SUPPLEMENTARY APPENDIX

Addition of the mammalian target of rapamycin inhibitor, everolimus, to consolidation therapy in acute myeloid leukemia: experience from the UK NCRI AML17 trial

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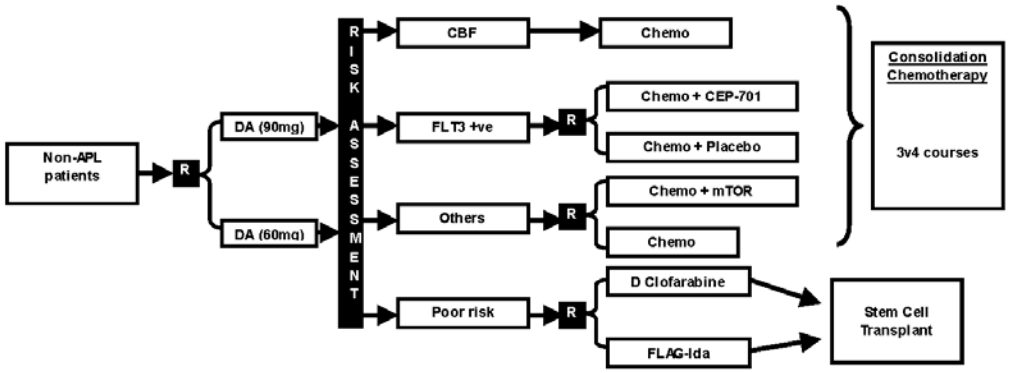
Supplemental Figure 1: Randomizations Addressed in AML17

Protocol Version 7

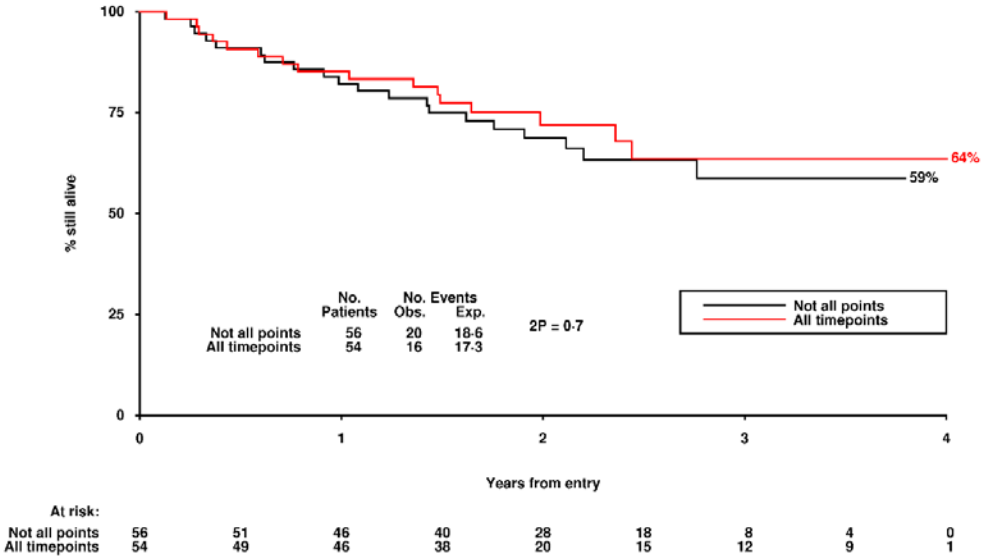


Gemtuzumab Ozogamicin 3mg/m^2 vs 6mg/m^2 (day 1 course 1)
ADE vs DA
DA (Daunorubicin 90mg/m^2) vs DA (Daunorubicin 60mg/m^2)
Lestaurtinib vs not (FLT3 mutant)
Everolimus vs not (courses 2-4)
3 vs 4 courses in total (if not high risk)
FLAG-Ida vs Daunorubicin/Clofarabine

