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Effects of anti-NKG2A antibody administration on leukemia and normal hematopoietic cells

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Conflict of Interest:
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Abstract:
Natural Killer cells represent key cells of the innate immune system. Natural Killer cell receptor repertoires are diversified by a stochastic expression of Killer-cell-immunoglobulin-like receptors and lectin-like receptors such as NKG2 receptors. All individuals harbour a subset of Natural Killer cells expressing NKG2A, the inhibitory checkpoint receptor for HLA-E. Most of the neoplastic and normal hematopoietic cells express HLA-E, the inhibitory ligand of NKG2A. Killing of tumor cells was induced by a novel anti-human NKG2A antibody, suggesting it may have utility for treatment of cancers expressing HLA-E.
Immunodeficient mice, coinfused with human primary leukemias or EBV cell lines and NKG2A+ Natural Killer cells, pretreated with anti-human NKG2A, are rescued from disease progression. Human NKG2A+ Natural Killer cells reconstitute in immunodeficient mice after transplantation of human CD34+ cells.
These Natural Killer cells are able to kill engrafted human primary leukemia or EBV cell lines by lysis after intraperitoneal administration of anti-human NKG2A. Thus, this anti-NKG2A may exploit the anti-leukemic action of the wave of NKG2A+ Natural Killer cells recovering after hematopoietic stem cell transplants or of the adoptive therapy with Natural Killer cell infusions from matched or mismatched familiar donors after chemotherapy treatment of acute leukemia patient, without the need to search for an NK alloreactive donor.
**Introduction**

Natural killer (NK) cells play a critical role in host defence against infections and tumours by secreting cytokines and killing infected or transformed cells. Activation of NK-cell effector functions is regulated by activating and inhibitory receptors that recognize ligands on potential target cells. NK cell–mediated killing is efficient when target cells abundantly express stress- or transformation-induced ligands for activating NK receptors, and few or no major histocompatibility complex (MHC)–class I molecules, which are ligands for inhibitory receptors on NK cells. In humans, a family of Killer cell Immunoglobulin (Ig)–like receptors (KIRs) binds distinct subgroups of HLA class I allotypes. KIRs are clonally expressed on NK cells, creating a repertoire of NK cells with specificities for different HLA class I molecules. Due to extensive genetic polymorphisms, there is significant variations in the repertoire of KIR⁺-NK cells among individuals in the population. Another inhibitory receptor, with broad specificity, the CD94–NKG2A complex, recognizes HLA-E, a non-classical MHC class I molecule. CD94-NKG2A and its HLA-E ligand exhibit very limited polymorphism. CD94-NKG2A is expressed primarily on NK cells that do not express an inhibitory KIR for a self-HLA class I, so it fills gaps in the KIR repertoire. However, some NK cells co-express CD94–NKG2A and one or more inhibitory KIRs with different MHC class I specificities (1-3). NKG2A receptor is also expressed on T cells.

Individuals harbour NK cells in their repertoire that may express as the only inhibitory receptor a single KIR that is inhibited by one self MHC class I KIR ligand. Target cells that lack this KIR ligand do not block NK cell activation, and are killed. The clinical relevance of such missing-self recognition was demonstrated in adult patients with acute myeloid
leukemia (AML) and in children with acute lymphoblastic leukemias (ALL) (4-9). Haploidentical stem cell transplantation from KIR ligand mismatched donors (NK alloreactive donors) was associated with reduced risk of relapse and increased survival rates (4-8). Unfortunately, NK alloreactive donors cannot be identified for about 50% of patients who express each of the main three groups of KIR ligands (HLA-C group 1 and 2 and Bw4 specificity) that block all the NK cells in the donor repertoire. To extend the benefits of NK cell alloreactivity to these patients another strategy had to be found. A human anti-KIR monoclonal antibody (mAb) (Lirilumab) was generated to bind to all KIR2D inhibitory receptors specific for groups 1 and 2 HLA-C alleles. In vitro and murine model studies showed Lirilumab efficiently promoted NK cell alloreactivity and killing of otherwise resistant HLA-C group 1+ or group 2+ targets, like normal and tumor cells (10-13). Phase I clinical trials demonstrated anti-inhibitory KIR mAb is safe (14). Phase II clinical trials with Lirilumab are ongoing.

