U.S. Department of Health and Human Services
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National Cancer Institute

16th Meeting
Frederick National Laboratory Advisory Committee

Summary of Meeting
June 27, 2019

Conference Room TE406, East Wing, Shady Grove Campus
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

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16th Meeting of the Frederick National Laboratory Advisory Committee
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Summary Minutes

The Frederick National Laboratory Advisory Committee (FNLAC) convened for its 16th meeting on June 27, 2019, in Conference Room TE406, East Wing, Shady Grove Campus, National Institutes of Health, Bethesda, MD. The meeting was open to the public on Thursday, June 27, 2019, from 9:30 a.m. to 3:40 p.m. The FNLAC Chairperson, Dr. Lawrence J. Marnett, Dean of Basic Sciences, Mary Geddes Stahlman Professor of Cancer Research, and Professor of Biochemistry, Chemistry, and Pharmacology, Vanderbilt University School of Medicine, presided.

FNLAC Members
Dr. Lawrence J. Marnett (Chair)
Dr. Catherine M. Bollard
Dr. Andrea Califano* (absent)
Dr. Lisa M. Coussens (absent)
Dr. Kevin J. Cullen
Dr. Raymond N. DuBois (absent)
Dr. Angela M. Gronenborn (absent)
Dr. Robert L. Grossman
Dr. Klaus M. Hahn (absent)
Dr. David I. Hirsh
Dr. Elizabeth M. Jaffee (absent)
Dr. Sanford D. Markowitz (absent)
Dr. Patrick Nana-Sinkam
Dr. Piermaria J. Oddone
Dr. Nilsa C. Ramirez-Milan
Dr. Lincoln D. Stein (absent)
Dr. Cheryl L. Willman (absent)
Dr. Jedd D. Wolchok (absent)

*pending appointment

***Ex Officio* Members**
Dr. Stephen J. Chanock
Dr. James H. Doroshow
Dr. Paulette S. Gray
Dr. Sara Hook
Dr. Anthony Kerlavage
Dr. Tom Misteli (absent)
Ms. Donna Siegle
Dr. Dinah S. Singer

Executive Secretary
Dr. Caron A. Lyman

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I. OPENING REMARKS—DR. LAWRENCE J. MARNETT

Dr. Lawrence J. Marnett, Chair, called to order the 16th meeting of the Frederick National Laboratory Advisory Committee (FNLAC) and welcomed the Committee members, National Cancer Institute (NCI) staff, and guests. Dr. Marnett reminded members of the conflict-of-interest guidelines and confidentiality requirements. Members of the public were welcomed and invited to submit to Dr. Caron A. Lyman, Executive Secretary, in writing and within 10 days, any comments regarding items discussed during the meeting.

Motion. A motion to approve the minutes of the October 29, 2018 FNLAC meeting was approved unanimously.

Dr. Marnett called Committee members' attention to the future meeting dates listed on the agenda, which need to be confirmed.

Motion. A motion to confirm the future meeting dates was approved unanimously.

II. REPORT FROM THE ACTING NCI DIRECTOR—DR. DOUGLAS R. LOWY

Dr. Douglas R. Lowy, Acting Director, NCI, welcomed the FNLAC returning and new members and attendees to the meeting. He provided updates on the NCI budget, new and ongoing activities, and the Research Program Grant (RPG) Pool and also noted the full agenda.

NCI-Frederick and Advancing Cancer Research. Dr. Lowy explained that the NCI will continue with new activities started during former NCI Director Dr. Norman E. Sharpless' tenure, such as the cell therapy manufacturing expansion at the Frederick National Laboratory for Cancer Research (FNLCR) on the campus of NCI-Frederick. He remarked that the FNLCR-led National Cryo-Electron Microscopy Facility (NCEF), which launched in 2017, has supported extramural investigators in collecting more than 200 high-resolution datasets, including the human inflammasome NLR family pyrin domain containing 3 (NLRP3) in complex with NIMA-related kinase family 7 (NEK7) reported in the June 2019 issue of *Nature* by the National Institutes of Health (NIH)–supported laboratory of Dr. Hao Wu at the Harvard Medical School. Dr. Lowy was pleased to announce that Dr. Sara Hook has been named Associate Director at NCI-Frederick and noted that she will be a strong addition to the leadership. Dr. Hook has been at the NCI since 2010 and has provided oversight to both the RAS Initiative and NCEF projects.

Dr. Lowy highlighted two advances in which NCI-Frederick and FNLCR have played a role. The recent *Annual Report to the Nation on the Status of Cancer*—a collaborative effort between the NCI, Centers for Disease Control and Prevention (CDC), American Cancer Society, and the North American Association of Central Cancer Registries—shows an overall decrease in the U.S. mortality rates of most cancers for men and women, with the exception of nonmelanoma skin cancer in men and liver intrahepatic bile duct cancer in women. The largest annual percentage decrease over the past 4 years has been in melanoma cancer, primarily due to the advances in therapies and improved patient responses.

The Cancer Moonshot℠ Initiative, which is in its third year of implementation, conveys NCI's commitment to improving the outcome for cancer patients. In an iterative process, the NCI has developed and approved concepts and issued funding opportunity announcements (FOAs) covering each of the 10 National Cancer Advisory Board (NCAB) Blue Ribbon Panel recommendations for fiscal years (FYs) 2017 to 2019. Many FOAs are proposed for FY 2020. Dr. Lowy expressed appreciation to Dr. Dinah Singer, Director, Division of Cancer Biology (DCB), who was NCI Acting Deputy Director during development of the Cancer Moonshot℠, and NCI staff for their efforts in implementing these activities. He congratulated Dr. Singer on her becoming NCI Deputy Director for Scientific Strategy and Development. The FNLAC

members are welcome to contact Ms. Christine Siemon, Scientific Program Specialist, DCB, for access to the interactive map of the NCI Cancer Moonshot [SM] landscape and activities.

NCI Budget and Appropriations. Dr. Lowy reminded the FNLAC members that the Cancer Moonshot [SM] appropriation of $400 million (M) for FY 2019 will be the highest of the $1.8 billion (B), 7-year funding period for the initiative. Beginning in FY 2020, the annual allotments will decrease by $200 M per year until the funding period ends in 2023. The NCI regular appropriations, similar to most other NIH Institutes and Centers (ICs) have steadily increased since FY 2015, which Dr. Lowy credits primarily to the continuing bipartisan support of Congress. The NCI/NIH multistep budget process for regular appropriations is currently at step two, in which Congressional Appropriations Committees are considering the President's budget proposal and preparing legislation. The House Appropriations Subcommittee on Labor, Health and Human Services, Education, and Related Agencies (L-HHS) approved its FY 2020 spending bill markup in May 2019 and includes a $2 B increase to the NIH and $310 M increase to the NCI, which is a 5 percent increase above the FY 2019 enacted budget; the Senate Appropriations L-HHS Subcommittee spending bill is expected in July 2019.

Ongoing and New Activities. Dr. Lowy informed the FNLAC members that the NCI has established two new working groups, the internal Screening and Early Detection Working Group, chaired by Dr. Debbie Winn, Acting Director, Division of Cancer Prevention (DCP), and the Board of Scientific Advisors (BSA) *ad hoc* Working Group on Prevention, co-chaired by BSA and NCAB members Drs. Graham Colditz and Judy Garber. The Screening and Early Detection Working Group will work to identify opportunities in screening and early detection that the NCI could support if the necessary resources were available. The BSA *ad hoc* Working Group on Prevention will focus on identifying gaps in cancer prevention, which can be formulated into recommendations for the NCI.

