JSKN022, a first-in-class multi-specific ADC targeting PD-L1 and ITGB6/8 with an innovative DNA topoisomerase I inhibitor demonstrates encouraging efficacy in preclinical models

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Abstract

Background

Monoclonal antibodies targeting PD-1/PD-L1 have significantly transformed cancer therapy. However, the majority of patients still cannot get benefit from this treatment or may develop resistance afterwards. The increased expression of PD-L1 in tumors makes it an attractive target for ADC development leading to the most advanced program in registration trials. We developed a multi-specific ADC targeting another set of antigens, ITGB6/8. Integrins are cell surface receptors that usually mediate cell to cell adhesion. Integrin β6/8 (ITGB6/8), which can only form a heterodimer with the αv integrin subunit, is highly expressed in various tumors. Utilizing our proprietary site-specific glycan conjugation, we generated JSKN022, an innovative PD-L1/ITGB6/8 targeting ADC, which structurally composed of a sdAb (single domain antibody) Fc fusion protein targeting PD-L1 and ITGB6/8, a cleavable linker and a novel DNA topoisomerase I inhibitor (T01, Alphatecan). In this study, the pharmacological activity of JSKN022 was evaluated using preclinical in vitro and in vivo models.

Methods

Based on Envafolimab, we designed a novel sdAb-Fc fusion protein which targeting both PD-L1 and ITGB6/8. Then T01 was homogeneously and stably conjugated to glycan located on Fc. The conjugated product (DAR4) was thoroughly characterized by LC-MS. The binding specificity of JSKN022 and its internalization rate were assessed. Pattern of payload released were analyzed using LC-HRMS. Serum stability was tested across various species. In vitro cell growth inhibition and in vivo antitumor efficacy of JSKN022 were examined using a range of cancer cell lines and CDX models with parental antibody ADC as control.

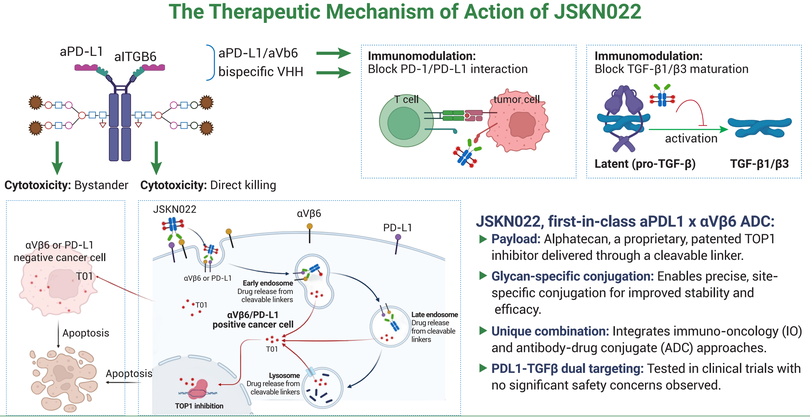
Results

JSKN022 demonstrated specific binding to the αvβ6/8 protein, without cross-reactivity with other members of integrin family (Figure 1). JSKN022 exhibited a consistent DAR4. Bridging ELISA indicated that JSKN022 can simultaneously bind to both PD-L1 and ITGB6/8 (Figure 1). Flow cytometry binding assays revealed that JSKN022 possessed a superior binding capacity compared to both parental antibodies (Figure 2). Correspondingly, the naked antibody of JSKN022 showed an increased internalization rate relative to parental antibodies in HCC4006 and Capan-2 tumor cells (Figure 3). JSKN022 effectively inhibits the proliferation of antigen-positive cancer cells and exhibited enhanced tumor suppression compared to the single-target ADCs (Figure 4 and 5). Furthermore, benefiting from glycan conjugation, JSKN022 demonstrated excellent stability in serum from rats, mice, monkeys, and humans, with minimal T01release (Figure 6).

Conclusion

This preclinical study suggests JSKN022, as a first in class multi-specific PD-L1/ITGB6/8 ADC, might potentially bring in novelty in the therapeutic approach for cancers that are refractory or resistant to PD-1/PD-L1 inhibitors.

