The Fusion Oncoproteins in Childhood Cancers (FusOnC2) Consortium

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IMAT Meeting Presentation

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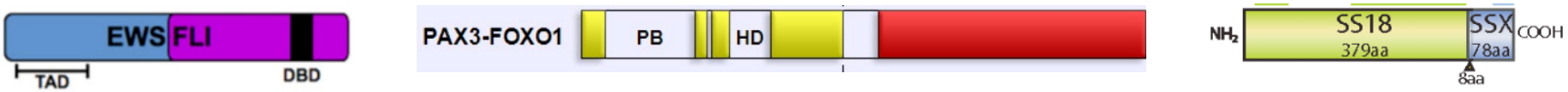
NIH NATIONAL CANCER INSTITUTE

NCI Cancer Moonshot℠ Blue Ribbon Panel Recommendations

A. Establish a network for **direct patient involvement**
B. Create a translational science network devoted to **immunotherapy**
C. Develop ways to overcome **resistance to therapy**
D. Build a national cancer **data ecosystem**
E. Intensify research on the major drivers of **childhood cancer**
F. Minimize cancer treatment's debilitating **side effects**
G. Expand use of proven **prevention and early detection** strategies
H. Mine past patient data to predict future **patient outcomes**
I. Develop a 3D **cancer atlas**
J. Develop new cancer **technologies**

Cancer Moonshot BRP Recommendation E



E. Intensify research on the major drivers of childhood cancers

Improve our understanding of fusion oncoproteins in pediatric cancer and use new preclinical models to develop inhibitors that target them.

Multidisciplinary team science approach:

- proteomics
- structural biology
- genomics/epigenomics
- medicinal chemistry
- experimental therapeutics
- cancer biology

To learn more about the molecular mechanisms of transformation driven by fusion oncoproteins and apply this knowledge to target identification, small molecule inhibition, and pre-clinical testing.

To Implement Recommendation E

The Fusion Oncoproteins in Childhood Cancers (FusOnC2) Consortium

- U54 program to support multi-disciplinary collaborative teams taking a comprehensive approach to understanding the biology of fusion oncoproteins and developing targeted therapeutics
- Focus on fusion oncoproteins found in tumors that have high risk of treatment failure and for which there has been little progress in identifying targeted agents

FY18 solicitation: RFA-CA-17-049

FY19 solicitation: RFA-CA-19-016 Open Now-

Receipt Date: December 7, 2018

U54 Components of the FusOnC2 Consortium (so far)

- **The Center for Therapeutic Targeting of EWS-oncoproteins**

  Kimberly Stegmaier and Scott Armstrong (Dana-Farber Cancer Institute)

  with Miguel Rivera, Nathanael Gray, Eric Fischer (Dana-Farber); John Bushweller (UVA); Andrew Kung (MSKCC); Alejandro Sweet-Cordero (UCSF)

- **An Integrated Approach to Analyze and Target EWS/FLI in Ewing Sarcoma**

  Stephen Lessnick (Nationwide Children's Hospital)

  with Xavier Darzacq and Robert Tjian (UC Berkeley)

- **Targeting SS18-SSX Biology in Pediatric Cancer**

  Kevin B. Jones (University of Utah)

  with Bradley Cairns and Jeffrey Yap (Utah); T. Michael Underhill and Torsten Nielsen (UBC); Scott Lowe and Marc Ladanyi (MSKCC)

- **The Center for Synovial Sarcoma Biology and Therapeutics**

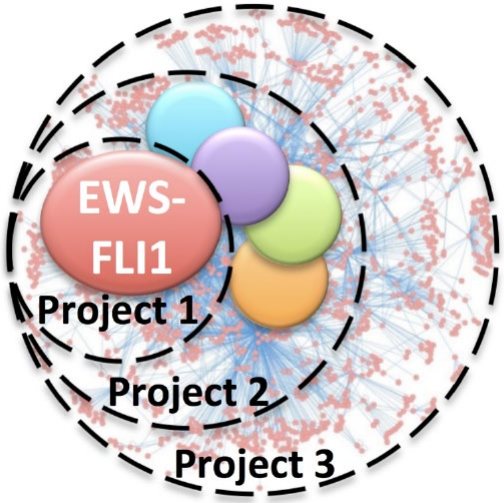
  Cigall Kadoch (Dana-Farber) and Ali Shilatifard (Northwestern)

The Center for Therapeutic Targeting of EWS-oncoproteins

Lead PIs: Kimberly Stegmaier, MD, and Scott Armstrong, MD, PhD
Dana-Farber Cancer Institute

Overall Goals

- **Specific Aim 1**: To develop novel small-molecule strategies for fusion-driven Ewing sarcoma.
- **Specific Aim 2**: To define critical chromatin associated complexes in Ewing sarcoma.
- **Specific Aim 3**: To define the key transcriptional circuitry of Ewing sarcoma.



