

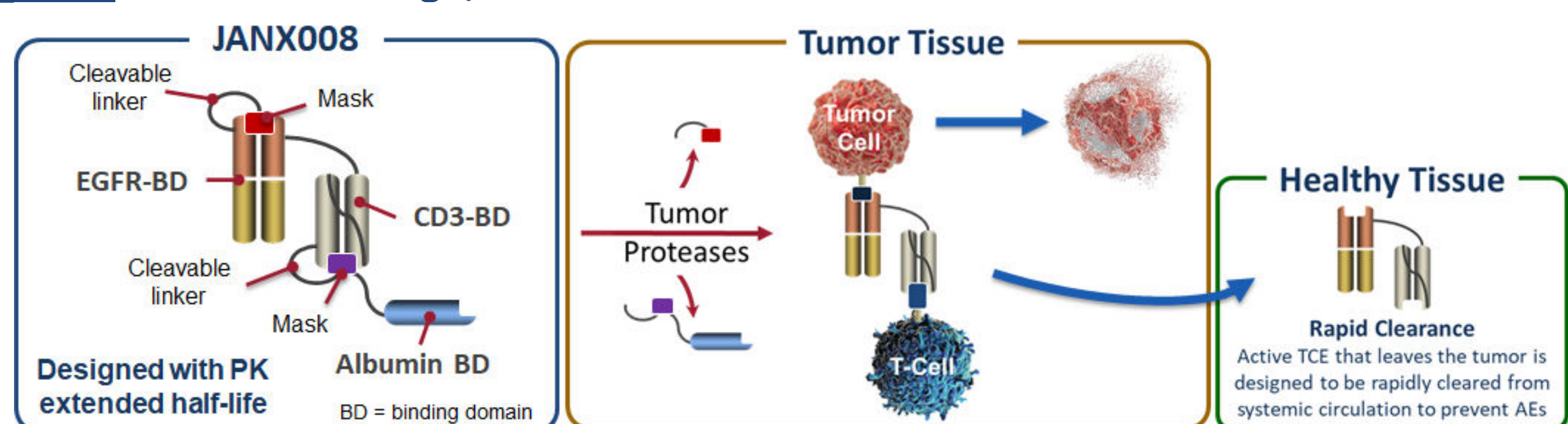
Preclinical Development of an EGFR-Targeted Tumor-Activated T Cell Engager with Enhanced Safety to Activity Multiple and Pharmacokinetics Profile

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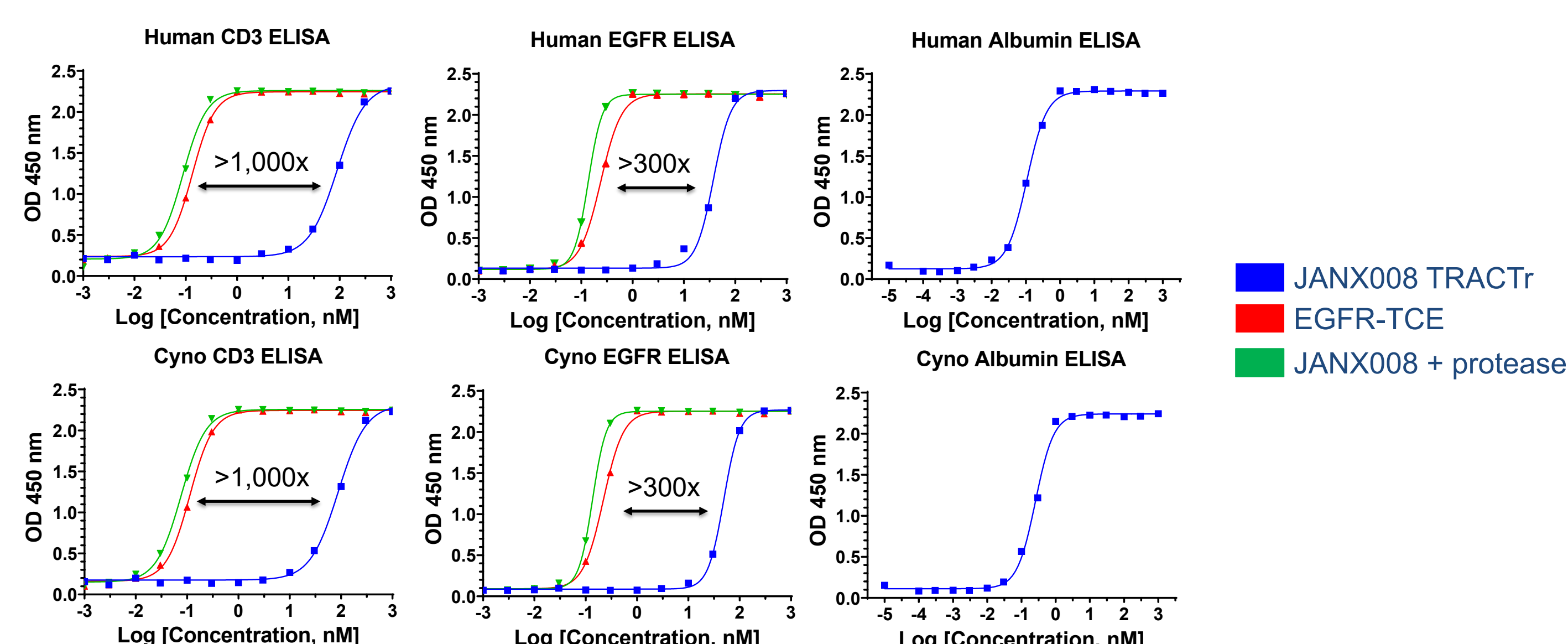
INTRODUCTION: Epidermal growth factor receptor (EGFR) is the most commonly expressed membrane oncogenic protein in human cancers. KRAS mutations are significant drivers of resistance to EGFR-targeted antibody therapies. Unlike other treatments, EGFR-targeting, CD3 bispecific T cell engagers (TCEs) retain activity against tumors bearing resistance mutations. However, cytokine release syndrome (CRS), on-target off-tumor 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 (TRACTr) featuring enhanced safety and PK profiles.

