TP53 immunohistochemistry correlates with *TP53* mutation status and clearance in decitabine-treated patients with myeloid malignancies

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Supplemental Text:

TP53 expression and cytogenetic abnormalities: *TP53* mutations are enriched in cases with complex cytogenetics, including chromosome 5, 7 and 17 abnormalities [1, S1]. In our cohort, information regarding karyotype was available in 70 of 72 cases. Complex cytogenetic profile (3 or more abnormalities) was more common in cases with elevated TP53 expression than in cases with baseline levels of TP53 (60% vs 18%, p = 0.00054). We found significant increase in TP53 staining in those with complex cytogenetic abnormalities (38 ± 6% vs. 13 ± 2%, p<0.0001, Supplemental Figure 1A). We also found significantly greater TP53 staining in cases with 5q deletion /monosomy 5(47 ± 5% vs.13 ± 2%, p<0.0001, Supplemental Figure 1B) and chromosome 17 abnormality (56 ± 5% vs.16 ± 2.5%, p<0.0001, Supplemental Figure 1C). No statistically significant differences in TP53 staining were associated with presence of 7q deletion/monosomy 7 (29 ± 7% vs. 19 ± 3%, p=0.1170).

Proportion of *TP53* truncating mutations: The proportion of truncating *TP53* mutations in myeloid malignancies reported in some individual studies is somewhat higher than that found in aggregated COSMIC database[S2], likely due to lower numbers of tested cases. The Cancer Genome Atlas (TCGA) study[8] performed on 200 AML cases identified only 16 samples with *TP53* mutations, with 9 samples (56.5%) containing mutations predicted to result in protein truncation. However, 4 of the samples had both missense and truncating mutations. In our study, 3 cases had both truncating and non-truncating *TP53* mutations, and all showed elevated TP53 staining (Supplemental Table 1). Thus, in the TCGA cohort, 5 cases which contained only truncating mutations (31%) are predicted to be undetectable by IHC. Papaemmannuil et al.[S3] reported 37 *TP53*-mutated cases, with 22% of these cases containing truncating mutations likely undetectable by IHC. Studies with larger numbers of *TP53*-mutated cases [S4, S5] reveal lower percentage of truncating *TP53* mutations that are likely to be missed by IHC analysis (18% total truncating variants with 14% of cases containing truncating variants only[S4]; 28% total

truncating mutations with 16% of cases containing truncating *TP53* mutations alone[S5]). In cases with multiple *TP53* mutations of different types including a truncating variant, serial molecular monitoring may be still needed in addition to TP53 staining to ensure that relapse with truncating clone alone is not missed by IHC.

Elevated TP53 expression in the absence of *TP53* mutation: We also observed two cases with elevated TP53 IHC (present in 58% and 45% of cells), but no mutations in *TP53,* similar to findings reported by others[11]. Additional FISH assays for 17p were performed on paraffin-embedded tissue using commercially available probes (Abbott Molecular, Abbott Park, IL, USA). One of the cases had partial loss of *TP53* locus, as determined by FISH for 17p (patient 1024), while the second case had no conclusive cytogenetic abnormalities (case 1021). Alternative mechanisms of TP53 stabilization in the absence of *TP53* mutation include overexpression of negative regulators such as MDM2 and/or its homolog MDM4 or inactivation of p14 ARF[S6].

***TP53* VAF and TP53 expression level:** Recent work has suggested that for MDS prognostic assessment *TP53* VAF measurements are superior to *TP53* mutational status alone [S7]. Previous investigation of relationship between *TP53* VAF and TP53 expression in MDS revealed strong association between these two measures[9]. In our cohort, Day 0 TP53 IHC, while elevated in *TP53*-mutated patients, exhibited no significant correlation with Day 0 *TP53* VAF, even when cases with truncating mutations and technical concerns were excluded ($r=0.3433$, $R^2= 0.0399$, $p=0.1630$, Supplemental Figure 1F).

Supplemental References:

S1. Haferlach C, Dicker F, Herholz H, Schnittger S, Kern W, Haferlach T. Mutations of the TP53 gene in acute myeloid leukemia are strongly associated with a complex aberrant karyotype. Leukemia. 2008;22143:1539–41.

