Optimizing Ex-vivo Expanded NK Cell-Mediated Antibody-Dependent Cellular Cytotoxicity (ADCC) Combined With NKTR-255 in Chronic Lymphocytic Leukemia (CLL), Follicular Lymphoma (FL), and Burkitt Lymphoma (BL)

Yaya Chu, PhD[1*], Susiyan Jiang, MS[1*], Jian Jiang, MD[1*], Meijuan Tian, PhD[1], Dean A. Lee, MD/PhD[2], Loui Madakamutil, PhD[3], A. Mario Marcondes, MD/PhD[3], Christian Klein, PhD[4], Mitchell Cairo, MD [1,5,6,7,8,9,10]
New York Medical College, Department of [1]Pediatrics, [5]Pathology, [6]Epidemiology and Community Health, [7]Medicine, [8]Microbiology and Immunology, [9]Cell Biology and [10]Anatomy, New York Medical College, Valhalla, New York; [2]Hem/Onc/BMT, Nationwide Children's Hospital, Columbus OH, [3]Nektar Therapeutics, San Francisco, CA, [4]Roche Pharmaceutical Research & Early Development, Roche Innovation Center Zurich, Switzerland.

Boston Children's Health Physicians
Until every child is well

PCRF
Pediatric Cancer Research Foundation

NEW YORK MEDICAL COLLEGE
CHARTERED 1860

Children and Adolescent Cancer & Blood Diseases Center
MARIA FARERI CHILDRENS HOSPITAL
WESTCHESTER MEDICAL CENTER
NEW YORK MEDICAL COLLEGE
Breakthrough personalized therapy through poineering research

I have nothing to disclose.

Background

- CD20 is a glycosylated phosphoprotein expressed on the surface of B cells on all developmental stages except for pro-B cells or plasma cells. It is also expressed in >98% of childhood, adolescent and adult mature B-cell NHLs and therefore is an attractive cancer therapeutic target.

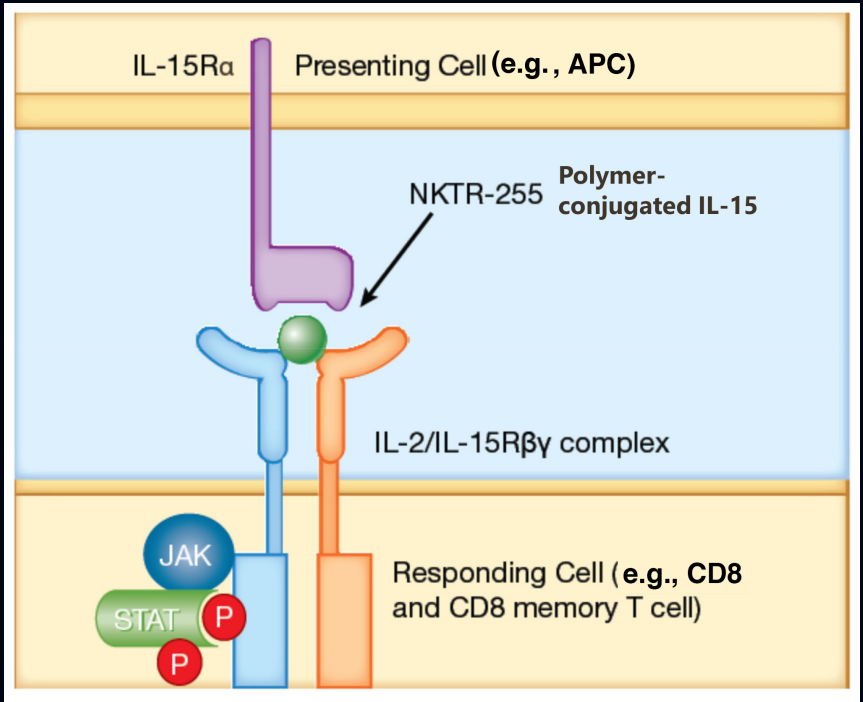
- Rituximab, a monoclonal chimeric anti-CD20 antibody, has been widely used as a chemoimmunotherapeutic regimen in the frontline therapy for patients with $CD20^{+}$ BL and diffuse large B-cell lymphoma. The addition of rituximab to the CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) backbone or to standard FAB/LMB therapy has greatly improved outcomes without significantly increasing toxicity in patients with B-NHL. However, patients who relapse have a poor clinical response to rituximab retreatment.

