ARTICLE

Sequential vs myeloablative vs reduced intensity conditioning for patients with myelodysplastic syndromes with an excess of blasts at time of allogeneic haematopoietic cell transplantation: a retrospective study by the chronic malignancies working party of the EBMT

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The optimal conditioning for patients with higher risk MDS receiving potentially curative allogeneic haematopoietic stem cell transplant(allo-HCT) remains to be defined. This is particularly the case for patients with excess of blasts at time of allo-HCT. Sequential (Seq) conditioning, whereby chemotherapy is followed rapidly by transplant conditioning, offers an opportunity to decrease disease burden, potentially improving outcomes allo-HCT outcomes. Herein we present the only analysis comparing Seq to myeloablative (MAC) and reduced intensity conditioning (RIC) specifically focussed on MDS patients with excess of blasts at allo-HCT. 303 patients were identified in the EBMT registry, receiving RIC ($n = 158$), Seq ($n = 105$), and MAC ($n = 40$). Median follow-up was 67.2 months and median age at allo-HCT was 59.5 years (IQR 53.5–65.6). For the entire cohort, 3 y overall survival (OS) was 50% (95% CI 45–56%) and relapse free survival (RFS) 45% (95% CI 40–51%). No significant differences in OS (log-rank $p = 0.13$) and RFS (log-rank $p = 0.18$) were observed between conditioning protocols. On multivariable analysis, lower performance status, worse IPSS-R cytogenetics, sibling donor (compared to 8/8 MUD) and ≥20% blasts at allo-HCT were associated with worse outcomes. In conclusion, the Seq protocol did little to influence the outcome in this high-risk group of patients, with outcomes mostly determined by baseline disease risk and patient characteristics such as performance status.

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INTRODUCTION

Whilst established that allogeneic hematopoietic cell transplantation (allo-HCT) remains the only curative option for patients with myelodysplastic syndrome (MDS), the optimal conditioning protocol has not been defined [1, 2]. Overall, outcomes remain suboptimal with less than 50% of patients achieving long-term cure, this is particularly pertinent for those with poor prognosis disease where relapse rates remain high. Myeloablative conditioning (MAC) regimens are associated with a lower risk of relapse, however often at the expense of higher transplant related morbidity and mortality. Reduced intensity conditioning (RIC) regimens while associated with higher rates of relapse, offer potentially curative options to those patients who would not otherwise tolerate MAC due to either advanced age or presence of co-morbidities. This is of pivotal importance given the advanced age of the majority of patients with MDS. In recent years the development of the so-called sequential approach (Seq), whereby transplant conditioning is delivered immediately after a course of chemotherapy to control pre-transplant disease burden, has appeared promising in some studies [3, 4]. This approach may be particularly useful for patients with higher risk MDS who may proceed to transplant despite an excess of blasts

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at the time of allo-HCT. No prospective data comparing this approach directly with MAC or RIC protocols focussed on such patients has been reported. We therefore compared outcomes after allo-HCT in MDS patients with ≥5% of marrow blasts conditioned using RIC, Seq or MAC protocols using multicentre data from the EBMT-registry.

METHODS

This was a retrospective, multicentre, registry-based analysis approved by the Chronic Malignancies Working Party of the EBMT. The EBMT is a non-profit, scientific society representing more than 600 transplant centres mainly in Europe. Data obtained from patients undergoing allo-HCT are entered, managed, and maintained in a central database. EBMT centers commit to obtain informed consent according to the local regulations applicable at the time in order to report pseudonymized data. Patients with a diagnosis of MDS included in the EBMT registry data quality initiative, whereby requests for updates on missing data had been previously made, undergoing first allo-HCT were selected. Only patients with ≥5% of marrow blasts at the time of allo-HCT and only first allo-HCT's between 2007 and 2014 were included in the final analysis. Data collected included demographics and disease variables. Disease was classified as per WHO 2016 criteria and risk status as per International Prognostic Scoring System (IPSS) [5, 6]. Cytogenetics was additionally classified as per IPSS-Revised (IPSS-R) cytogenetic classification system with the required information [7]. Conditioning intensity was defined as per standard EBMT criteria as MAC and RIC [8]. Patients who had received a non-myeloablative conditioning were excluded. Seq-conditioning regimens were those that included fludarabine, cytarabine and either amsacrine or clofarabine followed by RIC. The primary endpoint was overall survival (OS). Secondary end points included progression-free survival (PFS), cumulative incidence of relapse (CIR), non-relapse mortality (NRM), acute (grade II, III or IV) and chronic (limited or extensive) graft-versus-host-disease (GVHD) and GVHD relapse/progression-free survival (GRFS).

Statistical analysis

Patients' characteristics between groups were compared using the χ2 test for categorical variables and the Kruskal-Wallis test for continuous data. OS was defined as the time from allo-HCT to death from any cause. PFS was defined as the time between allo-HCT and relapse/progression of disease or death, whichever occurred first. GRFS was defined as the time from the date of allo-HCT to the date when the first of the following events occurred: acute GvHD (grade III or IV), extensive chronic GvHD, relapse or death.