S2. Forbes SA, Beare D, Boutselakis H, et al. COSMIC: somatic cancer genetics at high resolution. *Nucleic Acids Res*. 2017; **45**(D1): D777-D783.

S3. Papaemmanuil E, Gerstung M, Malcovati L, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood.* 2013; **122**(22):3616-3627.

S4. Papaemmanuil E, Gerstung M, Billinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med*. 2016; **374**(23):2209-2221.

S5. Lindsley RC, Saber W, Mar BG, et al. Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation. N Engl J Med. 2017; 376(6):536-547.

S6. Pomerantz J, Schreiber-Agus N, Lie NJ, et al. The Ink4a Tumor Suppressor Gene Product , p19 Arf , Interacts with MDM2 and Neutralizes MDM2 ' s Inhibition of TP53. *Cell.* 1998;**92**:713–723.

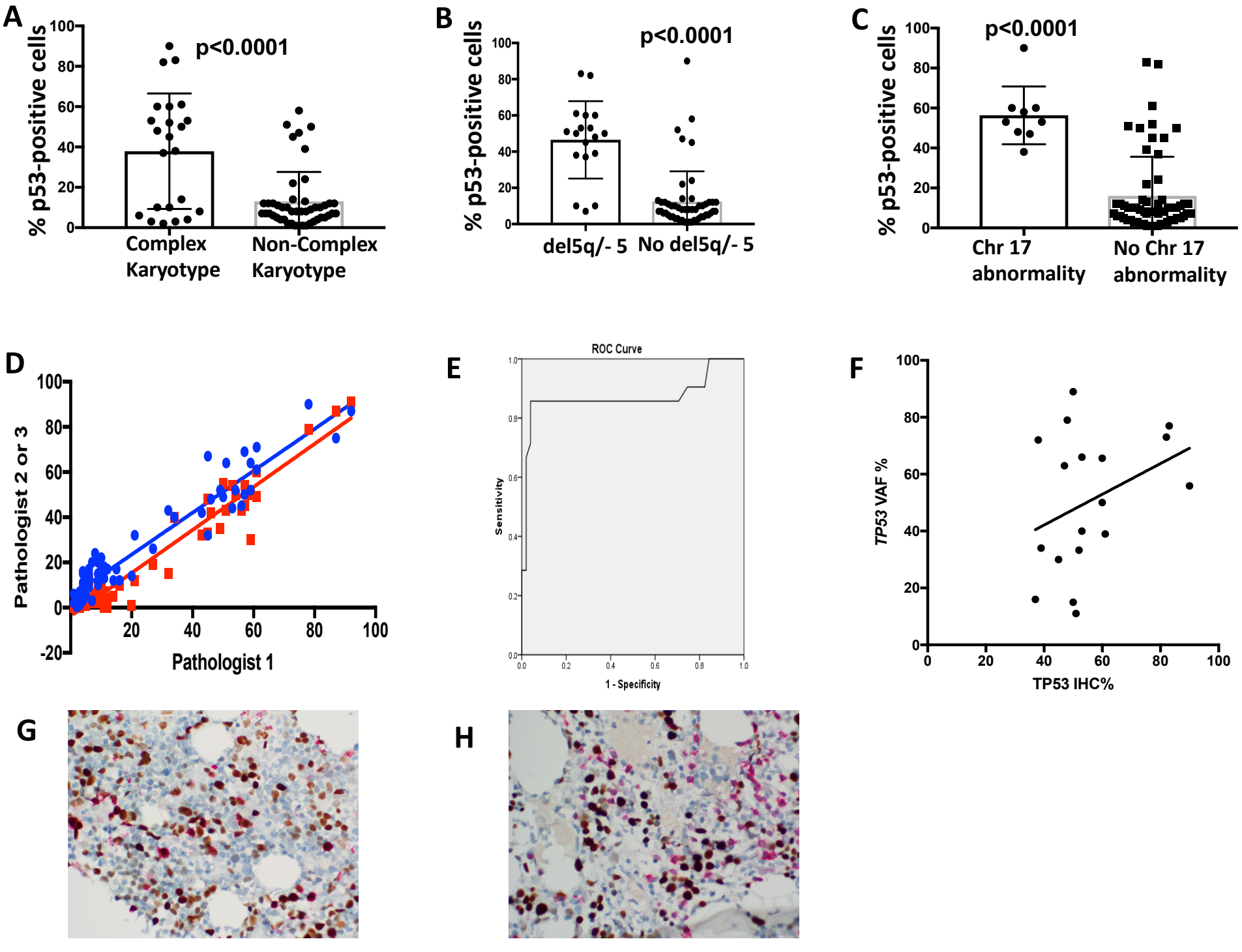
S7. Sallman DA, Komrokji R, Vaupel C, et al. Impact of TP53 mutation variant allele frequency on phenotype and outcomes in myelodysplastic syndromes. *Leukemia.* 2016;**30**(3):666–673.

