UNIVERSITY OF BIRMINGHAM University of Birmingham Research at Birmingham

Molecular MRD status and outcome after transplantation in NPM1-mutated AML

Dillon, Richard; Hills, Robert K; Freeman, Sylvie D; Potter, Nicola; Jovanovic, Jelena; Ivey, Adam; Kanda, Anju Shankar; Runglall, Manohursingh; Foot, Nicola; Valganon, Mikel; Khwaja, Asim; Cavenagh, Jamie; Smith, Matthew L; Ommen, Hans Beier; Overgaard, Ulrik; Dennis, Mike; Knapper, Steven; Kaur, Harpreet; Taussig, David C; Mehta, Privanka

DOI: 10.1182/blood.2019002959

License: None: All rights reserved

Document Version Peer reviewed version

Citation for published version (Harvard):

Dillon, R, Hills, RK, Freeman, SD, Potter, N, Jovanovic, J, Ivey, A, Kanda, AS, Runglall, M, Foot, N, Valganon, M, Khwaja, A, Cavenagh, J, Smith, ML, Ommen, HB, Overgaard, U, Dennis, M, Knapper, S, Kaur, H, Taussig, DC, Mehta, P, Raj, K, Novitzky-Basso, I, Nikolousis, E, Danby, RD, Krishnamurthy, P, Hill, K, Finnegan, D, Alimam, S, Hurst, E, Johnson, P, Khan, AB, Salim, R, Craddock, CF, Ruth Lilian Spearing, D, Gilkes, AF, Gale, RE, Alan Kenneth Burnett, Russell, NH & Grimwade, D 2020, 'Molecular MRD status and outcome after transplantation in NPM1-mutated AML', Blood, vol. 135, no. 9, pp. 680-688. https://doi.org/10.1182/blood.2019002959

Link to publication on Research at Birmingham portal

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

•Users may freely distribute the URL that is used to identify this publication.

•Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.

•User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?) Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Molecular MRD status and outcome after transplantation in *NPM1* mutated AML: results from the UK NCRI AML17 study

Richard Dillon^{1,4,14}, Robert Hills², Sylvie Freeman³, Nicola Potter^{1,4}, Jelena Jovanovic¹, Adam Ivey¹, Anju Kanda¹, Manohursingh Runglall¹, Nicola Foot⁴, Mikel Valganon⁴, Asim Khwaja⁵, Jamie Cavenagh⁶, Matthew Smith⁶, Hans Beier Ommen⁷, Ulrik Malthe Overgaard⁸, Mike Dennis⁹, Steven Knapper¹⁰, Harpreet Kaur¹¹, David Taussig¹², Priyanka Mehta¹³, Kavita Raj¹⁴, Igor Novitzky-Basso¹⁵, Manos Nikolousis¹⁶, Robert Danby¹⁷, Pramila Krishnamurthy¹⁸, Kate Hill¹⁹, Damian Finnegan²⁰, Samah Alimam^{1,14}, Erin Hurst²¹, Peter Johnson²², Anjum Khan²³, Rahuman Salim²⁴, Charles Craddock²⁵, Ruth Spearing²⁶, Amanda Gilkes¹⁰, Rosemary Gale⁵, Alan Burnett²⁷, Nigel Russell²⁸ and David Grimwade^{1,14}, on behalf of the UK National Cancer Research Institute Acute Myeloid Leukaemia Working Group*

- 1) Department of Medical and Molecular Genetics, King's College, London, UK
- 2) Nuffield Department of Population Health, University of Oxford, UK
- 3) Institute of Immunology and Immunotherapy, University of Birmingham, UK
- 4) Cancer Genetics Service, Viapath, Guy's Hospital, London, UK
- 5) University College, London, UK
- 6) Bart's Hospital, London, UK
- 7) University Hospital, Aarhus, Denmark
- 8) Rigshospitalet, Copenhagen, Denmark
- 9) Christie Hospital, Manchester, UK
- 10) Cardiff University, Cardiff, UK
- 11) Royal Hallamshire Hospital, Sheffield, UK
- 12) Royal Marsden Hospital, Sutton, UK
- 13) Bristol Haematology and Oncology Centre, Bristol, UK
- 14) Guy's Hospital, London, UK
- 15) Beatson Cancer Centre, Glasgow, UK
- 16) Heartlands Hospital, Birmingham, UK
- 17) Churchill Hospital, Oxford, UK
- 18) Addenbrookes, Hospital, Cambridge, UK
- 19) University Hospital, Southampton, UK
- 20) Belfast City Hospital, Belfast, UK
- 21) Royal Victoria Infirmary, Newcastle, UK
- 22) Western General Hospital, Edinburgh, UK
- 23) St James' Hospital, Leeds, UK
- 24) Clatterbridge Cancer Centre, Liverpool, UK
- 25) Queen Elizabeth Hospital, Birmingham
- 26) Christchurch Hospital, Christchurch, New Zealand
- 27) Blackwaterfoot, Isle of Arran, UK
- 28) Nottingham University Hospital, Nottingham, UK

* Members of the study group are listed in the Supplementary Appendix

Key points

Pre-transplant MRD level is highly predictive of outcome, thresholds of 200 copies / 10⁵ ABL in PB and 1000 copies in BM are discriminatory.