- Obinutuzumab is a humanized, type II anti-CD20 monoclonal antibody glycoengineered to enhance Fc receptor affinity. It has lower complement-dependent cytotoxicity than rituximab but greater ADCC, phagocytosis and direct B-cell killing effects.

- Our group has successfully expanded functional and active peripheral blood (PB) NK cells with irradiated feeder cells to target B-NHL. We previously demonstrated that obinutuzumab has significantly enhanced expanded PBNK mediated cytotoxicity against BL and pre-B-ALL cell lines compared to rituximab.

Chu/Cairo, BJH, 2016
Goldman/Cairo, *Leukemia*, 2013,
Coiffier et al, *NEJM*, 2002
Chu/Cairo, BJH, 2018
Chu/Cairo, et al, *Can Imm Res* 2015
Tiwari/Cairo et al, BJH, 2015

NKTR-255



- IL-15 is a pleiotropic cytokine with roles in innate and adaptive immunity.
- Identified by NCI as one of the most promising immuno-oncology agents.
- Key role in formation and maintenance of immunological memory.
- Essential factor for NK (Natural Killer) cells development and homeostasis.
- In vitro, IL-15 can reverse tumor-induced NK cell dysfunction.
- NKTR-255 is an IL-15 agonist designed to activate the IL-15 pathway and expand NK cells and promote the survival and expansion of memory CD8+ T cells without inducing suppressive regulatory T cells (Kuo/Zalevsky, Cancer Res. 2017).
- NKTR-255 stimulates proliferation and survival of NK, CD8+ T cells, and enhances long-term immunological memory which may lead to sustained anti-tumor immune response.