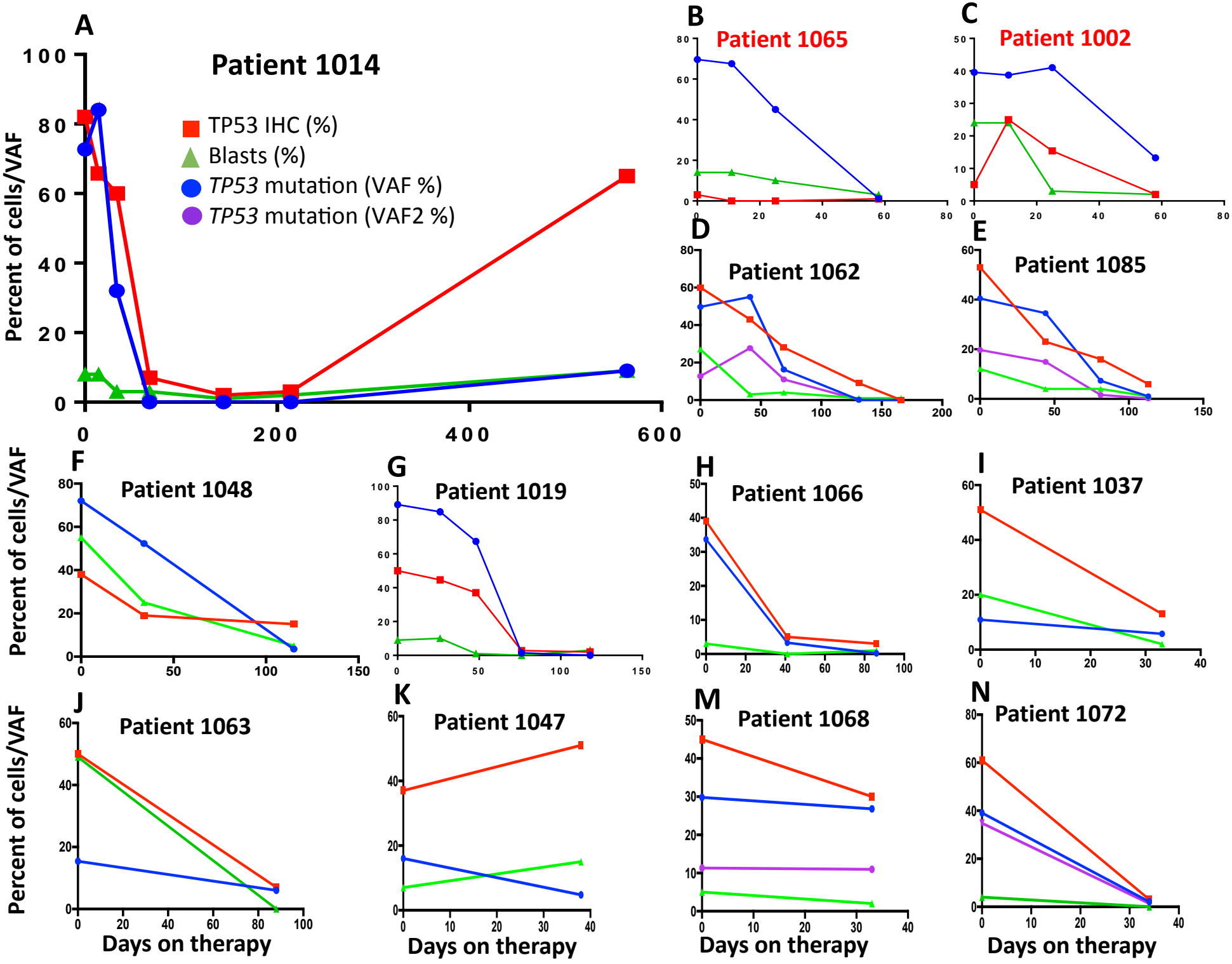
Supplemental Table 1: Clinical, cytogenetic, immunophenotypic and molecular findings for study patients.

