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Distinct factors determine the kinetics of disease relapse in adults transplanted for acute myeloid leukaemia

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Abstract. Craddock C, Versluis J, Labopin M, Socie G, Huynh A, Deconinck E, Volin L, Milpied N, Bourhis JH, Rambaldi A, Chevallier P, Blaise D, Manz M, Vellenga E, Vekemans M-C, Maertens J, Passweg J, Vyas P, Schmid C, Löwenberg B, Ossenkoppele G, Mohty M, Cornelissen JJ, Nagler A, for the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation and HOVON-SAKK (Queen Elizabeth Hospital, Birmingham, UK; Erasmus University Medical Center Cancer Institute, Rotterdam, The Netherlands; Hospital Saint Antoine; Sorbonne University, Paris; CHU, Toulouse; CHU, Besancon, France; HUCH Comprehensive Cancer Center, Helsinki, Finland; CHU, Nantes; Institute of Cancer, Villejuif, France; University of Milan, Milan, Italy; Centre of Cancer Research, Marseille, France; University Hospital Zurich, Zurich, Switzerland; University Medical Center Groningen, Groningen, The Netherlands; Saint-Luc University, Brussels; University Hospital Gasthuisberg, Leuven, Belgium; University of Basel, Basel, Switzerland; University of Oxford, Oxford, UK; University of Munich, Munich, Germany; University Medical Center, Amsterdam, The Netherlands; University UPMC, Paris, France; Tel Aviv University, Tel Aviv, Israel; ALWP office of the EBMT Hospital Saint Antoine, Paris, France). Distinct factors determine the kinetics of disease relapse in adults transplanted for acute myeloid leukaemia. *J Intern Med* 2018; **283**: 371–379.

Background. Disease recurrence remains the major cause of death in adults with acute myeloid leukaemia (AML) treated using either intensive chemotherapy (IC) or allogenic stem cell transplantation (allo-SCT).

Aims. The timely delivery of maintenance drug or cellular therapies represent emerging strategies with the potential to reduce relapse after both treatment modalities, but whilst the determinants of overall relapse risk have been extensively characterized the factors determining the timing of disease recurrence have not been characterized.

Materials and Methods. We have therefore examined, using a series of sequential landmark analyses, relapse kinetics in a cohort of 2028 patients who received an allo-SCT for AML in CR1 and separately 570 patients treated with IC alone.

Results. In the first 3 months after allo-SCT, the factors associated with an increased risk of relapse included the presence of the *FLT3*-ITD ($P < 0.001$), patient age ($P = 0.012$), time interval from CR1 to transplant ($P < 0.001$) and donor type ($P = 0.03$). Relapse from 3 to 6 months was associated with a higher white cell count at diagnosis ($P = 0.001$), adverse-risk cytogenetics ($P < 0.001$), presence of FLT3-ITD mutation ($P < 0.001$) and time interval to achieve first complete remission ($P = 0.013$). Later relapse was associated with adverse cytogenetics,

mutated NPM1, absence of chronic graft-versus-host disease (GVHD) and the use of *in vivo* T-cell depletion. In patients treated with IC alone, the factors associated with relapse in the first 3 months were adverse-risk cytogenetics ($P < 0.001$) and *FLT*3-ITD status ($P = 0.001$). The factors predicting later relapse were the time interval from diagnosis to CR1 ($P = 0.22$) and time interval from CR1 to IC ($P = 0.012$).

Discussion and Conclusion. Taken together, these data provide novel insights into the biology of disease recurrence after both allo-SCT and IC and have the potential to inform the design of novel maintenance strategies in both clinical settings.

Keywords: Acute myeloid leukaemia, stem cell transplantation, intensive chemotherapy, kinetics, maintenance therapy.

