Preclinical Development of an EGFR-Targeted Tumor-Activated T Cell Engager with Enhanced Safety to Activity Multiple and Pharmacokinetics Profile


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**INTRODUCTION:** Epidermal growth factor receptor (EGFR) is the most commonly expressed membrane oncogenic protein in human cancers. KRAS mutations are significant drivers of resistance to EGFR-targeted antibody therapies. Unlike other treatments, EGFR-targeting, CD3 bispecific T cell engagers (TCEs) retain activity against tumors bearing resistance mutations. However, cytokine release syndrome (CRS), off-target toxicities, and poor pharmacokinetic (PK) properties present major clinical limitations for these potent immunomodulators. To overcome these challenges, Janux has developed JANX008, an EGFR- and CD3-targeted tumor-activated T cell engager (TRACT) featuring enhanced safety and PK profiles.

**Figure 1: JANX008 – Design, Structure and Mechanism of Action**

- JANX008 is a tumor-activated T cell engager (TRACT) with EGFR- and CD3-binding domains, an albumin-binding domain to extend circulating half-life, and two protease cleavable masks that inhibit EGFR engagement on target cells and CD3 engagement on T cells, respectively.
- Tumor specific proteolysis separates the EGFR mask and the tandem CD3 mask with albumin-binding domain from JANX008. It enables tumor-restricted EGFR and CD3 binding and subsequent T cell activity against cancer cells.
- JANX008 exhibits a large safety multiple relative to EGFR- or CD3-binding by >300 and >1,000 fold.
- Target engagement is cleavage-dependent, >9 masks reducing EGFR- or CD3-binding by >300 and >1,000 fold.
- Treatment of JANX008 with protease enzyme enables potent binding comparable to non-masked EGFR-TCR.
- JANX008 exhibits potent binding to human and monkey albumin.
- Functional activity against KRAS mutant anti-EGFR resistant CRC and NSCLC cell lines in T cell co-culture assays is cleavage-dependent. Protease-treated JANX008 exhibits similar potency relative to non-masked EGFR-TCR.
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**Figure 2: Binding of JANX008 to CD3 and EGFR is cleavage- and dose-dependent**

**Figure 3: Functional activity of JANX008 is cleavage- and dose-dependent**

**Figure 4: JANX008 exhibits cleavage-dependent activity in a HCT116 mouse tumor model**

**Figure 5: JANX008 shows cleavage-dependent activity in human CRC organoids**

**Figure 6: JANX008 has extended half-life and enhanced safety profile in NHPs**

**Figure 7: JANX008 exhibits a large safety multiple relative to EGFR-TCR in NHP studies**

**JANX008 NHP toxicity studies support its enhanced PK, safety, and design**

- JANX008 exhibited once weekly for three weeks was well tolerated without clinical observations or notable changes in clinical chemistry, hematology, or pathophysiology.
- EGFR-TCR dosed by continuous IV infusion at 15 μg/kg/day had increased body temp & increased liver enzymes.
- Treatment with EGFR-TCR at 30 μg/kg/day by continuous IV infusion had to be terminated 24 hrs after dosing due to severe adverse events, including fever, skin rash, GI toxicities, and lack of responsiveness in the animals.
- Clinical observations, body temperatures, and clinical pathology measurements support the large safety multiple (>300x) for EGFR-TCR relative to non-masked EGFR-TCR.
- JANX008 reduces cytokine release and healthy tissue toxicities (GI, Liver, skin) at high exposures in cynomolgus monkeys despite broad tissue expression of EGFR.
- JANX008 No-Observed-Adverse-Effect-Level (NOAEL) ≥ 0.6 mg/kg/dose weekly IV bolus for three weeks.

**SUMMARY & CONCLUSIONS:**

- JANX008 TRACT exhibits enhanced safety and PK properties relative to the EGFR-TCR.
- The critical safety features of JANX008 are two tumor protease-cleavable peptide masks that inhibit EGFR and CD3 binding by >300 and >1,000x, respectively.
- Potent cleavage- and dose-dependent activity of JANX008 TRACT was demonstrated in multiple preclinical models, including EGFR antibody-resistant tumor and T cell co-culture assays, humanized mouse CRC model, and a fully human primary CRC tumor system with intact TME.
- Enhanced PK profile and high exposure of JANX008 were well tolerated in NHP safety studies with limited CRS and healthy tissue toxicities.
- GMP manufacturing complete to support phase 1 clinical trial.
- Preclinical data demonstrate key characteristics of JANX008 including cleavage-dependent activity, half-life extended PK, potential for superior safety, and manufacturability properties that could mitigate major limitations of TCEs and support JANX008 clinical development.

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