NCI RAS Initiative Evaluation Team (RIET) Ad Hoc Working Group Review Report on the FNLCR RAS Initiative

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Executive Summary

The RAS Initiative was launched in 2013 to explore in detail the biology of oncogenic RAS and to discover therapeutic approaches for this previously "undruggable" target, with the ultimate goal of developing new drugs. The Initiative utilizes a "hub-and-spoke" model where core elements that are located at FNLCR (hub) have multiple interactions (spokes) to the extramural academic and commercial communities. The Ras Initiative began by addressing critical knowledge gaps that had impeded the exploitation of RAS as a drug target, and significant investments in structural biology, biochemistry and biophysics, chemical screening, and engagement with the global RAS community yielded impactful outcomes during the inaugural period in FY14-18. A 5- year renewal for FY18-22 built on this success with two broad scientific goals being pursued: a. to develop small molecules that bind directly to KRAS and block its function, and advance these towards clinical evaluation, and b. to determine how KRAS proteins interact with plasma membranes and how they activate RAF kinases.

Under the leadership of Drs. McCormick and Nissley, a wonderful team of tremendously qualified scientists has been assembled, with an amazing breadth of expertise, both within the Frederick team or through established collaborations with companies or extramural labs. As a result of these efforts, small molecule inhibitors have been discovered and are being brought to the clinic in collaboration with a biopharmaceutical partner; new technologies have been developed to identify additional binding regions in RAS that might be drugged; approaches to targeting RAS effectors have been pursued. The Initiative has also allowed for know-how and reagents transfers though multiple avenues, including the organization of the *RAS symposium*, a fantastic way to unite the field.

The idea that RAS is undruggable, the very impetus for forming the RAS Initiative, is simply no longer true, with academia and industry providing a pipeline of inhibitors. Moreover, based on other targeted therapies and results from clinical trials, RAS inhibitors are unlikely to be curative, therefore future work will likely involve drug combination trials, based on in depth studies to delineate mechanisms of resistance and adaptation.

With the Initiative having successfully completed its critical task, it is recommended that NCI evaluate whether to continue exploration on RAS or begin winding this program down as originally designed, by providing for example, 3 years of support to complete the most exciting lines of investigation, and during that time, forming a committee that will work with the NCI on the identification of new initiative(s) that will leverage the unrivalled infrastructure established under the RAS Initiative and the talent of the exceptional investigators that have contributed to it.

Section 1: RAS Initiative Overview

The RAS Initiative was launched in 2013 to explore in detail the biology of oncogenic RAS and to discover therapeutic approaches for this previously "undruggable" target, with the ultimate goal of developing new drugs. The Initiative utilizes a "hub-and-spoke" model where core elements that are located at FNLCR (hub) have multiple interactions (spokes) to the extramural academic and commercial communities. The Ras Initiative began by addressing critical knowledge gaps that had impeded the exploitation of RAS as a drug target, and significant investments in structural biology, biochemistry and biophysics, chemical screening, and engagement with the global RAS community yielded impactful outcomes during the inaugural period in FY14-18. A 5- year renewal for FY18-22 builds on this success with two broad scientific goals being pursued: 1) develop small molecules that bind directly to KRAS and block its function, and advance these towards clinical evaluation, and 2) determine how KRAS proteins interact with plasma membranes and how they activate RAF kinases.

Under the outstanding leadership of Drs. McCormick and Nissley, the Initiative has made significant progress in the last 5 years, despite the recent COVID-19 challenges. Notable achievements include building a talented, cohesive, and dedicated multi-disciplinary team of scientists. As such, the Initiative's capabilities on biophysics, biochemistry and structural biology are truly exceptional and world leading. New investments have been made in additional technology capabilities such as medicinal and computational chemistry, NMR, cryo-EM, and proteomics. Important collaborations exist with a large network of partners from academia, NCI, DoE, as well as from biotech and pharma companies. Furthermore, the establishment of contractor Cooperative Research And Development Agreements (cCRADAs) has provided crucial access to various drug discovery platforms, compound libraries and drug development expertise through CROs and other partners. This collaborative approach has enabled the RAS Initiative to evolve into a virtual world-class "bench to bedside" drug discovery and development organization.

Most notable achievements during the last funding period include two novel therapeutic candidates – a covalent, active-form KRAS G12C inhibitor and a RAS/PI3Ka complex disruptor – that are expected to enter clinical development in 2023/2024. A very robust earlier-stage pipeline exists, including targeting different KRAS mutants, most notably KRAS G12D, as well as targeting RAS regulatory and effector proteins. A novel disulfide tethering library has enabled identification of pocket-specific binders for KRAS mutants. The Research Team has also solved several important protein structures including several "firsts" (such as first structure of RAF1 RBD/CRD bound to wild-type and mutant RAS) and has utilized insights from these structures to develop completely novel drug discovery programs. Additional important accomplishments include insights into the structure and function of neurofibromin. Further, the Team continues its efforts to produce insights into how KRAS interacts with the plasma membrane, and how KRAS activates RAF kinases.

In the next five years, current drug discovery projects towards the clinic will be continued, and additional new therapeutic options that have emerged will be pursued. Ongoing investigations into the role of RAS at the membrane, the characterization of KRAS alleles, and the structure and function of neurofibromin will continue. Several novel projects leveraging the Research

Team's experience, expertise and preliminary results obtained will also be launched. These project plans are reviewed and discussed later in this document.

In sum, as indicated below, the RAS Initiative has leveraged its unique organizational and funding model, and it has evolved into a dynamic, cohesive, and collaborative "center of excellence" in drug discovery and development. It is expected that the RAS Initiative will continue on a successful trajectory, if it continues to leverage its differentiating approaches and focus on pertinent questions not easily addressable by others or by using other funding mechanisms.

a. The Initiative has generated several unique, high-quality tools, reagents, and technological capabilities on RAS and RAS pathway proteins including SPRED and NF1. The RAS Initiative leadership, in collaboration and consultation with NCI, is advised to devise ways how to make these resources more readily available to the larger RAS community, and to more actively share the unblinded chemical compounds that are precompetitive in nature like the Cys tethering collection.
b. The Initiative would benefit from prioritization of projects, as well as definition of milestones and go/no go decisions for activities going forward. As noted above, the Initiative should continue to leverage its differentiating approaches and focus on projects and questions not easily addressable by others or by using other funding mechanisms.
c. Additionally, more transparency on the program is recommended as it would prevent unnecessary duplication of efforts and NCI resources elsewhere, as well as provide opportunities for collaborations within the broader NCI-funded research community.
d. Clinical advancement of the KRAS G12C inhibitor and the RAS/PI3Ka complex disruptor is largely expected to be carried out by TheRas/BridgeBio. It is hoped that the important role that the RAS Initiative has played as a driver of innovation for these programs will be recognized and that increased transparency with regard to the process used to execute cCRADAs with TheRas will ameliorate concerns about potential conflicts of interest.
e. The movement of other KRAS mutant (especially KRAS G12D) and pan-KRAS compounds toward clinical translation should continue to be prioritized within the Initiative, however it is expected that most of these activities and tasks, which focus on standard drug development/pre-IND activities, will be carried out by outside partners with drug development expertise. Given the increased competition in the field, the Initiative is advised to focus on pipeline programs where it can best differentiate itself from others.
f. With the advancement of the RAS Initiative drug discovery pipeline, and two approved anti-RAS agents in clinical use already, this is an opportune time to catalyze a robust assessment into mechanisms of drug response and resistance and biomarker development. This could involve translational studies on patient tissue samples and cell/organoid culture and preclinical in vivo models and utilize cutting-edge multi-omics

and other technologies available in the cancer biology community.

