Department of Health and Human Services
Public Health Service
National Institutes of Health
National Cancer Institute

7th Meeting of the Frederick National Laboratory Advisory Committee (FNLAC)
(Formerly NCI-Frederick Advisory Committee (NFAC))
September 30, 2014

Summary Minutes

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

National Cancer Institute
7th Meeting of the NCI-Frederick Advisory Committee (NFAC)
September 30, 2014

Summary Minutes

The NCI-Frederick Advisory Committee (NFAC) convened for its 7th meeting on 30 September 2014, at 31 Center Drive, Building 31, C Wing, Conference Room 6, Bethesda, MD. The meeting was open to the public on Tuesday, 30 September 2014, from 9:00 a.m. to 3:35 p.m. The NFAC Chairperson, Dr. Joe W. Gray, Gordon Moore Endowed Chair, Department of Biomedical Engineering, Director, OHSU Center for Spatial Systems Biomedicine, Oregon Health and Science University, presided.

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

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

Executive Secretary
Dr. Thomas M. Vollberg, Sr.

TABLE OF CONTENTS

I. Opening Remarks—Drs. Joe W. Gray and Harold E. Varmus 1
II. Update: Frederick National Laboratory for Cancer Research (FNLCR)—
Dr. David C. Heimbrook 1
III. Recognition of Retiring Members—Dr. Harold E. Varmus 4
IV. Report: *Ad Hoc* RAS Oversight Subcommittee and Lessons Learned—
Drs. Frank McCormick and Levi Garraway 4
V. Engaging the Larger Community—Dr. Edward E. Harlow 9
VI. National Molecular Microscopy Laboratory (NMML)—Dr. Sriram Subramaniam 10
VII. Ongoing and New Business—Dr. Joe W. Gray 12
VIII. Closing Remarks—Drs. Joe W. Gray and Harold E. Varmus 12
IX. Adjournment—Dr. Joe W. Gray 12

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

Dr. Joseph W. Gray, Chair, called to order the 7th meeting of the NFAC and welcomed the Committee members. Dr. Gray reminded members of the conflict-of-interest guidelines and confidentiality requirements. Members of the public were welcomed and invited to submit to Dr. Thomas M. Vollberg, Executive Secretary, in writing and within 10 days, any comments regarding items discussed during the meeting.

Dr. Harold E. Varmus, Director, NCI, welcomed Committee members and other attendees to a pivotal meeting of the NFAC and invited members to introduce themselves. Dr. Varmus briefly reviewed the history of the NFAC, which was established under the leadership of Dr. Zach Hall in response to concerns by the National Cancer Institute's (NCI) National Cancer Advisory Board (NCAB) to elucidate the activities at the Frederick National Laboratory for Cancer Research (FNLCR) and provide coordinated oversight by a multidisciplinary group. Members were reminded that the FNLCR is the only Federally Funded Research and Development Center (FFRDC) in the Department of Health and Human Services (HHS). FNLCR provides laboratory opportunities and contract services for the extramural community. He stated that leadership has learned lessons from other FFRDC national laboratories; the Frederick National Laboratory landscape has changed through establishment of large projects, such as the RAS Program, as well as through the leadership change in the FNLCR contractor, Leidos Biomedical Research. Dr. Varmus reviewed the agenda, noted the prominence of the RAS Program, which is headed by Dr. Frank McCormick, Director, University of California, San Francisco (UCSF) Helen Diller Family Comprehensive Cancer Center, and FNLCR RAS Program Consultant, with an oversight subcommittee led by NFAC member Dr. Levi Garraway, Assistant Professor of Medicine, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School. He encouraged members to consider a potential project in structural biology that would be presented and to help identify other potential signature projects.

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

Dr. David C. Heimbrook, Laboratory Director, FNLCR, provided an update on activities of the FNLCR, including support for the National Institute of Allergy and Infectious Diseases' (NIAID) Ebola vaccine efforts, contractor partnering authorities, and two National Laboratory programs: the AIDS and Cancer Vaccine Program (ACVP) and the Nanotechnology Characterization Laboratory (NCL).

The FNLCR is assisting the NIAID in its Ebola vaccine activities through the Vaccine Clinical Materials Program (VCMP). Dr. Heimbrook explained that the Vaccine Research Center (VRC) was created by an Executive Order in 1997, the need for a pilot plant was identified in 1999, and the Vaccine Pilot Plant (VPP) commenced GMP manufacturing by 2006. The VPP is operated by the FNLCR on behalf of the NIAID, and the state-of-the-art facility has 50,000 square feet of manufacturing and formulation-filling capability, as well as assay and quality control laboratories, a warehouse and dispensary section, and administrative offices. In addition to DNA-type vaccines such as DNA plasmid vaccines and adenovirus vector vaccine, VCMP platform technologies include virus-like particles from human cell culture to support alphavirus disease studies, such as the chikungunya virus vaccine, and biotherapeutic monoclonal antibody production.

