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Donor-Derived Cytokine-Induced Killer Cell Infusion as Consolidation after Nonmyeloablative Allogeneic Transplantation for Myeloid Neoplasms

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A B S T R A C T

Non-myeloablative conditioning, such as with total lymphoid irradiation and antithymocyte globulin (TLI-ATG), has allowed allogeneic hematopoietic cell transplantation (allo-HCT) with curative potential for older patients and those with comorbid medical conditions with myeloid neoplasms. However, early achievement of full donor chimerism (FDC) and relapse remain challenging. Cytokine-induced killer (CIK) cells have been shown to have antitumor cytotoxicity. Infusion of donor-derived CIK cells has been studied for hematologic malignancies relapsed after allo-HCT but has not been evaluated as post-transplant consolidation. In this phase II study, we prospectively studied whether a one-time infusion of 1×10^8/kg $CD3^+$ donor-derived CIK cells administered between day +21 and day +35 after TLI-ATG conditioning could improve achievement of FDC by day +90 and 2-year clinical outcomes in patients with myeloid neoplasms. CIK cells, containing predominantly $CD3^+CD8^+NKG2D^+$ cells along with significantly expanded $CD3^+CD56^+$ cells, were infused in 31 of 44 patients. Study outcomes were compared to outcomes of a retrospective historical cohort of 100 patients. We found that this one-time CIK infusion did not increase the rate of FDC by day +90. On an intention-to-treat analysis, 2-year non-relapse mortality (6.8%; 95% confidence interval [CI], 0-14.5%), event-free survival (27.3%; 95% CI, 16.8-44.2%), and overall survival (50.6%; 95% CI, 37.5-68.2%) were similar to the values seen in the historical cohort. The cumulative incidence of grade II-IV acute graft-versus-host disease at 1-year was 25.1% (95% CI, 12-38.2%). On univariate analysis, the presence of monosomal or complex karyotype was adversely associated with relapse-free survival and overall survival. Given the favorable safety profile of CIK cell infusion, strategies such as repeat dosing or genetic modification merit exploration. This trial was registered at ClinicalTrials.gov (NCT01392989).

INTRODUCTION

Reduced-intensity conditioning (RIC) and non-myeloablative (NMA) conditioning regimens are often used for patients with myeloid neoplasms (MNs) of older age or with concurrent comorbid conditions undergoing allogeneic hematopoietic cell transplant (allo-HCT) due to the high incidence of treatment-related toxicity with myeloablative conditioning. In an analysis of 61 patients with myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN), or therapy-related MN (t-MN) undergoing NMA conditioning combining total lymphoid irradiation (TLI) with antithymocyte globulin (ATG) [1–3], the 3-year overall survival (OS) was 41% with a 3-year non-relapse mortality (NRM) of 11% [2]. Although low rates of NRM with curative potential are attractive features of TLI-ATG and other NMA conditioning regimens, early achievement of full donor chimerism (FDC), which has been associated with disease control [3–5], remains a challenge for patients with MNs.

We have shown that cytokine-induced killer (CIK) cells can be derived ex vivo from peripheral blood culture with IFN-γ,

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IL-2, and anti-CD3 [6,7]. CIK cells harvested with this approach generally include populations of $CD3^+CD56^+$ as well as $CD3^+CD314/NKG2D^+$ (natural killer group 2, member D) cells [7,8]. $CD3^+CD56^+$ cells have been shown to have non-MHC-restricted antitumor cytotoxic activity [9]. NKG2D, a member of the C-type lectin-like receptor family [10], has been associated with antitumor immune activity [11].

CIK cells have been shown to have antitumor effects in vitro, in severe combined immunodeficiency murine models with tumor, and in clinical studies [7,12]. While the majority of CIK cell clinical studies have been in the autologous setting in hematologic neoplasms and solid tumors, a few studies have evaluated allogeneic donor-derived CIK cell infusion for patients with relapsed hematologic neoplasms after allo-HCT [8,13–15]. In the first reported phase I allogeneic CIK cell infusion study [13], 11 patients with hematologic disease relapse after allo-HCT received repeated infusions of CIK cells, with a median of 12.4×10^6/kg total CIK cells per patient. Two patients with mixed donor chimerism (MDC) subsequently converted to FDC, including 1 patient with chronic myelomonocytic leukemia and 1 patient with MDS, both of whom experienced a complete response [13]. In our previous phase I/II study of allogeneic CIK cells from matched related sibling donors in 18 patients with hematologic malignancies who relapsed after allo-HCT, we evaluated dose escalations from 1×10^7/kg (n = 4), 5×10^7/kg (n = 6), to the highest planned dose of 1×10^8/kg $CD3^+$ cells (n = 8). We found that CIK cells could be safely administered in the relapsed setting and were associated with a low incidence of acute graft-versus-host disease (aGVHD) [8].

We hypothesized that early post-transplant consolidation with donor-derived CIK cells would be well tolerated, have antitumor activity, and promote early donor chimerism without significantly affecting rates of aGVHD. As post-transplant consolidation with allogeneic CIK cells has not been previously studied, we chose a dose of a one-time infusion of 1×10^8/kg $CD3^+$ donor-derived CIK cells to be given between days +21 and +35 following TLI-ATG based allo-HCT for patients with MDS, MPN, t-MN, and secondary acute myeloid leukemia (s-AML). Our primary objective was to determine the proportion of patients achieving FDC by day +90 post-transplant. Our secondary objectives were to determine 2-year OS, 2-year event-free survival (EFS), aGVHD incidence, and to assess NKG2D ligand expression.

METHODS

Study Patients and Donors

This was a single-center, nonrandomized, open-label phase II trial (study protocol 217) evaluating the effects of a one-time infusion of donor-derived CIK cells following allo-HCT with TLI-ATG conditioning for patients with MNs. This study was approved by the Scientific Review Committee and Institutional Review Board of Stanford University and the US Food and Drug Administration (IND number 14307). It was performed in accordance with the principles of the Declaration of Helsinki. All subjects provided written informed consent and were treated at Stanford University Medical Center. Fifty-six patients provided informed consent; 12 patients were found to be ineligible, and 44 patients were treated on the study between March 2011 and February 2016. Data were censored using the last follow-up visit before August 2017 to allow for a minimum follow-up of 18 months.

