NX-1607, a small molecule inhibitor of CBL-B, enhances anti-PD-1-mediated tumor growth inhibition by reshaping intratumoral innate and adaptive immune responses

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Abstract

The E3 ubiquitin ligase Casitas B-lineage lymphoma B (CBL-B) is expressed in leukocytes and regulates signaling pathways in T and NK cells, significantly limiting their antitumor effector function. In T cells, CBL-B attenuates activation initiated by TCR engagement in part by mediating the requirement for CD28 co-stimulation, thus setting the threshold for T cell activation. In NK cells, CBL-B functions downstream of TAM receptors and negatively regulates cytokine production and target cell killing.
We previously reported that oral administration of NX-1607, a potent inhibitor of CBL-B, resulted in significant dose-dependent, single-agent inhibition of tumor growth in the subcutaneous CT26 colon carcinoma model. This inhibition was dependent on NK cells and T cells. When NX-1607 was combined with anti-PD-1, we observed a substantial increase in the median overall survival and the frequency of complete tumor rejections in this preclinical tumor model.

To gain a better understanding of how NX-1607 treatment affects different immune cell types and immune pathways within the tumor microenvironment, we conducted gene expression analysis of tumor samples obtained from mice treated with NX-1607 as monotherapy or in combination with anti-PD-1. Our analysis revealed that CT26 tumors from mice treated with NX-1607 exhibited significant changes in the immune cell density score and gene expression pathways related to innate and adaptive immune signaling, including antigen presentation, cytokine and chemokine signaling, and interferon-gamma response genes. When NX-1607 was combined with anti-PD-1 we observed further enhancement of most of the immune cell scores and immune gene signatures induced by NX-1607 monotherapy, consistent with the observed antitumor synergy of these agents.

In addition, we performed TCR repertoire analysis and found that the response to NX-1607 was associated with an expansion of unique T cell clones in the tumor microenvironment. This expansion was evidenced by a significant increase in the number of unique complementary determining region 3 (CDR3) sequences. The increased richness of TCR repertoire following NX-1607 treatment was similar to that observed with anti-PD-1 monotherapy.

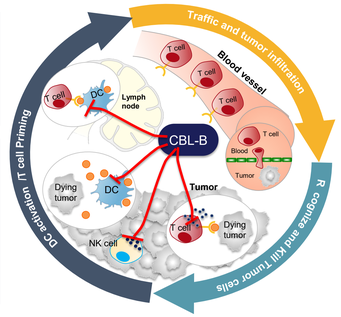
These results demonstrate that the response to NX-1607 in the CT26 tumor model is associated with increased density and function of innate and adaptive immune cells within the tumor. These effects are further amplified when NX-1607 is combined with anti-PD-1. These findings provide additional support for clinical development of this novel CBL-B inhibitor given as monotherapy or in combination with PD-1 blockade. A Phase 1 clinical trial of NX-1607 in patients with advanced tumors is ongoing (NCT05107674).

Introduction

CBL-B: A Modulator of T Cell Activation and a Novel Target for Immuno-oncology

- CBL-B E3 ligase is a master orchestrator of the immune response.
- CBL-B mediated mechanisms strongly restrains a productive antitumor response.

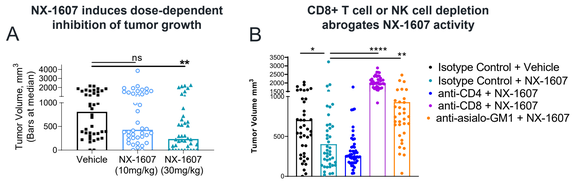
CBL-B inhibition increases:
- DC and NK infiltration and function
- T cell priming
- Cytotoxic T cells function
- Ability of T cells to resist tumor immunosuppressive mechanisms: Treg, MDSC, and TGF-β



NX-1607: Optimized CBL-B inhibitor for oral delivery. NX-1607 is currently in a Phase 1a clinical trial in patients with advanced solid tumors NX-1607-101 (NCT05107674).

