The CBL-B Inhibitor, NX-0255, Enhances Human Drug-Enhanced Tumor-Infiltrating Lymphocyte (DeTIL) Expansion and T Cell Function in Full-Scale Runs

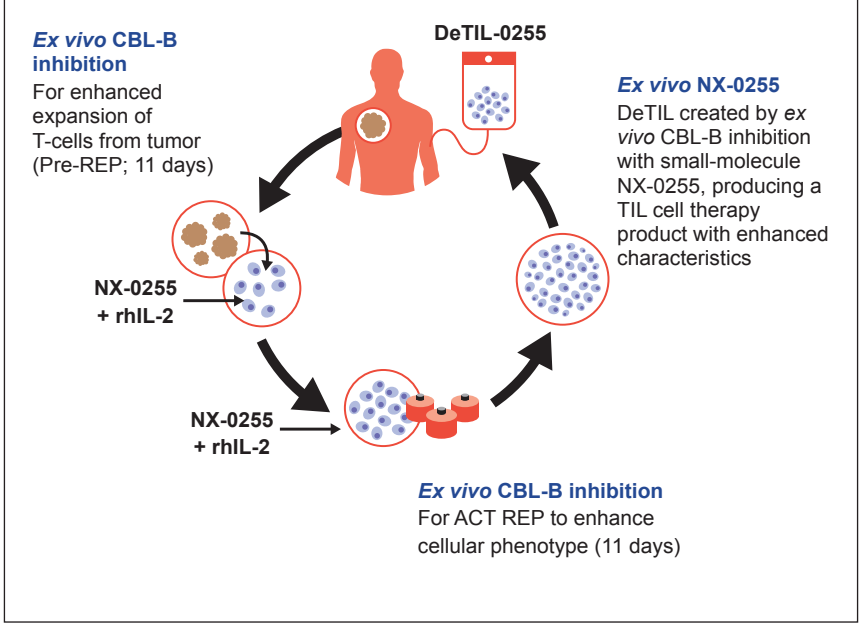
Xiaoyan Liang, Xianzhu Wu, Jeevitha Jeevan, Samuel C. Butler, Pranav Murthy, Arthur T. Sands, Michael T. Lotze

Nurix Therapeutics, San Francisco, CA and Pittsburgh, PA, USA

Introduction

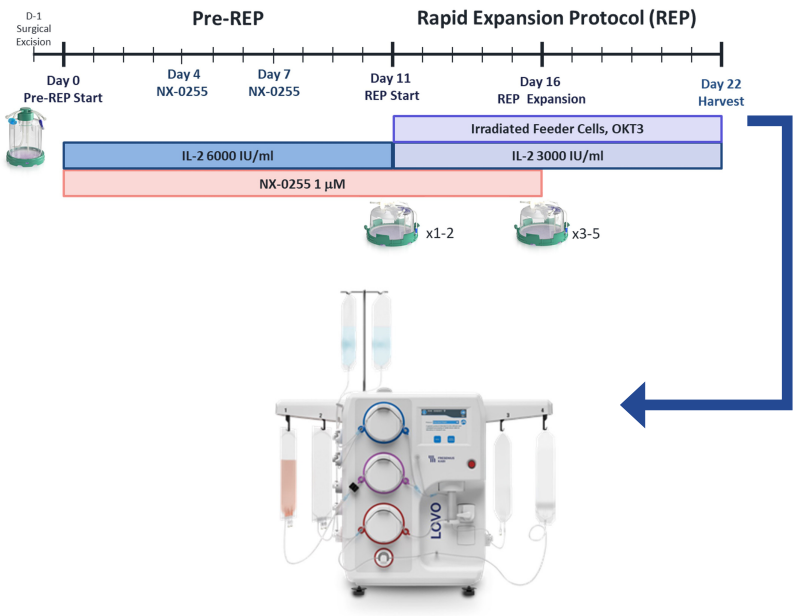
- Tumor-infiltrating lymphocyte (TIL) therapy is a promising adoptive cell therapy (ACT) that has demonstrated durable complete responses in patients with metastatic melanoma and several epithelial tumors.[1,2]
- Obtaining TIL with sufficient quality (increased memory phenotype and reduced exhaustion) during the manufacturing process is a major challenge.[3,4]
- The E3 ubiquitin ligase, Casitas B-lineage lymphoma B (CBL-B), limits T cell activation by inhibiting T cell receptor signaling in the absence of CD28 (signal 2) engagement[5,6] CBL-B also regulates NK, DC, and B cell activation and function.
- Addition of ex vivo NX-0255, a highly potent CBL-B inhibitor (CBL-Bi), significantly increases TIL growth with a favorable phenotype at research scale expansion (**Fig. 1**).