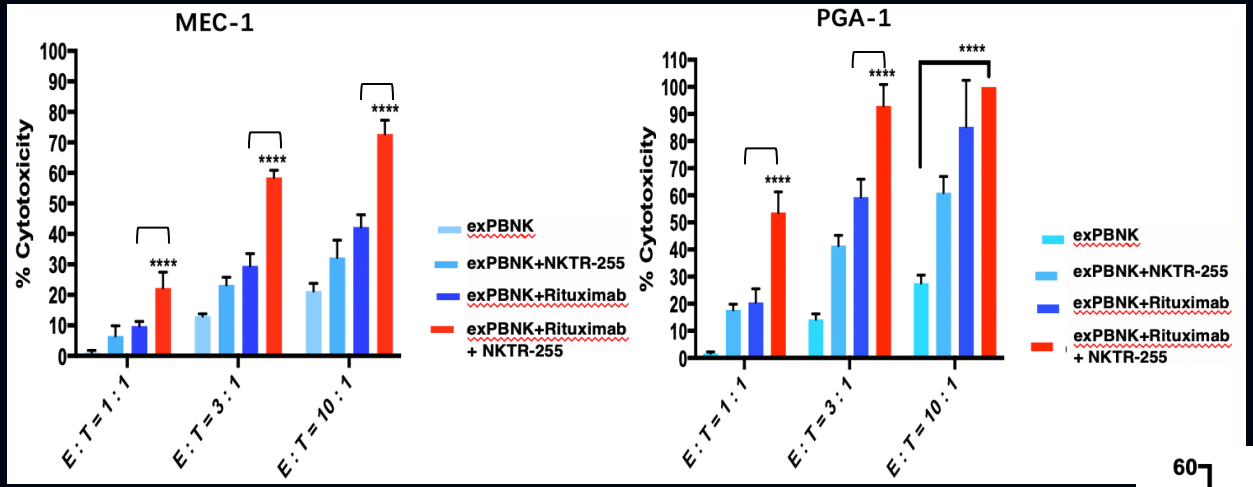
Objective

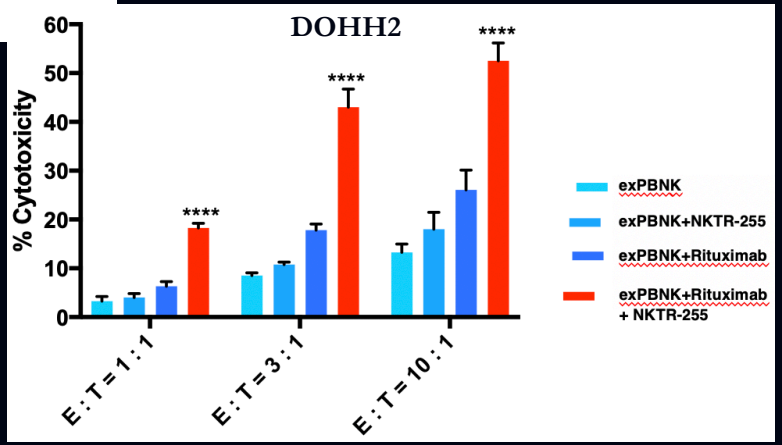
To investigate the effects of NKTR-255 on the ADCC of expanded NK cells with anti-CD20 type I and type II antibodies against CLL, FL and rituximab-resistant BL.

Methods

- NK cells were expanded with lethally irradiated K562-mbIL21-41BBL cells as previously described (Denman/Dean Lee, *PLoS One*, 2012).
- Expanded PBNK (peripheral blood NK) cells were isolated using Miltenyi NK cell isolation kit. NKTR-255 was generously provided by Nektar Therapeutics.
- In vitro cytotoxicity was examined using luminescence reporter-based assays. IFNγ, granzyme B and perforin levels were examined by standard enzyme-linked immunosorbent assays as previously described (Chu/Cairo, ASH, 2018).
- MEC-1 (CLL), PGA-1 (CLL), DOHH2 (FL) and rituximab-resistant BL cells Raji-2R and Raji-4RH were used as target cells.

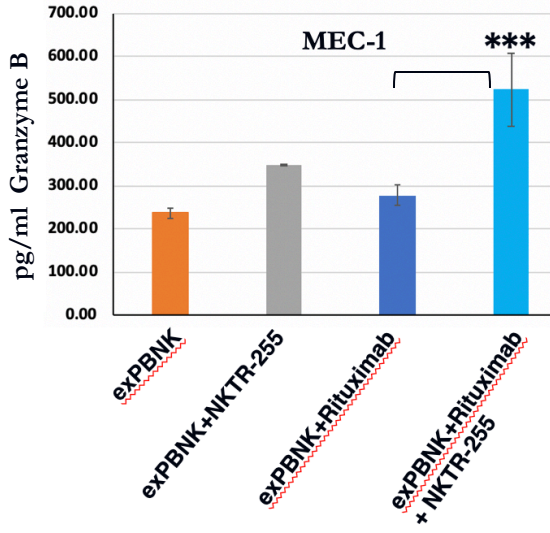
NKTR-255, when combined with rituximab, significantly enhanced the in vitro cytotoxicity of expanded NK cells against against CLL: MEC-1, PGA-1, and FL: DOHH2

