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Research article

Effects of Korean red ginseng on T-cell repopulation after autologous hematopoietic stem cell transplantation in childhood cancer patients

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ABSTRACT

Background: Although the survival outcomes of childhood cancer patients have improved, childhood cancer survivors suffer from various degrees of immune dysfunction or delayed immune reconstitution. This study aimed to investigate the effect of Korean Red Ginseng (KRG) on T cell recovery in childhood cancer patients who underwent autologous hematopoietic stem cell transplantation (ASCT) from the perspective of inflammatory and senescent phenotypes.

Methods: This was a single-arm exploratory trial. The KRG group (n = 15) received KRG powder from month 1 to month 12 post-ASCT. We compared the results of the KRG group with those of the control group (n = 23). The proportions of T cell populations, senescent phenotypes, and cytokine production profiles were analyzed at 1, 3, 6, and 12 months post-ASCT using peripheral blood samples.

Results: All patients in the KRG group completed the treatment without any safety issues and showed a comparable T cell repopulation pattern to that in the control group. In particular, KRG administration influenced the repopulation of $CD4^+$ T cells via T cell expansion and differentiation into effector memory cell re-expressing CD45RA (EMRA) cells. Although the KRG group showed an increase in the number of $CD4^+$ EMRA cells, the expression of senescent and exhausted markers in these cells decreased, and the capacity for senescence-related cytokine production in the senescent $CD28^-$ subset was ameliorated.

Conclusions: These findings suggest that KRG promotes the repopulation of $CD4^+$ EMRA T cells and regulates phenotypical and functional senescent changes after ASCT in pediatric patients with cancer.

1. Introduction

The survival rate of childhood cancer has shown significant improvement over the years, increasing from approximately 60% in 1990 to 85% in 2020 [1,2]. This remarkable progress can be attributed to advancements in chemotherapy, surgery, and radiotherapy, as well as

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enhanced supportive care. Consequently, there has been a steady rise in the number of childhood cancer survivors, accompanied by the emergence of various long-term complications [3,4]. Additionally, it has been observed that these survivors often experience immune dysfunction or delayed immune reconstitution to varying degrees [5,6]. Therefore, it is crucial to address the restoration of immune system function in childhood cancer survivors, particularly those who have undergone hematopoietic stem cell transplantation.

High-dose chemotherapy combined with autologous hematopoietic stem cell transplantation (ASCT) plays a crucial role in treating high-risk solid tumors, including neuroblastomas, brain tumors, and malignant lymphomas in pediatric patients [7,8]. While high-dose chemotherapy offers improved anti-tumor effects, it often leads to severe bone marrow suppression. ASCT serves as a valuable approach to mitigate this limitation. However, immune recovery following high-dose chemotherapy and ASCT is slower compared to chemotherapy alone, necessitating comprehensive post-transplant monitoring to prevent opportunistic infections in ASCT recipients.

Korean Red Ginseng (KRG) is widely used as a herbal medicine; it contains saponins and acidic polysaccharides and is known for its antioxidant properties and beneficial effects such as strengthening immunity, reducing fatigue, and enhancing memory [9–14]. Furthermore, a study conducted by Lee et al demonstrated that the administration of KRG extract could potentially contribute to the stabilization of inflammatory cytokines in pediatric patients with cancer following chemotherapy [15]. Their study included 19 patients who received KRG extracts over a one-year period. However, limited research exists on the effects of KRG specifically in pediatric patients undergoing ASCT.

We conducted this exploratory trial to analyze the patterns of T cell function recovery and the safety of KRG powder in patients with childhood cancers who underwent high-dose chemotherapy and ASCT.

Table 1
Patient Characteristics

Characteristics	KRG group (n = 15)	Control group (n = 23)	*p* value
Age at diagnosis, median, year (range)	7.5 (1.0-18.5)	7.3 (0.8-14.8)	0.552
Age at ASCT, median, year (range)	8.1 (2.7-19.1)	9.0 (1.7-15.6)	0.525
Sex			0.346
male	13 (86.7%)	17 (73.9%)	
female	2 (13.3%)	6 (26.1%)	
Body weight at ASCT, median, kg (range)	28.8 (11.7-113.1)	25.3 (10.5-63.7)	0.228
Body surface area at ASCT, median, m2 (range)	1.0 (0.6-2.4)	1.0 (0.5-1.7)	0.357
Diagnosis			0.87
Brain tumor	4 (26.7%)	4 (17.4%)	
Malignant lymphoma	3 (20.0%)	3 (13.0%)	
Osteosarcoma	3 (20.0%)	5 (21.7%)	
Ewing sarcoma	0 (0.0%)	3 (13.0%)	
Neuroblastoma	2 (13.3%)	3 (13.0%)	
Soft tissue sarcoma	1 (6.7%)	1 (4.3%)	
Hepatoblastoma	1 (6.7%)	1 (4.3%)	
Others	1 (6.7%)	3 (13.0%)	
Number of previous chemotherapy cycle, mean (range)	8.0 (5-21)	9.1 (5-21)	0.444
Number of previous HDC, mean (range)	0.7 (0-2)	0.6 (0-2)	0.832
HDC regimen			0.277
Melphalan + Etoposide + Carboplatin	7 (46.7%)	9 (39.1%)	
Busulfan + Melphalan (±Thiotepa)	1 (6.7%)	7 (30.4%)	
Carboplatin + Thiotepa	4 (26.7%)	2 (8.7%)	
Carmustine + Etoposide + (Cyclophosphamide or Melphalan/ Cytarabine)	3 (20.0%)	4 (17.4%)	
Melphalan + Cyclophosphamide	0 (0.0%)	1 (4.3%)	
Follow-up time from HSCT, year, range	2.3 (1.5-2.9)	2.6 (0.7-3.7)	0.826

KRG, Korea Red Ginseng; ASCT, autologous hematopoietic stem cell transplantation; HDC, high-dose chemotherapy.

