Department of Health and Human Services
Public Health Service
National Institutes of Health (NIH)
National Cancer Institute (NCI)

13th Meeting
Frederick National Laboratory Advisory Committee

Summary of Meeting
October 30, 2017

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

National Cancer Institute
13th Meeting of the Frederick National Laboratory Advisory Committee
to the National Cancer Institute
October 30, 2017

Summary Minutes

The Frederick National Laboratory Advisory Committee (FNLAC) convened for its 13th meeting on 30 October 2017 at 31 Center Drive, Building 31, C Wing, Conference Room 10, Sixth Floor, Bethesda, MD. The meeting was open to the public on Monday, 30 October 2017, from 9:00 a.m. to 3:52 p.m. The FNLAC Chairperson, Dr. Lawrence J. Marnett, Dean of Basic Sciences, University Professor, Mary Geddes Stahlman Professor of Cancer Research, and Professor of Biochemistry, Chemistry, and Pharmacology, Vanderbilt University, presided.

FNLAC Members
Dr. Lawrence J. Marnett (Chair)
Dr. Gail A. Bishop (absent)
Dr. Lisa M. Coussens
Dr. Kevin J. Cullen
Dr. Levi A. Garraway
Dr. Angela M. Gronenborn
Dr. Robert L. Grossman
Dr. Klaus M. Hahn
Dr. David I. Hirsh
Dr. Elizabeth M. Jaffee (absent)
Dr. Sanford D. Markowitz (absent)
Dr. Piermaria Oddone (absent)
Dr. Kenneth J. Pienta
Dr. Nilsa C. Ramirez-Milan
Dr. Cheryl L. Willman
Dr. Jedd D. Wolchok (absent)

***Ex Officio* Members**
Dr. Stephen J. Chanock (absent)
Dr. James H. Doroshow
Dr. Paulette S. Gray
Dr. Anthony Kerlavage
Dr. Kristen Komschlies McConville
Dr. Tom Misteli (absent)
Dr. 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 13th 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.

Dr. Marnett called Committee members' attention to future meeting dates listed on the agenda.

Dr. Marnett expressed condolences to the family and colleagues of FNLAC member, Dr. Janet A. Houghton, on her recent passing. Dr. Houghton had been a faculty member at St. Jude Children's Research Hospital for 29 years, was chair of a department at Cleveland Clinic's Lerner Research Institute for 10 years, and was currently in her third year as Senior Research Fellow and Endowed Chair in Cancer Biology at Southern Research Institute.

Dr. Marnett congratulated Dr. Douglas R. Lowy, Deputy Director, NCI, and Dr. John T. Schiller, Center for Cancer Research (CCR), NCI, on receiving the 2017 Lasker-DeBakey Clinical Medical Research Award for their research leading to the development of human papillomavirus (HPV) vaccines.

Motion. A motion to approve the minutes of the July 18, 2017, FNLAC meeting was approved unanimously.

II. REPORT FROM THE NCI DIRECTOR—DRS. NORMAN E. SHARPLESS, DOUGLAS R. LOWY, AND JAMES H. DOROSHOW

Dr. Norman E. Sharpless, Director, NCI, stated that he was pleased to have the opportunity to address the Committee in this capacity and expressed appreciation to the meeting organizers. He noted prior meetings with former NCI Directors who are well acquainted with the activities of the Frederick National Laboratory for Cancer Research (FNLCR), but said that this information is still not well understood in the extramural community. Dr. Sharpless reported that Dr. Lowy will provide the NCI Director's update for today's meeting and pointed out that his goal for today is to listen and learn from the discussions and hear from the Committee their thoughts on FNLCR.

Dr. Lowy welcomed new and continuing Committee members and other attendees. He updated the members on the NCI budget, appropriations, and Cancer Moonshot℠ initiatives. He was joined by Dr. James H. Doroshow, Deputy Director, Clinical and Translational Research, and Director, Division of Cancer Treatment and Diagnosis (DCTD), NCI, who provided an update on NCI's clinical and translational research activities.

Dr. Lowy announced that Dr. David C. Heimbrook will be stepping down as laboratory director of FNLCR and president of Leidos Biomedical Research, Inc., (Leidos). He expressed appreciation to Dr. Heimbrook for his commendable leadership during a period of considerable change and substantial improvement at the FNLCR and in spearheading the development of large-scale projects, including the RAS Initiative and the National Cryo-Electron Microscopy (Cryo-EM) Facility (NCEF), as well as the more recent Cancer Moonshot℠ initiatives. Dr. Lowy remarked on a few of Dr. Heimbrook's exemplary qualities, including his collegial spirit and efforts in improving communications and interactions between the NCI and Leidos and his desire to continue on as a Leidos consultant to enable a seamless transition to his successor, Dr. Ethan Dmitrovsky, former provost and executive vice president of the University of Texas MD Anderson Cancer Center.

The NCI Budget and Regular Appropriations. Dr. Lowy reported that there have been substantial increases in the NCI regular appropriations over the past 3 years; the Cancer Moonshot℠ allotment for fiscal year (FY) 2017 and beyond are separate from the regular appropriations. The NCI is operating under a continuing resolution (CR) that funds the government through December 8, 2017. The NCI/NIH budget process for the regular appropriations is currently between steps two and three of the four-step process for FY 2018. The House Appropriations Subcommittee on Labor, Health and Human Services, Education, and Related Agencies advanced its bill out of committee to increase funding to the NIH by $1.1 billion (B) and to the NCI by $82 million (M); the Senate advanced its bill out of committee to increase funding to the NIH by $2 B and to the NCI by $169 M. Also, $300 M was appropriated for the 21st Century Cures Cancer Moonshot℠ funding. Although more than the President's proposed FY 2018 budget, the House and Senate FY 2018 allowances are similar to the FY 2017 enacted appropriation.

Research supported by regular appropriations are largely non-overlapping with Cancer Moonshot℠ activities and include ongoing and new initiatives, such as the National Cryo-EM Facility and the Tomosynthesis Mammography Imaging and Screening Trial (TMIST), NCI's first large randomized controlled trial in this area in a number of years. The primary goal of TMIST, which is being conducted in collaboration with the Eastern Cooperative Oncology Group and the American College of Radiology Imaging Network (ECOG-ACRIN) Cancer Research Group, is to determine whether the cumulative rate of advanced breast cancer in women undergoing screening with tomosynthesis plus digital mammography is less than among those screened with digital mammography alone. Biannual screening of normal-risk menopausal women, if demonstrated to be effective without a loss of safety, could result in a one-third lifetime reduction in the number of screening mammograms a woman in the United States undergoes. The establishment of a biorepository also is a key feature.

