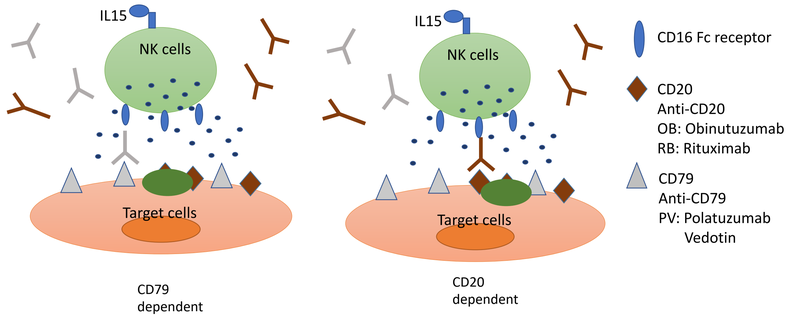
Optimizing Chimeric Antigen Receptor (CAR) Engineered NK Cell- Mediated Cytotoxicity Combined With anti-CD20 or anti-CD79 Therapeutic Antibodies and NKTR-255 in Burkitt Lymphoma (BL)

Yaya Chu, PhD[1*], Meijuan Tian, PhD[1*], Mario Marcondes, MD/PhD[2], Willem W. Overwijk, PhD[2], Dean A. Lee, MD/PhD[3], Christian Klein, PhD[4], Mitchell Cairo, MD [1,5,6,7]

[1]Department of Pediatrics, New York Medical College, Valhalla, NY 10595, USA. [2]Nektar Therapeutics, San Francisco, CA 94158, USA [3]Center for Childhood Cancer and Blood Diseases, Nationwide Children's Hospital, The Ohio State University, Columbus, OH 43205, USA. [4]Roche Pharmaceutical Research & Early Development, Roche Innovation Center Zurich, Switzerland. [5]Department of Cell Biology & Anatomy, New York Medical College, Valhalla, NY 10595, USA. [6]Department of Pathology, Microbiology & Immunology, New York Medical College, Valhalla, NY 10595, USA [7]Department of Medicine, New York Medical College, Valhalla, NY 10595, USA *Considered as Co-Primary First Authors

Background

Rituximab (Rb), a monoclonal chimeric anti-CD20 antibody (mAb), has been widely used with chemoimmunotherapeutic regimens in the frontline therapy for patients with CD20+ BL (Goldman/Cairo, *Leukemia*, 2013 and Leukemia, 2021). Obinutuzumab (Ob) is a humanized, type II anti-CD20 mAb glycoengineered to enhance Fc receptor affinity (Tiwari/Cairo et al, BJH, 2015). Polatuzumab Vedotin (PV) is an anti-CD79 mAb drug conjugate and has demonstrated significant preclinical activity against indolent CD79b+ NHL (Polson, et al, Cancer Res, 2009). Our group has successfully expanded and engineered peripheral blood NK cells with CAR to target B-NHL (Chu/Cairo, et al, *Can Imm Res* 2015). NKTR-255 is an investigational IL-15Rα-dependent, polymer-conjugated, recombinant human IL-15 agonist that retains the full spectrum of IL-15 biology, including expansion of NK cells (Miyazaki, JITC, 2021; Robinson, J Clin Invest, 2021).



Objective

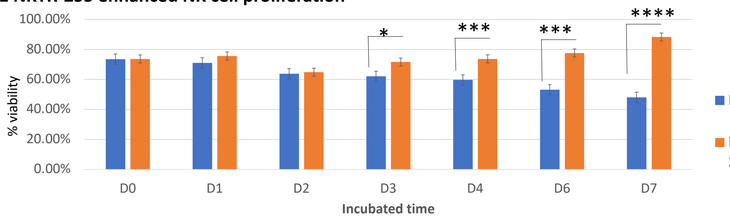
To investigate the anti-tumor effect of ex-vivo expanded anti-CD19 CAR NK cells combined with rituximab, Obinutuzumab, or PV with or without NKTR-255 against BL.

Method

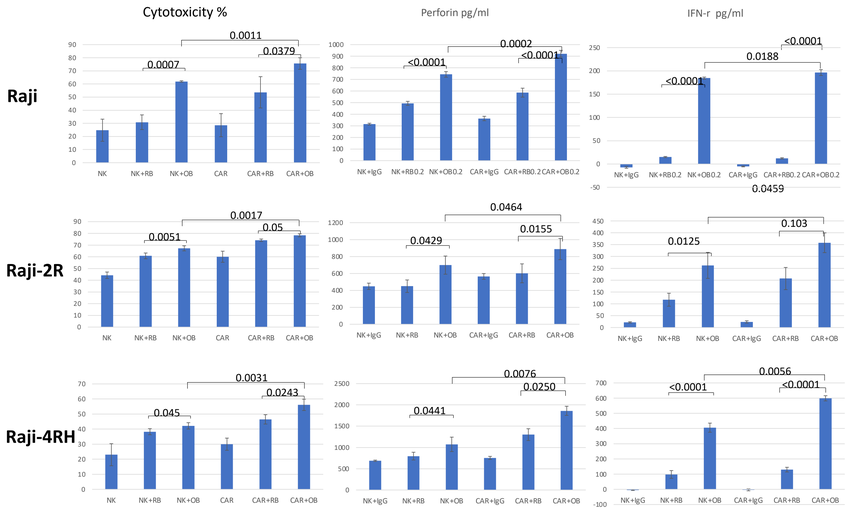
NK cells were expanded with lethally irradiated K562-mbIL21-41BBL cells and isolated as we previous described (Chu/Cairo, et al, *JITC* 2020). Anti-CD19 CAR mRNA was electroporated to expanded NK cells. 40ng/ml NKTR-255 and 1ug/ml antibody were used for in vitro cytotoxicity assays. IFN-r and perforin levels were examined by ELISA as we previous described (Chu/Cairo, et al, *JITC* 2021). Rituximab-sensitive Raji and -resistant Raji-2R and Raji-4RH were used as target cells.

