NX-0255, a Small-Molecule CBL-B Inhibitor, Expands and Enhances Tumor-Infiltrating Lymphocytes for Use in Adoptive Cancer Immunotherapy

Poster 98

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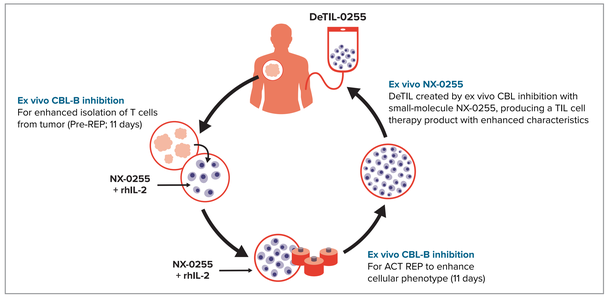
BACKGROUND

- Adoptive cell transfer (ACT) of tumor-infiltrating lymphocytes (TIL) effects durable responses in patients with melanoma and some epithelial tumors[1-4]
- It is thought that poor in vitro cell expansion and inefficient T-cell migration to the tumor limits the broader application of this approach[5,6]
- The E3 ubiquitin ligase, Casitas B-lineage lymphoma b (CBL-B) is expressed in T cells in which it functions as a regulator of immune cell activation, in part by requiring CD28 costimulation in addition to T-cell receptor activation[7,8]
- We have developed NX-0255, a highly potent, small-molecule inhibitor of CBL-B, demonstrating its ability to increase T-cell–derived cytokine secretion and proliferation in the presence or absence of costimulation
- Here, we investigated the effects of NX-0255 on the ex vivo growth and characteristics of human TIL to create drug-enhanced TIL (DeTIL-0255) as an ACT product for treating patients with cancer
- We hypothesize that CBL inhibition during TIL ex vivo expansion will promote the generation of a product with superior characteristics (**Figure 1**)

Figure 1. Schema for Expanding DeTIL-0255



ACT, adoptive cell transfer; DeTIL, drug-enhanced tumor infiltrating lymphocytes; CBL-B, Casitas B-lineage lymphoma b; REP, rapid expansion phase; rhIL-2, recombinant human interleukin-2.

METHODS

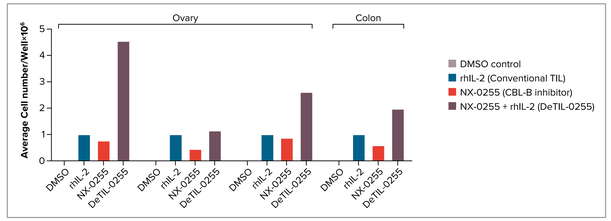
- To understand the role of NX-0255 during the expansion of TIL, tumor fragments from ovary and colon tumors were cultured with recombinant human interleukin-2 (rhIL-2) and compared in 2 experimental groups: NX-0255 without rhIL-2, or NX-0255 in combination with rhIL-2
  - After 28 days of culture, cell yield and viability were assessed
- After process development, cells were cultured from tumor fragments from ovary, colon, lung, head and neck, breast, and vulva carcinomas in G-REX flasks (Wilson Wolf, St Paul, MN) with either rhIL-2 alone (TIL) or rhIL-2 in combination with NX-0255 (DeTIL-0255) for a period of 22 days
  - After expansion, TIL and DeTIL-0255 were evaluated for yield (cell count), phenotype (flow cytometry), cytokine/chemokine secretion (cytometric bead assays), functionality (anti-CD3/CD28 stimulation), T-cell receptor (TCR) Vβ repertoire (RNA sequencing), and single-cell transcriptomics (10× Genomics)

