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Graft-versus-host disease and graft-versus-leukaemia effects in secondary acute myeloid leukaemia: a retrospective, multicentre registry analysis from the Acute Leukaemia Working Party of the EBMT

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Summary

We assessed the susceptibility of secondary acute myeloid leukaemia (sAML) to graft-versus-leukaemia effects. Data from 2414 sAML patients in first (n = 2194) or second (n = 220) complete remission were included. They were given grafts from human leucocyte antigen (HLA)-matched sibling (MSD, n = 1085), 10/10 unrelated donor (MUD, n = 1066) or 9/10 mismatched unrelated donor (MMUD, n = 263). The 100-day incidence of grade II-IV acute graft-versus-host disease (GVHD) was 25% while 2-year incidence of chronic GVHD was 38%. Relapse rates declined steadily by duration of follow-up and were significantly lower in patients with chronic GVHD ($P < 0{\cdot}001$). Limited (hazard ratio [HR] = 0·66, $P < 0{\cdot}001$) and extensive (HR = 0·52, $P < 0{\cdot}001$) chronic GVHD were associated with a lower incidence of relapse. Each grade III-IV acute (HR = 7·04, $P < 0{\cdot}001$) as well as limited (HR = 1·42, $P = 0{\cdot}03$) and extensive (HR = 3·97, $P < 0{\cdot}001$) chronic GVHD were associated with higher non-relapse mortality (NRM). This translated to better overall survival (OS; HR = 0·61, $P < 0{\cdot}001$) in patients with limited chronic GVHD. In contrast, grade III-IV acute and extensive chronic GVHD were associated with worse OS (HR = 3·16, $P < 0{\cdot}001$ and HR = 1·21, $P = 0{\cdot}03$, respectively). Further, in comparison to HLA-identical sibling recipients, MUD recipients had a lower risk of relapse (HR = 0·82, $P = 0{\cdot}03$) but higher NRM (HR = 1·38, $P = 0{\cdot}004$). In conclusion, these data demonstrate that sAML is susceptible to graft-versus-leukaemia effects.

Keywords: secondary AML, GVHD, graft-versus-leukaemia effects, reduced intensity conditioning.

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Secondary acute myeloid leukaemia (sAML) has been defined as AML that occurs after myelodysplastic syndrome or myeloproliferative neoplasm or AML following exposure to radiation or chemotherapy, and represents 10–25% of all AML (Hulegardh, *et al*, 2015; Zeichner & Arellano, 2015).

Prior studies have observed lower outcomes in sAML than in *de novo* AML patients. This has been partly attributed to a depletion of haematopoietic reserve that might prolong the duration of neutropenia after chemotherapy as well as to a high incidence of each poor-risk cytogenetic and tumour protein 53 gene (*TP53*) mutation (Rampal, *et al*, 2014; Takahashi, *et al*, 2017), which have been associated with a high risk of AML relapse in *de novo* AML.

While up to 60% of patients with sAML achieve a complete remission (CR) with induction-remission chemotherapy, 5-year survival after diagnosis in fit sAML patients has remained at approximately 25% (Granfeldt Ostgard, *et al*, 2015). Based on these observations, allogeneic haematopoietic stem cell transplantation (allo-HSCT) in first CR has been indicated in fit sAML patients without favourable risk cytogenetics while allo-HSCT has remained the best curative option for most sAML patients in second CR (Sengsayadeth, *et al*, 2018).

For adult patients with *de novo* AML, cure after allo-HSCT depends on both the intensity of the conditioning regimen given before transplantation (Scott, *et al*, 2017) and on immune-mediated graft-versus-leukaemia (GVL) effects (Weiden, *et al*, 1981; Baron, *et al*, 2005; Baron, *et al*, 2012). While previous studies have demonstrated that sAML is less susceptible than *de novo* AML to intensive chemotherapy, the susceptibility of sAML to GVL effects has not yet been in a large cohort of patients. This is unfortunate given that some of the characteristics associated with sAML, such as *TP53* mutations, might affect the susceptibility of tumour cells to immune-mediated cytolysis (Meslin, *et al*, 2007).

In the current retrospective study, we assessed the impact of graft-versus-host disease (GVHD) occurrence on transplantation outcomes as a surrogate for GVL effects. Further, we also assessed the impact of donor type (human leucocyte antigen [HLA]-identical sibling donor [matched sibling donor, MSD] vs. unrelated donor transplantation) on the risk of relapse, as the greater genetic diversity in the latter case might result in enhanced GVL effects (Martin, *et al*, 2017).

Methods

Data collection

This survey is a retrospective registry-based study performed by the Acute Leukaemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT). The EBMT is a non-profit, scientific society representing >600 transplant centres, mainly in Europe. Data are entered, managed and maintained in a central database with internet access; each EBMT centre is represented in this database. Quality control consist of confirmation of validity of the data by the reporting team, selective comparison of the survey data with minimum essential data A (MED-A) data sets in the EBMT registry database, cross-checking with the National Registries, and regular in-house and external data audits. Since 1990, patients have provided informed consent authorizing the use of their personal information for research purposes.

Inclusion criteria were adult patients aged $\geq$ 18 years, sAML, first or second CR, bone marrow (BM) or peripheral blood stem cells (PBSC) as stem cell source, MSD or 10/ 10 (matched unrelated donor, MUD) or 9/10 (mis-matched unrelated donor, MMUD) HLA-matched unrelated donor, no *ex vivo* T-cell depletion of the graft, achievement of neutrophil engraftment, data on GVHD, and first transplantation performed between 2005 and 2016. The date and severity (limited vs. extensive) of chronic GVHD (cGVHD; graded according to established criteria [Glucksberg, *et al*, 1974]) were prospectively collected using the EBMT Mimimum Essential Data-A form.

sAML was defined as AML occurring after an antecedent of a haematological malignant disorder, a bone marrow failure syndrome, or a solid tumour with prior chemo- and or –radio- therapy, as previously reported (Sengsayadeth, *et al*, 2018). Reduced intensity conditioning (RIC) was defined as fludarabine associated with <6 Gy total body irradiation, or busulfan $\leq$ 8 mg/kg or other nonmyeloablative drugs. All necessary data were collected according to EBMT guidelines.

Statistical analyses

Data from all patients meeting the inclusion/exclusion criteria were included in the analyses. Start time was date of transplant for all endpoints. Relapse was defined as the presence of five per cent bone marrow blasts and/or reappearance of the underlying disease. Non-relapse mortality (NRM) was

defined as death without evidence of relapse or progression. Overall survival (OS) was defined as the time from allo-HSCT to death, regardless of the cause.

Cumulative incidence functions were used to estimate relapse incidence and NRM in a competing-risks setting, because death and relapse compete with each other. To study acute GVHD (aGVHD) and cGVHD, we considered relapse and death to be competing events. OS was estimated using the Kaplan-Meier estimates.

