Yale School of Medicine

Yale@START
Closing Symposium

Thursday August 2, 2018
Jane Ellen Hope Building H-110
315 Cedar Street
The Yale@START program is grateful for the generous support of Robert J. Alpern, M.D., Dean of Yale School of Medicine; Alan Graham, M.D., Yale School of Medicine, Class of 1971; and Paul Rothman, M.D., Yale School of Medicine, Class of 1984.

We would also like to thank the Yale@START mentors for providing our students with outstanding research experiences.
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OVERVIEW OF THE Yale@START PROGRAM

Yale@START is a program to provide incoming M.D. and M.D-Ph.D. students with a mentored research rotation and associated educational and social activities during the summer before the first year.

Dr. Laura Ment, Associate Dean of Admissions & Financial Aid, YSM Student Affairs, Professor Pediatrics & Neurology – 2018 Yale@START faculty leader

Dr. Peter Aronson, CNH Long Professor of Internal Medicine and Physiology – first 2012 Yale@START faculty leader

Yale@START STEERING COMMITTEE:

Dr. Richard Belitsky, Harold W. Jockers Associate Professor of Psychiatry; YSM Deputy Dean for Education

Dr. Nancy Angoff, Associate Professor of Medicine (General Medicine); YSM Associate Dean for Student Affairs

Dr. John N. Forrest, Jr., Professor Internal Medicine; Director, YSM Office of Student Research

Dr. Barbara Kazmierczak, Associate Professor of Medicine (Infectious Diseases); Director, M.D.-Ph.D. Program, Yale University

Dr. Michael Schwartz, Associate Professor of Neuroscience; Director, Medical Studies in Neurobiology; Associate Dean for Curriculum, YSM

2018 Yale@START Student Mentors: June Criscione and Anna Lynn
Program Coordinator: Reagin Carney
## Presentation Schedule

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<td>Michael Flores, Francis Lee, “Analysis of the anti-cancer effects of ERK, CCL20, IL8 inhibition for osteolytic breast cancer,” Orthopaedics and Rehabilitation</td>
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<td>Shanin Chowdhury, Silvia Vilarinho, “Liver Disease Caused by Mutations in a Small GTPase,” Digestive Disease</td>
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<td>11:30 a.m.</td>
<td>Dennis Caruana, David Rimm, “Quantitative determination of estrogen receptor (ER) dynamic range in low-ER positive breast carcinoma,” Oncology</td>
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11:45 a.m.  Kedous Mekbib, Dr. Marcella Nunez-Smith, "Sociodemographic Differences in Physician-Patient Nonverbal Communication and Effect on Patient Adherence,” Internal Medicine

12:00-12:45 p.m.  Lunch

12:45-1:30 p.m.  Dr. David Hafler, William S. and Lois Stiles Edgerly Professor of Neurology and Professor of Immunobiology

1:30 p.m.  Zeyu Tang, Dr. Elizabeth Jonas, “Roles of Familial Parkinson’s Disease Gene DJ-1: Immunofluorescence Imaging of TH+ Nigrostriatal Neurons in a DJ-1 -/- Mouse Model; ATP Synthase Beta-subunit Transport Rate in DJ-1 Over-expressed Cell Line,” Endocrinology

1:45 p.m.  Amar Sheth, Dr. Flora Vaccarino, “A Review of Developmental Toxicity and Somatic Mosaicism,” Child Study Center

2:00 p.m.  Kevin Wang, Dr. Kristopher Kahle, “Role of TRIM71 in Human Congenital Hydrocephalus,” Neurosurgery

2:15 p.m.  Nicholas Diab, Dr. Aaron Ring, “Directed evolution of environmentally biased cytokines,” Immunobiology

2:30 p.m.  Closing reception
Temporally precise characterization of principle neuron activity in the basolateral amygdala during appetitive learning

Hannah Batchelor, Richard Crouse, Steven Pittenger, Ph.D., Marina Picciotto, Ph.D.

**Problem:** Learning associations between environmental cues and their outcomes allows animals to perform behaviors that promote positive events (like getting food) and avoid negative ones (like getting injured). Excitatory principle neurons (PN) in the basolateral amygdala (BLA) respond robustly to aversive stimuli and cues that predict them. This leads to a learned response that guides adaptive behaviors when cues signal different environmental conditions. BLA PNs can respond in similar ways to appetitive (positive) stimuli and their associated cues, however, little is known about how BLA PNs behave during appetitive learning. The studies presented here are designed to elucidate how cholinergic signaling in the BLA affects PN activity, and in turn affects appetitive learning. However, informed manipulation of this circuit requires knowledge of the temporally precise BLA PN activity during our unique behavioral task.