RESULTS

- Culturing of TIL in the presence of NX-0255 alone resulted in the expansion of TIL to a similar extent as standard rhIL-2. The combination of NX-0255 with rhIL-2 dramatically increased TIL expansion in 3 of 4 samples (**Figure 2**)
- DeTIL-0255 significantly increased the number of cells expanded from tumor tissue (ovary, lung, head and neck, melanoma, breast, and vulva carcinomas) compared with conventionally cultured TIL (n=16, *P*=0.004) (**Figure 3**)
- Flow cytometric analysis demonstrated that DeTIL-0255 CD8+ T cells were significantly less exhausted than TIL, as shown by the significant reduced expression of programmed cell death protein 1 (PD-1; *P*=0.02), and coexpressing PD-1+ TIM-3 (T cell immunoglobulin and mucin domain-containing protein 3; *P*=0.03) and PD-1+ LAG-3 (lymphocyte activation gene 3; *P*=0.03) (**Figure 4**)
- The functional capacity of DeTIL-0255 was differentially enhanced, with significant increases in the absolute numbers of CD8+ T-cells expressing intracellular perforin (*P*=0.001), granzyme B (*P*=0.005), and CD107a (*P*=0.01) when comparing DeTIL-0255 with TIL. An increase of CD8+ T cells expressing CD137/4-1BB, a biomarker of CD8+ T-cell tumor reactivity was also observed (*P*=0.03) (**Figure 5**)
- TCR repertoire analysis showed that DeTIL-0255 had a significant increase in diversity index (Di) and unique CDR3 β chains in comparison with TIL, suggesting an increase in CD8+ T-cell effector function and TCR diversity is associated with antitumor effects (**Figure 6**)
- Single-cell sequencing analysis demonstrated that DeTIL-0255 expanded in the pre-rapid expansion phase had increased expression of genes associated with stemness (CCR7+,CD62L+[SELL],CD127+[IL-7R]) and cytotoxicity (GNLY+,GZMB+,NKG7+) (**Figure 7**)

RESULTS

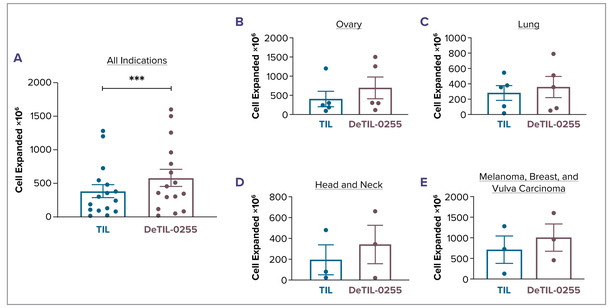
Figure 2. NX-0255 in Combination With rhIL-2 Yields Superior T-Cell Expansion



CBL-B, Casitas B-lineage lymphoma b; DeTIL-0255, drug-enhanced tumor-infiltrating lymphocyte; DMSO, dimethyl sulfoxide; rhIL-2, recombinant human interleukin-2; TIL, tumor-infiltrating lymphocyte.

- Culturing of TIL in the presence of NX-0255 alone resulted in the expansion of TIL to a similar extent as standard rhIL-2. The combination of NX-0255 with rhIL-2 dramatically increased TIL expansion in 3 of 4 samples

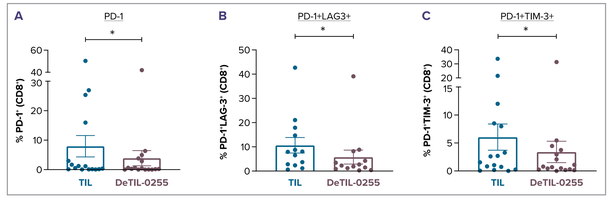
Figure 3. NX-0255 in Combination With rhIL-2 (DeTIL-0255) Significantly Increased the Number of T Cells Expanded From Tumor Tissue Compared With rhIL-2 Alone (TIL)



The number of T cells expanded from all indications (A); ovary tumor tissue (B); lung tumor tissue (C); head and neck tumor tissue (D); melanoma, breast and vulva carcinoma tissue (E). Statistical significance was assessed using 2-tailed Wilcoxon signed-rank test; *P<0.05, **P<0.005, and ***P<0.001. Error bars depict standard error of the mean (SEM).
DeTIL-0255, drug-enhanced tumor-infiltrating lymphocyte; rhIL-2, recombinant human interleukin-2; TIL, tumor-infiltrating lymphocyte.

