# #4583 Heterogeneity in immune biomarker expression: Detailed analysis of a case with SCLC transformation after EGFR-TKI treatment

Kenichi Suda[1,2], Isao Murakami[3], Hui Yu[1], Jihye Kim[1], Kim Ellison[1], Christopher J. Rivard[1], Tetsuya Mitsudomi[2], and Fred R. Hirsch[1]

[1]Division of Medical Oncology, University of Colorado Anschutz Medical Campus, United States; [2]Division of Thoracic Surgery, Department of Surgery, Kindai University Faculty of Medicine; [3]Department of Respiratory Medicine, Higashi-Hiroshima Medical Center, Japan.

## Summary

**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 epidermal growth factor receptor (EGFR) mutation, following acquisition of resistance to tyrosine kinase inhibitors (TKIs), is so far unclear.

After acquisition of resistance to EGFR-TKIs, heterogeneous 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 autopsy, comparing lesions with EGFR T790M secondary mutation and those with small cell lung cancer (SCLC) transformation.

**Methods:** A 76-year-old never-smoking female with EGFR mutated lung adenocarcinoma (AC) acquired resistance to gefitinib. After her death, autopsy revealed SCLC transformation and EGFR T790M mutation as mutually exclusive resistance mechanisms (**Figure 1**).

Two liver metastases (SCLC vs. AC with T790M) and two lymph node metastases (SCLC vs. AC with T790M) were analyzed to compare the expression status of immune markers by immunohistochemistry (IHC) and an immune-oncology (IO) gene expression panel (**Figure 2**).

**Results:** IHC analysis revealed that PD-L1 was expressed in 5% of tumor cells with AC histology (T790M) but not in tumor cells with SCLC transformation (**Figure 3** and **Table 1**). The liver metastasis with SCLC transformation showed negative stromal PD-L1 expression and scant tumor infiltrating lymphocytes, while the other lesions demonstrated stromal PD-L1 staining and infiltration of CD8-positive T cells (**Table 1**). PD-1 was positive in lymphocytes from lymph node metastases, but not in those from liver metastasis (**Figure 4** and **Table 1**).

Data generated using an IO panel indicated higher expression of galectin 9 and higher level of T cell co-stimulatory checkpoints in lesions with SCLC transformation (**Figure 5**). In addition, a bioinformatic analysis demonstrated lower expression of type I interferon regulated genes in lesions with SCLC transformation (**Figure 6**).

## Acknowledgements

The authors thank the patient and her legal guardians. We also appreciate Mark Stern and John Wineman at HTG Molecular Diagnostics Inc. for their technical and material assistance for this study.

This work is partly supported by a Young Investigator Award to K. Suda (2015 – 2017) from the International Association for the Study of Lung Cancer (IASLC).

## COI information

We declare no conflict of interest related to this study.

## Methods

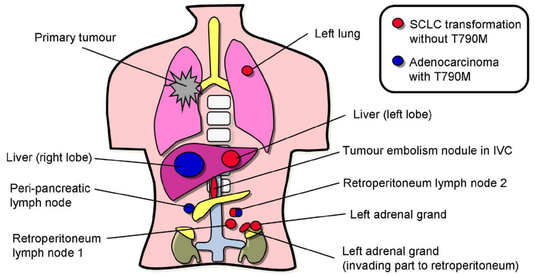
**1. Clinical course of the patient and findings at autopsy**

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 palliative radiation therapy for her cervical lymph node 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 adenocarcinoma histology harboring a T790M secondary EGFR mutation (**Figure 1**).

**Figure 1** Resistance mechanisms identified at autopsy




**2. Immunohistochemistry analysis**

FFPE tumor tissue was sectioned at a thickness of 4μm 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-1 (Mouse mAb #760-4895, Ventana Medical Systems, Inc.), and CD8α (Mouse mAb #70306, Cell Signaling Technology) antibodies were used to detect specific protein expression. The staining platform utilized the Ultraview development reagents (Ventana Medical Systems) or the Envision FLEX visualization system (Dako – Agilent Technologies). PD-L1 levels were assessed independently in both tumor and stromal cells.