Probabilities of OS, PFS and GRFS were computed using the Kaplan-Meier estimator and compared between groups by log-rank test. The crude cumulative incidence estimator was used for competing events (CIR and NRM; chronic GvHD, death before chronic GvHD; acute GvHD, death before acute GvHD) and Gray's test was used for the comparison of cumulative incidence rates between groups. Outcomes occurring after 5 years were artificially censored for OS, PFS, CIR, NRM, chronic GvHD and death before chronic GvHD and likewise at 100 days for acute GvHD and death before acute GvHD. The Cox Proportional Hazards (PH) regression model was used to investigate the association between conditioning intensity (Seq, MAC and RIC) and outcome after allo-HCT adjusted for (potential) confounders. These include the interval time between diagnosis and allo-HCT, age at allo-HCT (both as linear continuous variables), donor type (HLA identical siblings, matched unrelated donor (MUD)), Karnofsky score at allo-HCT (<90 and ≥90), IPSS-R cytogenetics (very poor/poor and intermediate/good/very good/missing), and blast percentage in bone marrow at allo-HCT (<20% and ≥20%). Source of graft (peripheral blood (PB) and bone marrow (BM)) was used in the analysis of acute and chronic GvHD. Cause specific hazard ratios were estimated for CIR, NRM, acute and chronic GvHD. Multivariable models were performed on the basis of compete cases (except for IPSS-R cytogenetics). We tested whether the association between outcomes and conditioning intensity differed according to the blast percentage in BM at allo-HCT by including interaction terms conditioning intensity x blast percentage in BM (as a continuous linear variable). The median follow-up was calculated using the reverse Kaplan–Meier estimator [9]. All p-values shown are from two-sided tests and the reported confidence intervals (CI) refer to 95% boundaries, a p-value < 0.05 was regarded as statistically significant.

RESULTS

Patients and transplant characteristics

A total of 303 patients were identified. The majority received RIC ($n = 158$, 52%) followed by Seq ($n = 105$, 35%), and MAC ($n = 40$, 13%) conditioning protocols. The median follow-up for the entire cohort was 67.2 months (IQR: 44.6–91.4 months). Table 1 shows patient, disease and transplant characteristics. Median age at time of allo-HCT was 59.5 years (IQR 53.5–65.6). A total of 186 (61%) patients were male and 117 (39%) female. Significant differences across protocols were observed with regard to age at allo-HCT (the median age in patients receiving Seq was lower compared to those receiving RIC but higher compared to those receiving MAC), use of in-vivo T-cell depletion (TCD) and graft source. Rates of in-vivo TCD were 91% for Seq, 25% for MAC, and 49% for RIC ($p < 0.001$). Patients receiving MAC were more likely to receive bone marrow as the graft source; 20% for MAC compared with 2% for Seq and 4% for RIC ($p = 0.006$), respectively. Importantly for this analysis, there was no significant difference across protocols for percentage bone marrow blasts at time of allo-HCT.

Engraftment

Median time (IQR) to neutrophil engraftment for patients undergoing RIC, Seq or MAC conditioning protocols was 17 (IQR 14 to 20), 13 (11 to 17) and 17 (14 to 22) days, respectively (Gray's test $p < 0.001$). Median time (IQR) to platelet engraftment (defined as $>20 \times 10 \wedge 9/L$) for patients undergoing RIC, Seq or MAC conditioning protocols was 15 (13 to 19), 13 (11 to 19) and 15 (12 to 19) days, respectively (Gray's test $p = 0.22$).

Overall survival and relapse free survival

For the entire cohort, OS was 50% (95% CI 45–56%) and RFS 45% (95% CI 40–51%) at three years. There was no significant difference in OS up to 3-years comparing the three conditioning protocols; 3-year OS: MAC 62% (95% CI 47–77%) vs. Seq 52% (95% CI 43–62%) vs. RIC 46% (95% CI 38–54); log-rank $p = 0.13$. There was also no significant difference in RFS observed; 3-year RFS: MAC 60% (95% CI 45–75%) vs. Seq 46% (95% CI 36–55%) vs. RIC 42% (95% CI 34–50%) log-rank $p = 0.18$ (Fig. 1).

CIR and non-relapse mortality

For the entire cohort the CIR and NRM at 3 years was 27% (95% CI 22–32%), and 28% (95% CI 23–33%), respectively. No significant differences existed between the three regimens for either CIR (MAC 18%, 95% CI 6–29% vs. RIC 25%, 95% CI 18–32% vs. Seq 33%, 95% CI 24–41%, Gray's test $p = 0.14$) or NRM (MAC 22%, 95% CI 10–35% vs. RIC 33%, 95% CI 26–40% vs. Seq 22%, 95% CI 14–30% ($p = 0.16$) (Fig. 1).

