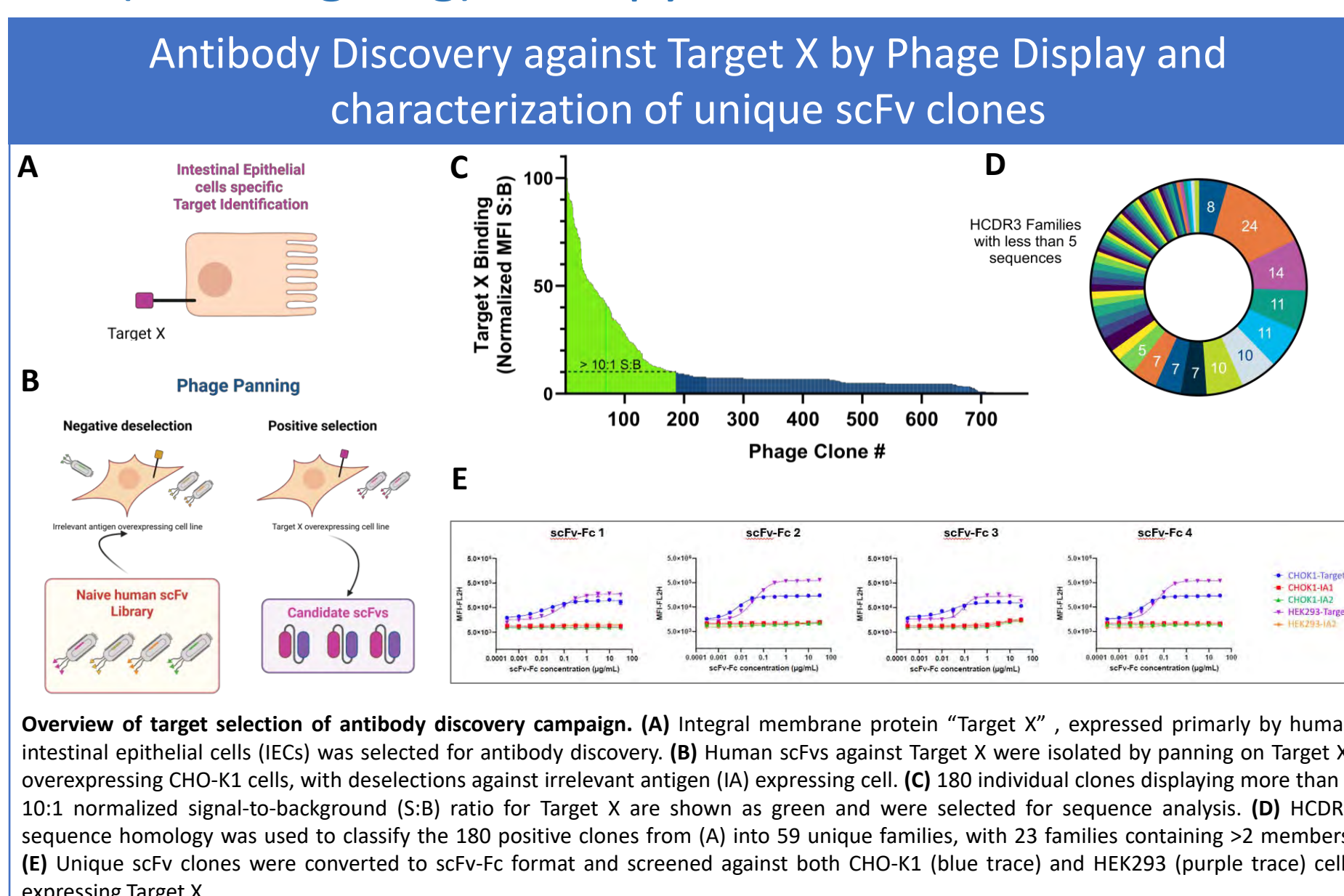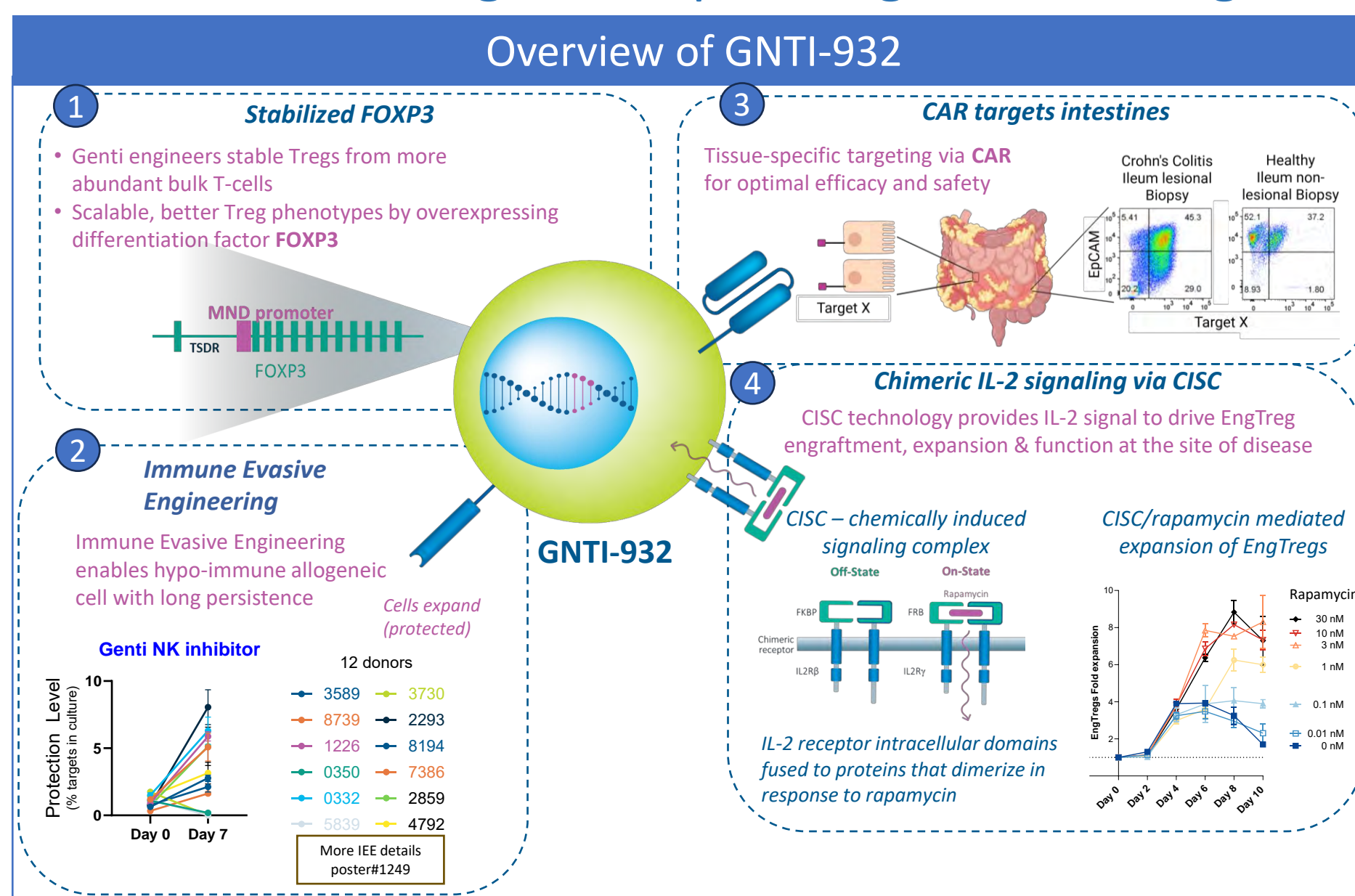# GNTI-932: A gut specific CAR-Engineered Regulatory T cell therapy to treat Inflammatory Bowel Disease

