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

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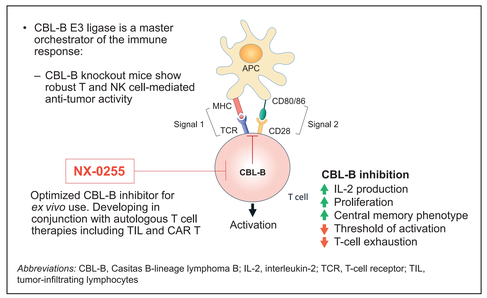
Background

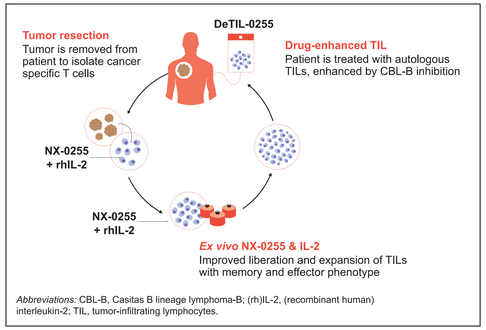
- *Ex-vivo* expanded autologous tumor-infiltrating lymphocyte (TIL) therapy is an adoptive cell therapy (ACT) that has demonstrated encouraging clinical responses in patients with melanoma and selected epithelial tumors.[1-6]
- Effective methods to obtain sufficient TIL with suitable quality and diversity from tumor samples remain a challenge, given the suppressive tumor microenvironment.[7]
- The E3 ubiquitin ligase, Casitas B lineage lymphoma B (CBL-B) is expressed in T cells where it regulates signaling through the T-cell receptor (TCR), limiting T-cell activation, differentiation, production of effector cytokines, and proliferation.
- Previous studies demonstrated that addition of NX-0255, a highly potent CBL-B inhibitor, during *ex-vivo* TIL expansion resulted in a favorable TIL phenotype and higher cell yields (**Figures 1 and 2**).[8]

Figure 1. CBL-B: A modulator of T-cell activation and novel target for immuno-oncology



Abbreviations: CBL-B, Casitas B-lineage lymphoma B; IL-2, interleukin-2; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocytes

Figure 2. Overview of drug-enhanced TIL therapy



Abbreviations: CBL-B, Casitas B lineage lymphoma-B; (rh)IL-2, (recombinant human) interleukin-2; TIL, tumor-infiltrating lymphocytes.

Objective

- To evaluate the effects of the CBL-B inhibitor NX-0255 on the expansion and phenotype of drug-enhanced TIL (DeTIL), in multiple full-scale (clinical scale manufacturing) processes.

Material and Methods

- Six full-scale studies were performed in parallel with TIL expanded:
  - in the presence of interleukin (IL)-2 (TIL arm).
  - in the presence of IL-2 and NX-0255 (DeTIL-0255 arm).
- Various tumor types were assessed, including endometrial (n=2), cervical (n=1), lung (n=1), colon (n=1), and melanoma (n=1) tumors.
- TIL and DeTIL-0255 were harvested following 22 days of expansion and assessed for total cell number, viability, phenotype, bulk transcriptome and function.

Statistical Analysis

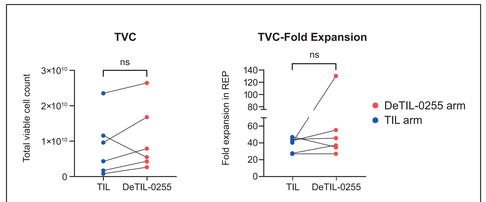
- All statistical analyses were performed using a two-tailed Wilcoxon matched-pairs signed-rank test.

Results

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

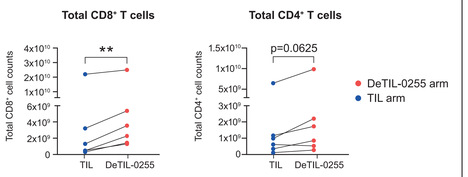
- Demonstrated a trend towards an increased number of total viable cells (**Figure 3**).
- Increased the total number of CD8$^+$ T cells (**Figure 4**).
- Increased the total number of CD8$^+$ & CD4$^+$ central memory T cells (**Figure 5**).
- Increased the number of CD8$^+$ CD39$^-$ CD69$^-$ 'stem-like' memory cells (**Figure 6**).
- Increased the intracellular expression of interferon (IFN)-γ in response to phorbol-12-myristate-13-acetate (PMA) and Ionomycin stimulation and granzyme B, in response to αCD3-αCD28 stimulation (**Figure 7**).
- Enhanced granulysin, granzyme B and memory-related CD27 gene expression, suggesting an increased cytolytic potential and memory phenotype (**Figure 8**).

Figure 3. DeTIL-0255 demonstrate a trend towards an increased number of total viable cells



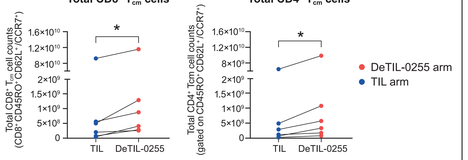
ns, no significant difference
All cell counts were obtained following harvest and LOVO wash/concentration on Day 22 using the Chemometec NC-200. Fold expansion in the rapid expansion protocol was calculated as Day 22 total viable cells divided by Day 11 seeded cell numbers; DeTIL, drug-enhanced TIL; TIL, tumor-infiltrating lymphocyte; TVC, total viable cell.