Another approach has been to generate and explore the role of an anti-human NKG2A antibody. Every individual possesses NKG2A+ NK cells which are always blocked by HLA-E. Since HLA-E is expressed by most of normal and neoplastic hematopoietic cells, these are protected from killing by CD94-NKG2A+ NK cells (1-3).

Stem cell transplantation remains the only curative treatment option for many patients with AL. Interestingly, in the immediate post-transplant period, most reconstituting NK cells are NKG2A+ (15). Nguyen and Godal already demonstrated in vitro anti-NKG2A antibody treatment is able to reconstitute NKG2A+ NK cell lysis against acute leukemia cells (16, 17). Administering an anti-NKG2A mAb could strengthen many of the benefits of NK cell alloreactivity and potentiate the anti-leukemic action of NK cells recovering after hematopoietic transplants or of NK
cell infusions from matched or mismatched familiar donors without the need to search for an NK alloreactive donor.

We have generated a novel, humanized anti-NKG2A therapeutic mAb that is being developed for treatment of solid tumors such as ovarian cancer and hematological malignancies. In this study, we investigated the potential clinical role of this new therapeutic mAb in vitro and in humanized mouse models.

**Methods**

**Therapeutic anti-NKG2A mAb**

The murine anti-human NKG2A mAb clone Z270 was generated and characterized as previously described (18). Details of the generation and characterization of humanized Z270 will be reported elsewhere. In brief, the murine Z270 mAb was humanized by grafting the Kabat CDRs onto a human acceptor framework, and expressed in CHO cells. Recombinant humanized clones were screened to identify those that retained binding to CD94/NKG2A with similar affinity as the original murine mAb. Clones were then counter-screened on CD94-NKG2C and –E, to ensure specificity for CD94-NKG2A. The selected humanized clone, designated humZ270, or IPH2201, was expressed as an IgG4 with a single point mutation in the Fc heavy chain to prevent formation of half-antibodies.

**Cell isolation**

All neoplastic cells were obtained from patients’ bone marrow aspirates or peripheral blood. All the lympho-hematopoietic normal cell types were obtained from healthy donors. Patients and donors provided prior written informed consent in accordance with the Declaration of Helsinki.
Neoplastic cells (if > 95% of all cells) were obtained from peripheral or marrow blood samples after a Ficoll-Hypaque gradient separation. Human T, B cells and monocytes were purified from peripheral blood mononuclear cells on a Ficoll-Hypaque gradient and enriched by human T, B isolation KITs or anti-CD14+ microbeads, respectively, and immunomagnetic selection (Miltenyi Biotec, Bergisch Gladbach, Germany). Dendritic cells (DCs) were obtained as described (19). Human NK cells were purified from peripheral blood mononuclear cells on a Ficoll-Hypaque gradient, enriched by human NK isolation KIT and immunomagnetic selection (Miltenyi Biotec). Single KIR+/NKG2A− NK cells were cloned and used as controls for NK cell alloreactivity assay as described (7). NKG2A+/KIR− NK cells were depleted of KIR2DL1/2/3+ and KIR3DL1+ cells using KIR2DL2/L3/S2 (clone CH-L, IgG2b) (BD Biosciences San José, CA), anti-KIR2DL1 (clone #143211, IgG1) (R&D Systems Inc., Minneapolis, MN) and KIR3DL1 (Miltenyi Biotech) Phycoeritrin (PE)-conjugated mAbs and negative selection by anti-PE immuno-magnetic microbeads (Miltenyi Biotech). NKG2A+/KIR− NK cells were stimulated by 1% phytohemagglutinin (PHA) (Biochrom, Berlin, Germany) and 250 UI/mL interleukin (IL)–2 (Novartis Farma S.p.A., Origgio, Italy), and expanded for up to 7 days. At the end of culture, before their use, final purity of NKG2A+NK cells was > 95%. CD34+ stem cells were obtained from healthy donors’ peripheral blood after mobilization with G-CSF growth factor, leukapheresis and positive selection by immunomagnetic microbeads conjugated with anti-human CD34+ mAb (Miltenyi Biotec).
EBV cell lines
HLA-E⁺ EBV-transformed B cell lines, which were resistant to NKG2A⁺ NK cell lysis, were kindly gifted by the European Collection for Biomedical Research (ECBR). Anti-human HLA-E-PE (IgG1, clone 3D12, eBioscience, San Diego, CA) was used to estimate HLA-E expression on EBV cell lines and all the other hematopoietic normal and neoplastic human cells by flow cytometry.

