Scientific Report Series: Understanding the Biology of Sex Differences

SOCIETY FOR WOMEN'S HEALTH RESEARCH

Sex Differences and the FDA Critical Path Initiative

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ABSTRACT

The FDA Critical Path Initiative calls for the use of modern research and analysis methods and tools to speed the development of new medical products. These include genetic, genomic, or proteomic markers; advanced medical imaging; and use of biomarkers to predict risk of side effects and response to treatment. The Society for Women's Health Research and the FDA Office of Women's Health convened a workshop to determine the best approaches to advance research efforts on sex and gender differences, and to better understand the contribution that knowledge of sex and gender differences can make to improve or enhance therapeutic product development. Workshop participants developed recommendations for the integration of analysis for sex differences research in the FDA Critical Path Initiative that encompass data collection and analysis, and research methodology.

INTRODUCTION

On November 13, 2006, the Food and Drug Administration Office of Women's Health (FDA OWH) and the Society for Women's Health Research (SWHR) convened a thought leaders' workshop on the FDA Critical Path Initiative. The workshop addressed the importance of understanding the biological differences between men and women in the context of developing tools to improve and accelerate the development and approval of drugs, biologics, and devices. The workshop featured presentations on the state of the art in pharmacogenomics, bioinformatics, and biomarkers research. Attendees participated in breakout sessions where they brainstormed and discussed ways to incorporate sex differences research into the Critical Path Initiative.

Advancing research into the biological basis of differences in the prevention, diagnosis, and treatment of disease in women and men is a significant part of the mission of the SWHR. SWHR's efforts led to the 2001 ground-breaking report by the National Academies of Science's Institute of Medicine, which concluded that the study of sex differences could lead to significant improvements in health for both women and men.[1] The report recommended that research on sex differences be conducted at every level—gene, cell, tissue, organ, and organism—and that sex differences be studied at every stage of life, from conception through death. This includes the study of sex differences in response to therapeutic agents.

"Since we know that there are [sex] differences in the processes of drug absorption and metabolism, and in some areas of safety and efficacy, we owe it to all patients to further explore these differences in current and future therapies," said Phyllis Greenberger, President and CEO of SWHR, in her opening remarks.

Dr. Kathleen Uhl, Assistant Commissioner for Women's Health at the FDA, noted that "the FDA's Critical Path Opportunities List, although quite extensive, has nothing that is unique to women's health, and nothing that is specific about sex and gender differences, hence the reason for the workshop." Both Dr. Uhl and Ms. Greenberger stressed that the purpose of the workshop is to advance the FDA's efforts on sex and gender differences research, resulting in a better understanding of the contribution that such differences can make to improve or enhance therapeutic product development.

FDA'S CRITICAL PATH INITIATIVE: DISCERNING SEX DIFFERENCES

Dr. Janet Woodcock, Deputy Commissioner for Operations and the Chief Operating Officer at the FDA described the agency's Critical Path Initiative and its implications for discerning sex differences in health and drug response. Dr. Woodcock detailed the shortcomings of the current drug development process that led to the creation of the Critical Path Initiative. She noted that despite a doubling of federal funding for biomedical research at NIH between 1993 and 2004, along with a doubling of research and development funding in the pharmaceutical industry, there has not been a corresponding increase in medical products. A bottleneck occurs in what the FDA terms the "critical path" from the discovery of a new potential medical product (drug, device, or biologic) and its development into a product that is used clinically.

As detailed in a 2004 FDA white paper[2] and discussed by Dr. Woodcock, there are several reasons for this bottleneck, including:

- High costs linked to drug development coupled with a high failure rate, and a low yield of useful information
- A lack of modern tools being used to assess safety and efficacy of drugs that would lead to information needed for practice decisions
- A lack of sharing of data and resources among industry, academia, and government

New drug development is extraordinarily expensive, in part because it is so prone to failure. Only about 10 percent of investigational drugs make it to the market. Because drug development is so expensive, many of the questions that would lead to useful clinical information are not addressed in clinical trials. Some of these questions relate to sex differences in drug response, while others could provide answers that would lead to more individualized treatment. The current drug development and testing process usually yields little that can be used to individualize treatment. Traditionally, efficacy is determined as population means that do not identify nor fine-tune the response by subgroups and consider what individual patient characteristics are linked to an effective and safe response to a drug.

Modern tools of analysis are not being routinely applied to animal or clinical studies of new medical products, adding to the expense of drug development. "We are using the evaluation tools and infrastructure of the last century or the 19th century to develop this century's advances," Dr. Woodcock said. Typically, drug tests rely on the empirical knowledge gained from animal toxicology or population-based observational studies, a laborious and time-consuming trial and error process. "We just try things, and wait months or years until we have the outcome. And then if it fails, we try again," Dr. Woodcock said. "We don't build airplanes, bridges or skyscrapers that way. Engineers design these on a computer and they usually work. They do not fall out of the sky or fall down because a large amount of scientific knowledge is applied in their development. We have to aim for a more mechanistic, scientifically-based development process than what we have now, and we have the tools to do that in our hands."

