An immune co-stimulatory vaccine, with adoptive transfer of natural killer cells and immune checkpoint blockade, after allogeneic bone marrow transplant, delays and reduces neuroblastoma tumor growth

Nicholas Mohrdieck1,2, Paul Bates1,2, Sean Rinella1,2, Chloe King1,2, Katherine Tippins1,3, Christian Capitini1,2,3
1University of Wisconsin – Madison School of Medicine and Public Health, 2Department of Pediatrics, 3Carbone Cancer Center

The AgN2a 4P vaccine, with adoptive transfer of donor-derived NK cells and immune checkpoint blockade, after bone marrow transplant, can serve as a novel treatment for established neuroblastoma, as it delays and reduces tumor growth.

BACKGROUND

The AgN2a 4P vaccine is an aggressive variant of the murine Neuro-2a neuroblastoma cell line engineered by nucleofection to express:

- CD4 (SCAM-1 adhesion molecule that binds LFA-1 and MAC-1 on T cells and NK cells)
- CD9 (co-stimulatory molecule for T-cell activation)
- CD80 (co-stimulatory molecule for T-cell activation)
- CD86 (co-stimulatory molecule for T cell and NK cell activation)

Figure 1. AgN2a 4P vaccine. AgN2a 4P is an aggressive variant of the murine Neuro-2a neuroblastoma cell line engineered by nucleofection to express:

METHODS

A. Transplant scheme for bone marrow transplant model that includes three vaccinations of irradiated AgN2a 4P with a single adoptive transfer of NK cells. On day 0, recipient mice were given 566 haplo-identical bone marrow cells, and 3x 3 haplo-identical T cells. On day 7, recipient mice were challenged with 2x 4 neuro-2a neuroblastoma tumor cells. On day 9, immune checkpoint therapy, via anti-PD1 (35µg), began and was repeated every 14 days. On days 14, 21, and 28, recipient mice received 2x 4 irradiated AgN2a 4P cells. On day 28, recipient mice received 2x 4 donor-derived NK cells.

B. Transplant scheme for bone marrow transplant model that includes three vaccinations of irradiated AgN2a 4P with three adoptive transfers of NK cells. On day 0, recipient mice were given 566 haplo-identical bone marrow cells, and 3x 3 haplo-identical T cells. On day 7, recipient mice were challenged with 2x 4 neuro-2a neuroblastoma tumor cells. On day 9, immune checkpoint therapy, via anti-PD1 (35µg), began and was repeated every 14 days. On days 14, 21, and 28, recipient mice received 2x 4 irradiated AgN2a 4P cells. On days 14, 21, and 28, recipient mice received 2x 4 donor-derived NK cells.

RESULTS

Figure 2. Bone marrow transplant scheme for study of efficacy of immune checkpoint blockade via anti-PD1. A. Transplant scheme for bone marrow transplant model that includes three vaccinations of irradiated AgN2a 4P with a single adoptive transfer of NK cells. On day 0, recipient mice were given 566 haplo-identical bone marrow cells, and 3x 3 haplo-identical T cells. On day 7, recipient mice were challenged with 2x 4 neuro-2a neuroblastoma tumor cells. On day 9, immune checkpoint therapy, via anti-PD1 (35µg), began and was repeated every 14 days. On days 14, 21, and 28, recipient mice received 2x 4 irradiated AgN2a 4P cells. On day 28, recipient mice received 2x 4 donor-derived NK cells.

CONCLUSIONS

- The AgN2a 4P vaccine with adoptive transfer of donor-derived NK cells and immune inhibition blockade significantly reduced neuroblastoma tumor growth in both allogeneic and syngeneic bone marrow transplant models, compared to vaccine and tumor only controls.
- A4P match is not required for NK cell activation with the AgN2a 4P vaccine; common neuroblastoma antigens will sensitize NK cells.
- Immune inhibition blockade, via anti-PD1, helps improve cytotoxic effects in T cells, but did not show any improved benefit in NK cells; however, both NK and T cells are needed for significant reduced tumor growth.
- Multiple adoptive transfers of NK cells, with vaccine treatment, significantly delays tumor onset.

ADDITIONAL KEY INFORMATION

Future Studies

- Testing the efficacy of anti-TIM3 in rescuing NK cell exhaustion after bone marrow transplant
- Potential to combine anti-PD1 and anti-TIM3 therapies to further enhance the anti-tumor effects of our treatment model.
- Testing the efficacy of donor NK cells and T cells, post-Agn2a 4P stimulation, in enhancing anti-tumor effects

Author Contact Information

Nicholas Mohrdieck – nmohrdieck@wisc.edu

Acknowledgements

AgN2a 4P was a gift from Dr. Bryan Johnson at Medical College of Wisconsin. This work was supported by grants from the St. Baldrick’s—Stand up to Cancer Pediatric Dream Team Translational Research Grant SU2C-AACR-DT17-13-JJ3/NIH FO1 CA250282, American Cancer Society Research Scholar grant RSG-18-180-01-CG, Hyundai Hope on Wheels and the MACC Fund (CA14-1). We would like to thank the UWSC core facilities, who are supported in part through NIH/NHLR FO1 CA250282, Stand up to Cancer is a division of the Entertainment Industry Foundation. Research Grants are administered by the American Association for Cancer Research, the Scientific Partner of SU2C.

Dr. Capitini declares honorarium from Nektar Therapeutics, who had no input in study design, analysis, reagents used or writing for this study. No other relevant conflicts of interest are reported.