GvHD

There was a trend (Gray's test $p = 0.08$) for a higher cumulative incidence of acute GvHD grades II-IV in patients receiving MAC (38% at 100 days, 95% CI 22–53%) and RIC (38%, 95% CI 30–46) compared to Seq (25%, 95% CI 17–34%). The D100 cumulative incidence for Grade III-IV acute GVHD, was 20% (95% CI 8–32%) in patients receiving MAC, 18% (12–24%) for RIC and 9% (3–14%) for Seq ($p = 0.08$). The cumulative incidence of any grade chronic GVHD at 3 years was highest in patients receiving MAC at 81% (95% CI 68–94%); this compares to 58% (50–66%) for RIC and 47% (38–57%) for Seq (Gray's test $p < 0.001$) (Fig. 2). The cumulative incidence of extensive chronic GVHD at 3 years was highest for MAC at 49% (95% CI 32–65%), compared to 30% (23–37%) for RIC and 24% (15–32%) for Seq ($p = 0.015$). The probability to be alive, without previous GVHD or previous progression/relapse at 3-years after allo-HCT was 34% (95% CI 25–43%) for Seq, 19% (13–25%) for RIC and 18% (6–29%) for MAC. The GRFS curves were hence significantly different between the 3 protocols, log-rank $p = 0.01$ (Fig. 3).

Table 1. Patient characteristics by regimen.

	Seq ($n = 105$) N (%)	RIC ($n = 158$) N (%)	MAC ($n = 40$) N (%)	Total ($n = 303$) N (%)	p-value
Sex					
Male	73 (70)	91 (58)	22 (55)	186 (61)	$p = 0.10$
Female	32 (30)	67 (42)	18 (45)	117 (39)	
Karnofsky performance status, (N missing = 1)					
<90	36 (35)	60 (38)	10 (25)	106 (35)	$P = 0.30$
90–100	68 (65)	98 (62)	30 (75)	196 (65)	
Age at allo-HCT in years, median (IQR)	59 (53–65)	62 (56–67)	55 (46–60)	59 (53–66)	$p < 0.0001$
IPSS at diagnosis, (N missing = 66)					
Low	7 (9)	6 (5)	2 (6)	15 (6)	$p = 0.72$
Intermediate-1	22 (27)	38 (30)	12 (39)	72 (30)	
Intermediate-2	26 (32)	48 (38)	9 (29)	83 (35)	
High	26 (32)	33 (26)	8 (26)	67 (28)	
IPSS-R cytogenetics, (N missing = 8)					
Good/Very good	51 (52)	79 (53)	16 (43)	146 (51)	$p = 0.78$
Intermediate	19 (19)	24 (16)	7 (19)	50 (18)	
Poor	14 (14)	22 (15)	9 (24)	45 (16)	
Very poor	14 (14)	25 (17)	5 (14)	44 (15)	
Donor type					
HLA-identical Sibling	34 (32)	71 (45)	18 (45)	123 (41)	$p = 0.11$
Matched 8/8 unrelated	71 (68)	87 (55)	22 (55)	180 (59)	
Graft source					
BM	2 (2)	8 (5)	8 (20)	17 (6)	$p = 0.0006$
PBSC	103 (98)	150 (95)	32 (80)	285 (94)	
In-vivo T-cell depletion					
No	9 (9)	81 (52)	30 (75)	120 (40)	$p < 0.0001$
Yes	96 (91)	77 (49)	10 (25)	182 (60)	
CMV status patient/donor, (N missing = 2)					
−/−	35 (34)	50 (32)	10 (25)	95 (32)	$p = 0.82$
−/+	12 (11)	12 (8)	3 (7.5)	27 (9)	
+/−	19 (18)	35 (22)	9 (22.5)	63 (21)	
+/+	38 (37)	60 (38)	18 (45)	116 (38)	
WHO disease category at allo-HCT					
EB1	14 (13)	38 (24)	9 (22)	61 (20)	$p = 0.22$
EB2	57 (54)	67 (42)	18 (45)	142 (47)	
Transformed to AML	34 (32)	53 (34)	13 (33)	100 (33)	
Interval diagnosis to HCT in months, median (IQR)	8.0 (4.2–23.6)	8.5 (4.8–19.7)	7.6 (4.7–16.4)	8.3 (4.6–19.6)	$p = 0.66$
% blasts in BM at allo-HCT in percentage, median (IQR)	14 (10–19)	12 (7–19)	10 (7–15)	12 (8–19)	$p = 0.10$

P-values were obtained using the $\chi 2$ test for categorical variables and the Kruskal-Wallis test for continuous data.
BM bone marrow, *IQR* interquartile range, *CI* confidence interval, *N* number.

Multivariate analysis (MVA)