Introduction

Disease relapse remains the major cause of treatment failure in adults with acute myeloid leukaemia (AML) receiving allogeneic stem cell transplantation (allo-SCT) or intensive chemotherapy (IC) delivered with curative intent [1, 2]. Although the factors determining overall relapse risk after both allo-SCT and IC have been well defined [3], it is unknown whether they contribute equally to the risk of early and late relapse or whether these represent distinct biological entities. Such information would inform both our understanding of the biology of disease relapse and the development of novel strategies designed to reduce the risk of relapse.

Allo-SCT remains the most effective curative option in adults with high-risk AML but up to 70% of patients still relapse [3]. Strategies which reduce the risk of disease recurrence are consequently urgently required [4]. Disease biology is an important determinant of overall relapse risk in patients treated with IC, consequent presumably upon genetically mediated chemoresistance [5], but whether distinct molecular or cellular factors determine relapse kinetics is not known. In patients allografted for AML, the potential mechanisms contributing to disease relapse and its kinetics are more complex. A number of distinct biological mechanisms have the potential to mediate disease recurrence after allo-SCT which can be broadly categorized as resistance of host malignant hematopoiesis to components of the conditioning regimen or the abrogation of a graft-versus-leukaemia (GVL) effect [6]. Disease- and transplant-specific factors, such as presentation karyotype and conditioning regimen intensity, respectively, have previously been shown to predict overall relapse risk after allo-SCT, but their impact on relapse kinetics has not been studied and the detailed mechanism by which they contribute to disease recurrence remains poorly understood [7,8]. Consequently, detailed characterization of the contribution of disease- and transplant-specific factors to the kinetics of disease relapse may provide additional insights into the biology of both early and late disease relapse after both allo-SCT and IC.

Scheduled administration of cellular interventions, such as donor lymphocyte infusions (DLI), or pharmacological therapies in the early post-transplant period represents one of the most promising novel approaches with the potential to reduce the risk of disease relapse after allo-SCT [9]. Similarly, administration of both pharmacological and immunotherapeutic maintenance therapies in patients treated with IC alone represents promising new treatment strategies and will plausibly be informed by a more detailed understanding of the factors determining the kinetics of relapse in this setting. We now report the first systematic study of factors determining the kinetics of disease relapse in patients with AML whose definitive therapy consisted either of allo-SCT or IC.

Patients and methods

Registries

This was a retrospective multicentre analysis. Data were provided and approved for this study by both the Acute Leukaemia Working Party (ALWP) of the EBMT and the HOVON-SAKK cooperative study group. The EBMT is a voluntary working group of more than 500 transplant centres that are required to report outcomes on all consecutive stem cell transplantations they perform and the HOVON_-SAKK a Dutch-Belgian-Swiss cooperative study group performing trials for adult patients with haematological malignancies. The study protocols were approved by the institutional review board at each site and complied with country-specific requirements. Long-term follow-up data from both the ALWP and HOVON-SAKK are reported on an annual basis, and audits are years routinely performed to determine the accuracy of the reported

data. The study was conducted in accordance with the declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent authorizing the use of their personal information for research purposes.

