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Robust expansion of human g-NK cells in NK MACS® GMP Medium

Austin Bigley, PhD, Shanae Spade, Nadia Agha, PhD, Mariam Khalil, Lyle Babcock, PhD, Jenna Recker, and Rachel Bates
Indapta Therapeutics, Houston, TX, US

Background

Natural killer (NK) cells comprise a population of cytotoxic innate lymphoid cells capable of direct recognition and elimination of virally infected cells and tumor cells. Equipped with the ability to elicit several cytotoxicity mechanisms and secrete pro-inflammatory cytokines, NK cells play a central role in anticancer immunity. One of the most potent cytotoxicity mechanisms displayed by NK cells is triggered by the recognition of antibody-coated cells through their FcγRIII (CD16) receptor, a process termed antibody-dependent cellular cytotoxicity (ADCC). Thus, many immunotherapy strategies aim to boost NK cell-mediated ADCC to achieve greater anti-tumor responses.

The newly described FcεRIγ-deficient NK cells (g-NK cells) can display an increased ADCC activity after CD16 crosslinking compared to conventional NK cells, holding great potential for cancer therapy in combination with monoclonal antibodies (mAbs).[1–2] In fact, g-NK cells have shown enhanced anti-myeloma ADCC responses and *in vivo* elimination of myeloma tumor burden when combined with the therapeutic monoclonal antibody daratumumab.[3] This population of g-NK cells represents a small fraction in the total NK cell compartment of only some cytomegalovirus (CMV)-seropositive individuals.[2–3] The low availability of g-NK cells implies the need for *ex vivo* expansion in order to generate sufficient numbers for clinical doses.

NK MACS GMP Medium (Phenol Red) is an animal-component free medium specifically developed for the optimal *ex vivo* expansion of isolated human NK cells or NK cells from PBMCs. This application note shows exemplary data from the work performed by Indapta Therapeutics on the GMP-compliant expansion of g-NK cells in NK MACS GMP Medium compared to another commercially available medium.

Materials and methods

g-NK cell expansion
Using Indapta's proprietary feeder cell line and pro inflammatory cytokines including IL-2, g-NK cells were expanded from magnetically sorted $CD3^-/CD56^+$ NK cells, in either NK MACS GMP Medium or another commercial GMP-compliant cell culture medium (Medium 1).
Each medium was supplemented with 5% AB serum. The cells were expanded for two weeks at 37 °C and 5% CO_2 supply. Cell numbers were determined at days 5, 7, 9, 11, and 14.

ADCC assays
ADCC potency assays were performed using the multiple myeloma (MM) cell lines LP1 (DSMZ) and KMS-34 (JCRB). Expanded NK cells were co-cultured with 3×10^4 CD71-stained MM target cells at 0.5:1 and 1:1 NK cell to target cell ratios in the presence or absence of 1 µg/mL daratumumab (anti-CD38) or 1 µg/mL elotuzumab (anti-SLAMF7). After a 4-hour incubation at 37 °C in a CO_2 incubator, the cells were washed and stained to quantify the number of NK cells, live target cells, and dead target cells by using a 4-color flow cytometry panel.[4]

Antibody-dependent degranulation, cytokine, and cytolytic enzyme expression
NK cells (2.0×10^5) were added to the same MM target cells at a 0.5:1 NK:MM ratio under three conditions: no mAb, 1 µg/mL daratumumab, or 1µg/mL elotuzumab.
The VioGreen™ Dye-conjugated anti-CD107a was added to the co-culture and incubated for 1 hour at 37 °C in a CO_2 incubator. Next, monensin (BD GolgiStop™) and brefeldin A (BD GolgiPlug™) were added to the co-culture, and the cells were incubated for an additional 5 hours.

Once harvested, the cells were washed and stained with anti-CD45, anti-CD3, and anti-CD56 antibodies. Finally, the cells were fixed and permeabilized using the Inside Stain Kit, and stained with anti-FcεRIγ, anti-perforin, anti-granzyme B, anti-interferon-γ (IFN-γ), and anti-tumor necrosis factor-α (TNF-α) antibodies for flow cytometric analysis.

