From GS KO to ADCC+: Expanding the CHOSOURCE™ Expression Platform for High-Potency Therapeutic Antibodies

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1 Abstract

Revvity's CHOSOURCE™ expression platform is a globally recognised solution for development and manufacturing of biological therapies. At the core of the CHOSOURCE expression platform is the Chinese hamster ovary (CHO) CHO-K1 suspension adapted host cell line with the Glutamine Synthetase (GS) gene knocked out (KO) and the CHOSOURCE TnT transposon technology. To further expand the capabilities of this CHOSOURCE expression platform, an additional CHO cell line has been developed: 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 glycosylated proteins completely devoid of 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.

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.

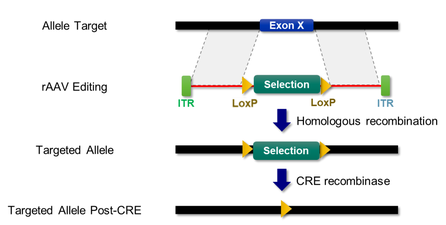

Fig. 1: Outline of the gene editing strategy using Revvity's proprietary rAAV technology.

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 (data not shown).

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 transiently 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 TnT Transposon Technology (transposase-based genetic integration) and placed under selection 48 hours post-transfection in absence of 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).

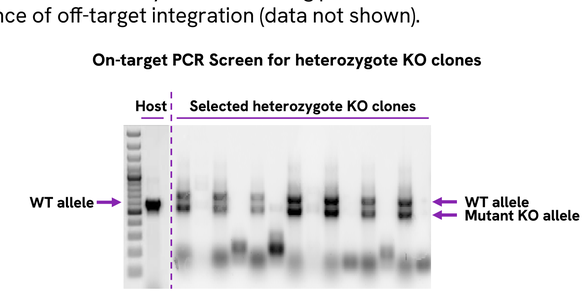

Fig. 2: DNA agarose gel shows presence of wild-type (WT) and mutant KO allele in the selected heterozygote clones, and only WT allele in CHOSOURCE GS KO cells.

Most heterozygote KO clones were shown to have comparable growth profiles to the CHOSOURCE GS KO cell line, and one clone was selected for targeting of the second allele.

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. 3**).

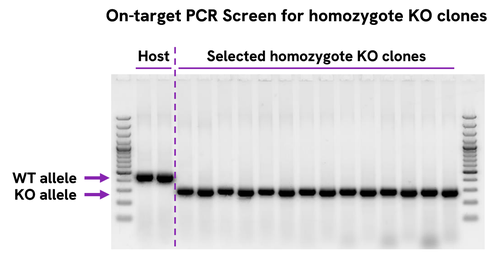

Fig. 3: On-target PCR validation confirming KO of both alleles in homozygote clones and WT alleles in CHOSOURCE GS KO host cell line.

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**).

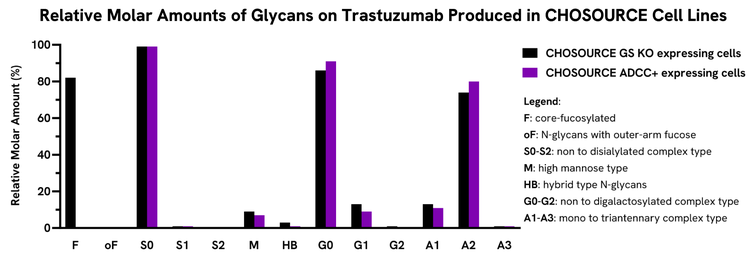

Fig. 6: N-Glycan profiling of antibody produced in CHOSOURCE ADCC+ cells shows 100% elimination of fucosylated species.

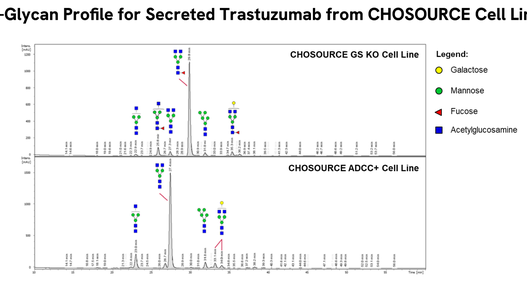

Fig. 7: N-Glycan profile shows that antibody produced in CHOSOURCE ADCC+ cell (bottom profile) completely lack fucose moieties (◀) in the glycan structure.

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**).

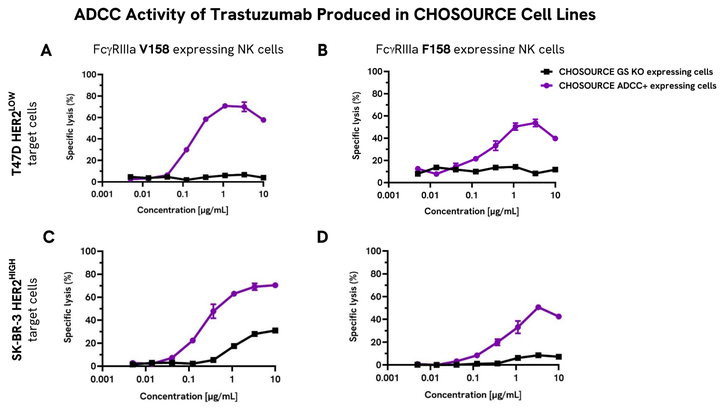

Fig. 8: Non-fucosylated TTZ produced in CHOSOURCE ADCC+ cells elicits substantially higher ADCC activity, than CHOSOURCE GS KO cells.