Patients

Using the EBMT Registry we identified 20 341 adult patients (age ≥ 18) with *de novo* non-APML AML in first complete remission (CR1) who underwent transplantation between 2000 and 2015, using bone marrow or GCSF mobilized peripheral blood stem cells from HLA-matched sibling or unrelated donors using either a myeloablative conditioning (MAC) or reduced intensity (RIC) regimen based on published criteria [10]. Cytogenetic data were available from a diagnostic bone marrow aspirate in 9218 patients permitting risk stratification according to MRC criteria [11]. Of these, information concerning the number of courses of induction chemotherapy was available in 6191 patients. Of this cohort, mutational analysis of the *NPM1* gene and information concerning the presence or absence of a *FLT*3-ITD was available in 2028 patients who are reported in this analysis (Table 1). The median age of the allo-SCT cohort was 51 (18–77) years. The white cell count (WBC) at diagnosis was 12.4 × 10^9/L. Eighty-five per cent of patients had good-/intermediate-risk cytogenetics at diagnosis. The interval from diagnosis to transplant was 151 (43–731) days. The interval from time of acquisition of CR to transplant was 98 (11–357) days. Of note, the time from diagnosis to acquisition of CR1 was correlated with the number of courses of induction chemotherapy delivered. Eight hundred and eighty-six patients were transplanted using a sibling donor and 1142 from an adult-matched unrelated donor (Table 1). One thousand and forty-one patients were transplanted using a myeloablative conditioning (MAC) and 987 a reduced intensity conditioning (RIC) regimen. Four hundred and thirty-eight patients received TBI as component of their conditioning regimen: 216 in the context of a MAC regimen and 222 as part of a RIC regimen. The commonest MAC regimens utilized were a myeloablative combination of busulphan (Bu) and cyclophosphamide (Cy) (n = 439), Bu and fludarabine (Flu) (n = 270) or Cy and TBI (n = 210). The most frequently utilized RIC regimens were a combination of Flu and BU (n = 535), Flu and TBI (n = 203), Flu and Melphalan (n = 101). One thousand one hundred and seventy-six patients received *in vivo* GVHD prophylaxis utilizing anti-thymocyte globulin (ATG) (n = 1093) or alemtuzumab (n = 83). No patients received *in vitro* T-cell depletion. Disease relapse was diagnosed using conventional morphological criteria. Pretransplant measurable disease (MRD) data were not available.

In a separate analysis, factors determining the kinetics of relapse were studied in a comparable cohort of adults treated on the HOVON-SAKK prospective clinical trials AML29, AML42, AML43, AML81 and AML92 which accrued patients during the time period 2000-2010**.** Postremission treatment was applied according to a risk-adapted strategy in the HOVON-SAKK studies: (i) patients with AML classified as favourable risk, according to cytogenetic and molecular analysis, were planned for a third cycle of chemotherapy; (ii) intermediate-risk patients were preferentially treated by allo-SCT using a human leucocyte antigen (HLA)-matched sibling donor or a fully HLA-matched unrelated donor if available; (iii) patients with adverse-risk AML proceeded to allo-SCT using either a sibling donor, unrelated donor, or cord blood grafts; (iv) patients alternatively received an auto-SCT or a third cycle of chemotherapy if no suitable donor was available.

A total of 570 adults who achieved remission (CR1) after induction chemotherapy whose subsequent treatment consisted of IC consolidation in the form of mitoxantrone 10 mg m^{-2} for 5 days and etoposide 100 mg m^{-2} for 5 days were included in this study (Table 2). The median age of patients in this cohort was 47 (16–77) years. The WBC at diagnosis was 12 × 10^9/L. Seventy-eight per cent of patients had good-/intermediate-risk cytogenetics at diagnosis. The median time from diagnosis to acquisition of CR1 was 35 days (19–140 days), and the median time from acquisition of CR to the commencement of IC was 59 days (0–370). Patients treated on HOVON studies after 2010 were not included because of a change in the intensive chemotherapy schedule after this date.

Statistical methods

A series of landmark analyses were performed at 3, 6 and 12 months post-transplant in order to identify prognostic factors of relapse for patients alive and well at the beginning of each time interval. The probabilities of relapse were calculated using the cumulative incidence estimator to accommodate for death as a competing risk. Factors predicting

Table 1 *(a) Characteristics of 1057 patients undergoing allo-SCT. (b) Transplant characteristics of allo-SCT patients*

	Allo-SCT (N = 2028)	
(a)		
Sex		
Male	1042	51%
Female	984	49%
Age (years)		
Median	51	
Range	18–77	
WBC at diagnosis		
Median	12.4	
Range	0.1–780	
Year of chemotherapy		
Median	2012	
Range	2000–2015	
Cytogenetics		
Good	41	2%
Intermediate	1679	83%
Adverse	308	15%
NPM1-FLT3-ITD		
Pos/Neg	153	8%
Pos/Pos	536	26%
Neg/Pos	278	14%
Neg/Neg	1061	52%
Time from CR to PRT (days)		
Median	98	
Range	11–357	
(b)		
Donor		
HLA-identical Sib	886	44%
MUD	1142	56%
Female donor to male recipient	327	16%
Conditioning		
MAC	1041	51%
RIC	987	49%
TBI		
Yes	438	22%
No	1590	78%
Stem cell source		
BM	418	21%
PB	1610	79%
CMV donor/recipient		
Pos/Neg	218	11%
Pos/Pos	738	37%
Neg/Pos	507	25%
Neg/Neg	547	27%
In vivo T-cell depletion		
No	847	42%
Yes	1176	58%

