Report and Recommendations from the RAS Ad Hoc Working Group on the NCI RAS Initiative

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Report and Recommendations from the RAS ad hoc Working Group on the NCI RAS Initiative

Executive Summary

The NCI RAS Initiative, the lead National Mission Program sponsored by the NCI and conducted in state-of-the-art facilities at FNLCR, was launched in 2013 to address the unmet medical needs of patients with RAS-driven cancers. The NCI RAS Initiative Research Team is co-led and directed by Drs. Frank McCormick and Dwight Nissley, and has been remarkably productive. Their research findings have advanced our understanding of RAS biology and catalyzed new intramural, academic, and private efforts to discover and develop therapeutics for RAS-mutant cancers and move them into the clinic. The Research Team has been leading several innovative strategies that will inform next-generation molecules. This includes developing non-traditional screening approaches for formerly "undruggable targets" and has broadened the scope of RAS-directed therapies to include multiple *RAS* alleles as well as RAS regulatory and effector proteins. Resources developed by the Research Team are used globally to advance RAS research, and symposia hosted by the RAS Initiative have fostered new academic and industry collaborations. The current momentum and trajectory of the Research Team promise significant and sustained impact. The RAS ad hoc Working Group makes the following recommendations for continuing and expanding this momentum: also

1. Continue efforts by the NCI RAS Initiative Research Team at FNLCR to translate internally generated RAS therapeutics.
2. Identify resistance mechanisms to recent RAS therapeutics and pursue efforts to overcome them.
3. Continue to catalyze the renaissance in RAS targeting approaches on a global scale, including efforts to understand the dynamics of RAS activation, and explore inhibitors of additional RAS family members.
4. Continue to foster collaborations with public and private institutions to advance the field and drug discovery efforts.

Introduction

The RAS ad hoc Working Group was established during the RAS Initiative's inaugural period of performance (FY14-18) and maintained thereafter to advise the Program on strategic, technical, and scientific initiatives, and to present its findings and recommendations to the Frederick National Laboratory Advisory Committee (FNLAC) and the FNLAC NCI RAS Initiative Evaluation Team (RIET). The specific goals of the Working Group are to provide regular, candid assessments of the Program, to make suggestions for improvements or pivots, and to ensure optimal connectivity between the FNLCR RAS Initiative and the extramural community. The Working Group met nine times between September 2018 and August 2022 to carry out this work.

The initial focus of the RAS Initiative was to address critical knowledge gaps that had impeded or discouraged the exploitation of RAS as a drug target. The *RAS* gene family (*KRAS*, *NRAS*, and *HRAS*) comprises the most common oncogenes in cancer, with mutations in *KRAS* being prevalent in three of the most lethal cancers (lung cancer, colorectal cancer, and pancreatic cancer). For more than 30 years, the conventional wisdom was that RAS was an "undruggable target". This notion changed following discussions led by Dr. Varmus with NCI and extramural cancer community leaders, who pointed out that new chemical approaches were being developed in industry and academia that renewed interest in targeting RAS, with an initial focus on KRAS. Indeed, in 2013 the Shokat group published a series of covalent, irreversible inhibitors of KRAS-G12C, a mutant *KRAS* allele found in lung cancer. These compounds bound KRAS-G12C in the inactive, GDP-bound state, blocked SOS-catalyzed nucleotide exchange, and inhibited KRAS-G12C association with RAF. This proof-of-principle study combined with the NCI's significant, 5-

year investment (and subsequent 5-year renewal) of the RAS Initiative, sparked renewed interest in mutant RAS—a target that had defied modulation for decades.

The RAS Initiative began by addressing persistent and challenging knowledge gaps pertaining to i), the structure of wild-type KRAS and its codon 12 mutations; ii), investigating how RAS proteins interact with each other in the membrane; iii), defining how RAS proteins activate their effectors; and, iv), determining which effectors are the most important in cancer. Critical investments in structural biology, biochemistry and biophysics, chemical screening, and engagement with the global RAS community have yielded several significant and impactful outcomes.