Cancer Moonshot℠ Initiatives. Dr. Lowy reported that the NCI funded the initiation of eight of the 10 Blue Ribbon Panel recommendations in FY 2017. Cancer Moonshot℠ Implementation Teams are continuing to develop initiatives for the remaining recommendations, which will be parlayed into new funding opportunities for FY 2018 and 2019. Further details and updates on the Cancer Moonshot℠ Request for Applications can be accessed on NCI's website.

NCI Clinical and Translational Update. Dr. Doroshow reported on two activities that are supported by FNLCR: the NCI-Molecular Analysis for Therapy Choice (NCI-MATCH) trial and the Cancer Immune Monitoring and Analysis Centers (CIMACs). The NCI-MATCH trial, which opened August 12, 2015, completed its initial phase in May 2017, 2 years ahead of schedule, and is one of the NCI's National Clinical Trials Network (NCTN) most rapidly completed accruals. The trial enrolled and tested tumor biopsies of approximate metastatic sites from 6,397 patients, and 794 were enrolled for treatment. At the time the initial phase ended, of the 30 active treatment arms, 50 percent were fully accrued, 25 percent were close to reaching complete accrual, and 25 percent needed additional accruals for rare mutations. In addition, six new treatment arms have been approved for subsequent studies within the second phase of the NCI-MATCH trial, the Rare Variant Initiative. The assay success rate remained high throughout the study, at 94 percent, partly because of the expertise of the Molecular Characterization Laboratory, FNLCR, which led training and quality control efforts within the network of clinical laboratories to ensure concordance between centers. Objective responses (i.e., measurable responses) have been observed for three of the initial treatment arms and are expected to be reported on at the Society for Immunotherapy of Cancer annual meeting being held November 10–12, 2017, in National Harbor, Maryland. The statistical analysis plan for the NCI-MATCH trial was designed to accrue 35 patients into each treatment arm, with a minimum of five objective responses per arm. After meeting these criteria, the treatment (i.e., targeted agent) would be deemed satisfactory for further evaluation in a Phase II study.

To address the 25 percent of treatment arms needing additional accrual for rare mutations, the NCI-MATCH trial was amended to continue in a second phase as the Rare Variant Initiative. The rationale is that 15 of the 30 treatment arms have mutational prevalence rates of less than 1 percent, and it is therefore challenging to reach accrual goals. Although mutation rates for the common cancers (e.g., breast, colorectal, non-small-cell lung, or prostate) could be estimated from The Cancer Genome Atlas (TCGA) and other sources with some degree of accuracy, the rates for uncommon cancers or low prevalence tumors could not be determined. Furthermore, 60 percent of the patients screened had less common forms of cancers, which was more than expected—the NCI-MATCH provides treatment options for these patients.

Initiating as the first phase ended, the NCI-MATCH Rare Variant trial, in collaboration with two commercial laboratories—Foundation Medicine, Inc. (FMI) and Caris Life Sciences—will refer patients with rare mutations that align with the NCI-MATCH trial treatment arms to one of the 1,100 approved study sites. FMI and Caris will use their own tumor testing platforms, but the results must be verified centrally (e.g., through the network of clinical laboratories) by the NCI-MATCH Oncomine® assay. Also, two clinical laboratories that perform testing on their own patients using their institutional assays—MD Anderson Cancer Center and Memorial Sloan Kettering Cancer Center—have agreed to refer patients. More than 60 patients have been enrolled since the trial began, and the NCI anticipates that this process will inform future precision medicine trials. Future possibilities for the NCI-MATCH trial include responsive phenotypes and new treatment arms.

Dr. Doroshow reported that the CIMACs were funded in October 2017 as part of the Precision Medicine in Oncology and Cancer Moonshot℠ initiatives to improve the standards for immunotherapy biomarker discovery in assay development and enhancements to existing markers. Four centers—the MD Anderson Cancer Center, Icahn School of Medicine, Dana–Farber Cancer Institute, and Stanford University—along with the Pharmacodynamics and Development Laboratory, FNLCR, will work to develop the expertise and assays that will be used across the network of laboratories to support NCI-funded early Phase I and II immunotherapy clinical trials.

Dr. Lowy added that the NCI regular appropriations enabled expanding the NCI-MATCH trial and establishing the Partnership for Accelerating Cancer Therapies (PACT), a public-private partnership with 11 leading pharmaceutical companies. He acknowledged Dr. Doroshow's leadership and the role of Dr. Francis S. Collins, Director, NIH, in this effort.

In discussion, the following points were made:

- The success of the NCI-MATCH trial and the establishment of CIMACs exemplify the strength of the FNLCR to coordinate these efforts to develop and standardize assays and collect data.

- Allowing the NCI-MATCH Oncomine® assay to be used as an initial clinical trial entry point could be leveraged by existing networks, such as the Oncology Research Information Exchange Network (ORIEN), and could increase patient accruals and broaden minority representation in trials.

- Consider the financial effect on and cost to laboratories external to the FNLCR that are already performing next-generation sequencing (NGS) and networks in the decision-making process to establish a centralized Clinical Laboratory Improvement Amendments-validation of the NCI-MATCH trial NGS Assay.

- Consider harmonizing data and leveraging the NCI-funded repositories and registries that may have correlative genomic data, such as the Center for International Blood and Marrow Transplant Research, to assist in establishing databases associated with the CIMACs.

- Develop a structure that would allow cloud-based data sharing without the loss of privacy for individual groups (e.g., Tribal Nations) that are sensitive to sharing their data.

- The NCI has made agreements with the American Association of Cancer Research project Genomics, Evidence, Neoplasia, Information, Exchange (GENIE) and a number of NCI-designated Cancer Centers to share, with appropriate security, their data sets in the Genomic Data Commons (GDC). Other groups, such as ORIEN, also are welcome to share data in this platform.

III. FNLCR OPERATIONAL OVERVIEW—DR. DAVID C. HEIMBROOK

Dr. Heimbrook, Laboratory Director, FNLCR, President, Leidos, provided an overview of the FNLCR operations and an update on new initiatives and also addressed questions raised in prior FNLAC meetings. He expressed appreciation to the NCI leadership, FNLCR staff, and FNLAC for collectively working to enhance the impact of FNLCR during his 6.5-year tenure as laboratory director, which will be ending soon. The scientific mission and support at the FNLCR encompasses a diverse customer base within the divisions, offices, and centers of the NCI and across the NIH. Activities include those found in the academic and pharmaceutical settings. Rather than discussing each of these activities separately, Dr. Heimbrook detailed the framework for mission support, which illuminates four broad categories of activities.

