Characterization of the anti-tumor activity of memory cytokine enriched NK cells (M-ceNK) against tumors with neuroendocrine features

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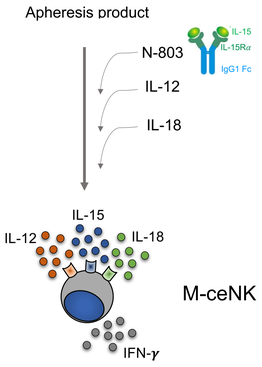
Background

Small cell lung cancer (SCLC) is an aggressive neuroendocrine (NE) carcinoma with few treatment options. Although immune checkpoint blockade (ICB) is approved in combination with chemotherapy in extensive stage disease, only a subset of patients experience an improvement in overall survival. Studies suggest that a lack of response to ICB is partially attributable to low expression of MHC-class I. In a recent report, our group found that the lack of MHC-class I can be utilized to enable targeting by NK cells stimulated with an IL-15 cytokine superagonist (N-803). These findings led us to hypothesize that cytokine stimulated memory-like NK cells (M-ceNK) may be effective in targeting SCLC.

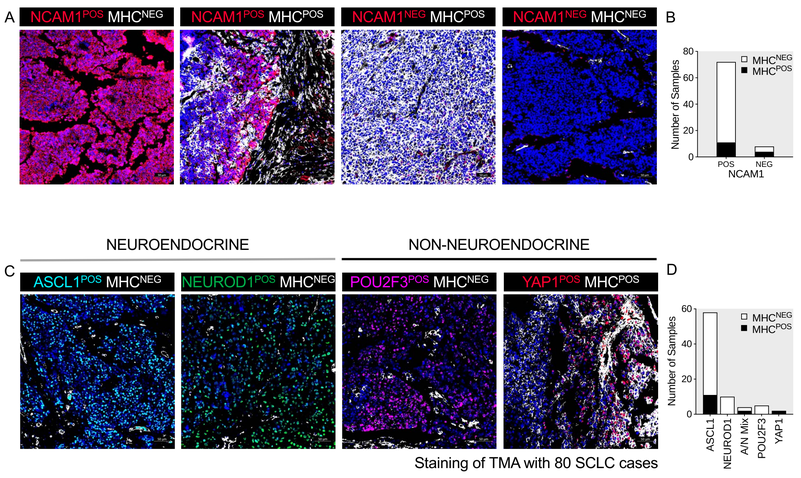
Methods

M-ceNK were derived from apheresis product from healthy donors via culture in the presence of cytokines including N-803 (a clinical-stage superagonist complex of a mutant human IL-15 combined with the sushi domain of hIL15Rα and fused to an IgG1 Fc domain), IL-12, and IL-18. Resulting M-ceNK were characterized by flow cytometry for expression of NK activating and inhibitory receptors as well as the intracellular expression of cytolytic mediators. Evaluation of the functional killing capacity of M-ceNK was assessed via 6-hour *in vitro* immune cytotoxicity assays against SCLC cell lines representative of each of the four molecular subtypes (ASCL1, NEUROD1, POU2F3, YAP1).



Foundation for the Study

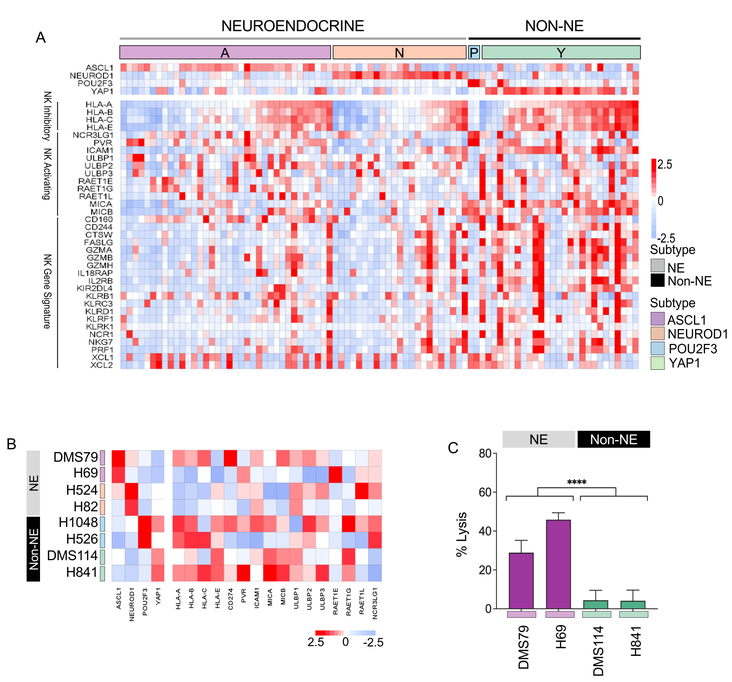
A majority of SCLC tumors lack expression of MHC-Class I, making them targetable by NK-driven therapeutic strategies