The impact of aGVHD and cGVHD on transplantation outcome was assessed using multivariate Cox models with GVHD modelled as a time-dependent covariate. Patients at risk of relapse or mortality were classified in 3 mutually exclusive conditions based on their histories of aGVHD or cGVHD: grades 0-I aGVHD without cGVHD (hereafter designated "no GVHD"), grades II-IV aGVHD without cGVHD (hereafter designated "aGVHD" group that was separated in some analyses between grade II acute and grade III-IV aGVHD), or cGVHD with or without grade II-IV aGVHD (hereafter designated "cGVHD" group that was separated in some analyses between limited and extensive cGVHD). All patients were first classified in the "no GVHD" group until the onset of aGVHD or cGVHD. Patients with grade II-IV "aGVHD" were classified in that condition, regardless of whether aGVHD had resolved, until the onset of cGVHD if it occurred. Patients with "cGVHD" were classified in that condition thereafter. Only cases of cGVHD occurring before relapse were taken into consideration.

The impact of GVHD on relapse, NRM and overall mortality was further illustrated by calculating the rate of relapse, NRM and mortality per patient-year for each condition within sequential 90-day intervals after transplantation, and smoothed estimates of the event rates obtained by fitting a Poisson regression model to the observed numbers of events, using cubic spline terms for time, as previously reported (Inamoto, *et al*, 2011).

All tests were two sided. The type I error rate was fixed at 0·05 for determination of factors associated with time to event outcomes. Statistical analyses were performed with SPSS 24·0 (SPSS Inc, Chicago, IL, USA) and R 3·4.0 (R Core Team (2017).

Results

Patients

A total of 2414 patients met the study inclusion criteria (Table I). Median patient age was 58 years (range, 18–77 years) and 40% of the patients were ≥ 60 years of age at transplantation. Status at transplantation was first CR in 2194 patients (91%) and second CR in the remaining 220 patients. Patients received either grafts from MSD ($n = 1085$), MUD ($n = 1066$) or MMUD ($n = 263$). Stem cell source was PBSC in 91% of the patients. Conditioning regimen was myeloablative (MAC) in 43% of the patients. In addition, 58% of the patients received *in vivo* T-cell depletion as GVHD prophylaxis. This includes 48% of the patients receiving antithymocyte globulin (ATG) and 10% of the patients given alemtuzumab.

Transplantation outcomes

Cumulative incidences of grade II and III-IV aGVHD were 25% (95% confidence interval [CI]: 23–26%) and 9% (95% CI: 8–11%), respectively. As expected, the cumulative incidence of grade II-IV aGVHD was lower in MSD (20%; range, 18–22%) than in MUD (28%; range, 25–30%) and MMUD (31%; range, 25–36%) recipients (global $P < 0·001$). In multivariate analyses, factors associated with higher incidence of grade II-IV aGVHD included MUD (in comparison to MSD; HR = 1·67, 95% CI: 1·36–2·04, $P < 0·001$) and MMUD (in comparison to MSD, HR = 1·94, 95% CI: 1·47–2·56, $P < 0·001$), while the use of *in vivo* T-cell depletion (HR = 0·69, 95%CI: 0·58–0·83, $P < 0·001$) was associated with a lower incidence of grade II-IV aGVHD.

At 2 years, the cumulative incidence of cGVHD was 38% (95% CI, 36–40%; including 17% extensive cGVHD). Median time for cGVHD occurrence was 177 days (interquartile range: 122–273 days). In multivariate analyses, factors associated with higher incidence of cGVHD included older patient age (HR per 10 years = 1·07, 95% CI: 1·00–1·14, $P = 0·05$) and PBSC as stem cell source (HR = 1·41, 95% CI: 1·11–1·81, $P = 0·006$), while recent year of transplantation (assessed as a continuous variable) (HR = 0·97, 95% CI: 0·95–0·99, $P = 0·01$) and the use of *in vivo* T-cell depletion (HR = 0·64, 95%CI: 0·55–0·74, $P < 0·001$) were associated with a lower incidence of cGVHD.

Two-year cumulative incidences of relapse and NRM were 31% (95% CI, 29–33%) and 19% (95% CI, 17–20%), respectively. Two-year OS was 56% (95% CI, 54–59%).

Impact of GVHD on relapse incidence

Relapse rates per patient year declined steadily by duration of follow-up (Fig 1). In multivariate time-dependent analyses, there was no impact of grade II or grade III aGVHD on the risk of relapse (HR = 0·85, 95% CI: 0·67–1·09, $P = 0·19$ and HR = 0·98, 95% CI: 0·69–1·39, $P = 0·90$, respectively) (Table II). The same was true when grade II-IV aGVHD was assessed as a single group (HR = 0·89, 95% CI: 0·72–1·10, $P = 0·26$). This is illustrated in Fig 1, showing no impact of grade II-IV aGVHD on the evolution of relapse rates per patient year.

Interestingly, limited (HR = 0·66, 95% CI: 0·52–0·84, $P < 0·001$) and extensive (HR = 0·52, 95% CI: 0·38–0·71, $P < 0·001$) cGVHD were each associated with lower risks of relapse. The same was true when all cases of cGVHD (limited or extensive) were assessed as a single group (HR = 0·61, 95% CI: 0·50–0·75, $P < 0·001$). This is illustrated in Fig 1, showing lower relapse rates per patient year in patients with cGVHD than in those without.

Table I. Patient characteristics

Variable	Overall (n = 2414)
Follow-up (reverse KM) months; median (IQR)	50·8 (24·6–83·7)
Patient age at transplant, years; median (min-max)	57·6 (18·1–77·5)
Patient sex, n (%)	
Male	1275 (52·8)
Female	1138 (47·2)
Missing	1
Karnofsky score, n (%)	
≤80	118 (5·2)
>80	2154 (94·8)
Missing	142
Diagnosis to transplant, months; median (min-max)	4·82 (0·07–177·1)
Previous diagnosis, n (%)	
Myelodysplastic syndrome/ myeloproliferative neoplasm	1300 (53·8)
Lymphoma	116 (4·8)
Other haematological malignancy	64 (2·7)
Aplastic anaemia	8 (0·3)
Breast cancer	162 (6·7)
Other solid tumour	113 (4·7)
Therapy-related (primary diagnosis not specified)	619 (25·6)
Other diagnosis	32 (1·3)
Disease status at transplant, n (%)	
First complete remission	2194 (90·9)
Second complete remission	220 (9·1)
Cytogenetics, n (%)	
Good*	52 (2·1)
Intermediate†	704 (29·2)
Poor‡	306 (12·7)
NA/failed	1352 (56·0)
Year of transplantation, median (min-max)	2012 (2005–2016)
Donor type, n (%)	
HLA-identical sibling	1085 (44·9)
HLA-matched unrelated donor 10/10	1066 (44·2)
1/10 HLA-mismatched unrelated donor	263 (10·9)
Female to male, n (%)	
No	2003 (83·3)
Yes	403 (16·7)
Missing	8
Cytomegalovirus donor/patient, n (%)	
−/−	553 (23·6)
+/−	218 (9·3)
−/+	572 (24·4)
+/+	1002 (42·7)
Missing	69
Cell sources, n (%)	
Bone marrow	228 (9·4)
PBSC	2186 (90·6)
Conditioning type, n (%)	
Myeloablative	1047 (43·5)
Reduced-intensity§	1362 (56·5)
Missing	5
In-vivo TCD, n (%)	
No	1003 (41·5)
Yes	1411 (58·5)
If yes, ATG/ campath	1162/249

ATG, antithymocyte globulin; HLA, human leucocyte antigen; IQR, interquartile range; KM, Kaplan-Meier; NA, not available; PBSC, peripheral blood stem cells; TCD, T-cell depletion.