Type 1, contractor-provided research support, involves partnering technical staff with principal investigators within the NCI Intramural Research Program (e.g., CCR) and other NIH ICs. Type 2, contractor-executed research projects, is the broadest category, providing collaborative research resources for government-directed research involving additional technical expertise and scientific leadership for core laboratories, including the Laboratory Animal Sciences Program (LASP). The infrastructures of Type 1 and Type 2 are tailored to match the research support activities and provide procurement support for core services. Type 3, contractor-executed subcontracted projects (e.g., Chemical Biology Consortium [CBC], NCI Experimental Therapeutics [NExT] Program), extend to extramural project support that is primarily executed through subcontracts to academic or commercial centers with technical project manager oversight. Specialized laboratories and computer infrastructure support the national laboratory-directed research function of Type 4, contractor-directed and -executed projects, which also includes partnership development. Examples include the RAS Initiative and the Nanotechnology Characterization Laboratory.

Dr. Heimbrook discussed the weighting of the NCI mission support types. The Leidos Operations and Financial Group led by Dr. Kathy Terlesky, Chief Operating Officer, developed detailed charts depicting the FNLCR total direct science support to the NCI for FY 2016 stratified by mission support type, yielding 33 subcategories spanning 200,000 projects and highlighting the division, office, or center. Type 2, research programs, accounts for approximately 43 percent of costs; research support (Type 1) and contractor-executed (Type 3) projects each account for 22 percent; and contractor-directed and -executed projects (Type 4) represented 12 percent of the total cost. The CCR is the primary center receiving research support and covers the broadest range of the divisions, offices, and centers. The Immediate Office of the Director is the predominate supporter of Type 3 programs, followed by the DCTD, which primarily sponsors Type 2 programs. Normalized to each FY's direct science costs as a percentage, growth in Type 3 and Type 4 programs is predominate, and this trend is expected to continue.

Dr. Heimbrook described new enabling initiatives within the LASP, an essential core capability that supports multiple divisions, offices, and centers at the NCI and other ICs. The facility is led by Dr. Steve Jones and consists of 315 LASP associates who manage NCI's 27 rodent and nonhuman primate research facilities located in Bethesda and Frederick, Maryland. In addition, LASP maintains 133,400 animals occupying 50,000 cages and supports 206 investigators on 551 active animal study protocols. Three

components recently added to facilitate state-of-the-art animal research include the Genome Modification Core (GMC), Small-Animal Imaging Program (SAIP), and the Gnotobiotics Facility (GF).

The GMC leverages existing LASP resources to provide a ready-to-operate centralized core to assist NCI investigators in the design, development, and validation of gene-editing processes (e.g., Clustered, Regularly Interspaced Short Palindromic Repeats [CRISPR]) from initial selection of DNA sequences of the gene of interest to generation of the genetically engineered mouse models.

The SAIP assists NCI investigators in the development of non-invasive monitoring of tumor-bearing genetically engineered and patient-derived xenograft mouse models of human cancers. The recent addition of hyperpolarized metabolic imaging (magnetic resonance imaging [MRI] technology) to this program will permit real-time analysis of biochemical and metabolic changes in these models as well.

The GF will help NCI investigators to develop and monitor germ-free or germ-specific mouse colonies; explore the role of microbiota in inflammation and the development of colon or gastric cancers; and examine the effects of the microbiota on mouse models of lung cancers. The GF is equipped with a biofilm sterilization unit, recently doubled its animal cage capacity, and performs Animal Biosafety Level 2 (ABSL-2) procedures within the isolators.

Dr. Heimbrook reported that the FNLCR received five non-servable (1-year awards) task orders and three servable (i.e., ongoing, long-term) projects supporting eight of NCI's research activities for the Cancer Moonshot℠ Initiative in the three areas of fundamental science, treatment and cures, and health promotion and disease prevention.

Dr. Heimbrook detailed other efforts that the FNLCR supports. The NCI–Department of Energy (DOE) high-performance computing collaboration, the Joint Design of Advanced Computing Solutions for Cancer (JDACS4C), which comprises 3-year pilot projects representing three distinct domains—preclinical (Pilot 1), molecular (Pilot 2), and clinical (Pilot 3)—was announced in 2016. A new public-private partnership, Accelerating Therapeutics for Opportunities in Medicine (ATOM), has emerged from the NCI–DOE collaborations to accelerate the development of more effective therapies for patients, aligning with the goals of the JDACS4C. It will leverage existing resources (biological data) in the biopharmaceutical industry. The founding members—FNLCR, GlaxoSmithKline (GSK), Lawrence Livermore National Laboratory (LLNL), and University of California, San Francisco (UCSF)—signed a Contractor Cooperative Research and Development Agreements (cCRADA) in October 2017 establishing the ATOM Consortium. Other interested parties are encouraged to join. It was explained that a governance board and oversight committee oversee ATOM. The NCI is planning to locate the Consortium near UCSF's Mission Bay Campus in time for a spring 2018 launch. Dr. Heimbrook discussed the ATOM workflow, platform vision, and elements of membership and highlighted contributions from the founding members. Additional details are available on the NCI-Frederick website: frederick.cancer.gov/science/atom.

Dr. Heimbrook introduced the new Laboratory Director, FNLCR and President, Leidos, Dr. Ethan Dmitrovsky. Dr. Dmitrovsky expressed appreciation to the NCI for this opportunity and is looking forward to serving the NIH and the public.

In the discussion, the following points were made:

- Members voiced concerns on the vetting process for establishing the ATOM agreement with the pharmaceutical companies and academic institutions. The process should be clear and transparent because it involves public funds such as the Cancer Moonshot℠ funding. It was noted that plans for the ATOM project were described in detail at the May 2017 FNLAC meeting.

- The NCI Division of Extramural Activities (DEA) is developing a FNLAC Orientation Book and will schedule an orientation session for new and incoming members at the next meeting.

- Provide ways to increase awareness in the extramural community and NCI-designated Cancer Centers about the GF. Having data on the microbiota and cancer publicly available would be an incentive to participate in the GF.

IV. UPDATE: NATIONAL CRYO-EM FACILITY—DR. SRIRAM SUBRAMANIAM

Dr. Sriram Subramaniam, Senior Investigator, Laboratory of Cell Biology (LCB), CCR, NCI, detailed the NCEF's scientific background, implementation, applications to cancer research, and community dissemination. The cryo-EM method as it relates to protein structure elucidation begins with the vitrification (i.e. rapid freezing) of minute amounts of biological samples, which are then subsequently maintained at cryogenic temperatures for electron microscopy. Advances in detection and image processing in the cryo-EM field have led to the development of systems capable of achieving near-atomic resolution, which has greatly improved the quality of images and sparked its use in biology. These improvements also have transitioned structure elucidation from the traditional cryo-EM targets (e.g., viruses or ribosomes) to smaller protein complexes and diverse targets, which are more representative of cellular interactions that are of interest to cancer research. Many researchers are beginning to include cryo-EM as a structural tool alongside X-ray crystallography. When demonstrating cryo-EM for the first time, Dr. Subramaniam's group was able to show the structure for beta-galactosidase and granular features (e.g., water molecules) at high resolution (2.2 angstroms).

