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8th Meeting of the Frederick National Laboratory Advisory Committee (FNLAC)
(Formerly NCI-Frederick Advisory Committee [NFAC])
February 3, 2015

Summary Minutes

Conference Room 10, C Wing, 6th Floor
Building 31
Bethesda, Maryland

National Cancer Institute
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The Frederick National Laboratory Advisory Committee (FNLAC) convened for its 8th meeting on 3 February 2015, at 31 Center Drive, Building 31, C Wing, Conference Room 10, 6th Floor, Bethesda, MD. The meeting was open to the public on Tuesday, 3 February 2015, from 8:30 a.m. to 3:54 p.m. The FNLAC Chairperson, Dr. Joe W. Gray, Gordon Moore Endowed Chair, Department of Biomedical Engineering, Director, OHSU Center for Spatial Systems Biomedicine, Oregon Health & Science University, presided.

FNLAC Members
Dr. Joe W. Gray (Chair)
Dr. Gail A. Bishop
Dr. David Botstein (absent)
Dr. Vicki L. Colvin (absent)
Dr. Levi A. Garraway
Dr. Robert L. Grossman
Dr. Beatrice H. Hahn (absent)
Dr. Elizabeth M. Jaffee (absent)
Dr. Alexandra L. Joyner
Dr. Lawrence J. Marnett (absent)
Dr. Jill P. Mesirov
Dr. Garry P. Nolan (absent)
Dr. Kenneth J. Pienta
Dr. Cheryl L. Willman

***Ex Officio* Members**
Dr. Stephen J. Chanock (absent)
Dr. James H. Doroshow
Dr. Paulette S. Gray
Dr. Douglas R. Lowy
Mr. Patrick McGarey
Dr. Alan S. Rabson (absent)
Dr. Craig W. Reynolds
Ms. Donna Siegle
Dr. Robert H. Wiltrout

Executive Secretary
Dr. Thomas M. Vollberg, Sr.

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I. OPENING REMARKS—DRS. JOE W. GRAY AND HAROLD E. VARMUS

Dr. Joseph W. Gray, Chair, called to order the 8th meeting of the FNLAC and welcomed the Committee members. Dr. Gray reminded members of the conflict-of-interest guidelines and confidentiality requirements. Members of the public were welcomed and invited to submit to Dr. Thomas M. Vollberg, Executive Secretary, in writing and within 10 days, any comments regarding items discussed during the meeting.

Dr. Harold E. Varmus, Director, National Cancer Institute (NCI), welcomed Committee members and other attendees. Dr. Varmus remarked on the effect that the concept of a National Laboratory has had at the NCI and expressed appreciation to the Committee for its influence in helping the NCI transform the Frederick National Laboratory for Cancer Research (FNLCR) under Leidos' leadership into a National Laboratory. He stated that the President's budget proposes a 3.2 percent increase for the National Institutes of Health (NIH) and includes $70 million (M) for a new Precision Medicine Initiative. Members were reminded about the challenges involved in making decisions about initiating new activities within a constrained budget circumstance.

Dr. Varmus noted that the FNLAC reviews two principal topics: (1) management of the National Laboratory by Leidos, including the RAS Project, which is overseen by the FNLAC's RAS *Ad Hoc* Working Group under the leadership of Dr. Levi Garraway, Dana-Farber Cancer Institute; and (2) expansion of or changes to the FNLCR's project pursuits. He reviewed the day's agenda, which included potential projects on cryo-electron microscopy (cryo-EM), in which other NIH Institutes may have interest; the validation of cancer targets of clinical interest; and possible expansion of the Nanotechnology Characterization Laboratory (NCL). Members were referred to a recent article by Drs. Varmus and Francis Collins, Director, NIH, on a new initiative on precision medicine (*NEJM*, 2015 Jan 30, Epub ahead of print), and were encouraged to suggest underrepresented disciplines or candidates to participate on the FNLAC.

In the discussion, the following point was made:

- Committee members were encouraged to look actively for and suggest new ideas for FNLCR projects.

II. FREDERICK NATIONAL LABORATORY FOR CANCER RESEARCH (FNLCR) OPERATIONAL UPDATE—DR. DAVID C. HEIMBROOK

Dr. David C. Heimbrook, Laboratory Director, FNLCR, provided an update on activities of the FNLCR, including assistance with the Ebola outbreak response, partnering initiatives, and national programs in the facility. Dr. Heimbrook also described Federally Funded Research and Development Centers (FFRDC) best practices that were being implemented in the FNLCR.

The FNLCR's support to the Ebola outbreak response has been primarily through the manufacture of a chimpanzee adenovirus Ebola vaccine drug product and support of vaccine clinical trials for the National Institute of Allergy and Infectious Diseases (NIAID). The Vaccine Clinical Materials Program completed the manufacture of approximately 6,000 vials of chimpanzee adenovirus vector vaccines. The vaccine candidate was developed by the FNLCR's Vaccine Research Center (VRC) in collaboration with Okairos, a European biotechnology company recently acquired by GlaxoSmithKline. Based on positive results of Phase I trials, the NIAID is continuing with plans for larger efficacy trials in West Africa, including a Phase II/III trial. For this effort, the FNLCR has established required infrastructure and operation centers, including staffing, procurement, and subject matter experts, in Monrovia, Liberia.

