New trial for kids with relapsed or refractory neuroblastoma

Neuroblastoma is the most common solid tumor of childhood occurring outside the central nervous system. Patients can be subdivided into different risk groups based on their age, stage, and biologic features of the tumor. While children classified as having low or intermediate-risk disease have an excellent prognosis (> 90% survival), patients with high-risk disease have significantly less favorable outcomes, despite use of aggressive combination chemotherapy, surgery, stem cell transplantation and external beam radiation.

However, a relatively recent and exciting development in the field has been the incorporation of anti-GD2 monoclonal antibody (mAb) therapy into the treatment regimen, which resulted in a 20% improvement in event-free survival\(^1\). The antibody presumably works by engaging cells of the innate immune system (e.g. NK cells, macrophages) via their Fc receptors (FcR), thereby bringing them in close proximity to the GD2-expressing neuroblastoma cells where cytotoxicity can be induced via perforin – granzyme, or through engagement of death receptors on the tumor surface, such as TRAIL and Fas. This immunotherapy approach has proven effective in the setting of minimal residual disease, but not for patients with grossly measurable tumor.

At the University of Wisconsin - American Family Children’s Hospital we have initiated a first in human clinical trial for children with relapsed or refractory neuroblastoma utilizing the combination of an anti-GD2 mAb and haploidentical ex-vivo activated and expanded NK cells (AE NK cells, NCT03209869). The antibody used in this trial is the hu14.18-IL2 immunocytokine (IC). This molecule has several potential advantages compared to the commercially available anti-GD2 mAb ch14.18 (dinutuximab). First, the IC has been humanized and therefore contains less mouse protein, which should reduce the likelihood of eliciting a neutralizing human anti-mouse antibody (HAMA) response. Secondly, the IL-2 component of the IC can help maintain the NK cells in an activated state since IL-2 stimulates NK cell proliferation, increases production of perforin and interferon gamma (IFN\(_\gamma\)), upregulates surface expression of NK cell activation receptors such as the natural cytotoxicity receptors (e.g. NKp30, NKp44, NKp46), NKG2D and DNAM-1, and enhances both natural cytotoxicity (i.e. antibody independent) and antibody dependent cellular cytotoxicity (ADCC). Moreover, binding of the IL-2 component of the IC to the IL-2 receptors present on NK cells offers another pathway by which the NK cells can form an immune synapse with the tumor (in addition to binding through their FcR)\(^2\). The Children’s Oncology Group has conducted phase 1 and 2 testing of the hu14.18-IL2 IC in children with relapsed/refractory neuroblastoma\(^3,4,5\). In both phase 2 trials, patients were subdivided into 2 strata: those with tumor measurable by standard radiographic criteria, and those with disease only
measurable by MIBG scan and/or bone marrow histology. Objective responses were only seen in the latter cohort, suggesting that treatment with the IC alone was insufficient to mediate anti-tumor activity in patients with grossly measurable disease. We hypothesized that the combined use of hu14.18-IL2 and adoptively transferred NK cells will illicit a more robust anti-tumor response.

The use of haploidentical NK cells in this trial may help to optimize their cytotoxic potential. NK cell activity is controlled through a series of complex interactions between activating and inhibitory receptors present on the NK cell and the corresponding ligands expressed by potential targets. One clinically important class of inhibitory receptors found on NK cells is known as killer immunoglobulin-like receptors or KIR. If an NK cell expressing a particular inhibitory KIR repertoire engages a target that lacks the corresponding KIR ligands, then a cytotoxic response may be elicited, provided that activating signals are also present. The ligands for KIR are class 1 HLA molecules. Consequently, by performing KIR typing of potential NK cell donors (e.g. the child’s parents) and HLA typing the patient, it may be possible to select donors whose NK cells are better equipped to destroy the patient’s cancer.

The clinical relevance of “KIR mismatching” was first demonstrated in a landmark study by Ruggeri et al, evaluating the impact of KIR ligand mismatch between donors and recipients, in the setting of haploidentical stem cell transplantation for AML. Patients who were KIR ligand mismatched with their donors had a substantially lower risk of post-transplant relapse compared with KIR ligand matched donor-recipient pairs (0% vs. 75%, P < .0008). Interestingly, a similar phenomenon has been observed in pediatric patients undergoing autologous transplant for high-risk neuroblastoma. The genes that code for HLA antigens on chromosome 6 segregate independently from the KIR genes located on chromosome 19. Consequently, it is possible for some individuals to be KIR – KIR ligand mismatched with themselves (i.e. express inhibitory KIR, but not the corresponding KIR ligands). The NK cells of such individuals might be more cytotoxic due to lack of inhibitory signaling. Venstrom et al found that the probability of survival following autologous transplant for neuroblastoma was significantly better in children who lacked any KIR ligands, compared to those in whom all ligands were present.

