Meeting minutes: SCLC Consortium WebEx

September 6, 2018 @ 1:00pm ET

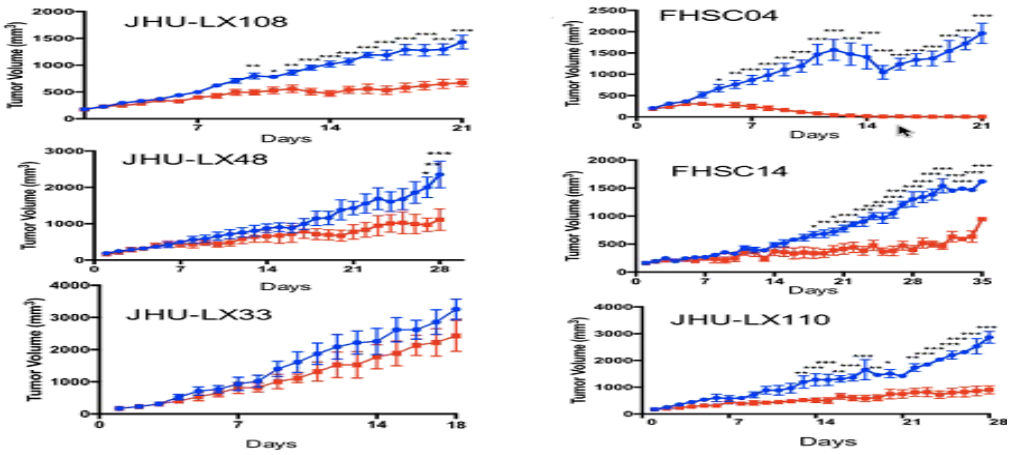
Announcements/Updates

- Three new grants funded in the UO1 network!
  - Nick Dyson and Ana Farago (MGH)-pt derived model and understanding drug resistance
  - Luigi Marchionni, Christine Hann and Phuoc Tran (Hopkins)-bioinformatic and chemical approach to credentialing molecular targets
  - Ramaswamy Govindan, Obi Griffith and Trudy Oliver (Washington University and University of Utah)-genomic and functional identifications chemo therapy resistant mechanisms

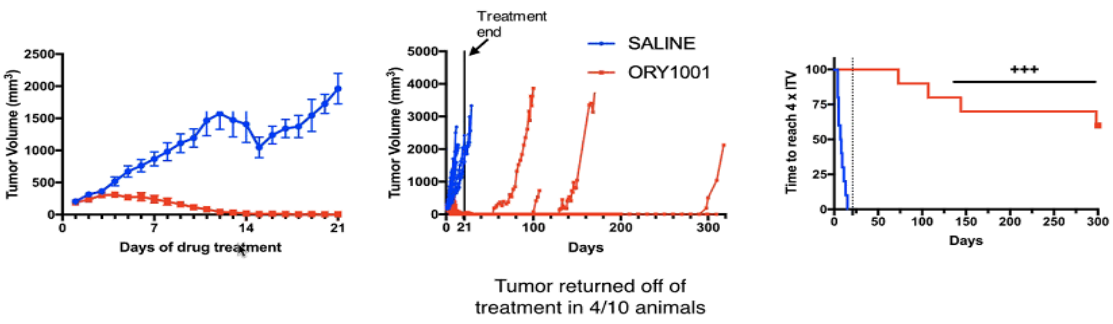
Title: Understanding Different Subsets of Small Cell Lung Cancer

Dr. David MacPherson- Fred Hutchinson Cancer Research Center

- Key regulators of transcriptional enhancers mutated in SCLC
  - KMT2D/MLL2 truncating mutations frequent in SCLC
  - Global reduction of H3K4me1 in cell lines with KMT2D mutation
- LSD1 Inhibition in SCLC
  - LSD1 demethylates histone H3K4
  - LDS1 inhibitors are being investigated clinically for SCLC
  - We hypothesize that SCLC tumor genotype, especially mutations in chromatin regulating genes, may influence response to LSD1 inhibition
  - ORY1001: a novel, highly potent and specific LSD1 inhibitor being developed by Oryzon for AML and SCLC (Maes et al, Cancer Cell 2018)
- Heterogeneity in Response to ORY1001 in SCLC PDX Models