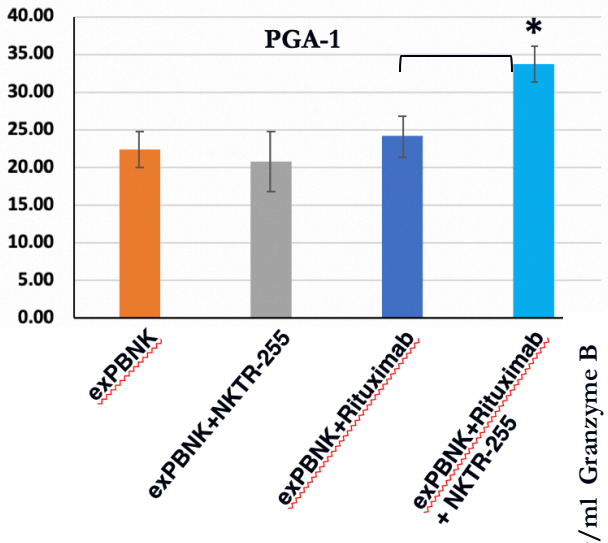


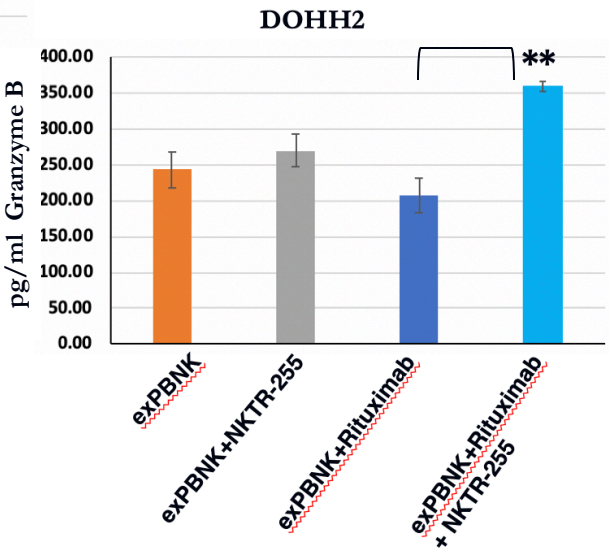


***, p<0.001; **, p<0.01; *, p<0.05

NKTR-255, when combined with rituximab, significantly enhanced the granzyme B release from expanded NK cells against CLL: MEC-1, PGA-1, and FL: DOHH2