relapse were studied using Cox regression model including time-dependent variables. The variables included in the regression analysis of the transplant cohort were age, WBC at diagnosis, time from diagnosis to CR, time from CR to transplant, female donor to male recipient, donor type, CMV status of patient and donor, conditioning regimen, NPM1 and FLT3-ITD mutation status, adverse-risk cytogenetics, *in vivo* T-cell depletion, stem cell source, previous acute GVHD grade II-IV and previous chronic GVHD. The variables included in regression analysis of the IC cohort were age, sex, WBC at diagnosis, adverse-risk cytogenetics, FLT3-ITD, NPM1, number of induction cycles to CR, year of chemotherapy, time from diagnosis to CR and time from CR to chemotherapy. A backward stepwise procedure was used for variable selection with a P-value of <0.05. The purpose of this study was to identify prognostic factors influencing relapse risk for patients alive at different time-points after allo-SCT or IC. Time post-transplant in smaller intervals or as a continuous parameter could not be studied since the number of events would be too low for analysis. In the transplant population, chronic GVHD was studied as a fixed variable in landmark analyses and only taken into account if it was documented prior to the specific landmark under examination.

Results

Relapse incidence according to time in patients with newly diagnosed AML treated with allo-SCT

With a median follow-up of 36 months, 519 (26%) of the 2028 informative patients relapsed after allo-SCT resulting in a 3-year cumulative incidence of relapse (CIR) of 26% [95% CI: 24–28]. The corresponding estimated 3-year cumulative incidence of

Table 2 *Characteristics of 570 patients treated with intensive chemotherapy alone*

	Chemotherapy (N = 570)	
Sex		
Male	296	52%
Female	274	48%
Age (years)		
Median	47	
IQ Range	16–77	
WBC at diagnosis		
Median	12	
IQ Range	0.3–510	
Year of chemotherapy		
Median	2004	
IQ Range	2000–2010	
Cytogenetics		
Good	112	18%
Intermediate	340	60%
Adverse	91	16%
Missing	27	5%
NPM1-FLT3-ITD		
Pos/Neg	54	9%
Pos/Pos	48	8%
Neg/Pos	25	4%
Neg/Neg	184	32%
Missing	259	45%
Time from diagnosis to CR (days)		
Median	35	
IQ range	19–140	
Time from CR to PRT (days)		
Median	59	
IQ range	0–370	

nonrelapse mortality was 15% resulting in a 59% 3-year probability of leukaemia free survival. The CIR in the first 3 months post-transplant was 7.0% (95% CI: 5.8–8.0%), 7.8% (95% CI: 6.6–9.1%) between 3 and 6 months, 7.4% (95% CI: 6.2–8.8%) between 6 and 12 months and 9.7% (95% CI: 8–11.6) beyond 12 months, respectively. Overall 73.7% of patients destined to relapse did so within the first year post-transplant.

Factors predicting relapse risk according to time after allogeneic SCT

The overall factors predicting disease relapse for the whole population were the presence of a *FLT*3-ITD mutation at diagnosis ($P < 0.001$), the absence of an NPM1 mutation ($P < 0.001$), adverse-risk cytogenetics at diagnosis ($P < 0.001$), time from acquisition of CR1 to transplant ($P < 0.001$), a higher WBC at diagnosis ($P = 0.005$), age at transplant ($P = 0.02$) and chronic GVHD studied as a time-dependent variable ($P = 0.001$). Of note conditioning regimen intensity as not correlated with relapse risk in the studied population.