- An Exceptional Response to ORY1001 in PDX Model in FHSC04



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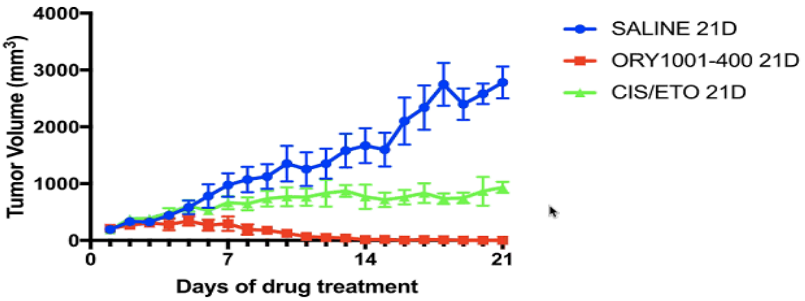
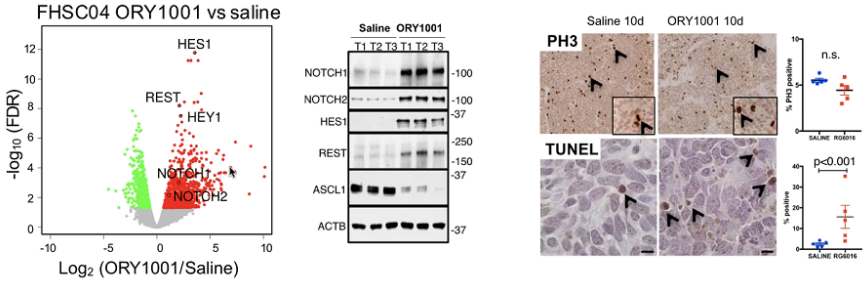


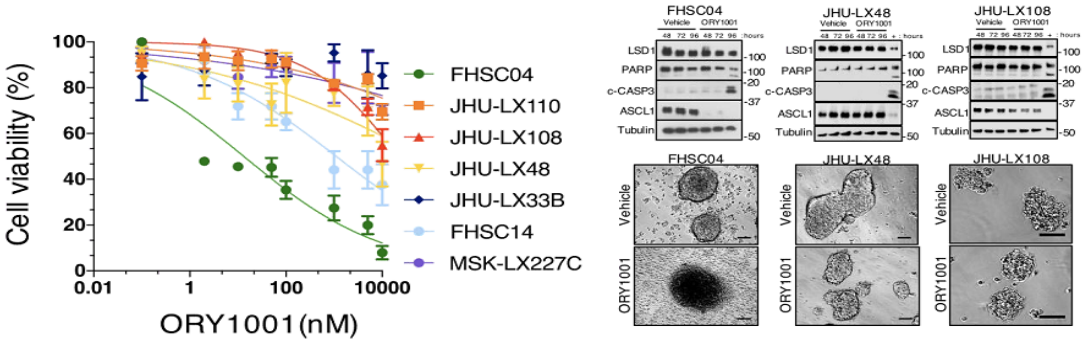
What underlies the strong response to LSD1 inhibition?
Understanding basis for this difference may help us to design SCLC clinical trials to direct therapies to right patient

- Upregulation of NOTCH pathway genes in ASCL1 Suppression in Response to ORY1001