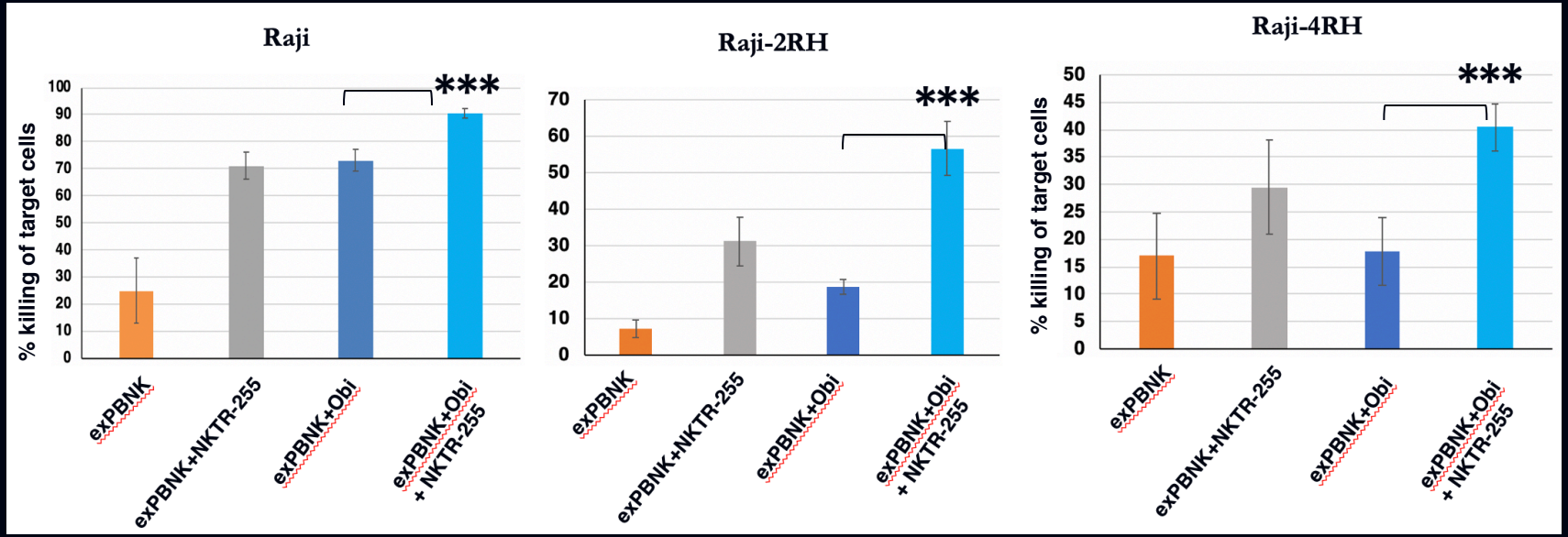






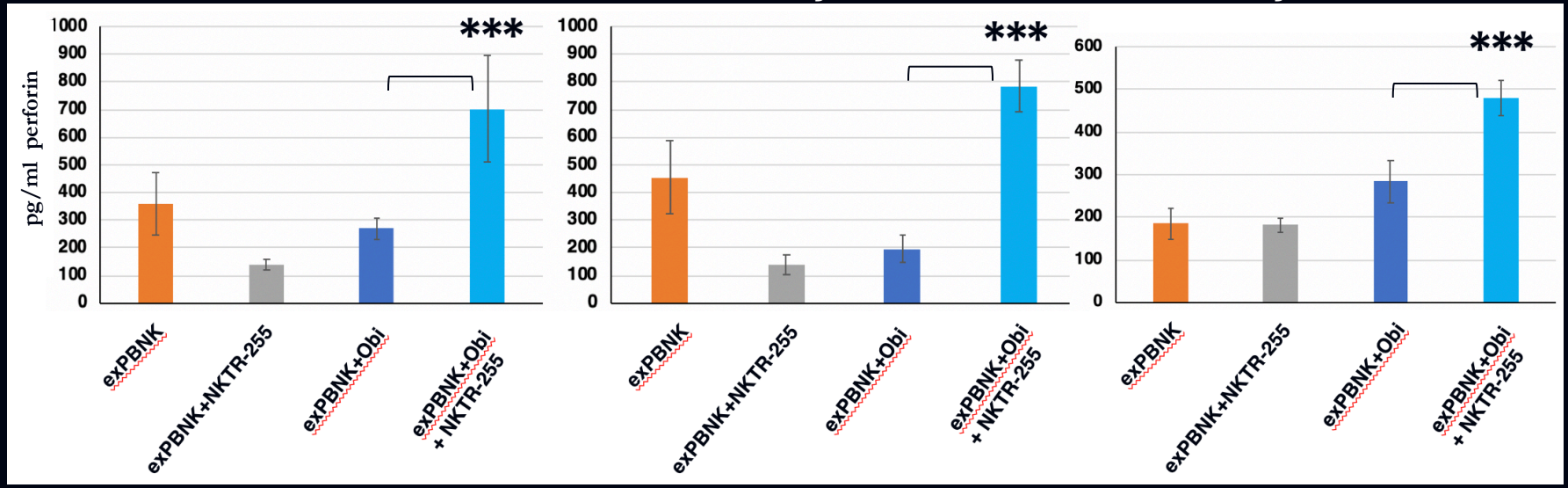
***, p<0.001; **, p<0.01; *, p<0.05

NKTR-255, when combined with obinutuzumab, significantly enhanced the in vitro cytotoxicity of expanded NK cells against rituximab-sensitive Burkitt lymphoma Raji and -resistant Raji-2R and Raji-4RH



