2888

DB-1418 (AVZO-1418[a]), a bispecific antibody-drug conjugate targeting EGFR and HER3, demonstrates superior and broad antitumor efficacy and favorable safety in preclinical models

Yunhua Zhou, Jun Yao, Yang Qiu, John Zhu, Haiqing Hua

Duality Biologics Ltd., Unit 1106, 868 Yinghua Road, Shanghai, P. R. China

a. Avenzo Therapeutics holds global rights to DB-1418 (AVZO-1418) outside of Greater China.

ABSTRACT

Background:

EGFR, an overexpressed oncogene in tumor cells, is a clinically-validated target for ADC development. HER3, a member of the ERBB family, preferentially forms heterodimers with HER2 or EGFR to facilitate signaling. Both EGFR and HER3 are co-overexpressed in a spectrum of tumors, including lung, breast, gastrointestinal, renal cell, ovarian, bladder, and prostate cancers. Importantly, HER3 expression is further enhanced upon inhibition of EGFR, suggesting that simultaneous targeting of both EGFR and HER3 could enhance tumor selectivity and improve therapeutic efficacy in EGFR tyrosine kinase inhibitor-resistant tumors. Here, we developed a novel bispecific ADC with strong and broad antitumor activity in preclinical studies.

Methods:

We generated DB-1418, an asymmetric IgG-like bispecific ADC, featuring a Fab arm targeting EGFR with medium affinity and a scFv arm targeting HER3 with high affinity. The antibody is connected via a knock-into-hole (KIH) Fc domain and conjugated with our clinically validated linker-payload P1021 at a drug-to-antibody ratio (DAR) of 6. We also produced a BL-B01D1 analog as a benchmark.

Results:

In the EGFR-dominant CAL-27 model, DB-1418 demonstrated a significant reduction in tumor size with a tumor growth inhibition (TGI) of 83% after an intravenous dose of 1.9 mg/kg every 3 weeks (Q3W), whereas the BL-B01D1 analog achieved only a 73% reduction at a dose of 3 mg/kg Q3W. In the HER3-dominant SW-620 model, DB-1418 at 10 mg/kg Q3W twice induced complete tumor regression in 4 out of 5 mice and persisted up to 63 days after administration. In contrast, BL-B01D1 analog treatment at the same dose and frequency led to a 9.24-fold increase in relative tumor volumes compared to baseline by the study end. Notably, in a validated osimertinib-resistant NSCLC xenograft model with the C797S mutation, DB-1418 induced tumor regression with a TGI of 90% at a dose of 3.8 mg/kg Q3W and 98% at a dose of 6 mg/kg Q3W, significantly higher than the BL-B01D1 analog at 6 mg/kg Q3W (TGI of 68%, p<0.01). DB-1418 demonstrated in vitro plasma stability in human, monkey, and rat models. In cynomolgus monkeys, the dose-range finding study showed favorable safety, with the highest non-severely toxic dose (HNSTD) reaching up to 45 mg/kg administered every 2 weeks (Q2W) for two cycles.

Conclusions:

DB-1418 has shown promising pharmacological and pharmacokinetic properties, differentiated from BL-B01D1 analog, a first-in-class EGFR-HER3 bispecific ADC on clinical trials. DB-1418 potentially offers additional treatment options for patients who have developed resistance to EGFR tyrosine kinase inhibitors (TKIs) and addresses other unmet medical needs.

RESULTS

DB-1418 is a bispecific ADC targeting EGFR and HER3 with outstanding plasma stability

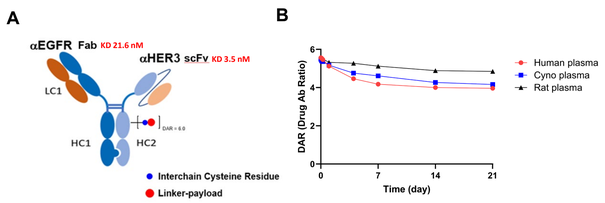


Figure 1. Characterization of DB-1418. A. an asymmetric IgG-like bispecific ADC, featuring a Fab arm targeting EGFR with medium affinity and a scFv arm targeting HER3 with high affinity. The antibody is connected via a KIH Fc domain and conjugated through a tetra-peptide based cleavable linker with our clinically validated topoisomerase I inhibitor (P1021) at a DAR of 6. **B.** DB-1418 was incubated with Human, Cynomolgus, Rat plasma for up to 21 days. The change of DAR was measured by LC/MS.

