bjh short report

Venetoclax induces rapid elimination of *NPM1* mutant measurable residual disease in combination with low-intensity chemotherapy in acute myeloid leukaemia

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Received 26 February 2020; accepted for publication 14 April 2020

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Summary

Based on promising results in older adults with acute myeloid leukaemia (AML), we treated patients with *NPM1*^{mut} measurable residual disease (MRD) using off-label venetoclax in combination with low-dose cytarabine or azacitidine. Twelve consecutive patients were retrospectively identified, including five with molecular persistence and seven with molecular relapse/progression. All patients with molecular persistence achieved durable molecular complete remission (CR_{MRD-}) without transplantation. Six of seven patients with molecular relapse/progression achieved CR_{MRD-} after 1–2 cycles of venetoclax. This paper highlights the promising efficacy of venetoclax-based therapy to reduce the relapse risk in patients with persistent or rising *NPM1*^{mut} MRD.

Keywords: AML, MRD, NPM1, venetoclax.

NPM1 mutations (mut) represent the largest (27–35%) molecular subgroup of acute myeloid leukaemia (AML). Suboptimal reduction in the level of *NPM1*^{mut} measurable residual disease (MRD) after chemotherapy is associated with relapse and poor overall survival.[1,2] Molecular relapse or progression after completion of therapy is invariably associated with clinical relapse.[1,3,4] Detection of *NPM1*^{mut}-MRD prior to allogeneic haematopoietic stem cell transplantation (HSCT) is associated with inferior survival.[5–8]

It is not known if pre-emptive intervention can alter the natural history of *NPM1*^{mut}-MRD positivity. In the NCRI AML17 trial, intensive chemotherapy was given to 27 patients with molecular relapse of whom 16 (59%) achieved MRD negativity.[5] In the RELAZA2 study, azacitidine

First published online 26 May 2020
doi: 10.1111/bjh.16722

suppressed *NPM1*^{mut}-MRD levels to <1% in 17/33 (52%) patients; relapse-free survival (RFS) was superior in patients rendered MRD-negative.[9]

Among elderly patients with *NPM1*^{mut} AML unfit for intensive chemotherapy, complete remission (CR/CRi) rates of 89 and 91% were achieved with venetoclax and low-dose cytarabine (LDAC) or a hypomethylating agent (HMA) respectively.[10,11] In addition, *NPM1*^{mut} molecular complete remission (CR_{MRD-}) was achieved rapidly and durably for >24 months.[12] We therefore hypothesised that venetoclax-based regimens could represent an effective low-intensity option for *NPM1*^{mut} patients with molecular failure.

Patients and methods

The participating hospitals routinely monitored *NPM1*^{mut} MRD. We retrospectively identified consecutive patients with *NPM1*^{mut} AML with: (i) molecular persistence (cohort 1): any detectable transcript level after completion of chemotherapy, and (ii) molecular relapse or progression (cohort 2): re-emergent or rising transcript level of ≥1 $\log_{10}$ confirmed by second sample, unless the level was ≥1%. Patients had received induction chemotherapy containing an anthracycline and cytarabine followed by 0–3 cycles of consolidation. This study was approved by local ethics committees in accordance with the Declaration of Helsinki. Informed consent from subjects was waived for this retrospective study.

Patients were treated at the discretion of clinicians using off-label venetoclax (400–600 mg PO daily for 14–28 days) in combination with LDAC (20 mg/m^2 SC daily for 7–10 days) or azacitidine (75 mg/m^2 SC daily for 5–7 days), between August 2017 and February 2019 (Table SI). Initial AML diagnoses were between October 2016 and January 2019. No dose ramp-up was used. If concurrent strong CYP3A4 inhibitor (e.g. posaconazole) was used, venetoclax was reduced to 100 mg daily.[13] Prophylaxis for tumour lysis syndrome was not given.

All *NPM1*^{mut} MRD cases were analysed from bone marrow source using mutant-specific reverse transcription quantitative polymerase chain reaction (RT-qPCR) as previously described,[1] according to Europe Against Cancer (EAC) criteria[14] and using *ABL* as housekeeping gene. MRD results are expressed relative to the diagnostic samples (%). The median sensitivity was 2×10^{-6} (range 5×10^{-5} to 3×10^{-7}). Other mutation testing was performed by certified molecular diagnostic laboratories with approximately 5% sensitivity.