M Sassone-Corsi, D Tucker, J Yam, A Doherty, N Zammit, A Landyut, M Rashkovskii, S Verma, N Ngwenyama, G Uenishi, SA Hernandez, T Guo, A Burgess, P Rompikuntal, C Moore, D Gaddis, D Gopal, V Catala, H Kellog, Y Agarwal, T Chan, A Del Rio, J Mellen, Y Lao, V DeVault, A Singh, T Crevier, C Patel, T Wickham, TF Chen
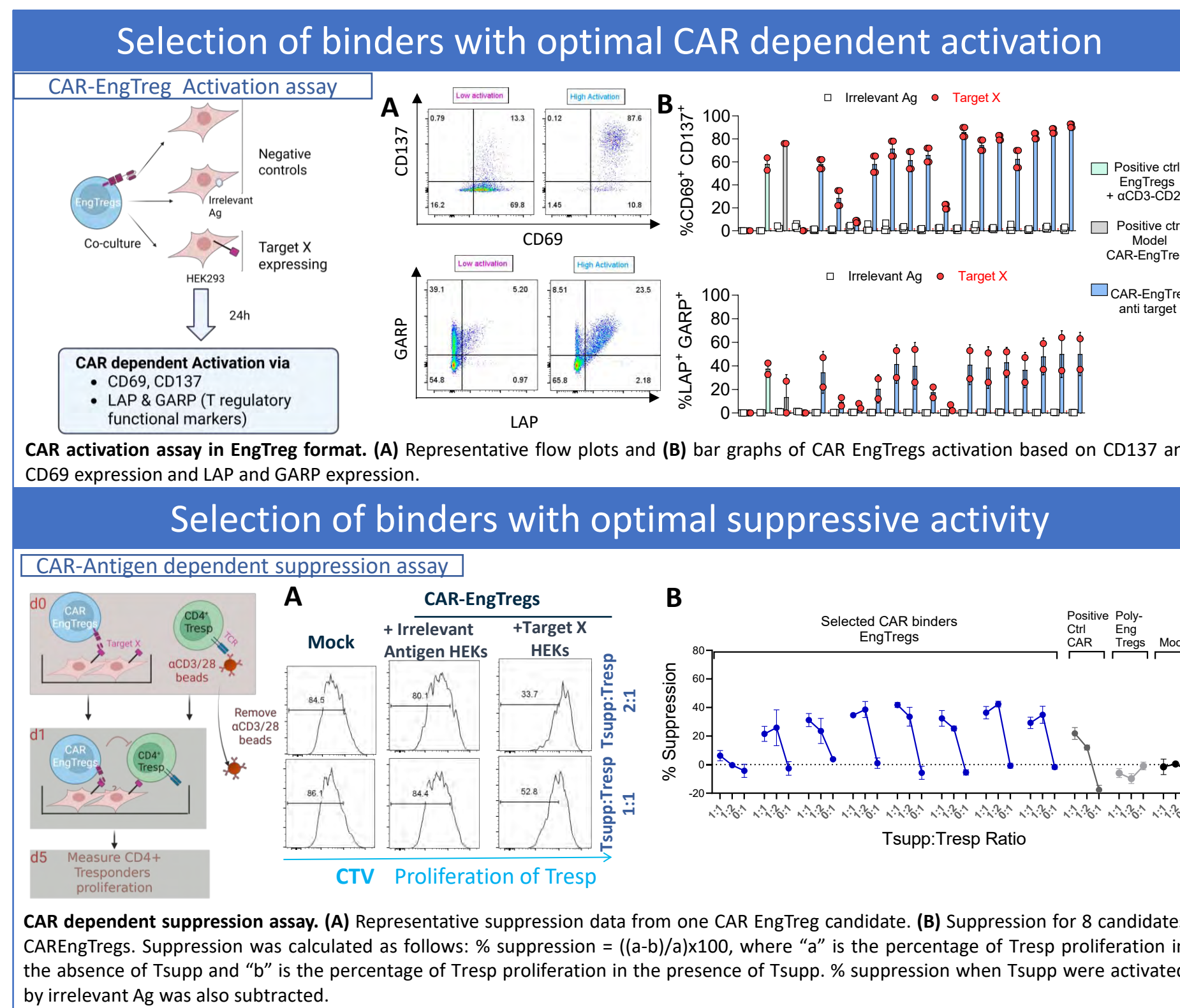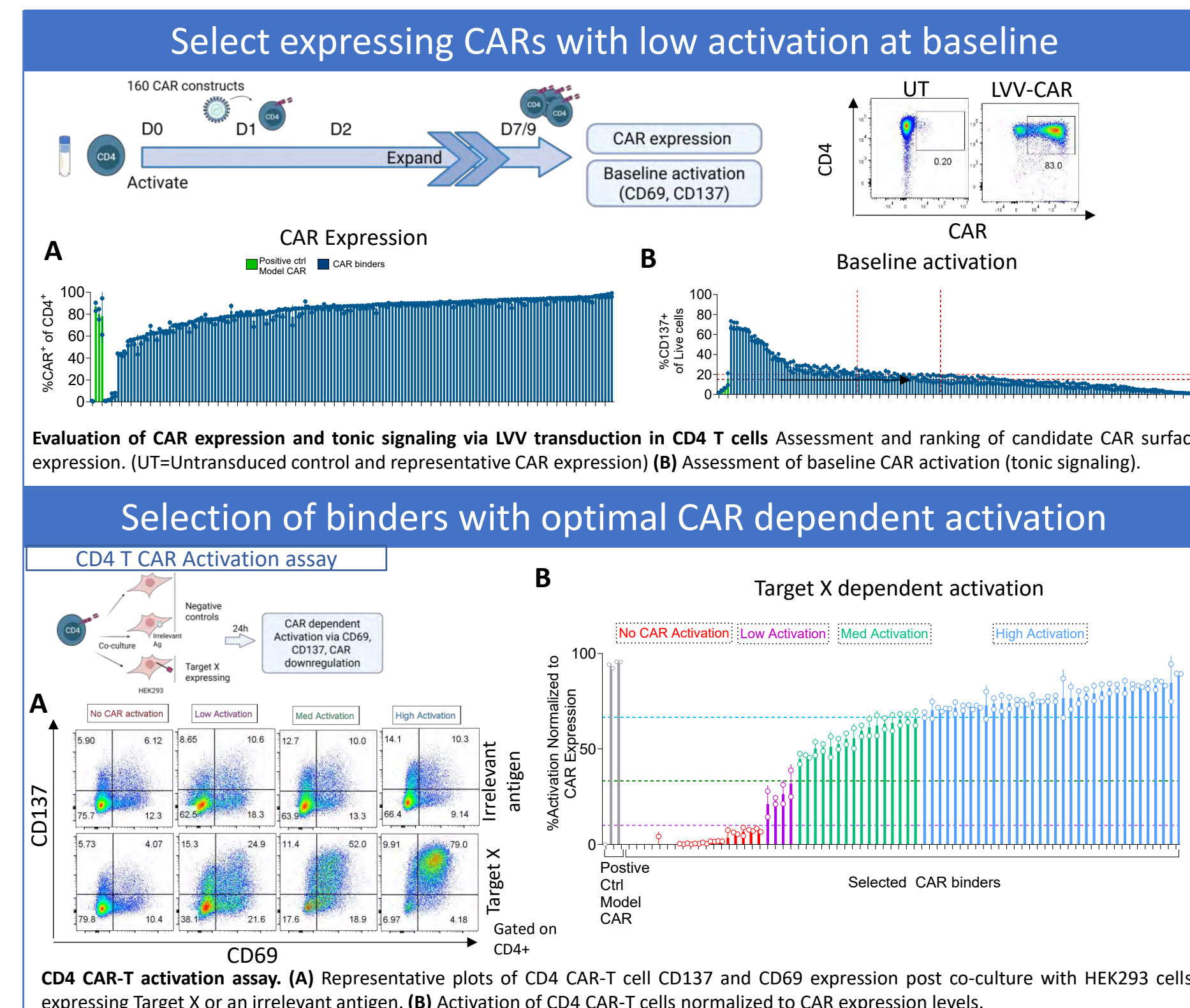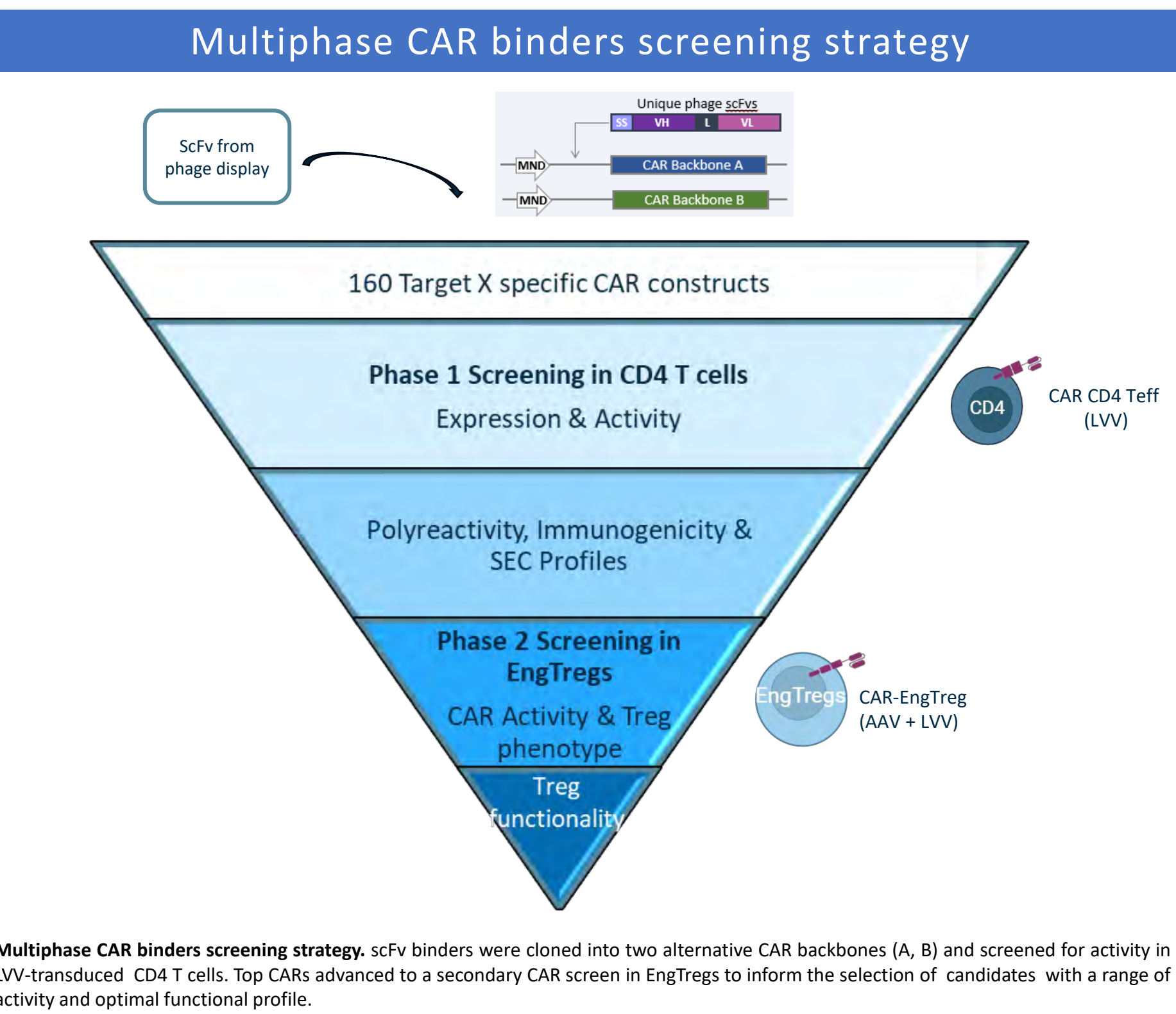
GentiBio, Inc., Cambridge, MA, USA

