Clonal hematopoiesis in the donor does not adversely affect long-term outcomes following allogeneic hematopoietic stem cell transplantation: result from a 13-year follow-up

Kyoung Ha Kim,[1,2*] TaeHyung Kim,[1,3,4*] Igor Novitzky-Basso,[1] Hyewon Lee,[1,5] Youngseok Yoo,[1] Jae-Sook Ahn,[4,6] Ivan Pasic,[1] Arjun Law,[1] Wilson Lam,[1] Fotios V. Michelis,[1] Armin Gerbitz,[1] Auro Viswabandya,[1] Jeffrey Lipton,[1] Rajat Kumar,[1] Jonas Mattsson,[7] Zhaolei Zhang,[3,4,8] Nathali Kaushansky,[9] Yardena Brilon,[9] Noa Chapal-Ilani,[9] Tamir Biezuner,[9] Liran I. Shlush[9#] and Dennis Dong Hwan Kim[1,10#]

[1]Division of Medical Oncology and Hematology, Princess Margaret Cancer Center, Toronto, Ontario, Canada; [2]Department of Internal Medicine, Soonchunhyang University College of Medicine, Soonchunhyang University Hospital, Seoul, Korea; [3]Department of Computer Science, University of Toronto, Toronto, Ontario, Canada; [4]The Donnelly Center for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada; [5]Division of Rare and Refractory Cancer, Division of Hemato-Oncology, and Center for Hematologic Malignancy Research Institute and Hospital, National Cancer Center, Goyang, Korea; [6]Department of Internal Medicine, Chonnam National University Hwasun Hospital, Chonnam National University, Gwangju, Korea; [7]Gloria and Seymour Epstein Chair in Cell Therapy and Transplantation, Department of Medicine, University of Toronto, Toronto, Ontario, Canada; [8]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; [9]Department of Immunology, Weizmann Institute of Science, Rehovot, Israel and [10]Institute for Medical Science, Faculty of Medicine, University of Toronto, Toronto, Canada

KHK and TK contributed equally as co-first authors.
#LS and DK contributed equally as co-senior authors.

Correspondence: D. D. H. Kim
dr.dennis.kim@uhn.ca

L. Shlush
liran.shlush@weizmann.ac.il

Received: July 24, 2022.
Accepted: January 26, 2023.
Early view: February 2, 2023.

https://doi.org/10.3324/haematol.2022.281806

Abstract

Donor clonal hematopoiesis may be transferred to the recipient through allogeneic hematopoietic stem cell transplantation (HSCT), but the potential for adverse long-term impact on transplant outcomes remains unknown. A total of 744 samples from 372 recipients who received HSCT and the corresponding donors were included. Bar-coded error-corrected sequencing using a modified molecular inversion probe capture protocol was performed, which targeted 33 genes covering mutations involved in clonal hematopoiesis with indeterminate potential (CHIP) and other acute myeloid leukemia-related mutations. A total of 30 mutations were detected from 25 donors (6.7%): the most frequently mutated gene was *TET2* (n=7, 28%), followed by *DNMT3A* (n=4, 16%), *SMC3* (n=3, 12%) and *SF3B1* (n=3, 12%). With a median follow-up duration of 13 years among survivors, the presence of CHIP in the donor was not associated with recipient overall survival (P=0.969), relapse incidence (*P*=0.600) or non-relapse mortality (*P*=0.570). Donor CHIP did not impair neutrophil (*P*=0.460) or platelet (*P*=0.250) engraftment, the rates of acute (*P*=0.490), or chronic graft-*versus*-host disease (*P*=0.220). No significant difference was noted for secondary malignancy following HSCT between the two groups. The present study suggests that the presence of CHIP in allogeneic stem donors does not adversely affect transplant outcomes after HSCT. Accordingly, further study is warranted to reach a clearer conclusion on whether molecular profiling to determine the presence of CHIP mutations is necessary for the pretransplant evaluation of donors prior to stem cell donation.

Introduction

Clonal hematopoiesis with indeterminate potential (CHIP) constitutes a part of the biological aging process,[1] and comprises the acquisition of somatic mutations in hematopoietic stem cells (HSC). The presence of CHIP in healthy individuals without any evidence of hematologic abnormalities is known to increase the risk of hematologic malignancy.[2] CHIP-related mutations within genes such as *DNMT3A*, or *TET2* can be detected in up to 95% of healthy individuals with a median age of 50 years when sequencing depth is enhanced up to 100,000× coverage,[3] which is at least a thousand times deeper (84× coverage) than that used in the original study which first described the CHIP.[1,4]

Nowadays, CHIP is no longer considered a rare phenomenon in healthy individuals, although its biological consequences are still under investigation.

In the context of allogeneic hematopoietic stem cell transplantation (HSCT), the potential transfer of CHIP from donor to recipient may raise concerning implications. An early anecdotal report described transfer of CHIP-mutated HSC to recipients through HSCT.[5] Another study suggested an increased risk of poor graft function with HSCT transfer of CHIP hematopoiesis.[6] These reports prompted further investigation into whether donors carrying CHIP are acceptable for HSC donation, and it remains unclear whether the use of HSC from donors carrying CHIP correlates with delayed engraftment of HSC after HSCT.

In addition to engraftment, the impact of the presence of CHIP in donors ("donor CHIP") on long-term outcomes following allogeneic HSCT remains to be fully elucidated, including overall survival, relapse incidence or non-relapse mortality (NRM).[7,8] Oran *et al.*[8] reported that donor-derived CHIP does not increase the risk of relapse, NRM or survival after allogeneic HCT, while Frick *et al.*[7] reported somewhat contradicting results, showing reduced incidence of relapse/progression when transplanted with donors with CHIP.

It is a matter of debate whether donor CHIP increases the risk of graft-*versus*-host disease (GvHD) following HSCT. Frick *et al.*[7] reported that patients who received HSC from a donor with CHIP had a comparatively higher incidence of chronic GvHD (cGvHD) compared to those having a donor without CHIP, while the risk of acute GvHD (aGvHD) was not different between the two groups. In contrast, Oran *et al.*[8] reported no difference in the risk of cGvHD according to the presence of CHIP in donors but found a higher risk of aGvHD in recipients of a donor with CHIP. This debate also prompted us to evaluate the impact of donor CHIP in detail not only on a/cGvHD incidence, but also on the severity and the extent of organ involvement by a/cGvHD.

Interestingly, other work has suggested that the presence of CHIP is associated with an increased risk of solid tumors. CHIP is more prevalent in patients with solid tumors, with a prevalence of approximately 30% in the blood of solid tumor patients compared with the general population.[9] A study of paired tumor/blood sequencing was performed in a large cohort of 8,810 patients with non-hematologic cancer using deep coverage. Although it was not completely clear whether shared risk factors, such as smoking, existed between solid cancer and CHIP, it suggested a potential relationship between CHIP and the risk of solid cancer.[9] Therefore, we examined whether donor CHIP was associated with the risk of secondary malignancy (SM) in HSCT recipients within our cohort. Given that the present study has a long follow-up duration of 13 years, this work presented a unique opportunity to evaluate whether donor CHIP affected the incidence of SM after allogeneic HSCT.

