AWARD NUMBER: W81XWH-20-1-0129

TITLE: Regulating Neurofibromin Through Degradation, Dimerization, and Binding to SPRED1

PRINCIPAL INVESTIGATOR: Frank P. McCormick, PhD

CONTRACTING ORGANIZATION: University of California, San Francisco, CA

REPORT DATE: May 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command

Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE
May 2022

2. REPORT TYPE
Annual

3. DATES COVERED
01Apr2021-31Mar2022

4. TITLE AND SUBTITLE
Regulating Neurofibromin Through Degradation, Dimerization, and Binding to SPRED1

5a. CONTRACT NUMBER
W81XWH-20-1-0129

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Frank McCormick, PhD

E-Mail: frank.mccormick@ucsf.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California, San Francisco, Helen Diller Family Comprehensive Cancer Center, San Francisco, CA 94158-9001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
We have previously shown that SPRED1 regulates localization of neurofibromin (the protein product of the *NF1* gene) to the plasma membrane where it functions as a negative regulator of RAS signalling. *NF1* mutations in neurofibromatosis cause loss of neurofibromin activity, resulting in hyperactivated RAS in patient cells and subsequent manifestation of symptoms. While our understanding of neurofibromin regulation has advanced in recent years, many questions regarding its meticulous control of RAS activity remain to be answered. Our objective is to find ways of increasing neurofibromin's GAP activity, thereby reducing levels of active RAS. NF1 mutations in a hotspot within the N-terminal domain of neurofibromin are known to cause severe phenotype disease. We propose that these mutations confer reduced protein stability. We hypothesize that re-stabilization of the mutant protein provides a therapeutic angle with which to treat severely affected patients, and understanding the pathways that degrade neurofibromin is key to developing this theory. We have identified that neurofibromin exists in an inactive homodimer conformation. Our objective is to understand how these dimers are regulated and how that contributes to neurofibromin activity. In line with this, we propose that activation of the receptor tyrosine kinase c-KIT is an important factor in regulating neurofibromin/SPRED1 complexes: we will determine how activation of c-KIT affects neurofibromin GAP activity.

15. SUBJECT TERMS
Neurofibromin, SPRED1, Ras GAP, c-KIT

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	45	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

	Page
1. Introduction………………………………………..…………………….	1
2. Keywords ……………………………………….……………………	1
3. Accomplishments………………………………..…………………	1
4. Impact……………………………………………...………………….	24
5. Changes/Problems.……………………………...……………..…….	24
6. Products…………………………………………………..…………	25
7. Participants & Other Collaborating Organizations……………………...	25
8. Special Reporting Requirements……………………………………………..	27
9. Appendices…………………………………………………………………..	27

1. Introduction

The overall goal of the project is to find ways of increasing neurofibromin activity, thereby reducing levels of active Ras responsible for pathologies associated with NF1 disease.

2. Keywords

Neurofibromin, SPRED1, Ras GAP, c-KIT

3. Accomplishments

Specific Aim 1

Major Task 1: Identify stability-related posttranslational modifications on neurofibromin protein and relevant enzymes/mechanisms

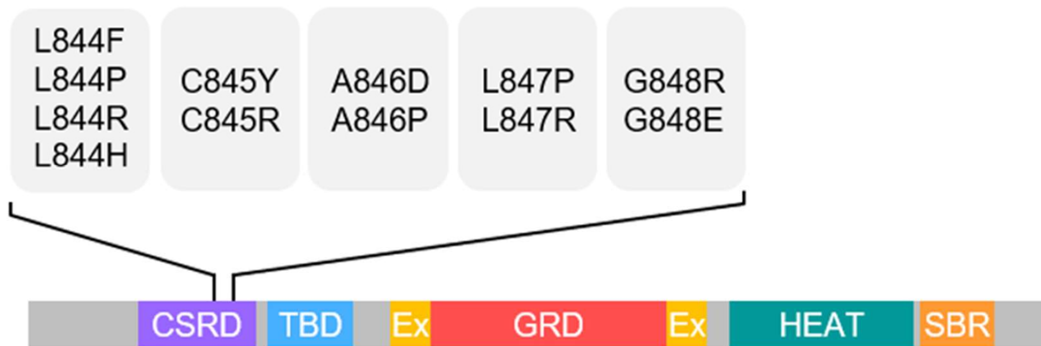


Figure 1. Mutations in neurofibromin that cause severe NF1 phenotypes. CSRD: Cysteine Serine Rich Domain. TBD: Tubulin Binding Domain. EX: Extra domains flanking the GAP Related Domain (GRD). HEAT: Helical Armadillo Repeat Region. SBR: Syndecan Binding Region.

We analyzed biochemical properties of neurofibromin mutants associated with a more severe phenotype (Koczkowska et al, *Am J Hum Genet* 2018). These occur in 5 adjacent codons within the Cysteine/Serine Rich Domain, as shown in Figure 1. We analyzed a representative from each codon.

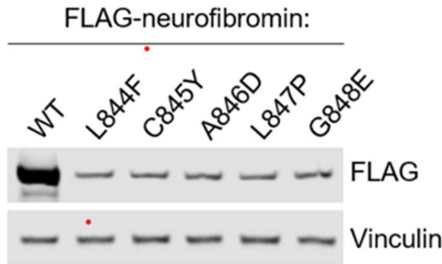


Figure 2. Expression of mutant neurofibromin proteins in 293T cells

We engineered cDNAs for each mutant with an N-terminal FLAG epitope tag, and transiently expressed each in 293T cells. Proteins were analyzed by Western blotting. Figure 2 shows that levels of expression of each mutant was significantly lower than wild type neurofibromin.

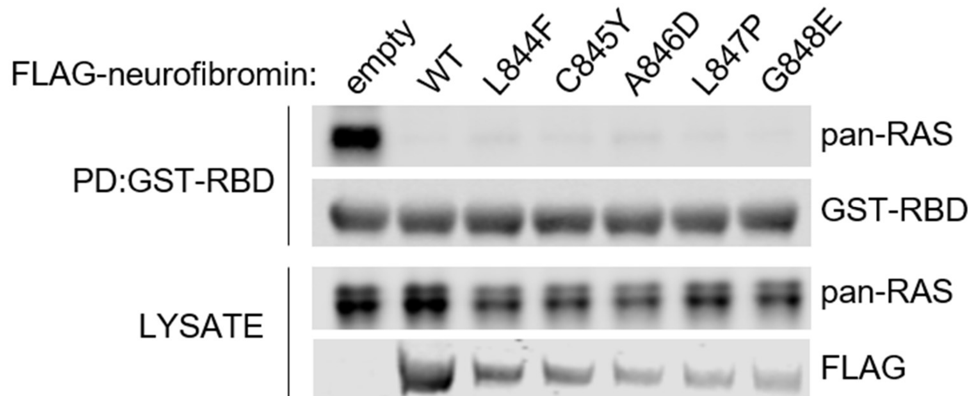


Figure 3. Levels of Ras.GTP in cells transfected with mutant neurofibromin proteins. Lysates were probed with beads coupled to the Ras-binding domain of Raf1 (RBD), beads were washed, eluted and bound Ras proteins detected by Western Blotting.

Despite lower levels of expression, each mutant was able to promote loss of Ras.GTP (Figure 3), showing that they retain GAP activity. We them performed a pulse chase experiment in which cells were treated with cycloheximide to block protein synthesis, and levels of neurofibromin were measured at various times after treatment. Figure 4 shows that one of the mutants tested, L847P, was lost more rapidly than wild type protein, with an estimated half life of about 6 minutes, compared to about 22 minutes for wild type protein (Figure 4).

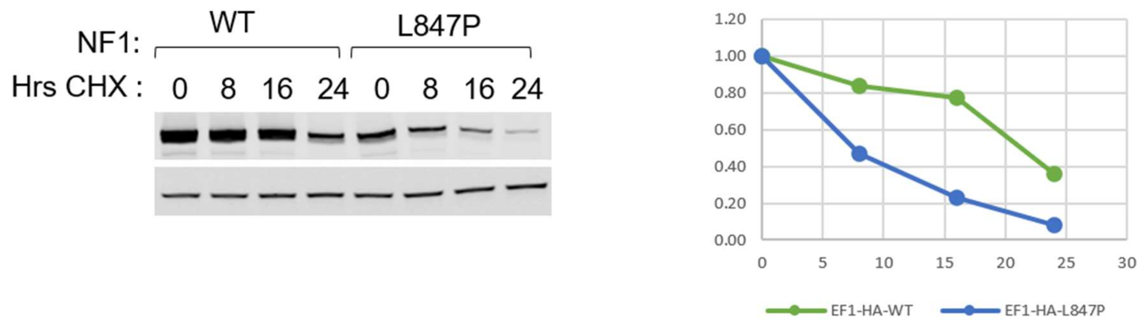


Figure 4. Turnover of wild type neurofibromin and a pathogenic mutant L847P. Cells were treated with cycloheximide (50 ug/ml) at the times shown and levels of proteins determined by Western Blot analysis (left) and digital scanning (right), relative to wild type (1.00).

To address the mechanism by which mutant neurofibromin might be degraded, we analyzed mutant proteins for modification by ubiquitin. We transfected 293T cells with each mutant, pull down the transfected protein through the FLA Tag and probed Western blots with an antibody that recognizes ubiquitin. Figure 5 shows that each mutant is ubiquitylated, each to approximately the same extent.

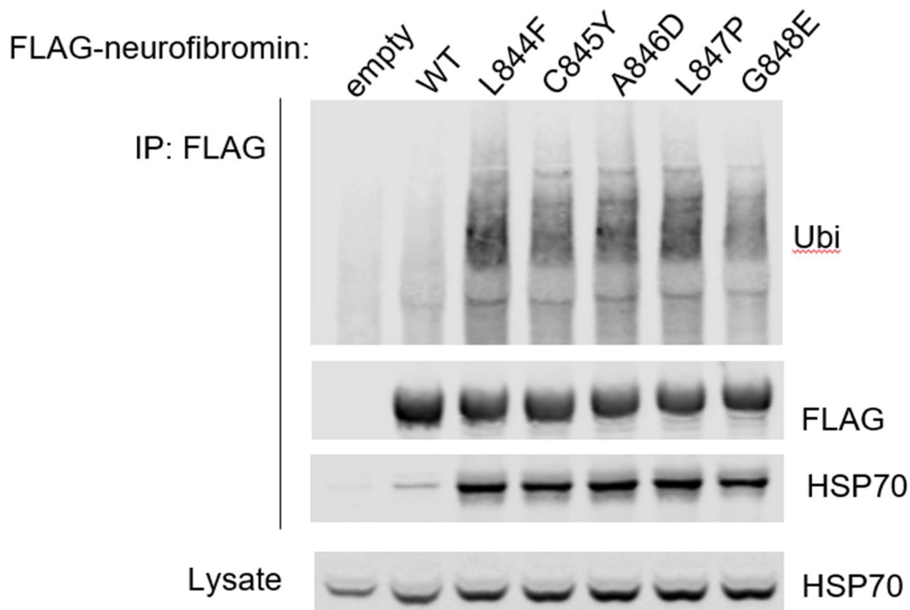


Figure 5. Pathogenic neurofibromin mutants are modified by ubiquitylation.

We also analyzed the G848E mutant protein by mass spectrometry. In comparison to wild type neurofibromin, the mutant protein was associated with components of the proteosome complex, Proteasome 20S Subunit Beta 2-5 (Table 1). In subsequent mass spec analysis, we also identified Hsp70 and Hsp90, and verified these interactions by Western blot analysis (Figure 6).

WT only		mostly WT		G848E only		mostly G848E	
YY2	transcription	HIST1H1T	nucleosome	OTUD4	deubiquitinase	PSMB2	proteolysis
		HIST1H1A	nucleosome	EIF3L	translation	PSMB4	proteolysis
		MYL12B	cell shape	VPS52	vesicle trafficking	PSMB5	proteolysis
		CCAR2	genome stability			EIF3M	translation
		MYL12A	cell shape			PABPC3	translation
		MYL9	cell shape			PABPC4	translation
		CDC42EP1	signal transduction			PABPC1L	translation
		AMOT	signal transduction			PSPC1	transcription
		DPY30	cell cycle regulation			ILF3	transcription
		SCAF4	transcription			EFTUD2	splicing
		SARNP	transcription			SF3B1	splicing
		RBM5	splicing			ALDH3A2	metabolism
		ZCCHC6	mRNA decay			PGRMC1	metabolism
		GPATCH4	nucleic acid binding			BABAM2	DSB repair
		DNAJC19	protein import			TAB2	signal transduction
		ATP12A	ion transport				
		BSG	metabolism				
		DCTPP1	metabolism				
		C16orf87	-				

Table 1. Proteins that bind to wild type or mutant neurofibromin, identified by mass spectrometry.

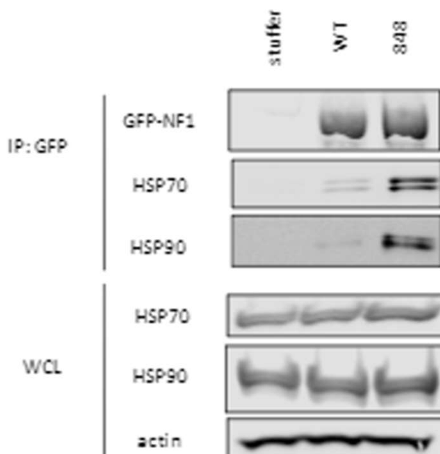


Figure 6. Binding of Hsp70 and Hsp90 proteins to G848E neurofibromin.

Major Task 2: Identify potential targets for therapeutic intervention that stabilize mutant neurofibromin protein

These experiments suggest that pathogenic mutants of neurofibromin such as G848E are degraded by a ubiquitination followed by proteasomal processing. We therefore attempted to stabilize the mutant proteome with proteasome inhibitors such as bortezomib. Figure 7 shows that while the drug was effective at stabilizing beta-catenin, as a positive control, no clear effect was seen on neurofibromin, either wild type or mutant. Similar effects were observed with a different proteasome inhibitor, carfilzomib. We are testing addition compounds in this assay system. This work is ongoing. We are also planning to screen for genes that regulate protein degradation using a CRISPR library constructed at UCSF by Dr David Toczyski.

