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Donor *IFNL4* genotype predicts non-relapse mortality after unrelated donor myeloablative hematopoietic cell transplantation for acute leukemia: A retrospective cohort study

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SUMMARY

Background.—*IFNL4* genotype regulates the immune response by controlling the production of IFN- λ 4, a type III interferon. We hypothesized that IFN- λ 4 may play a role in infection clearance or alloreactivity in patients with acute leukemia who received myeloablative 10/10 HLA-matched hematopoietic cell transplant (HCT).

Methods.—We conducted a two-stage study using the Center for International Blood and Marrow Transplant Research repository and database. A discovery set of 404 patients (HCT: January 9, 2004 - December 26, 2008): *IFNL4* polymorphisms (rs368234815, rs12979860, and rs117648444) were genotyped with TaqMan assays, and an independent validation of 1245 patients from the DISCOVeRY-BMT study (HCT: January 7, 2000 - December 26, 2008) with existing Illumina array genotype data. A combined analysis of 1591 was also performed.

Findings.—Donor, but not recipient *IFNL4*-positive genotype, was associated with increased risk of non-relapse mortality (NRM; HR-discovery=1.60, 95% CI=1.23–2.10, p=0.0005, HR-validation=1.22, 95% CI=1.05–1.40, p=0.007, and HR-combined =1.27, 95% CI=1.12–1.45, p=0.0001). The effect was driven by excess risk of deaths from graft-*versus*-host disease (HR-discovery= 2.0, 95% CI=1.05–3.84, p=0.03, and HR-validation=1.40, 95% CI=1.02–1.92, p=0.04, and HR-combined=1.46, 95% CI=1.11–1.94, p=0.008), and possibly infections (HR-discovery= 1.49, 95% CI=0.71–3.10, p=0.29, and HR-validation=1.54, 95% CI=1.13–2.10, p=0.006, and HR-combined=1.54, 95% CI=1.16–2.04, p=0.003). Associations with post-HCT overall survival (OS) were as follow: HR-discovery=1.24, 95% CI=1.02–1.51, p=0.03, and HR-validation=1.10, 95% CI=0.98–1.20, p=0.1, and HR-combined=1.11, 95% CI=1.02–1.22, p=0.02).

Interpretation.—Prioritizing HCT donors with *IFNL4*-Null genotype may decrease NRM and improve OS without significantly limiting the donor pool.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is an effective therapy for patients with acute leukemia because of the anti-leukemia effect driven by the donor graft, known as the graft-*versus*-leukemia effect.¹ The 3-year probabilities of matched unrelated donor post-HCT survival for adults with acute myeloid leukemia are 53%, 50% and 27% for patients in first, second remission and not in remission, respectively.² Despite recent improvements in HCT modalities, the risks of transplant-related complications and non-relapse mortality (NRM) remain high, with deaths primarily caused by infections, graft-*versus*-host disease (GvHD), and organ failure. Together, these causes account for approximately 60% and 40% of deaths occurring within and beyond the first 100 days post-HCT, respectively.²

The interferon lambda 4 gene (*IFNL4*) encodes IFN- λ 4, a recently discovered member of the type III interferon gene family.³ Protein expression of IFN- λ 4 is controlled by a dinucleotide genetic variant (rs368234815-TT/dG) located in the first exon of *IFNL4*. The rs368234815-dG allele supports the production of IFN- λ 4 protein, while the TT allele introduces a protein-abrogating frameshift. Thus, *IFNL4* genotype can be categorized into *IFNL4*-positive (those producing 1 or 2 copies of IFN- λ 4 protein), and *IFNL4*-Null (those not producing IFN- λ 4 protein).³ Another *IFNL4* polymorphism (rs12979860) is located within the first intron and can be used as a proxy when rs368234815 is not available. Individuals with *IFNL4*-positive genotype can be further genotyped for rs117648444 (a missense variant P70S in exon 2 of *IFNL4*) and categorized into those who can only produce functionally weak IFN- λ 4-S70 protein and those who carry at least one haplotype for producing functionally strong IFN- λ 4-P70 protein (reviewed in⁴; details are presented Appendix p10). *IFNL4*-Null individuals are more likely to clear hepatitis C virus (HCV) infection and less likely to reactivate cytomegalovirus (CMV) infection after solid organ transplantation.⁵ IFN- λ 4 activates the JAK/STAT pathway,³ and induces expression of negative regulators of cytokine and interferon signaling,⁶ which may have a broad impact on both the adaptive and innate immune responses, and explain diverse and generally negative clinical outcomes associated with its activity.⁷

We hypothesized that IFN- λ 4 might be important for outcomes of HCT by affecting infection clearance and/or alloreactivity. In this study, we evaluated the association between the recipient and donor *IFNL4* genetic polymorphisms with post-HCT survival outcomes in patients with acute leukemia.

METHODS

Study Design and Participants:

The Center for International Blood and Marrow Transplant Research (CIBMTR) maintains one of the world's largest databases and biorepositories serving HCT and cellular therapy-related studies. CIBMTR collects detailed information on almost all allogeneic HCT

performed in the United States. The research sample biorepository primarily includes samples collected in the context of unrelated donor transplants facilitated by the National Marrow Donor Program (NMDP/ Be The Match®). Data collected by the CIBMTR is thoroughly reviewed and audited to ensure high quality (details at <https://www.cibmtr.org/Data/Available/Pages/index.aspx>).

