Response to the November 2016 Subcommittee Review
Division of Systems Biology
NCTR/FDA

We appreciate the thorough examination and review of the Division of Systems Biology research by the Science Advisory Board (SAB) Subcommittee on November 3-4, 2016 chaired by Dr. Suresh Pillai and Dr. Diwakar Jain, both members of our SAB.

We also want to thank the team members who participated in this review: Dr. Nigel Greene (AstraZeneca), Dr. Timothy Ryan (Sano Informed Prescribing), Dr. Frank Barile (St. John's University), and Dr. Greg Lanza (Washington University School of Medicine).

Our appreciation also goes to those representatives of the FDA Centers who contributed their thoughts and comments: Drs. Dana van Bemmel (CTP), Phil Yeager (CTP), Jose Centeno (CDRH), Carol Linden (OCS), Tracy Chen (OCS), Steve Solomon (ORA), Paul Norris (ORA), David Strauss (CDER), Tim McGovern (CDER), Carolyn Wilson (CBER), and Hong Yang (CBER).

We are grateful for the positive and constructive nature of the comments and recommendations in the draft report. Your comments are valuable to us as we determine the best ways to address critical needs of the FDA as we move forward over the next 5 years.

The Subcommittee's review offered encouragement and insight, as well as comments that highlighted some points to be considered. We have tried to capture those points as main topics, along with comments in the review (copied or paraphrased here in italics) that illustrate them.

ORGANIZATION

"The present organizational structure maybe falling short in capturing the scientific potential of its staff. In today's big data-driven world, there is a compelling need for the integration of systems biology with computational technologies." Page 2

This is a valid observation that we concur with. Accordingly, we are reviewing the Divisional structure with an eye to how it empowers the Division's research. We are further reviewing collaboration opportunities within the NCTR and within the Agency that would address our data-integration / computational needs.

Theme 1: Clinical/Translational Metabolomics and Proteomics

BIOMARKER VALIDATION

"The review team feels that validation of candidate biomarkers should be a clear strategic focus of this group, recognizing that translation could involve early biomarker qualification steps with additional 'early adopting' preclinical and clinical collaborators." Page 4
"The review team feels that it is prudent to develop a strategic plan for biomarker validation and to temper the urge to identify more candidates rather than closing out the existing work." Page 4

The Division appreciates the Subcommittee's response to our questions regarding biomarker discovery versus validation. We agree that a great deal of effort to date has been focused on biomarker discovery. Historically this has been driven by the nature of the expertise and technology resources of the Division: systems biology, by one definition, is a collection of 'omics technologies that do operate in a discovery and hypothesis generating mode. Nonetheless, there are indeed resources within the Division that could be strategically utilized for biomarker development.

It is important to note the use of the phrase "biomarker development", as biomarker "validation" and/or "qualification" is a non-trivial enterprise, one, in fact, with legal overtones (see the 21st Century Cures Act). The Division Director has had extensive experience in regulatory biomarker qualification as it was developed both at the FDA and EMA, and is keenly aware of the considerations in such an enterprise. In agreement with the Subcommittee's recommendation, this is an area well worth taking seriously.

To that end, the Division has engaged a dialogue with the newly formed FDA Drug Development Tool Qualification team to identify biomarkers that may be of sufficient high priority to warrant a development (i.e., validation) effort. We have begun developing putative "Context of Use" (COU) statements that would be relevant to biomarker qualification, and such statements when reviewed by the team could allow prioritization. As an example, one biomarker with potential for development is plasma palmitoyl carnitine, which may be a translational marker of drug-induced mitochondrial injury (DIMI). We have reached out to not only FDA reviewers but experts in the field for input on its relevance and the steps required for its further development. We are also engaging the Predictive Safety Testing Consortium (PSTC) of the Critical Path Institute, and HESI for interest in this and other biomarker work. Indeed. collaborations with outside groups and especially consortia are critical not only for the extensive work required for biomarker development, but most importantly for confirming the relevance of such biomarkers to drug development or clinical practice. Furthermore, the steps required for any given biomarker validation generally must have consensus support from a larger scientific community. In short, we are taking the Subcommittee's advice seriously and are approaching biomarker development and/or validation in a strategic fashion.

"In the case of MALDI imaging, the group could quickly become field leading." Page 4

The Division greatly appreciates the kind words and encouragement to this effort. We do intend to leverage this technology and expertise within the FDA and greater biomedical research community.

Regarding sample and data quality: "These variables clearly contribute to the interpretation of proteomic and metabolomic data, and with the unique purview of the FDA, are necessary to objectively review data from disparate submission sources. Further, these activities support the overall FDA mission, especially as it pertains to data submission." Page 4

The Division appreciates the affirmation of this effort; we will indeed support and further it.

