NX-1607, an inhibitor of the CBL-B E3 ubiquitin ligase, promotes favorable local and systemic changes in infiltrating immune cells in multiple mouse tumor models

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Abstract

The E3 ubiquitin ligase Casitas B-lineage lymphoma B (CBL-B) is expressed in leukocytes, regulating 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. This sets 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.

Here we characterize the antitumor and immune effects of NX-1607, a potent, specific and orally bioavailable intramolecular glue inhibitor of CBL-B, in three individual mouse tumor models: the subcutaneous CT26 colon carcinoma, the metastatic 4T1 triple negative breast tumor and the A20 syngeneic B cell lymphoma. We show that oral administration of NX-1607 induced significant tumor growth inhibition in all three tumors. In the 4T1 tumor model, when administered in a neo-adjuvant setting, NX-1607 therapy induced durable complete responses (CRs) in 13 out of 24 animals surviving at 140 days, compared with 2 out of 24 surviving animals for the control. Mice treated with NX-1607 demonstrated immunological memory, with 92% of mice with CRs successfully rejecting tumor growth upon rechallenge with 4T1 tumor cells.
Immunophenotyping studies of tumor infiltrating lymphocytes (TILs) showed an increase in antigen-specific CD8+ T cells in 4T1 tumor-bearing mice treated with NX-1607. Tumor infiltrating antigen-specific CD8+ T cells displayed lower expression of the exhaustion markers TIM3 and LAG-3 in mice treated with NX-1607. Within the CT26 tumor, NX-1607 treatments doubled the ratio of CD8+ to Treg TILs and significantly increased the frequency of the cytotoxic marker Granzyme B within NK cell subsets.
Profiling of circulating cells from mice treated with NX-1607 showed increased antigen-experienced PD-1+ CD8+ T cells and an increased percentage of circulating CD8+ T cells co-expressing activation markers (CD44 and 4-1BB/CD137) and memory markers (CD44, CD127 and CD27). Moreover, we showed that following NX-1607 treatment, the observed increase in antigen-experienced CD8+ T cells in the blood correlated with antitumor response (day 19 post treatment, $p < 0.001$).

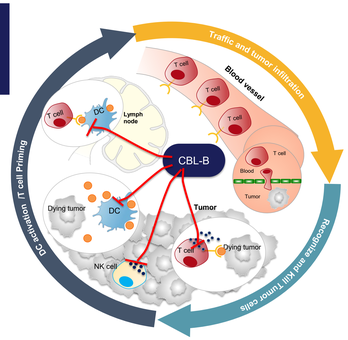
Collectively, these studies provide insight into the in vivo activity of this novel inhibitor of CBL-B, demonstrating that NX-1607 displays single agent antitumor activity in multiple preclinical tumor models and functions to enhance innate and adaptive immune responses that may help overcome a suppressive tumor microenvironment. These findings also deliver experimental support for clinical development of this novel CBL-B E3 ligase inhibitor, NX-1607. We have initiated a clinical trial with NX-1607 in patients with advanced solid tumors NX-1607-101 (NCT05107674).

Introduction

The CBL-B inhibitor, NX-1607, acts on multiple immune cells, addressing several antitumor resistance mechanisms

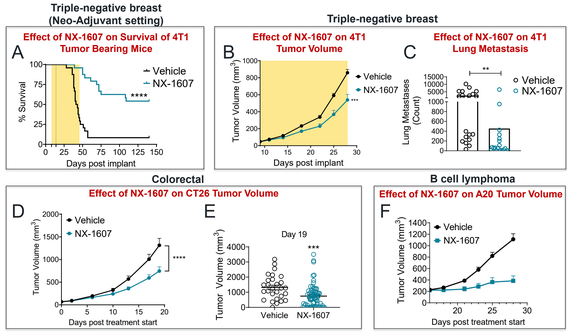
- CBL-B E3 ligase is a master orchestrator of the immune response.
- CBL-B mediated mechanisms strongly restrains a productive antitumor response.

