881 SIGNIFICANT THERAPEUTIC EFFECTS OF ANTI-ROR1 CAR NK AGAINST NEUROBLASTOMA BY ONCOLYTIC VIRUS ARMORED WITH IL-21 *IN-VITRO* AND *IN-VIVO*

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Background Metastatic and relapsed/refractory neuroblastoma (NB) has very poor diagnosis.[1] Novel therapies are desperately needed.[2] ROR1 is overexpressed in a variety of human cancers including NB.[3-5] Our group has successfully expanded peripheral blood NK cells (exPBNKs) and modified exPBNK cells to express chimeric antigen receptor (CAR).[6]. Oncolytic herpes simplex viruses (oHSVs) have been safely used in clinical trials for a wide range of cancers.[7]. IL-21 sustained the survival and increased the cytotoxicity of NK cells.[8] We sought to determine the anti-tumor effect of anti-ROR1 CAR engineered exPBNK cells (CAR-exPBNKs) against ROR1$^+$ NB and if the anti-tumor efficacy can be improved by oHSV engineered to express human IL21.

Methods NK cells were expanded and electroporated with anti-ROR1-CAR mRNA. oHSV C134 was modified to express hIL-21 gene (C021). In-vitro cytotoxicity and cytokines levels of CAR-exPBNKs against NB cell lines were examined as we previously described (2). In-vivo hIL21 secretion and anti-tumor effect of CAR-exPBNKs with or without the C021 was examined utilizing human NB xenografted NSG mice.

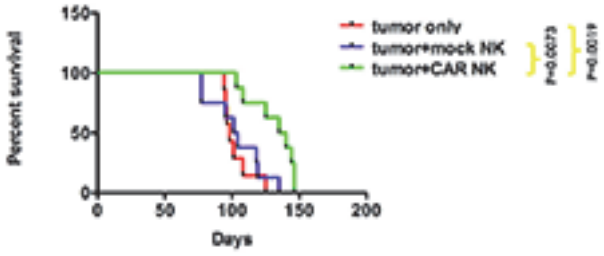
Results NK cells were significantly expanded by co-culture with irradiated K562-mbIL21 cells at 14 days (>2000 folds), and expanded NK cells were isolated with more than 95% purity. Anti-ROR1-CAR was expressed on >90% of exPBNK cells after CAR mRNA electroporation. CAR-exPBNK cells had significantly enhanced in-vitro cytotoxicity compared to Mock-exPBNK against ROR1+ SKNBE(2)N, CHLA-255, and SKNFI NB cells at different E:T ratios ($p<0.001$) regardless of MYCN amplification status. Expression of CD107a and IFN-g were significantly increased in CAR-exPBNK cells compared to Mock-exPBNK ($p<0.05$). CyTOF analysis showed that phosphorylation of STAT5 was enhanced in CAR-exPBNK when targeting NB as compared to exPBNK cells. In-vivo study showed that CAR-exPBNK significantly extended mice survival in human NB xenografted NSG mice ($p<0.01$) (figure 1). Furthermore, the combination of C021 and CAR-exPBNK significantly enhanced the killing of NB with significantly enhanced secretion of IFN-g, granzyme B and perforin and significantly enhanced expression of NK activating marker CD25 (all $p<0.05$) compared to controls. Our in-vivo animal study showed that NB infected with C021 secreted hIL21 and the combination of C021 and CAR-exPBNK cells reduced tumor burden in human NB xenografted NSG mice compared to the untreated group ($p<0.05$) and the CAR exPBNKs-treated group ($P=0.056$) (figure 2).

Conclusions Our data demonstrate the significant anti-tumor efficacy of combining C021 with anti-ROR1 CAR-exPBNKs to therapeutically target NB in-vitro and in-vivo. (Funded by U54 CA232561).

