Background Epidermal growth receptor (EGFR) is the most expressed membrane oncogenic protein in human cancers. KRAS and BRAF mutations are significant drivers of resistance to EGFR-targeted therapies. Unlike other treatments, EGFR-targeting, CD3 bispecific T cell engagers (TCEs) can potentially retain activity against tumors bearing resistance mutations. However, cytokine release syndrome (CRS), on-target off-tumor toxicities, and poor pharmacokinetics (PK) properties present significant clinical limitations for these potent immunomodulators. To overcome these challenges, Janux has developed JANX008, an EGFR- and CD3-targeted tumor-activated T cell engager (TRACTr). JANX008 is a humanized trispecific protein that contains EGFR- and CD3-binding domains, an albumin binding domain to extend circulating half-life, and two different peptide masks fused to the molecule through tumor protease cleavable linkers. One peptide mask inhibits EGFR engagement on target cells, and the other inhibits CD3 engagement on T cells. Once the cleavage sequences undergo proteolysis by tumor proteases, the EGFR and CD3 masks are released, and the resulting active molecule can bind EGFR and CD3 on target cells.

Methods Peptide masks against EGFR- and CD3-binding domains were identified via phage display. The efficiency of the masks was evaluated using human EGFR and CD3 ELISAs. JANX008-induced cleavage-dependent T cell killing was evaluated in human PBMC/tumor cell co-culture assays. Antibody efficacy of JANX008 was tested in multiple preclinical models, including EGFR antibody-resistant KRAS- and PIK3CA-mutant mouse colon cancer model (HCT116) and a fully human primary colorectal cancer (CRC) tumoroid system. The pharmacokinetic and safety profile of JANX008 was evaluated in non-human primate studies.

Results JANX008 target engagement was cleavage-dependent, where masking reduced EGFR and CD3 binding by >300x and >1,000x, respectively. JANX008 exhibited potent cleavage- and dose-dependent activity in multiple preclinical models, including EGFR antibody-resistant KRAS- and PIK3CA-mutant mouse colon cancer model (HCT116) and a fully human primary colorectal cancer (CRC) tumoroid system. The pharmacokinetic and safety profile of JANX008 was evaluated in non-human primate studies.

Conclusions Preclinical data demonstrate key characteristics of JANX008, including cleavage-dependent activity, half-life extended PK, the potential for superior safety, and manufacturability properties that could mitigate significant limitations of TCEs and support JANX008 clinical development.

Acknowledgements We acknowledge Marque Todd for providing insightful comments and help with the design and interpretation of NHP safety studies.

Ethics Approval All animal experiments were approved by the Institutional Animal Use and Care Committee of the institutions conducting the studies and in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.