Supplemental appendix

Contents

Supplemental Methods

Inclusion criteria

To be included into the study patients had to meet the following criteria:

1. Newly diagnosed AML with *NPM1* mutation
2. Frontline treatment with venetoclax combination
3. Achievement of complete remission with or without count recovery
4. At least one bone marrow MRD assessment in the first 4 cycles of therapy

Ethics

The study was conducted in accordance with the Declaration of Helsinki. Data collection for the project at UK sites was approved by the Central Bristol Research Ethics Committee (22/SW/0042). The study was approved by the Alfred Health (88/20) research ethics committee.

MRD analyses

MRD testing for *NPM1* mutations was performed as part of routine care in three central reference laboratories. Mutant-specific RT-qPCR was performed on RNA extracted from bone marrow aspirates or peripheral blood with *ABL1* as a control gene as previously described[11], according to Europe Against Cancer (EAC) criteria[12]. Samples were run in triplicate and those with inadequate input RNA (*ABL1* cycle threshold >30) were excluded. RT-qPCR results are expressed as a copy number normalised to 10^5 copies of *ABL1.*

Endpoints and statistical analysis

Continuous variables are summarised using medians and inter-quartile range (IQR) with groups compared using the Wilcoxon rank-sum test, while categorical variables are displayed as frequencies and percentages and compared using the Chi-squared or Fisher's exact tests. Median FU was calculated using the reverse Kaplan-Meier method. Overall survival (OS) was defined as per ELN criteria and measured from the day of starting therapy[13]. As all patients were subject to regular MRD monitoring, and subsequent therapies initiated at molecular relapse, event-free survival (EFS) included molecular progression or relapse as events, in addition to haematological relapse or death. Time-to-event variables were estimated using the Kaplan-Meier method and groups compared using the log-rank test. The impact of variables on OS was analysed with Cox regression, with the time of achieving MRD negativity entered as a time-dependent variable.

Patients who initiated another therapy, including allogeneic SCT, prior to meeting ELN criteria for molecular relapse or progression (n=7) were censored for EFS at the time of next therapy. They remained uncensored in OS calculations. All analyses were performed with R statistical software version 4.2.1 (R Core Development Team, Vienna, Austria).

Supplemental Table 1 – comparison of HMA and LDAC

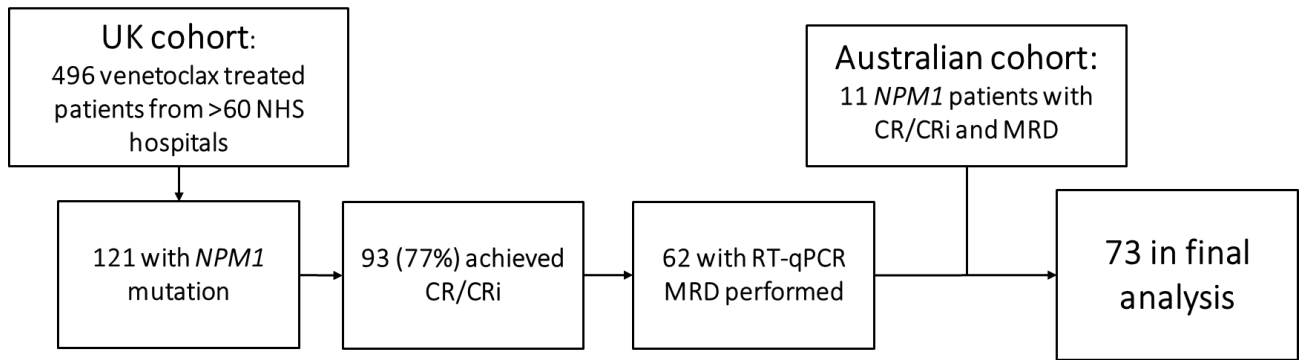| Characteristic	HMA, N = 46	LDAC, N = 27	p-value
Age (IQR)	71.90 (69.10 - 75.00)	72.90 (66.00 - 76.45)	>0.9
Female	22 (48%)	15 (56%)	0.5
Performance status			
0 – 1	30 (82%)	23 (92%)	
≥2	7 (18%)	2 (8.0%)	
Disease category			0.5
De novo	33 (72%)	23 (85%)	
Secondary	7 (15%)	2 (7.4%)	
Therapy-related	6 (13%)	2 (7.4%)	
Cytogenetic risk			0.5
Intermediate	2 (4.3%)	0 (0%)	
Adverse	42 (91%)	25 (93%)	
Failed	2 (4.3%)	2 (7.4%)	
FLT3 ITD	12 (26%)	6 (22%)	0.7
FLT3 TKD	6 (13%)	5 (19%)	0.5
DNMT3A	13 (36%)	6 (26%)	0.4
IDH1	3 (8.3%)	3 (13%)	0.7
IDH2	6 (17%)	6 (26%)	0.5
TP53	1 (2.8%)	0 (0%)	>0.9
Allogeneic transplant			
In first CR1	2 (4.3%)	2 (7.4%)	>0.9
After relapse	41 (89%)	23 (85%)	
No transplant	3 (6.5%)	2 (7.4%)	
MRD negative at any time	25 (54%)	15 (56%)	0.9
Best response in first 2 cycles			0.9
Negative	11 (25%)	6 (23%)	
> 4 log reduction	6 (14%)	5 (19%)	
< 4 log reduction	27 (61%)	15 (58%)	
No result	2	1	
Best response in first 4 cycles			0.8
Negative	21 (46%)	14 (52%)	
> 4 log reduction	10 (22%)	4 (15%)	
< 4 log reduction	15 (33%)	9 (33%)	
2-year overall survival	59%	65%	0.6
2-year event-free survival	49%	50%	0.7