Results

Expansion of g-NK cells is enhanced in NK MACS GMP Medium

Using Indapta's proprietary feeder cell line and cytokine cocktail, g-NK cells were expanded in either Medium 1 or in NK MACS GMP Medium. After 14 days in culture, a much higher expansion rate of the cells was achieved when cultured in NK MACS GMP Medium compared to Medium 1 (fig. 1).

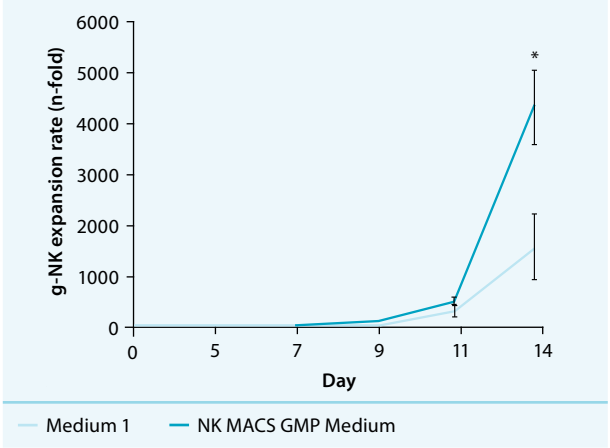

Figure 1: Expansion rate of g-NK cells after 14 days in culture. The expansion rate of g-NK cells was determined at different time points during the 14 days of culture, either in Medium 1 or in NK MACS GMP Medium. The values shown correspond to mean ± SE and *p<0.05 (n=7).

g-NK cells expanded in NK MACS GMP Medium show enhanced ADCC against different myeloma cell lines

After 14 days in culture, the functionality of the expanded g-NK cells was assessed via ADCC assay using two different mAbs (elotuzumab and daratumumab) targeting the myeloma cell lines KMS-34 and LP1. The g-NK cells expanded using NK MACS GMP Medium had a higher elotuzumab- and daratumumab-mediated ADCC response against KMS-34 and LP1 myeloma cells compared to g-NK cells expanded in Medium 1 (fig. 2).

The enhanced ADCC response of NK MACS GMP Medium-expanded g-NK cells correlated with a higher mAb-mediated degranulation (figs. 3A, 3B). Interestingly, the expression levels of perforin and granzyme B were comparable in the cells expanded with either medium (figs. 3C, 3D).