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



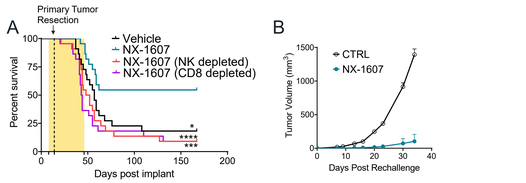
Results

Figure 1. Single-agent NX-1607 induces antitumor response in multiple tumor models



Percentage survival over time through day 140 (A) of mice bearing 4T1 tumors treated by administration of either: vehicle control daily, orally (po) or NX-1607 at 30 mg/kg daily, po (shaded area). Primary tumors were resected on day 14 and 15 (dotted line) when they reached a mean tumor volume of 145 mm^3. Statistical significance of differences in conditional survival between groups was evaluated using the Log-rank (Mantel-Cox) test. Group mean tumor volumes ± SEM (B) and number of 4T1 metastatic tumor cell colonies measured in lungs harvested on Day 28 (C) of mice bearing orthotopic 4T1 tumors were treated by administration of either: vehicle control given daily by oral route (po) or NX-1607 at 30 mg/kg given daily, po (shaded area). Statistical significance of differences in mean tumor volumes between groups was evaluated using a mixed-effects model and Dunnett's multiple comparisons test. Statistical significance of differences in the number of colonies between groups was evaluated using Kruskal-Wallis one-way ANOVA with Dunn's multiple comparisons test. Group mean tumor volumes ± SEM (D) and individual tumor volumes taken on Day 19 (E) of mice bearing CT26 tumors were treated by daily oral administration of either: vehicle or NX-1607 at 30 mg/kg. Statistical significance of differences was evaluated using two-way ANOVA with Bonferroni multiple comparisons test and Mann-Whitney test, respectively. Group mean tumor volumes ± SEM (F) of mice bearing A20 tumors treated (starting at Day 16 post tumor implant) by daily oral administration of either: vehicle or NX-1607 at 30 mg/kg. Statistical significance of differences between groups was evaluated using a mixed-effects model and Dunnett's multiple comparisons test. Statistical significance: not significant (ns) $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and **** $P \leq 0.0001$.

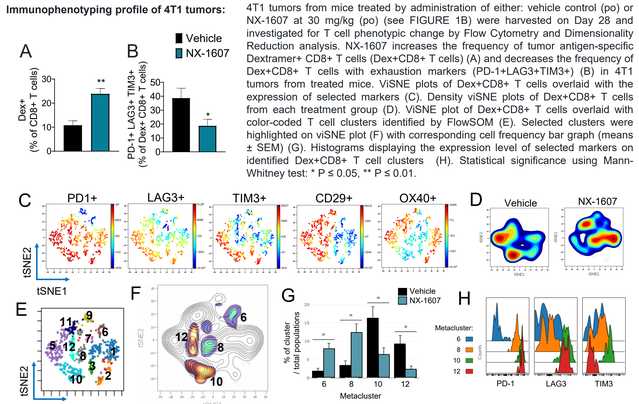
Figure 2. NX-1607 antitumor effect is NK and T cell-dependent and generates immunological memory



Percentage survival over time through day 170 (A) of mice bearing 4T1 tumors treated by administration of either: vehicle control daily, orally (po) or NX-1607 at 30 mg/kg daily, po (shaded area). Primary tumors were resected on day 14 and 15 (dotted line) when they reached a mean tumor volume of 145 mm^3. Two additional groups of mice were treated in the presence of depleting antibodies for NK cells or CD8+ T cells. Statistical significance of differences in conditional survival between groups was evaluated using the Log-rank (Mantel-Cox) test. Group mean tumor volumes ± SEM (B) of mice previously treated with NX-1607 and had complete tumor regression (from study in FIGURE 1A) were re-challenged with 4T1 cells 140 days after the initial implant. "CTRL" group of mice were age-matched naïve mice, inoculated with 4T1 cells. Statistical significance: * $P \leq 0.05$, *** $P \leq 0.001$, and **** $P \leq 0.0001$.

Figure 3. NX-1607 increases antigen-specific CD8+ T-cells with reduced exhaustion markers in the tumor

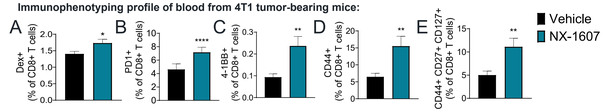
Immunophenotyping profile of 4T1 tumors:



4T1 tumors from mice treated by administration of either: vehicle control (po) or NX-1607 at 30 mg/kg (po) (see FIGURE 1B) were harvested on Day 28 and investigated for T cell phenotypic change by Flow Cytometry and Dimensionality Reduction analysis. NX-1607 increases the frequency of tumor antigen-specific Dextramer+ CD8+ T cells (Dex+CD8+ T cells) (A) and decreases the frequency of Dex+CD8+ T cells with exhaustion markers (PD-1+LAG3+TIM3+) (B) in 4T1 tumors from treated mice. ViSNE plots of Dex+CD8+ T cells overlaid with the expression of selected markers (C). Density viSNE plots of Dex+CD8+ T cells from each treatment group (D). ViSNE plot of Dex+CD8+ T cells overlaid with color-coded T cell clusters identified by FlowSOM (E). Selected clusters were highlighted on viSNE plot (F) with corresponding cell frequency bar graph (means ± SEM) (G). Histograms displaying the expression level of selected markers on identified Dex+CD8+ T cell clusters (H). Statistical significance using Mann-Whitney test: * $P \leq 0.05$, ** $P \leq 0.01$.