Dr. Lowy announced his plans for added emphasis in four areas for the NCI—childhood cancers, investigator-initiated research, health disparities, and therapeutic resistance—and elaborated on one of those areas that closely affects the FNLCR. Basic science and investigator-initiated research are important to cancer breakthroughs and are reflected in outcomes for patients. Although the advances in melanoma and immunotherapy have benefited many patients with metastatic disease, the prognosis for patients with glioblastomas is not as positive, signifying the need for more research. A Bentley University narrative review study led by its Center for Integration of Science and Industry published in the March 2018 edition of the *Proceedings of the National Academy of Sciences* reported that from 2010 to 2016, NIH funding contributed to all 210 U.S. Food and Drug Administration-approved new drugs. Basic research was involved in identifying the biological targets in preclinical studies, which could be translated into an intervention.

RPG Pool Update. Dr. Lowy presented a report on the NCI RPG Pool to the NCAB Planning and Budget Subcommittee on June 9, 2019, and to the Joint BSA/NCAB on June 10, 2019. A detailed NCAB Planning and Budget Subcommittee meeting summary can be accessed from the NCI website. Dr. Lowy conveyed NCI's strong support of the RPGs and described data compiled from FY 2013 to 2019. The NCI has increased funding to the RPG Pool since FY 2014 and by the end of FY 2019 will have added a total of $400 M. The allocations to support new competing (Type 2) and Noncompeting Continuation (Type 5) awards also have been increased from $400 to $500 M per year and the 7-year Outstanding Investigator Award (OIA) was established in FY 2014. The Early Stage Investigator awards were extended from 5 to 7 years by the Method to Extend Research Time (MERIT) award (R37) mechanism in FY 2018, and higher paylines for these investigators were preserved.

Consequently, the 2-year R21 grant awards have decreased, the R01 applications have increased by nearly 50 percent, and the paylines and funding success rates have decreased. In FY 2018, 41 percent of the NCI overall budget supported the RPG Pool and 13.9 percent was allocated to Research and Development contracts, which includes the FNLCR. Of the 41 percent, 55 percent funded traditional R01 grants. Data

from the NIH Research Portfolio Online Reporting Tools (RePORT) showed that NCI competing R01 applications increased by 46 percent from FY 2013 to FY 2018, which is 10 percent higher than any other NIH IC.

The NIH funding success rate by ICs was 16.8 percent in FY 2013 and 20.2 percent in FY 2018 and, in general, increased with the NIH budget. Conversely, for the NCI, the success rates went from 13.7 percent in FY 2013 to 11.3 percent in FY 2018, showing a significant disparity compared with most other ICs and decreasing despite the increased NCI budget. The gap between the NCI and the highest funded IC increased from 1 to 5 percent within this same period. Funding patterns for competing RPG grants showed a 15 percent success rate for unsolicited R01s in FY 2014, which had declined to 12 percent by FY 2018. The R01 RFAs' success rates increased slightly, from 13 to 14 percent.

Dr. Lowy called the FNLAC members' attention to the resources available on the RPG funding patterns, which can be accessed from the NCI Division of Extramural Activities (DEA) website, and additional information on the budget that can be found in the NCI *Budget Fact Book*. Although the NCI welcomes the increased excitement about opportunities in cancer research and the tremendous prospects for advances in translational and clinical sciences, Dr. Lowy remarked on continuing the focus on fundamental research and cited two main reasons. First, fundamental research is the main source of breakthrough observations that increase the cancer community's understanding of physiology and pathology. Second, fundamental research is needed for major advances in the improvement of health.

Leadership Appointments and Vacancies. Dr. Lowy announced that Ms. Anne Lubenow is Chief of Staff, Office of the Director (OD), Mr. Eric L. Cole is the new NCI Deputy Executive Officer, Dr. Tony Kerlavage is the new Director of the Center for Biomedical Informatics and Information Technology (CBIIT), Mr. Jeff Schilling is the new NCI Chief Information Officer, Dr. Jonas Almeida is now Chief Data Scientist in the Division of Cancer Epidemiology and Genetics (DCEG), and Mr. Weston Ricks is now NCI Budget Director. He noted NCI's ongoing recruitment efforts for directors for the Center for Global Health (CGH), Cancer Therapy Evaluation Program (CTEP), and DCP; and he expressed appreciation to Dr. Robert Croyle, Acting Director, CGH, Dr. Winn, Acting Director, DCP, and Dr. Margaret Mooney, Acting Associate Director, CTEP, for stepping in to fill these roles.

In the discussion, the following points were made:

- Many outstanding R01 proposals received in the NCI have not been funded in the current climate that includes an 8 percent payline and funding success rate of 11 percent. Dr. Lowy's intent is to improve the likelihood of funding in FY 2020 rather than explore alternatives to an R01.

III. RECOGNITION OF RETIRING FNLAC MEMBER—DR. DOUGLAS R. LOWY

On behalf of the NCI, Dr. Lowy recognized the contributions made by Dr. Piermaria J. Oddone, Director Emeritus, Fermi National Accelerator Laboratory, whose term on the FNLAC will terminate at the end of June. He expressed appreciation for Dr. Oddone's service and dedication over the course of his term.

IV. SPECTRUM OF SCIENCE CONDUCTED AT THE FREDERICK NATIONAL LABORATORY FOR CANCER RESEARCH (FNLCR)—DR. ETHAN DMITROVSKY

Dr. Ethan Dmitrovsky, Laboratory Director, FNLCR, President, Leidos Biomedical Research, Inc., (Leidos Biomedical Research) discussed FNLCR's range of current and future work. The FNLCR partners with the NCI, National Institute of Allergy and Infectious Diseases (NIAID), other NIH ICs, and other government agencies to prevent, diagnose, and treat AIDS, infectious diseases, and emerging public health challenges (e.g., Ebola and Zika virus epidemics). The spectrum of work includes the science (e.g., state-of-the-art research programs), advanced technologies, efficient and rapid response, and doing business with

outside organizations through such mechanisms as Contractor Cooperative Research and Development Agreements (cCRADAs) or Technical Service Agreements (TSAs).

The day-to-day operational delivery of the Leidos Biomedical Research contract involves both government and contractor leadership engagement. The NCI-Frederick administration consists of the Office of Scientific Operations (OSO), which manages the Leidos Biomedical Research infrastructure and business support functions, and the Management and Operations Support Branch (MOSB), which conducts the formal contracting interactions with Leidos Biomedical Research. Aside from administration and contract management, other formal interactions include monthly and quarterly meetings with Leidos Biomedical Research Clinical and Scientific Directorate and NCI Division heads to assess each project's milestones. These collaborative meetings and daily communications between both leaderships on the hypothesis-driven research address issues and review metrics and progress.