To investigate the various T cell functions, we measured the proportional changes in T cell populations, the expression of cell surface senescent markers, and cytokine production profiles; we also investigated patients' clinical courses and safety profiles.

2. Materials and methods

2.1. Patients

In this study, a total of 38 patients were included, with 15 patients in the KRG group and 23 patients in the control group. All participants were diagnosed with solid tumors or malignant lymphomas and had undergone high-dose chemotherapy and ASCT as consolidative therapy at the Seoul National University Children's Hospital. The KRG group consisted of patients who agreed to participate in the study, were able to receive KRG powder starting 1 month after ASCT, and underwent ASCT from March 2019 to July 2020. The control group consisted of patients who agreed to participate in a different biorepository study that collected post-ASCT peripheral blood for immunologic analysis from May 2018 to January 2021. Autologous peripheral blood stem cells were collected using granulocyte colony-stimulating factor (G-CSF) administration either in conjunction with chemotherapy or plerixafor [16]. High-dose chemotherapy regimens varied and included melphalan, busulfan, carboplatin, etoposide, thiotepa, carmustine, and cyclophosphamide, as outlined in Table S1. Post-ASCT supportive management followed institutional guidelines [17,18]. This study received approval from the Institutional Review Board, and informed consent was obtained from legal guardians (H-1812-126-997 and H-1702-058-831).

2.2. Study design and KRG powder

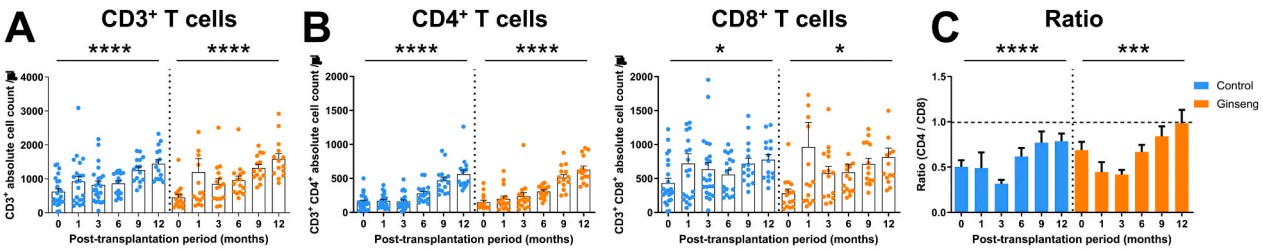
This was a single-arm exploratory trial that investigated the effects of KRG powder on T cell phenotypes and functions after ASCT in pediatric patients. To examine these effects, we compared the results of the KRG group with those of the control group. The KRG powder, which contained $>$ 5.5 mg/g of ginsenosides Rg1+Rb1+Rg3, was supplied by Korea Ginseng Corporation (Seoul, Korea). The KRG group was administered KRG powder (1.5 g/day and 3.0 g/day for patients aged $\leq$ 15 years and $>$ 15 years, respectively) from month 1 to month 12 post-ASCT. Patients who received anticoagulants, antiplatelet agents, antipsychotics, insulin, or other herbal medicines were excluded. Peripheral blood (10-15 mL) was collected for analysis at 1, 3, 6, and 12 months post-ASCT. The compliance of patients with and the safety of the KRG treatment were evaluated regularly (twice a month until 6 months post-ASCT and once a month until 12 months post-ASCT).

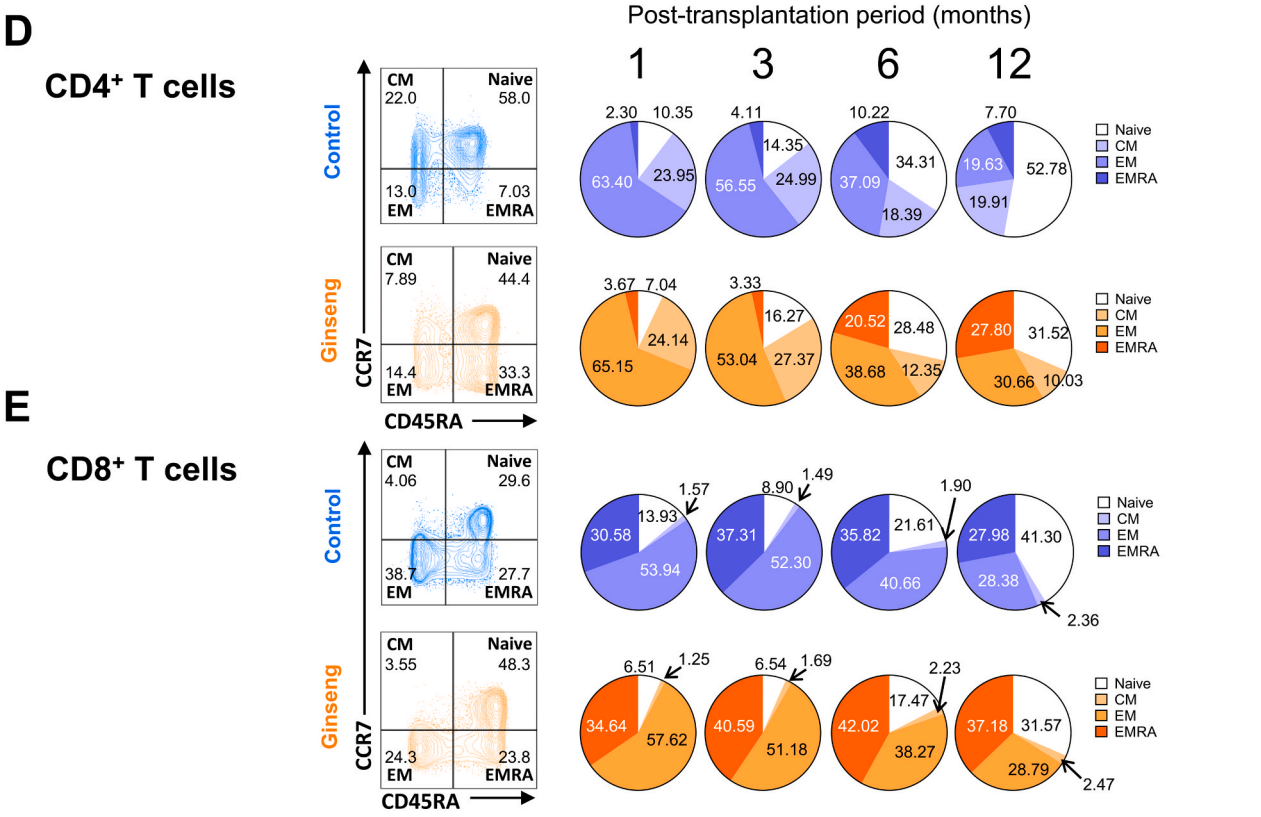
2.3. Cell preparation and flow cytometric analysis