Methods

Summary of the cohorts and transplant outcomes

A total of 744 samples were included from 372 recipients who received allogeneic HSCT from 2000 to 2007 at the Princess Margaret Cancer Center, Toronto, Canada, and the corresponding donors. GvHD prophylaxis and supportive care adhered to previously described institutional policies.[10-12] Genomic DNA samples from consenting donors and recipients were archived from blood samples taken 2-3 weeks prior to HSCT. This study was approved by the Institutional Ethics Board at the Princess Margaret Cancer Center. Patient characteristics are summarized in Table 1: male 59.9% (n=223); median age 48 years (range, 17-71); predominant use of myeloablative conditioning (n=267, 71.8%), HLA-matched related donor (n=272, 73.1%) and peripheral blood stem cells as a source of stem cells (n=259, 69.6%).

Bar coded error-corrected sequencing for CHIP detection

For the detection of CHIP, bar-coded error-corrected sequencing method was used which is a modified molecular inversion probe capture protocol,[13] performed at the Weismann Institute of Science (Rehovot, Israel). Details are provided in the *Online Supplementary Appendix*. In summary, we designed probes targeting 33 genes covering CHIP-related mutations along with other mutations related to AML: *FLT3*/ITD, *NPM1c* and *CEBPA*. The list of genes targeted is presented in the *Online Supplementary Table S1*. Bar-coded next-generation sequencing (NGS) library was generated and processed for sequencing with a 150 bp pair-end mode (NovaSeq, Illumina). Somatic variant calling analysis was performed using a customized computational pipeline.[14] CHIP mutations were confirmed if they were present with variant allele frequency (VAF) >0.005 in two technical duplicates, with the addition of other filters as described in *Online Supplementary Appendix*.[14]

Definition of statistical endpoints

The day of the stem cell infusion was defined as day 0. Overall survival (OS) duration was calculated as the time from day 0 until death from any cause or last follow-up. NRM was defined as the event of death not related to disease recurrence or progression. Recurrence was defined as recurrence of primary disease following HCT. Engraftment after HCT was determined as a peripheral neutrophil count of $\geq 0.5\times10^9$/L for 3 consecutive days, and a platelet count of $\geq 20\times10^9$/L for at least 3 consecutive days without requiring transfusions or growth factor support. aGvHD and cGvHD were diagnosed and graded using the aGvHD consensus conference criteria[15] and the NIH consensus criteria for cGvHD.[16] Documentation of secondary malignancy (SM) in the BMT in-house database was captured and summarized for tumor type, tumor site, tumor stage,

the time from day 0 to diagnosis of SM, and the presence of active GvHD at the time of diagnosis of SM.[17]

Statistical analysis

Patient baseline demographic and disease characteristics as well as transplant procedures are presented with descriptive statistics (Table 1) and were compared according to the presence of donor CHIP using chi-square test for categorical variables and Wilcoxon rank-sum test for continuous variables.

For OS, Kaplan-Meier method was used using the log-rank test, while Cox proportional hazard model was used for univariate and multivariate analysis. For the cumulative incidence analysis of relapse, NRM, engraftment of neutrophil/platelet, aGvHD, cGvHD or SM, Gray method was applied considering the competing events as appropriate. For example, the incidence of SM was defined as time from day 0 until documented date of clinical diagnosis of SM or last follow-up considering death not related to SM or relapse of primary disease as competing events. The Fine-Gray proportional hazard regression model was used for univariate and multivariate analysis of cumulative incidence. For multivariate analysis, stepwise selection procedure was applied including all variables significant in univariate analysis at *P* value ≤0.1. The presence of donor CHIP variable was mandated for inclusion in the final model throughout the study. Hazard ratio (HR) and 95% confidence interval (CI) were estimated using a predetermined reference risk of 1.0. *P* values of <0.05 were considered statistically significant. For statistical analyses, R statistical software 3.5.0 (the R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) were used. EZR (version 1.41) is a modified version of R commander18 (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html).

Results

Detection of clonal hematopoiesis-related mutation in donors and recipients

The mean on-target sequencing coverage was 8,540×. All sequencing data included in this study have been up-

Table 1. Demographic, disease, and transplantation characteristics of recipients according to the presence of donor CHIP.

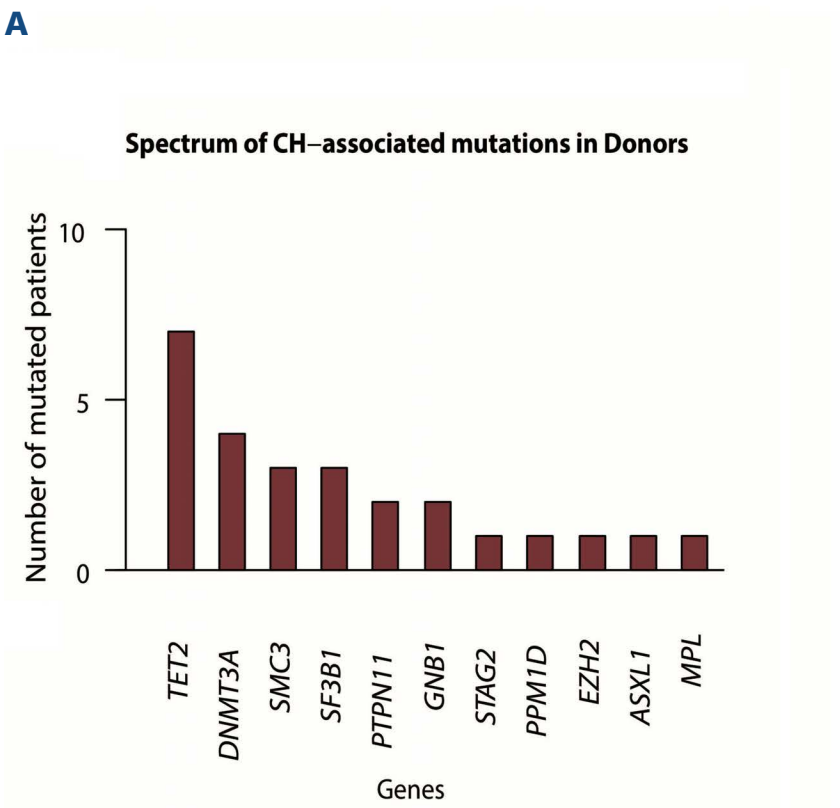
Patients, N (%)	CHIP in donor (N=25)	No CHIP in donor (N=347)	*P*
Donor age in years, median (range)	55 (24-70)	48 (11-75)	0.074
Recipient age in years, median (range)	51 (21-65)	47 (17-71)	0.158
Recipient sex			1.000
Male	15 (60.0)	208 (59.9)	
Female	10 (40.0)	139 (40.1)	
Diagnosis			0.594
Aplastic anemia	1 (4.0)	15 (4.3)	
AML/MDS/MPN	13 (52.4)/0 (0)/1 (4.0)	125 (36.0)/28 (8.1)/19 (5.5)	
ALL/CLL/NHL	1 (4.0)/1 (4.0)/5 (20.0)	50(14.4)/24 (6.9)/42 (12.1)	
CML/MM/Other†	3 (12.0)/0 (0)/0 (0)	36 (10.4)/4(1.2)/4 (1.2)	
Conditioning regimen by intensity			1.000
MAC	18 (72.0)	249 (71.8)	
RIC	7 (28.0)	98 (28.2)	
Source of stem cells			0.270
Bone marrow	10 (40.0)	103 (29.7)	
PBSC	15 (60.0)	244 (70.3)	
Donor type			0.049
HLA-matched related donor	16 (64.0)	256 (73.8)	
HLA-matched unrelated donor	4 (16.0)	70 (20.2)	
Alternative donor	5 (20.0)	21 (6.1)	
TBI			0.630
No TBI	7 (28.0)	82 (23.6)	
TBI (either low dose or myeloablative dose)	18 (72.0)	265 (76.4)	
GvHD prophylaxis			1.000
No TCD	22 (88.0)	303 (87.3)	
TCD	3 (12.0)	44 (12.7)	