Eligibility criteria for this study included: patients with acute myeloid leukemia (AML) or acute lymphocytic leukemia (ALL), who received HCT at any age from an unrelated 10/10 HLA-matched donor, with myeloablative conditioning regimen, between January 1, 2000 - December 31, 2008, and had available pre-HCT recipient and/or donor blood sample.

The discovery cohort (NCI set) used an available set (N=446) that was part of a previously published study to test this hypothesis.⁸ Patients in the original study were in first or second complete remission and had an available donor pre-HCT blood sample (since the original study focused on donor telomere length) in the CIBMTR biorepository. Of those, 410 recipient-donor pairs met study eligibility criteria; genotyping was completed for 404 donors and 319 recipients with available DNA.

A validation cohort was sought after promising results were obtained in the first set. We used genomic and outcome data from the DISCOVeRY-BMT study (DBMT; a genome-wide association study aiming to determine the influence of susceptibility variants on one-year mortality after HCT).⁹ The DBMT included patients with AML, ALL, and myelodysplastic syndrome (MDS) selected from the CIBMTR database and biorepository. Of the 1593 HCT recipients meeting study eligibility criteria, we excluded 348 patients who were already included in the NCI set. This left 1245 unique patients for an independent validation analysis. We also performed a combined analysis using the full DBMT set of 1593 eligible patients (independent validation + overlapping patients in the NCI set) to improve statistical power. Figure 1 presents the study flow chart.

The study was approved by the NMDP Institutional Review Board. All study participants provided written informed consent for participation in the CIBMTR repository and database.

Outcomes:

Non-relapse mortality (NRM) was defined as death during continuous complete remission. Overall survival (OS) was defined as time from date of HCT to death from any cause or last follow-up. Relapse definition was primarily based on not achieving complete hematologic recovery. Acute and chronic graft-*versus*-host disease (GvHD) were defined according to standard criteria.

Causes of death were ascertained at one year after HCT from adjudicated data generated as part of the DBMT study; detailed definitions are available elsewhere.⁹ Briefly, relapse mortality is death after leukemia relapse or disease progression. GvHD death is from severe acute or chronic GvHD under active treatment. Infection death included bacterial, viral, fungal, and/or protozoan infections causing end organ damage. Organ failure deaths were those not due to disease progression, GvHD or infection. The adjudicated causes of death data were available for 348 patients in the discovery set and all patients in the validation set.

Procedures:

In the NCI set, we genotyped *IFNL4* polymorphisms rs368234815, rs117648444 and rs12979860 using TaqMan assays as was previously described.³ Blinded technical duplicates (10% of all samples) showed 100% genotype concordance. Polymorphisms rs368234815 and rs12979860 were in near-complete linkage disequilibrium ($r^2=0.99$, Appendix p11), thus in the NCI set we focused on the functional rs368234815.

In the DBMT set, we used existing genotyping data from Illumina HumanOmniExpress-24 BeadChip available for the *IFNL4*-rs12979860, while rs117648444 was imputed using Haplotype Reference Consortium, hg19, via the Michigan Imputation server;¹⁰ only genotypes imputed with high confidence (>0.9) were used for analyses. Genotype concordance in the 348 overlapping samples between the NCI and the DBMT sets were 100% and 99% for rs12979860 and rs117648444, respectively.

Laboratory personnel were blinded to patient outcomes. Genotype and allele frequencies of all the markers in our study populations were similar to those from European populations in the 1000 Genomes Project (Appendix p. 2, 3). *IFNL4* genotype, haplotype and protein groups were assigned to all individuals as outlined in Appendix (p 4, 5, 10).

We analyzed a publicly available single-cell RNA-sequencing (scRNA-seq) dataset¹¹ to evaluate the expression levels of all human interferons, including *IFNL4*. The analysis was done in the dataset¹¹ of 32,333 bone marrow/progenitor CD34+ CD38- cells from 3 healthy (HIV, HCV and HBV-negative) bone marrow donors downloaded from Single Cell Expression Atlas (<https://www.ebi.ac.uk/gxa/sc/experiments/E-HCAD-6/downloads>). We used matrix.mtx, barcode and feature files for normalized scRNA-seq counts, and R packages “SingleCellExperiment” version 1.9.1 and “Scater” version 1.15.16 for the analysis. Expression values were computed as counts-per-million (CPM) and plotted using the R package “ggplot2”. Downloaded scRNA-seq BAM files were indexed using SAMtools and visualized with Integrative Genome Viewer against hg38 reference coordinates. *IFNL4* genotypes were scored from sequencing reads for rs73930703 located in 3'UTR of *IFNL4*.

