Molecular MRD assessment is strongly prognostic in patients with *NPM1*-mutated AML receiving venetoclax-based non-intensive therapy

Abstract
Assessment of measurable residual disease (MRD) by RT-qPCR is strongly prognostic in patients with *NPM1* mutated AML treated with intensive chemotherapy, however there are currently no data regarding its utility in patients undergoing venetoclax-based non-intensive therapy, despite high reported efficacy in this genotype. Here we analysed the prognostic impact of *NPM1* MRD using a multinational real-world cohort of 73 previously untreated patients with *NPM1* mutated AML achieving CR/CRi following treatment with venetoclax and azacitidine or low dose cytarabine. Forty patients (55%) achieved MRD negativity and a further 14 (19%) a reduction of <4 $\log_{10}$ from baseline as their best response. Most patients (90%) achieved their deepest response by the end of cycle 4. Patients achieving bone marrow MRD negativity by this time point had a 2-year overall (OS) of 80% compared to 44% in those remaining MRD positive. On multivariable analysis MRD status was the strongest prognostic factor. 21 patients electively stopped therapy after a median of X months in MRD negative remission with 2-year treatment-free RFS of 88%. In patients with *NPM1* mutated AML attaining remission with venetoclax combination therapies, *NPM1* MRD provides valuable prognostic information.

Introduction
Nucleophosmin *(NPM1)* mutations are the commonest recurrent genetic abnormality in adult acute myeloid leukemia (AML) and confer favourable prognosis in younger patients receiving intensive chemotherapy[1–3]. In older or less fit patients, venetoclax combinations have been shown to be particularly effective in this disease subgroup[4]. The addition of venetoclax to azacitidine improved composite complete remission (CR) rate in patients with *NPM1* mutation (mut) from 24% to 67%[5], and its combination with low-dose cytarabine (LDAC) improved composite CR from 57% to 79%[6].

Molecular measurable residual disease (MRD) by mutant specific RT-qPCR is strongly predictive of outcomes in patients with *NPM1*^{mut} AML treated with intensive chemotherapy[7,8] however to date only flow cytometric MRD has been systematically evaluated for patients receiving venetoclax combinations[9–11]. While this was prognostic for survival in the VIALE-A study, it showed limited power in the small subset of patients with *NPM1*^{mut} where a robust leukaemia-associated immunophenotype is often absent[9]. We therefore aimed to evaluate the prognostic utility of RT-qPCR in *NPM1*^{mut} patients undergoing treatment with venetoclax combinations.

Methods
Patients were identified from a national real-world cohort study in the UK and from patients treated in Melbourne, Australia (Figure S1). Patients were included in the study if they received venetoclax with either hypomethylating agents (HMA) or LDAC as first line therapy for *NPM1*^{mut} AML, achieved CR or CR with incomplete haematological recovery (CRi) and had at least one bone marrow (BM) MRD assessment in the first 4 cycles of therapy. RT-qPCR MRD was performed at one of three central reference laboratories using validated assays and was requested at the discretion of the treating clinician, no specific monitoring schedule was recommended. Time-to-event endpoints were measured from the time of starting therapy, and molecular relapses were included as an event-free survival (EFS) event (supplemental Methods)[12].

Results and discussion

73 patients were identified from 34 hospitals with a median age of 72.1 years (range 34–86, Table 1). They had undergone a median of 2 (range 1 – 6) BM MRD assessments within the first 6 cycles of therapy. There were no differences in depth of MRD response or outcomes for patients treated with LDAC or HMAs, therefore these groups were combined for subsequent analyses (Table S1).

