Background

- Antibody targeting Delta-like protein-3 (DLL3) has been used with success as an entryway to expose tumor cells to a toxic substrate.
- The effectiveness of these molecules seems closely related to the amount of DLL3 expressed by the tumoral cells.
- The prevalence of DLL3 expression in Small Cell Lung Carcinoma (SCLC), as well as the heterogeneity of expression in the same tumor and the corresponding metastases has not been fully evaluated.

Objectives

- To evaluate the immunohistochemical (IHC) expression of DLL3 in SCLC.
- To assess the evaluation agreement of DLL3 expression in SCLC between a pathologist and an image analysis software.

Methods

  - 35 cases: 2 representatives FFPE blocks
  - 4 cases: 1 FFPE block.
- Staining: DLL3 sp347 clone, Ventana BenchMark Ultra PLC.
- Evaluation: Digitalized slides by PGY3 pathology’s resident.
  - Classification of DLL3 expression: <1%, 1-49%, 50-74%, ≥75%.
  - Evaluation by an image analysis software (CaloPix, TRIBVN, Chatillon, France).
  - 33 cases were analysed. 6 cases were excluded due to technical difficulties with the image analysis software.

Statistics:

- Kramer’s V test was used to evaluate the association between the image analysis software’s and pathologist’s analysis.

Results

Table 1. DLL3 expression in SCLC by the pathologist:

<table>
<thead>
<tr>
<th>DLL3 expression</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1%</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>1-49%</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>50-74%</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>≥75%</td>
<td>26</td>
<td>67</td>
</tr>
<tr>
<td>TOTAL</td>
<td>39</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Comparison between pathologist’s and image analysis software evaluation:

<table>
<thead>
<tr>
<th>DLL3 expression</th>
<th>CaloPix</th>
<th>Pathologist</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-49%</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>50-74%</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>≥75%</td>
<td>29</td>
<td>88</td>
</tr>
<tr>
<td>TOTAL</td>
<td>33</td>
<td>100</td>
</tr>
</tbody>
</table>

The Cramer’s V statistic analyses comparing image software analysis versus pathologist analysis found a statistic value of 0.622 (p-value = 8.21e-03) which indicates a strong association and concordance.

Discussion

- In our study, 36 (82%) of SCLC on resection cases were found to be positive for DLL3 (expression ≥1%) by immunohistochemistry (IHC), and 27 (69%) showed high expression of DLL3 (defined as positive in at least 50% of the tumor cells) which is in agreement with current literature.
- Image software’s and pathologist’s analyses classified the cases in the same category of positivity in 88% of cases with a strong association found in the Cramer’s V test.
- Heterogeneity was observed within the same tumor in two cases that were classified using the mean DLL3 expression value.
- In 6 cases with DLL3 expression of less than 50%, the image software was not able to analyze the level of expression. Failure to achieve better tumor segmentation in low positive or negative cases could be easily addressed by improving nucleus counterstain or with improvement in the recognition algorithm.

Conclusions

- The majority of cases of SCLC on resection specimens express high level of DLL3.
- There was a strong concordance between the pathologist’s and the software image analysis.
- Our findings confirm the relevance of performing IHC validation and clinical-pathological correlation studies with the aim of better characterizing the DLL3 expression threshold as a predictive biomarker for DLL3 targeted agents.

Acknowledgments

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Figure 1. DLL3 expression in SCLC.

Figure 2. DLL3 Heterogeneity expression in SCLC.

Figure 2. Image software analysis steps.