CHOSOURCE™ ADCC+ cell line for enhanced therapeutic potency

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❶ Abstract

Horizon's CHOSOURCE™ expression platform is used globally for development of biological therapies. Until now the platform has consisted of the CHO-K1 suspension adapted cell line with the Glutamine Synthetase (GS) gene knocked out (KO), for the purpose of providing a robust industry standard selection system, and a dual promoter expression vector for expression of functional antibodies.

This CHOSOURCE™ GS KO cell line is currently used worldwide by over 100 pharma, biotech and Contract Manufacturing Organisations (CMO's) for the discovery, development and manufacture of human and animal biotherapeutics as well as diagnostics and vaccines. To continue to build on the capabilities of this CHOSOURCE expression platform, we have developed an additional CHO-K1 cell line; CHOSOURCE™ ADCC+ cell line.

CHOSOURCE ADCC+ cell line has been built on our existing CHOSOURCE GS KO cell line by eliminating the cell's natural fucosylation activity and therefore is able to express antibodies and other fusion proteins with glycoforms completely lacking fucose. The absence of fucose has been shown to increase Antibody Dependent Cellular Cytotoxicity (ADCC) activity.

The use of ADCC-enhanced therapeutics can result in increased potency, and by increasing its therapeutic window, may help reduce dosage requirements thereby reducing side effects for patients.

The data presented outlines the development of the CHOSOURCE ADCC+ cell line, functional validation, and cell line performance following transfection using CHOSOURCE™ TnT transposon technology.

❷ Method

The CHOSOURCE ADCC+ cell line was generated using Horizon's recombinant adeno-associated virus (rAAV) gene editing platform, where the existing CHOSOURCE GS KO cell line was used as a host cell line. The pipeline involved the following steps:

I- Gene Target & Vector Design

- Target gene copy number analysis, conducted using droplet digital PCR, revealed two copies of the target gene in the host cell line.
- Targeting vector was designed (**Fig. 1**) for gene editing in host genome.

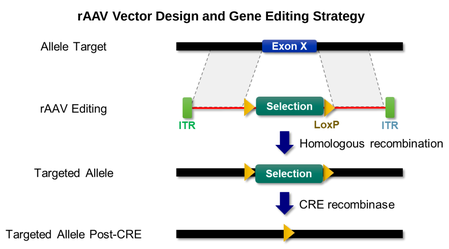

Fig. 1: Outline of the gene editing strategy using Horizon's proprietary rAAV technology.

II- First Allele Knockout Process

- CHOSOURCE GS KO cell line used as host cell line.
- rAAV gene editing technology used to create the first allele KO.
- On- and off-target PCR screens conducted to validate the KO allele.
- Growth profile of multiple heterozygous clones analysed in batch culture.

III- Second Allele Knockout Process

- Followed the same process as above, but a heterozygote KO clone was used as the starting host cell line, to generate homozygote clones.

IV- Functional Analysis

- **Glycan Profile Analysis** was performed on anti-HER2 (Trastuzumab, TTZ) samples produced from stably transfected CHOSOURCE ADCC+ pools, using HILIC-UPLC MS/MS. TTZ produced from CHOSOURCE GS KO expressing pools was used as control.

- **Antibody-Dependent Cellular Cytotoxicity (ADCC) Activity Analysis** of TTZ produced from the KO cells was tested as follows:
  - TTZ expressed in two different CHO cell backgrounds:
    - CHOSOURCE GS KO cell line (control)
    - CHOSOURCE ADCC+ cell line
  - Two target cell lines expressing different levels of HER2 antigen:
    - T47D cells (low antigen expressing cells, $HER2^{Low}$)
    - SK-BR-3 cells (high antigen expressing cells, $HER2^{High}$)
  - Two effector cell lines expressing two variants of the FcγRIIIa receptor:
    - Effector cells expressing FcγRIIIa Val158 (V158)
    - Effector cells expressing FcγRIIIa Phe158 (F158)

- **Productivity Performance Analysis** of CHOSOURCE GS KO and CHOSOURCE ADCC+ TTZ-expressing pools. Both cell lines were stably transfected using CHOSOURCE Transposon Technology (transposase-based genetic integration) and placed under selection 48 hours post-transfection (no MSX). Following recovery, pool productivity was assessed using Horizon's standard shake flask fed-batch process.

❸ Results

I- First Allele Knockout

Following transduction of CHOSOURCE GS KO host cell line with the rAAV vector, mini-pool selection and clone isolation, on- and off-target PCR screens were performed to identify clones showing presence of the mutant allele (**Fig. 2**), and absence of off-target integration (data not shown).

