SLFN11 and EZH2 Protein Expression and Localization in Circulating Tumor Cells to Predict Response or Resistance to DNA Damaging Therapies in Small Cell Lung Cancer

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**Background**
- A recent phase II trial in recurrent small cell lung cancer (SCLC) demonstrated that patients with high SLFN11 expression in tissue biopsies had improved survival when treated with PARP inhibition (PARPi)
- EZH2 is also highly expressed in SCLC tissue and is associated with chemoresistance through epigenetic silencing of SLFN11
- Here we developed a liquid biopsy test to explore the clinical feasibility of utilizing SLFN11 and EZH2 expression on circulating tumor cells (CTCs) to aid in the prediction of drug response/resistance to DNA damaging chemotherapy or PARPi in SCLC

**Methods**
26 Blood samples were drawn from 20 SCLC pts, including 11 treatment naive, 7 on-Tx, 8 relapse samples (5 pts had multiple draws). Samples were sent to Epic Sciences for analysis utilizing Epic Sciences’ EZH2 and SLFN11 immunofluorescence assays.

1) SLIDE PREPARATION
2) CELL STARING
3) SCANNING
4) SINGLE CELL DIGITAL PATHOLOGY

**Heterogeneous SLFN11/EZH2 Expression in SCLC**

- CTCs were detected in 85% (22/26) samples
  - SLFN11+ CTCs was detected in 73% (19/26) samples
  - Nuclear localized SLFN11+ CTCs was detected in 31% (8/26) samples
  - EZH2+ CTCs was detected in 38% (10/26) samples
  - Nuclear localized EZH2+ CTCs was detected in 35% (9/26) samples
  - 6 samples had both SLFN11 and EZH2 nuclear localized CTCs

**Dynamic Changes in Biomarker Expression by CTCs Collected Over Time**

<table>
<thead>
<tr>
<th>Sample Size</th>
<th>CTC/mL</th>
<th>SLFN11+ CTC/mL</th>
<th>% SLFN11+ CTC</th>
<th>% EZH2+ CTC</th>
<th>% SLFN11+ CTC/mL</th>
<th>% EZH2+ CTC</th>
<th>% SLFN11+ CTC</th>
<th>% EZH2+ CTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tx Naive</td>
<td>11</td>
<td>19 (0-140)</td>
<td>4 (0-52)</td>
<td>29% (0-63%)</td>
<td>4 (0-40)</td>
<td>23% (0-83%)</td>
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<tr>
<td>On-Tx</td>
<td>7</td>
<td>1.3 (0-61)</td>
<td>0 (0-25)</td>
<td>0% (0-00%)</td>
<td>0 (0-14)</td>
<td>0% (0-27%)</td>
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<td></td>
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<tr>
<td>Relapsed</td>
<td>8</td>
<td>8 (1-41)</td>
<td>2.5 (0-52)</td>
<td>30% (10-100)</td>
<td>0 (0-3.5)</td>
<td>0% (0-33%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>22 (0-140)</td>
<td>2.5 (0-52)</td>
<td>22% (0-100)</td>
<td>0 (0-40)</td>
<td>0% (0-83%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions**
- 5 patients had multiple samples analyzed
- MDA-SC66 had longitudinally increased CTC count and decreased SLFN1 suggesting the resistant mechanism
- USE of these assays is underway in SCLC patient samples to assess correlation with PARPi and platinum agent response

**Tx Naïve Pts are More Likely to Express SLFN11 in the Nucleus of CTCs**

- Fisher Exact Test: p=0.0299

**Longitudinal CTC and SLFN11+ CTC Changes Supporting Platinum Resistant Mechanism in SCLC**

- Inter- and intra-sample heterogenous SLFN11 and EZH2 protein expression was observed in CTCs from SCLC patients
- SLFN11+ and EZH2+ CTCs, especially nuclear localized biomarker+, significantly decreased in On-Tx and relapsed samples suggesting the Tx resistant mechanism

**SCLC SLFN11/EZH2+ CTC Cell Image Examples**