*Defined as t(8;21), t(15;17), inv or del (16), or acute promyelocytic leukaemia, these abnormalities only or combined with others.

†Defined as all cytogenetics not belonging to the good or high risk (including trisomias).

‡Defined as 11q23 abnormalities, complex karyotype, abnormalities of chromosomes 5 and 7, as well as 3q26 and 17p abnormalities, as defined previously (Baron, *et al*, 2019a).

We performed sensitivity analyses to assess whether the impact of cGVHD on the risk of relapse was consistent among various subgroups. The association between cGVHD and GVL effects was consistent among subgroups with the exception of the subgroup of patients with poor-risk cytogenetics (Fig 2).

Impact of GVHD on NRM

In multivariate time-dependent analyses, there was no impact of grade II aGVHD on NRM (HR = 0·97, 95% CI: 0·66–1·42, P = 0·88; Table II). In contrast, grade III-IV acute (HR = 7·04, 95% CI 5·40–9·19, P < 0·001) as well as limited (HR = 1·42, 95% CI 1·04–1·93, P = 0·03) and extensive (HR = 3·97, 95% CI 3·05–5·17, P < 0·001) cGVHD were each associated with higher NRM.

Impact of GVHD on OS

In multivariate time-dependent analyses, there was no impact of grade II aGVHD on mortality (HR = 0·92, 95% CI: 0·74–1·14, P = 0·43) (Table II). However, grade III-IV acute (HR = 3·16, 95% CI: 2·58–3·86, P < 0·001) as well as extensive (HR = 1·21, 95% CI 1·01–1·45, P = 0·03) cGVHD were each associated with a higher mortality. In contrast, limited cGVHD was associated with a better OS (HR = 0·61, 95% CI: 0·50–0·74, P < 0·001). This is illustrated in Fig 3, showing that mortality rates per patient year declined by duration of follow-up in each condition, and were lower in patients with cGVHD than in those without.

Impact of in vivo *T-cell depletion, donor type and conditioning intensity*

We then assessed the impact of other factors that might impact transplantation outcomes (such as conditioning

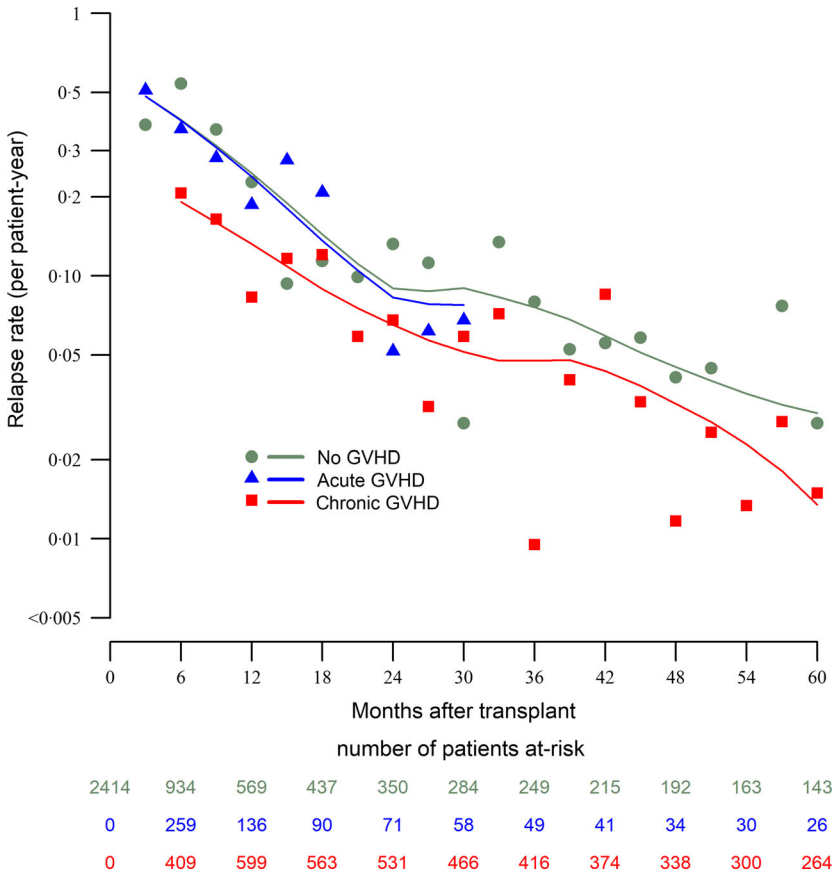

Fig 1. Evolution of relapse rates according to GVHD status. Rates were calculated within sequential 90-day intervals for patients without graft-versus-host disease (GVHD; shown in blue), for patients with grades II-IV acute GVHD (shown in green) or for patients with chronic GVHD (shown in red). Small symbols represent the actual relapse rates for each 90-day interval. The smoothed rates were plotted as curves for each condition

intensity, donor type and the use of *in vivo* T-cell depletion) in addition to GVHD occurrence. In multivariate analyses, in comparison to patients given MAC, RIC patients had a higher risk of relapse (HR = 1·49, 95% CI: 1·26–1·77, $P < 0·001$) and lower OS (HR = 1·24, 95% CI: 1·08–1·42, $P = 0·002$) (Table II). Further, in comparison to patients given grafts from HLA-identical siblings, those given grafts from MUD had a lower risk of relapse (HR = 0·82, 95% CI: 0·70–0·98, $P = 0·03$) but experienced higher NRM (HR = 1·38, 95% CI 1·10–1·71, $P = 0·004$), translating to comparable OS (HR = 1·06, 95% CI: 0·92–1·22, $P = 0·40$). MMUD patients had also higher NRM (HR = 1·62, 95% CI 1·20–2·17, $P = 0·001$). Interestingly, the use of *in vivo* T-cell depletion was not significantly associated with transplantation outcomes (besides lowering acute and cGVHD incidences; Table II). Finally, disease status at transplantation was not associated with any transplantation outcomes (Table II).