The NIAID's Ebola Vaccine Program was initiated in 2011 to enable the VCMP to support cGMP vaccine manufacturing, filling, finishing, and obtaining regulatory approval. Dr. Heimbrook stated that an investigational chimpanzee adenovirus vector vaccine was developed by the VRC in collaboration with Okairos (acquired by GlaxoSmithKline) and has shown promise in primate models. The FNLCR and VCMP supports this program by subcontracting the manufacture of the vaccine; conducting the formulation, fill, and finish of the drug product at the VPP; and supporting the filing of the investigational new drug (IND) application, with the first patient in the NIH clinical trial approximately 2 weeks following the IND submission. Ongoing efforts by NIAID include a companion booster vaccine that has shown interesting

preclinical activity, and scale up of the chimp adenovirus Ebola vaccine to support additional clinical trials in the United Kingdom and Africa.

Members were informed that contractor partnering authorities are being increasingly exploited to enable biomedical researchers in academia and the pharmaceutical industry. The Contractor Cooperative Research and Development Agreement (cCRADA) is a mechanism to support research collaboration involving intellectual and material contributions between FNLCR scientists and the external partners. Commonly used by U.S. Department of Energy (DOE) FFRDCs to foster strategic relationships, the cCRADA is useful for projects of long scope and duration, provides special protections for joint intellectual property (IP) that might emerge, and can include co-location of scientists. Dr. Heimbrook said that the FNLCR has executed six cCRADAs since receiving contractor partnering authorities in 2012, and six additional cCRADAs are in process; the median time from concept approval to final signature is approximately 5 months, and efforts to reduce the time will continue. The approved cCRADAs involve the ACVP, Cancer Research and Technology Program (CRTP), and Human Papillomavirus (HPV) Immunology Laboratory and encompass such subjects as protein scale-up, immunology for the HPV antibody, RAS, and next-generation sequencing. Other partnering agreements used by the FNLCR are the Materials cCRADA, which facilitates the transfer of incoming materials that require IP considerations; Collaboration Agreement, which does not involve the creation of joint IP or transfer of funds; and Technical Service Agreement (TSA), which allows FNLCR laboratories to provide well-defined and validated research services to the scientific community. Members were informed that partner contributions through the TSA mechanism has totaled $250,000 and $1.5 million (M) in FY 2013 and 2014, respectively. Technical services are available from many directorates, with the ACVP and Laboratory Animal Services Program (LASP) services most in demand.

Dr. Heimbrook stated that the ACVP and NCL are poised for expansion and evolution to fulfill their aspirations as National Programs within the FNLCR. He identified characteristics of an FNLCR National Program, including programs that are directed toward a coherent objective and focused on enabling scientific, technical, or medical advances in the broader biomedical community; scientific content that is fundamentally driven by teams of FNLCR scientists; and a program that is highly visible and impactful to the external scientific community. Two examples of such “National Programs” include the ACVP and the NCL.

The ACVP’s research focuses on HIV/AIDS and infections with cancer-associated viruses, developing novel research methods, analytical techniques and reagents, and proactively making these available to the broader research community. The Program includes unique research support cores and extensive interactions with academic and industry investigators outside of ACVP. Its cutting-edge science enables high-impact publications and creates collaborative demand; in FY 2014, ACVP executed TSAs with committed partner contributions totaling more than $1.9 M. The limitations of combination anti-retroviral treatment are recognized, and an increased emphasis on viral eradication and functional cure has been propelled by the example of the cured “Berlin Patient” Timothy Brown. More definitive treatments are needed, and various organizations such as the NIH, industry, and charitable foundations have expressed a strong commitment to move HIV therapy forward. Dr. Heimbrook remarked on the ACVP’s established state-of-the-art expertise and unique capabilities to assist in this effort. To fulfill the increased collaborative demand for ACVP services, the FNLCR requires additional primate space. Since no such space is currently available in Bethesda, the ACVP has identified a suitable offsite facility which can be leased to enable fulfillment of cCRADA opportunities.

The NCL is a collaboration started in 2004 between the NCI/FNLCR, National Institute of Standards and Technology (NIST), and U.S. Food and Drug Administration (FDA). The Laboratory’s Assay Work Flow includes both physicochemical *in vitro* and *in vivo* assays to help move compounds from the earliest stages into toxicology studies. The NCL is the only laboratory evaluating the wide variety of platforms (e.g., metals, dendrimers, emulsions, liposomes) used in nanomedicine, and in its 10-year history has characterized more than 300 different nanomaterials and generated more than 100 publications. The NCL has numerous collaborators, with an average of 15 active collaborations at a given time, and characterizes an average of 75 samples each year; 10 collaborations have moved products into clinical trials. The types of requests have

evolved as the nanotechnology field has progressed; initial efforts focused on requests for completing physicochemical and analytical characterization of the materials, followed by evaluation of nanomaterial activity and toxicity *in vitro* and *in vivo*. As the nanomedicine field evolves, so must the NCL. For example, as a National Program, the NCL should continue to provide assay cascade resources and mentoring for materials scientists and engineers; collaborate with industry on reformulation and cGMP; expand into non-oncology nanomedicines; work with instrument manufacturers on metrology and new methods, conduct basic research on grand challenges such topics as immunotoxicity and active targeting; and help inform FDA regulatory concerns as "nano-similars" and more complex compounds emerge. In addition, opportunities for translational collaboration exist; for example, the NCL serves as a reference for the European Union's nanomaterials program in how to conduct nanotechnology science effectively. Dr. Heimbrook expressed appreciation for NFAC's support, reiterated the need for NCL's evolution in response to evolving demands, and highlighted its status as a global resource for nanomedicine.