Figure 1. Overview of Drug-Enhanced TIL Therapy (DeTIL-0255)



Materials and Methods

- We assessed the impact of *ex vivo* CBL-Bi with NX-0255 on the expansion and phenotype of drug-enhanced TIL (DeTIL), in multiple full-scale processes.
- Various tumor types were assessed, including endometrial (n=2), cervical (n=1), lung (n=1), colon (n=1), and melanoma (n=1) tumors.
- Six full-scale studies were performed in parallel with TIL expanded either solely in the presence of IL-2 (TIL arm), or in the presence of IL-2 and NX-0255 (DeTIL-0255 arm).
- TIL and DeTIL-0255 were harvested following 22 days of expansion and assessed for total cell number, viability, phenotype, and function.



Statistical analysis

All statistical analyses were performed using a two-tailed Wilcoxon signed-rank test. *p<0.05, **p<0.01. Red color represents the DeTIL-0255 arm, and blue color represents the TIL arm.

References

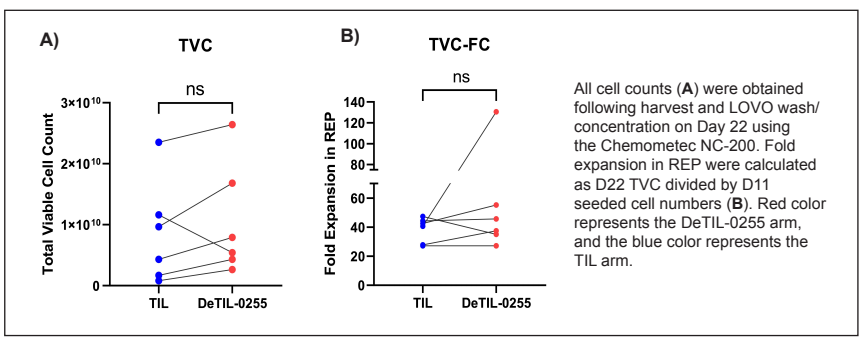
1. Rosenberg SA, et al. Clin Cancer Res. 2011;17:4550–7.
2. Zacharakis N, et al. Nat Med 2018; 24, 724–730.
3. Wang S, et al. BMC Medicine 2021,19:140–7.
4. Krishna S, et al. Science 2020;370:1328–34.
5. Lutz-Micoladoni C, et al. Frontiers in Oncology 2015;5:1–14.
6. Zhou X, et al. Signal Transduction And Targeted Therapy 2021;6:16.

Results

Compared with the TIL arm, the addition of NX-0255 in DeTIL-0255:

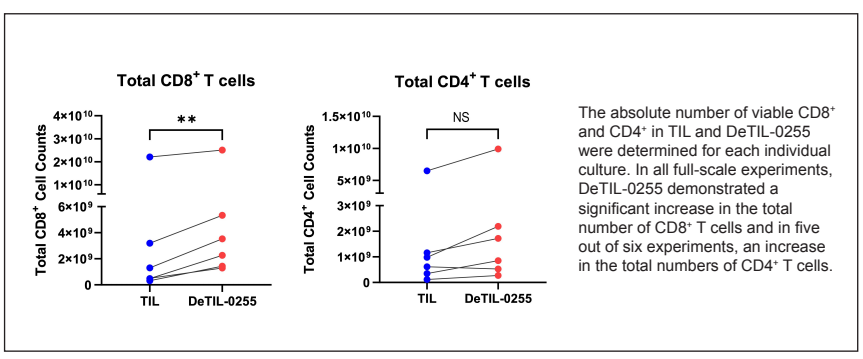
- Increases the number of total viable cells in five of six full-scale experiments (**Figure 2**).
- Significantly increases the total number of CD8⁺ T cells (**Figure 3**).
- Significantly increases the total number of CD8⁺ & CD4⁺ central memory T cells (**Figure 4**).
- Significantly increases the number of CD8⁺ CD39⁻ CD69⁻ 'stem-like' memory cells (**Figure 5**).
- Significantly increases the intracellular expression of IFN-γ in response to PMA and Ionomycin stimulation and granzyme B, in response to αCD3-αCD28 stimulation (**Figure 6**).

Figure 2. DeTIL-0255 demonstrate increased number of total viable cells in 5 of 6 full-scale manufacturing runs



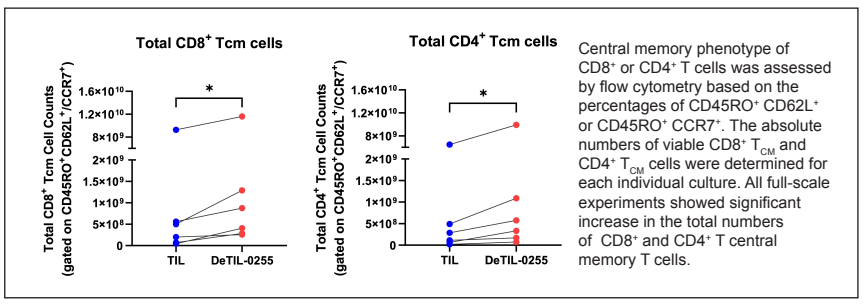
All cell counts (**A**) were obtained following harvest and LOVO wash/ concentration on Day 22 using the Chemometec NC-200. Fold expansion in REP were calculated as D22 TVC divided by D11 seeded cell numbers (**B**). Red color represents the DeTIL-0255 arm, and the blue color represents the TIL arm.