In multivariate analysis of OS, RFS and NRM, no significant differences were observed between the three conditioning protocols (Table 2). The cause specific hazard of relapse was also not significantly different between patients receiving MAC and RIC (hazard ratio (HR) MAC vs. RIC 0.54 (95% CI 0.23–1.23)) and between patients receiving Seq and RIC protocols (Seq vs. RIC HR 1.27, (95% CI 0.80–1.99)). The hazard of relapse was borderline significantly higher in patients receiving Seq protocols compared to MAC (HR 2.37, (95% CI 1.03–5.40)). Better performance status as reflected by a higher KPS, better IPSS-R cytogenetic status (Int, good or very good by IPSS-R classification), utilisation of a matched (8/8) unrelated donor (as compared to HLA-identical siblings) and having less than 20% blasts in the bone marrow at time of allo-HCT were significantly associated with better OS and RFS. For relapse, lower hazard ratios were estimated for matched unrelated donors compared to HLA-identical siblings (0.57, 95% CI 0.36–0.88, $p = 0.004$) and lower IPSS-R cytogenetic risk category (HR poor/very poor compared to intermediate/good/very good; 2.12, 95% CI 1.34–3.34 $p = 0.001$). For NRM significant associations were observed for KPS (HR < 90 compared to 90–100 1.74, 95% CI 1.12–2.70, $p = 0.01$) as well as IPSS-R cytogenetic risk category (HR poor/very poor compared to intermediate/good/very good 1.82, 95% CI 1.15–2.88, $p = 0.005$) and BM blast HR (≥20% vs <20% 1.60, 95% CI 1.01–2.54, $p = 0.01$). Age did not retain significance for NRM on MVA. There was no evidence that the hazard of death

differed according to the level of BM blast at allo-HCT in patients receiving MAC, RIC or Seq (test for interaction in the analysis of OS, RFS, relapse and NRM $p = 0.81$, $p = 0.47$, $p = 0.41$ and $p = 0.75$, respectively). The cause specific hazard of cGVHD was significantly lower when Seq was received [HR vs. RIC 0.66 (95% CI 0.47–0.94) $p = 0.02$)] and in turn, significantly higher when MAC was received [HR vs. RIC 1.84 95% CI, 1.15–2.923, $p = 0.01$]. No other variables in the model were significantly associated with the hazard of cGvHD. When the analysis was restricted to the 182 patients who received a T-cell depleted graft, we did not observe any significant differences in hazard of OS and RFS and cause specific hazard of relapse and NRM between patients receiving RIC or Seq. The cause specific hazard of cGVHD remained lower when Seq was received albeit not significantly [HR vs. RIC 0.71 (95% CI 0.47–1.06, $p = 0.12$)]. On MVA for GFRS this was improved for Seq compared to RIC [HR 0.70 (95% CI 0.52–0.94, $p = 0.02$)]. When the MVA for GFRS is restricted to the T-cell deplete population the effect of conditioning no longer retains significance (Supplementary Table 1).

Using the estimates from Table 2, we predicted survival for reference MDS patients receiving either a MAC, Seq or RIC conditioning protocol (Fig. 4). These reference patients were further assumed to be a 60-year old man at time of the allo-HCT, to have had an intermediate, good or very good cytogenetic risk score at diagnosis, to have less than 20% blasts in bone marrow and a KPS of 90 or 100. The allo-HCT was assumed to be 8 months after diagnosis with a graft obtained from an unrelated matched (8/8) donor. The predicted survival probability after five years after allo-HCT for a reference patient was highest with a MAC conditioning protocol (74%, 95% CI 62–88%), followed by Seq (67%, 95% CI 57–78%) and RIC (62%, 95% CI 52–73%). Details of different conditioning protocols that make up the MAC, RIC and Seq categories are included in Supplementary Table 2.