Study Eligibility

Eligibility criteria are described in more detail in Supplementary Methods. In brief, recipient eligible diagnoses included MDS, MPN excluding Philadelphia chromosome-positive chronic myeloid leukemia, MDS-MPN overlap syndromes, s-AML, and t-MN (including t-MDS, t-MPN, t-MDS-MPN overlap, and t-AML). To be eligible, recipients had to be age >50 years or deemed at high risk for toxicity from myeloablative conditioning, with availability of a fully HLA-matched or single antigen/allele-mismatched related or unrelated donor. Recipient exclusion criteria included uncontrolled central nervous system involvement, Karnofsky Performance Sale Status (KPSS) score <70, documented progressive infections, viral hepatitis, HIV positivity, pregnancy or lactation, or evidence of organ dysfunction.

Donors were considered eligible if they were age ≤75, had negative HIV and HBV nucleic acid testing within 30 days of collection, had adequate venous access for leukapheresis or be willing to undergo insertion of central venous catheter if needed, and willing to undergo a second donation of peripheral blood or bone marrow harvest for graft failure (GF). Donor exclusion criteria included identical twins for related donors, pregnant or lactating females, or any medical, physical or psychological factor that would increase the risk of complications from growth factor or leukapheresis.

Study Treatment

TLI was given as a total dose of 1,200 cGy in 10 fractions (given on days -11 to -7 and on days -4 to -1) [1–3]. Rabbit-derived ATG was given on days -11 to -7 at a daily dose of 1.5 mg/kg/day for a total dose of 7.5 mg/kg with solumedrol 1 mg/kg premedication before each dose. Peripheral blood hematopoietic progenitor cells were collected via G-CSF mobilization with apheresis on days -1 and 0. Although a $CD34^+$ cell dose of 5×10^6/kg of recipient body weight was targeted, transplants with less than target could still proceed. Additional apheresis was allowed for related donors if the target $CD34^+$ cell dose was not met. GVHD immunosuppression with cyclosporine (CsA) and mycophenolate mofetil (MMF) have been described previously [2]. Standard institutional guidelines were followed for infection prophylaxis and treatment. An aliquot (<5%) of freshly collected primary graft was used for CIK cell expansion. The CIK cell expansion method has been described previously [7,8] (see Supplementary Methods). CIK cultures were not initiated when graft $CD34^+$ cell dose was $<5 \times 10^6$/kg and discontinued for recipient grade II or higher aGVHD or infection before scheduled infusion. CIK cells were harvested and infused between day +21 and day +35 at a target dose of 1×10^8 $CD3^+$ cells per recipient kg. Cells were infused fresh without cryopreservation.

CIK Cell Immunophenotyping and In Vitro Cytotoxicity Assays

Aliquots of harvested CIK cells were evaluated by flow cytometry for immunophenotype and for in vitro antitumor cytotoxicity against 4 common tumor cell lines—Jurkat, OCI-Ly8, SU-DHL4, and DB—using CIK effector-to-tumor target (E:T) cell ratios of 0:1, 10:1, 20:1, and 40:1. Methods have been described previously [8] (see Supplementary Methods).

Laboratory Assessments

Weekly blood counts, blood chemistries, and liver function tests were obtained post-transplant until day +90 or more frequently as clinically indicated. Bone marrow aspirate/biopsy specimens were obtained in accordance with institutional guidelines generally at 3, 6, and 12 months post-transplantand then yearly until 5 years post-transplant. Donor chimerism was assessed as described previously [2,16] and performed no more than 48 hours before CIK infusion or by day +28 and again around day +56 and day +90 ± 10 days, and subsequently annually or more frequently at clinical discretion.

For the comparison historical cohort described below, donor chimerism data were notated retrospectively as available. FDC was defined as attainment of >95% peripheral donor-type $CD3^+$ cells. MDC was defined as having between 5% and 95% peripheral donor-type $CD3^+$ cells, and GF was defined as having <5% donor-type $CD3^+$ peripheral cells without concurrent relapse.

Historical Cohort

For outcome comparison, we retrospectively evaluated data from 100 patients with similar diagnoses consecutively enrolled on TLI-ATG-based NMA protocols (153, 9153, 168) between April 2004 through October 2015 (the historical cohort). The conditioning regimen included TLI with a total cumulative dose of either 800 cGy or 1,200 cGy in 10 fractions combined with ATG 1.5 mg/kg/day for 5 days. Donor $CD34^+$ graft source and dose, along with immunosuppression taper (CsA/MMF), have been described previously [1–3].

Measurement of Pretransplantation Characteristics

International Prognostic Scoring System (IPSS) [17] and revised International Prognostic Scoring System (R-IPSS) [18] scores were assigned to patients with de novo MDS and t-MDS. European LeukemiaNet prognostic status [19] was assigned for patients with AML. Types of pretransplant therapy, presence of known prior monosomal [20] or complex karyotype [19,21], and HCT-CI score [22] were noted. Pretransplant disease status was assessed using the International Working Group criteria for MDS and European LeukemiaNet criteria for AML [19,23], but with persistence of MN morphologically notated as persistent disease for secondary AML for statistical analyses. Grouping of pretransplant disease status and types of therapy received before transplant for statistical analyses are detailed in Supplementary Methods.

Statistical Analyses

The primary objective of the study was to determine the proportion of patients achieving FDC on or by day +90 after infusion of allogeneic CIK cells following allo-HCT with TLI-ATG conditioning. Secondary objectives included determination of 2-year OS rate, 2-year EFS rate, incidence of aGVHD, and assessment

of NKG2D ligand expression. The study was designed as a Simon-2 stage optimal phase II single-arm study with an enrollment target of 21 patients, using a 10% type I error and 80% power to detect an improvement in FDC from 25% (based on the historical rate) to 50% on or by day +90. Enrollment was increased to 44, because our initial goal did not clearly distinguish between phase I dose finding for unrelated donors (n = 4) and for patients who would not receive CIK cells owing to a low graft cell count, other illness, or logistical reasons.