Peripheral blood mononuclear cells (PBMCs) were isolated from human child blood by density gradient centrifugation (Biocoll Separating Solution; Biochrom, Cambridge, UK). Phenotypic staining was performed on the day of isolation, and the remaining cells were frozen in cryovials in liquid nitrogen until use. Freshly-isolated PBMCs were stained at 4 °C for 30 min with the following fluorochrome-conjugated antibodies: Alexa700-anti-CD4, V500-anti-CD8, APC-anti-CD28, PE-Cy7-anti-CCR7 (all four from BD Biosciences, Franklin Lakes, NJ, USA), APC-Cy7-anti-CD3, FITC-anti-CD45RA, APC-anti-PD-1, FITC-anti-LAG-3, PE-anti-TIM-3 (all five from BioLegend, San Diego, CA, USA), V450-anti-CD57, and PE-anti-CD85j (both from eBioscience, San Diego, CA, USA) antibodies. Stained cells were acquired using Fortessa-X20 or LSRFortessa (BD Biosciences) and analyzed using the FlowJo software (Tree Star, Ashland, OR, USA).

2.4. Intracellular staining

Thawed frozen peripheral blood mononuclear cells (PBMCs) were





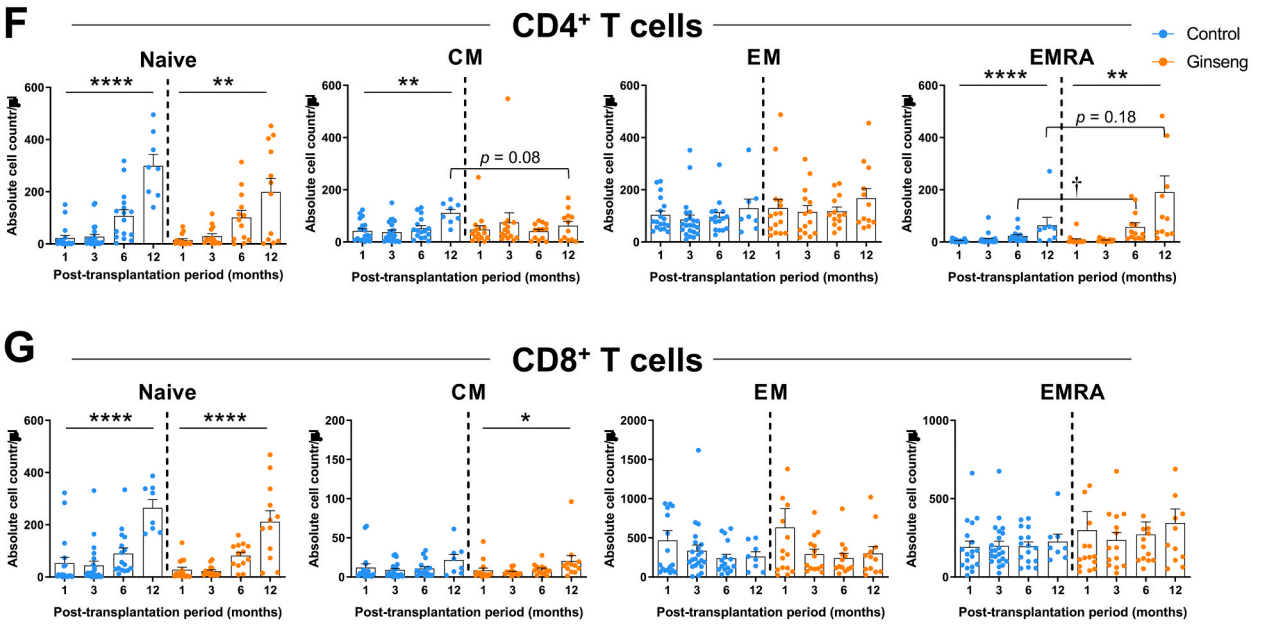


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Fig. 1. Effect of KRG treatment on the repopulation of T cells following ASCT. (**A, B**) Absolute number of $CD3^+$, $CD4^+$, and $CD8^+$ T cells in the control and KRG-treated patients after ASCT. (**C**) Ratio of $CD4^+$ to $CD8^+$ T cells in both groups. (**D, E**) Phenotypic analysis of T cells in freshly-isolated PBMC samples by flow cytometry. Distribution of functional $CD4^+$ and $CD8^+$ T cell subsets is defined by CD45RA and CCR7 expression: naive ($CD45RA^+CCR7^+$), central memory (CM; $CD45RA^-CCR7^+$), effector memory (EM; $CD45RA^-CCR7^-$), and $CD45RA^+$ effector memory (EMRA; $CD45RA^+CCR7^-$). Representative FACS plot of functional T cell subsets at 12 months post-ASCT (left) and time-dependent changes in the frequency of each subset (right) are shown. (**F, G**) Absolute number of functional T cells in the $CD4^+$ and $CD8^+$ subsets. Bar graphs show the mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ as determined by one-way ANOVA. †$P < 0.05$ as determined by two-tailed unpaired non-parametric *t*-test.

incubated overnight in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine at 37°C and 5% CO2. The cultured cells were then re-stimulated for 6 hours with PMA (50 ng/mL) and ionomycin (1 μg/mL) in the presence of PE-anti-CD107a antibody (BD Bioscience) and Brefeldin A (Cell Signaling Technology, Danvers, MA, USA) during the last 4 hours. Following stimulation, the cells were stained with APC-Cy7-anti-CD3 antibody (BioLegend), Alexa700-anti-CD4 antibody, V500-anti-CD8 antibody, and APC-anti-CD28 antibody (all from BD Biosciences). Fixation and permeabilization of the cells were performed using the BD Cytofix/Cytoperm Solution Kit (BD Biosciences). The fixed cells were further stained with V450-anti-IFN-γ antibody, PE-Cy7-anti-TNF-α antibody, and PerCP-anti-MIP1β antibody. The stained cells were then acquired using either Fortessa-X20 or LSRFortessa flow cytometers and analyzed using FlowJo software.

2.5. Statistical analysis

One-way analysis of variance was used to analyze the data, and the Mann-Whitney U test was performed when the number of patients was less than 20. The Prism 8 software (GraphPad Software Inc., La Jolla, CA, USA) was used as indicated in the figure legends. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