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



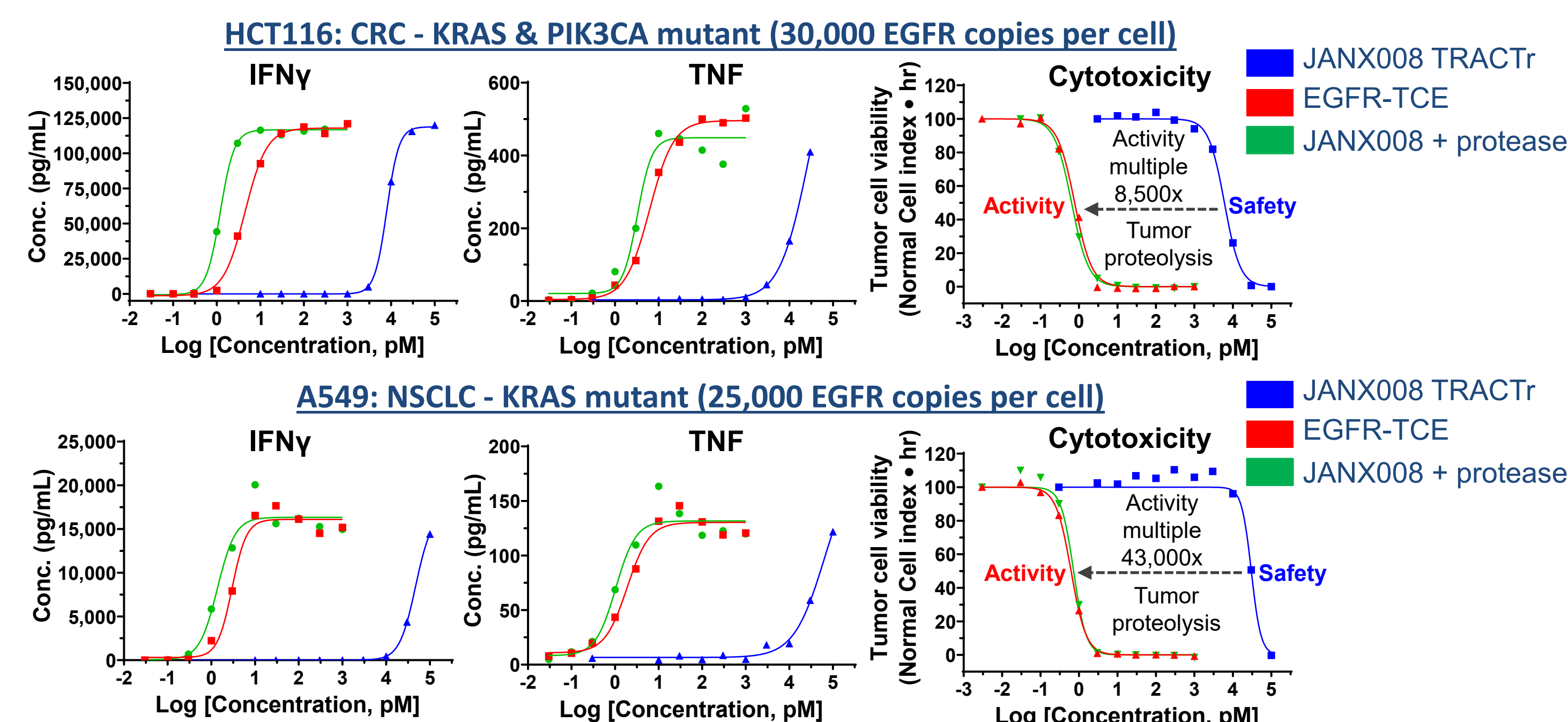
- JANX008 is a tumor-activated T cell engager (TRACTr) with EGFR- and CD3-binding domains, an albumin-binding domain to extend circulating half-life, and two protease cleavable masks that inhibit EGFR engagement on target cells and CD3 engagement on T cells, respectively.
- Tumor specific proteolysis 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.
- Loss of the albumin-binding domain likely potentiates that any activated JANX008 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: Binding of JANX008 to CD3 and EGFR is cleavage- and dose-dependent