g. Similarly, expansion of community engagement could be helpful when exploring the effects of microenvironment and the immune system on these therapeutic agents in RAS- driven cancers, especially in a metastatic setting. Likewise, the community could be engaged in pre-clinical and translational studies on combination therapies with the novel compounds.
h. Studies of RAS on the membrane brings the Initiative together with LLNL to explore and model the activation of RAF kinases. This study seeks to invent novel computational capabilities that will make it possible to model protein domain movement at time scales relevant for biology. Numerous cutting-edge experimental systems are in place to complement these efforts. This is a highly innovative area of biological research that could bring significant unique insights into RAS signaling and should be additionally prioritized.
i. Novel innovative strategies, such as targeting mutant RAS as a tumor specific neoantigen, are being developed in the academic community and would provide a new collaborative opportunity for the RAS Initiative programs.

Section 2: RAS Initiative Research Project Summaries

2.1: RAS Initiative reagents and analytics (RRA)

RRA remains a critical component of network engagement and is an exceptionally strong element of the initiative. RRA broadly supports the effort, developing a variety of tools (e.g., nucleic acids, proteins, cell lines) that enable a broad set of experiments and that introduce tremendous rigor and experimental consistency into the field. For example, mutations in clones or variation in tagging strategies can lead to experimental variation and the RRA has developed high-quality and standardized tools to propel the field. Overall, the RRA has been highly productive in developing high-quality reagents and new technologies for a broad swath of extramural RAS investigators. Selected achievements are summarized below:

Protein production: The team developed >2,500 large-scale protein preps for a variety of applications (e.g., NMR, RAS-related biochemistry involving FL proteins, biotinylated proteins for biochemistry and biophysics). The team has also developed an impressive number of plasmids and constructs for production. Innovation has come in the form of scaled up and consistent processes for both pure protein expression and multiprotein complexes (e.g., to enable structural biology efforts).

Nucleic acid production: This team broadly supported a wider set of groups, enabling basic biology (e.g., knockdowns, CRISPR), cell-based assays (e.g., biosensors), drug discovery (e.g., mutants, proteins for covalent tethering), biochemistry/biophysics (e.g., SPR, HTRF), and structural biology (e.g., scaled up protein production). All these activities are greatly supportive of the overall effort.

Cell line production: The CDG developed new lines while maintaining critical quality control activities. The team generated >1,500 cell lines in the last five years. The team generated or acquired an additional 600 MEF cell lines covering various mutations in the past five years. Notably, the MEFs have been distributed to a large swath of companies under agreement, and new proposed work relates to improving MEF reagents.

New technology development, assessment, or incorporation: The team optimized a new expression platform taking advantage of *vibrio natriegens*, enabling expression for some of the more challenging domains of interest to the community. Along the same lines, folding chaperone methodologies and cleverly identified SGT1 to assist in SHOC2 production and developed an engineered variant with broader utility in folding LRR proteins. The introduction of mass spectrometry is a strength for this group and other teams in the initiative, and enables protein quality control as well as RAS proteoform analysis, identification of novel RAS PTMs, and characterization of target:compound engagement, among others. The panel would like the MS team to expand beyond static snapshots and incorporate kinetic analyses in the future, but the team's work has been impressive to date. Many of the technological advances may have broader impacts that can enable studies and investigators beyond the RAS network.

Bioinformatics and analytics are also included, although the description of achievements is rather spartan. It's clear for the full proposal that informatics and analytics is critical to large-scale experimentation, but this section is not fully described. It's not entirely clear why analytics falls into this group. Most of the details related to informatics and computation fell

into individual research sections. The panel also wanted to know more about how AI/ML supports the wider Initiative, and if not, how this group intends to do so in the future.

The future activities for RRA are clearly outlined. A critical and valuable part of the proposal really boils down to 'more of the same' given the success and impact this group has had historically. One suggestion included expanding reagent development to also include reagent curation from the community and the development of a high-quality repository that included validated tools developed outside of the Frederick labs. Three specific areas of focus are discussed in more detail include the development of a second MEF panel due to issues related to maintenance of ploidy in the original reagent set. A new panel of mutant MEFs will now be generated to improve reagent consistency. The team plans to expand the panel beyond the RAS mutant isoforms to include signaling partners. The team is well suited to undertake this task, but the panel suggests that the team consider exploration of other model systems, including patient-derived models. Probably wisely, there is an increased interest in studying RAS-related multiprotein complexes as an alternative to individual and isolated proteins. This includes structural investigations of complexes involving >6 proteins. Rather than rely on reconstitution, the team will navigate complex co-expression strategies and aim to develop new strategies that may have general utility. The panel was enthusiastic about this new direction, which takes advantage of the sophisticated protein expression systems, state-of-the-art x-ray crystallography and cryo-EM methods developed within the Initiative. Finally, advanced proteomic methods and instruments will be utilized to characterize protein complexes as well as novel RAS proteoforms. All these studies are appropriate and form an important extension of work in the context of the overall RAS Initiative.

Overall, there were few weaknesses with the work to date and proposed strategies moving forward. The team is exceptional. The team should consider the RRA as a mechanism for distributing tool compounds, moving beyond biologic reagents and technologies. The model for RRA may have utility for other areas of cancer research or potentially transformative targets in oncology.

2.2: Targeting the RAS: PI3Ka interaction with molecular breakers

The RAS Initiative and their collaborators have successfully executed on an elegant approach to discover and develop molecules to specifically disrupt the interaction of RAS with PI3Ka. These efforts have established a drug discovery campaign entering the final phases of lead optimization with a goal of progressing a development candidate through IND-enabling studies and into the clinic within the next twelve months. This effort has the potential to bring a molecule to patients that is positively differentiated from the FDA approved PI3Ka inhibitor alpelisib on two points: the RAS:PI3Ka molecular breaker exhibits a significantly reduced risk of hyperglycemia, and furthermore has the potential for therapeutic benefit in a broader patient population, including patients with PIK3CA and KRAS G12 mutations.