The best MRD response at any time during therapy was MRD negative in 40 (55%), MRD positive with ≥4 log_{10} reduction from baseline in 14 (19%) and <4 log_{10} reduction in 19 (26%). 17 patients (24%) achieved MRD negativity by the end of cycle 2 and 35 (48%) by the end of cycle 4, with only 7 patients (10%) deepening their response beyond cycle 4 (Figures 1a and S2). In those reaching MRD negativity, the median time to first negative result was 101.5 days (IQR 75.5 – 147.5), and only 5 did so beyond 4 cycles (cycle 6, 8, 9, 10 and 11 respectively). Patients with *IDH1/2* co-mutations had a particularly high rate of *NPM1* MRD negativity whereas those with secondary AML and *FLT3* mutations had poorer responses (Figure 1E).

Median follow-up was 27 months (95% CI 24.5–29.8), with 2-year overall survival (OS) of 61% (95% CI 50–74) and 2-year EFS 49% (95% CI 38–64). The deepest MRD reduction achieved at any time during venetoclax therapy was strongly associated with survival, 2-year OS was 83% (95% CI 71–96) in those with undetectable MRD, 50% (95% CI 30–84) if detectable but ≥4 log_{10} reduction from baseline, and 20% (95% CI 7–53) if <4 log_{10} reduction (Figure S3). Cumulative incidence of relapse (CIR) was higher in those with poor MRD responses.

Using the lowest *NPM1* copy number achieved by the end of cycle 2 and cycle 4, we used maximally selected rank statistics to identify a predictive and clinically useful MRD threshold and time point (Table S2)[13]. Based on these results, and considering the high coefficient of variation in copy number calculation between laboratories[14], we selected a cut-off of 0 *NPM1* copies per 100 *ABL1* (i.e. MRD negative) by the end of cycle 4 as the optimal threshold. This threshold provided a sensitivity of 82%, specificity 84% and AUC 0.83 for 24-month EFS by receiver-operator characteristic curve analyses. Patients remaining MRD positive in the first 4 cycles had 2-year OS 44%, EFS 18% and CIR of 74%, whereas in those achieving MRD negativity 2-year OS and EFS were 80% and CIR 11% (Figure 1B-D). Multivariable analyses for OS showed achievement of MRD negativity in 4 cycles (as a time-dependent variable) to be the most important factor associated with survival with a hazard ratio of 0.22 (95% CI 0.08 – 0.60, p=0.003, Table S3). The MRD threshold at cycle 2 (<0.002 *NPM1* copies per 100 *ABL1*) also predicted outcome but did not discriminate as well as the cycle 4 threshold (Figure S4).

Thirty-eight patients also had at least one peripheral blood (PB) MRD sample for analysis, with 26 (68%) achieving MRD negativity in the first 4 cycles. Patients with persisting detectable MRD had poor outcomes (2-year OS 35%, EFS 19%, CIR 73%, Figure S5). As expected, PB was less sensitive than BM (Figure S6). There were 5 patients who achieved MRD negativity in the PB but not BM in the first 4 cycles. These patients had OS similar to those negative in both sample sources but significantly poorer EFS (Figure S7).

21 patients who achieved BM MRD negativity electively stopped therapy after a median of 8 cycles (range 3 – 18). With median follow-up from stopping therapy of 16.2 months, only 2 relapses and one non-relapse mortality have occurred, with 2-year treatment-free remission of 88% (Figure S8).

In this cohort of 73 patients with *NPM1*^{mut} AML achieving CR/CRi with venetoclax combinations, MRD by RT-qPCR was strongly associated with clinical outcomes. Achievement of *NPM1* MRD negativity in the BM within the first 4 cycles of therapy identified patients with excellent survival, independent of pre-treatment variables.

The prognostic importance of *NPM1* RT-qPCR in patients treated with intensive chemotherapy is well established, including at early time points[7,8], the end of therapy[15] and prior to allogeneic transplant[16,17]. It has not previously been demonstrated that deeper remissions, as measured by RT-qPCR, have prognostic importance when patients are treated with less intensive, continuous therapies such as venetoclax combinations. Here we show that venetoclax combinations are able to induce deep remissions, including MRD negativity in 55%, and that the depth of response clearly predicts outcomes. In those with CR/CRi but persistence of MRD, relapses are frequent and occur early, including while still on therapy.