**Hypothesis:** BLA PNs will be more active during distinct training phases and/or time points within our appetitive learning task.

**Methods:** We use fiber photometry to detect the composite activity of BLA PNs in mice during each phase of an appetitive learning task. Mice were trained to learn that an auditory cue signals the opportunity to poke their nose into a port to receive a reward. Entry into the port when the cue has not been presented is penalized by increased time until the next cue.

**Results:** Our preliminary data show that BLA PNs consistently increase activity following the cue onset and peak as the mouse successfully retrieves the reward.

**Conclusions:** These data provide us with a convincing framework for manipulating BLA PN’s to better understand this circuit’s involvement in appetitive learning.
**Problem:** Cardiovascular disease is the most common cause of death among adults in the United States. Since 1984, the annual CVD mortality rate has remained greater for women than for men, and the absolute numbers of individuals living with and dying of CVD in the United States are larger for women than for men.

**Purpose:** This retrospective study will identify and compare modifiable risk factors of ACS outcomes based on sex that may influence sex-specific treatment approaches.

**Methods:** In order to examine the totality of risk for ACS patients based on sex, we will use a database that includes information on over 500,000 anonymized patients. The primary endpoint in this study is the incidence of major adverse cardiac events (MACE), defined as a composite of cardiovascular death, non-fatal non-procedural Myocardial Infarction (MI), and non-fatal stroke. The components of MACE and any periprocedural bleeding will be studied as secondary endpoints.

We have identified three separate contributors to overall risk of MACE. We will evaluate the incremental impact of these risks by using 3 risk model adjustments in sequence. Model 1 will adjust for baseline patient demographics, Model 2 will adjust for baseline demographics + medical therapy (P2Y12 type) + interventional treatment (PCI vs no PCI), and Model 3 will adjust for baseline demographics + periprocedural complications. We will also study time trends in outcomes by sex and the impact of age on outcomes by sex using the unadjusted population and the population adjusted to the three models described above.

The results of this tiered risk analysis will enable us to hone in on the most impactful targets of therapy and their implications on sex-based outcomes.
Quantitative determination of estrogen receptor (ER) dynamic range in low-ER positive breast carcinoma

Dennis L. Caruana, David Rimm, M.D., Ph.D.

**Problem:** Although ASCO/CAP guidelines define ER positivity as 1% cell immunoreactivity in the presence of normal epithelial elements and external controls, there is currently no universally-accepted, quantitative method for determining ER status in breast carcinoma.

**Hypothesis:** Quantitatively comparing the dynamic range of ER expression between cancerous tissue and normal ducts in low-ER+ breast carcinoma may provide a valuable internal control in the determination of ER status.

**Methods:** ER expression was quantitated using computer-assisted image analysis software in archival, low-ER+ breast carcinoma tissue slides stained between 2010 and 2017 with either SP1 (32 cases) or 1D5 (2).

**Results:** At this stage—which is limited by, among other factors, the low quantity of cancerous tissue regions and normal ducts for which ER has so far been quantitated—there is considerable similarity between the dynamic range of ER between cancerous tissue and that of normal ducts in low-ER+ breast carcinoma. However, the interquartile range for ER expression is notably different between cancerous tissue and normal ducts, which may warrant further exploration.

**Conclusions:** Continued comparison of ER dynamic range between low-ER+ cancerous tissue and normal ducts is necessary to determine the utility of ER dynamic range as an internal control in the determination of ER status. Further, if such utility is demonstrated, additional studies will be required to validate that quantitative determination of dynamic range will better inform ER status assignment in routine practice.
Liver Disease Caused by Mutations in a Small GTPase

Shanin Chowdhury, Silvia Vilarinho, M.D., Ph.D.

**Problem:** Deficiency in a small GTPase causes nodular regenerative hyperplasia of the liver both in mice and humans.

**Hypothesis:** We posit that there are liver cell populations that express this small GTPase and in the absence of its expression, a homeostatic cell circuit is disrupted leading to liver pathology.

**Methods:** Using a mouse model, livers were perfused and non-parenchymal cells were isolated. Western blot was performed to assess protein expression across different cell populations. Flow cytometry and liver histology were used for further characterization of underlying liver pathology.

**Results:** Our data shows that resident hepatic macrophages are the sole expressing liver cells of this small GTPase and they appear to modulate expression of CD34, a capillarization marker, in liver sinusoidal endothelial cells.