Relapse in patients with pre-transplant MRD positivity below these levels is largely restricted to those with *FLT3* ITD.

Abstract

Relapse remains the most common cause of treatment failure for patients with acute myeloid leukaemia (AML) who undergo allogeneic stem cell transplantation (alloSCT) and carries a grave prognosis. Multiple studies have identified the presence of measurable residual disease (MRD) assessed by flow cytometry (FCM) prior to alloSCT as a strong predictor of relapse, but it is not clear how these findings apply to patients who test positive in molecular MRD assays which have far greater sensitivity. We analysed pre-transplant blood and bone marrow samples by reverse-transcription polymerase chain reaction (RTgPCR) in 107 patients with NPM1 mutant AML enrolled in the UK National Cancer Research Institute (NCRI) AML17 study. After a median follow-up of 4.9 years, patients with negative, low (<200 copies / 10⁵ ABL in the PB and <1000 copies in the BM) and high levels of MRD had an estimated 2y overall survival (OS) of 83%, 63% and 13% respectively (p<0.0001). Focussing on patients with low level MRD prior to alloSCT, those with FLT3 ITD had significantly poorer outcome (hazard ratio, HR, 6.14, p=0.01). Combining these variables was highly prognostic, dividing patients into two groups with 2y OS of 17% and 82% (HR 13.2, p<0.0001). T-depletion was associated with significantly reduced survival both in the entire cohort (2v OS 56% vs 96%, HR 3.24, p=0.0005) and in MRD positive patients (2v OS 34% vs 100%, HR 3.78, p=0.003) but there was no significant effect of either conditioning regimen or donor source on outcome. Registered at ISRCTN (http://www.isrctn.com/ISRCTN55675535).

Introduction

Optimal therapy for patients with cytogenetically normal acute myeloid leukaemia (AML) remains controversial, particularly regarding the use of allogeneic stem cell transplantation (alloSCT)¹⁻³. Many recent studies have identified the presence of measurable residual disease detected by polymerase chain reaction (PCR)⁴⁻¹⁰, flow cytometry (FCM)¹¹⁻¹⁶ or next-generation sequencing (NGS)¹⁷⁻¹⁹ as a powerful predictor of outcome and MRD status is increasingly used to allocate patients for transplantation²⁰⁻²³, however peri-transplant management of MRD positive patients remains highly challenging.

Multiple studies have identified the presence of measurable residual disease (MRD) assessed by FCM²⁴⁻³², abnormal gene expression^{33,34} and NGS^{35,36} immediately prior to alloSCT as a strong predictor of adverse outcome; patients who test positive using these methods have a relapse risk of up to 69%³². As relapse after alloSCT carries a grave prognosis³⁷ there is growing interest in peri-transplant interventions to reduce or eliminate MRD, which might diminish relapse risk^{31,38}. In this regard, the effect of different conditioning regimens on the outcome of patients who are MRD positive remains uncertain^{28,39,40}.

Although the great majority of studies of pre-transplant MRD in AML have utilised FCM, over half of patients with cytogenetically normal AML harbour mutations in the gene encoding nucleophosmin (*NPM1*)^{41,42}. The recommended method for MRD assessment in these patients is reverse-transcription quantitative PCR (RT-qPCR)⁴³ which affords a sensitivity of 1:10⁻⁵ - 1:10⁻⁶ i.e. 100-1000 fold greater than that achieved by FCM or NGS⁴⁻¹⁰. Thus, the strongly adverse outcome reported in patients who are MRD positive using FCM and NGS may not be applicable to *NPM1* mutated patients with low level positivity by PCR. Despite this, few studies have examined the impact of pre-transplant *NPM1* MRD status^{44,45}. Absence of robust outcome data for these patients is a barrier both to rational clinical decision making and to planning interventional studies in this setting.

In this study, we report the outcomes of a large cohort of patients with *NPM1* mutated AML treated on the NCRI AML17 protocol who had pre-transplant molecular MRD assessment.

Methods

Patients.

Between 2009-2014 the NCRI AML17 study (ISRCTN 55675535) enrolled 3215 patients with non-M3 AML aged 16-77 eligible for intensive chemotherapy. This study was approved by the Medical Research Ethics Committee for Wales, and conducted in accordance with the Declaration of Helsinki. The treatment protocol has been described previously⁴⁶. Central screening for NPM1 mutations was positive in 861/2949 (29%) and 530 of these provided serial samples for MRD monitoring. Paired blood (PB) and bone marrow aspirates (BM) were requested on regeneration after each cycle of chemotherapy and then every three months. Post-remission treatment was determined according to the validated NCRI risk score, with poor-risk patients recommended for allogeneic stem-cell transplantation (alloSCT) during first complete remission (CR1). Further information regarding calculation of the NCRI risk score is provided in the supplementary appendix. For patients receiving a transplant, additional samples were requested prior to alloSCT, at D+30 and D+100 and then at three-monthly intervals for at least two years. For this study, pre-SCT results were included if the sample was taken within 60 days before transplant and the patient had not received any further therapy between sampling and the start of conditioning. Results were issued to treating clinicians from June 2012 only (i.e. 51/107 patients) and after this time patients could be treated for confirmed re-emergent or persistent molecular positivity.

