A NOVEL MEDIUM FOR INCREASED CYTOTOXICITY AND SERUM-MINIMAL EX VIVO EXPANSION OF NATURAL KILLER (NK) CELLULAR IMMUNOTHERAPIES

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Background Despite significant clinical breakthroughs,^{1, 2} cellular immunotherapy remains unacceptably variable in performance³ and impractically expensive for mainstream adoption.⁴ These hurdles are particularly true for new cancer immunotherapies with natural killer (NK) cells. Culturing NK cells ex vivo is challenging due to short half-lives, reduced functionality, and increased exhaustion.⁵ Also, the use of serum introduces inconsistency and increases cost.⁶ Here we introduce a novel prototype medium specifically formulated for NK cells, which uses a unique combination of plant extracts and molecular ingredients to increase cytotoxic performance while allowing the reduction of serum by up to 90 percent.

Methods KHYG-1 NK cell line, or primary NK cells, were brought to 2% or 2.5% serum, respectively, in the prototype media. DMEM/F12 with 20% serum (for KHYG-1) or 10% serum (for primary cells) was the control. All media was supplemented with 100 U/mL IL-2. Cell numbers were periodically assessed, using a cell counter or flow cytometry. For cytotoxicity assays, K562 target cells were cocultured with KHYG-1 cells (effector:target ratio 20:1) for 5h, or with primary NK cells (4:1) for 18h. Cells were stained with annexin V and propidium iodide to determine the levels of apoptosis and necrosis by flow cytometry. Levels of secreted proteins were determined using Luminex. Expression of CD56 was determined by flow cytometry.

Results Following adaptation to 2% serum, growth of KHYG-1 cells in the prototype media stayed nearly on pace with the control for over 3 weeks. Human primary NK cells grew 10-fold over 10 days in the prototype media with 2.5% serum, without feeder cells; in contrast, primary cells did not proliferate in control medium (figure 1). Beta testing confirmed that the prototype media improved the proliferation of primary NK cells over a 12-day period, relative to a gold standard medium (figure 2). After culture in prototype media, both KHYG-1 cells and primary NK cells exhibited higher cytotoxic activity toward K562 cells, compared with the activity of the same cells cultured in control medium (figure 3). Secretion of interferon-gamma, perforin, and granzyme A was increased in KHYG-1 cells cultured in prototype media (figure 4). Additionally, culture in this media pushes NK cells toward a more CD56-dim phenotype (figure 5), which is associated with increased cytotoxic activity.

Abstract 210 Figure 1 Growth of NK cell line and human primary NK cells. (A) Growth of the KHYG-1 cell line, cultured in prototype media, kept pace with cells cultured in control medium. Orange indicates gradual adaptation to lower serum content; red indicates culturing in the formulation optimized for cell proliferation; blue indicates culturing in the formulation developed for NK cell activation. (B) Growth of primary NK cells was achieved in prototype media, but these cells did not grow in control medium.

A

B

Abstract 210 Figure 2 Primary NK cells grow better in prototype media

Abstract 210 Figure 3 NK cytotoxicity is enhanced by prototype media. (A and B) KHYG-1 cell line cocultured with K562 target cells. (C and D) Primary human NK cells cocultured with K562 target cells

Abstract 210 Figure 4 Secretion of interferon, perforin, and granzyme A

A

B

Abstract 210 Figure 5 Shift to CD56-dim phenotype

Conclusions The prototype media increases the cytotoxic activity of NK cells against cancer cells. Also, it supports proliferation of NK cell lines and primary NK cells, even at reduced serum content.

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