Acute lymphoblastic leukemia (ALL) and chronic lymphoblastic leukemia (CLL) are common forms of cancer worldwide. They still remain as incurable diseases in a significant number of cases despite the development of new therapeutic regimens. Chemotherapy, antibodies directed against different surface antigens, and allogeneic hematopoietic stem cell transplantation can be curative, but their application is not always successful. Clearly, alternative therapies are needed for treatments leading to the cure of refractory cancers.

Natural killer (NK) cells are CD3−CD56+ lymphocytes that play a role in the innate immune response against tumor development. NK cells have been described as promising effectors for adoptive immunotherapy against cancer. It has been demonstrated that NK cell alloreactivity can control relapse of acute myeloid leukemia (AML) without causing graft-versus-host disease (GVHD) in the setting of haploidentical stem cell transplantation (SCT). Moreover, haploidentical NK cell infusions might provide a less costly alternative to haploidentical SCT with no long-term side effects. However, only a few trials investigating adoptive NK cell infusions have been conducted to date. A major obstacle for NK cell infusions is the relatively small number of NK cells that can be isolated from regular leukapheresis products. Interestingly, a very recent report has described an alternative cytokine-based culture method with the capability of generating clinically relevant NK cell products with high cell numbers from umbilical cord blood derived hematopoietic stem cells (UCB-HSCs).

Another alternative source of NK cells is human induced pluripotent stem cells (hiPSCs), which are a type of pluripotent stem cell artificially derived from a non-pluripotent cell—an adult somatic cell—by inducing a “forced” expression of specific genes (Oct4, Sox2, cMyc, and Klf4). Because hiPSCs are developed from a patient's own somatic cells, it is believed that treatment with hiPSCs would avoid any immunogenic responses. Some groups have previously demonstrated the ability of hiPS-derived hematopoietic precursors to produce functional NK cells. Also, hiPS-derived NK cells could provide a “universal” source of allogeneic NK cells with known KIR-ligand mismatch and optimal KIR alloreactivity. The ability to modify hiPS-derived NK cells with tumor-specific receptors holds promise for using them against a variety of malignancies.

Chimeric antigen receptor (CAR) technology is a new alternative therapeutic treatment for cancer. Adoptive transfer of cytotoxic NK cells genetically modified to express a CAR can combine the beneficial effects of both antibody-based therapy and NK cell mediated immune responses. T cells expressing CARs targeting CD19 antigens have been developed to treat B-cell–derived malignancies, and clinical trials using this technology are currently ongoing in several international institutions. In the same manner, it is also possible to genetically modify NK cells to carry a CAR consisting of a specific scFv antibody fragment, that is, anti-CD19 or anti-CD20, which, via a flexible hinge region, is connected to the intracellular tail of a signaling moiety. The genetically modified NK cells
display markedly enhanced cytotoxicity toward NK-sensitive CD19/CD20 cancer cells, therefore being suitable for the development of effective cell-based therapeutics for the treatment of B-cell malignancies.

In the present study, the main goal is to investigate and determine the best cell source for the generation of CAR bearing NK cells for the treatment of hematological cancer. Also we will test the cytotoxic function of the different engineered CAR-NK cells against B-cell lines. The objectives are:

- To determine the best cell type and source in the generation of CAR-transduced NK cells.
- To determine the best cytotoxic effect of the engineered CAR-NK cells from different blood sources (allogeneic human cord blood stem cells, adult peripheral blood, or NK cells derived from hiPSCs).

Methodology:
1. Isolation and activation of blood cells

- NK cells from healthy donor peripheral blood will be isolated, expanded, and activated following several protocols.
- Mature NK cells from cord blood and CD34-derived NK cells from cord blood cells: NK cells will be derived from CD34⁺ HSCs using a well-established method of culturing cells with cytokines. The generation of mature and active NK cells will be determined by flow cytometric techniques.
- NK cells derived from hiPSCs from three sources, cord blood (CB-iPSCs), peripheral blood mononuclear cells (PBMC-iPSCs), and fibroblasts (F-iPSCs).

2. Generation of CD19/CD20-specific CARs

Retroviral vector for CAR engineering cytotoxic cells: A retroviral vector (pLXSN) expressing the anti-CD19/CD20 scFv (Leu 16) antibody fragment linked to the CD3ζ chain as a signaling moiety.

3. NK cell transduction: The NK cells from different sources will be transduced with the anti-CD19/CD20 CAR. Several protocols for transduction will be assayed, using non-integrative strategies (Sendai virus, adenovirus, and mRNA). The efficiency of transduction will be determined by flow cytometric analysis.

4. Cytotoxic assays by using tumor and primary cell lines. They will be performed using flow cytometric techniques and Europium release assays. In addition, cytokine production will be measured.

5. In vivo assays by using immunodeficient mice (NOD/SCID and/or RAG2-/-γc-/-).
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