Amplification of NPM1 mutated transcripts.

Samples were analysed by RT-qPCR as previously described⁴. Briefly, RNA was isolated using Trizol reagent (Life Technologies, Carlsbad, CA) and reverse transcribed using ThermoScript (Life Technologies). Primer and probe sets described by Gorello et al⁷ were used to amplify *NPM1* type A, B and D mutant transcripts and patient specific primers were designed to detect rare mutations. Samples were run in triplicate using an ABI 7900 thermocycler (Life Technologies) with parallel amplification of a control gene (*ABL*). Samples with *ABL* cycle threshold of \geq 30 were excluded. Criteria proposed by the Europe Against Cancer programme⁴⁷ were used to define MRD positivity (i.e. amplification in at least two of three replicates with cycle-threshold values of 40 or less using a threshold setting of 0.1). Molecular relapse was diagnosed if there were two consecutive positive samples showing increasing transcript levels in a patient who had previously tested MRD negative in a technically adequate sample, consistent with ELN guidelines⁴³. All *NPM1* expression levels are reported as the number of mutated transcripts per 10⁵ copies of *ABL*.

Analysis of FLT3 ITD status and allelic ratio.

PCR amplification of exons 14 and 15 of *FLT3* was performed using fluorescently labelled primers and analysed using capillary electrophoresis as previously described⁴⁸. The allelic ratio was determined by comparing the areas under the curves from the mutated and wild-type products.

Statistical Analyses.

Kaplan-Meier estimates were used to calculate survival percentages. Time to event analysis was performed using the log rank test. Thresholds were selected by identifying cut-offs providing the maximum discrimination between the low and high positive groups in terms of the hazard ratio for overall survival (supplementary figure 4). The threshold could not be zero and if a number of thresholds produced the same hazard ratio, the highest of these levels was selected. We used Cox regression with forward selection to identify independent prognostic factors. Categorical variables were analysed using Fisher's exact test. All reported P values are two-sided.

Results

In total 107/ 530 patients received alloSCT: 56 (52%) in CR1, 30 (28%) after molecular relapse (MR) and 21 (20%) in second remission after morphological relapse (CR2) (figure 1). Clinical and molecular details are shown in table 1. Median follow-up was 4.9 years from transplant (range 1.0 - 8.4y). Forty-two (39%) patients died, the cause of death was disease relapse in 21 patients, was not attributed to relapse in 19 and could not be determined in 2 patients. Overall survival 2 years from the date of transplant (2y-OS) was 68% for patients transplanted in CR1 without molecular relapse, 63% for those transplanted after a molecular relapse and 57% for those in CR2 at the time of transplant. There were no statistically significant differences in survival between these groups (p=0.25 for CR1 vs others, p=0.63 for molecular vs haematological relapse, p=0.22 for CR1 vs CR2, overall p=0.2 for trend, supplementary figure 1).

Evaluable pre-SCT PB and BM samples taken in the 60 days preceding SCT were available for 103 and 78 patients, both were available for 74 patients. The median time between sampling and transplant was 29 days (range 5-57 days). In total, 58 (54%) patients were MRD negative prior to SCT; 48 patients received additional chemotherapy prior to SCT for molecular (n=27) or haematological relapse (n=21) and 27/48 (56%) achieved MRD negativity (figure 1).

Survival according to pre-transplant molecular MRD status

Overall survival 2 years from allograft was 83% for MRD negative patients versus 45% for patients with any detectable MRD by RT-qPCR in the pre-transplant samples; median OS (mOS) was not reached (NR) and 10.5 months respectively (hazard ratio, HR, 3.60 95% confidence interval, CI, 1.92-6.77, p<0.0001, figure 2a). For patients with negative pre-SCT PB samples (n=73) 2y-OS was 81%, compared with 30% for patients with any PB positivity (n=30) (HR 8.30, CI 3.77-18.20, p<0.0001, fig 2b); mOS was NR and 7.4 months. Patients with a negative pre-SCT BM (n=37) had a 5y-OS of 84% compared with 49% if the BM was MRD positive (n=41); mOS was NR and 13.1 months (HR 3.17, CI 1.54-6.54, p=0.002, figure 2c).

For those patients who relapsed after transplant (n=21) the median time from relapse to death was 34 days (range 3-344 days, supplementary figure 2) and consequently overall and relapse-free survival times were similar. Relapse free survival curves are shown in supplementary figure 3.

