Molecular and Genomic Characterization of Small Cell Lung Cancer (SCLC)

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Abstract

Introduction: Small cell lung cancer (SCLC), strongly tobacco-associated, has been described to have a heavy mutation burden, harboring high rates of TP53 and RB1 alterations. While initially responsive to radiation and chemotherapy, SCLC is characterized by eventual progression and resistance to traditional therapy. We retrospectively analyzed a molecular profiling (MP) database to identify potentially actionable alterations using a multi-platform approach which includes massively parallel sequencing.

Experimental Procedures: SCLC patient samples were referred to a central CLIA laboratory (Caris Life Sciences, AZ) for MP (immunohistochemistry [IHC] and next generation sequencing [NGS]). Expression of PD1 (MRQ-22, ≥1+) on tumor infiltrating lymphocytes (TILs) and PDL1 (130021, SP142; ≥2+≥5%) in tumor cells was performed by IHC. Additional IHC (ERCC1, TOPO1) and NGS on 591 genes was performed on FFPE samples using the Illumina NextSeq platform in a subset of patient samples. All variants were detected with > 99 % confidence. Variants are described as follows: pathogenic, presumed pathogenic, variants of unknown significance and unclassified variants (excluding SNPs).

Results: 203 SCLC samples were identified, 48% were females (97) and 52% were males (106). Median age was 65 [range: 29-88]. Cancer cells expressed PDL1 in 2.5% of cases (5/203) and PD1+ TILs were detected in 38% (75/197). For comparison, internal PDL1+ in non-small cell lung carcinomas (NSCLC) was 31% (339/1098). Notable findings from IHC included ERCC1 negative status in 93% (14/15) and TOPO1 + in 70% (14/20). CNV and mutational analysis (NGS) was available for 10 and 22 patients, respectively. Amplifications were found in the following genes: CCND3, CRKL, FGF4, FGFR1 and NFKB1A (n=1, respectively), and CCND1, CCNE1, CDKN2A and FGF3 (n=2, respectively). As previously reported, the most frequently altered genes were TP53 (73%) and RB1 (68%). Clinically relevant pathogenic or presumed pathogenic variants included: EGFR (exon 19 deletion), BRAF (G469A), APC (T1556fs), NF1 (A1610fs, D699fs), NOTCH1 (E473fs, C332Y, G546X) and PTCH1 (N1351fs). It was thought the patient with EGFR mutation is a case of NSCLC transformation to SCLC. Variants with unknown significance or unclassified variants detected in genes with clinical relevance and of potential interest for targeted therapy in SCLC include: DDR2, cMET, RET, FGFR1/3, BRCA2, IGF1R, RICTOR and NTRK1.

Conclusion: Genomic and molecular characterization of SCLC samples reveals a heterogeneous population. Several potentially actionable targets are identified by NGS. Early reported trial data suggests activity of SCLC to checkpoint inhibitors. We observed higher levels of PD1+ TILs, however differences in antibody clones, thresholds or staining localization (tumor cells vs. stromal lymphocytes), may account for the observed overall low PD-L1 expression. Further efforts are needed to identify and validate new therapeutic targets in SCLC.

Background

- Nearly all cases of SCLC are attributable to cigarette smoking.
- Genomic studies have shown high rates of TP53 and RB1 mutations, leading to a loss of cell cycle control, rapid doubling time and early development of metastases
- SCLC is highly sensitive to initial chemo/radiotherapy, however most patients succumb to recurrent disease
- The discovery of new targets are highly desirable for this disease which is very difficult to treat in advanced stages.

Methods

246 patients with SCLC were included in the study and tested centrally at a CLIA laboratory (Caris Life Sciences, Phoenix, AZ). Tests included one or more of the following: gene sequencing, copy number variation (NextSeq Illumina) and protein expression (immunohistochemistry [IHC]. Antibodies and cutoffs can be obtained by request, or are provided in the figures.

**An additional 43 patients profiled after abstract submission were included in the final analysis

Results

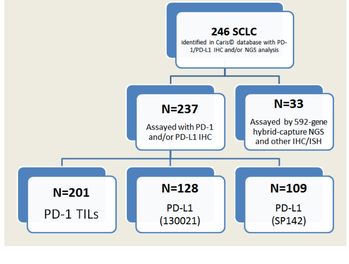


Figure 1. SCLC cases selected for immune checkpoint pathway and expanded next-generation sequencing analyses.