Dr. Dmitrovsky described the two types of work—severable and non-severable—supported by the FNLCR. Severable work is that in which benefits of services are recurring and involve annual appropriations, such as with the Center for Cancer Research (CCR) support. Under the Bridge contract (i.e., Leidos Biomedical Research's former contract to a Bridge award), the FNLCR will transition to Task Orders to conduct severable and non-severable work. Distinct multiyear projects lasting for up to 5 years comprise the non-severable work. The benefit of services is met at the completion of the project work; to date, 85 task orders from NIH ICs and NCI Divisions, Offices and Centers (DOCs) have been awarded. The Ebola epidemic response, Zika virus clinical trial support, and facility refurbishment projects are examples of non-severable projects. Dr. Dmitrovsky noted the NCI-Frederick Building 538 refurbishment project and renovations completed in collaboration with the FNLCR Facilities Maintenance and Engineering and subcontractors brought the facility up to its current standards. The FNLCR executive leadership held its annual retreat to discuss and align the scientific and administrative efforts. As a bidirectional hub-and-spoke model of serving both the intra- and extramural communities, the FNLCR also broadly addresses public health. Seventy-four percent of FNLCR funds support extramural research through subcontracts and/or procurements. Under the direction of its Chief Medical Officer, Dr. Barry L. Gause, the FNLCR supports the ICs with its Applied and Developmental Research and Clinical Monitoring Research Program Directorates, as well as the Clinical Research, Biopharmaceutical Development, and Vaccine Clinical Materials Programs.

Dr. Dmitrovsky highlighted examples of projects funded by the Division of Cancer Treatment and Diagnosis (DCTD), CCR, and NIH/NIAID Vaccine Research Center (VRC). The FNLCR provides the infrastructure needs, especially in chimeric antigen receptor (CAR)-T cell manufacturing to support the NCI CAR-T cell clinical trials. In a phased approach, a multisite pediatric acute myeloid leukemia (AML) trial will launch at the NIH Clinical Center late in 2019 and subsequently the Children's Hospital of Philadelphia and National Marrow Donor Program sites, all of which have prior experience with CAR-T cell therapy. A major effort of the FNLCR is to facilitate efficient and effective support of domestic and international clinical trial programs, including vaccine trials to combat HPV, Ebola and Zika viruses, and other diseases. To date, more than 400 Phase I to Phase III trials in 42 countries have been supported.

In rapid response to two simultaneous Ebola virus outbreaks in 2018 in the Democratic Republic of the Congo (DRC), one in Bikoro and one in Beni, the FNLCR is supporting the Ebola Trial, a multicenter, multi-outbreak, randomized control trial (RCT) being conducted in the North Kivu Province. Unlike the response to the 2014–2015 Ebola outbreak in Liberia, approximately 2,000 active Ebola cases are being treated in the DRC and are being supported by the FNLCR, requiring a medical relief team to be deployed into the nearby area to establish a clinic. The Ebola trial has enrolled 371 patients who are randomized to one of four treatment arms consisting of three antibodies—Zmapp, mAb114, and REGN3470-3471-3479—and one anti-viral agent, GS-5734 (i.e., remdesivir). Dr. Dmitrovsky pointed out the challenges of performing this case study/RCT in the political and militarily unstable Kivu region, which has seen active conflict dating back to 2001. Because of disputes with the local militia and based on daily threat assessment

data, the medical team was removed from the area. The FNLCR has since worked with NIAID, the World Health Organization, United Nations, DRC, Institut National de Recherche Biomédicale, U.S. State Department, and Leidos, Inc. international security experts to redeploy the medical team. Daily and electronic monitoring and rigorous safety measures are in place. Another clinical trial includes assisting the NIH/NIAID VRC in conducting the Zika 705 Phase II/IIB DNA vaccine RCT at 17 sites; 3 in the United States, 4 in Puerto Rico, and 10 in Latin America. Despite the early lag in accrual, the final enrollment included 2,333 participants, which can be credited to the VRC-FNLCR Working Group established to address the study barriers and concerns. The trial closeout procedures are in progress and are being led by a standing VRC-FNLCR committee. Ongoing efforts also are focused on the management of a diverse array of vaccine development and manufacturing projects, such as HIV and influenza.

Dr. Dmitrovsky introduced Dr. Leonard P. Freedman as the FNLCR Chief Science Officer who oversees five Directorates: Laboratory Animal Sciences Program (LASP), Biomedical Informatics and Data Science (BIDS), Basic Science Program (BSP), Cancer Research Technology Program (CRTP), and AIDS and Cancer Virus Program (ACVP), all of which have published findings in high-impact score journals. Dr. Freedman also manages the Laboratory Directed Exploratory Research (LDER) Program. The LASP is a core research animal facility funded by the NCI OD and supports both intramural and extramural investigators. The CRTP is funded by multiple NCI DOCs and manages specialized laboratories, core facilities, and large-scale projects, such as the NCEF and RAS Initiative. Major CRTP accomplishments include distributing RAS reference reagents, characterizing nanoparticles for drug development, and providing a user facility for collection of cryo-electron microscopy (EM) data. The FNLCR is seeking advice on ways to address the growing need to support the extramural community with cryo-EM sample preparation and image analysis, as well as ideas for new national initiatives.

The CCR-funded BSP supports a core facility and is home for six CCR and FNLCR principal investigators. Major accomplishments include next-generation sequencing for human leukocyte antigen (HLA) and killer immunoglobulin-like receptor; and cohort development. The BIDS Directorate is funded by multiple DOCs and supports resource centers and cores. Accomplishments include facilitating the Accelerating Therapeutics for Opportunities in Medicine (ATOM) and Joint Design of Advanced Computing Solutions for Cancer (JDACS4C) initiatives and developing deep learning models for histopathology imaging and other analyses. The ACVP consists of both intramural and extramural collaborations, and together their major contributions include developing diagnostic tools, optimizing nonhuman primate models of disease, and facilitating the first HIV testing to secure the safety of the American blood supply. In addition, the ACVP's efforts have contributed to the advent of such innovative treatments as antiretroviral therapy, which has moved HIV from a near fatal to a chronic disease.

Dr. Dmitrovsky described outreach activities focused on disseminating knowledge and information to the extramural community. The Leidos Biomed–Hood College Cancer Science Symposium titled "Imaging in Cancer Biology" was held June 21–23, 2019, and Dr. Otis Brawley, Bloomberg Distinguished Professor of Oncology and Epidemiology, The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University, and BSA member was the keynote speaker. The FNLCR Director's Distinguished Lecture Series was launched, the 2019 speakers have been confirmed, and sessions have been well attended. The FNLCR collaborates extensively with the extramural community and partners with 127 institutions to conduct its work; 42 percent are universities, 20 percent are the pharmaceutical industry, and 29 percent are research institutions.

In the discussion, the following points were made:

- No single government agency or nongovernmental organization, but a consortium, financially supports the Ebola medical team and clinical trial. The current FNLCR efforts in this context are funded by NIAID.

- The medical team consists of experts in the Ebola response, including Leidos Biomedical Research employees who selflessly address this public health crisis. Some clinicians are trained at NIAID and have returned to their respective countries.

- Although pediatric cases of AML are rare, the CAR-T cell clinical trial is addressing an area of unmet need, which is to treat orphan diseases and to take the lead on a national level.

- Three of the four Ebola trial treatments are available through external biopharmaceutical sources and one, mAb114, was developed in the NIAID VRC with support from the FNLCR.