In vitro cytotoxicity assays
NKG2A⁺/KIR⁻ NK cells were pre-treated with humanized anti-human NKG2A Ab or with a isotype control antibody (10μg/1x10⁶cells/ml). Single KIR⁺/NKG2A⁻ and KIR⁻/NKG2A⁺ NK cells were screened for alloreactivity by standard 51Cr release cytotoxicity assays at an increasing effector-to-target (E:T) ratio (from 1:1 to 20:1) against KIR ligand mismatched HLA-E⁺ B and T cells, monocytes, DCs, EBV cell lines, chronic lymphatic leukaemia cells (CLL), T cell acute lymphoblastic leukaemia cells (T-ALL), AML and multiple myeloma cells (MM).

Mouse models
Colonies of Non-Obese Diabetic–Severe Combined Immunodeficiency (NOD-SCID) mice and NOD-scidIL2rgtm (NSG) mice were bred at the University of Perugia Animal House. Breeders were obtained from the Jackson laboratory (Bar Harbor, Maine, USA).
All experiments were performed in accordance with the National Ethic Approval Document for animal experimentation.
Female ten week old mice were irradiated with 3.5 Gy. The next day NOD-SCID mice received intravenous co-infusion of primary AML cells (12x10⁶) or EBV-transformed B cell line (12x10⁶) and NKG2A⁺ non-
alloreactive, IL-2 activated NK cells \((1 \times 10^6)\) that had been pre-treated with anti-human NKG2A mAb \((10\mu g/1 \times 10^6\text{cells/ml})\) at the E:T ratio \(= 1:12\). Isotype control Ab-pretreated NK cells were infused in control mice at the E:T ratio \(= 1:12\).

Mice that succumbed to leukemia or EBV lympho-proliferative disease were assessed for AML or EBV organ infiltration by flow cytometry analysis with a specific panel of anti–human mAb which previously characterized the neoplastic cells (see below).

In a model of engrafted disease, we infused the same mouse strain with AML or EBV cell lines. When bone marrow engraftment was around 20-30\%, mice were given escalating doses of IL-2 activated NKG2A+NK cells \((1 \times 10^6)\) that had been pre-treated with anti-human NKG2A mAb \((10\mu g/1 \times 10^6\text{cells/ml})\) (from 1 to 10 million per mouse, intravenously).

Mice that died of leukemia or lymphoma were assessed for AML or EBV organ infiltration by flow cytometry analysis using a specific panel of anti–human mAb (see below).

In other mouse models, the day after irradiation female ten week old NSG mice were given intravenously \(10 \times 10^6\) human CD34\(^+\) hematopoietic stem cells. At day 20 mice were infused with \(5 \times 10^6\) intravenously HLA-E\(^+\) EBV cell line or AML cells. When CD34\(^+\) stem cells had differentiated into CD56\(^+\)/CD3\(^-\)/NKG2A\(^+\) NK cells, mice received intraperitoneal administration of 200, 250 or 300 \(\mu g\) anti-human NKG2A mAb. Control mice were left untreated or treated with same doses of isotype control Ab. From day 40 onwards mice were evaluated for EBV or AML engraftment with a combination of anti–human CD45 mAb and mAb specific for AML or EBV cell line (anti-CD45, anti-CD20, anti-CD19, anti-IgM, anti-kappa, anti-lambda, anti-CD23, anti-CD3, anti-CD33, anti-CD34, anti-CD56, anti-CD117, anti-CD8, CD4, CD34 mAbs, eBioscience).
Mice that succumbed to EBV lympho-proliferative disease or leukemia were assessed for EBV or AML organ infiltration by flow cytometry analysis with a combination of anti–human mAbs (see above). Mice that survived were sacrificed after 100 days, and tumor organ infiltration analyzed with the same antibody combination.