To initiate this program, the team took advantage of the discovery of a molecular "glue" that significantly increased the affinity of the interaction of KRAS with p110a. This compound allowed the formation of a stable KRAS:p110a ternary complex, which enabled the elucidation of the KRAS:p110a co-crystal structure. The team was then able to leverage structural insights from "glue" driven interaction between KRAS and p110a to successfully convert the "glue"

into a disrupter and block p110a interaction with KRAS. The subsequent drug discovery campaign has identified potent and selective orally bioavailable "breakers" with *in-vivo* efficacy in multiple PDX models at doses that do not affect glucose metabolism or insulin levels. While the data demonstrating on-target efficacy with limited impact on insulin signaling in multiple tumor xenograft models was very robust, it would have been helpful if the team had described in general terms the potential liabilities of these molecules. This information would be useful when assessing the potential for technical success for the current lead molecule and the need for a back-up or second-generation molecule, and ultimately ascertain whether there is a need for the RAS Initiative to remain engaged in this mature drug discovery effort.

This program provides a robust illustration of the strengths of the RAS Initiative in the arenas of biophysics, biochemistry, and structural biology. Furthermore, it serves to highlight the ability of the team from the RAS Initiative and their collaborators at Lawrence Livermore National Laboratory and Bridge Bio to rapidly progress a highly innovative drug discovery program with potential to bring forward a first-in-class molecule targeting the interaction of RAS and PI3Ka. This effort nicely illustrates the potential for the team to apply these skills and learnings to future challenges.

Given the advanced stages of these drug discovery efforts and the transfer to BridgeBio, it is assumed the RAS Initiative role will pivot. The future plans as laid out in the renewal request would benefit from more detail and clarity on the efforts to build a deeper understanding of the potential patient population that could derive benefit from a RAS:PI3Ka breaker. For example, within the KRAS mutant population, are there specific mutations or indications that are potentially more sensitive than others? The approach for combinations is not well articulated and would benefit from a more developed strategy highlighting opportunities that have the potential for greatest impact. Finally, a well-developed understanding the mechanisms of acquired and resistance to the "breaker" molecules will be critical to ensure success in the clinic. In summary, being well prepared to address these apparent gaps would seem to be an obvious future priority for the RAS Initiative when the focus is going to transition from a drug discovery campaign to more nuanced biology and translational questions.

The work undertaken by the RAS Initiative and their collaborators on the discovery of breakers of the PI3Ka:RAS interaction is impressive and has the potential for several high impact publications. However, it wasn't totally clear how the groundbreaking work from this program will be disclosed to the broader scientific community. Is this a decision for the RAS Initiative or Bridge Bio? It seems like this could be a great opportunity to showcase the work of the RAS Initiative in peer-reviewed journals to maximize the impact of the work undertaken to date. Finally, the RAS Initiative needs to ensure a mechanism is in place to share well-characterized tool compounds from this program with the broader community in the very near future.

2.3: Development of dual KRAS ON/OFF inhibitors

The RAS Initiative has developed innovative molecules that target both the active (GTP) and inactive (GDP) bound states of KRASG12C using both covalent and non-covalent mechanisms.

The application of structural biology and biophysics expertise to the non-covalent inhibitors was particularly noteworthy and highlights the strength of the group. These dual-inhibitors are expected to provide an advance over currently approved molecules that only have affinity for the inactive GDP-bound state. Indeed, compelling evidence is presented that shows superiority over compounds like AMG510. This project is an excellent example of how the capabilities and synergy of the group can be successfully applied to tackle a challenging problem. The report indicates that one compound is expected to enter clinical trials in mid-2023. However, missing were details about exactly what stage the project was at, what are next steps and what are go/ no go decision points for advancement to clinical trials later this year. Also unclear was how the RAS Initiative was contributing to these development efforts.

It appears that Mirati has reported on non-covalent compounds with a similar profile, suggesting related work is going on in the outside community. This is an area that deserved additional discussion, as well as a description of how the RAS Initiative could uniquely contribute now that compounds are at such an advanced stage and now that others are also pursuing similar approaches.

The future plans for this project are only briefly discussed, and the committee was looking for a pivot to a more biology-centric approach to answer key questions that will be essential for clinical development such as 1) are the compounds cytostatic or cytotoxic; 2) does resistance develop and what is that mechanism; 3) evaluation of key compounds in more sophisticated pre-clinical models; 4) given the goal to treat pancreatic cancer, evaluation of efficacy in these models. A second disappointment was that there was no mention of how/ when the compounds will be made available to the broader scientific community. Doing so is one way to address many of the questions above. Finally, there was little clarity on the roles and responsibilities of the RAS Initiative vs. TheRas for the next phases of this project.

There was a lack of clarity on the new scaffolds/ class of compounds mentioned in the future plans. With a proof of concept that such compounds are feasible, and with other groups pursuing similar approaches, one question is whether this is the most appropriate use of the unique RAS Initiative resources and whether this work is more of an incremental advancement rather than paradigm shifting.

The panel would have liked to see a disclosure/ publication plan that would optimize this work's impact and reflect and showcase the outstanding work of the RAS Initiative.

2.4: Construction and screening of a novel disulfide tethering library

The overarching idea, inspired by previous work targeting KRAS C185 and H95, is to survey all possible surface accessible residues on KRAS to identify new proximal binding pockets or 'ligandable' sites. The principal chemist is a highly skilled project lead for the covalent inhibition efforts and a new disulfide tethering library.

The library appears to be thoughtfully designed but there are no details on how the fragments are selected and given they are commercially available it is further unclear why these have not been shared as an SD file with the community. Some expansion of mol weight might be useful

to get better hit rates.

The linker design appears to be based around the principal chemist's prior experience at a highly regarded research university, and this is a critical element that was well addressed in Q&A. The team outlined future plans related to linkerology, emphasizing introduction of rigidity.

The library has been used in multiple contexts to date, suggesting that this investment can enable discovery in multiple ways that can impact RAS biology. There were a few suggestions about strategy for advancement. First, hit expansion using non-commercially available acids would be an interesting future activity. This proposal and screening efforts have lacked incremental chemistry efforts for advanced hit finding but rather has relied on HT docking to find related compounds to discover validated hits. Additionally, the replacement of a warhead with acrylamides is logical, but small changes in the hit structure via synthesis is missing and this activity could expand the wider library. While described not as a drug discovery focused effort, the team has leads and quantitative thresholds for advancement and clear go/no go included for optimization of tools.

The team should think seriously about the national library concept as there is increasing interest in Cys-reactive approaches or disulfide tethering approaches to map novel binding pockets around other targets of interest to the community and the NCI, including the FusOnc2 Network focused on highly undruggable oncofusion proteins implicated in pediatric fusions. This is a great example of how the RAS effort has developed valuable tools and resources that can be deployed more widely than originally envisioned. The nature of the collection including synthetic methods and design elements should be published as used by the RAS Initiative.