Using landmark analyses, the factors determining the relapse risk within the first 3 months post-transplant were patient age ($P = 0.012$), interval from CR1 to transplant ($P < 0.001$), the presence of a *FLT3*-ITD mutation at diagnosis ($P < 0.001$) and donor type ($P = 0.033$) with a lower risk of relapse noted in recipients of a MUD (Table 3). In allo-SCT recipients who relapsed between 3 and 6 months post-transplant, factors associated with relapse were higher WBC at diagnosis ($P = 0.001$), adverse-risk cytogenetics ($P < 0.001$), the presence of a *FLT3*-ITD mutation at diagnosis ($P < 0.001$) and the time interval from diagnosis to acquisition of CR1 ($P = 0.013$). The risk of relapse 6–12 months after transplant was associated with adverse cytogenetics ($P = 0.003$), the absence of an NPM1 mutation ($P = 0.013$) and the absence of chronic graft-versus-host disease (GVHD) ($P < 0.001$). Finally, a longer time from CR1 acquisition to transplant ($P = 0.016$), the absence of an *NPM1* mutation ($P = 0.018$), adverse cytogenetics ($P = 0.002$), the use of *in vivo* TCD ($P = 0.037$) and the absence of GVHD ($P = 0.037$) predicted for relapse risk for relapse more than 12 months post-transplant. Of interest when the analysis is restricted to the 987 patients transplanted using a RIC regimen many of the factors determining disease relapse remain the same but distinct factors emerge in this population. Thus, the factors determining relapse within the first 3 months after a RIC allograft were time from CR1 to transplant ($P = 0.02$), the utilization of *in vivo* T-cell depletion ($P = 0.013$), higher WBC at diagnosis ($P = 0.005$), the presence of a *FLT3*-ITD mutation at diagnosis ($P = 0.008$) and the absence of an NPM1 mutation ($P = 0.02$). For patients relapsing 6–12 months post-transplant, the factors were the absence of chronic GVHD before 6 months post-transplant ($P < 0.001$) and the absence of an NPM1 mutation ($P = 0.03$). Finally, the use of *in vivo* T-cell depletion was associated with a trend for a higher relapse rate after 12 months ($P = 0.09$).

Table 3 *Factors determining kinetics of disease relapse after allo-SCT and IC*

	P-value	HR	95% CI
Allo-SCT			
1. Factors influencing relapse within 3 months			
FLT3-ITD	<0.001	2.19	1.56–3.07
Age (per decade)	0.012	1.19	1.04–1.37
Time interval CR1 to transplant (months)	<0.001	0.79	0.70–0.88
Unrelated donor	0.033	0.69	0.49–0.97
2. Factors influencing relapse within 3–6 months			
WBC at diagnosis (per 10)	*0.001*	*1.02*	*1.01–1.04*
Adverse-risk cytogenetics	<0.001	2.47	1.73–3.51
FLT3-ITD	<0.001	1.85	1.35–2.53
Time interval diagnosis to CR1 (months)	0.013	1.15	1.03–1.29
3. Factors influencing relapse within 6–12 months			
cGVHD before 6 months	<0.001	0.29	0.19–0.46
Mutated NPM1	0.013	0.53	0.33–0.87
Adverse-risk cytogenetics	0.003	1.97	1.26–3.07
4. Factors influencing relapse after 12 months			
Time interval CR1 to transplant (months)	0.016	0.88	0.79–0.98
Adverse-risk cytogenetics	0.002	1.94	1.27–2.97
cGVHD before 12 months	0.019	0.66	0.46–0.93
Mutated NPM1	0.018	0.62	0.41–0.92
In vivo TCD	0.037	1.48	1.03–2.15
Chemotherapy			
1. Factors influencing relapse within 3 months			
Adverse-risk cytogenetics	<0.001	3.90	2.16–10.75
FLT3-ITD	0.001	4.82	1.76–8.68
Age (per decade)	0.059	1.43	0.99–2.07
WBC at diagnosis (per 10)	0.063	1.03	1.00–1.07
2. Factors influencing relapse within 3–6 months			
FLT3-ITD	<0.001	3.69	1.90–7.19
Adverse-risk cytogenetics	<0.001	3.29	1.78–6.08
Mutated *NPM1*	0.084	0.52	0.24–1.09
3. Factors influencing relapse within 6–12 months			
Time interval diagnosis to CR1 (months)	0.022	0.38	0.17–0.87
Time interval CR1 to chemo (months)	0.012	0.54	0.33–0.87