Supplemental Figure 1: Significant increase in % of TP53-positive cells in patients with complex cytogenetics (A), 5q abnormalities (B) and chromosome 17 abnormalities (C). Interobserver variability in TP53 % counts between hematopathologists (D). Receiver operator analysis for the detection of *TP53* mutation using p53 immunohistochemistry (E). Lack of association between % TP53 positivity and *TP53* VAF on Day 0 (F). Co-expression of TP53 in CD34-positive blasts (G) and CD3-positive T cells (H) (brown nuclear staining – p53, red staining – CD34 or CD3).

Supplemental Figure 2: Correlation between *TP53* VAF, TP53 IHC and blast percentages in *TP53*-mutated patient during the course of decitabine therapy (A). No increase in p53 staining is seen in serial samples from *TP53*-mutated patient with truncating mutation (B) but increase in p53 staining is noted in a case where original low p53 staining is thought to be due to processing (C). Correlation between *TP53* VAF, p53 IHC and blast percentages in the remaining *TP53*-mutated patients treated with decitabine (D-M).

Case	Diagnosis	Baseline blasts (%)	Baseline cytogenetics	Sequencing method	TP53 mutation	Variant detected	Day 0 *TP53* variant allele frequency	Day 0 p53 iHC average	Clinical response*
1002	AML with maturation (M2)	24	Del 7q 85%	exome	Detected	p.G245S	40	5	CRi
1004	AML with myelodysplasia related changes	57	Complex; del5q 80%, del17p 81%, del 13q 73%	exome	Detected	p.Y126C	66	53	CR
1013	AML with maturation (M2)	57	Complex; del5, del 6, -12, -16, -17, -18	RMG	Detected	p.V216M	79	48	CRi
1014	refractory anemia with excess blasts-2	8	Complex; 5q 74%	exome	Detected	p.L344P	73	82	CR
1017	AML not otherwise specified	37	Del17q 49%	exome	Detected	p.Y234N	63	47	CRi
1019	refractory anemia with excess blasts-2	9	Complex; del5q 80%, mono12p 76%	exome	Detected	p.R175G	89	50	mCR
1023	refractory anemia with excess blasts-2	11	Complex; del 5q-85%	exome	Detected	p.R273C	77	83	mCR
1037	AML with myelodysplasia related changes	20	Del5q 17.5%; monosomy 7 18%	RMG	Detected	p.R273H	11	51	CRi
1047	refractory anemia with excess blasts-2	7	Complex; -5, -7, -11, del12	RMG	Detected	p.H193R	16	37	mCR
1048	AML with minimal differentiation (M0)	55	Complex; XY, add(3), add(4), -5, -7, -10, -15, add(17), -18, -2	RMG	Detected	p.R282W	72	38	CRi
1062	AML with maturation (M2)	27	Complex; -5, -7, -11, -13, -14, -15, -16, -17, -18, -19, -20, -21	RMG	Detected	p.L257P	50	60	CRi
1063	refractory anemia with excess blasts-2	11	Del5q 41%; monosomy7 52%	exome	Detected	p.E286K	15	50	mCR
1065	refractory anemia with excess blasts-2	14	Complex; del 16q 73.5%	Ampliseq	Detected	p.T81fs	70	3	mCR
1066	refractory anemia with excess blasts-1	3	Del5q 63.5%; mono7 67%	Ampliseq	Detected	p.R248W	34	39	mCR
1068	refractory anemia	5	Tri5p,Tri8,del5q,del7q	Ampliseq	Detected	p.R273L & p.C141Y	30	45	mCR
