NX-1607, a small molecule inhibitor of CBL-B, is efficacious as a single agent and in combination with Rituximab in preclinical mouse models of lymphoma

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Abstract

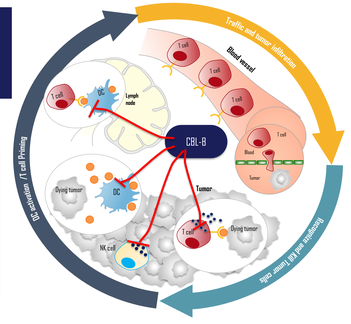
The E3 ubiquitin ligase Casitas B-lineage lymphoma B (CBL-B) is expressed in leukocytes and regulates signaling pathways in T and NK cells, significantly limiting their antitumor effector function. In T cells, CBL-B attenuates activation initiated by TCR engagement in part by mediating the requirement 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 cytotoxicity.
Here, we characterized the antitumor and immune effects of NX-1607, a potent orally bioavailable inhibitor of CBL-B, when used as a single agent in a murine A20 syngeneic B cell lymphoma model and in combination with Rituximab in a Raji cell xenograft model of Non-Hodgkin's Lymphoma (NHL).
We show that NX-1607 treatment of immunocompetent mice bearing subcutaneous A20 B cell lymphoma tumors leads to robust, T-cell dependent, tumor regression. All mice that achieved complete responses (CRs) after NX-1607 treatment successfully rejected tumor growth during a subsequent challenge with A20 tumors. Immunophenotyping studies of tumor infiltrating CD8+ T cells from A20 tumor-bearing mice treated with NX-1607 showed higher expression of multiple activation markers (CD25, CD69, PD-1), co-stimulatory markers (4-1BB, GITR, CD226/DNAM-1) and the cytotoxic marker Granzyme B. Profiling of circulating peripheral blood T cells from tumor bearing mice treated with NX-1607 showed an increased percentage of antigen-experienced PD-1+ CD8+ T cells with increased expression of activation markers (e.g., CD69), co-stimulatory markers (e.g., GITR) and the cytotoxic marker Granzyme B.
Moreover, we show in a Raji NHL model of disseminated disease that the combination of daily NX-1607 administration with Rituximab enhanced median overall survival when compared to single agent activity (p<0.0001). The survival benefit provided by NX-1607 was abrogated by depletion of NK cells, which suggests that NX-1607 enhances NK cell-mediated antibody dependent cellular cytotoxicity (ADCC) in vivo.
Collectively, these studies provide insights into the in vivo activity of this novel inhibitor of CBL-B, demonstrating that NX-1607 displays antitumor efficacy in preclinical lymphoma models by enhancing innate and adaptive immune responses. The observed synergistic antitumor effects of NX-1607 in combination with Rituximab support its potential as an adjunctive treatment to enhance antitumor efficacy of antibody therapy for patients with hematopoietic malignancies. NX-1607 is currently being investigated in patients with advanced cancer including solid tumors and malignant lymphoma, including large B cell lymphoma (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.

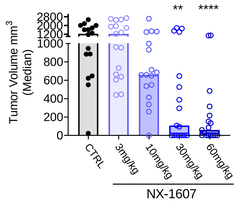
CBL-B inhibition increases:
- DC and NK infiltration and function
- T cell priming
- Cytotoxic T cells function
- Ability of T cells to resist tumor immunosuppressive mechanisms: Treg, MDSC, and TGF-β



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).

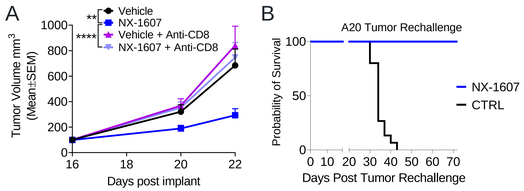
Results

Figure 1. NX-1607 induces dose-dependent antitumor response in the A20 lymphoma model