Theme 2: Doxorubicin/Cardiotoxicity markers

"There is definitely a need for more specific proteomic, metabolomics or genomic biomarkers, based on the biochemical, molecular and cellular injury specific to the anthracyclines." Page 5

"This is an interesting and well-designed study and is likely to provide important insights into the mechanism of

sunitinib cardiotoxicity and perhaps cardiotoxicity of other TKIs (tyrosine kinase inhibitors) as well. The panel feels that this is probably an interesting and appropriate way to study a relatively complex project." Page 6

The Division appreciates the affirmation of these efforts; we will indeed support and further them. We have designed this and other studies in collaboration with colleagues in FDA review divisions who are familiar with the issues of TKI-induced cardiotoxicity.

CLINICAL RESEARCH AND FOCUS

"The research by this team is highly relevant and needs to be extended to humans to identify genomic, proteomic and metabolomics markers to predict cardiotoxicity in clinical context." Page 5
"Perhaps, future investigations should focus on human studies." Page 5

The review team emphasized the importance of collaborations with clinical teams. This is an important point that we take very seriously. Historically, our interactions with clinical scientists has been episodic with a focus on applying discovery technologies to the clinician's sample set. While this has indeed been productive from a biomarker discovery standpoint, it does not play into a larger translational biomarker development strategy. As noted above, a comprehensive strategy is optimally developed in a consortium fashion, including input from target users. This might entail input solely from clinicians if the biomarker is solely targeted for clinical decision-making, but even there, a diagnostic test would require regulatory consideration. Thus, for each biomarker study a more intentional strategy is desired and the Division intends to incorporate such strategies going forward. As noted above, developing draft Context of Use (COU) statements promptly after a putative biomarker discovery provides the groundwork for a subsequent development strategy. In fact, developing a COU prior to initiating a discovery study would also focus the study design. Again, we greatly appreciate the Subcommittee's observations.

"Dr. Desai also made a presentation the development of a mouse model of doxorubicin-induced delayed onset cardiotoxicity… with increasing long-term survival of cancer patients, particularly in pediatric malignancy, this area is of significant clinical interest… Perhaps, parallel long-term clinical studies in patients undergoing doxorubicin therapy for clinical indications may need to consideration." Page 6

The Division appreciates the encouragement and takes seriously the challenges of approaching this question. In addition, the Division concurs that parallel clinical studies are important, and has established a collaboration with a pediatric oncology group at the Arkansas Children's Hospital.

GENETICALLY DIVERSE VS HOMOGENEOUS ANIMALS – ADDRESSING HUMAN VARIATION

"A unique feature of anthracycline cardiotoxicity in human is a very wide range of susceptibility to cardiotoxicity across different patients.."
"… animals in this study are genetically related to each other (littermates), one would anticipate them to behave fairly similarly or uniformly…over time. One possible variation would be to use animals derived from different strains or unrelated animals to introduce genetic variation in the model." Page 6

The review team noted the importance of considering variable responses in humans and genetic diversity in animal studies. We agree completely and can point to studies where using outbred animals results in variable responses to treatments. In any experimental design the inclusion or exclusion of genetic diversity must be considered in terms of its potential impact on the results. It is noted that in the case of mouse studies, samples from several animals must sometimes be combined to allow analyses, and in that case, genetic diversity is of no advantage. We believe

that you need a homogenous population to identify biomarkers, which can then be tested for individual responses in in a genetically diverse strain. We will consider animal models (perhaps rats) that would allow longitudinal and/or individual sampling and comparisons between outbred (e.g., Sprague-Dawley) and inbred strains (e.g., Fisher 344).

Theme 3: TKI Markers and Mechanisms

"Dr. Vijay made a presentation on the differential gene expression as a possible predictor of susceptibility to TKIs organ toxicity… This is an ambitious data-mining project and the panel encourages this study.
Inclusion of (drug) properties are more important when the investigators move into analyzing human data."
Page 7
We appreciate the encouragement and the Subcommittee's response to this question.

