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Brief Communication

Vemurafenib Enhances NK cell Expansion and Tumor-killing Activity for Cancer Immunotherapy

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Natural killer (NK) cells are innate immune cells and play important roles as the first immune cells to recognize and kill cancer. In patients with advanced and terminal cancer, NK cells are often inactivated, suggesting that NK cells may play important roles in cancer treatment. In particular, the proportion of NK cells among immune cells infiltrating tumor tissues is often low, which suggests that NK cells do not survive in tumor microenvironment (TME). In order to overcome these hurdles of NK cells in cancer treatment, it is critical to develop strategies that enhance the proliferation and cytolytic activity of NK cells. We applied Vemurafenib to NK cells and measured the degree of NK cell proliferation and functional activation. We obtained unexpected results of increased NK cell numbers and anti-tumor activity after Vemurafenib treatment. Although further investigation is required to uncover the detailed mechanisms, our results suggest that Vemurafenib is a promising candidate to increase the efficacy of cancer immunotherapy using NK cells.

Key Words: Vemurafenib, NK cell, Cancer, Immunotherapy

NK cells, which are CD3 negative and CD56 positive subsets, are innate immune cells responsible for a significant role in destroying both tumors and virus-infected cells (Shin et al., 2020). They make up around 5% to 15% of lymphocytes found in the human bloodstream (Berrien-Elliott et al., 2023). When stimulated, NK cells exhibit anti-tumor capabilities against tumor cells by releasing effector cytokines such as interferon (IFN)-γ, along with cytolytic granules that contain perforin and granzyme B (Shin et al., 2020; Wolf et al., 2023). The cytolytic activity of NK cells is controlled by a variety of activating and inhibitory receptors present on their cell surfaces (Shin et al., 2020). Furthermore, NK cells play a role in the process of apoptosis by regulating death receptors including TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FasL) (Prager and Watzl, 2019; Ramírez-Labrada et al., 2022). The diverse and immunosuppressive TME substantially impairs the activity of NK cells and their ability to penetrate tumors (Lim et al., 2021; Shin et al., 2023; Zhou et al., 2023). As a result, formulating a strategy to enhance cytotoxicity and proliferation of NK cells is essential for the success of cancer immunotherapy.

Vemurafenib is a specific inhibitor of mutated serine-threonine kinase v-Raf murine sarcoma viral oncogene homolog B (BRAF) V600E oncoprotein approved in the United States (US) and Europe for treatment of advanced melanoma (Kim et al., 2014; Swaika et al., 2014). The presence of the mutated BRAF V600E protein results in persistent activation of the mitogen-activated protein kinase (MAPK) signaling pathway (Davies et al., 2002; Ascierto

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et al., 2012). This, in turn, triggers cellular proliferation that is independent of growth factors, fuels oncogenic phenotype by reducing apoptosis, and augments invasiveness (Ascierto et al., 2012). The specific inhibition of mutated BRAF V600E by Vemurafenib interrupts the BRAF / mitogen-activated ERK kinase (MEK) / extracellular signal-regulated kinases (ERK) pathway to cause apoptosis in melanoma cells (Beck et al., 2013; Sanchez et al., 2018).

The anti-tumor effects of Vemurafenib have led us to propose that Vemurafenib may stimulate effector immune cells such as NK cells. In this study, we explored the effects of Vemurafenib on proliferation and cytotoxic activity of NK cells.

The following methods were employed. Peripheral blood mononuclear cells (PBMCs) were obtained from the peripheral blood of healthy donors using Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden). PBMCs were grown in RPMI 1640 medium enriched with 10% Fetal Bovine Serum (FBS) and recombinant human interleukin-2 (rhIL-2) at a concentration of 500 U/mL (rhIL-2, Proleukin; Novartis, Basel, Switzerland). K562 cells, which were exposed to gamma irradiation at a dose of 100 Gy, were co-cultured with PBMCs to serve as feeder cells. K562 (human leukemia cell line) and CEM (human leukemia cell line) cells were grown in RPMI 1640, contained 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin. Vemurafenib was purchased from Selleckchem (Houston, Texas, United States). The study was granted approval by the Institutional Review Board, and it was conducted with the informed consent of the donors (IRB# KUIRB-2021-0264-01).

The total count of NK cells was ascertained by multiplying the overall count of viable cells by the proportion of CD56+CD3- cells, which was assessed with flow cytometry. The fold increase in NK cell numbers was determined by dividing the count of viable NK cells at the end of the culture period by the count of viable NK cells at the outset of the culture.