Italicised text refers to gene mutations studied.

Relapse incidence according to time in patients with newly diagnosed AML treated with IC

In patients treated with IC alone, a total of 302 (53%) patients relapsed with a median follow-of 86 months. The CIR at 3 years was 54% [95% CI: 50–58). Two hundred and twelve (80%) patients relapsed within the first year after completion of IC. Factors significantly associated with relapse in the first 3 months after chemotherapy were adverse-risk cytogenetics ($P < 0.001$) and the presence of a *FLT3*-ITD mutation at diagnosis ($P = 0.001$) which were also the factors that predicted for relapse within 3 and 6 months (both $P < 0.001$). The time interval from diagnosis to CR1 and from CR1 to consolidation ($P = 0.012$) was inversely associated

with relapse between six and twelve months post-treatment.

Discussion

This analysis demonstrates that distinct leukaemia- and transplant-specific factors contribute to the risk of early and late relapse post-transplant. Notably, the clinical and genetic attributes of the leukaemia which are associated with an increased risk of early relapse post-transplant differ from those correlated with later relapse and are similar to those which predict the kinetics of relapse in patients treated with chemotherapy alone. It also appears that factors previously associated with an increased risk of relapse post-transplant, such as the absence of chronic GVHD, exert this effect at specific time-points post-transplant. These observations are consistent with disease relapse occurring as a dynamic interplay of tumour- and transplant-associated factors throughout the post-transplant period and identify specific, potentially manipulable contributors to relapse at distinct time-points postallograft.

The biology of disease relapse after allo-SCT remains poorly understood. Our data suggest that the specific characteristics of the leukaemia predispose to early relapse post-transplant, although the underlying biological mechanisms remain speculative. The increased risk of early relapse associated with the presence of an adverse-risk karyotype or *FLT*3-ITD may be consequent on either a higher level of pretransplant MRD [12] or rapid expansion of the tumour cells not eradicated by the conditioning regimen. Another possibility is that the potency of the allo-immune response is modulated by specific disease characteristics and that this contributes to both absolute relapse risk and its timing. In this context, it is of interest that mutations in IDH1 and other leukaemia-associated genes modulate DNA methylation in leukaemic blasts and potentially their ability to be recognized by the donor allo-immune response [13] consistent with this hypothesis. On the other hand, a previous HOVON analysis identified a similar reduction in relapse risk after allo-SCT in different AML risk categories indicating that the GVL effect is similarly exerted in adverse, intermediate and favourable risk AML [7] and determined by differences in minor and major HLA-antigens rather than an interplay between alloreactivity and disease biology. Nevertheless, absolute percentages of relapse are higher in poor-risk AML with the majority of relapses after allo-SCT occurring within the first year after transplantation. The similar determinants of relapse early after chemotherapy and after transplant, highlight the possibility that tumour growth kinetics, determined by specific mutations including the *FLT*3-ITD, may play a centrally important role in the early blunting of a GVL effect by outcompeting the expansion of alloreactive T cells in the immediate post-transplant period. Our data also demonstrate that the timing of relapse is driven by distinct transplant-specific factors. Of interest, the use of an adult unrelated donor is associated with a decreased risk of disease relapse in the first few months post-transplant consistent with a recent large analysis from the EBMT [14] Similarly, it is striking that the development of chronic GVHD reduces relapse risk within the first year post-transplant. When the analysis was extended to smaller population of patients allografted using a RIC regimen, although statistical power was lost broadly similar disease- and transplant-specific factors determining relapse kinetics were identified, although of interest the utilization of *in vivo* TCD emerged as a significant risk factor in this distinct setting. Of interest, the use of a RIC regimen was not associated with an increased risk of disease relapse which is consistent with two recent prospective randomized trials but at variance with the findings of a recently reported US CTN study [15–17]. Taken together, these data suggest the existence of a complex interaction between leukaemia- and transplant-specific factors in the maintenance of disease remission post-transplant and identify potential manipulable pathways at different stages post-transplant. The retrospective nature of this analysis limits its interpretation, and prospective studies will important to validate the observations we have made and limit selection bias. It is important also to note that the age of the cohort treated with IC in this study is lower than the transplant cohort, although this may be of less relevance given the increasing age at which allogeneic transplants can now be delivered with relative safety [18]. Future studies on larger patient cohorts with more detailed molecular analyses will provide important information concerning whether specific molecular abnormalities predict either relapse risk or its kinetics. Specifically, the prospective incorporation of pretransplant MRD assessment will provide important information concerning the relative importance of the contrasting mechanisms of early and late relapse we have identified.