For determination of the primary endpoint, patients achieving FDC on or by approximately day +90 among those receiving CIK cell infusion were counted, including those who achieved FDC but died before day +90. In cases where chimerism data availability were limited, the closest values before day +118 were used. Post-transplant persistent disease (including ≥10% morphological dysplasia for MDS and persistence of any disease-related cytogenetic abnormalities), disease relapse (including reappearance of circulating blasts, new extramedullary disease, and recurrence or evolution of disease-related cytogenetic abnormalities), and disease progression were grouped together as relapsed disease. The secondary endpoints of OS, EFS, and aGVHD incidence were analyzed in all enrolled patients. The OS rate was analyzed using death from any cause using the Kaplan-Meier (KM) estimator from the day of transplant with censoring time being the date of last follow-up or the date of second transplant. The median follow-up was estimated based on a reverse KM estimator [24]. For analyzing EFS rate, events were defined as relapse, grade III-IV aGVHD, or death.

Relapse-free survival (RFS) was defined using relapse or death as events. The cumulative incidence of relapse was calculated in a competing-risks model including GF or death as competing risks. Cumulative incidence of GF was calculated treating relapse or death as competing risks. Cumulative incidence of NRM was calculated treating relapse as a competing risk. OS, EFS, and RFS were compared between the study cohort and historical cohort using the log-rank method. aGVHD was graded using the modified Keystone criteria [25]. Cumulative incidence of aGVHD grade II-IV was calculated treating aGVHD maximum grade I, GF, or death as competing risks. Chronic GVHD (cGVHD) was graded using the National Institutes of Health scale [26]. The cumulative incidence of cGVHD was calculated using GF or death as competing risks. For comparison of characteristics between the study and historical cohorts, Fisher's exact test and the Mann-Whitney *U*test were used for categorical and continuous variables, respectively. Univariate analyses for RFS and OS were completed using the Cox proportional hazards method. Univariate analyses for CIK cell subset versus outcome was completed using the logistic regression method. The statistical significance level for all tests was set at 0.05. Statistical analyses were conducted using R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient Baseline Characteristics

The median age of the study cohort was 64.5 years (range, 37 to 74 years), with 48% females and 52% males, with most patients having a baseline KPSS score of 80 to 90. Baseline characteristics are summarized in Table 1. Disease diagnoses included MDS (61%), s- or t-AML (27%), MPN (5%), and MDS/MPN overlap syndromes (7%). Of the patients with MDS, 55% had refractory anemia with excess blasts (RAEB)-1 or RAEB-2 type disease, and 15% had t-MDS. All patients with t-MDS had IPSS higher-risk disease (Int-2 or High). Of the 23 patients with de novo MDS, 48% had IPSS higher-risk disease. Complex and monosomal karyotypes at disease diagnosis were noted in 21% and 16% of patients, respectively. In terms of therapy prior to transplant, 66% of patients had therapy with hypomethylating agents (HMAs) and/or immunomodulators (IMIDs), 14% had cytoreductive induction-type chemotherapy regimens, and 14% had both prior cytoreductive therapy and prior HMAs and/or IMIDs. The majority of patients with AML (8 of 12; 67%) were in complete remission (CR) at the time of transplant. Of the patients with MDS, 33% were in CR, 41% had a response less than CR, and 26% were either nonresponsive to prior therapy or had progressive disease at the time of allo-HCT.

Transplant Characteristics

Transplant characteristics are summarized in Table 2. Sixty-four percent of donors were unrelated, and 39% of patients received a sex-mismatched transplant. There was a cytomegalovirus donor/recipient seropositivity discordance of 30% (reactive/nonreactive or nonreactive/reactive). The median graft $CD34^+$ dose was 7.1×10^6/kg (range, 2.3 to 17×10^6/kg). Of the 44 patients treated on study, 31 received CIK cells at a median of 26 days after allo-HCT (range, 24 to 31 days) and are designated the CIK recipient subgroup. Characteristics and outcomes of the CIK recipient subgroup are detailed in the Supplementary tables. CIK cells were infused in the ambulatory setting, and no major infusion reactions were noted. As part of our phase I dose-finding assessment for patients with unrelated donors, the CIK $CD3^+$ cell dose was escalated from 1×10^7/kg (n = 1) to 3×10^7/kg (n = 1) to 1×10^8/kg (n = 2) among the first 4 patients with unrelated donors. Two other patients later received less than the target of CIK $CD3^+$ 1×10^8 cells/kg (range, 9.0 to 9.5×10^7/kg). Thirteen patients did not receive CIK cells, for the following reasons: 5 donor grafts had a $CD34^+$ cell dose below the threshold for CIK culture initiation, 4 patients had aGVHD prior to scheduled infusion, 1 patient had fever with concern for active infection, and in 3 patients there were logistical issues with initiating CIK cells (including transplant delay and reagent unavailability).

For comparison of study outcomes, we evaluated retrospective data obtained from a historical cohort of 100 patients who were consecutively treated with TLI-ATG-based allo-HCT for similar diagnoses. Baseline characteristics were mostly similar (Table 1); however, KPSS score and the type of therapy received before allo-HCT differed between the 2 groups. More patients in the historical cohort had received previous cytoreductive induction-type chemotherapy and other care/supportive care. The median duration of follow-up by the reverse KM estimator was longer in the historical cohort compared with the study cohort (2539 days versus 1375 days, respectively).

CIK Cell Immunophenotype by Flow Cytometry and In Vitro Cytotoxicity Assays

The majority of the CIK cells were $CD3^+$ (median, 97.3%; range, 59% to 99.8%) (Figure 1A, Supplementary Table 1). Among the viable $CD45^+$ cells, $CD3^+CD8^+NKG2D/CD314^+$ was the predominant cell type (median, 51.2%; range, 8.1% to 80.9%), with the majority of $CD8^+$ cells of the $CD45RO^+$ memory phenotype (Figure 1A). Comparing graft seed aliquot to final CIK cell culture, the median percentile fold expansion of $CD3^+CD56^+$ cells was 15.3 (Supplementary Table S1). Supplementary Figure 1 shows sample flow plots comparing the graft seed aliquot and final CIK cell culture for a representative patient.

The cytotoxic capability of CIK cells from 22 cultures were tested in vitro against 4 common tumor cell lines (Figure 1B). The mean tumor target cell killing at a ratio of 40:1 effectors to targets after a 1-hour coincubation at 37°C was as follows: 42.3% for Jurkat (range, 10.6% to 96.6%), 24.6% for OCI-Ly8 (range, 6.9% to 52.4%), 33.0% for DB (range, 6.8% to 91.4%), and 31.7% for SU-DHL4 (range, 8.3% to 80.4%).