**3. Comprehensive analysis for immune markers**

HTG EdgeSeq utilizes nuclease protection chemistry that can be applied to FFPE specimens. The Immuno-Oncology (IO) Assay (HTG Molecular Diagnostics, Inc.) was employed to compare expression levels of multiple immune markers between specimens according to the manufacture's protocol. Briefly, tumor cells were macro-dissected from a single 4μm FFPE specimen and dissolved in the provided lysis buffer. After incubation with proteinase K, lysates were processed using the HTG EdgeSeq platform. Pooled libraries were analyzed by MiSeq next generation sequencer. Parsed data for the HTG EdgeSeq IO panel provided raw counts of 549 immune-related genes. Data was normalized using total counts following the guidelines from HTG molecular for the comparison.

**Figure 2** Workflow of the HTG EdgeSeq assay


https://www.htgmolecular.com/chemistry/edgeseq-ngs-library-prep-chemistry

## 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.
- ✓ Our results suggest the following immuno-features in lesions with SCLC transformation after acquisition of resistance to EGFR-TKI:
  - Decreased PD-L1 expression in tumor cells.
  - PD-L1 expression in stromal cells and TILs are not conclusive.
  - Decreased type I IFN regulated genes.
  - Increased expression of T cell costimulatory checkpoints.
  - Increased expression of galectin 9.

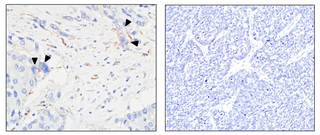
## Results

**Table 1** Summary of immune marker IHC staining in all specimens

| Specimens | PD-L1 (tumor) | PD-L1 (stroma) | TILs | CD8 (T cells) | PD-1 (T cells) |
|---|---|---|---|---|---|
| Liver metastasis (AC / T790M) | 5% | 5% | + | + | negative |
| Liver metastasis (SCLC) | negative | negative | scant | n.a. | n.a. |
| LN metastasis (AC / T790M) | 5% | 5% | + | + | + |
| LN metastasis (SCLC) | negative | 10% | + | + | + |

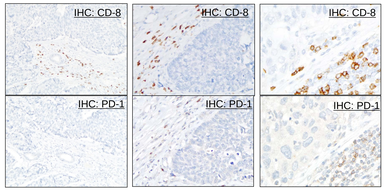
*TILs*, tumor infiltrating lymphocytes; *AC*, adenocarcinoma; *SCLC*, small cell lung cancer transformation; *LN*, lymph nodes; *n.a.*, not available

**Figure 3** IHC images for PD-L1 (40x)



*Left*, Adenocarcinoma (liver metastasis) and arrowheads indicate tumor cell staining.
*Right*, Lesion with SCLC transformation (liver metastasis).

**Figure 4** IHC images for CD-8 or PD-1




*Left*, Adenocarcinoma (liver metastasis, 10x).
*Middle*, Lesion with SCLC transformation (LN metastasis, 20x).
*Right*, Adenocarcinoma (LN metastasis, 40x).

**Figure 5** Relative gene expression of T cell inhibitory (left) and costimulatory (right) checkpoints

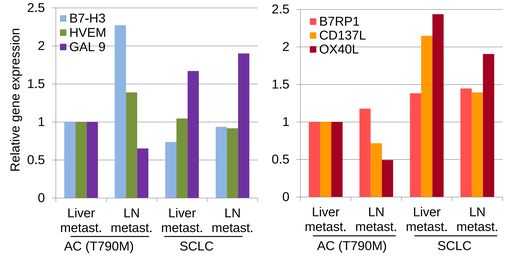
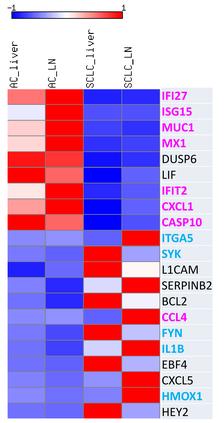



**Figure 6** A heatmap analysis




Genes upregulated by type I IFN
Genes downregulated by type I IFN