There is increasing recognition that post-transplant pharmacological or cellular intervention may

represent an important novel strategy by which the risk of disease relapse is reduced in patients undergoing allo-SCT as well as those treated with chemotherapy alone. Such approaches include both the administration of prophylactic DLI as well as the use of biologically targeted therapies such as *FLT*3 inhibitors or epigenetic therapies such as azacitidine and panobinostat [19–23]. Similarly, in patients treated with intensive chemotherapy alone, there have been encouraging data reported utilizing maintenance strategies employing both azacitidine and decitabine [24, 25]. A major challenge in the safe and effective delivery of novel drug and cellular therapies particularly after an allogeneic transplant is the toxicity associated with both modalities. Of particular, concern is the substantial risk of severe GVHD which is observed when DLI is administered early post-transplant, but it is also the case that the tolerability of pharmacological interventions in the form of maintenance therapy can be problematic in patients treated with intensive chemotherapy alone. Thus, the ability of our data to identify patients at particular risk of early and later relapse can be predicted to be of value in the design of novel treatment strategies particularly with regard to the timing of post-transplant interventions. Specifically, our data emphasize the importance of early intervention in patients allografted for AML associated with a *FLT*3-ITD or adverse-risk cytogenetics given the striking increase in relapse in the first 3 months post-transplant in this subgroup of patients. Consequently, the encouraging preliminary data reported using sorafenib in patients transplanted for *FLT3*-ITD-positive AML are encouraging – in particular the reported ability to administer this agent relatively early post-transplant [21]. In the light of the substantial risk of GVHD associated with the early administration of DLI and the practicalities of immunosuppression withdrawal such a group of patients are more likely to benefit from pharmacological intervention with agents such as sorafenib or DNMT inhibitors [26]. Alternatively, it may be possible to identify a population of patients likely to relapse later, for whom DLI is an important alternative intervention.

Our data provide novel insights into the mechanism of disease relapse and identify a complex interaction of factors determining the timing of disease relapse postallograft. Specifically, they demonstrate that distinct and potentially manipulable tumour and transplant-related factors play contrasting roles in the determining the timing of relapse post-transplant. These observations can inform the design of novel strategies aimed at reducing the risk of relapse postallograft and importantly imply that a nuanced approach should be taken with specific reference to the timing of intervention according to disease and transplant factors.

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Authorship contributions

CC, JV, ML, PV, MM, JC and AN designed the research and analysed the data. GS, AH, ED, LV, NM, JHB, AR, PC, DB, MM, EV, MCV, JM, JP, CS, BL and GO provided important clinical data. CC, JV and JC wrote the first draft of the manuscript, and all authors approved the final version of the manuscript.

Conflict of interest

The authors declare no competing financial interests.

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