Transplant Outcomes

Transplant outcomes are summarized in Table 3. For our primary endpoint, 6 of 31 CIK recipients (19%) achieved FDC by day +90, an outcome not significantly different from that in the overall study cohort (25%; p = 0.780) or the historical cohort (27%; p = 0.482), not meeting our target improvement of 50% with this one-time infusion intervention.

In terms of other clinical outcomes (Figure 2), the 2-year RFS was similar between the study and historical cohorts (27.3% (95% CI16.8-44.2%) versus 27.9% (95% CI20.4-38.3%); hazard ratio (HR) 0.938 [0.625, 1.408], p = 0.76). The 2-year EFS was also similar between the study and historical cohorts (27.3% (95% CI16.8-44.2%) versus 27.9% (95% CI20.4-38.3%); HR0.996 [0.663,1.494], p = 0.98). The 2-year NRM

Table 1
Patient Demographic Data and Disease Characteristics

Variable	Historical Cohort (N = 100)	Study 217 Cohort (N = 44)	p-value
Age, yr, median (range)	63 (46-74)	64.5 (37-74)	0.1688
Age, yr, n (%)			0.3202
<60	30 (30)	9 (20)	
60-69	61 (61)	28 (64)	
70-74	9 (9)	7 (16)	
Year of HCT, n (%)			3.303e-13
2001-2010	58 (58)	0 (0)	
2011-2016	42 (42)	44 (100)	
Sex, male/female, n (%)	55 (55)/45 (45)	23 (52)/21 (48)	0.8563
Pretransplant KPSS score, n (%)			0.02773
70	3 (3)	0 (0)	
80-90	70 (70)	37 (84)	
100	14 (14)	7 (16)	
NA	13 (13)	0 (0)	
HCT-CI score, n (%)			0.07767
0	60 (60)	18 (41)	
1-2	18 (18)	14 (32)	
≥3	22 (22)	12 (27)	
Disease type			
AML, n (%)	33 (33)	12 (27)	1.00
Secondary/t-AML	20 (61)/13 (39)	8 (67)/4 (33)	
MDS, n (%)	52 (52)	27 (61)	0.1537
RARS, RARS-T, or RCMD	16 (31)	8 (30)	
RAEB-1 or RAEB-2	17 (32)	15 (55)	
MDS, unclassifiable	3 (6)	0 (0)	
t-MDS	16 (31)	4 (15)	
MPN, n (%)	7 (7)	2 (5)	1.00
De novo/t-MPN	6 (86)/1 (14)	2 (100)/0 (0)	
MDS/MPN overlap, n (%)	8 (8)	3 (7)	0.05455
De novo/t-MDS/MPN overlap	8 (100)/0 (0)	1 (33)/2 (66)	
IPSS risk classification of de novo MDS, n (%)	36	23	0.3105
Low or int-1	18 (50)	11 (48)	
Int-2 or high	12 (33)	11 (48)	
Unknown	6 (17)	1 (4)	
IPSS risk classification of t-MDS, n (%)	16	4	1.00
Low or int-1	2 (12)	0 (0)	
Int-2 or high	14 (88)	4 (100)	
Unknown	0 (0)	0 (0)	
R-IPSS risk classification of de novo MDS, n (%)	36	23	0.4513
Very low or low	7 (19)	3 (13)	
Intermediate	10 (28)	8 (35)	
High or very high	13 (36)	11 (48)	
Unknown	6 (17)	1 (4)	
R-IPSS risk classification of t-MDS, n (%)	16	4	1.00
Very low or low	1 (6)	0 (0)	
Intermediate	1 (6)	0 (0)	
High or very high	14 (88)	4 (100)	
Unknown	0 (0)	0 (0)	
ELN classification for AML, n (%)	33	12	0.5275
Favorable	3 (10)	1 (8)	
Int-I or int-II	7 (21)	4 (33)	
Adverse	13 (39)	2 (17)	
Unassigned or unknown	10 (30)	5 (42)	
Complex cytogenetics, n (%)			0.8648
No/yes/unknown	63 (63)/22 (22)/15 (15)	30 (68)/9 (21)/5 (11)	
Monosomal karyotype, n (%)			0.8218

(*continued*)

Table 1 (*Continued*)

Variable	Historical Cohort (N = 100)	Study 217 Cohort (N = 44)	p-value
No/yes/unknown	67 (67)/18 (18)/15 (15)	32 (73)/7 (16)/5 (11)	
Therapies prior to transplant, n (%)			0.03261
Cytoreductive induction type therapy only	29 (29)	6 (14)	
Cytoreductive and HMA and/or IMID	6 (6)	6 (14)	
HMA and/or IMID	49 (49)	29 (66)	
Other prior care/supportive care	16 (16)	3 (6)	
Type of previous HMA, n (%)	45	32	0.1377
Decitabine alone	9 (20)	9 (28)	
Azacitidine alone	31 (69)	23 (72)	
Both azacitidine and decitabine	5 (11)	0 (0)	
Disease status at HCT, AML, n (%)	33	12	0.3366
Morphological CR	20 (61)	8 (68)	
Morphological CRi	10 (30)	2 (16)	
Persistent disease	1 (3)	2 (16)	
NA	2 (6)	0 (0)	
Disease status at HCT, MDS, n (%)	52	27	0.2975
CR	10 (19)	9 (33)	
PR or marrow CR+/-HI	4 (8)	4 (15)	
SD+/-HI	21 (40)	7 (26)	
Nonresponsive or progressive disease	17 (33)	7 (26)	
Disease status at HCT, MPN or MDS/MPN overlap	15	5	0.3661
CR	3 (20)	0 (0)	
PMR or some response	0 (0)	1 (20)	
Persistent or progressive disease	12 (80)	4 (80)	
Time from diagnosis to HCT, d, median (range)	247.5 (92-5502)	227.5 (97-2342)	0.5401

ELN, European Leukemia Net; NA, not available; PR, partial remission; PMR, partial marrow response; RARS, refractory anemia with ring sideroblasts; RARS-T, RARS with thrombocytosis; RCMD, refractory cytopenia with multilineage dysplasia.

was low and not significantly different between the study and historical cohorts (6.8% (95% CI0-14.5%) versus 6.0% (95% CI1.3-10.7%); HR1.063 [0.330-3.421], p =0.919). Whereas the median OS for the study cohort (689 days; 95% CI373-not reached) and CIK recipient subgroup (736 days; 95% CI373-not reached) trended higher than that for the historical cohort (521 days; 95% CI353-1036), the secondary endpoint of 2-year OS was not statistically significant (study cohort: 50.6% (95% CI37.5-68.2%) versus historical cohort: 42.9% (95% CI34.2-53.8%); HR0.858 [0.546,1.347], p = 0.51).

