Disclosure Information R.A. Eladwy: None. **D. Chang:** None. **C. Li:** None. **D. Bhuyan:** None.

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

[1]MA Garcia Marquez*, [1]M Thelen, [1]E Bauer, [1]K Wennhold, [1]J Lehmann, [1]D Keller, [1]L Maas, [1]M Nicolik, [1]J George, [1]T Zander, [1]W Schroeder, [1]P Müller, [1]C Bruns, [1]R Thomas, [1]B Gathof, [1]A Quaas, [1]AM Hillmer, [1]M Peifer, [2]M von Bergwelt-Baildon, [1]HA Schlößer. *[1]University Clinic of Cologne, Cologne, Germany; [2]University Hospital, LMU Munich, Munich, Germany*

10.1136/jitc-2022-ITOC9.68

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 ofHLA 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 esophago-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 M.A. Garcia Marquez: None. **M. Thelen:** None. **E. Bauer:** None. **K. Wennhold:** None. **J. Lehmann:** None. **D. Keller:** None. **L. Maas:** None. **M. Nicolik:** None. **J. George:** None. **T. Zander:** None. **W. Schroeder:** None. **P. Müller:** None. **C. Bruns:** None. **R. Thomas:** None. **B. Gathof:** None. **A. Quaas:** None. **A.M. Hillmer:** None. **M. Peifer:** None. **M. von Bergwelt-Baildon:** C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; Astellas, Roche, MSD. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Significant; BMS. **H.A. Schlößer:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Astra Zeneca.

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

L Falceto Font*, C Francis, D Jin, B DiVita Dean, A Reid, K Long-James, L Deleyrolle, C Flores. *University of Florida, Gainesville, FL, USA*

10.1136/jitc-2022-ITOC9.69

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. C57/BL6 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.