In the discussion, the following points were made:

- National Programs, such as the ACVP and NCL, and other activities of the FNLCR originate from an NIH sponsor. Accomplishments are incorporated in the review cycles of the principal investigators (PIs) and programs. For the ACVP, small research sections generally include a PI and two to four staff members.

- The new CRADA work streams in the ACVP will expand primate facilities but are not expected to affect other ongoing projects.

- The FNLCR is assisting with one part of NIAID's vaccine efforts in the international landscape responding to the Ebola outbreak in Africa. The FNLCR VCMP is helping to produce one of the vaccines being contemplated for clinical trials.

- Ten (10) nanocompounds are in clinical trials and may eventually move into Phase II and III studies. The type of requests has evolved from the physicochemical and analytical characterization of the nanomaterials to biology and toxicology to integration into multidisciplinary approaches.

- Promising compounds from extramural partners that might benefit from nanoformulation are brought into the FNLCR though an NCI review process. The FNLCR has helped companies and academic investigators at all stages of promising nanoparticle formulations.

- Approximately 60 percent of requests from investigators to the NCL are accepted at the current time. Funding for activities of the NCL is currently a single stream from NCI, and like other FNLCR activities is facilitated as a task order from an NCI sponsor. The acceptance rate of less than 100 percent is not caused by a ceiling or limit on the funds available to the NCL.

- FNLCR leadership was encouraged to market the FNLCR's capabilities to potential stakeholder scientists and institutions and continue to raise overall scientific community awareness about the FNLCR as a National Program, including its capacity to support research efforts of the NCI and NIAID.

- Products are poised for commercialization as a result of FNLCR efforts, such as an antibody manufactured for pediatric neuroblastoma trials which has been successfully transferred to an external company.

- Members discussed ways to raise awareness about the FNLCR's capabilities. The success and visibility of the RAS Project has been exemplary, and its website provides an ongoing conduit to

highlight the FNLCR capabilities. The sponsorship of Funding Opportunity Announcements that require the use of the FNLCR might be one way to engage relevant stakeholders who are otherwise unfamiliar with the FNLCR. Twitter and other social media venues could help raise awareness about the FNLCR, but communications should provide substantive information. The NCL has achieved many successes, but generally is not recognized as part of a national laboratory; nanotechnology activities in the EU are spurring the biomedical field, and marketing opportunities exist within the pharmaceutical industry. FNLCR's unique qualifications as an FFRDC, namely its ability to support research that cannot be conducted elsewhere, should be explicitly marketed.

III. RECOGNITION OF RETIRING MEMBERS—DR. HAROLD E. VARMUS

Dr. Varmus expressed appreciation to Dr. Steven T. Rosen, Provost and Chief Scientific Officer, Director, Comprehensive Cancer Center, and Irell and Manella Professor, City of Hope National Medical Center, for his service on the NFAC.

IV. REPORT: *Ad Hoc* RAS OVERSIGHT SUBCOMMITTEE AND LESSONS LEARNED—DRS. FRANK MCCORMICK AND LEVI GARRAWAY

Dr. McCormick provided an overview of the RAS National Program activities during the past year and was joined by Dr. Garraway, Chair of the *Ad Hoc* RAS Oversight Subcommittee, who presented recommendations from an August 2014 meeting of a RAS Working Group.

Overview of the RAS Program. Dr. McCormick reminded members that RAS mutations are prevalent in many human cancers, including pancreas, colorectal, lung, acute myeloid leukemia (AML), melanoma, and bladder, with an estimated 1 million people dying every year from cancers driven by RAS. With no effective targeting for RAS proteins and no drugs in development to attack RAS proteins directly, the RAS Program leads a significant research agenda at the FNLCR to provide the research community with information, tools, and technology to facilitate discovery. The importance of the RAS pathway in cancer is illustrated by The Cancer Genome Atlas (TCGA) data that showed approximately 75 percent of lung adenocarcinoma mutations in a dataset affect drivers in the RAS/RTK pathway, with 32 percent of the mutations involving KRAS directly. Challenges to targeting RAS cancers include the lack of structures of full-length KRAS, RAF, or mutant KRAS complexed with any effector or regulator. It is unknown how RAS activates RAF kinase, one of its major effectors. In addition, which KRAS cancers depend on KRAS *in vivo* and which effector pathways are critical *in vivo* are unclear.