Table 2
Transplantation Characteristics

Main Category	Historical Cohort (N = 100)	Study 217 Cohort (N = 44)	p-value
Donor age, yr, median (range)	47 (18-75)	31.5 (21-72)	0.1683
Donor type, n (%)			0.5549
Matched related	39 (39)	16 (36)	
Matched unrelated	46 (46)	24 (55)	
Mismatch unrelated	15 (15)	4 (9)	
Donor/recipient sex match, n (%)			0.4129
Female/female	25 (25)	11 (25)	
Male/male	27 (27)	16 (36)	
Female/male	28 (28)	7 (16)	
Male/female	20 (20)	10 (23)	
Donor/recipient CMV serostatus, n (%)			0.142
Reactive/reactive	31 (31)	18 (41)	
Nonreactive/nonreactive	20 (20)	13 (29)	
Reactive/nonreactive	18 (18)	3 (7)	
Nonreactive/reactive	31 (31)	10 (23)	
Graft $CD34^+$cell dose, $\times 10^6$/kg, median (range)	6.1 (1.8-21.9)	7.1 (2.3-17)	0.0644
Graft $CD3^+$cell dose, $\times 10^8$/kg, median (range)	2.9 (0.55-6.35)	2.62 (1.44-6.87)	0.3256

CMV indicates cytomegalovirus.

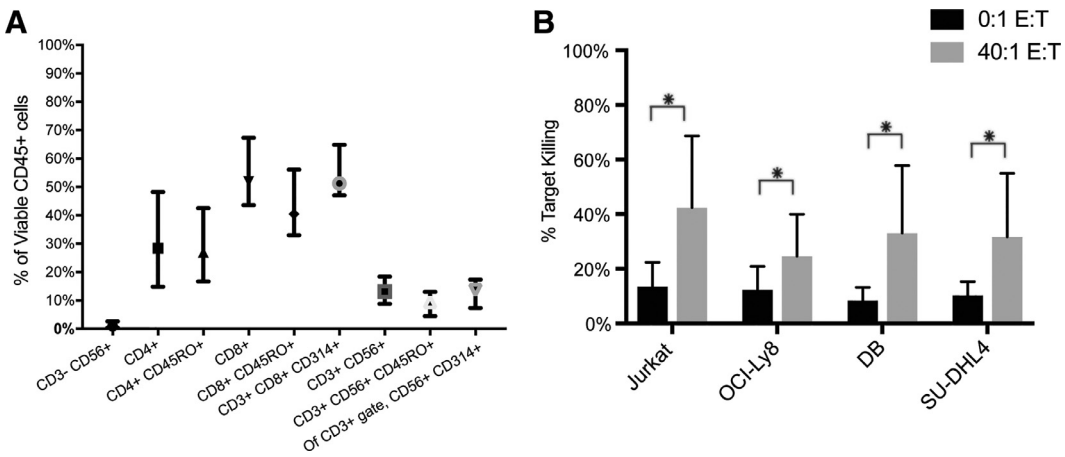

Figure 1. Characteristics of harvested CIK cells. (A) Immunophenotype of CIK cells by flow cytometry. Data are shown as median with 95% CI. (B) CIK cells from 22 patients were tested for in vitro cytotoxicity against 4 different tumor cell lines—Jurkat, OCI-Ly8, DB, and SU-DHL4—at varying effector CIK to tumor target (E:T) cell ratios. The percent of tumor target cell killing with 0:1 (no CIK cells) and 40:1 E:T (after 1 hour of CIK coincubation) is shown. Data are shown as mean ± SD. *indicates $p < 0.05$.

Table 3
Transplant Outcomes

Outcome	Historical Cohort (N = 100)	Study 217 Cohort (N = 44)
On/by day +90 chimerism, n (%)		p = 0.8194
NA	1 (1)	0 (0)
GF	1 (1)	0 (0)
GF with concurrent relapse	6 (6)	1 (2)
MDC	65 (65)	32 (73)
FDC	27 (27)	11 (25)
FDC by/before day +90, n (%)		p = 0.8406
Yes	27 (27)	11 (25)
No or NA	73 (73)	33 (75)
GF		*p* = 0.5858
No/yes, n	98/2	42/2
Cumulative incidence	1 per 279 person-yr	1 per 89 person-yr
Relapse		
Time to relapse, d, median (range)	89 (13-3290)	88 (25-600)
2-year cumulative incidence of relapse, % (95% CI)	66.1 (56.7-75.5)	65.9 (51.5-80.3)
2-year RFS, % (95% CI)	27.9 (20.4-38.3)	27.3 (16.8-44.2)
2-year NRM, % (95% CI)	6.0 (1.3-10.7)	6.8 (0-14.5)
2-year EFS, % (95% CI)	27.9 (20.4-38.3)	27.3 (16.8-44.2)
OS, days, median (range)	521 (353-1036)	689 (373-)
2-year OS, % (95% CI)	42.9 (34.2-53.8)	50.6 (37.5-68.2)

The 2-year OS for the CIK recipient subgroup was 52.6% (95% CI37.3-74.2%), which was not statistically significant compared to the historical cohort (p = 0.36). Because pretransplant recipient tumor samples were generally not available, we were unable to assess NKG2D ligand expression as part of our secondary objectives.

