Novel RNA Construct Increases Perforin and Granzyme B in NK cells and Cytotoxic T cells

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Abstract

Natural killer (NK) cells and cytotoxic T cells are currently tested in various clinical trials as a treatment against numerous cancer types. One of the critical factors for the success of adoptive immunotherapy may be the load of granzyme B and perforin in the effector cells. In addition, the capacity of an effector cell to kill multiple targets, termed serial killing, may have an impact on tumor burden. Stimulation of innate anti-viral defense mechanisms is a very attractive approach in order to boost the immune responses of lymphocytes. Activation of cytosolic RNA recognition receptors RIG-I and MDA5 will stimulate the synthesis of a broad range of antiviral effector molecules, cytokines and chemokines that have a crucial role for priming, expansion and polarization of immune cells. In this study we screened a panel of RNA constructs in silico predicted to bind to RIG-I or MDA5 in order to induce a type I IFN response. Interestingly, one of the RNA constructs did not induce a type I IFN response but inreased perforin and granzyme B levels in NK and T cells. We hereby report the *in vitro* and *in vivo* activity of this RNA construct and anti-tumor activity.

VyX, a novel RNA construct, increases NK cell functions *in vitro* and *in vivo*

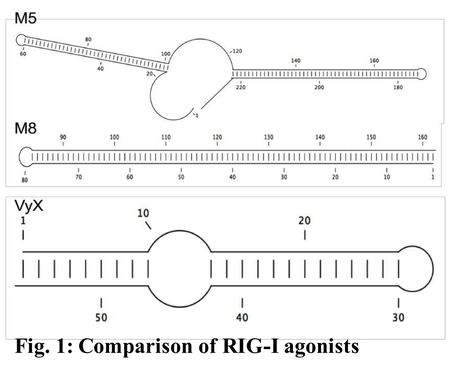
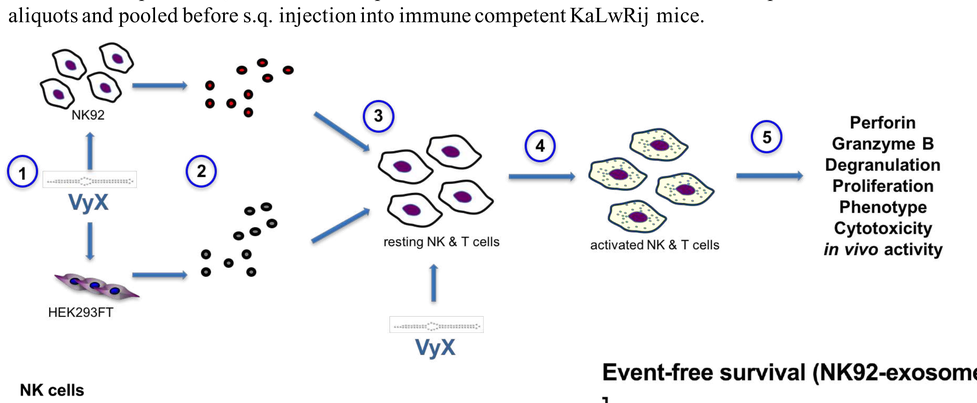


Fig. 1: Comparison of RIG-I agonists

The RNA sequence and secondary structure of VyX differs from the previously published RIG-I agonists M5 and M8. VyX is significantly shorter and shares no significant sequence homology. Vyx forms a hairpin and a loop structure.

Fig. 2: Delivery of VyX.

(1) HEK293FT cells or NK-92 cells were transfected with the RNA construct VyX. (2) Exosomes were isolated by ultracentrifugation and (3) VyX was delivered as pure RNA or RNA-ferrying exosomes to the effector cells. (4) Effector cells were stimulated for 4-16h *in vitro* before (5) effector function were assessed. For *in vivo* experiments, transfection of producer cells and exosome isolation were performed in several aliquots and pooled before s.q. injection into immune competent KaLwRij mice.



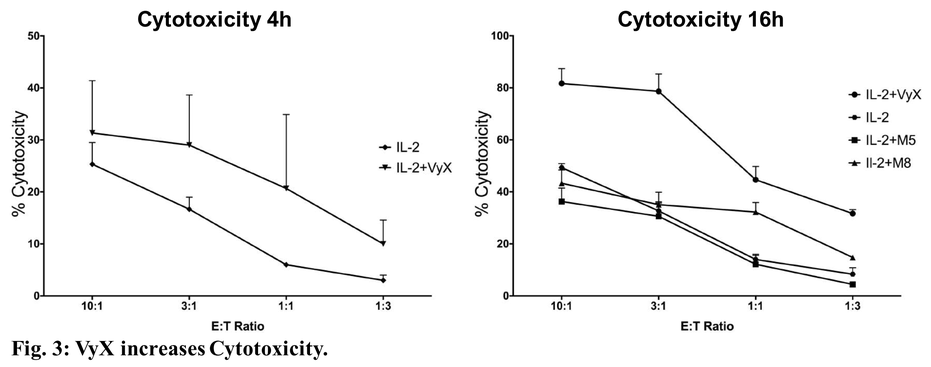


Fig. 3: VyX increases Cytotoxicity.