V. NATIONAL CRYO-EM FACILITY (NCEF) UPDATE—DR. ULRICH BAXA

Dr. Ulrich Baxa, Senior Microscopist, NCEF, FNLCR, provided an update on the NCEF, which has the mission of addressing the gap between the need for cryo-EM and access to expensive instrumentation. The NCEF opened on May 15, 2017, with one Titan™ Krios microscope. A Glacios screening microscope became operational in December 2018, and a second Krios was put into service in May 2019. All instruments will eventually be housed at the Advanced Technology Research Facility (ATRF) on the FNLCR campus. A third Krios will be added if demand increases. To date, the NCEF has supported 250 data collections for 60 different extramural research groups from 32 different U.S. academic institutions; overall user feedback has been positive. Many of the data sets have resulted in high-quality images—near-atomic resolutions are now achievable—useful for elucidating structures. User research is being published in high-impact journals, including *Nature Communications, PNAS, Nature,* and *Nature Structural and Molecular Biology*; the first article appeared only 10 months after the NCEF began operation.

Dr. Baxa described the organizational structure of the NCEF and acknowledged key personnel who will be responsible for daily operations as follows: as Senior Microscopist, Dr. Baxa serves as the technical lead for the facility and is supported by two other microscopists, Drs. Thomas Edwards and Adam Wier; Scientific Project Manager Helen Wang; and two Information Technology Support Specialists, Mr. Matt Hutchison and Mr. Joseph Finney. Dr. Sriram Subramaniam, Founding Director, NCEF, and former Program Advisor, will, after a 1-year separation of service from the NCI, return as a consultant.

Dr. Baxa explained that the overall NCEF workflow begins when potential users purify their protein or molecular complex of interest suitable for cryo-EM, optimize freezing conditions, and submit a sample information form (SIF). Once the SIF has been approved, users are permitted to send prepared samples as frozen EM grids to the facility, where they are screened for imaging quality and properly stored until the scheduled imaging date, which is determined by a dynamic scheduling queue. Users access the facility remotely, attend meetings via WebEx, and can view the images in real time and make comments. Each user is allotted a maximum of 48 hours of imaging time. Data are collected, compressed, and transferred after each session, downloaded via the Globus platform, and stored at the facility in a secure 100-terabyte server for up to 1 month. Data quality control (QC) is performed on each run in the form of global motion correction and contrast transfer function fit and results are live-streamed to a monitoring website made available to users.

Dr. Baxa described one example of a user project workflow highlighting the NCEF process from start to finish. Dr. Cynthia Wolberger at Johns Hopkins University School of Medicine recently published in *Cell* data collected in the NCEF to solve the structure of the disruptor of telomeric silencing 1-like (DOT1L) methyltransferase-histone 2B (H2B)-ubiquitinated nucleosome complex, which is implicated in leukemias. To begin the data collection process, a SIF containing general details on the nature of the cancer-related project was submitted on September 6, 2018 and approved on the same day because sample quality prescreen checks were satisfactory. The run date was set by the scheduler for 6 weeks after SIF approval, and the sample was delivered to the NCEF for imaging on October 31, 2018. A total of 2,284 images (i.e.,

movies) were collected over the 48-hour run period, and a detailed report containing the data acquisition parameters, QC data, and image ice thickness was automatically generated. On November 6, 2018, a link was provided to the user for data download. Dr. Wolberger's manuscript was submitted to *Cell* on December 14, 2018 and published on February 11, 2019.

Dr. Baxa highlighted other recent NCEF success stories—which can be accessed from the NCI website (https://www.cancer.gov/research/resources/cryoem/publications/ncef-publications)—and noted the NCEF's performance metrics and future plans. Since inception, the microscope has been operational for 96 percent of the time, based on a 365-day calendar. Of the 96 percent, 83 percent is spent on user projects, 8 percent on routine maintenance cycles, and 5 percent on installing and testing software. The microscope's actual downtime is just 4 percent. The Krios 1 will be relocated in July–August 2019 to the ATRF, outfitted with a Gatan BioQuantum K3 imaging filter, and returned to operation by October 2019. The NCEF has plans to increase the number of user projects and implement several outreach efforts.

In the discussion, the following points were made:

- The NCEF leadership should develop a procedure to ensure that users acknowledge in their publications that the data being reported were supported by the NCEF.

- The NCEF operational team could explore developing best practices on the start-up process and operation of a cryo-EM core facility, which could be shared with the structural biology research community.

VI. NCI/DEPARTMENT OF ENERGY (DOE) COLLABORATIONS WORKING GROUP UPDATE—DR. PIERMARIA J. ODDONE

Dr. Oddone updated the FNLAC on the accomplishments and lessons learned in the first 3 years of the NCI-DOE collaboration and conveyed the NCI/DOE Collaborations Working Group's (Working Group) opinions regarding specific aims and directions for years 4 and 5. He noted that the NCI-DOE partnership is designed to accelerate the use of high-performance computing, including artificial intelligence and deep neural networks. The NCI will apply these innovations to better understand cancer biology to develop more effective cancer therapies, whereas the DOE will leverage them to assist in implementing exascale computing infrastructure. The mission of the Working Group is to provide scientific evaluation of programs, projects, and activities formed in support of the NCI-DOE collaboration and to advise the FNLAC and NCI Director on related projects to enhance value to both agencies. The Working Group activities are to provide technical evaluation of the JDACS4C three Pilot projects (Pilots), including the uncertainty quantification (UQ) efforts. The Working Group also provides guidance on crosscutting initiatives—CANcer Distributed Learning Environment (CANDLE) and ATOM—and explores new collaborative possibilities to mutually advance the missions of the NCI and DOE. Dr. Oddone emphasized that each Pilot is a major research project involving significant collaboration and exploring difficult problems that will require sustained efforts over many years. Pilot 1 focuses on cancer at the cellular level, Pilot 2 at the molecular level, and Pilot 3 at the population level. Each Pilot team has refined or expanded its Specific Aims for the next 2 years, as well as the UQ efforts.

Pilot 1—Predictive Modeling for Pre-Clinical Screening. *Aim 1.* Advance state-of-the-art machine learning models for patient-derived-xenograft (PDX) and patient-derived organoid (PDOrg) drug response predictions. *Aim 2.* Develop low-data learning methods aimed at maximizing the value of high-cost experiments. *Aim 3.* Develop methods for interpretability of deep learning models for hypothesis formation and explainability. Dr. Oddone explained that Pilot 1's foundation has been established with the ongoing development of machine learning–based predictive models, followed by UQ analysis and design of optimal experiments. These processes will lead to hypothesis formation and mixed modeling. Methods and computer systems generated in Pilot 1 are available to the public through the CANDLE platform.

Dr. Oddone summarized the Working Group's observations and suggestions for Pilot 1. The limited use of cell line-based predictions suggests that PDX and PDOrg models are preferred. Because the availability of these models is limited, further understanding of machine learning and UQ in the context of limited statistical samples will be required. The main challenge is not algorithmic but related to data availability. A formal definition of the minimum requirements for a suitable machine learning data set in language that is informative to cancer biologists is necessary. The Pilot is advised to review the utility of transferring learning from cell lines to PDOrg and PDX.

