IL-2 and zoledronic acid therapy restores the in vivo anti-leukemic activity of human lymphocytes pre-exposed to simulated microgravity

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Abstract

Background: We have previously shown that the anti-tumor activity of human lymphocytes is diminished *in vitro* after 12-hours pre-exposure to simulated microgravity (SMG). Here we used an immunocompromised mouse model to determine if this loss of function would extend *in vivo*, and to also test the efficacy of IL-2 and zoledronic acid (ZOL) therapy as a potential countermeasure against SMG-induced immune dysfunction. We adoptively transferred human lymphocytes that were exposed to either SMG or 1G-control into NSG-Tg(Hu-IL 15) mice 1-week after they were injected with a luciferase-tagged human chronic myeloid leukemia (K562) cell line. Tumor growth was monitored 2x weekly with bioluminescence imaging (BLI) for up to 6-weeks.

Results: Mice that received lymphocytes exposed to SMG showed greater tumor burden compared to those receiving lymphocytes exposed to 1G (week 6 BLI: $1.8e^{10} \pm 8.07e^9$ versus $2.22e^8 \pm 1.39e^8$ photons/second; $p < 0.0001$). Peak BLI was also higher in the SMG group compared to 1G-control ($2.34e^{10} \pm 1.23e^{10}$ versus $3.75e^8 \pm 1.56e^8$ photons/second; $p = 0.0062$). Exposure to SMG did not affect the ability of human lymphocytes to engraft or evoke xeno-graft-versus-host disease in the mice. Additionally, we injected the mice with IL-2 and zoledronic acid (ZOL) to expand and activate the anti-tumor activity of NK cells and $\gamma\delta$ -T cells, respectively. This was found to revive the loss in anti-leukemic function observed *in vivo* when lymphocytes were pre-exposed to SMG.

Conclusion: Microgravity plays a contributory role in loss of tumor control *in vivo*. Immuno-stimulating agents like ZOL+IL-2 may offer an important countermeasure for immune dysregulation during prolonged spaceflight.

Background

Immune dysregulation has been reported in astronauts following both short (14–17 days)^{1–4} and long (5–6 months)^{5,6} duration spaceflight. This includes diminished cell mediated immunity with a Th-2 cytokine shift, with latent viral reactivation being a frequent manifestation of immune dysregulation in astronauts. Many factors including microgravity, space radiation, isolation and confinement stress, altered circadian rhythms, nutritional deficiencies, and the gut microbiome are believed to play a role in altering astronaut immunity during extended space voyages⁷.

A particular concern for astronauts is that space travel might impair anti-tumor immune surveillance, increasing cancer risk in future exploration class mission crew. We showed previously that the anti-tumor activity of natural killer (NK) cells was diminished in crewmembers onboard the International Space Station⁸. We further demonstrated that pre-exposing human lymphocytes to 12h of simulated microgravity (SMG), impaired NK cell killing against a range of hematological cancer cell lines *in vitro* when returned to the 1G environment⁹. The SMG exposed NK cells expressed lower levels of the cytolytic granules perforin and granzyme b, and were less capable of degranulation (CD107a+) and secreting effector cytokines (e.g. TNF- α and IFN- γ) when co-cultured with tumor target cells after being pre-exposed

to SMG. Exposing K562 chronic myeloid leukemia (CML) cells to SMG did not alter their susceptibility to NK cell killing, indicating that cancer cells might be more resistant to the effects of SMG compared to primary immune cells. This might tip the balance in the favor of tumor transformation during a long duration mission, particularly against a backdrop of continuous radiation exposure and further immune suppression due to latent viral reactivation.

A limitation of our previous work was the reliance on *in vitro* assays to assess the anti-tumor activity of human lymphocytes collected from astronauts in space or following exposure to SMG^{8,9}. Unfortunately, these assays fail to capture other key elements of the anti-tumor response unique to *in vivo* setting including extravasation, homing, tumor infiltration, and killing. Humanized mouse models offer an excellent platform to determine the anti-tumor activity of human immune cells *in vivo*. NSG-Tg(Hu-IL15) animals harbor Prkdc^{scid}¹⁰ and IL2rg^{tm1Wjl}^{11–13} mutations and an insertion of humanized IL-15 sequence (Tg(IL15)1Sz/SzJ) that allows improved engraftment of primary human cells¹¹ and increased survival of NK cells¹⁴. This mouse model also allows us to test the effectiveness of certain pharmacological countermeasures that have been proposed to mitigate spaceflight associated immune dysregulation¹⁵.

