NX-1607, a Small Molecule Inhibitor of the CBL-B E3 Ubiquitin Ligase, Promotes T and NK Cell Activation and Enhances NK-mediated ADCC in a Mouse Lymphoma Tumor Model

Marilena Gallotta, Jennifa Gosling, Austin Tenn-McClellan, Serena Ranucci, Jose Gomez Romo, Frederick Cohen, Gwenn M Hansen, Arthur T Sands, Cristiana Guiducci, Ryan Rountree

Nurix Therapeutics, San Francisco, CA, USA

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 describe the effects of NX-1607, an orally bioavailable intramolecular glue inhibitor of CBL-B, on primary human T and NK cells and assess NX-1607 in combination with Rituximab in a murine xenograft model of Non-Hodgkin's Lymphoma (NHL). Previously, we showed that NX-1607 enhances IL-2 and IFN-γ secretion in human T cells following TCR stimulation. Regulatory T cells (Tregs) produce multiple cytokines in the tumor microenvironment (TME) that work to counteract the antitumor response by suppressing T-cell activation. Proliferation of CD4+ effector T cells activated by anti-CD3/CD28 was suppressed when cultured 1:1 with Tregs or TGF-β. Addition of NX-1607 recovered the proliferative capacity of CD4+ effector T cells to levels equivalent to that of anti-CD3/CD28 stimulation alone. Therefore, in addition to enhancing T-cell activation, NX-1607 renders T cells resistant to Treg and TGF-β-mediated suppression.

In an in vitro ADCC assay, addition of NX-1607 significantly enhanced TNF-α and IFN-γ production in human primary NK cells. The efficacy of NX-1607 in combination with Rituximab was evaluated in a Raji NHL model where Raji cells were administered by IV to establish disseminated tumors followed by treatment with NX-1607 (30 mg/kg QD) and/or Rituximab (10 mg/kg). Both NX-1607 and Rituximab given as monotherapy provided a significant survival benefit. Combination of NX-1607 and Rituximab significantly enhanced tumor growth inhibition and stable rejections when compared to single agent activity. Importantly, the survival benefit provided by NX-1607 was abrogated by depletion of NK cells. Therefore, NX-1607 augments NK cell activity both in human NK cells and in mouse tumor models.

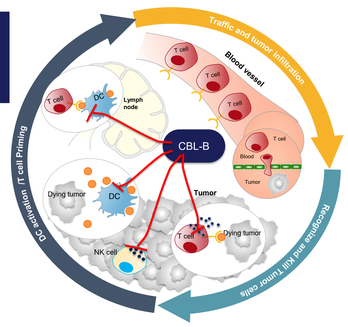
These studies provide insight into the antitumor activity of this novel, small molecule inhibitor of CBL-B, demonstrating that NX-1607 enhances both innate and adaptive immune responses, both of which are important for overcoming a suppressive TME. These studies also provide support for clinical development of NX-1607 as a monotherapy or in combination with antibody therapeutics to enhance ADCC antitumor effects. NX-1607 is currently in a Phase 1a clinical trial 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. NX-1607 Limits TGF-β Mediated T-cell Suppression

Primary human T cell TGF-β assay:

TGF-β assay
- Freshly isolated T-cells labelled with Cell Trace Violet.
- Pre-treated with 5ng/mL TGF-β for 30 minutes followed by addition of NX-1607 for another 30 minutes.
- 48-hour stimulation with anti-CD3 (10ug/mL) and anti-CD28 (5ug/mL).

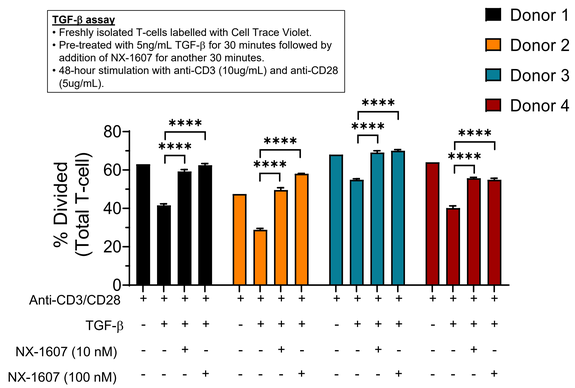


Figure 2. NX-1607 Limits Treg Mediated T-cell Suppression

Human Treg inhibition assay:

Treg assay
- nTreg (CD4+CD127lowCD25+) & Tresp isolated from same donor and rested overnight:
  - nTreg rested with 2ng/mL IL-2
  - Tresp rested with 1ng/mL IL-7
- nTregs labelled with Cell Trace Violet.
- Tresp labelled with CFSE.
- Tresp seeded at uniform density.
- nTregs seeded at indicated ratios.
- Cells pre-treated with 1uM NX-1607 for 30 minutes.
- After pre-treatment, cells stimulated with CD3/CD28 Dynabeads (8:1 Tresp:Dynabead).
- 3–5-day stimulation.