Supplemental Table 2 – identification of MRD thresholds using maximally selected rank statistics

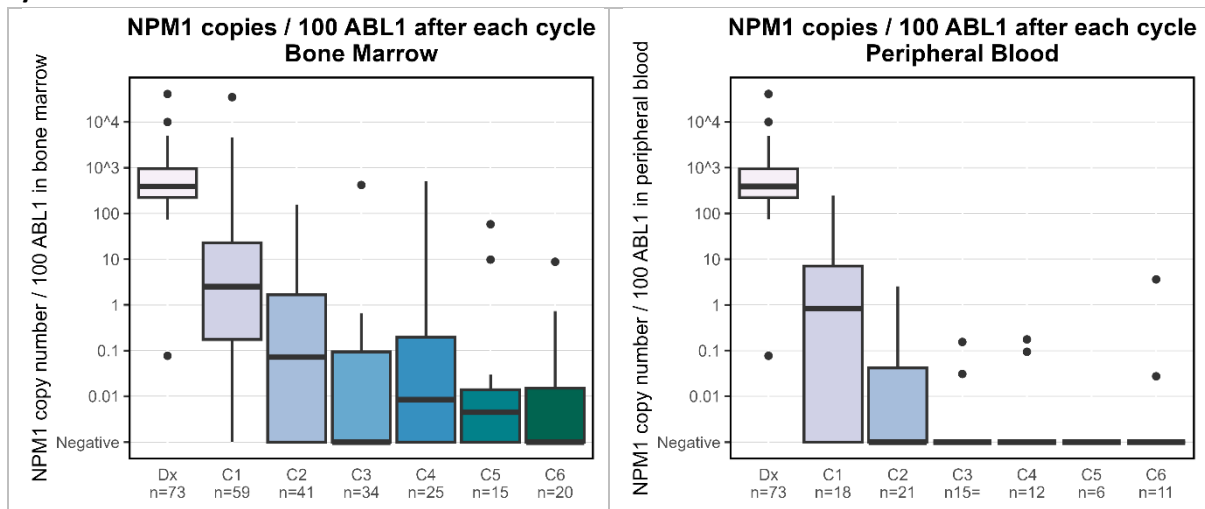|	Overall survival	Event-free survival
Lowest *NPM1* copy number in the first 2 cycles	Copy number threshold = 29 M = 2.37 p-value = 0.15	Copy number threshold = 0.002 M = 2.46 p-value = 0.12
Lowest *NPM1* copy number in the first 4 cycles	Copy number threshold = 0.005 M = 3.96 p-value = 8e-04	Copy number threshold = 0 M = 5.04 p-value < 2.2e-16