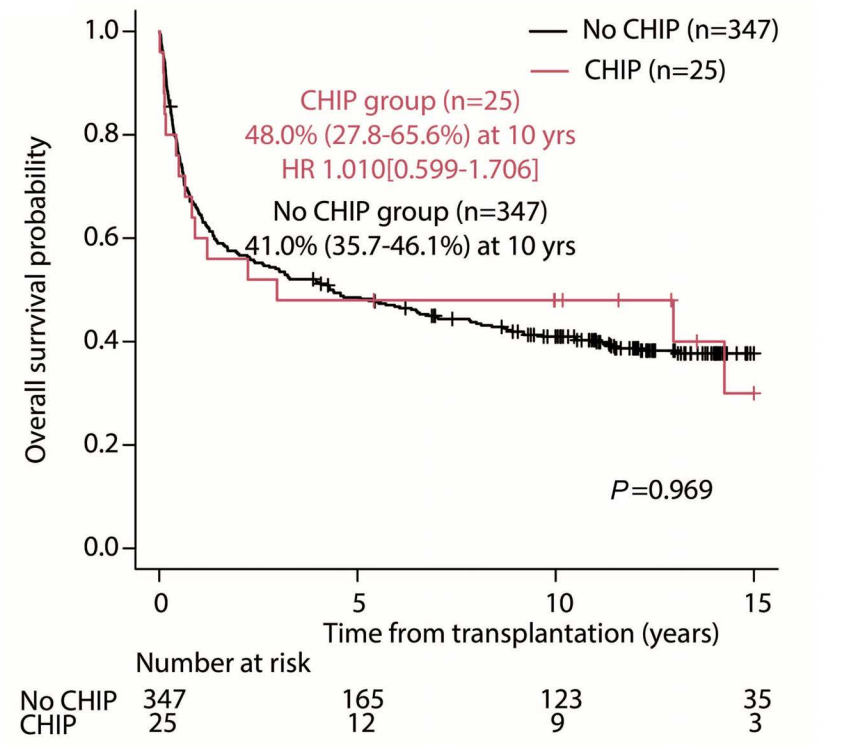
†Other diseases include prolymphocytic leukemia (N=1), NK-cell leukemia (N=1) and chronic eosinophilic leukemia (N=2). CHIP: clonal hematopoiesis of indeterminate potential; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; ALL: acute lymphoblastic leukemia; CLL: chronic lymphocytic leukemia; NHL: non-Hodgkin lymphoma; MM: multiple myeloma; CML: chronic myeloid leukemia; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; PBSC: peripheral blood stem cell; HLA: human leukocyte antigen; TBI: total body irradiation; GvHD: graft-*versus*-host diseases; TCD: T-cell depletion.

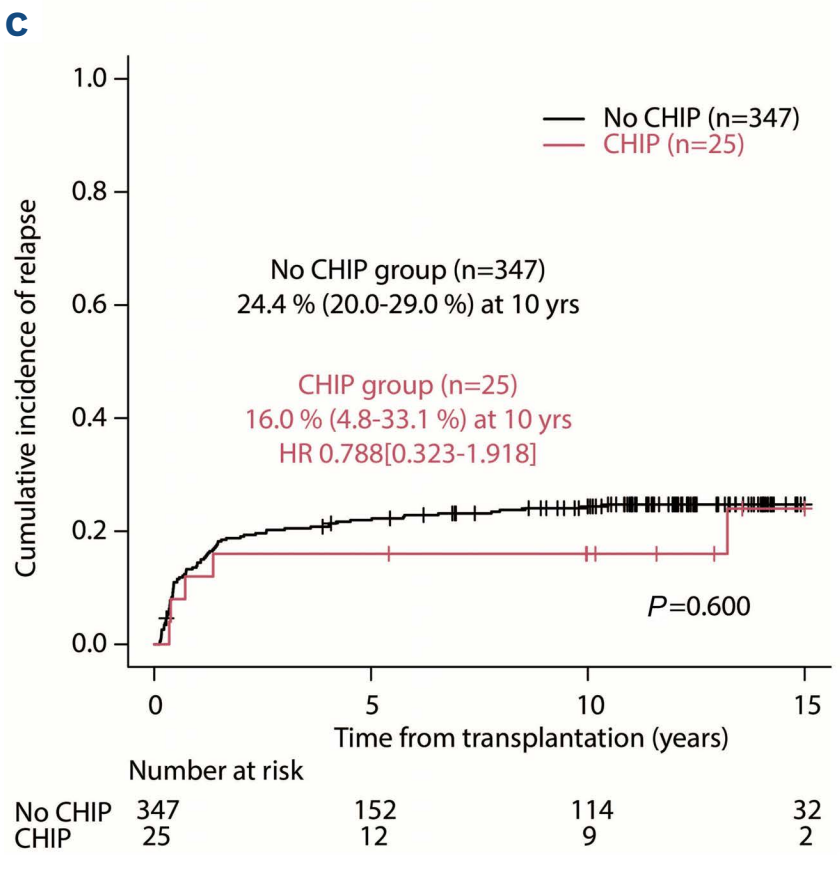
loaded into the European Nucleotide Archive. Read processing and variant calling procedures were performed as previously published.[14] Detailed descriptions are provided in *Online Supplementary Appendix*.

Analysis of 744 samples from 372 donor-recipient pairs, a total of 92 mutations were detected, of which 25 mutations came from 25 donors (6.7%) (Figure 1A). *TET2* was the most frequently mutated gene in donors (n=7, 28%), followed by *DNMT3A* (n=4, 16%), *SMC3* (n=3, 12%), and *SF3B1* (n=3, 12%). In the recipients, 67 mutations were detected from 55 recipients (18.0%). *DNMT3A* was the most frequently detected mutation in 16 recipients, followed by *TET2* (n=7). The median number of mutations was 1 (range, 0-1) in donors, and 1 (range, 0-3) in recipients, while median mutation VAF was 1.86% (range, 0.62-48.7%) in donors and 13.1% (range, 0.62-94.4%) in recipients.

