Cytotherapy, 2015; 17: 245–249

International Society for Cellular Therapy
ISCT®

REVIEW

Challenges of cancer therapy with natural killer cells

HANS KLINGEMANN

Tufts University Medical School and Conkwest Inc, Cambridge, Massachusetts, USA

Abstract

Background aims. Natural killer (NK) cells from peripheral or cord blood—especially if they are obtained from a human leukocyte antigen–mismatched (allogeneic) donor—are increasingly being considered for treatment of malignant diseases and to prevent or treat relapse after stem cell transplant. However, in addition to proving their efficacy, there are some more logistical and technical issues that must be addressed before NK cell infusions will be fully accepted by the medical community. *Methods.* Issues include (i) the expansion of sufficient numbers of cells under conditions suitable, (ii) cryopreservation and (iii) optimization/standardization of shipping conditions if the cells are used at distant sites. Because the patient's own autologous cells usually are not fully functional because of inhibition by "self" major histocompatibility complex expression, better methods must be developed to target NK cells to tumor cells and overcome self-inhibition. *Results.* Tumor-directed NK-cell therapy can be best accomplished through genetic engineering of NK cells expressing receptors for tumor antigens or combination with monoclonal antibodies that preferentially kill tumors through antibody-dependent cellular cytotoxicity. If allogeneic NK cells are used, T-lymphocytes in the cell collections that can cause acute graft-versus-host disease in the recipient must be removed. *Conclusions.* In addition to showing efficacy in clinical trials, the production of NK cells for treatment must be cost-effective to be eligible for reimbursement by third-party players.

Key Words: *cancer, CAR, cell expansion, cell therapy, cryopreservation, natural killer cells*

Introduction

For decades, natural killer (NK) NK cells existed as "non-specific" killer cells in the shadow of T cells. Recent discoveries that better explain how NK cells recognize and kill their targets and their ability to produce immune-active cytokines have made them more attractive tools for immunotherapy.

NK cells are considered part of the innate immune system, able to respond quickly to "invaders" without a "priming" period as required for T cells. They are operationally defined by their morphology (large granular lymphocytes) and surface marker expression (CD56+/CD3−). Only approximately 10% of all lymphocytes in the peripheral blood are NK cells, which pales in comparison to the number of T-lymphocytes, which is usually in the range of 50–70%. Unfortunately, the cancer patient's own (autologous) NK cells, even when they are activated with cytokines, are often dysfunctional and compromised by chemotherapy. In addition, we have learned that NK cells are trained to recognize "non-self" histocompatibility antigens (human leukocyte antigen, HLA) on the surface of cells through their killer cell immunoglobulin-like (KIR) receptors. NK cell activation is blocked through engagement of their KIR receptors when they encounter self (autologous) major histocompatibility complex (MHC) antigens [1]. Unless MHC antigens are mutated or missing on cancer cells, autologous NK cells will not recognize malignant cells. Consequently, clinical trials of infusions of the patient's own (autologous) NK cells have not shown any clinical benefit [2,3]. The only way to overcome this deficiency of autologous NK cells is to engineer them to express a tumor antigen–recognizing receptor (ie, chimeric antigen receptor [CAR]), which, on engagement, will override inhibitory signals. However, this has proven to be challenging because the

Correspondence: **Hans Klingemann**, MD, PhD, Conkwest Inc at LabCentral, 700 Main Street, Cambridge, MA 02139, USA. E-mail: hans.klingemann@tufts.edu

(Received 15 April 2014; accepted 16 September 2014)

http://dx.doi.org/10.1016/j.jcyt.2014.09.007

Table I. Challenges of cell therapy with NK cells.

- Unmodified autologous NK cells lack anti-tumor efficacy
- Yield of NK cells in leukaphereses collections is highly donor-dependent
- Allogeneic collections require removal of T cells to prevent graft-versus-host-disease
- Potential risk of hemolysis and EBV: lymphoma if B cells remain and T cells are removed
- Variable expansion of blood NK cells to clinical scale
- NK cells are sensitive to cold storage and lose some cytotoxicity after cryopreservation
- If product is used at distant facility: adhere to defined shipping conditions
- Need improved transfection efficiency to genetically engineer NK cells
- Costs of generating the product

transfection efficiency of blood NK cells even with viral vectors is low [4].

