



2011 Research Grant Program Winning Abstract

Effect of Lenalidomide on the Anti-Neuroblastoma Cytotoxicity of Human Natural Killer Cells Expanded from Blood Using K562-IL21 Feeder Cells

By Michael Sheard

Hypothesis. Therapy involving ex vivo expansion of human natural killer (NK) cells, combined with administration of an immune-modulating drug, may enable infusion of large numbers of NK cells that can be activated to eradicate neuroblastoma tumors.

Specific Aims. 1) Determine the activation status of patient NK cells expanded ex vivo with K562-IL21 feeder cells and treated with the immune-modulating drug lenalidomide in vitro, using 10-color flow cytometry panels and BD™ Cytometric Bead Array assays.

2) Demonstrate the therapeutic efficacy of combining infusion of ex vivo-expanded NK cells with in vivo administration of lenalidomide, in the treatment of luciferase-labeled human neuroblastoma xenografts grown in NOD-SCID immunodeficient mice.

3) Examine this therapeutic combination in human clinical trials under the auspices of the NANT consortium (www.nant.org).

Background and Significance. Cellular immunotherapy represents a promising avenue for targeting cancer cells. After expansion of a cancer patient's cytotoxic immune cells in the laboratory, the patient can be re-infused with his or her own NK cells. NK cells are activated by, bind to, and kill cancer cells that have little or no expression of MHC class I proteins. Neuroblastoma is a childhood cancer that rarely expresses detectable levels of MHC class I proteins, and is therefore a promising target for NK-cell-mediated therapies.

NK cells can be obtained from human blood but only in small numbers. NK cells can be induced to proliferate exponentially and become activated by incubating them with specially designed "feeder cells." K562 is a human leukemia cell line known to potently induce NK cells to proliferate and become primed for killing target cells. To further enhance their feeder cell function, K562 cells have previously been engineered to express interleukin-21 (IL21) on their cell surface. We and our collaborators have found that 14-day incubation of patient peripheral blood mononuclear cells (PBMCs) with K562-IL21 cells and low dose interleukin-2 (50 U/mL) is able to expand NK cell numbers 200-fold in the laboratory while simultaneously activating them to express surface receptors (NKG2D, DNAM-1, CD56) involved in the recognition and killing of target cells. Concurrently, monocytes and B cells fail to proliferate and are completely absent from the final product, and the small subset of T cells that persists has a typically low frequency CD4⁺CD25⁺ T-regulatory cell (0.1% of total cells).

Lenalidomide is a drug approved by the FDA for myelodysplastic syndromes and multiple myeloma. Lenalidomide has multiple, potentially beneficial effects on the anti-cancer immune response. We and others have also observed that daily doses of



lenalidomide for 3 weeks induce NK expansion and activation in patients in clinical trials. However, although lenalidomide is known to have these effects on unexpanded NK cells, it is unknown what its effect would be on the phenotype, functional status, and therapeutic efficacy of K562-IL21–expanded NK cells.

Research Design and Methods. To achieve Specific Aim 1: We will determine the activation status of patient NK cells expanded *ex vivo* with K562-IL21 feeder cells and treated with lenalidomide, employing 10-color flow cytometry panels, BD Cytometric Bead Array assays, and a well established *in vitro* cytotoxicity assay.

We will use 10-color flow cytometry to precisely identify cell surface expression of activation markers on relevant immune subsets, outlined as follows:

Activated NK cells: CD56, CD16, NKG2D, DNAM-1, NKp46, CD107a, CXCR4, CD45, CD3 & CD14 (in dump channel), propidium iodide

T cells: CD3, CD4, CD8, TCR-gamma/delta, CD56, NKG2D, CD16, CD107a, CD25, propidium iodide

Monocytes: CD14, CD16, Tie-2, CXCR4, CSF-1R, HLA-DR, CCR2, CD45, CD3 & CD56 (in dump channel), propidium iodide

We will measure secretion of IFN-gamma, IL6, IL10, IL-12, granzyme A, granzyme B, and MCP-1 from expanded NK cells after incubation *in vitro* with and without clinically achievable doses of lenalidomide with and without neuroblastoma cells. NK cytotoxicity against neuroblastoma cells will be examined by digital imaging microscopy (Frgala et al, 2007, *Mol Cancer Ther*) after loading neuroblastoma cells with the fluorescent dye calcein-AM.

To achieve Specific Aim 2: In NOD-SCID mice, we will examine whether intraperitoneal administration of lenalidomide at clinically relevant doses 5 days per week for 3 weeks can enhance the anti-neuroblastoma effect of K562-expanded NK cells administered once (or twice) per week. Our luciferase-labeled human neuroblastoma xenograft model has been established previously, and we have extensive experience infusing xenografts and *ex vivo*-expanded NK cells into NOD-SCID mice. The endpoints will be imaging and survival.

To achieve Specific Aim 3: We will propose a phase I clinical trial in collaboration with the New Approaches to Neuroblastoma Therapy (NANT) consortium (www.nant.org) to examine the combination of autologous K562-IL21–expanded NK cells with lenalidomide. Lenalidomide administered by itself has been found to be safe in a phase I trial of pediatric cancer patients (Berg et al, 2011, *J Clin Oncol*). In the clinical trial to be proposed, patient blood will be monitored at weekly intervals using the 10-color panel described previously, and plasma cytokine levels will be monitored using BD Cytometric Bead Array assays.



Potential Pitfalls. If the activity of lenalidomide is found to be less than ideal for our purposes, we can evaluate the next generation immune modulating drug, palmolidomide.

The BD Biosciences Research Grant Program aims to reward and enable important research by providing vital funding for scientists pursuing innovative experiments to advance the scientific understanding of disease.

Visit bdbiosciences.com/grant to learn more and apply online.