The panel would like to see more publications or outward facing presentations from this team to the wider community. The lead chemists should be presenting at AACR and other conferences about their approach to identify new binding pockets and the development of the library. We expect to see them advance a lead further.

2.5: RAS activation of RAF kinase

The team is to be commended for the truly outstanding structural biology and biophysics focused on the role of RAF-RAS interaction. Structural biology is high impact and extraordinarily informative. The biophysical studies of membrane interactions are well thought out and executed. The combination of the experimental studies with the simulations is viewed with substantial enthusiasm. The two can feed each other as well as provide validation. This project is viewed as a clear strength of the program.

The investigators have carried out a series of high-end biophysical measurements using SPR, neutron reflectometry, and NMR spectroscopy to garner insights into RAS and RAF interaction with membranes. Among the interesting findings were the lack of membrane binding by the RBD of RAF and the moderate membrane binding of the CRD domain of RAF, both of which likely have functional implications. These experimental measurements are being coupled with molecular dynamics simulations to garner further insights. This combination of computational approaches with appropriate experimental data is viewed as a powerful approach to model this complex process and develop testable hypotheses. As SPR is being used for these

measurements, it is also possible to probe the kinetics of the interactions with the liposomes. Similarly, the binding studies focused on KRAS and RAF binding would also benefit from analysis of the kinetics, particularly with respect to binding of isolated RBD and CRD domains to KRAS versus the tandem RBD+CBD. This applies both to the wildtype proteins as well as the mutants developed. Kinetics could have important functional implications, so this seems like a missed opportunity.

The RAS Initiative has had an outstanding track record of structure determinations that have greatly advanced our understanding of isolated proteins and more importantly the multi-protein complexes they function as part of. The determination of the structure of the KRAS-RAF(RBD+CRD) complex certainly adds to this outstanding legacy. This structure and the combined functional studies showing the importance of the CRD-RAS interaction for RAF activation is an important contribution. Indeed, the working model of RAF activation presented relies on insights gained from the structure and functional studies. The collaboration with Dr. Morrison is viewed as an outstanding development.

One caveat regarding the NMR studies of RAS membrane interactions is these are being done with nanodiscs of defined lipid composition. Nanodiscs are certainly a lipid bilayer mimetic, albeit one which cannot reproduce the curvature of the membrane and for which the composition is limited. The SPR studies utilized liposomes with varied lipid composition. This is much closer to a true bilayer environment and therefore would be a more appropriate system to evaluate the interactions with membranes. EPR spectroscopy has been used extensively to probe such interactions, for example by measuring depth of insertion of nitroxide labels introduced at appropriate sites on a protein. The team made clear they are aware of these capabilities and are exploring the use of this approach as well as EPR DEER measurements to probe conformational changes in the lifecycle of RAF activation in the lipid environment used for the SPR studies and utilizing the same lipid composition in the simulations. This approach is highly likely to yield important insights into RAF activation and is viewed with enthusiasm. Importantly, the team has a clear and testable working model for RAF activation which is guiding their experimental approach.

Future efforts include using cryo-EM to solve a structure of KRAS and RAF bound to a nanodisc. This will provide critical insight into the interactions with the membrane and membrane effects on the proteins. As stated above, the ongoing collaboration with the DOE to carry out simulations, guided by the experimental data, is a clear strength and there is significant enthusiasm for these ongoing efforts. The team has plans to transition to full-length RAF for their studies. This is an essential next step in these studies to garner the most physiologically relevant insights. The team also indicated their awareness of the various isoforms of 14-3-3 and the need to evaluate the effects of different 14-3-3 isoforms, highlighting their deep knowledge of the field. The plans for future efforts are well thought out and likely to yield important functional insights.

The only concern identified is the lack of an effort to use the elegant structural and biophysical data to guide functional studies to evaluate effects on RAS/RAF signaling.

2.6: RAS in membranes

The major goal of this project is to develop a molecular framework for understanding how the interactions of RAS with membranes dictates effector interactions and activation. Using state-of- the art imaging and analytic techniques such as super resolution microscopy, single particle tracking, diffusion analysis and multi-scale simulation, several important observations pertaining to the biochemical and biophysical interactions of RAS with membrane have been made. These include identification of structural and biochemical feature of KRas4b that contribute to its diffusion characteristics as well as the impact of lipid composition on effector binding. Notably, the team working on this project is comprised of exceptionally skilled scientists whose contributions to the advances made thus far deserve the highest praise. The pursuit of this project within the confines of the RAS Initiative offers unique advantages stemming from access to high-performance computing infrastructures and computational scientists afforded by the collaboration with DOE National Laboratory.

The efforts dedicated to this project have yielded a powerful experimental platform for addressing questions related to RAS behavior in membranes. However, it was not apparent how information gathered from inherently reductionist approaches can capture the complex biology that drives the interactions of RAS with membrane and the resulting effector pathways activation. A strategic plan that delineates how this gap might be closed (potentially leveraging the knowledge base within the extramural community) would be critical for achieving translational impact.

2.7: Structure and function of the SHOC2-MRAS-PP1C (SMP) complex

The RAS Initiative has had an outstanding track record of structure determinations that have greatly advanced our understanding of isolated proteins and more importantly the multi-protein complexes they function as part of. The determination of the structure of the SHOC2-MRAS-PP1C complex adds to this outstanding legacy in a highly contested area from Novartis and others. This structure determination also very effectively highlights the critical role of the protein production expertise embedded in the RAS Initiative, without which this structure would not have been solved. The clear value of having both protein production and structural biology intimately linked as key components of the RAS Initiative is made very clear with this structure determination. The progress in getting to a better understanding of SHOC2 structural biology is novel and interesting relative to better understanding with the SMP/SKP complex. This information should be shared with the community without any delay.

The focus on trying to target specific interfaces in this complex, particularly the SHOC2-MRAS interface, is well justified by the biology and toxicity considerations. However, the utility of this approach should be evaluated in cells via introduction of the appropriate mutations into SHOC2 and evaluation of effects prior to committing to a drug discovery effort. A potential critical residue R188 in the interaction with PP1C is an important discovery and should be published to better help the community in the work of potential drug design. This does not mean that this group shouldn't work on targeting that in parallel but making this available to

others is important.

There were no clear plans on how one would consider drugging this complex – not that this is critical with where the work is right now, but the group appears highly motivated to do drug discovery and the relative druggable nature of this interaction compared to other opportunities described in the overall proposal is not outlined. One approach to consider for therapeutic targeting of this complex could be the development of a ligand that binds to one protein of the complex and converting this to a PROTAC which directs ubiquitination of a separate member of the complex. In this way, selective destruction of the complex but not apo proteins might be achieved. Would targeting SHOC2 require an extended molecule that can engage multiple LRR motifs? These questions remain unanswered so it is not clear how one would move into ligand discovery.