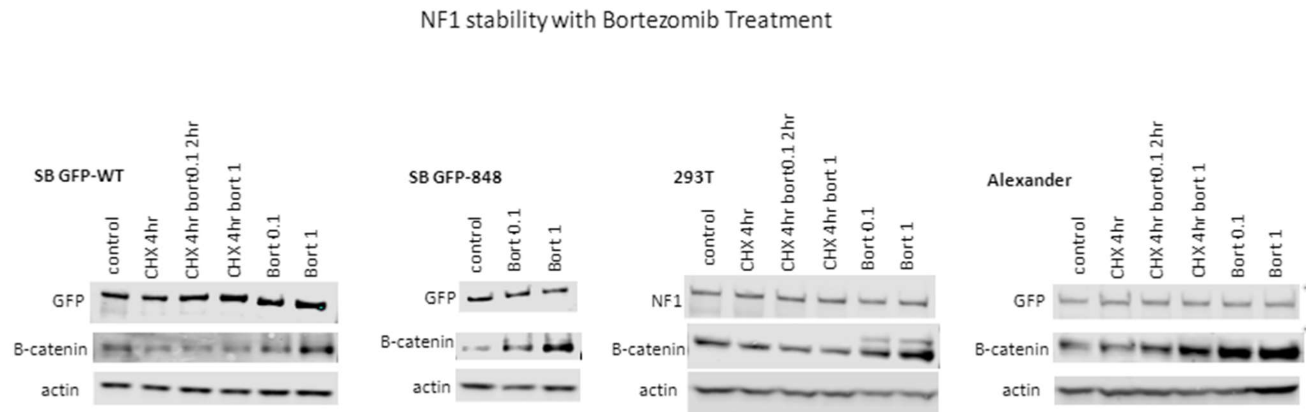


Figure 7. Bortezomib fails to stabilize wild type or mutant neurofibromin. Cells expressing wild type or mutant neurofibromin were exposed to 0.1 um or 1 uM bortezomib in the presence or absence of cycloheximide. Beta-catenin levels were monitored as positive controls for proteasome mediated degradation.

Major Task 3: Examine the effects of wild type neurofibromin on stability of the G848E mutant

Our data showing that mutant neurofibromin is modified by ubiquitylation, associates with proteasome components and is bound to Hsp70 and Hsp90 chaperone proteins (above) indicate this mutant protein is actively degraded. We have also shown the protein retains its ability to dimerize. This suggests the possibility that these high turnover mutants could bind wild type neurofibromin and promote its degradation. To test this, we co-expressed mutant and wild type proteins, each with a different epitope tag, and asked whether increasing doses of the mutant protein caused loss of the wild type. Figure 8 shows that this is indeed he case. Increased expression of either G848E or L847P mutants led to dose-dependent loss of wild type neurofibromin.

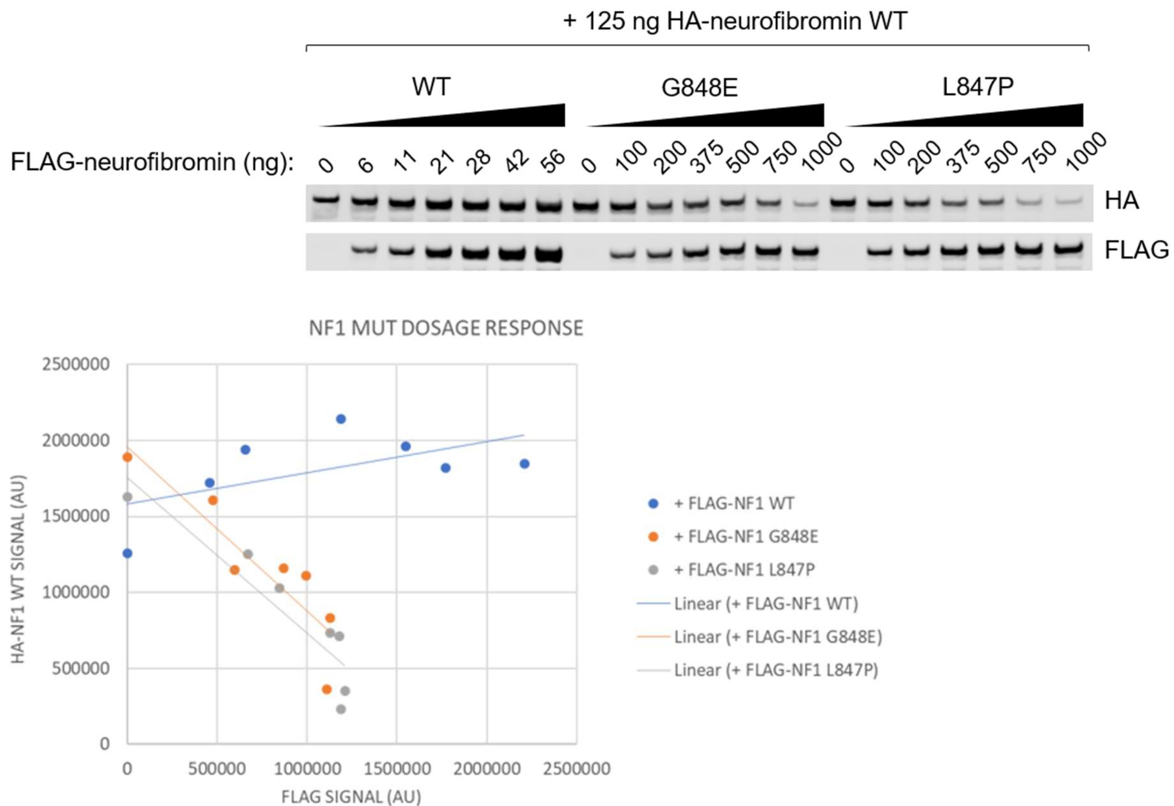


Figure 8. Increased expression of mutant neurofibromin causes loss of wild type protein. 293T cells were transfected with increasing amounts of plasmids expressing wild type or pathogenic HA-tagged mutants, in the presence of fixed amounts of FLAG tagged wild type neurofibromin. Proteins were analyzed by Western blots (top panel) which were analyzed quantitatively in the lower panel.

We sought to provide a structural basis for the severe phenotypic effects of mutations affecting residues 844-848. A recently derived 3.6 Å-resolution cryogenic electron microscopy (cryo-EM) structure of the "short" isoform of human neurofibromin from the Frederick National Lab, shows that ^{844}Leu-Cys-Ala-Leu-Gly^{848} form the C-terminal half of α-helix 34 (Figure 9). Except for Gly848, the side chains of the segment line the hydrophobic interfaces of the α33-α36 HEAT repeat (Figure 9B, 9C), thus stabilizing the repeat itself and presumably the distal half of the Cysteine-Serine-Rich Domain (CSRD) of neurofibromin. The GTPase-activating protein-related domain (GRD) sits atop the α33-α36 HEAT repeat, and structural modelling shows that the GRD is inaccessible to RAS binding in the context of full-length neurofibromin dimer due to steric clashes which would form between RAS and the α33-α36 HEAT repeat.

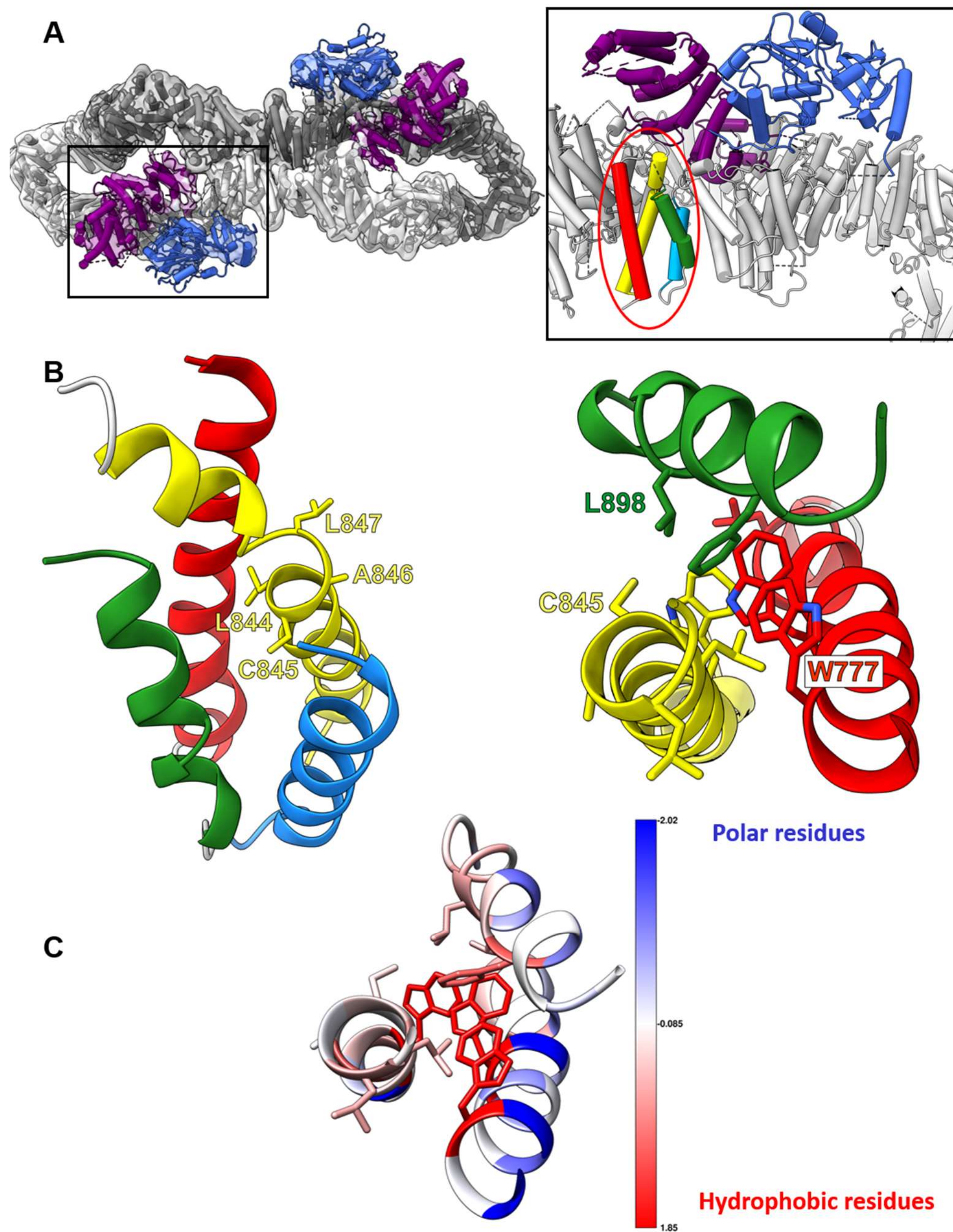


Figure 9. Cryo-EM structure of neurofibromin dimes and detailed view of the 844-848 region.

The above-described mutations localizing within α34 are substitutions of shorter hydrophobic residues with either bulky hydrophobic Phe and Tyr, bulky polar His, Arg and Glu, or a Pro. Introduction of any of the bulkier side chains would necessitate a shift in the position of the backbone atoms which would break the regular pitch of the helix and destabilize the rigid α33-a36 HEAT interface, whereas introduction of a Pro would directly compromise the fold of α-helix 34 and similarly abolish some of its structural rigidity. Thus, each of these severe phenotype

mutations would disrupt the tight packing of the helices and risk exposing the interfacial hydrophobic segments that are securely buried in the wild-type protein. These uncovered hydrophobic residues would be vulnerable to recognition by members of the protein quality control system, ultimately leading to degradation of the entire neurofibromin dimer and lower levels of protein, both mutant and wild type, in the cell. These structural predictions align with our biochemical analyses and explain why wild type neurofibromin expresses less well in the presence of a destabilizing mutant.

In order to test our structure-driven hypothesis we predicted substitutions in 844-848 residues that would be less disruptive to the α-helix 34 structure. Substitution of Leu847 to Ile and Gly848 to Ala do not cause expression levels to go down like their pathogenic counterparts when expressed in 293T and rescue protein solubility in baculovirus (Figure 10). A similar pattern is observed whereby 'mild' substitutions are less ubiquitylated and co-immunoprecipitate with less HSP70 than the pathogenic variants.

We hypothesized that N-terminal pathogenic substitution variants outside of the 844-848 region may be similarly disruptive to neurofibromin structure. We used our cryo-EM model to identify variants W837F, F894S and L898R (present in the ClinVar database) that may cause disruption to the helices, and at the same time predicted 'less-disruptive' versions as we had done for L847 and G848 (Figure 10). The helices that contain these variants are conserved between species as for the 844-848. All 3 pathogenic substitutions express less well than wild-type and can be 'rescued' by Trp837 to Phe, Phe894 to Ser and Leu898 to Arg in 293T cells (Figure 10), and change from insoluble to soluble protein in baculovirus (Figure 10B) and display the same pattern of ubiquitylation and HSP70-binding as for the 847 and 848 pairs. Despite lower expression levels, W837F, F894S and L898R also retain GAP activity and binding to SPRED1. Given the observation the 844-848 substitutions act in a dominant negative manner by destabilizing wild-type protein, we repeated a dose response curve and found that F894S and L898R behave in a similar way, solidifying our observations that variants that disrupt protein structure target wild-type protein for degradation through dimerization (Figure 10). During this process we have also identified other pathogenic variants which have the potential to disrupt structure but we do not always have consistent results between mammalian and insect cell expression and thus these variants may be the subject of further investigation.

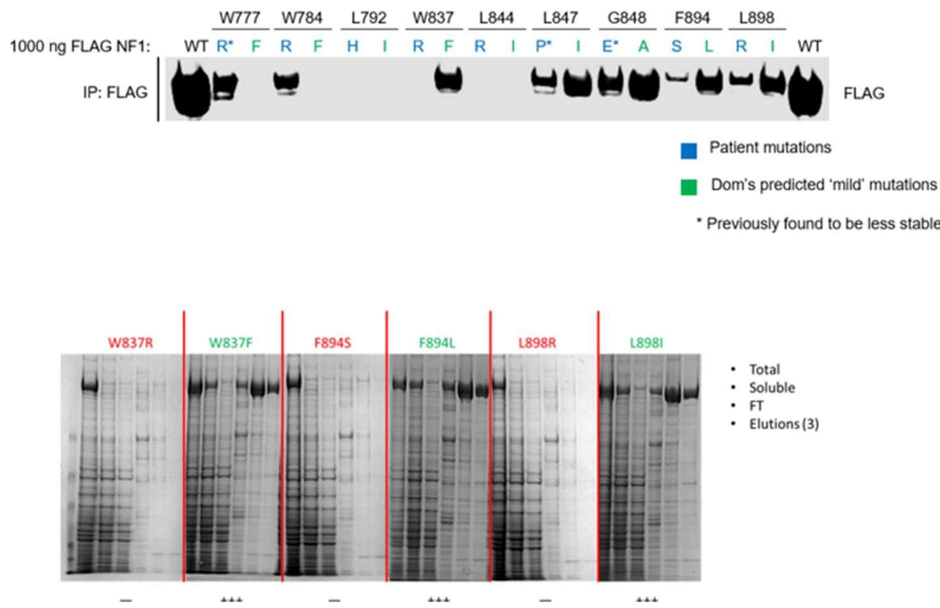


Figure 10. Stability of additional pathogenic mutants expressed in 293T cells (top panel) or in insect cells using baculovirus expression (lower panel).

We have identified a phosphorylation site on neurofibromin that has not been reported previously. It is at codon 864, adjacent to the 844-848 hot spot regions discussed above. None of the pathogenic mutants characterized here are phosphorylated on this site, as shown in Figure 11. We have made a phosphomimic and a phosphorylation defective mutant at this site and observe that lack of phosphorylation leads to unstable protein. The codon 864 kinase is predicted to be a member of the CDK family and we are currently investigating whether inhibitors of these enzymes affect neurofibromin stability.