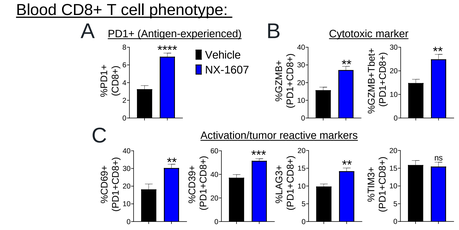
Individual tumor volumes taken on Day 28 of mice bearing subcutaneous A20 tumors treated by daily oral administration of either: vehicle control given daily by oral route (po) or NX-1607 at 3, 10, 30 or 60 mg/kg given daily, po. Statistical significance of differences was evaluated using one-way ANOVA with Dunnett's multiple comparisons 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 antitumor effect is T cell-dependent and generates immunological memory



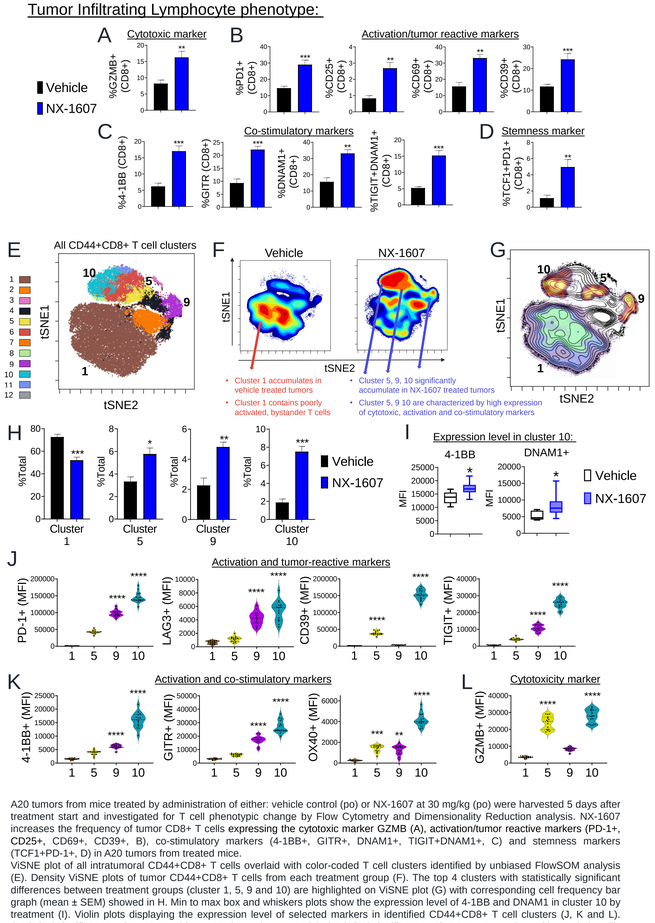
Group mean tumor volumes ± SEM (A) of mice bearing A20 tumors treated (starting at Day 16 post tumor implant) by daily oral administration of either: vehicle or NX-1607 at 30 mg/kg. Two additional groups of mice were treated in the presence of depleting antibodies for CD8+ T cells. Statistical significance of differences between groups was evaluated using two-way ANOVA model with Tukey's multiple comparisons test. Percentage survival over time through day 72 (B) of the mice previously treated with NX-1607 that had complete tumor regression which were then re-challenged with A20 cells 139 days after the initial implant. "CTRL" group of mice were age-matched naive mice, inoculated with A20 cells. Statistical significance: ** P ≤ 0.01, and **** P ≤ 0.0001.

Figure 3. NX-1607 increases the expression of activation and cytotoxic markers in circulating CD8+ T-cells in the A20 lymphoma model



Mice bearing A20 tumors were treated by administration of either: vehicle control daily, orally (po) or NX-1607 at 30 mg/kg daily, po. Five days post treatment start, phenotype of CD8 T cells was assessed in the blood by Flow Cytometry. NX-1607 increases the frequency of antigen-experienced CD8+ T cells (PD1+, A), and, within this population, the frequency of circulating CD8+ T-cells expressing cytotoxic markers (GZMB+, GZMB+Tbet+, B) and activation/tumor reactive markers (CD69+, CD39+, LAG3+, C), with no changes in TIM3 expression (C). Statistical significance using Mann-Whitney test: : * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001.

