JSKN021, an innovative site-specific dual-payload bispecific antibody drug conjugate targeting EGFR and HER3 exhibits potent preclinical activities

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Abstract

Background

EGFR and HER3, both belonging to the EGFR family, are widely overexpressed in human malignancies, rendering them promising targets for cancer treatment. Cancer is a heterogeneous disease. Inter-and intratumor heterogeneity is believed to be a major factor contributing to recurrence, metastasis and resistance to current SOCs. To address these therapeutic challenges, we generated JSKN021, a dual payload ADC targeting EGFR/HER3. Both payloads, a novel DNA topoisomerase I inhibitor (T01, Alphatecan) and MMAE, are conjugated to glycan on Fc via cleavable linkers. The conjugation processes were carried out via combination of glycan transferase and click reaction. The streamlined process generated site specific and homogeneous conjugation with DAR4 (T01) and DAR2 (MMAE). In this study, the pharmacological activity of JSKN021 was evaluated using relevant in vitro cell killing assays and in vivo models.

Methods

A novel Topo 1 inhibitor T01 and MMAE were stably conjugated to an EGFR/HER3 mAb using a glycan based site-specific manner. The conjugated products were characterized by LC-MS. Binding specificity of JSKN021 was confirmed by ELISA. In addition, release of payload were evaluated by LC-HRMS. At last, in vitro cell growth inhibitory and in vivo antitumor activities of JSKN021 were evaluated using different cancer cell lines and CDX models in comparison to single payload ADCs.

Results

After conjugation, LC-MS results showed that JSKN021 had a homogeneous DAR4 for T01 payload and DAR2 for MMAE. JSKN021 was demonstrated to bind EGFR and HER3 simultaneously. In addition, both T01 and MMAE were detected in cell culture supernatant and suspension of HCC827. Yet, no other metabolites were found detectable. JSKN021 demonstrated excellent stability in rats, mice, monkeys, and human serum, with minimal payload release. The stability was derived from the advantage of glycan based conjugation. JSKN021 inhibited the growth of cancer cells with either Her3 or EGFR or both expression, such as HCC827, MDA-MB-468,A431 and NCI-H1975. Furthermore, JSKN021 showed stronger tumor inhibition efficacy than mono payload ADCs in multiple CDX models.

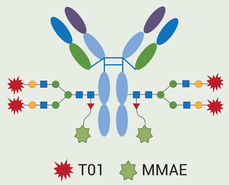
Conclusion

The preclinical studies suggest JSKN021, as a dual-payload EGFR/HER3 bispecific ADC, might be a promising novel antitumor therapeutic agent.

First-in-class EGFR x HER3-targeted dual-payload ADC

JSKN021 is a first-in-class (FIC) dual payload ADC consisting of an EGFRxHER3 bispecific antibody conjugated with TOP1 inhibitor and tubulin inhibitor.

Dual-action mAb against EGFR and HER3



Highlights

- **Optimized Dual-Action Binder:** Engineered with finely tuned binding avidity in both arms to address tumor heterogeneity while minimizing on-target, off-tumor toxicity.
- **Glycan-Specific Dual-Payload Platform:** Designed for enhanced stability and improved homogeneity.
- **Dual-Payload Approach:** Combines T01 (DAR 4) and MMAE (DAR 2) payloads to overcome non-response and resistance observed with single-payload strategies.

JSKN021 bound simultaneously to EGFR and HER3

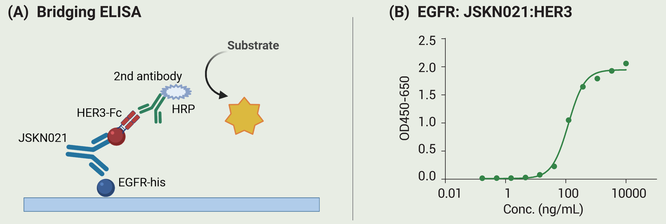


Figure 1. JSKN021 was demonstrated to bind EGFR and HER3 simultaneously. **(A)** Graphic illustration of bridging ELISA method to assess the capacity of JSKN021 to bind to EGFR and HER3 simultaneously. EGFR is immobilized on the plate, JSKN021 is added, and HER3-muFc binding is detected using a HRP-labeled secondary reagent. **(B)** The ELISA assay confirms JSKN021's dualtarget engagement and functionality through colorimetric absorbance measurement.