Statistical Analysis

We calculated hazard ratios (HRs) and 95% confidence intervals (CIs) for OS using multivariable Cox proportional hazard models and modeled cause-specific hazards for NRM and causes of death analyses. Competing events were relapse in NRM and cause of death other than that under study for cause-specific mortality. To select clinical factors predicting outcomes of interest to be included in the model, we used a stepwise forward-backward procedure in the NCI set with a p-value threshold of 0.05 for model entry and retention. All clinical factors in Table 1 were eligible to enter the model. For consistency, the same variables were used for the validation and combined analyses. Proportional hazards assumption was tested for all variables using a time-dependent approach. Factors violating the assumption were adjusted for through stratification. Final models were adjusted for donor and recipient age, GVHD prophylaxis, use of total body irradiation, and stratified by graft type. Follow-up started at the date of HCT and ended at the event of interest, death, or end of study (May 31, 2019). Associations with *IFNL4* genotype were tested under an

additive genetic model (based on 0, 1 or 2 copies of the risk alleles rs368234815-dG or rs12979860-T). The models calculated hazard ratios for NRM or OS per risk allele. For IFN- λ 4 protein analysis, we compared the IFN- λ 4-Weak and IFN- λ 4-Strong with the *IFNL4*-Null group as the reference, adjusting for the same set of clinical factors as in the main models.

All statistical analyses were performed using SAS software version 9.4 (Cary, NC, USA).

To provide guidance for clinical decision-making, we calculated the absolute risks (AR) of NRM and OS, and Mean Risk Stratification (MRS)¹² in relation to donor *IFNL4* genotype (online calculator is available at <https://analysistools.cancer.gov/biomarkerTools/#meanRiskStratification>). MRS is calculated based on the following equation ($MRS=2t(1-t) \times RD$) where RD is absolute risk difference, and t is *IFNL4* genotype frequency. As a measure of risk stratification, MRS is a more comprehensive metric for interpreting the potential value of a biomarker test than metrics of pure predictiveness, such as AUC (area under the curve).¹² We calculated MRS using genotype frequency and risk estimates from the full DBMT set, under 2 possible clinical decision scenarios: 1) accepting only donors with *IFNL4*-Null (approximately 50% of our donor pool and rejecting the others), or 2) rejecting donors homozygous for *IFNL4*-positive genotype (accepting approximately 90% of our donor pool). See Supplemental Online Methods for detailed MRS explanation. To interpret the clinical significance of MRS in the context of HCT, we calculated the MRS for NRM and OS, based on 7/8 vs. 8/8 HLA-matching of donors after standardizing this calculation using NRM and OS probability estimates from our study and risk estimates from previous report.¹³ We used 8/8 vs. 7/8 HLA matching as an example of an MRS cutoff point that resulted in a successful actionable clinical decision change in HCT. See Online Methods for calculation details (Appendix p. 15–18).

Role of Funding Source:

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

RESULTS

Table 1 summarizes the patient characteristics by study sets. The discovery and validation sets (NCI; N=404 and DBMT; N=1245; all 10/10 HLA matched) were similar in the demographics of recipients and donors, disease frequency, and predominance of tacrolimus-based regimens for GVHD prophylaxis. Notable differences between the two sets included: graft source (bone marrow grafts in 129 patients (31.9%) of 404 vs. 596 (47.9%) of 1245, for the NCI and DBMT, respectively), and disease status at HCT (404 patients (100%) vs. 802 (64.2%) of 1245 were in remission, respectively). The distributions of recipient and donor *IFNL4* genotypes were similar in both sets, such as 156 (49.4%) of 316 and 550 (44.9%) of 1225 of the NCI and DBMT recipients; and 177 (43.8%) of 404 and 556 (44.7%) of 1245 of the donors, respectively were *IFNL4*-Null (Table 1).

Results from both the discovery and validation sets showed no statistically significant association between recipient *IFNL4* genotype and post-transplant NRM (HR=1.12, 95% CI=0.84–1.51, p=0.44, and HR=1.11, 95% CI=0.95–1.28, p=0.19, respectively) or OS (HR=1.01, 95% CI=0.81–1.26, p=0.96, and HR=1.07, 95% CI=0.97–1.19, p=0.17). Analysis of the full DBMT set (combined set) showed similar results (Appendix p 6).

On the other hand, donor *IFNL4-positive* genotype was statistically significantly associated with an excess risk of NRM in both cohorts (HR=1.60, 95% CI=1.23–2.10, p=0.0005, and HR=1.22, 95% CI=1.05–1.41, p=0.007, in the discovery and validation sets, respectively) (Table 2). Similar result was noted in the combined set (HR =1.27, 95% CI=1.12–1.45, p=0.0001) (Table 2 & Figure 2A).

The observed higher risk of NRM in patients receiving HCT from donors with *IFNL4-positive* genotype resulted in statistically significant inferior OS in the discovery and combined sets (HR=1.24, 95% CI=1.02–1.51, p=0.03, and HR =1.11, 95% CI=1.02–1.22, p=0.02, respectively) (Table 2 & Figure 2B). The association did not reach statistical significance in the validation cohort (HR=1.10, 95% CI=0.98–1.20, p=0.10) (Table 2).

The associations between donor *IFNL4* genotypes and one-year post-HCT causes of death are summarized in Table 3. The data showed statistically significant associations between donor *IFNL4-positive* genotype and risk of death from GvHD in all study sets. A significant relationship with infection-related death was only noted in the validation, and combined sets. No associations were noted with death from organ failure (HR-combined=1.05, 95% CI=0.78–1.40, p=0.74) or leukemia relapse (HR-combined=0.96, 95% CI=0.82–1.12, p=0.60).