The Center for Therapeutic Targeting of EWS-oncoproteins

Lead PIs: Kimberly Stegmaier, MD, and Scott Armstrong, MD, PhD
Dana-Farber Cancer Institute

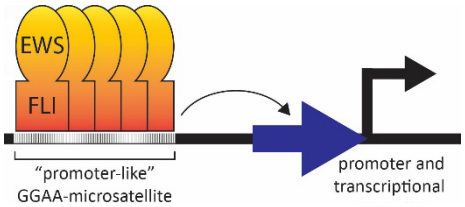
Technologies

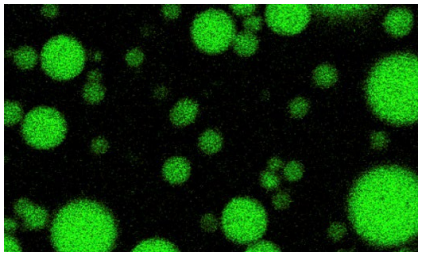
- Degrader technology – structural biology and medicinal chemistry to develop small molecule degraders of EWS-FLI
- dTAG technology – mimic small molecule-induced protein degradation
- Defining protein interaction networks – Quantitative IP-Mass spec, CRISPR domain scanning
- CRISPR-Cas9 dependency screening
- Defining core regulatory circuitry – ChIP-seq, RNA-seq, super enhancer calling, mapping transcription factor binding sites
- In vivo modeling – Ewing sarcoma PDX models, CRISPR-enabled Ewing sarcoma models, preclinical testing of novel therapeutic agents

An integrated approach to analyze and target EWS/FLI in Ewing sarcoma

Stephen Lessnick (Nationwide Children's) and Xavier Darzacq (UC Berkeley)

Focus on self-association properties of EWS/FLI in vitro and in vivo:





Goals

Project 1: To understand details of EWS/FLI binding to repetitive GGAA-microsatellites in the genome, how this binding modulates chromatin looping and other 3D changes, and how these effects modulate transcriptional output.

Project 2: To understand the dynamics of protein-protein interactions mediated by low-complexity domains within EWS/FLI and its partners using single-cell imaging.

An integrated approach to analyze and target EWS/FLI in Ewing sarcoma

Stephen Lessnick (Nationwide Children's) and Xavier Darzacq (UC Berkeley)

Approaches

Genomics: DNA localization studies (ChIP-seq, Hi-ChIP, etc.), 3D chromatin studies (HiC, single-cell ATAC-seq, etc.), RNA-seq

Biochemistry: *in vitro* analysis of protein-DNA interactions, purification of hard-to-purify proteins

Imaging: live single cell analysis of protein-DNA interactions, dynamic measurements

Technology Gaps

Computational/analytics: challenges in comparing orthogonal datasets

Biochemistry: low-complexity protein-protein interactions are weak and transient and are not retained using traditional IP type approaches

Low-complexity domains: few quantitative methods available to measure "phase-separation" and its consequences

Single-cell work: how best to compare imaging with gene-expression, epigenetics, etc.?

The Center for Synovial Sarcoma Biology and Therapeutics

Cigall Kadoch (Dana-Farber) and Ali Shilatifard (Northwestern)

PROJECT 1: SS18-SSX-MEDIATED HIJACKING OF MSWI/SNF (BAF) COMPLEXES: MECHANISMS AND FUNCTIONAL DEPENDENCIES

(1) Comprehensively define the targets of SS18-SSX-containing BAF complexes genome-wide and determine the relationship of this targeting to SS-specific gene expression, enhancer state, and chromatin topology;

(2) Determine the specific feature(s) on histones to which the SSX 78aa tail (and hence the SS18-SSX-bound BAF complex) tethers using biophysical assays; and

(3) Discover and mechanistically interrogate synovial sarcoma-specific synthetic lethal vulnerabilities that represent actionable therapeutic strategies.