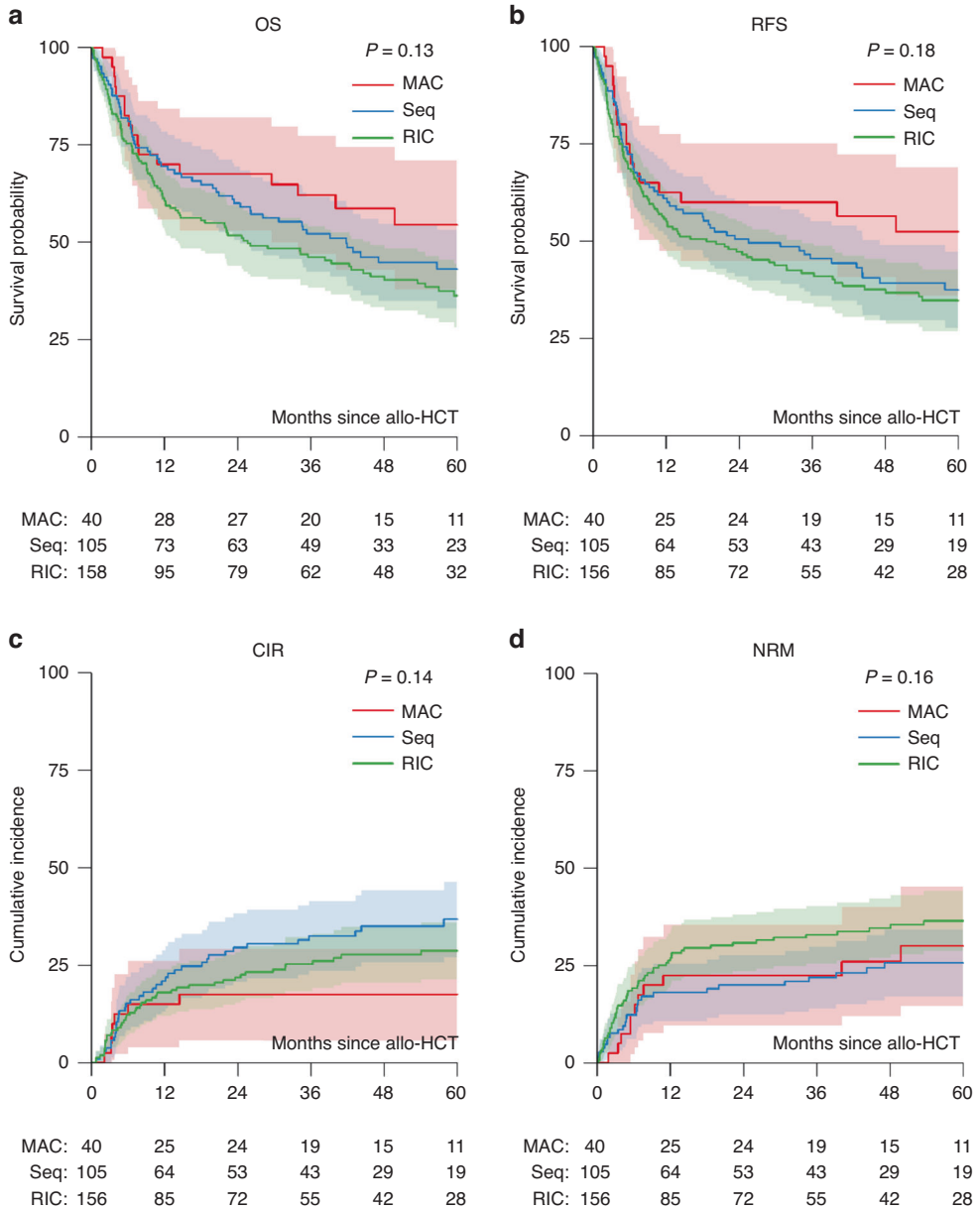

Fig. 1 OS, RFS, CIR and NRM for MAC, Seq and RIC protocols. a Probability of overall survival (OS), (**b**) probability of relapse free survival (RFS), (**c**) cumulative incidence of relapse (CIR) and (**d**) non-relapse mortality (NRM) per conditioning protocol in MDS patients after allo-HCT. Numbers below the graph indicate the number of patients at risk. Seq Sequential conditioning, RIC reduced intensity conditioning, MAC myeloablative conditioning.

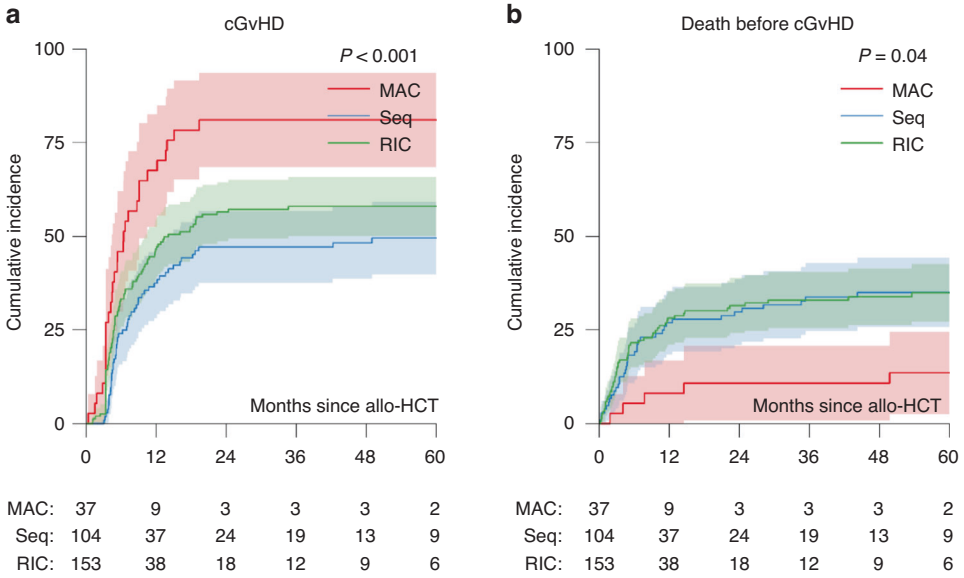

Fig. 2 Chronic GVHD and mortality not related to GVHD for MAC, Seq and RIC protocols. Cumulative incidence of (**a**) chronic GvHD (cGvHD) and (**b**) non-cGvHD mortality per conditioning protocol in MDS patients after allo-HCT. Numbers below the graph indicate the number of patients at risk. Seq Sequential conditioning, RIC reduced intensity conditioning, MAC myeloablative conditioning.

