

# Combining anti-VEGF-A and anti-PD-L1 therapy prolongs overall survival in small cell lung carcinomas

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**Purpose/Objectives:** SCLC accounts for approximately 15% of newly diagnosed lung cancer cases, is driven by RB1 and TP53 lesions and represents the most aggressive pulmonary carcinoma. Current chemotherapies are initially effective in SCLC patients, however tumors rapidly reoccur and patients die within a few months. Results from KEYNOTE-028 revealed that PD-L1 positive late stage SCLC patients who received a human-ized monoclonal anti-PD-1 antibody, showed 25% response rates. This finding is remarkable for SCLC, since targeted therapies for this tumor entity are rare. However, most SCLC patients harbor a primary resistance or acquire resistance during treatment by an immune suppressive microenvironment. Thus, there is a critical need to combine immune checkpoint inhibitors with other therapies to overcome these resistances.

**Materials/Methods:** We implemented a combined therapy concept including anti-VEGF-A and anti-PD-L1 monoclonal antibodies in an autochthonous SCLC mouse model. As a read out, we used X-ray computed tomography, flow cytometry and end-point immunohistochemistry.

**Results:** We found that combined anti-VEGF-A/anti-PD-L1 treatment synergistically improved overall survival, compared to monotherapies, and enhanced T cell infiltration into the tumor. Moreover, we identified in murine SCLC tumors which acquired resistance to anti-PD-L1, a significant increase in PD-1+/TIM-3+ exhausted CD4 and CD8 T-cells accompanied by significantly increased expression of Galectin-9, the major TIM-3 ligand, on TAMs. In addition, we observed a significantly enriched fraction of TAMs with a similar exhausted phenotype indicated by the expression of LAG-3 and CTLA-4 upon acquired resistance to PD-L1 blockade. Importantly, the exhaustion of T-cells and TAMs was reverted

upon combined anti-VEGF-A/anti-PD-L1 therapy. In line with the results obtained from the mouse model, we showed mechanistically in PBMCs of SCLC patients, that VEGF-A triggers co-expression of PD-1 and TIM-3 on T-cells, indicating an immunosuppressive function of VEGF-A in SCLC patients during anti-PD-1/PD-L1 therapy. Furthermore, we elucidated using CRISPR-Cas9 technology, that VEGF-A liberated from the tumor microenvironment is the likely mediator of acquired resistance to PD1/PD-L1 blockade in SCLC.

**Conclusion:** Taken together, in SCLC there is evidence for a substantial benefit of implementing combined anti-angiogenic and anti-immune checkpoint therapy approaches in the clinic in order to overcome acquired resistances delivered by T-cells and macrophages in the tumor microenvironment.

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