Small molecule inhibition of the ubiquitin ligase CBL-B with NX-1607 results in potent T and NK cell mediated anti-tumor response

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Introduction

- CBL-B is an E3 ligase that regulates the innate and adaptive immune system by ubiquitylating proteins involved in signaling.
- CBL-B attenuates T-cell activation initiated by TCR engagement in part by mediating the requirement for CD28 co-stimulation, thus setting the threshold for T cell activation.
- CD4+ and CD8+ T cells from mice deficient in *Cbl-b* have 5 to 10-fold enhanced secretion of IL-2 and IFN-γ when stimulated ex vivo with anti-CD3.
- *Cbl-b* deficient mice also demonstrate enhanced NK cell function.

Results

- NX-1607 inhibition of CBL-B enhanced IL-2 and IFN-γ secretion in primary human T cells stimulated with anti-CD3 antibodies, in both the presence and absence of CD28 co-stimulation, although to a lesser degree in the absence of co-stimulation.
- In vivo, once daily oral administration of NX-1607 in mice demonstrated significant anti-tumor activity in two colon carcinoma tumor models, CT26 and MC38, as well as a metastatic triple-negative breast tumor model, 4T1.
- Anti-tumor activity of NX-1607 is associated with infiltration of T cells and innate cells such as DC, macrophage, and NK cells.
- Depletion of CD8+ T cells or NK cells abrogated NX-1607 antitumor activity.
- The combination of NX-1607 and anti-PD-1 substantially increased the median overall survival and the frequency of long-term tumor rejections in all three tumor models.

Conclusions

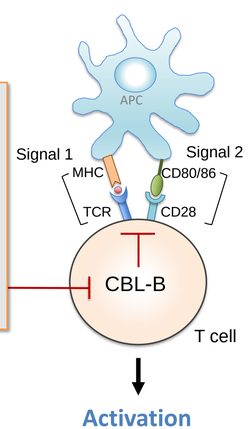
- NX-1607 mediates anti-tumor activity through both T cells and NK cells
- Preclinical tumor models support clinical development of NX-1607 as monotherapy or in combination with PD-1 blockade

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

NX-1607

Optimized CBL-B inhibitor for oral delivery.

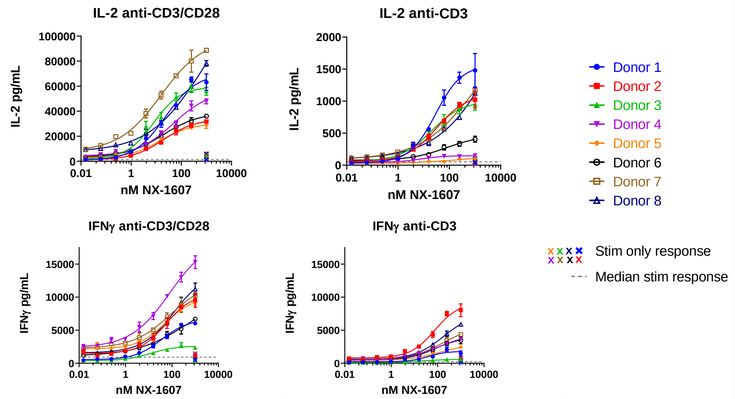
Developing as an oral intracellular checkpoint inhibitor for treating solid tumors.



CBL-B inhibition

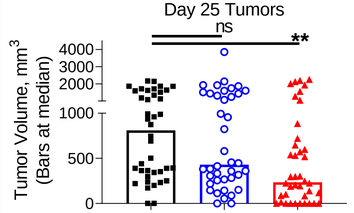
- ↑ IL-2 production
- ↑ IFN-γ production
- ↑ Proliferation
- ↑ Anti-tumor activity
- ↓ Threshold of activation

CBL-B Inhibitor NX-1607 Elevates IL-2 and IFN-γ in TCR Stimulated Primary Human T Cells



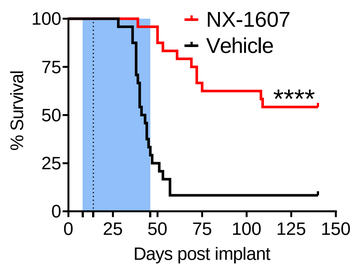
Effects of NX-1607 on total primary human T-cells was assessed by stimulating cells with plate bound αCD3 (right) or αCD3/αCD28 (left) in presence or absence of the indicated concentration of NX-1607. Release of IL-2 or IFN-γ were assessed by ELISA.

Effect of NX-1607 on CT26 Tumor Volume



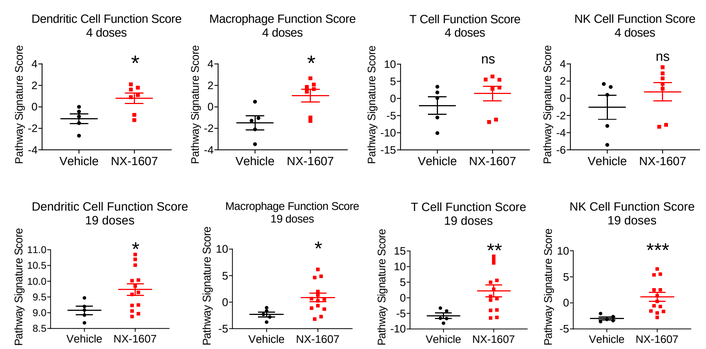
Mice bearing tumors on their left and right flanks were treated from Day 7 to Day 32 with daily oral NX-1607 at 10 mg/kg (blue circles) or 30 mg/kg (red circles) or Vehicle (black squares). Volumes at Day 25 are indicated. Stats were calculated with one-way ANOVA and Dunn's multiple comparisons test ** P ≤ 0.01.