Pilot 2—RAS Biology in Membranes. *Aim 1.* Pursue spatial, hierarchical multiscale modeling. Extend the two-scale simulation approach to a third scale that provides atomistic resolution and incorporate membrane curvature into the macroscopic model. *Aim 2.* Understand activation of the extended RAS complex. Deepen understanding of the RAS signaling cascade and RAF-RAS interactions and extend the workflow to enable simultaneous communication across three scales. *Aim 3.* Develop a machine learning-enabled dynamic validation approach to high-fidelity simulation. Define a machine learning approach to handle scale-transition decisions across three levels. Dr. Oddone explained that Pilot 2's extended aims for Years 4 and 5 align with the broad categories of their original specific aims. He summarized that the Working Group observed that Pilot 2 has substantially increased knowledge of RAS membrane interactions at the macroscopic and molecular scales. The structural data on various related proteins allow modeling interactions at the atomistic level. Expanding to include an atomistic level is necessary to understand in greater detail how KRAS4b binds to other molecules on the membrane lipid surface, thereby initiating the signal transduction cascade. Experiments on artificial membranes (e.g., nanodiscs) verify the Pilot's computational models, which are filling knowledge gaps that cannot be observed experimentally about interactions of KRAS4b with the lipid membrane and other proteins. The insights gained from the two-scale simulations have been informative. Adding a third scale will greatly expand the Pilot's capabilities. The Working Group highlighted the importance of fostering detailed and clear exchanges of information between the simulations and biological experiments so that the simulations can fill gaps in experimental knowledge and vice versa. Sustained *ab initio* (i.e., from the beginning) modeling of this complex experimental system is required to identify new potential RAS therapeutic targets.

Pilot 3—Population Information Integration, Analysis, and Modeling for Comprehensive Cancer Surveillance. *Aim 1.* Advance machine learning for scalable information extraction from unstructured clinical reports and medical images. *Aim 2.* Provide scalable and visual analytics to understand the associations of patient trajectories and the exposome with patient outcomes and to enable better matching of clinical trials. *Aim 3.* Produce precision data-driven modeling of patient trajectories with a focus on cancer recurrence. Dr. Oddone expressed that Pilot 3's natural language processing developments are being implemented in cancer registries; the Pilot team aims to scale up to include 35 percent of the U.S. population in the next 2 years. The ultimate goal—a Surveillance, Epidemiology, and End Results (SEER) Knowledge Graph—will automatically link cancer diagnoses and pathology reports with additional data sets in environmental, behavioral, and comorbidity categories. The Knowledge Graph will inform clinical trial matching and make available an individualized cancer risk score. The Working Group suggested that Pilot 3 distribute tools and convene a collaborative community similar to NCI's RAS Initiative. Within Aim 1, the Working Group suggested applying existing and novel deep-learning methods through CANDLE to pathology and medical images at scale. Within Aim 2, the Pilot can provide a framework and infrastructure for matching cancer patients to clinical trials if the trials and patient phenotypes are computable. A long-term goal within Aim 3 would entail building a predictive model for recurrence for all cancer patients.

UQ Efforts and Future Directions. The JDACS4C UQ team supports all three Pilots. UQ explores the uncertainty of individual predictions, assesses the quality of input data, improves model quality, and determines the quantity and quality of data needed. Dr. Oddone indicated that the UQ efforts have helped cancer researchers to consider uncertainty in their analyses. UQ plans for Years 4 and 5 are directed at transfer learning, addressing the limited quantity of data, and efficient abstention based on

constraints within the data. The Working Group provided two suggestions to the UQ team. (1) In terms of Pilot 2 multiscale simulations, consider some of the underlying principles of Bayesian optimal experimental design, which may share similarities to the challenges of healing coarse simulations, thus balancing exploration with exploitation. (2) Tracking UQ developments about the reliability of machine learning models in general, beyond the existing heteroscedastic approaches, would benefit the computer science community.

Year 3 Conclusions and Perspective on Future Directions. Dr. Oddone highlighted the Working Group's conclusions about the JDACS4C Pilots. All three Pilots have developed notably throughout the past 3 years, and the UQ work has been essential for the predictive models. The Pilots are helping to define the quality and types of data necessary to make progress. The focused aims for Years 4 and 5 resulted from the lessons learned from the first 3 years. In closing, Dr. Oddone provided his perspective on the future of the NCI-DOE collaboration, emphasizing that both agencies would need to determine how the collaboration evolves beyond Years 4 and 5. He suggested that the NCI and DOE perform an in-depth technical review of each Pilot prior to the end of Year 5 to facilitate the transition into NCI programs. The NCI should begin to consider how machine learning and deep neural networks can be applied to solving additional cancer problems beyond the Pilot research areas. The NCI and DOE will need to determine whether this collaboration will become a sustainable and stable partnership.

In the discussion, the following points were made:

- Identifying targets from cancer cell lines might work for subsets or certain cell types of cancers, but not for all cancer cell types. Data from cell lines have not translated into the development of PDX or PDOrg models.

- Pilot 3 plans to make de-identified data available to the broader cancer research community. Pilot 3 will make its algorithms available to all U.S. cancer registries, as well as to individual and commercial investigators.

- As clinical databases often contain imperfections, Pilot 3 data are curated by internal checks: Human readers can eliminate some errors, and remaining imperfections are analyzed by UQ.

- UQ is a very important component of Pilot 3's efforts. The Pilot team uses abstracted and manually curated information that is edited and reviewed for its initial algorithm development to support automation. The algorithm has been incorporated into six SEER cancer registries to date, and its performance is iteratively tested, reviewed, and modified by DOE scientists.

Motion. A motion to accept the report of the FNLAC A*d Hoc* NCI-DOE Collaborations Working Group was approved unanimously.

VII. CANCER MODELS AND THERAPEUTICS DEVELOPMENT WORKING GROUP UPDATE—DR. JAMES H. DOROSHOW

Dr. James H. Doroshow, Deputy Director, Clinical and Translational Research, and Director, DCTD, presented the update from the Cancer Models and Therapeutics Development Working Group and focused on the model development efforts and activities of the NCI Patient-Derived Models Repository (PDMR). He noted that the NCI is seeking advice on topics that could be addressed at future Working Group meetings. The Working Group met via teleconference on June 17, 2019, and discussed the issues, challenges, and activities of the PDMR. He acknowledged the Working Group members. Dr. Doroshow reminded the FNLAC members that the idea of a PDMR began in 2015–2016 and went live in May 2017, with the goal of collecting and developing a minimum of 1,000 clinically annotated and molecularly

characterized PDX models for the research community in collaboration with the NCI-Designated Cancer Centers and NCI Community Oncology Research Program (NCORP) Community and Minority/ Underserved Community sites. The expectation is to have 50 models in varying histologies, which would be made available to the extramural research community at a low cost compared with other distributors. Ultimately, tumor biopsies or surgical samples from a single patient could be used to generate multiple models, including the PDX, patient-derived tumor cell cultures (PDCs), cancer-associated fibroblasts (CAFs), and PDOrgs to support *in vitro* drug screening and *in vivo* molecular characterization of patient tumors.

Model development and characterization for the PDMR requires a multilaboratory effort. Dr. Doroshow and the DCTD provide leadership and scientific and technical oversight to the PDMR. Dr. Yvonne Evrard, PDMR, FNLCR, provides administrative supervision, scientific and technical oversight, contract management, and interface with clinical colleagues. Dr. Melinda Hollingshead and the DCTD Biological Testing Branch oversee the *in vivo* PDX development studies, *in vitro* studies, and preclinical efficacy studies. Dr. Dianne Newton, FNLCR, and the *In Vitro* Evaluation Group are responsible for cell line and organoid development. Dr. Mickey Williams, FNLCR, and the Molecular Characterization Laboratory perform next-generation sequencing and bioinformatics services. Dr. Doroshow pointed out that the PDMR currently has 233 PDX models publicly available that span several solid-tumor histologies; 211 additional models are awaiting a final QC; more than 300 models currently are in passage 1–4 (i.e., subcultures); and approximately 400 models are in passage 0. The PDMR distributes median passage (e.g., passage 2) clinically annotated and molecularly characterized models. Specimens collected include patient samples of primary tumor and metastatic disease and of both heavily pretreated and treatment-naïve cases.