Cytotoxicity of primary NK cells against K562 target cells was analyzed in a 4h or 16h chromium release assay. Cytotoxicity of NK cells was significantly increased compared to the previously published RIG-I agonists.

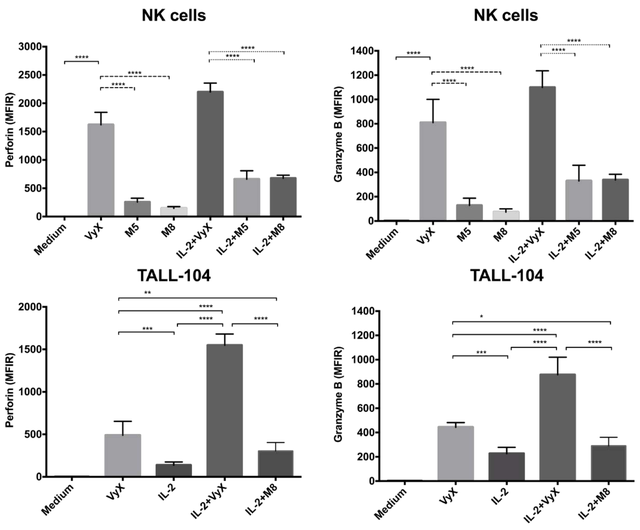


Fig. 4: Increases Perforin and Granzyme B expression.

After 4h of incubation with the RIG-I agonists, VyX, M5 and M8, the granzyme B and perforin expression of NK cells and TALL-104 T cells was analyzed by flow cytometry. A 1600 - 2000 fold increase in Perforin content and a 800 - 1100 fold increase in granzyme B could be observed. This increase in cytotoxic proteins synergized with IL-2 stimulation.

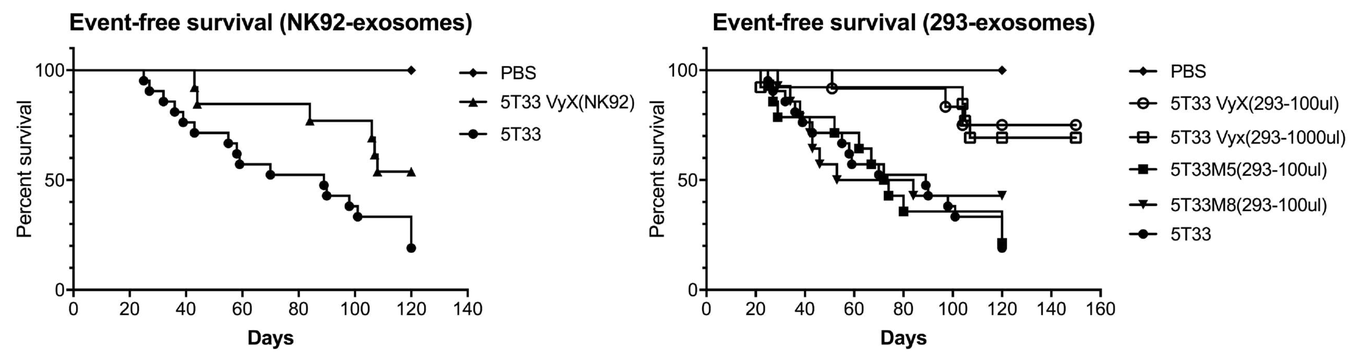


Fig. 5: Prolonged survivial of tumor-bearing immunocompetent mice after treatment with VyX

Administration of the RNA construct to a syngeneic immunocompetent multiple myeloma (MM) mouse model (C57BL/KaLwRij) increased survival of tumor-bearing mice. KaLwRij mice receiving 5T33 MM cells develop disease within 40 days. We have previoulsy shown that activated NK cells are critical for MM rejection in a dose-dependent manner. Mice were injected i.v. with 10.000 eGFP-5T33 MM and/or non-transduced 5T33 MM cells suspended in PBS. The following day, the mice received 5 doses s.q. of exosomes harvested from Vyx-treated NK-92 or HEK293FT cells. The animals were examined twice daily for the development of paraplegia. At weekly intervals and at the time of disease development, the mice were killed by CO2 inhalation and the spleens, livers, thymus and lymph nodes were excised and kept in PBS until processing for preparation of single-cell suspensions. Treatment of mice with VyX resulted in a significant delay in tumor development and increased survival compared to published RIG-I agonists. No off-target effects or severe adverse events were observed.

Outlook

In this study, we identified and characterized a novel RNA construct that is highly specific for RIG-I and significantly increases the expression of perforin and granzyme B in NK cells and cytotoxic T cells. Introduction of the RNA construct to NK cells using transfection or extracellular vesicle-mediated delivery resulted in a rapid increase of both molecules, which could be further boosted by IL-2. To our knowledge, this is the first report of an RNA construct leading to such a drastic difference in the proteomic profile of cytotoxic lymphocytes.

In summary, we have demonstrated that VyX RNA construct induces an increased perforin and Granzyme B expression, which may lead to higher serial selective cytotoxic capacity both *in vitro* and *in vivo*. Potential applications of this group of molecules can be a simple *ex vivo* treatment of cells before adoptive transfer or direct administration. Further studies to understand the safety and efficacy profile of these constructs are warranted.

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