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STRATEGIES TO IMPROVE PRODUCTIVITY OF CHO-S CELLS EXPRESSING AN ANTI-TNFα MAB WITH BIOSIMILAR POTENTIAL

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ABSTRACT

ADABUT CELL LINE GENERATION

Tumor necrosis factor alpha (TNFα) is a proinflammatory cytokine that mediates the homeostasis of immune responses; its exacerbated production is associated with the pathogenesis of autoimmune and chronic inflammatory diseases. TNFα antagonists have revolutionized the treatment of inflammatory conditions such as rheumatoid arthritis and Crohn's disease. Currently, a worldwide race is on stage for the production of biosimilars moved by patent expiration of monoclonal antibodies (mAbs), such as anti-TNFα adalimumab. This project is based on the first development stage for an adalimumab biosimilar candidate with potential for national production through the generation of a productive and stable cell line and assessment of its functionality. The robotic system ClonePix-FL was used for screening and isolation of colonies from transfected CHO-S stable pools plated in semisolid medium. Selected clones were expanded based on growth and productivity (Figure 1-2). Purified mAbs from different clones were tested for binding and functional activity. The binding affinity of the denominated adabut clones to TNFα (Figure 3) and FcRs, tested by surface plasmon resonance, did not differ statistically when compared to reference adalimumab. One functional activity assay demonstrated the antibody neutralization capacity of the cytotoxicity induced by TNFα in L929 murine fibroblasts. A second assay confirmed adabut as an antagonist of the TNFα activity by the inhibition of the cell adhesion molecule expression in HUVEC cultures (figure 4). The binding kinetics and functional analysis performed suggest a potential for further development of adabut as a biosimilar.

CULTURE CONDITIONS

The process of the cell line development for adabut generated data consisting of cell growth/viability, volumetric and specific productivity, antigen binding kinetics, functional assays and long-term stability, from which 3 clones were selected. One clone (123) was top ranked and 2 others (70 and 225) showed equivalent performance. To increase the basal productivity of clone 123, 0.6g/L, we run 24 experiments evaluating 6 basal media and 9 supplements from 4 vendors. A temperature shift to 32 °C on day 6 was applied on some conditions. The experiments were conducted in shaker flasks along 14 days or while cell viability was above 60%. The flasks were sampled daily for cell counting/viability by Vi-Cell XR cell counter and glucose monitoring. Samples were stored for additional metabolites and antibody concentration assessment. By testing 24 different fed-batch (FB) strategies we were able to increase the volumetric clone productivity while maintaining structure integrity. Table 1 shows the 7 best conditions for clone 123, with titer/IVCC shown on Figure 5. The mAbs were purified by protein-A capture from clarified supernatants taken on days 14, 16 or 17 of FB cultures. Analyses consisted of SDS-PAGE, SEC-HPLC, IEF and IEX-HPLC (Figure 6), in comparison to the reference. The FB condition 2 was elected to cultivate the 3 top clones (Figure 7). At day 14 the duplicate runs were stopped, presenting viability over 95%. The clarified supernatants were fully purified through protein A capture, acid pH hold, Q and S sepharose resins. The mAb integrity was evaluated by SDS-PAGE (reduced and non-reduced), IEF, SEC-HPLC and IEX-HPLC. The purity was above 99-100% for all samples. IEX-HPLC and IEF analyses allowed the identification of fractions very similar to the reference.

RESULTS

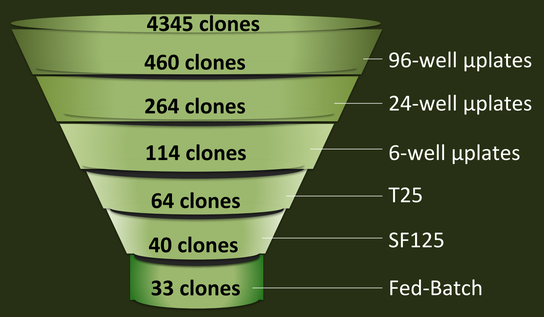

Figure 1: Expansion of the adabut selected clones based on growth and productivity. The clones were expanded in 24 and 6-wells plates, T25 and SF125 flasks, as confluence showed >70% and/or were chosen along the process based on specific productivity. The productivity was measured by ELISA or SPR (Surface Plasmon Resonance) in a BIAcore T-200 system (GE Healthcare).

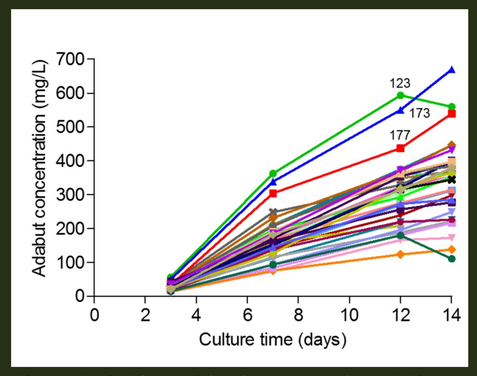

Figure 2: Batch - with glucose supplementation - experiment of 33 selected adabut clones in SF-125. Glucose was added to the cell culture on days 3 (4 g/L), 5 (4 g/L) and 7 (6 g/L). The concentration of adabut mAb in the supernatant was measured by SPR