We then used the combined set and complete the following secondary analyses: 1) restricted to patients who received HCT while in remission, this showed similar results to that observed in all patients (HR for NRM =1.27, p=0.001, and for OS=1.12, p=0.04); 2) stratified on graft source, the HR for NRM=1.36, p=0.0003; and HR=1.18, p=0.11, for peripheral blood vs. bone marrow stem cell transplants respectively, p-interaction=0.56); 3) stratified on donor-recipient pre-HCT CMV serostatus as a proxy for risk of post-HCT CMV viremia or use of anti-viral prophylaxis, and found no risk differences in NRM or OS in relation to donor *IFNL4* genotype by donor-recipient CMV matching or donor seropositivity (Appendix p.8).

In further analysis, compared with *IFNL4*-Null donors, a higher risk of NRM was observed in patients who received HCT from donors with IFN- λ 4-Strong, but not from donors with IFN- λ 4-Weak protein; similar results were noted in all study sets. Results for OS followed the same trend, but did not reach statistical significance in the validation cohort or the combined analysis (Table 2 & Appendix p.12).

To explain the association between *IFNL4* genotype and outcomes of HCT, we hypothesized that the transplanted graft is the source of *IFNL4*-expressing cells. We analyzed mRNA expression of all human interferons - *IFNL4* and 21 other transcripts in bone marrow samples of three healthy donors. We showed that the *IFNL4* was the main interferon transcript expressed in bone marrow of all three individuals tested, with expression detected

in 0.67% of all sequenced cells (range of expression of other interferons=0–0.07%), and in 83.1% of all interferon-expressing cells (range for other interferons=0–8.8%). Based on the *IFNL4* proxy variant rs73930703 within the transcribed region, all three individuals are likely to be *IFNL4*-Null (Appendix p 7&13).

Using risk stratification tools to guide donor selection strategy based on *IFNL4* genotype testing, we found that selecting *IFNL4*-Null donors was associated with an absolute risk reduction of post-HCT NRM of 6.4% (95% CI=1.2–11.6%) versus 11.0% (95% CI=2.7–19.4%) if rejecting donors homozygous for the *IFNL4*-positive genotype. The Mean Risk Stratification (MRS) provided by selecting HCT donors with the common *IFNL4*-Null status (~50% frequency) changed the average risk of NRM by MRS=3.2% (95% CI=0.67% to 5.74%). Our estimate for the corresponding MRS for 8/8 vs. 7/8 HLA-matching is 3.5% (95% CI=1.83%–5.23%). In contrast, the MRS of the rare homozygous *IFNL4*-positive genotype (frequency=12.1%) was only 2.4% (95% CI=0.56% to 4.15%), despite contributing the highest AR difference (11.0%). These results suggest that the strongest impact on population-average risk of NRM would be achieved by attempting to use donors with *IFNL4*-Null status rather than merely avoiding homozygous *IFNL4*-positive donors. For OS, the MRS for selecting donors with the *IFNL4*-Null status was 1.4% (95% CI= –1.3% to 4.1%), which is less than that for HLA matching (2.1%; 95% CI= 0.53% to 3.7%). See Supplemental Results for MRS and AUC comparison. (Appendix p. 19–24)

DISCUSSION

In this study of acute leukemia HCT recipients, we showed that donor *IFNL4* genotype is associated with patient risk of NRM. The observed risk reduction when selecting *IFNL4*-Null donors was beyond that already provided by the HLA matching since all patients in this study received an HCT from a 10/10 HLA-matched donor. Our findings suggest potential clinical utility for prioritizing 10/10 HLA-matched donors with *IFNL4*-Null genotype to further reduce patient risk of NRM and improve OS.

Donor selection is key for HCT success, with HLA matching being the most important factor. A CIBMTR study of 3,857 patients who received myeloablative regimens for malignant conditions showed an associated mortality risk of 1.25 with every single HLA mismatch.¹³ Growing evidence suggests a role for additional donor genetic factors in post-HCT survival. For example, longer donor telomere length was associated with an improved OS after unrelated donor HCT in patients with SAA.^{14–16} Yet, results for the possible post-HCT benefit of longer donor telomere length in acute leukemia are still inconclusive.¹⁷ Other examples include polymorphisms in Vitamin D receptor gene, cytokine genes, and HLA-DP permissiveness and expression.^{18–20} None of these markers made it to the donor selection clinical practice with the exception of considering HLA-DP.

Consistent with the unfavorable effect of *IFNL4*-positive genotype detected in our study, a large study of 840 solid organ transplant recipients showed a high incidence of CMV reactivation in patients with the rs368234815-dG/dG genotype (HR=1.46, p=0.05), while no association was noted in patients receiving antiviral prophylaxis.⁵ Two small studies evaluated the possible effect of *IFNL4* genotype on CMV viremia after HCT with

inconclusive results.^{21,22} The use of infection prophylaxis after HCT is a common practice. In 2009, a guideline for preventing infection-related complications after HCT was published in an effort to mainstream recommendations and practices.²³ In recent years, new and more effective drugs to prevent and treat infections- particularly CMV and invasive fungal disease- became available,^{24,25} leading to decrease in post-HCT infections and improving patient survival. In our current analysis, donor *IFNL4* genotype was associated with increased risk of death from infections, but detailed information on infections or infection prophylaxis was not available to further explore this question. We found no risk differences in NRM or OS in relation to donor *IFNL4* genotype by donor-recipient CMV matching or donor seropositivity, as a proxy for CMV risk of infection or need for prophylaxis. The use of actual detailed data is necessary to answer the question since strategies of infection prophylaxis may differ between transplant centers.

