NCTR Subcommittee Review

Subcommittee Site Visit Review, November 7-8, 2017

Finalized: February 6, 2018

Executive Summary

A Subcommittee of the NCTR's Science Advisory Board was tasked with reviewing some cross-cutting science being performed at this FDA Center. The areas under review included the Nanotechnology Core facility, bioimaging capabilities and bioanalytical imaging (imaging, large databases/informatics, and modeling). There was uniform agreement on the strengths and weaknesses of NCTR's capabilities. These provide valuable resources to the FDA. As configured, this effort provides a unique interdisciplinary approach to regulatory issues confronted by the FDA, its matrix is unique, and it is strongly recommended that this unique resource be preserved and supported in future years. Strengths include a collaborative relationship among scientists within NCTR, strong leadership, interdisciplinary programs, general responsiveness to the needs of the FDA, and focal areas of high relevance. There were clear differences in the quality of research being done at the individual laboratory level. The NCTR Director is encouraged to reassess specific projects and help frame more fundamentally sound research efforts in those areas that were viewed by the panel as needing improvements. Developing new research areas should be carefully considered to meet with expertise already inherent to the NCTR that is also congruent with FDA's mission. Exceling in multiple research domains with limited personnel and shrinking budgets seems unrealistic; accordingly, the panel strongly encourages the NCTR leadership to continue and strengthen existing research areas, and incorporating important emerging technologies and sciences as appropriate.

Overview

Review Subcommittee and Expertise

The names and affiliation of the site visit reviewers are provided in Appendix 1. Three members (Drs. Aschner, Stice and Lanza) of the NCTR Scientific Science Advisory Board (SAB) participated in the review. Drs. Aschner and Stice served as subcommittee chair and co-chair, respectively. All other reviewers participated as subject matter experts in content areas of interest to the Division of Bioinformatics and Biostatistics (DBB, referred to hereafter as "the Division"). The subcommittee members received a written overview of their charge in a memorandum dated May 23 from Daniel Acosta, Jr., Ph.D., NCTR Deputy Director for Research and Donna L. Mendrick, Ph.D., Associate Director for Regulatory Activities. The charge memo is attached in Appendix 2. Site visit reviewers were provided with project overviews divided into thematic areas on October 15, 2017 and at the site visit and a slightly revised version of these materials on October 31, 2017.

NCTR SAB and subcommittee present for the Presentations on Analytics

Name	Primary reviewer	Secondary reviewer
Steven Stice - SAB co-chair Sub committee	November 7th and 8th	
Michael Aschner– SAB co-chair Sub committee	November 7th and 8th	
Susan Felter – SAB		November 7-8th
John Michael Sauer – SAB		November 7- 8th
Greg Lanza – SAB	November 7th	
Richard Corley – SME Sub committee	November 7th and 8th	

Patrick McConville – SME Sub committee	November 7th and 8th	

The Subcommittees charge was the following (May 23 Letter)
1). How best to organize these activities for NCTR and the rest of the FDA?
2). What expertise are we missing? What physical plant changes do we need?
3). What might be on the horizon in these areas that we should explore?

1) How best to organize these activities for NCTR and the rest of the FDA?

Current Analytic organizational structure.
The units and groups we reviewed have developed organically as needs arose from internal and external projects. These projects are thoroughly reviewed and vetted both internally and externally. The NCTR Director and top-level staff are tireless in their efforts to provide the FDA with the most up to date analytical processes (equipment and facilities and expertise (staff) to address demands). Since much of the research and service are in response to approved projects it is difficult to conduct long term strategic planning which inhibits organizational structure. The result is that when short staffed in particular areas the projects are slowed or can't be initiated. Much of the equipment is state-of-the-art, but often staff resource limitation hampers projects. In particular, staff with image analysis expertise and experience are the critical resource, and there is a shortage of these individuals at NCTR.