Allogeneic KIR-mismatched NK cells, on the other hand, will recognize the discordant HLA antigen pattern on host cells and consequently will not be de-activated. The potential benefit of those KIR-mismatched NK cells became evident when the bone marrow transplant team in Perugia analyzed their outcome data on MHC haplotype–mismatched stem cell transplants for leukemia and noticed that patients with AML whose donors were KIR-mismatched had a lower relapse rate [5]. This finding was confirmed in some larger retrospective analyses [6,7]. Moreover, two additional single-arm trials in Minnesota and Memphis in which allogeneic NK cells were given to AML patients suggested a clinical benefit [8,9].

For allogeneic NK cell therapy to be effective, it is important to select the appropriate donor who is not only mismatched at the KIR loci, but, as recent data have shown, also has the appropriate KIR allelic polymorphism, which may consist of only one amino acid difference. To identify such a "perfect" KIR-mismatched donor requires sequencing of the KIR locus. This adds costs to the process, but the group at Memphis has shown that this extra effort seems to be paying off: some patients with active leukemia could be induced into a remission after KIR-selected NK cell infusions (Leung, oral communication, 2013). However, matching for the right KIR on NK cells is not sufficient to guarantee an anti-tumor effect. NK cells also express activating receptors that must "connect" with appropriate ligands on tumor cells that may not always be present or may be mutated.

Although is appears attractive to develop allogeneic NK cells for therapeutic infusions, this poses a different problem: the allogeneic T-lymphocytes in the cell collection can cause acute graft-versus-host-disease that can be responsible for significant morbidity and mortality. Hence, T cells must be removed before the cell product is infused into the recipient. This can be accomplished with the use of anti-CD3 monoclonal antibodies that are conjugated to an iron particle, which is then passed over a magnetic column resulting in the removal of the T cells (CliniMACS, Miltenyi Biotech).

Such a T-cell depletion step usually enriches—depending on the donor—NK cells to approximately 20–40% [10,11]. Some investigators may decide to use a second immunomagnetic column to specifically enrich for CD56+ NK cells (positive selection). This can result in some cell loss, and there is some suggestion that these highly enriched NK cell preparations actually may have less activity against cancer because they miss accessory cells such as monocytes that can support NK cell function [11]. However, there are some advantages of further enriching CD56+ NK cells beyond just T-cell depletion. The B-lymphocytes that are left behind after T-cell depletion, especially from blood group O+ donors, can cause hemolysis in immune-compromised recipients who carry red cells of group A or group B ("passenger lymphocyte syndrome"). There have also been occasional reports on EBV-driven lymphomas originating from B-lymphocytes that (because of T-cell depletion) can proliferate unchecked [11]. Both complications can be prevented either by positive CD56 selection or by an additional B-cell depletion.

Expansion of NK cells

The yield of blood NK cells from a single-donor leukapheresis that has been T-lymphocyte–depleted is highly variable, and the numbers are not sufficient for therapeutic infusions [11,12]. Hence, expansion and activation of NK cells becomes necessary, a process that usually takes 2–3 weeks of culture of NK cells in the presence of cytokines such as interleukin (IL)-2 that will also activate the NK cells. A feeder layer can optimize the expansion process, and the use of irradiated EBV-transformed lymphoblastic cell line or K562 cells engineered to express IL-15 or IL-21 along with an adhesion molecule [I-BB4] has resulted in more predictable expansion of blood NK cells [13], although the problem of donor variability with respect to cell numbers still remains an issue. Furthermore, these cell collections must be depleted of T cells, which is best done before culture expansion. Because K562 is a malignant cell line, quality control assays before infusion must document complete removal of these cells before infusion into a patient.

Expansion of NK cells can be performed in a variety of vessels or bioreactors. In addition to Teflon bags (Vuelife by Afc) or flasks (Wilson Wolf