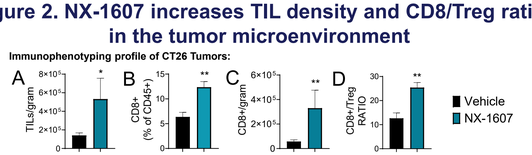
Results

Figure 1. Single-agent NX-1607 induces NK and T cell-dependent antitumor response



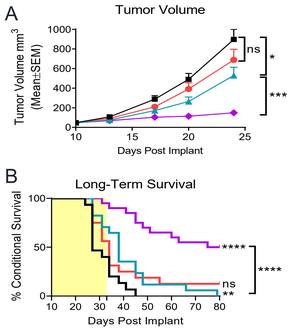
Individual tumor volumes taken on Day 19 (A) of mice bearing CT26 tumors treated by daily oral administration of: vehicle or NX-1607 at 10 or 30 mg/kg. NX-1607 exhibits single agent efficacy in the CT26 model (TGI=71%, p<0.01 at 30 mg/kg). In a second study, mice bearing CT26 tumors were treated daily from Day 9 to Day 25 with oral NX-1607 at 30 mg/kg in presence of depleting antibodies for CD4+ cells, CD8+ cells, or NK cells (anti-asialo-GM1). Individual tumor volumes at Day 25 are showed (B). Statistical significance of differences was evaluated using Mann-Whitney test. Statistical significance: not significant (ns) P > 0.05, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001.

Figure 2. NX-1607 increases TIL density and CD8/Treg ratio in the tumor microenvironment



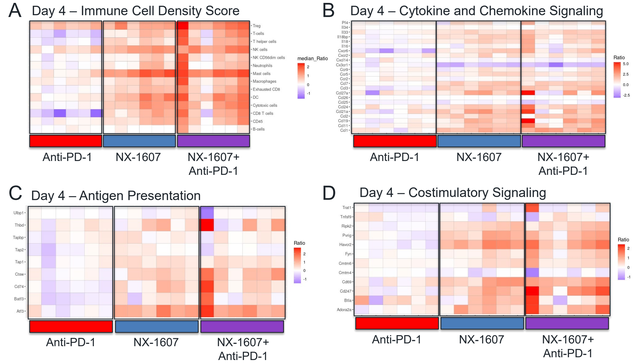
CT26 tumors from mice treated by administration of either: vehicle control (PO, n = 6) or NX-1607 at 30 mg/kg (PO, n = 12) (see FIGURE 1A) were harvested on Day 19 and investigated for T cell phenotypic change by Flow Cytometry. NX-1607 increases the number of tumor-infiltrating leukocytes (TIL) per gram of tumor (A), the frequency of total CD8+ T cells as a percentage of CD45+ leukocytes (B), the number of total CD8+ T cells per gram of tumor (C), and the CD8+ T cells to Tregs ratio (D) in CT26 tumors from treated mice. Statistical significance using Mann-Whitney test: * P ≤ 0.05, ** P ≤ 0.01.

Figure 3. NX-1607 and anti-PD-1 synergize to enhance antitumor effects and survival of mice



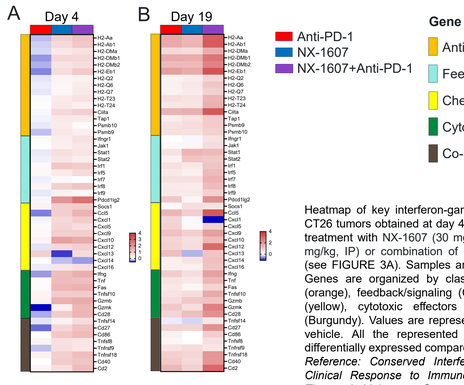
Group mean tumor volumes ± SEM (A) and percentage survival over time through day 80 (B) of mice bearing CT26 tumors were treated by: vehicle (n = 30), NX-1607 (30 mg/kg, PO, shaded area, n = 32), anti-PD-1 (10 mg/kg, IP, twice per week on Day 10, 13, 17, 20, 24, 27, and 31, n = 34) or combination of NX-1607 plus anti-PD-1 (n = 40). Statistical significance of differences in tumor volume was evaluated using two-way ANOVA with Tukey multiple comparisons test. Statistical significance of differences in conditional survival between groups was evaluated using the Log-rank (Mantel-Cox) test. Statistical significance: not significant (ns) P > 0.05, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001.