The FNLCR's partnering efforts include two newly signed contractor Cooperative Research and Development Agreements (cCRADAs and 24 Technical Service Agreements signed. Eight additional cCRADAs are under consideration, including an evaluation of the bioequivalence of nanomedicines and two activities concerning KRAS.

Dr. Heimbrook informed the members about several initiatives to be considered for FNLCR programs. One is the acquisition of a new primate facility by the AIDS and Cancer Vaccine Program (ACVP), which would enable the return of current primate housing to the Center for Cancer Research (CCR) and accommodate anticipated cCRADA demand from academia and industry for new AIDS primate models. In addition, new opportunities exist for the Nanotechnology Characterization Laboratory (NCL) based on the evolution of nanomedicine during the past decade; these were described in detail by Dr. Scott McNeil, FNLCR, later in the meeting.

Dr. Heimbrook described several key opportunities for the FNLCR identified from FFRDC best practices implemented by other FFRDC National Laboratories. A "user facility" capability would draw a continuous flow of external scientists to the FNLCR; the proposed National Molecular Microscopy Laboratory (NMML) addresses this opportunity and was presented by Dr. S. Subramaniam, NCI, later in the meeting. The FNLCR has begun strengthening ties with local universities, including through a strategic relationship with the University of Maryland and scientific collaborations with The Johns Hopkins University. Dr. Heimbrook mentioned that Leidos Biomedical Research has committed funding to a joint scientific symposium in 2015 to build scientist-to-scientist contacts with Johns Hopkins. Additional collaboration is underway through the Frederick Center for Research Education in Science and Technology (Frederick CREST), a collaborative educational and research hub in Frederick County, Maryland, between regional businesses and educational institutions to enhance economic development and job creation.

Another opportunity is to cultivate an "intrapreneurial" mindset in the FNLCR that provides freedom and financial support to create exploratory new programs. The U.S. Department of Energy (DOE) FFRDCs use a Laboratory Directed Research and Development (LDRD) fund as the primary vehicle to support this culture, which provides robust "venture" funding of pilot projects. All DOE LDRDs are funded through an appropriation-enabled tax on all funding. The FNLCR currently does not have an LDRD fund. Modest venture funding had existed prior to the RAS pivot, at a level of $3 million per year from the Office of Scientific Operations (OSO) for technology development within the Advanced Technology Program and at a level of $200,000–400,000 per year that derived from Leidos' earned award fee for one-time short-term research activities at the Laboratory Director's discretion. Dr. Heimbrook described a plan for an LDRD-like funding mechanism, with funding support from the NCI's Office of the Director, a "technical Fellows team" to define a strategic focus for initial proposals for proof-of-concept pilot projects, to be approved by NCI's Office of the Director, and an open solicitation process for 1-year awards with a firm-fixed budget. Proposal review and prioritization would be performed by a review team comprising the Technical Fellows and representatives from Johns Hopkins and U. of MD by, with funding decisions by the Laboratory Director. Program updates would be provided every 6 months, project reviews occur after 1 year or at expenditure of budgeted funds, and reports about LDRD accomplishments from the Laboratory Director to the NCI's OD and the FNLAC would occur annually.

In the discussion, the following points were made:

- The NIAID has used the FNLCR's services for many years with funding at approximately $90 M per year, particularly the FNLCR's Clinical Monitoring Research Program (CMRP) and Vaccine Pilot Plant (VPP). The NIAID, not the NCI, is funding the FNLCR's Ebola outbreak response efforts; one of the primary target populations for the vaccination trial are health care workers, who are at a higher risk for transmission than other people. The FNLCR's qualities allowed an accelerated process to provide the vaccines, with vaccine formulation, fill, and finish accomplished

within 3 to 4 months. Lessons from the vaccine manufacturing concerning the process and material transfer will be used in future responses to improve FNLCR nimbleness.

- Members encouraged the FNLCR to develop relationships with national universities as well as regional schools. Universities can bring funds to a project through a cCRADA.

- The LDRD is envisioned as a strategic avenue to encourage collaborations between the FNLCR and regional universities. CREST also provides an opportunity for the FNLCR to augment the scientific expertise and enthusiasm of its scientists, and ongoing interactions will help set future directions. State leadership appears attracted to the Laboratory's collaborative activities and could be a source for resources to enable university scientists to participate in collaborative interactions with the FNLCR.

- Other National Laboratories (Los Alamos, Sandia) have overcome interaction hurdles through an inter-institutional agreement that mandated that intellectual property (IP) rights from an LDRD project be shared equally; it has resulted in a large number of joint patents. This transformative approach eliminated the need for renegotiation with each project and removed barriers to scientific exchange.

- The FNLCR should involve postdoctoral researchers and other young investigators. DOE laboratories offer a student internship mechanism that allows students to become involved in new areas that they might not otherwise do, with a low cost to the laboratory and some prestige attached to the internship.

- Members expressed support for an LDRD model at the FNLCR to initiate pilot projects. The most impactful LDRD projects in Sandia and Livermore National Laboratories came from allowing a free platform of creativity rather than a top-down directed model. The Laboratories also created Grand Challenge LDRDs, which are directed but encourage intellectual adventurousness. The time from concept to launch of an LDRD project can be short, depending on the LDRD structure and breadth of the project focus; a balanced approach that promotes creativity with appropriate direction is important. An important consideration at the time of project initiation is the sustainability of successful projects beyond the proof-of-concept phase.

