Complete remission with incomplete count recovery (CRi) prior to allogeneic HCT for acute myeloid leukaemia is associated with a high non-relapse mortality.

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Introduction

Consolidation of chemotherapy with allogeneic haematopoietic cell transplantation (HCT) improves outcome in a large proportion of patients with acute myeloid leukaemia (AML) by reducing relapse risk and improving survival (1). Disease response is a powerful predictor of outcome, and is incorporated into standard risk stratification models such as the EBMT score (2). However, it is becoming increasingly clear that the group of patients categorised as complete remission (CR) encompasses a heterogenous population. The European LeukemiaNET (ELN)-2017 response criteria recognise this, and permits further sub-classification of CR patients into CR_{MDR-} for those with undetectable measurable residual disease (MRD), as well as CRi for those in remission but with incomplete hematologic recovery (3). The impact of MRD in AML is established (5,6) and its impact on HCT outcome has recently been shown (7,8), however the impact of incomplete count recovery is less clear, and unknown in the setting of HCT. We therefore compared the outcomes of patients undergoing HCT for AML in CR to those in CRi and active disease (AD).

Subjects and Methods

Study design and patient population

This single centre retrospective observational study Included all patients undergoing HCT for AML at our institution from January 2005 until December 2017. All patients gave informed consent for data collection and use for research in line with the

declaration of Helsinki. All patients met the WHO criteria for AML (4) and had received induction chemotherapy, either in line with the UK-NCRI AML study protocols or by physician choice of institutionally approved regimens (most commonly 2 cycles of daunorubicin and cytarabine-based regimens with or without consolidation with high dose cytarabine based regimens).

Timing of transplant, donor choice, and conditioning regimen were in line with UK-NCRI AML studies or at the discretion of the treating physician within the scope of institutionally approved protocols.

Definitions

Patients were grouped by disease status immediately prior to transplantation. CR was defined as less than 5% blasts on bone marrow (BM) examination with peripheral platelet count $\geq 100 \times 10^9$/L and neutrophils $\geq 1 \times 10^9$/L. CRi was defined as less than 5% blasts on BM examination with peripheral platelets $< 100 \times 10^9$/L and/or neutrophils $< 1 \times 10^9$. Any other disease status was categorised as active disease (AD).

Measurable residual disease (MRD) positivity was defined as detectable disease by a contemporaneously accepted standard methodology at the time of transplant (e.g. PCR, immunophenotyping or fluorescence in situ hybridization for known disease markers), with all other patients deemed MRD negative. Transplant risk was determined using the standard EBMT risk score (2) and grouped into low-(EBMT score 0-3) or high-(EBMT score 4-7) risk groups. The ELN-2017 guideline definitions of cytogenetic risk categories were used (3)

Statistical analysis and endpoints

The primary endpoint was survival, with non-relapse mortality and relapse risk being secondary endpoints. Probabilities of survival were calculated using the Kaplan-Meier method, with the log-rank test utilised for comparison of groups. Probabilities of non-relapse mortality (NRM) and relapse were calculated using the cumulative incidence procedure, with disease progression being the competing risk for NRM, and NRM the competing risk for relapse. Gray's test was used to compare groups. To adjust the probabilities of survival by disease status in the multivariate setting, a proportional hazards regression analysis was undertaken, detailed in supplemental methods.

Results

Patient characteristics

During the inclusion period 155 patients received an allogeneic HCT for AML. At the time of analysis, 65 patients were alive (41.9%) and the median follow-up of surviving patients was 3.4 years [0.3-12.7 years]. At the time of transplantation, 80 patients (52%) were in CR, 55 (35%) in CRi, and 20 (13%) had AD. Patient and transplant characteristics are provided in Table 1. As expected, AD patients had longer disease duration and higher EBMT risk scores. The AD and CRi groups contained more male patients, and more female-to-male donors. CRi-patients had received fewer cycles of chemotherapy than CR-patients. No other significant differences were identified in patient, disease or transplant features between CR and CRi groups.