Discussion

Several previous studies have demonstrated that AML is susceptible to GVL effects, including showing a lower incidence of relapse following allogeneic than after autologous or syngeneic transplantation (Horowitz, *et al*, 1990; Suciu, *et al*, 2003; Cornelissen, *et al*, 2007), a lower risk of relapse in patients who develop grade II-IV aGVHD and/or cGVHD (Weiden, *et al*, 1981; Horowitz, *et al*, 1990; Baron, *et al*, 2005; Baron, *et al*, 2012), the efficacy of allo-HSCT following very low intensity conditioning regimens (Baron, *et al*, 2005) and the ability of donor lymphocyte infusion to cure a proportion of patients suffering from AML relapse after allo-HSCT (Schmid, *et al*, 2007). In recent years, it has been shown that the biology of sAML was largely different from the biology of *de novo* AML. We reasoned that these differences might have an impact of the susceptibility of AML blasts to GVL effects. As example, while *TP53* mutations are frequently observed in sAML (Rampal, *et al*, 2014), it has been demonstrated that granzyme B-mediated cell death (a major player in GVL effects) was dependent of TP53 activation by tumour cells. In line with these observations, previous studies questioned the role of allo-HSCT in AML with abnormalities of chromosome 17p (location of *TP53*)(Poire, *et al*, 2017). These considerations prompted us to look at the susceptibility of sAML to GVL effects.

The multivariate Cox model with GVHD assessed as time-varying covariates has been the gold standard technique for assessing the impact of GVHD on transplantation outcomes (Storer, 2009). In addition of using this technique, we further illustrated the impact of GVHD on transplantation outcomes by assessing the event rates during sequential

Table II. Multivariate Cox models

Variable	Relapse HR (95% CI)	*P*	Non relapse mortality HR	*P*	Overall mortality HR	*P*
GVHD*						
aGVHD only grade 0-I (ref)	–	–	–	–	–	–
aGVHD only grade II	0·85 (0·67–1·09)	0·19	0·97 (0·66–1·42)	0·88	0·92 (0·74–1·14)	0·43
aGVHD only grade III-IV	0·98 (0·69–1·39)	0·90	7·04 (5·4–9·19)	<0·001	3·16 (2·58–3·86)	<0·001
cGVHD limited with or without aGVHD	0·66 (0·52–0·84)	<0·001	1·42 (1·04–1·93)	0·026	0·61 (0·50–0·74)	<0·001
cGVHD extensive with or without aGVHD	0·52 (0·38–0·71)	<0·001	3·97 (3·05–5·17)	<0·001	1·21 (1·01–1·45)	0·03
Patient age at transplant†	1·0 (0·99–1·01)	0·72	1·02 (1·01–1·03)	<0·001	1·01 (1–1·02)	<0·001
Disease status at HSCT: CR2 *versus* CR1	1·15 (0·89–1·49)	0·27	0·94 (0·69–1·28)	0·70	1·13 (0·92–1·38)	0·24
Cytogenetic						
Good (ref)‡	–	–	–	–	–	–
Intermediate§	1·41 (0·78–2·54)	0·25	4·58 (1·45–14·4)	0·009	2·17 (1·24–3·79)	0·007
Poor¶	3·28 (1·8–5·95)	<0·001	4·02 (1·24–13)	0·02	3·39 (1·92–6)	<0·001
NA/failed	1·58 (0·88–2·82)	0·12	4·12 (1·31–12·9)	0·015	2·2 (1·26–3·82)	0·005
Year of HSCT†	1·02 (0·99–1·05)	0·15	1·01 (0·97–1·04)	0·71	1·01 (0·99–1·03)	0·23
Donor type						
MSD (ref)	–	–	–	–	–	–
MUD 10/10	0·82 (0·70–0·98)	0·026	1·38 (1·1–1·71)	0·004	1·06 (0·92–1·22)	0·40
MMUD	0·80 (0·62–1·04)	0·096	1·62 (1·2–2·17)	0·001	1·12 (0·92–1·37)	0·27
Female to male: yes *versus* no	0·93 (0·76–1·13)	0·45	1·19 (0·94–1·49)	0·14	1·08 (0·93–1·26)	0·33
CMV patient positive: yes *versus* no	1·03 (0·88–1·21)	0·71	1·31 (1·07–1·6)	0·009	1·15 (1–1·31)	0·046
CMV donor positive: yes *versus* no	1·02 (0·87–1·19)	0·80	0·93 (0·77–1·12)	0·42	1·01 (0·90–1·15)	0·82
Cell source: PBSC *versus* BM	0·84 (0·66–1·06)	0·14	1·16 (0·83–1·62)	0·37	0·94 (0·77–1·15)	0·55
Conditioning typ: RIC *versus* MAC	1·49 (1·26–1·77)	<0·001	1·02 (0·83–1·24)	0·88	1·24 (1·08–1·42)	0·002
In-vivo TCD: yes *versus* no	0·96 (0·81–1·13)	0·60	0·97 (0·79–1·18)	0·76	0·91 (0·79–1·03)	0·14

aGVHD, acute GVHD; ATG, anti-thymocyte globulin; BM, bone marrow; cGVHD, chronic GVHD; CI: confidence interval; CMV, cytomegalovirus; CR, complete remission; GVHD, graft-versus-host disease; HR: hazard ratio; HSCT: haematopoietic stem cell transplantation; MAC, myeloablative conditioning; MMUD, 1/10 HLA-mismatched unrelated donor; MSD, HLA-identical sibling donor; MUD, HLA-matched unrelated donor; NA: not available; PBSC, peripheral blood stem cells; RIC, reduced-intensity conditioning (as defined by Baron, *et al*, 2016); TCD: T-cell depletion.

*GVHD was handled as a time-dependent covariate.

†Introduced as a continuous variable in the models.

‡Defined as *t*(8;21), *t*(15;17), inv or del (16), or acute promyelocytic leukaemia, these abnormalities only or combined with others.

§Defined as all cytogenetics not belonging to the good or high risk (including trisomias).

¶Defined as 11q23 abnormalities, complex karyotype, abnormalities of chromosomes 5 and 7, as well as 3q26 and 17p abnormalities, as defined previously (Baron, *et al*, 2019c).

90-day periods according to GVHD status. This last technique enables the evolution of event rates over time to be determined and is not impacted by competing risks (Inamoto, *et al*, 2011).

A first observation of our study was that occurrence of cGVHD was associated with lower risk of sAML relapse, clearly demonstrating a GVL effect in sAML. This was observed both with limited and extensive forms of cGVHD. The beneficial impact of cGVHD was consistent among various subgroups with the possible exception of the subgroup of patients with poor risk cytogenetics, although this observation should be interpreted with caution given the relative low number of patients in that subgroup. Indeed, donor *versus* no donor analyses have demonstrated a beneficial impact of having a HLA-identical sibling donor in (*de novo*) AML patients with poor-risk cytogenetics (Suciu, *et al*, 2003; Baron, *et al*, 2019b), including those with monosomal karyotype (Baron, *et al*, 2019c).

