Isolation, characterization and optimization of an anti-TNF antibody

Gómez-Castellano KM[1,2] , Arrieta-Oliva HI [1,2], Guzmán-Bringas UO[1,2], Camacho-Sandoval R[1,2],Vázquez-Leyva SK[1,2], Vallejo-Castillo LA[1,2], González-González E[1,2], Ávila-Flores S[1,2], 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.

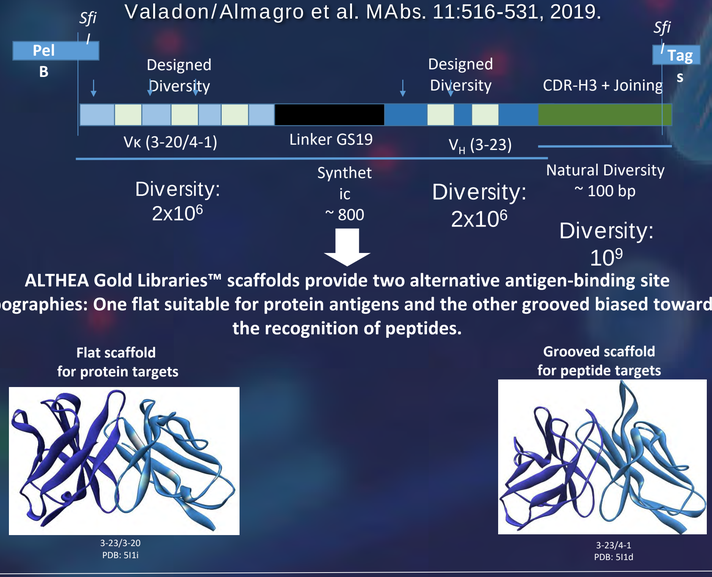
Abstract

The goal of the present study is to develop and optimize a promising anti-TNF-α antibody from ALTHEA Gold Libraries™, a platform previously shown to be a valuable source of high affinity, highly stable and biologically active specific antibodies in order to validate therapeutic targets. Panning with recombinant Tumor Necrosis Factor alpha (TNF-α) and screening through in vitro assays to test its biological activity, led to the isolation of nine antibody fragments with anti TNF-α specificity. One of these antibodies displayed ideal characteristics to be considered as the lead clone.

Later, a process to optimize the affinity was implemented leading to the selection of an antibody with higher affinity than the parental clone. The affinity maturation of the antibody correlated with an increase in the biological activity in vitro. The results suggest that the interaction with TNF-α is mainly governed by the VH domain, hence a deeper knowledge of such interaction could lead to design a strategy to obtain an antibody with similar or superior affinity compared with reference therapeutic antibodies.

ALTHEA Gold Libraries™ as a platform to discover therapeutic antibodies

Valadon/Almagro et al. MAbs. 11:516-531, 2019.



ALTHEA Gold Libraries™ scaffolds provide two alternative antigen-binding site topographies: One flat suitable for protein antigens and the other grooved biased towards the recognition of peptides.