Organizational recommendations:
NCTR should identify current resources and existing staff to implement a team of mission critical expertise, likely image analysts with domain knowledge who can imbed in or interact with multiple labs throughout NCTR to facilitate the productive use of the instrumentation resources. We believe that the first task is to determine the mission critical activities not based on history but rather a formal survey of other FDA centers and then determine which new hires need to be made to address these needs. The organizational structure should be team oriented with cross over expertise (Nanotechnology to Mass Spectrometry). The team of analyst members should:
- have a common flat organizational structure where they report
- facilitate knowledge sharing, pooling of resources
- have very direct connections to the teams/labs requiring their knowledge, technologies, software and services
- reduce the opportunity for silos: e.g., have internal transparency and awareness of project demand across the organization, and have enough cross-training such that the analyst team can be deployed efficiently/back each other up in specific project work

Given current budget issues, it is unlikely that a significant number of new permanent hires will be made. It is then imperative that opportunities for joint postdocs and students be initiated. Joint postdocs will provide an opportunity for more communication and knowledge capabilities across FDA centers and divisions within NCTR.

In several instances, it also appeared that resources outside of the NCTR that are amenable to ongoing in-house research were not utilized and explored. Consideration should be also given to the need for a go-to inventory of available partners, both Federal and in Academia. Finally, a silo structure should be considered as inhibitory to collaboration, and leadership should facilitate interaction between cores, bridging the perceived vertical silos.

To address the needs for more staff and reduce the impact of loss of key personnel on projects, we suggest that joint postdocs might provide better continuity through cross training and greater career opportunities in NCTR. Currently, NCTR invests training, expertise and a knowledge base in single post docs that have one focused project(s). This is acceptable when post docs do not move onto new positions prior to the project ending. This is rarely the case. We suggest and anticipate that more cross training/responsibilities through teams, if initiated, might reduce the effect of losing individual post docs and potentially greater job satisfaction. Joint post docs within NCTR but also among other Centers should be contemplated. These activities, if implemented, may lessen the significance of setbacks in any one project. Revolving postdocs is an issue in academic institutions as well and has been addressed in part by establishing new career tracks such as Research Scientists who have greater responsibilities and freedom, but may not be considered parament faculty or employees. These career tracks, if they can be established at NTCR, may lead to higher retention of "promoted" excellent post docs for additional periods of time.

2) Review and recommend changes to core areas of expertise and physical plant

Nanotechnology core expertise and capabilities.

The NCTR Nanotechnology program is among the elite in the Nation if not the world under the leadership of Dr. Anil Patri. Dr. Patri has both hands experience developing and characterizing dendrimeric nanotechnologies that were ultimately brought to the clinic, but also many years of experience beginning with the founding of the NIH NCI Nanotechnology Characterization Laboratory (NCL) until his recruitment to NCTR. At NCL Dr. Patri was heavily involved with organizing significant portions of the NCL as well as developing or leading the development of many of the current analytical procedures used in that program. He was instrumental in collaborating with NIST and guiding the development of procedural and reference standards. At the NCL, Dr. Patri interacted with the FDA, the various institutes within the NIH, the National Nanotechnology Initiative, and their foreign counterparts. At least 100 nanotechnology concepts were evaluated by NCL during his tenure and many received his personal attention in their evaluations and final reports. Importantly, these early nanomedicine forefront technologies and had many and varied flaws that provided a consolidated understanding into the problems and limitations of nanotechnology investigations and the issues surrounding their design, synthesis, characterization and testing. The NCTR under Dr. Patri's guidance builds upon this wealth of experience.

The NCTR nanotechnology core clearly has the equipment and know-how to address most issues that are currently on the FDA review horizon, which represent mostly old technologies being reworked into new applications. The laboratory is equipped with the most vital instrumentation available for its charter, including field-flow fractionation, FLPC, numerous types of Mass-Spectroscopy, dynamic particle size analysis, outstanding electron microscopy including the standard transmission and scanning electron microscopy (SEM), cryo-electron microscopy, and Low-voltage SEM, and 3D SEM. While the center has been very productive, it is handicapped by turnover of key personnel; often hired as transient post-docs on focused projects as mentioned above. As a consequence, sustained productivity and importantly institutional memory are suffering in a "two steps forward – one step back" fashion.