Interestingly, grade II aGVHD was not associated with lower risks of relapse. This is in contrast to what has been observed in patients with *de novo* AML given grafts after MAC or RIC regimens (Baron, *et al*, 2012) and might reflect differences in the biology of *de novo versus* secondary AML.

An important observation of our study is that, because of their association with NRM, all severe (grade III-IV acute and extensive chronic) forms of GVHD were associated with lower OS. This clearly indicates that, despite the high risk of relapse associated with sAML, strategies aimed at preventing severe GVHD are nevertheless warranted in that setting. ATG has been shown to efficiently prevent GVHD without increasing the risk of relapse in several prospective phase III trials, including mainly AML patients (Kroger, *et al*, 2016;

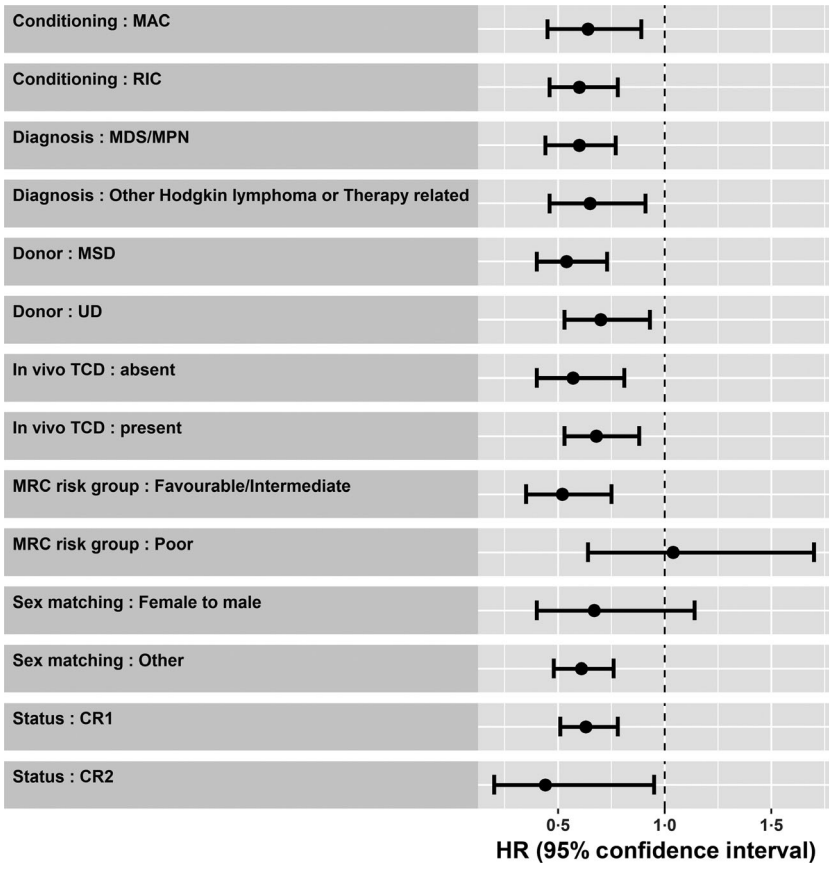

Fig 2. Sensitivity analyses looking at the association between chronic GVHD and the risk of relapse in various subgroups. The forest plot graph shows the adjusted HR and 95% CI. CI: confidence interval; CR, complete remission; HR: hazard ratio; MAC, myeloablative conditioning; MDS: myelodysplastic syndrome; MPN: myeloproliferative disease; MRC: Medical Research Council; MSD, HLA-identical sibling donor; RIC, reduced-intensity conditioning

Walker, *et al*, 2016; Finke, *et al*, 2017). In our large cohort of sAML patients, the use of *in vivo* T-cell depletion (mainly ATG) did not impact the risk of relapse but failed to significantly improve OS, as observed in prior phase III trials (Kroger, *et al*, 2016; Finke, *et al*, 2017).

In addition to provide evidence for GVL effects in sAML, the current study also showed a higher risk of relapse in patients given RIC regimens, translating to worse OS. This demonstrates that the intensity of the conditioning regimen matters in sAML patients offered an allo-HSCT. This observation is in line with observations made in *de novo* AML and in MDS patients, where patients randomized to receive a RIC regimen had higher risk of relapse, translating to a trend for lower OS in comparison to those offered a MAC regimen (Scott, *et al*, 2017).

Several studies have compared the strength of GVL effects after transplantation with MUD *versus* HLA-identical sibling donors, yielding conflicting results (Ringden, *et al*, 2009; Ho, *et al*, 2011). Indeed, there is greater genetic diversity in case of MUD transplantation that might result in enhanced GVL activity (Martin, *et al*, 2017). In concordance with this hypothesis, we observed lower relapse incidence in MUD recipients than in those transplanted with grafts from HLA-identical siblings, suggesting stronger GVL effects. However, we did not observe lower risks of relapse in male patients given grafts from female donors neither in the whole cohort of patients and nor the subgroup of patients given grafts from HLA-identical sibling (HR = 0·8, 95% CI: 0·7–1·1).

Despite the fact that this retrospective study included only data from patients in CR at transplantation, the relapse incidence was relatively high (30% at 2 years), underlying the poor prognosis of sAML patients. Assessing the relapse rates per patient-year over a 90-day period allowed clear demonstration that relapse occurrence declined over time, signifying that future strategies aimed at preventing disease progression should investigate early interventions. These interventions might include post-transplant administration of targeted therapies, such as demethylating agents (Goodyear, *et al*, 2012; Ehx, *et al*, 2017; Huls, *et al*, 2019). Another strategy for separating GVL effects from severe GVHD in the HLA-matched transplantation setting might comprise post-transplantation administration of cyclophosphamide (PTCy, 50 mg/kg on days +3 and +4) as a single GVHD-prophylaxis agent (Kanakry, *et al*, 2014; McCurdy, *et al*, 2019).

This study has some limitations. First, by using grade 0–1 aGVHD allo-HSCT recipients as the reference group, our study does not take GVL occurring either without clinical or with grade I aGVHD into account. Indeed, previous studies have demonstrated a lower incidence of relapse associated with grade I aGVHD. Secondly, cGVHD was stratified using the limited *versus* extensive classification and not with the recent NIH classification (Baron *et al*, 2012). Thirdly,