Parameters affecting the oncogenic activity of mutant KRAS that exists in either the GTP or off-GDP state include that GTPase-accelerating proteins (GAPs) have intrinsic rates that destabilize RAS GTP and convert it to RAS GDP. These rates are slow but measurable. RAS can return to an active state by releasing GDP and binding GTP, which can occur slowly by spontaneous release or, depending on the mutant RAS, by SOS mediation. Because individual RAS proteins have varying degrees of dependence on this step, they exist in different ratios of GTP and GDP in the cell; the precise ratio of RAS in the active state for any given mutant is not yet known. Other knowledge gaps exist, such as how effectors compete to bind to RAS in the GTP state and how RAS activates these effectors when they engage.

The Program is producing and analyzing in detail recombinant RAS proteins expressed in *Escherichia coli (E. coli)*; mutant behavior is important to understand, as the cancers driven by each of these different mutants vary in their responses to therapy. Biochemical characterization of the mutant proteins is underway, including rates to determine how much RAS is in the GTP or GDP state, as well as intrinsic hydrolysis rates because models are suggesting that a small increase in intrinsic GTPase would inactivate these proteins.

A short-term priority is to solve the structures of the mutant proteins to advance studies in inhibiting RAS activity. Protein crystallization has progressed, with a current focus on obtaining crystal structures of all the major mutants on their own and a plan to obtain them in the future in complex with other proteins. Members were told that a crystallography expert, Dr. Dhirendra Simanshu, has joined the FNLCR as an in-house expert in structural biology.

To find structures that might yield new ways of targeting RAS proteins in complexes (Project One), the Program is developing high-throughput biochemical and phenotypic assays, such as alpha screens for RAS binding to RAF. Parameters affecting oncogenic RAS activity primarily involve the plasma membrane, and the Program has invested substantial effort to produce significant amounts of fully processed KRAS. Dr. McCormick explained that KRAS is present in the membrane through its C-terminal tail, and studies suggest specific interactions between a phospholipid in the membrane and residues on the RAS protein that affect interaction through allosteric networks. It is unknown why RAS will not activate to bind RAF unless in the plasma membrane, although such binding can occur *in vitro*. Members were told that producing fully processed KRAS proteins has been technically challenging, as the processing reactions (through farnesyltransferase, protease, and methyltransferase enzymes) are complicated. The extramural community has interest in obtaining fully processed KRAS for biophysical and biochemical analysis in nanodiscs, liposomes, or other membrane structures. Studies have shown that KRAS proteins exist as dimers in the plasma membrane, suggesting a surface of interaction between RAS proteins that could be targeted with small molecules to disrupt this interaction. Several ways to disrupt RAS dimers look promising, such as using BRET, FRET, or other systems. Potential collaborations regarding RAS dimerization in membranes and other biophysical and biochemical analyses are being considered.

A system to develop RAS-less cells has been established that allows rescue of the cells with mutant or wild-type RAS proteins and discrimination between compounds that affect KRAS versus HRAS. It provides opportunities to examine the signaling properties of individual RAS proteins, identify the specificity of potential RAS drugs, and help to validate and credential potential RAS compounds, as well as primary screens for targeting RAS. For example, in RAS-dependent proliferation screening strategies, studies measuring the proliferation of HRAS or KRAS-4B in response to therapeutics found equal sensitivity to chemotherapy drugs and MEK inhibitors, but showed that only HRAS cells are sensitive to farnesyltransferase inhibitors.

Collaborations also are ongoing regarding assays of KRAS cell-line signaling nodes in colon, lung, and pancreatic cancers. The assays involve growth, proliferation rates, reactive oxygen species, apoptosis, cell size, and other aspects that can be measured with a cell sorter. In addition, a recent workshop considered different technologies to generate a list of proteins differentially associated with phenotypes of KRAS cells as part of the project to map the surface of KRAS cancer cells. Although the FNLCR has expertise in mass spectrometry, other approaches such as phage display and bioinformatics are needed, and project staff members are gathering information to focus these technologies on KRAS cancers.

Dr. McCormick expressed the Program's interest in processing data from TCGA and other KRAS-relevant sources. One example is the analysis of TCGA data to identify cells in which the ratio of 4A to 4B is exceptionally high and determine whether any genes of interest track with high or low 4A. Another idea is to conduct, with assistance from the extramural community, RAS-centric analysis of all TCGA data across all the different tissue sites. Members were referred to the RAS Program website, which provides an information gathering place called RAS Central for the RAS community. The bioinformatics project will solicit feedback from the community via RAS Central as a pilot project to encourage interactive discussion on RAS Central.

Recommendations of the RAS Working Group. Dr. Garraway stated that the purpose of the RAS Working Group is to provide the highest quality oversight to the technical aspects of the RAS Program and provide its findings and recommendations to the NFAC and the *Ad Hoc* RAS Oversight Subcommittee regarding the scientific goals, directions, priorities, and timelines of RAS research projects at the FNLCR as well as the engagement of the extramural community and industry by the RAS Program in sharing ideas,

reagents, and data. The Working Group met in July 2014 and reviewed major initiatives linked to all major RAS project components, provided feedback and suggestions to the RAS Program, candidly assessed areas working well and those needing work, and assessed connectivity with the extramural community. Members were reminded that the RAS Program has approximately 50 staff and operates on a $10 M budget drawn from reprioritized funds. The FNLCR's hub-and-spoke model, in which the FNLCR as the hub interacts with extramural NCI-supported academic laboratories, biotech companies, pharmaceutical companies, and contract research organizations, works well for the RAS Project. Examples of community interaction include a U01 mechanism announced in August 2014 for next-generation synthetic lethal screens beyond 2D culture as well as the FNLCR's postdoctoral fellows program.