The entire FDA benefits from the NCTR Nanocore and will continue to do so. There is little doubt that FDA reviewer issues relating to nanotechnology products or products incorporating nanotechnologies, whether food or medically oriented, will increasingly depend on NCTR for training, consultation, independent testing and evaluation. Although nanotechnologies can be viewed as just another drug, in many respects, the physical nature of these multicomponent

concepts and their breakdown exceeds the complexity of prior pharmaceuticals. Of particular concern are those particles greater than the 8nm renal clearance threshold, which incorporate heavy metals or solids and are non-degradable. (Note: rodents eliminate large particles (at 300nm) through the biliary system, which does not occur in humans, creating model translation issues.) The slow acceptance of nanotechnology in the pharmaceutical companies is related to the risk imposed by the complexity and challenges in developing and demonstrating GMP scalable, stable, and reproducible products versus simpler products with less ambitious CMC development pathways.

Imaging Assessment

MRI (with PET/CT), electron microscopy, and imaging mass spectrometry are cutting edge imaging technologies at the NCTR that should be developed into centers of excellence.

Other points for Bioimaging (MRI, PET/CT);

- Current focus is on neuroimaging and neurotoxicity and the imaging platforms are appropriate. In fact, the T2 MR imaging project is so well-developed that efforts should be expended to complete the project and move into translating the approach from animal models to humans.
- Development of new or novel imaging biomarkers of neurotoxicity is a more challenging endeavor, just as it is with any biomarker discovery program, especially with regard to biomarker selectivity and specificity for early, diagnostic adverse responses. Without a cyclotron or local producer for short-lived isotopes, PET/CT applications for biomarker discovery/evaluation will continue to be challenging. External collaborations may be necessary.
- To improve impact, the imaging team should be viewed as a critical core capability directly connected with multiple specific NCTR project that is focused upon a key adverse response or disease pathway involving multiple experimental capabilities and not just an island of research contained within the imaging group. There is simply too much for the limited personnel devoted to MR, PET/CT imaging to accomplish without such collaborations. These collaborations also have the benefit of broadening support for additional personnel and capabilities (e.g., image processing and analysis).
  A common theme clearly emerged as a significant challenge to increasing the impact of imaging at NCTR—image processing and image analysis. This was true for all imaging platforms presented (EM, MRI, PET/CT, IMS). There are no fit-for-all or turn-key applications that are capable of addressing the needs for all application areas. However, there are several tools that could form the basis for an NCTR core capability in image analysis. If each project or research group that benefits from imaging could contribute partial funding to staffing this core with the appropriate expertise (typically drawn from mathematics, physics, engineering (bioengineers, computer/software engineers)), this core group could be initially developed and supplemented with students and post docs with minimal initial impact to the number of full time equivalents (FTEs) at NCTR. Even with this core, it must be recognized that each platform or even each project will likely require adaptation of existing tools or development of new tools to achieve project goals. Creating the right balance of FTEs and limited-term employee (LTEs) would require strategic discussions at NCTR across groups and projects to prioritize those capabilities that are needed first, or are close to being useful and need only minor support to have impact, or have longer-term potential for project impact by improving efficiencies in data (imaging) analysis that is currently a drain on project budgets. It was not clear whether project PIs, investigators or groups at NCTR have already begun these levels of strategic discussion, especially within the context that foreseeable future budgets will limit the numbers of new hires of FTEs without sacrificing other areas of current need.

Such discussions are outside the domain of this subcommittee but are recommended for NCTR to consider.

