Discovery of anti-CD20 antibodies from Semisynthetic phage-scFv libraries: ALTHEA 4x4 Platinum Libraries™

Gómez-Castellano KM[1,2], Cortés-Paniagua MI, Guzmán-Bringas UO[1,2], Alvarez-Fosado TI, González-González E[1,2], Hernandez- Ruiz M., Pérez-Tapia SM[1,2], Almagro JC. [1,2,3]

1 Unidad de Desarrollo e Investigación en Bioterapéuticos (UDIBI), Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, 11340, México
2 Laboratorio Nacional para Servicios Especializados de Investigación, Desarrollo e Innovación (I+D+i) para Farmoquímicos y Biotecnológicos, LANSEIDI-FarBiotec-CONACyT, México
3 GlobalBio, Inc. 320 Concord Ave, Cambridge, MA 02138, USA.

Abstract

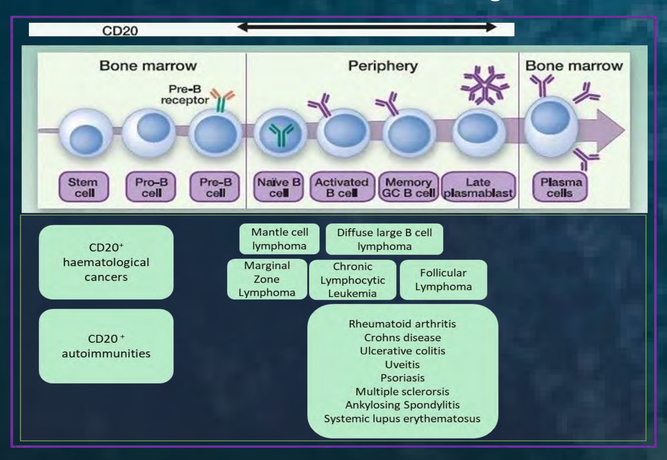
CD20 is non-glycosylated phosphoprotein expressed on the surface of mature undifferentiated B-cells. It is involved in B-cell receptor activation, proliferation, and Ca^{2+} transport. The high levels of expression of CD20 on activated and malignant B-cells makes CD20 a therapeutic target.

Patients with CD20-positive B-cell malignancies such as several autoimmunities lymphoma and leukemia have shown a significant improvement with the introduction of targeted therapy with the recombinant monoclonal antibodies. Fc receptors, mainly expressed on the surface of natural killer cells and macrophages, facilitates complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity/phagocytosis (ADCC/ADCP), induce antiproliferative and pro-apoptotic effects, resulting on better outcomes in patients with B-cell malignancies.

In order to isolate human antibodies that specifically recognize CD20, ALTHEA 4X4 Platinum Libraries TM was panned against Daudi Cells. ALTHEA 4x4 Platinum Libraries TM are Semi-synthetic human scFv phage display libraries (size ≈1 × 10^{10}) based on VH scaffolds cover 4 IGHV families (IGHV1, IGHV3, IGHV5 and IGHV6) out of the seven human IGHV gene families and VL scaffolds cover 3 IGKV families (IGKV1, IGKV3 and IGKV4) out of the six IGKV gene families. Further, the HCDR3 diversity was generated with an exclusive pool of natural H3J fragments obtained from 200 donors. Therefore, it is expected to generate a highly diverse set of well-expressed antibodies, recognizing diverse epitopes on CD20 molecule.

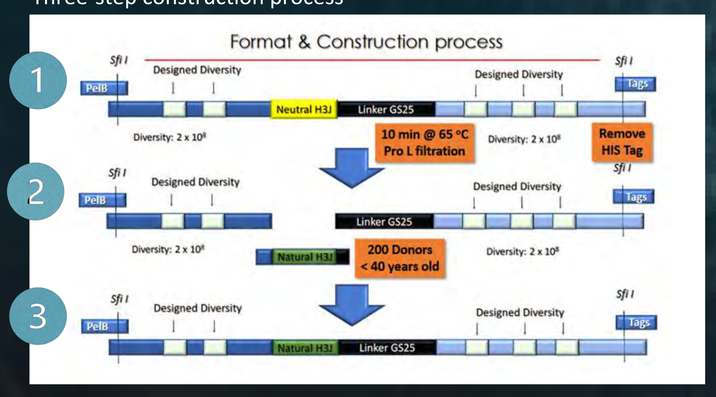
After three rounds of solution panning using Daudi cells as selector, a total of 69 clones (76%) were positives and were potential binders to CD20. The selection of the best clones is based on binding and functional assays.