When recipient characteristics were compared according to donor CHIP status as shown in Table 1, no difference was found between the two groups with respect to diagnosis, conditioning regimen intensity, source of stem cells,







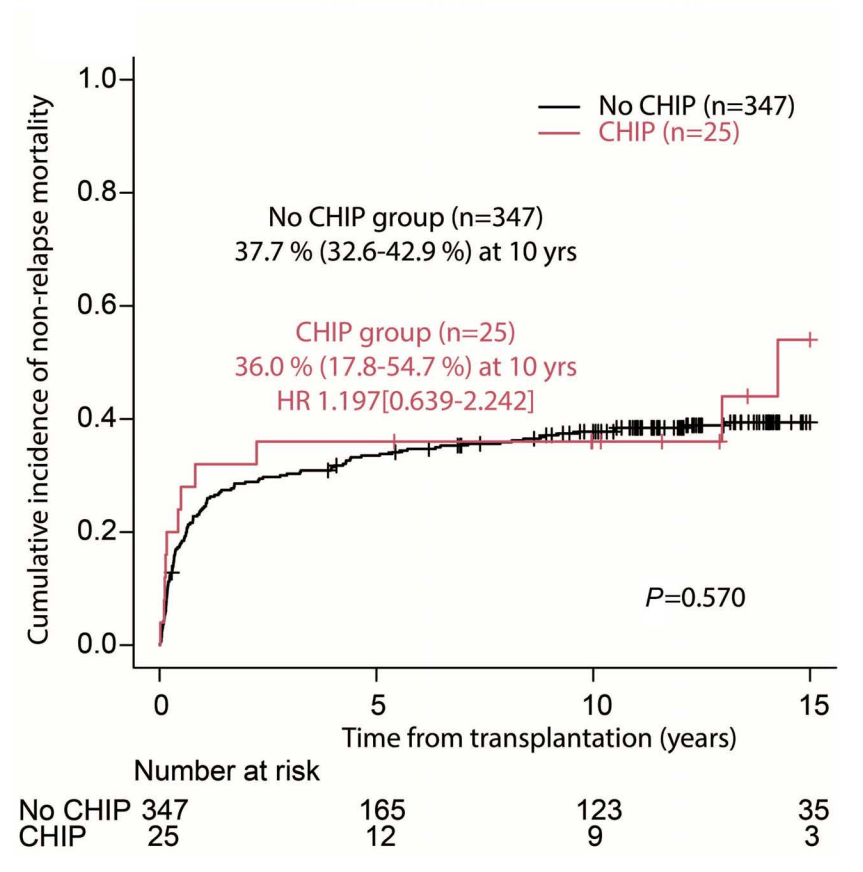


Figure 1. Donor CHIP and survival outcomes (N=372). (A). Frequency and type of clonal hematopoiesis of indeterminate potential (CHIP) mutation detected in donors and recipients (n=372). (B) Overall survival according to the presence of donor CHIP (n=372). (C) Cumulative incidence of relapse according to the presence of donor CHIP (n=372). (D) Cumulative incidence of non-relapse mortality according to the presence of donor CHIP (n=372). HR: hazard ratio; yrs: years.

donor type or GvHD prophylaxis. Of note, a statistical trend was found towards a higher age in donors with CHIP-related mutation (median 55 years) compared to donors without it (median 48 years; *P*=0.074 by Mann-Whitney U-test).

Overall outcomes following allogeneic hematopoietic stem cell transplantation

With a median follow-up duration of 13 years (range, 0.3-18.2 years) in the whole group, the 10-year rate of OS, relapse and NRM were 41.4% (95% CI: 36.4-46.4), 23.8% (95% CI: 19.6-28.3) and 37.6% (95% CI: 32.7-42.6), respectively (*Online Supplementary Figure S1*). Median day of neutrophil and platelet engraftment was 19 (range, 18-20) and 15 (range, 14-17), while the cumulative incidence of neutrophil and platelet engraftment at day 30 was 91.4% (95% CI: 88.0-93.8) and 81.5% (95% CI: 77.1-85.1), respectively. The cumulative incidence of any grade of aGvHD at day 100 and cGVHD at 3 years was 77.3% (95% CI: 72.6-81.2%) and 62.9% (95% CI: 57.8-67.6) (Table 2), respectively. The incidence of SM at 13 years was 14.3% (95% CI: 10.6-18.4).

No impact of donor CHIP on long-term outcomes following allogeneic hematopoietic stem cell transplantation including overall survival, relapse or non-relapse mortality

We next examined long-term outcomes following HSCT according to the presence of donor CHIP. Consistent with the results from the other studies, we did not find any significant difference in OS, relapse or NRM between the two groups. The 10-year OS rate was not different between the donor CHIP (48.0%) and no donor CHIP group (41.0%), HR=1.010; 95% CI: 0.599-1.706; *P*=0.969. The 10-year cumulative incidence of relapse was not different between the donor CHIP (16.0%) and no donor CHIP group (24.4%), HR=0.788; 95% CI: 0.323-1.918; *P*=0.60. In addition, the presence of CHIP in donors was not associated with 10-year NRM: 36.0% in the CHIP group compared to 37.7% in the no CHIP group, HR=1.197; 95% CI: 0.639-2.242; *P*=0.570 (Figure 1B-D; *Online Supplementary Table S2*), which was confirmed in multivariate analysis (Figure 2A).

No impact of donor CHIP on engraftment kinetics of neutrophils or platelets following allogeneic hematopoietic stem cell transplantation

A previous study reported that donor cell-derived CHIP is common amongst recipients who developed unexplained cytopenia after allogeneic HCT.[6] Thus, we hypothesized that HSC from donors with CHIP could adversely affect engraftment kinetics after allogeneic HSCT, thus increasing the risk of graft failure.[6] We examined median day of engraftment and the cumulative incidence rate of engraftment at 30 days after HSCT. Median day of neutrophil engraftment was 19 days (range, 14-24) in the donor CHIP group *versus* 19 days (range, 16-23) in the no donor CHIP group (*data not shown*). The cumulative incidence of neutrophil engraftment by day 30 was 88.0% in the donor CHIP group *versus* 91.6% in no donor CHIP group HR=0.843; 95% CI: 0.534-1.331; *P*=0.460. When considering other clinical risk factors associated with neutrophil engraftment in multivariate analysis, donor CHIP was not an adverse risk factor for delayed neutrophil engraftment (Figure 2B; *Online Supplementary Table S3*).

Median day of platelet engraftment was 15 days (range, 11-25) in the donor CHIP group *versus* 14 days (range, 11-23) in the no donor CHIP group. The cumulative incidence of platelet engraftment by day 30 was 72.0% in the donor CHIP group *versus* 82.1% in the no donor CHIP group HR=0.751; 95% CI: 0.461-1.224; *P*=0.250. Again, when considering other clinical risk factors associated with platelet engraftment, the presence of donor CHIP was not found to increase the risk of delayed platelet engraftment in multivariate analysis (Figure 2B; *Online Supplementary Table S3*).

No impact of donor CHIP on the risk of overall graft-*versus*-host disease and organ specific graft-*versus*-host disease

The present study also evaluated the impact of the pres-

Table 2. Summary of transplant outcomes in the overall population and according to the presence of donor CHIP.

Outcomes, % (95% CI)	Overall (N=372)	CHIP in donor (N=25)	No CHIP in donor (N=347)	*P*
10-year OS	41.4 (36.4-46.4)	48.0 (27.8-65.6)	41.0 (35.7-46.1)	0.969
10-year relapse	23.8 (19.6-28.3)	16.0 (4.8-33.1)	24.4 (20.0-29.0)	0.600
10-year NRM	37.6 (32.7-42.6)	36.0 (17.8-54.7)	37.7 (32.6-42.9)	0.570
Day 30 neutrophil engraftment	91.4 (88.0 -93.8)	88.0 (64.0-96.4)	91.6 (88.2-94.1)	0.460
Day 30 platelet engraftment	81.5 (77.1-85.1)	72.0 (48.8-86.0)	82.1 (77.7-85.8)	0.250
Day 100 aGvHD	77.3 (72.6-81.2)	80.0 (56.3-91.7)	77.1 (72.2-81.2)	0.490
3-year cGvHD	62.9 (57.8-67.6)	48.0 (26.9-66.3)	64.0 (58.7-68.8)	0.220
13-year secondary malignancies	14.3 (10.6-18.4)	6.0 (3.0-25.2)	14.8 (11.0-19.2)	0.370

CI: confidence interval; CHIP: clonal hematopoiesis of indeterminate potential; OS: overall survival; NRM: non-relapse mortality; aGvHD: acute graft-*versus*-host disease; cGvHD: chronic GvHD.