The Critical Path Initiative calls for modernizing clinical trials. This would involve:

- Moving towards a greater understanding of the mechanisms of action of a therapeutic agent with the aid of biomarkers or other means
- Investigating individual factors in drug response
- Developing new methods and approaches to evaluation
- Moving towards a more automated environment

The payoff in pursuing these improvements may be greater treatment effects in clinical trials, according to Dr. Woodcock. Biomarkers or other tools that predict which people are likely to respond to a specific new treatment will allow testing that treatment on those people, rather than the population at large. Biomarkers may also predict the risk of adverse effects of the new treatments so that drug safety could be improved by giving these treatments only to individuals not likely to have severe adverse reactions. The resulting better outcomes from clinical trials would lead to medical products coming to market more quickly, and with better information on how to use these products safely. "If we could put the science into the product development process and come out of it with more informed, better under-

stood products, we could improve health outcomes directly and rapidly," Dr. Woodcock said.

Modern tools of analysis include in vitro diagnostics such as genetic, genomic or proteomic tests; advanced medical imaging; and preclinical safety markers that use biomarkers to predict risk of side effects and/or response to treatment. However, these biomarkers are often not developed into useful clinical assays because such efforts fall outside the major domains of industry, academia, or government institutions. "Nobody is really in charge of this critical path area and that is one reason it has not advanced," Dr. Woodcock said. "What we need to do is collaborate because everybody has a stake in getting this right."

Recognizing these shortcomings in the drug development process, the FDA's Critical Path Initiative aims to improve the medical product development process by:

- Incorporating new scientific advances
- Sharing data and resources
- Developing data standards
- Qualifying new biomarkers

A guiding principle of the FDA's Critical Path Initiative is to foster more collaborative efforts in drug development among government, academia, industry, and patient groups. These efforts should focus more on infrastructure and "toolkit" development rather than product development, the Initiative specifies. To foster this shift in focus, more support needs to be given for academics that pursue the kind of research that will advance the critical path, Dr. Woodcock noted.

In a later question and answer session, Dr. Wendy Sanhai, Senior Scientific Advisor, Office of the Commissioner, FDA pointed out that the FDA has initiated and developed a number of consortia with academia, industry, professional societies, and other government agencies. These collaborative efforts provide the resources and expertise needed to implement specific programs under the Critical Path Initiative. One example of such a consortium is the Cardiac Safety Research Consortium[a].

Another discussant, Dr. Denise Faustman of the Harvard Medical School, pointed out that in academia, a major impediment to developing biomarkers is a lack of monetary rewards for such research. She noted that academic researchers are well funded for basic research and clinical research on a therapeutic, but it is difficult to get investment in such research on biomarkers from foundations and industry. "[There are] big differences in financial incentives

for working on diagnostics versus therapeutics," she said. "When we get a therapeutic patent, a lot of money comes back in to the university. When we get a diagnostic patent, it is hard to give away - it is hard to get the licenses for it just to cover the $200,000 worth of patent filing expenses."

Dr. Sanhai responded that the Critical Path is trying to counter that problem by tying the development of diagnostics and other tests to the development of therapeutics, so that methods for diagnosis, disease staging, treatment, and patient monitoring work well together. This codevelopment makes sense from a medical standpoint because "no matter how good the drug is, if you do not get it to the right patient, it really means nothing," she said. The codevelopment process also fosters sharing of the risks and benefits of product development among the diagnostics and therapeutics companies.

There also needs to be more sharing of existing knowledge and databases, Dr. Woodcock pointed out in her talk. Such sharing could be fostered by the development of data standards so researchers can do cross-trial analyses. Dr. Woodcock noted that the FDA holds the largest collection of primary clinical trial data in the world, but most of these data are not analyzable across studies or retrievable because of a lack of standardization and electronic entry of the data. "In other scientific areas, you build knowledge out of information. We do all these trials and basically we just have a lot of data. We do not build cumulative knowledge because we cannot do cross-study analyses, so that we do not have consensus standards to describe a condition, symptom, outcome, etc.," Dr. Woodcock said.

High up on the Initiative list of major opportunities for modernization is developing the information needed to understand a biomarker's clinical meaning in a given situation, also called "biomarker qualification." For example, a long QT interval on an electrocardiogram (ECG) is a biomarker for the development of arrhythmias, but how exactly it correlates with an adverse clinical outcome is not known in a quantitative way that is useful for clinical assessments. As Dr. Woodcock pointed out, "We probably have hundreds of thousands of biomarkers—it is easy to discover them and publish papers on them. It is not easy to develop the understanding of their clinical meaning, if any."

A big stumbling block to biomarker qualification is the current lack of consensus on the amount and type of data needed to qualify a biomarker for a regulatory, clinical, or other decision. The FDA plans to publish a guidance in this regard, which they will then modify based on the comments of stakeholders. The agency is also developing consortia of pharmaceutical and diagnostic companies for qualification

[a] http://www.cardiac-safety.org

of specific biomarkers. Although an attendee raised the question of whether such private industries would be reluctant to share their data as a way of protecting their trade secrets, Dr. Woodcock responded that "For pharmaceutical or device companies, their innovation is really their product and not necessarily their data. They are going to be willing to share data under certain circumstances." She pointed out that a number of private companies collaborated and shared data to qualify the surrogates for treatment outcomes in HIV patients. "What we need to do now is put together consortia where we can protect intellectual property but share information," Dr. Woodcock said.

