## ORT.18 - Screening of proteins related to the immunological checkpoint lymphocyte activation gene-3 (lag-3) through the BioID method.

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**Introduction:** Inhibitory receptors such as PD-1, LAG-3 and CTLA-4 have gained special attention as potential targets for immunotherapy, since manipulation of negative signals mediated by these receptors may provide new therapeutic options for several diseases, as cancer. LAG-3 was described as a cell surface molecule interacting with MHC class II molecules. Identifying how proteins transduce the signal from these receptors has been a challenge but, once identified, these molecules can also be targets for novel therapeutics. In 2012, a method called BioID was developed based on the fusion of a protein of interest to a mutated biotin ligase (R118G), which has the ability to add biotin to molecules that are at 20 nm or less from the protein of interest. Once biotinylated, the proteins can be recovered and identified by mass spectrometry.

**Objective:** To perform a screening of proteins interacting with LAG-3 through the BioID method.

**Methodology**: chimeric antigen receptors (CARs) were constructed with the anti-CD20 scFv fused to the intracellular domains consisting of: Lag-3 WT, Lag-3 Kmut, Lag3 EPdel (deleted EP domain), all fused to the BirA domain, with further induction of expression in the HEK293T and Jurkat cell lines. Flow cytometry analysis will be performed to verify the CAR's expression in the cells, and immunofluorescence assay to analyze the localization of the CARs.

**Results:** The CAR anti-CD20/Lag3 WT-BirA was electroporated in the HEK293T cells presenting 80% of expression. CARs Ep del and Kmut showed 39% and 41% of CAR expression. By immunofluorescence staining it was possible to observe the cytoplasmatic localization of the CARs. Analysis of the biotinylation pattern by Western blotting was performed for CAR anti-CD20/Lag3WT-BirA, and the expected ladder pattern of biotinylation was observed.

**Conclusion**: The constructed CARs were expressed in the target cell lines leading to the expected biotinilated patterns.

**Keywords:** BioID ; Chimeric antigens receptors; LAG-3