Characteristics of the patients in the KRG and control groups are presented in Table 1. The median age at the time of ASCT was 8.1 years (ranging from 2.7 to 19.1) in the KRG group and 9.0 years (ranging from 1.7 to 15.6) in the control group. The study included patients with malignant lymphomas and high-risk solid tumors such as brain tumors, neuroblastomas, and Ewing sarcomas who met the criteria for high-dose chemotherapy and ASCT. All patients in the KRG group underwent blood sampling for 1 year after ASCT, whereas 8 patients in the control group (34.8%) were unable to complete the sampling due to relapse or secondary malignancy. There were no significant differences in the age, sex, body weight, body surface area, and diagnosis of patients, the high-dose chemotherapy regimens, and the number of previous chemotherapy cycles or ASCT procedures between the two groups.

3.2. Repopulation of the $CD4^+$ and $CD8^+$ T cell subsets after ASCT

We analyzed the T cell repopulation patterns in patients who underwent ASCT for 1 year after ASCT. The total number of $CD3^+$ T cells increased significantly during the first 12 months after ASCT (Fig. 1A). In addition, the absolute number of $CD4^+$ T cells gradually increased during the first 12 months after ASCT, whereas that of $CD8^+$ T cells increased remarkably during the first month after ASCT and fluctuated over the last 11 months (Fig. 1B). In normal pediatric individuals, the CD4/CD8 ratio of T cells is > 1 [19]. Our data show that the CD4/CD8 ratio started to increase 9 months after the ASCT procedure in both groups and was close to 1 after 12 months in KRG-treated patients (Fig. 1C). These results indicate that ASCT increases the number of T cells, leading to the repopulation of $CD4^+$ and $CD8^+$ T cells, albeit in a different pattern, and restoration of the CD4/CD8 ratio to normal levels in KRG-treated patients.

3.3. Effect of KRG treatment on the repopulation of T cells after ASCT

To further investigate the effect of KRG administration on T cell repopulation in patients who underwent ASCT, time-dependent changes in the frequency and the absolute number of functional T cell subsets were compared between the two groups (Fig. S1). In the control group, the naive subset of $CD4^+$ and $CD8^+$ T cells and the effector memory cell re-expressing CD45RA (EMRA) subset of $CD4^+$ T cells expanded over time after ASCT, whereas the effector memory (EM) subset of both $CD4^+$ and $CD8^+$ T cells diminished during the first 12 months after ASCT (Fig. 1D and E, S2A, S2B). However, the central memory (CM) subset of $CD4^+$ cells declined gradually during the first 12 months after ASCT in the KRG group, with a significant difference when compared with the control group. Moreover, KRG-treated patients showed an increase in the $CD4^+$ EMRA population at 12 months compared to the control patients. In agreement with the frequency of functional T cell subsets, the administration of KRG powder significantly increased the number of $CD4^+$ EMRA cells and tended to reduce the number of $CD4^+$ CM cells at 6 and 12 months, respectively (Fig. 1F and G). These data show that KRG administration predominantly influences the repopulation of $CD4^+$ T cells via T cell expansion and differentiation into EMRA cells.