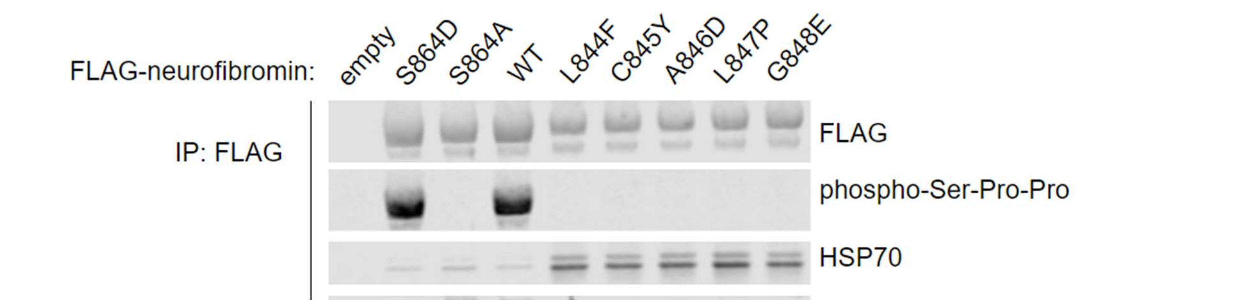


Figure 11. Identification of a novel site of phosphorylation in neurofibromin using a phospho-specific antibody that recognizes the P-Ser-Pro-Pro motif.

Specific Aim 2

Major Task 1: Biochemical analysis of neurofibromin dimers/monomers

Our work on the biochemical analysis of neurofibromin dimers has now been published (Sherekar et al, *J Biol Chem* 2019). The protein appears to be an obligate homodimer, with a dissociation constant of 1 nM, or lower. We have attempted to disrupt the homodimer by co-expressing neurofibromin with oncogenic RAS, and by exposing cells to MEK inhibitors, or AKT inhibitors, but have failed to observe any evidence of dissociation. Our structural analysis of the dimeric structure is nearly complete: this should allow us to test mutations that could affect dimerization and so allow us to assess the functional significance of dimerization on GAP activity. This work is being performed in collaboration with the Cryo-EM facility at the Frederick National Labs in Maryland. As published in Sherekar et al, the full-length dimeric protein is relatively inactive. We are attempting to understand how activation is regulated and whether dimerization is involved.

We have previously screened for genes which would affect cells in cells that express wild type neurofibromin, but not in cells lacking the protein. These efforts failed, partly because loss of neurofibromin upregulates Ras activity and makes cells resistant to regulatory proteins that are downstream of Ras, and therefore do not act through neurofibromin itself. We have now taken a different approach: we have searched for compounds that affect the MAPK pathway in the presence of neurofibromin but not in absence using P-ERK as a readout. Using this approach, we have discovered that drugs that inhibit PI 3' kinase-beta cause a decrease in P-ERK in wild type cells, but not in isogenic NF1-knockout cells, or in cells expressing oncogenic RAS. Figure 12 shows that exposure of MEFs expressing wild type KRas 4b to PI 3' kinase inhibitors that block the beta isoform (yellow boxes) inhibits P-ERK (panels 1 and 3, the latter being a repeat experiment identical to panel 1). In contrast, cells in which NF1 has been ablated by CRISPR knock-out are unaffected (panels 2 and 4).

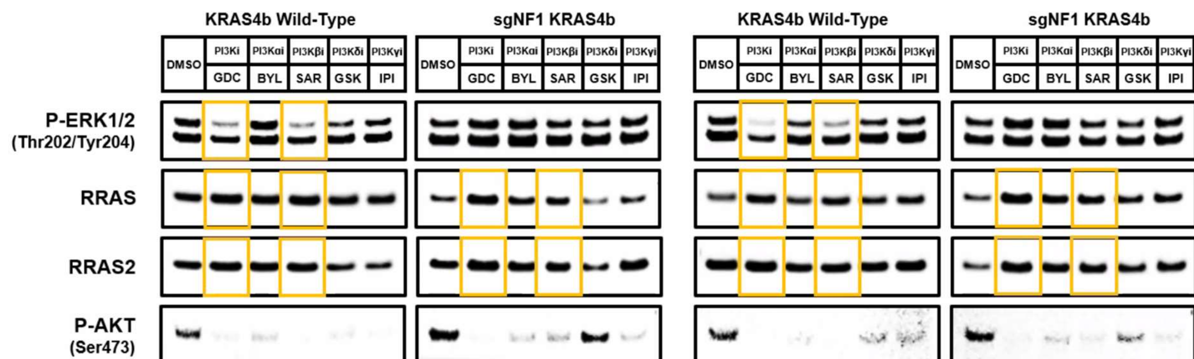
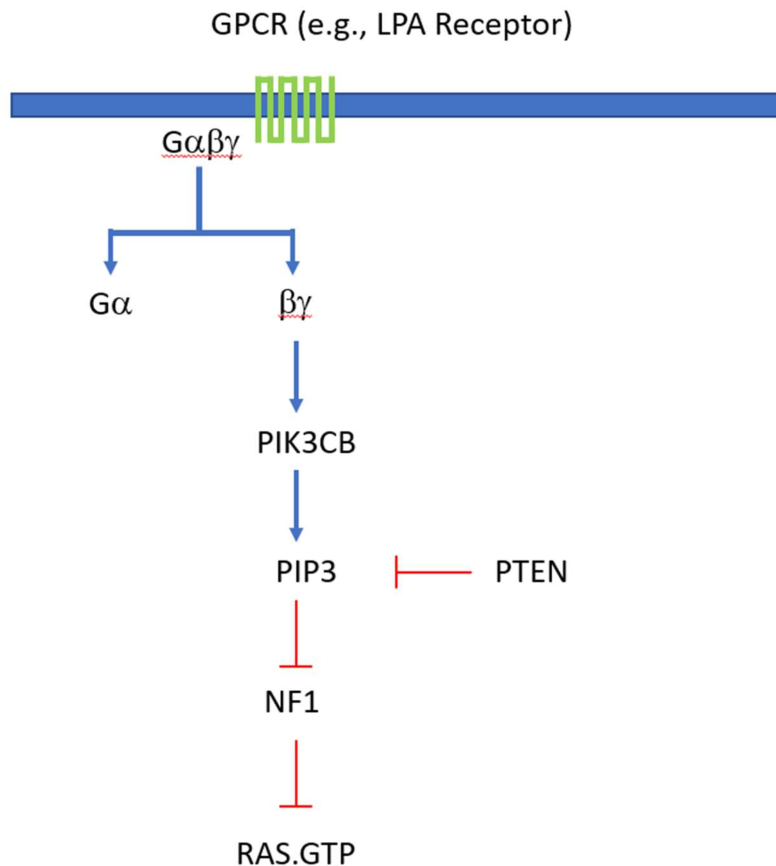


Figure 12. PI3 kinase inhibitors that inhibit the beta-isoform inhibit P-ERK in cells expressing neurofibromin, but not NF1 -/- cells. MEFs expressing KRas4b as the only Ras isoform, or isogenic counterparts in which NF1 has been knocked out by CRISPR were treated with a panel of PI3 kinase inhibitors: GDC: GDC-0941, pan PIK inhibitor. BY: BYL719, PIK-alpha specific inhibitor. SAR: PIK-beta inhibitor. GSK:GSK2126458, PIK/mTOR inhibitor. PIK3K gamma inhibitor. After 4 hours, cells were harvested and analyzed by Western blots.

These results lead to the following model, which we are currently testing:



In this model, activation of G-proteins known to regulate the MAPK pathway, such as the receptor for lysophatidic acid (LPA) leads to release of G-protein βγ subunits which activate PI 3' Kinase beta (PIK3CB) and generate PIP3. This inhibits neurofibromin allowing Ras.GTP to accumulate. Inhibition of PI3' kinase beta leads to activation of neurofibromin and suppression of Ras.GTP. We have shown that oncogenic mutants of KRAS (G12C and G12V) are resistant to the effects of PIK2 CA-beta inhibitors, supporting this model. Whether PIP3 acts directly on neurofibromin or via other intermediates remains to be determined. These data were generated in isogenic MEF cell lines and are now being expanded to physiological relevant cell types such as Schwann cells and melanocytes.

We have performed transcriptional profiling of MEFs in which we have knocked out NF1, or SPRED1 and 2, in the background of KRAS G12D. The results are summarized in figure 13. A set of genes associated with EMT was identified as significantly reduced in cells lacking either NF1 or SPRED1 and 2.

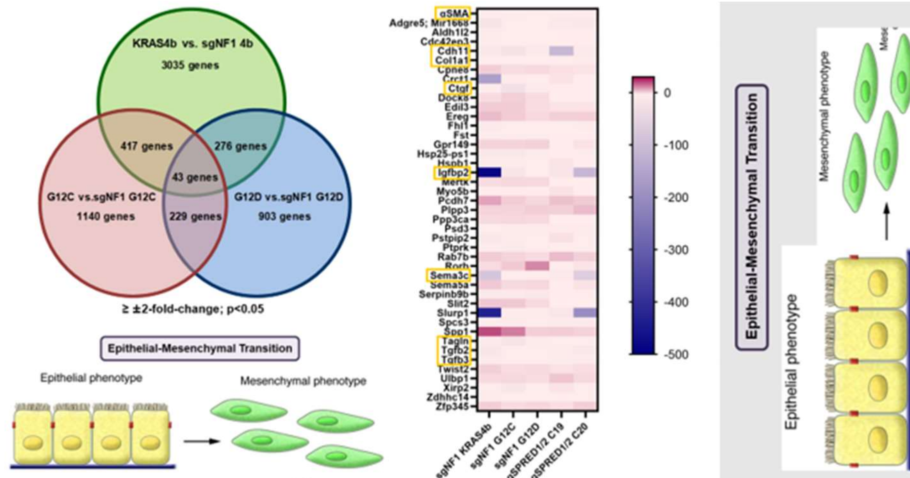


Figure 13. Gene expression analysis from MEFs. Three comparisons are shown: Green circle (left) genes differentially expressed in MEFs expressing only KRAS4b wild type, vs these cells in which NF1 was knocked out by CRISPR. Orange circle; single isoform KRAS G12C MEFs , before and after knockout of NF1. Blue circle, KRASG12D MEFs, before and after NF1 knock out. Shared genes between these treatments are listed in the middle panel. Many are genes known to be involved in EMT, the epithelial to mesenchyme transition.

To verify these data at the protein expression level, we performed Western blot analysis, using antibodies against representative genes down regulated at the level of mRNA expression, namely IGFBP2, COL1A1, CDH11, a-SMA and TAGLN. Of these, IGFBP2, COL1A1 and CDH11 were dramatically reduced following knock-down (Figure 14).

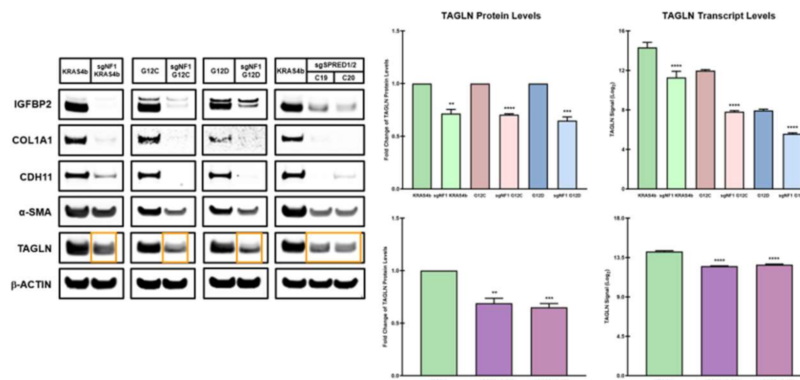


Figure 14. Genes whose expression is significantly reduced following knockout of NF1 or SPRED1 and 2, identified by Western blots from parental MEFs.

We then compared expression of these genes in human Schwann cells from an NF1 patient, and from a neurofibroma from the same patients and another neurofibroma from a second NF1 patient. In both neurofibromas, the NF1 gene has undergone LOH, and no neurofibromin is expressed. Figure 15 shows that, remarkably, the same genes were downregulated in neurofibromas derived from an NF1 patient as those derived from MEFs shown in Figure 14.

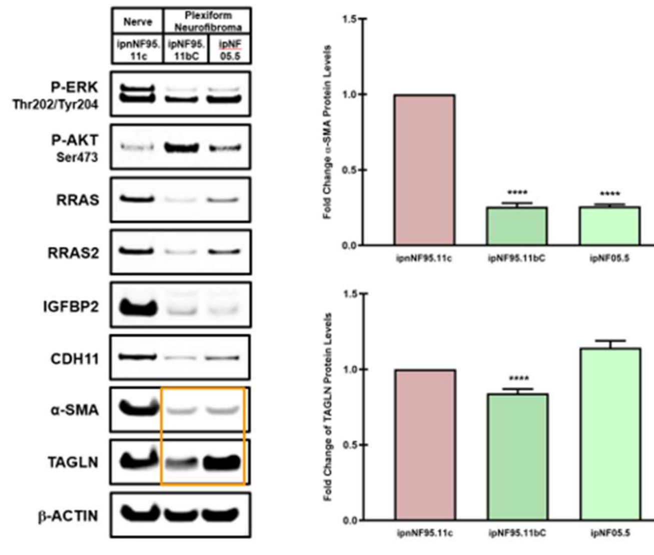


Figure 15. Loss of expression of specific NF1-regulated genes in human schwann cells and neurofibromas derived from an NF1+/- patient

The fact that a set of genes is down regulated following knockout of both NF1 and SPRED1 and 2 suggests these genes are regulated by neurofibromin GAP activity, since this activity is SPRED dependent. More importantly, these genes are affected by NF1 knock down in the presence of oncogenic RAS proteins, which are refractory to GAP regulation by neurofibromin. Loss of expression of these genes these genes is therefore independent of RAS driven MAPK activity and is likely to due to up-regulation of other RAS proteins such as RRAS2 and MRAS, which are expressed in these cells. Figure 16 shows the effects of MEK inhibition on cells following neurofibromin knock out.

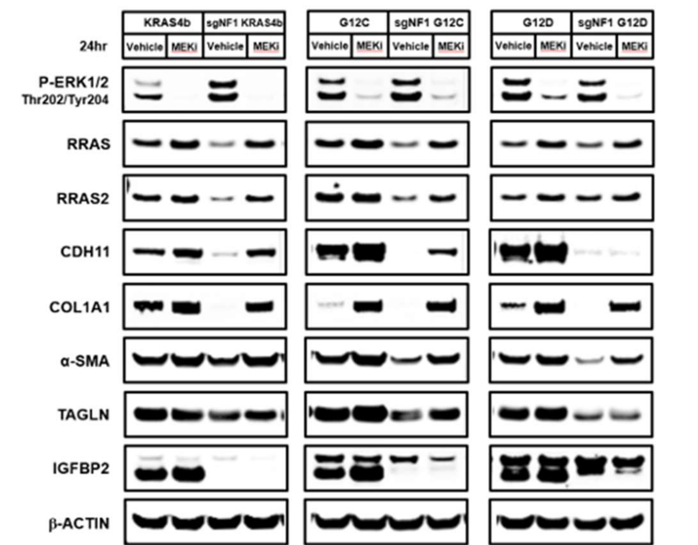


Figure 16. Effects of MEK inhibitors on gene expression following NF1 knock out in G12C or G12D KRAS single isoform MEFs.

