# Artificial Intelligence-Based Dynamic Single-Cell Imaging Reveals Enhanced Migration and Immune Synapse Formation by IDP-023, an Allogeneic G-NK Cell Product

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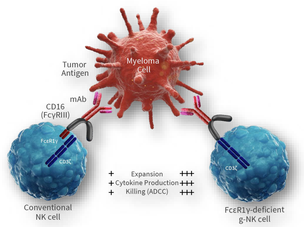
## Abstract

**Background:** FcεR1γ-deficient Natural Killer (g-NK) cells are a naturally occurring subset of NK cells that occur in a portion of individuals latently infected with cytomegalovirus. The lack of FcεR1γ-adapter protein results in exclusive signaling through CD3ζ and consequently, significantly enhanced CD16-mediated antibody-dependent cellular cytotoxicity (ADCC) compared to conventional NK (cNK) cells in bulk coculture assays with tumor cells. However, the resulting cellular mechanisms underlying this enhanced ADCC activity of g-NK cells are not fully understood.

**Methods:** Artificial intelligence-powered **T**ime-lapse **I**maging **M**icroscopy **I**n **N**anowell **G**rids (TIMING™) was applied to compare the interaction dynamics and cytotoxicity kinetics of cNK and g-NK cells at a single-cell level with a human multiple myeloma cell line (LP-1) with or without the anti-CD38 monoclonal antibody (mAb) daratumumab (dara). Nanowell grids were seeded with target and NK cells of either subtype at varying E:T ratios. Cell identity, migration, interaction dynamics, and cytotoxicity were quantified and statistically tested for the effect of mAb on cNK or g-NK cells.

## G-NK Platform

### Novel Subset of Therapeutic NK Cells for Cancer and Autoimmune Disease




**G-NK cells show markedly higher antibody-dependent cytotoxicity (ADCC) than conventional NK-cells**

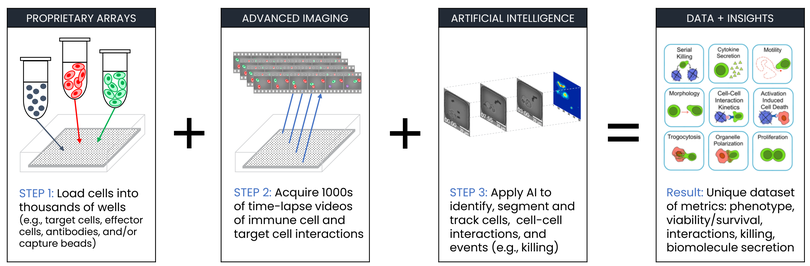
- Called ”G minus NK cell” or ‘g-NK’ because lack the FcεR1γ[1,2]

  CD16 engagement via Fc, triggers exclusive signaling through CD3ζ resulting in:
  - Increased cytokine secretion
  - Higher levels of cytolytic enzymes
  - Superior ADCC

  as compared to conventional *ex vivo* expanded NK cells (cNK).

## CELLCHORUS TIMING™ Technology

### AI-Powered Single Cell Imaging for Deep Insights into Cell Interaction, Function and Drug Activity




STEP 1: Load cells into thousands of wells (e.g., target cells, effector cells, antibodies, and/or capture beads)

STEP 2: Acquire 1000s of time-lapse videos of immune cell and target cell interactions

STEP 3: Apply AI to identify, segment and track cells, cell-cell interactions, and events (e.g., killing)

Result: Unique dataset of metrics: phenotype, viability/survival, interactions, killing, biomolecule secretion

Machine learning powers thousands of microscopy experiments in parallel.

## Results

### G-NK Cells Are More Efficient at Synapse Formation than cNK Cells, Both With & Without Targeting mAb

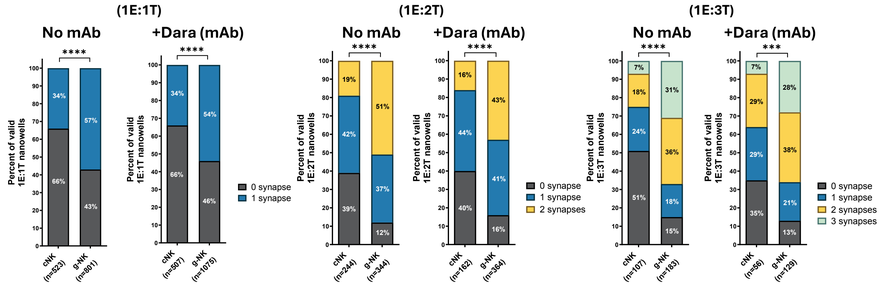



**Figure 1. Synapse event analysis (see Fig 3B) reveals synapse formation with target tumor cells was almost 2x higher for g-NK cells as compared to cNK cells.** The addition of Dara (mAb) did not alter the frequency of synapse formation in either cNK or g-NK cells suggesting that synapse formation occurs independent of CD16/mAb/Target engagement. P-value generated using Fisher’s exact test.