Survival and NRM

The 5-year probability of survival for the whole group was 37.1% (95%CI 29.4- 46.8) and was significantly different between the three cohorts: 51.3% (95%CI 40.2-65.4) for CR, 24.4% (95%CI 14.1-42.4) for CRi and 12.7% (95%CI 3.6-45.2) for AD ($p<0.001$) (Fig 1a). Hazard ratios (HR) after adjustment were 2.09 (95%CI 1.32-3.31, $p=0.002$) for CRi and 3.53 (95%CI 1.93-6.46, $p<0.001$) for AD respectively. NRM was significantly higher in the CRi and AD groups compared to the CR group with a cumulative incidence of 6.3%, 23.6% and 35.0% for CR, CRi and AD respectively at 100 days and 26.8%, 46.8% and 48.1% respectively at 5-years ($p<0.001$) (Fig. 1b). While these observed differences were significant in pairwise comparison, between CR and CRi ($p=0.007$) and CR and AD ($p=0.024$)), no such difference was observed between CRi and AD ($p=0.77$).

Relapse risk

While the relapse risk was higher, for those transplanted with active disease (AD), it was not significantly different between the CR and CRi groups (Fig. 1c). Cumulative incidence of relapse at 2 years was 20.6% for those in CR, 19.3% for those in CRi and 36.9% for those transplanted with active disease (CR v CRi $p=0.86$, CR v AD $p=0.08$, CRi v AD $p=0.19$).

Cause of death

Relapse was the leading cause of death for the group as a whole (n=31), followed closely by infection (n=30) (supplemental table 1). Notably the cumulative incidence of death from infection was significantly higher in CRi- compared to CR-patients (23.8% v 10.4% at 1 year and 32.6% v 18.5% at 3 years, p=0.035) (supplemental figure 1).

Engraftment and graft-versus-host disease

8 patients died before engraftment, 1 in CR group, 6 in the CRi group and 1 in the AD group. Time to engraftment was not significantly different for the three groups (18 [10-32], 21 [11-47] and 21 [14-34] days for CR, CRi and AD respectively). The rates of grade II-IV acute graft-versus-host disease (GvHD) were equal between the groups (29%, 28% and 22% for CR, CRi and AD respectively, p=0.78), as were chronic extensive GvHD (27%, 16% and 28% for CR, CRi and AD respectively p=0.27).

Impact of CRi in MRD negative patients

Patients with MRD (n=28) had a higher incidence of relapse compared to MRD negative patients (N=107) (two-year CI 34.5% vs 16.2% p=0.009), but there were no differences in the number of MRD positive patients between the CR and CRi groups (20% v 22%, p=0.80). In order to investigate the effect of incomplete count recovery in patients with lowest risk of relapse, we performed sub-group analyses on MRD negative patients. Within this sub-group, CRi-patients (N=43) had no increased risk of relapse compared to CR-patients (n=64) (2-year CI 15.4% vs 16.9% p=0.90), but had significantly worse overall survival (5-year overall survival of 26.1% vs 52.7%,

p=0.002) resulting from significantly higher NRM (25.6% vs 4.7% at 100 days and 52.7% vs 28.8% at 5 years p=0.005) (Fig. 1d).

Discussion

Response to induction chemotherapy is a powerful predictor of outcome in AML (1,8). However, often, composite CR end-points that combine both CR and CRi, frequently used in clinical trials, are transcribed into routine clinical practice and risk stratification models for HCT (9). The changes in response criteria in the ELN-2017 guidelines reflects the better understanding of the heterogeneous group of patients encompassed by defining all patients with less than 5% BM blasts as CR without considering additional factors such as MRD or count recovery. While our classification assesses disease status immediately prior to HCT, rather than directly after induction treatment, our data shows that patients undergoing HCT in complete remission but with incomplete count recovery have a poorer survival resulting from increased NRM rather than increased relapse risk, than patients transplanted in CR with count recovery. The observation that the increased NRM and poorer survival associated with incomplete count recovery persists in MRD negative patients suggests that incomplete count recovery independently predicts worse survival even in good risk patients.