Dr. Garraway said that the Working Group heard progress updates about the ongoing RAS projects and components, reviewed their goals and challenges, and presented the Working Group's recommendations for each.

Project 1 is focused on determining which RAS effectors are engaged by each of the mutant proteins, solving structures of mutant proteins in complexes with relevant effectors, and characterizing RAS post-translational processing. Dr. Garraway remarked that the structural biology component synergizes with the inherent capabilities of the FNLCR, including through the generation of high-quality reagents in a production-scale manner, determination of mutant structures, characterization of biophysical parameters of key KRAS interactions, and production of processed KRAS at high yield. Challenges in RAS structural biology activities include conditions around crystallization, the absence of a structural biologist in the RAS Program, and limited understanding of both RAS complexed to GTPase-activating proteins (GAP) and specific effectors bound by mutant RAS in cancer cells. The Working Group recommended that the project consider crystal structures of mutant RAS oncoproteins for early characterization and dissemination to the community, discuss collaborative activities with pharmaceutical companies that have RAS structural biology experience, and consider patient-derived pancreatic cancer cells for experiments. Other recommendations included that the RAS Program study the structure of multiple GAPs complexed to RAS to learn the "rules" of such interactions and support *in silico* screens for compounds that stabilize RAS-GAP, and establish clear project milestones.

RAS processing represents another component of Project 1 and involves generating prenylated RAS proteins through baculovirus technology and conducting structural and biophysical analyses in membranes, liposomes, and other contexts; this should enable crystallography, electron microscopy, and biochemical studies to characterize biochemical and biophysical parameters of prenylated RAS. RAS processing has been challenging, however, and human FTI was engineered because insect cells are not effective at farnesylation, a methyltransferase protein also needed to be co-infected, and a substantial amount of unprocessed KRAS remains after these manipulations. Suggestions include large-scale production of RAS proteins, particularly membrane-based liposomes, as well as the development of robust production quality standards and mechanisms for sharing these resources with the research community.

A third component of Project 1 is the study of KRAS-effector interactions and conduct of cell-based assays to develop drugs that could disrupt these interactions. RAS effector binding assays and read-outs of RAS dimerization are needed, and members were told that three secondary assays are in development: the cell-based AlphaScreen, bimolecular fluorescence complementation assay (constitutive mCherry for cell proliferation), and bioluminescence resonance energy transfer assay. The Work Group noted that concentrations of KRAS and C-RAF in cells are about 100-fold less than in the current assay conditions, encouraged the RAS Program to be mindful of potentially creating *in vitro* artifacts that might not be relevant *in vivo*, and suggested that other RAS/RAF isoforms be considered in terms of biochemical interplay. Additional recommendations were to retain compound hits that stabilize or destabilize RAS-effector interactions, avoid complicated primary screening assays, and access many screening libraries.

Project 2 addresses phenotypic assays to identify KRAS-selective compounds through RAS-less mouse embryonic fibroblasts (MEFs) to read out isoform-dependent proliferation. The project aims to

complete cell-line testing and develop a high-throughput screen for human epithelial cell lines using an engineered endogenous KRAS locus. The Work Group cautioned that false positives and negatives can occur in this assay approach, recognized technical challenges in the development of competitive growth assays, and encouraged staff to design controls to mitigate such issues.

Dr. Garraway described Project 3 efforts to systematically perturb RAS signaling nodes to create better models of RAS signaling and possible downstream targets. In a series of KRAS-mutant cancer cell line models, one goal is to knock-down KRAS and/or downstream signaling nodes and measure various hallmarks of cancer. The Work Group was enthusiastic about the project but noted challenges regarding the visualization component; potential assay variability; and confounders (e.g., eGFP and RAS signaling both can induce ROS) that could affect data interpretation. Clear metrics and deliverables were recommended to track and communicate progress.

Members were informed that Project 4 focuses on cell-surface components, specifically mapping the surface of KRAS-mutant cancer cells to identify antigens that could be targeted by immunotherapy or nanoparticles. Preliminary studies using mass-spectrometry based interrogation of wild-type and KRAS-driven cell lines and other tools have identified more than 650 cell surface proteins unique to MCF10A KRAS cells. The Work Group found this work to be interesting but preliminary; for example, concerns were expressed about confounder effects when comparing a KRAS-expressing, immortalized breast epithelial line to a B cell (KRAS negative) line, and the Work Group reflected on the challenges in designing an ideal experiment that would allow discovery of meaningful and generalizable KRAS-dependent cell membrane expression differences.