- The FNLCR was encouraged to adopt an LDRD model that allowed any university, Cancer Center, or scientists from the NIH Intramural Research Program (IRP) to develop a partnership with a FNLCR scientist.

- Dr. Heimbrook explained that proposals would originate from and require the participation of FNLCR scientists. It was noted that staff at other National Laboratories often hold joint appointments at universities, thereby facilitating scientific synergies; in these cases, the understanding is that the work is conducted at the National Laboratory. National Laboratories serve as a place to develop new capabilities rather than another grant system.

III. NCI RAS INITIATIVE UPDATE AND RAS WORKING GROUP REPORT— DRS. DWIGHT NISSLEY AND DHIRENDRA SIMANSHU

Dr. Dwight Nissley, Director of the Cancer Research Technology Program (CRTP), provided an update on the scientific progress of the RAS Initiative and related activities. Dr. Dhirendra Simanshu, RAS Initiative Structural Biology Lead, CRTP, presented an overview of the Initiative's structural biology efforts.

Overview of the RAS Initiative. Dr. Nissley stated that RAS mutations are a major driver of several cancers, particularly pancreatic, colorectal, and lung cancers. A significant portion of these cancers are driven by the KRAS protein, with more than 100,000 cases of KRAS-driven cancers arising each year. Prognosis for KRAS-driven cancers is poor, and no therapeutics exist that target KRAS directly. A major objective of the RAS Initiative has been to use modern technologies to investigate and characterize RAS proteins from a biophysical perspective. Classical techniques have previously shown that all RAS mutations encode small hydrolase enzymes that bind and hydrolyze guanosine triphosphate (i.e., GTPases). RAS GTPases are highly homologous in the first 164 amino acids, have a G-domain and a hyper-variable region, and are farnesylated and palmitoylated to facilitate binding to membranes. Dr. Nissley reminded the group that wild-type RAS binds to GTP, and in this GTP-bound form it binds to interacting GTPase Activating Proteins (GAPs), which drive signal transduction. Oncogenic RAS, however, ignores the presence of GAPs, constitutively binds GTP, and is always on for signaling.

Dr. Nissley remarked that one of the striking themes that has emerged from the study of RAS biology and clinical observations is the distinct allele specificity associated with different cancers. This allele-specific pathway signaling has instigated an in-depth analysis of RAS biology and biochemistry. Dr. Nissley stated that Dr. Andy Stephen is leading the KRAS biophysical characterization effort at the FNLCR. Several parameters of RAS proteins have been studied thus far, including the GAP-independent intrinsic hydrolysis activity of RAS. Dr. Nissley explained that while the presence of GAP stimulates RAS activity by about 10,000-fold, RAS also is active independently of GAP. Dr. Stephen has demonstrated a nearly 100-fold range in GAP-independent hydrolysis activity of RAS oncogenic alleles or variants. Q61 mutants showed very little activity, whereas some oncogenic alleles, such as RASG12C and RASG13D, are within several-fold of wild-type RAS intrinsic activities.

Researchers also have investigated the binding of RAS to GTP using a non-hydrolyzable homologue, GppNHp, tagged with fluorescent label. Because wild-type GTP dissociates at a specific rate, the dissociation rates of GTP from various proteins can be measured. Additional work has examined how RAS proteins bind with effectors. In collaboration with Dr. Kenneth Westover at the University of Texas, Southwestern, scientists are in the process of establishing dissociation constants for a large set of proteins that have not previously been characterized. The goal is to create a data packet for the proteins that, along with the results from Dr. Simanshu's structural studies, would be made publicly available to establish a baseline for future studies. In addition, interest in rare KRAS oncogenic mutants has spurred analyses of their GDP-bound structures, hydrolysis, and off-rates.

Dr. Nissley informed members that considerable effort is being undertaken to develop a "next-generation RAS." Potential drug therapies hope to target and disrupt the hypervariable region of RAS, which associates with the membrane. Dr. Dominic Esposito and his group at the FNLCR have generated processed and unprocessed RAS by achieving a hypervariable region that is appropriately prenylated. This was accomplished by expressing KRAS and overexpressing human farnesyltransferase and carboxymethyltransferase genes in insect cells. With newly developed separation technologies, studies can begin to investigate RAS in the context of lipid membranes or surrogates for membranes. These studies include work with liposomes; tethered or supported bilayers in collaboration with Dr. Jay Groves at the University of California, Berkeley; and nanodiscs with Dr. Stephen Sligar at the University of Illinois at Urbana-Champaign. With assistance from the University of Wisconsin's National Magnetic Resonance Facility at Madison, Dr. Sriram Subramaniam and colleagues are interested in conducting high-resolution cryo-EM studies of the nuclear magnetic resonance structure of RAS floating in a nanodisc.