Dr. Jeffery Cossman of the Critical Path Institute added that scientists from the 15 diagnostic or pharmaceutical companies participating in the FDA consortia on optimal methods for preclinical testing[b] were more than willing to share their data and testing strategies, despite being competitors. "This information is being used so that all of them can improve their own process. Company A will test Company B's method and vice versa to find the optimal methods for preclinical testing," he said. To further information sharing relevant to ECG biomarker qualification, the agency also developed a standard for a computer-stored ECG reading, and have made available to researchers 250,000 digital ECGs along with their relevant clinical data[c].

In a later question and answer session, Dr. Kathryn Sandberg, of Georgetown University and President of the Organization for the Study of Sex Differences, asked what the Critical Path Initiative is doing to motivate basic scientists to study female animals or tissues or cells from female animals. Dr. Sanhai responded that the funding and overseeing of basic research is more a mission of the NIH than the FDA. However, she added that FDA officials have numerous collaborations with NIH officials aimed at encouraging researchers to include sex and gender differences or pharmacogenomics in their studies. Such analysis may be meaningful in a regulatory context and may not have been the focus of many of their grants in the past.

SEX DIFFERENCES IN DRUG EFFECTS AND SIDE EFFECTS

Jeffrey Cossman, Chief Scientific Officer for the Critical Path Institute in Washington, D.C., discussed the systematic difference in form between individuals of different sex in the same species, known as sexual dimorphism. There are striking examples of sexual dimorphism in animals, such as the showy feathers of the male peacock that are missing in the female. Sexual dimorphism also occurs in humans; an obvious example of this is that men, on average, are taller

and heavier than women. However, there are many less obvious biological differences between men and women, he added. "The biology down to the level of the cell and the molecule is very different and that is the basis for differences in health and response to therapy," he said.

Sexual dimorphism occurs at the genetic level, and is not limited to the genes found on the sex chromosomes X and Y. Signals from genes on the X and Y chromosomes probably affect the activity of autosomal genes, and it is the genes on the autosomes that create the sex differences, including differences in the incidence or severity of disease. Dr. Cossman presented data from a recent study of gene expression in several organs in the mouse that revealed sex differences in the expression of several dozen genes in the liver alone. [3] "Many of these genes have no obvious connection at all to X chromosomes, Y chromosomes, or gonadal hormones," he said. Such differential gene expression occurs in many different tissues of the body, such as the kidney, muscle, heart, and the brain.

Some of the genes whose expression differs between men and women code for enzymes that metabolize drugs, which can lead to sex differences in drug metabolism. Men metabolize caffeine more rapidly than women, for example. Women are known to more rapidly clear from their bloodstream such drugs as erythromycin, cyclosporine, tirilazad, verapamil, and diazepam.[4] Because of differences in metabolism, "the same drug given to a man and a woman may be handled differently in those two. However, most drugs are approved as a single recommended dose, not in doses that differ according to the sex of the individual taking the drug.

Because of sex differences in drug metabolism or other traits, there are sex differences in the adverse effects of drugs. For example, women are more susceptible to developing long QT syndrome from drugs than men. This syndrome causes an electrical abnormality in the heart that can result in potentially fatal arrhythmias. A large number of drugs can cause long QT syndrome, and some have been pulled off the market because of this. Women are more susceptible to developing a certain type of drug-induced arrhythmia, torsades de pointes, which results from prolongation of the QT interval.[5] Dr. Cossman speculated that women's increased susceptibility to QT interval prolongation might be due to sex differences in the expression of genes that affect the electrical conduction system of the heart.

BIOMARKERS

Dr. Nicholas Dracopoli, the Vice-President of the Clinical Discovery Technologies division of Bristol-Myers Squibb

[b] http://www.fda/gov/oc/initiatives/criticalpath/projectsummary/consortium/html
[c] http://www.cardiac-safety.org

provided an overview of three broad categories of biomarkers: genomic biomarkers, dynamic biomarkers, and surrogate markers.

Inherited genomic biomarkers that are stable and do not change over time (except in the case of tumors) may indicate increased or decreased risk of developing a disease or condition. An example is the apoE4 allele that confers increased risk for the development of Alzheimer's disease. Dynamic biomarkers, such as changes in blood levels of a protein or metabolite, or in the level of expression of a gene, may change in response to interventions and, within the context of a clinical trial, must be assessed multiple times. Typically there is a baseline measurement of a dynamic biomarker, and then repeated measurements are made over time to evaluate response to therapy.

Surrogate markers provide outcome measures that can substitute for final outcomes in clinical trials.[6] There are very few FDA-sanctioned surrogate markers, but those that have been validated are widely used and well known. These include the use of HIV viral load as a surrogate for response to HIV treatment, and the use of serum levels of low-density cholesterol as a surrogate for response to treatments to prevent coronary artery disease (CAD). Surrogate markers "take many years to develop and many participants," said Dr. Dracopoli. "No single company or single academic group is ever going to establish a surrogate marker by itself."

Biomarkers have many different uses in drug development. They are used to help assess the safety and efficacy of drugs in preclinical, clinical, and post-approval testing. Dr. Dracopoli focused his talk on the use of biomarkers in clinical testing, specifically their use to select patients likely to respond or have an adverse reaction to a drug. Such selection can reduce the number of subjects in clinical trials as fewer patients are needed to demonstrate the benefit of an experimental therapy.

