Preclinical Activity and Safety Profile of JANX007, a Novel PSMA-Targeting Tumor-Activated T Cell Engager for Treatment of Metastatic Castration-Resistant Prostate Cancer

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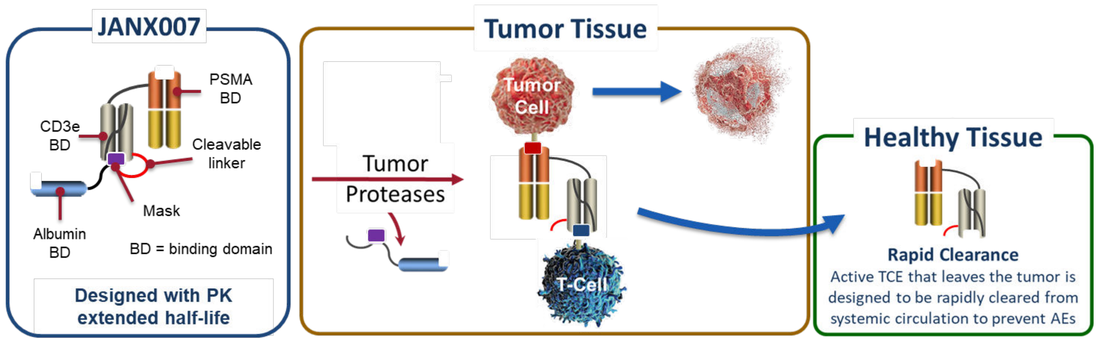
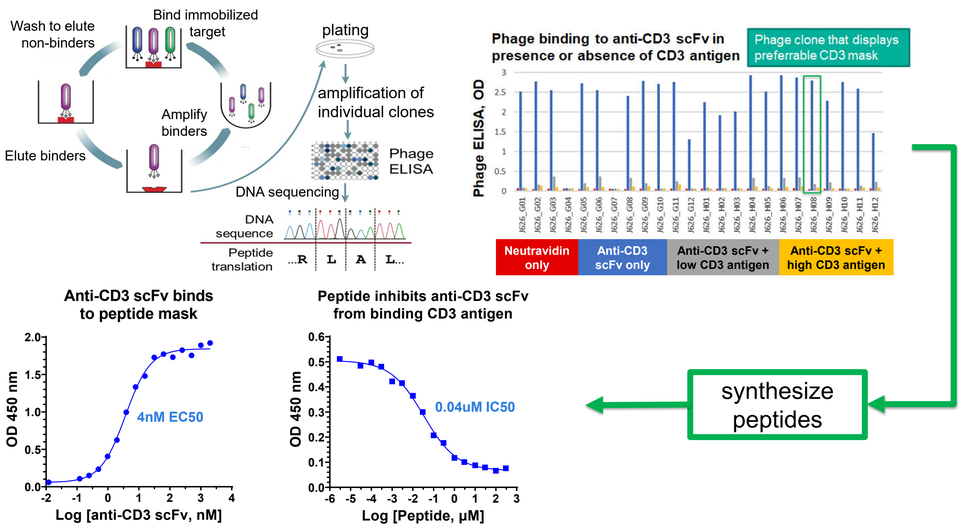
INTRODUCTION: Metastatic castration-resistant prostate cancer (mCRPC) remains an incurable disease. Bispecific T cell engagers (TCEs) targeting prostate-specific membrane antigen (PSMA) on prostate tumor cells and cluster of differentiation 3 (CD3) on T cells have demonstrated clinical efficacy for the treatment of mCRPC. However, cytokine release syndrome (CRS) and poor pharmacokinetic (PK) profile remain critical challenges that hinder this powerful drug class. To overcome these challenges, Janux has developed JANX007; a PSMA-targeted tumor-activated T cell engager (TRACTr) featuring enhanced safety and pharmacokinetics profiles. JANX007 is entering the clinic with FDA acceptance of its IND.