The function of this complex is still a work in progress and the recommendation is to continue this effort to better understand the role of this complex in disease. While some very nice work has been completed for the various isoforms and their relative binding – the functional consequence of that for specific tumor types will be important. What insights will be gleaned from structures of SMP complexes with H/K/NRAS that were not obtained with the MRAS structure and their relative role in tumor profiling? For example, what are the functional effects of SHOC2 mutations in LRR2 and/or LRR4? Have all of these been introduced into cells and RAS driven readouts assessed?

2.8: Neurofibromin (NF1) biochemistry and structural biology

The RAS Initiative project on NF1 succeeded in expressing the large NF1 protein and altering the large NF1 cDNA to make it stable; these are major accomplishments recognized in the community. They produced NF1 fragments and provided these to outside labs. The team also used numerous biophysical assays available to them to show that NF1 exists as an obligate dimer;-- another major accomplishment. The team developed negative staining assays to use to suggest the remarkable structure of the NF1 dimer, and most recently contributed to structural biology analysis of NF1 using cryo-EM.

We rank the prior accomplishments on the NF1 project as excellent.

While other, academic, groups more rapidly reported the accurate lemniscate structure of the NF1 dimer using cryo-EM, and positioned patient mutations in the dimer interfaces, the RAS Initiative team is now poised to use their strengths in crystallography and biophysical analyses to define NF1 interactions with several target proteins, in collaboration with the National Cryo-EM facility, and to better define unstructured domains containing phosphorylation sites. Specifically, structure/function studies and elucidation of interaction partners RAS, SPRED, and the RTK KIT are planned, taking advantage of their expertise in protein production, and working with protein complexes and artificial lipid combinations.

The team proposed several additional avenues for study. It will be important to define immediate and long-term goals of each project/subproject, and to prioritize among the diverse studies. Prioritization of those studies most relevant to RAS and cancer, versus RAS signaling in

Rasopathy disorders, was not discussed but will be an important future consideration.

The identification of NF1 as an obligate dimer and production of full length, stable, NF1 RNA and protein was significant and elucidation of the structure of NF1 with interacting proteins takes advantage of the unique capabilities of the FNLCR team. Other parts of the described future plans, such as elucidating the mechanism of neurofibromin activation may be redundant with work in the academic community, and study details were not defined. Dampening KRAS (oncogenic) activity by enhancing NF1 stability or augmenting NF1/RAS interaction was a second goal. Stabilizing NF1/RAS interaction takes advantage of the outstanding technology developed at FNLCR, and such stabilizing compounds might be useful in some cancers. Stabilizing neurofibromin itself using molecular glues/chaperones was also proposed. This was judged to be more relevant to RASopathies than cancer- taking on restoration of function approaches for NF1 would be an expansion of the RAS Initiative mission. Also, restoration of function in NF1 may be difficult, different contact points will require stabilization in individuals with specific mutations; as the team understands, NF1 tumor phenotypes require loss of heterozygosity, so it is unclear whether stabilization could be effective.

2.9: KRAS alleles

This section is based on the observation that particular mutants track with specific cancers, and that such differences may represent unique vulnerabilities, with an emphasis on characterizing the biochemical activity of different mutants. The approach is divided into six efforts 1) Deep mutational scanning of the KRAS $G_{12}D$ mutant, 2) Characterizing the ability of NF1 and RASA1 to stimulate the GTPase activity of KRAS mutants $G_{12}C/D/V$, $G_{13}D$, $Q_{61}H/L$, and $A_{146}T$, 3) Targeting active KRAS $G_{13}D$ via fragment screening, 4) KRAS G_{12} oncogenic mutations modulate protein conformation within the Switch II/a3 pocket, 5) Insights into the crosstalk between effector and allosteric lobes of KRAS from methyl conformational dynamics, and 6) Identification of an excited state in KRAS $G_{13}D$ with reduced dynamic complexity. This led to three papers, one of which the RAS Initiative led.

Strengths include exceptional biochemistry (binding assays, NMR, and crystallography), wonderful expertise in RAS biochemistry, important findings that dig deeper into RAS structure and the effect of mutants thereof, spectrum of research from saturation mutagenesis to crystallography, and the value of this research to the RAS community. There was a recommendation to focus on the strengths of this section, such as continuing the G_{13}-targeting strategy and develop the $G_{12}D/C$ comparison studies towards an allele-selective therapeutic strategy, as well as suggestions that the GAP studies be incorporated into the NF1 research.

Weaknesses include that this section appeared to be a set of targeted questions rather than a cohesive effort working towards a common objective, and in general, the research of this section was thinly spread over a number of very diverse directions. There was little to no crosstalk described within the different research directors or between other sections. While the approaches will add to our understanding of RAS, they do not necessarily break new ground. Future efforts were often not described in the summary, although the presentation

did provide some of this information. It was unclear how prioritization will be leveraged to focus on the most promising research, there was no appreciation of the value of extending the biochemical analysis (ostensibly through collaborations) to cellular signaling. Productivity in terms of publications was low. In general, this section was seen to be very good to excellent.

2.9.1: Deep mutational scanning of KRAS $G_{12}D$

The extramural labs of Drs. Hahn and Aguirre repeat saturation repeat saturation mutagenesis of KRAS, but this time with a $G_{12}D$ mutation, in a cell line in which RAS fosters proliferation versus another cell line that RAS inhibits transformed growth. Nearly 700 mutations at 41 different positions were identified, most mapping to the effector lobe, with half reducing protein stability. 14 mutants were biochemically studied (presumably by the RAS Initiative), some of which were defective in SOS-mediated stimulation, effector interaction, or both.

Strengths*:*

a. Productive collaboration between extramural labs specializing in saturation mutagenesis with the RAS Initiative providing extensive biochemical support. Each brought something unique to the table;

b. The idea of performing a screen in two systems in which oncogenic RAS either enhances or suppresses proliferation/transformation was clever;

c. The approach is well choreographed, with mutagenesis informing biochemistry;

d. The study is of a comprehensive nature.

Weaknesses:

a. The rationale of this screen was to determine why different mutations arise in different cancers (focusing on the idea that each mutant has a distinct activity), however this experimental approach does not address this question;
b. Reaching the point of limiting returns for saturation mutagenesis of RAS;
c. Adds to an already extensive existing literature, rather than uncovering anything entirely unexpected.

2.9.2. Mutant allele biochemistry ($G_{12}C/D/V$, $G_{13}D$, $Q_{61}H/L$, and $A_{146}T$)

Based on the observation that the EGFR inhibitor cetuximab has some effect in $G_{13}D$, but not $G_{12}D$ KRAS-mutant colorectal cancers, the ability of wild-type versus the $R_{1276}A$ mutant of the GAP domain of NF1 was compared to the GAP domain of another RASGAP, RASA1 for its ability to activate the GTPase activity of KRAS with different mutants ($G_{12}C/D/V$, $G_{13}D$, $Q_{61}H/L$, and $A_{146}T$). Results shed further insight into how NF1 stimulates different mutants via unique mechanisms. Interestingly, also found differences in which mutants are stimulated by NF1 versus RASA1.