Theme 4: Alternative Drug Safety Models

ALTERNATE MODELS BASED ON iPSC

"The research offers a possible model to support a mechanism-based biomarker discovery program which could lead to better preclinical safety evaluations... The model is acceptable particularly since the use of iPSCs has generated significant interest in toxicity testing.
…The model (cardiomyocytes derived from induced pluripotent stem cells – iPSC-CM) suffers from significant shortcomings. In vitro behavior of these cells necessitates precise manipulation of the growth, proliferation and differentiation properties. Additionally, the process of initially establishing iPSCs is cumbersome and not always successful…..the review team questions the predictive ability or practicality of an in vitro model in general."
Page 8

The Division appreciates the Subcommittee's encouragement as well as the critical insights. As a first principle, "All models are wrong but some are useful". Even a clinical study, by sampling a population, is a model, and may not pertain to the totality of humanity. We do take seriously an awareness of the limitations of and caveats applied to any given model. Cardiomyocytes derived from human stem cells are clearly not actual hearts embedded in a fully functional organism, responding to mechanical, hormonal and electrical cues. Furthermore, it is acknowledged that in the current state of the art, such iPSC-CMs do not have the full mature transcriptional profile of *in vivo* cardiomyocytes. We do not pretend to be experts in CM derivation, and accordingly we rely upon well-established commercial sources for cells. We appreciate the Subcommittee's reminder that an iPSC-CM model can address only certain questions. Results from such studies would then need further *in vivo* or clinical studies to confirm relevance.

EFFECTS OF OPIOID EXPOSURE ON NEURAL DEVELOPMENT

The Division thanks the Subcommittee for their support of this project, and their recommendation for defining direction. We do feel the primary focus should be "effects of opioid exposure on neural development". This is an area well in keeping with the Agency's efforts to address issues with the opioid use epidemic. As far as the concern for "investigating differentiation of stem cells to NPCs (neural progenitor cells), neurons and glial cells" (page 9) we understand that this is a difficult area. We are aware of the several approaches and issues, and will closely monitor the progress of the initial studies to gauge the direction of this project.

ALTERNATE MODELS OF MALE REPRODUCTIVE TOXICITY

"The review team feels that the objectives may need better definition. Overall, the review team notes that the different efforts have resulted in specialized laboratories with deep expertise in specific areas. However, the review team feels that although the laboratories are highly engaged, the overall directions and applications may need some fine-tuning." Page 9

We thank the review team for their helpful comments and will work to focus the work being done in this area. We are currently working to establish collaborations with other laboratories that would strengthen this research.

Theme 5: Food Safety Technology

The Division greatly appreciates the Subcommittee's positive comments on the RAPID-B technology, and its applicability in many arenas.

"The researchers should explore how their expertise in flow cytometry can enhance current genomic technologies. The CFSAN and the CDC relies on genomic detection platforms such as real-time PCR (RT-PCR) and whole genome sequencing to detect and characterize pathogens… It may be, therefore, worthwhile to explore whether the RAPID-B is applicable as a primary screening approach prior to genomic analyses." Page 10

The review team again notes the importance of coordinating the Division's efforts with those other Centers. Accordingly, early in 2017 we arranged for an ORISE fellow currently developing RAPID-B to relocate to a CFSAN laboratory in Maryland so as to integrate this technology with the other technologies CFSAN is pursuing. Indeed, CFSAN relies upon RT-PCR for pathogen detection and Whole-Genome Sequencing (WGS) for forensic characterization of existing outbreaks. Our hope is to demonstrate the complementarity of an ultra-sensitive detection tool (i.e., primary screening) that can turn around results in a realistic time frame. This collaboration with the CFSAN laboratory of Eric Brown affords that opportunity and valuable feedback on the needs not addressed by RT-PCR and WGS.

Theme 5: Computational Modeling

The Division posed a series of questions to the review team, and greatly appreciates the specific and detailed answers that were given. The responses are very helpful in moving forward in this field. Some other comments from the review team follow.

"The modelling performance for hERG was significantly impacted by the classification of the experimental data into a binary system of active or inactive. While this approach may be appropriate for some biological effects such as mutagenicity where the concentration where the effect is seen is considered irrelevant in a risk assessment context, most decisions in the determination of risk with drugs and chemicals in general are based on a comparison of exposure at which toxicity is observed versus the exposure administered to human subjects or patients. Therefore, in most cases there is a need to consider predictions of the concentration where adverse biological effects would be anticipated" Page 11

The Division agrees with the review team that classification of the experimental data into a binary system of active or inactive impacts the performance of computational models. We agree that the field often find themselves negating Paracelsus' mantra that "the dose makes the poison" and instead leaning toward a lay perspective of "toxic" or "non-toxic". The field of "prediction" is covered with these examples, so much so that to communicate one's work one is often obliged to translate one's models into this perspective. In the case of the phospholipidosis (PLD) modelling a single binary endpoint (active/inactive) per compound was provided by NCATS. The Division whole-heartedly agrees this is suboptimal and that "in using a binary classification system the subtle but important structural determinants of activity will be invisible to the modelling algorithm and hence unidentifiable." Ideally modelling data of toxicological concern can make use of continuous endpoints, and our intent is to avoid binary classifications as much as possible.