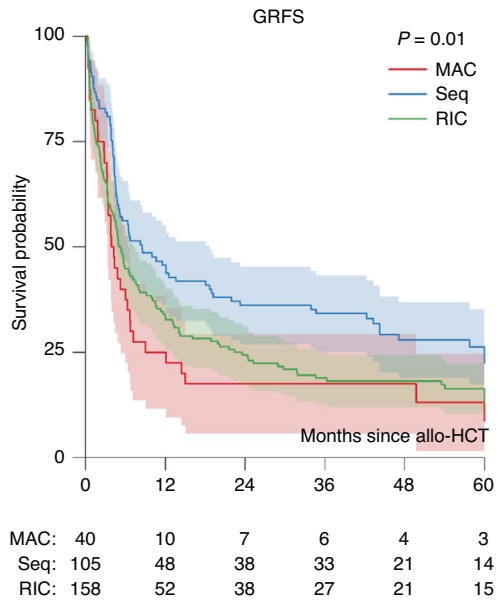

Fig. 3 GVHD free, relapse free survival per conditioning protocol in MDS patients after allo-HCT. Numbers below the graph indicate the number of patients at risk. Seq sequential conditioning, RIC reduced intensity conditioning, MAC myeloablative conditioning.

DISCUSSION

Herein, we present the largest analysis presented to date comparing the results of sequential based conditioning for MDS patients with an excess of blasts at the time of allo-HCT compared to both RIC and MAC approaches. Overall outcomes were acceptable for the entire high-risk cohort with a 3-year OS of 50%, and for those patients able to receive MAC protocols there was an estimated 3-year OS of 62%. Within our cohort, a group of patients were able to be identified with better outcomes post allo-HCT; these include patients with a KPS ≥ 90, the presence of 'Int, good or very good' cytogenetics as classified by IPSS-R, use of an unrelated donor and a blast percentage <20% at the time of allo-HCT. Despite this we demonstrate a lack of superiority for any particular conditioning approach for MDS patients with blasts >5% at the time of allo-HCT with regard to OS, with outcomes more dependent on patient- and disease related factors such as KPS and cytogenetic risk category.

Regarding the use of Seq conditioning in MDS, initially a small number of retrospective studies had been published [3, 4]. Schmid et al. were able to demonstrate an OS of 42% at 2 years in a cohort of high risk MDS and AML patients [4]. Subsequent studies have demonstrated high rates of survival when this approach is used up-front in patients with MDS without the need for prior chemotherapy. Suare et al. demonstrated a 2-year OS of 70% with an estimated event free survival (EFS) of 63% in this situation [3].

Two large prospective studies comparing RIC and MAC regimens did not include the Seq approach [10, 11]. In these, MAC approaches appeared to decrease the risk of relapse; it is notable that one of these studies closed early due to worse outcomes in terms of relapse in the high-risk group and the other study had difficulties in terms of recruitment, potentially somewhat limiting the conclusions that can be drawn from these cohorts. Only one study has prospectively compared the results of Seq versus other conditioning approaches. The FIGARO study analysed the results of the FLAMSA Seq regimen compared to a RIC regimen for patients with high-risk MDS ($n = 80$) and AML ($n = 164$) [12]. Similar to our analysis, FIGARO also demonstrated no advantage for the Seq conditioning approach. Although the patient group was different to this study, with the majority of patients having AML as opposed to MDS, the prospective nature of this study adds weight to the conclusions drawn. A particularly important finding of FIGARO was the observation that positive pre-transplant Minimal Residual Disease (MRD) measured by flow cytometry independently predicted inferior outcomes and that full donor chimerism at three months abrogated the adverse impact of positive pre-transplant MRD. Hourgian et al. has additionally been able to demonstrate an interaction with conditioning intensity and the presence of MRD detected by next-generation sequencing, showing that MAC was superior to RIC in those with genomic evidence of MRD prior to allo-HCT [13]. In the population studied for this analysis the question of MRD is not relevant as all patients had excess of blasts. Furthermore the retrospective nature of this study did not allow us to fully interrogate relationships between level of disease control and underlying

Table 2. Results from multivariable Cox proportional hazards regression on overall survival (OS, $n = 302$), relapse free survival (RFS, $n = 300$), relapse ($n = 300$), non-relapse mortality (NRM, $n = 300$) and chronic graft-versus-host-disease (cGvHD, $n = 293$).