Supplemental Table 3 – multivariable analysis for OS

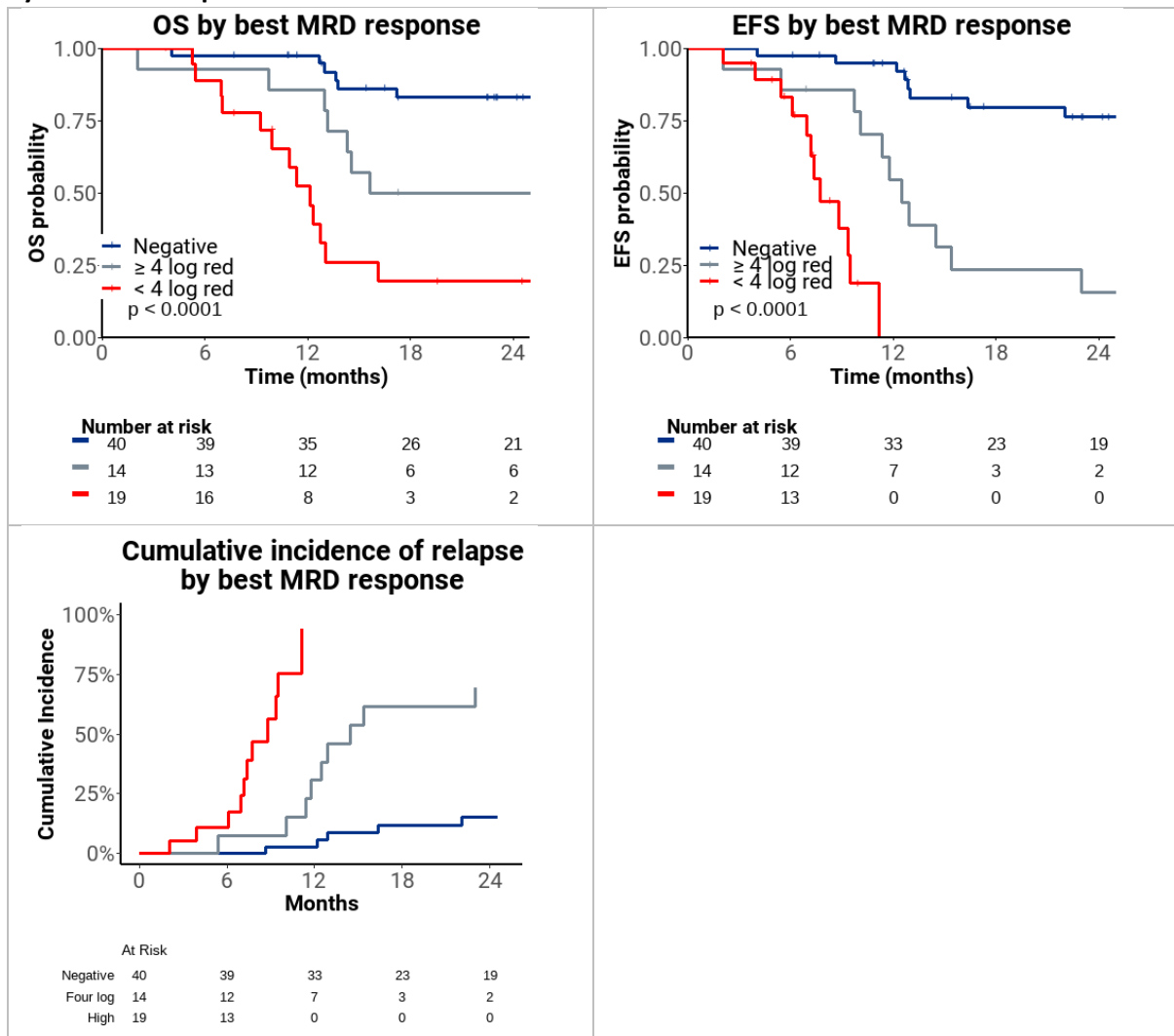| Characteristic	HR[1]	95% CI[1]	p-value
Age	1.03	0.99, 1.08	0.12
Performance status	1.09	0.56, 2.12	0.8
Secondary or therapy-related disease	0.99	0.36, 2.75	>0.9
Abnormal cytogenetics	1.50	0.43, 5.28	0.5
FLT3 mutation (ITD and/or TKD)	2.60	1.05, 6.44	0.04
Ven-LDAC (compared to ven-HMA)	1.13	0.45, 2.85	0.8
Achieving MRD negative in first 4 cycles (time-dependent variable)	0.22	0.08, 0.60	0.003

