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

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BACKGROUND

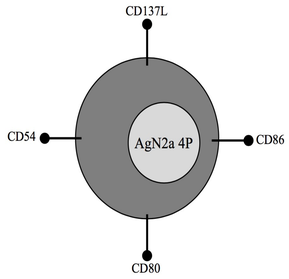

Figure 1. AgN2a 4P vaccine. AgN2a 4P is an aggressive variant of the murine Neuro-2a neuroblastoma cell line engineered by nucleofection to express:

CD54 (ICAM-1 adhesion molecule that binds LFA-1 and MAC-1 on T cells and NK cells)
CD80 (co-stimulatory molecule for T-cell activation)
CD86 (co-stimulatory molecule for T-cell activation)
CD137L (co-stimulatory molecule for T cell and NK cell activation)

Ref: Johnson BD et al. *J Immunother* 2005 Sep-Oct;28(5):449-60

- There are currently no vaccines present in clinic to treat neuroblastoma
- Immune checkpoint blockade has yielded mixed results in pediatric cancer models

METHODS

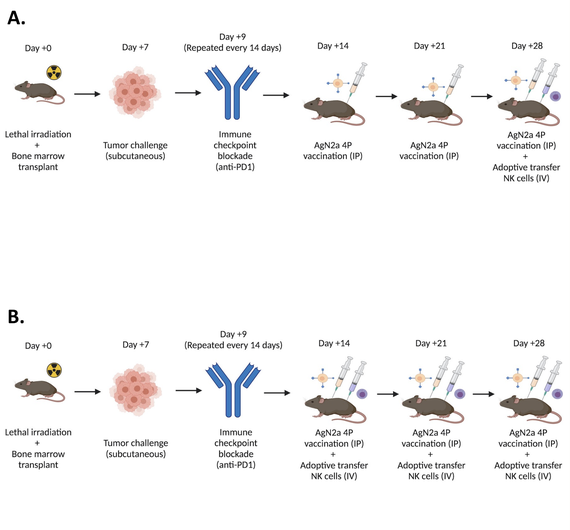

Figure 2. Bone marrow transplant schema for study of efficacy of immune checkpoint blockade via anti-PD1. A. Transplant schema for bone marrow transplant model that includes three vaccinations of irradiated AgN2a 4P, with a single adoptive transfer of NK cells. On day +0, recipient mice were given 5e6 haploidentical bone marrow cells, and 1e3 haploidentical T cells. On day +7, recipient mice were challenged with 2e6 neuro-2a neuroblastoma tumor cells. On day +9, immune checkpoint therapy, via anti-PD1 (250μg), began and was repeated every 14 days. On days +14, +21, and +28, recipient mice received 2e6 irradiated AgN2a 4P cells. On day +28, recipient mice received 2e6 donor-derived NK cells. **B.** Transplant schema 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 5e6 haploidentical bone marrow cells, and 1e3 haploidentical T cells. On day +7, recipient mice were challenged with 2e6 neuro-2a neuroblastoma tumor cells. On day +9, immune checkpoint therapy, via anti-PD1 (250μg), began and was repeated every 14 days. On days +14, +21, and +28, recipient mice received 2e6 irradiated AgN2a 4P cells. On days +14, +21, and +28, recipient mice received 2e6 donor-derived NK cells.

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.**

RESULTS

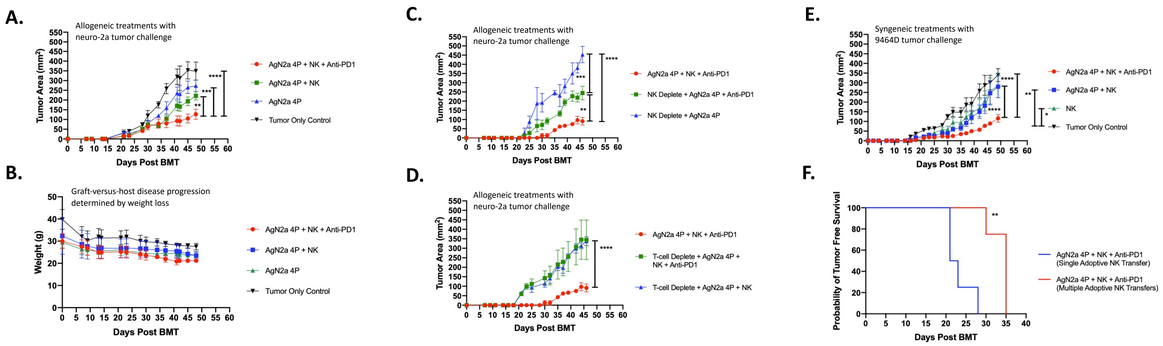

Figure 3. Results from anti-PD1 therapy when combined with AgN2a 4P vaccination and adoptive transfer of NK cells. A. Tumor area curve for an allogeneic bone marrow transplant, combined with the AgN2a 4P vaccine, a single adoptive transfer of NK cells, and anti-PD1 therapy. The vaccine develops a graft-versus-tumor effect *in vivo*, reducing tumor growth compared to control recipients of allogeneic bone marrow transplant without vaccine. An adoptive transfer of donor-derived allogeneic NK cells with the vaccine further reduces tumor growth compared to giving vaccine alone. Finally, anti-PD1 treatment combined with an adoptive transfer of donor-derived allogeneic NK cells and the vaccine significantly reduces neuroblastoma tumor growth. (P-value = **0.0071; ***0.0006; **** <0.0001). **B.** Weight curve for an allogeneic bone marrow transplant, combined with the AgN2a 4P vaccine, a single adoptive transfer of NK cells, and anti-PD1 therapy. There was slight GVHD observed (weight loss >10%), but treatment did not cause significant change in GVHD status. **C.** Tumor area curve for an allogeneic bone marrow transplant, with an NK cell depletion, combined with the AgN2a 4P vaccine, multiple adoptive transfers of NK cells, and anti-PD1 therapy. The absence of NK cells led to larger tumors. The addition of anti-PD1 partially rescued the anti-tumor effect, implying T-cell exhaustion is reversed by anti-PD1 therapy. (P-value = **0.0018; ***0.0004; **** <0.0001). **D.** Tumor area curve for an allogeneic bone marrow transplant, with a T cell depletion, combined with the AgN2a 4P vaccine, multiple adoptive transfers of NK cells, and anti-PD1 therapy. The absence of T cells led to larger tumors. The addition of anti-PD1 could not rescue the anti-tumor effect, implying NK cell exhaustion is not reversed by anti-PD1 therapy. (P-value = **** <0.0001). **E.** Tumor area curve for a syngeneic bone marrow transplant, combined with the AgN2a 4P vaccine, multiple adoptive transfers of NK cells, and anti-PD1 therapy. A similar trend in tumor reduction occurs to that of the allogeneic transplant model, which implies that Ly49 specificity is not required for activation and stimulation of donor NK cells. (P-value = *0.0249; **0.0047; **** <0.0001). **F.** Survival curve comparing the efficacy of a single adoptive transfer of NK cells to multiple adoptive transfers of NK cells. Multiple adoptive transfers signifies three adoptive transfers with three vaccinations of AgN2a 4P. The addition of three adoptive transfers of NK cells to three AgN2a 4P vaccinations significantly delays tumor onset. (P-value = **0.0062).

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
- Ly49 match is not needed 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

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