Specific Aim 3

Major Task 1: Determine the nature of different neurofibromin and SPRED1 complexes in cells

This task in progress.

Major task 2: Identify SPRED1 phosphorylation sites

293T cells transfected with FLAG tagged SPRED1 were treated with Stem Cell Factor (SCF) for up to 40 minutes. Cells were lysed, and P-Tyrosine on SPRED1 was detected by Western blot. Figure 17 shows 3 separate biologic replicates in which P-Tyrosine was detected at a maximal level about 30 minutes after stimulation. These replicated samples were analyzed by mass spectrometry.

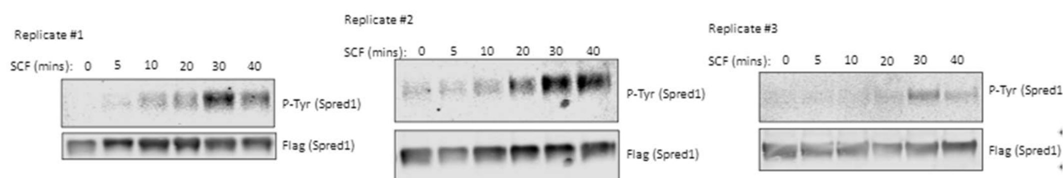
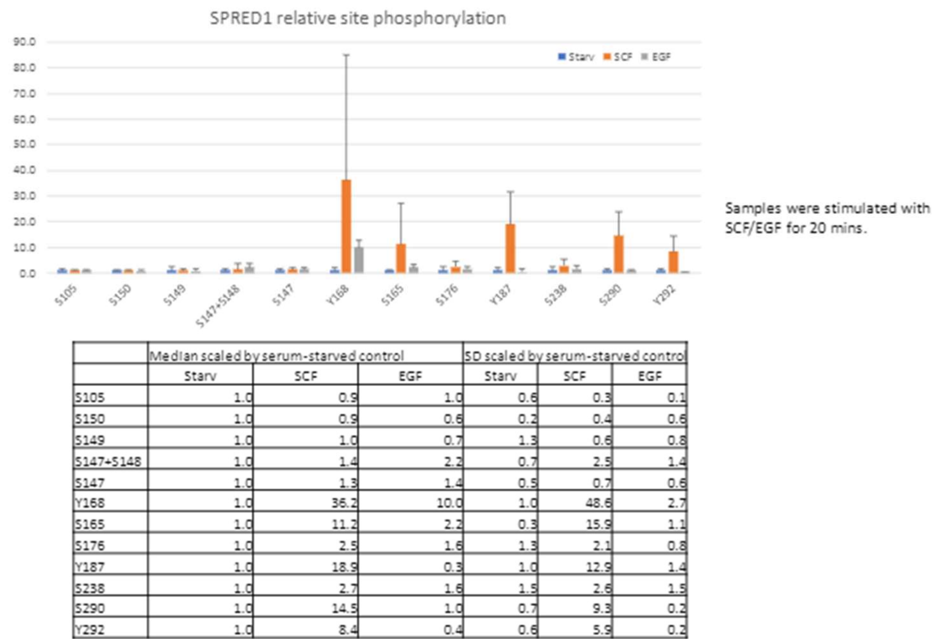


Figure 17. Detection of P-Tyrosine on SPRED1 after treatment with SCF.



	Median scaled by serum-starved control			SD scaled by serum-starved control		
	Starv	SCF	EGF	Starv	SCF	EGF
S105	1.0	0.9	1.0	0.6	0.3	0.1
S150	1.0	0.9	0.6	0.2	0.4	0.6
S149	1.0	1.0	0.7	1.3	0.6	0.8
S147+S148	1.0	1.4	2.2	0.7	2.5	1.4
S147	1.0	1.3	1.4	0.5	0.7	0.6
Y168	1.0	36.2	10.0	1.0	48.6	2.7
S165	1.0	11.2	2.2	0.3	15.9	1.1
S176	1.0	2.5	1.6	1.3	2.1	0.8
Y187	1.0	18.9	0.3	1.0	12.9	1.4
S238	1.0	2.7	1.6	1.5	2.6	1.5
S290	1.0	14.5	1.0	0.7	9.3	0.2
Y292	1.0	8.4	0.4	0.6	5.9	0.2

Figure 18. Phosphorylation sites on SPRED1 detected by mass spectrometry following treatment with SCF or EGF.

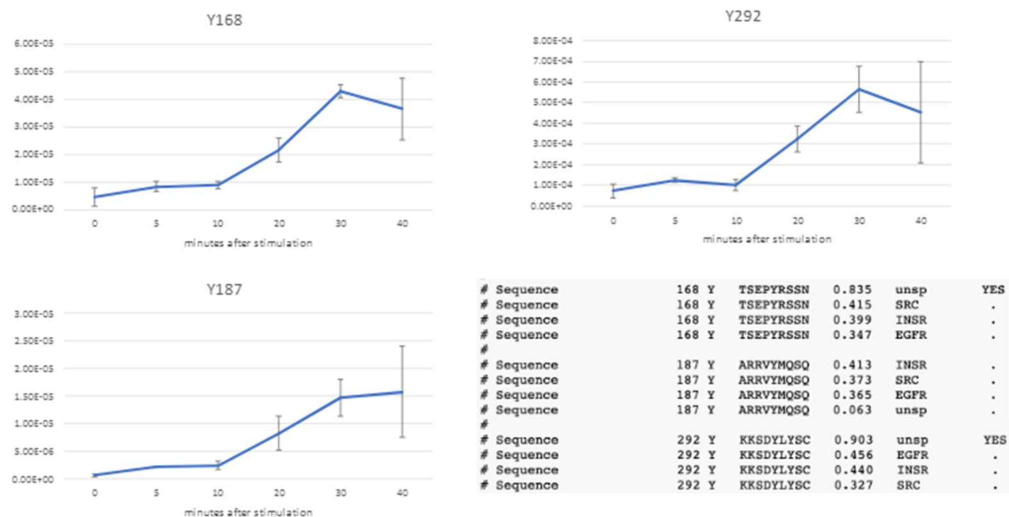


Figure 19. Time course of tyrosine phosphorylation on major sites after treatment with SCF.

Three major phosphorylation sites were identified on SPRED1 on response to SCF stimulation of c-KIT, Y168, Y187 and Y292, shown in Figures 18 and 19. These sites flank the Kit Binding Domain on SPRED1 (Figure 20). The EVH1 domain is the site of binding to neurofibromin (Yan et al, *Cell Rep* 2020). SPR is the Sprouty-related Domain, a region thought to be necessary for

plasma membrane binding of the SPRED and SPROUTY family of proteins (Lorenzo and McCormick, *Genes & Dev* 2020).

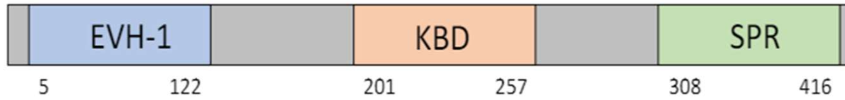


Figure 20. SPRED1 domains.

We have mapped the region of c-KIT that interacts with SPRED1 using a new technique developed in the McCormick laboratory that uses a modified yeast 2-hybrid technology coupled to next-gen sequencing (Figure 21). This method is described in Castel et al, *J Biol Chem* 2020. Using this novel method, we discovered that SPRED1 binds to the kinase insert region of c-KIT, a region previously identified as the site of binding of the p85 subunit of PIK3CA and the GRB2/SOS complex. We are currently investigating whether SPRED binding affects binding of these key signaling proteins.

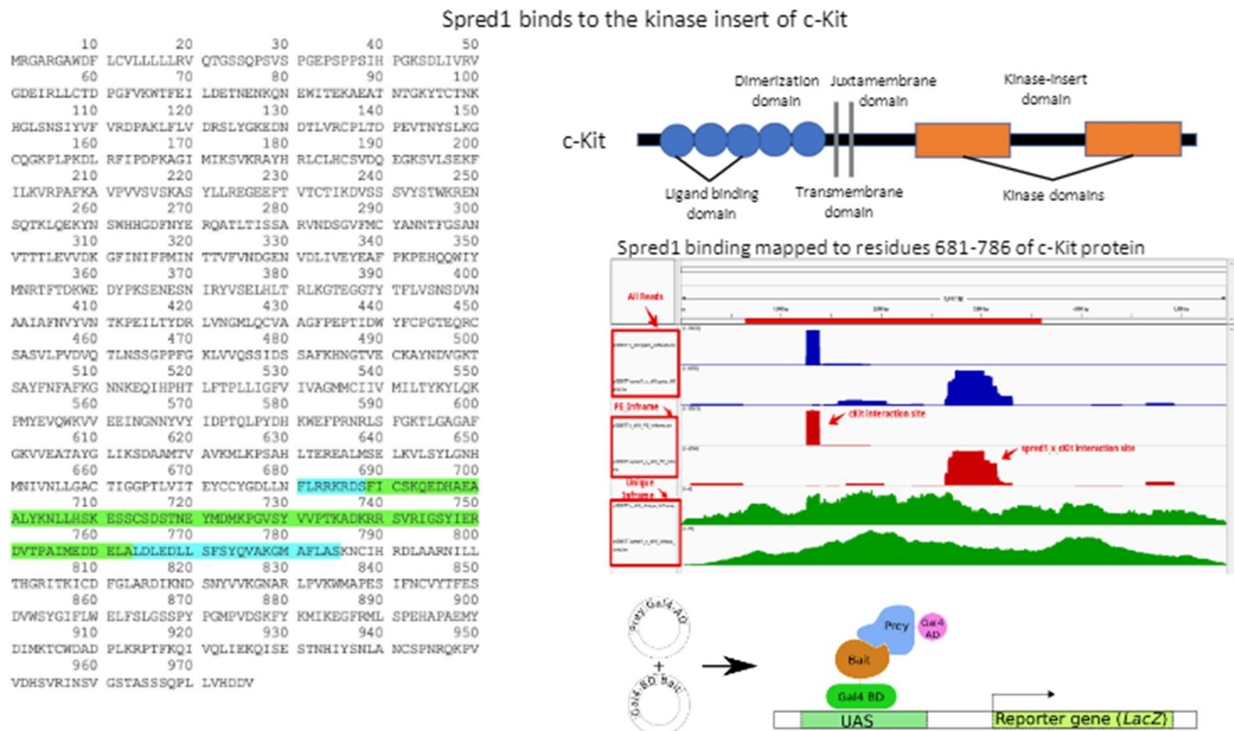


Figure 21. Mapping regions of c-KIT that bind to SPRED1 using Do-My-Seq technology. Fragments of c-KIT were expressed as a library in yeast. Fragments that bind SPRED1 grow in selected medium and are identified by DNA sequencing. The binding region maps to the kinase insert domain of the c-KIT receptor (highlighted as green in the sequence in the left panel).

SPRED1 binds to neurofibromin through the EVH1 domain (Yan et al, *Cell Rep* 2020). Figure 22 shows that a complex forms between neurofibromin, SPRED1 and c-KIT. In this experiment, we transfected SPRED1 null 293Tcells with neurofibromin and showed by immunoprecipitation that it associates with c-KIT in a SPRED1-dependent manner. We also knocked out endogenous SPRED2 protein to be sure that the trimeric complex was indeed dependent on SPRED and did not involve a potential heterodimer with SPRED2.

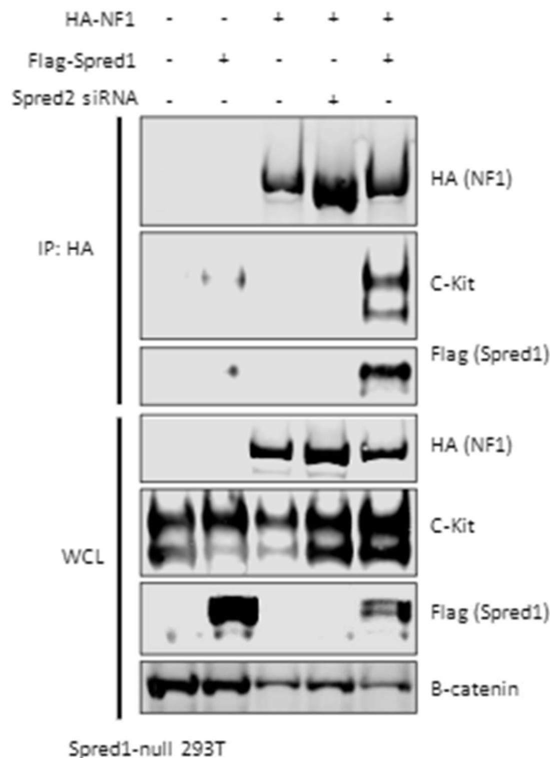


Figure 22. Neurofibromin associates with c-KIT in a SPRED1 dependent manner. Spred1 deficient 293T cells were transfected with neurofibromin and SPRED1, and association with endogenous c-KIT was detected by Western blot analysis.

We next tested whether SPRED binding to KIT is dependent on KIT tyrosine kinase activity. We transfected HA-tagged SPREDs 1, 2 or 3 and Flag tagged KIT, wither WT, activated or kinase dead mutants, as shown in Figure 23. We pulled down SPREDs through the HA tag and probed Western blots with antibodies against phosphor-tyrosine or FLAG. Association between SPREDs 1 and 2 and KIT was significantly higher in the kinase active KIT mutant (D816V) but was

detectable in cells expressing wild type KIT or kinase dead KIT (K623M). SPREDs 1 and 2 were strongly phosphorylated on tyrosine, but SPRED3 was not.