### Increased Synapse Formation Translates into Significantly Improved ADCC Activity of G-NK Cells

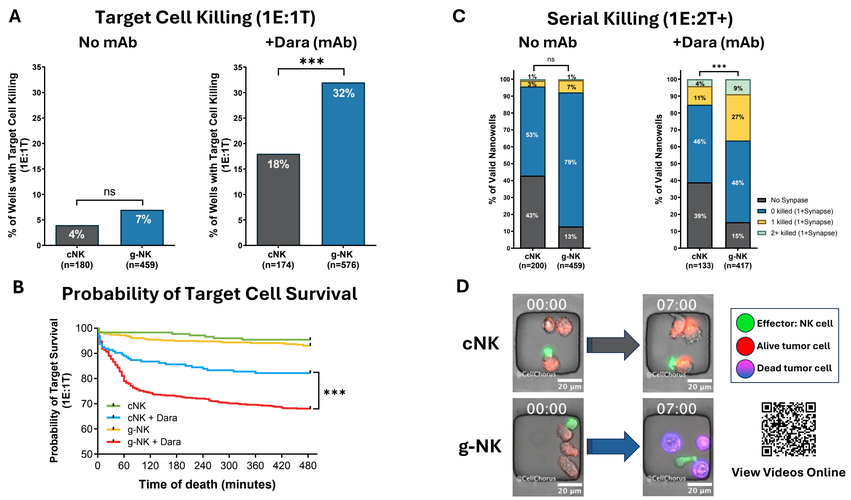



**Figure 2. ADCC activity of g-NK cells is superior to cNK cells and results in improved serial killing.** **(A)** ADCC target cell killing (at 1E:1T) was significantly higher for g-NK cells as compared to cNK. **(B)** Target cell killing was improved by addition of dara for both g-NK and cNK but killing of targets was significantly faster by G-NK cells (slope of curve) as observed in Target Survival Probability (1E:1T) curves. **(C)** Serial Killing (1E:2T+) by g-NK cells + mAb was significantly higher. Killing events plotted are those which occurred in wells with one or more synapses. **(D)** Representative Images of Nanowells. P-values determined by Fisher’s exact test. P-value generated using Kaplan-Meier analysis with log-rank tests (Mantel-Cox, trend, and Gehan-Breslow-Wilcoxon).

## G-NK Cells Migrate Faster than cNK

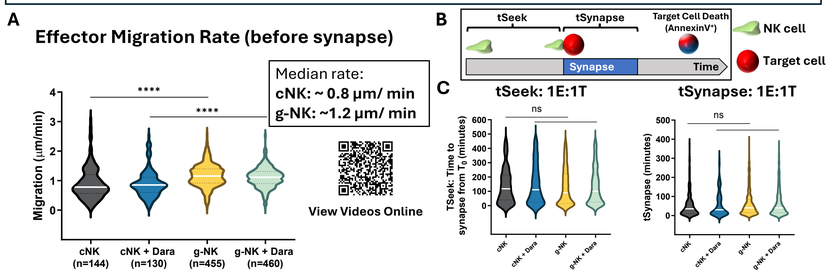



**Figure 3. G-NK cells exhibited a 1.5x higher rate of migration compared to cNK cells.**

**(A)** Migration of the NK cells were measured in nanowells over time. **(B)** Analysis Schematic **(C)** Interestingly, the rate at which g-NK cells found their targets (tSeek) and formed a synapse (tSynapse) was similar between g-NK and cNK suggesting that synapse formation and rate of migration may be independent variables in this system. P-value generated using Fisher’s exact test.

## Adhesion Molecule CD2 (LFA-2) is Enriched on G-NK Cells

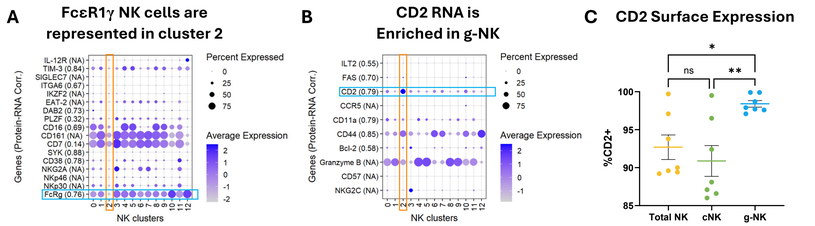



**Figure 4. Single cell RNA sequencing data from clear cell renal carcinoma biopsies[3] reveal the presence of endogenous g-NK cells enriched for CD2 which was confirmed by flow cytometry on ex vivo expanded g-NK.** **(A)** mRNA expression profile of NK cell subsets, FcRg negative g-NK cells are represented in cluster 2 **(B)** Select markers positively associated with g-NK cells showed CD2 (LFA-2) RNA was enriched in g-NK, Cluster 2. **(C)** CD2 protein expression on *ex vivo* expanded g-NK cells was confirmed by flow cytometry in total NK (CD56+), cNK (FcεR1γ+), and g-NK (FcεR1γ-) populations. *p<0.05, **p<0.01, One-way ANOVA, Tukey post-hoc test for multiple comparisons.

## Conclusions

- Enhanced ADCC of g-NK cells is driven in part by a higher frequency of synapse formation with target cells compared to cNK.
- Greater frequency of synapse formation leads to overall greater ADCC activity against at the single-target and serial killing level.
- CD2 (LFA-2), a protein involved in leukocyte adhesion and immune synapse formation was found to be enriched on g-NK cells consistent with their increased migration and cytotoxic functions consistent with findings in CAR-T[4].
- These results provide new mechanistic insights into how g-NK cells improve therapeutic efficacy of mAbs for the treatment of cancer or autoimmune disease.
- IDP-023 is currently in clinical trials for the treatment of advanced hematologic cancers (NCT06119685).

1. Bigley, A.B., et al (2021). Blood Adv, 5(15): 3021-3031.; 2. Dahlvang JD, Dick JK, Sangala JA, et al. *J Immunol.* 2023;210(8):1108-1122.; 3. Obradovic, A., et al (2021). Cell, 184(11): 2988-3005.; 4. Romain G., et al. *J Clin Invest.* 2022;132(17)

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