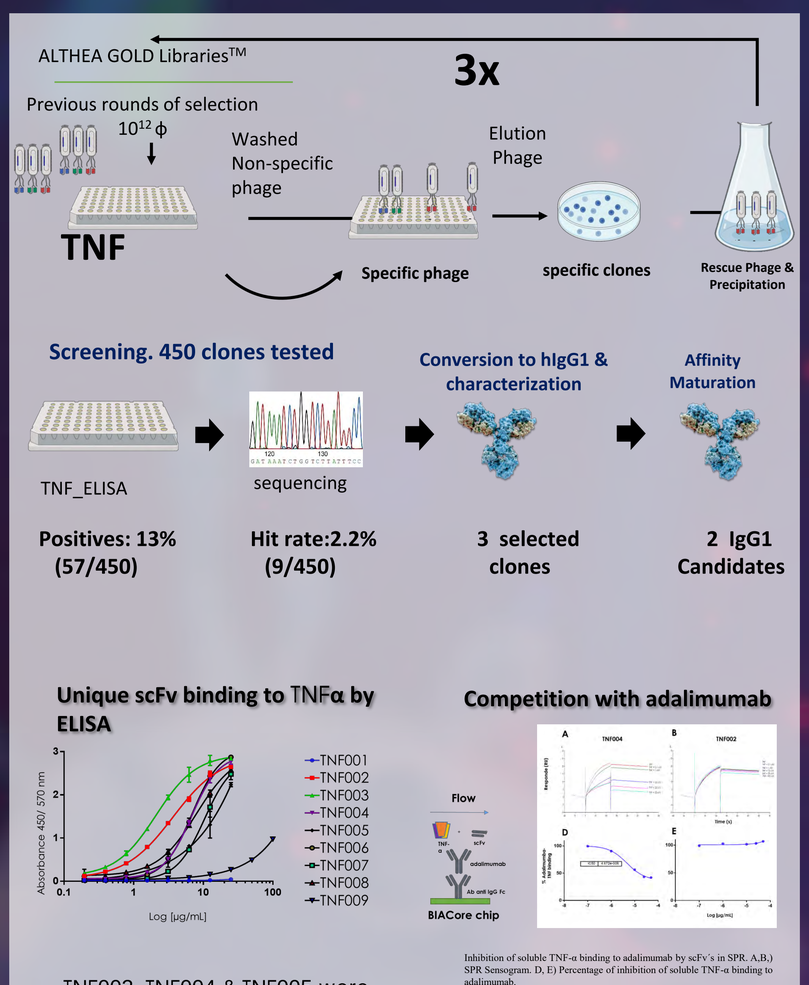
Flat scaffold for protein targets

3-23/3-20
PDB: 5i1i

Grooved scaffold for peptide targets

3-23/4-1
PDB: 5i1d

Panning and screening for scFv anti TNF-α



Unique scFv binding to TNFα by ELISA

TNF003, TNF004 & TNF005 were selected to be converted to hIgG1

Competition with adalimumab

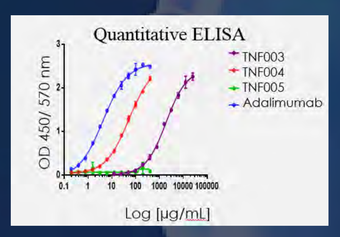
Inhibition of soluble TNF-α binding to adalimumab by scFv's in SPR. A,B,) SPR Sensogram. D, E) Percentage of inhibition of soluble TNF-α binding to adalimumab.

Characterization of the anti TNF-α IgG1

Table 1. Summary of the anti-TNF-α IgGs characteristics

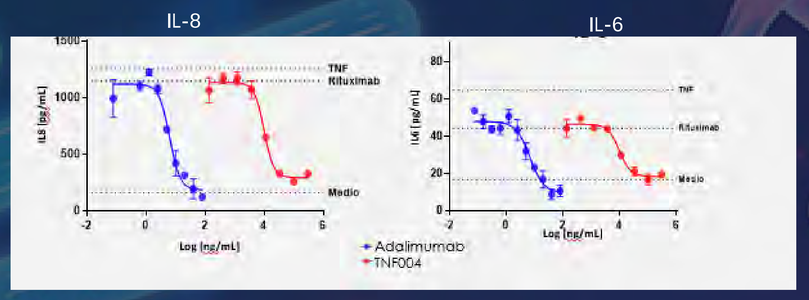
Clone	Yied HEK 293 (mg/L)	[1]EC_{50} (M)	[2]KD SPR (M)	[3]Biological activity
TNF003	26	$1.2x10^{-9}$	$1.2x10^{-8}$	*ND
TNF004	15	$3.4x10^{-10}$	$3.1x10^{-9}$	Yes
TNF005	23	*ND	*ND	*ND
Adalimumab	ND	$4.6x10^{-11}$	$9.4x10^{-11}$	Yes