Stable form of T01 and MMAE were released into the cell lysate and supernatant of JSKN021-treated HCC827 cells

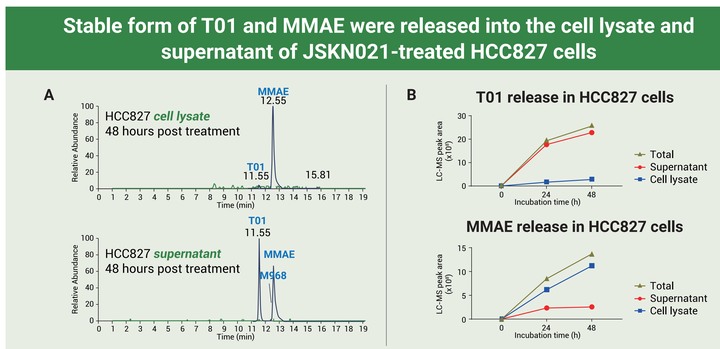


Figure 2. T01 and MMAE were released into the cell lysate and supernatant of JSKN021-treated HCC827 cells. HCC827 cells were treated with JSKN021 for 24 and 48 hours. The supernatant and cell lysates were collected to detect small molecules, including T01, MMAE, and other metabolites. **(A)** Both T01 and MMAE were detected in the cell culture supernatant and suspension of HCC827 cells after 48 hours, while no other detectable metabolites were observed. **(B)** Dynamic changes in T01 and MMAE levels in the supernatant and cell lysates of JSKN021-treated HCC827 cells.

JSKN021 demonstrated excellent stability in serum from rat, mice, monkey, and human, with minimal release of MMAE and T01

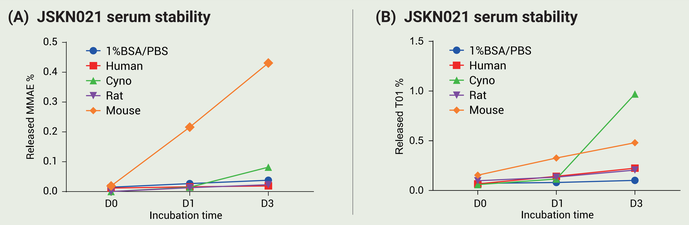


Figure 3. JSKN021 exhibited excellent stability, showing minimal payload release. This is indicated by the extremely low levels of MMAE **(A)** and T01 **(B)** detected in serum across various species after incubation for up to 3 days.

JSKN021 exhibited potent cytotoxicity in cancer cell lines expressing EGFR, HER3 or both

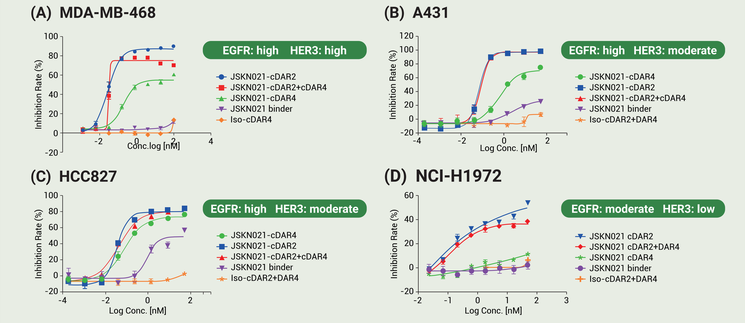


Figure 4. JSKN021 effectively suppressed the growth of cancer cells expressing Her3, EGFR, or both. **(A-D)** Dual payload ADC (JSKN021 DAR4+DAR2) exhibits superior cytotoxic potency compared to mono-payload ADC (JSKN021-DAR2 or -DAR4) across cell lines with a broad range of EGFR/HER3 expression levels.

JSKN021 showed strong tumor-suppressive efficacy in CDX models

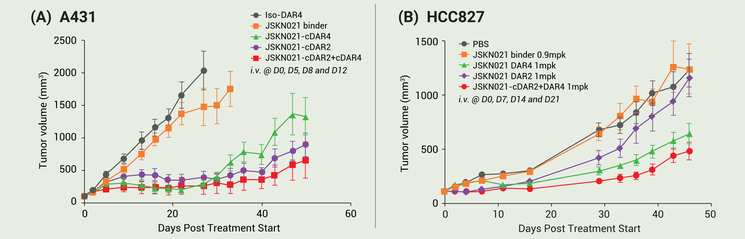


Figure 5. JSKN021 demonstrates excellent tumor-killing potency in CDX models. **(A-B)** JSKN021 payloads DAR2 (MMAE) and DAR4 (T01) show synergistic tumor growth inhibition in A431 (epidermoid carcinoma) and HCC827 (NSCLC) CDX models.

Summary

- **Conjugation and Stability:** JSKN021 demonstrated excellent stability in serum from rat, mice, monkey, and human, with minimal payload release owing to glycan-based conjugation.
- **Binding and Payload Release:** JSKN021 successfully bound to both EGFR and HER3, and its payloads (T01 and MMAE) were effectively released without detectable side metabolites.
- **In Vitro Efficacy:** JSKN021 significantly inhibited cancer cell growth in multiple cell lines expressing EGFR, HER3, or both (e.g., HCC827, MDA-MB-468, A431, NCI-H1975).
- **In Vivo Efficacy:** In multiple CDX models, JSKN021 showed superior tumor inhibition compared to single-payload ADCs