Administration of subcutaneous interleukin-2 (IL-2) has shown to improve anti-tumor activity of NK cells in pre-clinical mouse models^{16,17} and in various clinical trials^{18–20}. Zoledronic acid is an amino-bisphosphonate with clinical therapeutic applications including improving bone mineral density^{21,22} and anti-tumor immunity through suppression of T_{regs}^{23,24}. Zoledronic acid has also been shown to expand $\gamma\delta$ -T cells *in vivo*²⁵ and sensitize tumors to $\gamma\delta$ -T cell killing^{26,27}. As such, it is possible that ZOL + IL-2 could restore the anti-leukemic activity of human effector lymphocytes pre-exposed to SMG.

The aim of this study was to determine if pre-exposure to SMG would impair the anti-leukemic function of human lymphocytes *in vivo* using the NSG-Tg(Hu-IL15) mouse model (Fig. 1). K562 leukemia bearing mice were engrafted with human immune cells exposed to either SMG or 1G and monitored for tumor growth over 6-weeks via bioluminescent imaging. Additionally, we tested if ZOL + IL-2 administered *in vivo* could revive SMG-induced loss of anti-leukemic function.

Methods

1. Participants and blood collection

Healthy adults between the age of 18-49 years volunteered for the study. Each participant provided written informed consent and had a single blood sample drawn by standard phlebotomy. Blood was collected in acid citrate dextrose (ACD) tubes and peripheral blood mononuclear cells (PBMCs) were isolated using density gradient separation method with Ficoll (Sigma-Aldrich, St. Louis, MO, USA) and processed immediately. All study procedures were approved by the Institutional Review Board (IRB) at University of Arizona.

2. Rotary cell culture system

We exposed human PBMCs to simulated microgravity (SMG) or 1-G control as previously described⁹. Briefly, a rotary cell culture system (RCCS) (Synthecon, Houston, TX, USA) was used to simulate microgravity. PBMCs were resuspended in 10%FBS+RPMI media in 10ml high-aspect ratio vessels (HARVs) and exposed to simulated microgravity for 12-hours while rotating on a horizontal axis at 10RPM. A 1G-control HARV was rotated on a vertical axis exposing PBMCs to similar shear stress while experiencing 1G gravitational force.

3. NSG-Tg(Hu-IL15) mouse model

All mouse experiments were done in compliance with Institutional Animal Care and Use Committee (IACUC) guidelines at the University of Arizona under an approved protocol. NSG-Tg(Hu-IL15) animals were purchased from Jackson laboratory (Bar Harbor, ME, USA) and breeding colonies were maintained at the University Animal Care facility. Animals were housed for the duration of the experiments at the University Animal Care facility.

Animals were irradiated with 100 cGy in a Cesium¹³⁷ irradiator (Atomic Energy of Canada Limited, Chalk River, Canada) a day before they were injected with 1 million luciferase-tagged-k562 (chronic myeloid leukemia) cells (figure 1). One week later, they were injected with 10 million PBMCs from SMG or 1G control conditions. 'Tumor only' control group received only 1 million K562 cells with placebo (PBS) on the day of immune cells injection. Bioluminescent intensity, weight, morbidity, and GVHD were monitored twice a week. Engraftment data was also collected from another group of mice that received only immune cells without tumor cells to examine differences in engraftment in the absence of tumor. Blood draw was performed from the sub-mandibular vein once a week to examine engraftment dynamics. Animals were sacrificed when they reached sacrificial criteria or at the end of the experiment (6 weeks). Since we propose to study the effect SMG on *in vivo* anti-leukemic activity of human PBMCs, which is largely driven by NK cells, we used this NSG-Tg(Hu-IL15) mouse model, which enhances *in vivo* survival of human NK cells. Our experiments suggested that acute GVHD signs occur around 14 days after PBMC injection and severe GVHD occurs around day 28. Therefore, we chose to evaluate graft-versus-tumor effect before severe GVHD starts setting in to prevent GVHD associated immune modulation from eclipsing the graft-versus-tumor effect of human PBMCs. Acute GVHD scoring was based on 6 parameters in accordance with previously published grading criteria^{28,29}. All animals were terminated if they presented with a GvHD score of 4 or more or had lost more than 20% of their initial body weight.

Administration of zoledronic acid and interleukin-2: Subgroups of mice received an intraperitoneal injection of zoledronic acid (50µg/kg) with a subcutaneous injection of IL-2 (40,000 I.U) 1x weekly starting at day 7 (i.e. the day of PBMC transfer) of the experiment (fig.1).