Furthermore, in the initial phase 2 trial evaluating hu14.18-IL2 for patients with relapsed neuroblastoma, no responses were seen in the 14 children who were KIR-KIR ligand matched with themselves, whereas 7 of 28 children who were KIR-KIR ligand mismatched achieved a CR or demonstrated significant clinical improvement (p= 0.03). In addition, the importance of KIR and KIR ligands has been demonstrated in a large phase-III COG study of anti-GD2 mAb for newly diagnosed children with high-risk neuroblastoma. Collectively, these data suggest that NK cells play an important role in mediating a clinically significant anti-tumor effect for children with high-risk or relapsed neuroblastoma.

Various methodologies have been developed to expand autologous or allogeneic NK cells for the purpose of adoptive immunotherapy. In our trial, donors undergo a single leukopheresis procedure. The NK cells are activated and expanded up to ~200 fold in the Waisman Biomanufacturing facility under GMP conditions. Briefly, peripheral blood mononuclear cells are isolated and then co-cultured with irradiated K562 cells, which
have been transduced to express IL-15 and 41BBL, for 11 days in a WAVE bioreactor (figure). After the incubation period, the cells are concentrated by centrifugation and then undergo a CD3 depletion step using the CliniMACS® cell processing system. The final NK cell product is subjected to vigorous quality control testing and then divided into 4 aliquots. One aliquot is immediately infused into the patient while the remaining 3 aliquots are cryopreserved and infused at later time points.

The AE NK cells generated using this methodology have been well characterized and demonstrate increased expression of interferon gamma, granzyme B, and several NK cell activation markers including NKp30, NKp46 and NKG2D. The AE NK cells also demonstrate significantly enhanced killing capacity in vitro, compared to unmanipulated NK cells, both in natural cytotoxicity assays against K562 cells, and ADCC assays against neuroblastoma and melanoma cell lines with the addition of an anti-GD2 mAb. Importantly, the cryopreserved AE NK cells retain their cytotoxic activity. NK cells activated ex vivo by this process also retain regulation of NK function via KIR and KIR-ligand interactions, supporting the need for KIR/KIR-ligand genotyping in selecting optimal haplo-identical donors.

This clinical trial for relapsed/refractory neuroblastoma is the first study to administer haploidentical AE-NK cells to children in combination with an immunocytokine. Patients first receive lymphodepletive chemotherapy utilizing cyclophosphamide and fludarabine. Previous studies exploring the use of allogeneic NK cells for adoptive immunotherapy have demonstrated that administering lymphodepletive chemotherapy is necessary to ensure persistence of the infused cells for days to weeks. Following completion of chemotherapy, patients are treated with 1 x 10^7 AE NK cells/kg on “day 0”. The hu14.18-IL2 IC is then infused once daily on days 1 – 7. Patients may receive up to 4 infusions of AE-NK cells + hu14.18-IL2, with cycles being repeated every 28 – 42 days. Children who demonstrate at least stable disease after combination immunotherapy are eligible to receive 4 additional cycles of hu14.18-IL2 alone. The primary objective of this trial is to assess the feasibility and safety of administering multiple cycles of AE NK cell together with hu14.18-IL2 to children with relapsed or refractory neuroblastoma. Secondary objectives are to assess the pharmacokinetics and immunogenicity of hu14.18-IL2, determine persistence of the haploidentical AE NK cells after each infusion, and evaluate for anti-tumor efficacy mediated by this treatment regimen.

REFERENCES (Authors from our UW clinical or lab teams are bolded):

3. Osenga KL, Hank, JA, Albertini MR, Gan J, Sternberg AG, Eickhoff J, Seeger RC,


13. Wang W, Erbe AK, DeSantes KB, Sondel PM. Donor selection for ex vivo expanded

**Figure: Generation of Haploidentical AE NK Cells**

**NK Cell Expansion Process**

- **Component**: kopheresis Product
- **Steps**:
  - Density Gradient Isolation of PBMC
  - Co-Culture in WAVE Bioreactor
  - 11 Day Incubation

**Summery**: