BIO_04 - Structural modeling and design of scFv fragments from the antineoplastic antibody Brontictuzumab for enhanced binding to Notch1 NRR region

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Introduction: Dysregulation of the Notch pathway, involved in cell development and homeostasis, has been implicated in various diseases, including cancer. Several inhibitors targeting the Notch proteins have been developed to prevent the pathological overactivation of this pathway. For example, the Brontictuzumab monoclonal antibody (BRON) targets the NRR region of the Notch1 receptor, preventing its proteolytic activation by obstructing access to the S2 cleavage site. Thus, BRON emerges as a promising scaffold for developing antibodies in alternative formats targeting Notch1.

Objectives: Model and optimize a set of antibody fragments in the single chain Fragment variable (*scFv*) format derived from BRON targeting Notch1 NRR region.

Methodology: Three *scFv* fragments were modeled with RoseTTAFold and AlphaFold2. The validated 3D models were used to test the interactions with NRR (PDB: 3L95) via molecular docking using ClusPro Antibody Mode. The resulting complexes went through heated Molecular Dynamics (MD) to distinguish true binding modes from incorrect ones. Then, the scFv-NRR complexes were evaluated with two 400-ns independent MD replicates run with Amber18. MM/GBSA residue decomposition and mCSM-Ab2 alanine scanning were applied to explore the *scFvs* hot spots, and the mCSM-Ab2 saturation mutagenesis tool was used to propose affinity- enhancing mutations in the most accessed conformations for each MD trajectory.

Results: The proposed antibody fragments consisted of BRON's light and heavy variable chains connected by glycine-rich linkers with lengths of 9, 12, and 15 residues. Docking to NRR resulted in 90 complexes that, after initial selection, were submitted to heated MD. We obtained one stable complex for each *scFv* where the antibody fragment formed interactions with the S2 site in at least 30% of the simulated time. The three complexes also showed stability throughout the 400-ns MD and had interaction hot spots at positions 106, 161, and 223 of the *scFvs*. From nearly 1000 point mutations performed to each scFv conformation, four consensus mutations that increased the $\Delta\Delta G_{affinity}$ in at least 2 kcal/mol were found for the two tested conformations extracted from the MD replicates of the *scFv* with the 9-residues linker. In addition, five and four consensus mutations were observed for the 12-residue and 15-residue ones. Further *in silico* validation is required to determine the impact of the mutations on the interaction with NRR.

Conclusion: We proposed and optimized three antibody fragments based on BRON with a better predicted affinity towards the NRR region of Notch1 that could block the S2 site and prevent the activation of the receptor.

Keywords: Notch pathway, Cancer, Antibody fragments