[1]IgG1 EC_{50} determined by quantitative ELISA
[2]IgG1 KD determined by quantitative SPR
[3]Biological activity determined as neutralization of soluble TNF-α and binding to membrane TNF-α.
*ND: Not Determined



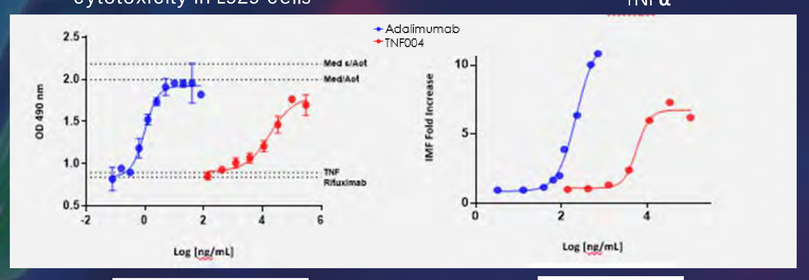
In vitro biological activity of anti-TNF-α TNF004 clone

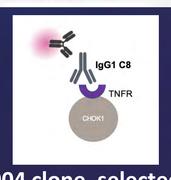
TNF-α induction of IL-8 and IL-6 in HUVEC cells is inhibited by anti TNF-α selected antibody



Inhibition of TNF-α induced cytotoxicity in L929 cells

Binding to transmembrane TNFα

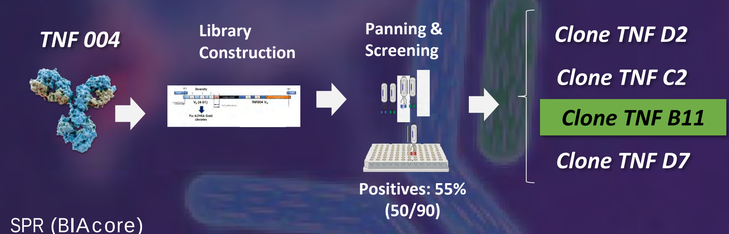




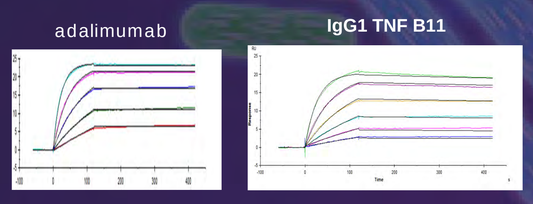
We performed affinity maturation of the IgG1 TNF004 clone, selected due to its capacity to neutralize TNF-α and bind to transmembrane TNF-α, to improved its potency

TNF004 Affinity Maturation by reshuffling diversity in the HCDR1 and HCDR2

A second set of antibodies was generated by reshuffling diversity of the HCDR1 and HCDR2 regions from the TNF004 clone. The four best clones, chosen based on its capacity to bind TNF-α were converted into hIgG1, and expressed in HEK293 cells



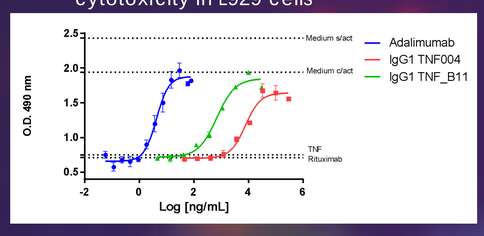
SPR (BIAcore)



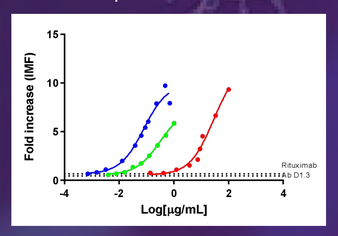
IgG1	KD (M)
Adalimumab	9.47E-11
TNF004	3.16E-09
TNF D2	3.75E-09
TNF C2	4.44E-10
TNF B11	5.33E-10
TNF D7	7.07E-10

