OTR5. GENOMIC EDITING, MEDIATED BY CRISPR / Cas9, OF PDCD1, CTLA4 E LAG3 *loci* IN T LYMPHOCYTES EXPRESSING CHIMERIC ANTIGENS RECEPTOR (CARS).

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INTRODUCTION The immune system plays an important role in tumor editing, being capable of eliminating this threat. The actual landscape of cancer treatment includes surgery, chemotherapy, radiotherapy and more recently immunebased approaches, such as the use of monoclonal antibodies, vaccines and adoptive immunotherapy. Different approaches to genetic modulate patients own immune response are being successfully used, such as CAR based treatment. However, occasionally tumor escapes. The Programmed Cell Death 1 (PD-1), Cytotoxic T-Lymphocyte-associated Protein 4 (CTLA-4) and Lymphocyte-activation gene 3 (LAG-3) pathways are constantly associated with cancer evasion from immune system. When associated with its ligands, PD-L1, CD80/86 and MHCII, respectively, these proteins inhibit T lymphocyte activation and proliferation, frequently blocking their effector function. Knockdown of PD-1, CTLA-4 and LAG-3 expression by genomic editing, may increase the antitumor functions of these cells. Recently, the Clustered Regularly Interspaced Short Palindromic Repeats system (CRISPR/Cas9) has emerged, as a new tool for site-specific genome editing.

OBJECTIVE We propose here a CRISPR-based genetic engineering system to knockdown PD-1, CTLA-4 and LAG-3 expression in T-cells rendering these cells resistant to checkpoint inhibitors mediated inhibition.

METHODOLOGY We designed gRNAs using the Optimized CRISPR Design program at *crispr.mit.edu* targeting the PDCD1, CTLA4 and LAG3*loci*. DNA sequences for the gRNAs were cloned into the CRISPR plasmid vector and electroporated into human peripheral blood mononuclear cells (PBMCs) and HEK293FT cell line. 24 hours later, DNA of CRISPR expressing cells was extracted by phenol-chloroform. A PCR was designed to amplify each target *locus*.

RESULTS The PCR products were cloned into the TA Cloning® Kit vector and the colonies were sequenced to validate the gRNAs. We are currently characterizing the functional edition of these *loci* by co-electroporating the CAR and the pool of gRNAs transcripts (*in vitro* transcription by MEGAshortscript™ kit), in PBMCs aiming tol evaluate the effective response of edited cells against CD19+, MHCII+, PD-L1+ and CD86+ cells by lysis assay, and their possible enrichment among the T cell population during *in vitro* lymphocyte expansion.

CONCLUSION We propose here a system to knockdown pathways largely used by tumors to inactivate the immune response. We will test the effects of PD-1, CTLA-4 and LAG-3 inactivation by CRISPR system in T Lymphocytes expressing a Chimeric Antigen Receptor.

KEYWORDS immunotherapy inhibitory receptors CRISPRCas9.