Accomplishments of the RAS Initiative since 2016 include key structural, biochemical, biophysical, and technological advances. The NCI RAS Initiative Research Team (Research Team) was the first to produce, purify, and generate a crystal structure of fully processed KRAS4b. This advancement enabled biophysical analyses of KRAS in synthetic membranes and showed how processing is critical in complexes interacting with chaperone proteins. The Research Team solved additional crystal structures of oncogenic KRAS proteins in complex with GTPase-activating proteins (GAPs). At the same time, the Research Team developed a range of cell-based, imaging, and biochemical screens to examine RAS activity, which have facilitated academic and industry collaborations. Technological advances by the Research Team include the development and characterization of a panel of “RAS-less” mouse embryo fibroblasts (MEFs) that were provided by Mariano Barbacid. These cell lines, along with expression vectors and single-molecule live-cell tracking systems developed by the Research Team, have facilitated efforts by the scientific community to understand the specificity of RAS protein signaling. Additionally, strategic high-performance computing alliances formed with the U.S. Department of Energy (DOE) National Laboratories have brought new insight into RAS membrane dynamics. Capitalizing on these advancements, the Research Team undertook two drug discovery efforts to target KRAS4b C185 and H95. These endeavors, though eventually repurposed, spawned exciting new drug discovery efforts.

The RAS Initiative’s success during its inaugural performance period (FY14-18) was followed by a 5-year renewal in which the following scientific goals were pursued: 1) identify and help advance compounds of translational potential, and; 2) develop a biophysical model of RAS activation and signaling from the membrane. The body of this report provides a high-level summary of scientific progress during this first renewal period (FY18-22). Key areas of advancement, include i) RAS membrane biology; ii) RAS and RAS effector structural biology; iii) RAS biophysics, molecular dynamics, and computational modeling; iv) RAS drug discovery and development and; v) RAS Initiative interactions with the scientific community. This report will also provide the Working Group’s overall recommendation for a second, 5-year renewal of the Program.

RAS Dependencies and Membrane Biology

The RAS initiative has advanced our fundamental understanding of RAS biology, biochemistry, genetics, and pharmacology over the past 5 years. Cell culture systems have identified distinguishing properties of RAS-mutant cancer cells and enabled focused efforts to target RAS dependency. For example, hyperactive glutamine and redox metabolism were shown to facilitate chemotherapeutic resistance in cultured, KRAS mutant pancreatic cancer cells. Additionally, the heightened intrinsic KRAS-G13D GTP exchange activity was shown to be additionally susceptible to extrinsic GAPs, pointing to GTP exchange inhibition as a potential therapeutic target in G13D mutant neoplastic cells. Finally, the NF1 tumor suppressor protein was shown to function as a dimer, explaining the “gain of function” signaling of multiple germline and somatic *NF1* alleles. Fundamental insights such as these are helping to direct novel therapeutic efforts for RAS-addicted cells, and are informing ongoing drug discovery efforts by the Research Team.

Top-down mass spectroscopy methods have enabled unprecedented analyses of intact KRAS-4B “proteoforms” in cancer cells, revealing distinct post-translational modifications (PTM) on subpopulations of KRAS oncoproteins. Proteoform analysis is now being performed for the KRAS-

4A splice form, HRAS, and NRAS. Whether PTMs enhance the transformation potential of KRAS-4B is a subject of ongoing investigations which could reveal additional therapeutic targets.

The Research Team also developed synthetic membrane systems, in which hypotheses about the lipid milieu of KRAS signaling could be tested. These studies identified lipid components that help anchor farnesylated KRAS-4B to the membrane and facilitate KRAS interactions with RAF1 and additional proteins. The Research Team also discovered that, before the assembly of higher-order structures, the membrane diffusion of KRAS-4B is dependent on both the globular domain and hypervariable region of the protein. Furthermore, a three-state model of the KRAS oncoprotein on a lipid bilayer was proposed based upon computational studies done through collaboration with the Lawrence Livermore Laboratory (LLL)/DOE and supported by direct measurements of KRAS membrane dynamics using high-resolution microscopy. These three states may represent different membrane/lipid environments or higher-order complexes and suggest a continuum of membrane-associated KRAS conformers each with distinct potential for chemical targeting.