The Therapeutic Mechanism of Action of JSKN022



JSKN022, first-in-class aPDL1 x αVβ6 ADC:

- **Payload:** Alphatecan, a proprietary, patented TOP1 inhibitor delivered through a cleavable linker.
- **Glycan-specific conjugation:** Enables precise, site-specific conjugation for improved stability and efficacy.
- **Unique combination:** Integrates immuno-oncology (IO) and antibody-drug conjugate (ADC) approaches.
- **PDL1-TGFβ dual targeting:** Tested in clinical trials with no significant safety concerns observed.

JSKN022 bound simultaneously to both PD-L1 and ITGB6, with minimal cross-reactivity with other members of the integrin family

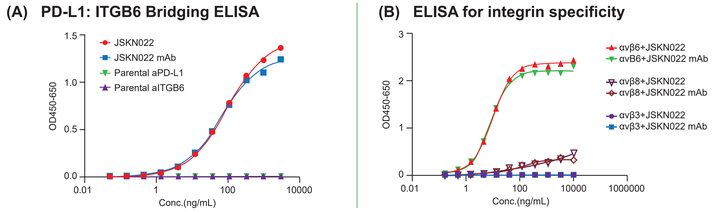


Figure 1. ELISA to detect the binding of JSKN022 to its two targets. **(A)** A bridging ELISA demonstrated that JSKN022 can simultaneously bind to both PD-L1 and ITGB6. In this assay, ITGB6 is immobilized on the plate, JSKN022 is introduced, and PD-L1-Fc binding is detected using an HRP-labeled secondary reagent specific for PD-L1 Fc. The assay confirms JSKN022's dual-target engagement and functionality through colorimetric absorbance measurement. **(B)** An ELISA assessing JSKN022's binding to various integrin family members confirmed its specificity for ITGB6. Different integrin proteins were immobilized on the plate, JSKN022 was added, and binding was detected using an HRP-labeled secondary reagent specific to JSKN022 Fc.

JSKN022 possessed a superior binding capacity compared to mono-target ADCs

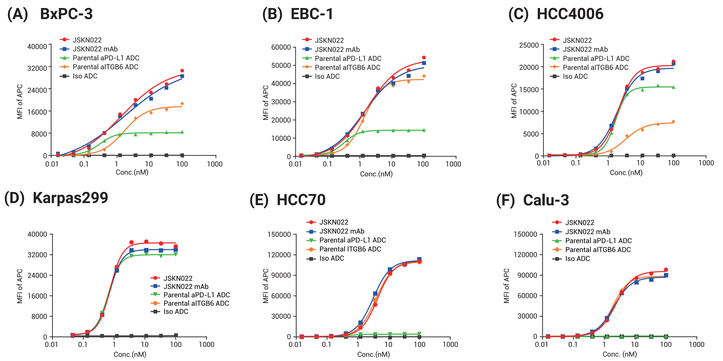


Figure 2. Binding of JSKN022 to cancer cells with expression of PD-L1 or ITGB6, or both. **(A-C)** JSKN022 demonstrates superior binding capacity to PD-L1+ITGB6+ cancer cells (BxPC-3, EBC-1, and HCC4006) compared to the parental mono-target ADCs (Parental aPD-L1 ADC, Parental aITGB6 ADC). **(D)** JSKN022 exhibits comparable cellular binding affinity to PD-L1+ITGB6- cancer cells (Karpas299) relative to the parental aPD-L1 ADC, while the parental aITGB6 ADC shows minimal binding activity. This highlights the specificity of the aITGB6-targeting component in JSKN022. **(E-F)** JSKN022 displays comparable cellular binding affinity to PD-L1-ITGB6+ cancer cells (HCC70 and Calu-3) relative to the parental aITGB6 ADC, while the parental aPD-L1 ADC exhibits minimal binding activity on these cells. This underscores the specificity of the aPD-L1-targeting component in JSKN022.