1072	AML with myelodysplasia related changes	30	Complex, mono 5 42%, tetrasomy 8 32%, trisomy 8 12%	Ampliseq	Detected	e4-2 & p.C124R	39	61	CRi
1076	AML without maturation (M1)	70	Complex; X, -Y, add(2), add(4), add(5), -7, -13, -17, -21, +1 ~	Ampliseq	Detected	p.R337C	56	90	CR
1085	refractory anemia with excess blasts-2	12	Complex; XX, -3, add(3), del(5), -6, add(9), -17, -18, -19, -20,	Ampliseq	Detected	e7-2 & p.P250L	40	53	mCR
1092	AML	15	Cyto-complex; FISH-complex	RMG	Detected	p.I195T & p.G187fs	33	52	CRi, CCR
1094	AML	88	Cyto-complex; FISH-Del 5q (89.5%), Del 17p (84.5%)	RMG	Detected	p.G244R	66	60	SD
1101	AML	85	Cyto-complex; FISH-complex	RMG	Detected	e6-1	42	3	PR
1001	refractory anemia with excess blasts-2	16	normal	exome	Not detected	n/a	0	12	PD
1003	Acute myelomonocytic leukemia (AMML - M4)	40	trisomy 8 85%	exome	Not detected	n/a	0	10	PR
1005	AML without maturation (M1)	56	CBFB 40%	exome	Not detected	n/a	0	10	PR
1008	AML with myelodysplasia related changes	35	Complex; inv(3), -7	exome	Not detected	n/a	0	10	PR
1009	Acute monoblastic (M5a) and monocytic (M5b)	57	AML1 80.5%	exome	Not detected	n/a	0	7	CR
1010	AML with maturation (M2)	54	Normal	exome	Not detected	n/a	0	8	CR
1011	AML with myelodysplasia related changes	22	Normal	exome	Not detected	n/a	0	4	CRi
1012	AML with minimal differentiation (M0)	69	Trisomy 13 80%	exome	Not detected	n/a	0	1	PD
1016	AML with minimal differentiation (M0)	40	Normal	exome	Not detected	n/a	0	1	PR
1018	AML without maturation (M1)	89	t(1;6), +8[18], +13[4]	exome	Not detected	n/a	0	4	SD
1021	AML with maturation (M2)	76	Normal	exome	Not detected	n/a	0	45	CRi
1022	AML with minimal differentiation (M0)	57	Normal cyto and FISH	exome	Not detected	n/a	0	11	SD
1024	AML with maturation (M2)	81	Normal cyto; FISH 17q-9% 17p- 25%	exome	Not detected	n/a	0	58	CRi
1025	refractory anemia with excess blasts-2	11	Normal	exome	Not detected	n/a	0	2	SD
1027	AML without maturation (M1)	65	Normal	exome	Not detected	n/a	0	1	SD
1028	refractory anemia	11	Normal	exome	Not detected	n/a	0	10	SD
1029	Therapy-related myeloid neoplasms	73	Normal	exome	Not detected	n/a	0	5	Not evaluable
1035	AML without maturation (M1)	35	Normal	exome	Not detected	n/a	0	14	CRi
1036	AML with myelodysplasia related changes	37	Trisomy 8-31.5%	exome	Not detected	n/a	0	7	CRi
1040	acute myeloid leukemia subtype M5a	21	Del 11q 48%	exome	Not detected	n/a	0	12	SD
1041	refractory anemia with excess blasts-2	9	XY, +2, t(3;21), +der21t(3;21)	exome	Not detected	n/a	0	2	CR
1042	refractory anemia with excess blasts-2	16	Del20q 92.5%	exome	Not detected	n/a	0	4	mCR
1043	AML not otherwise specified	14	Trisomy 4 12%, Trisomy 8 9%	exome	Not detected	n/a	0	5	SD
1044	Acute myelomonocytic leukemia (AMML - M4)	30	Tetrasomy 7 2.5%	exome	Not detected	n/a	0	12	CRi
1045	AML with myelodysplasia related changes	21	Normal/npm1 positive	exome	Not detected	n/a	0	7	CRi