Structural Biology and Biophysics. Dr. Simanshu further described the RAS Initiative's recent attempts to structurally characterize aspects of RAS biology. He explained that structural work on RAS proteins traditionally has focused on HRAS. There currently are approximately 120 structures of HRAS, whereas only approximately 36 exist for KRAS (about 30 of which were discovered in the last few years) and only one exists for NRAS. The RAS research community still lacks (1) a structure of KRAS (either wild-type or oncogenic mutant) with any effector or regulator; (2) structural and molecular insights about how RAS activates RAF kinase; (3) structural information on full-length processed RAS (HRAS, KRAS, or NRAS); and (4) structural information on full-length RAF, either free or in complex with RAS.

The RAS Initiative has four major goals related to structural biology. First, researchers will strive to characterize the structures of wild-type KRAS and oncogenic mutants in both their active and inactive states. To aid in structure-based drug design, scientists hope to determine the structures of KRAS complexes with various effectors and regulatory proteins. Third, efforts will be made to compare the structures of KRAS4A and KRAS4B. Finally, researchers will attempt to bind fully processed KRAS to a nanodisc. Collaborative efforts of the RAS Initiative include work on the KRASG12-GAP complex with Drs. Agnidipta Ghosh and Steven Almo at Albert Einstein College of Medicine and investigations of the calmodulin-KRAS complex with Dr. Carla Mattos at Northeastern University.

Targets and Assays. Dr. Nissley detailed some of the Initiative's ongoing work on RAS targets and assays. Dr. Matt Holderfield is spearheading research on new assays and the development of potential therapeutic drugs that target RAS or the interaction between RAS and downstream effectors. Dr. Holderfield has developed an AlphaScreen assay that is ready for pilot screening, and discussions are ongoing with pharmaceutical partners to potentially identify new inhibitors of the RAS–RAF interaction. Dr. Holderfield also has attempted to extend the AlphaScreen assay to fully processed RAS floating in a membrane, with encouraging initial results.

Another area of the screening program has focused on cellular assays based on RASless mouse embryonic fibroblasts (MEFs) developed by Dr. Mariano Barbacid. The technology was tested initially in KRASG12 and HRAS to provide isoform specificity, but the assays will be expanded to include many other KRAS oncogenic mutants, as well as a set of controls. Results from a RAS-dependent MEF pilot screen, in collaboration with the National Center for Advancing Translational Sciences (NCATS), have validated the use of KRASG12 and HRAS as an effective screening pair, with potential to move into a full screening campaign.

Two additional initiatives are ongoing in this area. Dr. Tommy Turbyville is leading an effort to assay for compounds that localize KRAS to the membrane. Another goal is to develop a primary assay to screen for inhibitors of KRAS multimerization using a bioluminescence resonance energy transfer (BRET) assay. Dr. Gordon Whiteley is leading a program on cell surface analysis. Its objective is to survey the surface of KRAS-driven cells to generate a list of proteins specifically associated with KRAS. These KRAS-associated cell surface determinants could represent new targets for antibody-mediated attack, immune-based therapy, and nanoparticle delivery.

RAS Community. One of the aspirations of the RAS Initiative has been to nucleate and enable a greater RAS research community. Toward this end, many resources have been made available to researchers. RAS reference reagents, many of which have been generated by Dr. Esposito's group, are provided upon request. Hundreds more have been generated for internal projects and will be made available to the external community as they become standardized and collected into sets. Forty "cancer toolkits" of pathway-activating clones are currently available, with more than 200 toolkits to come, and a RAS pathway clone set (181 clones) is underway.

A community website, commonly referred to as RAS Central or the RAS Initiative at Cancer.gov, was developed as a space for the RAS community to gather, exchange ideas, and have discussions.

Dr. Nissley shared an example of a discussion that ensued when a RAS signaling pathway diagram was posted to the website. Community members offered suggestions for amendments to the diagram, and a significantly more inclusive RAS signaling pathway diagram is currently posted on the website.

Additional ideas are being considered for engaging and stimulating a more interactive RAS community. Efforts to nucleate a RAS community have included organized seminars and workshops, collaborations with researchers at the NIH and externally, sharing of RAS reference reagents, and informal exchanges.

Oversight and Feedback. The RAS Initiative has generated significant attention from the NCI and beyond. The RAS Initiative *Ad Hoc* Working Group met on October 31, 2014, and made four recommendations to: (1) publish protocols for production of fully processed KRAS protein and associated structural studies and assay; (2) establish a process for collaboration and providing reagents to the community; (3)optimize the RASless MEF screen and to understand and eliminate sources of variation; and (4) collect and analyze the large data set from the node-knockdown-based (SiREN) approach for discussion at the next Working Group meeting.

Dr. Nissley expressed excitement about the success of the Pancreatic Action Network's collaboration with the FNLCR to advance KRAS research by awarding two year-long postdoctoral fellowships. The recipients of the fellowships were Dr. Lynn McGregor from Dr. Kevan Shokat's laboratory and Dr. John Hunter from Dr. Westover's laboratory. Dr. Nissley highlighted the availability of RAS Initiative resources to these and all RAS researchers.

In the discussion, the following points were made:

- The multimode analyses could be expanded to better understand RAS clonal heterogeneity and complexity, which has been seen in pediatric acute lymphomatic leukemia (ALL) relapses. In one ALL cohort study, multiple RAS mutations in different subclones were found in approximately 65 percent of children, and nearly all of the relapse clones were the predominant RAS clones that were the minority at diagnosis. Symposia planned for deep sequencing related to other RAS projects could encompass this issue to elucidate complicated biology.