	OS		RFS		Relapse		NRM		cGVHD	
	HR (95% CI)	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Conditioning regimen										
RIC	1.00	(0.22[a])	1.00	(0.28[a])	1.00	(0.11[a])	1.00	(0.24[a])	1.00	(0.0002[a])
MAC	0.66 (0.39–1.10)	0.11	0.65 (0.38–1.10)	0.11	0.54 (0.23–1.23)	0.14	0.76 (0.38–1.52)	0.57	1.84 (1.15-2.93)	0.01
Seq	0.83 (0.60–1.16)	0.28	0.92 (0.66–1.28)	0.63	1.27 (0.80–1.99)	0.31	0.66 (0.41–1.08)	0.10	0.66 (0.47–0.94)	0.02
Age at allo-HCT (per 10 years older)	1.11 (0.94–1.31)	0.20	1.01 (0.86–1.19)	0.93	0.97 (0.78–1.22)	0.81	1.04 (0.83–1.32)	0.73	1.05 (0.89–1.23)	0.58
Karnofsky score at allo-HCT										
≥90	1.00		1.00		1.00		1.00		1.00	
<90	1.51 (1.10–2.08)	0.01	1.54 (1.12–2.11)	0.008	1.34 (0.84–2.12)	0.21	1.74 (1.12–2.70)	0.01	0.90 (0.63–1.29)	0.56
IPSS-R cytogenetic score										
Intermediate/good/very good	1.00		1.00		1.00		1.00		1.00	
Poor/very poor	2.13 (1.55–2.94)	<0.0001	1.96 (1.42–2.71)	<0.0001	2.12 (1.34–3.34)	0.001	1.82 (1.15–2.88)	0.01	1.06 (0.75–1.50)	0.76
Donor type										
HLA-Identical sibling	1.00		1.00		1.00		1.00		1.00	
Matched (8/8) unrelated	0.71 (0.52–0.97)	0.03	0.69 (0.51–0.95)	0.02	0.57 (0.36–0.88)	0.01	0.84 (0.54–1.30)	0.43	1.29 (0.93–0.78)	0.12
Interval diagnosis - allo-HCT (per year longer)	1.06 (0.98–1.15)	0.15	1.10 (1.01–1.20)	0.02	1.09 (0.95–1.24)	0.21	1.12 (0.99–1.25)	0.06	0.94 (0.86–1.04)	0.22
BM blast % at allo-HCT										
<20%	1.00		1.00		1.00		1.00		1.00	
≥20%	1.55 (1.11–2.18)	0.01	1.39 (0.99–1.95)	0.06	1.16 (0.70–1.94)	0.56	1.60 (1.01–2.54)	0.05	1.10 (0.73–1.64)	0.66
Source										
BM									1.00	
PB									1.83 (0.92–3.62)	0.08

Cause specific hazard ratios are shown for relapse, NRM and cGvHD.
HCT hematopoietic cell transplantation, *RIC* reduced intensity conditioning, *MAC* myeloablative conditioning, Seq, *IPSS-R* international prognostic scoring system – revised, *BM* bone marrow, *PB* peripheral blood, *HR* hazard ratio, *CI* confidence interval.
[a]ANOVA Wald *p*-value.

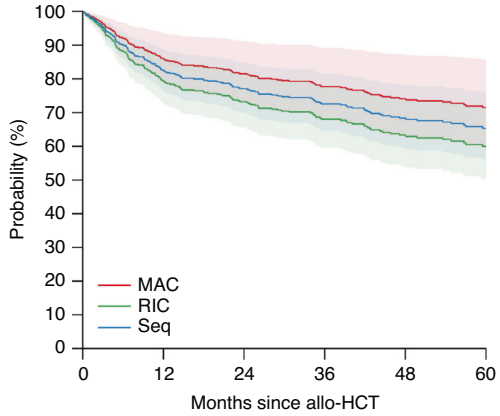

Fig. 4 Predicted survival probabilities based on a Cox model during the first 5 years after allo-HCT for a reference patient receiving either MAC, RIC or Seq conditioning. The reference patient is a male MDS patient, with an intermediate, good or very good cytogenetic risk score at diagnosis, 60 years of age, <20% blasts in bone marrow and KPS 90–100 at allo-HCT, 8 months after diagnosis, and an unrelated, matched (8/8) donor. Probabilities are calculated using the results from the Cox model for OS presented in Table 2. The shaded areas show the 95% confidence intervals.

genetic prognostic factors. It will be increasingly important to capture this data in future studies to understand how best to tailor conditioning approaches to individual patient scenarios. Adoptive immunotherapy with donor lymphocyte infusions (DLI) is one mechanism to induce full donor chimerism and planned DLI formed part of the original FLAMSA protocol. Unfortunately, in our analysis, DLI was used variably across protocols and numbers were too small for accurate analysis of effect. Prospective studies addressing the utility of DLI to improve outcomes in high-risk patients are ongoing in this area (PRO-T4, PRO-DLI) with recruitment completed and publication awaited.

In our analysis we were able to demonstrate an adverse effect of 'poor/very poor risk' cytogenetics (as defined by IPSS-R) on overall outcomes raising the additional question in regard to the prognostic value of somatic mutations. Given the registry-based nature of our study, we were not able to analyse the impact of somatic mutations. In a cohort of 62 patients receiving a seq allo-HCT for MDS, Christopeit et al. analysed the impact of somatic mutations and in fact demonstrated that none of the analysed mutations had prognostic impact [14]. This somewhat surprising finding, particularly with regard to *TP53*, differs to that reported in other larger studies documenting outcomes following MAC and RIC and requires further evaluation in the seq conditioned population [15, 16].

Chronic GVHD rates were high, especially for MAC regimens, with lower rates associated with the Seq conditioning approach. Interestingly on MVA we were able to demonstrate superiority of the Seq approach compared to MAC and RIC with respect to GRFS, predominantly a consequence of higher GVHD incidence in MAC protocols. This likely reflects a role of in-vivo T-cell depletion which was applied variably across protocols. Notably the difference was not present when GRFS analysis was restricted to the T-cell deplete population. A recent analysis by Forcade et al. [17] noted that type of TCD was important with both ATG and Alemtuzmab decreasing cGVHD but with an increased risk of relapse for alemtuzumab compared to ATG. It is important to note that the limitations of this analysis do not allow a detailed understanding of factors contributing to GVHD but do highlight this area as potential area to target for improving outcomes. This is important not just for survival outcomes but also quality of life.