Staining of TMA with 80 SCLC cases

(A) Immunofluorescent staining of the NE marker NCAM1 and MHC-class I (NCAM1 = red, MHC-class I = white, DAPI = blue). (B) Quantification of 80 SCLC tumors scored based on positivity for NCAM1 and MHC-class I expression demonstrated that **81% of SCLC lack expression of MHC-class I**. (C) Immunofluorescent staining of transcription factors ASCL1, NEUROD1, POU2F3, YAP1, and MHC-class I in SCLC tissues of a tumor microarray (ASCL1 = turquoise, NEUROD1 = green, POU2F3 = pink, YAP1 = red, MHC-class I = white, DAPI = blue). (D) Quantification of 79 evaluable tumors based on predominant transcription factor expression and MHC-class I within each case showed that there was **no correlation between SCLC subtype and the lack of MHC-class I.** *Fousek et al., J Thorac Oncol. 2023 Mar;18(3):350-368.*

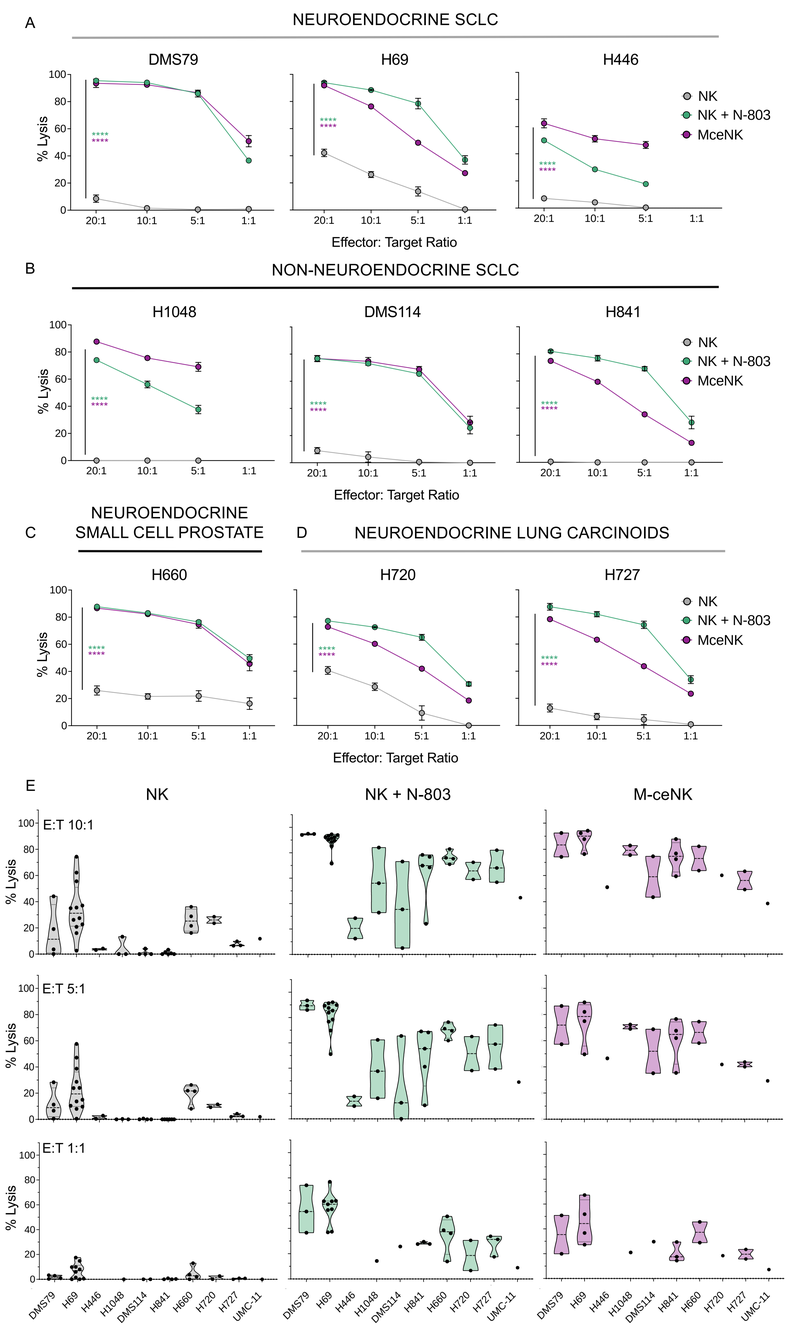
Analysis of metastatic SCLC tumor biopsies and a panel of SCLC cell lines showing expression of NK-related genes across the subtypes of SCLC