New genetic, genomic, and proteomic technologies are driving the discovery and use of biomarkers to select likely responders to new drugs. "One of the most extraordinary changes over the last few years is the ability to globally analyze biological systems," Dr. Dracopoli said. "We can use RNA profiling or gene expression profiling to look at essentially all of the genes in the genome in a single experiment. We are no longer reliant on looking at candidate genes or specific pathways, but we can actually mine the entire genome." These new technologies are "leading to a flood of new candidate biomarkers," he said.

There is a particularly pressing need for patient selection biomarkers for cancer therapies and other treatments, for which the consequences of the treatment failing are great.

Biomarkers may be used to predict the subpopulations of patients who are likely to respond to a drug that has a low degree of effectiveness in the general patient population, such as the cancer drug erlotinib. Paclitaxel is a drug that has been widely used for breast cancer for more than a decade. Recent studies reveal that response rates with paclitaxel combination therapy can be increased by a factor of two to three using predictive patient selection biomarkers.

"For a cancer therapeutic, where it is really important to get the patient on the optimal therapy at the earliest cycle of treatment, the consequences of therapeutic failure are very important," Dr. Dracopoli said. This is especially true when there are few alternative therapy options. Biomarkers may be particularly useful in applying a treatment for which either or both the pharmacodynamic and pharmacokinetic characteristics vary among patients.

Patient selection biomarkers are especially needed for targeted treatments, such as the new cancer drugs that target a particular pathology or abnormality in the tumor through a specific molecular mechanism of action. "If you are developing a drug against a particular target, you need to understand which tumors have abnormalities in that target or pathway and which ones do not," he said. "Because, theoretically, if that pathway is not involved in that particular disease, that patient will not benefit."

For example, the drug dasatinib is a tyrosine kinase inhibitor that is approved to treat certain leukemia patients. This drug targets several important oncogenic pathways, which raises the question, could dasatinib also effectively treat solid tumors such as breast cancers? Rather than first assessing this in breast cancer patients, researchers at Bristol Myer Squibb looked for a gene expression profile that was linked to response to the drug in breast cancer cell lines. This expression profile also predicted response to dasatinib in 150 breast cancer biopsy cells and is now being used to screen breast cancer patients prior to entering them in a clinical trial of this treatment.

Researchers at Duke University took a more mechanistic approach to finding a patient selector biomarker that could be used to predict which breast cancer patients would respond to dasatinib. These researchers used transfection assays to determine which known oncogenic pathways are activated in breast tumor cell lines. Those cell lines with active Src pathways were then tested with dasatinib, which is known to inhibit this pathway. The researchers discovered that having an activated Src pathway was strongly linked to response to dasatinib.

"All this work can be done before the first breast cancer patient is ever treated with the drug," said Dr. Dracopoli.

This allows clinical investigators to enroll only those patients who are likely to respond to the drug, which reduces the number of patients exposed to the potential risks or side effects of a new therapy.

Dr. Dracopoli summed up his talk by stressing that the use of biomarkers to identify patients who can benefit from a particular therapy is not new, as biomarkers have been used in clinical practice for many years. The improvements in biomarker development now allow a new approach to clinical trials, including the ability to analyze large amounts of clinical trials data to develop hypotheses for further testing. He concluded by stating "Biomarkers are a critical tool for critical path research and it is becoming increasingly important to use them, whether it is for sex-based differences, for age, for ethnic background, or for prediction of responders."

SEX DIFFERENCES IN PHARMACOGENOMICS

Dr. Wolfgang Sadee of Ohio State University College of Medicine provided examples of the use of pharmacogenomic analysis for development of biomarkers. Dr. Sadee first summarized the three main ways that genomic information is used in the pharmaceutical arena:

- To detect disease susceptibility genes that would enable early prevention or treatment efforts targeted to those individuals most likely to develop a particular disease.

- To aid drug discovery and the drug development process, as well as the clinical testing of drugs.

- To personalize medicine so treatments are better tailored to individuals.

Genomic information is currently used in assays for drug response. Currently there are over a dozen FDA-approved biomarker assays for predicting drug response, but only two are required in order to use specific approved medicines[d].[7] A 2004 study found that only two percent of drug package inserts contain pharmacogenomic data.[8] A more recent survey by FDA found pharmacogenomic data in 10 percent of drug labels.[9]

Sex is both a phenotype and a genetically-based biomarker that may predict disease susceptibility, onset and severity or response to therapy, and alter how doctors treat their patients. For example, it is well known that men suffer twice as many deaths from CAD than women. This is a phenotype difference, Dr. Sadee pointed out, "but a more subtle question would be: Do we have any genetic biomarkers [for CAD] that would differ depending upon whether you are male or female?" Dr. Sadee's laboratory and others are focused on determining the genetic differences that underlie phenotypic differences in sex that affect disease susceptibility or response

to drugs. Previously, researchers proposed a genetic marker that predicts HDL levels and response to the cholesterol-lowering drug pravastatin in males but not in females. Other work has suggested that different genetic markers combine to predict CAD in males, but not in females.