3.4. Effect of KRG treatment on the senescent phenotypes of T cells after ASCT

Next, we analyzed the senescent phenotypes of repopulated T cells (Fig. 2A, S1). Senescent T cells, especially $CD8^+$ T cells, are characterized by the loss of CD28 expression and gain of several NK cell receptors, such as CD57 and CD85j [20]. The frequency of $CD8^+$ T cells in the $CD28^-$ subset was higher than that of $CD4^+$ T cells. However, the frequency of $CD8^+$ T cells decreased during repopulation after ASCT (Fig. 2A). As $CD28^-$ cells mainly belong to the EM and EMRA subsets (Fig. 2B), we analyzed the time-dependent changes in the frequency of senescent T cells in these subsets in both patient groups (Fig. 2C). The frequencies of $CD28^-$, $CD57^+$, and $CD28^-CD57^+$ cells, but not that of $CD85j^+$ cells, decreased gradually in the EMRA subset of $CD4^+$ and $CD8^+$ T cells after ASCT. Moreover, KRG-treated patients showed a reduction in senescence phenotypes in the EMRA subset compared with the control patients. This reduction was more obvious in the $CD4^+$ EMRA subset, which was increased by KRG administration (Fig. S2A), suggesting that KRG alleviates senescent phenotypes. These data suggest that KRG administration influences the repopulation of $CD4^+$ T cells and regulates senescence.

3.5. Cytokine production profiles of senescent $CD28^-$ T cells

To determine whether the reduction in $CD28^-$ T cells in the KRG group alleviates the functional defects associated with senescence, we examined the cytokine production and cytotoxicity of T cells. The production of cytokines was generally reduced in $CD8^+$ and $CD4^+$ T cells in the KRG group compared to the control group (Fig. 3A and B). The frequency of $CD4^+$ T cells producing IFN-γ and MIP-1β in response to PMA/ionomycin stimulation was significantly lower in the KRG group. A similar trend was observed for IFN-γ and MIP-1β production by $CD8^+$ T cells. However, the administration of KRG powder increased the population of $CD107a^+CD4^+$ T cells 12 months after ASCT. To evaluate the effect of KRG powder on the functionality of senescent $CD28^-$ T cells, we analyzed cytokine production by examining $CD28^+$ and $CD28^-$ T cells