Strengths:

a. Careful and detailed biochemical analysis of different oncogenic mutants is critical to our understanding of why specific mutants are associated with defined cancers, and the potential to capitalize upon such differences for drug development;

b. All the reagents and expertise are in place, and quite a number of mutants were tested that are known to be biochemically quite distinct;

c. Both the use of the R_{1276}A mutant and another RASGAP were seen as strengths;

d. This research could inform and strength the NF1 studies described in sections 2.8.

Weaknesses:

a. Most of mutants studied have previously been characterized in vitro, although the addition of an R_{1276}A mutant and another RASGAP adds to the literature;

b. A very targeted approach (in contrast to, for example, section 2.9.1 that performed similar and additional analysis on some 17 mutants);

c. This seems more appropriate for section 2.8.

2.9.3: Targeting active KRAS G_{13}D via fragment screening

The argument was made that while much attention has been paid to the G_{12}D mutant of KRAS, the G_{13}D mutation is also common, yet understudied. To address this, fragment-based screening by high-throughput X-ray crystallography was performed on 965 fragments, identifying 34 fragments binding to 7 pockets. This number was reduced by a confirmational NMR screen to 23 fragments from three different pockets (Pg, Switch I/Switch II and a5/b2 pockets). Based on these fragments, 400 compounds were analyzed by NMR, followed by secondary analysis, including crystallography, to identify an unknown number of compounds with improved affinity.

Strengths:

a. Takes advantage of extensive structural studies already performed on RAS to jump right into a crystallography-based screen;

b. Screen was supported by a proposal to the XChem facility at the Diamond Light Source, which was seen as an innovative collaboration fostered by the RAS Initiative;

c. A large effort that has made amazing progress in a short amount of time;

d. While others have published on fragment-based screens of KRAS, this approach uniquely used crystallography;

e. This work could form the foundation for a robust line of future investigation into this understudied mutant;

f. Very innovative;

g. A nice example of using crystallography to do drug development.

Weaknesses:

a. The approach never addressed the specificity to the G13D mutant, or for that matter wild-type KRAS, or even other isoforms, so it is unclear if these fragments are mutant-specific;

b. At present, after considerable hit to lead efforts (400 compounds), the compounds still

have rather low affinity, so a lot of work remains to be completed and one questions whether these are indeed useful starting points;

c. No described go/no go strategy, and unclear how this approach fits into the broader effort of the RAS Initiative to target RAS (e.g. how integrated is this effort with others?.)

2.9.4: KRAS G_{12} oncogenic mutations modulate protein conformation within the Switch II/a3 pocket

To generate a more stable effector loop structure (switch I and II regions) of the open (effector deficient) conformation of KRAS, NMR was performed on wild-type, $G_{12}D$, and $G_{12}C$- mutant KRAS protein with a stabilizing $T_{35}S$ mutation, with the addition of another $C_{118}S$ mutation (presumably for stabilization?). Comparing the three versions of KRAS suggests greater conformational flexibility of the $G_{12}D$ compared to the $G_{12}C$ mutant.

Strengths:

a. Provides detailed analysis of the differences in effector loop structure between G12D and G12C mutants, shedding further light on how these mutants can have structural differences, which may find use in drug development;

b. Excellent expertise;

c. The observation of conformational differences between the two tested mutants could lead to the design of allele- selective chemical probes;

d. Future directions to further understand the difference of these two mutants could lead to allele-selective chemical probes was seen as a strength.

Weakness*:* Targeted question, which is not integrated into the larger effort of this section, for example, fragment-based discovery in section 2.9.3.

2.9.5: Insights into the crosstalk between effector and allosteric lobes of KRAS from methyl conformational dynamics

Because crystallography cannot capture the dynamics of RAS structures and NMR has only captured two major states in solution, NMR relaxation (methyl single-quantum CPMG relaxation dispersion) and other approaches were employed to search for other more dynamic conformations of the G-domain of GTP and GDP-bound KRAS in the wild-type and stabilizing mutant backgrounds ($T_{35}A/S$ and$V_{29}G$) in the absence and presence of the RBD of RAF1, revealing a relationship between binding and conformational dynamics.

Strengths*:*

a. Leverages the ultra-sensitivity of methyl SQ-CPMG to study conformational dynamics of RAS proteins, which will further our understanding of how RAS binds effectors;

b. Findings could be incredibly helpful in explaining why oncogenic mutants may differ in their engagement of effectors, and ultimately the impact that has on effector signaling;

c. Exceptional expertise.

2.9.6: Identification of an excited state in KRAS $G_{13}D$ with reduced dynamic complexity

Akin to section 2.9.5, it is argued that further work is needed to understand the conformational dynamics of KRAS, and in doing so by NMR across a panel of mutants a third intermediate conformational state unique to the $G_{13}D$ mutant was previously discovered whereby a hydrophobic cavity near A_{130} was exposed. Mutating this site ($A_{130}I$) increased the binding affinity of the RAF1 RBD to the $G_{13}D$ mutant. Such a pocket could be the basis for $G_{13}D$- specific inhibitors.

Strengths*:*

a. Leveraged work on conformational dynamics of different RAS mutants uncover a new pocket in the $G_{13}D$ mutant;

b. Very exciting observation.

Weaknesses:

a. While an exciting find, this work is preliminary;

b. A missed opportunity to inform on section 2.9.3.

Section 3: Interaction with the RAS Community

Seven efforts are described to foster interactions with the RAS community.

Strengths include multiple vehicles to reach the entire community, such as the RAS website (3.4) and especially the highly attended RAS symposium (3.6), a large number of DNA vectors and other reagents provided at discounted costs (3.3), 50 collaborations that led to 23 papers (3.1), and successful training of two postbacs (3.7).

Weaknesses include a lack of transparency on how collaborations are decided upon and how initiatives used to reach out to the RAS community are leveraged to foster such collaborations. Also, there was no description of how anti-RAS compounds or large datasets will be provided to the RAS research community. In general, the interaction with the RAS community was seen to be excellent, although there are missed opportunities.

3.1: Collaborations. 50 new collaborations are reported, and various exchanges of material and expertise are listed. This resulted in collaborative 23 papers, including two in the high-impact journals Cancer Discovery and Molecular Cell.

3.2: Contractor Cooperative Research and Development Agreement. A contract agreement was continued to allow 18 companies to use reagents and assays, particularly for screens. Of note, the director of the RAS Initiative is also listed as one of these contract agreements, the nature of which should be clarified.

3.3: RAS reagent distribution.

DNA: reagents are distributed by the non-profit Addgene, with 21 requests for complete RAS pathway kits and another 23 for RAS mutant kits, as well as requests encompassing 43 states, and 45 countries.