Dr. Garraway said that the Work Group also heard about additional RAS Program components, including RAS Central, an online resource to facilitate communications in the community and encourage collaborations. The RAS Program will build and maintain a data repository to house RAS project data, provide analytical mechanisms to integrate data with existing databases, and perform relevant data mining functions; Dr. Garraway shared an example of an initial study of the ratio of KRAS 4A and 4B isoforms in different settings, and he noted a recommendation that RAS bioinformatics personnel connect with The Cancer Genome Atlas (TCGA) investigators. Members were told that an important role of the RAS Program is to generate high-quality reference reagents (DNA vectors, cell lines, viruses, proteins, and antibodies) for distribution to the external RAS community through a facile mechanism. In addition, the FNLCR and pharmaceutical companies have met to discuss a consortium focused on the precompetitive space, and postdoctoral fellows could assist in such collaborations. Government regulations and other constraints to pharmaceutical collaborations should be clarified early, and awareness of the RAS Program should be expanded.

The Work Group identified immediate priorities for the RAS Program, including launching an FNLCR-industry consortium and a postdoctoral fellow program at the FNLCR; developing a community through RAS Central; and hosting RAS scientific conferences, including a workshop on immunotherapy in RAS cancers. In addition, RAS-focused analyses of crystal structures and TCGA data, as well as KRAS-based proteomic studies and characterization of tumor-infiltrating lymphocytes present in RAS tumors, were recommended. The Work Group encouraged the FNLCR to bring additional intellectual leadership to key areas, and to establish clear goals and objectives for RAS projects currently in a preliminary state.

In the discussion, the following points were made:

- Members encouraged leadership to recruit a computational chemist to serve as a member of the *Ad Hoc* RAS Oversight Subcommittee to assist regarding the linking of *in silico* screening and structural information. The Subcommittee should reflect deeply on the prioritization of the RAS Project's future staffing needs based on research trajectory.

- Prenylation is required for RAS protein activity. KRAS is normally farnesylated in cancer cells, but geranylgeranyltransferase takes over in the presence of farnesyltransferase inhibitors, explaining why farnesyltransferase inhibitors work well on HRAS, but not KRAS. Members encouraged the RAS Project to investigate the efficacy and toxicity of targeting both farnesyltransferase and geranylgeranyltransferase in KRAS cells. Toxicity is a concern because many proteins are modified by geranylgeranyltransferase.

- Members encouraged the RAS Project to use new techniques to evaluate whether a significant fraction of geranylgeranylated KRAS is present in normal cells. Another option is to develop *ex vivo* models of prenylated RAS in the membrane and characterize its activity as a basis to better understand the biology and screen potentially efficacious compounds.

- Dr. Jim Fagin at Memorial Sloan-Kettering has proposed a clinical trial to investigate the use of farnesyltransferase inhibitors on HRAS mutant cancers. Approximately 5 percent of thyroid cancers have HRAS mutations.

- The RAS Project could conduct pilot projects using clustered regularly interspaced short palindromic repeats (CRISPR) to correct KRAS mutations in human cell lines, determine whether cell lines with a RAS mutation are RAS-driven, and test prevalent ideas about RAS that lack a solid evidence base. In addition, discussions with the Clinical Proteomic Tumor Analysis Consortium (CPTAC), which has focused on the technology to identify differences in proteins between various types of colon cancer and includes a strong bioinformatics component, may help advance RAS research.

- Collaborative research opportunities may be found through the NIH Common Fund's Library of Integrated Network-based Cellular Signatures (LINCS) Project, which is developing experimental and computational infrastructure to perturb systems broadly. Investigator-initiated research project grants (R01s) could help fill knowledge gaps, and tracking the extent to which the R01 community is catalyzed could be a positive, measurable and outcome for the RAS Program.

- Members encouraged the FNLCR to consider awards to advance RAS understanding, such as challenges (e.g., a Dream Challenge) using bioinformatics tools; providing both the data and the computational resource to analyze the data at Frederick will encourage data sharing among the community. In addition, presentations at the American Association for Cancer Research (AACR) annual meeting or co-sponsored topical meetings could help raise awareness about the FNLCR's RAS activities; RAS meetings should be scheduled with the aim to engage the community even before significant results are available. The RAS Project should recruit bioinformatics staff as appropriate, with an emphasis on the ability to make extracted data useful to non-experts, and hold a symposium with bioinformatics experts to determine potential synergies in data architecture and other areas between RAS research and the bioinformatics field.

- The RAS Project is centralized in the FNLCR and operates separate from the NCI's intramural program.

- Dr. Varmus expressed appreciation to Dr. Garraway and the *Ad Hoc* RAS Oversight Subcommittee for its deep consideration of the RAS Project activities.