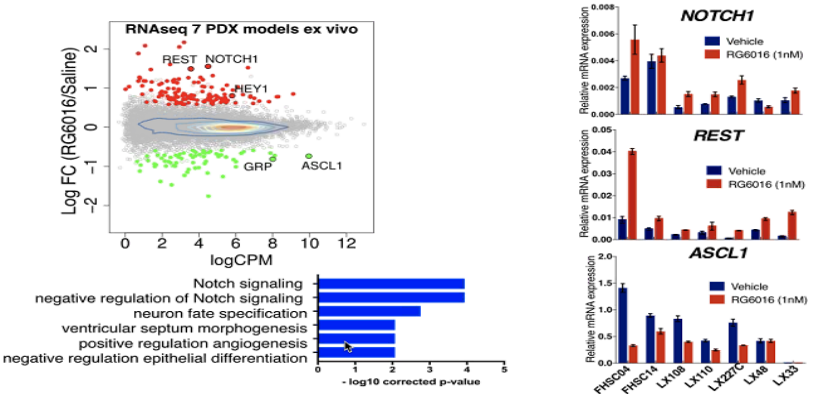


Analyses at day 10 of treatment

- Treatment of PDX tumors grown ex vivo recapitulates in vivo responses



- Ex vivo analyses of response to ORY1001 in PDX models



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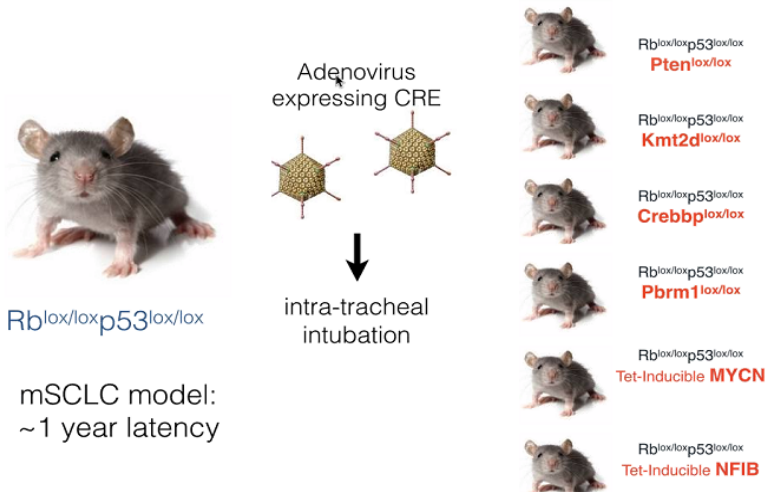
- Genetic suppression of LSD1 and ASCL1
  - LSD1 knockdown →activation of NOTCH and targets and suppression of ASCL1, cell death in sensitive models
  - ASCL1 knockdown→ loss of viability in sensitive models
- Gamma secretase inhibitors suppress NOTCH activation and effects of ORY1001
- LSD1 Inhibition in SCLC
  - Activation of NOTCH and suppression of ASCL1 occurs with ORY1001 but magnitude of these effects differ greatly-strong NOTCH activation and ASCL1 suppression associated with robust response to ORY1001
  - Need to understand underlying reasons for exceptional responses
  - Strongly responding FHSC04 model harbors inactivating mutations in CREBBP
    - Do mutations in this and/or other epigenetic regulators contribute to strong responses?
- Model Systems for SCLC
  - **PDX Models**-excellent model system for SCLC therapeutic studies, captures heterogeneity in mutations and therapeutic responses seen in patients
  - Ability to study these models ex vivo can facilitate studies of drug responses and help dissect mechanisms underlying response
  - High mutational burden/genetic heterogeneity may increase noise when trying to link a driver mutation to therapeutic vulnerability or uncover biology underlying driver function
  - **GEM Models**-genetically simplified system, ideal for uncovering how driver gene perturbation influences the biology of SCLC
  - Can also be used to link mutation to therapeutic susceptibility/resistance
  - Immunocompetent
- Sensitized mouse SCLC model to understand potential driver genes
  - Induction of small cell lung cancer by somatic inactivation of both Trp53 and Rb1 in a conditional mouse model



- Interrogating CREBBP tumor suppressor activity in SCLC
  - Is CREBBP a tumor suppressor in SCLC, and if so, how is this mediated?
    - Acetylation of histones can lead to
      - increased accessibility for transcription factors
      - binding sites for bromodomain containing proteins that include transcriptions co activators