Targeting RAS and RAS-Effector Interactions

The RAS Initiative's structural biology program is a key strength and the central pillar of its strategy to create small molecule RAS therapeutics. Early work of the Research Team afforded structures of full-length wildtype and mutant KRAS, which identified conformational changes associated with the GDP- (inactive, "state 1"; incompatible with effector binding) and GTP-bound (active, "state 2"; competent for effector binding) states of KRAS. Since then, the Research Team has used x-ray crystallography, cryo-electron microscopy, and NMR-based methods to solve additional KRAS structures in the presence and absence of tool compounds and early-stage RAS inhibitors. The Research Team's structural understanding of proprietary covalent, and later non-covalent binding inhibitors, has been instrumental translating multiple chemical hits into lead RAS inhibitors. The Research Team has expanded its current scope of structural work to include RAS binding partners, such as RAS effectors and regulatory proteins. As discussed below, these endeavors are likely to identify high-value drug targets.

RAFs are cytosolic serine/threonine kinases that serve as primary RAS effectors. RAFs are specifically central to the activation of MEK/ERK signaling in RAS-driven cancers. The RAS Initiative Research Team was the first to describe how the RAF1 RAS-binding domain (RBD) and its membrane-interacting cysteine-rich domain (CRD) bound wild-type and mutant KRAS. These data showed how the tandem RBD and CRD domains of RAF1 form one structural entity to interact with RAS. Furthermore, structural comparisons between autoinhibited and KRAS-bound RAF1 showed that the CRD stabilizes the autoinhibited and active states of RAF, and may also play a role in switching RAF kinase activity on and off. Knowledge of the KRAS-RAF1 structure has also unveiled new therapeutic opportunities that are being pursued by the Research Team in collaboration with Sanofi.

PI3Kα is another RAS effector that is commonly associated with tumorigenesis and drug resistance. While the selective PI3Kα inhibitor alpelisib is FDA approved for the treatment of breast cancer, it functions as an ATP-competitive inhibitor and thus causes expected side effects including severe hyperglycemia. PI3Kα is an obligate heterodimer, consisting of a catalytic (p110α) and a regulatory (p85) subunit and forms a weak complex with KRAS. The Research Team solved the first crystal structure of KRAS/PI3Kα (p110α/p85) complex using a small molecule "molecular glue" that binds to the target protein surface to enhance its affinity for the binding partner and enables the complex to be solved. Structural information derived from this complex is now being exploited to convert the glue molecule into an antagonist or complex breaker that can prevent KRAS binding to the PI3Kα heterodimer without impacting PI3Kα kinase activity. The Research Team has been extraordinarily successful in this endeavor, identifying over 35 RAS/PI3Kα co-structures that have been solved for this project, including co-structures with covalent and non-covalent binding inhibitors. The Research Team's internationally-leading structural knowledge of RAS and novel approaches differentiate their efforts from other research groups pursuing the same targets.

The Research Team has also improved our understanding of how RAS proteins are regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). The Research team was the first to demonstrate that full-length Neurofibromin 1 (NF1), a 320 kDa, multidomain GAP known to limit the MAP kinase pathway, can form high affinity dimers in human cells. Concomitantly, the Research Team discovered a ternary complex consisting of NF1 (GRD, GAP-related domain), SPRED1 (EVH1 domain), and active KRAS (GTP analog-bound). They showed that NF1 interacts with SPRED1 and KRAS via distinct interfaces. These data provide structural insights into how SPRED1 recruits NF1 to inhibit RAS-ERK signaling. This improved understanding of NF1 biology has allowed the Research Team to identify new therapeutic targets and launch efforts to pharmacologically validate these dependencies.

RAS Biophysics, Molecular Dynamics, and Computational Modeling

The Research Team identified prominent subpopulations of KRAS on the plasma membrane by applying a combination of NMR, fast photochemical oxidation of proteins (FPOP), and neutron reflexivity. A major subpopulation of KRAS conformers is displaced from the membrane and available to promote RAF1 activation via disassembly of the auto-inhibited RAF1 complex. The identification of this new subpopulation enabled the Research Team to propose a multi-state dynamic model of RAS activation, where some RAS molecules are in a latent conformation in contact with the membrane while others are free and competent for effector binding. This model predicts that RAS conformers may be regulated by additional processes, such as post-translational modifications, protein-protein, and protein-lipid interactions.

