**BIO_07 - Selection of human antibody fragments by phage display: development of new anti-CD19 molecules for CAR-T cell therapies**

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**Introduction:** Targeted immunotherapy approaches, such as chimeric antigen receptor (CAR) T cell therapy, are successful alternatives on the treatment of hematological malignancies such as leukemias and lymphomas. CD19, a transmembrane B-cell receptor, is an attractive target for immunotherapy because it is expressed on almost the entire B-cell life cycle. CAR structure consists of a single-chain fragment variable (scFv), which comprises antibody heavy and light chain variable domains, a transmembrane domain and at least two intracellular signaling/activation domains. Engineered CART-cells recognize the tumor antigen by scFv interaction and induce cancer cell death.

**Objective:** Select new anti-CD19 human antibody fragments, using a phage display approach, through biopanning against synthetic CD19 external loop peptide.

**Methodology:** The previously constructed Fab DNA library (variable chains cloned into pComb3XSS vector) was transformed into Escherichia coli (XL1-Blue) through electroporation. After regeneration in SOC medium, the culture titer was calculated and submitted to phage production by infection with helper phage. Phages were submitted to four rounds of selection against synthetic biotinylated human CD19 peptide. Two streptavidin Elisa plate coated wells were used for each round. Unbound phages were removed by progressively increased washes (5, 10, 15 and for rounds 1, 2, 3 and 4, respectively). After each round, phages were eluted using acidic solution and used to re-infect XL1-Blue for the following round. Titration of phages before and after each round was carried out by XL1-Blue infection to track selection, and the plasmid DNA of fifteen randomly selected bacteria of last round selection was purified for sequencing.

**Results:** The initial electroporation with the library phagemids rendered 2.64x108 CFU, twice the estimated library size. After each round of selection, the input (I) and output (O) titers were calculated and the ratio I/O of each round was $8.4 \times 10^5$, $1.8 \times 10^{10}$, $1.65 \times 10^9$, and $8.0 \times 10^9$, for rounds 1, 2, 3 and 4, respectively, suggesting phage selection after the 3rd round. Primers comprising the VL region of the selected Fab were used to amplify that region by PCR and cloned into pGEM-T plasmid. Sequencing of the fifteen clones showed only three different VL chain sequences, with a frequency of six, five and four clones. All sequences were compatible with consensus regions of immunoglobulin light chain regions.

**Conclusion:** Previous analyses revealed a highly diverse library of 1,08x108 clones, which emphasizes that the four phage display rounds selection were able to decrease diversity and select enriched three VL sequences out of this repertoire. Further experiments by New Generation Sequencing (NGS) will expand the analysis range and show all sequences that were preferably selected to bind to CD19 synthetic peptide, make it possible to design new scFv-format molecules (VH-linker-VL). These scFv molecules can be used to construct different antibody-based therapies, including new monoclonal antibodies and CAR-based cell therapy.

**Keywords:** Phage display; CAR-T cell; CD19