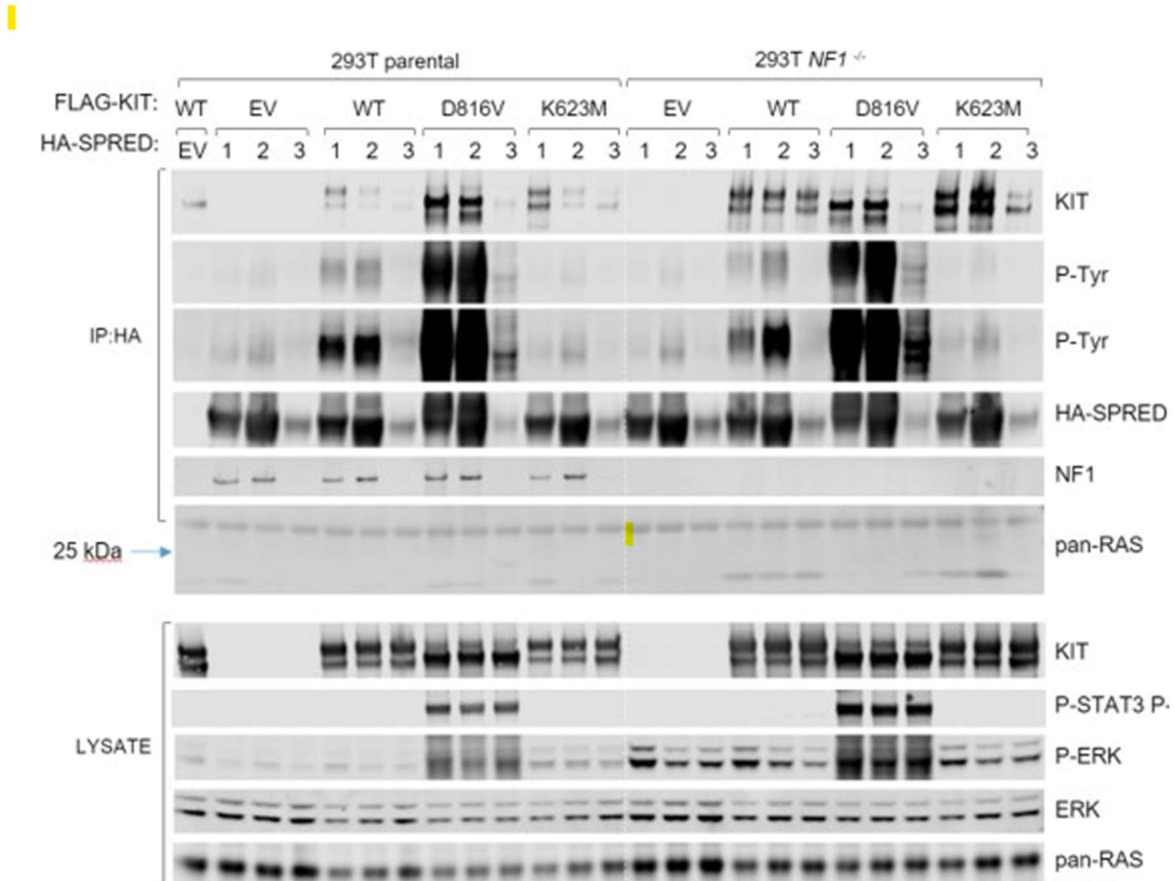


Figure 23. Association between SPREDs and KIT is enhanced by tyrosine phosphorylation.

Major task 3: BRET assay and co-IP studies for c-KIT/SPRED1 regulation of neurofibromin

Based on the data shown in Figure 21, we are investigating how the neurofibromin/SPRED1/c-KIT complex is regulated. To facilitate this analysis, we have developed a BRET based assay in which binding of SPRED1 and neurofibromin in cells is measured using energy transfer technology. Figure 24 shows that binding in cells can indeed be detected using BRET technology.

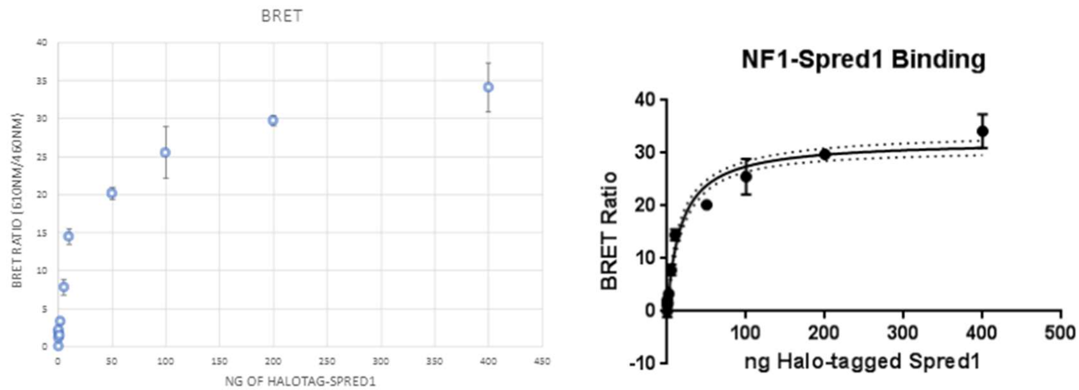


Figure 24. Interaction of SPRED1 and neurofibromin detected in cells using BRET technology.

We are now analyzing the role of SPRED1 phosphorylation on complex formation, and the effects of these interactions on neurofibromin GAP activity.

To identify proteins that bind to different members of the SPRED family, we performed mass spec analysis on epitope tagged SPRED1, 2 and 3 expressed in 293T cells. Surprisingly, SPRED3 was strongly associated with 14-3-3 proteins, indicating this member of the family is highly phosphorylated (Figure 25). However, of more interest, SPRED2 associated specifically with RSK2. Because this kinase plays a central role in signal transduction pathways involving both MAPK and PI 3 kinase pathways, we have pursued this further.

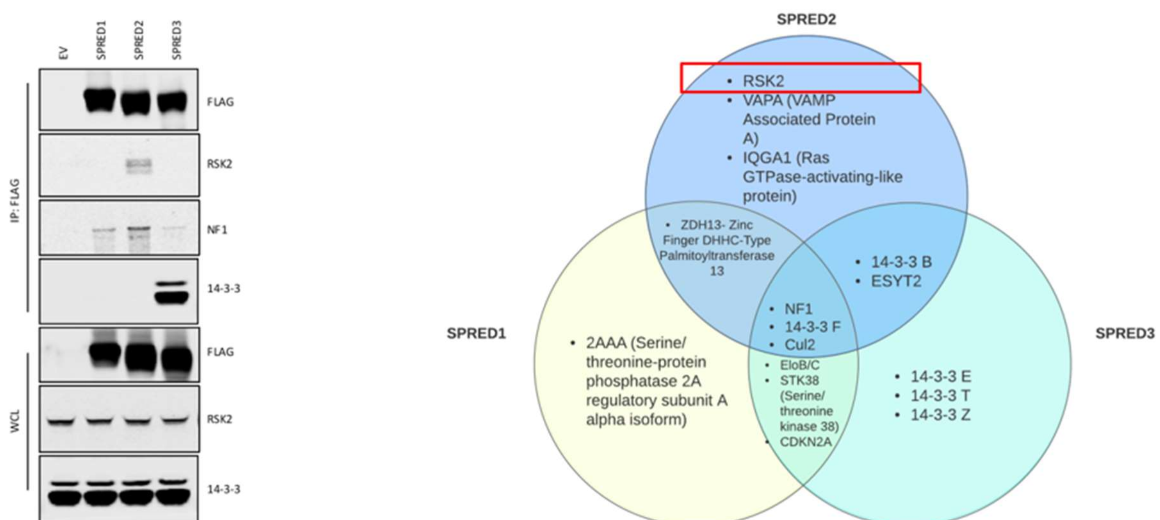


Figure 25. SPRED2, but not SPRED1 or SPRED3, binds to RSK2. Interaction was detected by immunoprecipitation with FLAG-tagged SPRED proteins and probed with antibodies against RSK2 to detect endogenous protein.

Next, we identified the region of SPRED2 to which RSK2 binds, by expressing a panel of deletion mutants in 293T cells, followed by pull-down on these mutants and probing for RSK2 on Western Blots, as shown in Figure 26. Amino acids 123-200 are necessary for binding.

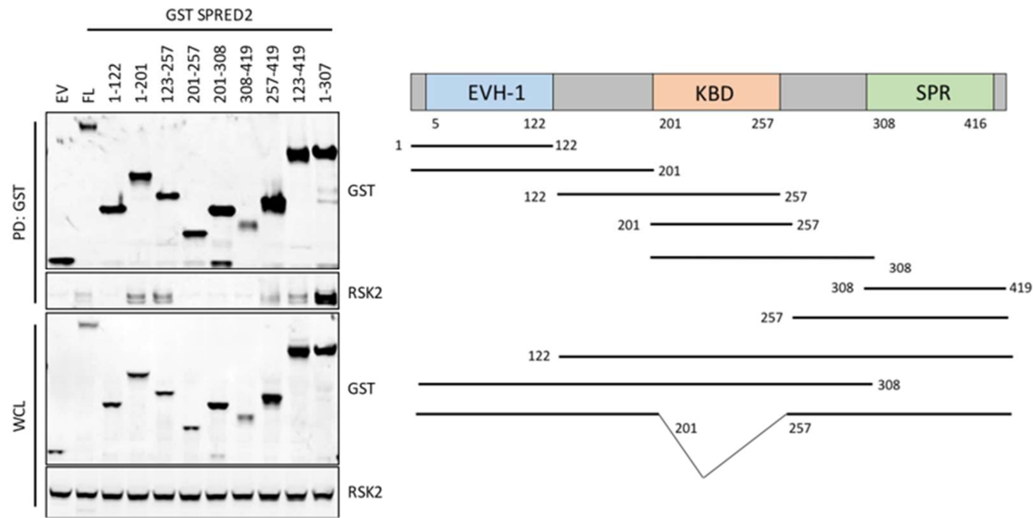
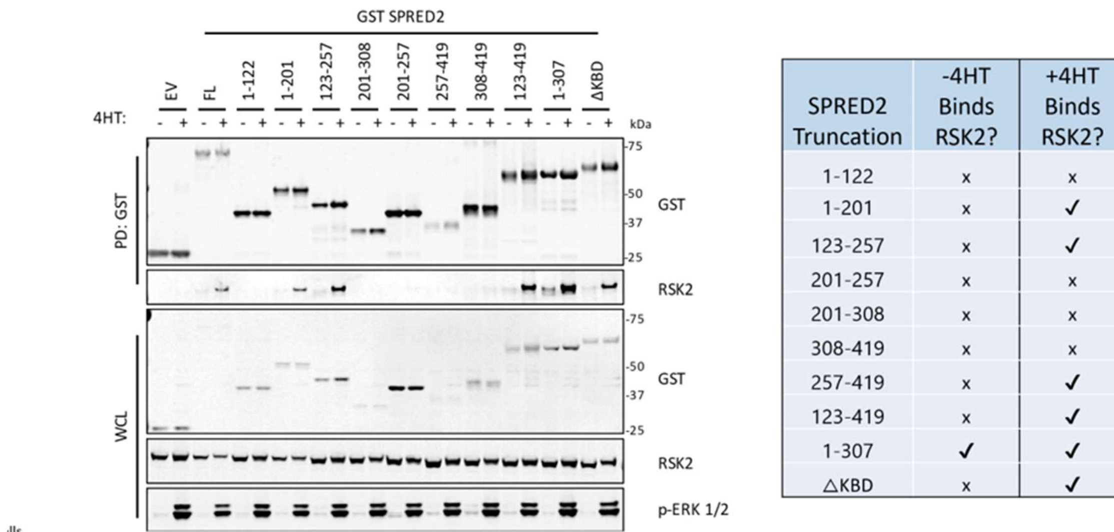


Figure 26. Mapping the region of SPRED2 to which RSK2 binds. A panel of mutants were transfected into 293T cells and endogenous RSK2 binding was detected by Western Blot.

We then repeated this experiment in cells in which the Raf MAPK has been activated by addition of 4-Hydroxy Tamoxifen (4HT). These cells express a RAF:ER fusion protein that is rapidly activated upon addition of 4HT. RSK2 was detected bound to SPRED2 mutants as in Figure 27. Binding is strongly dependent on 5HT as seen in Figure 26. This strongly suggests that RSK2 binding is a feedback loop that is activated when cells activate MAPK signaling.



SPRED2 Truncation	-4HT Binds RSK2?	+4HT Binds RSK2?
1-122	x	x
1-201	x	✓
123-257	x	✓
201-257	x	x
201-308	x	x
308-419	x	x
257-419	x	✓
123-419	x	✓
1-307	✓	✓
△KBD	x	✓

Figure 27. SPRED2 binding to RSK2 is enhanced by activation of the RAF MAPK pathway.

We then tested whether this novel interaction is inhibited by drugs that block MEK (trametinib), ERK (SCH772984) or RSK (LJH685). Figure 28 shows that interaction is completely blocked following inhibition of either MEK or ERK, but not RSK itself. The system used is the same as Figure 26.

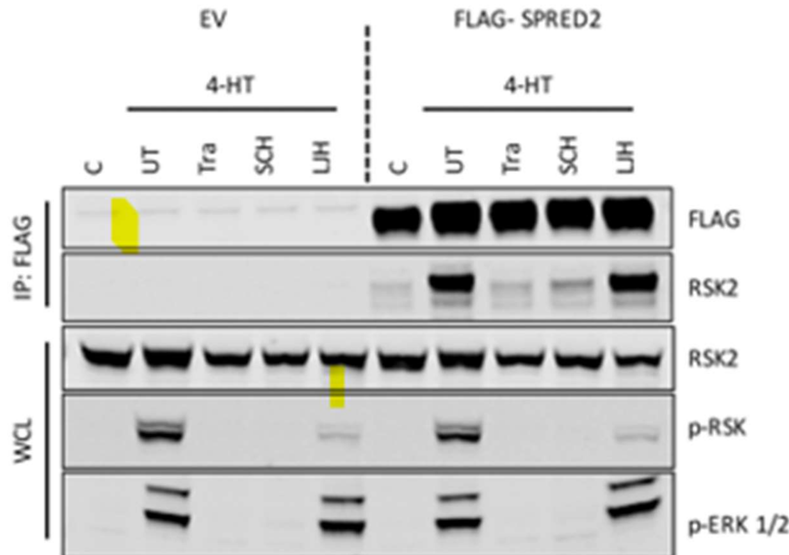


Figure 28. Inhibition of the MAPK pathway blocks RSK2/SPRED2 interaction.
Cells were transfected with FLAG-SPRED2 and probed for interaction with endogenous RSK2 following exposure to inhibitors of MEK (Tra), ERK (Sch) or RSK (LJH). UT is untreated, C is control, ie no induction with 4-HT.

Opportunities for training and professional Development

Post-doctoral scholar Dr. Lucy Young. Dr. Young meets with Dr. McCormick on a weekly basis either by Zoom or in-person to discuss project and career progress and goals. Dr. Young presented her research at lab group meetings. Dr. McCormick continues to run the NF1 Monthly Meetings between scientists and collaborators at Frederick National Laboratory for Cancer Research and the McCormick Lab. These meetings have offered the opportunity for trainees to participate in scientific discussions and collaborations between both groups. Topics have included updates on CRYO-EM analysis, progression of in biochemical analysis of neurofibromin and SPRED proteins and on biological consequences of neurofibromin loss. Dr. Young has presented on genotype-phenotype relationships that may point to particularly interesting regions of the protein that are critical for its function, as well as post-translational modifications identified by mass spectrometry.

Dr. Young has been a major contributor to these discussions and ongoing collaborations. NF1 Monthly Meetings usually include Drs. Young, Sale, Vasudevan, Francois and graduate students and trainees Cuevas-Navarro (Graduate Student TETRAD Program), Heuttemann (Rotation Graduate Student, TETRAD Program), Lopez (Graduate Student TETRAD Program), Martinez (Graduate Student, TETRAD Program) and Goldstein de Salazar (Jr Specialist) from the McCormick Lab. Additionally, Dr. Young has been co-mentoring and supervising the graduate trainees (including former graduate student Claire Lorenzo and Jr Specialist Goldstein de Salazar) in my Lab which entails daily and weekly supervision of their research projects. She continues her lab training education by attending training courses at UCSF and elsewhere as opportunities become available such as the UCSF Responsible Conduct of Research for Postdoctoral Scholars Training Courses.