The prevalence, severity, and response to treatment in central nervous system (CNS) disorders also differ by sex. Females are more prone to anxiety and depressive disorders, whereas males are more prone to antisocial disorders and Tourette's syndrome, for example. Women also require lower doses of anti-psychotic medicines and have more complications from them than men. In addition, females respond better to antidepressants that are monoamine oxidase inhibitors (MAOI's). Dr. Sadee then expanded on the research that is revealing specific genetic explanations for some of the sex differences related to disorders affected by monoamine oxidase (MAO). Monoamine oxidase A (MAOA) is an enzyme that affects the amount of serotonin in the brain and that has been linked to a number of CNS disorders including suicidal behavior. The gene that codes for MAOA is found on the X chromosome. "Here we have an X-linked gene and automatically there are major implications for the activity of this gene in females compared to males," said Dr. Sadee. Differences in MAOA between men and women appear to stem from genetic and epigenetic effects.

The amount of MAOA produced in women is determined, in part, by how much methylation occurs in a specific region (called CpG) of the promoter in the MAOA gene. Such CpG methylation is absent in males, whereas it appears to account for 50 percent of the variability in MAO gene expression in women. The remaining 50 percent seems to stem from different alleles in the promoter or other regions of MAOA.[10] Dr. Sadee summed up his talk by saying "Sex is a crucial consideration in the development of biomarkers for drug testing and approval. This will require populations and research cohorts that include adequate numbers of male and female subjects, as well as subjects from different ethnic populations."

BIOINFORMATICS

Dr. Armando Oliva, Deputy Director for Bioinformatics of the FDA's Office of Critical Path Programs identified the lack of data standards and standardized analytic tools or techniques for entering and analyzing clinical trials results as a crucial problem in the current bioinformatics infrastructure at FDA. Furthermore, there is no securely maintained central data repository that could ease information exchange, which makes it difficult, expensive, and time-consuming to analyze data across many trials, including analysis of data by sex.

[d]These are a test for the presence of epidermal growth factor receptor for use of cetuximab in the treatment of colon cancer, and a test for overexpression of HER2 protein for use of trastuzumab for treatment of breast cancer. See http://www.fda.gov/cder/genomics/genomic_biomarkers_table/htm

Table 1: FDA Resources for Data Collection and Analysis

ACRONYM	NAME	PURPOSE
CDISC	Clinical Data Interchange Standards Consortium	Develop data standards
CRIX	Clinical Research Information Exchange	Allow information exchange
	Janus	Central repository of standardized data
ESG	Electronic Secure Gateway	Secure submission of date via Internet
WebSDM	Web Submission Data Manager	Provide common analysis tools
SAFE	Secure Access for Everyone	Provide secure access to data
FIREBIRD	Federal Investigator Registry of Biomedical Informatics Research Data	Provide secure access to data

A number of programs have recently been developed to solve some of these problems in bioinformatics (see Table 1). The Clinical Data Interchange Standards Consortium (CDISC) is a non-profit, non-governmental organization that created standards for clinical trial data that the FDA adopted in 2004. However, there is no regulatory requirement that companies to submit data in the CDISC format, therefore much of the data the FDA receives is not standardized.

The Clinical Research Information Exchange[e] (CRIX) resulted from a collaboration among government, academia, and industry that began in 2005. This secure standards-based electronic infrastructure supports clinical research data sharing for faster and more efficient development of new drugs.

Janus is a pilot central repository of standardized clinical trial data that is being run jointly by the FDA and the National Cancer Institute (NCI).

The Electronic Secure Gateway (ESG) for secure electronic submissions of data via the Internet to the FDA is operational. The FDA and a private partner also developed the Web-based Submission Data Manager (WebSDM) to provide common analysis tools that enable scientists with little computer expertise to easily analyze large amounts of clinical data (similar to what is submitted to FDA for product approval).

To maintain the security of this information, a coalition of companies developed the Secure Access for Everyone (SAFE) initiative, which the FDA supports in an advisory capacity. SAFE led to FIREBIRD[f], a software application that enables clinical investigators to electronically store, maintain, and download information about their credentials and clinical trial experience. This information is accessible to trials sponsors and regulatory agencies. The information's security is ensured by verifiable digital signatures and smart card access. "This is the very first step of what we believe is the model for future solutions for information sharing in the clinical research community," Dr. Oliva said.

These bioinformatics programs will facilitate analysis of demographics data across clinical trials. Use of CDISC data standards will ensure that data set and variable names are consistent. Dr. Oliva noted that investigators and companies would enter standardized clinical trial data conforming to CDISC directly into Janus with CRIX, or indirectly via ESG.

"With the right bioinformatics infrastructure, we can do a much better job of monitoring the participation of women in clinical trials. It is a trivial problem with the right infrastructure," Dr. Oliva said. In a question and answer session following his presentation, Dr. Oliva noted that CDISC standards are already in place to track the menstrual status of women in clinical trials, including menstrual cycle stage.

Custom analysis tools such as WebSDM could then be used to quickly and easily extract demographic and other information on all the trials in the repository. "You can generate a report quickly and easily about how many women have participated either in a single clinical trial, or in clinical trials across an entire application or within an entire therapeutic class," he said.

The final step is to communicate the results of the data analyses, which will be aided by another FDA initiative called Structured Product Labeling (SPL). This is an initiative to convert all product labels from a paper format to a machine-readable format. This new format will include the conditions and limitations of the drug's use, such as those specific to women or to men. For example, if a woman is more likely to experience adverse side effects from a drug in the luteal phase of her menstrual cycle, that information will be in the label.

