Discovery of NTX-801, a Potent Cbl-b Inhibitor with Anti-tumor Activity in Syngeneic Models

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BACKGROUND

Casitas B-lineage lymphoma b (Cbl-b), a RING finger E3 ligase and a member of a highly conserved family of Cbl proteins, catalyzes the ubiquitination of substrate proteins to regulate multiple signaling events in a variety of cell types, including immune cells. In T cells, Cbl-b negatively regulates adaptive immune system signaling by establishing the threshold for the activation of antigen receptors. Additionally, Cbl-b regulates the function of other immune cell types, including NK cells, dendritic cells and monocytes. Cbl-b deficient T cells no longer require a costimulatory signal to be fully activated. Cbl-b KO mice spontaneously reject tumors via an enhanced immune response. Taken together, these findings point to Cbl-b inhibitors as having the potential to be highly efficacious immuno-oncology agents.

METHODS & RESULTS

A structure-based drug design approach was used to identify potent inhibitors of Cbl-b. The Cbl-b inhibitor NTX-801 potently binds to Cbl-b, preventing Cbl-b phosphorylation and activation with biochemical and cellular IC_{50} < 5nM. NTX-801 can enhance both T and NK cell activation, reinvigorate exhausted T cells, and inhibit the suppressive capacity of T regulatory cells. In vivo, Cbl-b inhibition enhanced cytokine production and increased T cell activation markers after a single dose of NTX-801. Furthermore, NTX-801 is shown to induce robust and statistically significant tumor growth inhibition in the CT-26 syngeneic model with increased T cell and NK cell signatures observed within the tumor. NTX-801 in combination with anti-PD1 resulted in robust anti-tumor activity, increased survival, and several complete responses, as defined by no measurable tumor.

FIGURE 1. NTX-801 Enhances Cytokine Expression from Purified Human T Cells and Human Whole Blood Stimulated with anti-CD3/28

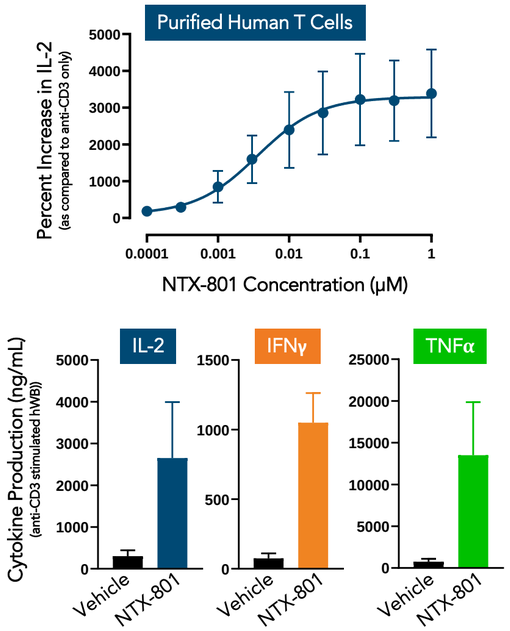


FIGURE 2. Cbl-b Inhibition Reinvigorates In Vitro Exhausted Human CD8⁺ T cells

Exhausted human CD8⁺ T cells show increased expression of immune checkpoint receptors and no longer respond to anti-CD3 stimulation

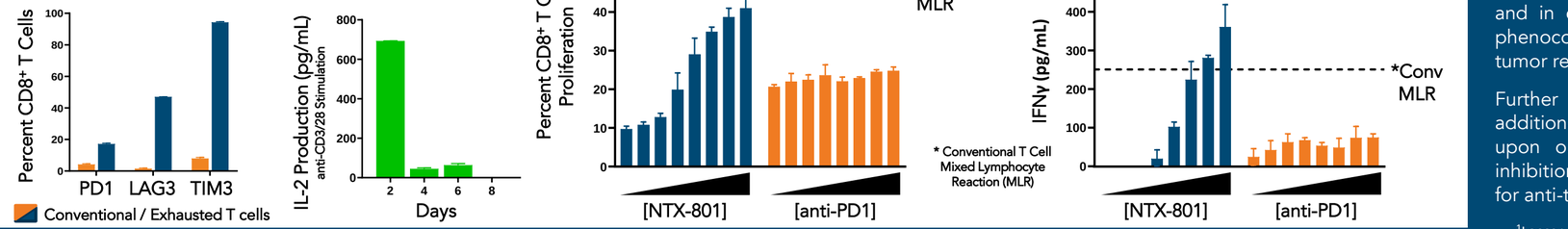


FIGURE 3. NTX-801 Inhibits the Suppressive Capacity of Human T Regulatory Cells

T regulatory cells were purified from multiple human donors and pretreated with NTX-801 or vehicle for 3hrs. Pretreated T regulatory cells were then co-cultured with T effector cells in a 1:1 ratio for 5 days. T regulatory cells were the only cell exposed to NTX-801 in these experiments.