1049	AML with myelodysplasia related changes	39	Normal	exome	Not detected	n/a	0	2	CR
1050	refractory anemia	5	7q	exome	Not detected	n/a	0	2	PD
1051	refractory anemia with excess blasts-1	7	11q 73.5%	exome	Not detected	n/a	0	7	mCR
1052	refractory anemia with excess blasts-2	8	Del5q 78%; mono7 72%	Ampliseq	Not detected	n/a	0	10	mCR
1057	refractory anemia	4	Mono 7 42.5%	RMG	Not detected	n/a	0	3	SD
1058	AML with myelodysplasia related changes	28	Trisomy 21	exome	Not detected	n/a	0	4	CRi
1059	AML with maturation (M2)	51	Trisomy 8	exome	Not detected	n/a	0	24	PR
1061	chronic myelomonocytic leukemia	11	Del5q 92%;Del7q 98.5%	Ampliseq	Not detected	n/a	0	7	mCR
1064	AML with myelodysplasia related changes	69	Trisomy 13	exome	Not detected	n/a	0	6	PR
1067	Therapy-related myeloid neoplasms	26	-18	Ampliseq	Not detected	n/a	0	12	CRi
1069	AML without maturation (M1)	11	Complex; Trisomy 10 (16.5%)	Ampliseq	Not detected	n/a	0	8	SD
1070	Acute myelomonocytic leukemia (AMML - M4)	46	Normal	Ampliseq	Not detected	n/a	0	13	PR
1073	AML with maturation (M2)	22	Normal cyto/normal FISH	Ampliseq	Not detected	n/a	0	22	CRi
1074	AML with maturation (M2)	8	Trisomy 7q 7.5%	Ampliseq	Not detected	n/a	0	11	CRi
1075	MDS	12	Complex; Del 5q 49%	RMG	Not detected	n/a	0	10	mCR, CCR
1077	refractory anemia with excess blasts-2	20	Normal	Ampliseq	Not detected	n/a	0	1	CRi
1078	refractory anemia with excess blasts-2	11	Normal	Ampliseq	Not detected	n/a	0	8	mCR
1079	Acute monoblastic (M5a) and monocytic (M5b)	50	Normal	Ampliseq	Not detected	n/a	0	9	CR
1081	MDS	5	Not done	RMG	Not detected	n/a	0	13	mCR, HI Hgb, HI PMN
1084	AML without maturation (M1)	46	-8	Ampliseq	Not detected	n/a	0	8	CR
1088	AML with minimal differentiation (M0)	75	Failed. Clinical FLT3, IDH, NPM1, DNMT3A neg	Ampliseq	Not detected	n/a	0	30	CRi
1089	refractory anemia with excess blasts-2	12	XY, +8[17], XY[3]	Ampliseq	Not detected	n/a	0	8	mCR
1090	Acute panmyelosis with myelofibrosis	2	Normal	Ampliseq	Not detected	n/a	0	12	SD
1093	AML	17	FISH-Mono 7 (15%)	RMG	Not detected	n/a	0	6	CRi
1095	AML	18	Cyto-complex; FISH-Del/mono 16q (57.5%)	RMG	Not detected	n/a	0	14	CRi, CCR
1098	AML	69	Cyto-complex; FISH-normal	RMG	Not detected	n/a	0	6	SD


A
% p53-positive cells
p<0.0001
Complex Karyotype
Non-Complex Karyotype
B
% p53-positive cells
p<0.0001
del5q/- 5
No del5q/- 5
C
% p53-positive cells
p<0.0001
Chr 17 abnormality
No Chr 17 abnormality
D
Pathologist 2 or 3
Pathologist 1
E
ROC Curve
Sensitivity
1 - Specificity
F
TP53 VAF %
TP53 IHC%
G
H


A
Patient 1014
TP53 IHC (%)
Blasts (%)
TP53 mutation (VAF %)
TP53 mutation (VAF2 %)
Percent of cells/VAF
B
Patient 1065
C
Patient 1002
D
Patient 1062
E
Patient 1085
F
Patient 1048
G
Patient 1019
H
Patient 1066
I
Patient 1037
J
Patient 1063
K
Patient 1047
M
Patient 1068
N
Patient 1072
Days on therapy