The impact of donor *IFNL4* genotype on HCT survival was less pronounced in the validation cohort where bone marrow grafts were more frequent than in the discovery set. Our evaluation suggested a possible higher risk of NRM associated with donor *IFNL4*-positive genotype in patients receiving peripheral blood than bone marrow stem cell source, although the difference was not statistically significant. This needs further evaluation since unrelated donor bone marrow transplants are associated with lower risk of NRM and improved OS, and may explain the observed difference in the impact of *IFNL4* genotype between study sets.

The donor *IFNL4* genotype was also associated with a higher risk of death due to GvHD (HR=1.54, p=0.003). This association may reflect a higher risk of developing severe forms of GvHD or a lower response to GvHD immunosuppressive therapies. We evaluated the risk of severe acute GvHD (grade 3–4) and chronic GvHD in relation to donor *IFNL4* genotype in the combined DBMT set. No statistically significant association was noted with acute GvHD grade 3–4 (HR=1.11, p=0.16), but a suggestive association with the risk of chronic GvHD existed (HR=1.12, p=0.03). Type III interferons, including IFN- λ 4, signal through a distinct receptor complex of IFNLR1 and IL-10R2, to activate the expression of IFN-stimulated genes (ISG) via the JAK/STAT signaling pathway (reviewed in²⁶). Donor *IL10R2* genotype and JAK/STAT signaling have been associated with risk of GvHD,²⁷ which was reduced by JAK inhibitors,²⁸ thus providing a plausible link between GvHD and type III interferon signaling.

It is intriguing that death from both immune complications of HCT – inefficient ability to clear infection and overreaction in GvHD - are associated with the genetically determined ability to produce IFN- λ 4. It is possible that this observation is attributed to ISG expression; furthermore, IFN- λ 4 expression was associated with enhanced negative regulator of IFN signaling upon exposure to viral infection,⁶ and interferon-related DNA damage resistance signature in prostate cancer.²⁹ Notably, *IFNL4* was the predominant interferon transcript detected by scRNA-seq in the bone marrow of healthy blood donors. It is possible that IFN- λ 4 produced in bone marrow in individuals with *IFNL4*-positive genotype can reprogram progenitor cells that later contribute to multiple negative phenotypes, especially when placed into an immunosuppressed environment of transplant recipients. The results suggest a novel

functional role of IFN- λ 4 in hematopoietic stem cells in normal and disease conditions; our scRNA-seq analysis was limited by the small sample size warranting future investigation.

IFN- λ 4-P70S status only affects IFN- λ 4 and not other IFN- λ s encoded in the same genomic region,⁴ and therefore can provide evidence of a direct relationship with *IFNL4*. Patients receiving HCT from donors with IFN- λ 4-Weak protein had NRM risk comparable to those with *IFNL4*-Null donors, while high NRM risk was observed in patients who received HCT from donors with IFN- λ 4-Strong protein. This is in line with increased interferon gene signature and impaired HCV clearance in patients with the IFN- λ 4-Strong compared to patients with IFN- λ 4-Weak protein and *IFNL4*-Null genotype.³⁰

Our calculated absolute risk difference (*RD*) and mean risk stratification (*MRS*) for NRM showed a potential benefit for selecting donors based on their *IFNL4* genotype in the current era of optimal HLA recipient-donor matching. Our data showed an additional 6.4% AR reduction in NRM in donors with *IFNL4*-Null genotype and 10/10 HLA matching. The effect of selecting donors based on their *IFNL4* genotype on the reduction of NRM risk was comparable to that of selecting 8/8 vs. 7/8 HLA matched donors (3.2% vs. 3.5%, respectively), but a lower *MRS* for risk of OS was noted (1.4% vs. 2.1%, respectively). Thus, our findings reinforce the primary importance of optimal HLA matching, while suggesting that using donors with *IFNL4*-Null genotype may further reduce NRM risk. A comprehensive study is needed to definitively decide which of the two strategies (merely avoiding donors homozygous for *IFNL4*-positive vs. actively selecting those with *IFNL4*-Null genotype) is more effective for clinical practice.

The strengths of this study include the availability of comprehensive clinical and outcome data for the study population, together with pre-HCT samples from both recipients and donors for a relatively large number of patients. Yet, the study was limited by the unavailability of information on infection prophylaxis regimens or episodes of post-HCT infections. Our results may not be generalizable beyond the target study population and need to be studied further for other HCT indications.