[e] http://crix.nci.nih.gov
[f] https://firebird-beta.nci/nih.gov/Firebird

CASE STUDY: SEX DIFFERENCES IN HIV TESTS

Dr. Hana Golding of the Center for Biologics Evaluation and Research of the FDA presented her research (funded by the FDA Office of Women's Health) on a novel HIV infection diagnostic. Acute HIV infections are often under-diagnosed in women for reasons that are not entirely understood. Viral load measurements during the initial phase of HIV infection tends to be about 30 percent lower in some women than what is typically seen in men during that phase of the infection.

Because of this known sex difference, Dr. Golding and her colleagues were careful to include both women and men in their tests of a new HIV assay designed to detect acute HIV infection in people who have already received HIV vaccines. There currently are 40 HIV candidate vaccines that will require testing in thousands of volunteers throughout the world. Because these second-generation vaccines have many of the virus' gene products, people who receive the vaccines are likely to test positive in most current antibody-based HIV tests.

To assess the efficacy of the vaccine, as well as to ensure those volunteers who become truly infected with HIV receive the proper treatment as soon as possible, investigators need an HIV test for acute infection that can distinguish between people who have a true HIV infection and those who have been vaccinated for the disease. Such a test is also needed to prevent discrimination against study volunteers who have received an experimental HIV vaccine and test positive for the disease on standard HIV diagnostics.

Dr. Golding and her colleagues discovered antibodies to two HIV sequences, found within the p6 protein and the in cytoplasmic tail of gp41, that are measurable within a month of HIV infection but do not appear to correlate with infectivity and thus are not part of most experimental vaccines. These proteins are highly conserved in all the known major clades and subtypes of the virus, so an HIV test that is based on them is likely to work throughout the world. The FDA researchers developed an HIV assay, called the HIV Selectest, which is based on response to gp41 and p6 antibodies.

Preliminary tests of this novel assay in people who were vaccinated against HIV indicated that it had a specificity of 99.3% for p6 and 100% for gp41. These tests revealed a high degree of cross-clade reactivity. Dr. Golding stressed that in all the trials of the HIV Selectest, researchers assessed whether there were any significant sex differences in test reactivity. Based on small numbers, there do not appear to be such differences, but more data are needed to assess this, she said. Future trials of the diagnostic are planned to be done in equal numbers of men and women so there are "large enough numbers of male and females to be able to actually do proper statistical analysis and to draw conclusions related to the sensitivity and specificity," Dr. Golding said.

RECOMMENDATIONS

Workshop attendees participated in one for three breakout sessions where they were asked to brainstorm and discuss two questions:

1. What resources and tools exist in this area for developing and applying knowledge of sex and gender differences?

2. What resources and tools are needed in this area to develop and apply knowledge of sex and gender differences?

Participants were asked to categorize their recommendations into short-term, medium-term, or long-term goals.

DATABASES AND SPECIMEN REPOSITORIES

The breakout group discussions reiterated Dr. Oliva's contention that data that could be useful in achieving the goals of the Critical Path Initiative is, for various reasons, unusable or inaccessible. The participants identified a number of existing databases and specimen repositories as important resources (see Table 2), and noted that these types of resources pose similar difficulties in their utility for advancing product development.

Existing databases include annotated indices of the biomedical research literature such as PubMed; databases containing clinical and clinical trials data; and databases holding biological data on genes and proteins. Specimen repositories include those that are funded and managed by NIH institutes and centers, by academic or private research centers, and by companies. A few databases and repositories are openly available to anyone who requests access, and some limit accessibility to subscribers, to researchers who provide data, to regulatory or funding agencies, or to employees or contractors of the organization that owns the data. Concerns about the proprietary nature of data and intellectual property rights are among the reasons for limiting access.

Participants agreed that wider availability of data and specimens would facilitate the use of bioinformatics tools in product development and the development of useful biomarkers. Some participants recommended the development of consortia of interested laboratories and institutions for the sharing of databases, and gave the Single Nucleo-

Table 2: Examples of Existing Databases and Repositories

Name	Contains	URL
PubMed	Published literature	www.pubmed.gov
GeneLogic	Microarray reference data	www.genelogic.com
NHLBI Biological Specimen Repository	Whole blood, serum, plasma, blood cells, DNA, RNA, bronchial/alveolar lavage fluid, urine	www.nhlbi.nih.gov/resources/medres/ reposit/reposit.htm
NIAID DAIDS Specimen Repositories	Peripheral blood mononuclear cells, serum, plasma, semen, saliva, vaginal washings, urine, placenta, and autopsy samples	www.niaid.nih.gov/reposit/over.htm
NCI Specimen Resources	Normal, benign, pre-cancerous, and cancerous tissue and microarrays	pluto3.nci.nih.gov/tissue/default.htm
NIDA Center for Genetic Studies	Data on family structure, age, sex, clinical status, and diagnoses; blood samples, DNA, immortalized cell lines; data derived from genotyping and other genetic analyses of these clinical data and biomaterials	drugabuse.gov/about/organization/ Genetics/tissuerep/index.html
NHGRI Databases	Includes sequence data, SNP consortium data, cDNA and expressed tags, model organism databases, 3-d structures	www.genome.gov/10000375
NIA SWAN Repository	Blood and urine samples from the Study of Women's Health Across the Nation	www.nia.nih.gov/ResearchInformation/ ScientificResources/Repository.htm
Genomic and Proteomic Databases	Microarray and gene expression data	established and held by individual companies, institutions, and government agencies
Clinical Research Studies Databases	Clinical data on study participants and study outcomes	established and held by individual companies, institutions, and government agencies

tide Polymorphism (SNP) Consortium, the International HapMap Project, and the Genetic Association Information Network (GAIN) as examples of successful consortia. Incentives for submission and sharing of data by consortium members include accessibility to data and product development tools.

