SUPPLEMENTAL DATA

Supplement to: Tiong IS, et al, Venetoclax induces rapid elimination of *NPM1* mutant measurable residual disease in combination with low intensity chemotherapy in acute myeloid leukaemia.

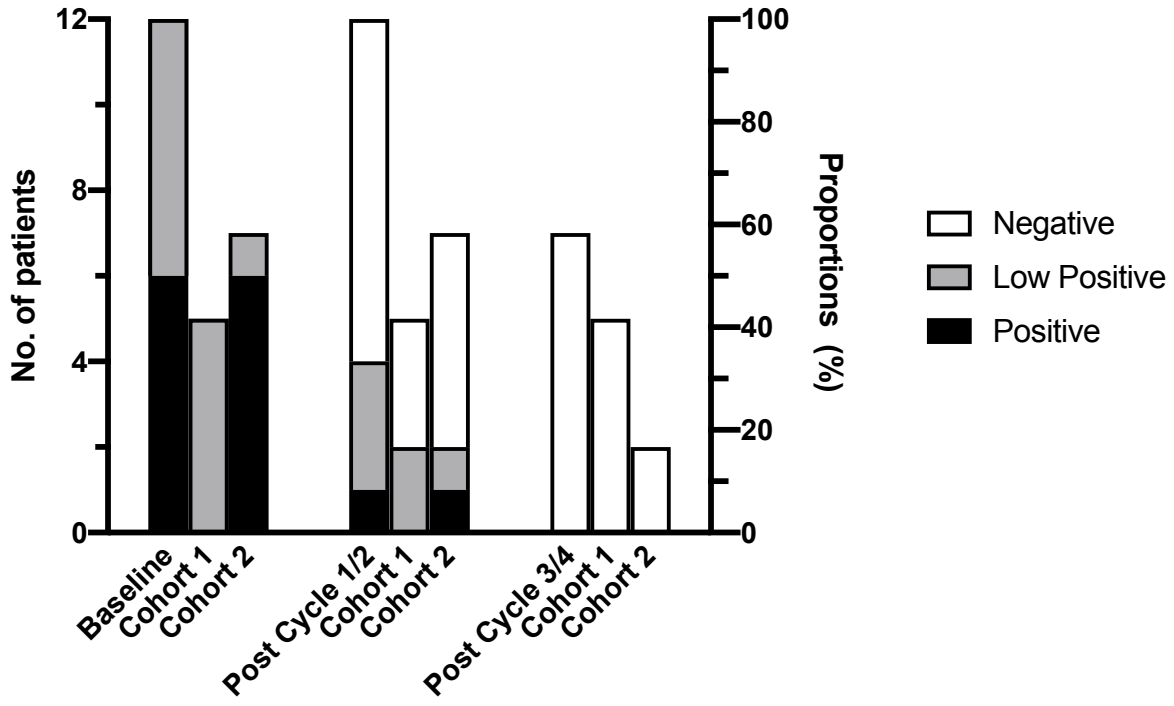
1. **Supplemental Table SI.** Venetoclax regimen, associated toxicities and dose adjustments.
2. **Supplemental Figure S1.** Proportion of NPM1mut MRD response following venetoclax in combination with low-intensity chemotherapy. Five patients (all from cohort 2) did not proceed beyond cycle 2: one patient 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.
3. **Supplemental Figure 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).
4. **Supplementary Figure S3.** Kaplan Meier plot of relapse-free survival for 6 patients with persistent *NPM1*^{mut}-MRD in the bone marrow post 4 courses of chemotherapy. Data reproduced from Ivey et al, *N Engl J Med* 2016.
5. **Supplementary Figure 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, *Blood* 2020.
6. **Case narratives** and serial NPM1mut MRD figures for cases #1 to #12.

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

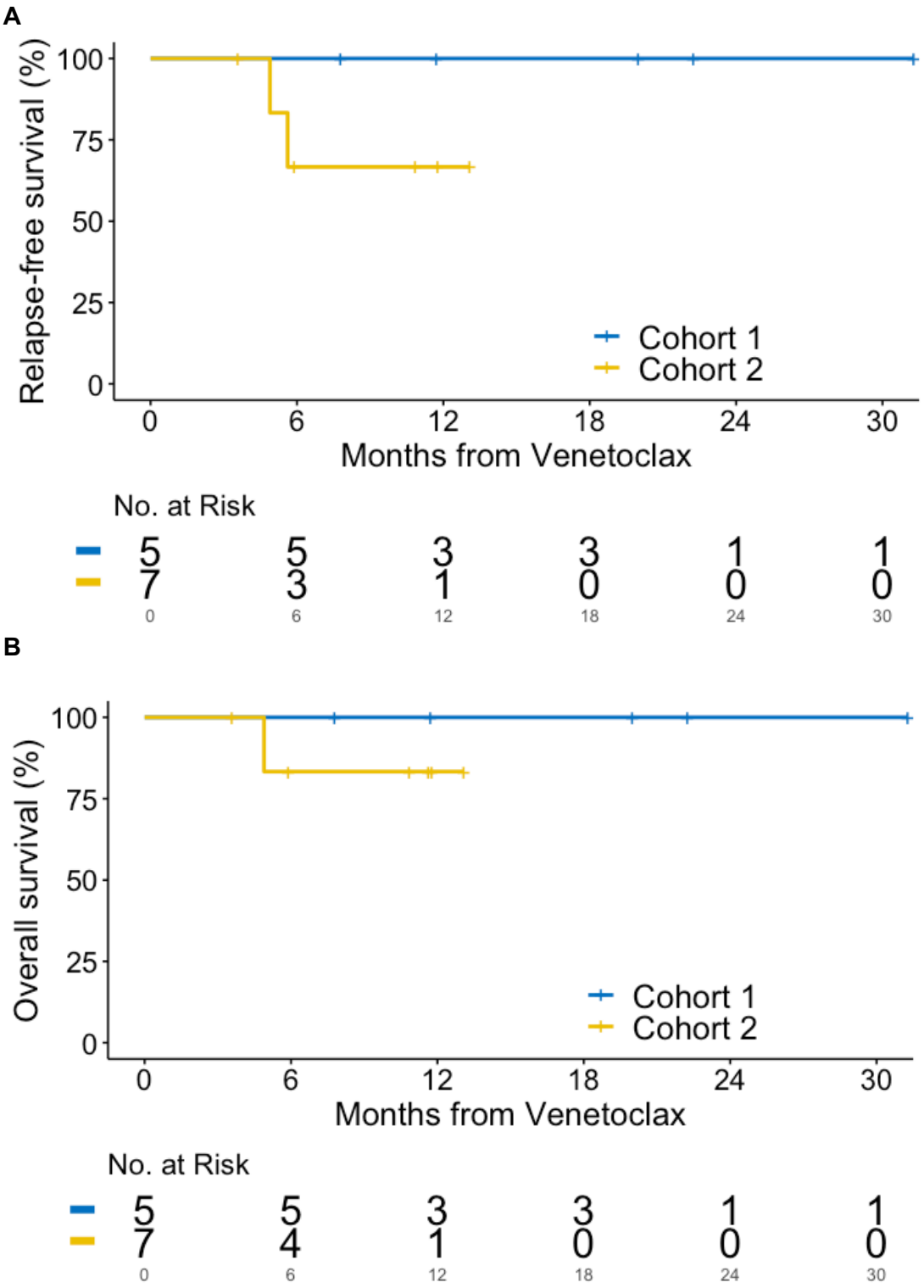
Case #	Venetoclax	Concurrent azole	Partner therapy	No. of Cycles	Grade 4 cytopenias	Dose adjustment	Reason for cessation
1	400mg x 28d	No (C5-6)	Aza 75mg/m^2 x 7d	6	Neut	Aza x5d in cycle 6 Ven 100mg during voriconazole	Invasive fungal infection
	400mg x 28d	No	-	10	-	-	Facial rash
2	100mg x 14d	Yes	LDAC 20mg/m^2 x 7d	3+	Both	-	Ongoing
3	100mg x 14d	Yes	LDAC 20mg/m^2 x 10d	4	Neut	Ven x 10d from cycle 3	Planned
4	100mg x 14d	Yes	LDAC 20mg/m^2 x 10d	4	Both	Ven x 10d from cycle 2	Planned
5	100mg x 14d	Yes	LDAC 20mg/m^2 x 10d	10+	-	Ven x 10d + LDAC x 7d from cycle 7; Ven x 7d + LDAC x 5d from cycle 9	Ongoing
6	100mg x 14d	Yes	Aza 75mg/m^2 x 7d	4+	Both	Aza x 5d from cycle 3 (febrile neutropenia and severe pneumonia requiring admission to intensive care unit)	Ongoing
7	100mg x 28d	Yes	LDAC 20mg/m^2 x 10d	2	Both	Ven x 17d in cycle 2	SCT
8	100mg x 14d	Yes	Aza 75mg/m^2 x 5d DLI	2+	-	-	Ongoing
9	100mg x 14d	Yes	LDAC 20mg/m^2 x 10d	2	-	-	SCT
10	100mg x 14d	Yes	LDAC 20mg/m^2 x 10d	2	Both	Ven x 28d in cycle 2 (increase)	SCT
11	100mg x 14d	Yes	LDAC 20mg/m^2 x 10d	1	Neut	-	SCT
12	600mg x 28d	No	LDAC 20mg/m^2 x 10d	7+	-	-	Ongoing