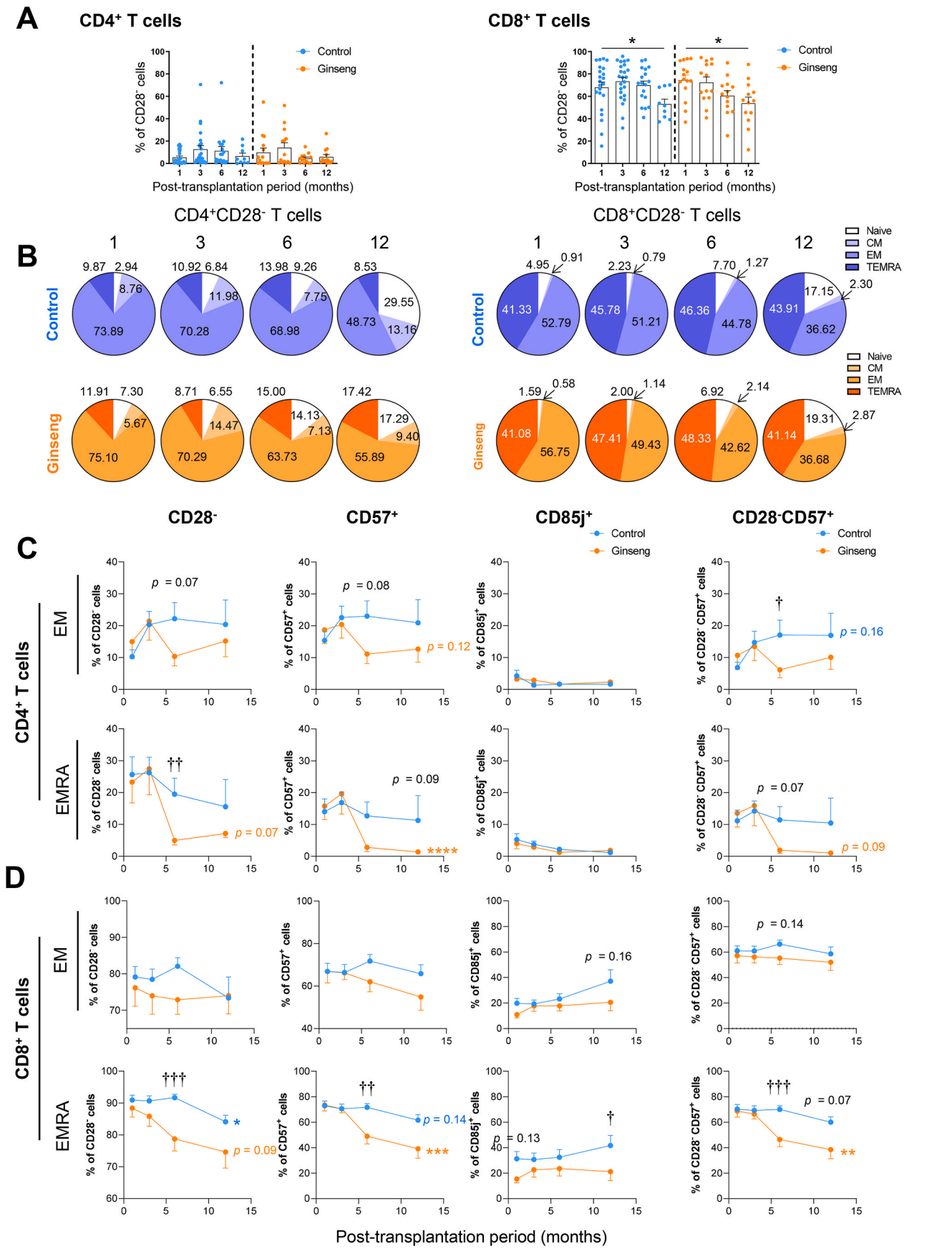

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Fig. 2. Effect of KRG treatment on the senescent phenotypes of T cells following ASCT. (**A**) Time-dependent changes in the frequency of $CD28^-$ cells among $CD4^+$ and $CD8^+$ T cells during the 12 months after ASCT were determined by flow cytometry. (**B**) Time-dependent changes in the frequency of naive, CM, EM, and EMRA subsets of $CD4^+CD28^-$ (left) and $CD8^+CD28^-$ (right) T cells in the control and KRG-treated patients. (**C, D**) Changes in the expression of senescence markers in the EM and EMRA subsets of $CD4^+$ and $CD8^+$ T cells. Frequency (%) of $CD28^-$, $CD57^+$, $CD85j^+$, or $CD28^-CD57^+$ cells in the EM and EMRA subsets of $CD4^+$ and $CD8^+$ T cells in both patient groups. Bar and line graphs show the mean ± SEM. Orange- or pale blue-colored *p*-values and asterisks were analyzed for the KGR or control group. Black-colored *p*-values and crosses were analyzed and compared at each time point between the KGR and control groups. $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ as determined by one-way ANOVA. $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, and $\dagger\dagger\dagger P < 0.001$ as determined by two-tailed unpaired non-parametric *t*-test.

(Fig. 3C). Consistent with previous findings [20], $CD28^-$ T cells produced more IFN-γ and CD107a upon stimulation than $CD28^+$ T cells in the $CD4^+$ subset in patients who underwent ASCT (Fig. 3D). Of note, the frequency of IFN-γ and MIP-1β-producing cells among the $CD4^+$ and $CD8^+$ T cells in the $CD28^-$ subset decreased following KRG administration. However, the frequency of CD107a-expressing cells among $CD28^-$ T cells increased in the KRG group. Our data show that the administration of KRG powder inhibited cytokine production in senescent $CD28^-$ T cells after ASCT, suggesting a reduction in senescence-dependent cytokine production.

3.6. Effect of KRG treatment on the proportions of exhausted $PD\text{-}1^+$ and $TIM3^+$ T cells after ASCT

T cell exhaustion and senescence are the two main dysfunctional states [21]. Thus, the expression of exhaustion markers in the repopulated T cell subsets was analyzed (Fig. S3). After 1 month of the ASCT procedure, the proportion of T cells expressing TIM3 was greater in the KRG group than in the control group. However, the proportion of $CD4^+$ and $CD8^+$ T cells expressing PD-1 and TIM3 decreased at 12 months post-ASCT in the KRG group and was similar to that in the control group. No significant changes were observed in the proportion of T cells expressing LAG3.

3.7. Safety profiles and comparison of ASCT complications

The post-ASCT complications were compared between the two groups and are summarized in Table 2. There were no significant differences in the incidence of hepatic and renal dysfunction or viral infections between the two groups. Moreover, no notable complications related to KRG administration were observed. All patients in the KRG group completed the KRG treatment period, which lasted 1 year after the ASCT procedure.

4. Discussion

This study examined the effect of KRG powder on the repopulation and functions of T cells in pediatric patients with cancer who received high-dose chemotherapy and ASCT. Upon comparing the KRG-treated group with the control group, we found that the KRG powder exerted beneficial effects on pediatric cancer survivors, including repopulation of $CD4^+$ T cells, regulation of senescence, decrease in the proportion of $PD\text{-}1^+$ and $TIM\text{-}3^+$ T cell populations, and amelioration of the capacity for senescence-related cytokine production in the $CD28^-$ T cell population. Moreover, the KRG powder was well-tolerated in this patient population. To the best of our knowledge, this is the first study to investigate the effects of KRG powder on pediatric patients with malignancies who underwent ASCT.

Our study showed that the administration of KRG powder led to the repopulation of both $CD4^+$ and $CD8^+$ T cells to an extent similar to that observed in the control group after ASCT. However, the administration of KRG powder predominantly influenced the repopulation of $CD4^+$ T cells via T cell expansion and differentiation into EMRA cells. The effect of KRG powder on T cell populations and functions may be explained by the ability of KRG to increase the pro-inflammatory activity of human T cells [22,23]. The repopulation of T cells, especially $CD4^+$ T cells, occurred in the patients through the accumulation of naive T cells and clonal expansion to the EMRA subset, which is known to express more senescence markers than other functional T cell subsets, such as naive and CM [20,24]. Therefore, when ASCT induces T-cell senescence, it may trigger greater chronic inflammatory responses than those in healthy controls. This might contribute to overcoming the lack of immunity in the early period after ASCT; however, the EMRA population has a senescent phenotype that shows abnormal immune responses.

Furthermore, the administration of KRG powder not only contributes to the repopulation of $CD4^+$ T cells but also alleviates senescent phenotypic changes in these cells. Clonal expansion induces replicative stress, which increases senescent changes, such as the loss of CD28 in T cells [25–27]. Moreover, $CD28^-$ T cells generally acquire both CD57 and CD85j, which contribute to the regulation of their functional activity [28–31]. We analyzed these senescent phenotypes in repopulated T cell subsets, which revealed that the administration of KRG powder reduced the senescence of $CD4^+$ T cells, suggesting that KRG powder regulates senescence while promoting the repopulation of $CD4^+$ T cells.