In vitro biological activity of anti-TNF-α B11 clone

Inhibition of TNFα-induced cytotoxicity in L929 cells



Binding to transmembrane TNFa expressed on CHO K1 cells

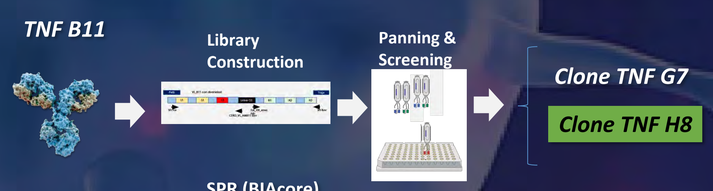


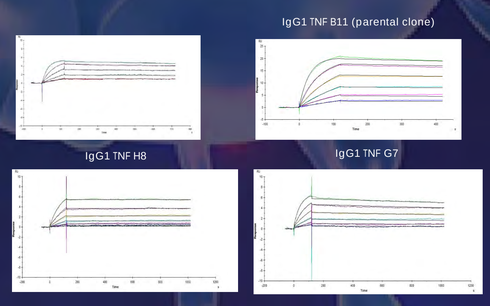
The increased in the affinity of anti TNF-α B11 correlates with an increase in the ability to:
1. Neutralize soluble TNF-α and
2. Bind to transmembrane TNF-α.

IgG1 TNF B11 was selected as the new lead candidate for a new round of affinity maturation

TNF B11 Affinity Maturation by reshuffling diversity in the LCDR3 region

Starting from TNF B11 a three set of antibodies was generated by reshuffling diversity in LCDR3. The clones with best ELISA binding to TNF-α were converted to hIgG1, expressed in HEK293 cells and further characterized yielding two final candidates.

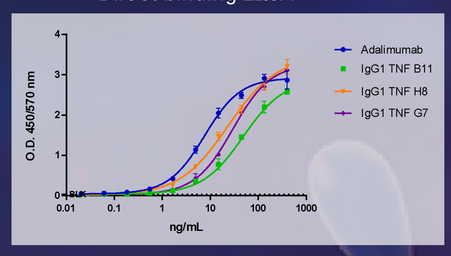




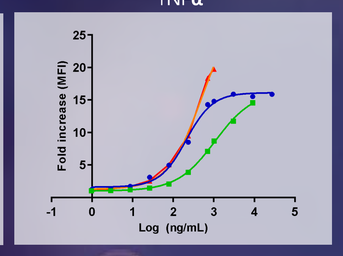
IgG1	KD (M)
Adalimumab	6.3758E-11
TNF B11	5.33E-10
TNF H8	1.82375E-11
TNF G7	5.53924E-10

Biological activity of anti-TNF-α antibodies

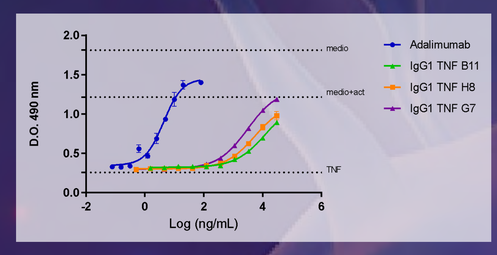
Direct binding ELISA



Binding to transmembrane TNFα



Inhibition of TNF-α induced cytotoxicity in L929



Summary

- Discovery of a panel of 9 scFvs specific for TNFα from ALTHEA Gold Libraries™
- The lead TNF004 inhibits IL-8 and IL-6 production induced by TNFα in HUVEC cells and inhibits cytotoxicity induced by TNFα in L929 cells.
- Affinity maturation of TNF004 clone resulted in the TNF B11 clone, which showed higher biological activity in vitro
- IgG1 TNF H8 was selected as the new lead candidate based on characteristics such as aminoacidic sequence, monomeric content, affinity and biological activity
- The strategy applied to discover scFv specific for TNF-a was successful as shown by the biological characterization. A new extended screening from TNF B11 and a new strategy of maturation are purposed

Reference

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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

The anti-TNFα lead antibody obtained from ALTHEA Gold Libraries™ is a promising candidate for therapeutic development.

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