The CD107a and IFN-γ levels were assessed according to the following protocols. NK cells, exposed to either Dimethyl sulfoxide (DMSO) or Vemurafenib, were co-cultured with K562 and CEM cells at an Effector:Target (E:T) ratio of 1:1 in a round-bottomed 96-well plate. Anti-

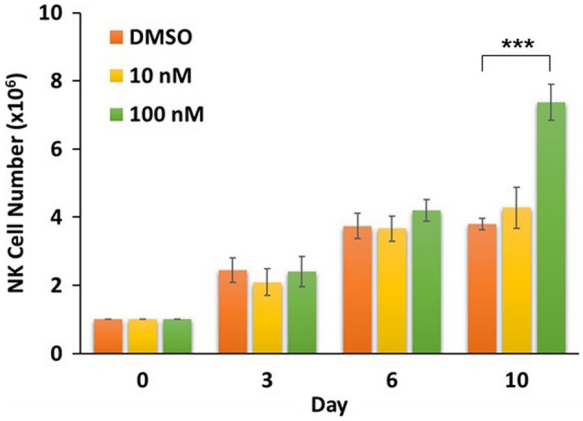

Fig. 1. The effect of Vemurafenib on the proliferation of NK cells was examined. Average cell counting results representing four different donors demonstrated that a concentration of 100 nM Vemurafenib resulted in an enhancement of NK cell proliferation. Data obtained from four different individual donors are presented as the mean ± SEM. ***, $P < 0.001$.

CD107a-FITC monoclonal antibody (mAb) and Golgistop (BD Pharmingen) were included, and the co-culture was incubated for 6 h. The samples were treated for fixation and permeabilization with the Cytofix/Cytoperm intracellular staining kits (BD Pharmingen). Subsequently, IFN-γ-PE mAb was introduced to quantify the intracellular IFN-γ of NK cells. Flow cytometric analysis was conducted with FACS CantoII (BD Bioscience), and CD3-PerCP negative and CD56-APC positive NK cells were selectively gated for analyzing with the FlowJo (Tree Star) software.

The cytotoxicity of NK cells exposed to Vemurafenib was assessed by employing the lactate dehydrogenase (LDH) cytotoxicity detection kit from Roche Diagnostics. Vemurafenib treated NK cells were co-cultured with K562 or CEM target tumor cells, and LDH activity released from the damaged tumor cells was quantified by spectrophotometer. The percentage of specific lysis was determined using the following calculation: [((absorbance (abs) 492 nm effector/target mix − abs 492 nm effector only) − abs 492 nm spontaneous)/(abs 492 nm maximum − abs 492 nm spontaneous)] × 100 (Lim et al., 2021).

The following results were observed. NK cells exposed to 100 nM Vemurafenib exhibited a remarkable average expansion of 7.360±0.529 fold by day 10, whereas NK cells cultured with DMSO showed limited average prolifer-