Various shared changes in T cell phenotype have been demonstrated in childhood cancer survivors and elderly individuals [32]; however, the increase of $CD4^+$ EMRA cells accompanied by a decrease in senescent markers is a unique finding within this population. In the context of normal aging, the $CD4^+$ EMRA T cell population remains relatively stable, whereas the $CD8^+$ EMRA T cell population increases progressively [33]. In an investigation involving neuroblastoma survivors, a persistent rise in $CD8^+$ EMRA T cells was observed post-treatment, while there was no definitive increase in $CD4^+$ EMRA cells. However, this trend became similar to the control group over a span exceeding 5 years [34]. Furthermore, an elevation in senescent markers of T cells was evident among survivors of childhood leukemia. Notably, $CD4^+CD57^+$ T cell counts exhibited a more pronounced increase among survivors than within the control cohort. This pattern was similar with those in elderly individuals and HIV patients, prompting the need for meticulous surveillance [6]. Recent research indicates that $CD57^-CD8^+$ EMRA cells are comparatively "young," characterized by heightened proliferative potential and versatile differentiation in contrast to their $CD57^+$ counterparts [35]. Importantly, the rapid and robust accumulation of EMRA cells in KRG-treated pediatric patients post ASCT showcases a marked reduction in CD57 expression and CD28 loss, indicative of "young" EMRA cells potentially advantageous for early-stage transplant-related host defense. While its positive impact remains uncertain, prioritizing ongoing and comprehensive immune monitoring is crucial for patients who exhibit an increased $CD4^+$ EMRA T cell population along with a decrease in senescent markers

Our data also showed that KRG administration regulates senescence-related pro-inflammatory cytokine production in $CD4^+$ and $CD8^+$ T cells. The EMRA subset, which is capable of inducing a similar level of immune response as the EM subset, has been linked to chronic inflammation in aging individuals. This is due to the chronic production of bystander IFN-γ by EMRA cells [36] and an increase in the concentration of MIP-1β (CCL4) and expression of CD107a with age, which further contribute to chronic inflammation [37]. The decrease in cytokine production following KRG administration can be related to an abnormal inflammatory response; however, reduced IFN-γ and MIP-1β production in $CD28^-$ cells might alleviate the excessive immune response of EMRA, which increased during repopulation after ASCT. Thus, KRG administration may provide the dual benefit of suppressing the accumulation of senescent CD28- T cells in patients while also reducing the secretion of inflammatory cytokines from these cells. Moreover, CD107a expression was significantly increased by the KRG treatment. CD107a is a