A threshold of 200 mutant *NPM1* transcripts / 10^5 ABL copies in the pre-SCT PB sample provided maximum additional discrimination (supplementary figure 4) and split patients into three groups with 2y-OS of 81% (negative, n=73, mOS NR), 54% (low, 0.1-200 copies, n=13, mOS NR) and 12% (high, >200 copies, n=17, mOS 6.5 months, HR by group 2.81, CI 1.96-4.02, p<0.0001, figure 2d, supplementary figure 3d).

In the BM, a threshold of 1000 copies provided maximum additional discrimination (supplementary figure 4) and defined 3 groups with 2y-OS of 84% (negative, n=37, mOS NR), 56% (low, 0.1-1000 copies, n=32, mOS NR) and 22% (high, >1000 copies, n=9, mOS 5.8 months, HR by group 2.87, CI 1.69-4.86, p<0.0001, figure 2e, supplementary figure 3e).

Overall (applying the higher level where there was a discrepancy between PB and BM results), 2y-OS was 83% (n=58, mOS NR) 63% (n=30, mOS NR) and 13% (n=19, mOS 6.5 months) for patients with negative, low and high levels of MRD (HR by group 2.83, CI 1.92-4.19, p<0.0001, figure 2f, supplementary figure 3f).

Impact of FLT3 status on post-transplant outcome

We next stratified patients according *FLT3* ITD status. Thirty-four patients were positive for *FLT3*-ITD at diagnosis and 73 were negative; 2y-OS was 62% and 67% respectively (HR 1.14, CI 0.59-2.19, p=0.7). *FLT3* ITD status was not associated with outcome in patients who were MRD negative before transplant (2y-OS ITD negative 77%, n=40, ITD positive 94%, n=18, HR 0.42, CI 0.14-1.28, p=0.13) or those who had high levels of MRD (2y-OS ITD negative 0%, n=11, ITD positive 25%, n=8, mOS 5.8 vs 6.8 months, HR 0.71 CI 0.26-1.92, p=0.5). In contrast for patients with low levels of MRD, *FLT3* ITD status was strongly associated with outcome: 2y-OS was 77% for ITD negative (n=22, mOS NR) and 25% for ITD positive patients (n=8, mOS 7.1 months, HR 6.14 CI 1.50-25.13, p=0.01, figure 3).

Owing to small numbers (n=8), we were unable to reliably assess the effect of *FLT3* ITD allelic ratio. Although a trend for better survival for patients with an allelic ratio <0.5 was apparent, this was not statistically significant (p=0.25, supplementary figure 5).

Impact of first line post-induction MRD status on post-transplant outcome

Peripheral blood MRD status after the second induction cycle of first line therapy (PBPC2) has previously been shown to be highly prognostic⁴ and retained power in this cohort (2y OS 76% vs 33% for PBPC2 negative and positive patients, mOS NR vs 9.6 months, HR 4.93, Cl 2.05-11.90, p=0.0004). There was an association between PBPC2 and pre-SCT MRD negativity (p=0.002, table 1). Of those patients who were PBPC2 negative and experienced molecular or haematological relapse, 60% (21/35) achieved MRD negativity following salvage therapy and a further 14% (5/35) were MRD positive at levels below the thresholds defined above and were *FLT3* WT; 2y OS for these patients was 88%.

Multivariable model for prediction of post-transplant outcome

We performed a multivariate analysis taking into account remission status at time of transplant (CR1 vs other), age at time of transplant, *FLT3* ITD status, PBPC2 status and pre-transplant MRD level (negative, low or high). The only factors which retained independent prognostic power were age (HR per decade 1.54, CI 1.08-2.19 p=0.02) and pre-transplant MRD level (HR 3.02, CI 1.97-4.62, p<0.0001).

We developed a two-group prognostic model incorporating MRD status (negative, low or high) and *FLT3* ITD (positive or negative). Patients who had high levels of MRD were allocated to the high-risk group together with patients with low levels of MRD who had *FLT3* ITD at diagnosis. The remaining patients were allocated to the low risk group. Patients with a negative PB and absent BM sample could not be reliably allocated to a risk group and were excluded from this analysis. There was sufficient information to assign a risk group in 83 patients. In the low-risk group (n=56) 2y-OS was 82% compared to 17% in the high-risk group (n=27, mOS NR vs 6.5 months, HR 13.2, CI 5.80-30.2, p<0.0001, figure 4, supplementary figure 6).

When risk group (low or high) was introduced as a candidate variable into the multivariable model described above, the only factors to retain prognostic significance were age at time of transplant (HR per decade 1.60, CI 1.08-2.37, p=0.02) and risk group (HR 9.5, CI 4.24-21.42, p<0.0001).

Effect of transplant-related factors on outcome according to MRD status

Donor source was a matched sibling in 43 patients, a volunteer unrelated donor (VUD) in 63 and umbilical cord blood in 1. Although a trend for greater overall survival in patients whose donor was a sibling compared to a VUD was noted, this was not statistically significant (2y-OS 72% vs 62%, HR 1.81, CI 0.97-3.35, p=0.06, figure 5a).

