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)

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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. IFN$\gamma$, 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 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.
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 \textit{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 \textit{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 \textit{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.