NCTR Response to SAB Subcommittee Review of November 7-8, 2017

We thank the Subcommittee for their careful review of and suggestions for our nanotoxicology, bioanalytical, and bioimaging capabilities. We thank the chair and co-chair (Drs. Michael Aschner and Steven Stice), Drs. Gregory Lanza, Susan Felter, John Michael Sauer, and Richard Corley for their expert analysis and suggestions. We also want to thank our colleagues from the other FDA Centers for their participation. The Subcommittee suggested that Dr. Slikker focus on areas that need improvement, reassess specific projects, and carefully consider new research areas. We take this advice seriously and will continue to focus on FDA programmatic needs, moving FDA forward, and performance-based activities.

There were several themes throughout the meeting including 1) cross-training and establishing a core imaging facility 2) adding to existing staff, and 3) outreach to available partners in other Federal agencies and in academia.

1. **Cross Training and Core Imaging Facility:** Training someone in electron microscopy, mass spectrometry, MRI, etc. would be daunting. It similarly would be difficult for someone to be proficient in every aspect of imaging because of the many different technologies. However, it may be possible to train someone in the common, overlapping areas of image analysis using computational tools through collaborations with academic and government partners — we will explore options. We discussed the possibility of collaborating with Advanced Biomedical Computing Center (ABCC) at the Frederick National Laboratory for Cancer Research (FNLCR). They have advanced knowledge and work with consortia to develop tools for image analysis. We are making progress in the infrastructure needed to support an image repository and software. More details are below in the "Image Analysis, Modeling, and Computational Analytics. "

2. **Adding to existing staff.** We acknowledge there are three areas that could benefit from additional staff.
    a. Individuals with expertise in the technology that can produce the data and keep the machines running. Experts trained in these areas can be difficult to recruit but we will continue to pursue.
    b. Postdocs to work on specific projects. Some postdoc positions are currently open and we need to find well-suited recruits.
    c. Individuals that work on the data storage, etc. We feel there are sufficient staff members in this area, but we may need to build additional infrastructure and provide additional training.

3. **Outreach:** We have ongoing collaborations with multiple groups and are reaching out to others at FDA, other federal agencies and academia.

Nanotechnology and Nanomaterial Assessments

Thank you for the wonderful assessment of our facility and the leadership by Dr. Anil Patri. Common themes in the NCTR Subcommittee Review evaluation are 1) the NCTR budget shortfall for the foreseeable future and 2) the expected impact this will have on manpower along with the downstream

effect on workflow through the NCTR/ORA Nanotechnology Core Facility (NanoCore). These are legitimate concerns.

Ideally, recognized experts in these nanotechnologies can be hired and maintained to provide core support on:

- NCTR research projects
- Projects from other Centers
- Externally funded research, such as through the National Toxicology Program (NTP).

The transient nature of postdoctoral researchers that are recruited for a specific project requires that we have several permanent principle investigators on staff to retain knowledge within the Nanocore. In addition, the emerging challenges in regulatory science with respect to innovative approaches to treat disease using nanotechnology and other emerging technologies requires an advanced understanding of the material through characterization, and their biological response. This will allow FDA to be better prepared to address regulatory issues and requires recruitment of permanent staff that are agile and flexible to the emerging needs. This is an opportunity for NCTR to expand recruitment in these emerging regulatory areas to maintain a world-class program.

Dr. Anil Patri is very active in both national and international coordination efforts in nanotechnology. His active participation in the following meetings and conferences allow him to ascertain the regulatory-science research needs in emerging technologies. The knowledge Dr. Patri gains from these interactions has allowed him to direct the Nanocore research to include areas that can have a significant impact for FDA. He participates in:

- National Nanotechnology Initiative interagency monthly meetings
- International regulators meetings
- Standards meetings
- International nanotechnology conferences

The projects described below are examples of the work being performed in the NanoCore. These also have potential to attract external funding through which the NanoCore may be able to recruit permanent staff to supplement NCTR's budget for Nanocore.

1) International Documentary Standards development through a) review of standards that come through the ISO/ASTM process and b) propose/co-develop new international standards relevant to FDA from a prioritized list of cross-center Nanotechnology Standards Sub-Committee of the Nanotechnology Task Force at FDA. Already one standard on Cryo-TEM of liposomes has been adopted as an "ASTM E56 Standard" that is available for public use. There are seven additional standards that Nanocore proposed and are working on, with support from NTP, that will eventually become standard guides and practices.
2) The NanoCore is in the process of establishing expertise in the immunotoxicology area. Specifically, emerging translational research in immunotherapies alone, and in combination with nanomaterial. This research effort will facilitate filling some of the knowledge gaps that exist in this area of regulatory science.
3) The NanoCore is conducting a study in collaboration with and support from the Center for Drug Evaluation and Research (CDER) that is comparing existing generic drugs containing nanomaterial. To ascertain the validity of clinical data in the public domain that points to

efficacy differences between these products, the NanoCore is conducting extensive advanced characterization in addition to *in vitro* and *in vivo* studies.