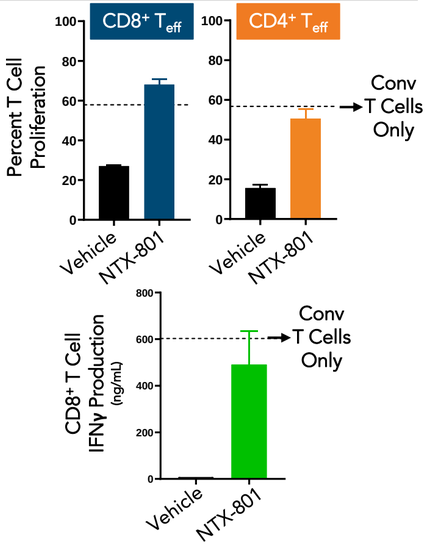


FIGURE 4. Cbl-b Inhibition Enhances Serum Cytokine Production in an In Vivo anti-CD3 PD Model

NTX-801 was administered orally 30min prior to IV administration of 2μg anti-CD3. Serum cytokines were assessed 8hrs after compound treatment

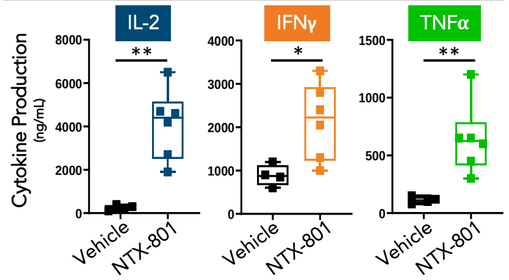


FIGURE 5. NTX-801 Increases Immune Cell Transcriptional Signatures in CT-26 Treated Tumors

Tumors from NTX-801 treated CT-26 syngeneic animals were processed for NanoString cell type profiling analyses

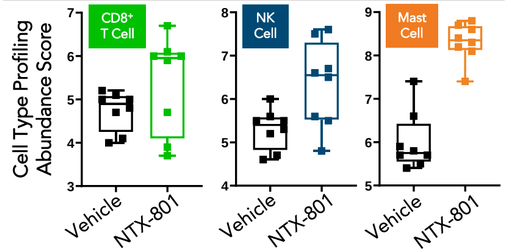
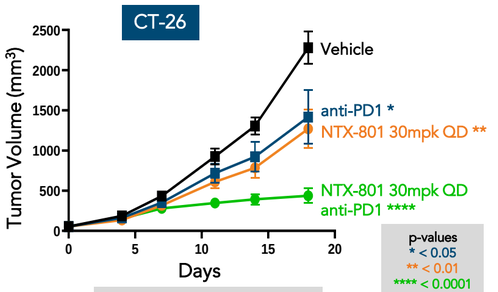
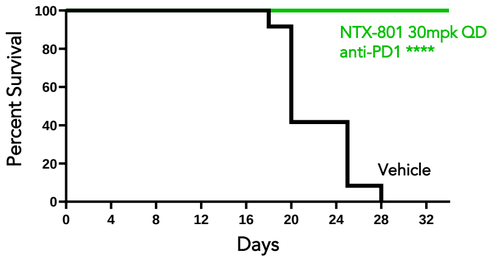


FIGURE 6. NTX-801 Induces Robust Tumor Growth Inhibition in Combination with anti-PD1 in the CT-26 Syngeneic Tumor Model



3 CRs (no measurable tumor) observed in combination group



CONCLUSIONS AND FUTURE DIRECTIONS

We show here that a potent small molecule Cbl-b inhibitor demonstrates strong immune cell activation and robust anti-tumor activity in a mouse syngeneic tumor model, as a single agent and in combination with anti-PD1. These data phenocopy the broad immune activation and tumor rejection data observed in Cbl-b KO mice.[1]

Further evaluation of these potent inhibitors in additional in vivo studies will continue to build upon our mechanistic understanding of Cbl-b inhibition as a novel immunomodulatory approach for anti-tumor immunity.

[1]Loeser, S. et al., JEM 2007 v204(4): 879-91