PROJECT 2: UNDERSTANDING AND TARGETING WILD-TYPE SS18 FUNCTION AND STABILITY IN SYNOVIAL SARCOMA

(1) Characterize the precise molecular mechanisms as to how rescue of wildtype SS18 blocks synovial sarcoma proliferation, by defining crucial functional domains, transcriptional targets and the SS18 gene-dosage required to arrest sarcoma growth.

(2) Identify targets and develop small molecules aimed at stabilizing wildtype SS18 in synovial sarcoma cells.

The Center for Synovial Sarcoma Biology and Therapeutics

Cigall Kadoch (Dana-Farber) and Ali Shilatifard (Northwestern)

Technologies

(1) Genome-wide chromatin-centered methodologies
- ChIP-seq
- RNA-seq
- ATAC-seq
- Hi-C/Hi-ChIP

(2) Biochemistry and proteomic mass spectrometry
- protein complex-informed immunocapture/immunotagging
- proteomic mass-spectrometry and density gradient mass spectrometry
- protein expression and purification (recombinant)
- biophysical binding assays (ITC, NMR, etc)
- protein half life measurements, labeling

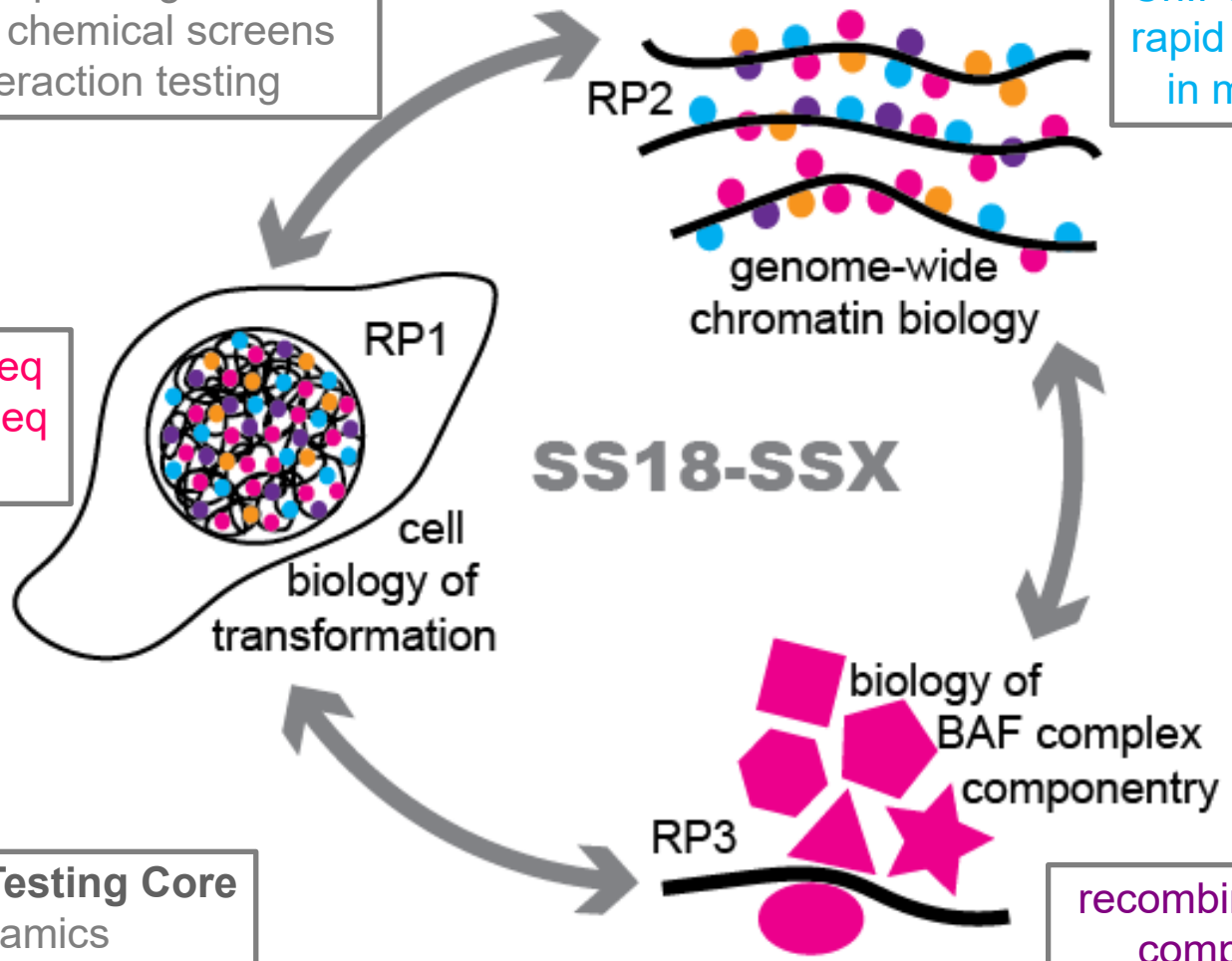
(3) Large-scale screening approaches
- shRNA and CRISPR/Cas9-based screens (synthetic lethal and fitness correlations)
- protein stabilization/degradation screens (quantification of fluorescence)