Figure 3. Addition of NX-0255 increases the total number of CD8⁺ T cells



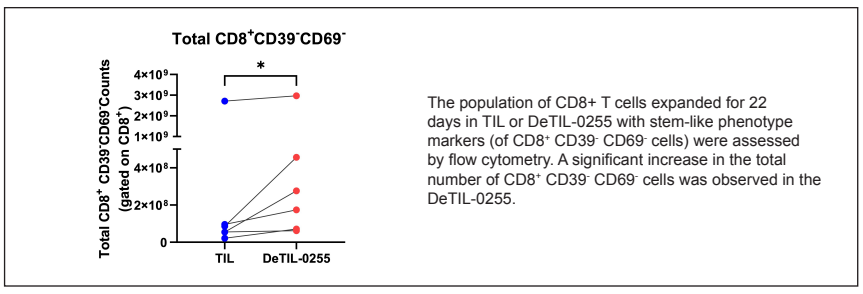
The absolute number of viable CD8⁺ and CD4⁺ in TIL and DeTIL-0255 were determined for each individual culture. In all full-scale experiments, DeTIL-0255 demonstrated a significant increase in the total number of CD8⁺ T cells and in five out of six experiments, an increase in the total numbers of CD4⁺ T cells.

Figure 4. DeTIL-0255 demonstrate significantly increased CD4⁺ and CD8⁺ central memory T cells compared to TIL



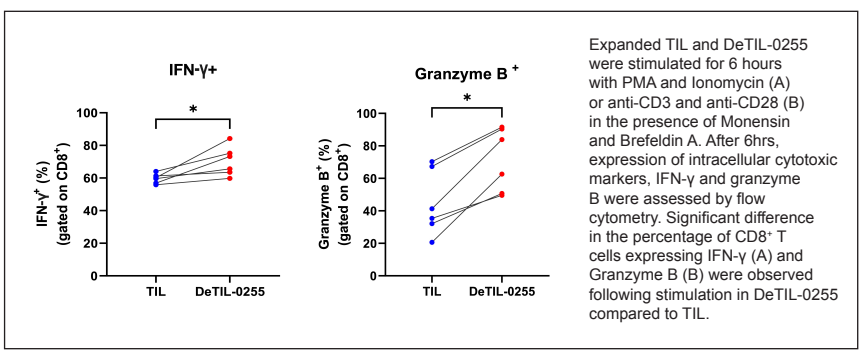
Central memory phenotype of CD8⁺ or CD4⁺ T cells was assessed by flow cytometry based on the percentages of CD45RO⁺ CD62L⁺ or CD45RO⁺ CCR7⁺. The absolute numbers of viable CD8⁺ T_{CM} and CD4⁺ T_{CM} cells were determined for each individual culture. All full-scale experiments showed significant increase in the total numbers of CD8⁺ and CD4⁺ T central memory T cells.

Figure 5. DeTIL-0255 significantly increased CD39⁻CD69⁻T stem-like CD8⁺ T cell when compared with TIL



The population of CD8+ T cells expanded for 22 days in TIL or DeTIL-0255 with stem-like phenotype markers (of CD8⁺ CD39⁻ CD69⁻ cells) were assessed by flow cytometry. A significant increase in the total number of CD8⁺ CD39⁻ CD69⁻ cells was observed in the DeTIL-0255.

Figure 6. DeTIL-0255 demonstrate significant increases in the proportion of CD8+ T cells expressing intracellular IFN-γ and granzyme B following stimulation



Expanded TIL and DeTIL-0255 were stimulated for 6 hours with PMA and Ionomycin (A) or anti-CD3 and anti-CD28 (B) in the presence of Monensin and Brefeldin A. After 6hrs, expression of intracellular cytotoxic markers, IFN-γ and granzyme B were assessed by flow cytometry. Significant difference in the percentage of CD8⁺ T cells expressing IFN-γ (A) and Granzyme B (B) were observed following stimulation in DeTIL-0255 compared to TIL.

Conclusions

Manufacturing of DeTIL-0255 is feasible and, in comparison to conventional TIL, demonstrates a superior phenotype that is suitable for clinical evaluation. Accordingly, we have initiated a clinical trial with DeTIL-0255 in patients with gynecologic malignancies (ovarian cancer, endometrial cancer, and cervical cancer), having treated our first patient (NCT05107739).

Presented at the 24th Annual TRCCC Meeting, June 6-8, 2022, Seven Springs PA, USA