Dr. Subramaniam noted that cryo-EM was recognized with a 2017 Nobel Prize and highlighted some of its applications in cancer research, including visualizing the detailed structure of the inhibitor binding site of the cancer target, p97 (NExT Program); identifying the atomic structure of human 26S proteasome to enable better understanding of the asymmetric docking of the regulatory particle onto the core; dissecting small-molecule binding and cofactor regulatory mechanisms of the anaphase-promoting complex; solving the structure of the desensitized glutamate receptor kainite subtype; and solving structures of a CRISPR-Cas surveillance complex.

Dr. Subramaniam remarked on the exponential growth in solving cryo-EM structures at medium and high resolutions, which has been increasing in recent years. In 2014, the NCI realized this growth and sought ways to address the increased interest in cryo-EM and began discussions on establishing a national facility. This effort became a part of a broader NIH initiative to establish state-of-the art regional user cryo-EM facilities. A proposal to establish a facility at the FNLCR that could catalyze the growth and adoption of cryo-EM for cancer research was approved by the FNLAC, and a series of actions to establish the NCEF began. The organizational structure of the NCEF—which became operational on May 15, 2017—includes key personnel who are responsible for daily operations: Dr. Subramaniam, Program Advisor; Dr. Heimbrook, Laboratory Director, FNLCR; Dr. Dwight Nissley, Director, Cancer Research Technology Program, FNLCR; Dr. Ulrich Baxa, Senior Microscopist and Technical Lead; Dr. Thomas Edwards, Junior Microscopist; and Ms. Helen Wang, Scientific Project Manager. An information technology and microscopy support specialist soon will join. Dr. Subramaniam acknowledged members of the *ad hoc* NCEF Oversight Working Group who have overseen the technical aspects of the NCEF.

To gain access to the NCEF, prospective users who have quality samples for imaging submit requests with prescreening images and a sample information form, which is linked to the NCEF website. Applicants are asked to provide details on the imaging conditions and expectations for their project. Within 48 hours of submission, NCEF staff will notify the applicant whether the samples have been accepted or rejected. Approved samples are shipped as frozen EM grids to the facility and properly stored until the

scheduled date of analysis. Each user is entered into a flexible scheduling queue and is alerted 24 to 48 hours prior to the date of their imaging time. Drs. Baxa and Edwards advise users on data collection procedures. Data are transferred immediately following each session and are stored at the facility for one month to address any issues. Users are not required to identify their sample or its specific relevancy to cancer; it only needs to address cancer. The current wait time from submission to analysis is less than one month, but will become progressively longer over the coming year.

The NCEF cumulative projects have increased steadily since the May 15, 2017, launch. Early performance indicators show that the growth is stable, with an average of two extramural projects per week. Roughly two-thirds of the time is spent supporting extramural user projects; the remaining time is dedicated to software/hardware installation and methods testing. To date, the facility has supported 40 extramural user projects from 11 different academic institutions. User feedback on the application process, shipping, data collection, data transfer, and data quality has been favorable. Customer satisfaction has ranked at 100 percent for each feedback category.

The plans for FY 2018 include constructing a new microscope facility at the Advanced Technology Research Facility (ATRF) on the FNLCR campus by June 2018; installing a second Krios microscope in July 2018; relocating the first Krios microscope to the ATRF when the second unit is operational; increasing core staff by one or two members by the summer of 2018; continuing efforts to provide users access to the latest cryo-EM technologies; and adding a third microscope in 2019, if demand increases. In addition, the NCEF team will be considering new directions for increasing the impact of the facility and welcomes advice and suggestions from the FNLAC.

In the discussion, the following points were made:

- Three different groups of users likely to benefit from the NCEF were identified early on with input from the FNLAC and the cancer research community: (1) researchers already experienced in cryo-EM technology; (2) structural biologists in adjacent disciplines, such as X-ray crystallography or nuclear magnetic resonance (NMR); and (3) researchers who have interesting cancer-related targets but are new to cryo-EM technology. Of the three groups, the experienced users, who are primarily early stage investigators and comprise the largest group contributing to the rapid growth of the field, drove the decisions for the initial NCEF customer base to serve at this time.

- As a service facility solely focused on addressing the structural biology needs of cancer researchers in the extramural community, the NCEF has leveraged the expertise of experienced users for a rapid launch. In the first 5 months of operation, more than half of users have been crystallographers who are just adopting cryo-EM and who have postdoctoral fellows or graduate students who are learning to use the publicly available data processing methods.

- As the scope expands, consultations with existing facilities in U.S. cities (e.g., New York, San Francisco, and Boston) would be one way to address scaling up to data processing and sample preparation at the NCEF, but developing a measured approach and hiring additional core staff would need to be considered first. Discussions on the FNLCR interactions with the proposed regional facilities being solicited through the National Institute of General Medical Sciences are yet to be scheduled.

- Unlike the streamlined work flow of X-ray crystallography, a cryo-EM project can take more than 2 years from the 2-day data collection performed at NCEF to a result. Prior to data collection, researchers would have spent 6 months or more identifying possible targets. Experienced users in a collaborative environment such as the CBC sometimes can complete projects within 1 year. Developing ways to accelerate the use of the data (i.e., final phase of the workflow) would be one strategy to consider for the NCEF in the future.

- The NCEF does not have an obligation or commitment to the NCI Intramural Research Program under this current structure, thereby maintaining the boundary between intramural and extramural support. Established intramural investigators, experienced crystallographers or not, have the opportunity to collaborate with the LCB to collect cryo-EM data. This type of collaboration could be formalized or addressed on a case-by-case basis to ensure that these projects are equally supported.

V. UPDATE ON RAS INITIATIVE—DRS. FRANK MCCORMICK AND LEVI A. GARRAWAY

Dr. Frank McCormick, Professor, Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco (UCSF), Scientific Advisor, NCI RAS Initiative, provided an update on the accomplishments of the RAS Initiative with highlights of the past year. He was joined by Dr. Levi Garraway, Senior Vice President, Global Development and Medical Affairs, Eli Lilly and Company, *ad hoc* RAS Working Group Chair, who commented on the progress since inception of the project. The major goals of the Initiative are to discover small molecules that directly bind to RAS or disrupt RAS interactions with its primary effectors.

The RAS Initiative is structured as a hub-and-spoke model, in which the Initiative acts as hub to interact with collaborators (i.e., spokes) in biotechnology and pharmaceutical companies and NCI-supported academic laboratories. Most of the drug discovery efforts are partnered through cCRADAs that involve projects to increase understanding of RAS biology and interactions in the plasma membrane, identify new targets, and validate those targets. Current and completed RAS cCRADAs with Sanofi, Daiichi Sankyo, and Eli Lilly and Company include projects for primary screening of new compounds that directly bind RAS. Other projects—conducted by small biotechnology companies, including Tosk, Inc., Kyras Therapeutics, Inc., and a biotechnology group funded through the Beatson Institute for Cancer Research— involve validation of compounds that have been shown in preliminary screening to interact with RAS and potentially further development of these compounds.