Figure 4. The addition of NX-0255 enhances the total number of CD8$^+$ T cells



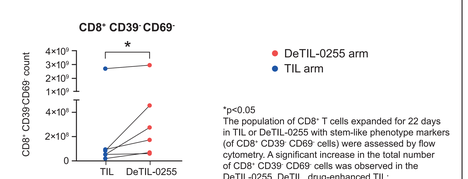
**p<0.01
The proportions of CD8$^+$ and CD4$^+$ T cells were assessed by flow cytometry. The absolute number of viable CD8$^+$ and CD4$^+$ T cells in TIL and DeTIL-0255 were determined for each individual culture. In all full-scale experiments, DeTIL-0255 demonstrated a significant increase in the total number of CD8$^+$ T cells and in five out of six experiments, an increase in the total numbers of CD4$^+$ T cells.
DeTIL, drug-enhanced TIL; TIL, tumor-infiltrating lymphocyte.

Figure 5. DeTIL-0255 demonstrate significantly increased CD8$^+$ and CD4$^+$ central memory T Cells



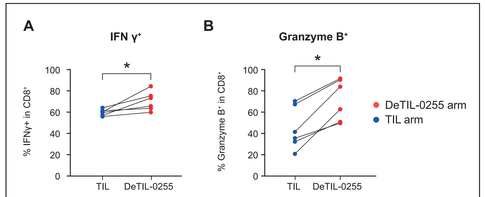
*p<0.05
Central memory phenotype of CD8$^+$ or CD4$^+$ T cells was assessed by flow cytometry based on the percentages of CD45RO$^+$ CD62L$^+$ or CD45RO$^+$ CCR7$^+$. The absolute numbers of viable CD8$^+$ T$_{CM}$ and CD4$^+$ T$_{CM}$ cells were determined for each individual culture. All full-scale experiments showed significant increase in the total numbers of CD8$^+$ and CD4$^+$ T central memory T cells.
DeTIL, drug-enhanced TIL; T$_{cm}$, central memory T cell; TIL, tumor-infiltrating lymphocyte.

Figure 6. DeTIL-0255 demonstrate significantly increased CD39$^-$ CD69$^-$ stem-like CD8$^+$ T-cells when compared with TIL



*p<0.05
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. DeTIL, drug-enhanced TIL; TIL, tumor-infiltrating lymphocyte.

Figure 7. DeTIL-0255 demonstrate significantly increased proportions of CD8$^+$ T cells expressing intracellular IFN-γ and granzyme B following stimulation



*p<0.05
Expanded TIL and DeTIL-0255 were stimulated for 6 hours with (**A**) PMA and Ionomycin or (**B**) anti-CD3 and anti-CD28 in the presence of Monensin and Brefeldin A. After 6 hours, expression of intracellular cytotoxic markers, IFN-γ and granzyme B were assessed by flow cytometry. Significant differences in the percentage of CD8$^+$ T cells expressing IFN-γ (**A**) and Granzyme B (**B**) were observed following stimulation in DeTIL-0255 compared with TIL. DeTIL, Drug-enhanced TIL; IFN, interferon; TIL, tumor-infiltrating lymphocyte.

Figure 8. DeTIL-0255 demonstrate an increased cytotoxic and memory gene expression profile

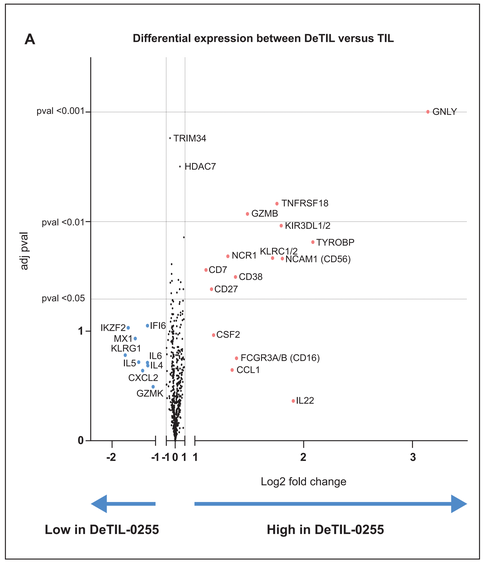
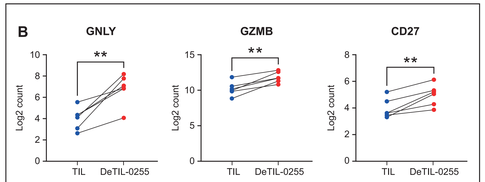


Figure 8 (cont.) DeTIL-0255 demonstrate an increased cytotoxic and memory gene expression profile



* p<0.05, ** p<0.01
Gene expression profile of DeTIL-0255 and TIL were evaluated by Nanostring and analyzed by nSolver analysis software. (**A**) Differential expression between DeTIL-0255 and TIL. (**B**). Log2 probe counts confirm Increase in cytotoxic gene Granulysin and Granzyme B, and memory related gene CD27 expression.
DeTIL, Drug-enhanced TIL; GNLY, granulysin; GZMB, granzyme B; TIL, tumor-infiltrating lymphocyte.

Conclusion

- Manufacturing of DeTIL-0255 is feasible, and compared with conventional TIL, demonstrates a superior phenotype and function that is suitable for clinical evaluation.
- A clinical trial has been initiated with DeTIL-0255 in patients with gynecologic malignancies (ovarian cancer, endometrial cancer, and cervical cancer; NCT05107739).

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Other DeTIL-0255 posters presented at SITC 2022

#671 Girda E, et al. A phase 1 adoptive cell therapy using drug-enhanced, tumor-infiltrating lymphocytes, DeTIL-0255, in adults with advanced malignancies

#361 Murthy P, et al. Universal expansion of CBL-B–inhibited tumor-infiltrating lymphocytes, DeTIL-0255, from women with ovarian cancer: process validation

#331 Gallotta M, et al. A novel small molecule inhibition of CBL-B shows potent antitumor activity in combination with Pmel-1 adoptive cell transfer in an aggressive mouse melanoma model