BIO_07 - Study, by Molecular Dynamics simulation, of structural determinants of single-chain M971 antibody fragments for an anti-CD22 CAR-T cell

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Introduction: The Chimeric Antigen Receptor (CAR) is recombinant protein expressed in T cells. The Single Chain Variable Fragment (scFv) formed by the VH and VL portions of a monoclonal antibody and the *linker*, which connects the two domains, is the CAR structure capable of detecting tumor antigens. Two anti-CD22 CARs that are already in clinical trials use the M971 derived scFv, one using a short *linker* scFv (GGGGS) and the other using a long *linker* scFv (GGGGS)4, with the short *linker* scFv having the highest affinity for the CD22, since the short *linker* in this scFv favors the formation of nanoclusters. Knowing this, it becomes necessary, to begin with assessing whether the *linker* size in the M971 scFvs affects the structural equilibrium of the scFv, using Molecular Dynamics (DM) simulation.

Objectives: Model the structure of two M971 scFvs and submit them to DM simulation, in order to assess whether *linker* size change in M971 scFvs affects structural equilibrium in aqueous solution.

Methodology: 3D scFv structures have been constructed with VH and VL derived from the M971 antibody (code PDB 7O52) connected with a short and long *linker* in *Modeller 10.1 software* using the addition of missing residues protocol. Then, each scFv model have been simulated at 500 ns in the *GROMACS 2018.3* package, using the CHARMM36m force field, with TIP3P water model and 0.15 M concentration of Na+ and Cl- ions. The structural equilibrium was determined by the Root Mean Square Deviation (RMSD).

Results: The RMSD profiles show that in the short *linker* scFv the VH+VL, VH and VL portions reach structural equilibrium after 50 ns of simulation (RMSDs 0.19 ± 0.02, 0.15 ± 0.01 and $0, 12 \pm 0.01$ nm, respectively) and in the long *linker* scFv the VH+VL, VH and VL portions reach structural equilibrium after 100 ns of simulation (RMSDs 0.21 ± 0.02, 0.13 ± 0.02 and 0.17 ± 0.02 nm, respectively). The two types of *linker* were not considered in the RMSD analyzes because of their structural flexibility.

Conclusion: The analysis of the RMSD profiles suggests that the scFv with short *linker* presented greater apparent structural equilibrium, since the VH+VL and VL portions had less structural modifications. However, the VH domain presented more conformational changes compared to the VH portion of the scFv of long *linker*. These results indicate that the lower structural movement of the VH+VL domain of the short *linker* scFv compared to the long *linker* scFv can justify the formation of nanoclusters. Lastly, both scFvs can be used to study the interaction with CD22, in order to analyze whether the same structural behavior of the scFvs will persist and how the size of the *linker* in the scFvs will influence the interaction of the scFv/CD22 complexes.

Keywords: CAR anti-CD22, scFv from M971, Molecular Dynamics