PDX models externally deposited in the PDMR consist of 179 models from the Cancer Moonshot℠–funded PDX Development and Trial Centers Research Network (PDXNet), 126 models from the DCTD administrative supplement grantees, and 27 models from the extramural academic centers. The specimen intake process involves a required implantation and expansion with mouse isolators to minimize pathogen contamination, and batch implantation by the contributing center is monitored for growth. The issues are that isolator space is limited and only one specimen per contributor can be supported. Despite the internal processing precautions, three external sites submitted models with pathogen contaminations and additional quarantine and testing have been implemented in the PDMR.

Dr. Doroshow remarked that the PDX tumor take-rates (i.e., growth in culture) from tissue implantations into host mice align with the published data but vary across disease states and histologies. Colorectal cancer has a 60 to 70 percent tumor take-rate, for example, whereas hormone-responsive breast cancers, prostate cancers, and renal cancer tumor take-rates range from 7 to 18 percent. Surprisingly, the PDX tumor take-rate assessment by tumor source was similar for specimens collected from surgical resection, tumor biopsy, and malignant effusion. In response to the FNLAC's prior recommendations on collaborating with institutions to collect specimens from warm autopsies, the PDMR team is partnering with Johns Hopkins University and University of Nebraska Rapid Autopsy Programs (RAPs) to obtain autopsy specimens from primary tumors and metastatic disease. To date, 316 RAP specimens collected from 50 to 60 patients have been submitted to the PDMR.

Overall, a significantly high number of understudied cancer histologies (e.g., Merkel Cell carcinoma) are represented in the available PDX models. The inferred ancestry of the PDMR based on whole-exome sequencing and self-reporting reveals a disproportionately high European representation compared with minority populations. The NCI has since funded two minority PDXNet sites and the minority NCORP sites are now enrolling patients in their trials. Currently, 73 two-dimensional PDCs and 134 CAFs are publicly available. Both will require specialized, defined media and culturing conditions. The first 46 PDOrg models are publicly available, and 30 are awaiting a final QC. The goal, whenever possible, is to develop all four models for comparative preclinical studies. A total of 26 matched PDX, PDOrg, PDC,

and CAF models are contained in the PDMR. Some models generated from matched specimens have demonstrated a clear heterogeneity with respect to a therapeutic drug response. The PDMR has distributed 688 total models to more than 22 different U.S. laboratories and 3 biotechnology companies via a Material Transfer Agreement (MTA). Investigators must not redistribute the models received under the signed MTA to others and are required to inform the PDMR team about the use of data generated from models for publications.

Dr. Doroshow detailed the Working Group discussion and input. Two recent challenges/issues were highlighted. (1) The newly identified Mouse Kidney Parvovirus (MKPV), which was first described in 2018 as causing murine nephropathy, was found in more than 20 breast cancer specimens received from external sources. The PDMR team revised all aspects of how externally derived PDX deposits are controlled within the DCTD Biological Testing Branch. All PDMR model recipients were notified of the issue, as were PDXnet members. The corrective actions included discontinuing distributions for 2 months, checking all stocks for MKPV, and developing standard operating procedures (SOPs) with details of commercial testing availability for MKPV. The SOPs are posted on the NCI/PDMR website, and MKPV testing is now routine in the PDMR. (2) Because more than 100 colon adenocarcinoma PDX models are available, the PDMR has stopped accepting these specimens for model development. Specimens with unique histologies or mutational status will be reviewed on a case-by-case basis.

The Working Group discussed ways to improve knowledge of model availability in the research community. Current efforts include blast emails to DCB and DCTD grantees each time a substantial set of new models is released. Also details of new models are announced on the NCI Treatment Twitter account. Regarding organoids, the PDMR team is attempting to make organoids from tumor types that traditionally are not thought to make organoids. One strategy is to promote new SOPs for nontraditional organoid propagation. The NCI is seeking advice on the best use and market for CAFs, preclinical assessment, and uses for Epstein-Barr virus-transformed diffuse large B-cell lymphoma-like models. The Working Group discussed other PDMR-related activities, such as PDXNet clinical trials, efforts to support the NCI-DOE collaborations in predictive model development, and imaging studies.

In the discussion, the following points were made:

- Although HLA-typed PDX models are currently not being developed in the PDMR, other investigators who receive the models via the MTA could be considering this type of study and they are expected to report back data to inform future studies.

- The NCI PDMR is a worthwhile resource and a significant cost savings to cancer researchers.

Motion. A motion to accept the report of the FNLAC *Ad Hoc* Working Group on Cancer Models and Therapeutics Development was approved unanimously.

VIII. ACCELERATING THERAPEUTICS FOR OPPORTUNITIES IN MEDICINE (ATOM): COMPUTATIONALLY-DRIVEN DRUG DISCOVERY—DR. LEONARD P. FREEDMAN

Dr. Freedman updated the FNLAC on the ATOM research approach, data and modeling capabilities, pilot projects, and current and future milestones. ATOM, a public-private partnership that began in 2017, integrates high performance computing, diverse data, and emerging technologies in a new platform designed to accelerate cancer drug discovery. Launched under a cCRADA, the goal of ATOM is to shorten the time of preclinical drug development from 6 years to 1 year and, in parallel, reduce costs and the rate of drug failures. ATOM's four founding members—FNLCR, Lawrence Livermore National Laboratory (LLNL), GlaxoSmithKline (GSK), and the University of California, San Francisco (UCSF)—are actively soliciting additional collaborators. The main operations are centrally located near UCSF's Mission Bay

Campus. The ATOM-FNLCR Team includes Drs. Dmitrovsky and Stahlberg, ATOM Governing Board; Dr. Nissley, ATOM Joint Research Committee; Dr. Izumi Hinkson, ATOM Operations and Research and Development; and Drs. Beth Winger and Benjamin Madej, ATOM Technical Team. Dr. Freedman noted that he serves as a Governing Board observer and brings a different perspective to the ATOM activities.

The ATOM research and development approach integrates data computation, experimentation, and active learning. Four integrated project teams focus on either pharmacokinetics, safety, efficacy, or chemistry design. These teams incorporate compound, cellular assay, protein, and clinical data, which are curated to develop predictive models. Experimentation fills data gaps and validates predictions. The workflow consists of a high-speed *in silico* multiparameter optimization loop, which proposes new molecules with optimized properties, in parallel with molecular feature simulations and an empirical experimentation loop fed by active learning. ATOM's active learning approaches will accelerate the drug discovery timeline. Currently, drug discovery after target identification requires 1.5 years for lead discovery, 3 years for lead optimization of molecules, and 1.5 years for preclinical experiments. By 2021, ATOM aims to reduce the lead discovery and lead optimization portions of drug discovery to less than a year and 1 year, respectively. ATOM will complete a major proof-of-concept project to validate this goal.

