**BIO_03 - Evaluation of the affinity between single-chain M971 antibody fragments and the CD22 membrane glycoprotein for a CAR-T Cell**

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**Introduction:** Acute Lymphoid Leukemia (ALL) is one of the most aggressive types of cancer. The CD22 antigen is one of the glycoproteins expressed by leukemic B cells. CAR-T cell therapy is an example of one of the immunotherapies developed in order to achieve remission of this malignancy. This immunotherapy includes the step of expressing a Chimeric Antigen Receptor (CAR) in the patient's T Cell, which has the function of resizing cellular action, making it capable of recognizing specific antigens on the surface of tumor cells. The structure of the CAR is composed of several domains, including the one responsible for antigen recognition, such as the single chain variable fragment (scFv), which is formed by the VL and VH domains of an antibody, linked by a sequence of residues called a linker. Recently, results of clinical trials of two anti-CD22 CARs called short and long linker scFv CARs, both using the VH and VL domains of M971, began to be published. It has been described that the scFv with a short linker has a greater affinity to CD22 than the scFv with a long linker.

**Objectives:** To understand the structural differences in the short and long scFv/CD22 interface that lead to differences in affinity and to create a protocol that can be used to propose mutations in these scFvs to improve cell efficiency of cell CAR-T.

**Methodology:** Experiments using Molecular Dynamics (MD) simulations were performed in triplicate, with the trajectory analyzed using the MM/PBSA, to investigate the binding free energy (ΔG) between CD22 and scFvs. The structure of the Fab fragment of M971 and the D6-D7 domains of CD22 is deposited in the PDB 7O52. The scFv/CD22 complexes, with CD22 inserted into a lipid membrane model, were constructed from the crystallographic structure, with missing regions modeled and subjected to minimization and thermalization protocols to produce trajectories by MD in triplicates.

**Results:** As a result, it was observed through RMSD and PCA analyzes that the short and long scFv/CD22 complexes had similar structural stability. The average ΔG of the scFv/CD22 systems are close to the experimental ΔG. Therefore, the use of the membrane model in the simulations influenced the ΔG result with greater accuracy. Finally, analyzes also made it possible to identify residues in the scFvs that most contribute to the attractive and repulsive interaction, making it possible to suggest mutations to enhance affinity and specificity, with residues R52 and R56 being the main candidates for mutation.

**Conclusion:** The results achieved generated a protocol that can be used to propose mutations in scFvs to enhance affinity to CD22 and propose more effective CARs for CAR-T Cell therapy.

**Keywords:** scFvs from M971 anti-CD22; Molecular Dynamics; MM/PBSA