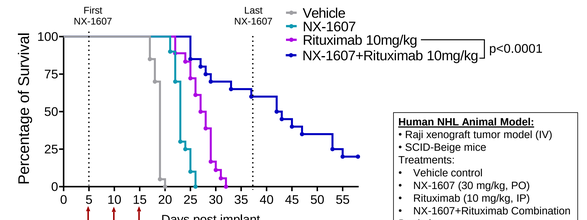
Figure 4. Response to NX-1607 is associated with tumor CD8+ T cell subsets characterized by high expression of cytotoxic, activation and co-stimulatory markers



A20 tumors from mice treated by administration of either: vehicle control (po) or NX-1607 at 30 mg/kg (po) were harvested 5 days after treatment start and investigated for T cell phenotypic change by Flow Cytometry and Dimensionality Reduction analysis. NX-1607 increases the frequency of tumor CD8+ T cells expressing the cytotoxic marker GZMB (A), activation/tumor reactive markers (PD-1+, CD25+, CD69+, CD39+, B), co-stimulatory markers (4-1BB+, GITR+, DNAM1+, TIGIT+DNAM1+, C) and stemness markers (TCF1+PD-1+, D) in A20 tumors from treated mice.
ViSNE plot of all intratumoral CD44+CD8+ T cells overlaid with color-coded T cell clusters identified by unbiased FlowSOM analysis (E). Density ViSNE plots of tumor CD44+CD8+ T cells from each treatment group (F). The top 4 clusters with statistically significant differences between treatment groups (cluster 1, 5, 9 and 10) are highlighted on ViSNE plot (G) with corresponding cell frequency bar graph (mean ± SEM) showed in H. Min to max box and whiskers plots show the expression level of 4-1BB and DNAM1 in cluster 10 by treatment (I). Violin plots displaying the expression level of selected markers in identified CD44+CD8+ T cell clusters (J, K and L). Statistical significance using Mann-Whitney test (A, B, C, D, H and I). Statistical significance using one-way ANOVA with Kruskal-Wallis test (J, K and L). * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001.

Figure 5. NX-1607 strongly potentiates Rituximab-directed NK Cell ADCC against tumor cells in a human NHL animal model

NX-1607-mediated NK activation potentiates Rituximab antitumor activity:

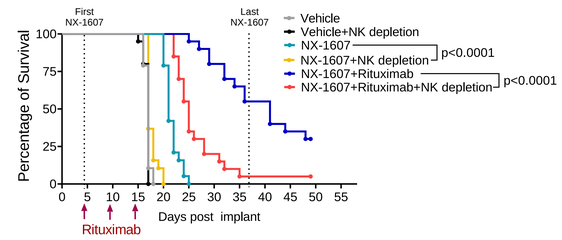


Human NHL Animal Model:
- Raji xenograft tumor model (IV)
- SCID-Beige mice

Treatments:
- Vehicle control
- NX-1607 (30 mg/kg, PO)
- Rituximab (10 mg/kg, IP)
- NX-1607+Rituximab Combination

Depleting treatments:
- Anti-Asialo GM1 (3 doses, IP)

NK depletion abrogates NX-1607 and NX-1607 + Rituximab antitumor activity:



Conclusions

- The CBL-B inhibitor, NX-1607, acts on multiple immune cells, addressing several antitumor resistance mechanisms that render it a potential next generation IO agent.
- Single-agent NX-1607 induces dose- and T cell-dependent antitumor response in the A20 lymphoma model and increases the expression of activation and cytotoxic markers in circulating CD8+ T cells.
- Response to NX-1607 is associated with intratumoral CD8+ T cell subsets characterized by high expression of cytotoxic, activation and co-stimulatory markers.
- NX-1607 strongly potentiates Rituximab-directed NK Cell ADCC against tumor cells in a human NHL animal model.
- These studies also provide support for clinical development of NX-1607 as a monotherapy or in combination with antibody therapeutics to enhance ADCC antitumor effects. We have initiated a clinical trial with NX-1607 in patients with advanced solid tumors NX-1607-101 (NCT05107674).

Presented at the CRI-ENCI-AACR Seventh International Cancer Immunotherapy (CICON 2023) Conference. September 20-23, 2023.