Accelerating the antibody development by an integrated platform with AI molecular assessment and site-specific integration (SSI) cell line development

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Introduction

◆ The gap between PCC (preclinical candidate compounds) and CMC: **Druggability**

- ✓ Unknown molecular defects: aggregation, unstable, etc.
- ✓ Different product activity from transient platform and stable cell line

◆ Solutions

- ✓ **Druggability** assessment using AI
- ✓ **Site-specific integration** platform for fast cell line development

Materials and Methods

◆ AI modeling and analyzing for the charge distribution of the given sequence

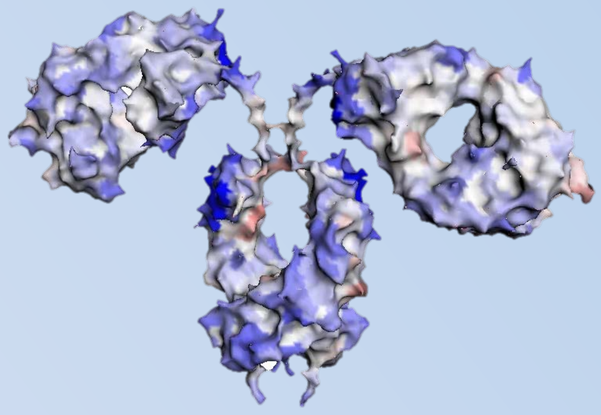


Fig.1 The structure of the antibody was generated using AI. The druggability related attributes, including humanize score, hydrophobic patch, charge patch, etc. were rated (Table 1).

- ✓ The distribution of hydrophobic (Red) and hydrophilic (Blue) domains were marked in the structure (Fig.1).
- ✓ The structures were modelled and analyzed by in-house algorithm.
- ✓ Free trial for the assessment tool:

https://www.greatbay-bio.com/services.html#dw1

◆ Cell line development using site-specific integration platform

Fig.2 The transfection of the GOI into the high productivity site in CHO-K1 cell. The AI selected high productivity site was marked by GFP.

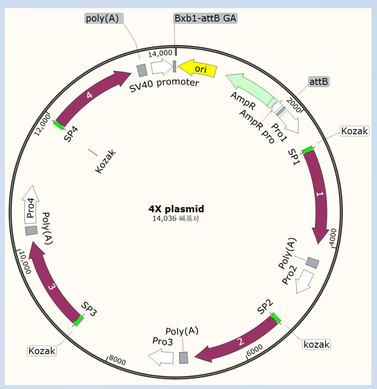
- ✓ The high productivity site was screened and marked with GFP gene.
- ✓ Electrophoresis was applied and the gene of interest (GOI) was integrated by a homologues recombinant manner (Fig.2).
- ✓ The GFP gene was substituted by GOI, and the non-fluoresces cell was selected as transformant.

◆ Cell line and products

- ✓ CHO-K1
- ✓ Molecular: 3 chains bispecific antibody
- ✓ Vector: single vector with four ORFs

◆ Transfection and culture media

- ✓ Electrophoresis
- ✓ Media: Advance+7a7b



◆ Cell line development in 5 weeks

- ✓ Three different chains were constructed into the single vector
- ✓ Transient expression in CHO was used to select the best design (Fig.4)

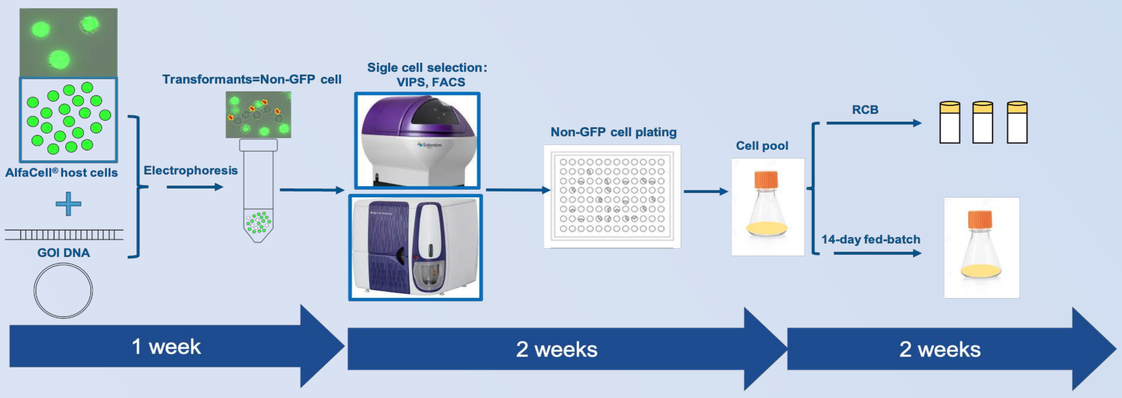


Fig.3 The 5-week cell line development process using the site-specific integration platform. The monoclonal cells were directly selected from the bulk pool using VIPS or FACS.