Graduate student Claire Lorenzo returned from Medical Leave Fall 2021 quarter and her last registered quarter was Winter 2022. Ms. Lorenzo was given one-on-one mentorship by Dr. McCormick which included at least bi-weekly meetings by Zoom to discuss project and career progress and goals. Ms. Lorenzo presented her research at lab group meetings by Zoom and in-person. Ms. Lorenzo decided to change direction in her career and was recently awarded a Master's (MS) Degree in Biochemistry and Molecular Biology. Her official graduation date was March 27, 2022. Ms. Lorenzo is now employed as Research Scientist at Bristol Myers Squibb (BMS), Redwood City.

Dissemination of Results

a. **Lucy Young:**
   1. Podium, UCSF Neurofibromatosis Conference, August 23, 2021

b. **Frank McCormick Presentations:**
   1. University of Miami, Sylvester Comprehensive Cancer Center Distinguished Lecture Series, May 14, 2021, Virtual, ***Invited Speaker***: *"RAS Proteins in Human Disease"*

   2. 2021 NCI RAS Initiative/Frederick National Laboratory for Cancer Research, RAS Symposium, May 24-May 28, 2021, Virtual, ***Session Chair**, RAS Biology*

   3. 1st JCA (Japanese Cancer Association)-AACR Precision Cancer Medicine International Conference, September 10-12, 2021, Virtual, ***Invited Speaker***, *Drug Resistance: RAS Proteins in Human Disease*

   4. 3rd RAS Targeted Drug Development Summit: ***Keynote speaker***, September 23, 2021, Virtual, *Progress in Targeting RAS*

5. Cincinnati Children's Hospital Medical Center: Molecular & Developmental Biology Seminar Series, October 20, 2021, Virtual, ***Invited Speaker***, *RAS Proteins in Human Disease*

6. 2nd AACR-KCA (Korean Cancer Association) Joint Conference on Precision Medicine in Solid Tumors, Nov 10, 2021, Virtual, ***Keynote speaker***: *Progress in targeting KRAS directly*

7. University of Chicago: Biomedical Sciences Cluster Committee on Cancer Biology Seminar Series, December 3, 2021, Virtual, ***Invited Speaker***, *RAS Proteins in Human Disease*

8. Baylor College of Medicine, Clinical Translational Research, Certificate of Added Qualification (CTR-CAQ) Program, Bench to Bedside Seminar, December 16, 2021, Virtual, ***Invited Speaker***, *Successes and failures targeting RAS oncogenes*

9. 2022 The Japanese Society of Medical Oncology (JSMO) Annual Meeting, February 17-20, Virtual, ***Invited Speaker****, Progress in treating RAS driven cancers*

10. NTAP Cutaneous Neurofibroma Symposium, March 2-4, 2022, In-person meeting, Therapeutic Targets for cNF Treatment Session, ***Invited Member***, Team 2: Therapeutic Targets for Cutaneous Neurofibroma Treatment

11. MERCK, March 10, 2022, Virtual, ***Invited Speaker****, Targeting RAS*

12. AACR Annual Meeting, Major Symposium, AACR KRAS Anniversary Session: Novel Mechanisms for Targeting KRAS, April 11, 2022, Virtual, ***Invited Speaker****, How RAS Causes Cancer*

c. **Abstracts:**

1. 1st JCA (Japanese Cancer Association)-AACR Precision Cancer Medicine International Conference, September 10-12, 2021, Virtual, ***Invited Speaker***, *Drug Resistance: RAS Proteins in Human Disease*

2. 2nd AACR-KCA (Korean Cancer Association) Joint Conference on Precision Medicine in Solid Tumors, Nov 10, 2021, Virtual, ***Keynote speaker***: *Progress in targeting KRAS directly*

3. 2022 The Japanese Society of Medical Oncology (JSMO) Annual Meeting, February 17-20, Virtual, ***Invited Speaker****, Progress in treating RAS driven cancers*

d. **Publications:** ***Reported May 2021***

1. **Lorenzo, C**. and **McCormick, F**. 2020. SPRED proteins and their roles in signal transduction, development, and malignancy. *Genes & Dev*. 34: 1410-1421. doi: 10.1101/gad.341222.120.

2. **Castel. P.,** Holtz-Morris, A., Kwon, Y., Suter, B.P., **McCormick, F.,** 2020. DoMY-Seq: A yeast two-hybrid-based technique for precision mapping of protein-protein interaction motifs. *J Biol Chem* 23:296:100023. doi: 10.1074/jbc.RA120.014284.

3. Yan, W., **Markegard, E**., Dharmaiah, S., Urisman, A., Drew, M., Esposito, D., Scheffzek, K., Nissley, D.V., **McCormick, F**., Simanshu, D.K. 2020. Structural Insights into the SPRED1-Neurofibromin-KRAS Complex and Disruption of SPRED1-Neurofibromin Interaction by Oncogenic EGFR. *Cell Rep*. Jul 21;32(3):107909. doi: 10.1016/j.celrep.2020.107909.

4. Sherekar, M., **Han, SW**., Ghirland, R., Messing, S., Drew, M., Rabara, D., Waybright, T., Juneja, P., O'Neill, H., Stanley, CB., Bhowmik, D., Ramanathan, Ar., Subrimaniam, S., Nissley, DV., Gillette, W., **McCormick, F**., Esposito, D. 2019. Biochemical and structural analyses reveal that the tumor suppressor neurofibromin (NF1) forms a high-affinity dimer. *J Biol Chem*. doi: 10.1074/jbc.RA119.010934

4. Impact

Nothing to report.

5. Changes/Problems

While the Aims of the project remain unchanged, we experienced delays with the project due to the COVID-19 pandemic. Post-doctoral scholar, Dr. Lucy Young, has registered with the Synapse platform and she is currently working with Sage Bionetworks in setting up the project on Synapse and registering the study on the NF Data Portal. We expect to have data uploaded to the NF Data Portal in the coming months.

6. **Products:**
    - ***Manuscript: In preparation and to submit to Nature Genetics***

    Working Title: *Cryo-EM structure of neurofibromin dimer explains basis of dominant negative missense variants*

    Authors: Lucy C. Young, **Ruby Goldstein de Salazar**, Zi Yi Stephanie Huang, Sae-Won Han, Alan Merk, Joseph Darling, Reinhard Grisshammer, Mukul Sherekar, Matthew Drew, Dwight V. Nissley, Dominic Esposito, Jana Ognjenovic†, **Frank McCormick†**

    †Co-senior authors

7. **Participants & Other Collaborating Organizations**

Participants: ***No change for key personnel. Graduate student Claire Lorenzo has graduated with a Master's Degree and is now a Research Scientist with BMS.***

Name:	Frank McCormick
Project Role:	PI

Name:	Lucy Young
Project Role:	Post-doctoral scholar

Name:	**Claire Lorenzo**
Project Role:	**Graduate Student**
Nearest Person Month Worked:	3.0
Contribution to Project:	***Graduated, Master's Degree***
Funding Support:	This award

Other collaborating organizations

We are collaborating with scientists at the Frederick National Laboratories for Cancer Research, Frederick, MD to solve the structure of neurofibromin using Cryo-EM and (for small domains) by conventional crystallography.

Changes in PI and Key Personnel support since Award Made:

FRANK MCCORMICK

ACTIVE

15R35CA197709 McCormick (PI) 04/01/2016-06/30/2023
NIH/NCI
New Ways of Targeting K-Ras
The overall goal is to find new ways of suppressing the function of hyper-active Ras proteins through a better understanding of how they function and how they are regulated.
No changes to report; award continuation

W81XWH2010129 McCormick (PI) 04/01/2020 – 03/30/2023
DOD/CDMRP
Regulating neurofibromin through degradation, dimerization and binding to SPRED1
Our objective is to identify ways of increasing neurofibromin's GAP activity that could lead to new therapeutic strategies for treating Neurofibromatosis Type 1, and potentially other syndromes caused be elevated levels of Ras.GTP, such as Noonan Syndrome and specific types of autism.
No changes to report; award continuation

(no grant #) McCormick (PI) 10/10/2020-06/10/2022
Boehringer Ingelheim RCV GmbH & Co KG
Direct targeting of SOS1 in SOS1-mutant cancer
The goal of the project is to identify potentials drugs targets in SOS1-mutant cancers.
Award continuation; no effort to report; no overlap with DOD grant

PREVIOUS

no grant #) McCormick (PI) 01/01/2021-12/31/2021
Oncogenuity Inc.
Oncogenic inhibition using peptide nucleic acids
Our objective is to identify ways of increasing neurofibromin's GAP activity that could lead to new therapeutic strategies for treating Neurofibromatosis Type 1, and potentially other syndromes caused be elevated levels of Ras.GTP, such as Noonan Syndrome and specific types of autism.
Previously active funding and now closed

Key Personnel:

LUCY YOUNG

<u>ACTIVE</u>

W81XWH2010129 McCormick (PI) 04/01/2020 – 03/30/2023
DOD/CDMRP
Regulating neurofibromin through degradation, dimerization and binding to SPRED1
Our objective is to identify ways of increasing neurofibromin's GAP activity that could lead to new therapeutic strategies for treating Neurofibromatosis Type 1, and potentially other syndromes caused be elevated levels of Ras.GTP, such as Noonan Syndrome and specific types of autism.
No changes to report; award continuation

8. **Special Reporting Requirements**

   We have experienced delays with the project due to the COVID-19 pandemic. Since our last report in May 2021, we have reestablished subcontract work with Sage Bionetworks and we are working with them to meet the requirements for the Data and Resources Sharing Plan. Post-doctoral scholar, Dr. Lucy Young, has registered with the Synapse platform and is currently working with Sage to get the data onto Synapse and onto the NF Data Portal.

9. **Appendices**
   A. Presentations

      1. Frank McCormick Abstracts
      2. Frank McCormick Presentations

1. Abstract:

1st JCA (Japanese Cancer Association)-AACR Precision Cancer Medicine International Conference, September 10-12, 2021, Virtual, Invited Speaker, Drug Resistance: RAS Proteins in Human Disease

RAS proteins in human disease

Frank McCormick, Helen Diller Family Comprehensive Cancer Center, University of California San Francisco, USA.

The role of RAS oncogenes in human cancer has been recognized for almost 40 years. The first compounds that target one form of oncogenic RAS, the G12C mutant, Sotorasib or AMGEN 510 has been approved for treatment of lung adenocarcinomas expressing the KRAS G12C protein. Development of covalent inhibitors targeting G12C was based on the discovery of Shokat and colleagues in 2013, and appeared to solve the problem of finding compounds that bind to KRAS with sufficient affinity to block its function. However, recent data have emerged suggesting that non-covalent approaches may also be effective, at least against the G12D allele and, indeed, clinical trials of such compounds are expected in the near future. We can be optimistic that compounds that target other common alleles, most notably the G12V allele which is second to G12D in overall prevalence, will emerge in the next few years.

Beyond their role as oncogenic drivers of many cancers, RAS proteins participate directly in other diseases. Neurofibromatosis Type 1 (NF1), for example, is caused by loss of the RAS GAP neurofibromin, which maintains RAS proteins in a relatively inactive state in normal cells. Resistance to the effects of Ras GAPs is the basis of most oncogenic RAS mutants. In the case of NF1 disease, loss of a single allele causes symptoms that include behavioral and cognitive problems and development delays. Sporadic loss of the second allele causes focal benign tumors which can often progress to more severe neoplasms including malignant peripheral nerve sheath tumors. This step is usually driven by loss of the Polycomb repressive complex PRC2. Structural and biochemical features of the neurofibromin protein will be discussed. The NF1 gene is frequently mutated in sporadic cancers in addition to the NF1 syndrome. As many as 15% of all human tumors may be driven by loss of NF1 and subsequent activation of wild type RAS proteins.

Activation of the RAS/MAPK pathway in the germline causes a group of diseases referred to as Rasopathies. These include NF1 and Noonan Syndrome, both of which are common in the human population, accounting for 1 in 3500 and 1 in 1000 births, respectively. Less common Rasopthies include Costello Syndrome, usually caused by the G12S mutation in HRAS, and CFC, usually caused by mutations in MEK or BRAF genes, details of which will be discussed. These syndromes affect development and carry risk of malignancy. In addition, they generally affect cognitive functions. In one striking case, loss of the RAS GAP SynGAP causes severe autism, some aspects of which can be reversed in mouse models through use of MEK inhibitors.

Opportunities for treating RAS related diseases through targeting downstream pathways such as the MAPK and PI 3’ kinase pathways are currently being developed and tested and represent addition ways of treating patients suffering from RAS drive disease, whether driven by mutant RAS or hyperactive forms of the wild type proteins.

AACR American Association for Cancer Research | 대한암학회 Korean Cancer Association

2nd AACR-KCA Joint Conference on Precision Medicine in Solid Tumors

in conjunction with the 24th KCA Fall Symposium

Title

Progress in targeting KRAS directly

Author(s)

- *Presenting author's name should be underlined and be without degrees or titles.*

Frank McCormick

Affiliation(s)

- *Author(s) should be listed by department, institution, city and country.*

UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, USA/Frederick National Laboratory for Cancer Research, Maryland, MD, USA

Content

The first direct KRAS inhibitor, sotorasib ("LUMAKRAS") from Amgen was approved by the FDA in 2021. This drug binds covalently to KRAS G12C and locks it in its inactive state. Multiple combination therapies are being tested to improve further its clinical activity, and other similar drugs are under evaluation. These studies, as well as studies suggesting mechanisms of resistance, will be summarized. The most common mutation in KRAS is G12D, followed by G12V. These mutations do not offer the opportunity for covalent engagement as first discovered by Dr Kevan Shokat and colleagues for G12C. However, compounds have recently been described that bind with high affinity to G12D forms of KRAS, and are active in cancer cell lines with high potency and specificity. These data suggest that compounds specific for G12V and other common alleles, may soon be developed, without the restrictive need for covalent binding. In addition, progress has been made targeting KRAS for degradation using Protac related technology.

The protein interfaces between KRAS and its primary effectors, RAF kinase and PI 3' kinase, offer additional opportunities for therapeutic intervention. The structure of a complex between KRAS and the RAS-binding domain and Cysteine-rich binding domains of Raf1 reveals an interface that appears amenable to small molecule intervention. This interface is of relatively low affinity, is distinct been Raf isoforms and exhibits pockets to which small molecules could bind. These new opportunities will be discussed. In addition, approaches to preventing KRAS activation of PI 3' kinase alpha will be discussed.

Keyword(s)

KRAS Targeted therapy, Raf kinase, PIK3CA

*** Please keep the length of the abstract within 1 page using Times New Roman 11 pts.**

3. Abstract:

S

2022 The Japanese Society of Medical Oncology (JSMO) Annual Meeting, February 17-20, Virtual, Invited Speaker, Progress in treating RAS driven cancers

Progress in treating RAS driven cancers

Frank McCormick, UCSF Helen Diller Comprehensive Cancer Center, San Francisco, California.