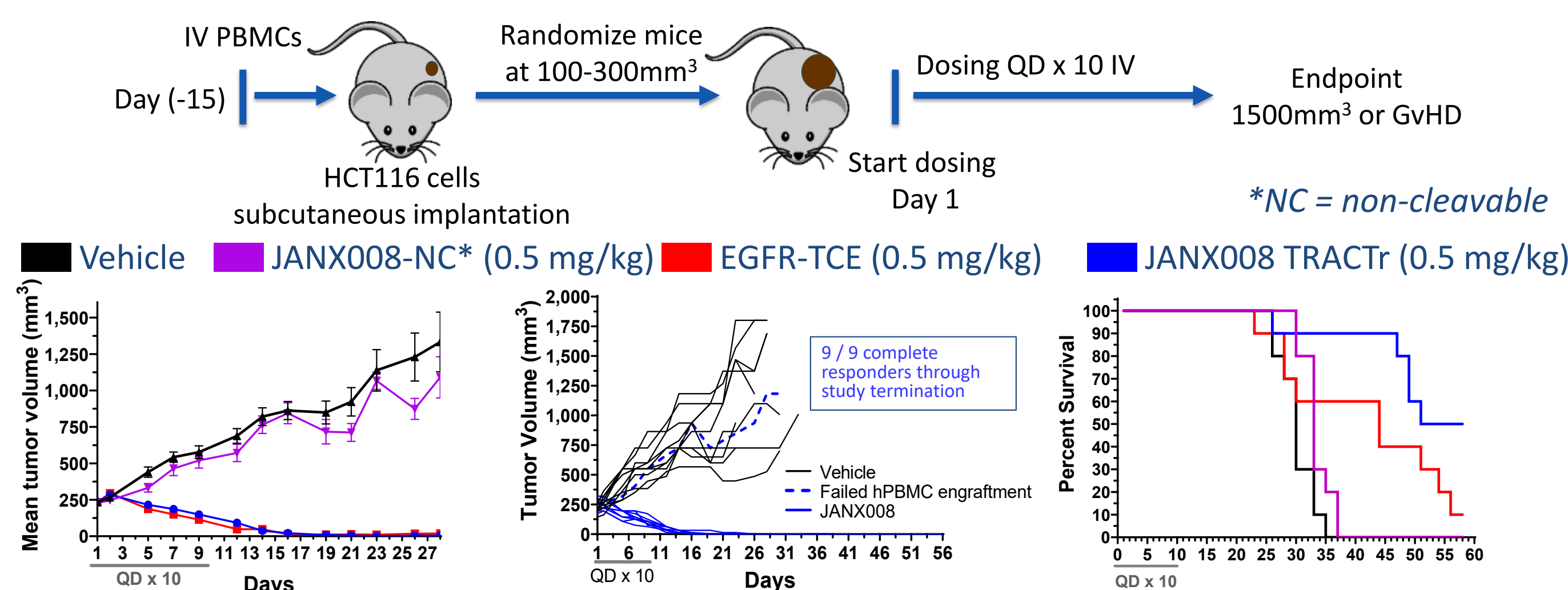
- Target engagement is cleavage-dependent → masking reduces EGFR- & CD3-binding by >300 and >1,000 fold.
- Treatment of JANX008 with protease enzyme enables potent binding comparable to non-masked EGFR-TCE.
- JANX008 exhibits potent binding to human and monkey albumin.