Supplemental Figure 1 – patient flow diagram

UK cohort:				Australian cohort:
496 venetoclax treated patients from >60 NHS hospitals				11 *NPM1* patients with CR/CRi and MRD

121 with *NPM1* mutation → 93 (77%) achieved CR/CRi → 62 with RT-qPCR MRD performed → 73 in final analysis
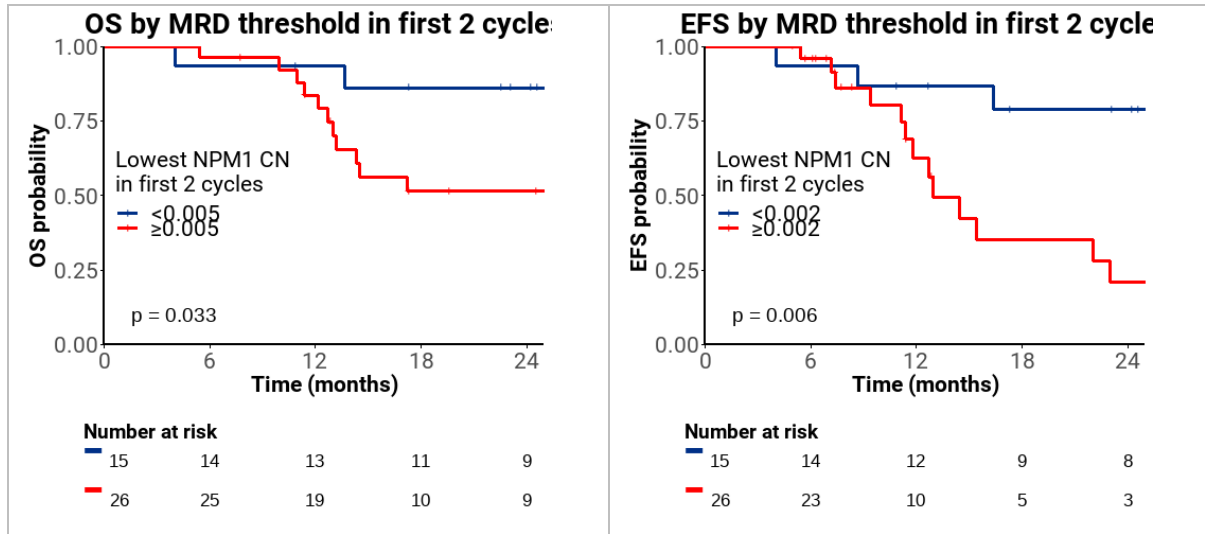
Supplemental Figure 2 – NPM1 copy number in bone marrow and peripheral blood after each cycle

Supplemental Figure 3 – overall survival, event-free survival and cumulative incidence of relapse by best MRD response

Supplemental Figure 4 – outcomes by MRD threshold after 2 cycles

OS by MRD threshold in first 2 cycles

OS probability

Lowest NPM1 CN
in first 2 cycles
— ≤0.005
— ≥0.005

p = 0.033

Time (months)

Number at risk

15	14	13	11	9
26	25	19	10	9

EFS by MRD threshold in first 2 cycles

EFS probability

Lowest NPM1 CN
in first 2 cycles
— ≤0.002
— ≥0.002

p = 0.006

Time (months)