Abbreviations: DLI: donor lymphocyte infusion; LDAC: low-dose cytarabine; Neut: neutropenia; SCT: stem cell transplantation.

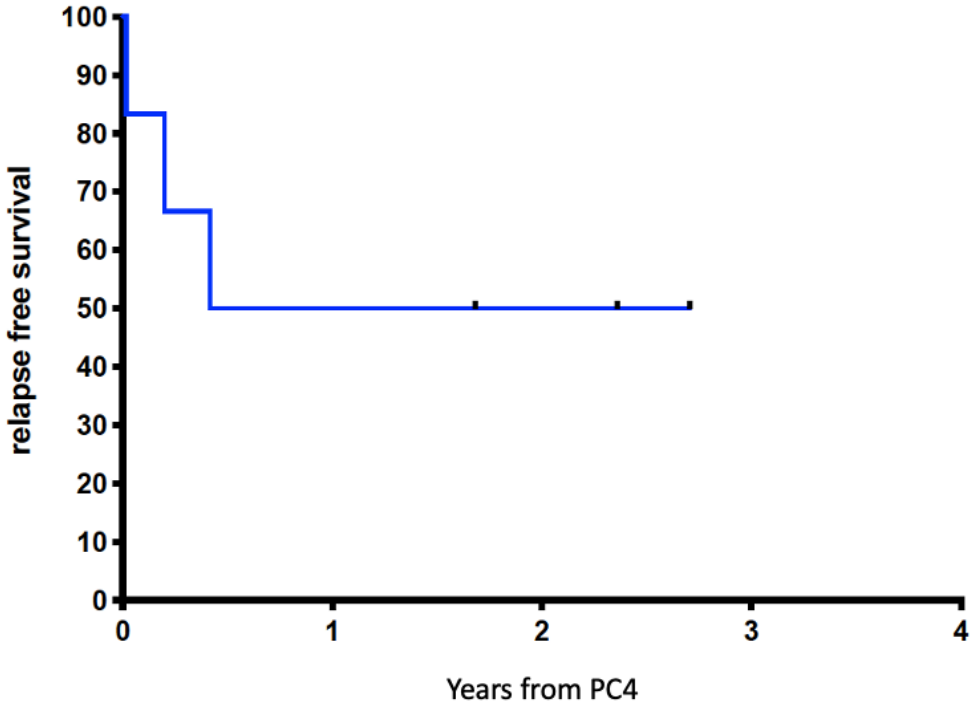
Supplemental Figure S1. Proportion of *NPM1*^{mut} MRD response following venetoclax in combination with low-intensity chemotherapy. Five patients (all from cohort 2) did not proceed beyond cycle 2: one patient ongoing post cycle 1 and four patients underwent HSCT (one post cycle 1 and three post cycle 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.



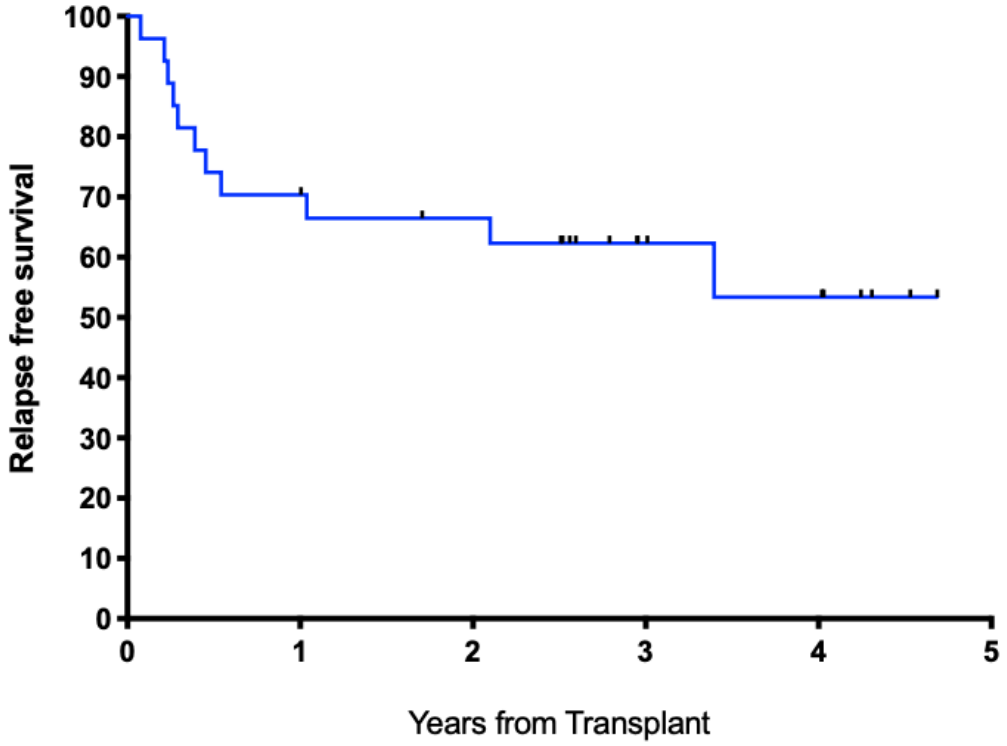
Supplemental Figure S2. Kaplan-Meier plots of (A) relapse-free and (B) overall survival for patients treated with venetoclax in combination with low-intensity chemotherapy for molecular persistence (cohort 1) and progression (cohort 2).



Supplementary Figure S3. Kaplan Meier plot of relapse-free survival for 6 patients with persistent *NPM1*^{mut}-MRD in the bone marrow post 4 courses of chemotherapy. Data reproduced from Ivey et al, *N Engl J Med* 2016.



Supplementary Figure 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, *Blood* 2020.



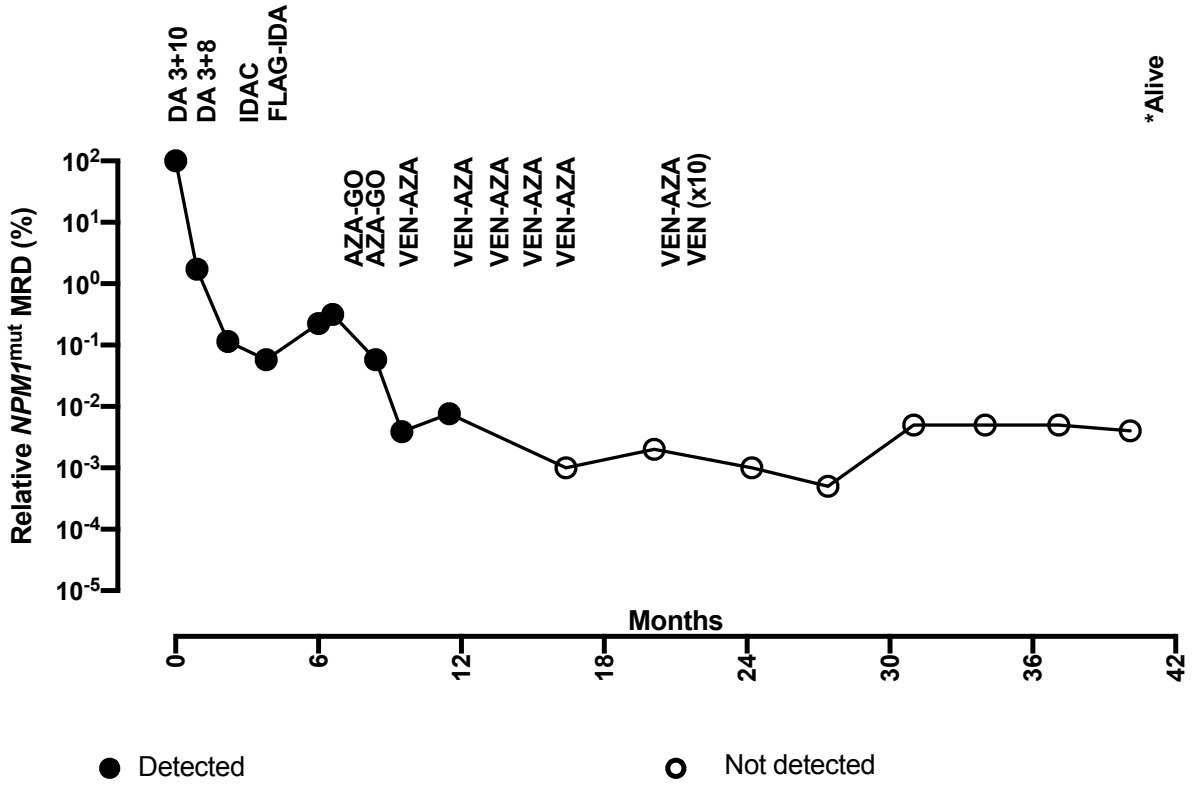
Case Narratives

Case #1

A 71-year-old male was diagnosed with AML with a normal karyotype and co-mutations in *DNMT3A*, *TET2* and *FLT3* (N676K). He was treated with DA3+10 then DA3+8. He achieved CR and a 2-log reduction in *NPM1* mutant transcripts after cycle 1. After cycle 2 there was a further 1 log reduction in *NPM1* mutant transcripts in the bone marrow and these were not detected in the peripheral blood. He received intermediate dose cytarabine (1.5mg/m^2 x 6) for cycle 3 with no further reduction in transcript level and subsequently received FLAG-Ida with a 50% dose reduction for cycle 4 after which there was a 0.6 log increase in expression, confirmed on a second sample consistent with molecular persistence of disease.

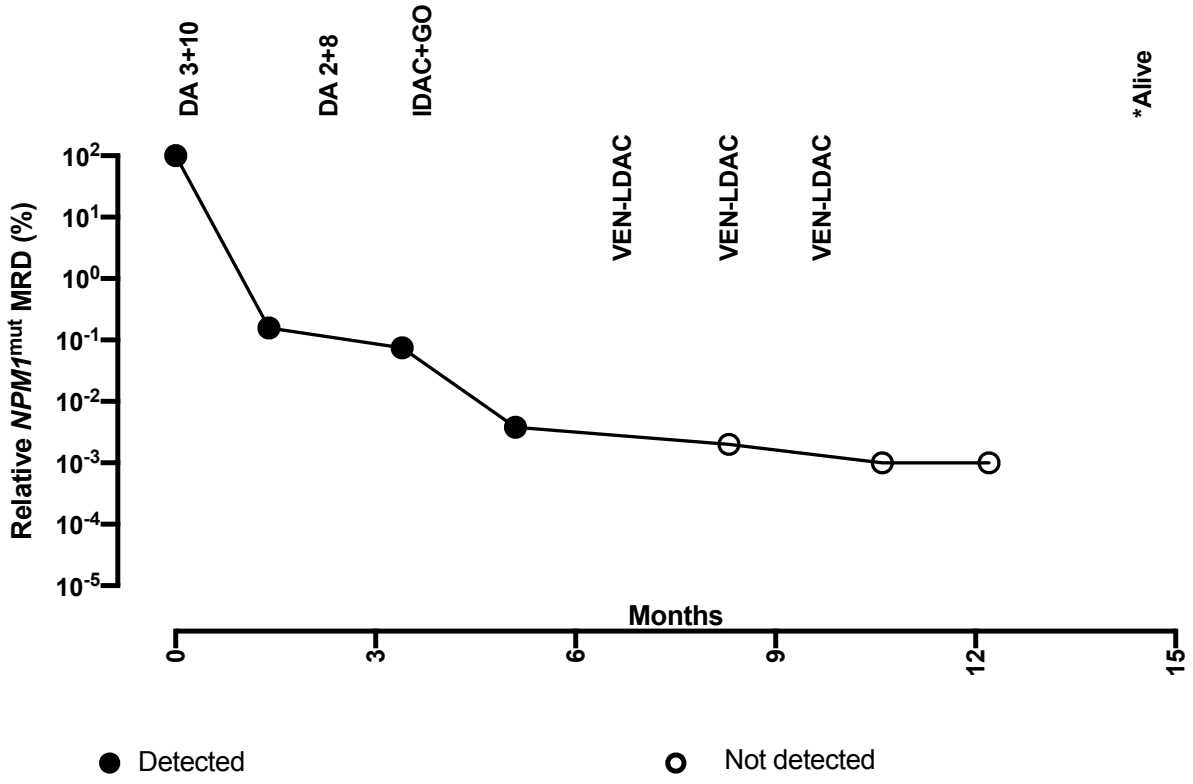
He then received two cycles of azacitidine + gemtuzumab ozogamicin resulting in a >2-log reduction in transcript levels which remained positive. This treatment was poorly tolerated and was stopped. He then received venetoclax (400mg daily) + azacitidine (75mg/m^2 x 7d), achieving molecular complete remission after the second cycle. He received a further five cycles of venetoclax + azacitidine. The 5th cycle was complicated by presumed pulmonary invasive fungal infection requiring admission to hospital. The azacitidine cycle length was reduced to 5 days for the 6th cycle. He then received venetoclax monotherapy for a further 10 cycles following which all therapy was stopped due to the development of a pustular skin rash affecting the face which completely resolved within one month of treatment cessation.

At the time of last follow up 31 months after initiation of venetoclax + azacitidine the patient remains alive and well and in ongoing molecular complete remission.



Case #2

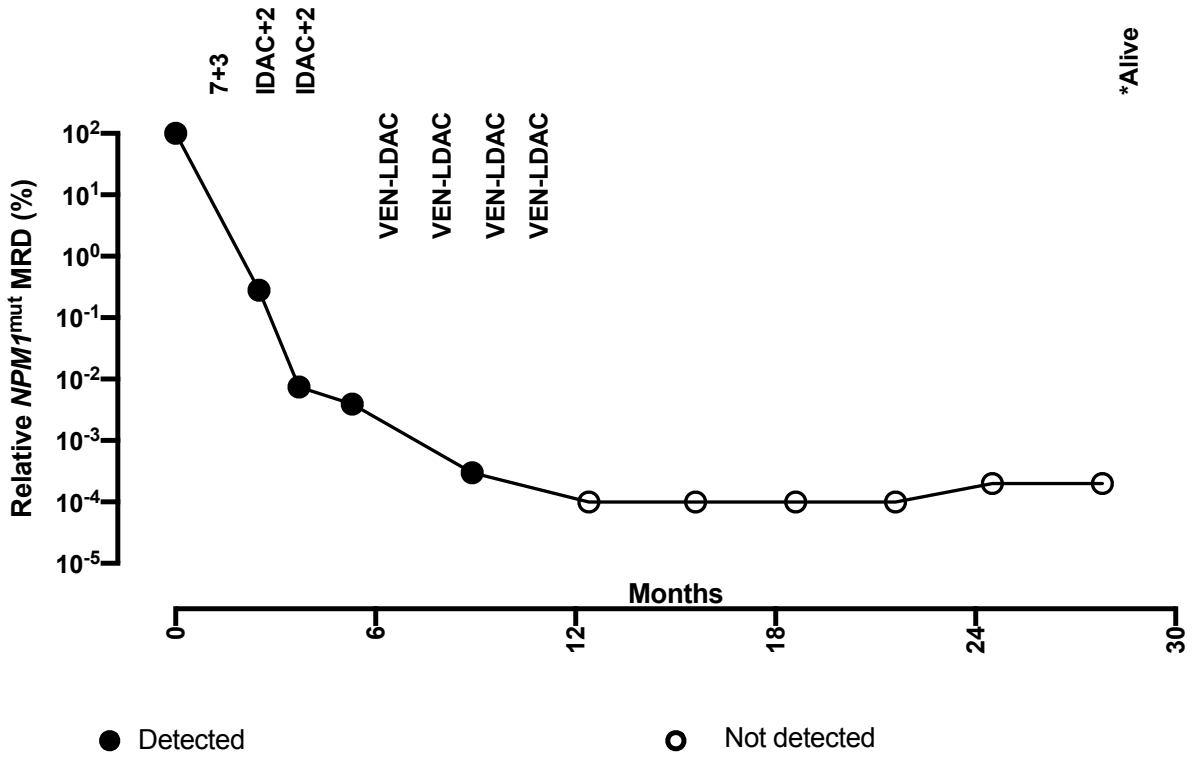
A 79-year-old female was diagnosed with normal karyotype *NPM1* mutated AML. No co-mutations were detected. She entered the NCRI AML18 clinical trial and received induction therapy with DA3+10 which was extremely poorly tolerated with life threatening bacterial and fungal infections and cardiomyopathy presumed secondary to anthracycline exposure. She achieved CR after cycle 1 and there was a 3-log reduction in *NPM1* mutant transcript levels. She received DA2+8 for the second cycle which was reasonably tolerated but there was no further reduction in transcript levels. Consequently, she received intermediate dose cytarabine (1 g/m^2 IV once daily x 5 days) with gemtuzumab ozogamicin for cycle 3 which was complicated by marked derangement in liver function tests. *NPM1* mutant transcripts remained detectable at the end of cycle 3 and treatment with venetoclax (100 mg once daily x 14 days with posaconazole) + cytarabine (20 mg/m^2/d x 7 days by continuous infusion) was initiated. This was well tolerated without infection or hospitalisation. Molecular complete remission was achieved after the first cycle and at the time of last follow-up she had received two further cycles with a plan for ongoing therapy.



Case #3

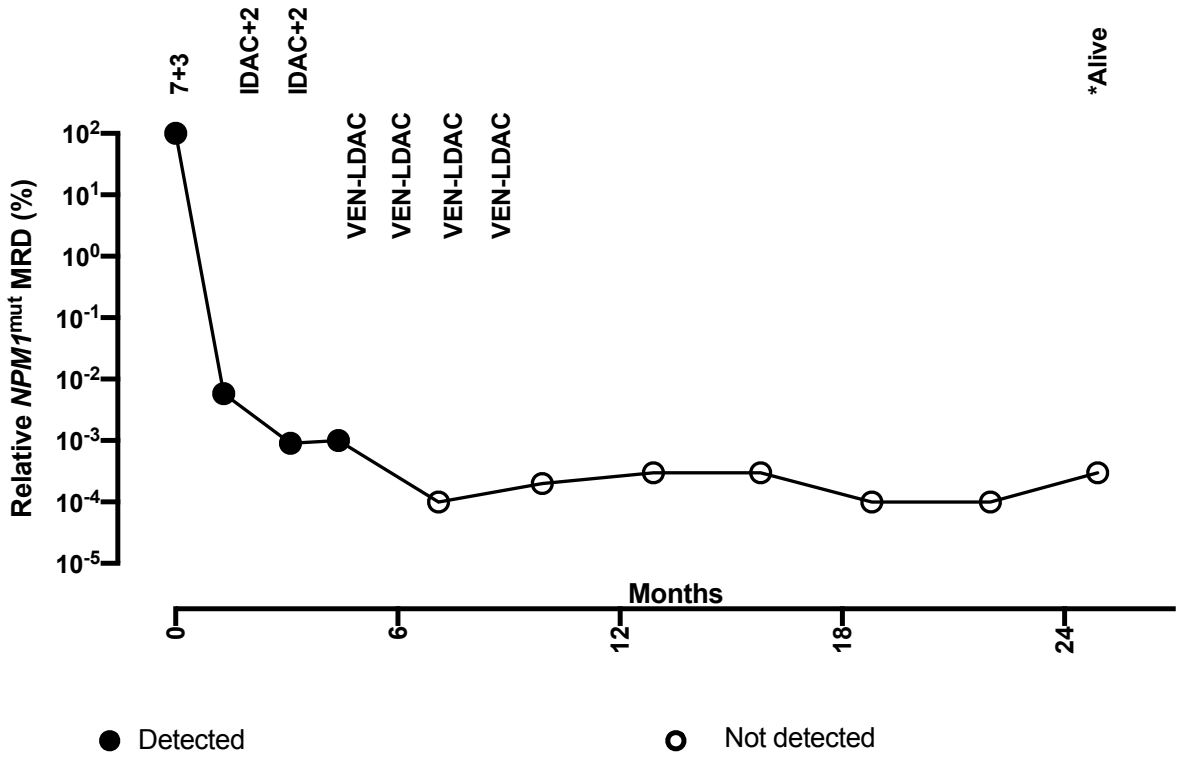
A 67-year-old female was diagnosed with pancytopenia and leucoerythroblastic blood picture. Bone marrow was hypercellular with 9.4% blasts, dysplastic changes with MF-2 reticulin fibrosis, consistent with an MDS/MPN syndrome. Molecular panel demonstrated *NPM1* and *DNMT3A* Met880Val mutations. Patient was treated as AML with 7+3 induction (2.6 log reduction) and 2 cycles of IDAC+2 consolidation (persistent low positive levels at end of therapy). Best response was CR with incomplete platelet recovery (CRp).

She then received 4 cycles of venetoclax (100 mg once daily x 14 days with posaconazole) with low-dose cytarabine, with successful eradication of *NPM1* mutant transcripts. In addition, patient made full hematologic recovery (CR). Treatment course was complicated by oral ulcers but otherwise well tolerated. Patient remained well at 22.2 months post venetoclax initiation.



Case #4

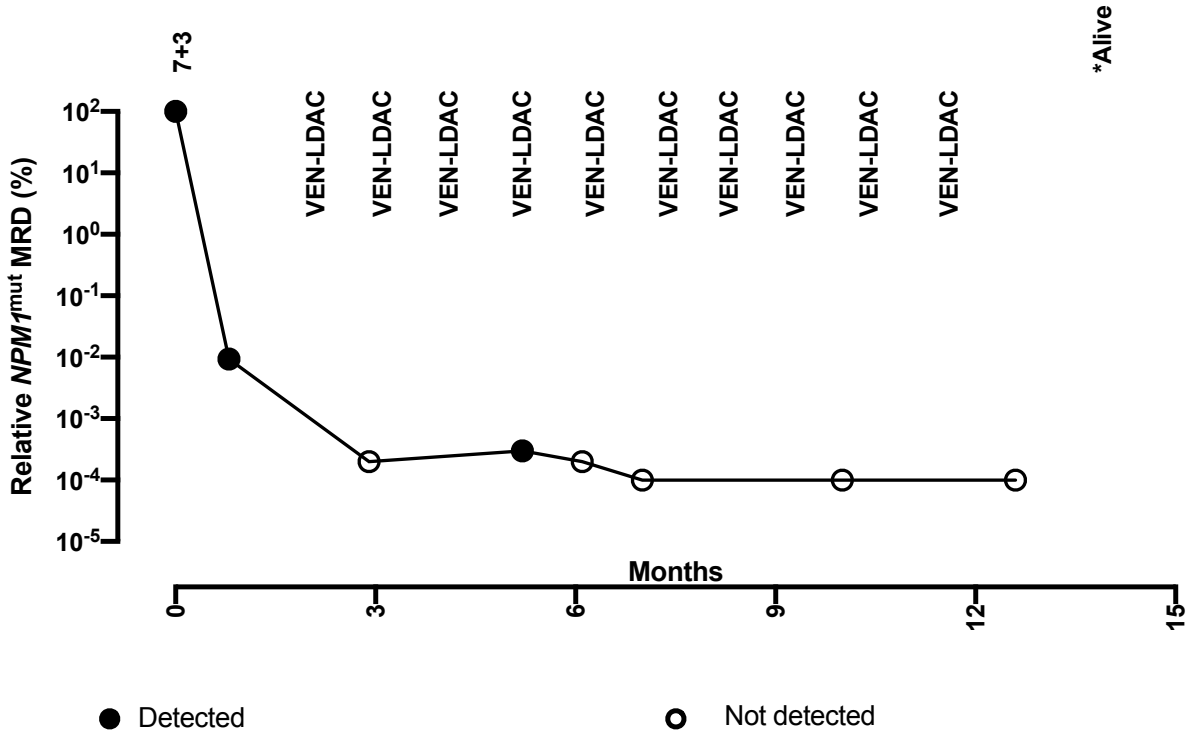
A 61-year old female was diagnosed with normal karyotype AML with mutations in *NPM1* and *IDH2*-R140. This was treated with 7+3 induction with a 4.2-log reduction. However, following further 2 cycles of IDAC+2 consolidation, *NPM1* mutant transcript persisted at low copy numbers. She then received 2 cycles of venetoclax (100 mg once daily x 14 days with posaconazole) with low-dose cytarabine. *NPM1* mutant MRD was eradicated following two cycles of therapy and patient went on to receive two further cycles. She remained well at 20 months post VEN initiation.



Case #5

A 62-year-old male presented with WCC > 100 x 10^9/L and diagnosed with AML: trisomy 21, and mutated *NPM1*, *FLT3* Asp839Glu, *DNMT3A* Arg882Cys, *TET2* Arg1354fs and *TET2* Arg1440fs. Induction (7+3 with midostaurin) was complicated by tumour lysis syndrome, oliguric acute kidney injury requiring renal replacement therapy, methicillin-sensitive Staphylococcal aureus sepsis, and intensive care admission. Complete remission was achieved post induction with a 4-log reduction in *NPM1*^{mut} MRD level.

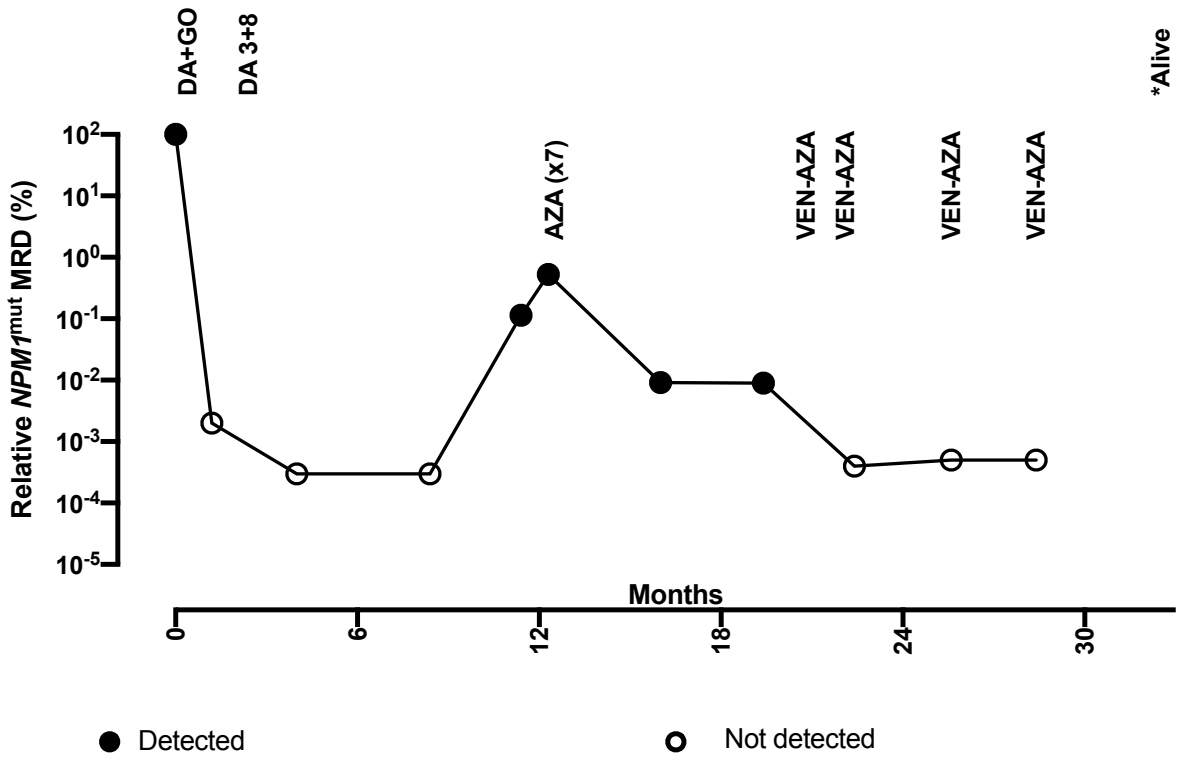
Patient was deemed unfit for further consolidation chemotherapy, but instead was commenced on venetoclax (100 mg once daily x 14 days with posaconazole) with low-dose cytarabine, at 65 days post induction. *NPM1*^{mut} MRD was rendered negative following first cycle of therapy. Post cycle 3 patient was hospitalised for viral upper respiratory tract infection and deranged liver biochemistry. A low level *NPM1*^{mut} MRD was also noted. Posaconazole was ceased from cycle 4-6 and venetoclax increased to 400 mg once daily. Cycle 7 onwards, itraconazole was initiated and venetoclax dose reduced back to 100 mg once daily. Subsequent MRD levels were undetectable and patient remained well with ongoing therapy (cycle 10+).



Case #6

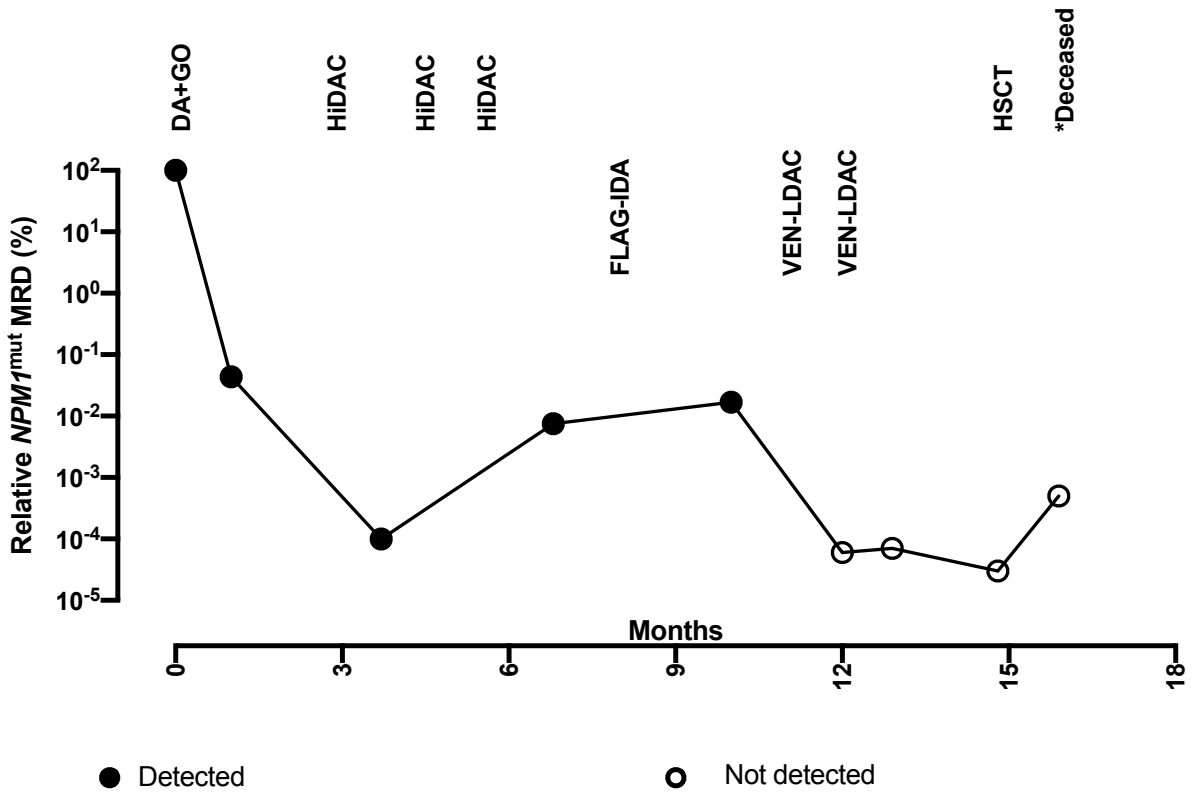
An 80-year-old male was diagnosed with AML with normal karyotype and co-mutations in *IDH1* (R132C) *FLT3* (D835E) and *SRSF2* (P95R). He entered the NCRI AML18 clinical trial and received induction therapy with DA3+10 with two doses of gemtuzumab ozogamicin. He achieved a morphological and molecular complete remission after cycle 1 and received DA3+8 for cycle 2 which was complicated by severe mucositis leading to the decision to withhold further treatment.

One year after his initial diagnosis, two consecutive bone marrow samples tested positive for *NPM1* mutant transcripts with a rising level and he was diagnosed with molecular relapse. He was treated with azacitidine monotherapy which resulted in an initial reduction in transcript levels which subsequently reached a plateau. He then commenced treatment with venetoclax (100mg od with posaconazole x 14d) + azacitidine (75mg/m^2 x 7d), achieving a molecular complete remission after the first cycle. The second cycle was complicated by neutropenic sepsis and severe pneumonia requiring intensive care admission for non-invasive ventilation. The length of azacitidine was reduced to 5 days for subsequent cycles and these were well tolerated. At last follow-up 11.8 months after initiation of venetoclax treatment, the patient remains alive and well in molecular complete remission with a plan for ongoing treatment.



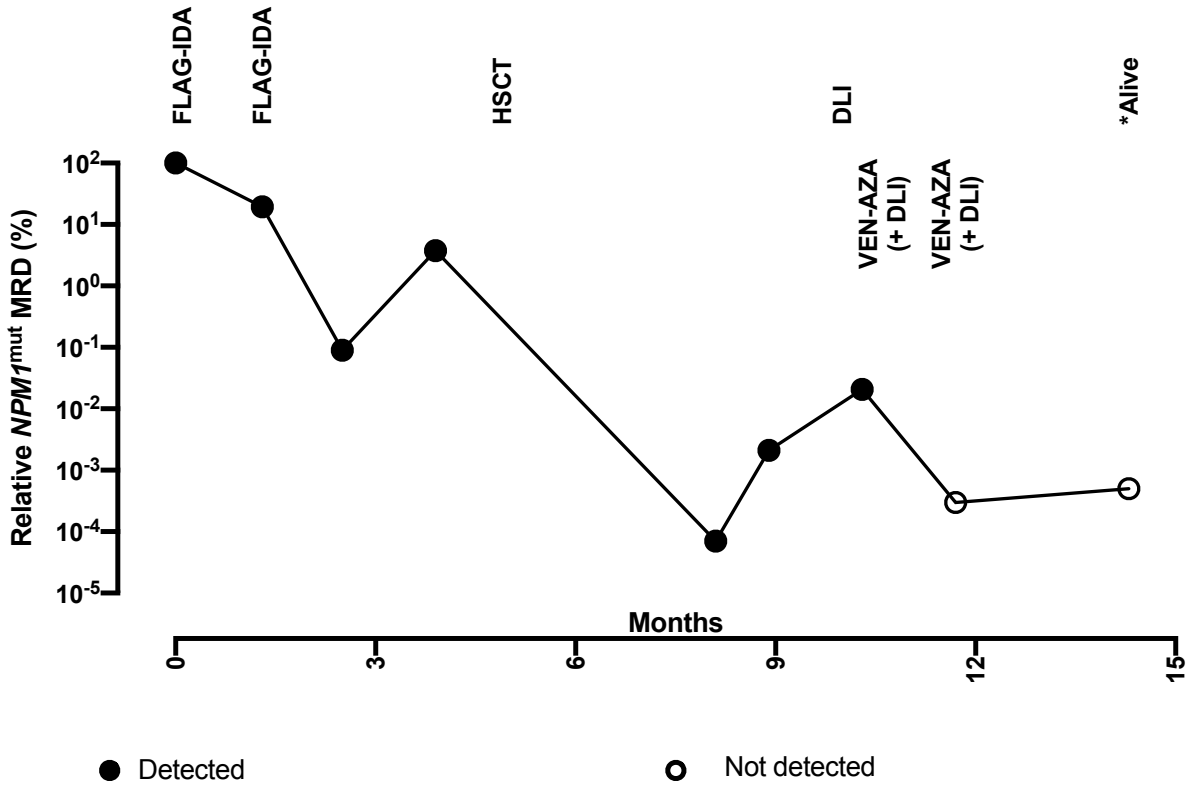
Case #7

A 40-year-old female was diagnosed with AML with a normal karyotype and mutations in *NPM1* and *FLT3* (D835Y). She entered the NCRI AML19 clinical trial and received induction therapy with DA3+10 and one dose of gemtuzumab ozogamicin. This was complicated by eosinophilic myocarditis thought to be caused by the gemtuzumab ozogamicin. She achieved CR after the first cycle with a 3.4 log reduction in *NPM1* mutant transcript levels and received consolidation with 3 cycles of high dose cytarabine (3 g/m^2 x 6). At the end of treatment, MRD assessment showed a 1.9 log increase in transcript levels consistent with molecular progression. She received FLAG-IDA as salvage therapy; this was poorly tolerated with life-threatening infection and on regeneration there had been no reduction in transcript levels. She then received venetoclax (100 mg od x 28d + posaconazole) + cytarabine (20 mg/m^2/d x 10d) and achieved complete molecular remission, grade 4 neutropenia and thrombocytopenia but no infection. Second cycle of therapy was continued without waiting for neutrophil and platelet recovery to grade <4, with venetoclax ceased at day 18. She went on to receive HSCT and MRD assessment pre-transplant and at D+30 showed ongoing molecular complete remission. Unfortunately, she was readmitted with sepsis after transplant having fully engrafted and subsequently died.



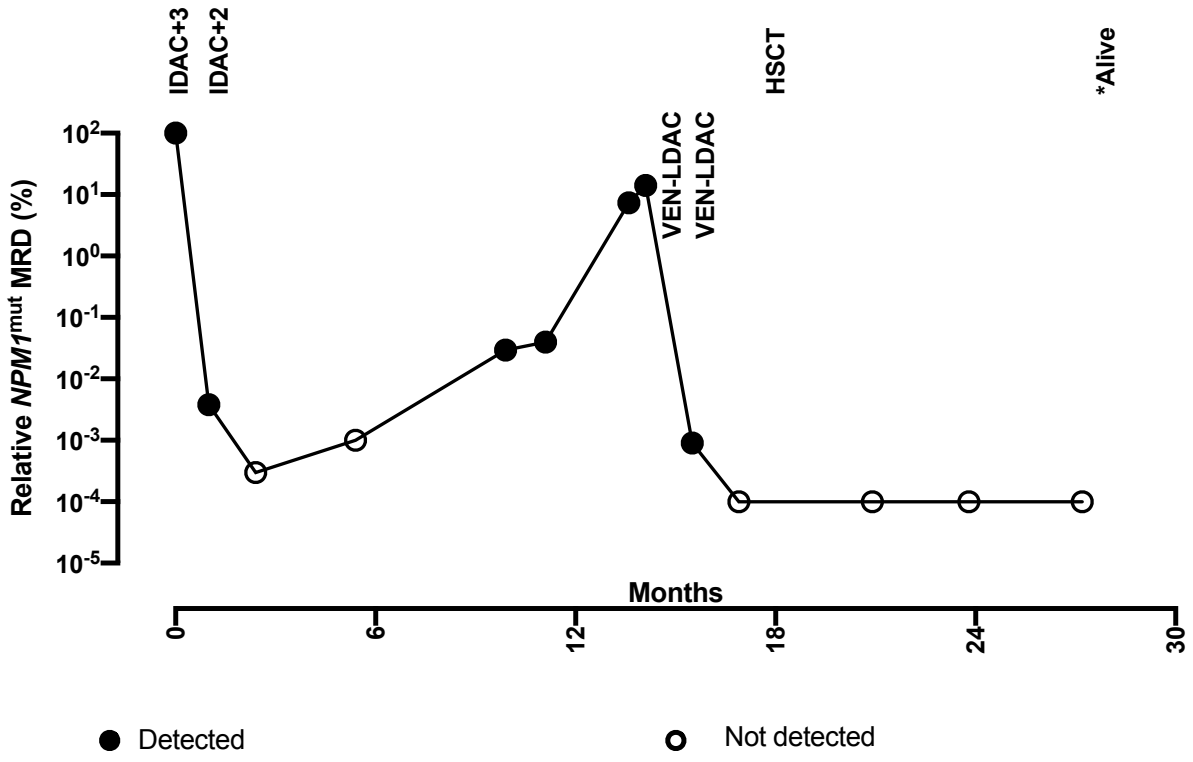
Case #8

A 28-year-old man presented with relapsed AML 7 years after his original diagnosis, having originally presented in 2012 with AML with fibrosis and an *NPM1* mutation. At relapse, in addition to persistent *NPM1*, he was also found to harbour a *FLT3* TKD (D835) mutation. He received two cycles of FLAG-IDA salvage therapy resulting in CR and a 3-log reduction in transcript levels. Post allogeneic HSCT, the D+100 bone marrow MRD assessment was positive. Despite withdrawal of immunosuppression and donor lymphocyte infusion (DLI; 10^6 cells), *NPM1*^{mut} levels increased in two consecutive samples consistent with molecular progression. Venetoclax (100 mg od x 14d) + azacitidine (75mg/m^2 for 5 days) was given resulting in molecular complete remission after one cycle of therapy in conjunction with DLI (10^6 cells). Venetoclax and azacitidine was well tolerated without grade 4 cytopenias, infection or hospital admission. Second cycle was delivered with DLI 4.7 x 10^6 cells. The patient remains alive and well with a plan for ongoing therapy.



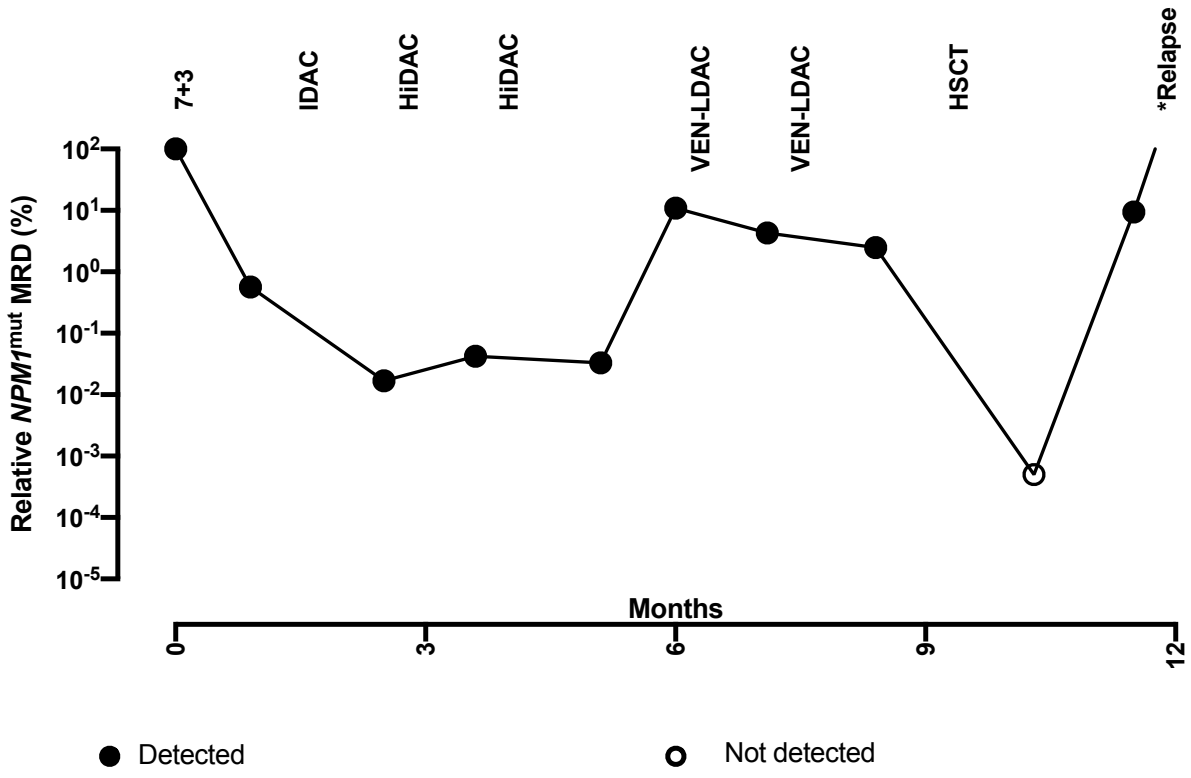
Case #9

A 52-year-old male was diagnosed with AML with normal karyotype and mutated *NPM1* (no other co-mutations). He achieved excellent response following IDAC+3 induction (cytarabine 1.5 g/m^2 x 8 and idarubicin 12 mg/m^2 x3) with a 4.4-log reduction in *NPM1*^{mut} MRD, and molecular complete remission after an IDAC+2 consolidation (cytarabine 1 g/m^2 x6 and idarubicin 12 mg/m^2 x2). During surveillance, *NPM1*^{mut} transcript levels were noted to be rising after 9 months post CR and peaked at 14% (~13 months post CR). Venetoclax (100 mg x 14d with posaconazole) and low-dose cytarabine were initiated with 4.2 log reduction post 1st cycle and molecular complete remission post 2nd cycle. Patient underwent allogeneic HSCT and remained alive at 9.9 months post HSCT.



Case #10

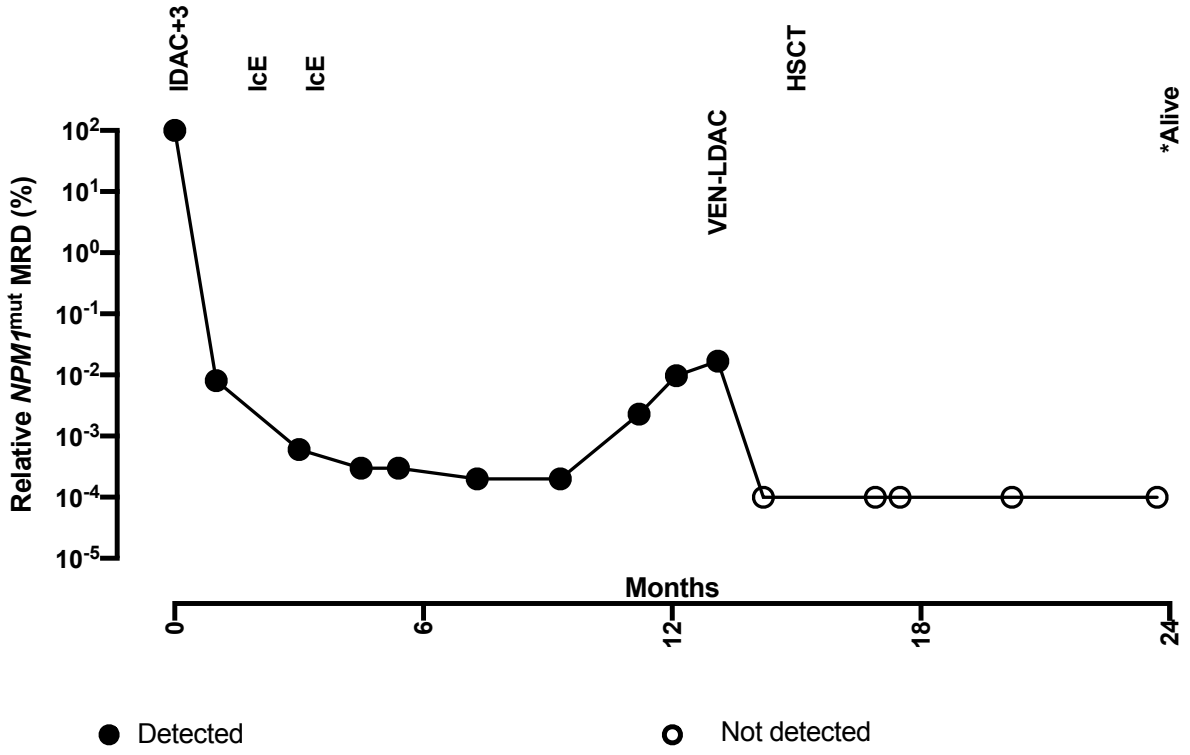
A 35-year-old female was diagnosed with AML with normal karyotype, mutated *NPM1*, *IDH2* R140 and *DNMT3A* R882. Post induction (7+3) response was suboptimal (2.2 log reduction), followed by three cycles of HiDAC consolidation (3 g/m^2 x 6) with persistent low copy numbers at approximately 3.5 log from baseline levels. Two months following completion of chemotherapy, molecular progression was evident. Two cycles of venetoclax (100 mg x 14d during cycle 1 and x 28d during cycle 2) with low-dose cytarabine were attempted with minimal response (0.6 log reduction). Patient went to have a myeloablative allogeneic HSCT but relapsed 78 days post HSCT with a newly acquired *FLT3*-ITD at allelic ratio 0.09. Baseline mutations (*NPM1, IDH2* and *DNMT3A*) were all persistent at the time of relapse. She was subsequently treated with gilteritinib.



Case #11

A 51-year-old male was diagnosed with AML with normal karyotype, *FLT3*-ITD (allelic ratio 0.32) and mutated *NPM1*. He entered ALLG AMLM16 trial and underwent induction with IDAC+3 (cytarabine 1.5 g/m^2 x8 and idarubicin 12 mg/m^2 x3) and sorafenib/placebo. Post induction assessment revealed CR with a 4.1-log reduction in *NPM1*^{mut} MRD. This was followed by two cycles of IcE consolidation (idarubicin 9 mg/m^2 x2, cytarabine 100 mg/m^2 x5 and etoposide 75 mg/m^2 x5), with persistent low copy numbers of *NPM1*^{mut} transcripts.

NPM1^{mut} MRD was initially stable during surveillance but molecular progression occurred at six months post completion of chemotherapy. After one cycle of venetoclax (100 mg x 14d with posaconazole) and low-dose cytarabine, *NPM1*^{mut} MRD was rendered undetectable and patient underwent reduced intensity conditioning HSCT. He remained alive and well at 8.9 months post HSCT.



Case #12

A 69-year-old male was diagnosed with AML with normal karyotype, mutated *NPM1* and *DNMT3A* R882. Post induction (7+3) response was suboptimal (2.8 log reduction), followed by two cycles of IDAC+2 consolidation (cytarabine 1 g/m^2 x 6 and idarubicin 12 mg/m^2 x2) with persistent low copy numbers at end of therapy.

NPM1^{mut} transcript levels were initially stable but molecular progression ensued eight months post completion of chemotherapy, followed shortly by early morphological relapse (5% bone marrow blasts) 3.1 months later. Patient entered a clinical trial receiving venetoclax in combination with a MCL1 inhibitor and achieved a 2.1-log MRD reduction but was withdrawn after one cycle due to toxicity. He subsequently received venetoclax (600 mg once daily x28d) and low-dose cytarabine, with a further 2.6-log reduction after one cycle and achieved molecular complete remission after 3 cycles. Patient remained on therapy (7+ cycles) and well at last follow up.