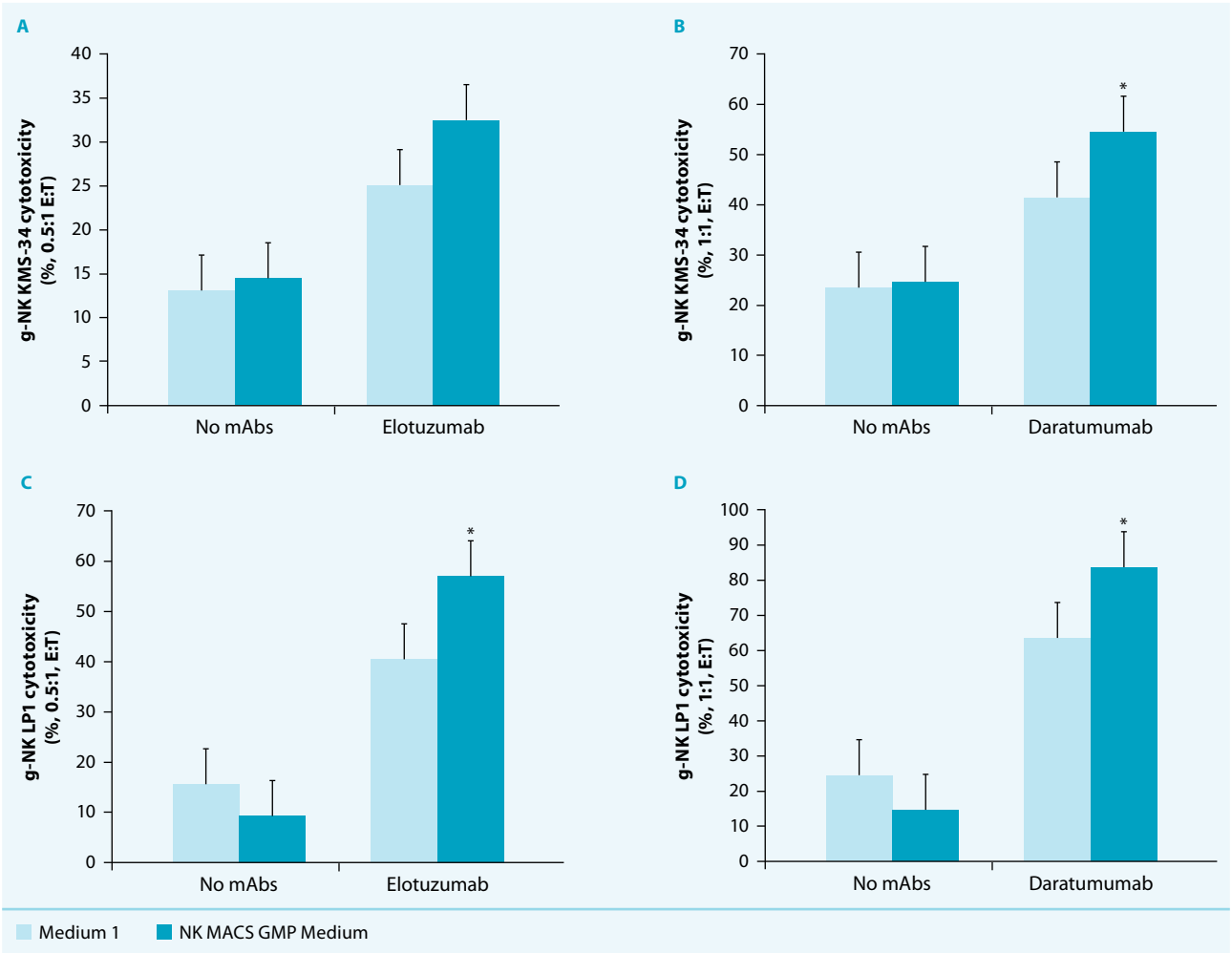

Figure 2: *In vitro* ADCC activity of expanded g-NK cells against myeloma cell lines. The cytotoxic activity of expanded g-NK cells in Medium 1 or in NK MACS GMP Medium was evaluated against KMS-34 (A and B) and LP1 (C and D) myeloma cell lines in the presence or absence of the mAbs daratumumab or elotuzumab. Values shown correspond to mean ± SE and *p<0.05 (n=7).

