Optimizing Ex-vivo Expanded NK Cell- Mediated Cellular Cytotoxicity by Obinutuzumab Combined With NKTR-255 in Burkitt Lymphoma (BL)

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INTRODUCTION

We have previously reported the success of treating children with Burkitt lymphoma (BL) resulting in a ≥90% long-term EFS utilizing short intensive chemotherapy and rituximab containing chemoimmunotherapy (Cairo et al, Leukemia, 2002; Cairo et al, Blood, 2007; Goldman /Cairo et al, Leukemia, 2021). However, the prognosis is dismal in children who are refractory or progress after chemoimmunotherapy (Cairo et al *Br J Haematol*. 2018). The mechanisms associated with this poor prognosis are mainly due to chemoradioimmunotherapy resistance and an immunosuppressive tumor microenvironment (TME) (Cairo et al *Br J Haematol*. 2016; Cairo et al *Br J Haematol*. 2019) and represent an urgent unmet need. Combinatorial immunological therapeutic approaches including both targeted humoral and cellular therapy are urgently needed to increase the survival of poor-risk pediatric patients with BL. The CD20 molecule is universally expressed by normal B cells in all stages of development, from the pre-B cell up to the mature plasma cell as well as by most B cell malignancies including CLL, FL and BL (Chu/Cairo, BJH, 2016). 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 (Chu/Cairo, BJH, 2018). Obinutuzumab has been successfully utilized in front-line therapy in FL (Marcus, et al, NEJM, 2017) and CLL (Goede, et al, NEJM, 2014; Moreno, et al, Lancet, 2019). Our group has successfully expanded functional and active peripheral blood NK cells PBNKwith irradiated feeder cells to target B-NHL (Chu/Cairo, et al, *Can Imm Res* 2015). We previously demonstrated that Obinutuzumab (OB) had significantly enhanced expanded PBNK mediated cytotoxicity against BL and pre-B-ALL cell lines compared to rituximab (Tiwari/Cairo et al, BJH, 2015). NKTR-255 is an IL-15 receptor 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.

AIM

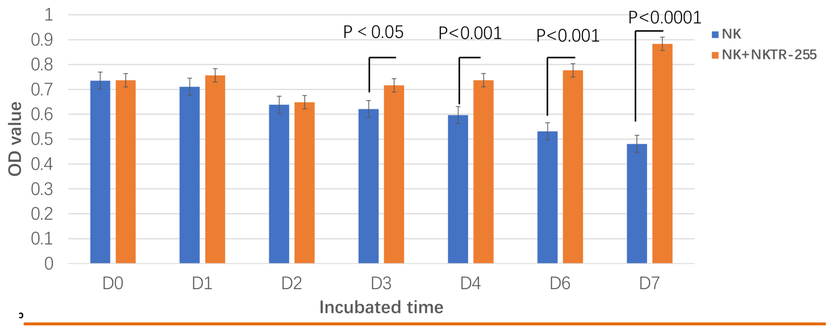
To investigate the effects of NKTR-255 on the ADCC of expanded NK cells with Obinutuzumab against rituximab-resistant BL.

METHOD

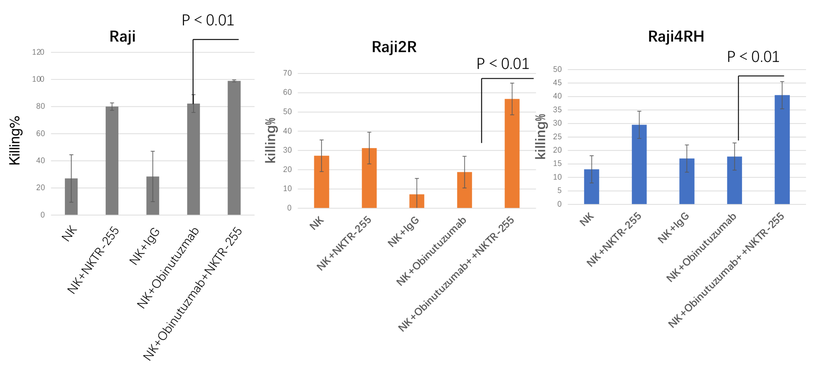
- NK cells were expanded with lethally irradiated K562-mbIL21-41BBL cells as previously described (Denman/Dean Lee, *PLoS One*, 2012).
- Expanded PBNK cells were isolated using Miltenyi NK cell isolation kit.
- NKTR-255 was generously provided by Nektar Therapeutics.
- In vitro cytotoxicity was examined using luminescence reporter-based assays.
- IFN-γ and granzyme B levels were examined by standard enzyme-linked immunosorbent assays as we previously described (Chu/Cairo, ASH, 2018). Rituximab-sensitive Raji, and -resistant Raji-2R and Raji-4RH were used as target cells.
- Luciferase expression Raji-4RH cells were xenografted to NSG mice.