4) The NanoCore is also interested in pursuing interagency efforts on a significant emerging challenge and knowledge gap with exposure and safety of micro- and nano-plastics in food and water.

We appreciate the comments from the sub-committee that Quality-by-Design (QbD) must be emphasized when supporting the reviews of sponsor applications. We agree with the comments: "NCTR should lead on understanding the key product variability issues to support reviewers with less nanotechnology experience. Their informed evaluation of submissions will lead to meaningful review and suggestions rather than make-work questions that reflect a lack of knowledge in the field. The FDA is positioned through the NCTR to know more about nanotechnology than most of the sponsors and in that way, be helpful in guiding these efforts efficiently to successful conclusion – partners versus adversaries." Currently the Nanocore conducts hands-on nanotechnology training for review staff from different product Centers, in collaboration with scientists from other regulatory Centers of FDA, to translate the lessons learned from internal and external research in public domain, so that the review process is further improved. This is a continuous learning process as novel products come through submissions through innovative science and advanced technologies with new instrumentation, posing challenges. The Nanocore concentrates on the reality-based regulatory science research on critical quality attributes (CQA) of nanotechnology products. We agree that slight changes in the physico-chemical attributes of nanotechnology products can have significant impact on unexpected biological outcome – safety and efficacy of these products. With advanced, additive, and continuous manufacturing already in the industrial domain with submissions to FDA, we must constantly update our knowledge in this field. Addition of permanent, well-qualified staff would facilitate regulatory science research to keep up with these demands.

We thank the review committee for providing excellent feedback on emerging areas that we should consider and agree with the assessment that "NCTR is not prepared to deal with the breadth of development likely to come forward in the next 5 years in a timely fashion" in Nanotechnology. We noted that Nanomedicine based vaccine development and polymeric, or non-liposomal based lipids for immunotherapy are two areas with great potential in the next 5 years, where "particle design, epitope type and presentation motifs, including antigen surface density and many features are critical and must be reproducible developed". We also noted that "since these agents typically require repeat injection, typically at high doses, the body's response both in terms of acute complement activation, adaptive immunity, clearance, organ impacts secondary to particle vascular entrapment versus MPS clearance will need to be evaluated."

In general, external collaborations with experts in the field is a key to leverage existing resources, as we will not be able to support all the emerging areas that are important for FDA in a timely fashion. We currently have several proposals in pipeline in collaboration with other Centers at FDA and one currently approved protocol to develop targeted nanomaterial where we are varying targeting ligand density on the particles, along with extensive controls, to investigate how these variations will influence *in vitro* cell-binding, uptake, and radiation therapy response. We made significant progress on this project

through developing methods to investigate heterogeneity, inhomogeneity, impurity analysis, optimization of methods, and started conducting *in vitro* studies. We hope that the research outcome from this project can be utilized in training our reviewers on new targeting approaches that are being submitted through investigational new drug submission (IND) process. We are also going to start a new project in collaboration with CDER on how shape changes will influence complement activation on liposome-based drugs and proceed with *in vivo* studies for biodistribution changes due to aspect ratio change of these nanomaterials. This will nicely fit with the priority area of complement activation standards development identified by the Subcommittee.

We appreciation the comments "perhaps the most impressive virtue of the nanotechnology research program at NCTR is its strength to tackle virtually any chemistry-biochemistry characterization project in drug delivery beyond nanotoxicology. Sans the serious issue with adequate personnel, Dr. Patri himself brings extensive synthetic and analytical chemistry expertise to NCTR allowing him to lead programs that complement any of the center needs. The Nanotechnology program has most of the key equipment in place to tackle diverse questions in the drug delivery in the generics, biological, small molecule, and with the correct further support, nucleic acid based system space. Their strength goes well beyond liposome/emulsion drug delivery. "We currently recruited one excellent chemist from one of the pioneering groups of Nanotechnology to a staff fellow position, through support from NTP, to augment the efforts in structure activity relationships on nanomaterial towards standards development. At the agency level, through the Nanotechnology Task Force, we prioritize developing consensus standards through stakeholder involvement is key to help industry and FDA as we venture into regulating novel emerging products that involve nanotechnology.

Generic biologics are strictly considered as 'biologics' at FDA and are not considered as nanotechnology products, even though they may fall in the nano-size range. However, as pointed out, the tools we have for nanomaterial assessment can be applied to understand product variations as new generics that enable affordability of medicines are approved. The Nanocore has recently acquired a low-voltage electron microscope, the first of its kind anywhere, to investigate soft nanomaterial and biologics in their native state without staining. We are slowly, but steadily, developing the utility of this technology to investigate areas that have not been investigated before.

We appreciate the comments from the sub-committee in the summary and agree that the NanoCore under Dr. Patri has made great strides yet suffers from a lack of technical staff. As suggested, we will work to resolve this by …" contracts and collaborations with external experts…" while remaining …" cognizant of technologies trending toward the clinic in emerging sectors of this diverse field to anticipate and prepare reviewers based on credible research experience." As part of this we will take your advice and use the seminar series and pilot funds from NCTR to strength our work and enhance collaborations within and outside the FDA.