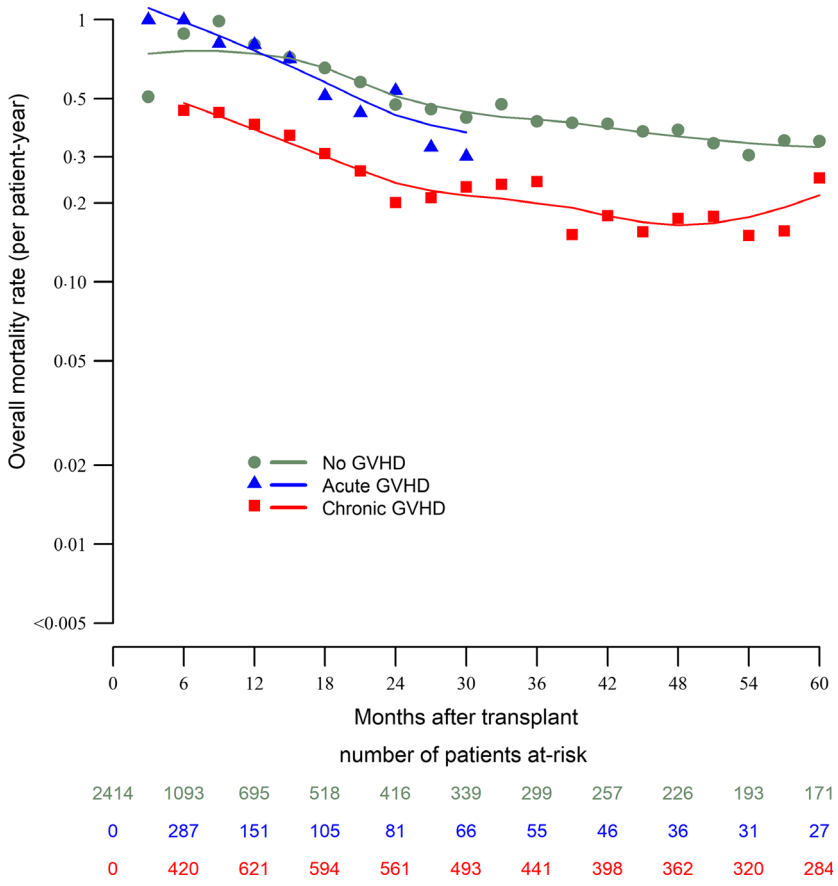

Fig 3. Evolution of mortality rates according to GVHD status. Rates were calculated within sequential 90-day intervals for patients without graft-versus-host disease (GVHD; shown in blue), for patients with grades II-IV acute GVHD (shown in green) or for patients with chronic GVHD (shown in red). In contrast to the classical Kaplan-Meier curve, lower rates translate better survival. Small symbols represent the actual relapse rates for each 90-day interval. The smoothed rates were plotted as curves for each condition

different RIC and MAC regimens were used, some of which might have greater anti-tumour activities than others (Baron, *et al*, 2015). Similarly, there was some heterogeneity in the methods used for GVHD prophylaxis that might also have an impact on AML relapse.

In summary, in this large cohort of sAML patients in CR at transplantation we observed GVL effects associated with cGVHD. While there was better OS in patients who developed limited cGVHD, extensive cGVHD and grade III-IV aGVHD were each associated with increased NRM and overall mortality. In addition, while MUD recipients had a lower risk of relapse, they had higher NRM offsetting any potential OS benefit. Further studies should focus at optimizing the conditioning regimens and/or administering post-transplant maintenance therapies in an attempt to further reduce post-transplantation relapses.

Ethical considerations

The current study has been approved by the scientific board of the ALWP of the EBMT.

Consent for publication

Not applicable.

Availability of data materials

ML, MM and AN had full access to all the data in the study.

Data sharing

Data request should be addressed to Myriam Labopin myriam.labopin@upmc.fr.

Author's contribution

FBa wrote the manuscript, designed the study and interpreted the data. ML and EB designed the study, performed the statistical analyses, interpreted the data and edited the manuscript. MM and AN designed the study, interpreted the data and edited the manuscript. BS, ME, VP, NK, DB, GS, MIR and MB reviewed the manuscript and provided clinical data. All authors approved the final version of the manuscript.

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Conflict of interest

FB has received travel grants from Celgene, Abbvie, Novartis and Sanofi. The other authors declare that they have no relevant conflict of interest.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. List of participating centers.

References

Baron, F., Maris, M.B., Sandmaier, B.M., Storer, B.E., Sorror, M., Diaconescu, R., Woolfrey, A.E., Chauncey, T.R., Flowers, M.E.D., Mielcarek, M., Maloney, D.G. & Storb, R. (2005) Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *Journal of Clinical Oncology*, **23**, 1993–2003.

Baron, F., Labopin, M., Niederwieser, D., Vigouroux, S., Cornelissen, J.J., Malm, C., Vindelov, L.L., Blaise, D., Janssen, J.J., Petersen, E., Socie, G., Nagler, A., Rocha, V. & Mohty, M. (2012) Impact of graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation for acute myeloid leukemia: a report from the Acute Leukemia Working Party of the European group for blood and marrow transplantation. *Leukemia*, **26**, 2462–2468.

Baron, F., Labopin, M., Peniket, A., Jindra, P., Afanasyev, B., Sanz, M.A., Deconinck, E., Nagler, A. & Mohty, M. (2015) Reduced-intensity conditioning with fludarabine and busulfan versus fludarabine and melphalan for patients with acute myeloid leukemia: a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Cancer*, **121**, 1048–1055.

Baron, F., Ruggeri, A., Beohou, E., Labopin, M., Sanz, G., Milpied, N., Michallet, M., Bacigalupo, A., Blaise, D., Sierra, J., Socie, G., Cornelissen, J.J., Schmid, C., Giebel, S., Gorin, N.C., Esteve, J., Ciceri, F., Savani, B.N., Mohty, M., Gluckman, E. & Nagler, A. (2016) RIC versus MAC UCBT in adults with AML: A report from Eurocord, the ALWP and the CTIWP of the EBMT. *Oncotarget*, **7**, 43027–43038.

Baron, F., Labopin, M., Ruggeri, A., Volt, F., Mohty, M., Blaise, D., Chevallier, P., Sanz, J., Fegueux, N., Cornelissen, J.J., Rambaldi, A., Savani, B.N., Gluckman, E. & Nagler, A. (2019a) Cord blood transplantation is associated with good outcomes in secondary Acute Myeloid Leukaemia in first remission. *Journal of Internal Medicine*, **285**, 445–454.

Baron, F., Efficace, F., Cannella, L., Willemze, R., Vignetti, M., Muus, P., Marie, J.P., Ferrero, D., Fazi, P., La Sala, E., Bourhis, J.H., Fabbiano, F., Bosi, A., Sborgia, M., Martinelli, G., Wittnebel, S., Trisolini, S., Petti, M.C., Halkes, C.J.M., van der Velden, W., de Witte, T., Amadori, S., Zittoun, R.A. & Suciu, S. (2019b) Long-term follow-up of a trial comparing post-remission treatment with autologous or allogeneic bone marrow transplantation or intensive chemotherapy in younger acute myeloid leukemia patients. *Haematologica*, https://doi.org/10.3324/haematol.2019.221333.

Baron, F., Stevens-Kroef, M., Kicinski, M., Meloni, G., Muus, P., Marie, J.P., Halkes, C.J.M., Thomas, X., Vrhovac, R., Albano, F., Lefrere, F., Sica, S., Mancini, M., Venditti, A., Hagemeijer, A., Jansen, J.H., Amadori, S., de Witte, T., Willemze, R. & Suciu, S. (2019c) Impact of induction regimen and of allogeneic hematopoietic cell transplantation on outcome in younger adults patients with acute myeloid leukemia with a monosomal karyotype. *Haematologica*, **104**, 1168–1175.

