

## **BIO.12 - *In silico* directed evolution in antibody engineering: a promising approach to improvement antitumor biopharmaceuticals.**

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**Introduction:** The successful introduction of antibody-based immunotherapies into the arsenal of treatments for cancer patients has been reinvigorated by antibody engineering technology. New antitumor strategies include enhancement of T-cell responses provided by monoclonal antibody activation of costimulatory molecules present on T-cell surface. OX40, a member of the TNF receptor superfamily (TNRFS4), is a key T-cell costimulator and a promising cancer immunotherapy target. Currently, several pharmaceutical companies have invested in clinical studies using mAb anti-OX40 for cancer and/or autoimmune treatment. In this context, *in silico* antibodies engineering emerges as a promising approach to develop novel biopharmaceuticals with improved specificity and affinity.

**Objective:** To develop an *in silico* strategy for novel biopharmaceutical development, using OX40 mAb as a model.

**Methodology:** Heavy and light chain amino acid sequences of anti-OX40 antibody were obtained from a patent prospection in the Integrity database (patent number: WO2018/178074). The human-scFv (single-chain variable fragment) model was constructed by comparative molecular modeling through the Modeller software. Predicted model quality was evaluated using Molprobit and Verify3D servers. ScFv model was subjected to molecular docking against the OX40 structure (PDB: 2HEV) on Cluspro server. The best complex according to Cluspro parameters was submitted to Robetta Alanine Scanning server to identify hotspots. Point mutations were defined using DUET server and performed on specific amino acids in order to increase the interaction and stability of the complex. Thereafter, a new molecular docking with mutated scFv's was performed in order to compare the results before and after the mutations.

**Results:** Anti-OX40 antibody was constructed by comparative modeling using as model PDB archive 6EHY that presented 84% amino acids sequence homology. An initial docking was performed. The scFv+OX40 complex was submitted to alanine scanning server. Seven important hotspots for the complex stability were identified in the scFv CDR's (complementarity determining region). Besides these, four possible mutation points in the CDR's were also identified. The choice of amino acids substitute was performed on the DUET server. Three substitutions were inferred as possibly being able to increase the complex stability (SER>ASP, SER>MET, ASP>LEU). This step originated seven variants of scFv. After a new docking with all possible mutants scFv's, it was possible to observe an increase in predicted complex interaction in 4 of 7 models tested, according to the parameters defined by the Cluspro server (members and weighted score). In the best result, with only one amino acid mutation, it was observed an increase in the Cluspro score from 124 to 249 members and, from -309,9 to -355,1 (Weighted Score) when compared to native molecule.

**Conclusion:** The proposed workflow resulted in improved predicted antibodies that showed increased *in silico* stability and better interaction with its correlated antigen, when compared to native molecule. *In silico* methods emerge as a promising approaches for antibodies rational design.

**Keywords:** cancer; monoclonal antibody; bioinformatics