"one of the requirements for a QSAR model to be considered acceptable in a regulatory setting is the ability to determine if a chemical lies within the applicability domain of the model... That said, the challenge here is how to appropriately represent the extent of the applicability domain of a model." Page 12

The Division whole-heartedly agrees that a careful approach to developing an applicability domain metric is essential to the SDAR modeling approach; we are exploring several avenues to this.

Theme 6: Precision Medicine

TRANSCRIPTOMICS-BASED PREDICTIONS OF SUSCEPTIBILITIES

"Understanding the intrinsic and extrinsic variables inherent to individual patients could ultimately improve real-world medication effectiveness. The patient variables of gender, age, and obesity are not typically part of sponsor-conducted efficacy trials, yet critical to medication tailoring. The NCTR is a logical institution to not just participate in, but lead this field of research, and the review team is highly supportive of these efforts." Page 13

"The review team notes the following areas of strength in this thematic area, namely, 1) pioneering proposal to use animal models to address important precision medicine questions, 2) efficient and thorough transcriptomic data mining, and 3) the overall project scope aligns with precision medicine, yet differentiates from rest of field that focuses on genetics." Page 15

We greatly appreciate the Subcommittee's affirmation and support of the direction we are taking.

"Therefore, answering this obvious question on preclinical species translatability raised by the data is of interest to the scientific community….The problem as stated is important, but the preliminary data and addition of a new variable (model cell culture system with different drug metabolizing capability) make the 'next steps' difficult to justify as proposed." Page 14

The pivotal goal of the current project is to test the hypothesis that hepatic transcription profiles could be used to predict age- and/or sex-related differences in drug or chemical disposition and downstream adverse events. As a first step, the approach is to first test this hypothesis in an *in vitro* rat model system. The use of an *in vitro* rat hepatocyte model, with its caveats, is driven by the practical issue of testing several drugs. We admit that this has its limitations.

We are aware of the literature documenting sex differences in human metabolism. As for translating our results to the clinical situation, if our central hypothesis is supported by our work, our plan is to next mine the growing human gene expression data in a parallel fashion.

OBESITY AND DRUG TREATMENT

"There was a uniform support and enthusiasm from the committee for this project."
The review team raised the question of how the current obesity model compares with other obesity models, and how different models may play into the research program. The team also felt that several organ systems should be considered in their response in an obesity model. The team also suggested that "drugs with narrow therapeutic safety or efficacy ranges may be studied, because even small variation in drug metabolism and pharmacokinetics may have larger clinical impacts in these cases." Page 8

The Division appreciates the Subcommittee's support for this effort and the clear encouragement. We also appreciate their insight in raising important questions impacting these studies. Suffice it to say that with this area of research is so relatively new that many of these questions remain to be addressed, and all have real impact. The choice of an obesity model is critical, but one must start somewhere. Likewise, the choice of pharmaceutical intervention to initially examine is challenging; in our case, we chose to key off a model that the Division has experience with and hence has historical comparators. Examining effects in tissues other than heart is indeed important and we will take the Subcommittee's advice seriously. Needless to say, the breadth of work that should be considered (different obesity models, other drugs) extends beyond the study described. This is a truly important field in what is truly personalized/precision medicine, and the review team points to a long-term research effort we hope to pursue.

"The review team feels that the Doxorubicin approach seems to be force fit to align with other DSB activities. The questions Dr Varma is looking to answer do not require insult with a model toxin. Multiple animal models of obesity are available, and Dr. Varma did mention several of these in her slides. Furthermore, focusing on chronically administered drugs, such as those to treat metabolic syndrome, may be a better group of agents to pursue in testing the hypothesis and producing results that would impact obese, high-risk healthcare patients." Page 15

Indeed, our intent was to use our historical experience with the doxorubicin model to ground the study of doxorubicin effects in obese mice. There is indeed evidence for effects of obesity on doxorubicin toxicity in the clinic. There are many questions to ask, and many models to consider, but as we have noted above, one must chose a starting point and in this case, we keyed off our prior studies. Our collaborations with several CDER laboratories

give us the opportunity to expand this work to address other questions (e.g., pharmacokinetics, immune function) that might be altered in obese models.

Summary

The Division and its Director owes the Subcommittee review team a debt of gratitude for the effort they put into our review and the insight and suggestions they have shared. We truly appreciate their and are confident that incorporating their thoughts into our work will help our research better impact the field of science and the FDA's mission.

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