The Initiative engages strategic collaborations with other governmental entities, including several DOE national laboratories—Argonne National Laboratory, Lawrence Livermore National Laboratory (LLNL), Oak Ridge National Laboratory, and Los Alamos National Laboratory—and such organizations as the Pancreatic Cancer Action Network. In addition, technology-based industry collaborations and a diverse range of academic collaborations are in place to either exchange resources or work to advance RAS discovery in each of these different institutions.

Efforts led by Dr. Matthew Holderfield and the RAS Drug Discovery Group have been focused on developing novel cell-based and biochemical assays, and image-based screens directed by Dr. Tommy Turbyville and the RAS Imaging Group have sought to identify compounds that bind directly to RAS. Analysis of RAS in nanoparticles has been conducted by Dr. Andrew Stephen and the Biophysical Group. Dr Anna Maciagand and colleagues have used screens which involve disulfide tethering and covalent inhibitors, to discover compounds that bind RAS via novel binding pockets that appear to be present in the protein's structure. New RAS structures solved in the Structural Biology Group directed by Dr. Dhirendra Simanshu have led to identification of new pockets that could be exploited for drug discovery using in silico screening methods. Dr. McCormick acknowledged Dr. Dominic Esposito and the RAS Reagents Group, Dr. Bob Stephens and the RAS Informatics Group, Dr. Rachel Bagni and the RAS Models Development, and other FNLCR staff for their support.

RAS Inhibitor Screens. The FNLCR drug discovery strategy encompasses the biochemical and cell-based assays, biophysical and *in silico* screens, and the cCRADAs. High-quality cell-based and screening assays developed and validated as part of the Initiative have received favorable responses from

the collaborators. Autogenic cell lines, RASless mouse embryonic fibroblasts (MEFs), were generated and validated through primary high-throughput screening (HTS) focusing on the drugs that react directly with the RAS protein and distinguishing between the different mutant alleles and subtypes of RAS. Preliminary data revealed (1) a preferential activity of wild-type HRAS and KRAS MEFs to receptor tyrosine kinase (RTK) inhibitors; (2) that HRAS, but not KRAS, is sensitive to farnesyl transferase inhibitors (FTI); and (3) a resistance of oncogenic MEFs to RTK and MEK inhibitors. The validated cell lines for cell-based assays have been made available to the RAS community of researchers in academia and industry to improve the quality of their validation processes.

Tethering and Covalent Inhibitors. Dr. McCormick described a project to identify compounds that block KRAS processing (i.e., farnesylation and geranylgeranylation) by targeting C185 in the C-terminus region of the protein using the disulfide tethering approach. He called attention to a prior tethering screen conducted in his laboratory at UCSF using a fragment-based screening library built at UCSF, which identified hit compounds that bind C185, suggesting the location of a noncovalent binding pocket. These efforts were continued in the RAS Initiative. Dr. Anna Maciag now leads this project at the FNLCR, with chemistry support from Dr. David M. Turner; both are new researchers to the RAS Initiative.

The next step was to replace the thio-reactive group with an electrophile (e.g., vinyl sulfonamides to form a thio-ether linkage) instead of a labile disulfide bond. Electrophiles that exhibited high potency in an additional screen were identified and were shown to prevent KRAS processing in cells and decreased membrane localization. At this phase of the project, synthesis of analogues to the lead compound 994566 was outsourced to a contract research organization. The ability of these second-generation compounds to knock down KRAS in cells and covalently bind RAS was measured using matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS). These compounds were superior to the lead compound in knocking down KRAS in cells and did so at notably lower concentrations. Also, the binding affinities were improved compared to the original hit and selectively blocked cell proliferation driven by different RAS alleles. These data bring the Initiative closer to identifying a KRAS-specific or FTI equivalent to KRAS that is not active to HRAS.

In collaboration with LLNL, three separate model simulations of these data suggest that the hypervariable region (HVR) occupies a groove between helix three and four, adjacent to amino acid residue histidine 95 (H95) and functions as a binding pocket for 994566. The fact that H95 appears to be part of this newly identified pocket suggests that compounds that bind are likely to be KRAS-selective, because H95 is unique to KRAS not HRAS or NRAS—a feature important to RAS drug design. Crystal structures will need to be solved, and experiments to validate these findings are ongoing. The progress made on this and other projects in the Initiative can be attributed partly to in-house medicinal chemistry and chemistry capabilities established at the FNLCR in 2016–2017.

Structural Biology and *In Silico* Screens. Dr. McCormick reported that analysis of solved structures of active forms (i.e., bound to GTP/5'-guanylyl imidodiphosphate [GMPPNP] and Mg) of wild-type KRAS and six mutant forms (G12C, G12D, G12V, G13D, Q61L, and Q61R) revealed new binding pockets in the switch I and switch II regions of the G12C, G12D, and Q61R mutants that small molecules could bind. Furthermore, the group also solved the crystal structure of the KRAS Q61R-RASA1 complex. Because of the long side chain, the arginine residue points toward the protein surface and reveals a void space not present in the wild-type structure. A cCRADA has been established between the FNLCR and Dr. Nir London at the Weizmann Institute for Science to perform *in silico* screening to identify potential KRAS binding compounds based on these new structural and biophysical characteristics. The RAS Initiative is considering hiring *in silico* screening experts to coordinate this effort, which Drs. Stephen and Simanshu also will be supporting.

RAS Membrane Interactions. Dr. McCormick described efforts to address a molecular description of RAS/RAF signaling complexes in membranes. In an iterative process, use of high-tech

imaging processes to measure RAS proteins in live cells and biochemical and biophysical analysis of recombinant RAS proteins on synthetic lipid bilayers (i.e., nanodiscs) will inform the DOE *in silico* modeling, which is the basis for the JDACS4C Pilot 2 project. The goal is to build models that accurately depict RAS interactions in plasma membranes. Preliminary DOE simulations of KRAS4b in mammalian plasma membranes revealed that the RAS proteins readily cluster and rapidly associate with aggregated charged lipids in the plasma membrane.

FNLAC *Ad Hoc* RAS Working Group. Dr. McCormick acknowledged new Working Group members: Drs. Nader Fotouhi, Chief Scientific Officer, TB Alliance; Nathanael S. Gray, Professor, Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Professor, Cancer Biology, Dana–Farber Cancer Institute; and Roger K. Sunahara, Professor, Department of Pharmacology, University of California, San Diego.

