Background Metastatic castration-resistant prostate cancer (mCRPC) remains an incurable disease. Bispecific T cell engagers (TCEs) targeting prostate-specific membrane antigen (PSMA) and CD3 on T cells showed great clinical potential for the treatment of mCRPC. However, cytokine release syndrome (CRS) and poor pharmacokinetic (PK) profile hinder their further development. To overcome these challenges, Janux has developed JANX007, a tumor-activated T cell engager (TRACTr) with enhanced safety and PK properties. JANX007 is a humanized trispecific protein that contains PSMA- and CD3-binding domains, an albumin binding domain to extend circulating half-life, and a CD3 inhibitory peptide mask fused to the molecule through tumor protease cleavable linker. Only when tumor-resident proteases cleave the TRACTr and enable mask separation can the resulting active molecule bind CD3. This cleavage-dependent CD3 agonism can potentially limit systemic toxicity associated with broad T cell activation.

Methods Peptide masks against the CD3 binding domain were identified via phage display. Mask efficiency was evaluated using human CD3 ELISAs. Masking and cleavable linker stability was characterized in human (healthy and mCRPC donor) and cynomolgus monkey serum. JANX007-induced cleavage-dependent activation of T cells was evaluated in human PBMC/prostate tumor cell in vitro co-culture assays. The pharmacokinetic and safety profile of JANX007 was evaluated in non-human primate (NHP) studies.

Results Engagement of CD3 target by JANX007 was shown to be cleavage dependent where masking reduced CD3 binding by >600x. In vitro, JANX007 exhibited a ~500x decrease in potency to activate T cells and induce T cell-mediated tumor cell killing relative to non-masked TCE. JANX007 was highly stable in healthy and mCRPC human donor serum, with ≤1% cleavage per day. While proteolytic cleavage of JANX007 in the tumor microenvironment is expected to drive anti-tumor activity, the maintenance of masking in the blood compartment is expected to mitigate the safety risks associated with potential off-tumor toxicity and CRS. JANX007 was found to be highly stable in NHPs with minimally detectable cleavage. The lack of TCE accumulation in NHPs mitigated on-target healthy tissue toxicities and minimized CRS. Clinical chemistry, hematology, and pathology data package support No-Observed-Adverse-Effect-Level (NOAEL) ≥1.5 mg/kg/dose. Finally, the cleavable albumin-binding domain extended the circulating half-life of JANX007 to ~120h in NHPs, relative to the 2h half-life of non-masked TCE, supporting its projected once-weekly clinical dosing.

Conclusions Cleavage-dependent activity, half-life extended PK, the potential for superior safety and manufacturability properties of JANX007 support its further development as an attractive mCRPC therapeutic.

Acknowledgements We acknowledge Marque Todd for providing insightful comments and help with the 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.