- In addition to needing core capabilities in platform-specific image analysis, co-registration capabilities will also be needed (e.g., across platforms such as PET with CT, or IMS with Histology/CT or MRI, or repeated imaging over time).
- Clearly, MRI and to some degree PET/CT are recognized areas of strength at NCTR. These imaging platforms provide critical information on 3D/4D organ structure and function that cannot be obtained by any other means. These capabilities are also invaluable resources for translating animal research to human clinical applications in support of FDA's mission areas. As such, the future need for expertise in MRI and PET/CT at NCTR will be a critical resource for multiple FDA Centers. While neurotoxic applications have understandably been a major emphasis for imaging applications, other organ systems will need to be expanded or incorporated to meet the needs for future regulatory science at FDA. Each organ system requires its own additional expertise and potentially unique needs for image analysis tools. Collaborations within and outside of NCTR will be needed to expand into these new research areas.

PET/CT is important, but as a "massless probe" the tracer health risk is typically low, and from a regulatory perspective, applications may be less in demand for issues such as:

- confirming proposed PET/CT biomarkers.
- Investigating whole body biodistribution, clearance and off target accumulation for labeled biologics (oligos to Abs), therapeutic cells and nanoparticles. This may be particularly important in immunotherapies that are most commonly involving biologics or therapeutic cells. In vivo/translational imaging (in particular PET) presents one of the only robust in vivo methods for tracking biologics and nanoparticles that are intended for therapeutic purposes and that can be used in human patients.

[Note also that Cherenkov radiation drive by the use of 18F is a promising theranostics approach that is gaining interest. This technology is often associated with the use of metals, such as Titanium, to generate free radicals in the presence of the radioisotope. What may be a concern in this arena is that the dose of 18F required may be well beyond the imaging dose and could border on toxicity ranges. This topic wasn't discussed but the literature has many high impact journal references and the topic is being presented in plenaries and keynote speeches worldwide.]

The electron microscopy (EM) core is an extremely impressive facility with multiple state of the art instruments. Applications for NCTR and broad and many. The major challenge for this facility is (as referenced above) on the data processing, analysis and reporting and related speed, efficiency and turnaround times. Perhaps more than any other lab, the EM facility is in need, and would benefit from a shared analytical resource and/or implementation of multi-purpose image storage, and analysis technology and software.

From the MRI perspective, the NCTR instrumentation is outstanding and the results to date have been excellent, frankly more than could be expected from the staff number available. This productivity reflects a high level of dedication that will be hard to sustain for years without frustration setting in leading to the further loss of key employees. Careful attention must be applied to ensuring the most efficient and optimal utilization of this resource – e.g., through careful/creative staffing and use of platform technologies and software that drive efficiencies and enable more to be obtained from collected data, with less time spent. As an example, Dr. Lanza's (Subcommittee member) laboratory at Washington University (WU) manages to

maintain under severe financial stress imposed by the NIH granting system 1) a part-time RF MSEE engineer designing and building specialized coils, amplifiers, and integrated scanner interfaces, 2) a BME PhD and MSBME engineers capable of scanning and designing pulse sequences for MR equipment from 1.5-11.7T field strengths and more importantly developing semi-automatic objective image post-processing programs to address each different experimental problem we study, and 3) a part-time (previously full-time) clinical MR tech familiar with multiple clinical imaging systems as well as now being trained in high field imaging on animal scanners. His supporting group includes an animal surgeon, who maintains all of the animal protocols and performs most of the smaller animal procedures, and a full morphology lab that performs histology and immunohistochemistry strictly tailored to match questions relating to the MRI (and other studies) being performed in his program. WU maintains the instrumentation and provides ancillary help through their small animal core for a fee. The importance of more permanent/retained staff in such a facility cannot be underestimated, and equally important the cross training of such individuals to be able to operate and handle instrumentation and image data across modalities (e.g., MRI, PET and CT).