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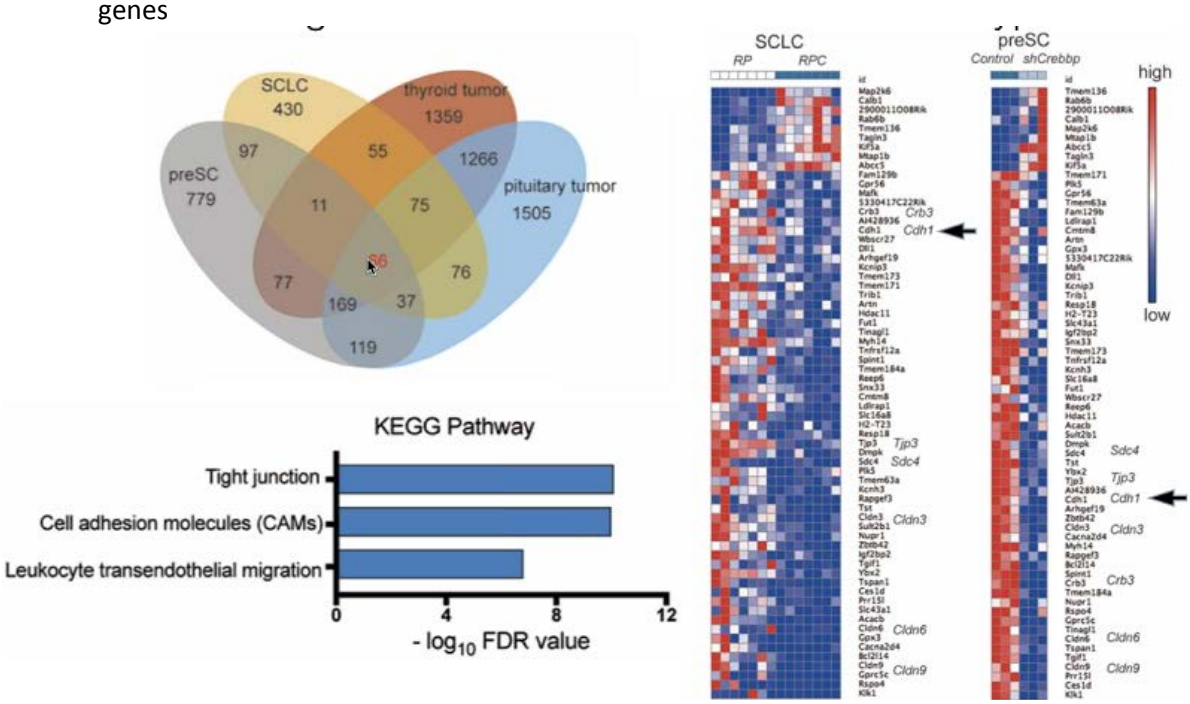
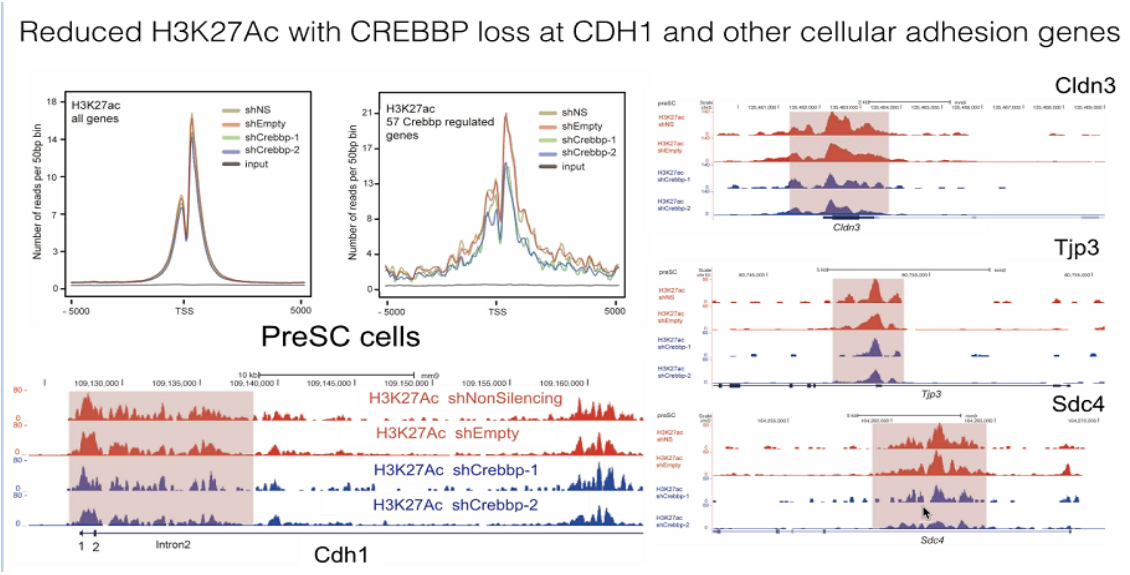
- Inactivation of Crebbp accelerates SCLC in mouse model
- Inactivation of Crebbp promotes transformation in model of early stage SCLC tumorigenesis
- Ascl-Cre-ERT2 mediated deletion of Crebbp cooperates with Rb/p53 deletion to promote pituitary and thyroid neuroendocrine tumors
- Crebbp inactivation in SCLC, pituitary carcinoma and thyroid c-cell tumors leads to changes in cell cycle related gene sets
- Cross tumor analyses to identify transcripts that are consistently CREBBP regulated across neuroendocrine tumor types
    - 66 genes commonly CREBBP regulated with strong enrichment in adhesion related genes