With the development of increasingly sensitive MRD detection techniques, we are progressively able to better predict relapse risk post-transplant (7,8). While our finding should be validated in an independent dataset, these data suggests that

combining count recovery status with MRD data may offer the best strategy to predict survival by combining risks of relapse and NRM. Failing do so therefore overlooks valuable prognostic information.

Perhaps these data should also add a note of caution to the prospective interpretations of MRD results in AML patients that are planned to undergo HCT. While retrospective data suggests patients who are MRD positive prior to HCT fair as poorly as those with active disease (7,8) the question of depth of remission prior to HCT should be achieved remains unanswered. Perhaps MRD negativity prior to HCT should not necessarily be pursued at all cost, particularly if that risks marrow toxicity and incomplete count recovery.

Finally, the high NRM seen in those with CRi should focus attention on optimising supportive care in these patients. While, all patient in this study received a standard approach to infection prophylaxis, these data would suggest that perhaps CRi-patients need more aggressive infection prophylaxis.

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Authors contributions

AJI, JP and RS conceived, designed, performed the research and wrote the manuscript. PW, SL, FF and DB collected and collated data, and AJI and RS performed statistical analysis. PM, ENM and EYF performed MRD analysis. RP, DM, EO and JFA provided guidance on the research strategy. All authors reviewed and edited the manuscript.

Conflict of Interest Disclosures

The authors have no competing financial interests to disclose.

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232 **Legends**

233

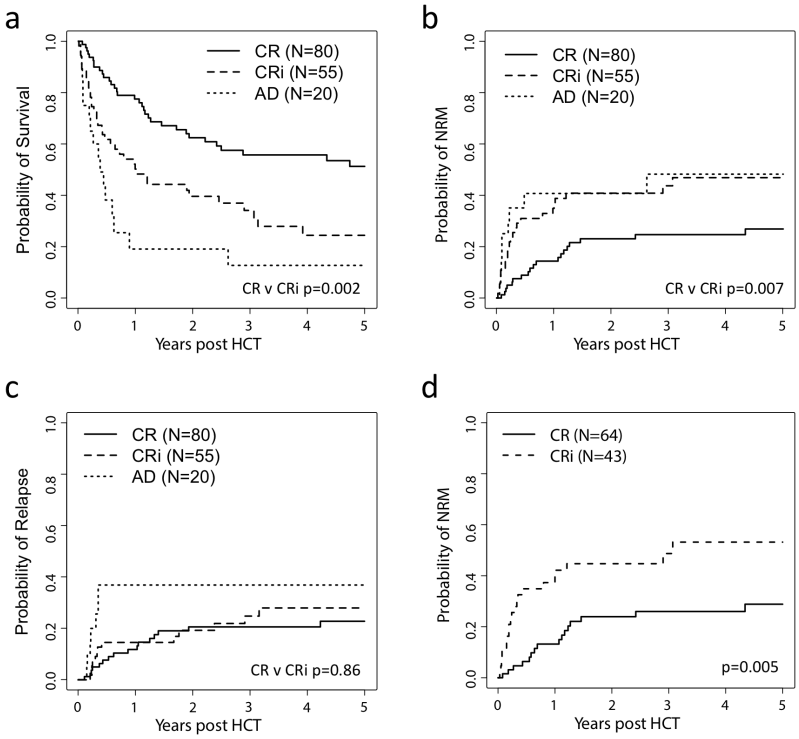
234 Table 1 – Patient demographics

235

236 Figure 1 - Outcome stratified by pre-transplant disease status a) probability of
237 survival, b) cumulative incidence of non-relapse mortality, c) cumulative incidence of
238 relapse d) cumulative incidence of non-relapse mortality in patients without
239 detectable measurable residual disease (MRD).