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



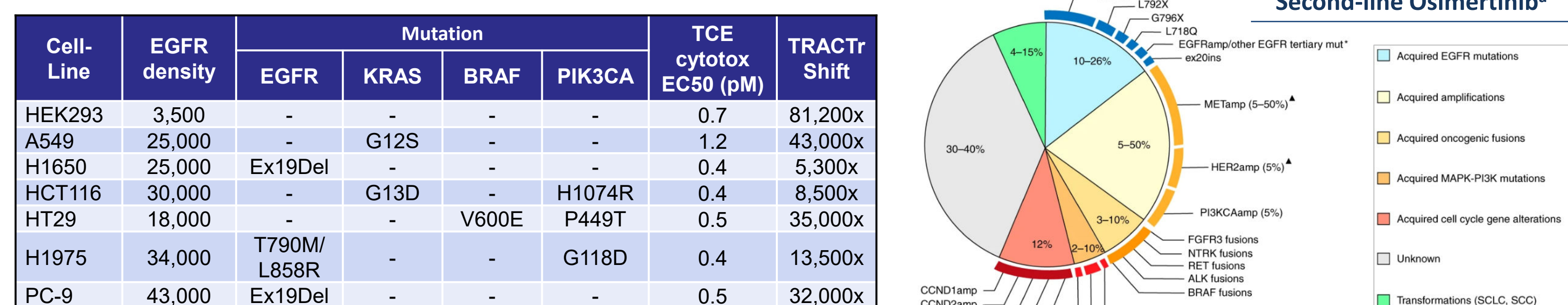
- Masking of JANX008 EGFR and CD3-binding domains reduces the capacity to induce cytokine release.
- Functional activity against KRAS mutant anti-EGFR resistant CRC and NSCLC cell lines in T cell co-culture assays is cleavage-dependent. Protease-treated JANX008 exhibits similar potency relative to non-masked EGFR-TCE.

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



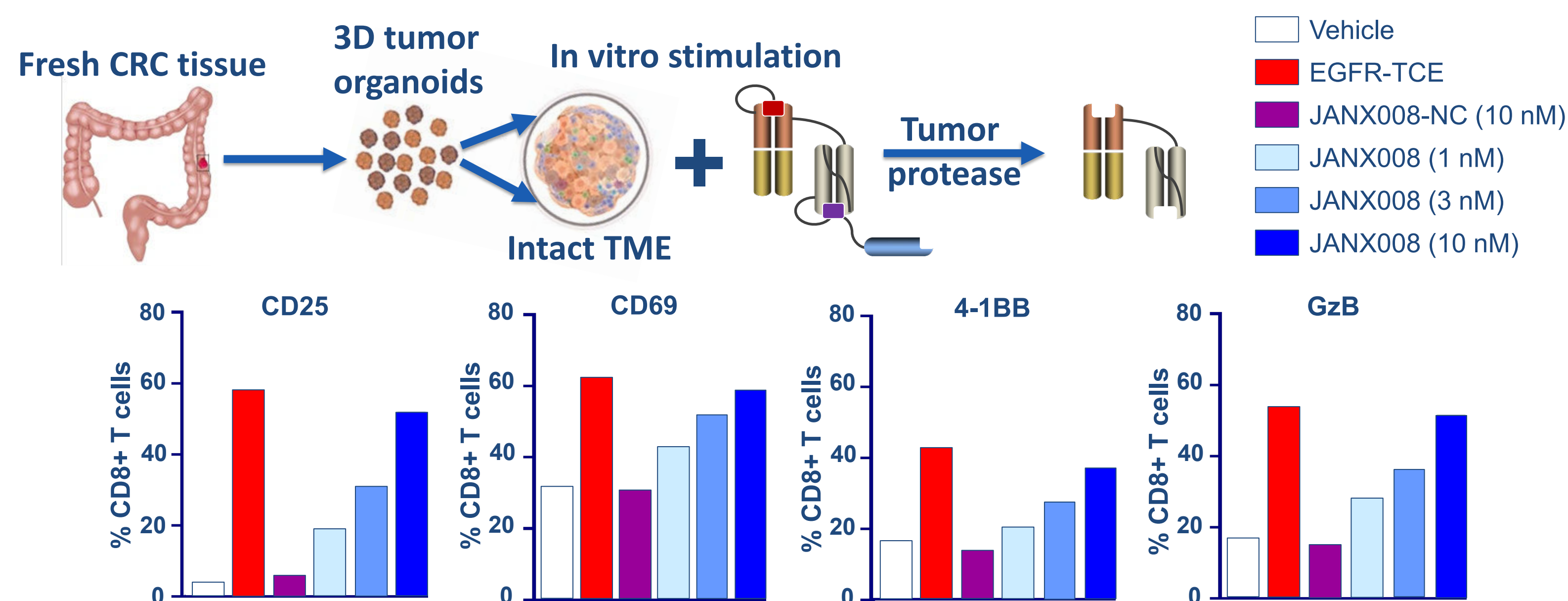
- Cleavable JANX008 TRACTr and EGFR-TCE at equivalent dose levels induce complete tumor eradication in human PBMC engrafted mice implanted with anti-EGFR resistant KRAS- and PIK3CA-mutant HCT116 tumor cells.
- JANX008 anti-tumor activity is tumor protease cleavage dependent (non-cleavable TRACTr is inactive).