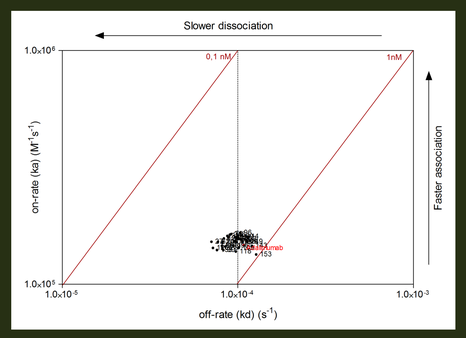

Figure 3: Binding affinity of 33 adabut clones to recombinant TNFα. The graphic represents the relationship between constants of association (ka), expressed in 1/M.s, and dissociation (kd), in s-1 determined by SPR in two independent experiments. The diagonal lines correspond to the affinity constant (KD), expressed in nM. Highlighted in red is the result obtained for the reference adalimumab

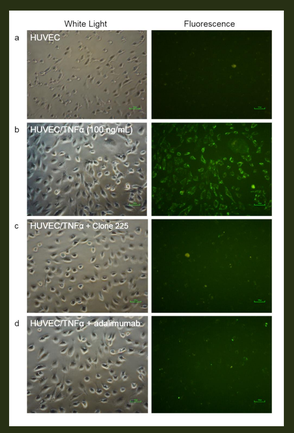

Figure 4: Inhibition of the Endothelial cell Leukocyte Adhesion Molecule-1 (ELAM-1) expression by HUVECs in response to anti-TNFα treatment. The induction of ELAM-1 expression by HUVEC was verified by cell surface fluorescence with a FITC-conjugated anti-ELAM-1 antibody. (a) HUVEC as negative control and (b) HUVEC/TNFα as positive control of the assay. (c) Example of purified anti-TNFα adabut mAb and (d) adalimumab. Scale 100 µm.

Table 1- Best culture conditions for clone 123 at FB in SF

Condition	Medium (Gln) + Feed (regimen)	Max viable cell density (x10⁶)/mL	Days of culture (% viability)	Glucose min-max (g/L)	Max lactate (g/L)	Max ammonia (mM)	Titer (mg/mL) by SPR (day 14)
1	M 1 (8mM) + F A/F B (day 3 to the end)	28.95 (day 11)	14 (93.5)	0.06-6.76	2.70 (day 8)	5.47 (day 4)	2.75
2	M 2 (8mM) + F A/F B (day 3 to the end) TºC shift	23.66 (day 8)	14 (97)	0.07-5.79	2.11 (day 6)	1.25 (day 3)	2.39
3	M 3 (6mM) +F 1 5% (even alternate days)	13.75 (day 10)	14 (79.7)	0.5-5.49	1.48 (day 7)	5.39 (day 14)	2.36
4	M 3 (4mM) + F 1 5% (even alternate days)	14.09 (day 10)	14 (83.6)	0.76-5.34	1.34 (day 3)	5.02 (day 12)	1.77
5	M 3 (8mM) + F 1 5% (asymmetric feed)	12.02 (day 8)	14 (73.2)	0.6-5.38	1.72 (day 7)	6.77 (day 8)	1.27
6	M 3 (8mM) + F 1 5% (day 3 to the end – 5 to 7.5%)	12.51 (day 7)	14 (64.5)	0.5-5.56	1.96 (day 8)	5.71 (day 5)	1.18
7	M 4 (8mM) + F A/F B (day 3 to the end)	40.25 (day 11)	14 (89.8)	0.07-5.95	2.35 (day 14)	2.55 (day 9)	1.14

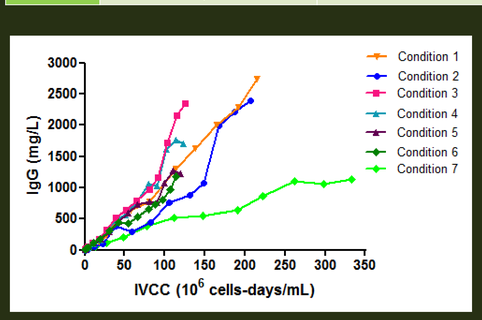

Figure 5: Adabut clone 123 titer in relation to cellular density along the FB cultivation. Seven different combinations of basal media and feeds were followed for 14 days. An anti-human Fc immobilized sensor was used for SPR titer evaluation

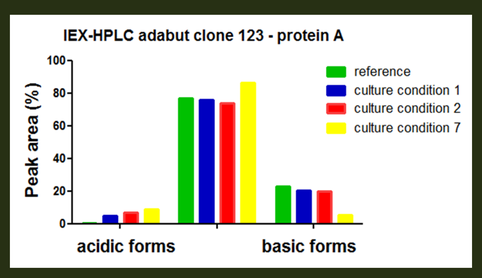

Figure 6: IEX-HPLC from protein-A purifed adabut clone 123 obtained by variations of feeding strategy, in comparison to the reference

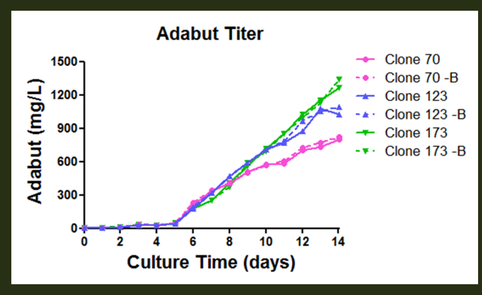

Figure 7: Adabut titers measured along 14 days of FB cultivation on condition 2 for 3 top clones. A protein-A immobilized sensor was used for SPR titer evaluation. The letter B stands for duplicate runs.

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