OVERVIEW | GNTI-932, a novel hypo-immune gut-specific chimeric antigen receptor engineered T regulatory cell (CAR-EngTreg) therapy
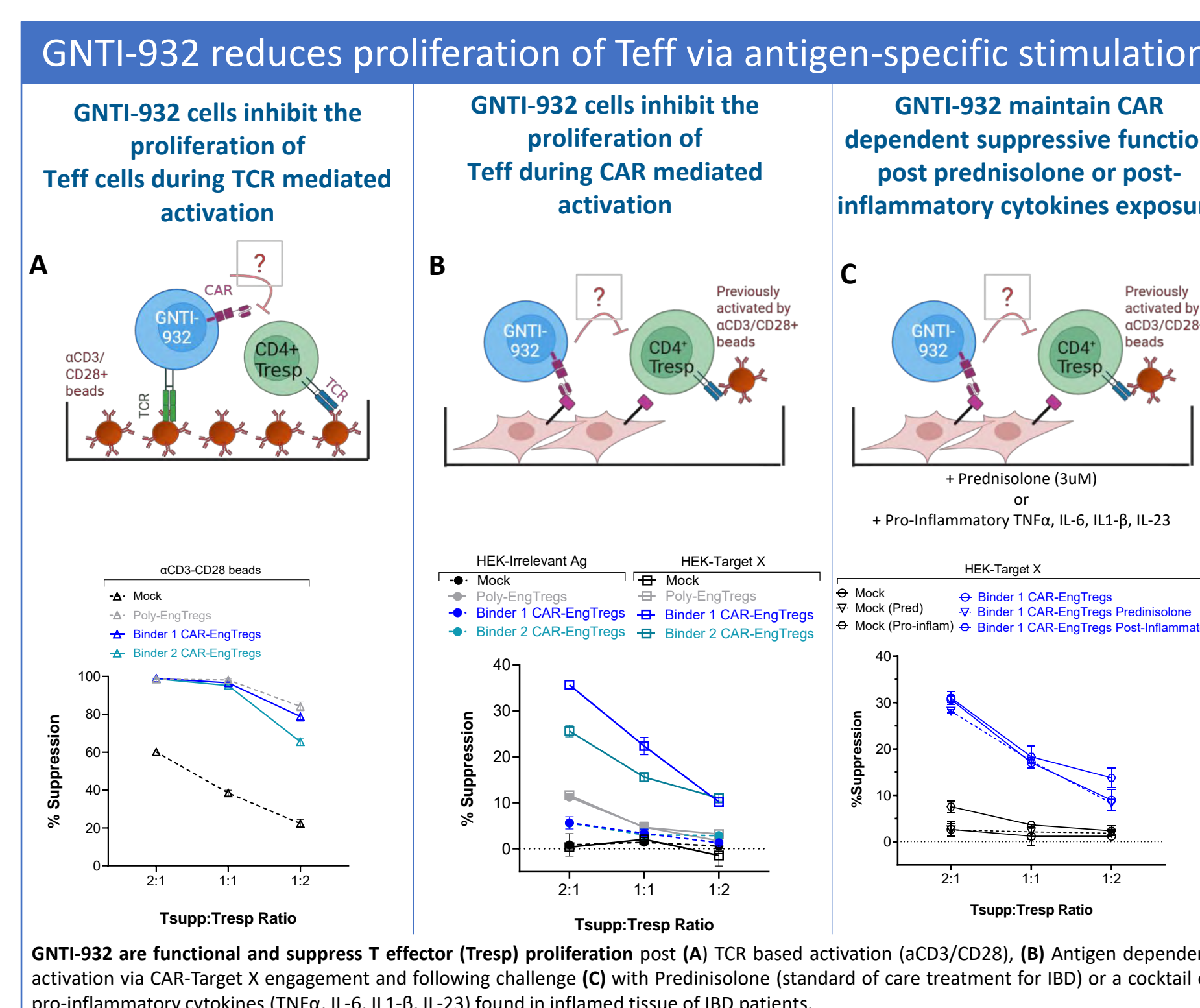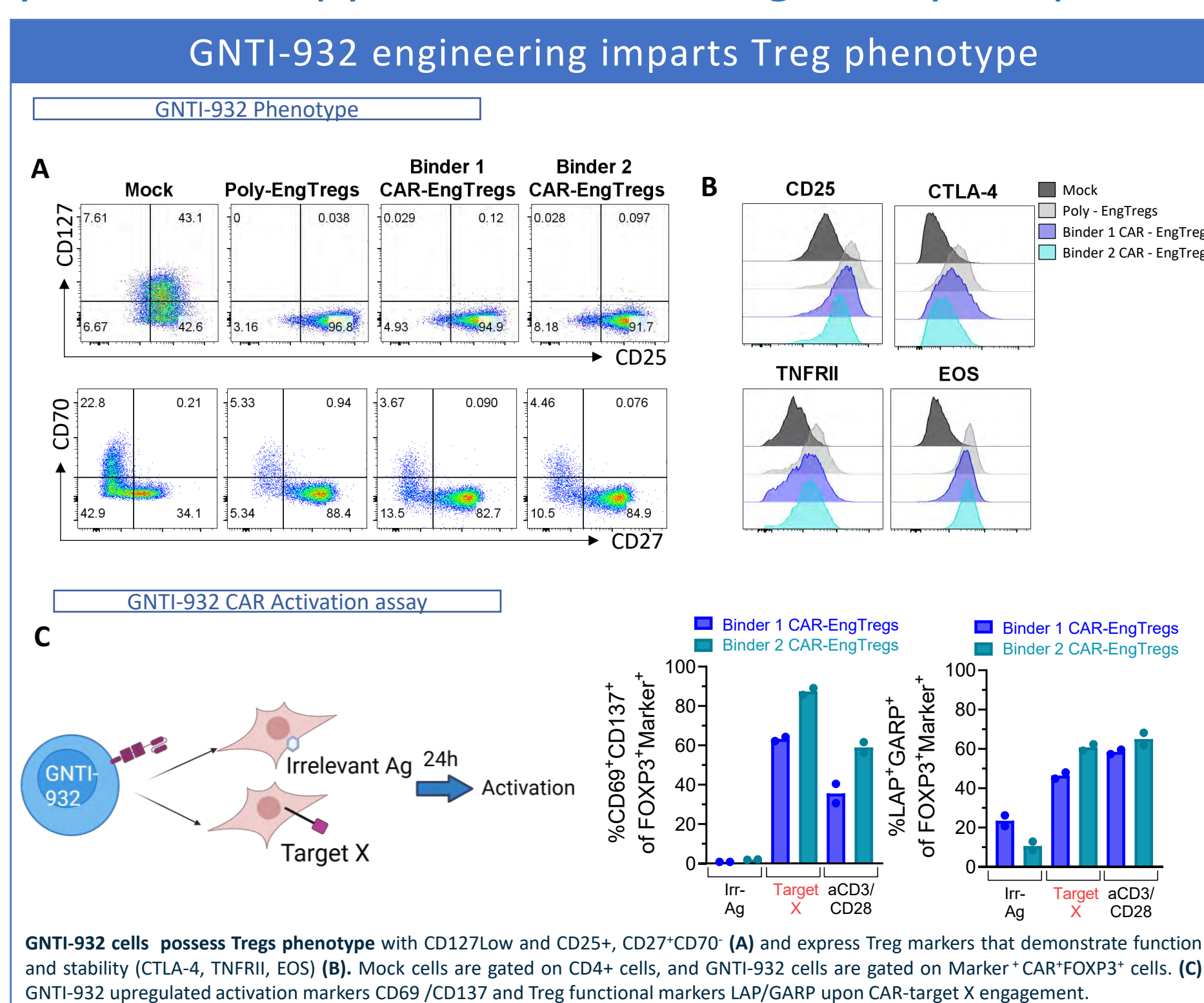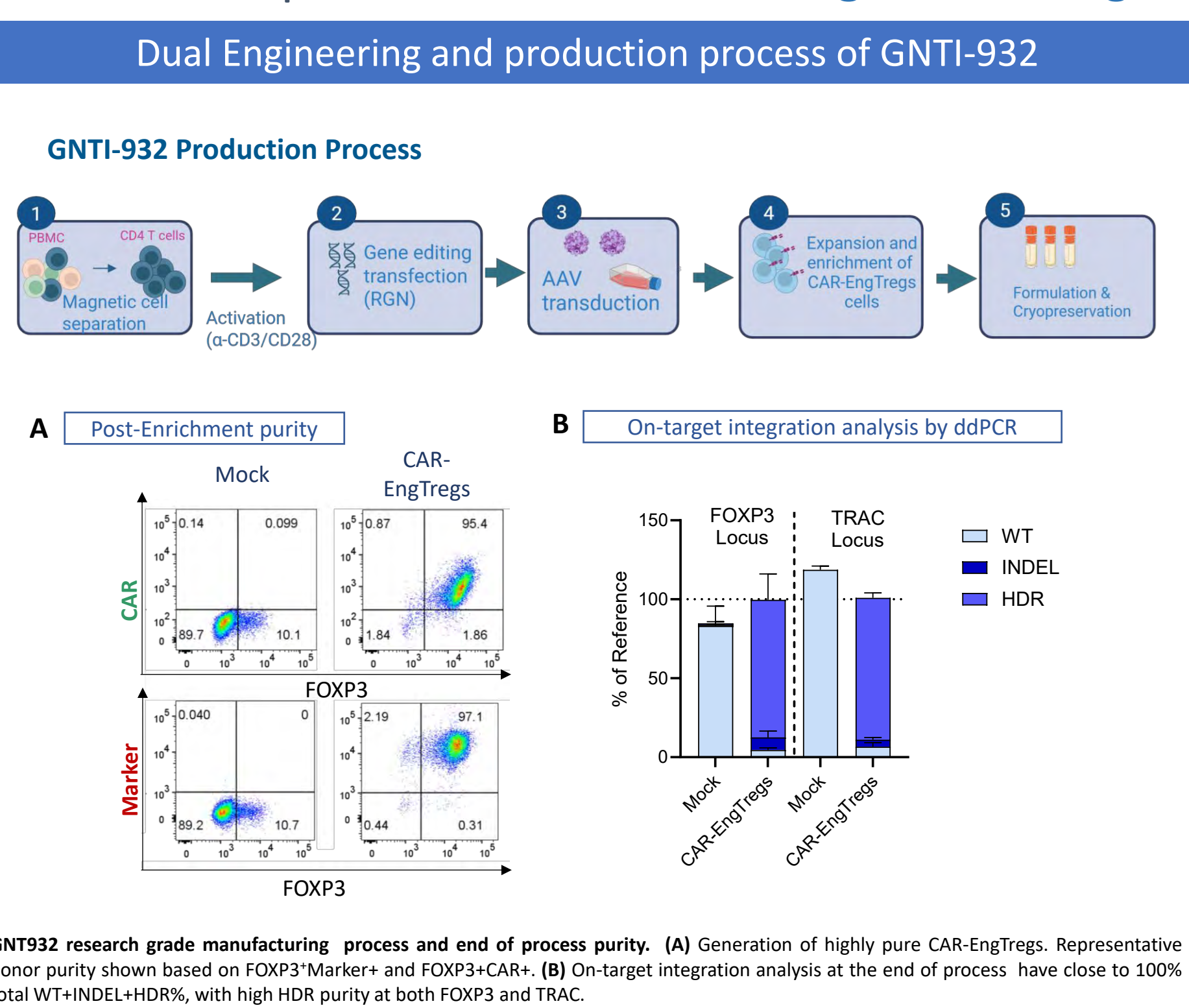
Summary

