FLOW CYTOMETRY IMMUNOPHENOTYPING OF NECK LYMPH NODES SAMPLED BY FINE NEEDLE ASPIRATION (FNA) IN PATIENTS SUFFERING FROM ADVANCED CUTANEOUS SQUAMOUS CELL CARCINOMA: A PILOT STUDY


Background Cutaneous Squamous cell cancer (cSCC) is one of the most common forms of cancer and has the highest incidence increase in Sweden. cSCC has a good prognosis, however around 5% develops advanced cSCC which is difficult to treat. Check point inhibitor (CPI) treatment is promising, but many patients do not show long-lasting remission. Therefore, we need to understand how the CPI modulation works to increase its clinical benefit.

Methods We analysed the lymphocyte phenotypes in peripheral circulation (PBMC), unfixed tumour tissue and sentinel lymph node samples from advanced cSCC patients with flow cytometry. The sentinel nodes were of particular interest since they are rarely analysed in this context but are essential for a well-functioning anticancer immune response. The lymphocytes were phenotypically characterized and analysed with the focus on activation markers and CPI expression.

Results In this pilot, we analysed samples from six patients. Sentinel node samples had a higher percentage of CD4+ T cells compared to PBMC. Furthermore, the number of activated CD4+CD69+ T cells was higher in sentinel nodes (32.4%) compared to PBMC (1.1%). A smaller amount of CD4+ cells in sentinel node expressed PD-1 compared to CD4+ cells in the tumour samples. CTLA-4 was expressed on CD4+ cells in sentinel nodes at low levels compared to the tumour samples.

Conclusions Our results confirm that the sentinel node is rich in highly activated cells that express immune checkpoint molecules. Further experiments are needed to investigate how the systemic treatment with immune checkpoint inhibitors influence the phenotypes and function of lymphocytes in sentinel nodes. In this project, we are going to look into correlation between flow cytometry findings (activation level, expression of CPI molecules) and clinical outcome of our patients (locoregional control, survival, response to the treatment). In the future, this could also help us discover biomarkers predicting response to CPI treatment. Furthermore, the results might also strengthen the role of flow cytometry analysis in clinical assessment and staging of cSCC patients.

Ethics Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Regional Ethics Committee Approval: 2019-03518.