- CDH1-a tumor suppressor gene that regulates EMT transitions
    - CDH1 encodes E-Cadherin, a tumor suppressor mutated in gastric, breast and other cancer types
    - CDH1 expression correlated with sensitivity to cisplatin in SCLC (Stewart et al, 2017, Oncotarget)
    - In normal development of lung neuroendocrine cell bodies, PNECs transiently suppress CDH1 and increase EMT markers while undergoing cell migration
- CREBBP inactivation associated with partial suppression of CDH1 and upregulation of EMT markers, while maintaining ASCL1 expression
    - Tissues in the mouse models→decreasing in E-CADHERIN, increasing in N-CADHERIN and SLUG and no changes in ASCL1
    - Knocked down CREBBP in preSC system→decrease in E-CADHERIN, upregulation of EMT type transcription factors (ZEB1 and SLUG)
    - Deleted with CRISPR→CREBBP in DMS53→drops in E-CADHERIN and upregulation of ZEB1
- Restoration of CREBBP in null human SCLC cells
    - Increased E-CADHERIN, decrease ZEB1, ASCL1 does not change→decreased proliferation and decreased anchorage independent growth and decreased colonies at low density

- LU505 cells derived for SCLC PDX model (homozygous CREBBP deletion)→upregulation of CHD1, downregulation of ZEB1 and SLUG→decreased proliferation, decreased migration

- Inactivation of Cdh1 promotes transformation in preSC model of early stage SCLC tumorigenesis
  - Lead to increased colonies at low density and increased anchorage in independent growth
- Reduced H3K27Ac with CREBBP loss at CDH1 and other cellular adhesion genes
  - PreSC cells with 2 CREBBP knockdowns-no difference globally
  - PreSC cells with 57 Crebbp regulated genes→decrease in acetylation



- If key targets of CREBBP are positively regulated by histone acetylation, could HDAC inhibition reverse effects of CREBBP loss?
  - If treat LU505 with Pracinostat→increased acetylation, upregulation of E-CADHERIN and downregulation of ZEB1
  - HDAC inhibitor Pracinostat activity correlates with CREBBP expression in SCLC cells
- Pracinostat treatment leads to strong regressions in a subset of Rb/p53/Crebbp mutant animals and exhibits efficacy in Rb/p53 model

Questions:

- Tried LSD inhibitions in context of triple mutant gem?
  - Grant being reviewed in a few weeks to test!

Reminders:

- Next Call: October 4th
  - Dr. Thomas O'Dorisio and Dr. Bryan Allen-University of Iowa
- November 1st call will be from 1:30-2:30pm EST (calendar invites will be updated)
- Annual Spring Meeting will be evening of April 3rd–April 5th 2019
  - Planning committee: Charles Rudin, JT Poirier, Trudy Oliver, Julie George and Taofeek Owonikok