Dr. Garraway commented on the progress of the RAS Initiative from his perspective as Working Group chair. When starting this project, the NCI thought that, at best, the Initiative's success would be measured in its ability to develop and validate RAS assays, catalyze the research, establish one or two strategic cCRADAs, and disseminate important new data on RAS biology to the community within 2 to 3 years of inception. The project has far exceeded those goals and expectations, partly due to the unique ability of the FNLCR to integrate the various enabling capabilities. The Initiative has successfully fostered collaborations and spurred new activities, including the NCI–DOE partnership. Key features that have made the Initiative progress well is the clear value of the question being addressed and the ability to undertake activities that could not be readily replicated elsewhere. The depth and breadth of the assays developed, as well as the high standard of quality the team insists on, are exemplary. New insights into RAS biology and discoveries of novel binding pockets and novel mechanisms of action have resulted from the efforts of the RAS Initiative. More important, the visionary leadership of Dr. McCormick, a leading RAS researcher, and the talented team of scientists at the FNLCR, have made this possible. This initial large-scale project for the FNLAC remains an outstanding effort and a model to emulate.

In the discussion, the following points were made:

- Develop a mechanism to orient and update new members on the progress and achievements of the RAS Initiative.

- The FNLAC, with the assistance of the *ad hoc* RAS Working Group, conducts in-depth reviews of the progress of the program, provides specific recommendations on new directions, and submits a report to the NCI. The RAS Initiative and FNLCR leadership address the recommendations.

- The Working Group is a model of how an extension from FNLAC can mediate and help accelerate large-scale projects, such as the RAS Initiative.

- The histidine95 compound provided valuable insight into the biophysical characteristics and will lead to the development of analogues that could be further interrogated.

- Existing NCI initiatives that have developed patient-derived xenograft models of RAS mutations (e.g., Therapeutically Applicable Research to Generate Effective Treatments [TARGET] Acute Myeloid Leukemia projects) could be leveraged to test promising new compounds.

- Consider cataloging the matching investments of the strategic, industry, and academic collaborations within the RAS Initiative that can be shared with the extramural community.

VI. CHEMICAL BIOLOGY CONSORTIUM—DRS. JAMES H. DOROSHOW, CHI V. DANG, RAYMOND J. DESHAIES, MICHELLE R. ARKIN, AND STEPHEN W. FESIK

Chemical Biology Consortium. Dr. Doroshow presented an overview of the NCI Experimental Therapeutics (NExT) Program and the Chemical Biology Consortium (CBC), the discovery engine. The NExT Program began in 2009 to focus on high-risk projects and engage the academic community in a consortium that would provide data and project-specific resources that are not commonly available for academic projects.

NExT advances compounds through a streamlined pipeline from early stage discovery, late discovery, and full development, from which promising candidates advance to potential first-in-human studies. Projects may enter the pipeline at any stage in the discovery and development process and are reviewed on a 4-month schedule by a Special Emphasis Panel (SEP). Of the projects reviewed to date, 66 percent investigated small molecules, and 31 percent studied biologics; 42 percent originated from academia; 12 percent, from the nonprofit sector; and 36 percent, from small biotechnology companies.

In the spring of 2016, the CBC network expanded to 23 centers, which includes 15 comprehensive and eight specialty centers. The comprehensive centers provide support for High Throughput Screening (HTS), complex chemistry applications, and a repository for new compounds. Specialty centers are organizations that provide additional expertise to assist investigators and allow access to new libraries and other resources that facilitate the NExT.

Since its inception, NExT has received 650 applications; 85 percent were reviewed by the SEP and did not move forward; of the remaining 15 percent, 5 percent moved into the pipeline. The evaluation process involves a “trust but verify” set of experiments. Roughly one-third of the projects fail even though compelling scientific data was the basis for the application. Following approval by the SEP, highly ranked projects enter the CBC specialty and comprehensive centers. Multidisciplinary teams that include the principal investigator are formed for each project. This team science approach is the driver for successful NExT projects. The breadth and depth of scientific experience in small-molecule drug discovery and the principal investigator’s knowledge of the target’s biology are essential for projects to progress to first-in-human studies.

Project applications are submitted to NExT in three cycles each year. The SEP reviews, evaluates, and selects the top 10 to 12 projects; highly ranked projects are reviewed and endorsed by the CBC Steering Committee, which is composed of members representing the 23 centers in the network. Selected projects enter a planning process, with scientific verification and validation. The pipeline is an actively managed discovery portfolio of dynamic and diverse projects; new projects are supported continuously.

The annual NExT portfolio review involves prioritization via SEP voting and ranking of the discovery, preclinical, and development projects using a three-tiered ranking system that assesses the feasibility of and confidence in the mechanism. Projects are prioritized or deprioritized based on the competitive landscape. Bottom-tier projects are either re-evaluated in a 6-month performance review or decommissioned. The 2017 CBC portfolio evaluation completed in August showed significant progress in top-tier and middle-tier ranked projects. Three NExT principal investigators will present on their projects: Drs. Chi V. Dang, Scientific Director at the Ludwig Institute for Cancer Research and Professor at The Wistar Institute; Michelle R. Arkin, Co-director, Small Molecule Discovery Center, UCSF; and Stephen W. Fesik, Orrin H. Ingram, II Chair in Cancer Research, Vanderbilt University Medical Center.

Lactate Dehydrogenase A Inhibitors. Dr. Chi Dang, Scientific Director at the Ludwig Institute for Cancer Research and Professor at The Wistar Institute, summarized the NExT Lactate Dehydrogenase A (LDHA) Inhibitors project, which has the objective of discovering and developing an LHDA inhibitor as an anti-neoplastic agent. This concept was developed based on prior observations from the Dang laboratory,

which showed that the c-Myc oncogene regulated lactate dehydrogenase (LDH) at the transcriptional level and that gene knock-down of LDHA diminished the ability of Burkitt lymphoma cells to establish colonies; these cells also showed some activity in xenograft animal models. Dr. Dang and his group also demonstrated that ectopic expression of c-Myc transformed cells and resulted in sensitization to glucose deprivation, suggesting that targeting LDHA might be of therapeutic interest to cancer research. In the past 10 years, researchers have been able to show that, mechanistically, Myc drives deregulated biosynthesis, and cells locked in this oncogenic state become dependent on nutrients. Interrupting specific enzymes, such as LDHA, can therefore disrupt tumorigenesis. Yet, targeting tumor energy pathways is not a new concept and dates to the Warburg hypothesis postulated by Nobel Laureate Dr. Otto Warburg in 1924.

Following application approval in 2010 and establishment of the scientific team (formulation chemists, biologists, and crystallographers) in 2011, a small molecule was interrogated as an LDHA inhibitor, but was unsuccessful. Over the next 5 years, the team performed HTS and identified and validated lead compounds, which currently are undergoing preclinical studies; the next step will be to conduct Investigational New Drug (IND) -enabling studies. In 2018, the NExT CBC, through team science, has completed development of a first set of compounds that are soon to be published. They range in LDHA inhibitory concentrations from micromolar (μm) levels of the active hit compounds to nanomolar (nm) levels for compound derivatives.