Results

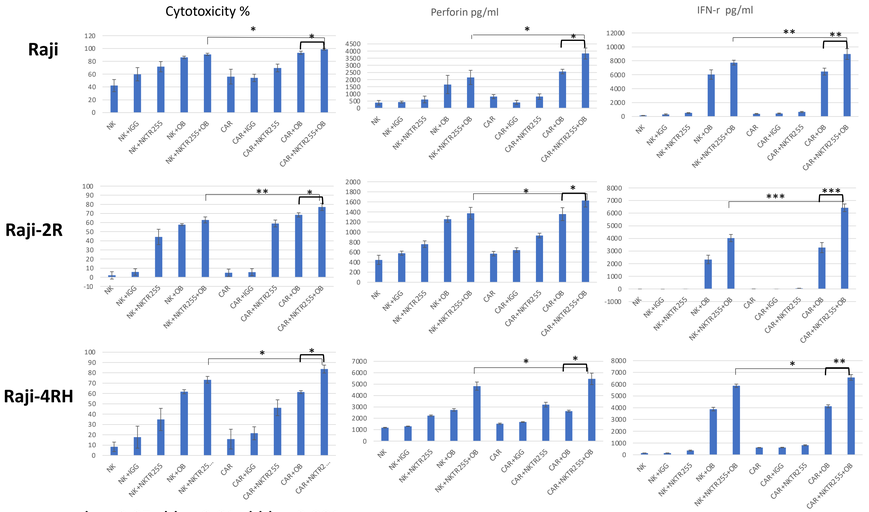
1 NKTR-255 enhanced NK cell proliferation



2 Obinutuzumab vs Rituximab mediated antibody dependent anti-tumor cytotoxicity of CAR/NK cells.

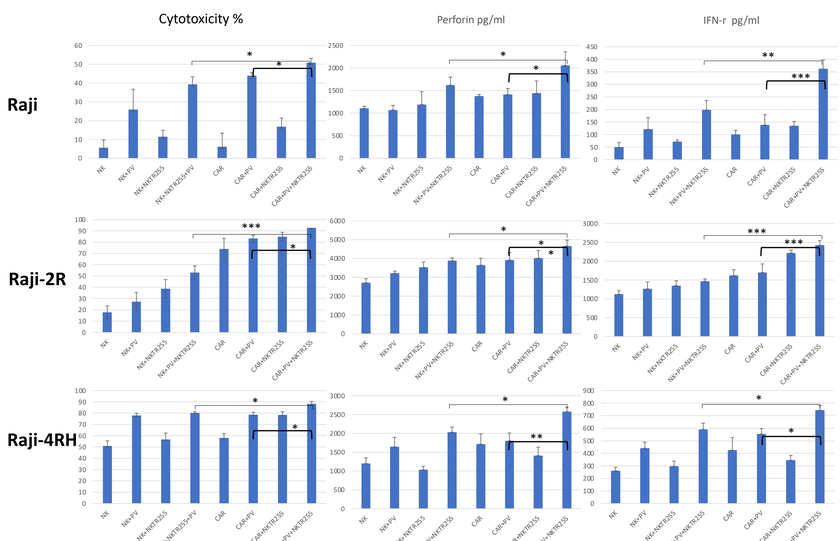


3 NKTR-255 enhanced OB(Obinutuzumab) dependent anti-tumor cytotoxicity of CAR/NK cells.



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

4 NKTR-255 enhanced OB(Obinutuzumab) dependent anti-tumor cytotoxicity of CAR/NK cells.



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

Results

Compared to controls, NKTR-255 + Ritux significantly enhanced both the in vitro cytotoxicity of anti-CD19 CAR NK against Raji (p=0.006) as well as the release of perforin (p=0.003) and IFN-g (p<0.01). We further confirmed the results utilizing Raji-2R and Raji-4RH cells. NKTR-255 + Ob significantly enhanced the in vitro cytotoxicity of anti-CD19 CAR NK compared to controls against Raji (p<0.0081) as well as release of perforin (p<0.05), IFN-g (p<0.001) and granzyme B (p<0.01). We further confirmed the results utilizing Raji-2R and Raji-4RH cells. NKTR-255 + PV significantly enhanced the in vitro cytotoxicity of anti-CD19 CAR NK cells compared to control groups such as expanded NK cells +NKTR-255+PV against Raji (p<0.0001), Raji-2R (p<0.0003), and Raji-4RH (p<0.0311) (Figure 1), as well as enhanced release of IFN-g and perforin.

Conclusion

We found that NKTR-255 + Ritux, Ob or PV significantly enhanced the in vitro cytotoxicity of anti-CD19 CAR NK cells against BL cells. The *in vivo* effects of NKTR-255 with anti-CD19 CAR NK cells and Ritux, Ob or PV against rituximab-sensitive and -resistant BL cells using humanized NSG models are under investigation.

Acknowledgements

This work is funded by Children's Cancer Fund Laboratory at New York Medical College and Rally foundation.