- The RAS Program has refined its focus to emphasize protein biophysics, process KRAS, structural biology, and development of new targets and assays. Several of the initial RAS projects no longer being pursued include synthetic lethality and cell surface analysis.

- A Program Announcement (PA) soliciting research assistance from the external community was published in November 2014, and a second PAR is planned for release in mid 2015.

- The RAS *Ad Hoc* Working Group has been enthusiastic about the Program's direction, including the focus on structural biology, biophysics, and siRNA and RNAi screening data, and has encouraged the FNLCR to provide a set of RAS Initiative deliverables to facilitate Program monitoring and evaluation. The Program also should address signal-to-noise aspects of some assays to elucidate types of variation and reduce issues regarding reproducibility. Progress should be communicated to the broader research community to highlight emerging areas of study and promote available reagents and bioinformatics tools available at the FNLCR to the extramural community.

IV. REPORT ON WORKSHOP FOR NATIONAL MOLECULAR MICROSCOPY LABORATORY (NMML)—DR. SRIRAM SUBRAMANIAM

Dr. Sriram Subramaniam, Laboratory of Cell Biology, CCR, presented a proposal for an NMML and provided an update on recent advances and opportunities in cryo-EM. Dr. Subramaniam described

traditional methods for protein structure determination, including X-ray crystallography and NMR spectroscopy. X‑ray crystallography needs well-ordered three-dimensional (3D) crystals and generally requires that flexible regions of proteins are truncated or modified; in addition, the structure in the crystal may not reflect the solution structure. NMR spectroscopy enables structure determination in solution, but is limited primarily to small proteins. In contrast, cryo‑EM provides a new tool for determining 3D structures of cells, viruses, and molecules. Atomic resolution cryo-EM emerged in 1990 with 2D protein crystals, and the field advanced into near-atomic resolution of viral structures in 2008 and membrane protein structures in 2013. Numerous structures have been reported since then at resolutions between 3 and 4 Angstroms.

The state of the cryo-EM field was discussed at an NCI-sponsored workshop in December 2014, covering recent progress, the value of hybrid approaches, the need for centers with advanced instrumentation, the NIH perspective, and research community participation. Questions addressed included the impact of recent advances for non-cryo-EM structural biologists, barriers to training and broader dissemination of the technology, applicability of the synchrotron model, ancillary needs for a cryo-EM center, the advantages and disadvantages of having one National Laboratory at the FNLCR versus multiple centers across the United States, effective ways to engage companies with computing expertise to support the cryo-EM community, and sustainability of a cryo-EM laboratory at the FNLCR. With recent significant technical advances, including direct electron detectors, and publicly accessible software, cryo-EM provides the ability to solve structures intractable by other modalities, but methods in cryo-EM are not yet developed for general use by non-experts.

Challenges to the growth of cryo-EM in the United States include the lack of adequate access of laboratories (with or without cryo-EM expertise) to advanced instrumentation, limited number of scientists trained in all aspects of cryo-EM structure determination, and outdated instrumentation. Recommendations from the workshop were to: (1) set up several (3-4) user-friendly state-of-the-art national cryo-EM facilities, possibly at synchrotron sites, using the facility at the UK Diamond synchrotron as a model; (2) support a number of institutions throughout the United States with more modern equipment with matching support from a host institution, such as seen at the New York Structural Biology Center; and (3) develop a robust training program to introduce X-ray, NMR, and biological investigators to cryo-EM and tomography. Dr. Subramaniam referred members to recommendations from the National Institute of General Medical Sciences (NIGMS) about regionally shared resources, computing needs, and infrastructure investments needed in local universities and institutions to support research of structure determination of a macromolecular assembly.

Likely users of cryo-EM methods include cryo-EM specialists who need access to advanced instrumentation, structural biologists (X-ray crystallographers and NMR spectroscopists), and biologists who work on problems that could benefit from cryo-EM analysis but need specialized training. Without access to specialized knowledge at the interface between biochemistry and EM, biologists may not be able to take advantage of advances in the field. Structural advances by cryo-EM have required close collaboration between microscopists and biochemists, and structural biochemistry is needed as each class of proteins has unique challenges. Structure determination can be a slow, iterative process. Dr. Subramaniam a described case study, where cryo-EM technology was used to distill the structure of a glutamate receptor; this example described challenges in preparing useful cryo-EM specimens from purified protein. In addition, the ability to use the technology to move from structure image at 50 Angstrom resolution to 7 Angstrom resolution has been used in new, ongoing studies of the p97 AAA ATPase; cryo-EM has shown distinct conformations in p97, a conformationally dynamic protein, and can localize ATP within the complex.

Key expertise elements necessary for biologists to become users of cryo-EM technology include knowledge and resources for state-of-the-art molecular biology; knowledge of diverse expression systems; effective purification strategies, along with biochemical readouts of function and biophysical readouts of

stability; expertise in reconstitution as well as cryo-EM image processing and structure determination; high-throughput screening; and resources for high-speed computing and data storage.