Core Team, R. (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Cornelissen, J.J., van Putten, W.L., Verdonck, L.F., Theobald, M., Jacky, E., Daenen, S.M., van Marwijk, K.M., Wijermans, P., Schouten, H., Huijgens, P.C., van der Lelie, H., Fey, M., Ferrant, A., Maertens, J., Gratwohl, A. & Lowenberg, B. (2007) Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? *Blood*, **109**, 3658–3666.

Ehx, G., Fransolet, G., de Leval, L., D'Hondt, S., Lucas, S., Hannon, M., Delens, L., Dubois, S., Drion, P., Beguin, Y., Humblet-Baron, S. & Baron, F. (2017) Azacytidine prevents experimental xenogeneic graft-versus-host disease without abrogating graft-versus-leukemia effects. *Oncoimmunology*, **6**, e1314425.

Finke, J., Schmoor, C., Bethge, W.A., Ottinger, H., Stelljes, M., Volin, L., Heim, D., Bertz, H., Grishina, O. & Socie, G. (2017) Long-term outcomes after standard graft-versus-host disease prophylaxis with or without anti-human-T-lymphocyte immunoglobulin in haemopoietic cell transplantation from matched unrelated donors: final results of a randomised controlled trial. *Lancet Haematol*, **4**, e293–e301.

Glucksberg, H., Storb, R., Fefer, A., Buckner, C.D., Neiman, P.E., Clift, R.A., Lerner, K.G. & Thomas, E.D. (1974) Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*, **18**, 295–304.

Goodyear, O.C., Dennis, M., Jilani, N.Y., Loke, J., Siddique, S., Ryan, G., Nunnick, J., Khanum, R., Raghavan, M., Cook, M., Snowden, J.A., Griffiths, M., Russell, N., Yin, J., Crawley, C., Cook, G., Vyas, P., Moss, P., Malladi, R. & Craddock, C.F. (2012) Azacitidine augments expansion of regulatory T cells after allogeneic stem cell transplantation in patients with acute myeloid leukemia (AML). *Blood*, **119**, 3361–3369.

Granfeldt Ostgard, L.S., Medeiros, B.C., Sengelov, H., Norgaard, M., Andersen, M.K., Dufva, I.H., Friis, L.S., Kjeldsen, E., Marcher, C.W., Preiss, B., Severinsen, M. & Norgaard, J.M. (2015) Epidemiology and clinical significance of secondary and therapy-related acute myeloid leukemia: a national population-based cohort study. *Journal of Clinical Oncology*, **33**, 3641–3649.

Ho, V.T., Kim, H.T., Aldridge, J., Liney, D., Kao, G., Armand, P., Koreth, J., Cutler, C., Ritz, J., Antin, J.H., Soiffer, R.J. & Alyea, E.P. (2011) Use of matched unrelated donors compared with matched related donors is associated with lower relapse and superior progression-free survival after reduced-intensity conditioning hematopoietic stem cell transplantation. *Biology of Blood and Marrow Transplantation*, **17**, 1196–1204.

Horowitz, M.M., Gale, R.P., Sondel, P.M., Goldman, J.M., Kersey, J., Kolb, H.J., Rimm, A.A., Ringden, O., Rozman, C., Speck, B., Truitt, R.L., Zwaan, F.E. & Bortin, M.M. (1990) Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*, **75**, 555–562.

Hulegardh, E., Nilsson, C., Lazarevic, V., Garelius, H., Antunovic, P., Rangert Derolf, A., Mollgard, L., Uggla, B., Wennstrom, L., Wahlin, A., Hoglund, M., Juliusson, G., Stockelberg, D. & Lehmann, S. (2015) Characterization and prognostic features of secondary acute myeloid leukemia in a population-based setting: a report from the Swedish Acute Leukemia Registry. *American Journal of Hematology*, **90**, 208–214.

Huls, G., Chitu, D.A., Havelange, V., Jongen-Lavrencic, M., van de Loosdrecht, A.A., Biemond, B.J., Sinnige, H., Hodossy, B., Graux, C., Kooy, R.V.M., de Weerdt, O., Breems, D., Klein, S., Kuball, J., Deeren, D., Terpstra, W., Vekemans, M.C., Ossenkoppele, G.J., Vellenga, E., Lowenberg, B. & Dutch-Belgian Hemato-Oncology Cooperative (2019) Azacitidine maintenance after intensive chemotherapy improves DFS in older AML patients. *Blood*, **133**, 1457–1464.

Inamoto, Y., Flowers, M.E., Lee, S.J., Carpenter, P.A., Warren, E.H., Deeg, H.J., Storb, R.F., Appelbaum, F.R., Storer, B.E. & Martin, P.J. (2011) Influence of immunosuppressive treatment on risk of recurrent malignancy after allogeneic hematopoietic cell transplantation. *Blood*, **118**, 456–463.

Kanakry, C.G., Tsai, H.L., Bolanos-Meade, J., Smith, B.D., Gojo, I., Kanakry, J.A., Kasamon, Y.L., Gladstone, D.E., Matsui, W., Borrello, I., Huff, C.A., Swinnen, L.J., Powell, J.D., Pratz, K.W., DeZern, A.E., Showel, M.M., McDevitt,

M.A., Brodsky, R.A., Levis, M.J., Ambinder, R.F., Fuchs, E.J., Rosner, G.L., Jones, R.J. & Luznik, L. (2014) Single-agent GVHD prophylaxis with posttransplantation cyclophosphamide after myeloablative, HLA-matched BMT for AML, ALL, and MDS. *Blood*, **124**, 3817–3827.

Kroger, N., Solano, C., Wolschke, C., Bandini, G., Patriarca, F., Pini, M., Nagler, A., Selleri, C., Risitano, A., Messina, G., Bethge, W., Perez de Oteiza, J., Duarte, R., Carella, A.M., Cimminiello, M., Guidi, S., Finke, J., Mordini, N., Ferra, C., Sierra, J., Russo, D., Petrini, M., Milone, G., Benedetti, F., Heinzelmann, M., Pastore, D., Jurado, M., Terruzzi, E., Narni, F., Volp, A., Ayuk, F., Ruutu, T. & Bonifazi, F. (2016) Antilymphocyte Globulin for Prevention of Chronic Graft-versus-Host Disease. *New England Journal of Medicine*, **374**, 43–53.

Martin, P.J., Levine, D.M., Storer, B.E., Warren, E.H., Zheng, X., Nelson, S.C., Smith, A.G., Mortensen, B.K. & Hansen, J.A. (2017) Genome-wide minor histocompatibility matching as related to the risk of graft-versus-host disease. *Blood*, **129**, 791–798.

