Introduction: Expression of immune-markers is of scientific interest due to their potential roles as predictive biomarkers for immunotherapy. However, our current understanding of the immune-markers expression on non-small cell lung cancer (NSCLC) patients with epithelial growth factor receptor (EGFR) mutation, following acquisition of resistance to tyrosine kinase inhibitors (TKIs), is as yet unclear.

After acquisition of resistance to EGFR-TKIs, heterogenous distribution of resistance mechanisms sometimes occurs between lesions within a patient. In this study, we analyzed the expression of immune-markers in isogenic lesions obtained at different metastatic sites and histological transformation. This indicates that a biopsy from one lesion may not be representative of immune-marker status for all lesions.

Our results suggest the following immune-features in lesions with SCLC transformation after acquisition of resistance to EGFR-TKIs:
- Decreased PD-L1 expression in tumor cells.
- PD-L1 expression in normal cells and TILs are not conclusive.
- Decreased type I IFN regulated genes.
- Increased expression of galectin 9.

Key Findings

Expression of immune-markers is heterogeneous depending on the metastatic sites and histological transformation. This indicates that a biopsy from one lesion may not be representative of immune-marker status for all lesions.

Methods

A 76-year-old never-smoking female with c-Stage IIIB NSCLC was initially treated with platinum-doublet chemotherapy with concurrent radiation. Fifteen months later, she experienced tumor relapse (pulmonary metastases) and was treated with gefitinib monotherapy because her initial lung biopsy harbored an EGFR exon 19 deletion mutation. Following an initial partial response, the patient developed acquired resistance at 5 months. Gefitinib was continued for an additional 3 months until her death with progressive metastases. After her death, tumor specimens were obtained by autopsy in accordance with ethical guidelines with written informed consent from her legal guardians. Autopsy revealed SCLC transformation (without T790M mutation) in the majority of lesions, while other lesions were determined to be adenosquamous carcinoma harboring a T790M secondary EGFR mutation (Figure 1).

For the HTG EdgeSeq platform. Pooling libraries were analyzed by MiSeq next generation sequencer. Results for the HTG EdgeSeq ID panel provided new counts of 549 immune-related genes. Data was normalized using total counts following the guidelines for HTG Myriad for the interpretation.

Figure 5

IHC images for PD-L1 (4X) (Vented Medical Systems) or the Envision FLEX Systems, Inc.) or the Link 48 Autostainer (Dako – Agilent Technologies). PD-L1 (22C3 pharmDx, Dako – Agilent Technologies), PD-L2 (Mouse mAb #70306, Cell Signaling Technology) antibodies were employed to compare expression levels of multiple immune markers between specimens according to the manufacturer’s protocol. Briefly, tumor cells were macro-dissected from a single 4-μm FFPE section and deparaffinized in the provided lyso buffer. After incubation with proteinase K, lysates were processed using m and mounted on glass slides. All

Figure 1

Resistence mechanisms identified at autopsy

Table 1

Summary of immune marker IHC staining in all specimens

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<tr>
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<tbody>
<tr>
<td>Liver metastasis (SCLC)</td>
<td>negative</td>
<td>n.a.</td>
<td>negative</td>
<td>-</td>
<td>n.a.</td>
<td>n.a.</td>
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<tr>
<td>Liver metastasis (AC)</td>
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<td>n.a.</td>
<td>negative</td>
<td>-</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>LN metastasis (AC)</td>
<td>negative</td>
<td>n.a.</td>
<td>negative</td>
<td>-</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>LN metastasis (SCLC)</td>
<td>negative</td>
<td>n.a.</td>
<td>negative</td>
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<td>n.a.</td>
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2. Immunochemistry analysis

FFPE tissue was cut at a thickness of 4-μm and mounted on glass slides. All staining was performed on the Benchmark XT automated stainer (Ventana Medical Systems, Inc.) or the Link 48 Autostainer (Dako – Agilent Technologies). PD-L1 (22C3 pharmDx, Dako – Agilent Technologies), PD-L2 (Mouse mAb #70306, Cell Signaling Technology) antibodies were employed to compare expression levels of multiple immune markers between specimens according to the manufacturer’s protocol. Briefly, tumor cells were macro-dissected from a single 4-μm FFPE section and deparaffinized in the provided lyso buffer. After incubation with proteinase K, lysates were processed using m and mounted on glass slides. All

Figure 2

Workflow of the HTG EdgeSeq assay

Figure 3

IHC images for PD-L1 (4X) (Vented Medical Systems) or the Envision FLEX Systems, Inc.) or the Link 48 Autostainer (Dako – Agilent Technologies). PD-L1 (22C3 pharmDx, Dako – Agilent Technologies), PD-L2 (Mouse mAb #70306, Cell Signaling Technology) antibodies were employed to compare expression levels of multiple immune markers between specimens according to the manufacturer’s protocol. Briefly, tumor cells were macro-dissected from a single 4-μm FFPE section and deparaffinized in the provided lyso buffer. After incubation with proteinase K, lysates were processed using m and mounted on glass slides. All

Figure 4

IHC images for CD-8 or PD-1

Figure 5

Relative gene expression of T cell inhibitory (left) and costimulatory (right) checkpoints

Figure 6

A heatmap analysis

Genes upregulated by type I IFN

Key Findings

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