Number at risk

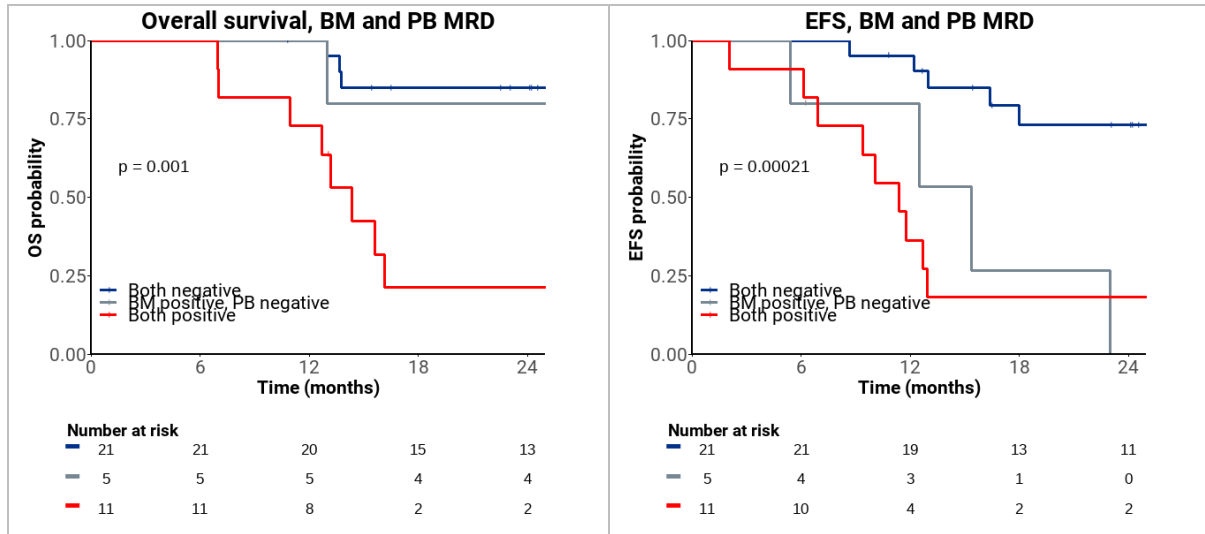|				
15	14	12	9	8
26	23	10	5	3

Supplemental Figure 5 – OS, EFS and cumulative incidence of relapse by peripheral blood MRD negativity within first 4 cycles
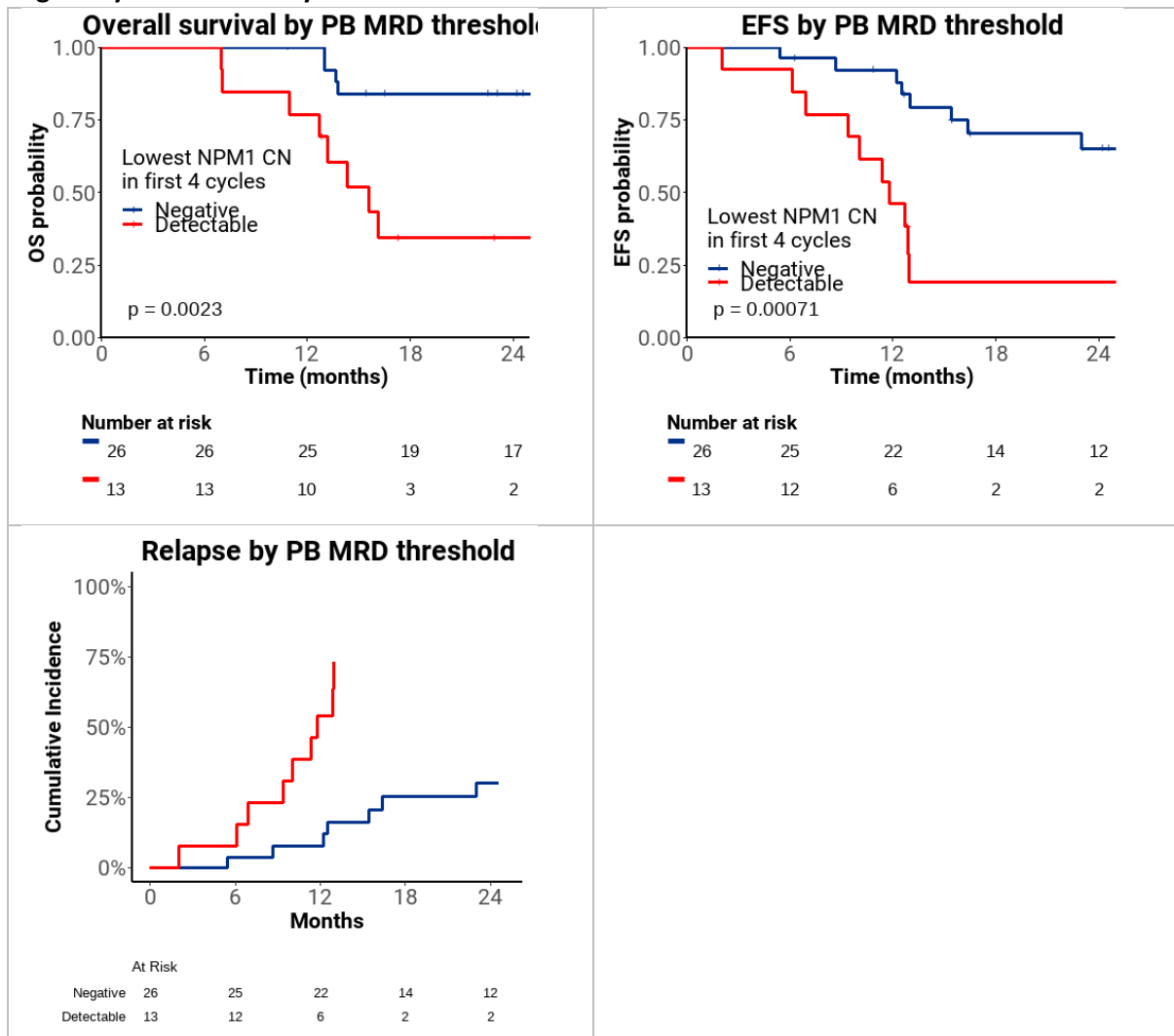
Supplemental Figure 6 – comparison of PB and BM results from samples taken concurrently