V. ENGAGING THE LARGER COMMUNITY—DR. EDWARD E. HARLOW

Dr. Edward E. Harlow, Special Advisor to the NCI Director, discussed potential interactions among the RAS initiative and the scientific community and future initiatives. Dr. Harlow reminded members that the RAS Program has been interacting with the community in several ways, including by making available

research reagents such as DNA clones from RAS mutant alleles and RAS pathway proteins. In addition, the RAS website identifies access to these reagents, and shares information and fosters discussions through RAS Central, which centralizes resources for the RAS community, including blogs, videos, forums, papers, dataset sharing, and other resources. The Program has held a series of workshops focused on current FNLCR RAS research goals; three meetings have focused on next-generation synthetic lethal screens, measuring and modeling the RAS pathway, and the surface of RAS tumor cells. Dr. Harlow noted that workshop follow-up led to a funding opportunity announcement (PAR) to further engage the community. Collaborations and CRADAs with experts in the RAS community have been established, and the RAS Program is hosting visits by major players ("rock star visits") to discuss RAS-related issues and establish research collaborations. Other interactions include three postdoctoral programs for RAS-related problems, growing interest in a pharmaceutical/biotechnology consortium, and the hiring of staff to deliver specific services. Dr. Harlow reminded members that although expectations are that the RAS Program should be characterized by a nimbleness similar to a biotechnology company, the lack of some tools and the need to provide process funding through contractual channels has delayed some interactions with the community.

Dr. Harlow reflected on general features of a national initiative for the FNLCR. A national initiative should be formed around a scientific or technical issue that is recognized as an important problem, be the right scale, not be solvable through other mechanisms, and have sufficient data to suggest success. Success should provide a major advance, be measured through tangible outputs of success, and require interactions with the community. The initiative should be difficult enough not to guarantee immediate success. Potential sources for initiative ideas include NCI staff, advisory groups, Directors of NCI-designated Cancer Centers, special panels of national experts, or a meeting similar to a Provocative Questions (PQ) initiative workshop. Dr. Harlow described his experience of involving the scientific community in the PQ workshops, including the increased attention of the community on various issues, credibility in the process as the community helps to build the subject, challenges to participants in preparing for a workshop, and the reaching of new or deeper understanding about topics through workshop interaction. For example, at recent PQ workshops in Boston, MA, participants advocated for a re-thinking about cancer prevention from an economic basis as research costs surge, a link of epidemiologically identified events to cancer cell biology and genetics, the development of drugs for transcription factors, and drug targeting of the gene MYC.

In the discussion, the following points were made:

- Members lauded the PQ initiative for its success in enveloping the broad research community and encouraged that there may be opportunities for FNLCR to partner with private industry and foundations on pancreatic and other cancers. They agreed that engaging the Directors of NCI-designated Cancer Centers in dialogue would be beneficial to both the FNLCR and the Cancer Centers.

- Members suggested that the PQ solicitation include a question on the relationship between inflammation and cancer, with emphases on precancer initiating events, mechanistic studies, and the microbiome and metabolism.

- The FNLCR's vaccine capabilities may have a role in advancing immunotherapy. For example, use of the mutant cysteine in the RAS pocket as a covalent interaction site to introduce a new epitope that could be preimmunized for that setting was suggested as a potential research idea at the initial RAS workshop in San Francisco, CA.

- The NCI was encouraged to provide direct feedback to PQ workshop participants regarding results of the questions discussed during the workshops.

VI. NATIONAL MOLECULAR MICROSCOPY LABORATORY (NMML)—DR. SRIRAM SUBRAMANIAM

Dr. Sriram Subramaniam, Laboratory of Cell Biology, CCR, presented a proposal for a National Molecular Microscopy Laboratory (NMML). Dr. Subramaniam described gaps in biomedical imaging that are not addressed by conventional electron microscopy or traditional imaging technologies. These included entities that are either too heterogeneous or large to be crystallized, such as protein and signaling complexes, membranes, some viruses, and whole organisms. During the past 10 years, work in molecular microscopy has considered proteins in isolated form and native context as well as viruses in whole, bacterial and mammalian cells in heterogeneous and pleomorphic (HIV, influenza, Ebola) entities. For example, activities focused on 3D mapping of cancer cells, special architecture of signal transduction, mechanisms of HIV entry, and protein complexes in metabolism. Members were told that the cryo-electron microscopy (EM) field currently is working in obtaining protein structures without crystallizing them but at near-atomic resolution.

Dr. Subramaniam highlighted recent developments and applications of imaging tools in cancer research that describe the landscape of the microscopy field and provided context for the NMML proposed project. The focused ion beam scanning electron microscopy tool was adapted from the semiconductor arena and now is routinely used to examine the outer structure of cells. It has elucidated the architecture of how various organelles are arranged in cells, been used to correlate fluorescent microscopy, and has brought scientists to a state where they can begin to localize molecules in the context of the large cell.

Electron tomography was used to develop computational tools to visualize whole bacterial cells as well as the structures of proteins that made up signaling arrays. This led to the development and use of tools to examine molecules in intact cells and predict the behavior of bacteria as conditions in the growth medium changed. In addition, cryo-electron tomography has been applied to HIV, providing a 3D image of a single variant. To address low-resolution images, however, tools were developed to average density maps for individual spikes; models for large entities studied at low resolution were developed that could combine the information in crystallography. Members were informed about the cryo-EM field's strong interest in automating the determination of protein structures without the use of crystallography.