In conclusion, our study showed that donor *IFNL4*-positive genotype is associated with an increased risk of NRM and inferior OS in patients with acute leukemia who received a 10/10 HLA-matched unrelated donor myeloablative HCT. If confirmed, prioritizing donors with the *IFNL4*-Null genotype may improve HCT survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed from English articles published between January 1, 2007 and June 28, 2020 evaluating the association between *IFNL4* genotype and outcomes after hematopoietic cell transplant (HCT). We used the following search terms: (IFNL4) or (IFNL3) or (IL28B) and (allogeneic cell transplant). Two reports were identified focusing on cytomegalovirus (CMV) viremia with inconclusive results, mostly because of the small sample size.

Added value of this study

Allogeneic hematopoietic cell transplantation (HCT) is an effective therapy for patients with acute leukemia, but patients still suffer from high morbidity and mortality. The discovery of new molecular and genetic markers that can guide donor selection and/or provide risk stratification tools are essential to improve patient survival. We explored the possible association between *IFNL4* genotype, a common genetic variant with a strong functional role in host immune modulation and viral clearance, and survival outcomes after 10/10 HLA-matched unrelated donor HCT in patients with acute leukemia. Our results showed that donor *IFNL4*-positive genotype was associated with excess risk of post-HCT non-relapse mortality (NRM) driven by increased deaths from infections and graft-*versus*-host disease.

Implications of all available evidence

Our findings suggest potential clinical utility for *IFNL4* genotype in donor selection.

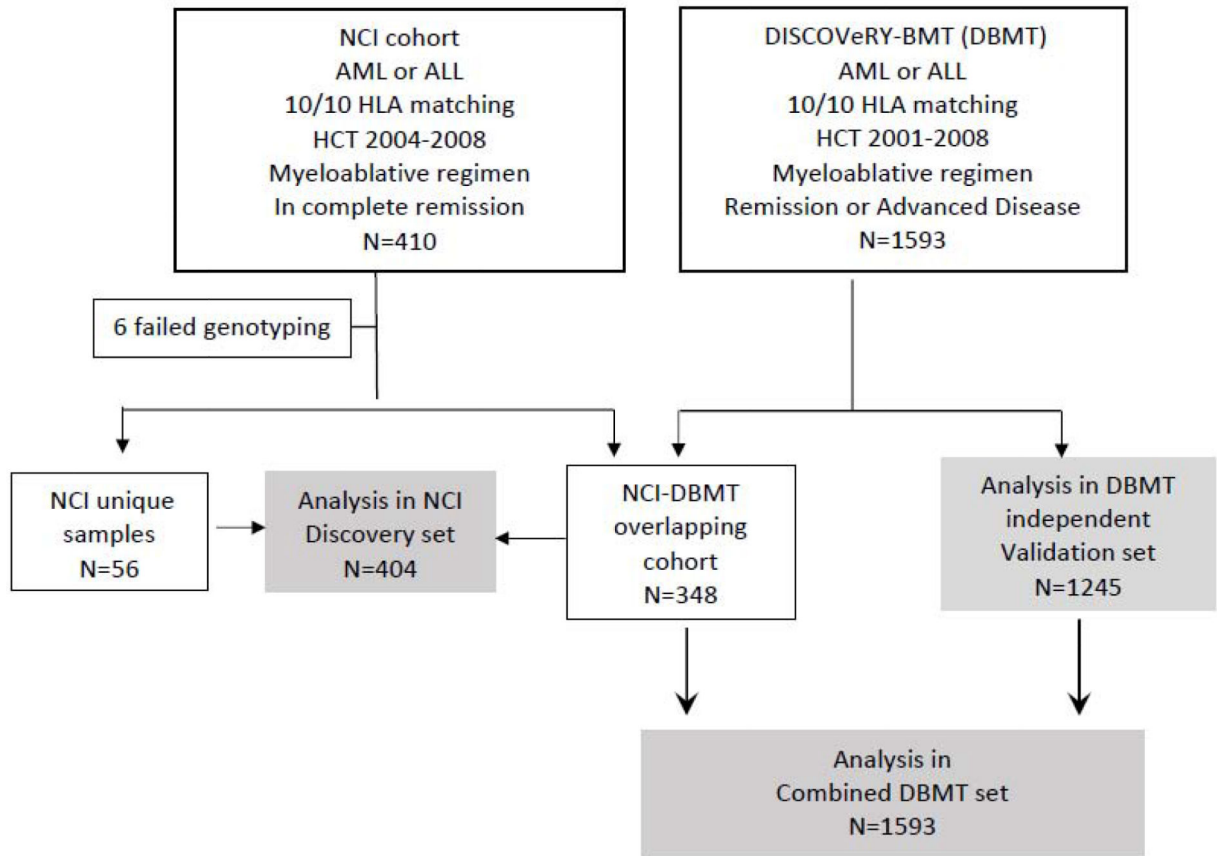


Figure 1:
Study flow chart.