Figure 1: JANX007 – Design, Structure and Mechanism of Action



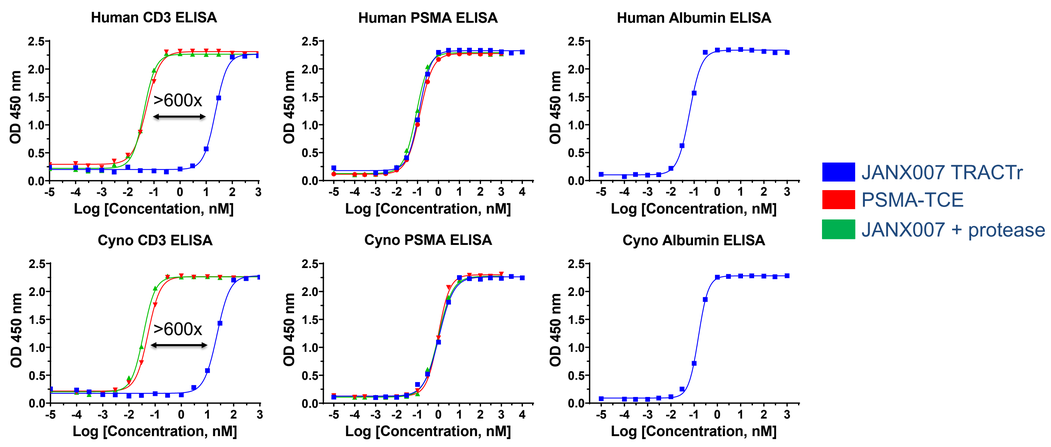
JANX007 is a tumor-activated T cell engager with PSMA- and CD3-binding domains, a peptide mask that inhibits CD3 engagement on T cells, an albumin-binding domain appended to the mask to extend circulating half-life, and a tumor protease cleavable linker. Tumor-specific proteolysis of the cleavable linker in the tumor microenvironment (TME) separates the tandem mask and albumin-binding domain from JANX007. It enables TME restricted CD3 binding and subsequent T cell activation against PSMA expressing prostate cancer cells. Loss of the albumin-binding domain likely potentiates that any activated JANX007 that migrates out of the tumor will be cleared rapidly and reduces its potential accumulation in healthy tissues that can contribute to safety risks.

Figure 2: Mask discovery by peptide phage display



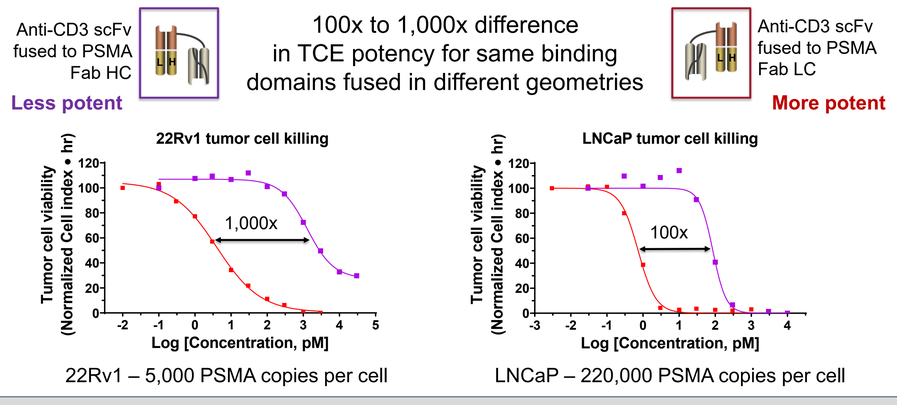
- Phage displaying peptide libraries were screened for binding to surface-immobilized anti-CD3 scFv.
- After several bind, elute, and amplify cycles, clonal phages were screened for CD3 competitive binding by ELISA.
- Selected clonal phage sequences were synthesized as peptides and screened for binding and inhibition properties against anti-CD3 scFv. Peptide inhibitors were then incorporated into TRACTr designs.

Figure 3: Binding of JANX007 to CD3 is cleavage- and dose-dependent



- JANX007 CD3 target engagement is cleavage dependent where masking reduces CD3 binding by >600 fold.
- Treatment of JANX007 with protease enzyme enables potent CD3 binding comparable to non-masked PSMA-TCE.
- JANX007 exhibits potent binding to human and monkey PSMA and albumin.