Members heard about the evolution of the cryo-EM field during the past 2 decades, starting with the work of Dr. Richard Henderson to determine the near-atomic resolution structure of bacteriorhodopsin, expanding to many structures captured in medium resolution, and significant changes in resolution in 2013 provided by the development of direct detectors, greater stability of microscopes, and improved computational tools that process signals. He noted that many of the laboratories in the cryo-EM field are well established, and breakthroughs in reaching near-atomic resolution through cryo-EM for many smaller proteins and ion channels were reached in 2013. The strategic vision for the proposed NMML would encompass: (1) an expansion of the imaging landscape particularly to involve structural biologists, crystallographers, biochemists, and others who are not in the cryo-EM field; and (2) consideration of how to drive imaging technology forward to provide more useful information through higher resolution technologies and application to interesting protein complexes and other biological and therapeutic concerns.

Dr. Subramaniam reflected on the overall NMML proposed project, describing it as an infrastructure for recruitment where the latest microscopes are operated by cryo-EM experts and provides a base for users from universities to come for training to collect the data so that they can then get answers to questions. A second mission component might be to support the development of reproducible workflows and new tools and technology through partnerships with companies. He recognized the importance of engaging the community of developers to ensure that they are in accord with the types of problems to be solved. In addition, training in the microscopy language would encourage smaller scale investments by local institutions in cyro-EM and other high-resolution imaging technologies. He also provided examples of experiments that users might bring to or take from the National Laboratory: a glutamate receptor as a prototype of a membrane protein purified in the NMML; and high-resolution imaging of the structure of glutamate dehydrogenase, a metabolic enzyme

important to cancer, to reveal components of 20,000 molecules, such as side chains, salt bridges, and localized ligands. Members were told that annual costs for the National Laboratory are estimated at $10–20 M, including biochemical, microscopy, and computing staff. Several high-end microscopes are anticipated, at an approximate cost of $4–5 M each. Dr. Subramaniam indicated that partnerships with microscope manufacturers, pharmaceutical companies, and other NIH Institutes and Centers (ICs) could reduce costs and offer greater synergies in the molecular microscopy landscape.

In the discussion, the following points were made:

- The proposed project would be broadly applicable as an enabling tool through a focus on complexes such as membrane proteins that are not solved but where solving them would be important, particularly in structural biology. Initial emphasis on describing the conformational landscape of the complexes without having to crystallize them in near-native conditions could have a profound effect in advancing the field.

- Members noted the rapid development and turnover of technological equipment in this field (12 months) and supported the project intent to interface with technology companies to elucidate the biological challenges faced and better influence technology development.

- In response to member concerns about having only one facility to meet the potential high demand for services, NCI leadership cited Dr. Subramaniam's lengthy experience in driving technology in partnership with industry. The availability of a place to support high-resolution imaging needs would provide a valuable service to the community.

- Increased automation, data handling, and sophisticated computing are components that would add value for the NMML and push the state-of-the-art in instrumentation. The proposed National Laboratory likely would integrate different aspects of sample preparation.

- The challenge is less to create a unique resource to send samples and more to move the field to the combination of interactions with companies and high-quality computing. It will bring interesting and important biological problems to the FNLCR so that the technology actually solves those kinds of problems.

- Possible routes to improve the use of microscopy technology are: (1) technology improvements; (2) onsite services for the community; (3) training opportunities to build capacity of other institutions; and (4) sample preparation.

- Members encouraged leadership to hold a workshop that engaged microscopy stakeholders such as user groups, biochemists and other technical experts, and software and large microscopy manufacturers to further discuss the NMML concept as a potential project.

- Dr. Gray requested a detailed presentation about the FNLCR's capabilities and work in computational biology at a future NFAC meeting.

VII. ONGOING AND NEW BUSINESS—DR. JOE W. GRAY

Motion. A motion to change the name of the NCI-Frederick Advisory Committee (NFAC) to the Frederick National Laboratory Advisory Committee (FNLAC) was approved unanimously.

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

Dr. Gray encouraged the NCI to continue efforts to obtain community input regarding potential projects for the FNLCR at such venues as PQ workshops and a Cancer Centers Directors meeting. He noted that none of the potential projects presented at the February 2014 meeting resounded to the participants and suggested that a follow-through mechanism may be needed. Members were referred to the February 2014 meeting minutes to review proposed projects, and urged to champion promising projects. Dr. Varmus said that the DOE National Laboratories have committees that nurture scientific projects. He added that he and Dr. Heimbrook have held discussions with regional universities about collaborative opportunities.

Future Agenda Items. Dr. Gray reflected on the meeting and requested that the next FNLAC meeting include a description of other FNLCR components beyond the RAS project, including the review process and evaluative mechanisms.

In the discussion, the following points were made:

- Constituencies could help identify future FNLCR projects through working lunch sessions at PQ workshops or a separate meeting of PQ "all-stars."

- Results of the PQ workshops can be seen in the form of solicitation questions provided on the PQ website. Each workshop generally yields 1–2 questions that are incorporated into a solicitation. The NCI was encouraged to provide direct feedback to PQ workshop participants.

VIII. ADJOURNMENT—DR. JOE W. GRAY

Dr. Gray thanked the Committee members and other invitees for attending. There being no further business, the 7th meeting of the FNLAC was adjourned at 3:35 p.m. on Tuesday, September 30, 2014.

_____ _____

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

_____ _____

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