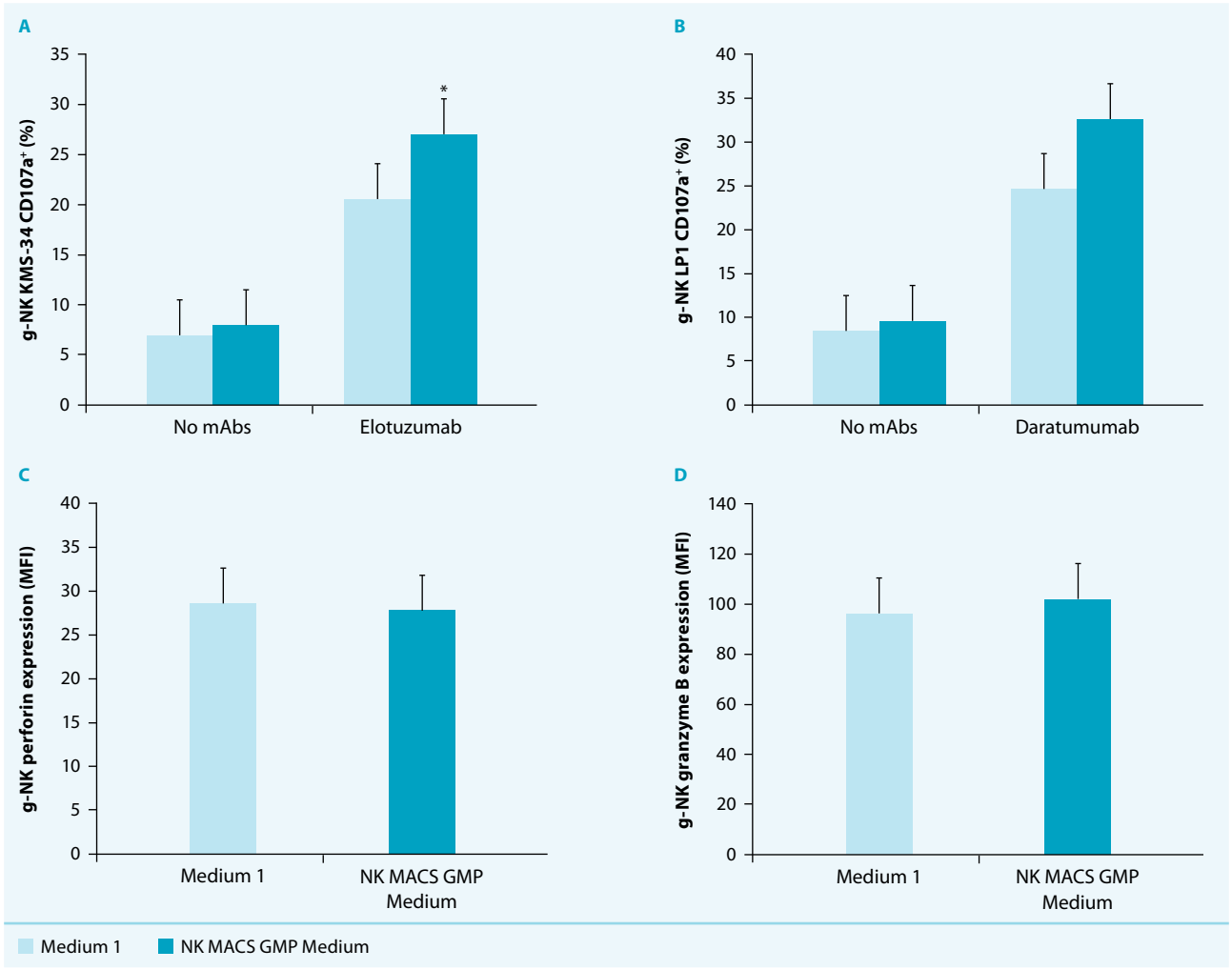

Figure 3: Analysis of degranulation rates and levels of perforin and granzyme B of expanded g-NK cells upon ADCC against myeloma cell lines. The degranulation rate of g-NK cells was determined by CD107a expression upon elotuzumab- and daratumumab-mediated ADCC (A and B) against KMS-34 and LP1 cells, respectively. The levels of perforin (C) and granzyme B (D) were determined at baseline by mean intensity fluorescence (MFI). The values shown correspond to mean ± SE and *p<0.05 (n=7).

g-NK cells expanded in NK MACS GMP Medium maintain their potential for cytokine secretion

Expanded g-NK cells showed an increased production of IFN-γ and TNF-α upon targeted ADCC against KMS-34 and LP1, using either elotuzumab or daratumumab (fig. 4). Slightly decreased responses of IFN-γ (figs. 4A, 4B) and TNF-α (figs. 4C, 4D) production were detected on g-NK cells expanded in NK MACS GMP Medium, which may be attributed to a slightly higher baseline activation of Medium 1-expanded g-NK cells as there was no difference in the antibody response.