V- Functional Analysis – Productivity Performance and Stability

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

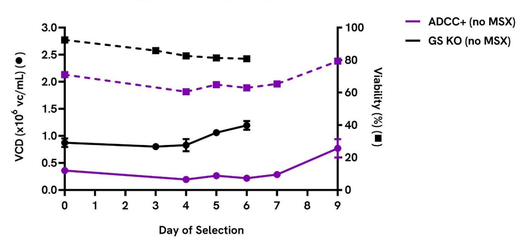

Fig. 9: CHOSOURCE ADCC+ cells transfected using CHOSOURCE TnT technology display fast selection recovery.

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 phenotypes.

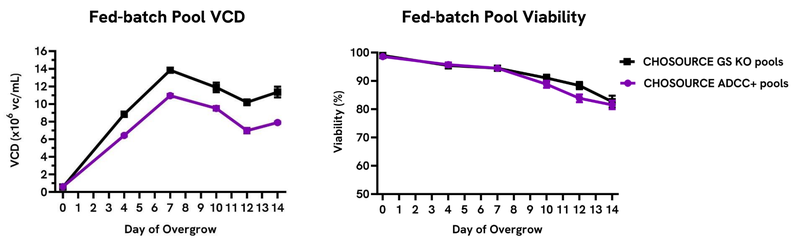

Fig. 10: Growth profiles of CHOSOURCE cell lines, in unoptimised fed-batch process, following transfection using CHOSOURCE TnT transposon technology.

CHOSOURCE ADCC+ expressing cells convey satisfactory productivity and performance under unoptimised process conditions, compared to CHOSOURCE GS KO expressing cells (**Fig. 11**). Productivity of clones isolated from the pools were also assessed using the standard fed-batch process. Titers obtained were comparable to the pool titers assessed previously (data not shown).

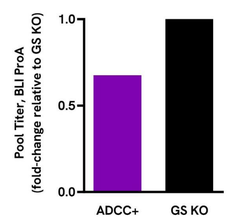

Fig. 11: Pool productivity of CHOSOURCE GS KO pools and ADCC+ pools, following biolayer interferometry (BLI) analysis.

VI- Clone Performance using CHOSOURCE ADCC+ cell line and TnT Transposon Technology – Stability (Customer Case Study)

Clones isolated from CHOSOURCE ADCC+ TnT pools expressing a monoclonal antibody were cultured for 60 generations and a 14-day fed-batch assessment was conducted to assess stability of the clones. Comparison of the titer from Generation 0 *vs.* 60 showed that all clones tested were stable (**Fig. 12**)*.

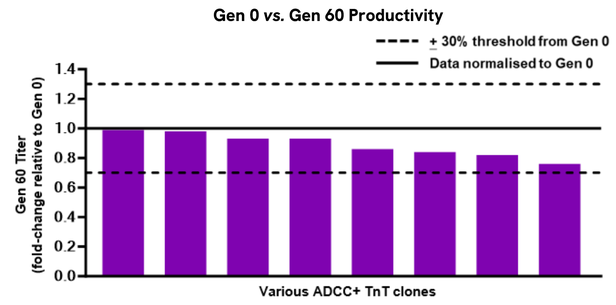

*Fig. 12: Productivity stability profile of clones, where Gen 60 titer is normalised to Gen 0 titer (*changes between Gen 0 and Gen 60 are ± 30%).*

Therefore, with the high clonal stability achieved using CHOSOURCE ADCC+ cell line in combination with the CHOSOURCE TnT Technology, consistent batch to batch production can be ensured. With the CHOSOURCE ADCC+ cell line derived from CHOSOURCE GS KO cell line processes can be transferable between the cell lines reducing time spent on process optimisation.

4 Conclusion

- **CHOSOURCE ADCC+ cell line produces molecules completely devoid of fucosylation, while maintaining a comparable glycosylation profile, growth characteristics, and selection profile to the parental line.**
- **Products expressed in this cell line demonstrate enhanced effector function, leading to increased drug potency, an expanded therapeutic window, and reduced dosage requirements.**
- **Reliable production of non-fucosylated glycoproteins eliminates variability linked to fucose glycan composition, reducing product quality deviations and failed batches. This removes the need for costly process control measures, making biopharmaceutical manufacturing more robust and cost-effective.**
- **CHOSOURCE ADCC+ cell line can be applied across a broad range of therapeutic areas, including oncology, infectious diseases, and autoimmune disorders.**

The CHOSOURCE™ Expression Platform is available for research, clinical, diagnostic, and commercialization applications, including services, under specific licenses from Revvity.