CHOSOURCE™ ADCC+ cell line for enhanced therapeutic potency

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1 Abstract

Revvity'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.

2 Method

The CHOSOURCE™ ADCC+ cell line was generated using Revvity'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.

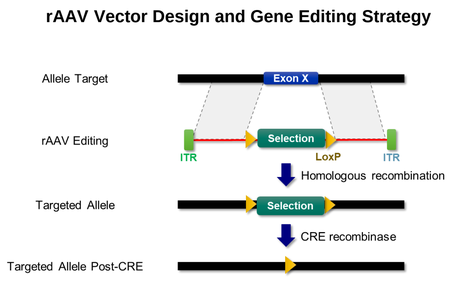

Fig. 1: Outline of the gene editing strategy using Revvity's proprietary rAAV technology.

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, HER2Low)
    - SK-BR-3 cells (high antigen expressing cells, HER2High)
  - 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 TTZ-expressing pools 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 methionine sulfoximine, MSX). Following recovery, pool productivity was assessed using Revvity's standard shake flask fed-batch process.

3 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).

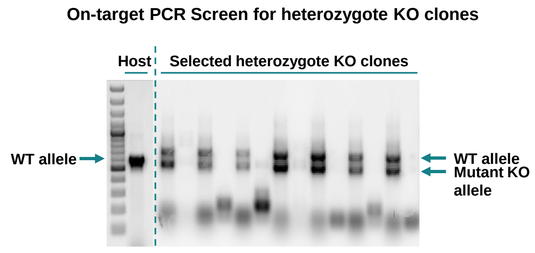

Fig. 2: DNA agarose gel shows presence of wild-type (WT) and mutant KO allele in the selected heterozygote clones, and only WT allele in CHOSOURCE™ GS KO cells.

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.

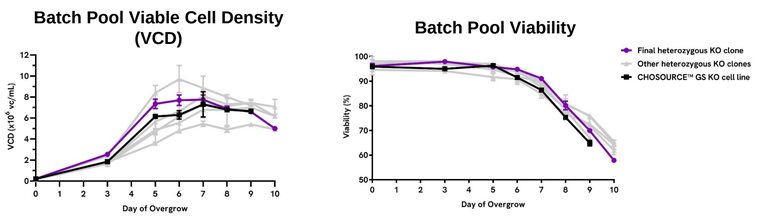

Fig. 3: Growth performance of selected heterozygote KO clones and CHOSOURCE™ GS KO cell line in standard batch overgrow conditions.

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**).

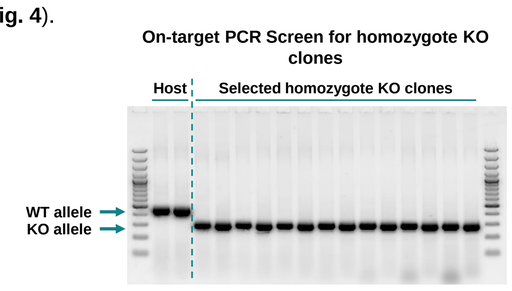

Fig. 4: On-target PCR validation confirming KO of both alleles in homozygote clones and WT alleles in CHOSOURCE™ GS KO host cell line.

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**).

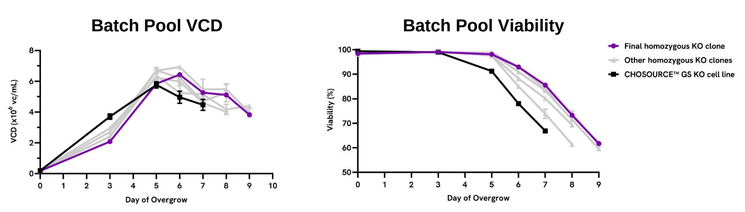

Fig. 5: Growth performance of selected KO clones and CHOSOURCE™ GS KO cells in batch overgrow conditions.

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**).

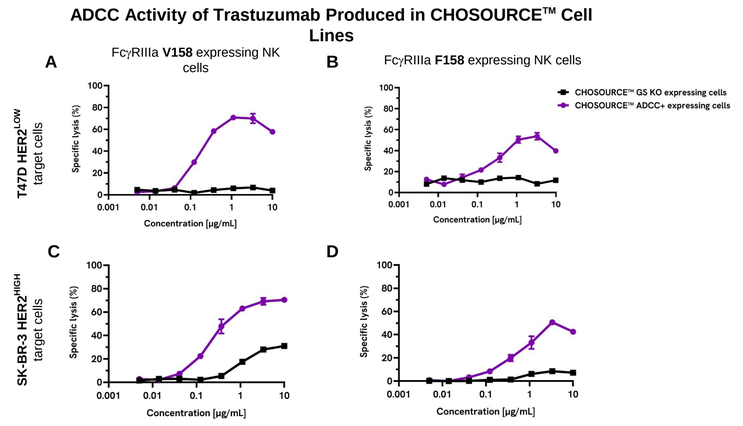

Fig. 8: Non-fucosylated TTZ produced in CHOSOURCE ADCC+ cells elicits substantially higher ADCC activity, than CHOSOURCE™ GS KO cells.

V- Functional Analysis – Productivity Performance

CHOSOURCE™ ADCC+ cell line was transfected using CHOSOURCE™ TnT technology for the stable expression of a non-optimized IgG reference molecule. CHOSOURCE™ ADCC+ cells transfected using CHOSOURCE™ TnT technology displays comparable fast selection recovery during bulk pool selection, compared to CHOSOURCE™ GS KO expressing cells (**Fig. 9**).

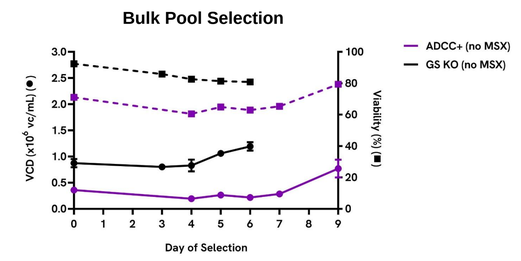

Fig. 9: CHOSOURCE™ ADCC+ cells transfected using CHOSOURCE™ TnT technology display fast selection recovery.

Pools were enrolled in a standard fed-batch process, for the assessment of pool performance**.** The growth pattern displayed by CHOSOURCE™ ADCC+ expressing cells is comparable to that of CHOSOURCE™ GS KO cells (**Fig. 10**). The difference seen between the two profiles is as expected for these particular phenotypes.

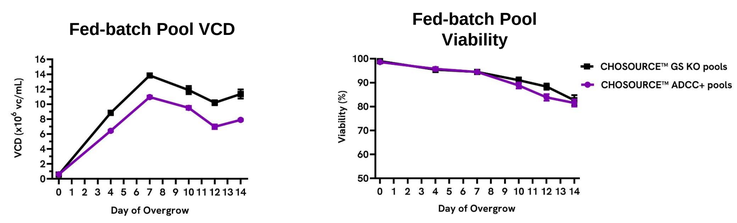

Fig. 10: Growth profiles of CHOSOURCE™ cell lines, in unoptimized fed-batch process, following transfection using CHOSOURCE™ TnT transposon technology.

CHOSOURCE™ ADCC+ expressing cells convey comparable productivity and performance, compared to CHOSOURCE™ GS KO expressing cells (**Fig. 11**).

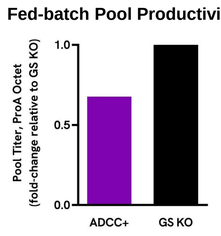

Fig. 11: Comparative productivity of CHOSOURCE™ GS KO pools and ADCC+ pools.

4 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, 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.**