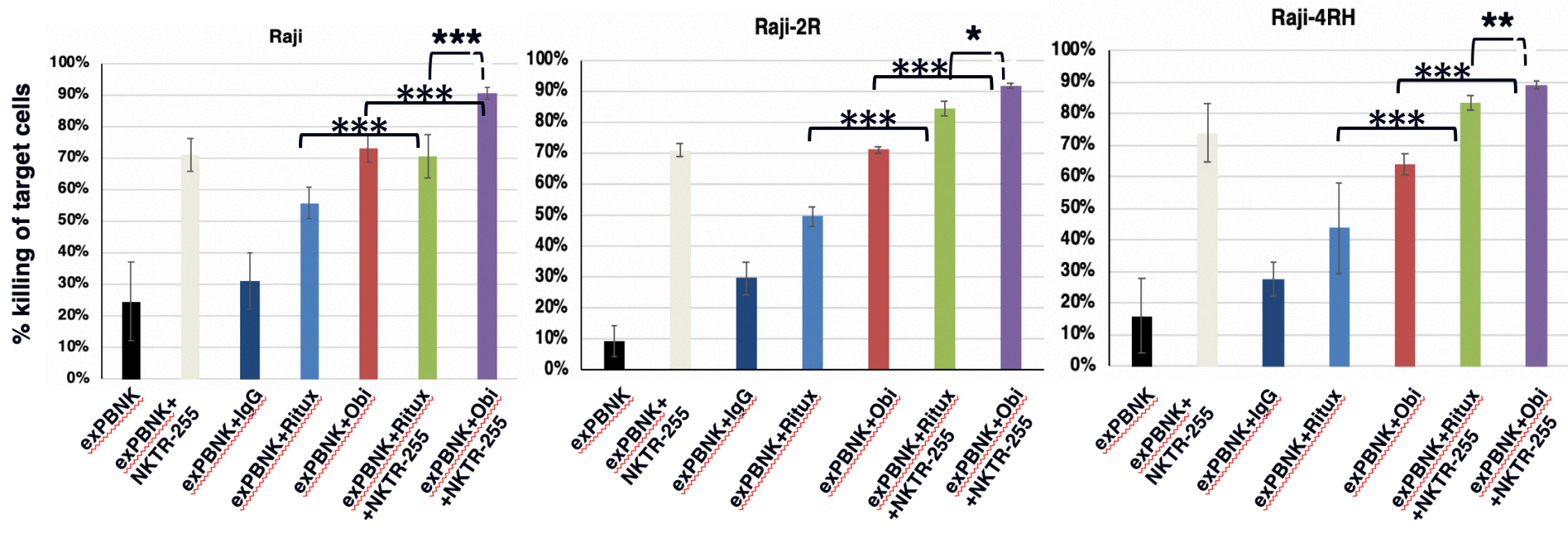
***, p<0.001

NKTR-255, when combined with obinutuzumab, significantly enhanced the perforin release from expanded NK cells against rituximab-sensitive Raji and -resistant Raji-2R and Raji-4RH cells



***, p<0.001

NKTR-255 + obinutuzumab enhanced the in vitro cytotoxicity of expanded NK cells against Burkitt lymphoma to a greater extent than the combination of NKTR-255 + rituximab



***, p<0.001; **, p<0.01; *, p<0.05

Conclusions

- NKTR-255 significantly enhanced the ADCC of expanded NK cells with the anti-CD20 type I antibody rituximab against CLL, FL *in vitro*.
- NKTR-255 significantly enhanced the ADCC of expanded NK cells with the type II antibody obinutuzumab against rituximab-sensitive and resistant BL cells *in vitro*.
- NKTR-255 + obinutuzumab enhanced the in vitro cycotoxicity of expanded NK cells against BL to a greater extent than NKTR-255 + rituximab.
- The *in vivo* effects of NKTR-255 with expanded NK cells and anti-CD20 type I and type II antibodies against CLL, FL and rituximab-resistant BL cells using humanized NSG models are under investigation.
- The presented data supports the further exploration of the proposed combination in the clinical setting.