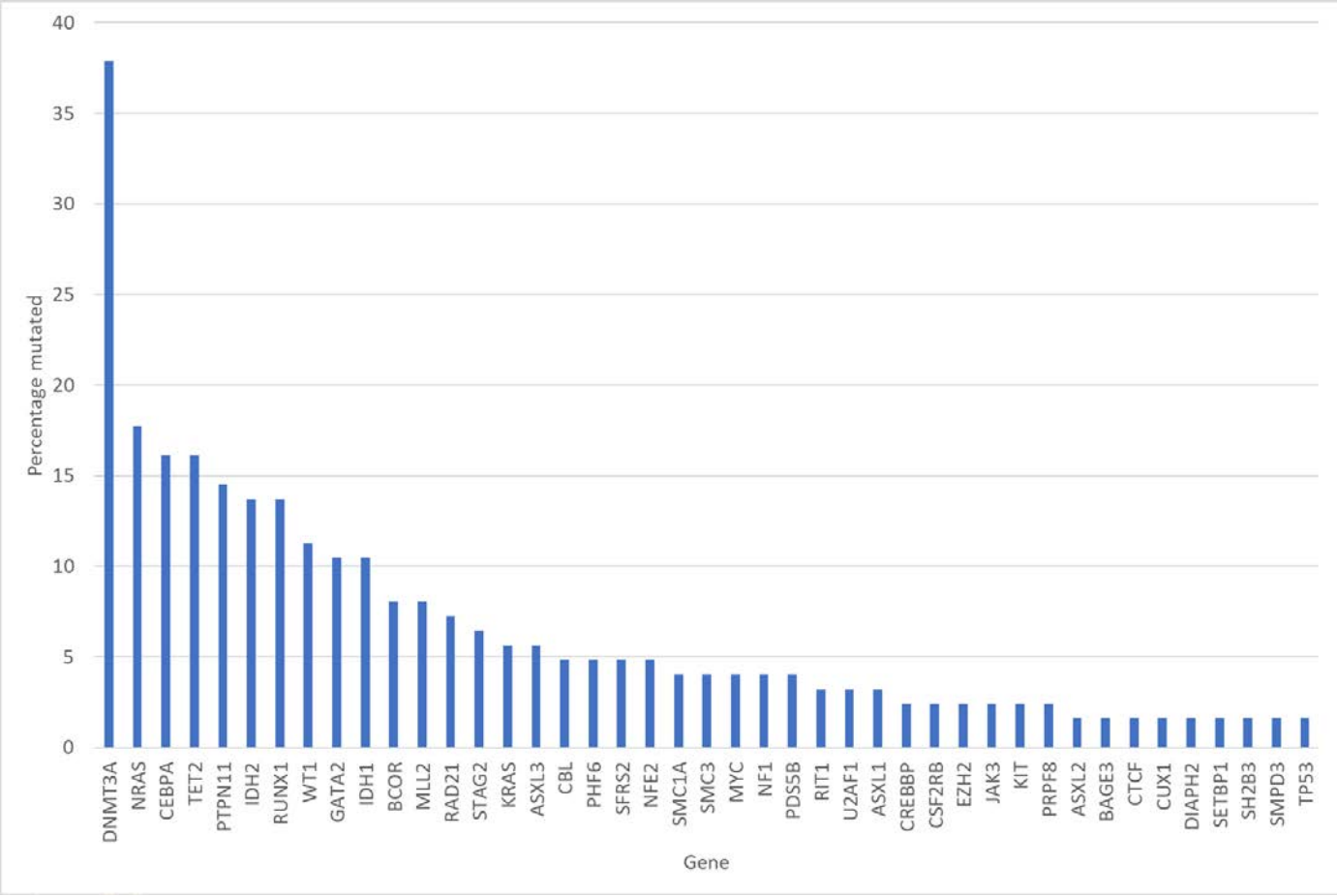
Supplementary Figure 2: Plasma Inhibitory activity (PIA) Measurement.