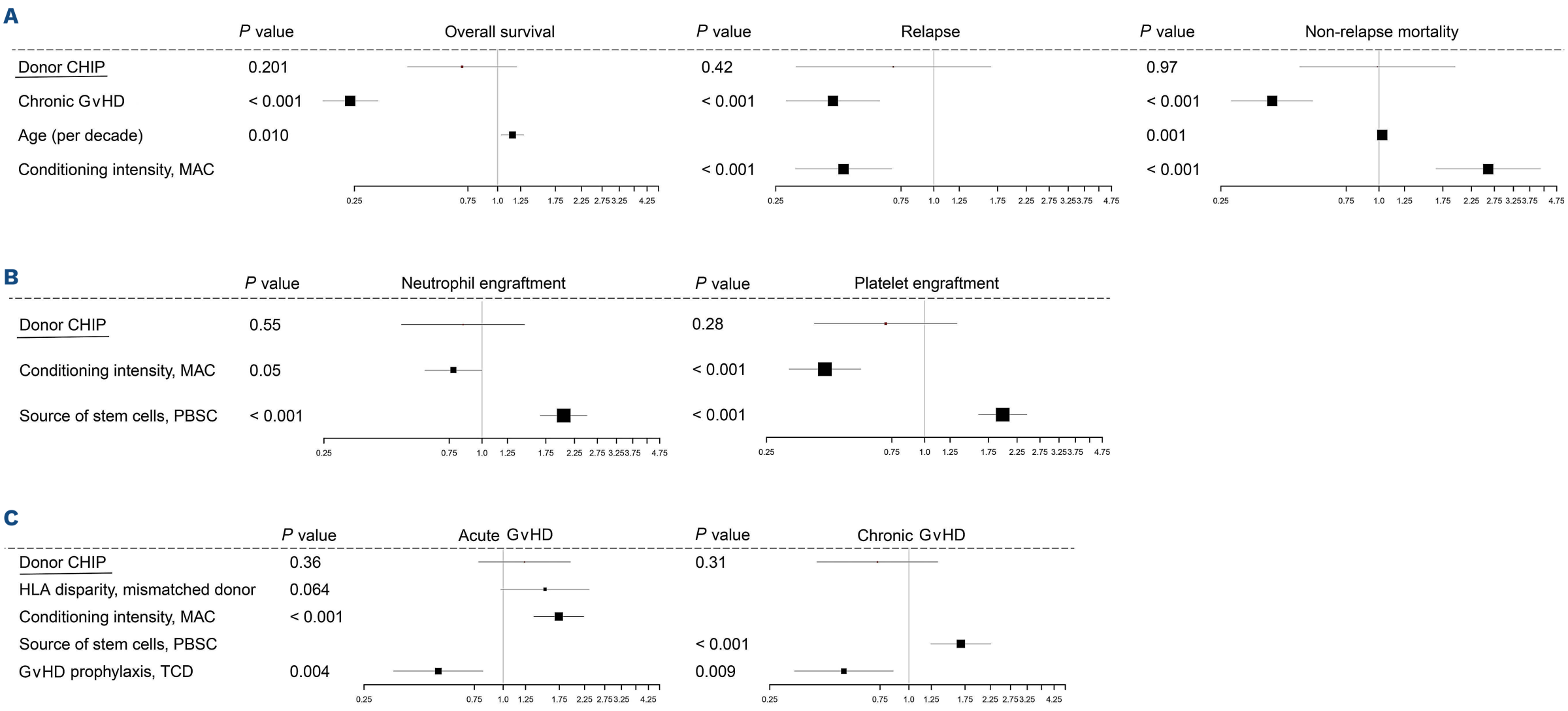


Figure 2. Risk factor analysis of transplant outcomes (N=372). (A) Multivariate analysis for risk factor of overall survival, relapse incidence, and non-relapse mortality (n=372). (B) Multivariate analysis for risk factor of neutrophil and platelet engraftment (n=372); (C) Multivariate analysis for risk factor of acute graft-*versus*-host disease (GvHD) and chronic GVHD (n=372). CHIP: clonal hematopoiesis of indeterminate potential; HR: hazard ratio; CI: confidence interval; MAC: myeloablative conditioning; PBSC: peripheral blood stem cell; HLA: human leukocyte antigen; TCD: T-cell depletion.

ence of donor CHIP on the risk of aGvHD and cGVHD. The presence of donor CHIP was not associated with the incidence of grade 1-4, 2-4 or 3/4 aGvHD. Similarly, aGvHD grade was not statistically different between the two groups (Figure 3). The incidence of grade 1-4 aGvHD at day 100 was 80.0% in the donor CHIP group *versus* 77.1% in the no donor CHIP group (*P*=0.490); grade 2-4 aGvHD was 77.0% in the donor CHIP group *versus* 69.1% in the no donor CHIP group (*P*=0.30). Likewise, there was no difference in grade 3/4 aGvHD between the two groups (*P*=0.110). In terms of the organ involvement by aGvHD, no difference was noted between the two (*Online Supplementary Table S4*; *Online Supplementary Figures 3* and *S4A*).

The donor CHIP group showed a trend toward lower incidence of 3-year cGvHD. The CHIP group showed 48.0% of cGvHD which was lower than that in the no CHIP group showing 64.0% of cGvHD incidence at 3 years (*P*=0.220). However, in multivariate analysis, the presence of donor CHIP was not associated with cGvHD (Figure 2C). The distribution of cGvHD severity was similar between the two groups (*P*=0.389; Figure 3). cGvHD organ involvement was not also significantly different between the two groups (*Online Supplementary Table S4*). Multivariate analyses confirmed that the presence of donor CHIP was not associated with the risk of acute or chronic GvHD (*Online Supplementary Tables S4* and *S5*; *Online Supplementary Figure S3* and *S4B*).

The risk of secondary malignancies following allogeneic hematopoietic stem cell is not associated with the presence of CHIP in donor

With a median follow-up duration of 13 years, we identified 56 cases (15.1%) of SM after HSCT, with a median latency of 8.4 years from HSCT (*Online Supplementary Table S6*). The most common types of SM were non-melanoma skin (n=27, 48%), lung (n=5, 8.9%), prostate (n=5, 8.9%), and hematological cancers (n=5, 8.9%; Figure 4).