Dr. Freedman detailed ATOM's major milestones toward the proof-of-concept project, including milestones already accomplished and those remaining. ATOM already has developed significant data and modeling capabilities in its first year. Its integrated platform has incorporated 2 million compounds from GSK. In 2018, ATOM researchers curated 169 model-ready data sets into usable formats for machine learning, a substantial effort. The team completed and validated the data-driven modeling pipeline, built baseline pharmacokinetics and safety models, and generated new data to expand models. One example application of the multiparameter optimization loop began with two preset design criteria for a cardiac safety marker. The loop ran for 30 cycles and successfully generated molecules *in silico* that fit the design criteria. In 2019, the team completed and performed initial validation of a human system physiologically based pharmacokinetic (PBPK) simulation model. ATOM's machine-learned models predict a designed molecule's parameters and input these into a PBPK simulator. The simulations generate time-concentration profiles, leading to summaries of pharmacokinetic parameters in various organ systems, plasma, and tumors.

Dr. Freedman described a promising pilot project on the generative molecular design of aurora kinase inhibitors that has further exercised ATOM's active learning components in 2019. The aurora kinase family of cell proliferation regulators is associated with tumorigenesis and has undergone numerous prior studies. The initial 24-hour design loop run generated more than 600,000 compound structures *in silico*. The machine-learning pipeline predicted the potency and selectivity for each compound. There were 150 compounds that met the nine predetermined design criteria of the pilot project. ATOM researchers will continue to validate these results. Efforts also are focused on generating new data and models for the active drug-induced liver injury program and scaling up the design loop architecture. In 2020, the ATOM active learning platform will be integrated and further validated to selectively drive mechanistic simulations and experimentation. The proof-of-concept project will initiate for expected delivery in 2021.

Dr. Freedman conveyed that ATOM scientists have published one article titled "Artificial Intelligence and Pharmacometrics: Time to Embrace, Capitalize, and Advance?" in the April 2019 issue of the *CPT: Pharmacometrics & Systems Pharmacology*. The ATOM Team has submitted and is in the process of writing additional papers. ATOM has expanded its engagement among members and collaborators, with the goal of adding additional partners. Dr. Freedman emphasized that the NCI views the ATOM platform as a resource for the research community. ATOM models could be employed in the future for precision medicine using patient-specific data, developing biotherapeutics, or even conducting diagnostic imaging tests.

In the discussion, the following points were made:

- ATOM's noteworthy advantages include computational power, infrastructure resources of LLNL, and GSK's compound library, but its most unique and novel feature is the public-private partnership.

- Information on molecular structures, molecular dynamics, simulations, and experimental observations are incorporated into the ATOM workflow. The platform uses several different models to understand the interactions between the molecules and potential targets.

- ATOM and the JDACS4C Pilot 1 team communicate with each other; some members overlap. The ATOM platform could eventually be applied to assist the RAS Initiative, but RAS was too challenging a topic to develop initially. Kinase inhibitors were better for ATOM to engage early because the cancer research community has focused extensively on these compounds for drug development.

- The ATOM platform is in place, but the collaborators would be open to other computational workflow ideas from new partners. The platform is designed to be flexible, supporting multiple models that may emerge and evolve over time.

- Each of the ATOM partners has contributed to the 25 full-time staff stationed at UCSF Mission Bay. The FNLCR and other partners contribute additional staff resources as needed.

IX. PROGRESS IN TARGETING KRAS: UPDATE FROM THE FNLCR RAS INITIATIVE—DR. FRANK MCCORMICK

Dr. Frank McCormick, Professor, Helen Diller Family Comprehensive Cancer Center, UCSF, provided an update on the RAS Initiative progress in targeting KRAS for therapeutic drug design. He announced new additions to the RAS team to strengthen capabilities in several key areas in response to prior FNLAC recommendations: RAS researcher, Dr. Caroline DeHart, formerly of Northwestern University; nuclear magnetic resonance (NMR) specialist, Dr. Alok Sharma, formerly of Warpdrive; NMR specialist, Dr. Gabriel Cornilescu, formerly of the University of Wisconsin–Madison; and computational drug designer, Dr. Trent Balius, formerly of UCSF. Aside from expanding NMR capabilities in staffing, a Brucker 700-megahertz NMR spectrometer has been purchased to support the RAS Initiative and is currently being installed at the ATRF.

Dr. McCormick reported that quality publications are being generated, with manuscripts in various stages of completion. A recent publication on KRAS and calmodulin binding by Dr. Constance Agamasu, a postdoctoral fellow in the RAS Biochemistry and Biophysics Group, was featured on the cover of the March 2019 issue of the *Biophysical Journal*. A publication on KRAS recruitment to the plasma membrane by senior author Dr. Frantz Jean-Francois and colleagues was featured on the cover of the December 2018 issue of the *Journal of Biological Chemistry*. In addition, a manuscript on the membrane interaction of the G-domain and KRAS hypervariable (HVR) region by Dr. Debanjan Goswami and colleagues in the RAS Image-Based Screens Group has recently been accepted for publication.

Structural Biology. FNLAC members were reminded that the crystal structures for active forms of wild-type KRAS and six oncogenic mutant forms of KRAS have been solved by Dr. Dhirendra Simanshu and the RAS Structural Biology Group. Three of the KRAS mutants—

G12C, G13D, and Q61L—are in the state I conformation and provide new hydrophobic pockets for drug discovery. These structures are being investigated using *in silico* screening techniques in an ongoing collaboration with Dr. Nir London at the Weizmann Institute for Science (WIS). Recent efforts are focusing on using fragment screening by crystallography for compounds that bind the KRAS G13D mutant. Compounds identified in the Diamond Light Source Ltd. (United Kingdom) fragment screen are being further evaluated in biophysical analysis at the FNLCR. Inside the mammalian cell, KRAS undergoes N-terminal processing, resulting in a lack of the initiator methionine (Met) and an acetylated N-terminal state. Dr. Simanshu solved the crystal structure of N-acetylated KRAS-GDP for the first time at a resolution of 1 angstrom (Å). Surprisingly, removing Met from KRAS resulted in structural changes, including a loss of magnesium, an extended form of the switch I region, and a new beta-strand.

Covalent Inhibitors. Dr. McCormick explained that Dr. Anna Maciag and her team conducted experiments focused on identifying covalent inhibitors (1) targeting KRAS4b-C185, which is a site of KRAS processing; and (2) histidine (H) 95, a unique residue in KRAS. The covalent binders to C185 in KRAS4b, identified using a UCSF tethering library, inhibit post-translational processing and proliferation of KRAS4b G12D in mouse embryo fibroblasts (MEFs), but not myristolated (Myr) KRAS G12D-C185S MEFs. The KRAS protein level in cells was decreased, and the accumulation of KRAS at the membrane was disrupted, suggesting that the compounds are on target. Experiments evaluating target engagement in live cells using mass spectrometry (MS) analysis showed an increased molecular mass in KRAS4b (1-188) labeled with the compound FNL0175, a covalent binder, introduced into human embryonic kidney (HEK) 293 cells compared with unlabeled HEK 293 cells. Investigating on-target versus off-target effects of the compounds directly binding KRAS using KRAS4b G12D MEFs showed inhibition of cell growth at 1 to 2 micromolar concentrations compared with off-target effects at concentrations 50 times higher in Myr-KRAS G12D-C185S MEFs. The next steps will be to analyze these compounds for drug-like properties using *in vivo* animal models.