Figure 5: JANX008 activity against tumors with resistance mutations



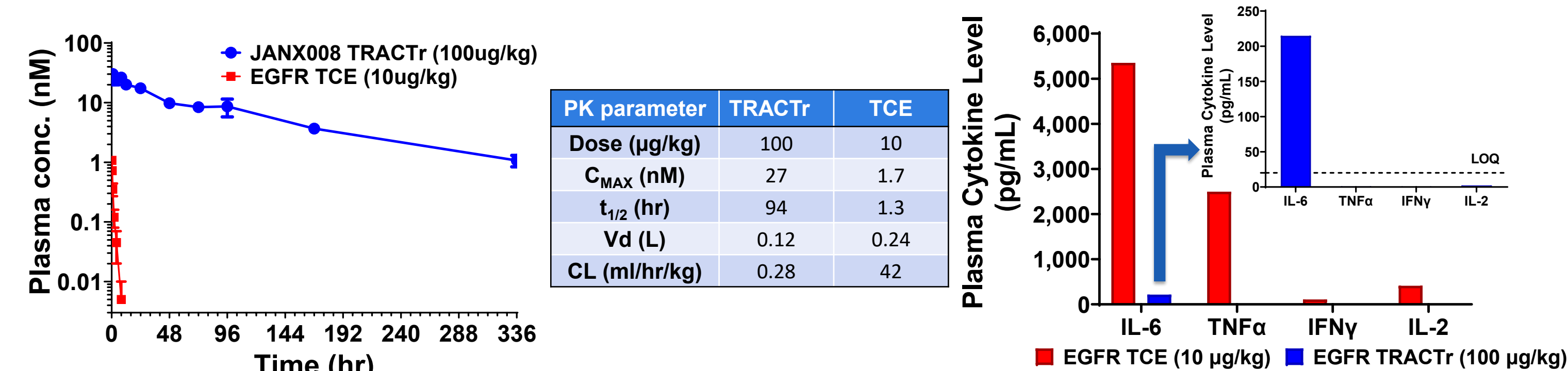
- JANX008 activity against tumor cells with KRAS, BRAF, PIK3CA, and EGFR mutations provides support to evaluate clinical responses in these patient populations with high unmet medical need

