An AI-Assisted Selective CD16A-targeting NK Cell Engager Showed Superior Anti-tumor Activity *In Vitro*

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Booth #306

Opportunities

NK cell bispecific antibodies represent a promising new approach to cancer immunotherapy by harnessing the power of the immune system to recognize and kill cancer cells more effectively.

The development of an NK cell engager (NKCE) that selectively targets CD16A but not CD16B holds promise as a potential cancer immunotherapy, without inducing CD16B-mediated adverse effects on neutrophils.

Challenges

Due to the mere 2-amino-acid difference in the extracellular domain between CD16A and CD16B, obtaining antibodies with higher specificity poses a significant challenge.

Conclusions

In this study, our XploreSeq™ platform rapidly generated several antibodies specifically targeting CD16A within just 8 weeks, utilizing AI-assisted large repertoire exploration, evaluation, and hit recommendations. Then, NK bispecific antibodies targeting EGFR tumors were generated by hit Msb021 in different formats.

In particular, BsAb03 and BsAb04 exhibited significantly higher NK activation and NK-mediated killing efficacy on a tumor cell line with moderate EGFR expression and showed a comparable cytokine release panel compared to front runner AFM24, which is an EGFR x CD16A bispecific antibody and currently in phase 1.

XploreSeq™ Platform: Rapid discovery of CD16A-specific antibodies

AI-empowered Antibody Discovery Platform XploreSeq™ were utilized for high-throughput hit identification from 2 mice immunized with CD16A.

The streamlined process, from next-generation sequencing to AI-powered evaluation, enables the rapid identification of antigen-specific antibodies for this NKCE campaign.

Step 1

Integrating single-cell and bulk NGS to sequence millions of B-cell receptors (BCRs), dramatically expanded the explored antibody sequence space.

Step 2

A rigorous bioinformatics pipeline assured high-quality BCR calling and data integrity at both cellular and sequence levels.

Step 3

AI-driven models, employing self-supervised and supervised learning, processed this data to evaluate antibody candidates for binding, affinity, function and developability.

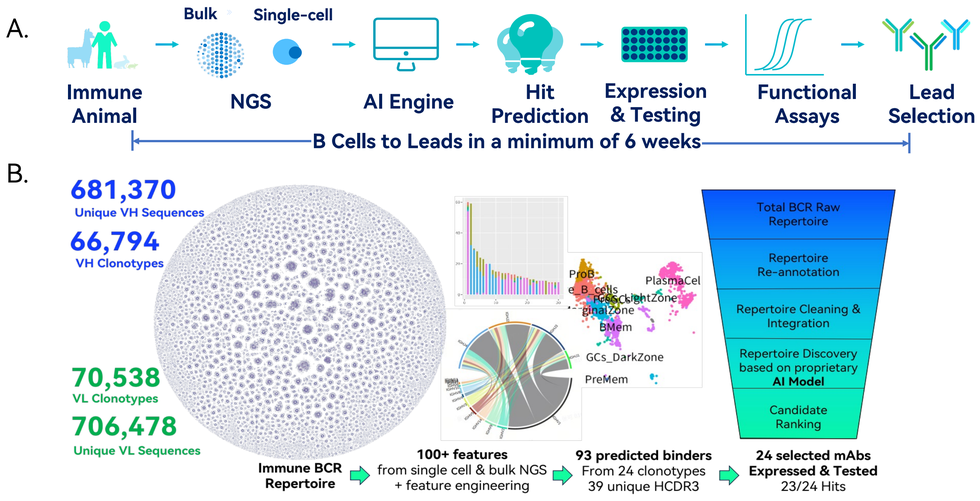
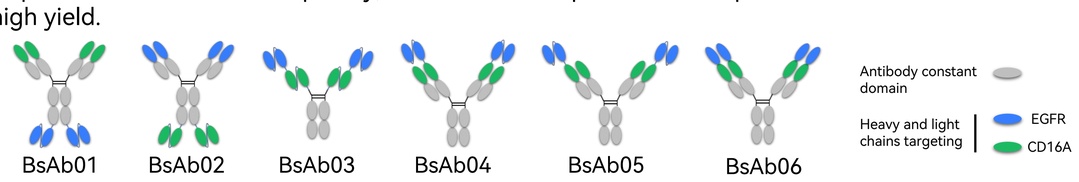


Figure 1. A. XploreSeq™ Platform Workflow; B. XploreSeq™ for NKCE Discovery

NKCE Platform: Engineering various formats of BsAbs with a high yield

In the development of the XtalPi NKCE platform, we engineered BsAbs to dual-target EGFR, which is expressed on tumor cells, and CD16A, which bridges and activates NK cells. The bivalent antibody arms, in either scFv or Fab form, were engineered into symmetrically structured, Fc-silenced IgG1 bispecific antibodies. Subsequently, the BsAbs were produced and purified in various formats with a high yield.



