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**P09.12 HEREDITARY HOMOZYGOSITY AND ALLELIC IMBALANCE OF HLA AS COMMON IMMUNE ESCAPE MECHANISMS IN ESOPHAGEAL ADENOCARCINOMA**

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**Background**

The human leukocyte antigen (HLA) genotype of an individual defines the repertoire of peptides which can be presented to T cells. In the tumor microenvironment, neoantigen presentation can be abrogated by alterations of HLA class I molecules, which have a direct impact in immune surveillance. This study aims to elucidate the impact of hereditary homozygosity and imbalanced expression of HLA-I loci on the repertoire of immunogenic peptides that are presented in esophageo-gastric adenocarcinoma patients (EGA).

**Materials and Methods**

High-resolution clinical-grade HLA-genotyping was performed using the NGS method (n=80). Whole exome sequencing (WES) was done on tumor and blood (n=39) to call for somatic mutations. The amount of potential high affinity binders derived from 10 tumor-associated antigens (TAAs) frequently expressed in EGA and non-synonymous mutations obtained from WES data were determined using an in-silico approach for MHC-binding (IEDB.org). Gene expression profiling was done with 3`RNAseq (n=39). Whole RNAseq data was used to detect imbalanced or loosed expression of HLA-A/B/C alleles (n=19).

**Results**

We compared the frequency of HLA homozygosity in EGA patients to an HLA-matched reference population derived from a large cohort of bone marrow donors (n=7.615). We demonstrate that homozygosity of HLA-I loci is significantly enriched in the germline of EGA patients compared to the control population (35% vs. 19%, corresponding to an odds ratio (OR) for homozygosity of 2.282 (95% CI 1.442–3.615, p<0.001)). We then aimed to estimate the influence of HLA-homozygosity in the context of tumor immune surveillance. Predictions by IEDB analysis resource tool indeed showed a reduced repertoire of high and moderate-affinity MHC-binders (both TAA-derived and mutation-derived peptides) in the homozygous cohort. Our findings demonstrate a reduced amount of potentially immunogenic peptides in EGA patients with HLA-homozygosity for at least one locus, which may result in impaired cancer immunosurveillance. In line with this observation, artificial restoration of the genotype of homozygous patients to a heterozygous genotype, resulted in a set of predicted good-binding peptides of comparable size to the heterozygous cohort. While 35% of EGA patients showed germline homozygosity of HLA-I loci, quantification of allele-specific expression of HLA-I revealed altered expression in 9 out of 12 heterozygous patients (75%). 3 of these patients showed complete loss of heterozygosity, whereas the others had altered expression of one or two HLA-I molecules. The allelic imbalance was significantly higher in heterozygous compared to homozygous were only 2 patients showed altered expression of one HLA-I molecule (28.6%, p=0.0240). None of the patients with allelic imbalance carried genetic mutations associated with HLA-I genes, underlying epigenetic regulation.

**Conclusions**

The high frequency of genomic HLA-I homozygosity observed in the EGA cohort suggests that limitation of neoantigen presentation might have a role during EGA development and may reflect an increased cancer risk for these patients. Moreover, the results herein highlight that despite having a complete set of HLA-I alleles on a genomic level, the majority of EGA patients carry allelic imbalance that limit the repertoire of neoantigens for presentation to immune cells.

**Disclosure Information**

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**P09.13 IMMUNE GEOSPATIAL PROFILING AND CHARACTERIZATION OF GLIOMA MICROENVIRONMENT AFTER ADOPTIVE CELL THERAPY SHOWS DIFFERENTIALLY EXPRESSED ANTIGEN PROCESSING AND CROSS-PRESENTATION GENES SIGNATURES**

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**Background**

Our group has previously shown that Adoptive Cellular Therapy (ACT) is efficacious against CNS malignancies including medulloblastoma, brain stem glioma, and glioblastoma. Here, we used GeoMx Digital Spatial profiling platform to perform in situ characterization and spatial distribution of the glioma microenvironment in tumor-bearing mice after ACT. Our goal is to elucidate the underlying cell dynamics occurring within the tumor microenvironment and determine what immune cells and gene signatures are playing a major role in overcoming treatment resistance. This method enabled us to define differences in antigen processing and cross presentation gene signatures in the ACT-treated tumors compared to untreated tumors. We also observed differences in gene expression within T cell subsets in the treated versus untreated tumors including changes in gene expression of regulatory T cell (Treg) associated genes, such as Runx1 and TGF-β. We also found a significant increase in Batf3 expression associated with therapy.