Figure 4: PSMA-TCE potency depends on structure and orientation

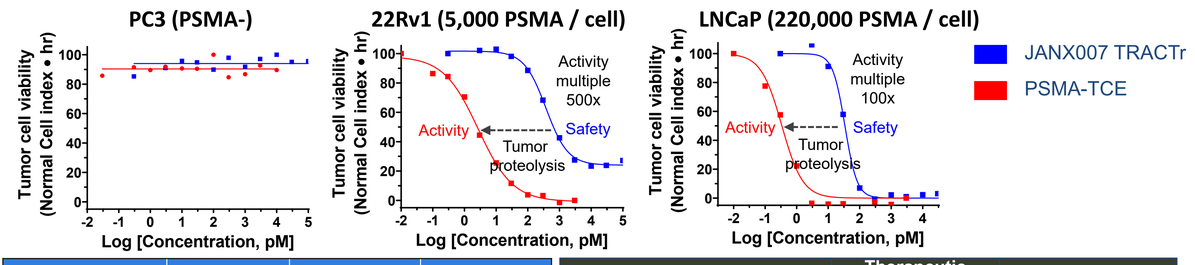


22Rv1 – 5,000 PSMA copies per cell

LNCaP – 220,000 PSMA copies per cell

- PSMA-TCE activity depends on the connecting geometry of PSMA and CD3 binding domains.

Figure 5: Activity of JANX007 is cleavage-, dose- and PSMA expression-dependent



	22Rv-1	PCa-2b	LNCaP
TCE EC50 (pM)	2	0.2	0.3
PSMA Density	5,000	90,000	220,000
AR-V7	(+)	(-)	(-)
AR Mutant	H875Y	L702H, T878A	T878A
BRCA(1/2) Mutant	(-)/(+)	(-)/(-)	(+)/(+)
MSI-H/dMMR	(+)	(-)	(+)

Key Resistance Drivers	Therapeutic Options	TRACTr Activity
Androgen Receptor Variants *18K yearly incidence*	No approved therapies	Responsive to PSMA-TCE
Androgen Receptor Mutants *8K yearly incidence*	No approved therapies	Responsive to PSMA-TCE
BRCA Mutants *5K yearly incidence*	PARP inhibitors	Responsive to PSMA-TCE
MSI-H, dMMR Mutants *2K yearly incidence*	Checkpoint antibodies	Responsive to PSMA-TCE

- Functional activity in prostate cancer and T cell co-culture assays is dependent on masking and PSMA expression.
- JANX007 activity exhibits a robust T cell functional shift that demonstrates potential for an enhanced safety profile.
- JANX007 activity provides broad coverage against key mCRPC resistance drivers that create unmet medical needs, such as AR, BRCA, and MSI-H/dMMR mutations.

Figure 6: Janux cleavable linkers feature rapid proteolysis and high serum stability

Apparent cleavage rate of TRACTrs harboring different cleavable linkers					
Tumor Proteases	**CL-1 (ISSGLLSGRSDNH)**	**CL-2**	**CL-3**	**CL-4**	**CL-5**
MMP2					
MMP7					
MMP9					
MMP13					
MMP14					
uPa					
MTSP1					
Hepsin					

Rate (M^{-1} s^{-1})	
> 10^5	
10^4 < r < 10^5	
2.5x10^3 < r < 10^4	
≤ 2.5x10^3	

Janux linkers are cleaved faster than that harbored by Pacmilimab (CL-1) AND maintain stability in human serum

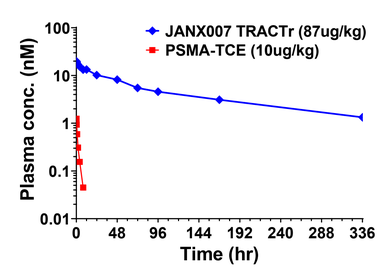
Relative TRACTr serum stability (cleavage rate - % per day)					
	CL-1 (ISSGLLSGRSDNH)	**CL-2**	**CL-3**	**CL-4**	**CL-5**
Human Serum	Stable; < 1% per day	Stable; < 1% per day	Stable; < 1% per day	Stable; < 1% per day	Stable; < 1% per day

Relative JANX007 (CL-5) serum stability in donor serum samples (cleavage rate - % per day)		
Normal pooled human serum	**Normal pooled cyno serum**	**mCRPC donor serum (n=6)**
< 1%	6%	1%