The 100-day cumulative incidence of grade II-IV aGVHD in the study cohort was 20.5% (95% CI8.4-32.5%) versus 12.0% for the historical cohort (95% CI5.6-18.4%, p = 0.244) with a 1-year cumulative incidence of grade II-IV aGVHD of 25.1% (95% CI12.0-38.2%) for the study cohort versus 16.0% (95% CI8.8-23.2%, p = 0.242) for the historical cohort (Table 4). In the CIK recipient subgroup, the 100-day and 1-year cumulative incidences of grade II-IV aGVHD were 9.7% (95% CI0-20.3%) and 16.3% (95% CI2.9-29.7%). In the study cohort, 6 of 44 patients experienced grade III (5 patients) or IV (1 patient) aGVHD within the first year, with 4 of these cases occurring within the first 100 days. Although there was a statistical difference in 100-day and 1-year aGVHD grade III-IV cumulative incidence between the study and historical cohorts (Table 4), given the small sample size and number of events, the p value and confidence interval need to be interpreted with caution. The 2-year cumulative incidence of cGVHD for the study cohort (28.2%, 95% CI14.4-42.1%) was similar to the historical cohort (30.2%, 95% CI21-39.3%, p = 0.829).

Statistical Analysis

In univariate logistic regression analyses evaluating the percentile of CIK cell subsets in harvested cells ($CD3^+$, $CD3^+CD56^+$, $CD3^+CD8^+CD314^+$, $CD8^+$, $CD8^+CD45RO^+$, $CD4^+$, $CD4^+CD45RO^+$, $CD3^-CD56^+$, and $CD3^+CD56^+CD314^+$), while there was no association of cell subsets with day +90 chimerism, the percentile of $CD3^+CD56^+$ and $CD3^+CD56^+CD314^+$ was significantly associated with increased odds of achieving post-transplant remission (CR or CRi) at day +90 (see Supplementary data). We also evaluated the impact of several pretransplant variables on RFS and OS. In univariate analysis for the study cohort (Table 5), prior therapy with cytoreductive induction-type chemotherapy was associated with increased RFS compared to HMAs and/or IMIDs (HR0.233, p = 0.049) (Figure 3). The presence of monosomal or complex karyotype was associated with an increased risk of relapse or death (HR2.846 for RFS, p = 0.00884). Having MPN or MPN/MDS overlap compared to MDS also appeared to be less favorable for RFS (HR2.971, p = 0.044). However, the only univariate that appeared to significantly affect OS for the study cohort was the presence of monosomal or complex karyotype (HR3.075, p = 0.009). Multivariate analysis using disease diagnosis, type of therapy prior to transplant, disease status at remission, presence of de novo versus secondary/t-MN, cytogenetics at disease diagnosis (monosomal and/or complex versus not),

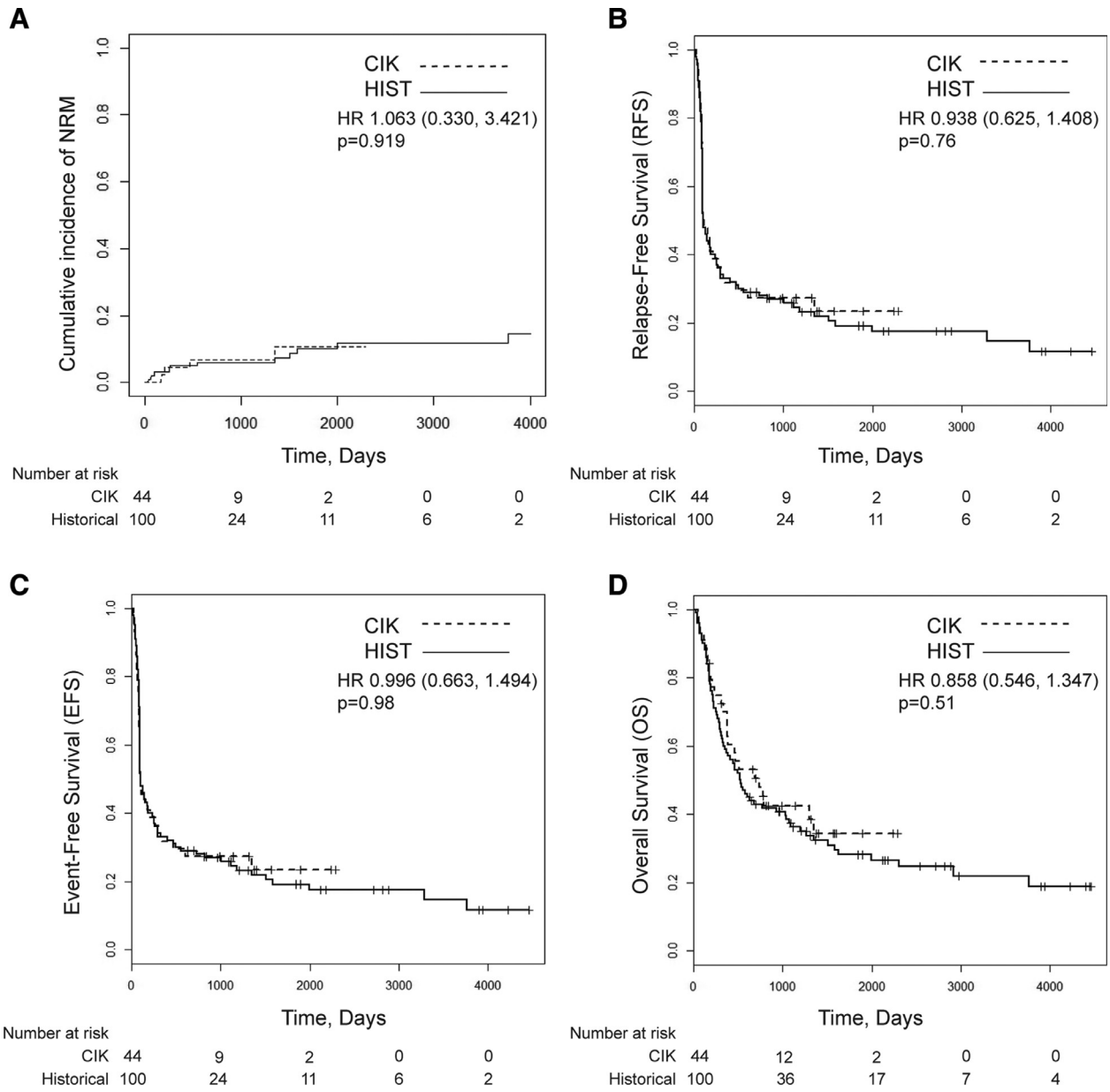

Figure 2. Transplant Outcomes. Cumulative incidences of NRM (A), RFS (B), EFS (C), and OS (D).