Fundamental studies carried out over the past five years have deepened our appreciation of how the plasma membrane facilitates RAS activation. The Research Team used molecular dynamic approaches and surface plasmon resonance to measure and predict the behavior of the RAS-RAF complex with constituents of the lipid bilayer. This work suggested an important role for anionic lipids in stabilizing RAS-RAF complexes at the membrane. Furthermore, empiric modification of the membrane components modulated the distribution of KRAS subpopulations, with certain anionic lipids restricting RAF access to KRAS. Alternatively, other lipid fingerprints favor states that are effector-binding competent, while PIP2 promoted the stabilization of KRAS4B in membrane mimetics.

A multi-year collaborative project between the LLL and biophysicists at the RAS Initiative used matched supercomputing and machine learning approaches to predict the dynamics of RAS and its effector proteins on a lipid bilayer. This project led to the proposal of a multiscale computational model capable of simulating RAF interaction with 100's of KRAS molecules on a plasma membrane. The time scale of this simulation enabled the formulation of hypotheses about higher-order KRAS complexes and signaling cascades using statistically relevant observations. Such hypotheses have been reproduced experimentally by the Research Team, which is now applying a similar machine learning approach to study mutant KRAS and to discern the molecular details surrounding the release of RAF autoinhibition to generate active RAF kinase.

RAS Drug Discovery and Development

Over the past 5 years, the RAS Initiative has matured beyond assay development and early fundamental work into deriving a diverse portfolio of small molecule RAS inhibitors. The Research Team's deep expertise in structural biology, biochemistry, biophysics and unique chemical matter (particularly covalent inhibitor tethering libraries) has enabled the development of affinity-based screening platforms that are ideal for identifying lead compounds for traditionally "undruggable targets" like RAS. The Research Team used this approach in collaboration with strategic partners in the private sector to rapidly create an impressive pipeline of KRAS and KRAS regulator/effector inhibitors. These novel inhibitors span the discovery, preclinical, and IND-enabling stages of drug development. The Research Team's two most advanced preclinical assets are anticipated to enter human clinical trials in 2023.

The Research Team's highest priority target was KRAS-G12C, a mutant allele found in 46% of RAS mutant lung adenocarcinomas. This KRAS allele appeared more chemically tractable, as inhibitors could be designed with a warhead that reacts with cysteine, but not glycine, to form a covalent bond. Mass spectrometry-based screening of full-length KRAS-G12C against a small, covalent library proprietary to the FNLCR identified a series of novel hits. A strategic partnership with BridgeBio Pharma and Theras accelerated the optimization of this series, resulting in a lead that engages KRAS-G12C more rapidly and potently than other first-generation KRAS-G12C inhibitors. This lead compound drives potent and sustained inhibition of G12C signaling in cells and is capable of inducing tumor regression in xenograft models without significant side effects. A unique feature of this clinical candidate is its ability to bind KRAS-G12C in both the active (GTP) and inactive (GDP) states. This contrasts with the standard of care (sotorasib) and other first-generation G12C inhibitors (adagrasib) that do not inhibit the active, GTP-bound form of the protein.

Histidine 95 is another potentially reactive amino acid present in K- but not H- or N-RAS. Based on structural studies, the Research Team hypothesized that this residue might be susceptible to covalent modification and lead to the identification of pan-KRAS inhibitors. While chemical screens identified strong electrophilic compounds, these molecules were too non-specifically reactive for further development. However, further structural analysis suggested that non-covalent binding compounds with an affinity for His-95 and the H3/S2 pocket could be developed. This observation launched two projects that are currently in preclinical development. The first project targets the oncogenic KRAS-G12D allele found in 45% of RAS-mutant pancreatic and colorectal cancers. Medicinal chemistry optimization has produced multiple lead compounds that non-covalently bind KRAS-G12D in both the active (GTP) and inactive (GDP) states. Biochemical studies indicate that these leads are extremely potent inhibitors (IC_{50}'s in the low nM range) that can disrupt KRAS-G12D effector interactions and exhibit a preference for KRAS-G12D over HRAS and NRAS. Structural analyses show that these compounds induce a KRAS conformation that does not support effector binding. A second project features multiple series of pan-KRAS, noncovalent inhibitors that are in an earlier stage of preclinical development. These compounds provide selectivity for KRAS over HRAS and NRAS, stronger non-covalent binding affinity, and improved pharmacokinetic properties. Both projects are driving towards the nomination of an orally bioavailable lead compound with suitable properties to support *in vivo* pharmacokinetic/pharmacodynamic and efficacy studies.