The PI of this analytical function needs time to interact with other investigators within and beyond the NCTR environment, communicate in written and oral forms internally and beyond, and importantly build an understanding and collaborations beyond NCTR. There is too much to do and the best may be to be loosely involved through collaborations at some academic sites on emerging topics of potential regulatory importance and to focus on the hardcore regulatory science issues with limited staff at and support at NCTR. What these hardcore questions are beyond the gadolinium and TBI issues, need to be fleshed out better.

On the EM side, the instrumentation is again outstanding and all of the right systems, but the staffing too low and transient to build and grow the program to a support ready status for FDA. Beyond the inherent bureaucratic challenges required for approving and initiating a project, the current structure of the NCTR Nanotechnology core essentially requires halting one program to do another. There is simply no bandwidth and the expertise developed in post-docs is lost after a year or two. There is an institutional brain drain that must be ended, at least for areas projected to be centers of excellence long-term.

Further complicating this particular program is the need for advanced image processing that is atypical of what is used in most labs but which does and has existed in other fields, such as MRI. In MRI techniques to accelerate imaging by compression and reconstruction of images from sparse data sets are now well developed. Similarly, issues regarding motion correction and 3D elastic registration are also highly developed in some of these circles. The technical expertise for these techniques is typically invested in mathematicians and physicists with extensive experience in numerical methods and imaging reconstruction from sparse data using parallel and numerous GPUs. The NCTR cannot hire this expertise permanently but through connections obtainable through other national laboratories and universities that have worked on these or related problems, a connection could be made. Universities' math departments working in conjunction with imaging equipment manufacturers, should be able to address some of the high terabyte image processing projects. Thought should also be given toward image storage, project management and image analysis platforms that are seeing broad and heavy use in industry and academia and are creating great efficiencies in multi-instrument/multi-modality imaging labs. For a relatively small expense, systems can be implanted to enable highly efficient access, analysis, archiving/backup and reporting of image data (the very things that tend to be the larger bottlenecks and more poorly addressed issue in today's state of the art imaging centers). Since the data itself are not specifically patient or sponsor related, much could be archived in the cloud for a price and smaller compressed subsets of the dataset used for routine

analysis at NCTR. Clearly, this is another potential center of excellence for NCTR within the FDA and a decision is required internally to develop and support the program or allow it to be hobbled in its current state.

Organs have highly developed spatial organization critical to their specific cellular and molecular functions and ignoring such structure in toxicity, disease, and therapeutic interactions can be erroneous at best or completely misleading at worst. Imaging Mass Spectrometry (IMS) has emerged as a powerful complementary tool to standard medical imaging modalities (MRI, CT, PET/SPECT, etc.) by providing critical 2D and 3D structural information on organ and cellular-level distribution of specific molecules including drugs or drug metabolites as well as endogenous macromolecules (proteins, metabolites, lipids) that are important to understanding toxicity and disease. Instrumentation for IMS, specifically MALDI-based platforms at NCTR is outstanding. Dr. Schnackenberg provided several collaborative examples where MALDI IMS has provided value to understanding to drug distribution and toxicity in animal models as complement to histopathology and MRI. Examples were also provided where IMS was used to study endogenous metabolites and lipids in response to toxicity as well as future directions in obesity, biomarker discovery and FDA Center-specific applications. As with the other imaging capabilities at NCTR, IMS suffers from a lack of image analysis resources and expertise. Expanding external collaborations, working with other funding agencies like NIH to push for research focused upon the development of image analysis tools that move the entire medical imaging field forward, or participating in/contributing to an imaging analysis core capability at NCTR are potential ways to move forward within budgetary limitations.