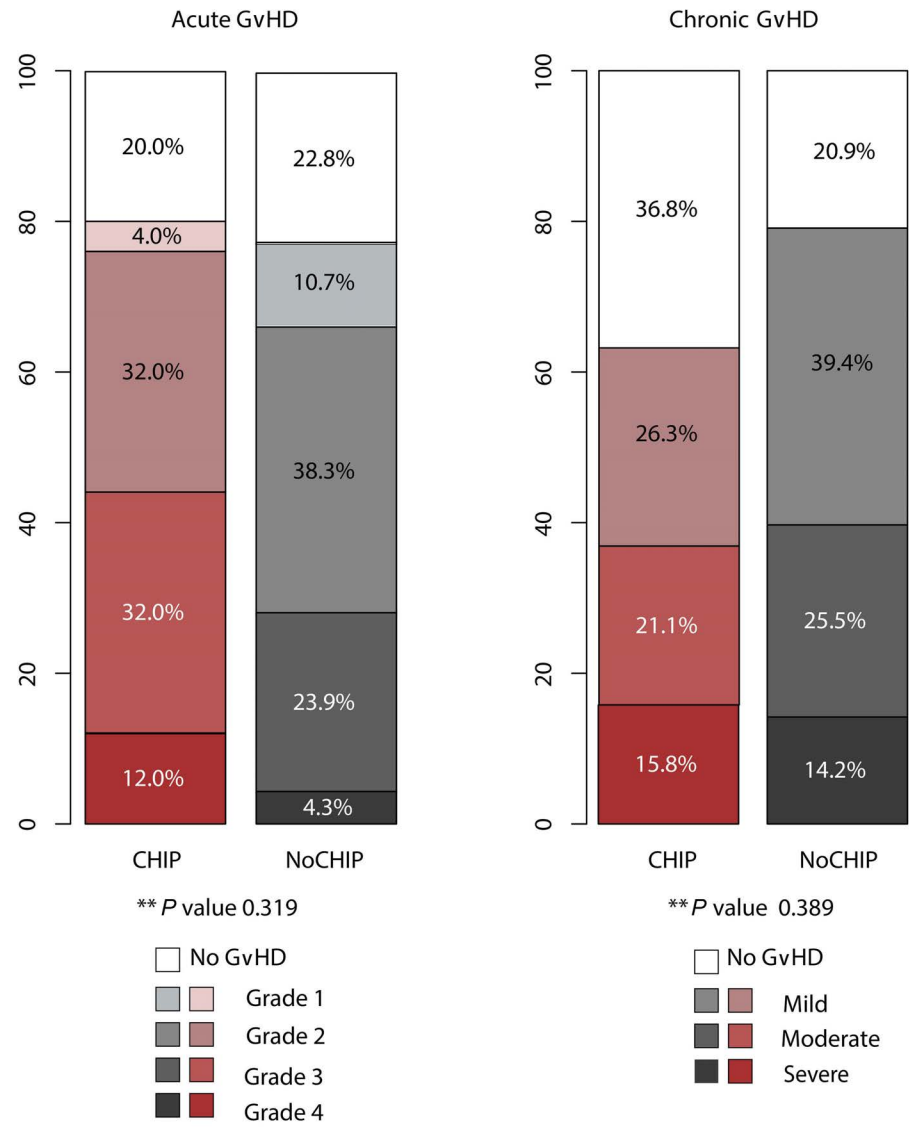


Figure 3. Development and severity of acute/chronic graft-versus-host disease according to the presence of CHIP in the donor (N=372). GvHD: graft-*versus*-host disease; CHIP: clonal hematopoiesis of indeterminate potential.

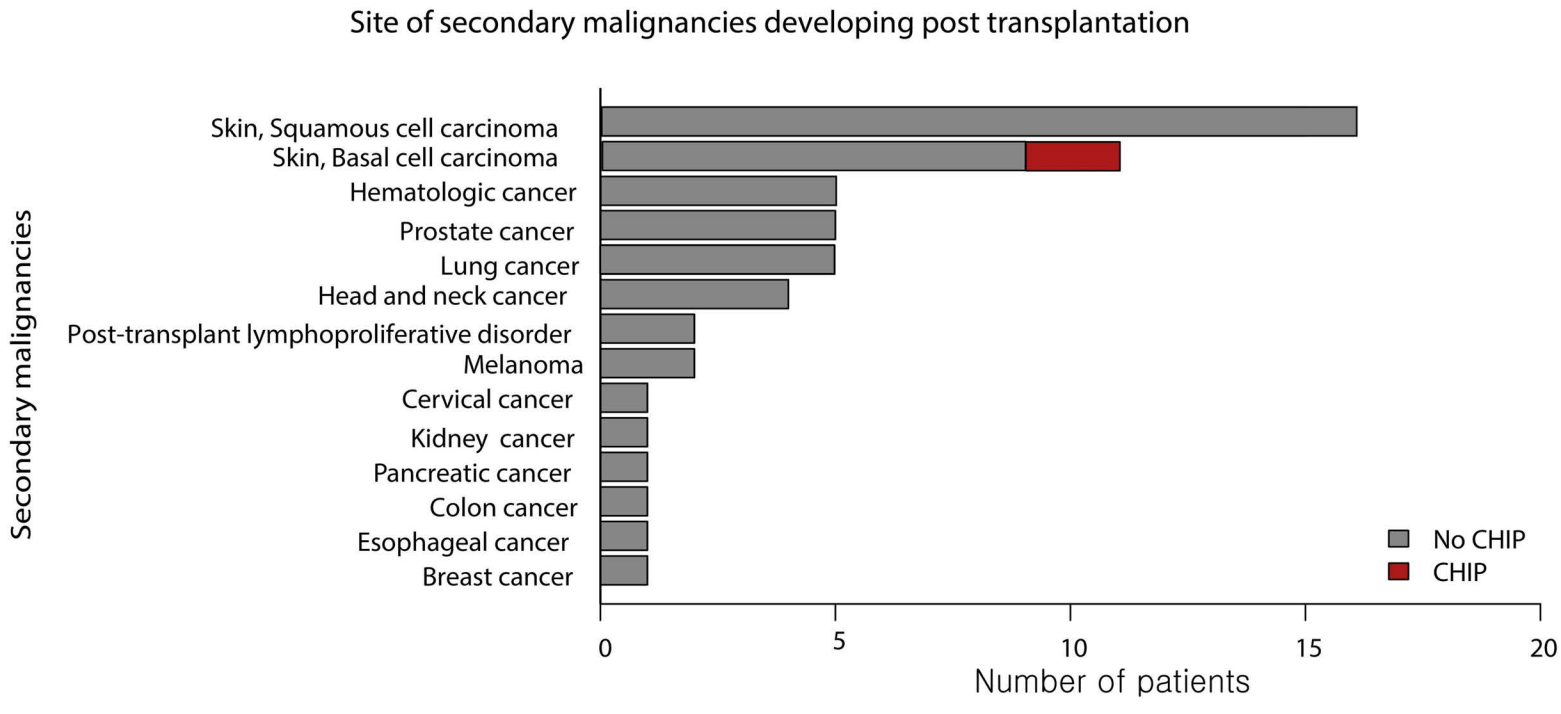


Figure 4. Secondary malignancies after allogeneic hematopoietic stem cell transplantation (N=372). CHIP: clonal hematopoiesis of indeterminate potential.

The cumulative incidence of SM was 8.9% at 10 years and 16.0% at 15 years post-HSCT, respectively.

Out of 56 patients with a confirmed diagnosis of SM post-HSCT, only two patients were in the donor CHIP group: two patients had received HSCT from a donor carrying CHIP (n=2/25, 8.0%), while the remaining 54 patients received HSCT from a donor without CHIP (n=54/347, 15.6%). Due to the small number of patients who developed secondary malignancies and the donor CHIP group, it was difficult to obtain reliable statistical measures. The cumulative incidence of SM at 13 years was 6.0% in the CHIP group *versus* 14.8% in the no CHIP group.