DB-1418 demonstrated additive binding capacity on EGFR/HER3 co-expressing cells

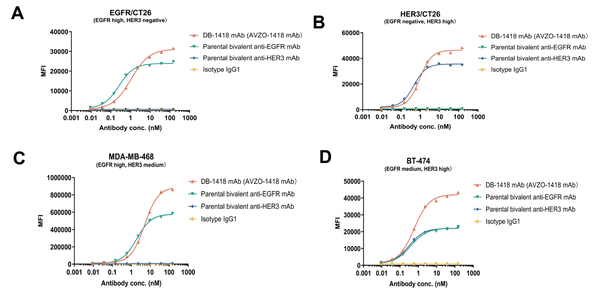
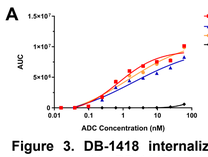


Figure 2. Binding of DB-1418 and parental bivalent mAbs on different expression EGFR/HER3 cell lines. A. In EGFR only cells, DB-1418 mAb binds weaker than parental anti-EGFR mAb. **B.** In HER3 only cells, DB-1418 mAb binds weaker than parental anti-HER3 mAb. **C-D.** In EGFR/HER3 co-expressing tumor cells, the span of DB-1418 mAb significantly enhanced.

DB-1418 has higher internalization capability than parental bivalent ADC on EGFR/HER3 co-expressing cells



B

	EC_{50} (nM)
DB-1418 (AVZO-1418)	0.78
Parental bivalent anti-EGFR ADC	1.95
Parental bivalent anti-HER3 ADC	1.49

Figure 3. DB-1418 internalization on HCC-827/ERBB3. A. HCC827/ERBB3 cells were incubated with DB-1418 and parental ADCs. The full-time course of internalization was visualized and automatically quantified every 2 hours over 48 hours using Incucyte real-time live-cell analysis. Each data point represented the total red fluorescence object area under curve (AUC) (N = 1). **B**. EC50 values for internalization of DB-1418 and parental ADCs.

DB-1418 exhibits synergistic cytotoxicity against EGFR/HER3 co-expressing tumor cells while sparing non-cancerous cells

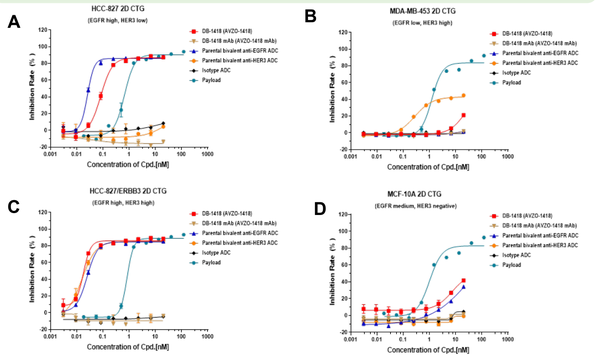


Figure 4. In vitro cytotoxic effect of DB-1418 on cell lines with different EGFR/HER3 expression levels. A-D. HCC-827 (lung adenocarcinoma), MDA-MB-453 (breast cancer), HCC-827/ERBB3 (overexpression of HER3 in HCC-827), and MCF-10A cells (normal mammary gland epithelial cell) were cultured with DB-1418, parental mAb, parental bivalent anti-EGFR/HER3 ADC, and isotype control ADC for 7 days, and then cell viability was examined by CTG luminescence. Each value represents the mean and SEM (N = 2).