**Conclusion:** Collectively, our findings suggest that resident liver macrophages that lack this small GTPase are deregulated and contribute to capillarization of liver sinusoidal endothelial cells, subsequent nodular regenerative hyperplasia of the liver and portal hypertension. Additional experiments are ongoing to determine the factors that disrupt this macrophage-endothelial cell circuit in the diseased liver.
Directed evolution of environmentally biased cytokines
Nicholas Diab, Aaron Ring, M.D. Ph.D.

**Problem:** Cytokines engage receptors on diverse cell types, making it difficult to employ cytokines as targeted therapies against restricted cell populations. Cytokines have unlocked therapeutic potential that could be tapped by biasing cytokine activity towards certain cells in an environmentally sensitive manner.

**Hypothesis:** We hypothesized that cytokines could be engineered to sense properties of their environment.

**Methods:** We used directed evolution to engineer environmentally biased cytokines. We displayed cytokines of interest on the surface of yeast, and used purified cytokine receptor to select environmentally sensitive variants.

**Results:** We found that it was possible to engineer environmentally biased cytokines.

**Conclusion:** Continuing studies are needed to evaluate the clinical potential of environmentally sensitive cytokines. Current data demonstrate that it is possible to engineer cytokines that sense environmental cues. Future studies will continue to explore the mechanisms whereby cytokines can be tuned to sense properties of the signaling environment. Additionally, using animal models of disease to evaluate the clinical potential of biased cytokines is an important next step in this work.
Problem: Temporal lobe seizures with impaired conscious awareness greatly impair quality of life and sometimes cannot be stopped by medications, surgery, or responsive neurostimulation. Consequences include risk of motor vehicle accidents, drowning, poor (work/school) performance, social stigmatization, and, rarely, death due to post-seizure compromised breathing.

Hypothesis: Preclinical studies in our lab have shown that stimulation of the intralaminar central lateral nucleus, a region of the thalamus that modulates arousal, can improve electrographic and behavioral markers of arousal during and after temporal lobe seizures. Our goal is to reverse the adverse effects of temporal lobe seizures on conscious arousal to improve quality of life for patients suffering refractory epilepsy.

Methods: We will use the Activa RC+S neurostimulator (Medtronic, Inc.) to design and implement a novel treatment that combines conventional responsive neurostimulation with deep brain stimulation of the central lateral thalamus, delivering the latter treatment if real-time sensing reveals that focal responsive neurostimulation fails to abort the seizure.

Results: We are collaborating with the Mayo Clinic group and Medtronic to adapt the (hardware/software) capabilities of the RC+S for our proposed treatment. Once our device is granted an investigational exemption from the FDA, a small, six patient feasibility-trial will commence.

Conclusions: We have prepared a submission for an Investigational Device Exemption from the FDA, a critical first step in what will eventually be a translational clinical trial.
Analysis of the anti-cancer effects of ERK, CCL20, and IL8 inhibition for osteolytic breast cancer

Michael Flores, Francis Lee, M.D., Ph.D., FAAOS

Problem: Bone metastases have been associated with inflammation. Screening experiments from a previous study revealed ERK1/2 as one of the most highly activated kinases in aggressive osteolytic breast cancer (OBC) cell lines. Species-specific Q-PCR screening results of the same study identified that the expressions of ERK pathway chemokines CCL20 and IL8 were increased in these cell lines compared to control cells.

Hypothesis: Inhibiting ERK1/2, CCL20, and IL8 will lead to anti-cancer effects in OBC metastases both in vitro and in vivo.

Methods: A murine model was used to assess the viability of treating OBC through ERK inhibition. Four metastatic bone cancer cells lines were studied in infected mice by treatment with Trametinib, an ERK inhibitor currently in use to treat melanoma. Tumor growth was measured. Screening of ERK pathway inflammatory cytokines was done in vitro. Both CCL20 and IL8 knock out osteolytic breast cancer cell lines were co-cultured with osteoblasts in order to measure osteolytic inhibition. Furthermore, human osteolytic breast cancer samples were analyzed using immunohistochemistry in order to identify the presence of CCL20, IL8, and other inflammatory cytokines secreted from breast cancer cells which trigger inflammatory osteoblasts.

Results: In vivo ERK inhibition effectively decreased tumor growth in the osteolytic breast cancer cell lines, but not in osteoblastic cell lines. Co-culturing revealed that targeting CCL20 and IL8 did inhibit cancer growth and stimulated bone growth. Both CCL20 and IL8 were identified in the human osteolytic breast cancer samples.