Figure 4. NX-1607 increases antigen-specific CD8+ T-cells and the expression of activation and memory markers in circulating CD8+ T-cells

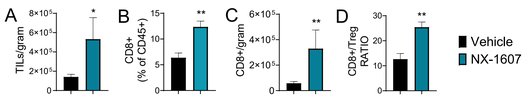
Immunophenotyping profile of blood from 4T1 tumor-bearing mice:



Mice bearing 4T1 tumors were treated by administration of either: vehicle control daily, orally (po) or NX-1607 at 30 mg/kg daily, po (see FIGURE 1B). At Day 28, frequency and phenotype of immune cells was assessed in the blood by Flow Cytometry. NX-1607 increases the frequency of tumor antigen-specific Dextramer+ CD8+ T cells (Dex+CD8+ T cells) (A), the frequency of CD8+ T cells that express the activation markers PD1 (B), 4-1BB (C) and CD44 (D), and the frequency of circulating CD8+ T-cells with memory phenotype (CD44+CD27+CD127+) (E) in the blood of treated 4T1-tumor-bearing mice. Statistical significance using Mann-Whitney test: : * $P \leq 0.05$, ** $P \leq 0.01$, and **** $P \leq 0.0001$.

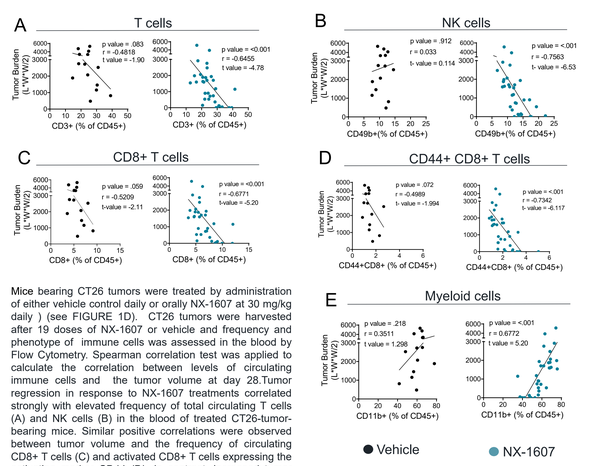
Figure 5. NX-1607 increases TILs density and CD8/Treg ratio in the tumor microenvironment

Immunophenotyping profile of CT26 Tumors:



CT26 tumors from mice treated by administration of either: vehicle control (po) or NX-1607 at 30 mg/kg (po) (see FIGURE 1E) 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: * $P \leq 0.05$, ** $P \leq 0.01$.

Figure 6. Following NX-1607 treatment, level of circulating T and NK cells correlates with antitumor response



Mice bearing CT26 tumors were treated by administration of either vehicle control daily or orally NX-1607 at 30 mg/kg daily) (see FIGURE 1D). CT26 tumors were harvested after 19 doses of NX-1607 or vehicle and frequency and phenotype of immune cells was assessed in the blood by Flow Cytometry. Spearman correlation test was applied to calculate the correlation between levels of circulating immune cells and the tumor volume at day 28.Tumor regression in response to NX-1607 treatments correlated strongly with elevated frequency of total circulating T cells (A) and NK cells (B) in the blood of treated CT26-tumor-bearing mice. Similar positive correlations were observed between tumor volume and the frequency of circulating CD8+ T cells (C) and activated CD8+ T cells expressing the activation marker CD44 (D). In contrast, increased tumor growth inhibition correlated with decreased levels of circulating myeloid cells, characterized by the expression of the CD11b marker (E).

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 antitumor response in multiple tumor models.
- NX-1607 antitumor effect is NK and T cell-dependent and generates immunological memory.
- NX-1607 increases antigen-specific CD8+ T-cells with reduced exhaustion markers and increases TIL density and CD8/Treg ratio in the tumor microenvironment.
- NX-1607 increases circulating antigen-specific CD8+ T-cells with enhanced expression of activation and memory markers.
- Following NX-1607 treatment, the level of circulating T and NK cells correlates with antitumor response.
- These results support the rationale for the use of NX-1607 in clinical trials in patients with advanced solid tumors NX-1607-101 (NCT05107674).

Disclosures

All authors are past or current employees of Nurix Therapeutics and hold company stock or stock options.

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