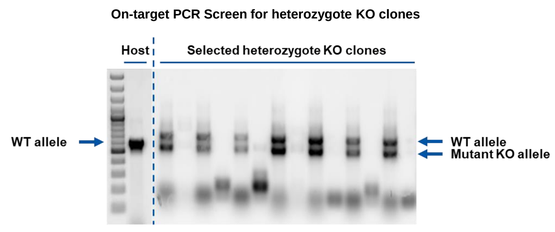

Fig. 2: DNA agarose gel shows presence of wild-type (WT) and mutant KO allele in the selected heterozygote clones, and only WT allele in CHOSOURCE GS KO.

The majority of heterozygote KO clones were shown to have comparable growth profiles to the CHOSOURCE GS KO cell line (**Fig. 3**). The clone shown in dark blue was selected for targeting of the second allele.

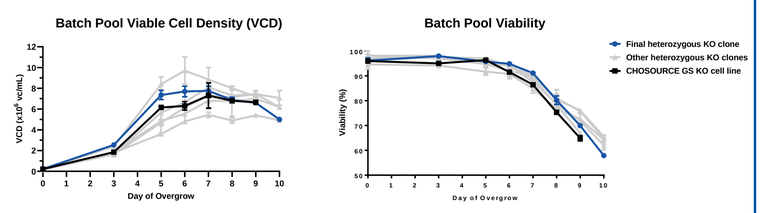

Fig. 3: Growth performance of selected heterozygote KO clones and CHOSOURCE GS KO cell line in standard batch overgrow conditions.

II- Second Allele Knockout

For the generation of the second allele KO, a similar process to that described above was followed. PCR validation confirmed KO of both alleles at the genomic level (**Fig. 4**).

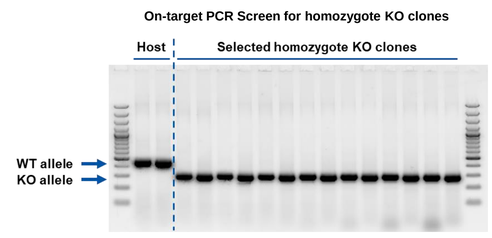

Fig. 4: On-target PCR validation confirming KO of both alleles in homozygote clones and WT alleles in CHOSOURCE GS KO host cell line.

The growth profiles for isolated KO clones were analysed in a batch overgrow setting, and compared to CHOSOURCE GS KO host cell line. All KO clones were shown to outgrow the host (**Fig. 5**).

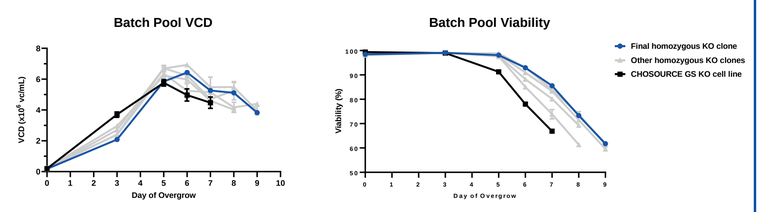

Fig. 5: Growth performance of selected KO clones and CHOSOURCE GS KO in batch overgrow conditions.

III- Functional Analysis – N-Glycan Profile

Glycan analysis of TTZ, produced in both CHOSOURCE cell lines, shows that antibody produced in CHOSOURCE ADCC+ cells is completely non-fucosylated (**Fig. 6** and **7**).

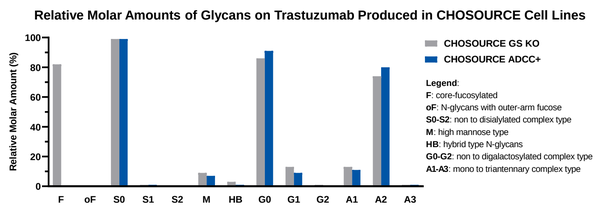

Fig. 6: N-Glycan profiling of antibody produced in CHOSOURCE ADCC+ cells shows 100% elimination of fucosylated species.

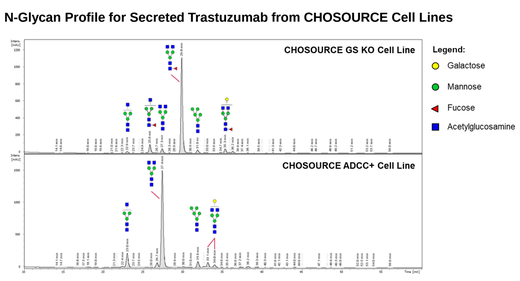

Fig. 7: N-Glycan profile shows that antibody produced in CHOSOURCE ADCC+ cell (bottom profile) completely lack fucose moieties (◀) in the glycan structure.