Cell lines: 1,000 (particularly MEFS) have been distributed to 80 researchers.

Protein: small amounts are provided under a collaborative agreement, but appropriate plasmids are provided.

Advice: provided but no statistics on this.

Antibodies: multi-institute collaboration to produce antibodies against 29 proteins that generated 96 antibodies for a nominal fee.

Strength*:* The RAS Initiative makes most reagents they develop easily accessible, and many of these reagents are used by the scientific community.

Weakness: It is not clear how small molecules targeting RAS or large datasets derived from the research of the RAS Initiative are to be made available to the RAS community.

3.4: RAS interactome.

RAS website: provides an online forum and information gateway.

RAS Lab: provides a more interactive site to foster discussion.

3.5: RAS Synthetic Lethal Network (RSLN). Supported six extramural labs from Sloan Kettering, Stanford, Beth Israel, the Broad and UNC-CH to regularly meet and have annual meetings. No

description of how this group was selected and assembled, what the metrics of success are, what support they receive, and what the future of this network will be.

3.6: Ras Initiative Symposia. Supported the second RAS symposia in 2017 that reached capacity, then a third virtual that logged in more than 1,700 participants. A fourth was held in-person in Fall 2022 that again reached capacity. These symposia were seen as a particular strength of the RAS Initiative's efforts to interact with the research community.

3.7: Training.

Postbac fellows: Two postbacs each generated two middle-author papers and went on to either a position in the NCI or a PhD program, with now three current fellows. Postbac training, while small, was productive.

Postdocs: Five postdocs were trained between 2016-2021. Four published 1-2 first author papers with two publishing in high impact journals (IF >10). One postdoc trained for two years and did not publish during this time. Upon completing their postdocs; three took staff scientist positions in industry or research institutes, one started a second postdoc, and one accepted a tenure-track associate professor position overseas.

Section 4: Future Plans

The RAS Initiative has two drug candidates that will be first in class headed for the clinic, a KRAS G12C binder that can bind to GTP and GDP bound KRAS and a KRAS/PI3Ka protein-protein interaction inhibitor. These have unique properties that distinguish them from existing agents targeting RAS and are therefore important contributions. They have published 75 manuscripts, including seminal contributions to the structural biology and functional understanding particularly of the multiprotein complexes that the RAS proteins are components of. Approximately 40% of the publications had a RAS Initiative research leader as first or last author. They provided a very large number of critical reagents to the field that are playing a key role in advancing RAS biology in a wide swath of labs. Their efforts have successfully de-risked KRAS as a target and stimulated extensive pharma efforts to target RAS proteins.

Future efforts are described in the following areas:

4.1: Development of pan-KRAS inhibitors

The biology clearly supports the development of pan KRAS inhibitors as long as they are selective for KRAS versus HRAS and NRAS. The data presented for the new class of pan KRAS inhibitors shows good potency against several G12 mutant forms of KRAS and sustained duration of action in cells, so this is all encouraging. The path forward was not clearly delineated, particularly with respect to examining whether these inhibitors are cytostatic or cytotoxic as well as the development of resistance. It is not clear how this effort differs from ongoing efforts in pharma. It is the view of the committee that the RAS Initiative should focus specifically on work that can only be uniquely driven by this group (rather than routine drug development work that can be outsourced) and not on work being done by the extramural community nor largely duplicated in pharma. To that end, there should be clear go/no go decision points for projects that include how the same work could not be done elsewhere, and those not meeting the criteria should be wound down. It was not clear this is in place for this project nor any of the other projects.

4.2: Targeting other small GTPases

As was stated in in the review by the Ad Hoc Working Group on the NCI RAS Initiative, efforts to target additional small GTPases should be pursued only if additional resources to support these efforts are available. Otherwise, there is a very real risk of diluting the successful KRAS targeting efforts. Proposed efforts and some pilot data are presented for targeting NRAS, RAC1, and RAC1b. There is real concern about dilution of efforts by trying to tackle too many targets. At most, one of these targets should be selected and actively pursued. Due to proximity to current efforts with KRAS and the very clear biological significance, NRAS would seem to be the most appropriate target to pursue further. Again here, clear go/no go decision points need to be put in place.

4.3: Biochemistry and structural biology of signaling complexes

The outstanding capabilities in protein production, particularly the use of chaperones to assist in the production of the multiprotein complexes RAS proteins function in, presents unique opportunities for structure determination of important multiprotein complexes that mediate RAS function. Structural efforts targeting several of these are proposed, all of which will greatly aid in

our understanding of RAS function and are viewed with enthusiasm. It will be important going forward to focus the structural efforts, where possible, on the use of full-length proteins to garner the most possible information on function.

4.4: RAS activation of RAF (ADMIRRAL)

The combination of experimental measurements in combination with computational approaches is the only way to decipher the mechanism of RAF activation by RAS in meaningful detail, so this approach is viewed with significant enthusiasm. The proposed experimental measurements should provide important guidance to the simulations as well as a check on the validity of the simulation results. This is a highly unique effort that could not be tackled easily by extramural investigators, so this is an excellent example of the unique scientific capabilities of the RAS Initiative. This is viewed as a strength of the RAS Initiative.

4.5: Second generation disulfide tethering

The nature of RAS as a challenging target certainly requires novel approaches to screening. The proposed disulfide tethering approaches could certainly help to identify appropriate fragment molecules that could be linked and/or elaborated to develop potent binders. It is unclear whether the structural diversity of the compound library developed is adequate for this purpose. More importantly, this library has the potential to be applied to many possible cancer targets which the extramural community has identified. The lack of effort to-date to make this library available for screening other classes of targets by extramural investigators and the unclear plan for doing so going forward is disappointing. Reagents developed by a national lab for one project with the potential to be useful in numerous others should be expeditiously made available for screening use by the extramural community.

4.6: Top-down proteomic analysis of RAS proteoforms from malignant cell lines

The technical capabilities and technical development presented in this area was impressive. The team is to be commended for their efforts. That said, it is less clear for these studies that this is something that can only be done by the RAS Initiative team and not by extramural investigators. In addition, to date these efforts have not been informative in terms of identifying new possible therapeutic approaches. As a result, the utility of these efforts is unclear.

Summary and Conclusions

The review committee was extraordinarily impressed with the Frederick National Lab personnel working on the RAS Initiative. They clearly demonstrated technical excellence, commitment to the project, deep knowledge of the field, and the ability to execute in this highly multi-disciplinary effort. They are to be commended for their outstanding work.

The committee believes that the RAS Initiative has made important contributions to the field. Most importantly, they have helped to de-risk RAS as a target and facilitate the widespread pharma efforts now underway on RAS. The outstanding protein production, biochemical studies, and structural biology are obvious strengths of the group. This has resulted in critical structural insights into RAS function and the ability to develop assays for inhibitor discovery and development. The introduction into the clinic of the KRAS G12C inhibitors targeting both GDP and GTP bound states as well as the PI3Ka:RAS breaker based on the science done in the group are seminal achievements and validate the initial investments in this effort.