Conclusions: Inhibiting ERK1/2 was shown to exhibit anti-cancer effects in osteolytic breast cancer metastases. Furthermore, CCL20 and IL8 were shown to be effective targets for inhibiting the ERK signaling pathway. These cumulative findings are promising evidence for the future possibility of osteolytic breast cancer treatment through CCL20 and IL8 inhibition.
Separating Transport and Signaling Functions in PIT1 to Understand Mechanism of Phosphate Sensing

Bryan Ho, Clemens Bergwitz, M.D.

**Problem:** Disorders in phosphate homeostasis cause symptoms including rickets and vascular calcifications. However, phosphate sensing is poorly understood in mammals. The inorganic phosphate transporter PIT1 possesses a phosphate transport and a MAPK signaling function and thus may meet the criteria for a phosphate sensor.

**Hypothesis:** PIT1’s phosphate signaling function is independent of its phosphate transport function. Adenoviral transduction permits us to test mutant PIT1 transporters in primary bone and muscle cell cultures obtained from PIT1/2 double-knockout (DKO) mice.

**Methods:** PIT1’s phosphate transport function was knock-downed by site-directed mutagenesis and introduced into adenovirus by Gateway Cloning. We generated V5 fusion vectors permitting transduction of MAPK pathway components. Rat UMR106 osteocyte-like cells and mouse C2C12 muscle cells are used to study regulation of FGF23 transcription and muscle ATP synthesis downstream of PIT1, respectively.

**Results:** Adenoviral infection does not affect phosphorylation of ERK1/2 after phosphate stimulation in UMR106 cells, and wild-type and transport-deficient PIT1 overexpression enhanced ERK1/2 phosphorylation to the same extent. Polybrene greatly enhanced adenoviral infection of C2C12 cells, which permits similar experiments in muscle cells. Adenoviral constructs were generated to permit us to determine whether PIT1 co-immunoprecipitates with MAPK pathway members.

**Conclusions:** Adenoviruses can be used as transduction agents since adenoviral infection does not interfere with MAPK pathway activation by phosphate stimulation. Our preliminary findings in UMR106 and C2C12 cells suggest that our adenoviral constructs permit us to test PIT1’s phosphate signaling function in primary cell cultures obtained from our PIT1/2 DKO mice to further elucidate the mechanism of phosphate sensing.
Sociodemographic Differences in Physician-Patient Nonverbal Communication and Effect on Patient Adherence

Kedous Mekbib, Carole Oladele, Ph.D., M.P.H., Marcella Nunez-Smith, M.D., M.H.S.

Problem: Health care disparities plague the United States with increasing research suggesting physician communication significantly influences the quality of care experienced by different sociodemographic groups. The purpose of this study was to measure differences in physician nonverbal communication with patients of varying sociodemographic groups and identify whether nonverbal communication is associated with patient adherence.

Hypothesis: We hypothesized that physician nonverbal communication is poorer for non-white patients, for patients with lower levels of education, and for patients with less income; and that poorer physician nonverbal communication results in lower rates of patient adherence.

Methods: Items from the Patient-reported Experiences of Discrimination in Care Tool (PreDict) survey were used to measure the patient-reported quality of physician nonverbal communication by asking if physicians: stayed physically distant, maintained eye contact, or were distracted when talking with patient. Patient adherence was measured by asking the patient if they were “following the discharge instructions” and “taking the medications prescribed” to them.

Results: The statistically significant $\chi^2$ results demonstrated that: lower income and non-white patients reported their physician maintained less eye contact and was more distracted when talking to them. Logistic regression models found that less eye contact was strongly associated with a reduced likelihood for the patient to follow discharge instructions while controlling for gender, insurance, education, age, and race.

Conclusions: In conclusion, this study demonstrates that lower income and non-white patients report poorer nonverbal communication and that poorer nonverbal communication is associated with poorer patient adherence; paving a promising avenue for combatting racial and socioeconomic disparities in health care.
Multidisciplinary Management of Infective Endocarditis Complicated by Ischemic Stroke

Alaa Mohamedali; Aakshi Agarwal; Kevin Sheth, M.D.; Emily Gilmore, M.D.; Stacy Chu, M.D.

Problem: Management strategies for infective endocarditis (IE) are heterogeneous and uncertainty exists as to the optimal timing and degree of multispecialty involvement.

Hypothesis: Among patients with ischemic stroke (IS) in the setting of IE, the timing and degree of neurologic specialty involvement are variable.