Effect of NX-1607 on Survival of 4T1 Tumor Bearing Mice



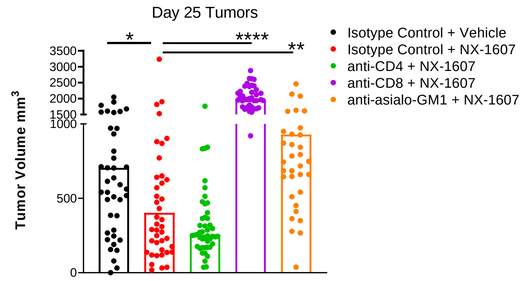
Mice were treated with NX-1607 at 30 mg/kg, PO QD, from Day 7 to Day 46 (shaded area). 4T1 primary tumors were surgically removed on Day 15 (dotted line). Survival of mice bearing 4T1 metastases. Log-rank (Mantel-Cox) test; **** P ≤ 0.0001

NX-1607 Promotes Infiltration of CT26 Tumors with Activated T and NK Cells



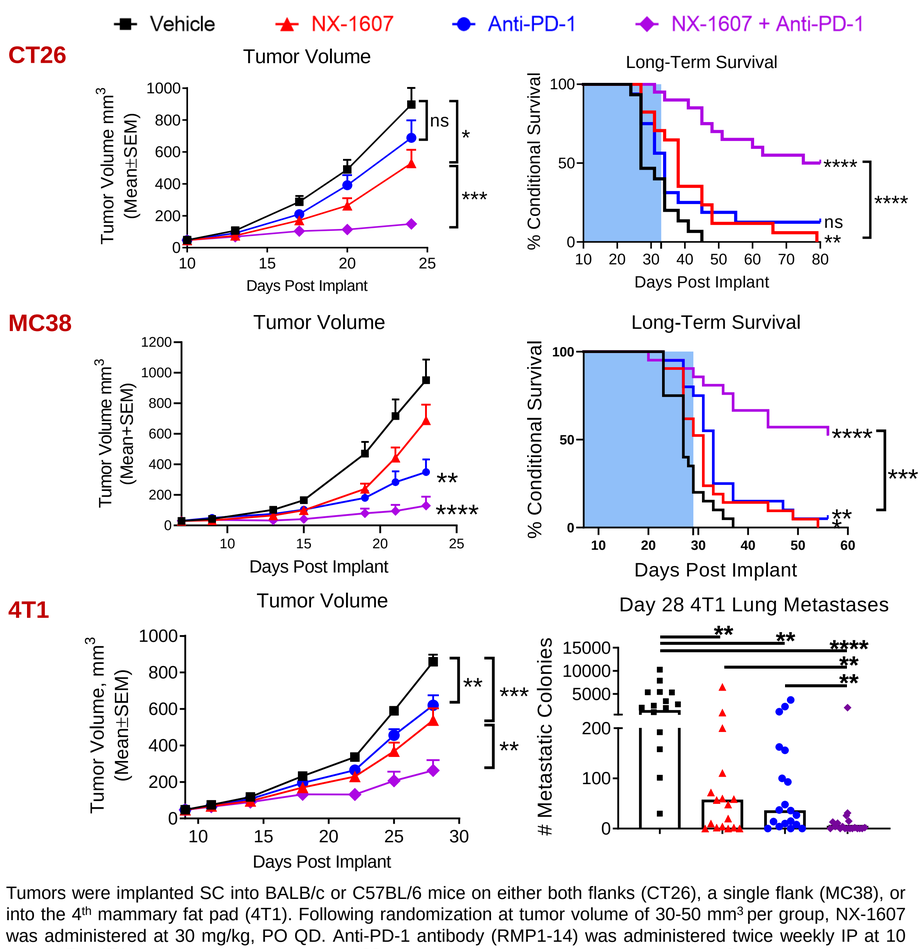
Effects of orally administered NX-1607 on TIL was assessed in CT26 tumors after 4 or 19 daily doses of NX-1607 at 30 mg/kg, PO QD, by analyzing RNA extracted from tumors with the NanoString nCounter PanCancer Mouse Immune Profile panel. Statistics used Mann-Whitney test (* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001).

NX-1607 Antitumor Efficacy is Abrogated By CD8+ T or NK Cell Depletion



Mice bearing CT26 tumors on their left and right flanks were treated from Day 9 to Day 25 with oral NX-1607 at 30 mg/kg, PO QD, in the presence of depleting antibodies for CD4+ cells, CD8+ cells, or NK cells (anti-asialo-GM1). Tumor volume at Day 25 is indicated. CD8+ T cell or NK cell depletion abrogates NX-1607 activity. Stats calculated with Mann-Whitney (Vehicle vs. NX-1607) or one-way ANOVA with Dunn's multiple comparisons test (NX-1607 vs. Depletion groups); * P ≤ 0.05, ** P ≤ 0.01, **** P ≤ 0.0001.

Combination of NX-1607 and Anti-PD-1 Synergize to Enhance Anti-Tumor Effects and Survival of Mice with CT26, MC38, or 4T1 Tumors



Tumors were implanted SC into BALB/c or C57BL/6 mice on either both flanks (CT26), a single flank (MC38), or into the 4th mammary fat pad (4T1). Following randomization at tumor volume of 30-50 mm³ per group, NX-1607 was administered at 30 mg/kg, PO QD. Anti-PD-1 antibody (RMP1-14) was administered twice weekly IP at 10 mg/kg. For 4T1, lung tissue was dissociated 28 days after tumor implant and cultured with 6-thioguanine to select and enumerate 4T1 metastatic colonies. Statistical analysis used one- or two-way ANOVA with corrections for multiple groups or Log-rank tests for survival curves. Shaded area: NX-1607 (30 mg/kg, PO QD) dosing period.