

373

COMBINATION VACCINE, ADOPTIVE NK CELL TRANSFER AND CHECKPOINT BLOCKADE REDUCE MURINE NEUROBLASTOMA PROGRESSION AFTER BONE MARROW TRANSPLANT

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Background Despite use of intense multimodal therapy for treatment of high-risk neuroblastoma, poor survival outcomes persist. While autologous stem cell transplant (SCT) is standard of care for high risk patients, allogeneic SCT has been explored to produce a graft-versus-tumor (GVT) effect but has been hampered by tumor relapse and graft-versus-host disease (GVHD). We propose enhancing GVT by combining adoptive Natural Killer (NK) cells, anti-PD1 checkpoint blockade, and a neuroblastoma vaccine engineered to express CD54, CD80, CD86, and CD137L (AgN2a-4P) to stimulate donor T and NK cells.

Methods NK cells or T cells were isolated from C57BL/6 (B6) mice and expanded *in vitro* with IL-15/IL-15R α alone (NK), IL-2 (T cells) with/without AgN2a-4P for 7 days. All groups were analyzed by flow cytometry. For *in vivo* studies, on day +0, recipient mice received lethal radiation and either syngeneic (B6 to B6) or allogeneic (B6 to B6AJF1) SCT. Mice were inoculated with 9464D or NXS2 neuroblastomas to model relapse, and then treated with anti-PD1 antibody. Select groups received irradiated AgN2A-4P and/or donor-derived NK cells on days 14, 21, and 28. B6 Rag1^{-/-} mice were used as bone marrow donors to analyze the role of donor B and T cells, while select B6 recipients were injected with anti-NK1.1 every 4 days to analyze the role of NK cells. All groups were analyzed for tumor growth, GVHD and overall survival. Tumors were also analyzed for relative gene expression by bulk RNA-Seq.

Results T cells exposed to AgN2a-4P showed a significant increase in CD69, PD-1 and TIM-3 after 7 days, while NK cells exposed to AgN2a-4P expressed high levels of NKG2D and Nkp46 *in vitro*. After autologous and allogeneic SCT, combination NK cells, anti-PD1 and AgN2a-4P led to a significant decrease in tumor growth and increase in survival. Multiple NK infusions were superior to a single infusion. Usage of Rag1^{-/-} donors abrogated the benefits of combination immunotherapy on tumor growth, whereas depleting NK cells abrogated the benefits of AgN2a-4P alone more than AgN2a-4P and anti-PD1.

Conclusions AgN2a-4P vaccine activates both T and NK cells *in vitro*. Combination NK cell therapy with anti-PD1 and AgN2a-4P vaccination significantly decreases neuroblastoma tumor growth *in vivo*. T and NK cells are required for therapeutic benefit, and modulation of myeloid gene expression is observed within the TME. These studies provide the first evidence that adoptive NK cell therapy, checkpoint blockade and tumor vaccines show safety and efficacy in combination after SCT.

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Ethics Approval The animal study M005915 was reviewed and approved by University of Wisconsin-Madison IACUC.

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