which conditions adjuvant formulations, which usually have more than one component, require a new, or more than one IND. Dr. Morris noted that if there are only minor changes to the adjuvant, a new IND may not be necessary; each product would be reviewed on a case-by-case basis, however. A similar question focused on a product in which the expression system was modified after completion of Phase 1 studies. Dr. Brennan noted that licensing a product has three tiers for changes to products and processes; these allowances do not apply to early studies, however. Each product change would be assessed on a case-by-case basis.

SESSION V: OPEN DISCUSSION

An initial question about review of the Gambia studies was posed to Dr. Hill, who noted that the regulatory agencies in the United Kingdom have no authority over studies conducted in Gambia, which, in turn, has no official or state health regulatory authority.

Much of the remaining open discussion focused on the "Koch phenomenon" and its impact on research; testing and use of various TB vaccine formulations in certain populations; and regulatory issues, guidance, and constraints.

Dr. Jerald Sadoff, Aeras Global Tuberculosis Vaccine Foundation, inquired about animal models and preclinical studies for Koch responses, and whether a grading system for these responses could be established. He noted that aspects of the phenomenon can be generated in some animals, but these features are not necessarily predictive of the clinical experience. Dr. Brennan replied that FDA and others are reviewing proposed animal models and appropriate endpoints and standards for each vaccine.

As the discussion continued, attendees pointed out that although the initial Koch responses were seen following vaccination of individuals with active disease, the current focus is on persons who are PPD-positive and may have latent disease. Some studies have shown lower efficacy in PPD-positive, vaccinated groups versus negative controls. However, whether the reduced efficacy in this group was

due to prior sensitization or something else is not clear. It was noted that a variety of factors can influence efficacy; for example, the efficacy of hepatitis B vaccines is two-fold less in smokers versus non-smokers.

Two well-conducted studies on BCG skin test (which is WHO approved) were cited. In one trial conducted in South India (Narain, 1976), all participants received a PPD skin test on the same day. Those with a negative skin test were given vaccine or placebo, as were those with a positive skin test 3 days later; a third group had positive skin test with active disease. Disease acceleration was seen in positive test groups, but no differences in response were observed between the placebo or vaccine group with the negative skin tests. A second study in support of the WHO recommendation included 12,000 Kenyan adolescents never administered BCG; half of the individuals with a positive skin test were subsequently given BGC, and the other half received a placebo. Evidence of AEs, including activation of disease, was associated with the vaccine. Dr. Brennan described preclinical studies that showed necrosis in lungs of previously infected animals previously and suggested that such models could be used for observation of and to develop tests for pre-existing (latent) disease.

Dr. Sizemore commented on reports from the Vaccine Conference in Montreal, which raised some questions about how to address Koch reactions, from a scientific perspective versus a clinical/safety perspective. Dr. Hill's suggestion to study participants in a progressive fashion (healthy adult volunteers then various special populations) may be one approach. Awaiting availability of animal models could slow vaccine development even further. Scientific developments and human response should not necessarily be dependent upon each other.

A key question among attendees was whether product development research would be able to proceed should any questionable/Koch-like reactions occur, and if so, how would those investigations move forward. For example, if there were no AEs in the animal model, would clinical trials still proceed with rigorous monitoring and careful surveillance? And specifically, how does necrosis <u>in animals</u> impact the decision to proceed to clinical trials?

Dr. Whalen noted that there was no evidence of the Koch phenomenon in the Uganda studies, despite household contacts and exposures to TB. Data suggested a different strain caused primary infection compared with a positive skin test without disease, producing more questions than answers. Does re-infection produce the Koch phenomenon? Do some persons have a dual infection? Is a latent infection re-activated to produce Koch reactions?

Dr. Sizemore suggested that human studies could be designed to monitor participants for Koch reactions and then intervene early if needed. Dr. Ballou offered one possible design for immunizing persons with active disease: Start patients out with an effective treatment until they become negative (about 8 weeks), then vaccinate and monitor for differences in responses at baseline and postvaccination. It was noted that revaccination (with BCG) of large populations has been studied in various settings; one such study in Peru involving a series of double and triple vaccinations reported no Koch phenomenon. In studies of pediatric TB, sensitivity is more likely to be a problem, rather than Koch reactions. Dr. Hill commented that latently infected individuals may have decreased immune responses to other mycobacteria, while their response to antigen increases.

One ethical issue that must be considered is how to proceed with persons who have positive skin tests; should they be routed directly into treatment or should blood be drawn, all participants vaccinated and blood tested for positive/negative status after vaccination? With one-third of the world's population possibly harboring *M. tuberculosis* infections, and about 5 percent of treated TB cases relapsing, reactivation could occur when the immune system is compromised. The two-pronged approach of prevention and treatment clearly should be considered in the study of some populations. Research to develop vaccines to prevent infection, to prevent disease following infection, and to prevent recurrent disease is needed.

SESSION VI: MEETING SUMMARY *Norman Baylor – FDA*

Dr. Baylor summarized key issues raised and points made during the workshop. The FDA's primary goal in providing regulatory oversight of vaccine research and licensing is to facilitate the development of safe and effective TB vaccines while protecting humans against undue risk and harm. In this role, the FDA recognizes that TB vaccine development is an evolving field, and the agency is making efforts to be open and flexible while maintaining its role and responsibilities. Dr. Baylor pointed out that both NIH and FDA promote interdisciplinary dialogue and collaborations across the spectrum of vaccine research development. He encouraged all sides to "listen to each other" and echoed many of the presenters by encouraging investigators to engage in dialogue with FDA staff from the very early stages of product development through licensing and into the post-licensing phase, as needed.

Regulations for proper cGMPs and manufacturing processes are especially relevant to Phase 1 and Phase 2 studies. Researchers and manufacturers should characterize their candidate vaccines thoroughly as possible in these earlier stages of product development. Toxicity studies contribute significantly to product characterization; the continual development of new assays to test toxicity push the science forward, and investigators and the FDA need to remain current in this evolving field.

Clinical trials issues continue to move forward in identifying the appropriate populations to study, including special populations and vulnerable groups, and then prioritizing those populations for further study. The testing and use of U.S.-developed and –regulated products in other countries raises a host of interesting questions regarding regulatory authority and requirements, ethical standards and considerations, risk-benefit analyses, study design and logistics, manufacturing and related issues such as product handling and storage, and feasibility and access.

Dr. Baylor encouraged investigators to submit available preclinical and clinical data related to the Koch phenomenon with their INDs so that issues related to clinical trials and vulnerable populations may be addressed and clinical trials may move forward. These issues may best be resolved through advisory groups.

Finally, as this field evolves and grows, the research and regulatory communities will need to address unique issues specific to the types of vaccines under development, including assays to test and validate the safety and efficacy of candidate products, as they arise.