1463 THE INTRA-TUMORAL SPATIAL HETEROGENEITY OF T CELL ANTIGENS IN GLIOBLASTOMA: AN INTEGRATED MULTI-OMICS APPROACH

[1,2,3]Marcel Wacker*, [4,5]Gioele Medici, [2,3,6,7]Marissa Dubbelaar, [2,3,6]Jens Bauer, [2,3,6]Annika Nelde, [7]Friederike Hanssen, [7]Carolin Schwitalla, [4]Luca Regli, [5]Michael Weller, [2,3,8]Hans-Georg Rammensee, [4,9,10]Marian Christoph Neidert, [2,3,6,11]Juliane S Walz. *[1]University and University Hospital Tübingen, Tübingen, Germany; [2]Institute for Cell Biology, Department of Immunology, University of Tübingen, Tübingen, Germany; [3]Cluster of Excellence iFIT (EXC2180) 'Image-Guided and Functionally Instructed Tumor Therapies', University of Tübingen, Tübingen, Germany; [4]Clinical Neuroscience Center and Department of Neurosurgery, University Hospital Zürich, University of Zürich, Zürich, Switzerland; [5]Laboratory of Molecular Neuro-Oncology, Department of Neurology and Brain Tumor Center, University Hospital Zürich, Zürich, Switzerland; [6]Department of Peptide-based Immunotherapy, University and University Hospital Tübingen, Tübingen, Germany; [7]Quantitative Biology Center (QBiC), University of Tübingen, Tübingen, Germany; [8]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ) partner site Tübingen, Tübingen, Germany; [9]Department of Neurosurgery, Cantonal Hospital St. Gallen, St. Gallen, Switzerland; [10]Neuroscience Center Zürich (ZNZ), University of Zürich and ETH Zürich, Zürich, Switzerland; [11]Clinical Collaboration Unit Translational Immunology, German Cancer Consortium (DKTK), Department of Internal Medicine, University Hospital Tübingen, Tübingen, Germany*

Background Glioblastoma is the most common primary malignant neoplasm of the central nervous system in adults. Current treatment options comprise maximal surgical resection followed by radiation and/or chemotherapy with temozolomide. These procedures are unable to eliminate all tumor cells, leading to disease recurrence and accounting for the poor prognosis. Glioblastoma is a highly infiltrative tumor with recurrence originating from the unresectable peritumoral infiltration zone (INF). Thus, novel treatment options specifically targeting tumor cells in the INF zone are needed to prevent relapse and enable long-lasting remission.

Methods In this work, we performed a multi-omics spatial analysis of the necrotic center (NEC), the gadolinium contrast-enhancing region (T1), and the INF zone integrating mass spectrometry-based immunopeptidome analysis with next-generation sequencing methods (whole exome and RNA sequencing) to assess immunologically relevant aspects of tumor heterogeneity. For the multi-omics analysis, HLA-restricted peptides and genetic material from 15 glioblastoma patients were analyzed from the three zones NEC, T1, INF. Additionally, adjacent benign (BEN) brain tissue was analyzed from four patients.

Results A total of 31,655 unique HLA class I and 10,071 unique HLA class II peptides were identified in 12 of the 15 glioblastoma patients. Comparative profiling of peptides from our study and a benign tissue database (HLA ligand atlas (https://hla-ligand-atlas.org)) identified 38% of HLA class I and 70% of HLA class II peptides as tumor-associated antigens (TAAs) originating from either INF, T1 or NEC zones, respectively. Of these HLA class I TAAs, 17%, 19%, and 17% and of the HLA class II TAAs 16%, 19%, and 43%, were exclusively presented in the INF, T1, or NEC zones, respectively. Five INF-associated HLA class I ligands were frequently presented in 42% of glioblastoma immunopeptidomes, whereas nine INF-associated HLA class II ligands were presented in up to 62% of glioblastoma immunopeptidomes. Of note, the glioblastoma-associated proteins BAALC, NCAN, and SLC20A1A were identified among these INF-associated ligands. Furthermore, integrated RNA/DNA sequencing enabled a greater understanding of spatial tumor antigen presentation. It led to the identification of 1,287 and 923 predicted possible INF-specific HLA class I and HLA class II neoepitopes, respectively, which are derived from tumor-associated mutations. The immunogenicity of these peptides will be further validated in upcoming T cell assays of tumor-infiltrating lymphocytes.

Conclusions In summary, intra-tumoral regional heterogeneity of tumor antigens was identified, which could be used for specific immunotherapy approaches targeting the INF zone of glioblastoma.

Ethics Approval Written informed consent was obtained for all patients following the Declaration of Helsinki protocol and the local review board (Kantonale Ethikkomission Zürich; KEK-ZH-Nr.2015–0163)

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