Results

Venetoclax rapidly eliminates NPM1^{mut} *molecular persistence (cohort 1)*

Five patients (age 59–79 years) had persistent *NPM1*^{mut}-MRD (median 0·0039%, range 0·001–0·009%) at the end of chemotherapy (Table I), including 3/5 with concurrent *DNMT3A* mutations and one with a concurrent *IDH2* mutation. One patient (case 5) had a complicated course after a single cycle of 7 + 3 induction and received no further chemotherapy.

All five patients achieved CR_{MRD-} in response to venetoclax with either LDAC (n = 4) or azacitidine (n = 1) following one (n = 2), two (n = 1) or four cycles (n = 2; one did not have MRD assessment between cycles 1 and 4) of therapy (Fig 1 and Figure S1). The median RFS was not reached after a median follow-up of 20 months (range 7·8–31·3 months); no patient experienced disease progression (Figure S2) and none received an HSCT. Case narratives are shown in the Supporting information.

A variable number of cycles were received: two received four planned cycles, one received 16 cycles (six in combination with azacitidine) and treatment is ongoing in two patients (3+ and 10+ cycles).

Venetoclax rapidly eliminates re-emergent and/or rising NPM1^{mut} *MRD (cohort 2)*

Seven patients (age 25–81 years) with *NPM1*^{mut} molecular progression (n = 5) or relapse (n = 2) after prior intensive chemotherapy were treated with venetoclax with either LDAC (n = 5) or azacitidine (n = 2) (Fig 1). One patient (case 8) was in second morphological remission. MRD-directed approaches prior to venetoclax included HSCT (case 8), fludarabine, cytarabine, idarubicin, and granulocyte colony-stimulating factor (FLAG-Ida) (case 7) and azacitidine (case 6). Another patient (case 12) received venetoclax with an MCL1 inhibitor on a clinical trial, achieved 2·1 log reduction, but was withdrawn from trial due to toxicity. The median *NPM1*^{mut}-MRD level prior to venetoclax-based intervention was 0·0208% (range 0·0090–14·1264%) (Table I).

CR_{MRD-} was achieved in 6/7 patients (86%) after 1–2 cycles of therapy. Case 10 was the only patient not rendered molecularly negative; this patient subsequently underwent a myeloablative HSCT while MRD-positive but relapsed soon thereafter with a newly emergent *FLT3*-ITD clone (allelic ratio 0·09); baseline mutations (*NPM1*, *IDH2* and *DNMT3A*) were persistent at relapse. Three patients received HSCT while MRD-negative: one (case 7) died from non-relapse mortality and two (cases 9 and 11) remained alive 10 and nine months post-HSCT respectively. One patient (case 8) received a donor lymphocyte infusion after one cycle of venetoclax and azacitidine, with plan for ongoing venetoclax therapy. Two other patients (age 69 and 81 years) were deemed unfit for HSCT and continued to receive venetoclax.

None of the six responding patients had subsequently experienced molecular or haematological relapse after a median of 10·8 months follow-up (range 3·6–13·1 months) (Figure S2). Case narratives are shown in the Supporting information.

Tolerability

Venetoclax in combination with LDAC/HMA was well tolerated with cytopenias manageable by dose reduction in six

Table I. Summary of patient characteristics and response to venetoclax in combination with low-intensity chemotherapy.

Case #	Age/sex	Regimen (no. cycle)	Other molecular features	Prior chemotherapy	Prior MRD intervention	MRD level pre-Ven (%)	Best MRD response (%)	Subsequent HSCT	RFS (months)
Cohort 1: *NPM1*^{mut} MRD molecular persistence									
1	71/M	VEN-AZA (×6) VEN (×10)	NK, *DNMT3A*, *TET2, FLT3*-TKD	DA3+10 DA3+8 IDAC FLAG-Ida	GO+ Aza	0.0039	MRD Neg	—	31.3*
2	79/F	VEN-LDAC (3+)	NK	DA3+10 DA2+8 IDAC+GO	—	0.0038	MRD Neg	—	7.8*
3	67/F	VEN-LDAC (4)	NK, *DNMT3A*	7+3 IDAC+2 (×2)	—	0.0039	MRD Neg	—	22.2*
4	59/F	VEN-LDAC (4)	NK, *IDH2* R140	7+3 IDAC+2 (×2)	—	0.0010	MRD Neg	—	20.0*
5	62/M	VEN-LDAC (10+)	+21, *DNMT3A*, *TET2, FLT3*-TKD	7+3	—	0.0093	MRD Neg	—	11.7*
Cohort 2: *NPM1*^{mut} MRD molecular progression/relapse									
6	81/M	VEN-AZA (4+)	NK, *IDH1, SRSF2* *FLT3*-TKD	DA+GO DA3+8	Aza	0.0090	MRD Neg	—	11.8*
7	41/F	VEN-LDAC (2)	NK, *FLT3*-TKD	DA+GO HiDAC (×3)	FLAG-Ida	0.0168	MRD Neg	Yes	4.9†
8	29/M	VEN-AZA (2+)	NK, *FLT3*-TKD	FLAG-Ida (×2)	HSCT + DLI	0.0206	MRD Neg	DLI	3.6*
9	52/M	VEN-LDAC (2)	NK	IDAC+3 IDAC+2	—	14.1264	MRD Neg	Yes	12.4*
10	35/F	VEN-LDAC (2)	NK, *DNMT3A* *IDH2* R140	7+3 IDAC HiDAC (×2)	—	10.8569	2.4916	Yes	5.6^{Rel}
11	51/M	VEN-LDAC (1)	NK, *FLT3*-ITD	IDAC+3 IcE (×2)	—	0.0168	MRD Neg	Yes	10.8*
12	69/M	VEN-LDAC (7+)	NK, *DNMT3A*	7+3 IDAC (×2)	Ven-MCL1i	1.4518	MRD Neg	—	5.9*

Aza, azacitidine; DA, daunorubicin and cytarabine; DLI, donor lymphocyte infusion; FLAG-Ida, fludarabine, cytarabine, G-CSF and idarubicin; GO, gemtuzumab ozogamicin; HiDAC, high-dose cytarabine; HSCT, allogeneic hematopoietic stem cell transplantation; IcE, idarubicin (9 mg/m^2 D1–2), cytarabine (100 mg/m^2 continuous infusion D1–5) and etoposide (75 mg/m^2 D1–5); IDAC, intermediate dose cytarabine; IDAC+2, cytarabine (1–1.5 g/m^2 BD D1,3,5) and idarubicin (12 mg/m^2 D1-2); IDAC+3, cytarabine (1–1.5 g/m^2 D1,3,5,7) and idarubicin (12 mg/m^2 D1–3); ITD, internal tandem duplication; LDAC, low-dose cytarabine; MCL1i, MCL1 inhibitor; MRD, measurable residual disease; Neg, negative; NK, normal karyotype; Rel, relapse; RFS, relapse-free survival; TKD, tyrosine kinase domain; Ven, venetoclax.

*Ongoing therapy/alive.

†Deceased.

(50%) patients. Patients were treated as outpatients and rarely needed transfusions. Febrile neutropenia occurred in two patients (17%): one developed invasive fungal infection and another grade 4 lung infection (Table SI). Grade 4 neutropenia occurred in eight patients (67%; 6/9 for LDAC and 2/3 for azacitidine): median duration was 8·5 days [interquartile range (IQR) 6–13 days]. Grade 4 thrombocytopenia occurred in five patients (42%; 4/9 for LDAC and 1/3 for azacitidine): median duration was eight days (IQR 3–22·5 days). Case 7 was heavily pretreated including a cycle of FLAG-Ida before receiving venetoclax and developed prolonged grade 4 neutropenia (49 days) and thrombocytopenia (72 days).

Discussion

Venetoclax with either LDAC or azacitidine had encouraging activity in eradicating persistent or relapsing *NPM1*^{mut}-MRD after intensive chemotherapy. None of the 11 (92%) patients achieving CR_{MRD-} have relapsed with a median follow-up of 11·8 months (range 3·6–31·3 months). The treatment was deliverable in the outpatient setting, did not require tumour lysis prophylaxis and was well tolerated with grade ≥ 3 non-haematological toxicities observed in two (17%) patients.

There is currently no consensus on how to manage patients with detectable MRD at end of chemotherapy. In the NCRI AML17 trial, *NPM1*^{mut}-MRD remained detectable

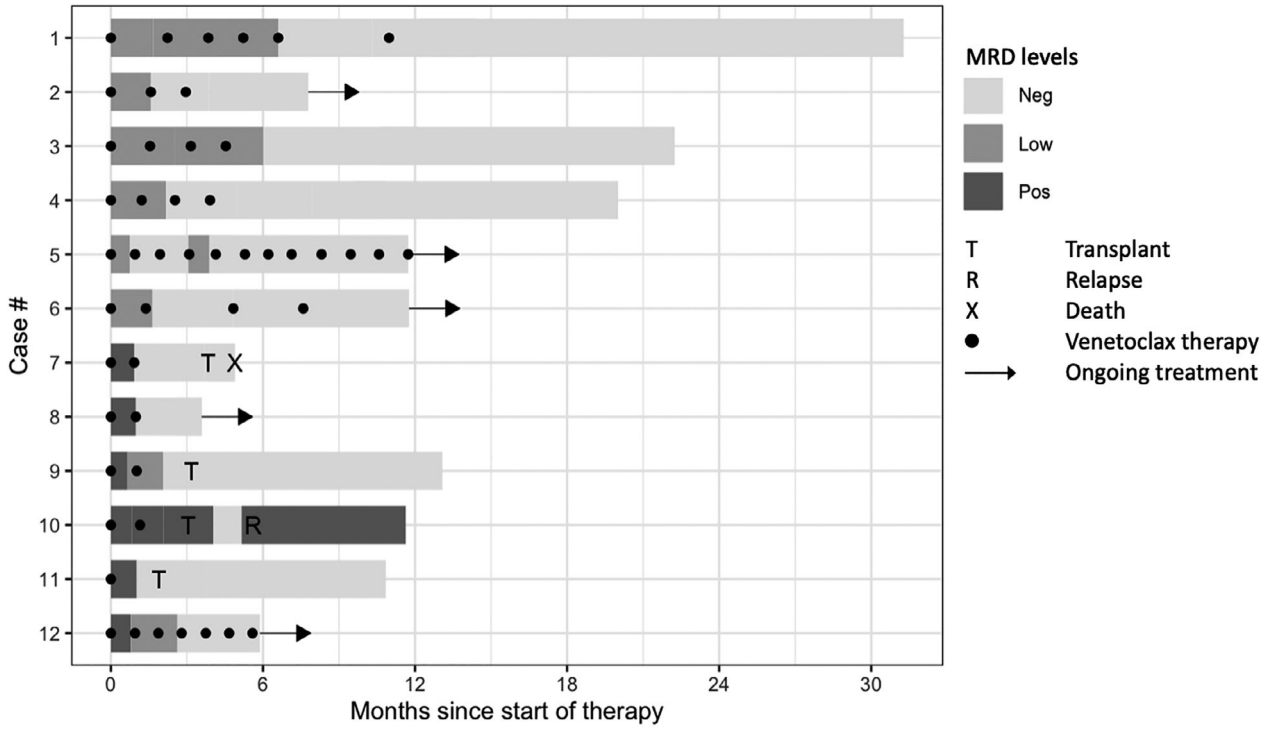

Fig 1. Swimmer plot of patients treated with venetoclax in combination with low-intensity chemotherapy for molecular persistence (cohort 1: cases 1–5) and progression (cohort 2: cases 6–12).

in the bone marrow in approximately 25% of patients post four courses of chemotherapy;[1] two-year RFS was 50% for these patients (Figure S3). Here we demonstrated all five patients in first cohort with persistent *NPM1*^{mut} successfully achieved negative MRD and remained free of molecular or morphological progression after follow-up of 7·8–31·3 months without HSCT.

Serially rising *NPM1*^{mut} transcript levels are inevitably associated with haematological relapse.[1,3,4] In NCRI AML17, 27 patients received salvage chemotherapy [16 (59%) attained CR_{MRD-}] followed by HSCT for molecular relapse;[5] estimated RFS was 66% at two years (Figure S4). In our second cohort, 6/7 (86%) patients attained CR_{MRD-} despite failure of prior regimens including high-dose cytarabine (n = 6), gemtuzumab ozogamicin (GO; n = 2), azacitidine (n = 1) or HSCT (n = 1). RFS was 67% at median follow-up of 10·8 months: one morphological relapse and another non-relapse mortality, both occurring post-HSCT. Two elderly patients considered unfit for HSCT remain alive at 6 and 12 months respectively. As detectable *NPM1*^{mut}-MRD levels prior to HSCT are associated with inferior outcomes,[6–8] it would seem reasonable to consider venetoclax-based therapy to try to attain a molecular CR before transplantation.[9,15]

One patient had persistent *NPM1*^{mut} MRD after venetoclax-based therapy and evolved a new *FLT3*-ITD clone at relapse post-HSCT, consistent with the role of *FLT3*-ITD in mediating resistance to venetoclax-based therapy.[12] Two patients received azacitidine [case 1 (with GO) and 6] for two and seven cycles and achieved minor response (1–2 log reduction) prior to addition of venetoclax. It is possible that prolonged azacitidine monotherapy may deepen the molecular response: 17/33 patients (52%) in the RELAZA2 study had reductions in *NPM1*^{mut}-MRD; however, the CR_{MRD-} rate was not reported for this subgroup.[9]

Limitations of our study include the small cohort size, retrospective nature of the study and relatively short follow-up. Selection bias is unavoidable as patient selection was up to the treating clinician and dependent on the availability of venetoclax and/or fitness for HSCT. Confirmation of our findings in prospective and ideally randomised studies together with longer follow-up of the current cohort will be necessary to prove whether responses are durable, including in the absence of an allograft. Other issues include whether concomitant mutations predict for later relapse and the optimal number of cycles of treatment required beyond attainment of molecular negativity.

This study highlights the promising efficacy of venetoclax in combination with either LDAC or HMA as an MRD-directed therapy for *NPM1*^{mut} AML. These combinations may replace salvage chemotherapy/HSCT for this molecularly defined subgroup of patients.

Acknowledgement

AHW is funded by the Leukemia & Lymphoma Society (LLS) Specialized Center of Research (SCOR) (Strasser) and

by the Medical Research Future Fund (MRFF). DCT is funded by the NIHR RM/ICR Biomedical Research Centre.

Author contributions

AHW, IST and RD designed the research, performed research, analysed data, and wrote the paper. AI, TT, PN, NC, DCT, AL, NP, MR, NHR, KR, APS, CYF and APG contributed patients or analytical tools, analysed data and approved the final manuscript.

Competing interests

AW is a former employee of the Walter and Eliza Hall Institute which receives milestone and royalty payments related to venetoclax. AW received payments from WEHI related to venetoclax. AW is a medical adviser and receives research funding and honoraria from Abbvie. RD has served as an advisory board member for and received speaker fees from Abbvie. CYF has served as an advisory board member for Abbvie.

Supporting Information

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

Table SI. Venetoclax regimen, associated toxicities and dose adjustments.

Fig S1. Proportion of *NPM1*^{mut}-MRD response following venetoclax in combination with low-intensity chemotherapy. Five patients (all from cohort 2) did not proceed beyond cycle 2: one patient ongoing post cycle 1 and four patients underwent HSCT (one post cycle 1 and three post cycle 2). Overall molecular response rate was 92% by end of second cycle. Low positive: detectable at >4-log reduction from baseline; positive: detectable at <4-log reduction from baseline.

Fig S2. Kaplan–Meier plots of (A) relapse-free and (B) overall survival for patients treated with venetoclax in combination with low-intensity chemotherapy for molecular persistence (cohort 1) and progression (cohort 2).

Fig S3. Kaplan–Meier plot of relapse-free survival for six patients with persistent *NPM1*^{mut}-MRD in the bone marrow post four courses of chemotherapy. Data reproduced from Ivey *et al.*, 2016.

Fig S4. Kaplan–Meier plot of relapse-free survival for 27 patients with molecular relapse who received salvage chemotherapy and subsequent allogeneic stem cell transplantation. Data reproduced from Dillon *et al.*, 2020.

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