Conclusions

- NK MACS GMP Medium is a specialized formula that supports the robust *ex vivo* expansion of NK cells such as g-NK cells
- As shown, g-NK cells expand at a higher rate in NK MACS GMP Medium compared to another commercially available GMP-compliant cell culture medium
- NK MACS GMP Medium also preserves the functionality of the cells as demonstrated by the functional assays performed after g-NK cell expansion

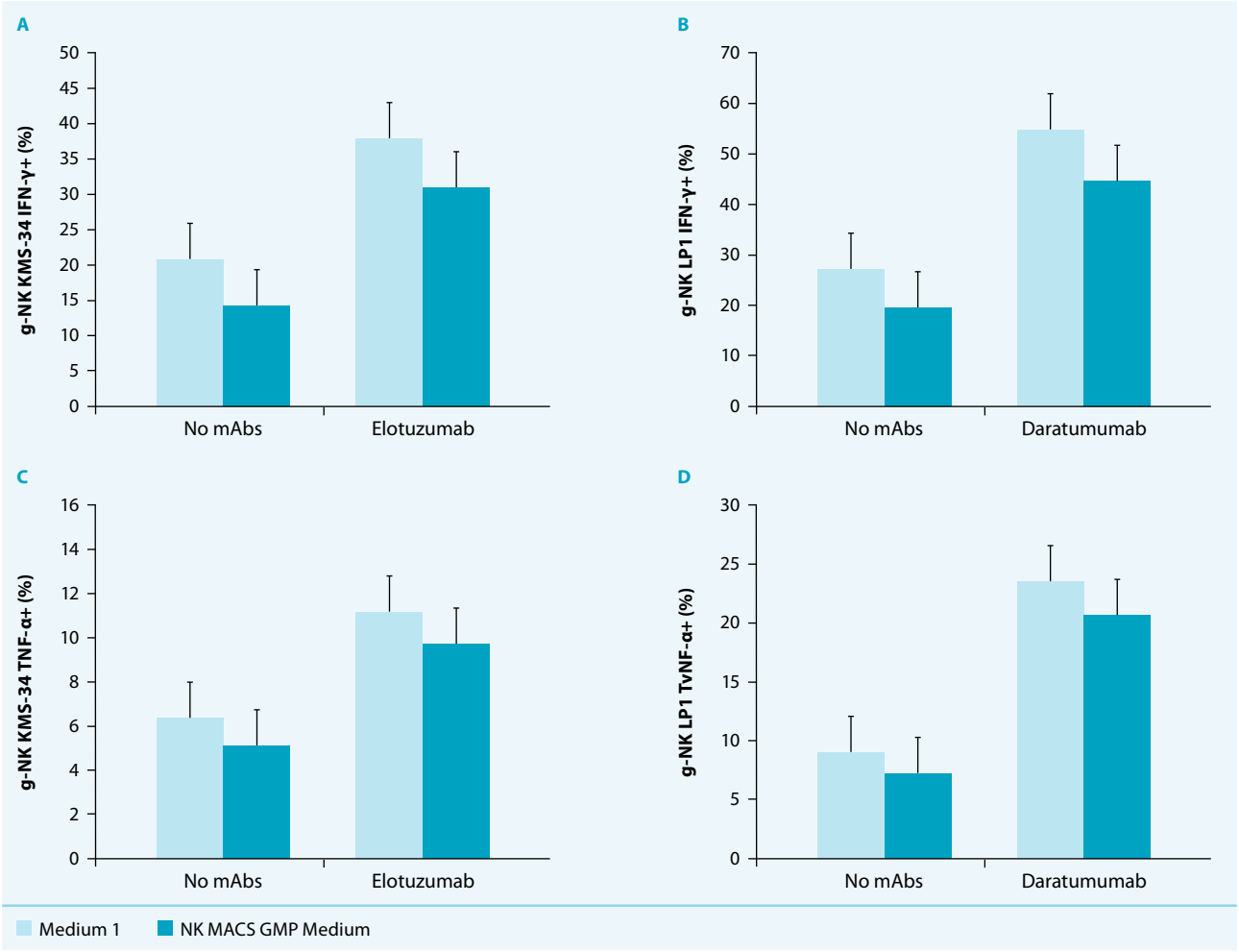

Figure 4: Analysis of IFN-γ and TNF-α production of expanded g-NK cell upon ADCC against myeloma cell lines. The frequency of cells expressing IFN-γ and TNF-α was analyzed upon elotuzumab- and daratumumab-mediated ADCC against KMS-34 (A and C) and LP1 (B and D) cell lines, respectively. The values shown correspond to mean ± SE (n=7).

References

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Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**

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