**Statistical analyses**

Student’s t test was used to compare variables and was applied by Graphpad Prism 5. Kaplan-Meier method evaluated murine survival. All $P$ values are two-sided and were considered significant at $P < .05$. 

Results

In vitro treatment with anti-human NKG2A antibody triggers NKG2A⁺ NK cell lysis of HLA-E⁺ hematopoietic lineage targets.

In order to assess the susceptibility of normal and neoplastic haematopoietic lineage targets to alloreactive NK cell lysis, we generated single inhibitory KIR⁺/NKG2A⁻ NK cell clones and evaluated their ability to kill KIR ligand missing targets such as B and T cells, monocytes, DCs, EBV cell lines, CLL, T-ALL, AML and MM cells. These normal and neoplastic hematopoietic lineage cells expressed HLA-E and were resistant to NKG2A⁺ NK cells. Figure 1 represents most of the acute leukemia (AL) express HLA-E⁺. All HLA-E⁺ lympho-hematopoietic cell types were targets of alloreactive NK cell killing when they did not express the appropriate inhibitory KIR ligand for the single inhibitory KIR receptor expressed by alloreactive NK cell clones (Figure 2A). We pretreated NKG2A⁺ NK cells with anti-human NKG2A Ab and assessed their ability to kill otherwise resistant HLA-E⁺ hematopoietic lineage cells. Treatment with anti-NKG2A mAb converted NKG2A⁺/KIR⁻ NK cells into cells that were functionally “alloreactive” against HLA-E⁺ lympho-hematopoietic cells i.e. killed B and T cells, monocytes, DCs, EBV cell lines, CLL, T-ALL, AML and MM cells. The most effective lysis was obtained from an effector-to-target (E:T) ratio 15:1 (Figure 2B).

Each cytotoxicity assay was repeated with 3 targets for each category of cells and mean ± SD is shown.
In vivo treatment with the anti-human NKG2A antibody eradicates HLA-E⁺ leukaemia and lymphoma.

In order to evaluate the in vivo efficacy of anti-NKG2A mAb to trigger NKG2A⁺ NK cells to kill neoplastic cells, we developed xenogenic murine models of human neoplastic disease. NOD-SCID mice that received the HLA-E⁺ EBV-cell lines or AML cells died of high grade lymphoma or AML. In these mice, co-infusion of human NKG2A⁺ non alloreactive NK cells did not prevent engraftment of EBV or AML cells and mice died of the diseases.

In contrast, infusion of NKG2A⁺ NK cells that had been pretreated with anti-NKG2A mAb, prevented engraftment of human EBV cell lines and AML cells and mice survived without symptoms or signs of tumor localization (Figure 3 A). In fact, mice were sacrificed 100 days post cell infusion and cytofluorimetric analysis confirmed no neoplastic infiltration. We pooled results of 8 experiments with 4 mice per group for each experiment.

NKG2A⁺NK cell elimination of engrafted human AML or human EBV cell lines was evaluated in escalating dose experiments. At least pre-3x10⁶ treated with anti-NKG2A NKG2A⁺NK cells per mouse were necessary to rescue 80% mice. Repeating intraperitoneal doses of Ab did not improve the results because the autologous NK cell killing (fratricide effect) (Figure 3 B).

In order to assess the ability of endogenously generated NKG2A⁺ NK cells to cure leukaemia or lymphoma, we transplanted NSG mice with 10 x 10⁶ human CD34⁺ hematopoietic cells. The transplanted CD34⁺ hematopoietic stem cells differentiated into various hematopoietic lineage cells, including NKG2A⁺ NK cells (20). Twenty days after the CD34⁺ cell infusion, mice received HLA-E⁺ EBV cell lines or AML tumor cells.
On day 30 after the CD34+ cell infusion, when the numbers of NKG2A+ NK cells reached a plateau value in the bone marrow and spleen we treated three groups of mice with 200, 250 or 300 µg of the anti-NKG2A mAb. Control mice treated with isotype control mAb, and mice that received 200 µg of anti-NKG2A mAb, succumbed to EBV lymphoproliferative disease or leukaemia. In contrast, mice that received 250 or 300 µg of anti-NKG2A mAb survived (Figure 4, A-B respectively). NKG2A+ NK cells totally ablated EBV cell line or AML cells in the bone marrow (Figure 4, C-D respectively) and spleen (Figure 4, E-F respectively). Therefore, treatment with anti-human NKG2A mAb enabled endogenously generated human NKG2A+ NK cells to kill lethal EBV lymphoproliferative disease or leukemia. We pooled results of 3 experiments with 5 mice per group for each experiment.