In conclusion, we demonstrate no clear OS or RFS advantage of Seq regimens over either MAC or RIC regimens in this large cohort of MDS patients with an excess of blasts at allo-HCT. Patients who are able to receive a MAC with certain pre-defined characteristics tended to have better outcomes; however, this is not an approach that can be applied to the majority of patients due to age or co-morbidities. Performance status as determined by KPS, and disease risk were major determinants of outcome in this analysis, as was the use of an unrelated donor which contributed to improved OS Understanding these factors and personalising transplant approaches are likely required to improve outcomes. Prospective studies that also incorporate strategies to optimise performance status pre-transplant, and target the high risk population with post-transplant maintenance therapies are required to determine the optimal transplant platform.

DATA AVAILABILITY

The final analysis dataset will be available upon specific request to the working party chair.

REFERENCES

1. Garcia-Manero G, Chien KS, Montalban-Bravo G. Myelodysplastic syndromes: 2021 update on diagnosis, risk stratification and management. Am J Hematol. 2020;95:1399–420.
2. Kröger N. Induction, bridging, or straight ahead: the ongoing dilemma of allografting in advanced myelodysplastic syndrome. Biol Blood Marrow Transpl. 2019;25:e247–9.
3. Saure C, Schroeder T, Zohren F, Groten A, Bruns I, Czibere A, et al. Upfront allogeneic blood stem cell transplantation for patients with high-risk myelodysplastic syndrome or secondary acute myeloid leukemia using a FLAMSA-based high-dose sequential conditioning regimen. Biol Blood Marrow Transpl. 2012;18:466–72.
4. Schmid C, Schleuning M, Ledderose G, Tischer J, Kolb H-J. Sequential regimen of chemotherapy, reduced-intensity conditioning for allogeneic stem-cell transplantation, and prophylactic donor lymphocyte transfusion in high-risk acute myeloid leukemia and myelodysplastic syndrome. J Clin Oncol. 2005;23:5675–87.
5. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89:2079–88.
6. Arber DA, Orazi A, Hasserjian R, Borowitz MJ, Beau MM, Bloomfield CD, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–406.
7. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120:2454–65.
8. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transpl J Am Soc Blood Marrow Transpl. 2009;15:1628–33.
9. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials. 1996;17:343–6.
10. Scott BL, Pasquini MC, Logan BR, Wu J, Devine SM, Porter DL, et al. Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. J Clin Oncol. 2017;35:1154–61.
11. Kröger N, Iacobelli S, Franke G-N, Platzbecker U, Uddin R, Hübel K, et al. Dose-reduced versus standard conditioning followed by allogeneic stem-cell transplantation for patients with myelodysplastic syndrome: a prospective randomized Phase III study of the EBMT (RICMAC Trial). J Clin Oncol. 2017;35:2157–64.
12. Craddock C, Jackson A, Loke J, Siddique S, Hodgkinson A, Mason J, et al. Augmented reduced-intensity regimen does not improve postallogeneic transplant outcomes in acute myeloid leukemia. J Clin Oncol. 2021;39:768–78.
13. Hourigan CS, Dillon LW, Gui G, Logan BR, Fei M, Ghannam J, et al. Impact of conditioning intensity of allogeneic transplantation for acute myeloid leukemia with genomic evidence of residual disease. J Clin Oncol. 2020;38:1273–83.
14. Christopeit M, Badbaran A, Alawi M, Zabelina T, Zeck G, Wolschke C, et al. Correlation of somatic mutations with outcome after FLAMSA-busulfan sequential conditioning and allogeneic stem cell transplantation in patients with myelodysplastic syndromes. Eur J Haematol. 2016;97:288–96.
15. Ciurea SO, Chilkulwar A, Saliba RM, Chen J, Rondon G, Patel KP, et al. Prognostic factors influencing survival after allogeneic transplantation for AML/MDS patients with TP53 mutations. Blood. 2018;131:2989–92.
16. Lindsley RC, Saber W, Mar BG, Redd R, Wang T, Haagenson MD, et al. Prognostic mutations in myelodysplastic syndrome after stem-cell transplantation. N. Engl J Med. 2017;376:536–47.

17. Forcade E, Chevret S, Finke J, Ehninger G, Beelen D, et al. Impact of in vivo T-cell depletion in patients with myelodysplastic syndromes undergoing allogeneic hematopoietic stem cell transplant: a registry study from the Chronic Malignancies Working Party of the EBMT. Bone Marrow Transpl. 2022;57:768–74.

AUTHOR CONTRIBUTIONS

VP conceived and designed the study proposal, acquired data, interpreted results, drafted and revised manuscript, and approved final version. MR, DM, IYA conceived and designed the study proposal, interpreted results, revised manuscript, and approved final version. LG, LK, LdW acquired data, completed statistical analysis, interpreted results and approved final version. NK, KS, AG, JF, HLW, RPdL, YK, US, LSF, PJ, TS, JT, MA, MPC, PH, KR, JDS, CS acquired data, interpreted results and approved final version.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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