The Research Team's focus on target tractability and affinity-based RAS inhibitors was supported by the outcomes of a traditional small molecule screening study conducted in collaboration with Sanofi. A high throughput phenotypic screen for cellular viability was performed in a KRAS-mutant cell line using a proprietary biopharmaceutical library of nearly 1 million compounds. Over 125 hits belonging to five distinct chemical clusters were confirmed, but none of these compounds bound directly to KRAS. Additional follow-up on these indirect KRAS blockers indicated that CDK9 dependency is an underlying mechanism that could potentially impede certain RAS-mediated cancers. The absence of direct-acting KRAS inhibitors in this screen and others conducted by academic and private groups suggests that traditional phenotypic screens using the chemical space of most small molecule libraries may bias towards more "traditional" drug targets. However, the Research Team found that one inhibitor identified by Boehringer Ingelheim (BI-2852) induced a nonfunctional KRAS dimer that occluded the interface needed for RAF-binding. Thus, the blockade of the interface between KRAS and its effectors could constitute an innovative, non-traditional mechanism to neutralize or ablate KRAS signaling.

Clinical trials of the first-generation KRAS G12C inhibitors, sotorasib and adagrasib, in patients with relapsed/refractory lung adenocarcinoma have demonstrated the near-universal development of resistance in patients with relapsed/refractory lung adenocarcinoma. Studies of the underlying resistance mechanisms indicate a need to restore RAS signaling, providing additional validation for targeting oncogenic RAS in cancers with multiple additional driver mutations. Potential strategies for overcoming the fundamental problem of resistance include developing more potent and selective inhibitors and pursuing rational drug combinations. Accordingly, the Research Team is evaluating orthogonal mechanisms to block RAS signaling that could be used independently or in combination with direct RAS inhibitors. One example of this

approach is a unique collaboration developed with BridgeBio Pharma and Theras that has the goal of preventing PI3Kα-RAS interactions without negatively impacting PI3Kα kinase activity. This is an entirely novel approach with the potential to inhibit RAS-driven PI3Kα activation without adverse effects on glucose metabolism. The Research Team hypothesized that their previously identified PI3Kα-RAS glue compound could be modified to act as a complex breaker. A concerted medicinal chemistry effort has successfully converted the glue compound into both covalent and non-covalent binding molecules that disrupt the RAS/PI3Kα interaction. Several chemical series have been identified, and two lead compounds are undergoing medicinal chemistry optimization. A development candidate for IND submission and clinical testing is anticipated for early 2023.

The Research Team is also exploring early chemical matter associated with multiple discovery-level projects. Two projects using cysteine tethering and KRAS G13D fragment screens have identified multiple, new pockets in KRAS. Two projects that involve initial hits are being characterized with mass spectrometry and NMR-based affinity assays while medicinal chemistry optimization is underway. A second direct-acting approach is the development of KRAS proteolysis-targeting chimeras (PROTACs) which have one distinct end that binds to KRAS and another end that binds an E3 ubiquitin ligase. Once KRAS and E3 ligase are bound together, the E3 ligase will ubiquitinate KRAS and target it for destruction via the cell's ubiquitin-dependent proteolysis pathway. This research is being conducted with cCRADA partner, Progenra, with a focus on developing assays and lead molecules for proof-of-principle experiments.

RAS-effector interaction projects are also underway. The first is being conducted in collaboration with Sanofi and seeks to identify small molecules that disrupt the interaction between RAS and RAF1. The Research Team is currently assessing hits from an initial screen with the intent of generating lead compounds that address this high-value target for RAS-driven cancers.