Msb021 exhibited a 10-fold higher affinity for specifically targeting CD16A compared to AFM24 anti-CD16A arm, while not interacting with CD16B

A. SPR binding kinetics of Msb021 interaction with human CD16A-V158 and CD16A-F158 variants

CD16A binding moiety	hCD16A-V158				hCD16A-F158			
	ka(1/Ms)	kd(1/s)	KD(M)	KD Fold	ka(1/Ms)	kd(1/s)	KD(M)	KD Fold
Msb021	1.70E+06	3.36E-03	1.98E-09	10.9	1.85E+06	2.94E-03	1.59E-09	10.8
AFM24 anti-CD16A arm	3.18E+05	6.83E-03	2.15E-08	/	3.52E+05	6.05E-03	1.72E-08	/

KD Fold = $KD_{(AFM24\ anti\text{-}CD16A\ arm)}$ / $KD_{(Msb021)}$

B. SPR binding kinetics of Msb021 interaction with human CD16B-NA1 and CD16B-NA2 variants

CD16A binding moiety	hCD16B-NA1			hCD16B-NA2		
	ka(1/Ms)	kd(1/s)	KD(M)	ka(1/Ms)	kd(1/s)	KD(M)
Msb021	N.B	N.B	N.B	N.B	N.B	N.B
AFM24 anti-CD16A arm	N.B	N.B	N.B	N.B	N.B	N.B

N.B = No Binding

Figure 2. Evaluation of Msb021 binding kinetics by Surface Plasmon Resonance (SPR) Assay

NK bispecific antibodies showed a high binding affinity profile for both EGFR and CD16A overexpressed cells

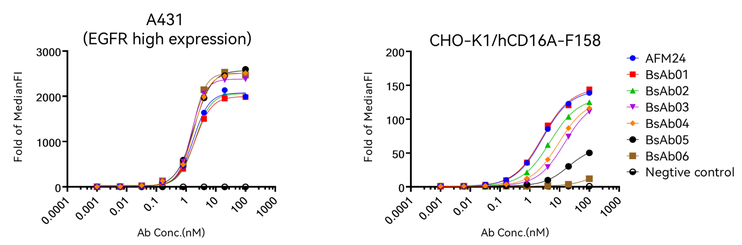


Figure 3. Binding affinities of NK BsAbs against EGFR and CD16A, which were individually evaluated on cells overexpressing EGFR and CD16A by flow cytometry (FACS) .

BsAb03 and BsAb04 showed significantly stronger activities in activating NK cells and promoting NK-mediated cytotoxicity, particularly against HT-29 cells with moderate EGFR expression, in comparison to AFM24

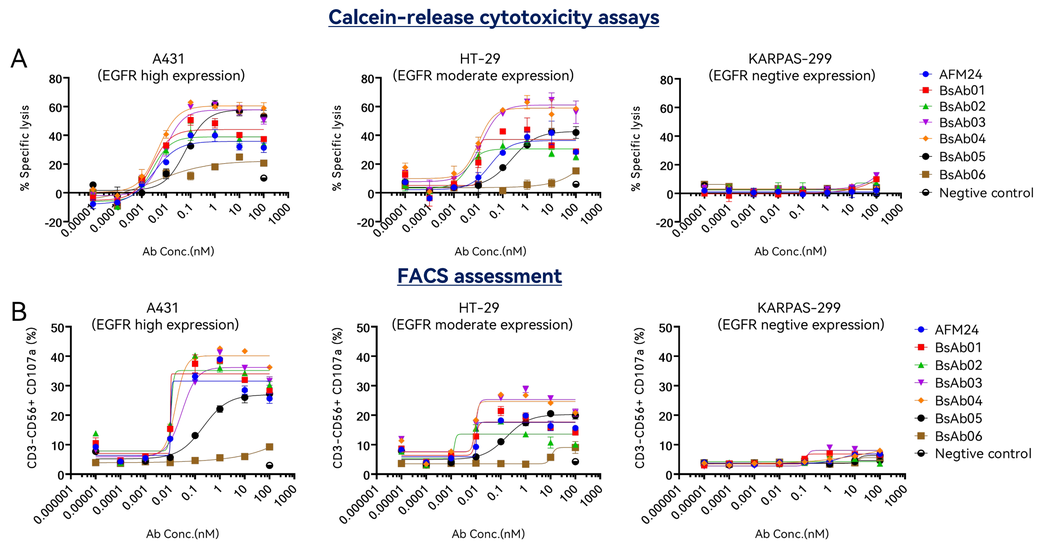


Figure 4. NK activation and NK-mediated cytotoxicity against tumor cells exhibiting high, moderate and negative EGFR expression levels were assessed in the presence of EGFR x CD16A bispecific antibodies. NK-mediated cytotoxic effect on tumor cells was assessed using calcein-release cytotoxicity assays, with human peripheral blood mononuclear cells (PBMCs) serving as effector cells at an effector-to-target (E:T) ratio of 50:1 (**A**). CD107a, the cell surface marker of activated NK cells was assessed by FACS (**B**).