The two cases diagnosed with SM after HSCT received from a donor carrying CHIP developed non-melanoma skin cancer (n=2) at 13 years and 11 years after HCT, respectively. The CHIP-related mutations in the donors were *TET2* (n=1) and *EZH2* (n=1), and their HCT indications were AML and NHL, respectively (*Online Supplementary Table S7*).

Discussion

Allogeneic HSCT involves the transfer of HSC from the donor to the recipient for reconstitution of hematopoiesis.[19] Therefore, there are concerns that CHIP mutations from the donor may be engrafted to the recipient through allogeneic HSCT and may affect clinical outcomes after HCT adversely. Previous work has shown that CHIP clones are associated with chronic inflammation and tissue damage.[20-23] Donor monocytes and immune cells derived from engrafted HSC carrying CHIP could theoretically promote pro-inflammatory cytokine production and altered epigenetic regulation, thus potentially provoking alloreactivity and GvHD.[24-26] This led to concerns that the risk of GvHD and other post-transplant complications might increase when HSC from a donor carrying CHIP are used for HSCT. [7,8] In addition, it was unclear whether HSC from donors with a CHIP-related mutation could affect long-term outcomes of survival, NRM, GvHD and other measures such as engraftment and SM. If the presence of CHIP is concluded to affect transplant outcomes, genetic testing of CHIP should be a part of donor evaluation prior to stem cell donation or prior to donor selection for HSCT. The current study concluded that i) the presence of CHIP in donors does neither reduce OS nor increase risk of relapse or NRM with the observation duration of 13 years of follow-up (range, 0.3-18.2 years); ii) the presence of donor CHIP does not impair engraftment of neutrophil or platelet after HSCT; iii) donor CHIP does not seem to increase the risk of aGvHD or cGvHD; iv) donor CHIP does not increase the risk of SM following HSCT. Accordingly, our result does not support the use of molecular tests to detect donor CHIP mutations during predonation testing.

Previous work reported that patients with CHIP prior to autologous stem cell transplantation (ASCT) had adverse survival after ASCT compared to those without CHIP.[27] The same study demonstrated an increased risk of therapy-related myeloid neoplasm (TMN) in patients carrying CHIP. In addition, an increased risk of NRM, but not risk of relapse, was found to be associated with both higher allele burden and a greater number of CHIP mutations. However, allogeneic HSCT, which may include the transfer of CHIP from healthy donors to recipients, differs from ASCT for several reasons. Donor HSC carrying CHIP will not have been exposed to cytotoxic agents during the HSCT procedure but instead face different and likely stronger immunologic cellular stressors.[28-30] In addition, in contrast to ASCT, where HSC carrying CHIP were prepopulated in the marrow prior to ASCT, more time for donor HSC carrying CHIP will be required to expand and become a predominant clone in a new marrow niche of the allogeneic recipient.[7] Thus, the prognostic impact of donor CHIP on long-term outcomes following allogeneic HSCT would not be as robust as that after ASCT. Boettcher *et al.*[19] reported that, in the cases of donor-engrafted CHIP, there was a significant increase in clonal size of CHIP as measured with VAF in recipients after HSCT. However, this increase in VAF was only relatively modest (i.e., 2.3-fold in median VAF between donors and recipients, with VAF in most recipients being ~0.1). This finding indicates transfer of a single CHIP clone, although it is engrafted in the recipient, does not seem to repopulate quickly and dominate recipient's hematopoietic system quickly.[31] Thus, there remains uncertainty on the fate of donor CHIP after transfer to donor hematopoietic system following allogeneic HSCT.

Similarly to the two previous studies,[7,8] our study confirmed lack of prognostic impact of donor CHIP on OS (P=0.969) or NRM (P=0.570) following HSCT, concluding that the presence of donor CHIP does not increase the risk of overall mortality or NRM after HSCT. Also, in accordance with the previous work,[7] the presence of donor CHIP does not negatively affect platelet engraftment rate or engraftment speed following HSCT.

The impact of donor CHIP on relapse risk remains controversial. Frick *et al.*[7] reported reduced risk of relapse in recipients transplanted from a donor with CHIP (HR=0.633; 95% CI: 0.41-0.98; P=0.042), while Oran *et al.*[8] reported no impact of donor CHIP on relapse risk (HR=0.97; 95% CI: 0.6-1.5; P=0.9), which is in agreement with our result (HR 0.788; 95% CI: 0.323-1.918; P=0.60). The protective effect of donor CHIP from relapse risk observed in the Frick *et al.*'s study[7] can be explained with the finding of higher incidence of cGvHD in patients transplanted with donor CHIP compared to others (HR=1.65; 95% CI:1.15-2.36; P=0.008). However, Oran *et al.*[8] reported no difference in cGvHD incidence between the two groups, similar to our result, with no impact of donor CHIP on relapse risk. In another study by Newell *et*

al.,[32] donor-derived CHIP was not associated with relapse or OS; however, patients with donor-derived CHIP were more likely to develop cGvHD, necessitating systemic immunosuppressive therapy (IST) (*P*=0.045) and less likely to discontinue IST (*P*=0.03) compared with controls without donor-derived CHIP. Thus, the differential impact of donor CHIP on relapse risk may not be directly from a putative biological CHIP-related protection from relapse but may instead be related to the occurrence of GvHD, which can indirectly affect the risk of relapse. In the present study, we did not find any difference in cGvHD incidence between the two groups (HR=0.685; *P*=0.220). Furthermore, Gibson *et al.*[33] recently reported that donor DNMT3A was associated with reduced relapse (HR=0.59; *P*=0.014), and increased cGvHD (HR=1.36; *P*=0.042).

In terms of aGvHD, while the present study and another study[7] have reported that the presence of CHIP do not affect the aGvHD incidence, Oran *et al.*[8] reported that donor CHIP increased the risk of grade 2-4 (*P*=0.001) and 3-4 aGvHD (*P*=0.008). This different result can result from the different population characteristics and/or transplant procedures which could affect the risk of aGvHD, such as the source of stem cells, GvHD prophylaxis or conditioning regimens. In order to reach a clear conclusion on this issue, further study is strongly warranted to include a larger number of homogenous population transplanted with less diverse conditioning regimen and/or GvHD prophylaxis.

One of the important long-term complications after HSCT is secondary malignancy, which sometimes we miss its importance on its negative impact on survival and quality of life in the transplant survivors. It usually occurs 3-10 years after HSCT. The risk of malignancy is 2-fold higher among recipients of allogeneic HSCT compared to that of the general population.[34] In our previous report evaluating the incidence of SM in 2,415 consecutive patients after HSCT, SM were diagnosed in 8.7% of HSCT recipients overall with SM incidence of 6.3% at 10 years[17] with a median follow-up duration of 127 months. We here evaluated the impact of donor CHIP on the risk of SM after allogeneic HCT with a median follow-up duration of 13 years. In the present study, follow-up duration was quite long, sufficient to observe SM events occurring after allogenic HSCT. However, because only two patients in the donor CHIP group developed SM, it was difficult to definitively conclude the statistical association between the presence of donor CHIP and the risk of SM after HSCT. Based on the result presented here in the current study, we were unable to observe the increased occurrence of SM in the donor CHIP group.