The LDHA project provides many clinical opportunities for investigation, including tumors that exhibit high glycolytic flux, hypoxic tumor microenvironments, and tumors with inherent defects in mitochondrial phosphorylation. Despite these opportunities, the LDHA team has faced key challenges, including approaches to mitigate the high cellular levels of LDHA/B, which placed constraints on the pharmacokinetics (PK) for extended periods, necessitating the development of compounds with high affinity for the target, and metabolic plasticity that often leads to drug resistance. To address these challenges, the team screened for and developed two high-target, long-engagement lead compounds, CBC 006 and CBC 737, which can be administered orally and have outperformed leading contenders. Furthermore, CBC 006 and CBC 737 significantly inhibited glycolysis *in vitro* using cell lines and *in vivo* in a pancreatic cancer model dosed intravenously (IV) with CBC 006 in experiments led by team member Dr. Leonard M. Neckers, CCR, NCI. The *in vivo* hyperpolarized carbon 13 labeled pyruvate (^{13}C-pyruvate) magnetic resonance spectroscopy (MRS) method also is one way to measure glycolysis inhibition.

Using a Ewing's sarcoma xenograft model, the team screened 15 LDHA inhibitors from the series of compounds generated to interrogate target engagement directly. Results showed a durable response that correlated to the tumor drug levels. The pattern of LDH inhibition corroborated the MRS imaging studies, and similar levels of LDH were demonstrated in human pancreatic cell line MIA PaCa-2 and human promyelocytic leukemia cell line HL60 tumor xenografts dosed IV with CBC 006. Also, tumor growth suppression and a therapeutic response was demonstrated in MIA PaCa-2 xenografts dosed IV with CBC 006 on a variable schedule regimen and in Myc-inducible lymphoma xenografts dosed IV with CBC 737. Combining LDHA inhibitors with metabolism inhibitors phenformin or metformin further enhanced efficacy *in vivo*.

Dr. Dang remarked on new applications for the use of CBC LDHA inhibitors, which include testing the effects on Myc-dependent Thymus (T) cell activation and ameliorating graft versus host disease (GVHD) in organ transplantation models by decreasing lactate production. The next steps will be to repeat and extend the initial efficacy findings in the GVHD, pancreatic cancer, and Ewing's sarcoma models. In addition, efforts will be focused on testing the compound in models suitable for oral administration, such as the hepatocellular, glioblastoma multiforme, and the disseminated liquid tumor models. Dr. Dang detailed the prior year's accomplishments and future goals for the LDHA project.

P97 ATPase Inhibitors. Dr. Michelle Arkin, Professor at the Department of Pharmaceutical Chemistry at UCSF, presented on behalf of principal investigator Dr. Raymond J. Deshaies, Visiting

Associate, Division of Biology and Biological Engineering, California Institute of Technology, who joined by telephone. She discussed the approaches, technologies, and capabilities for the development of allosteric inhibitors of p97 in the NExT CBC. Cancer cells possess high levels of ubiquitin proteasome system (UPS) stress due to mutated genomes. Inhibiting UPS using proteasome inhibitors results in apoptosis, which has been clinically validated in liquid cancers. The p97 protein, an ATPases Associated with diverse cellular Activities ATPase, and master regulator of protein homeostasis, is one such inhibitor. The opportunity exists for developing first-in-class p97 inhibitors and investigating their effectiveness in both hematological and solid tumors. One proof-of-concept agent, CB-5083, an ATP-competitive p97 inhibitor developed by Cleave Biosciences (first initiated in NIH's Molecular Libraries Probe Production Centers Network [MLPCN]), has been shown to be more effective in solid tumor xenografts than U.S. Food and Drug Administration (FDA) -approved proteasome inhibitors. Phase I clinical trials with CB-5083 have been halted due to an off-target effect in the retina mediated through phosphodiesterase 6. Because the proof of concept is valid, this project's objective is to develop a p97 inhibitor with a slightly different mechanism of action (MOA) and reduced toxicity.

The team solved ATP-bound (active state) and ADP-bound (inactive state) crystal structures of p97 by Cryo-EM (with Subramaniam at NCI), providing new insight into the conformational coupling of ATPase and protein-protein interaction (PPI) domains that could be manipulated with small molecules. Using an HTS approach to investigate the allosteric inhibition of the D2 ATPase domain of p97, the phenylindole scaffolds were identified as the most active class of compounds. With medicinal chemistry, the binding affinity significantly increased; Michaelis–Menten kinetics confirmed the compounds as uncompetitive inhibitors of p97. Biophysical characterization, mutagenesis and NMR analysis showed that this first series of compounds interacted with the D2 domain of p97. Furthermore, in 2016, the team reported the first high resolution cryo-EM structure of full-length p97 bound to an allosteric inhibitor, which was previously highlighted by Dr. Subramaniam, a p97 team member.

Dr. Arkin pointed out that the first series of compounds exhibited unexpected cellular activity in which they were effective in inhibiting autophagy, but exhibited modest proteasome inhibition. The team transitioned to a second series of compounds that were effective proteasome inhibitors with minimal activity in blocking autophagy. Multiple analogs in this series were developed and exhibited five-fold higher potency in blocking degradation of ubiquitinated proteins compared to CB-5083. Key biochemical, cell-based, pharmacodynamic, and *in vivo* assays were developed, and these compounds have been tested in established cell lines, including the NCI-60 and Massachusetts General Hospital (MGH) 1000 panels. She noted that many of these experiments were supported by the FNLCR.

Summarizing the past year's progress, the team completed medicinal chemistry optimization of p97 allosteric inhibitors, which resulted in compounds with superior potency compared to CB-5083 *in vitro,* and resistance in cell lines confirmed target engagement (i.e., binding). *In vivo* proof-of-concept studies are being initiated. The goals for the next 12 months will be to identify a predevelopment candidate; perform optimizations, potency, and therapeutic index experiments; and identify the most sensitive cell lines from the MGH 1000 panel.

To address the different cellular activities or MOA of series one (uncompetitive p97 inhibitors/ autophagy inhibitors) and series two (ER-associated degradation and ubiquitination blockers/ noncompetitive p97 inhibitors) compounds, the team used the NCI-60 panel to stratify agents with similar MOA to a specific fingerprint and found a variability in cell line sensitivities between the two series. Recent findings suggest that p97 acts as an Ufd1/Npl4 (UN) foldase and could explain the different MOA of the two series of compounds.

Dr. Arkin pointed out that the CBC p97 program has catalyzed new basic research projects to address remaining questions regarding which functions of p97 are critical for different diseases and whether the structural conformations of p97 alter its function. The team next could focus efforts to determine

function-specific modulators; develop ways to modulate PPI networks through allosteric and orthosteric binding; conduct high-definition conformational analysis; and design new conformational locks. The long-term goal is to develop context-specific modulators of p97 function. Dr. Arkin briefly described a Taspase 1 project, a previously undrugged threonine protease. The CBC team has developed the first potent, bona fide inhibitors of Taspase 1, and is developing these inhibitors to validate Taspase 1 as a target for breast cancer and T-ALL.