Results and Discussion

1. The druggability assessment of the candidates using AI-modeling platform

Table 1 The AI analyzed attributes related to druggability and the assessment.

Attributes / Molecular	Pos-charge Patch (Range 0~1.2)	Neg-charge Patch (Range 0~0.8)	Charge distribution (Range -4~12)	PI	Hydrophobic Patch (Range 0~12)	Humanize score (Range 0.5~1)	Stability (Range 56~60)	Assessment Result
A001	0.12	1.19 ↑↑	-6↓	9.02	2.4	0.72	54.1 ↓↓	·Aggregation ·Unstable
A002	0.12	0.89 ↑	4	6.97	4.5	0.03 ↓↓	51.7↓↓	·Aggregation ·Unstable ·Immunogenicity
A003	0.27	0.11	2	8.16	5.1	0.55	58.3	·Pass
A004	0.12	1.19 ↑↑	-6↓	8.12	3.4	0.72	54.1 ↓↓	·Aggregation · Unstable

2. Vector constructed of the selected: different designs

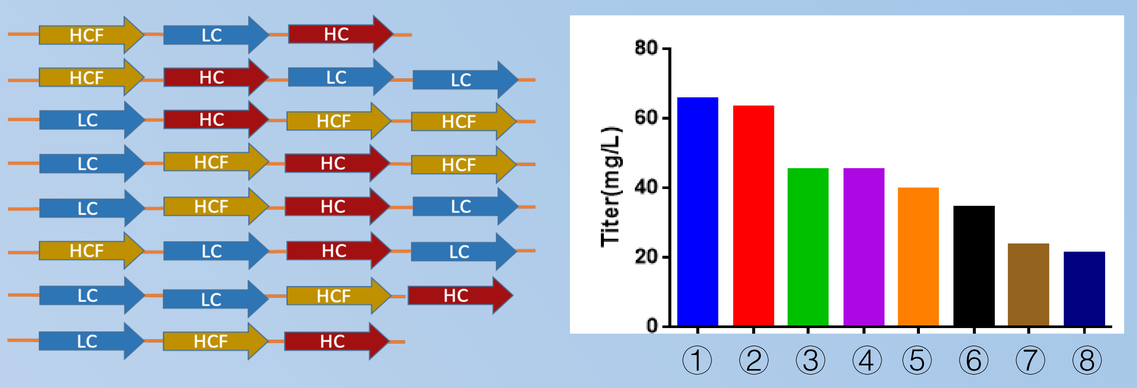


Fig.4 The different arrangement of the 3 chains and their transient expression titers.

- ➢ **Three different chains of the** BsAb were arranged in different manner
- ➢ **One vector** with separated 4 ORFs were constructed and transfected
- ➢ **Different titers** were observed in different vector designs
- ➢ **Top 3 vectors** were further used for stable cell line development

3. Site- specific integration cell line development in 5 weeks

Table.2 The 14-day fed-batch results of the monoclonal cell for the BsAb production.

Design	mRNA expression ratio: C1:C2:C3	14 days Fed batch titer (g/L)	Correct paring (%)
1	**2:3:3**	**5.9**	87.95
2	**3:2:2**	**4.8**	40.7
3	**3:2:4**	4.3	78.0

- ➢ **Precise adjustment** of the expression rate of the individual chains improved the CLD performance
- ➢ **6 g/L** of tier of the BsAb was obtained in 14 days fed-batch
- ➢ **Correct paring rate >87%**

Conclusions

- ➢ The stable cell line for a complex BsAb was successfully developed by integration of the *in silico* druggability assessment, vector design and site-specific cell line platforms
- ➢ Faster and better result was obtained by the application of AI technology in bioprocessing

Reference

[1] Shin S W , Lee J S . CHO Cell Line Development and Engineering via Site-specific Integration: Challenges and Opportunities[J]. Biotechnology and Bioprocess Engineering, 2020, 25(5).