Conditioning regimens were considered myeloablative (MAC) in 30 patients (28%, BuCy 4, CyTBI 20, FB4C 6) and reduced-intensity (RIC) in 77 (72%, FluMel 48, FluBu 11, FLAMSA-Bu 8, FluTBI 6, FluCy 2, FluCyTBI 2). Patients who received MAC were significantly younger (mean 43 vs 56 years p<0.0001). There was no difference in overall survival according to conditioning regimen type (2y OS MAC 71%, RIC 63%, HR 1.18, CI 0.61-2.29, p=0.6, figure 5b).

Alemtuzumab was given to 70 (65%) and anti-thymocyte globulin (ATG) to 12 (11%) patients for T-depletion; 2y-OS was 56% for these patients with no difference by T-depletion agent, compared to 96% in patients who did not receive T-depletion (n=25, HR 3.24, Cl 1.69-6.42, p=0.0005, figure 5c). Patients who received T-depletion were older (mean 54 vs 47y, p=0.0028) and were more likely to have been transplanted using a VUD (67% vs 33% for non-T-depleted, p=0.004) and with RIC (80% vs 44% for non-T depleted, p=0.0008). Cumulative incidence of relapse at 5 years was 24% in patients who underwent T-depletion compared with 4% in those who did not (p=0.0149). Cumulative incidence of non-relapse mortality at 5 years was 23% in patients who underwent T-depletion compared with 4% in patients who did not (p=0.0148).

Considering patients who were MRD positive prior to allograft, there was a trend for lower OS in patients who had received a VUD transplant (2y-OS 38% vs 55% for SIB, HR 1.94, CI 0.92-4.08, p=0.08, figure 5d). There was no association between OS and type of conditioning (2y OS 50% for MAC vs 43% for RIC, HR 1.22, CI 0.54-2.76, p=0.6, figure 5e). Specifically, MRD positive patients treated with the sequential FLAMSA protocol had no difference in overall survival. Patients who were MRD positive and who received T-depletion showed inferior overall survival than those who did not (2y-OS 34% vs 100%, mOS 7.8m vs NR, HR 3.78, CI 1.57-19.2, p=0.003, figure 5f).

Discussion

Patients with *NPM1* mutated AML who test MRD negative by RT-qPCR prior to transplant have an excellent chance of long-term survival regardless of other risk factors including *FLT3* status and independent of the intensity of the transplant conditioning regimen.

As expected, *NPM1* MRD positivity had an overall adverse effect on transplant outcome, but in contrast to patients who are MRD positive by FCM or NGS, patients who test positive for *NPM1* mutant transcripts prior to alloSCT do not have a universally poor outcome, indeed many become long-term survivors. Factors associated with adverse outcome are high levels of MRD (above 200 copies / 10^5 *ABL* in the PB or 1000 copies in the BM) and the presence of a *FLT3* ITD mutation at diagnosis. Patients who are MRD positive before transplant and have one or both of these features have a poor prognosis due to a high risk of disease relapse. Our data do not exclude the possibility that MRD positive patients with a low *FLT3* ITD allelic ratio may have a somewhat better outcome and larger studies will be required to address this.

Interestingly the threshold we identified of 1000 copies / 10^5 ABL in the bone marrow is the same as that selected by Kayser and colleagues⁴⁵ for their study of 39 patients with *NPM1* mutated AML in first or second morphological complete remission prior to transplant. In that study, the outcomes of patients with levels exceeding the threshold was the same as patients who were not in remission. No effect of either *FLT3* ITD status or allelic ratio was identified however we speculate that this may have been due to the sample size and indeed the only relapse observed in the group with MRD levels below the threshold was in a patient with *FLT3* ITD which would be consistent with our findings. Bill and colleagues⁴⁶ also report a significant difference in outcome according to molecular MRD status in 51 patients with *NPM1* mutated AML. In this study a lower threshold equivalent to 10 copies / 10^5 *ABL* was selected based on the technical characteristics of the digital droplet PCR platform employed, however this was applied to both PB and BM samples, and alternative cut-off levels were not comprehensively evaluated. Comparative studies of these two highly sensitive platforms would be of great interest.

Shayegi and colleagues¹⁰ identified post-transplant MRD levels equivalent to 10,000 copies / 10⁵ ABL as strongly predictive of relapse. In the present study we did not investigate post-transplant MRD levels as these were not available for all patients, however approximately half of patients had post-transplant MRD results provided to their treating physician and these were used to inform interventions such as immunosuppressive therapy and donor

lymphocyte infusion. These manipulations may have affected the outcome for some patients and it is possible that they reduced the overall survival difference between the MRD positive and negative groups. Nevertheless, we did not observe a difference in overall survival between patients who did or did not have post-transplant results returned (data not shown).