It is still a matter of debate if we have to routinely test the presence of CHIP in the allogeneic donor's HSC.[35,36] As the upper age limit of HCT recipients continues to increase, now we see the use of elderly related donors more frequently, which inevitably raises the concern of using an elderly donor with respect to the transfer of CHIP to the recipient.[37] The present result suggests that molecular testing for CHIP mutations may not need to be a part of routine donor evaluation prior to stem cell collection based on its neutral impact on transplant outcomes. However, there are still restricted clinical situations where mutational testing on the donor can be indicated and would be potentially helpful. Further study is warranted to reach a clearer conclusion on these questions.

In summary, based on our analysis as well as those of others, the presence of CHIP in the donor does not seem to increase the risk of adverse outcomes following HSCT. Donor CHIP does neither affect the risk of relapse, survival, GvHD or engraftment adversely, nor does it increase the risk of SM following allogeneic HSCT.

Disclosures

No conflicts of interest to disclose.

Contributions

TK, DK and LS designed the study. INB, HL, YY, JSA, IP, AL, WL, FVM, AG, AV, KL and RK collected samples and performed experiments. TK, ZZ, NK, YB, NCI, TB, and LS analyzed the sequencing data and performed computational analysis. KHK, TK, JSA and DK interpreted the data and statistical analyses. KHK, TK and DK wrote the paper.

Funding

The present study was supported by the Leukemia & Lymphoma Society of Canada (New Idea Award) and by the Princess Margaret Cancer Foundation.

Data-sharing statement

The dataset generated and analyzed during the current study is available in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena/browser/home) under accession number E-MTAB-12472. Code is available on GitHub under https://github.com/ShlushLab.

References

1. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488-2498.
2. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371(26):2477-2487.
3. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. Nat Commun. 2016;7:12484.
4. Abelson S, Collord G, Ng SWK, et al. Prediction of acute myeloid

leukaemia risk in healthy individuals. Nature. 2018;559(7714):400-404.
5. Rojek K, Nickels E, Neistadt B, et al. Identifying inherited and acquired genetic factors involved in poor stem cell mobilization and donor-derived malignancy. Biol Blood Marrow Transplant. 2016;22(11):2100-2103.
6. Gibson CJ, Kennedy JA, Nikiforow S, et al. Donor-engrafted CHIP is common among stem cell transplant recipients with unexplained cytopenias. Blood. 2017;130(1):91-94.
7. Frick M, Chan W, Arends CM, et al. Role of donor clonal hematopoiesis in allogeneic hematopoietic stem-cell transplantation. J Clin Oncol. 2019;37(5):375-385.
8. Oran B, Champlin RE, Wang F, et al. Donor clonal hematopoiesis increases risk of acute graft versus host disease after matched sibling transplantation. Leukemia. 2022;36(1):298.
9. Coombs CC, Zehir A, Devlin SM, et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. Cell Stem Cell. 2017;21(3):374-382.
10. Gupta V, Daly A, Lipton JH, et al. Nonmyeloablative stem cell transplantation for myelodysplastic syndrome or acute. Biol Blood Marrow Transplant. 2005;11(10):764-772.
11. Sibai H, Falcone U, Deotare U, et al. Myeloablative versus reduced-intensity conditioning in patients with myeloid malignancies: a propensity score-matched analysis. Biol Blood Marrow Transplant. 2016;22(12):2270-2275.
12. Khalil M, M,I,, Messner HA, Lipton JH, et al. Fludarabine and busulfan plus low-dose TBI as reduced intensity conditioning in. Ann Hematol. 2018;97(10):1975-1985.
13. Hiatt JB, Pritchard CC, Salipante SJ, O'Roak BJ, Shendure J. Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. Genome Res. 2013;23(5):843-854.
14. Biezuner T, Brilon Y, Arye AB, et al. An improved molecular inversion probe based targeted sequencing approach for low variant allele frequency. NAR Genom Bioinform. 2022;4(1):lqab125.
15. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on acute GVHD grading. Bone Marrow Transplant. 1995;15(6):825-828.
16. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21(3):389-401.
17. Michelis FV, Kotchetkov R, Grunwald RM, et al. Long-term incidence of secondary malignancies after allogeneic hematopoietic cell transplantation: a single-center experience. Biol Blood Marrow Transplant. 2017;23(6):945-951.
18. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48(3):452-458.
19. Boettcher S, Wilk CM, Singer J, et al. Clonal hematopoiesis in donors and long-term survivors of related allogeneic hematopoietic stem cell transplantation. Blood. 2020;135(18):1548-1559.
20. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science. 2017;355(6327):842-847.
21. Fuster JJ, Walsh K. somatic mutations and clonal hematopoiesis: unexpected potential new drivers of age-related cardiovascular disease. Circ Res. 2018;122(3):523-532.
22. Sano S, Oshima K, Wang Y, et al. Tet2-mediated clonal hematopoiesis accelerates heart failure through a mechanism involving the IL-1beta/NLRP3 inflammasome. J Am Coll Cardiol. 2018;71(8):875-886.
23. Savola P, Lundgren S, Keranen MAI, et al. Clonal hematopoiesis in patients with rheumatoid arthritis. Blood Cancer J. 2018;8(8):69.
24. Nakata K, Gotoh H, Watanabe J, et al. Augmented proliferation of human alveolar macrophages after allogeneic bone marrow transplantation. Blood. 1999;93(2):667-673.
25. Eisenbarth SC. Dendritic cell subsets in T cell programming: location dictates function. Nat Rev Immunol. 2019;19(2):89-103.
26. Abegunde SO, Buckstein R, Wells RA, Rauh MJ. An inflammatory environment containing TNFα favors Tet2-mutant clonal hematopoiesis. Exp Hematol. 2018;59:60-65.
27. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. J Clin Oncol. 2017;35(14):1598-1605.
28. Ogawa S. Clonal hematopoiesis in acquired aplastic anemia. Blood. 2016;128(3):337-347.
29. Schoettler ML, Nathan DG. The pathophysiology of acquired aplastic anemia: current concepts revisited. Hematol Oncol Clin North Am. 2018;32(4):581-594.
30. Stanley N, Olson TS, Babushok DV. Recent advances in understanding clonal haematopoiesis in aplastic anaemia. Br J Haematol. 2017;177(4):509-525.
31. Fabre MA, Vassiliou GS. Home and away: clonal hematopoiesis in sibling transplants. Blood. 2020;135(18):1511-1512.
32. Newell L, Williams T, Liu J, et al. Engrafted donor-derived clonal hematopoiesis after allogenic hematopoietic cell. Transplant Cell Ther. 2021;27(8):662.
33. Gibson CJ, Kim HT, Zhao L, et al. Donor clonal hematopoiesis and recipient outcomes after transplantation. J Clin Oncol. 2022;40(2):189-201.
34. Atsuta Y, Suzuki R, Yamashita T, et al. Continuing increased risk of oral/esophageal cancer after allogeneic hematopoietic stem cell transplantation in adults in association with chronic graft-versus-host disease. Ann Oncol. 2014;25(2):435-441.
35. DeZern AE, Gondek LP. Stem cell donors should be screened for CHIP. Blood Adv. 2020;4(4):784-788.
36. Gibson CJ, Lindsley RC. Stem cell donors should not be screened for clonal hematopoiesis. Blood Adv. 2020;4(4):789-792.
37. Randall J, Keven K, Atli T, Ustun C. Process of allogeneic hematopoietic cell transplantation decision making for older adults. Bone Marrow Transplant. 2016;51(5):623-628.