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

**Healthy Tissue**

**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 albumin 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 ensures 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 phage 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 >500 fold.
- Treatment of JANX007 with protease enzyme enables potent CD3 binding comparable to non-masked PSMA-TRACTr.
- JANX007 exhibits potent binding to human and monkey PSMA and albumin.

**Figure 4: PSMA-TRACTr potency depends on structure and orientation**

- 200x to 1,000x difference in TCE potency for same binding domains fused in different geometries
- JANX007 TRACTr exhibits enhanced safety and PK properties relative to the PSMA-TRACTr
- The critical safety feature of JANX007 is a tumor protease-cleavable, inhibitory peptide mask, which decreases JANX007 binding to human CD3 by >500x, 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-TRACTr.
- JANX007 TRACTr shows an enhanced safety profile in NHPs, featuring a decrease in cytokine CRS-associated proinflammatory cytokines with NOAE2 2.5 mg/kg iv bolus QW x5.
- Albumin-binding domain extends the circulating half-life of JANX007 to ~120h in NHPs, relative to 2 hr half-life of non-masked TCE, supporting the TRACTr’s projected once weekly clinical dosing.
- GMP Drug Substance and Drug Product production completed to support Phase 1 clinical trial.
- Cleavage-dependent activity, half-life extended PK, potential for superior safety, and manufacturability properties of JANX007 support its further development as an attractive mCRC therapeutic.

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

<table>
<thead>
<tr>
<th>JANX007 PSMA-TRACTr</th>
<th>PSMA-TECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04 uM IC50</td>
<td>0.01 uM IC50</td>
</tr>
<tr>
<td>0.01 uM IC50</td>
<td>0.005 uM IC50</td>
</tr>
<tr>
<td>&lt;0.005 uM IC50</td>
<td>&lt;0.0025 uM IC50</td>
</tr>
</tbody>
</table>

**Figure 6: JANX007 has extended half-life and enhanced safety profile in NHPs**

- JANX007’s toxicity studies support enhanced PK, safety, and drug design.
- JANX007 was dosed at ~1.5 mg/kg iv bolus Q4W in cynomolgus 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.
- Dose escalation and repeat dosing of JANX007 approached L60 in non-human primate (NHP) PK studies, demonstrating its potential as a repeat dosing therapeutic.

**Figure 7: JANX007 NOAE2 > 10 mg/kg iv bolus QW x5**

- JANX007 NOAE2 > 10 mg/kg iv bolus QW x5
- JANX007 and JANX007 TRACTr exhibit enhanced safety and PK properties relative to the PSMA-TRACTr
- The critical safety feature of JANX007 is a tumor protease-cleavable, inhibitory peptide mask, which decreases JANX007 binding to human CD3 by >500x, 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-TRACTr.
- JANX007 TRACTr shows an enhanced safety profile in NHPs, featuring a decrease in cytokine CRS-associated proinflammatory cytokines with NOAE2 2.5 mg/kg iv bolus QW x5.
- Albumin-binding domain extends the circulating half-life of JANX007 to ~120h in NHPs, relative to 2 hr half-life of non-masked TCE, supporting the TRACTr’s projected once weekly clinical dosing.
- GMP Drug Substance and Drug Product production completed to support Phase 1 clinical trial.
- Cleavage-dependent activity, half-life extended PK, potential for superior safety, and manufacturability properties of JANX007 support its further development as an attractive mCRC therapeutic.

**Summary & Conclusions:**

- JANX007 TRACTr exhibits enhanced safety and PK properties relative to the PSMA-TRACTr.
- The critical safety feature of JANX007 is a tumor protease-cleavable, inhibitory peptide mask, which decreases JANX007 binding to human CD3 by >500x, 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-TRACTr.
- JANX007 TRACTr shows an enhanced safety profile in NHPs, featuring a decrease in cytokine CRS-associated proinflammatory cytokines with NOAE2 2.5 mg/kg iv bolus QW x5.
- Albumin-binding domain extends the circulating half-life of JANX007 to ~120h in NHPs, relative to 2 hr half-life of non-masked TCE, supporting the TRACTr’s projected once weekly clinical dosing.
- GMP Drug Substance and Drug Product production completed to support Phase 1 clinical trial.
- Cleavage-dependent activity, half-life extended PK, potential for superior safety, and manufacturability properties of JANX007 support its further development as an attractive mCRC therapeutic.