In this study 27/48 (56%) of patients with a haematological or confirmed molecular relapse achieved MRD negativity with salvage chemotherapy and a further 8/48 (17%) became low risk as defined in our risk score. These 35 patients had an excellent outcome with an overall survival of 80% at 2 years. Additionally, 74% of patients who tested negative for MRD in the PB after second induction (PBPC2) and who subsequently relapsed achieved MRD negativity or low-risk status after salvage and had a 2y OS of 88%. This supports the approach adopted in the current NCRI AML19 protocol where such patients are not recommended for transplantation in CR1 and are offered sequential MRD monitoring to allow early detection and treatment of relapse.

Selection of transplant protocol remains controversial, particularly for patients who are MRD positive, and a key question is whether augmented conditioning can eliminate MRD and thereby improve outcome. Studies to date have provided conflicting results and have not examined the effect of transplant related factors specifically in *NPM1* mutated patients^{28,39,40,49}. Surprisingly, we observed no effect on survival according to conditioning type, either overall or in patients who were MRD positive. In contrast, we observed a strong association between use of T-depletion and adverse outcome. Relatively few patients who were MRD positive received T-replete transplants (n=8) and this retrospective non-randomised analysis clearly has significant limitations, however these results highlight T-depletion as a potentially critical factor which has not been consistently reported in other studies to date.

These findings require independent validation, however patients at highest risk of relapse identified here may benefit from an alternative approach prior to transplant such as the use of *FLT3* inhibition to reduce the level of MRD below the thresholds identified. Alternatively, augmentation of the graft-versus-leukaemia effect (e.g. through avoidance or minimisation of T-depletion, early withdrawal of immunosuppression and / or donor lymphocyte infusion) may be considered. Randomised studies to investigate these approaches are urgently required.

Acknowledgements

This study was funded by a programme grant from the UK National Institute for Health Research (NIHR) and a Clinical Trial Award from Cancer Research UK. In addition, RD is supported by Bloodwise and JJ is supported by Children With Cancer. We gratefully acknowledge assistance from the Cardiff University Centre for Trials Research (Ian Thomas, Sophie Betteridge, Laura Upton, Leona Batten, Melanie Varley) and sincerely thank all clinicians and patients for their participation in NCRI AML17.

Authorship contributions

Molecular analysis and interpretation: RD, NP, JJ, AK, MR, NF, MV, AG, RG, NR, DG. Statistical analysis: RD, RH. Clinical data collection: AK, JC, MS, HBO, UMO, MD, SK, HK, DT, PM, KR, INB, MN, RD, PK, KH, DF, SA, EH, PJ, AK, RSa, RSp, AB, NR, CC. Trial coordination: NR, AB, SK, MD. Manuscript preparation: RD, SF, NR, RH

Disclosure of conflicts of interest

The authors have no relevant conflicts of interest to declare.

Data sharing

Publication-related data are available on reasonable request by email from the corresponding author.

References

1. Burnett AK, Goldstone A, Hills RK, et al. Curability of patients with acute myeloid leukemia who did not undergo transplantation in first remission. J Clin Oncol 2013;31:1293-301.

2. Cornelissen JJ, Blaise D. Hematopoietic stem cell transplantation for patients with AML in first complete remission. Blood 2016;127:62-70.

3. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424-47.

4. Ivey A, Hills RK, Simpson MA, et al. Assessment of Minimal Residual Disease in Standard-Risk AML. N Engl J Med 2016;374:422-33.

5. Balsat M, Renneville A, Thomas X, et al. Postinduction Minimal Residual Disease Predicts Outcome and Benefit From Allogeneic Stem Cell Transplantation in Acute Myeloid Leukemia With NPM1 Mutation: A Study by the Acute Leukemia French Association Group. J Clin Oncol 2017;35:185-93.

6. Kronke J, Schlenk RF, Jensen KO, et al. Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. J Clin Oncol 2011;29:2709-16.

7. Gorello P, Cazzaniga G, Alberti F, et al. Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. Leukemia 2006;20:1103-8.

8. Chou WC, Tang JL, Wu SJ, et al. Clinical implications of minimal residual disease monitoring by quantitative polymerase chain reaction in acute myeloid leukemia patients bearing nucleophosmin (NPM1) mutations. Leukemia 2007;21:998-1004.

9. Schnittger S, Kern W, Tschulik C, et al. Minimal residual disease levels assessed by NPM1 mutation-specific RQ-PCR provide important prognostic information in AML. Blood 2009;114:2220-31.

10. Shayegi N, Kramer M, Bornhauser M, et al. The level of residual disease based on mutant NPM1 is an independent prognostic factor for relapse and survival in AML. Blood 2013;122:83-92.

11. Freeman SD, Hills RK, Virgo P, et al. Measurable Residual Disease at Induction Redefines Partial Response in Acute Myeloid Leukemia and Stratifies Outcomes in Patients at Standard Risk Without NPM1 Mutations. J Clin Oncol 2018;36:1486-97.

12. Freeman SD, Virgo P, Couzens S, et al. Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. J Clin Oncol 2013;31:4123-31.