The Research Team is also independently exploring molecular glues and using computational approaches and *in silico* screens to identify compounds that could drive the association of KRAS with Neurofibromin-1 (NF-1), a GTPase activating protein (GAP) that inactivates RAS. Tool compounds suitable for evaluation in proof-of-principal studies are being actively sought.

Fostering Interactions with the Scientific Community

A critical goal of the RAS Initiative is to promote and mediate the sharing of knowledge and resources among RAS researchers in the academic, government, and private sectors. The Research Team has made a remarkable impact on the field through its success in taking on some of the most challenging questions in RAS biology. Dissemination of these results through public presentations, website blogs, and more than 66 high-impact publications in FY18-22 alone has de-risked RAS as a therapeutic target and ignited a renaissance in RAS research. These efforts have also spawned renewed biopharmaceutical investment in the development and clinical testing of novel drug candidates targeting mutant RAS.

The RAS Initiative has also become a major contributor and hub for the development of RAS resources such as protocols, plasmid vectors, tools, proteins, and cell lines. These high-quality reagents are provided free of charge to the community through a third-party distributor (Addgene). Since FY18, nearly 12,000 plasmids, 63 proteins, and approximately 1,000 cell lines have been provided by the Research Team to more than 1,300 investigators.

The RAS Initiative is highly collaborative, having established nine (9) targeted partnerships during the FY18-22 period with academic and private sector laboratories via cCRADA. These strategic collaborations expand the capabilities of the Research Team by providing access to proprietary technology, applied science, or assistance with therapeutic discovery.

The RAS research community has benefitted from the NCI RAS Initiative's promotion of scientific discourse and dissemination of information about breakthroughs in the field. The RAS Initiative Symposia play a central role in fostering the exchange of ideas between academic and industry researchers in the field to accelerate the pace of discovery. The NCI RAS Initiative has planned two symposia during the current renewal period, a virtual meeting held during the global COVID-19 pandemic (May 2021) and an in-person meeting to take place in October 2022. In

addition to these symposia, the NCI RAS Initiative supports scientific communication and interaction with the research community through the RAS Initiative website (https://www.cancer.gov/research/key-initiatives/ras) located on NCI's homepage. A website update is currently underway to streamline its format, enhance its accessibility for the lay public, and update its scientific content. All publications from the NCI RAS Initiative are accessible on a dedicated webpage and include a link to the PubMed PMID reference. The NCI RAS Initiative website also includes a RAS Dialogue blog, webinars, and provides a gateway to the RAS Lab Discussion Forum. The RAS Lab Discussion Forum is an online discussion forum featuring over 1,000 members that provides an open and informal scientific discussion platform encompassing all aspects of RAS biology.

Overall, the NCI RAS Initiative outreach program has been exceptionally successful. External engagement measured by publication metrics and citations, number and scope of reagents provided to the external community, year-to-year RAS Symposia attendance and participation, and RAS Initiative website usage trends suggest that the RAS research community is vibrant, enthusiastic, and highly collaborative.

Conclusions

In summary, the NCI RAS Initiative has exceeded its objectives since its inception, and for FY18-22 despite the ongoing COVID-19 pandemic since the program was last renewed. Their first clinical candidate, generated in cooperation with BridgeBio Pharma and Theras, is headed for an IND to treat KRAS-G12C mutant cancers. There is research-based optimism from the experts of the RAS Initiative ad hoc Working Group that this new KRAS-G12C compound could be superior to the current FDA-approved KRAS-G12C therapies produced by Amgen and Mirati because it targets the GTP-bound conformation of KRAS-G12C. Importantly, the methodology of generating this compound is being applied to the more common KRAS-G12D and KRAS-G12V oncoproteins, and preclinical lead compounds are emerging. The establishment of exceptional capabilities in structural, biochemical, and biophysical approaches has enabled the success of the Research Team. These methods have been generalized to create additional means to target RAS, such as a first-in-class "complex breaker" molecule that binds PI3Kα to prevent engagement with KRAS-GTP. Furthermore, RAS family members and effectors such as RAC1 and PI3Kα are now being assessed for chemical tractability.