McCurdy, S.R., Kanakry, C.G., Tsai, H.L., Gojo, I., Smith, B.D., Gladstone, D.E., Bolanos-Meade, J., Borrello, I., Matsui, W.H., Swinnen, L.J., Huff, C.A., Brodsky, R.A., Ambinder, R.F., Fuchs, E.J., Rosner, G.L., Jones, R.J. & Luznik, L. (2019) Development of Grade II Acute Graft-versus-Host Disease Is Associated with Improved Survival after Myeloablative HLA-Matched Bone Marrow Transplantation using Single-Agent Post-Transplant Cyclophosphamide. *Biology of Blood and Marrow Transplantation*, **25**, 1128–1135.

Meslin, F., Thiery, J., Richon, C., Jalil, A. & Chouaib, S. (2007) Granzyme B-induced cell death involves induction of p53 tumor suppressor gene and its activation in tumor target cells. *Journal of Biological Chemistry*, **282**, 32991–32999.

Poire, X., Labopin, M., Maertens, J., Yakoub-Agha, I., Blaise, D., Ifrah, N., Socie, G., Gedde-Dhal, T., Schaap, N., Cornelissen, J.J., Vigouroux, S., Sanz, J., Michaux, L., Esteve, J., Mohty, M. & Nagler, A. (2017) Allogeneic stem cell transplantation in adult patients with acute myeloid leukaemia and 17p abnormalities in first complete remission: a study from the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT). *J Hematol Oncol*, **10**, 20.

Rampal, R., Ahn, J., Abdel-Wahab, O., Nahas, M., Wang, K., Lipson, D., Otto, G.A., Yelensky, R., Hricik, T., McKenney, A.S., Chiosis, G., Chung, Y.R., Pandey, S., van den Brink, M.R., Armstrong, S.A., Dogan, A., Intlekofer, A., Manshouri, T., Park, C.Y., Verstovsek, S., Rapaport, F., Stephens, P.J., Miller, V.A. & Levine, R.L. (2014) Genomic and functional analysis of leukemic transformation of myeloproliferative neoplasms. *Proc Natl Acad Sci U S A*, **111**, E5401–5410.

Ringden, O., Pavletic, S.Z., Anasetti, C., Barrett, A.J., Wang, T., Wang, D., Antin, J.H., Di Bartolomeo, P., Bolwell, B.J., Bredeson, C., Cairo, M.S., Gale, R.P., Gupta, V., Hahn, T., Hale, G.A., Halter, J., Jagasia, M., Litzow, M.R., Locatelli, F., Marks, D.I., McCarthy, P.L., Cowan, M.J., Petersdorf, E.W., Russell, J.A., Schiller, G.J., Schouten, H., Spellman, S., Verdonck, L.F., Wingard, J.R., Horowitz, M.M. & Arora, M. (2009) The graft-versus-leukemia effect using matched unrelated donors is not superior to HLA-identical siblings for hematopoietic stem cell transplantation. *Blood*, **113**, 3110–3118.

Schmid, C., Labopin, M., Nagler, A., Bornhauser, M., Finke, J., Fassas, A., Volin, L., Gurman, G., Maertens, J., Bordigoni, P., Holler, E., Ehninger, G., Polge, E., Gorin, N.C., Kolb, H.J., Rocha, V. & EBMT Acute Leukemia Working Party (2007) Donor lymphocyte infusion in the treatment of first hematological relapse after allogeneic stem-cell transplantation in adults with acute myeloid leukemia: a retrospective risk factors analysis and comparison with other strategies by the EBMT Acute Leukemia Working Party. *Journal of Clinical Oncology*, **25**, 4938–4945.

Scott, B.L., Pasquini, M.C., Logan, B.R., Wu, J., Devine, S.M., Porter, D.L., Maziarz, R.T., Warlick, E.D., Fernandez, H.F., Alyea, E.P., Hamadani, M., Bashey, A., Giralt, S., Geller, N.L., Leifer, E., Le-Rademacher, J., Mendizabal, A.M., Horowitz, M.M., Deeg, H.J. & Horwitz, M.E. (2017) Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. *Journal of Clinical Oncology*, **35**, 1154–1161.

Sengsayadeth, S., Labopin, M., Boumendil, A., Finke, J., Ganser, A., Stelljes, M., Ehninger, G., Beelen, D., Niederwieser, D., Blaise, D., Dreger, P., Mufti, G., Chevallier, P., Mailhol, A., Gatwood, K.S., Gorin, N., Esteve, J., Ciceri, F., Baron, F., Schmid, C., Giebel, S., Mohty, M., Savani, B.N. & Nagler, A. (2018) Transplant outcomes for secondary acute myeloid leukemia: acute leukemia working party of the european society for blood and bone marrow transplantation study. *Biology of Blood and Marrow Transplantation*.

Storer, B.E. (2009) Statistical considerations in studies of late effects in HCT. *Biology of Blood and Marrow Transplantation*, **15**, 25–28.

Suciu, S., Mandelli, F., de Witte, T., Zittoun, R., Gallo, E., Labar, B., De Rosa, G., Belhabri, A., Giustolisi, R., Delarue, R., Liso, V., Mirto, S., Leone, G., Bourhis, J.H., Fioritoni, G., Jehn, U., Amadori, S., Fazi, P., Hagemeijer, A. & Willemze, R. (2003) Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR32): an intention-to-treat analysis of the EORTC/GIMEMAAML-10 trial. *Blood*, **102**, 1232–1240.

Takahashi, K., Wang, F., Kantarjian, H., Doss, D., Khanna, K., Thompson, E., Zhao, L., Patel, K., Neelapu, S., Gumbs, C., Bueso-Ramos, C., DiNardo, C.D., Colla, S., Ravandi, F., Zhang, J., Huang, X., Wu, X., Samaniego, F., Garcia-Manero, G. & Futreal, P.A. (2017) Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. *The lancet Oncology*, **18**, 100–111.

Walker, I., Panzarella, T., Couban, S., Couture, F., Devins, G., Elemary, M., Gallagher, G., Kerr, H., Kuruvilla, J., Lee, S.J., Moore, J., Nevill, T., Popradi, G., Roy, J., Schultz, K.R., Szwajcer, D., Toze, C., Foley, R., Canadian, B. & Marrow Transplant, G. (2016) Pretreatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with haematological malignancies undergoing haemopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. *The lancet Oncology*, **17**, 164–173.

Weiden, P.L., Sullivan, K.M., Flournoy, N., Storb, R. & Thomas, E.D. (1981) Antileukemic effect of chronic graft-versus-host disease. Contribution to improved survival after allogeneic marrow transplantation. *New England Journal of Medicine*, **304**, 1529–1533.

Zeichner, S.B. & Arellano, M.L. (2015) Secondary Adult Acute Myeloid Leukemia: a Review of Our Evolving Understanding of a Complex Disease Process. *Current Treatment Options in Oncology*, **16**, 37.