13. Terwijn M, van Putten WL, Kelder A, et al. High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study. J Clin Oncol 2013;31:3889-97.

14. Ravandi F, Jorgensen J, Borthakur G, et al. Persistence of minimal residual disease assessed by multiparameter flow cytometry is highly prognostic in younger patients with acute myeloid leukemia. Cancer 2017;123:426-35.

15. Othus M, Wood BL, Stirewalt DL, et al. Effect of measurable ('minimal') residual disease (MRD) information on prediction of relapse and survival in adult acute myeloid leukemia. Leukemia 2016;30:2080-3.

16. Buccisano F, Maurillo L, Spagnoli A, et al. Cytogenetic and molecular diagnostic characterization combined to postconsolidation minimal residual disease assessment by flow cytometry improves risk stratification in adult acute myeloid leukemia. Blood 2010;116:2295-303.

17. Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular Minimal Residual Disease in Acute Myeloid Leukemia. N Engl J Med 2018;378:1189-99.

18. Morita K, Kantarjian HM, Wang F, et al. Clearance of Somatic Mutations at Remission and the Risk of Relapse in Acute Myeloid Leukemia. J Clin Oncol 2018;36:1788-97.

19. Klco JM, Miller CA, Griffith M, et al. Association Between Mutation Clearance After Induction Therapy and Outcomes in Acute Myeloid Leukemia. JAMA 2015;314:811-22.

20. Craddock C, Raghavan M. Which patients with acute myeloid leukemia in CR1 can be spared an allogeneic transplant? Curr Opin Hematol 2018.

21. Ossenkoppele G, Schuurhuis GJ. MRD in AML: does it already guide therapy decision-making? Hematology Am Soc Hematol Educ Program 2016;2016:356-65.

22. Rowe JM. AML in 2017: Advances in clinical practice. Best Pract Res Clin Haematol 2017;30:283-6.

23. Buccisano F, Dillon R, Freeman SD, Venditti A. Role of Minimal (Measurable) Residual Disease Assessment in Older Patients with Acute Myeloid Leukemia. Cancers (Basel) 2018;10.

24. Anthias C, Dignan FL, Morilla R, et al. Pre-transplant MRD predicts outcome following reduced-intensity and myeloablative allogeneic hemopoietic SCT in AML. Bone Marrow Transplant 2014;49:679-83.

25. Araki D, Wood BL, Othus M, et al. Allogeneic Hematopoietic Cell Transplantation for Acute Myeloid Leukemia: Time to Move Toward a Minimal Residual Disease-Based Definition of Complete Remission? J Clin Oncol 2016;34:329-36.

26. Walter RB, Gooley TA, Wood BL, et al. Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. J Clin Oncol 2011;29:1190-7.

27. Walter RB, Buckley SA, Pagel JM, et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. Blood 2013;122:1813-21.

28. Walter RB, Gyurkocza B, Storer BE, et al. Comparison of minimal residual disease as outcome predictor for AML patients in first complete remission undergoing myeloablative or nonmyeloablative allogeneic hematopoietic cell transplantation. Leukemia 2015;29:137-44.

29. Bastos-Oreiro M, Perez-Corral A, Martinez-Laperche C, et al. Prognostic impact of minimal residual disease analysis by flow cytometry in patients with acute myeloid leukemia before and after allogeneic hemopoietic stem cell transplantation. Eur J Haematol 2014;93:239-46.

30. Buckley SA, Wood BL, Othus M, et al. Minimal residual disease prior to allogeneic hematopoietic cell transplantation in acute myeloid leukemia: a meta-analysis. Haematologica 2017;102:865-73.

31. Buckley SA, Appelbaum FR, Walter RB. Prognostic and therapeutic implications of minimal residual disease at the time of transplantation in acute leukemia. Bone Marrow Transplant 2013;48:630-41.

32. Zhou Y, Othus M, Araki D, et al. Pre- and post-transplant quantification of measurable ('minimal') residual disease via multiparameter flow cytometry in adult acute myeloid leukemia. Leukemia 2016;30:1456-64.

33. Goswami M, McGowan KS, Lu K, et al. A multigene array for measurable residual disease detection in AML patients undergoing SCT. Bone Marrow Transplant 2015;50:642-51.

34. Jentzsch M, Bill M, Grimm J, et al. High BAALC copy numbers in peripheral blood prior to allogeneic transplantation predict early relapse in acute myeloid leukemia patients. Oncotarget 2017;8:87944-54.

35. Getta BM, Devlin SM, Levine RL, et al. Multicolor Flow Cytometry and Multigene Next-Generation Sequencing Are Complementary and Highly Predictive for Relapse in Acute Myeloid Leukemia after Allogeneic Transplantation. Biol Blood Marrow Transplant 2017;23:1064-71.

36. Thol F, Gabdoulline R, Liebich A, et al. Measurable residual disease monitoring by NGS before allogeneic hematopoietic cell transplantation in AML. Blood 2018;132:1703-13.