**In vivo treatment with the anti-NKG2A antibody transiently depletes non-neoplastic lympho-hematopoietic lineage cells.**

In order to evaluate the impact of anti-NKG2A mAb on the various lympho-hematopoietic lineage cell subsets in vivo, NSG mice were transplanted with human CD34+ hematopoietic stem cells and the differentiated lympho-hematopoietic cell subpopulations were analysed. One month after CD34+ cell infusion, human CD4+/CD8+ double positive thymocytes in the thymus, and myeloid lineage cells, B, NK cells and DCs in the bone marrow and in the spleen reached a plateau value (Figure 5). At this time point mice were treated with anti-human NKG2A mAb. Monitoring of human myeloid, B, and DC subpopulations in the bone marrow and spleen and human thymocytes at different time points after anti-NKG2A treatment showed all these hematopoietic lineage cells were transiently depleted. They returned to pre-treatment values within ten
days (Figure 5). Analysis of TCR repertoire in the thymocytes displayed it was polyclonal (data not shown). Therefore, in vivo treatment with anti-human NKG2A mAb did not induce persistent ablation of normal hematopoietic cells. We pooled results of 3 experiments with 5 mice per group for each experiment.

Discussion

The present investigation into the clinical potential of a recently developed humanized anti-NKG2A antibody showed it converted NKG2A⁺ NK cells into effector NK cells able to kill most HLA-E⁺ NK resistant lympho-hematopoietic cells, including B and T lymphocytes, dendritic and myeloid cells, leukaemic cells (CLL, T-ALL and AML), high-grade lymphoma and MM cells. We also demonstrated in mouse models that pre-treatment of NKG2A⁺ NK cells with anti-human NKG2A mAb prevented engraftment of otherwise lethal EBV cell lines or AML cells.

Interestingly, the repertoire of each individual expresses a certain percentage of NKG2A⁺ NK cells and, after haematopoietic stem cell transplantation, a large population of reconstituting NK cells expresses the CD94-NKG2A inhibitory receptor (15). Consequently, the use of the humanized anti-NKG2A antibody could enlarge the NK cell population that exerts an anti-tumor effect to the benefit of patients with hematological malignancies.

Potential side effects such as autoreactivity against hematopoietic stem cells and subsequent cytopenia could develop, particularly after transplantation. To test this hypothesis, we transplanted mice with human CD34⁺ stem cells and then leukemic cells, which engrafted because the stem cells could not develop into mature T cells or alloreactive single
KIR+/NKG2A- NK cells (20). Human hematopoietic stem cells could, however, develop NKG2A⁺ NK cells (20). Anti-NKG2A antibody treatment reconstituted NKG2A⁺ NK cell-mediated lysis of HLA-E⁺ engrafted leukemic cells, rescuing mice from death. Side effects appear slight as cytopenia of normal hematopoietic cells was transient and mice recovered quickly. The slight, transient cytopenia in the committed myeloid line may be due to either alloreactive NK cell fratricide or to CD34⁺ cell conservation. In fact, recurrent dosing does not seem to reduce CD34⁺ cells in number as engraftment was always successful (data not shown). One might hypothesize that they are not a target of alloreactive NK cells.

Interestingly these in vitro and in vivo results are in accordance with previous report of Lirilumab which bound to all KIR2D inhibitory receptors for groups 1 and 2 HLA-C alleles and blocked NK cell inhibitory recognition of self HLA-C. It activated NK cell killing in vivo, eradicating tumours in mice (10-13). In fact, clinical trials of this fully human anti-KIR antibody as single agent are on-going in patients with acute leukaemia (14).