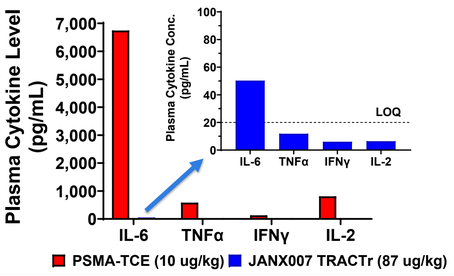
JANX007 serum stability was determined via CD3 binding experiments using an Octet RED instrument after incubation of JANX007 in different serum samples

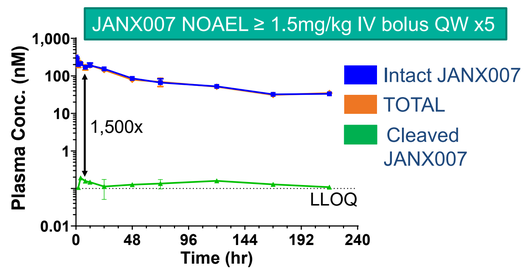
- Serum proteolytic activity is greater than blood and likely represents a conservative assessment of *in vivo* stability
- Janux cleavable linkers are cleaved rapidly by a panel of recombinant tumor proteases leading to enhanced de-masking in the TME and anti-tumor activity.
- JANX007 exhibits high stability in healthy and mCRPC human donor serum with ≤ 1% cleavage per day.
- While proteolytic cleavage of JANX007 in the TME is expected to drive anti-tumor activity, a critical safety feature of JANX007 is its stability in the blood compartment, where maintenance of masking is expected to mitigate the safety risks associated with potential healthy tissue toxicity and CRS.

Figure 7: JANX007 has extended half-life and enhanced safety profile in NHPs



PK parameter	TRACTr	TCE
Dose (ug/kg)	87	10
C_{MAX} (nM)	18	1.7
$t_{1/2}$ (hr)	119	2
Vd (L)	0.18	0.2
CL (ml/hr/kg)	0.34	25





JANX007 toxicity studies support enhanced PK, safety, and drug design

- JANX007 administered at 1.5 mg/kg IV bolus QW x5 in cyno monkeys achieved high exposures and long half-life without clinical signs or notable changes in clinical pathology measurements.
- JANX007 was highly stable in vivo with minimally detectable cleavage.
- Cleaved JANX007 approached LLoQ while intact (masked) JANX007 achieved 1,500x exposure multiple above its minimally detectable metabolite.
- Total (masked + unmasked) and intact JANX007 exhibited overlapping PK profiles further supporting JANX007 in vivo stability.
- Rapid clearance of TCE minimizes cleaved JANX007 accumulation and in turn minimizes risk of on-target healthy tissue toxicities and CRS.

PSMA-TRACTr (JANX007) GLP toxicity study summary in non-human primates

- Once weekly dosing, 0.1, 0.3, 1.5 mg/kg, with a 4-week recovery.
- No microscopic histopathology findings were observed.
- Lack of TCE accumulation in vivo mitigated on-target healthy tissue toxicities and minimized cytokine release.
- Clinical chemistry, hematology and pathology data package support No-Observed-Adverse-Effect-Level (NOAEL) ≥ 1.5 mg/kg/dose.

SUMMARY & CONCLUSIONS:

- JANX007 TRACTr exhibits enhanced safety and PK properties relative to the PSMA-TCE.
- The critical safety feature of JANX007 is a tumor protease-cleavable, inhibitory peptide mask, which decreases JANX007 binding to human CD3 by >600x, restricting T cell activation to the TME.
- In vitro, JANX007 TRACTr exhibits up to 500x decrease in potency to activate T cells and induce T-cell mediated tumor cell killing relative to non-masked PSMA-TCE.
- JANX007 TRACTr shows an enhanced safety profile in NHPs, featuring a decrease in cytokine CRS-associated proinflammatory cytokines with NOAEL ≥ 1.5 mg/kg/dose IV bolus QW x5.
- Albumin-binding domain extends the circulating half-life of JANX007 to ~120hr in NHPs, relative to 2hr half-life of non-masked TCE, supporting the TRACTr's projected once weekly clinical dosing.
- Cleavage-dependent activity, half-life extended PK, potential for superior safety, and manufacturability properties of JANX007 support its development as an attractive mCRPC therapeutic.
- GMP Drug Substance and Drug Product production completed to support Phase 1 clinical trial. FDA has accepted the IND for JANX007.

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