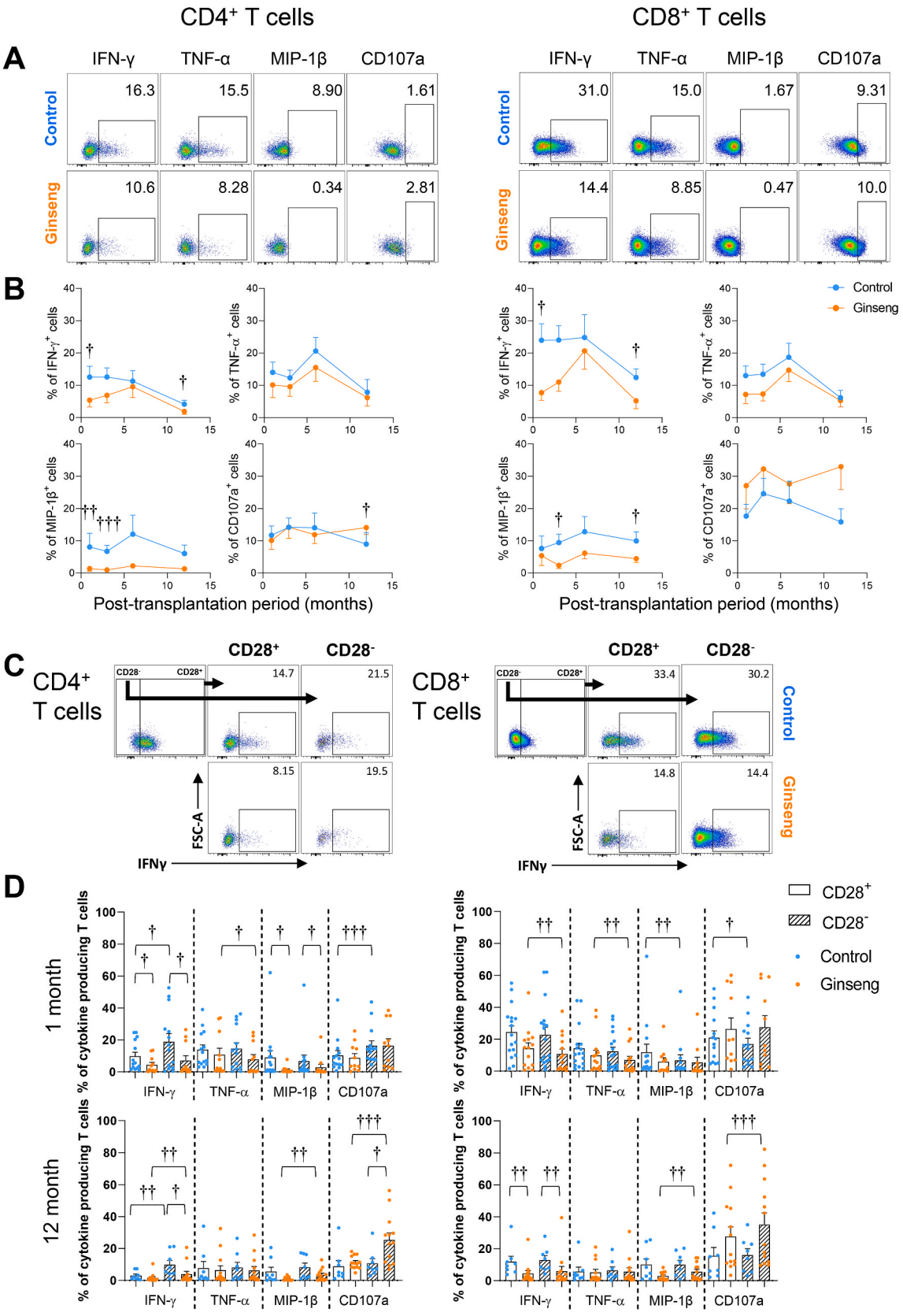

Fig. 3. Cytokine production profiles of senescent CD28⁻ T cells. PBMCs of patients were stimulated with PMA and ionomycin for 6 h ($n = 8 \sim 18$ for each time point), followed by intracellular cytokine staining (ICS). (**A**) Representative FACS plot of cytokine-producing $CD4^+$ and $CD8^+$ T cells following ASCT in the control and KRG-treated patients at 1-month post-ASCT. (**B**) Time-dependent changes in the frequency (%) of cytokine-producing $CD4^+$ (left) and $CD8^+$ (right) T cells. (**C**) Representative FACS plot of cytokine-producing $CD28^+$ or $CD28^-$ cells in the $CD4^+$ and $CD8^+$ populations at 1-month post-ASCT. (**D**) Frequency (%) of cytokine-producing $CD28^+$ or $CD28^-$ cells in $CD4^+$ and $CD8^+$ populations in the control and KRG-treated patients at 1 and 12 months after ASCT. Bar and line graphs show the mean ± S.E.M. †$P < 0.05$, ††$P < 0.01$, and †††$P < 0.001$ as determined by two-tailed unpaired non-parametric *t*-test.

Table 2
Comparison of Post-ASCT Complications

Complications	KRG group (n = 15)	Control group (n = 23)	p value
Liver enzyme elevation			0.225
Grade 1	13 (86.7%)	17 (73.9%)	
Grade 2	2 (13.3%)	2 (8.7%)	
Grade 3	0 (0.0%)	4 (17.4%)	
Total bilirubin elevation			0.346
Grade 1	0 (0.0%)	1 (4.3%)	
Grade 2	0 (0.0%)	2 (8.7%)	
Serum creatinine elevation			0.631
Grade 1	1 (6.7%)	4 (17.4%)	
Grade 2	1 (6.7%)	1 (4.3%)	
Grade 3	0 (0.0%)	1 (4.3%)	
CMV reactivation	2 (13.3%)	6 (26.1%)	0.346
EBV reactivation	0 (0.0%)	1 (4.3%)	0.413
Respiratory virus infection	0 (0.0%)	3 (13.0%)	0.145
Other complications			0.332
Thrombotic microangiopathy	0 (0.0%)	2 (8.7%)	
Evans syndrome	0 (0.0%)	1 (4.3%)	
Pancreatitis	0 (0.0%)	1 (4.3%)	
Hepatic veno-occlusive disease	0 (0.0%)	2 (8.7%)	
pneumonitis	0 (0.0%)	1 (4.3%)	
Hypercalcemia	1 (6.7%)	1 (4.3%)	
Hyperuricemia	2 (13.3%)	0 (0.0%)	

ASCT, autologous hematopoietic stem cell transplantation; KRG, Korea Red Ginseng; CMV, cytomegalovirus; EBV, Epstein-Barr virus.

functional marker of the activity of cytotoxic T lymphocytes and natural killer cells, which are critical for the host defense against infections. Therefore, the increase in CD107a expression may contribute to overcoming vulnerable immune responses during the early stages of transplantation.

Our study, which was not a randomized comparative trial and included a relatively small number of patients, has limitations owing to its experimental design. Although all patients completed the treatment at the time of enrollment, it was not possible to randomize the administration of KRG powder owing to safety concerns and the unwillingness of patients. However, to compare the effects and safety of KRG powder, patients who only agreed to participate in the collection of serial blood samples for a biorepository study were also studied as controls. Therefore, additional studies with larger sample sizes and randomized designs should be conducted to address these limitations and provide stronger evidence.

In conclusion, our study suggests that administering KRG powder to pediatric patients with high-risk malignancies who undergo high-dose chemotherapy and ASCT may be beneficial in terms of T cell regulation and safety. The KRG group showed a fast and robust repopulation of $CD4^+$ EMRA T cells, accompanied by regulation of the senescence process. To fully understand the long-term benefits of KRG powder in this population, which is at risk of developing complications or sequelae after treatment, further well-designed long-term follow-up trials are needed.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of the Seoul National University Hospital, and informed consent was obtained from legal guardians (H-1812-126-997 and H-1702-058-831).

Authors' contributions

HYS and HJK initiated the project; KTH and YJK participated in study design, data collection, statistical analysis/interpretation, manuscript drafting, and manuscript revisions. KTH, YJK, and WWL performed all the analyses. JYC, YJY, IMC, and HYS participated in manuscript editing. HJK and WWL participated in study design and manuscript editing. All authors have read and approved the manuscript.

Declaration of competing interest

The authors have no conflicts of interest to disclose.

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List of abbreviations

KRG	Korean Red Ginseng
ASCT	autologous hematopoietic stem cell transplantation
EMRA	effector memory cell re-expressing CD45RA
PBMCs	peripheral blood mononuclear cells
FBS	fetal bovine serum
EM	effector memory
CM	central memory

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2023.09.001.

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