We might hypothesize about using the humanized anti-NKG2A antibody as an alternative to chemotherapy. Some studies demonstrated safety and a promising clinical role of haploidentical alloreactive NK cell infusions in combination with chemotherapy for treatment of elderly or pediatric patients with high risk acute leukaemias (22-23). We speculate that humanized anti-NKG2A may be useful in similar settings, in order to reconstitute lysis by NKG2A⁺ NK cells obtained from non-alloreactive haploidentical or identical donors.

The role of the NKG2A receptor in auto-immune diseases is controversial. Activated NK cells with NKG2A downregulation may play a role in Psoriasis pathogenesis (24). However, since reconstituted NK
cell lysis by means of the anti-NKG2A antibody is also directed against
activated autologous T and B cells which mediate autoimmune diseases,
the antibody might also be envisaged as therapy against human
autoimmune diseases. In a murine model of rheumatoid arthritis, an anti-
murine NKG2A (Fab) antibody selectively increased lysis of autologous
TH17 and TFH cells that are the mediators of rheumatoid arthritis. The
Ab blockade of the inhibitory interaction between the NKG2A receptor
and its Qa-1 ligand enhanced the NK cell-dependent elimination of
pathogenic T cells, resulting in blocking disease onset or progression
(25).
In vitro and in vivo findings suggest that the humanized anti-NKG2A
antibody described here constitutes a unique, relatively safe, therapeutic
approach to malignant hematologic and autoimmune diseases. Phase I-II
clinical trials with anti-human NKG2A Ab are ongoing in patient with
tumor types known to express HLA-E, including chronic lymphocytic
leukemia (ClinicalTrials.gov: NCT02557516), head and neck cancer
(ClinicalTrials.gov: NCT02331875) and ovarian cancer
(ClinicalTrials.gov NCT02459301) (26) in order to validate present
observations and provide hope for 50% of patients with haematological
and solid malignancies who cannot find alloreactive NK cell donors.

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Authorship contributions
L.R. designed study, analyzed data and wrote the paper. E.U. performed experiments. P.A. designed study and analyzed data. A.M., A.T., F.T. contributed to cytofluorimetric analysis, NK cell cloning and cytotoxicity assays. M.B. contributed to write the paper. F.R., N.W and A.V. designed study, analyzed data and wrote the paper.

Conflict-of-interest disclosure

L.R. is a Leukemia and Lymphoma Society Scholar in Clinical Research. Anti-human NKG2A was kindly given by Innate Pharma

References


Legends

Figure 1. HLA-E expression on acute leukemia cells.
A-C: HLA-E expression on 3 AML cells
D-F: HLA-E expression on 3 ALL cells (1 T-ALL and 2 B-ALL)

Figure 2. In vitro treatment with anti-human NKG2A mAb reconstitute NKG2A+ NK cell lysis against HLA-E+ normal and neoplastic lymphohematopoietic cells

A. Percent of lysis of KIR ligand mismatched HLA-E+ B and T cells, monocytes, DCs, EBV cell lines, CLL, T-ALL, AML and MM cells mediated by single KIR+ alloreactive NK clones at the E/T 15:1 in a standard 51Cr release cytotoxicity assay.
B. Percent of lysis of HLA-E+ B and T cells, monocytes, DCs, EBV cell lines, CLL, T-ALL, AML and MM cells mediated by activated and cultured in IL2 NKG2A+/KIR- NK cells at the E/T 15:1 after treatment with anti-human NKG2A mAb (10μg/1x10^6cells/ml) in a standard 51Cr release cytotoxicity assay.
Lysis mediated by NKG2A+ NK cells after anti-human NKG2A mAb is comparable to lysis mediated by single KIR+ alloreactive NK cell clones. Each cytotoxicity assay was repeated with 3 targets for each category of cells and mean ± SD is shown.

Figure 3. Pre-treatment of human NKG2A+ NK cells with the anti-human NKG2A mAb prevents engraftment of human EBV cell lines and AML cells and cure engrafted disease in NOD-SCID mice.
A. One million NKG2A⁺/KIR⁻ NK cells were pre-treated with anti-human NKG2A mAb (10μg) and coinfused with EBV cell line (■) or AML (○) expressing HLA-E at E/T= 1:12. Control mice were coinfused with isotype control antibody-pretreated NKG2A⁺/KIR⁻ NK cells and EBV cell line (●) or AML (▲) expressing HLA-E at E/T= 1:12. Mice coinfused with human EBV cell lines or human AML cells and treated with isotype control Ab-pretreated NKG2A⁺ NK cells died of disease progression. The anti-human NKG2A mAb pre-treatment prevented disease engraftment and all mice survived. We pooled results of 8 experiments with 4 mice per group for each experiment.