(A) Heatmap expression of ASCL1, NEUROD1, POU2F3, YAP1, and genes relevant to NK activity in 88 SCLC tumor biopsies obtained from 62 patients with metastatic SCLC previously analyzed by RNA-Seq analysis. **NK activating receptor and NK gene signature expression are variable across tumors, and YAP^{POS} tumors are enriched in NK inhibitory receptors.** (B) Heatmap of expression of selected NK activating and NK inhibitory ligand genes as determined by quantitative reverse transcription PCR across a **panel of 8 SCLC cell lines chosen for use in in vitro experiments** (NE = neuroendocrine; non-NE = non-neuroendocrine). Values illustrated correspond to $\log_2$-transformed gene expression relative to the control gene GAPDH in each cell line. (C) Neuroendocrine (DMS79, H69) and non-neuroendocrine (DMS114, H841) SCLC cells were assayed for susceptibility to NK cells at an effector to target (E:T) ratio of 10:1 in a 24-hour assay. **Non-NE SCLC was completely refractory to lysis by NK cells**. ****p<0.0001 comparing NE versus non-NE by unpaired t-test. Data illustrated are representative of n = 8 healthy NK donors. *Fousek et. al., J Thorac Oncol. 2023 Mar;18(3):350-368*

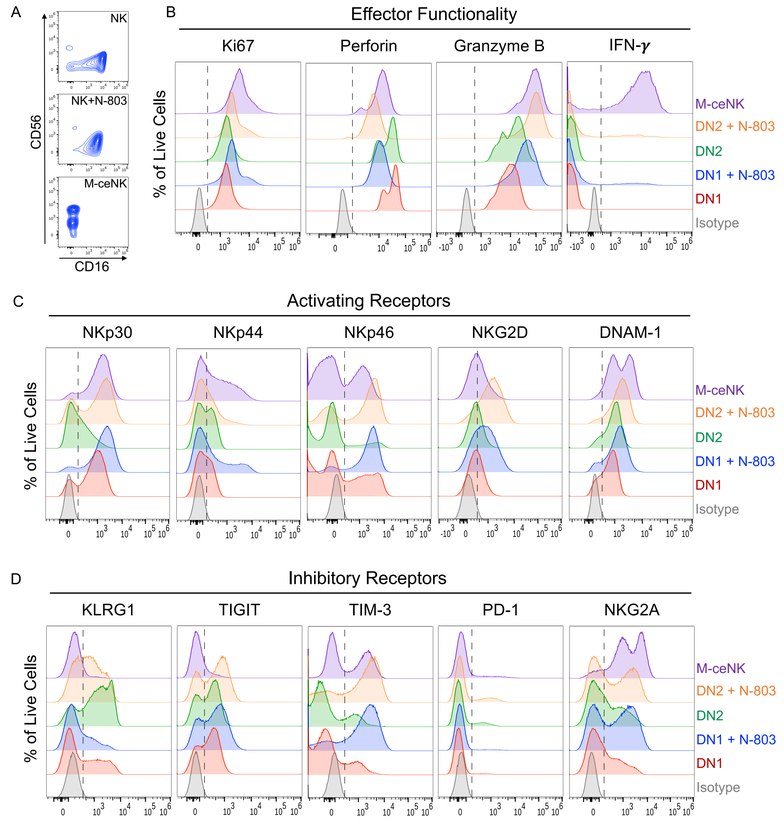
Results

SCLC and other neuroendocrine cancer cell modes are highly susceptible to lysis by M-ceNK



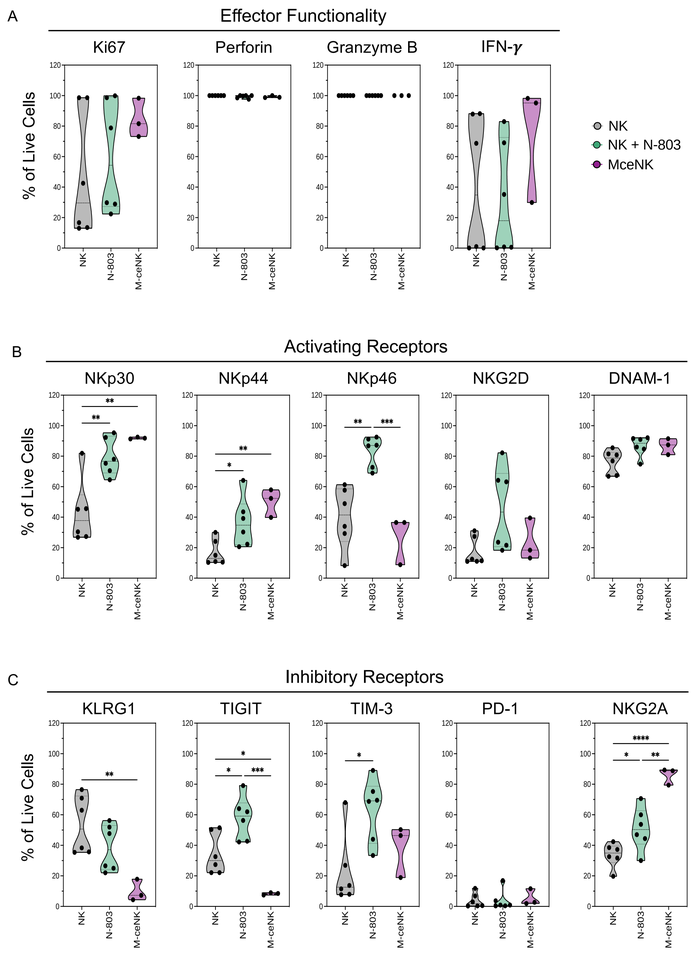
Six-hour NK lysis assay of (A) neuroendocrine SCLC, (B) non-neuroendocrine SCLC, (C) neuroendocrine small cell prostate, or (D) non-small cell neuroendocrine lung carcinoid cell lines using donor M-ceNK or NK cells freshly isolated or pre-incubated with 50 ng/mL N-803 for 48 hours before the cytotoxic assay, at the indicated E:T ratios. ****p<0.0001 by two-way ANOVA. Multiple comparisons to NK control represented by green for NK + N-803 condition and purple for M-ceNK condition. (E) Percent lysis achieved with NK cells +/- 50ng/mL N-803 (48-hour pre-incubation) or M-ceNK cells from multiple donors, across all cell lines at E:T ratios as indicated. Each data point indicates % lysis from an individual donor used in experiments. **M-ceNK demonstrate substantial lysis activity against all NE and non-NE target cell lines evaluated.**