HCT-CI score, donor-recipient sex mismatch, and age did not identify factors that were significantly associated with OS.

DISCUSSION

In our previous experience with TLI-ATG conditioning for MDS and MPN, we found a 6-month median time to FDC post-transplant, with decreased relapse rates in patients achieving early donor reconstitution [2]. We evaluated a one-time infusion of CIK cells as post-transplant consolidation to determine whether this intervention could promote early FDC achievement and potentially decrease relapse rates. Our study population included a relatively high-risk population, including 80% of patients age ≥60, 27% with HCT-CI scores of ≥3, and 23% with t-MN, with the majority of patients with MDS having intermediate or higher risk disease by R-IPSS. Sixty-one percent of patients had either a response less than CR or persistent/progressive disease at the time of transplant.

We found that CIK cell infusion was safe and feasible to infuse in the ambulatory setting as early consolidation therapy, with similar rates of 100-day and 1-year grade II-IV aGVHD. One limitation of our study was its nonrandomized design. Thus, study results were compared to retrospective historical data. However, the study and historical groups were broadly similar at baseline, with the backbone of TLI-ATG conditioning for similar diagnoses of MNs using the same GVHD prophylaxis, which facilitated an overall comparison of outcomes. The rate of FDC by day +90 following CIK infusion was not higher in our study population compared to the historical cohort. The cumulative incidence of relapse was similar between the study and historical cohorts, with relapse being the main cause of treatment failure and the majority of relapse events occurring within the first-year post-transplant, similar to what has been described for patients with AML and MDS [27,28]. We observed low rates of 1- and 2-year NRM (<10%), which compares favorably to recent 3-year transplant related mortality

Table 4
GVHD Outcomes

Characteristic	Historical Cohort (N = 100)	CIK Study Cohort (N = 44)	CIK Recipient Subgroup (N = 31)	p-value
Cumulative incidence of aGVHD grade II-IV, % (95% CI)	100-d: 12.0 (5.6-18.4)	100-d: 20.5 (8.4-32.5)	100-d: 9.7 (0-20.3)	Study versus historical
	1-yr: 16.0 (8.8-23.2)	1-yr: 25.1 (12.0-38.2)	1-yr: 16.3 (2.9-29.7)	100-d: 0.244
				1-yr: 0.242
				CIK recipient versus historical
				100-d: 0.660
				1-yr: 0.484
Cumulative incidence of aGVHD grade III-IV, % (95% CI)*	100-d: 1.0 (0-3.0)	100-d: 9.1 (0.5-17.7)	100-d: 3.2 (0-9.6)	Study versus historical
	1-yr: 2.0 (0-4.8)	1-yr: 13.7 (3.3-24.2)	1-yr: 9.9 (0-20.8)	100-d: <0.001
				1-yr: <0.001
				CIK recipient versus Historical
				100-d: 0.142
				1-yr: 0.006
Cumulative incidence of cGVHD, % (95% CI)	1-yr: 26.0 (17.3-34.7)	1-yr: 25.8 (12.4-39.2)	1-yr: 30.5 (13.4-47.7)	Study versus historical
	2-yr: 30.2 (21.0-39.3)	2-yr: 28.2 (14.4-42.1)	2-yr: 34.1 (16.3-51.9)	1-yr: 0.982
	3-yr: 32.6 (23.2-42.0)	3-yr: NA	3-yr: NA	2-yr: 0.829
				CIK recipient versus historical
				1-yr: 0.666
				2-yr: 0.718

* For comparison of aGVHD grade III-IV, given the small sample size and number of events, results including p-values and 95% CIs need to be interpreted with caution.

Table 5
Study 217 Cohort: Univariate Analysis for RFS and OS (N = 44)

Variable	RFS			OS		
	HR	95% CI	p-value	HR	95% CI	p-value
Therapy before transplantation						
HMA/IMID (reference)	1.00			1.00		
Cytoreductive induction-type therapy	0.233	0.055-0.994	0.049*	0.311	0.072-1.341	0.117
Cytoreductive and previous HMA/IMID	0.811	0.281-2.343	0.699	0.824	0.244-2.789	0.756
Other previous care/supportive care	1.053	0.315-3.513	0.933	0.840	0.195-3.615	0.815
Disease diagnosis						
MDS (reference)	1.00			1.00		
MPN/MDS overlap or MPN	2.971	1.028-8.586	0.044*	0.492	0.114-2.122	0.341
AML	0.724	0.306-1.712	0.462	0.674	0.267-1.703	0.404
Disease remission status at transplantation						
CR (reference)	1.00			1.00		
Response less than CR	1.261	0.518-3.072	0.609	0.884	0.346-2.259	0.797
Nonresponsive/progressive disease	1.753	0.689-4.461	0.239	1.042	0.377-2.880	0.937
Disease cytogenetics at diagnosis						
Nonmonosomal/noncomplex (reference)	1.00			1.00		
Monosomal or complex	2.846	1.301-6.227	0.00884*	3.075	1.316-7.182	0.009*
HCT-CI score						
0 (reference)	1.00			1.00		
1 or 2	1.101	0.493-2.461	0.815	2.249	0.883-5.728	0.089
3+	1.082	0.461-2.539	0.857	2.024	0.757-5.414	0.160
Donor/recipient sex mismatch						
No mismatch (F/F or M/M) (reference)	1.00			1.00		
Yes mismatch (F/M or M/F)	0.953	0.475-1.911	0.892	0.822	0.373-1.811	0.627
Age: 1 yr older	1.015	0.964-1.07	0.567	1.02	0.951-1.094	0.582
Disease type						
Secondary or therapy- related (reference)	1.00			1.00		
De novo	0.915	0.454-1.842	0.803	1.145	0.524-2.501	0.734