B. Mice engrafted with AML or EBV cell lines (20-30% of bone marrow infiltration) were infused with escalating dose of NKG2A⁺/KIR⁻ NK cells, pre-treated with anti-human NKG2A mAb (10μg/million of NK cells). Control mice were coinfused with isotype control antibody-pretreated NKG2A⁺/KIR⁻ NK cells and EBV cell line or AML. At least 3x10⁶ pretreated with anti-human NKG2A mAb NKG2A⁺/KIR⁻ NK cells cure 80% of mice with EBV or AML. Treatment of engrafted mice with at least 4x10⁶ pre-treated NKG2A⁺NK cells rescued 100% of mice affected by EBV (■) or AML (○).

Mice engrafted with human EBV cell lines (●) or human AML cells (▲) and infused with more than 4x10⁶ isotype control Ab-pretreated NKG2A⁺ NK cells died of disease progression. The anti-human NKG2A mAb pre-treatment cure engrafted diseases. We pooled results of 8 experiments with 4 mice per group for each experiment.

Figure 4. In vivo treatment with the anti-human NKG2A mAb rescues NSG mice engrafted with human CD34⁺ hematopoietic stem cells and HLA-E⁺ human AML or EBV cell line.
After 3.5 Gy TBI, mice were infused with $10 \times 10^6$ human CD34$^+$ hematopoietic stem cells. After 20 days they were infused with EBV cell line or AML cells. When NKG2A$^+$ NK cells differentiated from CD34$^+$ cells, mice were treated with anti-human NKG2A mAb. Mice that received 250 (○) or 300 µg (●) of anti-human NKG2A mAb survived, control mice (isotype control Ab) (■) or mice that received 200 µg of the Ab (▲) succumbed to EBV lympho-proliferative disease (A) or AML (B). NKG2A$^+$ NK cells ablated the EBV cell line in bone marrow* (C) and spleen (E) and AML cells in bone marrow* (D) and spleen (F). The normal human CD45$^+$ hematopoietic population, which developed from CD34$^+$ cells, was transiently depleted after administration of human anti-NKG2A Ab. We pooled results of 3 experiments with 5 mice per group for each experiment.

* Bone marrow cell numbers are from two femurs per mouse

Figure 5. Treatment with the anti-human NKG2A mAb transiently depleted HLA-E$^+$ autologous myeloid, B, T, NK and DC subpopulations in NSG mice engrafted with human CD34$^+$ hematopoietic stem cells.

After 3.5 Gy TBI, mice were infused with human CD34$^+$ hematopoietic stem cells. One month after, when NKG2A$^+$ NK cells differentiated from CD34$^+$ cells reached a plateau value, mice were treated with 300 µg of anti-human NKG2A mAb. Transient depletion of human myeloid, B, DCs, NK subpopulations in the bone marrow* (A) and spleen (B) and double negative (DN), single CD8$^+$ or CD4$^+$, CD4$^+$/CD8$^+$ double positive (DP) thymocytes (C) was followed by recovery of all cell subsets within

22
ten days. We pooled results of 3 experiments with 5 mice per group for each experiment.

* Bone marrow cell numbers are from two femurs per mouse
Fig. 2

A

- Non alloreactive NK cells
- Alloreactive NK cells

B

- Isotype control Ab
- Anti-NKG2A Ab

% lysis vs. cell type:
- B cells
- T cells
- Monocytes
- Dendritic cells
- EBV cell line
- CLL
- T-ALL
- AML
- MM
Fig. 3
Fig. 4
A Bone marrow

- Myeloid Lineage
- B cell lineage
- Dendritic cells
- NK cells

B Spleen

- Myeloid Lineage
- B cell lineage
- Dendritic cells
- NK cells

C Thymus

- DN Thymocytes
- DP Thymocytes
- SP Thymocytes

**Fig. 5**