CD20 B cell associated malignances

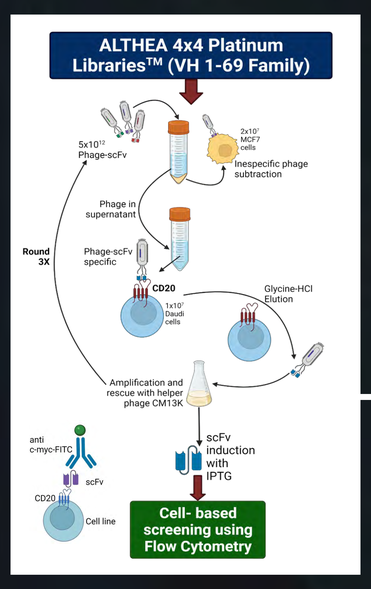


Generation of ALTHEA 4x4 Platinum Libraries™

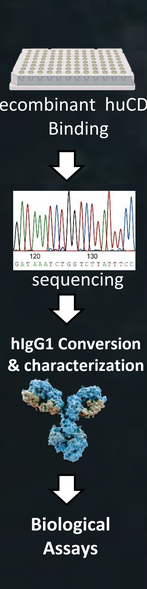
Three-step construction process



Biopanning & Screening

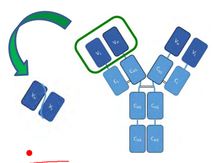


Next Steps

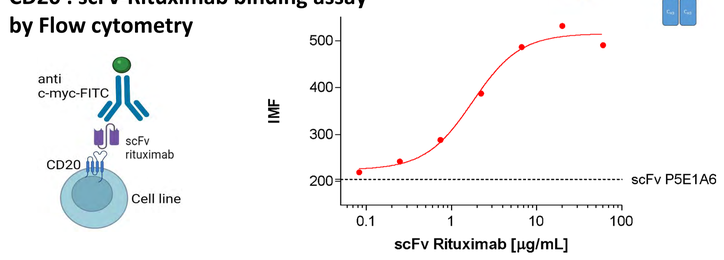


Generation and expression of scFv-Rituximab (positive control)

Rituximab is a chimeric monoclonal antibody employed for the treatment of CD20-positive B-cell malignancies.



CD20 : scFv-Rituximab binding assay by Flow cytometry



scFv-Rituximab was generated and validated as positive control for the following anti-CD20 (scFv format) screening. scFvP5E1A6 was used as negative control.

Phage-Antibody Selection

Table 1. Output of the panning of the phage-displayed ALTHEA 4x4 Platinum libraries on Daudi cells.

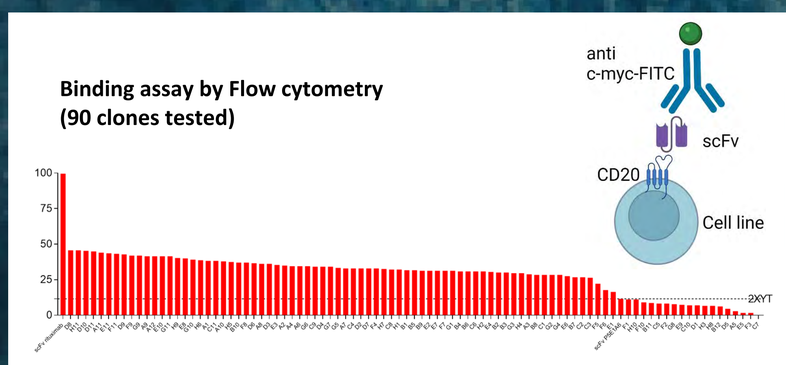
Experimental conditions			QC	Results
Round number	Target Concentration	INPUT (Total virions)	PCR control (scFvs)	Output Titre Total CFU***
Round 1	1x10⁷ Daudi cells	ALTHEA 4x4 Platinum Libraries (Fam 1-69) (5 x10¹² virions)	10/10	6.4x10⁵ CFU
Round 2	1x10⁷ Daudi cells	Phages Round 1 (5 x10¹² virions)	10/10	8.5x10⁴ CFU
Round 3	1x10⁷ Daudi cells	Phages Round 2 (5 x10¹² virions)	10/10	1.02x10⁸ CFU

After 3rd round of panning an increase in the phage titer was observed, suggesting enrichment for specific clones

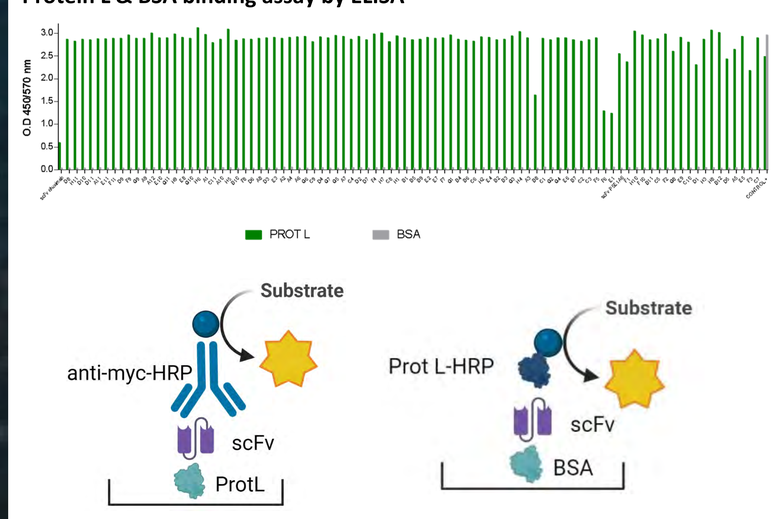
scFv Expression and Specific Binding to Daudi cells

The supernatants containing the IPTG-induced scFvs were tested in three primary assays: 1.- Binding to Daudi Cells 2.- scFv expression (Protein L) 3.- Unspecific binders (BSA)

Binding assay by Flow cytometry (90 clones tested)



Protein L & BSA binding assay by ELISA



Clones with a read out of 25% or more, as compared with the scFv-Rituximab positive control, were considered potential binders.
We got 77% positive individual clones by flow cytometry
Clones will be selected based on the sequence of specific and unique scFvs.