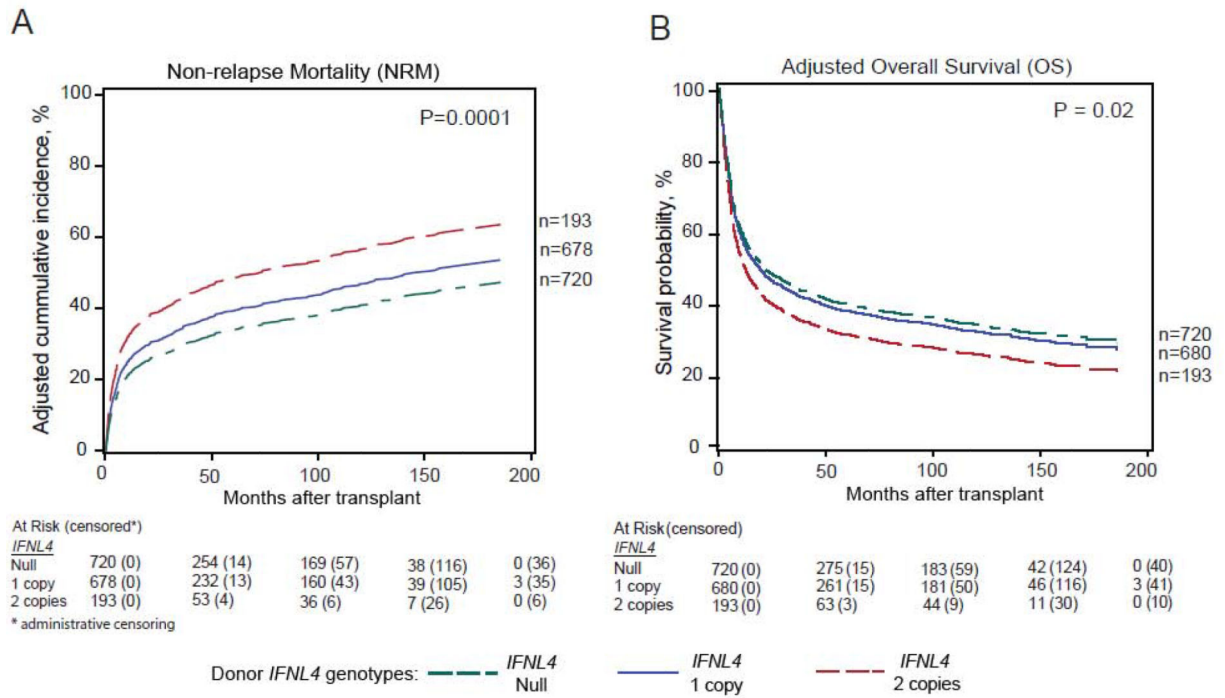


Figure 2:
IFNL4 genotype and hematopoietic cell transplant: Donor *IFNL4* genotype and the probability of post-transplant non-relapse mortality (A); Overall survival (B) in the combined DBMT set.

Table 1:

Characteristics of study participants in the discovery NCI and validation DBMT sets

	Discovery NCI Total, N=404	Validation DBMT Total, N=1245	P-value
	N (%)		
Patient Age			0.70
<20	73 (18.07)	254 (20.40)	
20–<40	140 (34.65)	420 (33.73)	
40–50	90 (22.28)	294 (23.61)	
50+	101 (25.00)	277 (22.25)	
Male Gender	222 (54.95)	689 (55.34)	0.93
Patient Race			0.68
Caucasian	374 (92.57)	1157 (92.93)	
African American	12 (2.97)	28 (2.25)	
Other	18 (4.46)	60 (4.82)	
Donor Age			0.08
18–30	169 (41.94)	463 (37.19)	
31–<50	203 (50.37)	705 (56.63)	
50+	31 (7.69)	77 (6.18)	
Donor Race			0.33
Caucasian	361 (89.36)	1084 (87.07)	
African American	10 (2.47)	28 (2.25)	
Other/unknown	33 (8.17)	133 (10.68)	
Disease Subtype			0.65
AML	263 (65.10)	793 (63.69)	
ALL	141 (34.90)	452 (36.31)	
Disease status at HCT			<0.001
1 st complete remission	253 (62.62)	406 (32.61)	
2 nd complete remission	151 (37.38)	396 (31.81)	
Not in remission/advanced disease	0 (0)	463 (37.19)	
Recipient-donor CMV Match			0.34
Both negative	131 (32.43)	342 (27.47)	
Donor negative-recipient positive	143 (35.40)	426 (34.22)	
Donor positive-recipient negative	55 (13.61)	181 (14.54)	
Both positive	75 (18.56)	259 (20.80)	
Karnofsky Score			0.001
<90	88 (21.78)	355 (28.51)	
90–100	285 (70.54)	755 (60.64)	
Missing	31 (7.67)	135 (10.84)	
Graft Type			<0.001