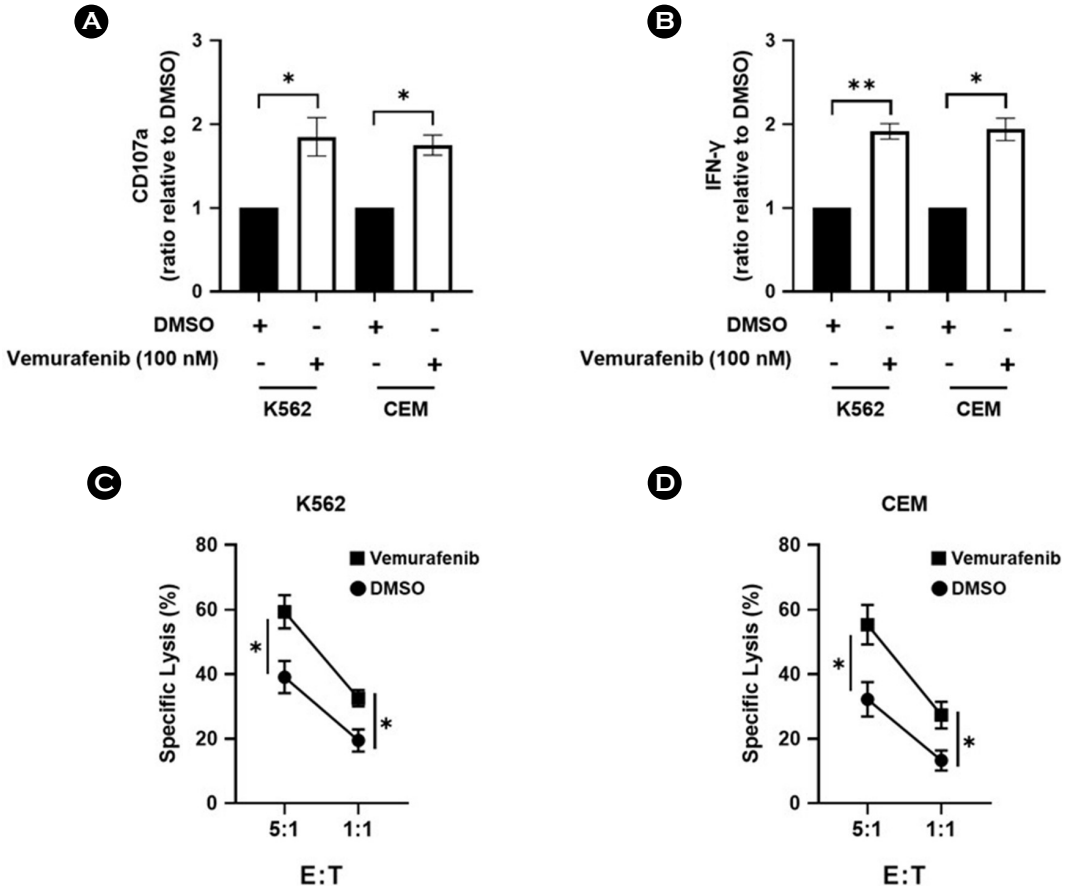

Fig. 2. Assessing the effect of Vemurafenib on NK Cell Anti-Tumor Activity. (A, B) Evaluating the degranulation and cytokine release of NK cells when challenged with K562 and CEM tumor targets. This was done by staining for surface CD107a and intracellular IFN-γ. (C, D) NK cell cytotoxicity against tumors was quantified by measuring LDH activity. The results are derived from three separate experiments, each employing PBMCs from different donors, and are presented as the mean ± SEM. *, $P < 0.05$; **, $P < 0.01$.

ation of 3.797±0.168 fold by day 10 from four different donors (Fig. 1, ***, $P < 0.001$). We subsequently investigated the effect of Vemurafenib on cytotoxicity against tumor cells and secretion of cytokines by NK cells. Following the administration of Vemurafenib, we assessed the degranulation of CD107a and the production of IFN-γ by NK cells when exposed to two distinct tumor targets: a human leukemia cell line, K562 and CEM. The proportion of CD107a-positive NK cells was increased in NK cells treated with Vemurafenib when exposed to K562 and CEM target tumor cells (Fig. 2A; for K562; 1.85 fold higher in Vemurafenib relative to DMSO, and for CEM; 1.75 fold higher in Vemurafenib relative to DMSO, *, $P < 0.05$). The Vemurafenib-treated group exhibited an elevated percentage of IFN-γ-positive NK cells when interacting with K562 and CEM target tumor cells (Fig. 2B; for K562; 1.9154 fold higher in Vemurafenib relative to DMSO, and for CEM; 1.940 fold higher in Vemurafenib relative to DMSO, *, $P < 0.05$; **, $P < 0.01$). The Vemurafenib-treated group exhibited an elevated level of NK cell tumor cytotoxicity, as measured by LDH cytotoxicity kit. This suggests that Vemurafenib augmented the anti-tumor capabilities of NK cells against both K562 and CEM tumor cell lines (Fig. 2C, D, *, $P < 0.05$).

Our findings uncover a previously undiscovered function of Vemurafenib that can improve the proliferation and functional characteristics of NK cells, thus offering promise for their application in adoptive cellular therapy for cancer treatment. These findings indicate that Vemurafenib represents significant potential as a promising candidate for repur-

posing to enhance the anticancer activity by improving the function of immune cells. Additional research is required to investigate the specific cellular and molecular mechanisms associated with how Vemurafenib functions in the activation of NK cells.

Abbreviations

Absorbance (abs)
Dimethyl sulfoxide (DMSO)
Effector:Target (E:T)
Extracellular signal-regulated kinases (ERK)
Fas ligand (FasL)
Fetal Bovine Serum (FBS)
Interferon (IFN)
Lactate dehydrogenase (LDH)
Mitogen-activated ERK kinase (MEK)
Mitogen-activated protein kinase (MAPK)
Monoclonal antibody (mAb)
Natural killer (NK)
Peripheral blood mononuclear cells (PBMCs)
Recombinant human interleukin-2 (rhIL-2)
TNF-related apoptosis-inducing ligand (TRAIL)
Tumor microenvironment (TME)
United States (US)
v-Raf murine sarcoma viral oncogene homolog B (BRAF)

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None.

CONFLICT OF INTEREST

The researcher claims no conflicts of interest.

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