- DeTIL-0255 significantly increased the number of cells expanded from tumor tissue (ovary, lung, head and neck, melanoma, breast, and vulva carcinomas) compared with conventionally cultured TIL (n=16, *P*=0.004)

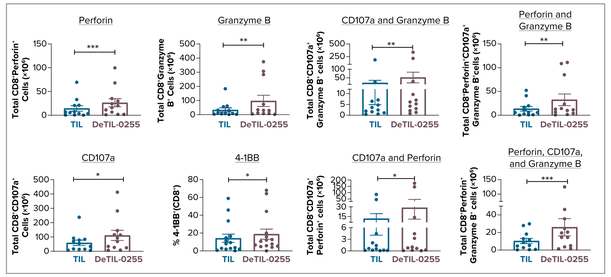
Figure 4. CD8+ T DeTIL-0255 Are Less Exhausted Than CD8+ TIL



Flow cytometry analysis of CD8+ T cells for PD-1 (A), PD-1 and LAG-3 (B), PD-1 and TIM-3 (C) expression. Statistical significance was assessed using 2-tailed Wilcoxon signed-rank test; *P<0.05, **P<0.005, and ***P<0.001. Error bars depict standard error of the mean (SEM).
DeTIL-0255, drug-enhanced tumor infiltrating lymphocyte; LAG-3, lymphocyte-activation gene 3; PD-1, programmed cell death protein 1; TIL, tumor-infiltrating lymphocyte; TIM-3, T-cell immunoglobulin and mucin domain-containing protein 3.

- (A) Flow cytometric analysis demonstrated that DeTIL-0255 CD8+ T cells were significantly less exhausted than TIL CD8+ T cells, as shown by the significant reduced expression of PD-1 (*P*=0.02) (B) and coexpressing PD-1+TIM-3+ (*P*=0.03) and (C) PD-1+LAG-3+ (*P*=0.03)

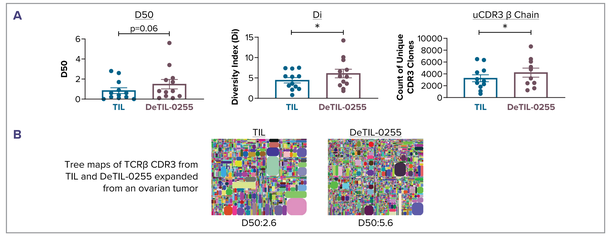
Figure 5. DeTIL-0255 Are Significantly More Cytotoxic Than TIL



Statistical significance was assessed using 2-tailed Wilcoxon signed-rank test; *P<0.05, **P<0.005, and ***P<0.001. Error bars depict standard error of the mean (SEM).
DeTIL-0255, drug-enhanced tumor-infiltrating lymphocyte; TIL, tumor-infiltrating lymphocyte.

- The functional capacity of DeTIL-0255 was differentially enhanced, with significant increases in the absolute numbers of CD8+ T cells expressing intracellular perforin (*P*=0.001), granzyme B (*P*=0.005) and CD107a (*P*=0.01) when comparing DeTIL-0255 with TIL. An increase of CD8+ T cells expressing CD137/4-1BB, a biomarker of CD8+ T-cell tumor reactivity, was also observed (*P*=0.03)

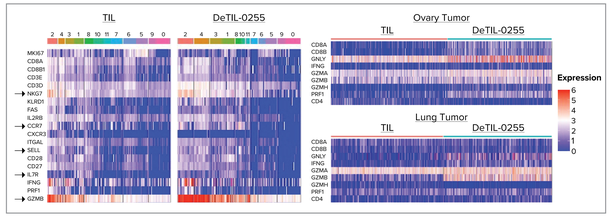
Figure 6. DeTIL-0255 Exhibit Significantly Increased TCR Diversity Compared With TIL



TCR repertoire diversity is quantified (A) and illustrated in tree maps where each rounded rectangle represents a unique entry: V–J–uCDR3, and the size of the spot denotes the relative frequency (B). Error bars depict standard error of the mean (SEM).
D50, diversity 50 value; DeTIL-0255, drug-enhanced tumor-infiltrating lymphocyte; Di, diversity index; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

