Background Upregulation of inhibitory checkpoint molecules (ICM) on T-Cells and their ligands on AML blasts may be a mechanism of AML relapse after allogeneic stem cell transplantation (SCT). Better understanding of relapse biology may improve treatment efficacy.

Materials and Methods We examined peripheral blood (PB) and bone marrow (BM) samples of 5 AML patients (PTs) relapsing after SCT and PB from 5 healthy individuals, including 2 stem cells donors. ICM (PD-1, CTLA-4) ligand (CD86, PD-L1, PD-L2) expression on T-Cells and blasts was assessed by flow cytometry. PTs' PB was cultivated with 'KitM' (GM-CSF+PGE1) and without (Ctr) to generate leukemia-derived dendritic cells (DClea), followed by MLC enriched with PTs'/donors' T-Cells. After MLC, immune activation and functionality (degranulation, intracellular cytokine production, blast lysis) was assessed.

Results All PTs showed high expression of PD-1 on T-Cells, additional overexpression of CTLA-4 correlated negatively with responses to relapse treatment. Expression of ICM was low on T-Cells of 4/5 healthy individuals.

Influence of KitM on ICM/ligand expression:

a. ICM/ligand expression on uncultured T-Cells/blast: Contrary to H, PTs presented high expressions of CTLA-4 and PD-1 on PB T-Cells. PTs also showed high frequencies of PB/BM blasts expressing CD86.

b. DC/DClea in PB: Generation of DClea in AML and generation of DC in H was successful with KitM pretreated PB vs. Ctr.

c. ICM expression on T-Cells after MLC: MLC of KitM treated PB enriched with unstimulated PTs' T-Cells resulted in reduced frequencies of ICM-positive T-Cells in 3/5 PTs and increased frequencies of activated (leukemia specific) T-Cells in 3/5 PTs. Blast lysis was improved in 4/5 samples treated with KitM vs. Ctr.

Possible impact of ICM on clinical outcome (case study): PT1 suffered early relapses after 2 SCTs from her healthy father. A role of ICM in AML relapse was suggested by CTLA-4/PD-1 expression on her T-Cells and CD86 expression on her blasts. Also, >90% of the father's T-Cells expressed CTLA-4/PD-1, which might have contributed to treatment failure. In contrast, T-Cells from PT1's mother presented with low ICM levels, suggesting that she may have been a better donor. Stimulation of PT1's PB cells with KitM generated DClea decreased ICM expression and increased T-Cell activity. KitM pretreated samples showed improved blast lysis after MLC.

Conclusions Concisely, T-Cells and blasts of AML PTs relapsing after SCT uniformly expressed ICM and their ligands, possibly leading to inferior immune responses. High aberrant ICM expression on donor T-Cells (particularly CTLA-4) may be a reason for relapse after SCT by inhibiting antileukemic immune reactions. Further, generation of DClea through KitM triggers immune responses in MLC along reduced ICM expression on T-Cells, possibly reducing their inhibitory effects and thereby improving antileukemic responses.

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/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.

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