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Primary and recurrent head and neck squamous carcinomas are strikingly different regarding their immune microenvironment

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Purpose/Objectives: The tumor immune microenvironment (TIME) has a crucial impact on cancer progression and patients´ survival for various cancer entities. In head and neck squamous cell carcinoma (HNSCC) the development of recurrences in up to 60% is one main factor leading to poor prognosis. We aimed to reveal changes in the TIME of primary tumours and their corresponding recurrences, hypothesising that recurrent HNSCC have successfully escaped anti-tumor immune response.

Materials/Methods: The TIME of 70 formalin fixed paraffin embedded (FFPE) tissue samples of HNSCC primary tumours and their corresponding local recurrences was characterised via immunohistochemical staining for the markers CD4, CD8, CD20, FOXP3, CD1A, PD1, CD68 and CD56 and semi-automated quantification with Definines software. RNA was extracted using the Maxwell® 16 LEV RNA FFPE Purification Kit for a subgroup of 18 patients, who underwent (chemo-)radiation therapy between the resection of the primary tumour and the development of a local recurrence. RNA expression levels of 770 immune related genes were identified by nanostring nCounter® PanCancer Immune Profiling Panel and assessed with the nSolver™ Analysis Software.

Results: The immunohistochemical TIME analysis demonstrated a loss of CD20+ B lymphocytes (p= 0.0006), an increase in CD1A+ dendritic cells (p= 0.017) and a decrease in the CD8/FOXP3 T-cell ratio (p= 0.106) in HNSCC recurrences compared to their corresponding primary tumours. Nanostring analysis confirmed the depletion of B

lymphocytes and additionally revealed a strong decrease in the total number of tumor infiltrating lymphocytes (TILs) as well as an increase in dendritic cells, mast cells, neutrophils and macrophages in HNSCC recurrences. In summary, 141 genes of the nanostring panel were identified to be significantly differentially expressed with the majority being downregulated in the HNSCC recurrences. Significantly downregulated chemokines included CXCL13 and CXCR5, which are involved in B-cell chemotaxis; whereas one of seven upregulated genes, Osteopontin, plays a major role in myeloid cell chemotaxis.

Conclusion: Our results reveal significant differences in the TIME of HNSCC primary tumours and recurrences, characterised by a loss of B lymphocytes and an overall shift from anti-tumor immune response to an increase in pro-tumor immune factors in recurrences.

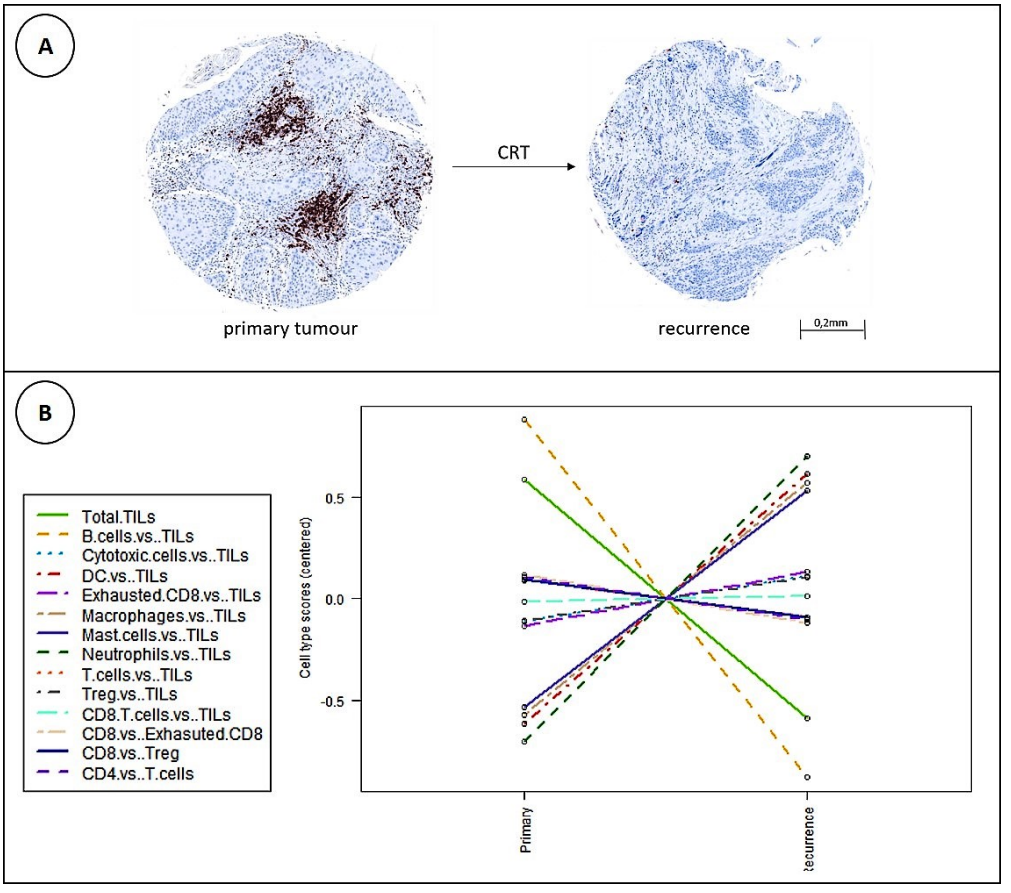

Figure 1: Characterisation of the tumor immune microenvironment in HNSCC primary tumours and recurrences: A: Representative immunohistochemical staining for CD20+ B-cells with a B-cell depletion in the recurrence after chemoradiation therapy of the primary tumour. B: Quantification of tumor infiltrating leukocytes (TILs) via RNA expression profiling of immune related genes in 18 primary tumours with corresponding recurrences. Recurrences show a decrease in total TILs and relative numbers of B-cells as well as an increase in relative numbers of dendritic cells, neutrophils, macrophages and mast cells compared to their primary tumours.