Comments on the common issue of operating and maintaining cutting edge instrumentation that was a concern to most presenters:

- Maintenance agreements are expensive. This is a concern at all research laboratories—how much project specific funding vs. institutional support is expended to keep critical instruments functioning. Most institutions use a combination of approaches and NCTR will have to continually prioritize budgets for institutional support and advocate for resources from sponsors to maintain scientific leadership.
- Reliance solely on limited term employees like post-docs to maintain and operate instruments creates the inevitable loss of ‘corporate memory’ and can be disruptive when post-docs move on to full-time employment. Without support from FDA Centers that recognize the need for maintaining state-of-the-art science and instrumentation to accomplish their mission, there’s no easy solution other than to maintain some level of permanent staffing vs. LTE within the existing organizational culture and budgets. Lab management can be advocates for new resources, but strategic impact assessments would be required. This is not something our Subcommittee can help with.

<u>Nanomaterial Assessments</u>

Awareness of the NCTR about the materials being employed in nanotechnologies is well established, at least as they relate to those agents currently being presented or likely to be presented to the other FDA center in the near future. Characterization and stability of raw materials that comprise most of the nanotechnologies is not questioned, but there is a broad and highly varied array of how these parts can be synthesized into a whole. Everything must be done repeatedly and precisely, and small deviations can result in significant efficacy and safety problems. While on one hand highly uniform particles are desired, in practice polydispersed nanosystems may be more efficacious and more scalable. The issue becomes what are the critical variations that have significant impact in the final product.

From the NCTR Nanotechnology perspective, approaches to Quality-by-Design (QbD) must be emphasized when supporting the reviews of sponsor applications. In the Nanotechnology world, it is likely that changes in material sources and impurities, even water sources, equipment changes in process, processing environments, etc. can all lead to unexpected product changes. A more focused QbD effort in nanotechnology will help address inevitable issues in the scale-up and expansion of technologies to understand the where acceptable variability can be accepted and where tight precision must be maintained. Inevitably, small changes will need to be implemented to address process issues or unexpected biological responses that will hopefully not stall the sponsor's development effort. NCTR should lead on understanding the key product variability issues in order 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, being helpful in guiding these efforts efficiently to successful conclusion – partners versus adversaries.

Image Analysis, Modelling, and Computational Analytics

NCTR has a long history in pharmacokinetics and the development of physiologically based pharmacokinetic models, especially in developmental toxicology. With retirements over the past decade in this area of research, the addition of Dr. Jeffrey Fisher, a highly regarded researcher in PBPK modeling, will serve NCTR well in keeping pharmacokinetics current and moving forward as new technologies are developed to meet the needs of multiple FDA Centers. While many examples that Dr. Fisher presented consisted of the development of PBPK models during animal and human development, the movement towards incorporating new imaging technologies that provide spatial information on organ function and molecular locations is timely. It is not yet clear how well-integrated pharmacokinetic/pharmacodynamic (PK/PD) modeling is with other research programs at NCTR (vs. self-contained projects), but considering PK/PD components in most, if not all, future projects will enhance the value and translation of research with *in vitro* systems or animal models to humans.

Today, NCTR image analysis and modelling has demonstrated good productivity across a number of relevant/high priority projects. However, the use of this resource is highly project-specific, and potential exists to create a broader more far reaching and higher impact resource even with the existing technologies, software and personnel. As referenced above, it is recommended that careful thought be given to adjusting or redeploying these resources to create a more centralized core that is highly connected to each of the user labs (including the imaging labs importantly), but that is not siloed in any way, and can easily move across areas/support challenges across areas through commonality that exists in such challenges.

For example, data storage, reporting and image analysis have to a large extent, common issues regardless of modality, platform and application. These commonalities should be leveraged. In doing this, it is recommended that already available products (e.g., hardware and software) be assessed carefully – particularly those offered on a commercial scale with inherent supports – to avoid reinvention of the wheel, save time and ultimately save budget. Small up-front investment may be important in doing this. Systems that are as scalable and broadly applicable as possible should be focused on, and features including cloud storage, automated reporting/generation of image snapshots/movies, automated reporting of parameters/endpoints and integration with advanced image processing package(s), should be considered. It is recommended that existing, affordable products be assessed and considered before decision to internally build such systems (at much greater time and expense). Rapidly advancing new approaches including

machine learning and AI/deep analysis should be included in assessment and development of image analysis resources. External collaborations and increasing connections to other entities are likely to be key in advancing NCTR image analysis resources.