4. Bioluminescent imaging

K-562-luc2 (ATCC® CCL-243-LUC2™) (American Type Culture Collection, Manassas, VA, USA), which is a K562 chronic myeloid leukemia cell line that has been transfected with luciferase, was used for the *in vivo* experiments^{30,31}. Tumor burden was measured immediately after D-luciferin (GoldBio, St Louis, MO) was injected intraperitoneally. A LagoX spectral imager (Spectral Instruments Imaging, Tucson, AZ) was used to quantify bioluminescent intensity (BLI) scores.

5. Flow cytometry

Antibodies (CD8 Vioblue, CD3 Viogreen, vγ9 vδ2 FITC, CD4 PE, CD45 Per-CP, vγ9 vδ1 APC, CD56 APC-Vio770, CD16 Vioblue, NKG2D PE, PD-1 Per-CP, CD158b PE-Vio770, NKG2A APC, TCR-γδ APC-Vio770, Nkp30 PE) (Miltenyi Biotec, San Diego, CA) were added to 25μL of whole blood at the proper titrated volumes and allowed to incubate for 30 minutes at room temperature in the dark. Blood samples were lysed with 1X Red Blood Cell Lysis solution (Miltenyi Biotec, San Diego, CA), washed twice in phosphate-buffered saline (PBS), and resuspended in 200μL PBS.

6. Statistical analysis

Statistical analysis was performed on Graphpad Prism 8.4.3 software (Graphpad, San Diego, CA). For tumor progression, bioluminescent intensity data was log-transformed for normality as previously indicated³². A mixed effects model was used to analyze differences between tumor growth with either 3 (e.g. SMG, 1G, vehicle) or 5 (e.g. SMG, SMG+ZOL+IL-2, 1G, vehicle, vehicle+ZOL+IL-2) 'condition' levels included in the model, along with a main effect for 'time' and 'condition*time' included as an interaction effect. When the interaction effect was significant (indicating that tumor growth differed between conditions as the experiment progressed), systematic pairwise comparisons were conducted with Bonferroni correction to identify which conditions were significantly different from each other over time. Peak BLI was analyzed using Friedman test for paired comparisons. GVHD scores and engraftment data were analyzed using a linear mixed model or a two-way RM ANOVA. A log-rank (Mantel-Cox) test was used for survival analysis. Statistical significance was set at $p < 0.05$.

Results

Exposure to SMG impaired the anti-leukemic activity of human immune cells *in vivo*.

To evaluate the effect of SMG on anti-leukemia activity of human immune cells *in vivo* we compared tumor growth between mice that were injected with PBMCs exposed to SMG (TUMOR + SMG PBMCs) or 1G-control (TUMOR + 1G PBMCs). TUMOR + vehicle (PBS) was used as a control reference for unrestrained tumor growth. Representative BLI images showing tumor dynamics *in vivo* are shown in Fig. 2. For tumor growth, statistically significant effects were found for time, condition, and

time*condition ($p < 0.0001$) (Fig. 3a). Post-hoc analyses revealed these effects to be driven by a lower tumor burden in the mice transferred with 1G exposed PBMCs compared to mice transferred with SMG exposed PBMCs and vehicle. Tumor growth kinetics were similar between SMG exposed PBMCs and vehicle control ($p > 0.05$). Peak tumor growth (as determined by highest BLI score) was significantly higher in the mice receiving SMG exposed PBMCs and vehicle compared to mice receiving 1G exposed PBMCs. There were no significant differences for peak tumor growth between the SMG exposed PBMC and vehicle conditions ($p > 0.05$). Overall survival up to 6-weeks did not differ significantly across the conditions ($\chi^2 = 2.934$, $p = 0.231$) (Fig. 3c). For GvHD/morbidity score, an expected significant effect was seen for time ($p < 0.0001$), but not 'condition' ($p = 0.764$) or 'condition*time' ($p = 0.981$) (Fig. 3d).

Exposure to SMG did not affect human immune cell engraftment in vivo.