M-ceNK exhibit a highly activated CD56^{HIGH} phenotype



(A) Representative flow cytometry plots depicting expression of CD56 and CD16 in healthy donor NK (untreated or pre-treated with 50ng/mL N-803 for 48 hours) and M-ceNK. **M-ceNK display a CD56^{HIGH} CD16^{NEG} phenotype.** (B-D) Flow cytometry histogram plots depicting the surface expression of markers of (B) proliferation and effector function, (C) NK activation, and (D) NK inhibition. Each plot displays data collected for two matched NK donors +/- N-803 pre-treatment (50ng/mL for 48 hours) and a single donor M-ceNK product. **M-ceNK exhibit a highly activated phenotype with minimal expression of inhibitory receptors.**

M-ceNK cells are highly activated and primed for killing

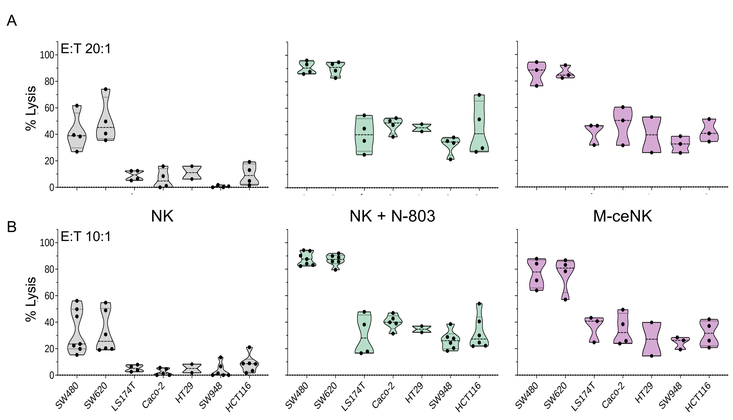


(A-C) Quantification of % of live cells expressing each indicated marker, assessed by flow cytometry in NK cells isolated from several matched healthy donors with or without N-803 exposure (50 ng/mL for 48 hours) as well as M-ceNK derived from three healthy donors. **Cytokine exposure increases the activation profile of NK cells, but only M-ceNK cells maintain a low expression of inhibitory markers.** Each dot represents a different healthy donor. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by one-way ANOVA with Tukey's multiple comparisons post-test.

Ongoing Studies

In vitro evaluation of M-ceNK in the context of other ICB resistant cancers

We aim to expand evaluation of the efficacy of M-ceNK to target additional models which are known to have poor responses to immune checkpoint blockade (ICB). Meta-analyses have shown that the overall response rate of colorectal cancer (CRC) to ICB is approximately 20%; moreover, CRC is described as a "cold" tumor with low expression of HLA-Class I. We have begun evaluating M-ceNK therapy in the context of CRC within *in vitro* models.



Percent lysis achieved against various colorectal cancer cell line models at an effector to target ratio of (A) 20:1 and (B) 10:1 with NK cells isolated from multiple healthy donors and cultured with or without N-803 (50 ng/mL for 48 hours) and M-ceNK derived from multiple healthy donors Each data point indicates % lysis from an individual donor used in experiments.

Future Studies

- Evaluation of the potential of the combination of M-ceNK and N-803 to provide efficacy in xenograft models of SCLC and other neuroendocrine tumors. The studies are being conducted in NSG-MHC I/II KO mice; tumor models of interest are H69, H841, H727.
- Additional studies are ongoing to determine the contribution of low MHC-class I or other tumor ligands to the mechanism of action enabling lysis by M-ceNK in the context of NE tumor models.

Summary and Relevance

These data demonstrate the potential for M-ceNK based approaches for the treatment of neuroendocrine tumors, including all molecular subtypes of SCLC.

SCLC is an aggressive disease with poor outcomes and few treatment options; while SCLC is the most well known neuroendocrine tumor type, these tumors also derive from many sites within the body, including small cell of the breast, prostate, colon, etc. Currently, most neuroendocrine tumors are treated with therapeutic regimens designed for SCLC, and although immunotherapy options are approved, they only provide modest improvements in survival to a small subset of patients.

These findings propose that M-ceNK may provide benefit to most patients with SCLC as well as patients with other types of neuroendocrine tumors. Furthermore, M-ceNK may provide an additional line of therapy in other cases of immunologically cold tumors lacking MHC expression after checkpoint blockade therapy.