JSKN022 mAb showed an increased internalization rate relative to mono-target antibodies in HCC4006 and Capan-2 cancer cells

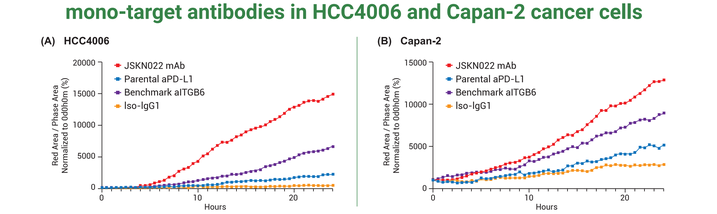


Figure 3. Incucyte was used to test the internalization of JSKN022 antibodies. **(A-B)** JSKN022 exhibits superior internalization capacity on cancer cells expressing PD-L1 and ITGB6 compared to the mono-target ADCs, including the parental aPD-L1 ADC and the investigational benchmark aITGB6 (Sigvatatug-vedotin).

JSKN022 effectively inhibited the proliferation of antigen-positive cancer cells

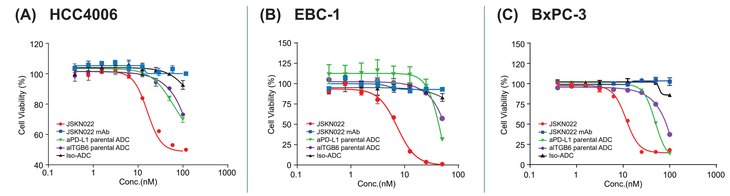


Figure 4. Cytotoxicity of JSKN022 on antigen-positive cells. **(A-C)** JSKN022 exhibits superior cytotoxicity against cancer cells co-expressing PD-L1 and ITGB6 when compared to the parental single-target ADCs (aPD-L1 parental ADC and aITGB6 parental ADC).

JSKN022 demonstrated excellent efficacy in inhibiting tumor growth in tumor models

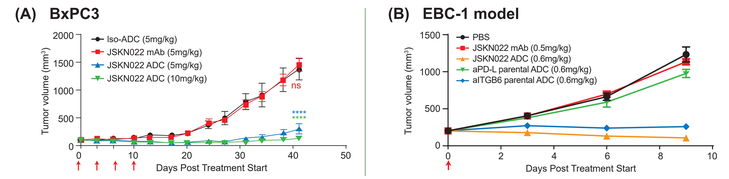


Figure 5. JSKN022 demonstrated excellent efficacy in inhibiting tumor growth in CDX models. **(A)** JSKN022 demonstrated excellent efficacy in inhibiting tumor growth in BxPC-3 (PDAC) CDX model. **(B)** JSKN022 shows superior efficacy in inhibiting tumor growth in EBC-1 (NSCLC) CDX models compared to single-target parental ADCs. In both models, JSKN022 mAb exhibited minimal effects, emphasizing that the cytotoxicity is dependent on the ADC.

JSKN022 demonstrated excellent stability in serum from rat, mice, monkey, and human, with minimal release of T01

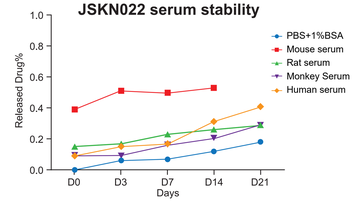


Figure 6. JSKN022 demonstrated excellent stability with very slow payload (T01) release, as evidenced by the extremely low T01 levels in serum across different species after incubation of 21 days.

Summary

- **Binding and Specificity:** JSKN022 demonstrated specific binding to the αvβ6 protein without cross-reacting with other integrin family members. It showed the ability to bind simultaneously to PD-L1 and ITGB6/8, confirmed by bridging ELISA.
- **Internalization:** Incucyte assay revealed superior internalization capacity in HCC4006 and Capan-2 cancer cells compared to mono-target antibodies.
- **Conjugation and Stability:** JSKN022 maintained a consistent DAR4, with excellent serum stability (rat, mice, monkey, human) and minimal payload (T01) release, attributed to glycan-based conjugation.
- **Efficacy:** JSKN022 effectively inhibited the proliferation of antigen-positive cancer cells and demonstrated greater tumor suppression than single-target ADCs in preclinical cancer models.