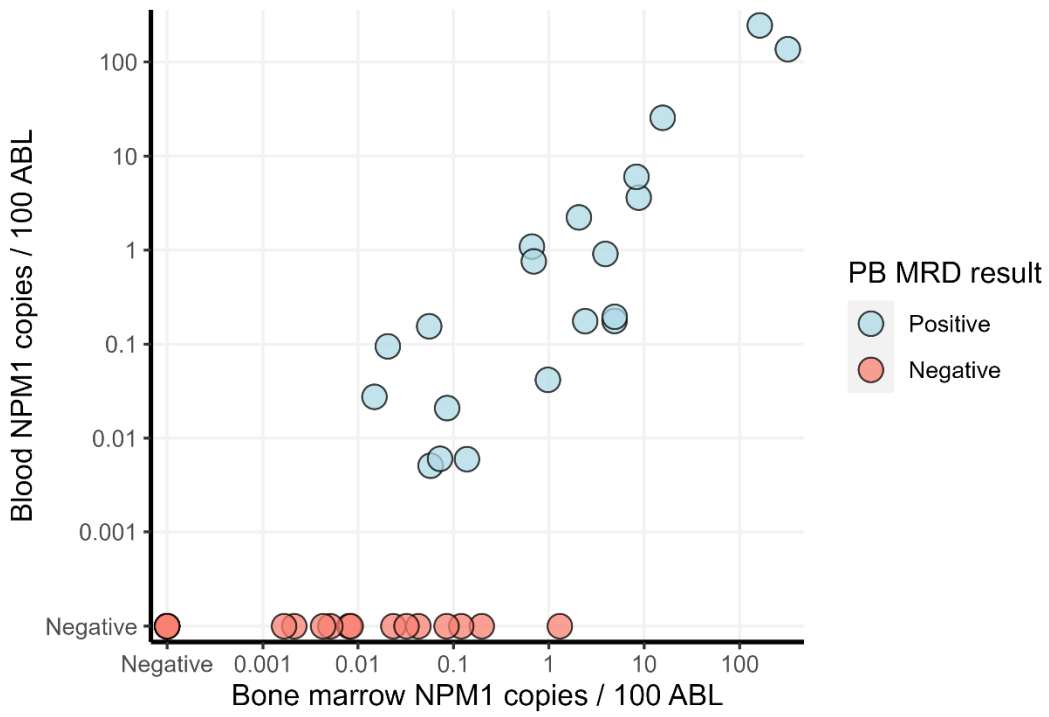
Supplemental Figure 7 – outcomes by combined PB and BM MRD negativity in first 4 cycles

Supplemental Figure 8 – swimmer plot showing outcomes in patients electively stopping therapy in MRD negative remission

Time on treatment and duration of treatment-free remission