Going forward, the following points should be considered, ahead of future decisions:

a. It is critical to view this program in terms of the generous support by the NCI (Direct costs of 10.5M/year supporting 73 FTE), how long they have been going at this (over a decade), the productivity of the group with regards to novel discoveries, papers, and compounds moving to the clinic, and likelihood that this work could not be completed by start-ups, big pharma, and academia.

b. While critically contributing to generating interest and commitment to the field, the Initiative has not led the field in drugging RAS, which started with the seminal discoveries conducted in laboratories in academia and industry, leading to the FDA approvals of two novel inhibitors, which are now used to treat lung cancer, with eight new inhibitors in clinical trials.

c. The RAS Initiative is not the only entity pursing the development of additional KRAS inhibitors; there are others in preclinical development and clinical trials, including inhibitors targeting the more common KRAS mutants G12V and G12D.

d. There seem to be diffuse objectives for the teams, and a better delineation of critical goals would be needed for future success. It would be important to prioritize future projects, include go/ no go decision points, outline how projects align with overall RAS Initiative goals and take advantage of the unique resources at the Initiative, specifically consideration of the advantage of conducting activities that are not being pursued elsewhere and determine quantifiable metrics of success. Incorporation of go/no go decision points is crucial, to enhance resource allocation to critical milestones.

e. It would be important to clarify for the community at large, how the transition of projects to commercial entities is managed. In the instance of transfer of the PI3Ka:RAS breaker and G12C inhibitors to BridgeBio, the fact that Dr. McCormick is both the Initiative's Scientific Consultant and the Co-Founder and Chairman of Oncology at BridgeBio (https://bridgebio.com/people/) calls for increased transparency regarding the process used to

execute cCRADAs with TheRas to ameliorate concerns about potential conflicts of interest.

f. It would also be important to delineate a process to ensure wide distribution of compounds and know-how to the external community, including the recently developed cysteine tethering library, which could be of tremendous utility for the community at large.

g. Publication output has been moderate, 75 papers attributed to the RAS Initiative, ~32 first or last authored by the 10 RAS Initiative research leaders (3 papers/team leader/5 years). There have been few high-impact discoveries published, just 4 primary research papers in very high- impact journals (IF ~20 or better: Science, Mol Cell, Cancer Discov, and Nat Struct Mol Biol) that are suggestive of discoveries of wide appeal and interest.

Based on the above considerations, there were differing views on the review committee as to the future of the RAS Initiative.

For some members of the committee, the assessment is that the program should continue as the results have been outstanding and there is a high likelihood that important contributions will continue to be made. However, even for this group of committee members, there is a clear view that the RAS Initiative needs to evolve and operate differently going forward. Suggested changes include the following:

a. There is a clear need for the RAS Initiative Team to focus on things that only this group can effectively tackle, not efforts that are, or will, be carried out by the extramural community and/or pharma. Consistent with this, there need to be clear approaches to assess whether projects meet that uniqueness criteria and clear go/no go decision points for decisions about sunsetting specific efforts. This is likely best achieved by constituting an advisory board in a manner such that they can make objective assessments about when to sunset specific projects based on uniqueness and progress towards goals.

b. There is a clear need to make reagents developed in this Initiative broadly available to the research community. Particularly in the cases of the inhibitors being made and the disulfide tethering library developed, mechanisms to provide these to the extramural community are needed.

c. There is a clear need for greater transparency on the process for engaging with pharma via the cCRADA mechanism to avoid the appearance of conflicts of interest.

d. There is a need to engage the extramural community to take advantage of the outstanding biochemistry and structural biology findings to guide relevant functional studies of RAS.

e. Given the rapid development of resistance for the first KRAS inhibitors introduced into the clinic, there is a clear need to engage in studies of resistance for all inhibitors being developed and to engage the extramural community to further studies of resistance, but it is unclear whether RAS Initiative Team might best contribute to this effort, given their core expertise.

For other members of the committee, there is a sense that with substantial pharma investment now in RAS as a target, the goals of the RAS Initiative have been largely met and it is time for a phased sunsetting of the program to make the extraordinary capabilities of the FNLCR available for other efforts. Pharma is now fully engaged in developing small molecule inhibitors targeting RAS and the compounds that will be deployed clinically in the future are more likely to come from them. Indeed, there are multiple mutant specific and pan KRAS inhibitors in development already, making it challenging for the RAS Initiative to compete on those fronts. Furthermore, the rapid development of resistance to the first KRAS inhibitors introduced into the clinic would suggest this is likely to be a recurring theme and that figuring out the mechanisms of this and the combinations that can be deployed to address innate or acquired resistance will be a critical effort going forward. As constituted now, the RAS Initiative is not tooled for such studies nor are they proposing such efforts. Therefore, this need will be met elsewhere.

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NCI RAS Initiative Evaluation Team Ad Hoc Working Group

CO-CHAIR

John H. Bushweller, Ph.D.
Professor
Department of Molecular Physiology and Biological Physics
Department of Chemistry
School of Medicine
University of Virginia
Charlottesville, Virginia

CO-CHAIR

Giulio F. Draetta, M.D., Ph.D.
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Sewell Family Distinguished University Chair
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Houston, Texas

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and Molecular Pharmacology
Department of Biochemistry
and Molecular Pharmacology NYU
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New York, New York

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Vice President, Medicinal Chemistry
CALIBR
Global Health Initiative
Scripps Research
San Diego, California

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George Barth Geller Distinguished Professor
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Department of Pharmacology and Cancer
Biology
School of Medicine
Duke University
Associate Director of Basic Research Duke
Cancer Institute
Durham, North Carolina

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Department of Pharmacology
and Chemical Biology
Professor
Department of Hematology and Medical
Oncology
Emory University School of Medicine
Leader
Discovery and Developmental Therapeutics
Program
Winship Cancer Institute
Atlanta, Georgia

Paul Hughes, D. Phil.
Executive Director
Amgen
Thousand Oaks, California

Donna M. Huryn, Ph.D.
Professor
Department of Pharmaceutical Sciences
School of Pharmacy
University of Pittsburgh
Pittsburgh, Pennsylvania

Angela N. Koehler, Ph.D.
Kathleen and Curtis Marble Professor
in Cancer Research
Department of Biological Engineering
Massachusetts Institute of Technology
Associate Director, Kosh Institute
for Integrative Cancer Research at MIT
Institute Member
Board Institute of MIT and Harvard
MIT Center for Precision Cancer Medicine
Cambridge, Massachusetts

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Professor
Department of Pediatrics
Cincinnati Children's Hospital
Cincinnati, Ohio

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President
Sanford Burnham Prebys Medical Discovery
Institute
La Jolla, California

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