We will take action on recruiting permanent staff, expand our collaborations to strengthen the science at NCTR nanocore, prioritize emerging challenges that we can address in the coming 5 years to focus on major challenges in regulatory science.

Electron Microscopy

With regards to electron microscopy (EM), the Subcommittee concluded that the low number of staff members and the transient nature of postdoctoral personnel do not support the start of an EM Core while also maintaining the NanoCore. The EM core needs permanent staff, but we do not have the funds to fill positions. The Subcommittee suggested an Imaging-Processing Core Facility be created, but this can be difficult for EM. The imaging data are very challenging and labs that have been successful in this area (e.g., NCI laboratory in Bethesda, the MRC in Europe, and NCMI in Houston) have been successful because they maintained two sets of scientists that worked together in their labs — one group dedicated to collecting the best image data possible and the other group devoted to developing the necessary software to process those data. Due to the existing challenges in data analysis, and the inability to recruit full-fledged staff to support EM work, we discussed the possibility of collaborating with the Advanced Biomedical Computing Center at the Frederick National Laboratory for Cancer Research, where existing researchers have tremendous experience in the field.

EM data are, by nature of the physics of the electron microscope, noisy and flawed. There are:

- phase flips that switch densities from black to white and from white to black at specific frequencies
- missing frequencies due to contrast transfer function where no structural data exists
- spherical aberrations that distort the images
- chromatic aberrations that impact resolution, etc.

These labs had to understand and model these defects to develop the necessary software to accurately process that data to high resolution. At NCTR, we have EM and other imaging techniques. Turnkey software like NIH ImageJ is usable but limited with EM. Spider, Imagic, EMAN1, EMAN2 were all software packages developed specifically to process EM data from high resolution and well-funded EM facilities.

Imaging Assessment

We appreciate the support of the Subcommittee in the current MALDI mass spectrometry and MRI imaging endeavors and their encouragement in continuing to further develop the technologies into Centers of Excellence. The Subcommittee encouraged the imaging groups to conduct cross-Center and cross-FDA collaborations to meet future FDA regulatory needs. The Subcommittee recommended that collaborations are necessary to develop appropriate analysis tools for all imaging modalities. These collaborations would also provide molecular probes for PET due to lack of a cyclotron or local producer for short-lived isotopes. The review of the imaging capabilities highlighted some of the challenges, which are addressed below.

1. The Subcommittee particularly noted that efforts should be made to translate the T2 MR imaging from animal models to humans. To that point, we are currently preparing the formal submission of the Letter of Intent to Biomarker Qualification Program at CDER for consideration of T2 MRI as a possible qualified biomarker of neurotoxicity in preclinical context of use. This will provide the necessary guidance from FDA on how to continue the program and will be a stepping stone in bringing this methodology to drug makers and possible clinical translation.

2. The Subcommittee praised the work that has come out of the MRI facility especially with the limited number of staff. The MRI group has added an animal handling specialist and is working to hire senior staff with radio-frequency electronic and pulse-sequence design expertise. As noted above, leveraging collaborations within and outside NCTR/FDA is also an important strategy for the MRI group. It is also agreed that MRI applications need to be expanded into non-brain areas as suggested by the Subcommittee. In fact, during the last year, two papers from the MRI group were published that described liver and heart-valve work.

3. Image processing and analysis was mentioned by the Subcommittee as "a significant challenge to increasing the impact of imaging at NCTR" and specifically the need for image co-registration capabilities between platforms was emphasized. We agree that such ability to co-register spectra or images from different modalities would make it possible to better assess and understand adverse responses or disease pathways. Additional computing power and storage will be needed to fully recognize the capabilities of the imaging technologies — especially with the recent purchase of a Bruker ScimaX that couples MALDI MS to a Fourier transform ion cyclotron resonance (FT-ICR) mass analyzer for high resolution analyses.

Image Analysis, Modeling and Computational Analytics

Thank you for the nice comments about our pharmacokinetics/pharmacokinetic modeling and of Dr. Fisher specifically. We agree that there is a lack of strategic thinking about Modeling & Simulation (M&S) at NCTR beyond individual projects or 'stovepipe' approaches to projects.

We appreciate the recommendations for improvements to the repertoire of imaging tools, management, and storage. Throughout the review, the Subcommittee makes recurring suggestions that NCTR address the image analysis and storage needs common among the technologies in use (review pp 4 thru 8).

We agree these are pressing issues and continue to work with the research laboratories to determine current and projected storage requirements for NCTR. Further, as cloud-based storage becomes available to FDA, the FDA's Scientific Computing Branch will perform an alternatives analysis to assess the cost, accessibility, and performance of cloud storage versus onsite storage.

In addition to the raw storage space, we are investigating image repository and management software (e.g., OMERO) and analysis applications that are image type and platform agnostic. We understand there are no 'fit-for-all' solutions; however, high-level tools will provide a foundation on which to build. We also note it is essential to marshal our resources as soon as possible as each additional terabyte of data (imaging or otherwise) intensifies the issue.