The FNLCR's unique features would facilitate a cryo-EM national center focused on structural biochemistry and high-throughput cryo-EM screening. These include an active CRADA with a major cryo-EM manufacturer, stellar biochemistry and biophysics infrastructure with structural biology expertise relevant to cancer signaling, strong local partnerships, and a magnet for cancer biologists and industry scientists who seek collaborative opportunities in structural biology studies of cancer-relevant protein complexes.

Dr. Subramaniam described the 5-year plan for cryo-EM at the FNLCR in two proposed phases. The first phase would be a 2-year process involving 10 staff and an estimated $7.5 M to establish a framework for structural biochemistry, install a microscope for high-throughput screening, develop partnerships, streamline cryo-EM sample preparation for selected targets, demonstrate effective gene-to-cryo-EM specimen workflows, and generate a well-defined set of potential targets for structural analysis. The second phase would occur over 3 years and include 15-20 staff, with an estimated cost of $21 M. This phase would introduce advanced instrumentation for increased throughput, lay a foundation to facilitate visits and biochemistry collaborations with external researchers, continue to develop solutions for sample preparation problems, and advance work on structures of important cancer-relevant complexes.

In the discussion, the following points were made:

- Challenges to the cryo-EM field include limited funding, the need for high-quality software, sample preparation, and training. The launch of the cryo-EM facility at UK Diamond involved $25 M grant funding that facilitated the purchase of two advanced and two ancillary microscopes, support staff, and expertise in structural biology. Although high-quality, open software is available, the software component of the field is in an early phase of development, which underlies the importance of training.

- The national centers would be easiest to launch at synchrotron sites as structural biologists are already located there and are accustomed to handling relevant samples.

- The NMML should be constructed to galvanize the community around vexing issues in cancer in a transformative way. Members requested that the plan be refined to clearly indicate the NMML's local, regional, and national scope at the FNLCR. The plan should describe the special attributes at Frederick that would make it the ideal location for the NMML (e.g., specialized expertise); the potential tomographic application of cryo-EM; strategies to solve methodological issues (e.g., a focal laboratory versus multiple laboratories); ability to solve problems in sample preparation; and support from other NIH Institutes that have an interest in cryo-EM.

- Dr. Subramaniam explained that the concept has two phases encompassing the development of a physical laboratory and projects. The vision for the second phase is to develop methods that are generalizable and so facilitate the preparation of kits that researchers can apply to their studies. In addition, the FNLCR will have an important role in supporting research into the sample preparation "structural biochemistry" project.

V. A NATIONAL LABORATORY TO VALIDATE CANCER TARGETS OF CLINICAL INTEREST—DR. MARIANO BARBACID

Dr. Mariano Barbacid, Director, National Oncological Research Centre, Spanish National Cancer Research Center, presented a proposal for a National Laboratory to validate targeted therapies in experimental models of cancer with unmet medical needs with the goal of guiding the design of future

clinical trials. Dr. Barbacid stated that because deep sequencing of human cancer genomes has revealed that solid tumors contain dozens of mutations that affect multiple signaling pathways, such as the RAS and MAP Kinase pathway and the PI3Kinase/PTEN/Akt pathway, the blocking of several signaling pathways is needed to induce significant and durable anti-tumor responses. Targeted therapies such as erlotinib and Gleevec® have provided clinical benefit for specific cancers but fail primarily due to the appearance of resistance mechanisms caused by secondary mutations in the target or by activation of alternative pathways. Many advanced tumors appear to be a heterogeneous mix of "cancer clones" that share only a limited number of common mutations, and carefully designed drug combinations can provide an effective way to block as many mutated or altered signaling pathways as possible and result in tumor regression. The precise combination of targets needed to block progression/maintenance of each tumor type, however, is unknown.

Preclinical animal models such as Patient Derived Xenograft (PDX) and the Genetically Engineered Mouse (GEM) tumor models offer the greatest chance for success. PDX models have a human origin and are fairly representative of the actual tumor at least for a few passages, but tumor variability, need for large cohorts of tumors to draw meaningful conclusions, and dependence on available suitable inhibitors are disadvantages. GEM models have a mouse origin and are less likely to accumulate as many mutations/alterations as human tumors, but they are reproducible, allow systematic analyses of suitable targets and pathways, and make it possible both to validate targets by genetic means and to combine genetic with pharmacological validation. Dr. Barbacid focused on the use of the GEM models of human cancer for the proposal.

The aim is to complement ongoing efforts at the FNLCR's Center for Advanced Preclinical Research on translational research using available GEM models of cancer that represent a few relevant tumor types to validate targets of potential therapeutic value. Target combinations that can eradicate advanced tumors without significantly affecting normal homeostasis will be devised, and pharmaceutical companies will be engaged to translate these results to a pharmacological scenario using high-quality drugs that have entered or will enter clinical trials. The program will not conduct basic research and will focus on targets that affect tumor cell proliferation or survival. The GEM models should faithfully reproduce the natural history of human cancer, be readily available, and be amenable to "scaling up" tumor aggressiveness by adding additional driver mutations; in addition, non-invasive techniques will be used for tumor detection.