Venetoclax combinations are currently administered as indefinite therapy, and long-term follow-up has not clearly demonstrated a survival plateau to suggest than patients may be cured[18]. However, it has been shown that MRD negative patients with *NPM1* or *IDH2* mutations can have prolonged remissions off treatment[19]. The excellent results in our cohort in those achieving MRD negativity, with a 2-year OS of 80%, suggest that the disease may indeed be eradicated in some of these patients. Prospective trials examining MRD directed treatment deintensification or cessation are warranted. Our preliminary data indicate that peripheral blood MRD may provide an alternative to bone marrow sampling to identify these good responders, however this requires further study due to the small numbers included in our analysis.

Patients not achieving MRD negativity in this cohort had poor outcomes and future studies should investigate whether treatment changes (such as increasing treatment intensity, or the use of other targeted agents) can improve survival for these patients.

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Tables

Table 1 – patient demographics

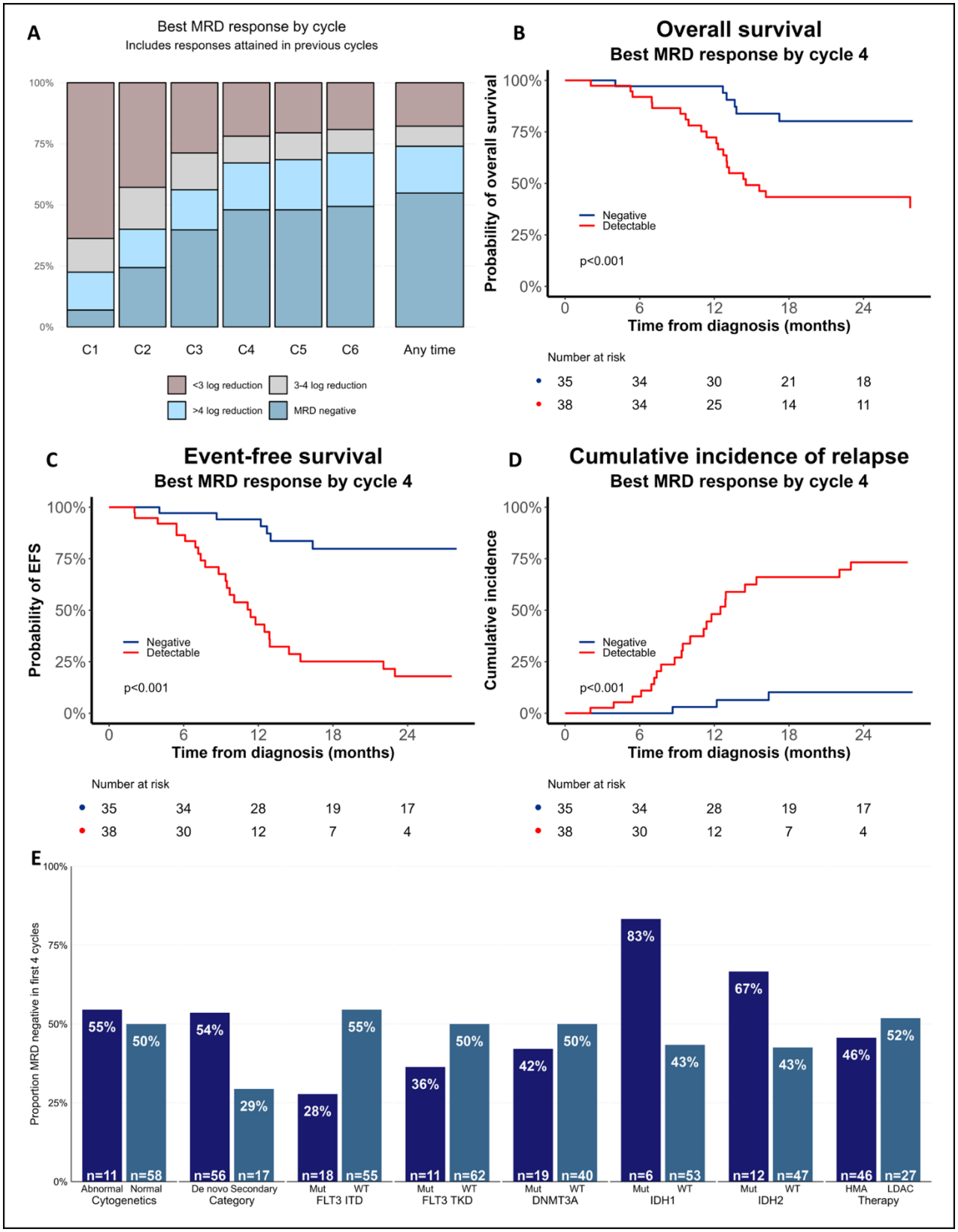
Characteristic	**N = 73**
Age at diagnosis (range)	72.1 (34 – 86)
Female	37 (51%)
Performance status	
0 – 1	53 (86%)
≥2	9 (15%)
Unknown	11
Disease category	
De novo	56 (77%)
Secondary	9 (12%)
Therapy-related	8 (11%)
Cytogenetics	
Normal	58 (84%)
Other Intermediate	9 (13%)
Adverse	2 (2.9%)
Failed	4
FLT3 ITD	18 (25%)
FLT3 TKD	11 (15%)
DNMT3A mutation*	19 (32%)
IDH1 mutation*	6 (10%)
IDH2 mutation*	12 (20%)
TP53 mutation*	2 (3.4%)
Therapy	
Azacitidine	44 (60%)
Decitabine	2 (2.7%)
Low dose cytarabine	27 (37%)
Best morphological response	
CR	69 (95%)
CRi	4 (5.5%)
Allogeneic transplant	
In first CR1	4 (5.5%)
After relapse	5 (6.8%)
No transplant	64 (88%)