DB-1418 showed superior antitumor activity in HNSCC, CRC, and EGFR TKI resistant NSCLC CDX or PDX models

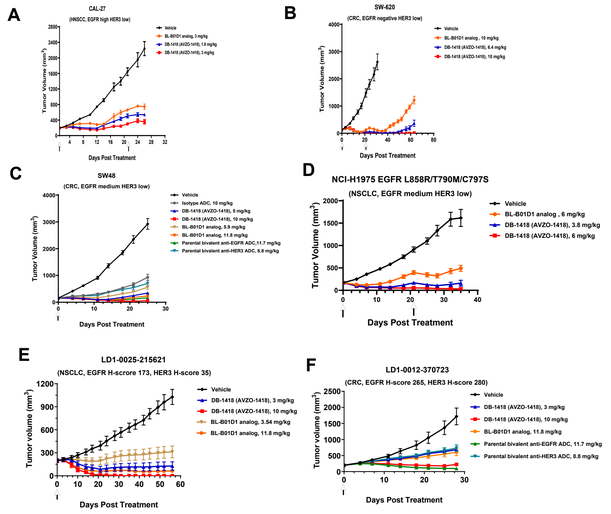


Figure 5. DB-1418 inhibits tumor growth in vivo. Adult immune-deficient mice were subcutaneously inoculated with CAL-27 head and neck squamous cell carcinoma **A**, SW-620 colon cancer cells **B**, SW-48 colon cancer cells **C**, harboring C797S NCI-H1975 non-small cell lung cancer cells **D**, LD1-0025-215621 non-small cell lung adenocarcinoma patient-derived tumor xenograft **E** or LD1-0012-370723 colon cancer patient-derived tumor xenograft **F** following with DB-1418 and other control ADCs single dose or every 3 weeks intravenous injection. The doses were based either on equal ADC molar or mass concentration, or equal payload molar concentration. Each value represents the mean and SEM (N=5 or 6).

DB-1418 showed better efficacy than parental bivalent ADC in NSCLC, RCC models

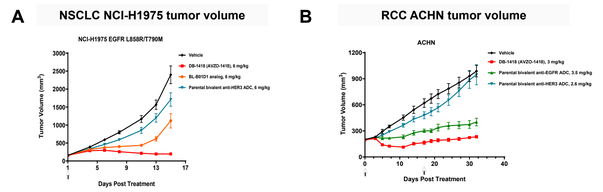


Figure 6. In vivo efficacy of DB-1418 compared to parental ADCs. A. Adult immune-deficient mice bearing subcutaneous NCI-H1975 were treated with DB-1418, parental anti-HER3 ADC and benchmark at equal mass concentration intravenous injection. Each value represents the mean and SEM (N=6). **B.** Adult immune-deficient mice bearing subcutaneous ACHN were treated with DB-1418 and parental ADCs at equal payload molar concentration intravenous injection. Each value represents the mean and SEM (N=4).

DB-1418 demonstrated tolerable safety in cynomolgus monkey

Toxicology	Study design	Key findings
Dose range finding toxicity study in Cynomolgus monkeys	20/45, 30 mg/kg Q2Wx3, 1M/1F/group	• No mortality • Loose feces • Decreased blood RET, RBC, WBC and the associated parameters • Increased ALT and LDH • Thymic atrophy • **HNSTD= 45 mg/kg**

CONCLUSIONS

- **DB-1418 (AVZO-1418) is a novel bispecific ADC targeting EGFR with medium affinity and HER3 with high affinity, conjugated with a topoisomerase I inhibitor with high stability and good developability.**
- **DB-1418 (AVZO-1418) shows additive binding affinity in EGFR/HER3 co-expressing tumor cells.**
- **DB-1418 (AVZO-1418) has higher internalization capability than parental ADC on EGFR/HER3 co-expressing cells.**
- **DB-1418 (AVZO-1418) shows better in vivo efficacy in lung cancer, colon cancer, head and neck cancer models than BL-B01D1 analog.**
- **DB-1418 (AVZO-1418) shows better efficacy than parental bivalent ADC in NSCLC and RCC models.**
- **DB-1418 (AVZO-1418) is on track for IND submission in the first half of 2025.**