The three steps in the program are: (1) genetic validation of individual targets; (2) genetic validation of combinations of targets; and (3) pharmacological validation using selective inhibitors. For individual targets, targets will be ablated in tumor-bearing mice, genetically inactivated to better mimic drug activity, and ablated or inactivated systematically to determine potential toxic effects. Targets to be validated should be as druggable as possible. Compound GEMs that facilitate ablation or inactivation of as many targets as possible (e.g., 4 to 5 targets) at one time in tumor-bearing mice will be generated for the combinations of targets. These complex compound strains will be generated with the help of the CRISPR editing technology either *in vivo* in embryonic stem cells derived from compound mice, and anti-tumor activity will be evaluated by adding sequential driver mutations. For the pharmacological validation using selective inhibitors step, selective inhibitors for those targets validated genetically will be used, and the pharmaceutical industry will be engaged to test their best-in-class compounds. The GEM strains used for genetic validation studies will be treated with the corresponding inhibitors to compare pharmacological and genetic outcomes and evaluate the specificity and potency of each drug candidate as well as the overall anti-tumor effect of the drug combinations. These studies also will reveal their (putative) undesirable off-target effects. Whenever possible, the drug combinations defined in these studies should be tested in PDX models, and for cases in which no suitable inhibitors/drugs might be available for all the targets included in the combination, genetic targeting will be combined with drug treatments.

Dr. Barbacid described results from studies of several tumor types that have used the same experimental approach proposed for this project. Initial tumor types to be studied include pancreatic ductal adenocarcinoma, triple negative breast tumors, glioblastoma, KRAS mutant lung adenocarcinoma, and

colorectal adenocarcinoma. Resources are estimated at four to six full-time equivalents (FTEs) per tumor model, with the assumption that some core support (e.g., animal care takers, imaging support) would be available.

In the discussion, the following points were made:

- Models for target discovery, such as PDX or cultured cell lines, should be considered, as disadvantages of the GEM model include that the GEM system is less ideal for studies of resistance to therapy and has not yielded new discoveries in comparison to earlier models. In addition, GEM models include a set of genomic abnormalities that are comparatively clean, whereas human abnormalities involve a more complex genome. Members agreed that a systematic model to test a high combination (3–4) of drugs would be helpful and encouraged the use or leverage of parallel or alternate tumor models along with GEM, such as the use of clustered regularly interspaced short palindromic repeats (CRISPR) libraries to search for loss of variability in given PDX lines.

- The project goal is to identify a combination of targets that, by blocking, eliminating, or inactivating those targets, will reduce or eradicate an aggressive tumor. The proposed project steps involve genetics, drugs, and drug combinations, and challenges include identification of a combination of four or five drugs that is effective but not toxic, and that drug combination studies may not have the same result as genetic studies. The combination of targets with genetics can lead to a cure.

- Dr. Barbacid's group has signed two contracts this year that put combinations of drugs together when the drugs come from different companies.

- The goal is full eradication of tumors in mice, with the assumption that results will be better in mice than humans, as studies in humans will involve drugs, not genetics. Eradication of a tumor in mice will not necessarily translate into similar results in humans.

VI. NANOTECHNOLOGY CHARACTERIZATION LABORATORY (NCL) AS A NATIONAL MISSION—DR. SCOTT MCNEIL

Dr. Scott McNeil presented a proposal to move the NCL into a National Program by expanding its activities beyond its services as an assay cascade resource into six additional topics: reformulation and cGMP, nanomaterials, metrology and new materials, basic research and grand challenges, informing the regulatory process, and transnational collaboration. The NCL currently subjects particles to a three-phase assay cascade that involves physical chemical, *in vitro*, and *in vivo* characterization following guidance. The Laboratory provides independent verification of results for the research and development community, and pharmaceutical mentorship for materials scientists and engineers. Because of the NCL's experiences with the U.S. Food and Drug Administration (FDA), it also provides submitters a preview of the FDA's potential concerns.

The NCL will continue to provide assay cascade services. Testing links physiochemical properties to biological outcomes and is tailored to platform properties, API, route of administration, and intended therapeutic outcome of the individual nanomedicine. The NCL has characterized more than 300 nanomaterials and a wide range of platforms, and has 10 collaborators with products in clinical trials. An average of 15 active collaborations is ongoing at any given time, and the Laboratory characterizes an average of 75 samples per year.

Nanotechnology reformulation can increase the solubility of hydrophobic drugs, alter the pharmacokinetics profile, and reduce drug toxicity. In collaboration using a legacy docetaxel drug, which previously had been shown to increase the distribution of the half-life from 30 minutes to 9 hours, the NCL raised the dose four times and increased animal survival, and so demonstrated an additional advantage for

systemic controlled release formulations available through nanotechnology. Challenges remain for the nanomedicine industry as nanoformulations involve complex, multi-step processes and often require specialized testing (e.g., orthogonal characterization, bioassays to demonstrate equivalence) per the FDA. Few manufacturers have the capabilities for cGMP manufacturing of nanomedicines, and many nanomedicine cGMP lots fail. Dr. McNeil noted the NCL's ongoing collaborations with the pharmaceutical companies and proposed to expand production for a larger portfolio of nanomedicines, meet demand for reformulation and cGMP collaboration, and address previously disqualifying toxicity or missed metrics.

The NCL's assay cascade resource is relevant to nanomaterials for non-oncology applications. Dr. McNeil shared a case study of physicochemical characterization of nanomaterials performed for a National Institute of Environmental Health Sciences (NIEHS) program. Expansion into non-oncology nanomaterials would meet demand and leverage NCL characterization resources in support of all NIH and Department of Health and Human Services (HHS) efforts in nanomedicine.