BsAb03 and BsAb04 showed comparable release levels of TNFα, and IL-6 as compared to AFM24

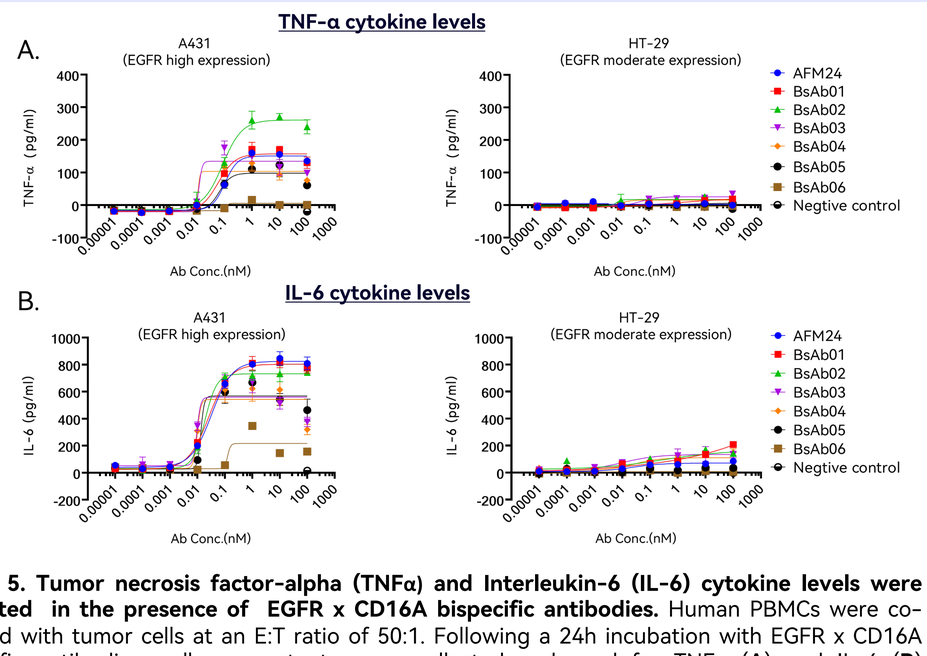


Figure 5. Tumor necrosis factor-alpha (TNFα) and Interleukin-6 (IL-6) cytokine levels were evaluated in the presence of EGFR x CD16A bispecific antibodies. Human PBMCs were co-cultured with tumor cells at an E:T ratio of 50:1. Following a 24h incubation with EGFR x CD16A bispecific antibodies, cell supernatants were collected and used for TNFα (**A**) and IL-6 (**B**) detection.

Discussion: Assisted by XploreSeq™, our NKCE platform engineered BsAbs with superior anti-tumor activity *in vitro*.

XploreSeq™ Platform

We utilized the XploreSeq™ for anti-CD16A antibody generation, obtaining over 680,000 heavy chain sequences and 700,000 light chain sequences.

From these, 93 candidate antibodies were identified, and 24 were selected for expression and evaluation. **A remarkable 95.8% (23/24) were confirmed as binders.**

The hit antibody Msb021, which specifically targets CD16A but not CD16B, was generated in just **8 weeks** and demonstrated a **10-fold higher** affinity for human CD16A in an SPR assay.

XploreSeq™ enables the rapid identification of antigen-specific antibodies for this NKCE campaign.

NKCE Platform

Following engineering with Msb021, the EGFR x CD16A bispecific antibodies demonstrated significantly enhanced NK-mediated efficacy in an EGFR-dependent manner.

Compared to AFM24, BsAb03 and BsAb04 exhibited significantly enhanced NK activation activity and NK-mediated cytotoxicity, particularly against HT-29 cells with moderate EGFR expression. This suggests our NKCEs may offer an opportunity to target tumor types even with moderate EGFR expression.

Our NKCEs offer a robust bispecific platform featuring **a diverse range of format options, potent NK-mediated efficacy, and an acceptable cytokine release profile**.

In future, we will leverage the strengths of our AI and biology experimental teams to further develop the NKCE platform. This will entail improving *in vivo* drug efficacy assessment, druggability evaluation and modification, and other aspects to progress the NKCE platform's products towards clinical applications.

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