	Discovery NCI Total, N=404	Validation DBMT Total, N=1245	P-value
	N (%)		
Bone marrow	129 (31.93)	596 (47.87)	
Peripheral blood	275 (68.07)	649 (52.13)	
Use of Total Body Irradiation	206 (50.99)	761 (61.12)	<0.001
GVHD Prophylaxis			<0.001
Tacrolimus-based	313 (77.48)	772 (62.0)	
Cyclosporine-based	91 (22.52)	392 (31.49)	
Other	0 (0)	81 (6.51)	
Year of HCT			<0.001
2000–2003	0 (0)	424 (34.06)	
2004–2006	230 (56.93)	532 (42.73)	
2007–2008	174 (43.07)	289 (23.21)	
Donor <i>IFNL4</i> genotype			0.88
<i>IFNL4</i> -Null	177 (43.81)	556 (44.66)	
1 copy <i>IFNL4</i> -positive	179 (44.31)	535 (42.97)	
2 copies <i>IFNL4</i> -positive	48 (11.88)	154 (12.37)	
Recipient <i>IFNL4</i> genotype*			0.32
<i>IFNL4</i> -Null	156 (49.37)	550 (44.90)	
1 copy <i>IFNL4</i> -positive	123 (38.92)	531 (43.35)	
2 copies <i>IFNL4</i> -positive	37 (11.71)	144 (11.76)	
Donor -rs117648444 genotype			0.71
G/G	328 (81.38)	1019 (82.11)	
G/A	70 (17.36)	212 (17.08)	
A/A	5 (1.24)	10 (0.81)	
Donor IFN-λ4 protein status			0.91
IFN- λ 4-Null	177 (43.92)	556 (44.80)	
IFN- λ 4-Weak	56 (13.89)	163 (13.13)	
IFN- λ 4-Strong	170 (42.18)	522 (42.06)	

* based on non-overlapping 316 recipients from the discovery NCI set and 1225 recipients from the DBMT validation set with genotype information for rs368234815 in NCI set and rs12979860 in DBMT.

Table 2:Risk of non-relapse mortality and overall survival in relation to donor *IFNL4* genotype

	Non-relapse Mortality (NRM)		Overall Survival (OS)	
	N ^a events/total	HR ^b (95% CI) P-value	N events/total	HR ^b (95% CI) P-value
Discovery NCI set^c (N=404)				
<i>IFNL4</i> genotype (per risk allele) ^d	115/402	1.60 (1.23–2.10) 0.0005	223/404	1.24 (1.02–1.51) 0.03
IFN-λ4 protein status				
IFN- λ 4-Null	38/177	reference	91/177	reference
IFN- λ 4-Weak	14/55	1.32 (0.71–2.48) 0.38	30/56	1.06 (0.69–1.62) 0.79
IFN- λ 4-Strong	62/169	2.18 (1.43–3.30) 0.0002	101/170	1.39 (1.04–1.86) 0.03
DBMT validation set^c (N=1245)				
<i>IFNL4</i> genotype (per risk allele) ^d	407/1244	1.22 (1.05–1.41) 0.007	875/1245	1.10 (0.98–1.20) 0.10
IFN-λ4 protein status				
IFN- λ 4-Null	165/556	reference	390/556	reference
IFN- λ 4-Weak	50/163	1.07 (0.77–1.47) 0.6	112/163	1.01 (0.82–1.2) 0.86
IFN- λ 4-Strong	191/521	1.29 (1.05–1.60) 0.01	371/522	1.08 (0.93–1.24) 0.28
DBMT full set (overlapping + validation; N=1593)				
<i>IFNL4</i> genotype (per risk allele) ^d	518/1591	1.27 (1.12–1.45) 0.0001	1078/1593	1.11 (1.02–1.22) 0.02
IFN-λ4 protein status				
IFN- λ 4-Null	211/720	reference	482/720	reference
IFN- λ 4-Weak	65/212	1.11 (0.84–1.47) 0.44	140/212	1.03 (0.85–1.25) 0.70
IFN- λ 4-Strong	241/655	1.37 (1.14–1.65) 0.0007	454/657	1.13 (0.99–1.28) 0.06

^aNumber of events and at risk by genotype is available in Supplemental Appendix p 9

^bAdditive genetic models (HR presented per risk allele), unless specified. All models were adjusted for: donor and recipient age, GvHD prophylaxis, use of total body irradiation, and stratified by graft type

^c348/404 samples were also genotyped in the DBMT (overlapping set)

^d*IFNL4* genotype risk allele is defined as the presence of rs368234815-dG allele in NCI set and rs12979860-T allele in DBMT

Table 3:Post-HCT one-year cause-specific mortality in relation to donor *IFNL4* genotype in the DBMT set

Primary cause of death	NCI validation set ^a N=348		DBMT independent set N=1245		Combined DBMT sets N=1953
	N ^b events	HR ^b (95% CI)	N ^b events	HR ^c (95% CI) p-value	HR (95% CI) p-value
GvHD	20	2.0 (1.05–3.84) P=0.03	78	1.40 (1.02–1.92) P=0.04	1.46 (1.11–1.94) 0.008
Infections	14	1.49 (0.71–3.10) 0.29	80	1.54 (1.13–2.1) 0.006	1.54 (1.16–2.04) P=0.003
Organ failure	13	1.80 (0.83–3.91) P=0.14	86	0.91 (0.65–1.26) 0.57	1.05 (0.78–1.40) P=0.74
Relapse	52	0.89 (0.58–1.37) P=0.60	313	0.97 (0.82–1.14) P=0.68	0.96 (0.82–1.12) P=0.60

^a Patients with adjudicated causes of death data generated as part of the DBMT study were included in this analysis

^b Number of events and at risk by genotype is available in Supplemental Appendix p 9

^c Cause-specific hazards accounting for competing mortality of causes other than those under study. HRs are calculated based on additive genetic models in relation to *IFNL4* genotype (rs12979860-T allele; risk allele) and adjusted for donor and recipient age, GvHD prophylaxis, use of total body irradiation, and stratified by graft type.