The protocol requested, with patient agreement, the collection of blood samples pre-dose on day 14 of each course of everolimus treatment. To assess mTOR inhibitory activity, 400µl patient plasma was incubated in triplicate with $5x10^5$ HEL cells for 1h at 37°C in a humidified incubator with 5%CO_2. The approach was similar to those reported for other inhibitory assays.[18] A standard curve of phospho-S6 ribosomal protein (pS6-RP) PIA versus everolimus concentration was generated by spiking healthy volunteer plasma with everolimus that produce clinically-relevant concentrations ranging from 1 to 200ng/ml. In this context an estimate of plasma inhibition of phospho-S6 ribsomal protein (p S6-RP) in response to patient plasma was measured in cell lysates by immunoblotting and ELISA. The results were expressed as a percentage reduction of pS6-RP inhibitory activity compared to the maximum inhibition achieved by a 200ng/ml everolimus concentration which was run in parallel along with a no drug control.

Overall survival from Everolimus randomization: Inhibition at all vs not at all timepoints (1+ timepoints measured)



Supplementary Figure 3: Resolution of Sanger data with AML17 mTOR randomisation (n=124)

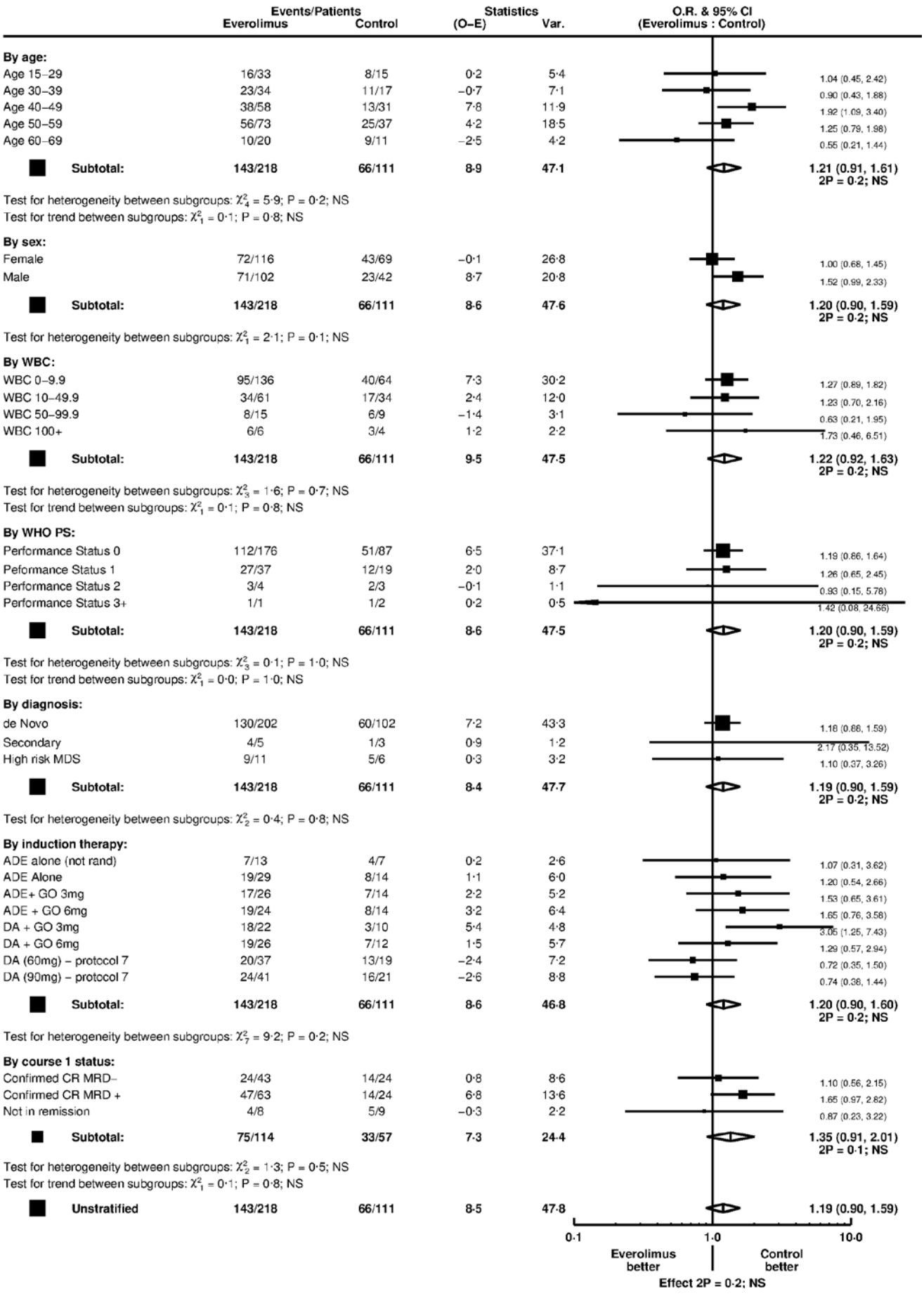


Mutations in the following genes were found in only one patient and are not shown in the graph:

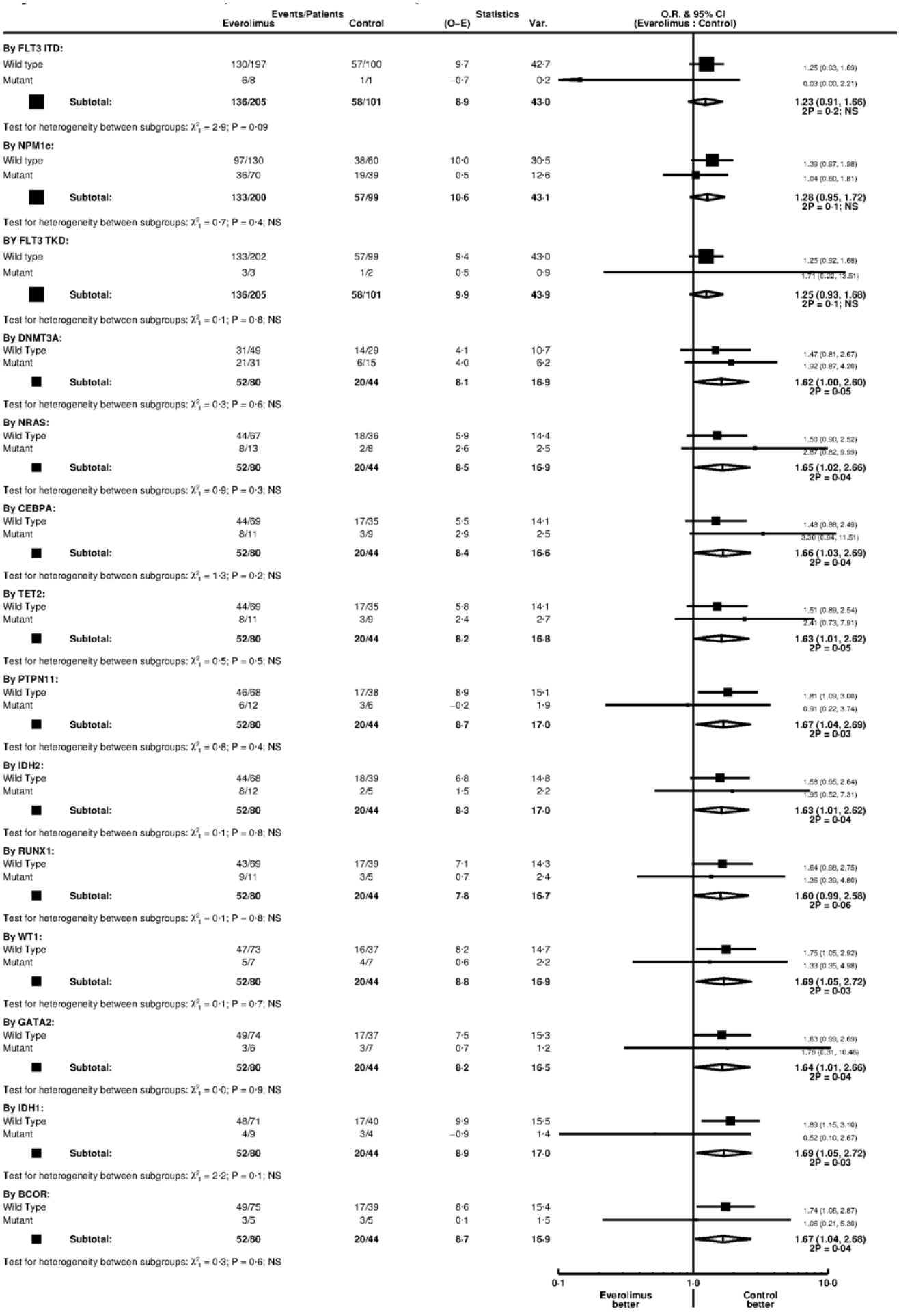
ATRX	**ERCC2**	**KIAA1267**	**PTEN**	**SMG1**
CBFB	**FBXW7**	**LUC7L2**	**PTPRF**	**STAG1**
CBLB	**GATA1**	**MED12**	**SF1**	**U2AF2**
CBLC	**KDM6A**	**MYH11**	**SF3B1**	**ZRSR2**
CSF3R				

