Acquired chemoresistance and EZH2

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SCLC2018 NCI meeting
• Modeling
  • Epi/Genetics of acquired resistance to chemotherapy
  • Identifying recurrent changes in chemosensitive relapse

• SLFN11 as an EZH2-regulated gene in SCLC
  • SLFN11 expression correlates with clinical response
  • EZH2 down-regulates SLFN11 following DNA damage

• EZH2 inhibition as a therapeutic strategy
  • Rescue of SLFN11 expression & gene body methylation
  • Potent activity with SOC in multiple models

• What happens to chromatin w/ EZH2i
  • What is permitting SLFN11 re-expression?
  • How does chromatin in the SLFN neighborhood change w/ EZH2i?
Why model acquired resistance in PDXs?

FDG-PET scans of MSK-LX40 patient

Pre-treatment

Post-treatment (*6)

Ideal Setting…
✧ repeat biopsies
✧ multi-site sampling
✧ compare genomics
✧ basket trial inclusion
✧ guide decisions at relapse

• Repeat biopsies may not be feasible/possible
• No definitive biomarkers of response/resistance
• >90% of patients w/ ES-SCLC relapse
• Characterize paired models by RNA-seq, WES and targeted (IMPACT) sequencing
• Emphasis placed on chemonaive models that showed initial response to C/E

Pooled Differential Changes by RNA-sequencing

- Recurrent defined as occurrence in at least 3 of 10 models
- Up-regulation of \textit{TWIST1} and down-regulation of \textit{SLFN11} were mutually-exclusive

SLFN11 is high in primary SCLC & lower post-treatment

SLFN11 – SCLC cell lines (CCLE)

SLFN11 – primary SCLC (TCGA)

SLFN11 is significantly decreased in previously-treated SCLC models

Memorial Sloan Kettering Cancer Center
**EZH2 in SCLC – RB1 loss and E2Fs**

- EZH2 inhibitors have activity in SCLC
- SWI/SNF mutations *may* create synthetic lethality
- Ras/MAPK activation confers resistance (rare in SCLC)

**EZH2 is highest in SCLC (TCGA)**

**EZH2 is higher in tumor vs. adjacent lung**

20% of SCLC have mutated SWI/SNF

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Chemical Inhibition of EZH2 can restore SLFN11

- Broad DNA methylation inhibitors do not appear to rescue SLFN11 in SCLC
- Histone modifications may be at the core of SLFN11 silencing

**Hypothesis:** If SLFN11 can be re-expressed, then we can chemosensitize to DNA damage.

The greater the re-expression w/ EZH2 inhibition, the greater the chemosensitization.

We hypothesize that EZH2 is responsible for global silencing events in SCLC that permit resistance to chemotherapy and chemical inhibition of EZH2 can partially reverse and/or prevent this resistance in vivo.
Efficacy in SLFN11\textsuperscript{HIGH} / SLFN11\textsuperscript{LOW} PDX models

**Ideal scenario** – combining a topoisomerase 1 poison w/ an EZH2 inhibitor in SLFN11\textsuperscript{HIGH}, chemosensitive relapse (MYC status appears to trump SLFN11)

~15-20% of SCLC cases have inactivating BAF mutations

**BAF** (hSWI/SNF) **euchromatin** “open”

**BAF** (hSWI/SNF) **heterochromatin** “closed”

**BAF** (hSWI/SNF) **PRC1/2**

**MSK-LX40 (SLFN11^{HIGH}) SMARCD1-mut**

- BAF^{MUT} SCLC may benefit from EZH2i **maintenance** post-chemotherapy
- Combination efficacy (EPZ/IRI) must be administered **together, chronically**
ChIP-sequencing suggest global rescue of TSS silencing

- H3K27me3 ↑ and K27Ac ↓ near TSS in resistant disease
- EZH2 chemical inhibition reverses both of these changes

Chemical EZH2 inhibition sustains SLFN11 expression during cycles of DNA damage \textit{in vivo}

SLFN11 expression in tumors

H3K27me3 / Ac ChIP-qPCR

SLFN11 expression is \textbf{maintained \textit{in vivo}} during cycles of chemotherapy with EZH2 inhibition

What regulates the *SLFN11* locus in human SCLC?


Gardner EE *et al.* *Cancer Cell* 2017

Gardner EE *et al.* unpublished

RNA-seq EZH2i time course for re-expression

ATAC-seq +/- EZH2i

ChIP-seq +/- EZH2i – define bivalency, enhancers, etc.

Proteomic Trapping – HALO-dCas9 purifications

CRISPR positive selection screens

Not all SCLC can “re-express” *SLFN11* under EZH2i

A bunch of NSG experiments is not mechanism nor is it direct evidence…


Gardner EE *et al.* *Cancer Cell* 2017

Gardner EE *et al.* unpublished
EZH2i takes time to rescue SLFN11 expression

**Western Blot** sampling of daily treatment of NCI-H82 cells with 1uM EZP/d for 10-ON / 10-OFF days
SLFN11 is not clearly a bivalent PRC2 target in SCLC

ChIP-sequencing of the SLFN “neighborhood” after one week of EZH2 chemical inhibition

Gardner EE et al. unpublished
EZH2i does not open local SLFN11 chromatin

**ATAC-sequencing** to assess local and global changes in chromatin accessibility to Tn5 transposase


Gardner EE *et al.* unpublished
Summary of EZH2’s role in remodeling the SCLC epigenome…

- EZH2 promotes global silencing in SCLC during acquired resistance to SOC.
- Chemical (catalytic) EZH2 inhibition can rescue/prevent some of these…
- SLFN11 locus is not a PRC2 bivalent region, nor opened (ATAC) with EZH2i.
- Rescue of SLFN11 may be regulated outside of local chromatin remodeling.
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Credentialing responsiveness of PDX models

Focused analyses on chemoresponsive PDXs where we observed some measurable response to C/E

Targeting **TWIST1** does not affect resistance in Hu/Mu-SCLC

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**mSCLC cell lines**

![Image of mSCLC cell lines]

**mSCLC allograft from GEMM**

![Graph showing percent survival over days elapsed]

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**Conditional suppression / expression**

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<th>Condition</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
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<td>Chemonaïve TKO allograft (A)</td>
<td>148.7 nM</td>
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<tr>
<td>TKO + DOX</td>
<td>393.7 nM</td>
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<tr>
<td>TKO-K145E + DOX</td>
<td>532.3 nM</td>
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<tr>
<th>Condition</th>
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<tr>
<td>Chemoresistant TKO allograft (AR)</td>
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<td>AR-shRenilla + DOX</td>
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<td>AR-shTWIST1.1 + DOX</td>
<td>2.21 μM</td>
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<tr>
<td>AR-shTWIST1.1 + DOX</td>
<td>1.65 μM</td>
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**TWIST1 is up-regulated across mSCLC systems**

![Graph showing log2 fold change]

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- **TWIST1** up-regulation may be associated w/, but not a determinant of resistance

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TWIST1<sup>HIGH</sup> resistant TKO-A cells are less aggressive in vivo.