Discovery of Subnanomolar Inhibitors of Myeloid Cell Leukemia 1 (MCL-1). Dr. Stephen Fesik, Professor of Biochemistry, Pharmacology, and Chemistry in the Biochemistry Department of Vanderbilt University School of Medicine, discussed the Vanderbilt University (VU) MCL-1 inhibitor program. MCL-1 is an anti-apoptotic member of the BCL-2 family of proteins and is a highly validated target. It is amplified in human cancers, decreases survival when overexpressed, and is implicated in drug resistance; gene knock-down sensitizes cells to apoptosis. The 1996 proof-of-concept studies conducted by Dr. Fesik and colleagues who were investigating anti-apoptotic proteins Bcl-2 and Bcl-XL used a then-new drug discovery approach, structure-activity relationship (SAR) by NMR, to screen for small-molecule inhibitors. These studies resulted in the initial lead compound, dual Bcl-2/Bcl-XL inhibitor, ABT-737. Further modifications and optimizations led to ABT-263 and finally to ABT-199, a selective Bcl-2 inhibitor (i.e., venetoclax), which recently was approved for chronic lymphoid leukemia. These studies are a validation of this approach to drug design and discovery and suggest that small-molecule inhibitors that target the Bcl-2 family of anti-apoptotic proteins, including MCL-1, can be successful in treating cancer.

Dr. Fesik explained that the first step to establishing the MCL-1 program at Vanderbilt was the development of a proprietary screening fragment library consisting of approximately 15,000 compounds. Next, the MCL-1 protein was cloned, expressed, purified, and screened against the fragment library using SAR NMR; 13 structurally distinct series of molecules were identified. A merged fragment approach to developing a compound and a structure-based design to further improve active hits identified in the fragment screen were used. In September 2015, a milestone compound with high binding affinity, VU0661013 (or VU013), was discovered. Selective cellular activity in MCL-1-sensitive and -insensitive cells was observed, and on-mechanisms activity assays were completed. Further validations at the NCI showed a dose-dependent activity in tumor models *in vivo*.

Dr. Fesik described additional studies of VU013. In collaboration with Dr. Michael R. Savona, Vanderbilt Ingram Cancer Center, a systemic animal model of heme malignancies that recapitulates human disease showed statistically significant inhibition of *in vivo* expansion of leukemia cells in the blood, bone marrow, and spleen of animals treated at the high dose of VU013 compared to controls. In xenograft tumor models, VU013 decreased tumor growth and had minimal effects on body mass. In combination with the FDA-approved breast cancer drugs docetaxel or doxorubicin, VU013 further decreased tumor growth in a triple-negative breast cancer tumor model. To determine which tumor types were most likely to respond to a MCL-1 inhibitor, more than 750 cancer cell lines were screened at Massachusetts General Hospital (MGH), in collaboration with Dr. Cyril Benes, using VU013 as a single agent. In these experiments, VU013 exhibited the highest potency against hematologic malignancies, and the highest activity was observed in breast and non-small-cell lung cancers. These preliminary data are similar to published studies, and experiments are being repeated.

Dr. Fesik pointed out that medicinal chemistry optimizations, fragment-based methods, and structure-based design efforts were focused to improve potency and drug-like characteristics of VU013, thus moving closer to developing an acceptable drug candidate. Newer generation compounds show significant enhanced binding affinities, and active hits were 12-fold more potent than VU013. Improved PK was observed not only in the acidic series, but also in a sub-class of MCL-1 inhibitors that are a zwitterionic, neutral, and basic series. One approach to selecting a potential candidate inhibitor compound is to determine the *in vivo* characteristics. The PK/pharmacodynamics (PD) screen, a time course biomarker measurement study developed and tested in collaboration with the NCI, is one such approach. The PK/PD studies

assessed the BAK-BAX dimerization and/or caspase activation; the new generation compounds showed dramatic improvement in PD compared to VU013.

Dr. Fesik summarized the progress of the Vanderbilt MCL-1 program to date. In medicinal chemistry and structural biology, five patents were filed for four different series of compounds, approximately 2,500 compounds were synthesized, and 64 X-ray co-crystal structures were solved. Development and optimization of cell biology assays and experiments—including a fluorescence polarization assay, a time-resolved fluorescence resonance energy transfer assay, on-mechanism activity assays, proliferation assays, and *in vivo* model experiments—were completed. In the drug metabolism and PK and animal efficacy model, 440 compounds were tested in an early absorption, distribution, metabolism, and excretion (ADME) screen in collaboration with the National Center for Advancing Translational Sciences (NCATS) Chemical and Genomics Center; PK studies conducted in collaboration with the NCI tested 22 compounds in mouse models, and 102 compounds were tested in rat models at VU. Also, *in vivo* efficacy studies were initiated and are ongoing, and patient selection biomarkers and PD biomarkers have been discovered. In the animal safety and assessment model, *in vitro* cardiac myocytes assay and multiple dosing maximum tolerated dose (MTD) studies were completed in collaboration with the NCI.

Dr. Fesik remarked that the ultimate goal of the project is to select a clinical candidate. Selections are being narrowed, with a decision to be made within the next 3 to 6 months. Based on the data, IV formulations appear to be the most effective route for administering the optimal compound. Parallel to the selection of a clinical candidate and IND-enabling studies will be the development of an oral formulation. The NExT program provides the necessary funding, infrastructure, and resources for drug design and discovery in an academic setting. The Vanderbilt MCL-1 inhibitor program is one that has benefitted.

In the discussion, the following points were made:

- Small-molecule-based proteolysis-targeting chimeras (PROTACS) have been used as an approach to reduce the high cellular levels of LDHA/B and are aggressively being tested.

- Although MCL-1 inhibitor lead compounds are effective and increase survival in xenograft animal models, testing these compounds in immunocompetent *de novo* cancer syngenic animal models and models of metastatic disease before planning clinical studies is advised.

- Successful compounds in the NExT program that progress to the clinical stage are open to licensing if industry interest exists and if it is the wish of the intellectual property rights owners, which are the academic institutions and the principal investigators.

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

Dr. Marnett requested input from the committee members regarding any remaining issues for discussion.

In the discussion, the following points were made:

- Establishing the Patient-Derived Xenograft (PDX) Centers and the Patient-Derived Models Repository (PDMR) will help standardize animal models across programs and academic centers and also would extend to the NExT Program projects. The FNLCR is supporting these efforts.

VIII. ADJOURNMENT—DR. LAWRENCE J. MARNETT

Dr. Marnett thanked the Committee members and other invitees for attending. There being no further business, the 13th meeting of the FNLAC was adjourned at 3:52 p.m. on Monday, 30 October 2017.

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