To verify that exposure to SMG did not impair the PBMCs' ability to engraft and expand in a xenograft; mice were injected with PBMCs only and not tumor. Engraftment was measured as a proportion of CD45human+ (CD45h+) cells in total immune cells in mice blood collected at weekly intervals from week 2–5. The engraftment dynamics of PBMCs in the mice were not statistically different between SMG and 1G conditions (Fig. 4). This was true for total PBMCs as defined by human CD45 expression as a percentage of the total human + mouse CD45 expressing cells (% $p = 0.175$; cells/uL, $p = 0.628$, Fig. 4a, 4c.) and for NK-cells (CD3-/CD56+) expressed as a percentage of CD45h+ cells (% $p = 0.219$; cells/ μ L, $p = 0.478$, Fig. 4b, 4d.). Similar human immune cell engraftment dynamics were observed in mice with tumor (Fig. 5).

Zoledronic acid and IL-2 therapy improved anti-leukemia activity of human immune cells in vivo.

In an attempt to 'rescue' the anti-leukemic effects of PBMCs pre-exposed to SMG prior to adoptive transfer, we injected mice with ZOL + IL-2 on the day of PBMC transfer and once weekly thereafter. For tumor growth, statistically significant effects were found for time, condition, and time*condition ($p < 0.0001$) (Fig. 6a). As observed in the previous experiment, 1G exposed PBMCs significantly inhibited tumor growth compared to SMG exposed PBMCs. Post-hoc analysis revealed a significant interaction effect ($p = 0.0004$) between TUMOR + SMG PBMCs and TUMOR + SMG PBMCs + ZOL + IL2, while no interaction effects were found for TUMOR + SMG PBMCs + ZOL + IL2 versus TUMOR + 1G PBMCs ($p = 0.567$). There were also no significant differences for condition ($p = 0.947$) or time ($p = 0.221$) between these groups. This revealed that ZOL + IL2 administration enabled SMG-exposed PBMCs to control tumor growth rate as efficiently as 1G-exposed PBMCs. As expected, ZOL + IL-2 had no effect on tumor growth dynamics in the absence of PBMCs ($p > 0.05$). Peak tumor growth (as determined by highest BLI score) was significantly higher in the mice receiving SMG exposed PBMCs and vehicle (with or without ZOL + IL-2) compared to mice receiving 1G exposed PBMCs and SMG exposed PBMCs + ZOL + IL-2. There were no significant differences for peak tumor growth between the 1G exposed PBMCs and the SMG exposed PBMCs + ZOL + IL-2 ($p > 0.05$), again indicating that the administration of ZOL + IL-2 rescued the anti-leukemic effect of PBMCs pre-exposed to SMG. Overall survival up to 6-weeks did not differ significantly

across the conditions ($\chi^2 = 2.429$, $p = 0.6575$) (Fig. 6c). Administration of ZOL + IL2 therapy did not affect GVHD/morbidity progression ($p > 0.999$) (Fig. 6d).

Zoledronic acid and IL-2 therapy did not alter the number of NK and $\gamma\delta$ -T cells in blood.

To determine if ZOL + IL2 therapy increased the number and proportion of NK cells and $\gamma\delta$ -T cells in peripheral blood, weekly human leukocyte engraftment checks were performed after adoptive transfer (Fig. 7). No significant interaction*time effects were found for total CD45h+ (% $p = 0.713$; cells/uL, $p = 0.419$, Fig. 7a, 7b), NK cells (% $p = 0.99$; cells/uL, $p = 0.941$, Fig. 7c, 7d), or $\gamma\delta$ -T cells (% $p = 0.915$; cells/uL, $p = 0.40937$, Fig. 7e, 7f), indicating that ZOL + IL-2, despite increasing tumor control, did not expand these leukocyte populations in peripheral blood.

Discussion

The overarching aims of this study were to determine if the loss of NK-cell function against a leukemic cell line *in vitro* due to exposure to simulated microgravity (SMG) would extend to an *in vivo* model, and to also test the efficacy of zoledronic acid and IL-2 (ZOL + IL-2) therapy as a countermeasure to SMG-induced immune dysfunction. Using a human K562 tumor bearing immunocompromised mouse model, we found that pre-exposing human PBMCs to SMG for 12h inhibits their ability to control leukemic growth *in vivo* after adoptive transfer, but systemic administration of ZOL + IL-2 'rescued' this SMG induced impairment of anti-leukemic activity. These findings indicate that microgravity likely plays a contributory role to immune system impairment during long duration space travel. Further, ZOL + IL-2 (a treatment that is currently used to expand and increase the anti-leukemic activity of NK-cells and $\gamma\delta$ -T cells in cancer patients^{19,20,33}) could serve as an effective countermeasure to mitigate deficits in anti-tumor immunity due to prolonged exposure to microgravity during extended space missions.