Supplementary Figure 4: Stratified analysis of Relapse Free Survival. A) Demographics; B) Mutation status (minimum 10 mutant patients with RFS data)

A)



B)


Events/Patients
Everolimus
Control
Statistics
(O–E)
Var.
O.R. & 95% CI
(Everolimus : Control)
By FLT3 ITD:
Wild type 130/197 57/100 9·7 42·7 1.25 (0.93, 1.69)
Mutant 6/8 1/1 −0·7 0·2 0.03 (0.00, 2.21)
Subtotal: 136/205 58/101 8·9 43·0 1.23 (0.91, 1.66) 2P = 0·2; NS
Test for heterogeneity between subgroups: χ^2_1 = 2·9; P = 0·09
By NPM1c:
Wild type 97/130 38/60 10·0 30·5 1.39 (0.97, 1.98)
Mutant 36/70 19/39 0·5 12·6 1.04 (0.60, 1.81)
Subtotal: 133/200 57/99 10·6 43·1 1.28 (0.95, 1.72) 2P = 0·1; NS
Test for heterogeneity between subgroups: χ^2_1 = 0·7; P = 0·4; NS
BY FLT3 TKD:
Wild type 133/202 57/99 9·4 43·0 1.25 (0.92, 1.68)
Mutant 3/3 1/2 0·5 0·9 1.71 (0.22, 13.51)
Subtotal: 136/205 58/101 9·9 43·9 1.25 (0.93, 1.68) 2P = 0·1; NS
Test for heterogeneity between subgroups: χ^2_1 = 0·1; P = 0·8; NS
By DNMT3A:
Wild Type 31/49 14/29 4·1 10·7 1.47 (0.81, 2.67)
Mutant 21/31 6/15 4·0 6·2 1.92 (0.87, 4.20)
Subtotal: 52/80 20/44 8·1 16·9 1.62 (1.00, 2.60) 2P = 0·05
Test for heterogeneity between subgroups: χ^2_1 = 0·3; P = 0·6; NS
By NRAS:
Wild Type 44/67 18/36 5·9 14·4 1.50 (0.90, 2.52)
Mutant 8/13 2/8 2·6 2·5 2.87 (0.82, 9.99)
Subtotal: 52/80 20/44 8·5 16·9 1.65 (1.02, 2.66) 2P = 0·04
Test for heterogeneity between subgroups: χ^2_1 = 0·9; P = 0·3; NS
By CEBPA:
Wild Type 44/69 17/35 5·5 14·1 1.48 (0.88, 2.49)
Mutant 8/11 3/9 2·9 2·5 3.30 (0.94, 11.51)
Subtotal: 52/80 20/44 8·4 16·6 1.66 (1.03, 2.69) 2P = 0·04
Test for heterogeneity between subgroups: χ^2_1 = 1·3; P = 0·2; NS
By TET2:
Wild Type 44/69 17/35 5·8 14·1 1.51 (0.89, 2.54)
Mutant 8/11 3/9 2·4 2·7 2.41 (0.73, 7.91)
Subtotal: 52/80 20/44 8·2 16·8 1.63 (1.01, 2.62) 2P = 0·05
