Background 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.

Methods 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).

Results 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.

Conclusions 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. We have initiated a clinical trial with NX-1607 in patients with advanced solid tumors (NCT05107674).