Chemical priming of natural killer cells
with branched polyethylenimine for cancer immunotherapy

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## Supplementary Data

### Table S1. Antibodies used in this study

| Antibody       | Fluorochrome | Vendor     | Cat.    |
|----------------|--------------|------------|---------|
| CD16           | PE           | BD         | 556619  |
| CD45           | PE           | Invitrogen | 12-0459-42 |
| NKG2A          | PE           | BioLegend  | FAB1059P |
| KIR2DS1        | PE           | R&D Systems | FAB8887P |
| KIR2DL4        | PE           | R&D Systems | FAB2238P |
| KIR2DL1/S1/S3/S5 | PE     | BioLegend  | 339505  |
| CD226          | PE           | BD         | 559789  |
| CD314          | PE           | BD         | 557940  |
| CD335          | PE           | BD         | 557991  |
| CD336          | PE           | BD         | 558563  |
| CD49b          | PE           | BioLegend  | 359308  |
| CD69           | PE           | BD         | 560968  |
| Isotype control | PE           | BD         | 551436  |
| CCR5           | FITC         | Invitrogen | MA1-20282 |
| CCR7           | FITC         | BD         | 561271  |
| CD18           | FITC         | BD         | 559223  |
| CD2            | FITC         | eBioscience | 11-0029-42 |
| CXCR1          | FITC         | eBioscience | 11-1819-42 |
| CD56           | FITC         | BD         | 562794  |
| KIR2DL1        | FITC         | BD         | 556062  |
| KIR2DL2        | FITC         | BD         | 559784  |
| KIR3DL1        | FITC         | BD         | 555966  |
| Isotype control | FITC         | BD         | 556649  |
| CD107a         | APC          | BD         | 560664  |
| perforin       | APC          | BioLegend  | 353312  |
| granzyme B     | APC          | BioLegend  | 372203  |
| KIR3DL3        | Alexa Fluor 647 | R&D Systems | FAB8919R |
| Isotype control | APC          | BD         | 551414  |
| CD57           | PE Cy7       | BioLegend  | 359624  |
| CCR3           | PC Cy5.5     | BD         | 564189  |
| CXCR3          | PC Cy5.5     | BioLegend  | 353714  |
| Isotype control | PC Cy5.5     | BD         | 45-4031-80 |
27  **Figure S1.**

29  The viability of NK92MI cells exposed to the indicated concentration of 13 chemicals was analyzed by 30  7AAD staining and flow cytometry analysis.
Figure S2.

The cytotoxicity of NK92MI cells exposed to the 13 chemicals was analyzed by CFSE-7AAD assay. NK92MI cells were co-incubated with MDA-MB231 at E:T ratio of 10:1.
Figure S3.

Cytotoxicity of NK92MI cells treated with 25KbPEI for the indicated time (from 12 h to 72 h). Control (C_NK) or 25KbPEI-treated NK92MI cells were co-cultured for 4 h with MDA-MB231 cells; the cytotoxicity of NK cells was then analyzed in an CFSE-7AAD assay. Data are presented as the mean ± SD. Statistical comparisons with C_NK were performed using one-way ANOVA.
Figure S4.

The effect of 25KbPEI on the cytotoxicity of primary NK cells that were primed by cytokines. Primary NK cells were cultured in the basic culture medium containing IL-2 (10 ng/ml), IL-15 (10 ng/ml), IL-27 (10 ng/ml) and IL-18 (10 ng/ml) in the presence or absence of 25KbPEI. Control (C_pNK) or 25KbPEI-treated (Chem_pNK) primary NK cells were co-cultured with MDA-MB231 cells at E:T ratio of 10:1 for 4h; the cytotoxicity of NK cells was then analyzed in an CFSE-7AAD assay. Data are presented as the mean ± SD and Two-way ANOVA and Sidak's multiple comparison tests.
Figure S5

Cytotoxicity of CD56 bright (pink highlight) vs dims (yellow highlight) (a), and immature dims (NKG2A⁺KIR⁺CD57⁺: green highlight) vs mature dims (NKG2A⁺KIR⁺CD57⁺: blue highlight) (b) primary NK cells. Human primary NK cells were sorted into indicated populations using CytExpert SRT, and then each cell population was treated with 25KbPEI for 12 h. Their cytotoxicity against MDA-MB231 was analyzed by CFSE-7AAD assay.
Figure S6

a. Immunoblot analysis of perforin and granzyme levels in control (C_NK) and 25KbPEI-treated (Chem_NK) NK92MI cells. GAPDH was used as a loading control (left panel). The band intensities of the immunoblot were quantified using Image Lab (right panel). Data are presented as the mean ± SD. For significance, two-way ANOVA with correction for multiple comparisons using Sidak’s test was used.

b. Flow cytometry analysis of perforin accumulation in control (C_pNK) and 25KbPEI treated (Chem_pNK) primary NK cells. Statistical comparisons between C_pNK and Chem_pNK was performed using unpaired t-test.
**Figure S7**

Flow cytometry analysis of KIRs, NKG2A level in control (C_NK) and 25KbPEI treated (Chem_NK) NK cells. Statistical comparisons between C_NK and Chem_NK was performed using two-way ANOVA.
Figure S8

Bioluminescence images of A2780-Luc xenograft tumor-bearing nude mice on the indicated days. Mice were injected intravenously with DPBS, control (C_NK), or 25K bPEI-treated (Chem_NK) NK92MI cells on the indicated days.
Figure S9

Bioluminescence images of MDA-MB231-Luc orthotopic tumor-bearing nude mice on the indicated days. Mice were injected intratumorally with DPBS, control (C_NK), or 25K bPEI-treated (Chem_NK) NK92MI cells on the indicated days.
**Figure S10**

$^{13}$C-NMR analysis of 25KbPEI and 25KfbPEI. The fluorocarbon and primary amine peaks are marked in cyan and red, respectively.
Figure S11

a. Gel retardation assay assessing the DNA binding affinity of bPEI and fbPEI.

b. Quantitative analysis of the gel retardation assay used to analyze the DNA binding affinity of bPEI and fbPEI. ***P<0.001.
**Figure S12**

Time-lapse flow cytometry observation of calcium influx of primary NK cells that were treated with DPBS (C_pNK), 25KbPEI (Chem_pNK) or 25KbPEI (fChem_pNK). Fluorescent calcium indicator, Fluo-4, was added to the culture medium of primary NK cells and calcium influx (MFI) was monitored at the indicated times.
**Figure S13**

**a.** Flow cytometry-based quantitative analysis of control (C_pNK) or 25KbPEI treated (Chem_pNK) primary NK cells containing fluorescent Fluo-4 in the presence or absence of the TRAM2 inhibitor 2-aminoethoxydiphenyl borate (2-APB).

**b.** Quantitative flow cytometry analysis of perforin protein levels in C_pNK and Chem_pNK cells in the presence or absence of 2-APB Data are presented as the mean ± SD. Statistical comparison were performed using two-way ANOVA Sidak’s multiple comparison tests.
Supplementary video
Supplementary video 1

A confocal time-lapse live cell imaging system was used for real-time monitoring of the cytotoxic effects of control (C_NK), 25K bPEI-treated (Chem_NK), and fluorinated 25K bPEI-treated (fChem_NK) NK92MI cells against MDA-MB231 cells.
Supplementary video 2

A confocal time-lapse live cell imaging system was used for real-time detection of calcium influx into control (C_NK), 25K bPEI-treated (Chem_NK), and fluorinated 25K bPEI-treated (fChem_NK) NK92MI cells. Intracellular calcium influx was detected using the fluorescent calcium indicator Fluo-4.