In addition to meeting the main overall therapeutic objective of the NCI RAS initiative, the program has continued to provide impactful discoveries about RAS biochemistry, including the finding that NF1 forms homodimers. This discovery explains why certain *NF1* alleles can act in a dominant-negative fashion. Furthermore, computational modeling approaches with the DOE and time-lapse microscopy have revealed that RAS exists in dynamic structures/states on the plasma membrane, that impact RAS biology. These pieces of information regarding activity and action have unlocked new strategies to attack mutant RAS signaling. Finally, the function of RAS compounds being generated such as the "complex breaker compounds" will provide valuable tools to address key questions for the field.

Future Plans and Recommendations

During the FY18-22 period, the NCI RAS Initiative Team, under the exceptional leadership of Drs. Frank McCormick and Dwight Nissley, has demonstrated its ability to work as a cohesive drug discovery unit, interfacing with both the academic and private sectors. The NCI RAS ad hoc Working Group provides the following recommendations to continue the remarkable trajectory of this group and maximize its impact over the next five years:

1) Continue efforts to translate homegrown NCI RAS Initiative compounds to the clinic in the state-of-the art facilities at FNLCR. The KRAS G12C inhibitor developed by the Research Team has unique properties (increased affinity, ability to bind GTP and GDP states) that set it apart from first-

generation clinical compounds. The movement of this compound and other KRAS-G12D and pan-KRAS compounds toward clinical translation should continue to be prioritized.

2) Understanding and discovery of mechanisms to combat therapeutic resistance. Therapeutic resistance will remain a challenge in treating RAS-mutant tumor types. Therefore, the NCI RAS Initiative ad hoc Working Group encourages the Research Team to continue the pursuit of secondary targets such as the PI3Kα-RAS “breaker” compound for use as salvage or combination therapy. Additionally, research to understand common mechanisms of resistance to homegrown and up-and-coming RAS inhibitors should be pursued with the goals of defining predictive biomarkers and preventing relapse.

3) Continue to catalyze the renaissance in RAS targeting approaches on a global scale, including the dynamics of RAS activation, and the exploration of inhibitors for additional RAS family members. Discoveries related to movement and dynamics of RAS at the membrane are re-shaping how the Research Team thinks about therapeutic development. Creative new initiatives come from tackling questions that are inaccessible to the general research community and partnerships between the RAS Initiative and DOE exemplify the types of research possible only at a governmental level. Secondly, the exploration of inhibitors for additional RAS family members altered in cancer is timely and important. The Research Team has developed the methods and expertise required to rapidly identify and accelerate lead compounds towards the clinic. These efforts have primarily focused on KRAS and downstream targets of KRAS signaling. While some of the compounds developed in-house may have efficacy for treating other cancers driven by other RAS family members, the Research team would need to develop additional resources to pursue these targets. Therefore, the committee recommends that these targets are directly pursued only if additional resources can be provided to the Research Team to allow for expansion without taking away from successful ongoing efforts to target KRAS-mutant disease.

4) Expansion of community engagement to further the impact of the NCI RAS Initiative. The NCI RAS Initiative has made a remarkable impact on the field, catalyzing a renaissance in RAS research. As previewed in the field of immunotherapy, the early success of RAS therapeutics is likely to catalyze an extraordinary expansion of the field. Important initiatives like the RAS Symposia and website may require additional financial administrative support to keep up with community demand. The COVID-19 pandemic has also highlighted the need for effective communication with the lay public and support to help the RAS initiative disseminate knowledge to these sectors would be useful. Finally, efforts should be made to ensure diversity in the current and future RAS research community. This could be achieved by actively seeking and promoting the work of underserved populations in the NCI RAS community, or through workshops and training events that engage such individuals in the efforts of the RAS Initiative Research Community.

In summary, it is clear to the NCI RAS ad hoc Working Group that the NCI RAS Initiative has made an incredible impact over the past reporting period and is now poised to develop creative new therapeutic strategies and rapidly accelerate several promising therapeutics to the clinic. The Initiative serves as a central hub in a growing network of academic and industry researchers worldwide. Sustained support is needed to ensure the continuation of this upward trajectory and meet the needs of a growing area of significant potential to impact the lives of cancer patients.

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Appendix A

NATIONAL INSTITUTES OF HEALTH

National Cancer Institute

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