Nanoformulation can affect drug-protein interactions. An unbound drug, such as Taxol®, can be in equilibrium with both the formulation components and protein. Bioequivalence studies require the evaluation of drug release and unencapsulated drug fraction. The NCL has developed novel assays for physicochemical characterization, immunology, and to assess nanoparticle stability and drug release. Components of a National Program would include collaborations with instrument manufacturers, development of drug-release methods with the pharmaceutical industry, and collaborations with the FDA to better inform the regulatory process.

The NCL's basic research and structure-activity relationship (SAR) studies on trends have informed the nanomedicine community and influenced the field through more than 100 publications and staff scientists who are internationally recognized experts. Examples of basic research include studies of immunotoxicity of nanoformulations compared with traditional formulations, hematology, and immune cell function. In support of basic research on mechanisms of toxicity, the NCL developed an assay to determine the degree of PEGylation in a 14-day absorption, distribution, metabolism, and excretion (ADME) toxicology study in rats; it was found that PEG was dissociating from the particles over time and ending in the solution. The differences found in coatings was subtle and not detected by routine physicochemical study. In the basic research and grand challenges component, the NCL would serve as a hub for the nanomedicine research community; host meetings and working groups to identify grand challenges; and leverage resources to solve scientific problems to advance translation.

Dr. McNeil stated that the NCL currently has quarterly interactions with the FDA to maintain collaboration, including for specific scientific areas conducted through Inter-Agency Agreements (IAAs). The Laboratory provides a preview to the FDA of the nanotechnology investigational new drug (IND) or investigational device exemption (IDE) applications that are in the pipeline. The NCL's collaborations with the FDA have addressed specific concerns such as immunological reactions to nanomaterials, dermal penetration of nanomaterials in sunscreens and cosmetics, endotoxin, and methods of sterilization for devices. Additional interactions occur through FDA representation on NCL's scientific oversight committee and NCL participation in relevant FDA public meetings. The NCL also will inform the regulatory process through interactions with international regulatory bodies. Dr. McNeil noted that a mechanism is needed for the NCL to submit research proposals to the FDA and other government agencies.

Transnational collaboration includes a "mirror" NCL in Europe that includes a consortium of eight academic, industry, and government laboratories distributed throughout the European Union (EU). The EU NCL is been funded for 2015. The U.S. NCL is providing historical knowledge to help the EU NCL reduce the risk of adverse events as well as leverage and scale up resources. This collaboration will expand the visibility of nanomedicine to users, the pharmaceutical industry, research and development community, and regulatory agencies. By working with the European Medicines Agency (EMA) and FDA, both NCLs can inform regulatory policy and improve international coordination in nanotechnology.

Dr. McNeil described the resources recommended to support the NCL national mission, including $1.5-2 M per year for eight FTEs, three postdoctoral researchers, and capital equipment, with revenues expected in year 3. In addition, a $15 M GMP facility for nanomaterials would involve milligram to gram-level scaleup, address the Phases I and II levels, and fund the infrastructure. Extensive collaboration and financial support from the extramural community through CRADAs, IAAs, and grants are expected. NCL expansion into a multi-component national program ensures that the Laboratory is recognized as a unique international resource and projects the FNLCR's impact as an FFRDC.

In the discussion, the following points were made:

- The NCL provides unique core services depended upon by academic partnerships funded by the NCI depend, as well as independent validation of toxicity sought by the FDA.

- The proposed NCL GMP facility involves a physical expansion. Members encouraged the FNLCR to consider a user-fee model and startup support from other NIH Institutes and industry, and they encouraged the development of a business plan that outlines recoverable investment and long-term sustainability. Given the need and demand, the facility is expected to return initial investments quickly.

- A NCL GMP facility would focus on vectors and antibodies. Similarities with other clean facilities such as found at Sandia National Laboratory include air handling and the qualification, but instrumentation would differ.

- Members commended the NCL's mentorship of material scientists and pharmaceutical staff.

- The NCL and the ACVP are the FNLCR's most visible and impactful projects to the external community.

- The HHS FFRDC limits the FNLCR's interaction to support of one agency. Funding a grand challenge with the FDA would involve an IAA between the FDA and the NCI.

VII. CLOSING REMARKS—DRS. JOE W. GRAY AND HAROLD E. VARMUS

Dr. Gray reflected on the FNLCR's updates and proposals provided during the meeting, expressed members' support for the direction of the RAS Program and anticipation of a set of deliverables to track progress, noted enthusiasm for a laboratory to validate cancer targets, and stated that the FNLAC looks forward to a further refined microscopy concept and a business plan for the NCL. He stated that progress is being made in defining what the National Laboratory should look like in the future. Dr. Varmus thanked members for their participation in a productive and educational meeting.

VIII. ADJOURNMENT—DR. JOE W. GRAY

Dr. Gray thanked the Committee members and other invitees for attending. There being no further business, the 8th meeting of the FNLAC was adjourned at 3:54 p.m. on Tuesday, February 3, 2015.

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Date Joe W. Gray, Ph.D., Chair

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Date Thomas M. Vollberg, Ph.D., Executive Secretary