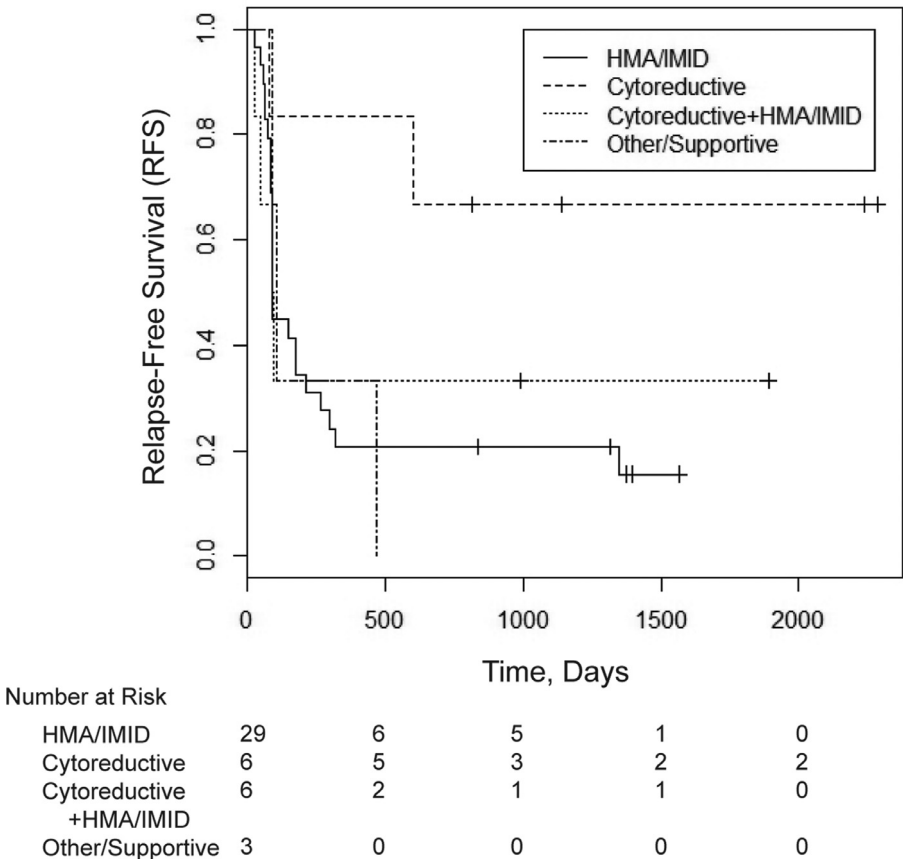

Figure 3. Impact of pretransplant therapy on RFS.

data reported from the Center for International Blood and Marrow Transplant Research for allo-HCT for patients with AML (20-22%) and MDS (26-37%) collected between 1998-2011 [27].

Two-year OS data in this study was also generally similar to that previously reported for MDS and AML [27–29]. While the median OS trended higher in our study cohort compared to historical cohort (689 days versus 521 days), the difference in 2-year OS was not statistically significant. Furthermore, a significant proportion of patients in the study group did not receive the planned CIK cell infusion (13 of 44) for a variety of reasons. For this reason, we analyzed the subgroup of patients who did receive CIK cells and found that although these patients showed a trend toward improved OS, relapse rates were not reduced.

The reasons for failure to see improved outcomes are potentially complex. Although previous work suggested that immunosuppressive medications such as CsA predominantly affect anti-CD3-based degranulation but not cytotoxicity [30], it is possible that CsA or MMF may have impacted CIK cell function and proliferation, or that CIK cells did not survive for a sufficiently long period. Although the effects of residual ATG on CIK cells are a possibility, our previous finding of residual ATG not being detected in recipient serum at 1 week after TLI-ATG conditioning [3] makes this less likely. The expression of exhaustion markers on healthy donor-derived CIK cells after infusion has not been well studied. In a study of checkpoint expression in autologous CIK cells derived from lung cancer patients [31], the expression of several markers, including LAG3 and PD-L1, became elevated during early culture points and remained elevated above baseline over the course of a 21-day culture; whereas expression of others, such as PD-1 and CTLA-4, returned toward baseline at the end of the culture. Because the CIK cells in our study were not modified with a marker, we were unable to follow their survival and evaluate checkpoint expression postinfusion, but monitoring such expression may be helpful in the future. A one-time infusion of CIK cells also may have been insufficient against the clonal burden present. Repeat dosing strategies have been evaluated for disease relapsed after allo-HCT [13]. Given the overall safety of CIK infusion as early post-transplant consolidation that we found in this study, repeat dosing schedules may be considered at this time point as well.

Other strategies to enhance CIK cell efficacy also merit exploration. Several groups have evaluated substitution of the use of IL-2 during CIK culture to other cytokines, such as IL-15 [32], as a potential way to increase CIK cell cytotoxicity and down-regulate T regulatory cells within the CIK culture. CIK cells modified with chimeric antigen receptors are being developed against both lymphoid and myeloid targets [33–35]. Interestingly, CIK cells modified using an oncolytic virus showed tumor regression in mouse models [36]. Strategies enhancing antitumor-specific targeting without increasing NRM and GVHD are likely to improve overall clinical outcomes.

In univariate analyses evaluating the effect of pretransplant variables on RFS and OS, type of prior therapy, presence of monosomal or complex karyotype, and disease type were found to impact RFS, with monosomal or complex karyotype impacting OS. Our multivariate analysis did not identify any variables with a significant association to OS. In several studies, monosomal karyotype has been associated with poor survival in patients with MDS and AML [27,37–40]. The impact of pretransplant therapy type in patients with MDS on post-transplant outcomes has been mixed [41–46] and may be confounded by age, fitness at diagnosis, and cytogenetic/molecular

features. Current consensus guidelines [47] currently recommend consideration of pretransplant therapy for higher-risk patients with MDS with >10% blasts but without a recommendation for optimal type of pretransplant disease-modifying therapy [47]. Intriguingly, in a prospective trial evaluating hypomethylation therapy with azacitidine for early post-transplant relapse in AML and MDS [48], pretransplant intensified chemotherapy was associated with increased response to salvage azacitidine and improved OS on multivariate analysis. How pretransplant induction-type chemotherapy affects post-transplant outcomes specifically in non-chemotherapy-based NMA conditioning regimens will be important to better understand as NMA regimens are increasingly being used in older patients with AML and MDS.

In summary, in this relatively high-risk population, we confirmed that TLI-ATG-based conditioning has a relatively low risk of NRM with a survival benefit in patients with MNs. Although we did not see earlier FDC or improved relapse rates with a one-time CIK cell infusion following TLI-ATG conditioning, we found that overall, such an infusion was safe as post-transplant consolidation. Varied dosing strategies or CIK cell modification may enhance the antitumor efficacy of this treatment modality.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2019.03.027.

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