382 TIM-3 BLOCKADE COMBINED WITH ADOPTIVE THERAPY OF EX VIVO ACTIVATED NK CELLS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANT REDUCES PROGRESSION OF RELAPSED MURINE NEUROBLASTOMA

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Background High-risk neuroblastoma (NBL) is an aggressive extra-cranial pediatric solid tumor with poor overall survival. Prior studies testing allogeneic hematopoietic stem cell transplant (allo-HSCT) for NBL patients were limited by graft-versus-host-disease (GVHD) and disease progression. Ex-vivo stimulated allogeneic Natural Killer (NK) cells can enhance the graft-versus-tumor (GVT) effect without exacerbation of GVHD. Because TIM-3 is expressed on both exhausted NK and T cells, immune checkpoint blockade may enhance the GVT effect after allo-HSCT. Here we investigate adoptive transfer of ex-vivo activated NK cells and TIM-3 checkpoint blockade after T cell depleted allo-HSCT for relapsed NBL.

Methods NK cells from C57BL/6 (B6) mice were expanded with soluble IL-15/IL-15Rα alone or with an irradiated murine neuroblastoma cell line transfected to over-express CD54, CD80, CD86 and CD137L, called AgN2a-4P, at a 1:1 ratio for 10–12 days. AgN2a-4P-stimulated allogeneic NK (Ag4P-NK) cell phenotype, cytokine production, and cytotoxic activity against murine NBL cell lines Neuro2a and NXS2 in the presence of anti-TIM-3 antibody were analyzed in vitro. B6AJF1 mice received a T cell depleted allo-HSCT from B6 donors followed by NBL inoculation to model relapse. Select groups received anti-TIM-3 antibody every 9 days and/or infusions of B6 Ag4P-NK cells on days 14, 21, and 28. In select groups, T cells or NK cells were depleted to determine their relative contribution on the GVT effect. All groups were analyzed for tumor growth, GVHD and survival.

Results NK expansion with AgN2a-4P and IL-15/IL-15Rα resulted in increased expression of Ki-67 and multiple markers of NK cell activation. Ag4P-NK cells showed enhanced cytotoxicity in vitro against NBL compared to IL-15/IL-15Rα activated NK cells, and this effect was augmented by TIM-3 blockade. Exposure to TIM-3 blockade further increased Ag4P-NK degranulation against Neuro2a compared to NXS2, due to a higher frequency of TIM-3 ligands present on Neuro2a. In vivo, the combination of Ag4P-NK cells and anti-TIM-3 after allo-HSCT resulted in significantly improved survival and reduced NBL tumor burden without inducing GVHD, as compared to mice treated with Ag4P-NK cells or anti-TIM-3 alone. Depletion of NK cells led to substantial increase in tumor burden, while depletion of T cells did not affect therapy efficacy.

Conclusions T cell-depleted allo-HSCT can be a viable treatment platform for treating relapsed NBL when combined with adoptive transfer of ex-vivo-stimulated NK cells and TIM 3 checkpoint blockade. These studies demonstrate that multimodal approaches incorporating combination immunotherapy are needed for NBL and that immunotherapies that enhance NK cell function should be prioritized.

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