Figure 6: JANX008 shows cleavage-dependent activity in human CRC organoids



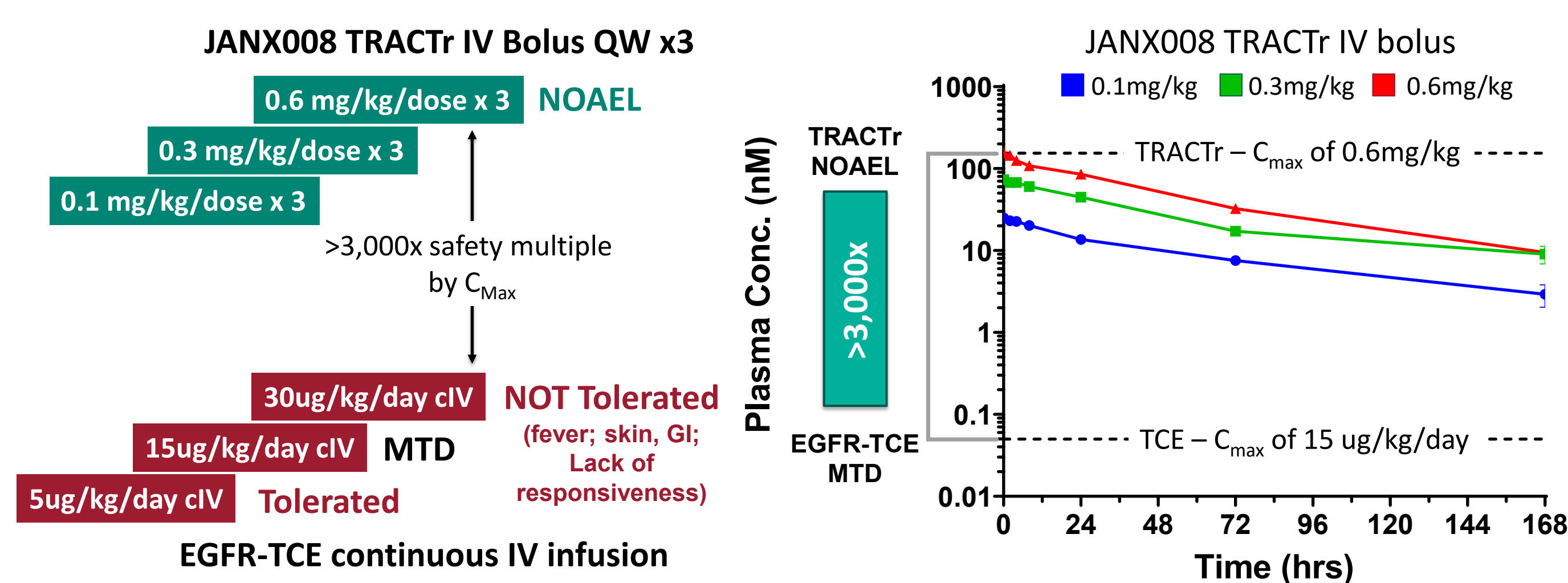
- JANX008 TRACTr exhibits dose- and cleavage-dependent activation of CD8+ TILs (non-cleavable molecule is inactive) in a fully human primary CRC tumoroid system with an intact non-manipulated immune compartment.

Figure 7: JANX008 has extended half-life and enhanced safety profile in NHPs



- JANX008 TRACTr exhibits an extended PK profile with a 94hr terminal half-life compared to the rapidly cleared EGFR-TCE with a 1.3hr half-life in cynomolgus monkeys.
- JANX008 also reduced cytokine induction in cynomolgus monkeys relative to non-masked EGFR-TCE.

Figure 8: JANX008 exhibits a large safety multiple relative to EGFR-TCE in NHP studies



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

- JANX008 administered once weekly for three weeks was well tolerated without clinical observations or notable changes in clinical chemistry, hematology, or pathology.
- EGFR-TCE dosed by continuous IV infusion at 15ug/kg/day had increased body temp & increased liver enzymes.
- Treatment with EGFR-TCE at 30ug/kg/day by continuous IV infusion had to be terminated 24hrs after dosing due to severe adverse events, including fever, skin rash, GI toxicities, and lack of responsiveness in the animals.
- Clinical observations, body temperature, and clinical pathology measurements support the large safety multiple (>3,000x) for EGFR-TRACTr relative to non-masked EGFR-TCE.
- 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 TRACTr exhibits enhanced safety and PK properties relative to the EGFR-TCE.
- The critical safety features of JANX008 are two tumor protease-cleavable peptide masks that inhibit EGFR and CD3 binding by >300x and >1,000x, respectively.
- Potent cleavage- and dose-dependent activity of JANX008 TRACTr 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.

¹Leonetti, A., Sharma, S., Minari, R. et al. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. Br J Cancer 121, 725–737 (2019). <https://doi.org/10.1038/s41416-019-0573-8>