In 2021, the first drug was approved for treating cancers driven my oncogenic KRAS. This drug, Sotorasib (Lumakras™, Amgen, Inc.), binds covalently and specifically to the G12C allele of KRAS, a mutation characteristic of lung adenocarcinomas caused by cigarette smoke. This landmark ends a long history of failed efforts to target KRAS cancers directly, or by targeting downstream pathways, specifically the RAF/MAPK pathway and the PI 3 kinase pathway, some of which will be discussed.

Despite the importance of this approval, the clinical effects of Sotorasib are not dramatic. Activation of upstream pathways off sets the potency. This was expected, based on previous efforts to target this pathway: therefore, combinations with inhibitors of upstream targets such as EGF-Receptor and SHP2 are likely to increase efficacy. Furthermore, drugs that block KRAS are expected to render tumors more susceptible to immune therapies, and combinations with checkpoint inhibitors are also under investigation for this reason.

The most common mutations in KRAS are G12D and G12V, which account for the majority of cases of pancreatic cancers and many lung and colorectal cancers. Efforts to target these proteins are underway, using strategies that do not depend on covalent binding. Some of these drug candidates are likely to be selective for specific mutants, but some may be “pan-KRAS” drugs. Such drugs would inhibit both oncogenic and wild type KRAS, but need to spare NRAS and HRAS to be safe and well tolerated.

Another approach that is being actively pursued involves preventing oncogenic KRAS activating its downstream targets (RAF and PI 3 kinase). These drugs do not block the downstream targets directly: they interfere with process of activation. Progress in these approaches will be discussed.

Finally, indirect approaches that target KRAS cancers by blocking immune recognition or by preventing KRAS expression or processing will be discussed. Together, these efforts give us reasons to be optimistic that patients suffering from KRAS cancers will have good therapeutic options in the near future.

Speakers

May 24

11:00 am - 11:10 am	**Introduction & Welcome from** NCI Director - Ned Sharpless RAS Scientific Consultant - Frank McCormick
11:10 am - 11:40 am	**Keynote Speaker:** Charles Swanton *Francis Crick Institute*
11:40 am - 2:00 pm	**RAS Biology (Session Chair: Frank McCormick)** 11:40 am: Mariano Barbacid *Spanish National Cancer Research Center* 12:10 pm: Andy Aguirre *Harvard University* 12:40 pm: Pablo Rodriguez-Viciana *University College London* 1:10 pm: Marie Evangelista *Genentech* 1:40 pm: **Short Talk:** Presented by Ms. Laura Adams *Northwestern University*
2:00 pm - 3:00 pm	Break
3:00 pm - 5:40 pm	**Structure (Session Chair: Dwight Nissley)** 3:00 pm: Dhirendra Simanshu *Frederick National Laboratory for Cancer Research* 3:30 pm: Debbie Morrison *National Cancer Institute* 4:00 pm: Michael Eck *Harvard University* 4:30 pm: Chris Marshall *Princess Margaret Cancer Center* 5:00 pm: **Short Talk:** Presented by Dr. Jana Ognjenovic *Frederick National Laboratory for Cancer Research* 5:20 pm: **Short Talk:** Presented by Dr. Timothy Wendorff

	Genentech

May 25

11:00 am - 1:40 pm	**RASopathies (Session Chair: Dom Esposito)** 11:00 am: Kate Rauen *University of California, Davis* 11:30 am: Brigitte Widemann *National Cancer Institute* 12:00 pm: Maria Kontaridis *Harvard University* 12:30 pm: Pau Castel *University of California, San Francisco* 1:00 pm: **Short Talk:** Presented by Antonio Cuevas Navarro *University of California, San Francisco* 1:20 pm: **Short Talk:** Presented by Dr. Russell Spencer-Smith *National Cancer Institute*
1:40 pm - 3:00 pm	Break
3:00 pm - 6:10 pm	**Therapeutics (Session Chair: Caroline DeHart)** 3:00 pm: David Turner *Frederick National Laboratory for Cancer Research* 3:30 pm: Craig Crews *Yale University* 4:00 pm: Rusty Lipford *Amgen* 4:30 pm: Kevan Shokat *University of California, San Francisco* 5:00 pm: Karen Cichowski *Harvard University* 5:30 pm: **Short Talk:** Presented by Dr. Isabel Fernandez-Pisonero *University of Salamanca, Spain* 5:50 pm: **Short Talk:** Presented by Dr. Luca Gerosa *Harvard Medical School*

May 26

11:00 am - 1:40 pm	**Therapeutics II (Session Chair: Tommy Turbyville)** 11:00 am: Mollie Leoni *Kura Oncology* 11:30 am: Darryl McConnell

FRANK MCCORMICK PRESENTATIONS

	Boehringer Ingelheim 12:00 pm: Piro Lito *Memorial Sloan Kettering Cancer Center* 12:30 pm: Bob Nichols *Revolution Medicines* 1:00 pm: **Short Talk:** Presented by Dr. Oleksii Rukhlenko *University College Dublin, Ireland* 1:20 pm: **Short Talk:** Presented by Dr. Matthias Drosten *Spanish National Cancer Research Center*
1:40 pm - 3:00 pm	Break
3:00 pm - 5:30 pm	**RAS Biology II (Session Chair: Andy Stephen)** 3:00 pm: Dave Tuveson *Cold Spring Harbor Laboratory* 3:30 pm: Channing Der *University of North Carolina* 4:00 pm: Bill Hahn *Harvard University* 4:30 pm: Arvin Dar *Icahn School of Medicine at Mount Sinai* 5:00 pm: Christian Burd *Ohio State University*

An asynchronous week-long poster session will be available to all registered participants for the full week: May 24th – May 28th. An email will be sent with those details after registration closes on May 15, 2021.

FRANK MCCORMICK PRESENTATIONS

JCA-AACR PRECISION CANCER MEDICINE INTERNATIONAL CONFERENCE

September 10-12, 2021 (US) / September 11-12, 2021 (Japan)
Virtual Meeting

PROGRAM

PROGRAM TOPICS AND SPEAKERS

SEPTEMBER 11, 2021, 8:00 AM-9:00 AM, JST (SEPTEMBER 10, 2021, 7:00 PM-8:00 PM, EDT)

KEYNOTE ADDRESSES

Hiroyuki Mano, National Cancer Center, Tokyo, Japan (JCA)
Elaine R. Mardis, Nationwide Children's Hospital, Columbus, Ohio (AACR)

SEPTEMBER 11, 2021, 9:00 AM-10:45 AM, JST (SEPTEMBER 10, 2021, 8:00 PM-9:45 PM, EDT)

This website uses cookies to ensure the best user experience. Learn More

ACCEPT

Takayuki Yoshino, National Cancer Center Hospital East, Chiba, Japan
Dennis Lo, Chinese University of Hong Kong, Hong Kong,China-Hong Kong
Lillian L. Siu, UHN Princess Margaret Cancer Centre, Ontario, Toronto, Canada
Wei Guo, University of Pennsylvania, Philadelphia, Pennsylvania

15-MINUTE BREAK

SEPTEMBER 11, 2021, 11:00 AM-12:30 PM, JST (SEPTEMBER 10, 2021, 10:00 PM-11:30 PM, EDT)

SESSION: SOMATIC MUTATIONS IN PRECANCEROUS TISSUE

Seishi Ogawa, Kyoto University, Kyoto, Japan
Kenichi Yoshida, Wellcome Sanger Institute, Saffron Walden, United Kingdom
Benjamin L. Ebert, Dana-Farber Cancer Institute, Boston, Massachusetts

SESSION: NOVEL PLATFORMS FOR PRECISION MEDICINE

Shohei Koyama, National Cancer Center, Tokyo, Japan
Shinichi Yachida, Osaka University, Osaka, Japan
Jeffrey W. Tyner, OHSU Knight Cancer Institute, Portland, Oregon

15-MINUTE BREAK

SEPTEMBER 11, 2021, 12:45 PM-2:15 PM, JST (SEPTEMBER 10, 11:45 PM-SEPTEMBER 11, 1:15 AM, EDT)

SPONSORED SESSIONS TO BE ANNOUNCED

15-MINUTE BREAK

SEPTEMBER 11, 2021, 2:30 PM-4:00 PM, JST (1:30 AM-3:00 AM, EDT)

SESSION: DRUG RESISTANCE

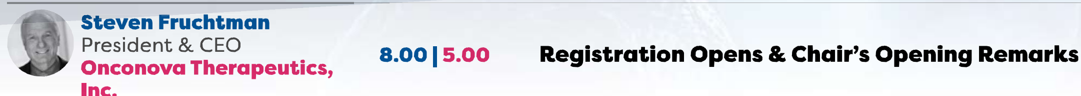
Ryohei Katayama, The Cancer Chemotherapy Center, JFCR, Tokyo, Japan
Frank McCormick, UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, California
Reuben J. Shaw, Salk Institute, La Jolla, California

This website uses cookies to ensure the best user experience. Learn More

ACCEPT

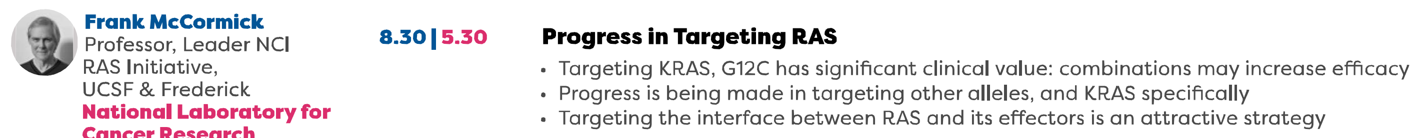
Conference Day Two
Thursday September 23, 2021

3rd Annual RAS-Targeted Drug Development
September 21-23, 2021 | 100% Online
8.00am - 5.30pm EDT | 5.00am - 2.30pm PDT



Steven Fruchtman
President & CEO
Onconova Therapeutics, Inc.

8.00 | 5.00 **Registration Opens & Chair's Opening Remarks**



Keynote Plenary Sessions

Frank McCormick
Professor, Leader NCI RAS Initiative,
UCSF & Frederick
National Laboratory for Cancer Research

8.30 | 5.30 **Progress in Targeting RAS**

- Targeting KRAS, G12C has significant clinical value: combinations may increase efficacy
- Progress is being made in targeting other alleles, and KRAS specifically
- Targeting the interface between RAS and its effectors is an attractive strategy

8.55 | 5.55 **Impact of G12C KRAS Inhibitory Drugs on the Tumor Microenvironment**

Julian Downward
Senior Group Leader &
Associate Research Director
Francis Crick Institute

- KRAS G12C inhibitors have proved effective on many G12C KRAS mutant tumor in vitro, in vivo and in clinical trials, but acquired and pre-existing resistance is a fundamental limitation to the clinical efficacy of these drugs
- Several approaches have been taken to establish how best to combine these drugs for more effective treatment of KRAS G12C mutant cancers and minimisation of resistance, but those reported to date largely delay rather than eliminate the development of resistance
- The response of KRAS G12C mutant lung tumor with these inhibitors profoundly alters the tumor immune microenvironment, allowing ingression of various effector immune cells: studying the phenotype of these cells suggests optimal combinations between KRAS G12C inhibitors and immunotherapies and suggests approaches to preventing the acquisition of therapy resistance

Namratha Sastry
Manager, Medical Affairs
Biodesix

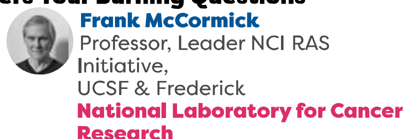
9.20 | 6.20 **Real-World Data: Importance of Monitoring KRAS Mutations in Blood**

- Why is it important to monitor KRAS in blood with ddPCR?
- Analytical, clinical, and real-world data for KRAS G12C mutations in NSCLC
- Bringing blood-based diagnostics from initial discovery to the clinic

9.45 | 6.45 **Live Panel Q&A – Ask Speakers Your Burning Questions**

Moderator:

Steven Fruchtman
President & CEO
Onconova Therapeutics, Inc.



Frank McCormick
Professor, Leader NCI RAS Initiative,
UCSF & Frederick
National Laboratory for Cancer Research

Julian Downward
Senior Group Leader & Associate Research Director
Francis Crick Institute

Namratha Sastry
Manager, Medical Affairs
Biodesix

10.00 | 7.00 **Morning Break & Speed Networking**

Reinventing the face-to-face networking in the virtual world. We will pair you up with fellow attendees to break the ice and make new and lasting connections with other RAS experts from industry and academia

LAST CHANCE TO SECURE YOUR PLACE

WELCOME TO THE 3RD ANNUAL RAS-TARGETED DRUG DEVELOPMENT SUMMIT
WHAT'S NEW FOR 2021
YOUR EXPERT SPEAKERS
AGENDA AT A GLANCE
PRE-CONFERENCE FOCUS DAY
PRE-CONFERENCE WORKSHOP DAY
CONFERENCE DAY ONE
CONFERENCE DAY TWO
OUR PARTNERS
PARTNER WITH US
YOUR DIGITAL EXPERIENCE
HOW TO JOIN

REGISTER NOW

2nd AACR-KCA Joint Conference on Precision Medicine in Solid Tumors

(KST) November 11–12, 2021 / (EST) November 10–11, 2021
In-Person & Virtual

PROGRAM COMMITTEE COCHAIRS:
Dong-Wan Kim, Seoul National University Hospital, Seoul, Korea
William C. Hahn, Dana-Farber Cancer Institute, Boston, MA

PROGRAM COMMITTEE:
Peter K. Jackson, Stanford University, Stanford, CA, USA
Carla F. Kim, Boston Children's Hospital, Boston, MA, USA
Eui-Cheol Shin, Korea Advanced Institute of Science and Technology, Daejeon, Korea
Jeeyun Lee, Sungkyunkwan University School of Medicine, Suwon, Korea
Kyo Young Song, The Catholic University of Korea College of Medicine, Seoul, Korea
Tae Min Kim (Secretary General), Seoul National University College of Medicine, Seoul, Korea

Abstract Review Committee Members (KCA)
Hyo Sup Shim, Yonsei University College of Medicine
Jung Kyoon Choi, Korea Advanced Institute of Science and Technology
Sae Byeol Choi, Korea University College of Medicine
Sung Ho Moon, National Cancer Center
+ Program Committee Members (Eui-Cheol Shin, Jeeyun Lee, Kyo Young Song)

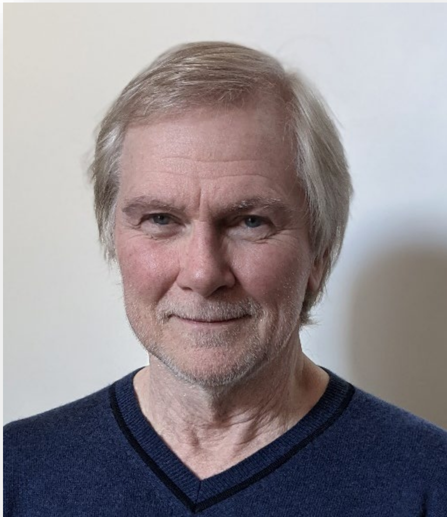
(KST) THURSDAY, NOVEMBER 11 / (EST) WEDNESDAY, NOVEMBER 10

Opening Remarks & Keynote Lecture
08:00-08:40 KST / 18:00-18:40 EST
Room A

Progress in targeting KRAS directly
Frank McCormick, UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, USA