- GentiBio is advancing the development of GNTI-932, a novel hypo-immune gut-specific chimeric antigen receptor engineered T regulatory cell (CAR-EngTreg), to restore immune homeostasis in patients with Inflammatory bowel disease.

- GNTI-932 is designed with constitutive FOXP3 expression and a rapamycin-activated IL-2 signaling complex for stability and scalability, a potent immune evasive engineering technology (IEE) for persistence and a CAR to enhance gut localization and antigen-specific suppression.

- A multi-phase CAR screening strategy was implemented, to enable high throughput discovery and selection of the best binders that maintain Treg phenotype and functionality.

- Preclinical studies using murine CAR-EngTreg surrogates targeting the mouse ortholog of the human target gut antigen demonstrated preferential gut localization, proliferation, and significant therapeutic efficacy in colitis models.

- These findings suggest that CAR-EngTregs like GNTI-932, targeting tissue-specific antigens, hold promise for improving the effectiveness of Treg therapies for IBD.
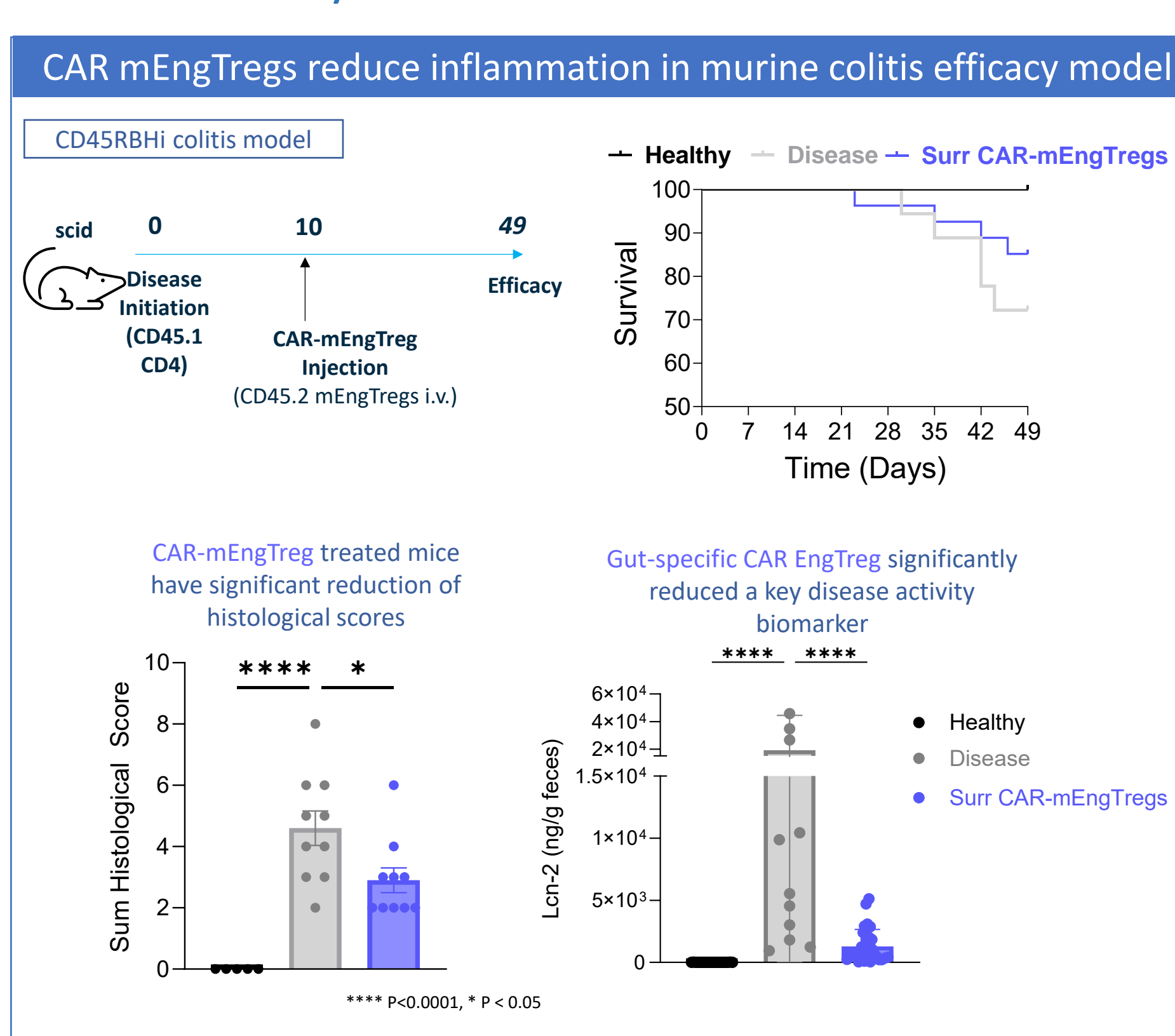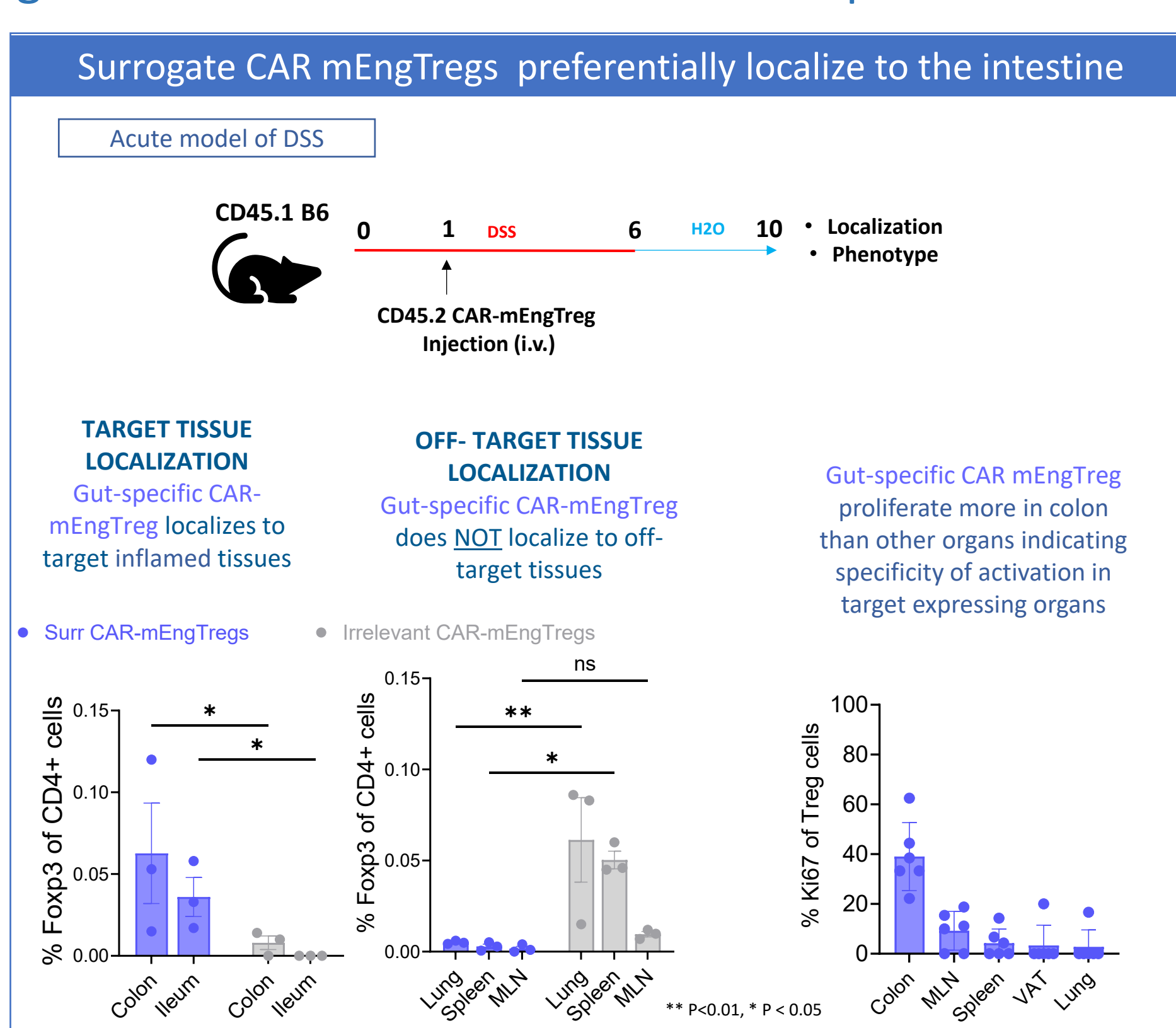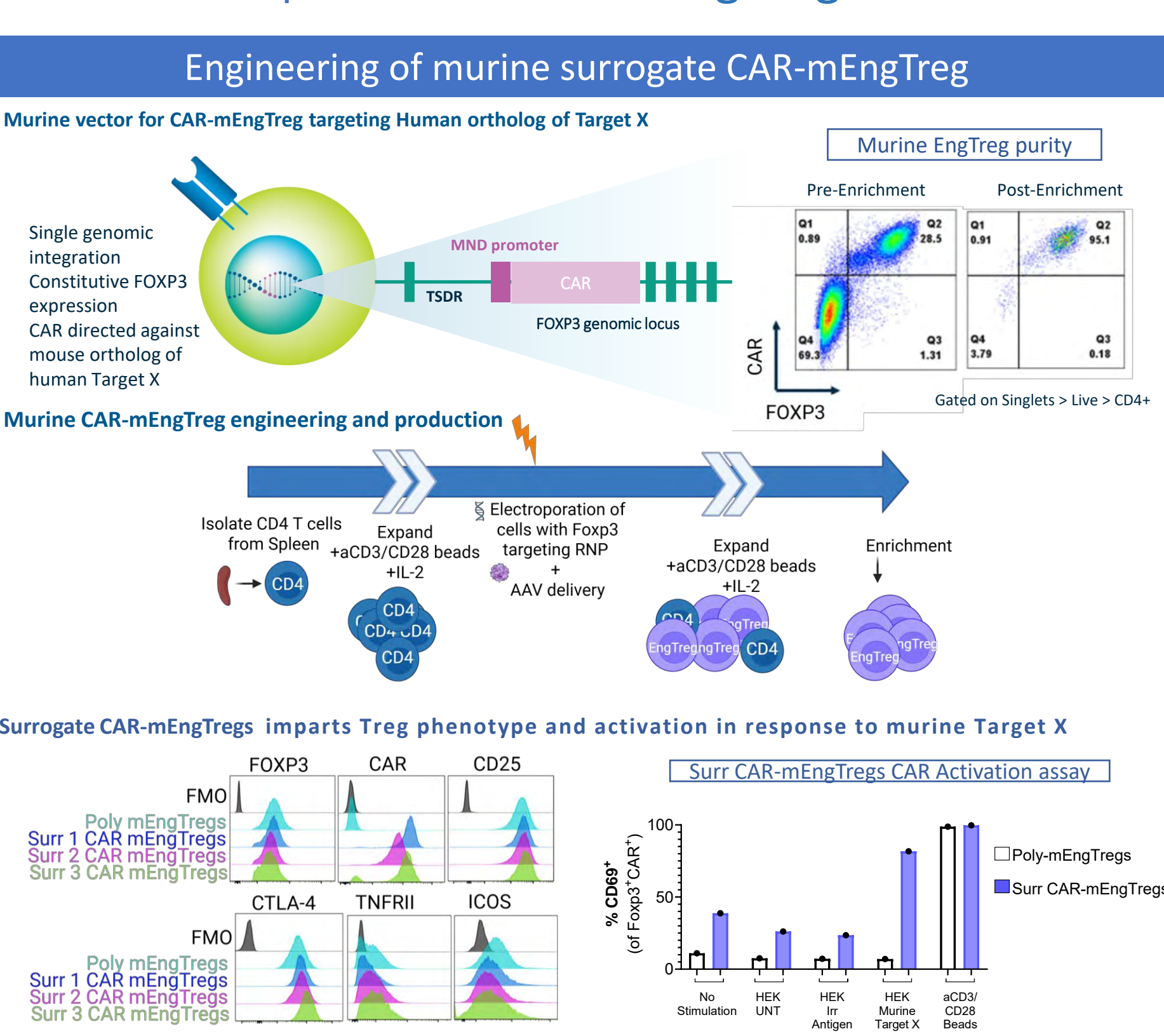
Overview of GNTI-932

