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# Harnessing the Antitumor Potential of Natural Killer Cells

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Natural killer (NK) cells are large granular lymphocytes of the innate immune system with effector and immunoregulatory functions. Circulating NK cells surveil for virally-infected and transformed cells and exert cytotoxic activity in the absence of prior antigenic sensitization. Unlike T lymphocytes, NK cells do not require antigen presentation by major histocompatibility complex class I (MHC-I). In fact, NK cells are "educated" to recognize self and remain quiescent by engaging MHC-I while simultaneously becoming activated to lyse target cells that lack or downregulate this expression.[1] They are distinguished by surface expression of CD56 and lack of surface CD3 expression.

Exploitation of NK cells for their antitumor potential has led to human clinical trials in adoptive immunotherapy for solid and hematologic malignancies. Allogeneic haploidentical NK cells have the ability to overcome the self-tolerance seen with autologous NK cells and can produce a graft-versus-tumor effect without apparent graft-versus-host disease.[2,3] Thus far, the greatest benefit has been shown in patients with acute myelogenous leukemia, including as salvage therapy for relapsed or refractory disease, consolidation therapy following hematopoietic cell transplant, and NK donor lymphocyte infusions for relapse or graft rejection.[4-9]

We recently reported our 15-year single institutional experience in clinical-scale production of NK cells under cGMP conditions for use in early phase human trials.[10] Briefly, non-mobilized peripheral blood mononuclear cells were collected by apheresis and incubated with immunomagnetic microbeads (anti-CD3, anti-CD56, or anti-CD19) prior to loading on a column in an automated cell selection system. The enriched NK cell products were incubated overnight in cytokine (IL-2 or IL-15) to promote cytotoxic activation and administered to patients following successful lot release testing. See figures 1 and 2 for comparative manufacturing processes and product characterization.

Patients receiving our initial T (CD3+) cell-depleted NK cell products achieved clinical remissions; however, complications related to B cell contamination caused us to move to a higher level of NK cell purification using sequential CD3 depletion and CD56 enrichment.[4] Unfortunately, this 2-step process led to significant cell loss and poor NK cell recovery. Despite these shortcomings, we continue to explore the efficacy of the more highly purified NK cell product with an open prospective randomized controlled trial comparing CD3/CD19 depleted to CD3 depleted/CD56 enriched haploidentical NK cell therapy (clinicaltrials.gov, NCT03152526). Presently our primary method of simultaneous CD3/CD19 depletion effectively minimizes T cell and B cell contamination in a single manipulation without compromise to NK cell recovery, and clinical remissions have been achieved.[5] For a more in-depth review, please refer to the full study in Transfusion.[10]

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Figure 1. Comparative manufacturing of clinical-scale, cGMP-grade NK cell products.[10]

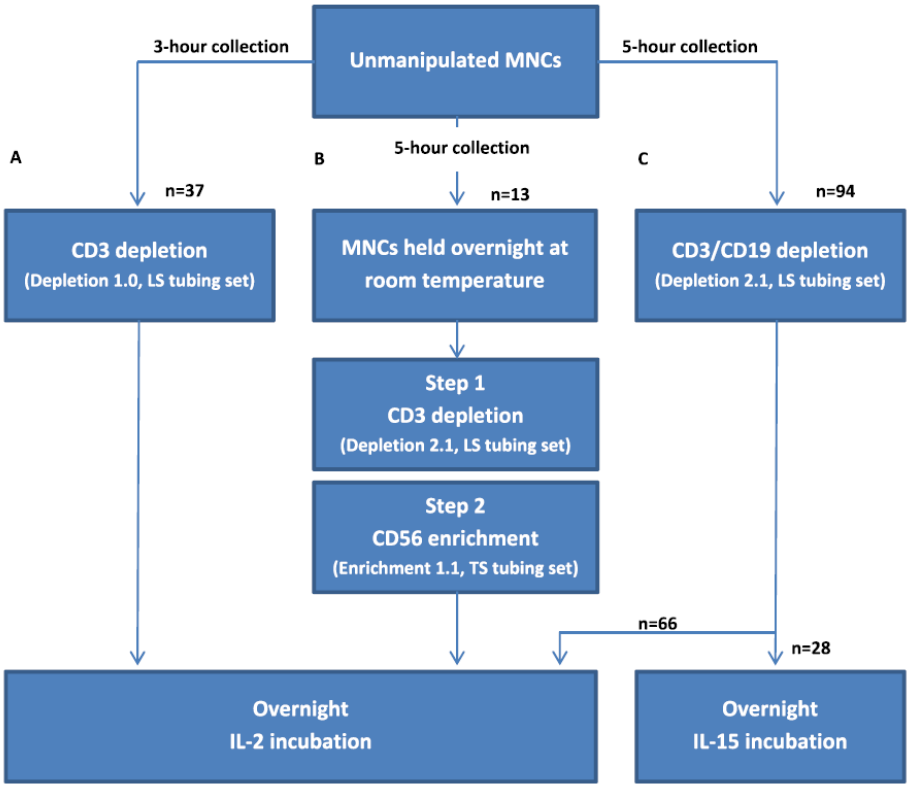

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Figure 2. Comparative flow cytometry characterization of the unmanipulated apheresis mononuclear cells and the NK cell products from each manufacturing process.[10]