Central Nervous System Acute Lymphoblastic Leukemia: role of Natural Killer Cells. Liron Frishman-Levy et al.

Supplementary File
**Figure S1:** IL-15 secretion from T-25 cells stably transfected with IL-15 expression vector (ELISA).
Figure S2: BALB/C mice were i.p. injected with T-25-IL15 (n=4) or T-25 (n=4) cells as control. Representative scatter plots demonstrating increased proportion of NK cells (upper panel) as well as NK activation state (lower panel) in the T-25-IL15 group (ii and iv respectively) compared to the control group (i and iii).
**Figure S3:**

10⁶ primary bone marrow cells (>90% blasts) from 2 patients with BCP-ALL were injected in parallel into NSG and NOD-SCID mice by intrafemoral injections. Mice were sacrificed when they showed leukemic symptoms. Patient A was t(1;19) positive and had a CNS 2b status at initial diagnosis. Patient B had no known cytogenetic changes and a CNS 3 status. (A) The percentage of leukemic blasts in the peripheral blood of mice was assessed by FACS analysis of human CD19/human CD45/mouse CD45 when mice started showing neurological symptoms (day 26 for patient A, day 46 for patient B). (B): Post-mortem splenic volume in the xenograft mice as assessed by the formula longest length x broadest width x highest height (cm³). (C) Total number of spleen cells (>90% blasts) recovered from the xenograft mice. (D) Histological scoring of CNS infiltration according to the algorithm published by Krause et al.: Negative (-), no detectable cells in the space between skull bone and brain tissue; intermediate (+), infiltration of the Dura but no subdural infiltration; positive (++), infiltration of the dura and the subdural space. (E) Representative examples of brain sections of one NSG and one NOD-SCID xenograft mouse for each patient. Hematoxylin/Eosin staining, 100x magnification view. Statistics were performed using Student’s t-test. P-values <0.05 were considered statistically significant.

**Figure S4:** Bioluminescence imaging of tumor growth in 018Z-engrafted NSG mouse model. Day 0, 7, 13 and 20 bioluminescence imaging data of mice treated with PBS (controls) and PBNK cells (PBS group: n=11; PBNK group: n=12). Quantification of bioluminescence and statistical analysis as in Figure 5A.
Figure S5: Staining of 018Z and REH cell lines with human Fc-fusion proteins of KIR2DL1 and KIR2DL2 followed by labeling with APC goat anti-human IgG. Non-specific binding was detected by staining with human Fc-protein (Filled Gray). Human Fc-fusion-KIR2DL1 (Black line), human Fc-fusion-KIR2DL2 (Dashed line).

**HLA class I and KIR genotyping:**

NK source: HLA: A*01, A*30, B*53, B*57, Cw*04 and Cw*06

REH cell line: HLA: A*23, A*32, B*35, B*50, Cw*04 and Cw*06

018Z cell line: HLA: A*01, A*02, B*08, B*15, Cw*04 and Cw*07

KIR genotyping of NK source used in this study: 2DL1, 2DL2, 2DL3, 2DL4, 2DL5B, 2DS2, 2DS4\textsc{full}, 2DS4\textsc{del}, 3DL1, 3DL2, 3DL3, 2DP1, 3DP1\textsc{del}.

HLA-KIR genotype comparison was performed using the EMBL-EBI website (http://www.ebi.ac.uk/ipd/kir/ligand.html).

018Z and REH expressed ligands for KIR2DL1 (Cw*04 and Cw*06) and 018Z expressed in addition HLA-Cw*07, a KIR2DL2/3 ligand

HLA-B alleles of NK source were both Bw4 (B*53/B*06) while both REH and 018Z cell lines HLA-B alleles were Bw6 (B*35/B*50, B*08/B*15 respectively).