This is the first study, to our knowledge, to show that pre-exposing primary human lymphocytes to SMG inhibits their ability to control leukemic growth *in vivo*. Not only were the SMG exposed PBMCs less effective at controlling leukemic growth in comparison to their 1G exposed counterparts, tumor growth kinetics were identical between the SMG exposed and vehicle control conditions indicating that SMG completely incapacitates the anti-leukemic activity of human lymphocytes. This corroborates our previous findings that pre-exposing human lymphocytes to SMG impairs their ability to kill various hematologic cancer cells lines *in vitro*³⁴. While the mechanisms underpinning this response are not fully known, our prior *in vitro* studies showed that SMG impairs several anti-tumor properties of NK-cells, including their ability to deliver 'lethal hits' through cytotoxic degranulation and secretion of effector cytokines³⁴. Although it is possible that SMG could have impaired human cell engraftment in the mouse, we somewhat alleviated this potential confounder by showing similar levels of human leukocyte engraftment between the SMG and 1G conditions. While blood levels of immune cells might not be reflective of engraftment levels in tissues or in mice with tumor, this showed that human immune cells (including NK cells) thrived similarly in mice after exposure to 1G and SMG. *In vivo* expansion of (CD45h + CD3-CD56+) human NK cells in the initial weeks was to be expected in an NSG-tg(hu-IL15) mouse

model. NSG-tg(hu-IL15) mice sustain IL-15 blood levels of 7.1 ± 0.3 pg/ml³⁵. These levels are considerably higher than the usual undetectable levels in human blood (≈ 1 pg/ml)³⁶. This mouse model was precisely chosen to sustain and expand NK cells, since a loss of NK cell function was deemed the underpinning reason why SMG evokes impaired anti-tumor responses of human lymphocytes. NK cell levels in blood fell in the later weeks when the differences in BLI between the groups reached discernible levels. It remains unclear if NK cells became exhausted in the later weeks (due to sustained IL-15 stimulation) or if they simply traffic away from the peripheral blood and are more abundant in the tissues and areas of sustained tumor growth.

Hematological tumor lysis in a 4-hour *in vitro* killing assay using mixed PBMCs is majorly attributed to the anti-tumor activity of NK cells; however, the ability to control tumor growth *in vivo* over a 6-week period might be a composite effect of all effector lymphocytes. While other effector lymphocytes like CD8 + T cells could have played a role, we deem this unlikely as GvHD scores (a manifestation of the human CD3 + $\alpha\beta$ T-cells attacking xenogeneic tissue in the mouse) were not different between the SMG and 1G exposed PBMC conditions indicating that SMG did not affect the alloreactivity of CD8 + $\alpha\beta$ T-cells. Past evidence shows *in vitro* T-cell activity is suppressed after exposure to SMG³⁷. However, in our *in vivo* model, there might be a recovery of the lost function over the duration of the experiment, particularly because the immunocompromised mice we used have a human IL-15 knock in that could have restored any detriments in $\alpha\beta$ T-cell function due to SMG. Since clinical symptoms of GvHD did not manifest until 3–4 weeks into the experiment, this might reflect similar GVHD onset patterns between the groups. While similar recovery is possible in NK cells too, failure to control tumor growth in the initial weeks might be contributing to tumor burden reaching an uncontrollable level before NK cells regain their activity. The more efficient 1G-exposed PBMCs could be more adept at killing K562 tumor cells *in vivo* during the initial weeks, which might have resulted in stunted tumor growth in the later weeks.

There is a critical need to identify effective countermeasures that can mitigate immune dysregulation during long duration spaceflight. Subcutaneous IL-2 injections are commonly used as an immunotherapeutic strategy to enhance graft-versus-leukemia effects in bone marrow transplant recipients by accelerating NK-cell reconstitution³⁸. Additionally, zoledronic acid, an amino-bisphosphonate that is used to prevent osteoporosis²¹ and currently being considered as a countermeasure to prevent decreases in bone mineral density due to weightlessness³⁹, has also been shown to expand $\gamma\delta$ -T cells *in vivo* and increase their anti-tumor activity in graft recipients⁴⁰. We show here for the first time that that systemic administration of ZOL + IL2 'rescued' the anti-leukemia activity of SMG-exposed PBMCs *in vivo*. Indeed, while SMG exposed PBMCs were rendered completely ineffective against K562 leukemic growth *in vivo*, the tumor growth kinetics in mice that received SMG exposed PBMCs were identical to those that received 1G exposed PBMCs when ZOL + IL-2 was administered. This indicates that ZOL + IL-2 has the potential to completely reverse the negative impact of SMG on the anti-leukemic activity of human lymphocytes. It should be noted, however, that all PBMCs were returned to the 1G environment after adoptive transfer and we do not currently know if ZOL + IL-2 is capable of maintaining anti-leukemic activity during periods of microgravity. Nevertheless, these results indicate that

ZOL + IL-2 could at least be an effective strategy to restore human lymphocyte function in conditions of 1g (e.g. on return to Earth after a mission) or partial (e.g. on arrival at the Martian surface) gravity following prolonged periods of weightlessness.