1. **Stabilized FOXP3** — Genti engineers stable Tregs from more abundant bulk T-cells. Scalable, better Treg phenotypes by overexpressing differentiation factor FOXP3

2. **Immune Evasive Engineering** — Immune Evasive Engineering enables hypo-immune allogeneic cell with long persistence

3. **CAR targets intestines** — Tissue-specific targeting of CAR for optimal efficacy and safety

4. **Chimeric IL-2 signaling via CISC** — CISC technology provides IL-2 signal to drive EngTreg engraftment, expansion & function at the site of disease. CISC – chemically induced signaling complex. IL-2 receptor intracellular domains fused to proteins that dimerize in response to rapamycin. CISC/rapamycin mediated expansion of EngTregs.

Antibody Discovery against Target X by Phage Display and characterization of unique scFv clones

Overview of target selection of antibody discovery campaign. (A) Integral membrane protein "Target X", expressed primarily by human intestinal epithelial cells (IECs) was selected for antibody discovery. (B) Human scFvs against Target X were isolated by panning on Target X-overexpressing CHO-K1 cells, with deselections against irrelevant antigen (IA) expressing cell. (C) 180 individual clones displaying more than a 10:1 normalized signal-to-background (S:B) ratio for Target X are shown as green and were selected for sequence analysis. (D) HCDR3 sequence homology was used to classify the 180 positive clones from (A) into 59 unique families, with 23 families containing >2 members. (E) Unique scFv clones were converted to scFv-Fc format and screened against both CHO-K1 (blue trace) and HEK293 (purple trace) cells expressing Target X.