37. Schmid C, Labopin M, Nagler A, et al. Treatment, risk factors, and outcome of adults with relapsed AML after reduced intensity conditioning for allogeneic stem cell transplantation. Blood 2012;119:1599-606.

38. Craddock C, Hoelzer D, Komanduri KV. Current status and future clinical directions in the prevention and treatment of relapse following hematopoietic transplantation for acute myeloid and lymphoblastic leukemia. Bone Marrow Transplant 2019;54:6-16.

39. Milano F, Gooley T, Wood B, et al. Cord-Blood Transplantation in Patients with Minimal Residual Disease. N Engl J Med 2016;375:944-53.

40. Hourigan CS, Haferlach T, Hokland P. Cord-Blood Transplantation in Patients with Minimal Residual Disease. N Engl J Med 2016;375:2204.

41. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 2005;352:254-66.

42. Schlenk RF, Dohner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med 2008;358:1909-18.

43. Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. Blood 2018;131:1275-91.

44. Bill M, Grimm J, Jentzsch M, et al. Digital droplet PCR-based absolute quantification of pre-transplant NPM1 mutation burden predicts relapse in acute myeloid leukemia patients. Ann Hematol 2018;97:1757-65.

45. Kayser S, Benner A, Thiede C, et al. Pretransplant NPM1 MRD levels predict outcome after allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia. Blood Cancer J 2016;6:e449.

46. Knapper S, Russell N, Gilkes A, et al. A randomized assessment of adding the kinase inhibitor lestaurtinib to first-line chemotherapy for FLT3-mutated AML. Blood 2017;129:1143-54.

47. Gabert J, Beillard E, van der Velden VH, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. Leukemia 2003;17:2318-57.

48. Gale RE, Green C, Allen C, et al. The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood 2008;111:2776-84.

49. Ustun C, Courville EL, DeFor T, et al. Myeloablative, but not Reduced-Intensity, Conditioning Overcomes the Negative Effect of Flow-Cytometric Evidence of Leukemia in Acute Myeloid Leukemia. Biol Blood Marrow Transplant 2016;22:669-75.

Table Legends

Table 1.

Clinical, molecular and transplant-related variables in each MRD-defined group. Spearman correlation *p* value is provided for the age comparison and Mantel-Haenszel *p* value is provided for all other variables. CR1 first complete remission. PB peripheral blood

Table 1

Pre-transplant MRD status	High n=19	Low n=30	Negative n=58	р
Median age, years Range	53 40-69	53 17-65	54 24-66	1.0
FLT3 ITD positive	8 (42%)	8 (27%)	18 (31%)	0.5
FLT3 ITD allelic ratio >0.5	4 (21%)	3 (10%)	7 (12%)	0.4
PB Post #2 MRD positive	6/17 (35%)	11/26 (42%)	4/53 (8%)	0.002
Transplanted in CR1	5 (26%)	20 (67%)	31 (53%)	0.16
Myeloablative conditioning	8 (42%)	6 (20%)	17 (29%)	0.5
Sibling donor	6 (32%)	14 (47%)	23 (40%)	0.7
T cell depletion	16 (84%)	25 (83%)	41 (71%)	0.15

Figure Legends

Figure 1.

CONSORT diagram showing the number of patients in each part of the trial, therapy given prior to transplant and outcomes in each group. CT chemotherapy, MRD measurable residual disease, NRM non-relapse mortality, REL relapse, UNK unknown cause of death.

Figure 2.

Overall survival from date of transplant according to pre-transplant molecular MRD status.

Panels A-C show the difference in survival between patients with positive and negative MRD (A) overall (B) in the peripheral blood, (C) in the bone marrow. Panels D-F show the difference in survival between patients with negative, low and high levels of MRD (D) in the peripheral blood using a cut-off at 200 copies per $10^5 ABL$ (E) in the bone marrow with level of >1000 copies and (E) with either, defining "high level" MRD. Percentages indicate estimated 2 year OS.

Figure 3.

Effect of *FLT3* ITD on outcome according to pre-transplant MRD status. (A) Hazard ratio and 95% confidence intervals for *FLT3* ITD mutation in each group. (B-D) Overall survival from transplant for patients with high (B), low (C) and negative (D) pre-transplant MRD. Percentages indicate estimated 2 year overall survival.

Figure 4.

Overall survival from transplant according to the risk group. The risk group was derived from *FLT3* ITD status and pre-transplant MRD level. Patients with high levels of MRD, and those with low levels who had the *FLT3* ITD mutation were allocated to the high-risk group. All other patients were allocated to the low-risk group. Percentages indicate estimated 2 year overall survival.

Figure 5.

Effect of transplant-related factors on overall survival. Panels A-C show the effect of transplant related variables in the entire cohort, panels D-F show their effect in patients who were MRD-positive prior to transplantation. (A,D) Effect of donor source. (B,E) Effect of conditioning type. (C,F) Effect of T-cell depletion. SIB sibling donor, VUD volunteer unrelated donor, MAC myeloablative conditioning, RIC reduced intensity conditioning.