IV- Functional Analysis – ADCC Activity

TTZ expressed in CHOSOURCE GS KO cells show:

- Weak to no ADCC activity with FcγRIIIa V158 effector cells, when using $HER2^{High}$ or $HER2^{Low}$ target cells, respectively (**Fig. 8A** and **8C**).
- No relevant ADCC activity with FcγRIIIa F158 effector cells, when using $HER2^{Low}$ or $HER2^{High}$ target cells (**Fig. 8B** and **8D**).

TTZ expressed in CHOSOURCE ADCC+ cells show:

- Strong ADCC activity with FcγRIIIa V158 effector cells, when using $HER2^{Low}$ or $HER2^{High}$ target cells (**Fig. 8A** and **8C**).
- Moderate ADCC activity with FcγRIIIa F158 effector cells, when using $HER2^{Low}$ or $HER2^{High}$ target cells (**Fig. 8B** and **8D**).

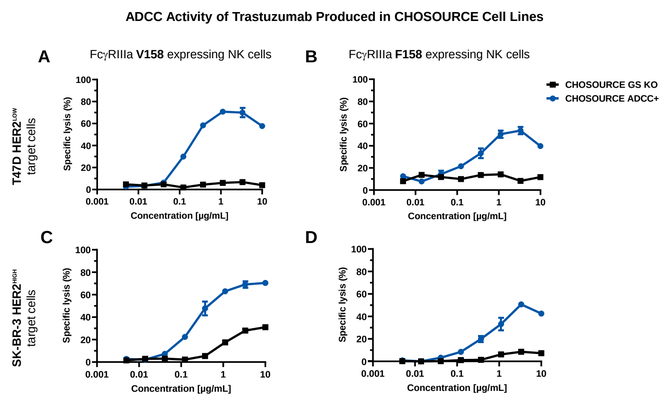

Fig. 8: Non-fucosylated TTZ produced in CHOSOURCE ADCC+ cells elicits substantially higher ADCC activity, than CHOSOURCE GS KO cells.

V- Functional Analysis – Productivity Performance

Both CHOSOURCE cell lines were stably transfected using CHOSOURCE TnT transposon technology, for expression of TTZ. Pools were enrolled in a standard fed-batch process, for the assessment of pool performance.

CHOSOURCE ADCC+ expressing cells reached a lower peak VCD, but comparable culture viability, compared to the CHOSOURCE GS KO expressing pools (**Fig. 10**).

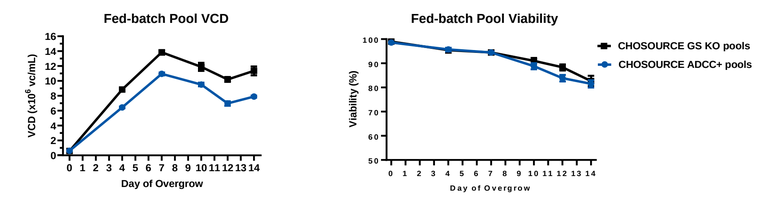

Fig. 9: Growth profiles of CHOSOURCE cell lines, in unoptimized fed-batch process, following transfection using CHOSOURCE TnT transposon technology.

CHOSOURCE ADCC+ expressing cells display similar product titer to the host cell line (around 3 g/L) and comparable overall cell specific productivity (Qp) to CHOSOURCE GS KO expressing cells (**Fig. 10**).

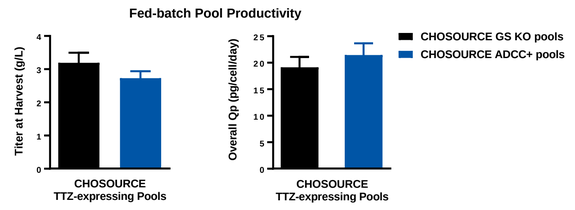

Fig. 10: Comparative productivity of CHOSOURCE GS KO and ADCC+ pools.

❹ Conclusion

- **CHOSOURCE ADCC+ cell line produces molecules without any fucosylation but otherwise comparable glycosylation profile to parental line.**
- **Products expressed in this cell line show enhanced effector function which in turn increases drug potency, expands therapeutic window and reduces dosage requirements, whilst potentially reducing undesirable side effects in patients.**
- **Consistent production of non-fucosylated glycoproteins eliminates product quality deviations and failed batches linked to variability in fucose glycan composition. Thus, removing costly process control methodologies, making biopharmaceutical manufacturing more robust, cost-effective, and safer to patients.**
- **As CHOSOURCE ADCC+ cell line has been built on a GS KO background (unique in the market), and due to the vector technology used, cell line development does not require the use of MSX during selection.**
- **CHOSOURCE ADCC+ cell line can be utilized in a growing number of applications to treat diseases, such as in the areas of oncology, infectious disease, and auto-immune disease.**

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