The structure for the group(s) involved in data analysis and image analysis should be such that they are directly and regularly connected to the labs that can benefit from their resource, and less siloed in overly project specific work, that while productive, is not as broad reaching and scalable across NCTR.

3) What are the new areas of emerging needs that NCTR should explore

A recent but important trend in nanomedicine has been their use for vaccine development both against pathogens but also to manipulate autoimmunity: examples include selective suppression of T-cell or B-cell immune responses or by inducing immune responses to key receptors, such as PCSK9 in cholesterol metabolism. Immunotherapy will expand in multiple forms, and typically are involving polymeric, nonliposomal lipid based, and genetically engineered viruses. (see Irvine, Chemical Reviews, DOI: 10.1021/acs.chemrev.5b00109 for a start). The potential for some of these areas is immense but the data already show that particle design, epitope type and presentation motifs, including antigen surface density and many features are critical and must be reproducible developed. 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. Also, although the antigen presented may be specific for the receptor at low Kd, unintended immune responses to the immunogen could occur as lower affinity clones are triggered because the antigen exposure is much higher. Also, genetic variability in susceptibility to these approaches will also need to be considered.

Additionally, as referenced above, labeling (in particular radiolabeling) of biologics, cells and nanoparticles may be a very critical technology to focus on in order to better understand biodistribution, off target accumulation, safety, clearance and residence times for the immmunotherapies that are rapidly becoming the standards for cancer treatment. Secondarily, biomarkers for detecting uncontrolled immune response to immunotherapies (e.g., "cytokine storm" after T cell therapies) that presents a safety issue may also be important for NCTR research – through a variety of detection/biomarker strategies.

Clearly, the potential questions presented to the nanotechnology and analytic lab are myriad and exceed the bandwidth of the program, particularly in manpower capacity and available expertise. A judicious effort to prioritize needs based on present strengths of NCTR's technology core must be undertaken and staffed to meet the current and the 5 yr. anticipated needs. Additional expertise in partnerships with other FDA related centers and labs must be sought and leveraged to address some of the emerging areas of development, currently not envisioned, that are presented by sponsors requesting pre-IND meetings or submitting applications. To reiterate on comment made by the CDRH representative, reviewers cannot be put in the position of not knowing how to respond in meetings with sponsors, and even the current excellence of the NCTR is not prepared to deal with the breadth of development likely to come forward in the next 5 years in a timely fashion.

The issue of macrophages phagocytosis was once considered a major barrier impairing nanotechnology success. This concept led to considerable research regarding the physicochemical properties that encouraged or slowed premature nanoparticle clearance.

While macrophage phagocytic system (MPS) clearance remains an issue, the more salient point has emerged that prolonged circulation to overcome extravascular diffusion barriers was ineffective. Ultimately, size and particle composition have come to the fore and the bottom line is to reach the targets rapidly and have excess particles and drugs cleared by the MPS. More recently, the importance of macrophages and their influence in inflammatory diseases, including cancer, atherosclerosis, arthritis, asthma, etc. has become recognized. In these contexts, the polarization of macrophages and the plasticity between M1 and M2 classes have gained increasing interest. Targeting of specific classes of macrophages is now proceeding as mechanisms to encourage tumor rejection, inhibit epithelial-mesenchymal transition, support distant metastasis, and so on. NCTR should consider expanding and enhancing the effort on macrophage phagocytosis and targeting to include better phenotypic characterization of the cells used with flow, and expanding studies to include different polarized fractions, than can be induced in culture. Aligned with is the interest in nitric oxide (NO) as a mediator of inflammatory effects. However, NO is now known to suppress local macrophage induced inflammation. The process involves increased macrophage autophagy flux that eventually leads to a decrease in NFκB and local cytokine liberation. This approach is now recognized in the context of direct neoendothelial targeted anti-angiogenesis which leads to apoptosis of the targeted cells with liberation of high levels of NO, leading to local macrophage down-regulation. New nanotechnologies are being designed and reported along this path of investigation. In conjunction with current experts in the roles of macrophages in these diseases, NCTR could consider updating the related standard protocols to these technologies.