		Total (N=155)	CR (N=80)	CRi (N=55)	AD (N=20)	P-value (3- groups)	P-value (CR vs CRi)
Patient factors							
Age, y (median, range)		51.0 (21.0-72.8)	50.7 (21.0-69.6)	56.6 (27.7-72.8)	50.1 (21.8-66.3)	0.09	0.15
Patient sex (N,%)	Male	86 (56%)	36 (45%)	35 (64%)	15(75%)	0.017	0.033
	Female	69 (44%)	44 (55%)	20 (36%)	5 (25%)		
Disease factors							
Disease status at transplant (N,%)	CR1	108 (70%)	65 (81%)	43 (78%)	0 (0%)	<0.001	0.66
	CR2	27 (17%)	15 (19%)	12 (22%)	0 (0%)		
	Higher	20 (13%)	0 (0%)	0 (0%)	20 (100%)		
Secondary AML	Yes	41 (26%)	16 (20%)	17 (31%)	8 (40%)	0.12	0.15
Cytogenetic Risk group (N,%)	Favourable	8 (5%)	5 (6%)	3 (5%)	0 (0%)	0.52	0.97
	Intermediate	98 (63%)	48 (60%)	34 (62%)	16 (80%)		
	Adverse	49 (32%)	27 (34%)	18 (33%)	4 (20%)		
Duration of disease (N,%)	≤12 months	116 (75%)	65 (81%)	42 (76%)	9 (45%)	0.004	0.49
	>12 months	39 (25%)	15 (19%)	13 (24%)	11 (55%)		
MRD Status	MRD +ve	28 (21%)	16 (20%)	12 (22%)	NA		0.80
Cycles of chemotherapy (N,%)	1-2	62 (41%)	26 (33%)	29(53%)	7(44%)	0.022	0.001
	3-4	59 (39%)	41 (51%)	14 (25%)	4 (25%)		
	> 4	30 (20%)	13 (16%)	12 (22%)	5 (31%)		
Transplant factors							
Patient / donor sex (N,%)	M / F	36 (23%)	12 (15%)	17 (31%)	7 (35%)	0.041	0.027
	Other	119 (77%)	68 (85%)	38 (69%)	13 (65%)		
Patient / donor CMV (N,%)	- / +	11 (7%)	5 (6%)	6 (11%)	0 (0%)	0.15	0.17
	Other	144 (93%)	75 (94%)	49 (89%)	20 (100%)		
Conditioning (N,%)	MAC	69 (44%)	38 (48%)	21 (38%)	10 (50%)	0.49	0.28
	RIC	86 (56%)	42 (52%)	34 (62%)	10 (50%)		
Donor type (N,%)	HLA-identical Sibling	60 (39%)	30 (37%)	23 (42%)	7 (35%)	0.069	0.23
	Mismatch-Sibling	6 (4%)	2 (2%)	1 (2%)	3 (15%)		
	HLA-identical VUD	48 (31%)	27 (34%)	16 (29%)	5 (25%)		
	Mismatched VUD	17 (11%)	6 (8%)	10 (18%)	1 (5%)		
	Haploidentical	24 (15%)	15 (19%)	5 (9%)	4 (20%)		
EBMT Risk Group (N,%)	0-3	102 (66%)	65 (79%)	37 (67%)	2 (10%)	<0.001	0.14
	4-7	53 (34%)	17 (21%)	18 (33%)	18 (90%)		
Pre-transplant counts (median, range)	Neutrophils ($x10^9$/L)	2.30 [0-11.0]	2.80 [1.1-11.0]	1.50 [0-8.4]	-		<0.001
	Platelets ($x10^9$/L)	129 [7-461]	174 [111-352]	62 [7-461]	-		<0.001

Table 1 – Patient demographics


a
Probability of Survival
CR (N=80)
CRi (N=55)
AD (N=20)
CR v CRi p=0.002
Years post HCT
b
Probability of NRM
CR (N=80)
CRi (N=55)
AD (N=20)
CR v CRi p=0.007
Years post HCT
c
Probability of Relapse
CR (N=80)
CRi (N=55)
AD (N=20)
CR v CRi p=0.86
Years post HCT
d
Probability of NRM
CR (N=64)
CRi (N=43)
p=0.005
Years post HCT