Conclusion

In summary, we have demonstrated that exposure to SMG impairs the anti-leukemia activity of human effector immune cells *in vivo* as well as *in vitro*, and that ZOL+IL2 therapy improved the anti-leukemia activity of human effector immune cells after exposure to SMG. Limitations of the present study include the short-term exposure to microgravity, not identifying the lymphocyte subtypes affected by SMG, and the fact that all functional experiments were conducted in 1G. We conclude that microgravity plays a contributory role in the loss of tumor control and should be considered a risk factor for impaired anti-tumor immune responses during prolonged space voyages. Immuno-stimulating agents like ZOL+IL-2 may help mitigate clinical risks associated with immune dysregulation during prolonged spaceflight and its effectiveness as an immune system countermeasure in humans warrants investigation.

Abbreviations

SMG, simulated microgravity; PBMC, peripheral blood mononuclear cells; NK cell, Natural Killer cell; MHC, major histocompatibility complex; GVHD, graft-versus-host-disease; ZOL, zoledronic acid; IL-2, interleukin-2; IL-15, interleukin-15; TNF α , tumor necrosis factor- alpha; IFN γ , interferon-gamma; BLI, bioluminescent intensity.

Declarations

Ethics approval and consent to participate:

All study procedures were approved by the Institutional Review Board (IRB) at University of Arizona. All mouse experiments were done in compliance with Institutional Animal Care and Use Committee (IACUC) guidelines at the University of Arizona under an approved protocol.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RJS, PLM and EK designed the research. PLM, FLB, DMD and GMN performed the research. PLM, RJS, EK, MMM and BEC analyzed and/or interpreted the data. PLM and RJS wrote the manuscript. All co-authors edited the manuscript and approved its submission.

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Figures

Figure 1.

Figure 1

Experimental design

Figure 2.

Figure 2

Bioluminescent intensity (BLI) images. Days 1, 12, 19, 22 and 28 of the experiment. Lane 1: tumor control, lanes 3, 5: tumor+ 1G PBMCs, lane 6: tumor+ SMG PBMCs, lane 8: PBMC control.

Figure 3.

Figure 3

Effect of SMG on effector immune cell function *in vivo*. Exposure to SMG impaired effector immune cell ability to control tumor growth in an *in vivo* model (3a.) (****= $p<0.0001$). Exposure to SMG also significantly increased peak BLI score during the experiment (3b.) (* denotes $p<0.05$, ** denotes $p<0.01$). Exposure to SMG did not affect survival (3c.) and GVHD incidence (3d.). N=12, MEAN $\pm$ SEM.

Figure 4.

Figure 4

Effect of exposure to SMG on human cell engraftment dynamics *in vivo* in the absence of tumor. Exposure to SMG did not impair human PBMCs' (4a., 4c.) and NK cells' (4b., 4d.) ability to engraft in the presence of tumor. N=10, MEAN±SEM.

Figure 5.

Figure 5

Effect of SMG on human cells engraftment *in vivo* in the presence of tumor. Exposure to SMG did not impair human PBMCs' (5a., 5c.) and NK cells' (5b., 5d.) ability to thrive *in vivo*. N=5, MEAN±SEM.

Figure 6.

Figure 6

Effect of zoledronic acid+IL2 (ZOL+IL2) therapy on SMG induced suppression of *in vivo* anti-leukemia activity. ZOL+IL2 therapy abrogated SMG induced loss of anti-leukemia activity *in vivo* (6a.), did not affect peak BLI (6b.), survival (6c.), GVHD incidence (6d.). N=5, MEAN $\pm$ SEM.

Figure 7.

Figure 7

Effect of SMG and ZOL+IL2 therapy on human cells engraftment in vivo in the presence of tumor. ZOL+IL2 therapy did not alter human PBMCs' (7a., 7b.) and NK cells' (7c., 7d.) and $\gamma\delta$ -T cell (7e., 7f.) engraftment dynamics. N=5 MEAN $\pm$ SEM.