Methods: Consecutive records of adult patients admitted to Yale-New Haven Hospital between January 2013 and May 2018 with IE and IS are reviewed. We use descriptive statistics to characterize the study population.

Results: Among 716 patients admitted with IE (mean age 63 [SD 18], male n=450 [62.8%]), 209 had IS (29.2%) and 192 (26.8%) had neurologic consultation. Patients with IS had higher mortality (20.1% vs. 11.1%, p=0.001) and readmission rates (43.1% vs. 29.9%, p=0.001). Rates of valve surgery were similar (24.4% vs. 19.3%, p=0.13). Only 113 of 209 (54.1%) patients with IS had neurologic consultation. Among patients with IS, those with neurologic consultation more often had additional specialty services involved (cardiology 60.2% vs. 39.6%, cardiothoracic surgery 60.2% vs. 31.2%, neurosurgery 19.5% vs. 3.1%, and palliative care 15.9% vs. 5.2%, all p<0.03) and longer mean lengths of stay (25.0 days vs. 15.1 days, p=0.004). They were more often in intensive care units (21.2% vs. 4.2%, p=0.001), had higher rates of valve surgery (32.7% vs 14.6%, p=0.004), and trended towards higher mortality (24.8% vs. 14.6%, p=0.10).

Conclusions: Neurologic specialty involvement at our institution is not consistent across patients with IE complicated by IS, and appears to be reserved for generally sicker patients. Further research will uncover the patient and disease level variables associated with specialty involvement and outcomes.
A Review of Developmental Toxicity and Somatic Mosaicism

Amar Sheth, Flora Vaccarino, M.D.

Problem: Exposure to toxins during prenatal development, such as ingestion of alcohol and heavy metals, contributes to the infant morbidities, developmental delays, and mortality in the world. Somatic mosaicism refers to the occurrence of two genetically distinct population of cells caused by de novo mutational events during postzygotic development.

Hypothesis: By assessing random somatic mutagenesis in fetal alcohol syndrome disorders (FASD) and heavy metal poisoning, we can better understand how somatic mosaicism facilitates the effects of phenotypic aberrations caused by prenatal exposures to toxins and teratogens.

Methods: We conducted a review of the current literature on effects of ethanol exposure, hypoxia, and heavy metals on neural development. We are working to improve the protocol for growing clonal neurosphere cultures from single human neural progenitor cells (hNPCs) using fluorescence activated cell sorting (FACS) and mechanical separation of neurospheres.

Results: Developmental markers expressed in our primary neurosphere cultures confirm that in vitro neurospheres successfully imitate early development of ventricular zone. Experiments in murine models have shown that aldehyde-related genotoxicity restricted to a pool of hematopoietic stem and progenitor cells results in cell cycle aberrations and Fanconi anaemia. Moors et al. also found mercury containing neurotoxicants aberrate phenotypic development of primary neurospheres in vivo. Together, these findings point to a genotoxic etiology of developmental aberrations in FASD.

Conclusions: Our success rate of growing clonal populations from single neurospheres was 6%. We must continue to successfully clone neurosphere populations from only single cells after exposure to ethanol to ensure we are able to detect low frequency somatic mutational events.
Roles of Familial Parkinson’s Disease Gene DJ-1: Immunofluorescence Imaging of TH+ Nigrostriatal Neurons in a DJ-1 -/- Mouse Model; ATP Synthase Beta-subunit Transport Rate in DJ-1 Over-expressed Cell Line

Zeyu Tang, Elizabeth Jonas, M.D.

Special Acknowledgements to Rongmin Chen, Pawel Licznerski, Shobana Subramanian, Harshvardhan Rolyan

**Problem:** Parkinson’s disease (PD) is a progressive neurodegenerative disorder that includes motor and non-motor symptoms. The specific causal mechanisms behind PD are not well understood. We currently study familial PD gene DJ-1, which affects 1% of familial PD, through human mutations and a DJ-1 -/- mouse model. There are two topics I intend to investigate.

First, loss of dopaminergic neurons is a hallmark of PD progression in humans, but it is uncertain whether there is a loss of nigrostriatal dopaminergic neurons in the DJ-1 -/- mice.

Second, we surprisingly found that the ATP synthase complex stoichiometry is disturbed in DJ-1 -/- neurons and patient cells; this raises the question of whether DJ-1 is required for the translocation of the beta subunit of the ATP synthase in mitochondria to improve ATP synthase function, ATP production, and cell growth.