For Information, please contact:

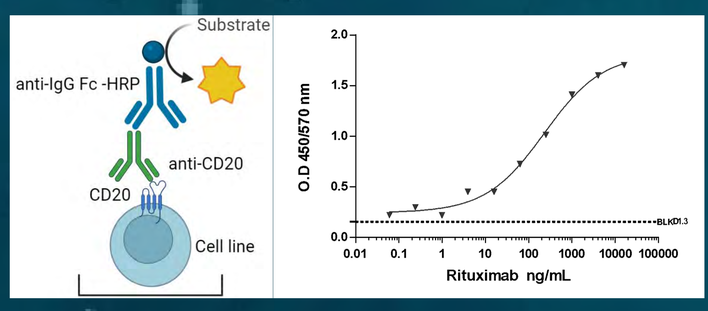
Juan C. Almagro, Ph.D.
juan.c.almagro@globalbioinc.com
Tel: +1.617.710.4487
www.udidi.com.mx

Sonia Mayra Perez Tapia, Ph.D.
Mayra.Perez@udibi.com.mx
Tel: +55.52.55.5498.6682
www.udidi.com.mx

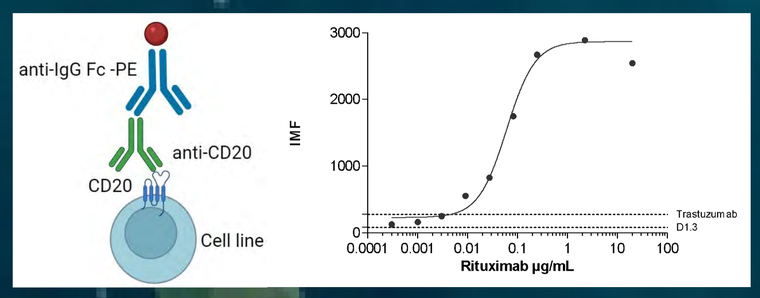
Antibody Functional Assays

We have developed and validated biological assays to test the mechanisms of action of anti-CD20 selected clones:
1) Binding to CD20 on Daudi cells by ELISA
2) Binding to CD20 on Daudi cells by Flow cytometry
3) CDC Assays (Complement-Dependent Cytotoxicity)

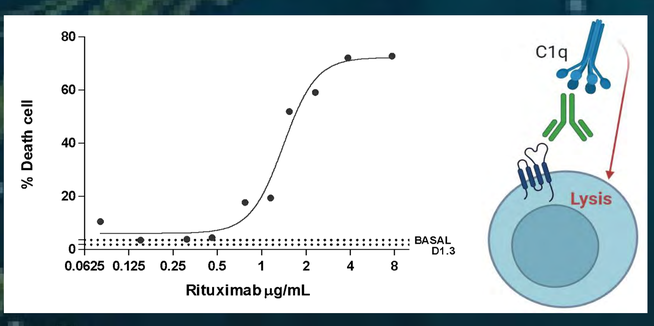
CD20 binding assay by ELISA



CD20 binding assay by Flow cytometry



Anti-CD20 mediated cytotoxicity assays



Rituximab mechanisms of action comprise the binding of its Fab domain to CD20⁺ B-lymphocytes for the induction of apoptosis, either directly or throughout the recruitment of immune effector functions by its Fc domain, thus mediating B-cell lysis through complement-dependent cytotoxicity mechanism (CDC), after binding to C1q, or antibody-dependent cellular cytotoxicity mechanism (ADCC) once is recognized by the Fcγ receptors (FcγRs) of effector cells.

Summary

- **CD20 is associated to several malignances and therefore one of the most important therapeutic targets.**
- **CD20 expression is specific, since it is present on B cell stages that give rise to autoimmunities, Chronic Lymphocyte Leukemia and B cell lymphomas, but not in other essential hematopoietic stem cells.**
- **A whole cell panning strategy was used to increase the probability to isolate clones recognizing the three-dimensional structure of a complex molecule such as CD20.**
- **The assays to test one of main mechanisms of action (CDC) of the anti-CD20 candidates are well stablished in our laboratory. ADCC assay and apoptosis assays are being optimized.**
- **Mexico is one of the countries with higher prevalence on rheumatoid arthritis. 75% of positive cases are females while 25% are males. Other susceptibility risk factors include genetics, overweight, obesity and smoking.**
- **Developing anti-CD20 therapeutic antibodies is essential for the Mexican population, since according to the latest WHO data published in 2018 RA deaths in Mexico reached 1,432 or 0.25% of total deaths. The age adjusted Death Rate is 1.26 per 100,000 of population ranks Mexico #3 in the world.**

References

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