**Materials and Methods**

To characterize the cellular dynamics behind the efficacy of this therapy, we performed in situ GeoMx Digital Spatial Profiling (DSP) and whole genome transcriptomics (RNA) of murine glioma KR-158B-luciferase tumors after adoptive cellular therapy. C57BL/6 mice were orthotopically implanted with KR158B-luciferase cells and subsequently treated with ACT. Histological slides of brain sections were sent to be stained for CD45, CD3, GFP (marks hematopoietic stem cell-derived cells) and nuclei, and processed following GeoMx DSP workflow for RNA.
Gene set enrichment analysis (GSEA) was performed to determine differentially expressed gene signatures in treated vs control mice. Gene expression clustering profiles were conducted using a Principal Component Analysis (PCA), and relative gene expression differences were analyzed by unpaired t-tests.

**Results** Our data shows differentially expressed antigen processing and cross presentation gene signatures in the ACT-treated compared to untreated tumors by GSEA analysis. We also observed that tumors treated with ACT cluster separately from control tumors in PCA analysis and they have several differentially expressed genes. Furthermore, we found downregulation of genes associated with the suppressive properties of regulatory T cells (Runx1 and TGF-β), and upregulation of dendritic cell characteristic genes (Batf3) in ACT-treated tumors vs control.

**Conclusions** These findings provide insights into the immune cell dynamics and specific genes and gene signatures that are differentially expressed within the tumor microenvironment after treatment. This could help improve current adoptive cellular therapies to treat brain tumors.

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**P09.14 THE ROLE OF IDO1 AND CELLULAR SENESCENCE IN THE EFFICACY OF PD-1 PATHWAY BLOCKADE AND RADIOThERAPY IN AGED MICE WITH GIOBLASTOMA**

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**Background** Glioblastoma is the most common primary brain tumor in adults with a median overall survival between twelve to fifteen months.1,2 The prognosis is worse for patients older than 65 years of age.3 Aging is associated with senescence, which leads to secretion of proinflammatory cytokines and increased levels of the immunosuppressive enzyme indoleamine-2,3-dioxygenase 1 (IDO1).4 Increased levels of IDO1 at an advanced age may attenuate the efficacy of immunotherapy.

**Materials and Methods** One hundred and seven mice between 79 and 92 weeks of age with differential Ido1 expression were intracranially injected with syngeneic murine GL261 cells and treated with PD-1 monoclonal antibodies and radiotherapy. The mice have been monitored for ninety days. U87 glioblastoma cells were cultured and treated with interferon-γ and NU223612.

**Results** PD-1 blockade and irradiation were less effective when Ido1 expression was preserved. Mouse lines with Ido1 knock-out in dendritic and endothelial cells had better survival. There was a higher senescent cell burden within the brain tumor than the extra-tumoral tissue in mice reaching the humane endpoint. In U87 glioblastoma cells, interferon-γ induced upregulation of PD-L1 and IDO1 in a similar pattern. IDO1 degradation resulted in concomitantly lower levels of PD-L1.

**Conclusions** Taken together, these results suggest that the treatment protocol with PD-1 pathway blockade plus radiotherapy should be combined with IDO1 inhibitors and potentially with senolytics to achieve a better therapeutic outcome in the elderly population with glioblastoma.

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**REFERENCES**


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**P09.15 PATIENT-DERIVED HEAD AND NECK TUMOR SLICE CULTURES – A VERSATILE TOOL TO STUDY ONCOLYTIC VIRUS ACTION**

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**Background** Translating preclinical data from cell-based in vitro systems and syngeneic mouse tumor models to the clinically heterogeneous ecosystem of human tumors is a challenging task. Especially head and neck squamous cell carcinomas (HNSCC) show a highly diverse and complex architecture which makes the prediction of a treatment outcome quite difficult. To bridge this gap, we have established and employed a patient-derived HNSCC slice culturing system to assess immunomodulatory effects as well as permissivity and oncolytic virus (OV) action.

**Materials and Methods** HNSCC tumor biopsies from 12 patients were sectioned using a vibratome and cultured under different conditions for 48hrs. Tumor content and viability of the cultured slices was assessed by two independent pathologists. Characterization of the morphology and tumor microenvironment was done by immunofluorescence stainings. Presence and activation of T-cells upon stimulation either with a bispecific EpCAM-C3D3 antibody or a combination of α-CD3/α-CD28 antibodies was analyzed with flow cytometry and measuring IFNγ secretion. Permissivity of an oncolytic virus, VSV-GP, in these HNSCC slices was tested using a GFP-tagged OV by following the infection for 48hrs. Co-immunofluorescent studies were performed to analyze which cell populations could be infected by the OV.

**Results** The heterogeneous morphology of a human tumor could be retained in these slice cultures including the preservation of different cell types like tumor cells, immune cells and cancer-associated fibroblast. Upon stimulation with different antibodies the remaining cytotoxic T-cells showed functionality and could be activated. In addition, more than half of the HNSCC slice cultures were permissive to VSV-GP, so they were not only susceptible to the OV but could also produce new progeny. VSV-GP could not only infect epithelial tumor cells but also cells of the tumor microenvironment like stromal cells.

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