BIO_29 - Reconversion of rituximab's scFv into FvFc: a suitable format to understand CDR mutations of monoclonal antibodies

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Introduction: Antibody-based therapies (immunotherapies) have been used to treat diseases such as cancer and autoimmune disorders. Phage display is often used to develop new antibodies by panning an antibody fragment from a diverse library against a specific target. Different selection strategies can be used to generate entirely new sequences or to improve the affinity of an existing molecule. CD20 is a target and several molecules have been developed against this receptor. The mechanism of action of such molecules is influenced by their affinity to the target. Understanding antibody affinity is relevant to propose therapies such as CAR-T cells and monoclonal antibodies.

Objectives: The aim of this work was to obtain mutated versions of the variable region of a commercial antibody (rituximab) to improve its affinity to CD20.

Methodology: A library was constructed using error-prone PCR to amplify and randomly mutate the scFv sequence of rituximab. This library was expressed on the surface of phages and the phages were selected against the epitope of rituximab (synthetic peptide). Three rounds of selection were performed, with low binding phages washed out after incubation with the peptide. Sequences from round 3 of the selection were obtained by next- generation sequencing on the Illumina® platform. Using an AuTomated Tool for Immunoglobulin Analysis (ATTILA), heavy and light variable chain (V H and VL) sequences were analyzed and selected based on their enrichment (compared to the original library).

Results: The size of the library was 1.4x105 CFU. A highly enriched clone (Mut1) carrying a HCDR3 mutation was analyzed. DNA sequences of wild-type rituximab (WT) and Mut1 were cloned into a mammalian expression vector and produced using the Expi293® system in an scFv-Fc format. CD20 binding of both WT and Mut1 was confirmed by flow cytometry to a similar extent. A flow cytometry-based cell viability assay showed an increase in 7-AAD (viability dye) staining of 6%, 16.7%, 15% and 17.3% for untreated (buffer), MabThera®, wild-type rituximab and Mut1, respectively.

Conclusion: Further characterization by biolayer interferometry and other Fc-dependent effector functions will elucidate the effect of CDR mutations and affinity on the CD20+ cell depleting capacity of rituximab.

Keywords: Cancer immunotherapy; Phage display; Antibody fragments