- TCR repertoire analysis showed that DeTIL-0255 had a significant increase in diversity index (Di) and unique CDR3 β chains in comparison with TIL, suggesting that an increase in CD8+ T-cell effector function and TCR diversity is associated with antitumor effects

Figure 7. DeTIL-0255 Are Less Differentiated and More Cytotoxic Than TIL Cells



CCR, C-C Motif Chemokine Receptor; CXCR, C-X-C chemokine receptor; CD, cluster of differentiation; DeTIL-0255, drug-enhanced tumor-infiltrating lymphocyte; FAS, Fas cell surface death receptor; GNLY, Granulysin; GZM, granzyme; IFN, interferon; IL, interleukin; KLR, Killer Cell Lectin Like Receptor; MKI, marker of proliferation; NKG, Natural Killer Cell Granule Protein; PRF1, Perforin 1; SELL, selectin L. TIL, tumor-infiltrating lymphocyte.

- Single-cell sequencing analysis demonstrated that DeTIL-0255 expanded in the pre-REP had increased expression of genes associated with stemness (CCR7+,CD62L+[SELL],CD127+[IL-7R]) and cytotoxicity (GNLY+,GZMB+,NKG7+)

Table 1: Summary of DeTIL-0255 Expansion Studies Compared With TIL

Exhaustion	
Marker	% of CD8+
Total PD-1+	↓
Total PD-1+ TIM-3+	↓
Total PD-1+ LAG-3+	↓

Cytotoxic Function	
Marker	Absolute No. of CD8
CD107a+	↑
GrB+	↑
Perforin+	↑
CD107a+ GrB+	↑
CD107a+ Perforin	↑
GrB+ Perforin	↑
GrB+ Perforin CD107A+	↑

Chemokine Secretion	
Secretion	pg/mL
RANTES	↑
MCP-1	↑
IL-8	↑

Cytokine Secretion	
Secretion	pg/mL
7 CRS-associated cytokines (IL-2, IL-4, IL-6, IL-9, IL-10, IFN-γ, TNF-α)	–

Tumor Reactivity	
CD8	% of CD8+
Total 41BB+	↑

Arrows indicate a statistically significant (*P*<0.05) change in DeTIL-0255 compared with TIL.
CRS, cytokine release syndrome; DeTIL-0255, drug-enhanced tumor-infiltrating lymphocytes; GrB, granzyme B; IFN-γ, interferon gamma; IL, interleukin; LAG-3, lymphocyte-activation gene 3; MCP-1, monocyte chemoattractant protein 1; PD-1, programmed cell death protein 1; TIL, tumor-infiltrating lymphocytes; TNF-α, tumor necrosis factor alpha.

CONCLUSIONS

- Culturing of TIL in the presence of NX-0255 without rhIL-2 resulted in the expansion of cells, with numbers comparable with that of conventionally cultured TIL with rhIL-2
- Furthermore, the addition of NX-0255 in combination with rhIL-2 significantly increased the number of cells expanded compared with conventionally cultured TIL
- DeTIL-0255 expansion resulted in T cells that were significantly less exhausted and more cytotoxic than conventional TIL.
  - TCR repertoire and single-cell sequencing analysis showed that DeTIL-0255 had increased TCR diversity and validated that DeTIL-0255 expressed genes associated with stemness and cytotoxicity
- DeTIL-0255 significantly increased chemokine and cytokine secretion upon TCR and CD28 costimulation but did not elicit significantly more cytokines than TIL, alleviating additional safety concerns with the addition of NX-0255
- Increased expansion, reduced exhaustion, increased cytotoxicity, increased TCR diversity, and gene expression consistent with stemness all predict broader functional activity of DeTIL-0255 compared with conventional TIL, potentially conferring improved antitumor activity.
- Taken together, these data support the clinical development of DeTIL-0255 for the treatment of patients with cancer

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