Targeting SS18-SSX Biology in Synovial Sarcomagenesis

Kevin B. Jones (Huntsman Cancer Institute, University of Utah)

Human Specimen Validation Core
tumor epigenomic profiling
high-through-put chemical screens
in situ protein interaction testing

methyl-seq
ChIPseq
rapid gene targeting
in mice

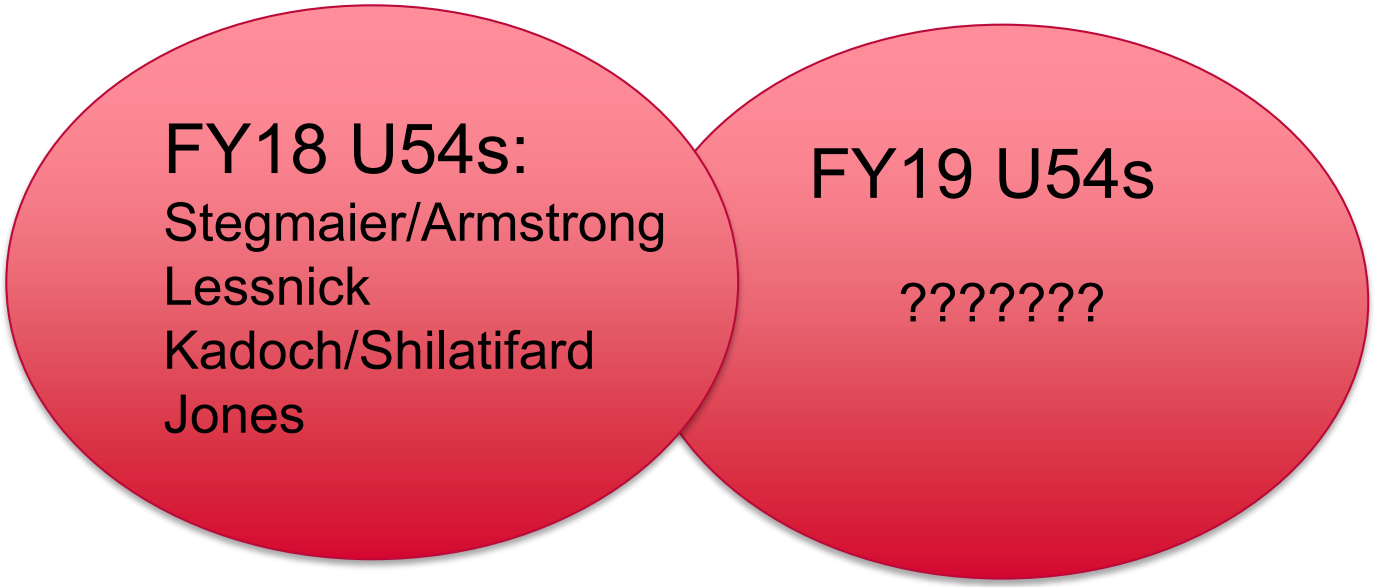


single cell RNAseq
single cell ChIPseq
lineage tracing

Pre-clinical Testing Core
pharmacodynamics
pharmacogenomics
novel PET imaging probes

recombinant protein
complex isolation
gRNA CRISPR screens
in vivo complex isolation

Collaborators Welcome!



If you are interested in working with any FusOnC2 Consortium members, either contact them directly or email me at witkinkeren@mail.nih.gov.

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