RESULTS

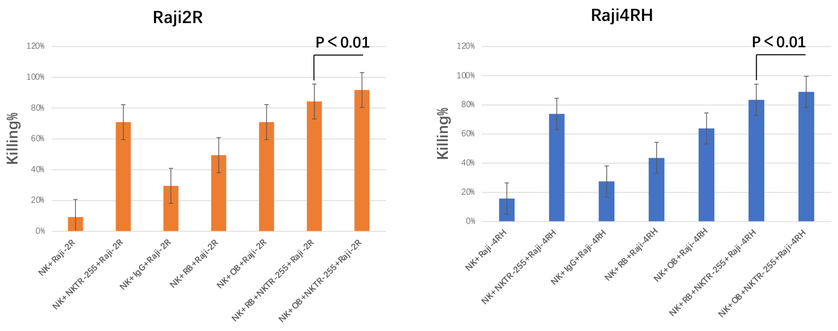
NKTR-255 significantly promoted expanded NK proliferation



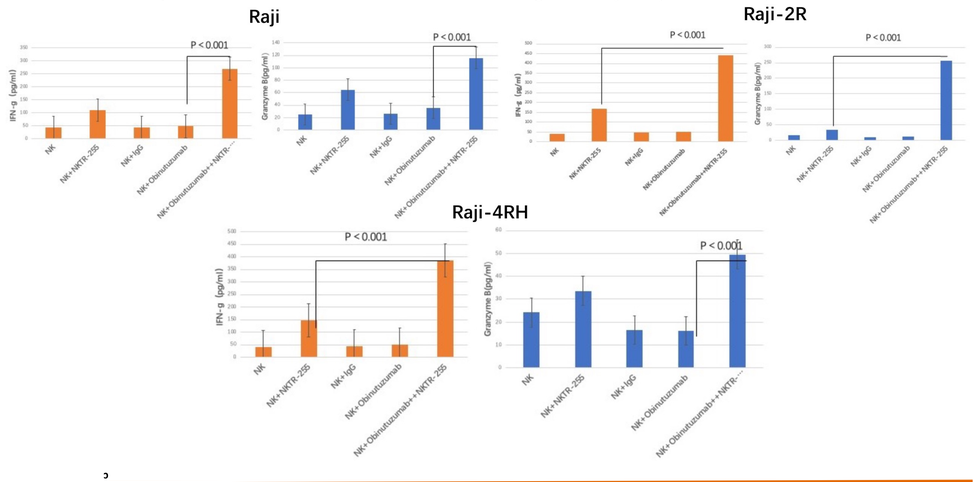
NKTR-255 significantly enhanced the *in-vitro* cytotoxicity of expanded NK cells when combined with Obinutuzumab against Raji, Raji-2R, and Raji-4RH



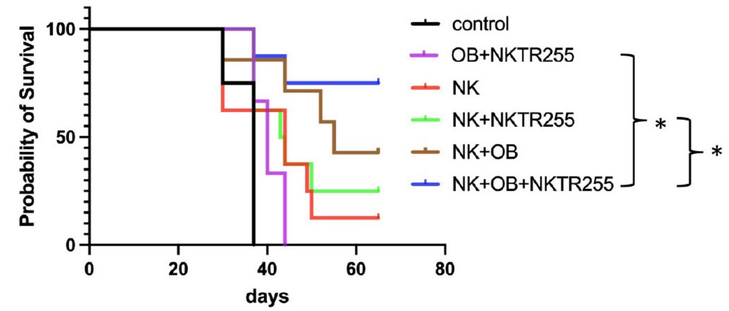
NKTR-255 significantly enhanced the *in-vitro* cytotoxicity of expanded NK cells when combined with Obinutuzumab (OB) vs rituximab (RB) against Raji-2R, and Raji-4RH



NKTR-255 (NKTR) also significantly enhanced IFN-γ and Granzyme B release from expanded NK cells when combined with OB against Raji, Raji-2R and Raji-4RH



The combination of NKTR-255, expanded NK cells and OB significantly extended Raji-4RH xenografted mice survival compared to NK alone



CONCLUSIONS

NKTR-255 significantly enhanced the ADCC of expanded NK cells with Obinutuzumab against rituximab-resistant BL cells *in-vitro* with enhanced IFN-γ and granzyme B release. The *in-vivo* effects of NKTR-255 with expanded NK cells and Obinutuzumab against rituximab-resistant BL cells using humanized NSG models are very promising. Mechanisms studies of BL relapsed from the combination therapy are under investigation. (The study is supported by HHOW and St Baldrick grants).

CONTACT INFORMATION

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