Further discussion led to the recommendation that all data that would be useful for the development of clinical trials methods, FDA guidance documents, and other tools for product development should, ultimately, be in the public domain - including much that is now regarded as proprietary or to which access is limited. For this to happen, the ethical and legal issues involved in getting permission to place data in the public domain will need to be addressed.

The use of technology to "de-identify" data will be crucial in this effort.

Beyond issues of access, the participants noted the challenges posed by the diversity in data collection and storage methods, terminology, and even computer languages. Such diversity prevents combining and analyzing data across databases. One participant noted that not only does the structure and terminology of data submitted to FDA differ among the different companies that submit the data, but database structure and terminology sometimes differs among different drug development phases of the same drug within the same company. Similarly, specimen repositories lack standardization and often have incomplete clinical data to accompany individual specimens.

The use of these resources to uncover and analyze sex differences is impeded by lack of standardization in data collection. Much of the discussion focused on the need for standardized methods for storing and accessing clinical data and repository specimens, including the need for consistent and accurate recording of both sex and gender[g]. Even though sex is considered a basic demographic item, it is frequently not included or listed as "unknown" in clinical data sets. Specimen repositories may or may not include the sex of the tissue or sample, depending on the use for which the repository was created. Participants agreed that all databases and repositories should include a field for recording sex, but that gender should be optional at this point because of the lack of a standard method to quantify and measure the phenotype of gender. The use of the terms "sex" and "gender" as synonyms is a basic problem that, for example, makes it difficult to ensure that the results of literature searches are accurate and complete.

Participants also discussed the value of including chronobiological information in databases and specimen repositories, which involves recording time of day, month, year, and reproductive or hormonal status. Chronobiology studies, including the exploration of when in the life span sex differences emerge or disappear, would be facilitated by proactively ensuring that appropriate data are collected from clinical studies.

The overall recommendation for bioinformatics is the standardization of data sets (see Table 3). This includes the use of standard case-report forms that specify all fields of information needed for a complete database record. Regulation, training, and education of users would be required to ensure consistent application of standards. Such standardization will require consensus on what data are necessary or useful for achieving the goals of the Critical Path Initiative, and how to make such data accessible without compromising intellectual property.

A crucial recommendation is to standardize both the collection of human specimens for repositories and the collection of data to accompany these specimens. Specimens should be made available for basic, applied, and clinical research, developing and validating biomarkers, and conducting proper statistical analyses on these biomarkers.

Standardization of data and specimen collection will allow for easier use of bioinformatics tools by permitting the combining and comparison of data across studies. These tools include pathway analysis of genetic information, principal component analysis to assess sex differences, other types of mathematical and statistical modeling, and meta-analysis.

DEVELOPMENT OF BIOMARKERS

Participants identified a number of laboratory techniques and methods that are being used or could be used to develop useful and effective biomarkers. These include genomic and proteomic microarrays, gas chromatography or tandem mass spectroscopy, high performance liquid chromatography, and tissue culture. The application of these techniques to the understanding of sex differences will require accurate knowledge of the sex of the source of the samples under analysis.

Applied research on biomarkers relies on animal models, and pharmacogenomic, pharmacodynamic, and pharmacokinetic studies. If both male and female animals are studied, sex differences in a biomarker might be detected; however, often only males are studied because of concern about the complicating influence of ovarian cycles. Applied research also often uses human biologic specimens and database information about those specimens. Sex differences can be detected only if both male and female specimens are used. None of this research is useful to clinicians unless it results in diagnostic tests or imaging modalities, and participants recommended seeking a change in FDA procedures to include consideration of sex differences in the evaluation of devices as it is currently in the evaluation of drugs and biologics. The FDA's Office of In Vitro Diagnostics requires testing in both male and female specimens and checking for sex differences before approving a new in vitro diagnostic test, unless the test is only going to be used in a single sex (e.g. measurement of prostate-specific antigen levels). Sex differences were not necessarily looked for or recorded for diagnostics and other devices that are not currently regulated by the FDA. Furthermore, for imaging diagnostics the FDA only regulates the machines and software used but not the use of imaging biomarker itself.

As mentioned earlier, the development of a centralized bank of well-annotated human specimens is critical to the development of clinically useful biomarkers. This specimen bank should include a wide range of specimen types; not just serum, or formalin-fixed and paraffin-embedded specimens but also clinical imaging results and other recordings such as electrocardiograms.