Clinical Translational Research
Certificate of Added Qualification

BENCH-TO-BEDSIDE SEMINAR SERIES



Frank McCormick, PhD, FRS, DSc (Hon)
Professor, UCSF Helen Diller Family Comprehensive Cancer Center
David A. Wood Distinguished Professor of Tumor Biology & Cancer Research
Scientific Director, NCI RAS Initiative, Frederick, Maryland

"Successes and Failures Targeting RAS Oncogenes"

Thursday, December 16
12:00-1:00

Zoom Meeting ID: 980 5702 1267

Password: 878848

https://bcm.zoom.us/j/98057021267?pwd=cytXVjhTMHZNVlVHcEY4R0ZoVVJaQT09

ALL INTERESTED PERSONS ARE INVITED AND WELCOME
For more information, please contact
Kelly Levitt, 713-798-4267
klevitt@bcm.edu

Baylor College of Medicine

MoS10 On-site + Live

7:20～8:10　第13会場（ザ・プリンス 京都宝ヶ池　B2階　ゴールドルーム）

Asian collaboration in various cancer types and the latest RWE in Melanoma
様々ながん種におけるアジアコラボレーションおよびメラノーマの最新Real World Evidence

Chair: Naoya Yamazaki（National Cancer Center Hospital, Department of Dermatologic Oncology）
司会：山﨑　直也（国立がん研究センター中央病院 皮膚腫瘍科）

Imagine, the cancer research within Asia, by Asian for Asians
アジアのアジアによるアジアのためのがん研究

Kan Yonemori（National Cancer Center Hospital, Department of Medical Oncology）
米盛　勧（国立がん研究センター中央病院 腫瘍内科）

Therapeutic Strategy for BRAF-mutation-positive Melanoma from view point of RWE
RWEから紐解くBRAF陽性Melanomaの治療戦略

Takeo Maekawa（Jichi Medical University, Department of Dermatology）
前川　武雄（自治医科大学 皮膚科学教室）

Co-host：Novartis Pharma K.K.／ノバルティス ファーマ株式会社

February 19 (Sat) Morning Seminar／モーニングセミナー11～22

MoS11 On-site + Live

7:20～8:10　第2会場（国立京都国際会館　1階　Annex 1）

Chair: Kazuhiko Nakagawa（Kindai University）
司会：中川　和彦（近畿大学医学部 内科学腫瘍内科部門）

Carcinoma of Unknown primary: Principles of practical management
原発不明がん～「わからない」を「わかる」にかえる朝練～

Kan Yonemori（National Cancer Center Hospital）
米盛　勧（国立がん研究センター中央病院 腫瘍内科）

Co-host：Bristol-Myers Squibb K.K. / ONO PHARMACEUTICAL CO., LTD.
ブリストル・マイヤーズ スクイブ株式会社／小野薬品工業株式会社

MoS12 On-site + Live

7:20～8:10　第3会場（国立京都国際会館　1階　Annex 2）

Tumor-Agnostic Approach for NTRK Fusion Gene-Positive Solid Tumors
NTRK融合遺伝子陽性固形癌に対する臓器横断的治療

Chair: Noboru Yamamoto（National Cancer Center Hospital, Department of Experimental Therapeutics）
司会：山本　昇（国立がん研究センター中央病院）

Targeting NTRK-fusions as the Oncogenic Driver of Many Cancer Types: Putting Tumor Agnostic Approach into Practice

George D. Demetri（Dana-Farber Cancer Institute and Ludwing Center at Harvard Medical School, USA）

Genome Medicine and Tumor-agnostic Approach
ゲノム医療と臓器横断的治療

Akira Kawai（National Cancer Center Hospital, Department of Musculoskeletal Oncology）
川井　章（国立がん研究センター中央病院）

Co-host：CHUGAI PHARMACEUTICAL CO., LTD.／中外製薬株式会社

MoS13　7:20〜8:10　第4会場（国立京都国際会館　2階　Room A）

On-site + Live

New Treatment (PRRT) for Neuroendocrine Neoplasm
神経内分泌腫瘍における新規治療(PRRT)の導入について

Chair: Junji Furuse（Department of Medical Oncology, Kyorin University Faculty of Medicine）
司会：古瀬　純司（杏林大学医学部 腫瘍内科学）

How will PRRT change NEN practice? - Focusing on pancreatic neuroendocrine
PRRTはNEN診療をどう変えていくか？　－膵神経内分泌腫瘍を中心に－

Susumu Hijioka（Department of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital）
肱岡　範（国立がん研究センター中央病院 肝胆膵内科）

Therapeutic Strategies for Neuroendocrine Tumors of the Gastrointestinal Tract
消化管神経内分泌腫瘍に対する治療戦略

Hidekazu Hirano（Department of Gastrointestinal Medical Oncology, National Cancer Center Hospital）
平野　秀和（国立がん研究センター中央病院 消化管内科）

Key points for implementing PRRT - Efforts required for radiopharmaceuticals
PRRTを導入・実施するためのポイント　－放射性医薬品ならではの取り組み－

Yasutake Ishikawa（Department of Radiological Technology, National Cancer Center Hospital）
石川　泰丈（国立がん研究センター中央病院 放射線技術部）

Co-host：FUJIFILM Toyama Chemical Co., Ltd. / Novartis Pharma K.K.
富士フイルム富山化学株式会社／ノバルティス ファーマ株式会社

MoS14　7:20〜8:10　第5会場（国立京都国際会館　2階　Room B-1）

On-site + Live

Chair: Motoko Yamaguchi（Department of Hematological Malignancies, Mie University Graduate School of Medicine）
司会：山口　素子（三重大学大学院医学系研究科 先進血液腫瘍学講座）

Treatment of peripheral T-cell Lymphoma in the era of CD30 antibody ADC
CD30抗体ADC時代の末梢T細胞リンパ腫の治療

Ritsuro Suzuki（Department of Oncology and Hematology, Shimane University Hospital）
鈴木　律朗（島根大学医学部 血液・腫瘍内科学）

Co-host：Takeda Pharmaceutical Company Limited／武田薬品工業株式会社

MoS15　7:20〜8:10　第6会場（国立京都国際会館　1階　Room D）

On-site + Live

Chair: Ayumu Hosokawa（Department of Clinical Oncology, University of Miyazaki Hospital）
司会：細川　歩（宮崎大学医学部附属病院 臨床腫瘍科）

Chemotherapy and supportive care for advanced gastrointestinal cancer
進行消化器癌における化学療法と支持療法

Yoshito Komatsu（Division of Cancer Chemotherapy and CancerBoard, Hokkaido University Hospital Cancer Center）
小松　嘉人（北海道大学病院 腫瘍センター 化学療法部・CancerBoard部）

Co-host：Nippon Zoki Pharmaceutical Co., Ltd.／日本臓器製薬

MoS16 On-site + Live 7:20～8:10 第7会場（国立京都国際会館　1階　Room E）

Chair: Akito Hata（Division of Thoracic Oncology, Kobe Minimally Invasive Cancer Center）
司会：秦　　明登（神戸低侵襲がん医療センター 呼吸器腫瘍内科）

Elucidation of intra-individual heterogeneity of EGFR+ NSCLC and perspectives for drug therapy
EGFR肺癌の個体内heterogeneityの解明と薬物療法の展望

Kei Morikawa（Division of Respiratory Diseases, Department of Internal Medicine, St. Marianna University School of Medicine, Japan）
森川　　慶（聖マリアンナ医科大学 呼吸器内科）

Co-host：Nippon Boehringer Ingelheim Co., Ltd.／日本ベーリンガーインゲルハイム株式会社

MoS17 On-site + Live 7:20～8:10 第8会場（国立京都国際会館　1階　さくら）

Chair: Hiroji Iwata（Aichi Cancer Center Department of Breast Oncology）
司会：岩田　広治（愛知県がんセンター 乳腺科部）

Current standard of care for HER2-positive metastatic breast cancer
HER2陽性進行再発乳癌の治療戦略

Junji Tsurutani（Advanced Cancer Translational Research Institute）
鶴谷　純司（昭和大学 先端がん治療研究所）

Co-host：DAIICHI SANKYO COMPANY, LIMITED／第一三共株式会社

MoS18 On-site + Live 7:20～8:10 第9会場（国立京都国際会館　1階　スワン）

Chair: Eiji Oki（Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University）
司会：沖　　英次（九州大学大学院医学研究院 消化器・総合外科）

All about MSI-high colorectal cancer - an early bird catches the worm -
MSI-high大腸がんのすべて　～早起きは三文の徳～

Hiroya Taniguchi（Department of Clinical Oncology, Aichi Cancer Center Hospital）
谷口　浩也（愛知県がんセンター 薬物療法部）

Co-host：Bristol-Myers Squibb K.K. / ONO PHARMACEUTICAL CO., LTD.
ブリストル・マイヤーズ スクイブ株式会社／小野薬品工業株式会社

On-site + Live：On-site + Live Streaming　On-demand：On-demand Streaming　: Simultaneous Interpretation (E&J)

FRANK MCCORMICK PRESENTATIONS

MoS19 On-site + Live

7:20～8:10　第10会場（ザ・プリンス 京都宝ヶ池　1階　ロイヤルルーム）

Chair: Kazuhisa Takahashi（Department of Respiratory Medicine Juntendo University Graduate School of Medicine）

司会：髙橋　和久（順天堂大学大学院医学研究科 呼吸器内科学）

Treatment strategy for non-small cell lung cancer in the era of immunotherapy
免疫療法時代の非小細胞肺がん治療戦略
～PD-L1だけじゃない！患者背景に応じた個別化のエッセンス～

Yukari Tsubata（Department of Internal Medicine, Division of Medical Oncology & Respiratory Medicine, Shimane University Faculty of Medicine）

津端由佳里（島根大学医学部附属病院 呼吸器・化学療法内科）

Treatment of Small-Cell Lung Cancer

Roy S. Herbst（Yale Cancer Center and Smilow Cancer Hospital, USA）

Co-host：CHUGAI PHARMACEUTICAL CO., LTD.／中外製薬株式会社

MoS20 On-site + Live

7:20～8:10　第11会場（ザ・プリンス 京都宝ヶ池　B2階　プリンスホール1）

Chair: Hiroyuki Shibata（Department of Clinical Oncology, Graduate School of Medicine, Akita University）

司会：柴田　浩行（秋田大学大学院 医学系研究科 臨床腫瘍学講座）

What lies beyond cure of cancer - Meaning of ambulatory for cancer patients-
がんを治す、その先にあるもの －歩くことは生きること－

Hirotaka Kawano（Department of Orthopaedic Surgery, Teikyo University School of Medicine）

河野　博隆（帝京大学医学部 整形外科学講座）

Co-host：Pfizer Japan Inc.／ファイザー株式会社

MoS21 On-site + Live

7:20～8:10　第12会場（ザ・プリンス 京都宝ヶ池　B2階　プリンスホール2）

Chair: Tetsuya Mitsudomi（Kindai University Faculty of Medicine）

司会：光冨　徹哉（近畿大学医学部外科学教室 呼吸器外科部門）

Progress in treating RAS driven cancers

Frank McCormick（University of California, San Francisco, USA）

Co-host：Amgen K.K.／アムジェン株式会社

MoS22 On-site + Live / On-demand

7:20～8:10　第13会場（ザ・プリンス 京都宝ヶ池　B2階　ゴールドルーム）

Chair: Akifumi Takaori（Department of Hematology / Oncology, Graduate School of Medicine, Kyoto University）

司会：髙折　晃史（京都大学大学院医学研究科 血液・腫瘍内科学）

Cutting edge of leukemia precision medicine and molecular targeted therapy
白血病プレシジョンメディシンと分子標的療法の最先端

Yosuke Minami（Department of Hematology, National Cancer Center Hospital East）

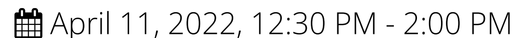
南　　陽介（国立がん研究センター東病院 血液腫瘍科）

Co-host：CMIC Co., Ltd./ CMIC ShiftZero K.K.／シミック株式会社／シミック・シフトゼロ株式会社

AACR American Association for Cancer Research ANNUAL MEETING 2022 New Orleans
JOIN US APRIL 8-13!

SESSION **SY20 - KRAS Anniversary Session: Novel Mechanisms for Targeting KRAS**

Add to My Itinerary


April 11, 2022, 12:30 PM - 2:00 PM

Hall B-C, Convention Center

DESCRIPTION

This year marks the 40th anniversary of the discovery of mutationally activated *RAS* genes in human cancer. In 1982, three laboratories, those of Weinberg, Wigler and Barbacid, working independently, cloned the first oncogene. This discovery not only stimulated intensive research efforts in understanding the protein structure of RAS and its role in cancer progression, but also laid the foundation for other areas of scientific discoveries. The origins of cancer could now be traced to a molecular level, paving the way for the discovery of genes mutated in cancer that now number in the thousands.Subsequent efforts led to the identification of specific *RAS* genes. One in particular, the Kirsten rat sarcoma viral oncogene homolog, or *KRAS* was later identified to be one of the most prevalent oncogenes in cancer. Found to be mutated in approximately 25% of all human cancers, KRAS has been the target of drug development since its discovery. For decades, scientists have faced challenges in directly targeting the protein. Once KRAS is activated, its exceptionally strong binding affinity for GTP render the protein constitutively active as this nucleotide is difficult to displace. However, recently scientists have made promising discoveries that have transformed our view of KRAS from one once considered undruggable into a druggable protein. This session will discuss the current state of preclinical and clinical research into the KRAS oncogene and existing challenges. It will cover mechanisms of resistance to KRAS inhibitors and the comparative merits of KRAS inhibition using sotorasib, as an example, versus KRAS degradation using a genetic model of KRAS ablation. Different mechanisms for oncogenic activation for KRAS will be presented, including how these mutations affect dynamics of signaling and engagement of effectors and lead to new therapeutic approaches. Also covered in this session will be the discovery of a druggable pocket on KRAS G12C that ultimately led to the field's first approved KRAS G12C inhibitor. Ongoing investigations focusing on drugging other oncogenic mutants of KRAS, including both reversible ligands for the most frequent KRAS G12D allele as well as covalent drugs targeting less common variants, will also be discussed. Finally, the session will conclude with clinical development strategies that have been investigated for targeting KRAS and the challenges that remain.

Session Type
Major Symposium

Track(s)
Molecular/Cellular Biology and Genetics,Drug Development

CME Credits Available
1.5

7 Presentations

12:30 PM - 12:31 PM

- Chairperson
Patricia M. LoRusso. Yale School of Medicine, New Haven, CT

Add to My Itinerary

12:30 PM - 12:34 PM

- Introduction
Patricia M. LoRusso. Yale University, Hamden, CT

Add to My Itinerary

12:34 PM - 12:54 PM

- Targeting KRAS: Light at the end of the tunnel
Mariano Barbacid. Spanish National Cancer Research Center (CNIO), Madrid, Spain

Add to My Itinerary

12:54 PM - 1:11 PM

- How Ras proteins cause cancer
Frank McCormick. UCSF Helen Diller Family Comprehensive Cancer Ctr., San Francisco, CA

Add to My Itinerary