To determine the compound docking site, computational analyses via the DOE molecular dynamic (MD) simulations of KRAS4b without compound were used to show that the HVR stably interacts with the G-domain of the protein. The NMR analysis of two KRAS4b constructs, 1-169 and 1-188, revealed that the HVR binds between helix three (H3) and helix four (H4). Additional MD simulations also revealed that the HVR interactions with the G-domain in the H3/H4 region are specific to KRAS4b and not NRAS, suggesting a selective means for targeting KRAS. Use of small-angle X-ray scattering (SAXS) methods to analyze KRAS4b188 without a compound and KRAS4b188 tethered to a covalent inhibitor, FNL0184, showed that the compound stabilizes the HVR and also suggested a conformational change in KRAS4b. These data were corroborated using chemical shift and NMR analysis.

Regarding H95 targeting, Dr. McCormick pointed out that novel small-molecule compounds targeting H95 have been identified by Dr. Maciag and the FNLCR's medicinal chemistry group. The DOE MD simulations suggest a binding pocket between H3 and the switch 2 region, which was confirmed by NMR. There is evidence for target engagement in cells, and specificity for the KRAS G12D mutant exists. He noted that this work is being performed in collaboration with the KRAS cCRADA, which supports 15 full-time medicinal chemists; five at FNLCR. Dr. McCormick was happy to report that the RAS Structural Biology Group solved crystal structures of the KRAS H95/switch 2 pocket in January 2019, which they further optimized in March 2019. Active drug design based on the actual structures, rather than animations and projections, is now possible.

Disulfide Tethering Library. Dr. McCormick introduced a new initiative being led by Dr. David Turner and the FNLCR medicinal chemistry group, which is focused on developing a

disulfide tethering library in-house that leverages the UCSF work. The aim is to develop a library of 1,500–2,000 high-quality and chemically diverse fragments and minimize overly complex molecules. Clustering-based methods are being used to determine the degree of library diversity and chemical space. The molecular properties of the tethering library have been evaluated to optimize the fragment-based portions of the molecules in terms of molecular weight, hydrogen bond acceptors, and chiral centers for efficient screening assays. In addition, a proprietary virtual library with a 5-year exclusivity agreement consisting of 13,000 carboxylic acid building blocks coupled virtually with a disulfide tether is being developed in collaboration with a contract research organization. The FNLCR disulfide tethering library will serve as an internal resource for the NCI and expand collaborations with academic groups and potential biotechnology and pharmaceutical partners.

Cysteine Mutant Library. FNLAC members were informed that the project to develop a KRAS cysteine mutant library is underway and is being led by Dr. Dominic Esposito and the RAS Reagents Group. The project goals are to use cysteine surface saturation mutagenesis to generate a library of KRAS proteins that contain single-point mutations to cysteine; validate the functionality of purified mutants in terms of their stability, proper folding, and activity; and utilize mutants for drug discovery and for understanding of RAS biology. The library was defined using the parental protein, KRAS4b (1-169) G12D/C118S and two methods, designed by Drs. Simanshu and Simon Messing, were utilized for determining KRAS surface residues. For the surface accessibility metric, a cutoff of 95 clones was selected. The distribution of cysteine mutations in KRAS4b (1-169) G12D/C118S was validated using NMR. A synthetic library of DNA clones was obtained for all 95 mutants, *Escherichia coli* expression was performed on a small scale for all mutants, the protein was successfully purified, and large-scaled production of all 95 proteins was completed in April 2019. Cysteine residues located in close proximity to KRAS binding pockets of interest will be further validated in screening assays using the FNLCR tethering library.

Modeling RAS and RAF on Membranes. Dr. McCormick remarked that the JDACS4C Pilot 2 described earlier in the meeting has been a very productive collaboration involving the DOE computational analysis and prediction of KRAS membrane interactions coupled with FNLCR biophysical and biochemical analysis of KRAS on synthetic membranes. To build the computational models, the RAS Biochemistry and Biophysics Group led by Dr. Andy Stephen investigated KRAS membrane orientation in neutron reflectivity and paramagnetic relaxation enhancement NMR (PRE-NMR) experiments. The results revealed that the KRAS center of mass is approximately 30 Å from the lipid headgroups. Protein footprinting identified several residues within the HVR that directly contact the membrane, but not within the G-domain. No chemical shift perturbations are observed between free-soluble KRAS or KRAS bound to lipid nanodiscs in two-dimensional NMR experiments.

PRE-NMR analysis identifies membrane bound states for KRAS. In fact, docking KRAS on a 2-lipid membrane system using Haddock revealed three conformations: (1) exposed conformation, which consists of the switch I region and RBD; (2) occluded conformation, in which switch I is not available for binding; and (3) the semi-exposed conformation, in which switch I is close to the membrane but no binding occurs. The DOE has developed a framework to couple macroscale (atomistic) and microscale (coarse grain) simulations. Macro model simulations capture the influence of the membrane on RAS in an 8-lipid dynamic membrane with KRAS molecules parameterized into hyper-coarsened particle beads. Additional investigations of the predicted and observed frequencies showed that enrichments for specific lipids correlated to the monomers, dimers, and multimers of RAS.

Efforts also have been focused on a new strategy for blocking RAS signaling, which involve solving the crystal structures of RAF bound to the RAS binding domain (RBD) and the

cysteine-rich domain (CRD) and mapping CRD binding regions. In NMR experiments of RAF1-CRD, results showed that the CRD hydrophobic residues are oriented toward the membrane, the K148 residue is pointing toward the membrane, and other basic residues are close to the membrane. In addition, NMR analysis of spin-labeled lipids revealed that the CRD residues L149, F158, and L160 are deeply inserted in the membrane lipid bilayer. Surface plasmon resonance analysis suggests that RBD-CRD binds more tightly to the membrane than does CRD alone. These data will inform the next phase of Pilot 2 simulations.

In discussion, the following points were made:

- DOE simulations suggest specificity in lipids in the KRAS membranes interactions. Limited data indicate that KRAS4b and CRD show a preference for phosphatidylserine residues.

- The decrease in KRAS protein level in cells with the covalent binders to C185, which is irreversible, is a result of protein degradation.

- The promising RAS inhibitors identified in the RAS Initiative are under the purview of the cCRADAs, and the sponsor would be the one to advance preclinical development.

X. ONGOING AND NEW BUSINESS—DR. LAWRENCE J. MARNETT

Dr. Marnett requested input from the Committee regarding any remaining issues. Dr. Catherine M. Bollard, Director, Center for Cancer and Immunology, Director, Program for Cell Enhancement and Technologies for Immunotherapy, Children's Research Institute, Children's National Health System, suggested establishing a FNLAC Cell Therapy Development Working Group, which could advise on new NCI initiatives.

Future Agenda Topics. FNLAC members discussed future agenda topics including how FNLCR interacts with the extramural community. Dr. Nana-Sinkam also recommended considering strategies for forming partnerships in the communities for addressing issues pertaining to vulnerable populations. Dr. Marnett reminded members to send any additional potential agenda topics for future FNLAC meetings to Dr. Caron Lyman; in the interim, he will forward the complete list of the previously identified FNLAC agenda topics to the members for review.

The next FNLAC meeting is scheduled for October 24, 2019 and it was suggested that FNLAC members visit the FNLCR in Frederick if schedules allow.

XI. ADJOURNMENT—DR. LAWRENCE J. MARNETT

Dr. Marnett thanked the Committee members and other invitees for attending. There being no further business, the 16th meeting of the FNLAC was adjourned at 3:40 p.m. on Thursday, June 27, 2019.

Date	Lawrence J. Marnett, Ph.D., Chair
Date	Caron A. Lyman, Ph.D., Executive Secretary