Finally, it is encouraging that NCTR has completed the development of analytical methodology to evaluate nanoparticle acute complement activation in a standardized and versatile manner, which is topic of international regulatory interest, particularly given the variability and noise levels of current ELISA kits for complement. While substantial expertise in the analytical method exists in NCTR, more consultation support into complement regulation and activation/suppression by nanoparticles, which is highly complex, could lead to a key influence paper guiding sponsors on elements of nanoparticle designs that might lead to complement activation and design features, particularly size, that may circumvent these issues.

Perhaps the most impressive virtue of the nanotechnology research program at NCTR was the 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.

One particular area in the emergent biologicals space are exosomes, which are highly complex and heterogeneous entities when produced at lab scale and are likely to be even more variable when produced at commercial scale. A complex interplay of biologically acting components (lipids, proteins, and nucleic acid) interacting within heterogeneous vesicle mixture contribute to desired and the adverse events but there is very little evidenced based understanding about these systems and what elements are critical and what is inert. The Nanotechnology group has the equipment and skills to parse and characterize these particles. Relatively common molecular biology enhancements added from within NCTR or developed by the Nanotechnology program could address this growing unmet need and assist the Center of Biologics in this difficult challenge.

Generic biologicals, such as monoclonals and proteins, are the current new drug to enter the clinic, which may be another area for the Nanotechnology group to broaden support the biological division. These drugs are currently extraordinarily priced, which creates a demand for cheaper alternatives. The nanotechnology chemistry program could contribute assistance in the physico-chemical and bioanalytical characterization of these emergent technologies. The Nanotechnology groups has most of the key analytical and imaging tools available anywhere to bring to bear on these questions.

Home and send-out testing of genetic based and even metabolism monitoring systems will only become more prevalent in the next decade. The veracity and reproducibility of the systems, and their comparability need to be rigorously assessed and validated. False positive and false negative diagnoses due to sensitivity and specificity thresholds for relating to different chemical designs and components will require rigorous scrutiny. Nucleic acids, proteins, and the like are chemically speaking complex polymers. Multidisciplinary perspectives that the Nano group has to offer may circumvent barriers of bias and dogma that sway the fundamental assumptions of longstanding investigators within any field.

Ultimately, the direction for expanding the purview of the Nanotechnology program is dependent on the perceived unmet needs of the centers, but the Nanotechnology core program in place at NCTR can be highly effective and diversified in its contributions on the chemistry/biochemistry/imaging side of the ledger to any program requirement of the FDA.

4. Summary

The NCI Nanotoxicology core was received with very high appreciation for what has been achieved in terms of the development of the center, the hiring of Anil Patri as the successor to Paul Howard. The core staff is very capable but clearly under supported with technical staff. NCTR will need to judiciously hire new support permanent scientists to stabilize this program and to maintain institutional experience. Many of the problems and issues confronting the Nano core will be transient that may be resolved through contracts and collaborations with external experts. The NCTR Nanotechnology group needs to continue to be cognizant of technologies trending toward the clinic in emerging sectors of this diverse field in order to anticipate and prepare reviewers based on credible research experience.

Additional Potential Actionable Items:

- Use the seminar series to strengthen "areas of interest" with invitees from outside NCTR, particularly those at other federal agencies with overlapping and or complimentary expertise.

- Pilot funds from NCTR are essential for enhancing collaborations across campus and other institutions. Solicit ideas from NCTR scientists and use external reviewers for input and funding recommendations.