**Hypothesis:** There is decreased density of dopaminergic neurons and axonal projections in the striatum of DJ-1 -/- mice. DJ-1 will increase the localization of beta-subunit into mitochondria.

**Methods:** Striatal sections were obtained from age- and gender-matched WT or DJ-1 -/- mice. Immunofluorescence staining used antibodies for tyrosine hydroxylase (TH), an enzyme necessary for dopamine synthesis; the nuclear stain DAPI was used as a positive control. HEK-293 cells over-expressing FLAG-tagged beta-subunit were incubated with DJ-1 or a green fluorescent protein control; the relative presence of beta-subunit in cytosol or mitochondria was visualized with western blot.
Results: The TH stain could not be successfully visualized in WT and DJ-1 -/- mice, while the DAPI stain could be visualized. These results indicate poor antibody penetration into neurons and suggest the staining protocol requires improvement.

Conclusions: No conclusions regarding dopaminergic neuron density could be drawn. Future work is needed to optimize this protocol, specifically by exploring antibody penetration through thick sections and by determining the correct times of incubation of, and concentration of, primary anti-TH antibody.
Role of TRIM71 in Human Congenital Hydrocephalus

Kevin Wang, Kristopher Kahle, M.D., Ph.D.

Problem: Congenital hydrocephalus is classically defined as the primary accumulation of cerebrospinal fluid causing an increase in the size of the brain’s ventricular system. Recent exome sequencing has linked de novo mutations to congenital hydrocephalus in several genes, one of which is TRIM71, a key regulator of stem cell growth. However, the mechanism by which mutations in TRIM71 cause hydrocephalus is unknown.

Hypothesis: Human mutations in TRIM71 cause congenital hydrocephalus by disrupting the balance between neural progenitor cell proliferation and differentiation in embryonic development, resulting in decreased neurogenesis and secondary dilatation of the cerebral ventricles.

Methods: CRISPR-Cas9 was used to generate a mouse with a knocked-in human TRIM71 hydrocephalus mutation (R608H). Cross-linking immunoprecipitation and quantitative PCR (CLIP-qPCR) was used to assess binding of the TRIM71 mutants to known RNA binding partners p21 and EGR1.

Results: In contrast to the heterozygous form, the homozygous TRIM71 R608H mutation was embryonic lethal. TRIM71 R608H and R796H disrupt the protein’s binding to target mRNAs p21 and EGR1, critical regulators of the cell cycle and cellular growth.

Conclusions: Homozygous TRIM71 R608H mutation is embryonic lethal and disrupts binding to critical mRNA targets which regulate neural progenitor cell growth. We hypothesize that TRIM71 R608H homozygotes die from progressive hydrocephalus, which results from neural progenitor cell dysregulation. Further work is needed to elucidate these phenotypes and their mechanisms.
Generating NEK9 Mutant Cell Lines with the CRISPR/CAS9 System

Elton Zhou, Keith Choate, M.D., Ph.D.

**Problem:** Nevus Comedonicus (NC) is a severe, localized form of acne that appears in infancy as linear patches. Previous research has shown that NC is a mosaic disorder caused by somatic mutations in the NEK9 gene. To better understand the function of NEK9, cell lines containing mutant NEK9 are needed. However, attempts to create stable mutant cell lines containing these NEK9 mutations have failed.

**Hypothesis:** Using the CRISPR/Cas9 system of gene editing, stable mutant NEK9 cell lines can be created to test the role of NEK9. Additionally, we predict that other cases of NC are a result of gain-of-function mutations in the NEK9 gene.

**Methods:** Plasmids containing the Cas9 coding gene complexed with guide RNA templates were generated. Mouse fibroblast cells (3T3) were nucleofected with the plasmid and oligonucleotides containing a NEK9 mutation. Cells were sorted using fluorescence activation of green fluorescence protein.

Human embryonic kidney cells (HEK293) with NEK9 mutations were generated. Gel electrophoresis and western blotting were performed to determine the presence of NEK9 phosphorylated at Thr210.

**Results:** Based on GFP fluorescence, 3T3s were transfected at a rate of 3-28%. Through western blotting, cells containing the NEK9 mutant construct contained more NEK9 phosphorylated at Thr210 than cells transfected with wild-type NEK9.

**Conclusions:** Preliminary data shows that CRISPR may be a possible method of generating stable cell lines containing the NEK9 mutation. Further research is being conducted to confirm complete incorporation of the NEK9 mutation into 3T3 cells. More thorough tests are needed to confirm gain-of-function behavior in the NEK9 mutation.