*NGS results available in 59 patients

Figure Legends

Figure 1

A) Best MRD response by the end of each cycle, and overall
B) OS by achievement of MRD negativity in first 4 cycles
C) EFS by achievement of MRD negativity in first 4 cycles
D) CIR by achievement of MRD negativity in first 4 cycles
E) Rates of MRD negativity in patient subgroups


A
Best MRD response by cycle
Includes responses attained in previous cycles
100%
75%
50%
25%
0%
C1
C2
C3
C4
C5
C6
Any time
<3 log reduction
3-4 log reduction
>4 log reduction
MRD negative
B
Overall survival
Best MRD response by cycle 4
Probability of overall survival
Negative
Detectable
p<0.001
0
6
12
18
24
Time from diagnosis (months)
Number at risk
35 34 30 21 18
38 34 25 14 11
C
Event-free survival
Best MRD response by cycle 4
Probability of EFS
Negative
Detectable
p<0.001
Time from diagnosis (months)
Number at risk
35 34 28 19 17
38 30 12 7 4
D
Cumulative incidence of relapse
Best MRD response by cycle 4
Cumulative incidence
Negative
Detectable
p<0.001
Time from diagnosis (months)
Number at risk
35 34 28 19 17
38 30 12 7 4
E
Proportion MRD negative in first 4 cycles
55%
50%
54%
29%
28%
55%
36%
50%
42%
50%
83%
43%
67%
43%
46%
52%
n=11
n=58
n=56
n=17
n=18
n=55
n=11
n=62
n=19
n=40
n=6
n=53
n=12
n=47
n=46
n=27
Abnormal
Normal
Cytogenetics
De novo
Secondary
Category
Mut
WT
FLT3 ITD
Mut
WT
FLT3 TKD
Mut
WT
DNMT3A
Mut
WT
IDH1
Mut
WT
IDH2
HMA
LDAC
Therapy