Figure 4. NX-1607 drives rapid changes in the immune cell density scores and immune function gene signatures



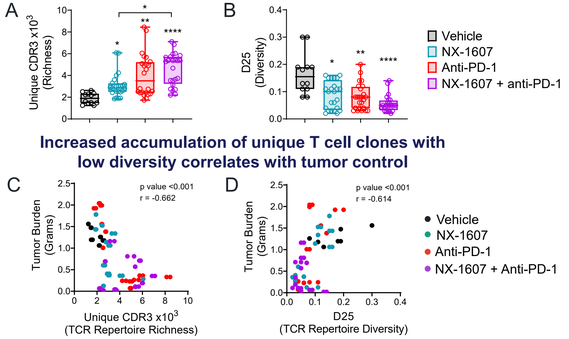
CT26 tumors from mice treated by administration of NX-1607 (30 mg/kg, PO), anti-PD-1 (10 mg/kg, IP) or combination of NX-1607 plus anti-PD-1 (see FIGURE 3A) were harvested on Day 4 and investigated for gene expression analysis. Heatmaps of genes represented as median ratio to vehicle for the immune cell density score (A) and gene expression pathways related to innate and adaptive immune signaling, including cytokine and chemokine signaling (B), antigen presentation (C) and costimulatory signaling (D).

Figure 5. NX-1607 induces rapid changes in key interferon-gamma response genes



Heatmap of key interferon-gamma response genes in CT26 tumors obtained at day 4 (A) and day 19 (B) post treatment with NX-1607 (30 mg/kg, PO), anti-PD-1 (10 mg/kg, IP) or combination of NX-1607 plus anti-PD-1 (see FIGURE 3A). Samples are ordered by treatment. Genes are organized by class: antigen presentation (orange), feedback/signaling (Cyan), chemoattractants (yellow), cytotoxic effectors (green), co-stimulatory (Burgundy). Values are represented as median ratio to vehicle. All the represented genes are significantly differentially expressed compared to vehicle.
Reference: Conserved Interferon-g Signaling Drives Clinical Response to Immune Checkpoint Blockade Therapy in Melanoma, Grasso et al., 2020, Cancer Cell.

Figure 6. NX-1607, similarly to anti-PD-1, induces expansion of unique T cell clones with reduced TCR diversity



CT26 tumors from mice treated by administration of NX-1607 (30 mg/kg, PO), anti-PD-1 (10 mg/kg, IP) or combination of NX-1607 plus anti-PD-1 (see FIGURE 3A) were harvested on Day 19 and investigated for TCR repertoire analysis. Richness of repertoire (A, unique CDR3) and diversity estimated with clonal proportion D25 index (B, the percentage of unique clonotypes that account for greater than 25% of the total number of sequences). Statistical significance of differences was evaluated using Kruskal-Wallis test. Spearman correlation test was applied to calculate the correlation between TCR repertoire richness and diversity and the tumor burden at day 19. Tumor regression in response to all immunotherapy treatments (NX-1607 in cyan, anti-PD-1 in red and combination in purple) correlates strongly with increased number of unique CDR3 (C) and reduced diversity index D25 (D). Statistical significance: not significant (ns) P > 0.05, * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001.

Conclusions

- The CBL-B inhibitor, NX-1607, acts on multiple immune cells, addressing several antitumor resistance mechanisms that render it an optimal next generation IO agent.
- Single-agent NX-1607 induces dose-dependent and NK and T cell-dependent antitumor response.
- NX-1607 increases TIL density and CD8/Treg ratio in the tumor microenvironment.
- NX-1607 and anti-PD-1 synergize to enhance antitumor effects and survival of mice.
- NX-1607 drives rapid changes in the immune cell density scores and immune function gene signatures.
- NX-1607 induces rapid changes in key interferon-gamma response genes.
- NX-1607, similarly to anti-PD-1, induces expansion of unique T cell clones with low TCR diversity that correlates with decreased tumor burden.
- These results support the rationale for the use of NX-1607 in clinical trials in patients with advanced solid tumors NX-1607-101 (NCT05107674).

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