Test for heterogeneity between subgroups: χ^2_1 = 0·5; P = 0·5; NS
By PTPN11:
Wild Type 46/68 17/38 8·9 15·1 1.81 (1.09, 3.00)
Mutant 6/12 3/6 −0·2 1·9 0.91 (0.22, 3.74)
Subtotal: 52/80 20/44 8·7 17·0 1.67 (1.04, 2.69) 2P = 0·03
Test for heterogeneity between subgroups: χ^2_1 = 0·8; P = 0·4; NS
By IDH2:
Wild Type 44/68 18/39 6·8 14·8 1.58 (0.95, 2.64)
Mutant 8/12 2/5 1·5 2·2 1.95 (0.52, 7.31)
Subtotal: 52/80 20/44 8·3 17·0 1.63 (1.01, 2.62) 2P = 0·04
Test for heterogeneity between subgroups: χ^2_1 = 0·1; P = 0·8; NS
By RUNX1:
Wild Type 43/69 17/39 7·1 14·3 1.64 (0.98, 2.75)
Mutant 9/11 3/5 0·7 2·4 1.36 (0.39, 4.80)
Subtotal: 52/80 20/44 7·8 16·7 1.60 (0.99, 2.58) 2P = 0·06
Test for heterogeneity between subgroups: χ^2_1 = 0·1; P = 0·8; NS
By WT1:
Wild Type 47/73 16/37 8·2 14·7 1.75 (1.05, 2.92)
Mutant 5/7 4/7 0·6 2·2 1.33 (0.35, 4.98)
Subtotal: 52/80 20/44 8·8 16·9 1.69 (1.05, 2.72) 2P = 0·03
Test for heterogeneity between subgroups: χ^2_1 = 0·1; P = 0·7; NS
By GATA2:
Wild Type 49/74 17/37 7·5 15·3 1.63 (0.99, 2.69)
Mutant 3/6 3/7 0·7 1·2 1.79 (0.31, 10.46)
Subtotal: 52/80 20/44 8·2 16·5 1.64 (1.01, 2.66) 2P = 0·04
Test for heterogeneity between subgroups: χ^2_1 = 0·0; P = 0·9; NS
By IDH1:
Wild Type 48/71 17/40 9·9 15·5 1.89 (1.15, 3.10)
Mutant 4/9 3/4 −0·9 1·4 0.52 (0.10, 2.67)
Subtotal: 52/80 20/44 8·9 17·0 1.69 (1.05, 2.72) 2P = 0·03
Test for heterogeneity between subgroups: χ^2_1 = 2·2; P = 0·1; NS
By BCOR:
Wild Type 49/75 17/39 8·6 15·4 1.74 (1.06, 2.87)
Mutant 3/5 3/5 0·1 1·5 1.06 (0.21, 5.30)
Subtotal: 52/80 20/44 8·7 16·9 1.67 (1.04, 2.68) 2P = 0·04
Test for heterogeneity between subgroups: χ^2_1 = 0·3; P = 0·6; NS
0·1
1·0
10·0
Everolimus better
Control better

***Supplementary Figure 5: Relapse Free Survival Related to Treatment Compliance*. Events within 30 days of course 3 are excluded**