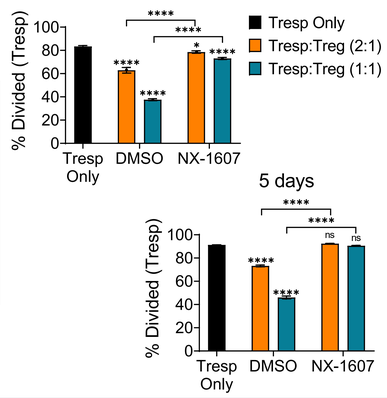
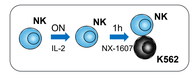
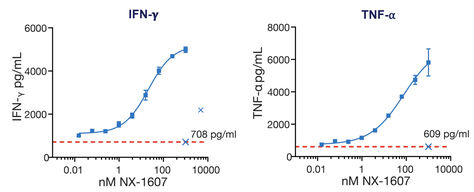


Figure 3. NX-1607 Increases Secretion of Pro-Inflammatory Cytokines in Human NK Cells

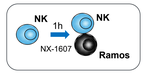
NK-K562 Assay:



NK-K562 Assay
- K562 (NucRed) target cells
- NK cells isolated and pre-conditioned with 60ng/mL IL-2 O.N.
- 1-hour compound pre-treatment prior to addition of K562s
- 6-hour NK/K562 coculture



NK-Ramos Assay:



NK-Ramos Assay
- Ramos (CT Violet) target cells
- 1-hour pre-treatment prior to addition of Ramos
- 4-hour NK/Ramos coculture

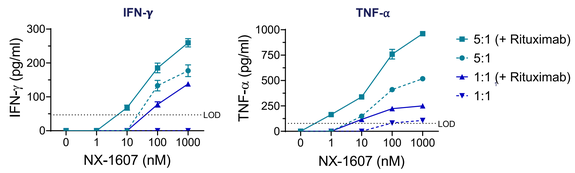
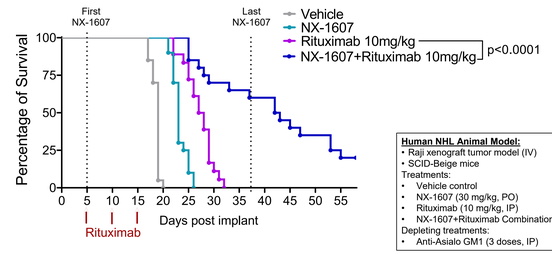


Figure 4. NX-1607 Strongly Potentiates Rituximab-Directed NK Cell ADCC Against Tumor Cells in a Human NHL Animal Model

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

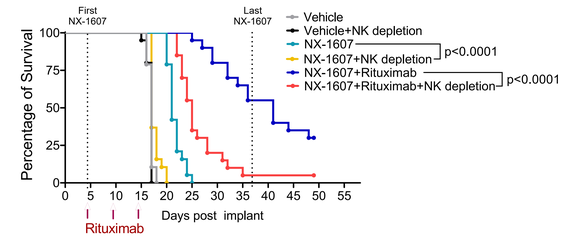


Human NHL Animal Model:
- Raji xenograft tumor model (IV)
- SCID-Beige mice

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.
- NX-1607 limits TGF-β and Treg mediated T-cell suppression.
- NX-1607 increases secretion of pro-inflammatory cytokines in human NK cells.
- 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).

Other NX-1607 posters presented at SITC 2022

#777 (Nov. 10th) Whelan S, et al. *Initial clinical characterization of novel proximal biomarkers for NX-1607, a first-in-class oral CBL-B inhibitor, in patients with advanced malignancies.*

#331 (Nov. 11th) Gallotta M, et al. *A novel small molecule inhibitor of CBL-B shows potent antitumor activity in combination with Pmel-1 adoptive cell transfer in an aggressive mouse melanoma model.*

Presented at the 37th SITC Annual Meeting, Nov. 8–12, 2022