RESULTS | 1st Phase screening in CD4 T cells for CAR activity and 2nd Phase for selection of top CAR-EngTregs with optimal Treg phenotype and functionality

Multiphase CAR binders screening strategy

scFv from phage display

- 160 Target X specific CAR constructs
- **Phase 1 Screening in CD4 T cells** — Expression & Activity — CAR CD4 Teff (LVV)
- Polyreactivity, Immunogenicity & SEC Profiles
- **Phase 2 Screening in EngTregs** — CAR Activity & Treg phenotype — CAR-EngTreg (AAV + LVV)
- Treg functionality

Multiphase CAR binders screening strategy. scFv binders were cloned into two alternative CAR backbones (A, B) and screened for activity in LVV-transduced CD4 T cells. Top CARs advanced to a secondary CAR screen in EngTregs to inform the selection of candidates with a range of activity and optimal functional profile.

Select expressing CARs with low activation at baseline

Evaluation of CAR expression and tonic signaling via LVV transduction in CD4 T cells Assessment and ranking of candidate CAR surface expression. (UT=Untransduced control and representative CAR expression) (B) Assessment of baseline CAR activation (tonic signaling).

Selection of binders with optimal CAR dependent activation

CAR activation assay in EngTreg format. (A) Representative flow plots and (B) bar graphs of CAR EngTregs activation based on CD137 and CD69 expression and LAP and GARP expression.

Selection of binders with optimal CAR dependent activation

CD4 CAR-T activation assay. (A) Representative plots of CD4 CAR-T cell CD137 and CD69 expression post co-culture with HEK293 cells expressing Target X or an irrelevant antigen. (B) Activation of CD4 CAR-T cells normalized to CAR expression levels.

Selection of binders with optimal suppressive activity

CAR dependent suppression assay. (A) Representative suppression data from one CAR EngTreg candidate. (B) Suppression for 8 candidates CAREngTregs. Suppression was calculated as follows: % suppression = ((a-b)/a)x100, where "a" is the percentage of Tresp proliferation in the absence of Tsupp and "b" is the percentage of Tresp proliferation in the presence of Tsupp. % suppression when Tsupp was activated by irrelevant Ag was also subtracted.

RESULTS | GNTI-932 is a Dual-Engineered Regulatory T Cell Therapy Product with T regulatory cell phenotype and function

Dual Engineering and production process of GNTI-932

GNTI-932 Production Process
1. Magnetic cell separation
2. Gene editing transfection (RGN) — Activation (αCD3/CD28)
3. AAV transduction
4. Expansion and enrichment of CAR-EngTreg cells
5. Formulation & Cryopreservation

GNT932 research grade manufacturing process and end of process purity. (A) Generation of highly pure CAR-EngTregs. Representative donor purity shown based on FOXP3*Marker+ and FOXP3+CAR+. (B) On-target integration analysis at the end of process have close to 100% total WT+INDEL+HDR%, with high HDR purity at both FOXP3 and TRAC.

GNTI-932 engineering imparts Treg phenotype

GNTI-932 cells possess Tregs phenotype with CD127Low and CD25+, CD27*CD70* and express Treg markers that demonstrate function and stability (CTLA-4, TNFRII, EOS) (B). Mock cells are gated on CD4+ cells, and GNTI-932 cells are gated on Marker * CAR*FOXP3* cells. (C) GNTI-932 upregulated activation markers CD69 /CD137 and Treg functional markers LAP/GARP upon CAR-target X engagement.

GNTI-932 reduces proliferation of Teff via antigen-specific stimulation

- **A** GNTI-932 cells inhibit the proliferation of Teff cells during TCR mediated activation
- **B** GNTI-932 cells inhibit the proliferation of Teff during CAR mediated activation
- **C** GNTI-932 maintain CAR dependent suppressive function post prednisolone or post-inflammatory cytokines exposure

GNTI-932 are functional and suppress T effector (Tresp) proliferation post (A) TCR based activation (αCD3/CD28), (B) Antigen dependent activation via CAR-Target X engagement and following challenge (C) with Prednisolone (standard of care treatment for IBD) or a cocktail of pro-inflammatory cytokines (TNFα, IL-6, IL1-β, IL-23) found in inflamed tissue of IBD patients.

RESULTS | Murine CAR-mEngTregs GNTI-932 surrogate localize in the intestine and improve outcomes in vivo efficacy models

Engineering of murine surrogate CAR-mEngTreg

- Single genomic integration
- Constitutive FOXP3 expression
- CAR directed against mouse ortholog of human Target X

Murine CAR-mEngTreg engineering and production
- Isolate CD4 T cells from Spleen → Expand αCD3/CD28 beads +IL-2 → Electroporation of cells with RGN targeting RNP → AAV delivery → Expand αCD3/CD28 beads +IL-2 → Enrichment

Surrogate CAR-mEngTregs imparts Treg phenotype and activation in response to murine Target X

Surrogate CAR mEngTregs preferentially localize to the intestine

Acute model of DSS
CD45.1 B6 — DSS (0–6), H2O (6–10) — CD45.2 CAR-mEngTreg Injection (i.v.) — Localization, Phenotype

TARGET TISSUE LOCALIZATION — Gut-specific CAR-mEngTreg localizes to target inflamed tissues

OFF-TARGET TISSUE LOCALIZATION — Gut-specific CAR-mEngTreg does NOT localize to off-target tissues

Gut-specific CAR mEngTreg proliferate more in colon than other organs indicating specificity of activation in target expressing organs

** P<0.01, * P < 0.05

CAR mEngTregs reduce inflammation in murine colitis efficacy model

CD45RBHi colitis model
scid → Disease Initiation (CD45.1 CD4) (0) → CAR-mEngTreg Injection (CD45.2 mEngTregs i.v.) (10) → Efficacy (49)

CAR-mEngTreg treated mice have significant reduction of histological scores

Gut-specific CAR EngTreg significantly reduced a key disease activity biomarker

**** P<0.0001, * P < 0.05

CONCLUSIONS

- A multi-phase CAR screening strategy facilitated high-throughput discovery and selection of optimal binders for maintaining Treg phenotype and functionality.
- GNTI-932 exhibits key Treg characteristics and demonstrates antigen-dependent suppressive function against effector T cells while maintaining stability under inflammatory conditions and standard IBD treatment.
- Preclinical studies using murine surrogates of GNTI-932 demonstrated effective gut localization, proliferation at the site of inflammation, and significant therapeutic benefit in experimental colitis models, highlighting the potential for in vivo efficacy.
- In summary, Targeting IBD with CAR-engineered Tregs (GNTI-932) offers a novel approach to overcome the limitations of polyclonal Treg therapy by enhancing target specificity and persistence through its unique design.

We make Tregs. *Better.*

28TH ANNUAL MEETING — MAY 13–17, 2025 — NEW ORLEANS