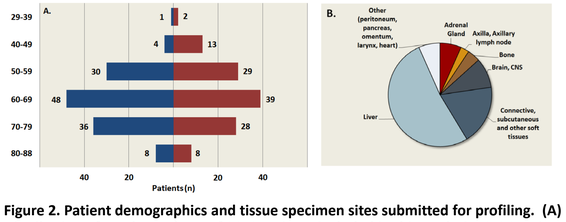
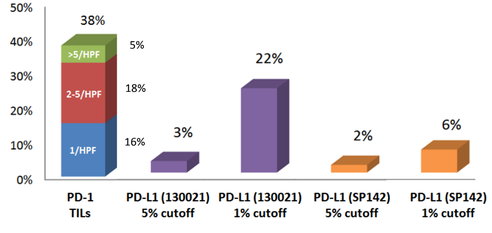


Figure 2. Patient demographics and tissue specimen sites submitted for profiling. (A) Distribution of gender and age of SCLC patients included in this analysis. (B) Specimen sites submitted for molecular profiling, excluding lung, mediastinal and thoracic regions.

Results, contd.

Figure 3. Immune Checkpoint Pathway
PD-1 positive TILs and PD-L1 tumor expression rate



n	201	128	128	109	109

Figure 3. Distribution of PD-1 positive tumor infiltrating lymphocytes (TILs) and PD-L1 positive expression in tumor cells. Antibodies: PD-1 Ventana (NAT105) [multi-color bar], R&D systems B7H1/PDL1 (130021) [purple bars] and Spring Bioscience (SP142) [orange bars].

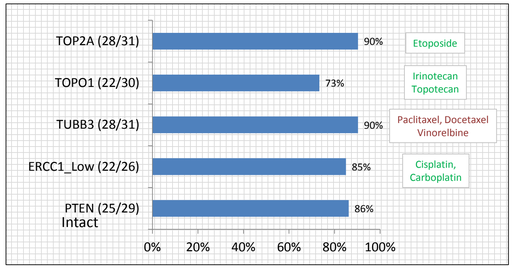


Figure 5. Protein expression rates of select biomarkers in SCLC.

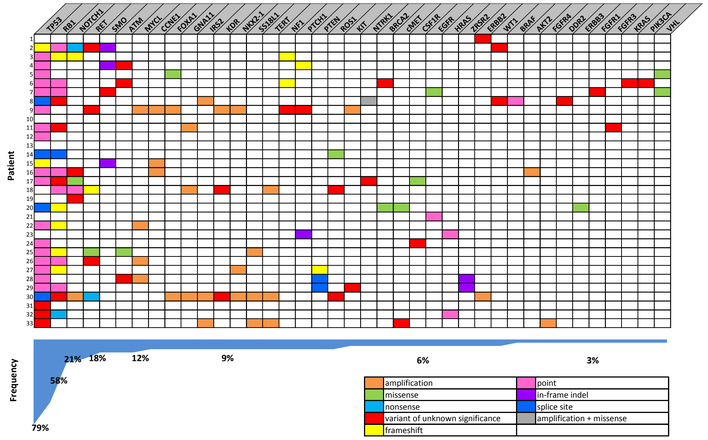


Figure 4. Most common genomic alterations in SCLC detected by next-generation sequencing.

Conclusions

- PD-1 immune checkpoint pathway shows over one-third of SCLC have PD-1 positive TILs and up to 22% PD-L1 tumor expression. Antibody clones and thresholds impact the frequencies observed in SCLC.
- Next-generation sequencing confirms previously observed high rates of TP53 and RB1 mutations, as well as NOTCH1.
- Mutations in additional, potentially actionable genes included RET, SMO, ATM, however whether these mutations are passenger or driver events remains to be determined.
- Genotyping may reveal mutations in known oncogenic drivers from other cancers, for example, BRAF, PIK3CA, PTEN, VHL, cKIT, etc.
- Further efforts are needed to identify and validate new therapeutic targets in SCLC.

References

1. National Comprehensive Cancer Network. Small Cell Lung Cancer (Version 1.2016) http://www.nccn.org/professionals/physician_gls/PDF/sclc.pdf : Accessed April 5, 2016.
2. George, J., Thomas, R.K., et al. (2015). Comprehensive genomic profiles of small cell lung cancer. Nature 524:47-5