Molecular Subtyping of Circulating Tumor Cells in Patients with Small Cell Lung Cancer

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INTRODUCTION
- Small Cell Lung Cancer (SCLC) comprises approximately 10-15% of all lung cancers.
- Most patients are diagnosed with metastatic/extensive stage disease where median survival is just 1 year, and 5-year overall survival rate is < 2%.
- While SCLC is initially sensitive to conventional platinum doublet chemotherapy, most patients subsequently relapse.
- Unlike NSCLC, tumor mutational profiling and the rational implementation of targeted therapies into the care of the patients with SCLC has not yet proven effective in this disease.
- Barriers to the study of SCLC stem from limited amounts of tumor biopsy samples and limited access to longitudinal tumor samples to understand tumor evolution.
- Recently, a new molecular classification of SCLC, defined by expression of four key transcription regulators - ASCL1, NEUROD1, YAP1, and POU2F3 has been proposed.
- Of these four transcription factors, ASCL1 and NEUROD1 are classified as neuroendocrine markers (NE) and YAP1 and POU2F3 are non-neuroendocrine markers (non-NE).
- These molecular subtypes may confer differential biology and therapeutic vulnerabilities, however, studies to date have been constrained by the paucity of available longitudinal tumor biopsy samples obtained from patients with SCLC.
- Circulating tumor cells (CTCs) were prospectively evaluated in the Treatment naïve, just 1 year, and 5 years of treatment samples.

METHODS
- Informed consent was obtained from patients with SCLC using an IRB approved protocol (RBM303763).
- We collected blood samples from 28 patients, including treatment naive samples from the entire cohort as well as on treatment and relapsed samples for processing using the RareCyte® platform (median of 4 collections per patient).
- The RareCyte® rare-cell analysis platform combines density-based collection of nucleated blood cells with automated staining, high-resolution episcopic imaging, and image analysis to quantify and isolate CTCs.
- CTCs were detected based on their expression of CK/EpCAM positive and CD45 negative staining.
- Each sample was also evaluated for expression of neuroendocrine markers (ASCL1, NEUROD1) and non-neuroendocrine markers (YAPI, POU2F3). Each set of neuroendocrine and non-neuroendocrine markers were co-stained along with the CTC markers.
- Antibodies were validated using SCLC cell lines known to be positive and negative for each of the markers and cell line spike-offs for positive and negative control.
- Mean fluorescence intensities of each marker were extracted using a python program, and intensity cut-offs for positive expression was based on the cell line data.

WORKFLOW FOR QUANTIFICATION OF CTCs FROM BLOOD SAMPLES
- Each blood collection tube with 7.5ml of blood was processed to obtain CTC counts using the RareCyte® platform.
- Patient’s blood was collected and processed to obtain CTC counts using the RareCyte® platform. Each tube was processed to obtain CTC counts from the buffy coat isolation tubes.
- Workflow for quantification of CTCs from blood samples. Each blood collection tube with 7.5ml of blood was processed to obtain CTC counts. Each patient had two blood collection tubes, each containing 7.5ml of blood.

OBJECTIVES
- To quantify CTC counts in patients with SCLC at initial diagnosis and throughout their treatment course, in order to monitor therapeutic resistance and progression of disease.
- To determine subtype marker expression on CTCs at the time of diagnosis and to evaluate for dynamic changes in marker expression throughout treatment course expression during treatment in patients with SCLC.

TABLE 1: QUANTITATION OF CTC counts and corresponding biomarker expression

Table 1: Quantitation of CTCs and corresponding biomarker expression on CTCs in patients treated with platinum/etoposide/atezolizumab therapy. Each CTC count is the number of CTCs identified and quantified in each patient's sample.

SUMMARY
- We developed an assay to evaluate the expression of NE (ASCL1, NEUROD1) and non-NE (YAPI, POU2F3) markers on CTCs.
- We collected blood samples from 28 SCLC patients and obtained data on CTC counts at different treatment naïve, on-treatment and at relapsed timepoints.
- CTCs were detected in 20/28 (71%) patients. Of these, ASCL1 was positive in 16/28 (57%), NEUROD1 was positive in 11/20 (55%), YAP1 was positive in 9/18 (50%) and POU2F3 was positive in 12/18 (67%) of samples.
- Of the three relapse samples analyzed to date, 2/3 had increase in CTC counts and decrease in ASCL1 expression from treatment naïve timepoint to relapsed timepoint. The third sample had decrease in CTC count and lost the expression of all biomarkers in the few CTC detected at the relapsed timepoint.

FUTURE DIRECTIONS
- We will correlate the expression of biomarkers on CTCs with the tumor biopsy tissues from the same cohort.
- We will correlate the biomarker expression on CTCs and biopsy tissue with treatment outcomes and evolution of disease for the patients.
- We have n=28 SCLC patients with longitudinal blood collections that have been processed and slides banded (sample size=136, median4) that are in the process of being analyzed.

REFERENCES

We thank Cindy Lowe, Lindsay Meyer and all members of the Translational Pathology, Shared Resources Core Thoric Biorepository Team, VUMC US4 CA217405-01 US1 CA224276-01 IASLC Lori Monroe Scholarship

THANKS TO ALL THE PATIENTS WHO SUPPORTED THIS WORK

ACKNOWLEDGEMENTS

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