A primary short-term goal in biomarker development is to profile and document sex differences (if any) in qualified biomarkers used in medical product development. In addition to the qualified biomarkers previously mentioned by other speakers, the group added ischemia/angina as a biomarker for CAD, and FDG-PET to image non-small cell lung carcinoma and non-Hodgkin's lymphoma. A

[g] Sex is the " . . . classification, generally as male or female, according to the reproductive organs and functions that derive from the chromosomal complement." Gender is ". . . a person's self-representation as male or female, or how that person is responded to by social institutions on the basis of the individual's gender presentation." (Wizeman and Pardue, 2001)

Table 3: Characteristics of Useful Resources for Sex Differences Research
Clinical Research Study Databases • Standardized structure and data terminology • Standardized case report forms and data entry • Structured for bioinformatics analyses • Separation of sex and gender as individual characteristics • Include chronobiology data • Include genetic polymorphism data • Include drug metabolic pathway data for pharmacogenomic analysis **Human Specimen Repositories** • Well-annotated specimens • Wide range of specimen types; not just serum, or formalin-fixed and paraffin-embedded Specimens (ECGs or imaging results, for example) • Standardized procedures for specimen collection and storage • Standardized specimen and specimen data collection and entry

medium-range goal is to identify research-grade biomarkers that "are close to the finish line," such as those that are currently in the pipeline, and to ensure that the investigation of sex differences is included in their development and qualification. Finally, a long-term goal is to capture data on sex and gender differences prospectively in the context of safety and efficacy when developing new biomarkers. This will require better use of mathematical modeling and statistical resources and expertise, particularly as needed for analyses of data by sex.

Professional organizations, healthcare payers, and the federal government (e.g., the Centers for Medicare and Medicaid Services) are potential funding sources for biomarker development projects. Incentives to prompt companies to undertake sex or gender-based biomarker development are needed, such as tax breaks or extended exclusivity options.

Existing research efforts should be leveraged and expanded to explore sex differences. For example, participants felt strongly that the current selective serotonin reuptake inhibitor study by the National Institute for Mental Health, and the Alzheimer's Disease Neuroimaging Initiative of the National Institute of Aging should include an exploration of sex differences.

PHARMACOGENOMICS

Resources and tools specific to the advancement of pharmacogenomics methods include large-scale genotyping, pathway analysis tools for interpreting the biological significance of genetic data; mathematical modeling tools; flow cytometry; nanotechnology; and protein assays, endocrine assays, and ELISA's. These existing tools and resources could be used to discern sex and gender differences if adequate numbers of women were included in the testing of new drugs, biologics, and devices to allow for statistically sound safety and efficacy analyses by sex. This is particularly important for studies of diseases or therapies that exhibit a known sex difference. The group also recommended taking a systems biology approach to pharmacogenomics such that not just gene expression is considered, but also protein levels, metabolites, etc.

Participants also recommended the development and use of a priori prospective standards for data analysis by sex in clinical studies. If an experimental drug is metabolized by a particular enzyme, for example, then the prevalence of genetic variants of that enzyme in the study subjects should be noted. The group also suggested addressing the importance of sex differences relative to other factors such as age, ethnicity, etc.

Table 4: Recommendations to Advance Knowledge of Sex Differences in Product Development
• Expand accessibility of databases and repositories
• Develop database and repository standards that include sex as a variable
• Separate sex and gender as demographic values in medical records study forms
• Develop methods to accurately assess gender
• Profile and document sex differences in currently used biomarkers
• Include sex differences in biomarker development
• Develop models for pre-clinical pharmacogenomics testing that include sex differences

Participants also recommended developing new pre-clinical models that consider sex differences in pharmacogenomics, and determining sex differences in the molecular mechanisms of drug action useful for drug development.

Additional recommendations included adding to the FDA's biomarkers table[h] a table of genetic polymorphisms known to influence clinical outcomes differently in men and women as they are identified and qualified. This polymorphism data should be used to improve clinical trial design. In other words, if a genetic polymorphism is likely to influence a response to a drug, then researchers should collect data on the distribution of that polymorphism in the study population. Within this time frame, participants suggested using biomarkers for predictive outcomes that vary by sex, and providing more education, expertise, and software for statistical and mathematical analyses of data by sex.

CONCLUSIONS

Table 4 summarizes the recommendations that emerged from the presentations and discussion at the workshop. Implementation of these recommendations will require the efforts of, and cooperation among, FDA, NIH, the Centers for Disease Control and Prevention, the Centers for Medicare and Medicaid Services and other federal agencies; academic and research institutions; and pharmaceutical, device,

and biological therapeutics companies. The development of effective consortia and other public-private cooperative endeavors will be key to advancing knowledge of sex as a key biological variable in product development.

Overall, the workshop presenters and participants concurred with the FDA's Critical Path Initiative analysis regarding the importance of bioinformatics methods and the use biomarkers, including pharmacogenomic markers, to improve and accelerate the evaluation and regulation of drugs, devices, and biologics. The question of sex differences – including the analysis of outcomes by sex – must be considered at every step in the development of drugs, devices, and biologics. Failure to consider sex differences during data collection and analysis will severely limit the usefulness of clinical data in the regulatory evaluation of product safety and efficacy, and may jeopardize the applicability of study results to clinical practice and personalized medicine. Incorporating knowledge of basic biological differences between men and women into study design and data analysis will ultimately improve clinical care for both men and women. Greater knowledge and understanding of sex differences at the time of evaluation and marketing approval decisions will enable sponsors and the FDA to usher in the era of individualized medicine more efficiently, and will ultimately benefit all patients and consumers.

[h] http://www.fda.gov/cder/genomics/genomic_biomarkers_table/htm

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