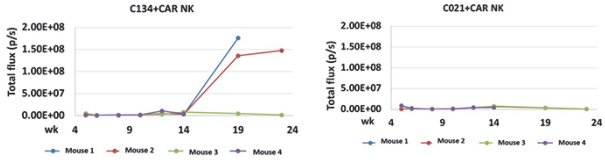
REFERENCES

1. Ambros PF, Ambros IM, Brodeur GM, Haber M, Khan J, Nakagawara A, *et al*. International consensus for neuroblastoma molecular diagnostics: report from the International Neuroblastoma Risk Group (INRG) biology committee. *Br J Cancer*. 2009;**100**(9):1471–82. Epub 2009/04/30. doi: 6605014 [pii]. 10.1038/sj.bjc.6605014. PubMed PMID: 19401703; PubMed Central PMCID: PMC2694415.
2. Chu Y, Nayyar G, Jiang S, Rosenblum JM, Soon-Shiong P, Safrit JT, *et al*. Combinatorial immunotherapy of N-803 (IL-15 superagonist) and dinutuximab with ex vivo expanded natural killer cells significantly enhances in vitro cytotoxicity against GD2(+) pediatric solid tumors and in vivo survival of xenografted immunodeficient NSG mice. *J Immunother Cancer*. 2021;**9**(7). Epub 2021/07/11. doi: 10.1136/jitc-2020-002267. PubMed PMID: 34244307; PubMed Central PMCID: PMCPMC8268924.
3. Matsuda T, Nomi M, Ikeya M, Kani S, Oishi I, Terashima T, *et al*. Expression of the receptor tyrosine kinase genes, Ror1 and Ror2, during mouse development. *Mech Dev*. 2001;**105**(1-2):153–6. Epub 2001/06/29. doi: S0925477301003835 [pii]. PubMed PMID: 11429290.
4. Zhang S, Chen L, Wang-Rodriguez J, Zhang L, Cui B, Frankel W, *et al*. The onco-embryonic antigen ROR1 is expressed by a variety of human cancers. *Am J Pathol*. 2012;**181**(6):1903-10. PubMed PMID: 23041612.
5. Hudecek M, Lupo-Stanghellini MT, Kosasih PL, Sommermeyer D, Jensen MC, Rader C, *et al*. Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. *Clin Cancer Res*. 2013;**19**(12):3153–64. Epub 2013/04/27. doi: 1078-0432.CCR-13-0330 [pii]. 10.1158/1078-0432.CCR-13-0330. PubMed PMID: 23620405; PubMed Central PMCID: PMC3804130.
6. Chu Y, Hochberg J, Yahr A, Ayello J, van de Ven C, Barth M, *et al*. Targeting CD20+ Aggressive B-cell Non-Hodgkin Lymphoma by Anti-CD20 CAR mRNA-Modified Expanded Natural Killer Cells In Vitro and in NSG Mice. *Cancer Immunol Res*. 2015;**3**(4):333–44. Epub 2014/12/11. doi: 10.1158/2326-6066.CIR-14-0114. PubMed PMID: 25492700.
7. Ghonime MG, Saini U, Kelly MC, Roth JC, Wang PY, Chen CY, *et al*. Eliciting an immune-mediated antitumor response through oncolytic herpes simplex virus-based shared antigen expression in tumors resistant to viroimmunotherapy. *J Immunother Cancer*. 2021;**9**(10). Epub 2021/10/03. doi: 10.1136/jitc-2021-002939. PubMed PMID: 34599026; PubMed Central PMCID: PMCPMC8488720.
8. Skak K, Frederiksen KS, Lundsgaard D. Interleukin-21 activates human natural killer cells and modulates their surface receptor expression. *Immunology*. 2008;**123**(4):575–83. Epub 2007/11/17. doi: 10.1111/j.1365-2567.2007.02730.x. PubMed PMID: 18005035; PubMed Central PMCID: PMCPMC2433320.

Ethics Approval NSG mice were bred, treated, and maintained in the animal facility of New York Medical College with NYMC International Animal Care and Use Committee approved protocols. The animal experiments were conducted in accordance with the recommendations of the Guide for Care and Use of Laboratory Animals.



Abstract 881 Figure 1 Anti-ROR-1 CAR NK significantly prolonged the survival of NSG mice xenografted with human NB cells



Abstract 881 Figure 2 C021+CAR-NK appeared to have a better anti-tumor effect than C134+CAR-NK in human NB xenografted NSG mice

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