Treatment with ibrutinib does not induce a *TP53* clonal evolution in chronic lymphocytic leukemia

Luciana Cafforio,[1] Sara Raponi,[1] Luca Vincenzo Cappelli,[1] Caterina Ilari,[1] Roberta Soscia,[1] Maria Stefania De Propris,[1] Paola Mariglia,[1] Gian Matteo Rigolin,[2] Antonella Bardi,[2] Nadia Peragine,[1] Alfonso Piciocchi,[3] Valentina Arena,[3] Francesca Romana Mauro,[1] Antonio Cuneo,[2] Anna Guarini,[4] Robin Foà[1] and Ilaria Del Giudice[1]

[1]Hematology Section, Department of Translational and Precision Medicine, Sapienza University, Rome; [2]Hematology Section, Department of Medical Science, Azienda Ospedaliero-Universitaria Arcispedale S. Anna, University of Ferrara, Ferrara; [3]GIMEMA Data Center, GIMEMA Foundation, Rome and [4]Department of Molecular Medicine, Sapienza University, Rome, Italy

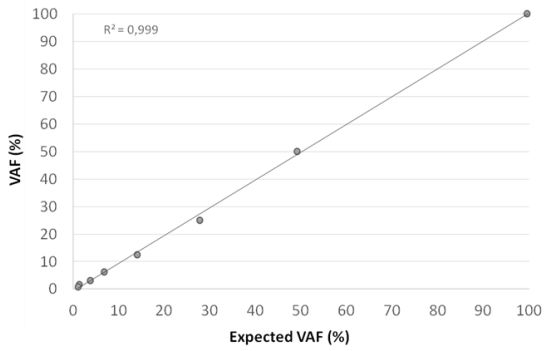
Correspondence: ILARIA DEL GIUDICE - delgiudice@bce.uniroma1.it

doi:10.3324/haematol.2020.263715

SUPPLEMENTARY DATA

Supplementary Figure 1. Dilution experiment to determine the sensitivity of deep-sequencing. **A)** The gDNA of SW837 cell line, harboring the TP53 c.742C>T p.R248W mutation in hemizygous status was serially diluted (1:2; 1:4; 1:8; 1:16; 1:32; 1:64; 1:128; 1:256) into a TP53 WT gDNA from a healthy donor. Starting from an input of 100 ng of gDNA (~15000 genomes equivalent), we detected the TP53 minor mutation up to 1:128, with a limit of detection (LOD) of 1% VAF. Plot shows a good correlation between allele frequency of the detected mutation and serial dilutions. **B)** A cancer cell enrichment experiment was performed starting from a sample of CLL viable cells carrying a *TP53* mutation with a VAF 90.4% in the absence of del(17p) (case 10714). Enriched CD19+/CD5+ cells were diluted with scalar concentration of peripheral blood mononuclear cells (PBMC) of a healthy donor, for a total of 5 dilutions. For each dilution, flow cytometry and ultra-deep sequencing were tested and CCF was calculated. Log2 FC was calculated comparing the CCF between each dilution point and the first (CLL 96%, VAF 98.7%). The sensitivity in identifying the mutation is high, since it was detected even for a CLL infiltration equal to 2%. The calculated CCF remains acceptable respect to the expected of 100%, with an underestimation when the CLL infiltration lowers. However, this does not impact on the trend evaluation with the Log2-fold change.

A)



Dilution	Expected VAF (%)	VAF (%)
1	100	99.55
1:2	50	49.05
1:4	25	27.67
1:8	12.5	13.97
1:16	6.25	6.75
1:32	3.13	3.65
1:64	1.56	1.32
1:128	0.78	0.98
1:256	0.39	n.d

n.d. non-detected

B)

Patient	Dilution	*TP53* Mutation	CLL flow (%)	VAF (%)	CCF (%)	Log2 FC (trend)
10714	100	c.584 T>C; p.I195T	96	98.7	102.81	
10714	50	c.584 T>C; p.I195T	53	52.7	99.43	-0.05 (Stable)
10714	25	c.584 T>C; p.I195T	31	25.8	83.23	-0.30 (Stable)
10714	10	c.584 T>C; p.I195T	11.1	8.4	75.68	-0.44 (Stable)
10714	5	c.584 T>C; p.I195T	6	4.7	78.33	-0.39 (Stable)
10714	2	c.584 T>C; p.I195T	2.3	1.8	78.26	-0.39 (Stable)

Supplementary Table 1: Dynamics of major and minor *TP53* mutations in TN patients, during ibrutinib treatment. Patients were evaluated every 6 months from therapy initiation (T= month). In light grey, the samples evaluated also for BTK and PLCγ2 mutations.

Sample ID	IGVH	FISH	FISH lesion %	Karyotype	Other mutations	*TP53* Mutation	CCF T0 (%)	CCF T8 (%)	CCF T14 (%)	CCF T20 (%)	CCF T26 (%)	CCF T32 (%)	CCF T38 (%)	CCF T44 (%)	TREND of CCF	Ibrutinib discontinuation
9615	Unmutated	del17p	87	Complex karyotype (≥3 lesions)	*SF3B1*	c.742C>T; p.R248W	97.6	100.0	66.1	26.2	75.6	-	56.7	59.4	DECREASED	no
9675	Unmutated	del17p	81	Complex karyotype (≥3 lesions)		c.626_627delGA; p.R209Kfs*6	98.0	93.2	54.7	83.6	63.4	72.8	51.9	49.1	DECREASED	no
9795	Unmutated	del17p	90	Complex karyotype (≥3 lesions)		c.844C>T; p.R282W	79.5	69.7	85.6	94.1	73.9	64.1	81.8	-	STABLE	yes (progression)
9878	Unmutated	del13q	94	Complex karyotype (≥3 lesions)	*NOTCH1*	c.375+2T>G; p.?	99.8	81.6	100.0	89.0	98.8	-	75.5	-	STABLE	no
9915	Mutated	normal	na	1 or 2 lesions	*NOTCH1*	c.377A>C; p.Y126S	8.7	11.1	17.5	WT	WT	WT	WT	-	UNDETECTABLE	no
10042	Mutated	del11q	67	1 or 2 lesions		c.809T>C;p.F270S	23.9	22.3	29.8	44.9	32.7	32.8		-	STABLE	no
10064	Unmutated	del17p	96.5	Complex karyotype (≥3 lesions)		c.673-2A>G; p.?	91.7	92.4	62.8	51.8	72.5	86.2	51.9	-	DECREASED	no
10191	Mutated	del17p	96	na		c.641A>G; p.H214R	89.7	70.3	26.9	-	-	-	-	-	DECREASED	no
10239	Unmutated	del17p	97	Complex karyotype (≥3 lesions)	*BIRC3*	c.733G>A; p.G245S	100.0	92.8	68.0	96.5	-	38.3	-	-	DECREASED	yes (progression)
10240	Unmutated	normal	na	na	*NOTCH1*	c.835G>T; p.G279W	45.5	24.9	36.3	82.4	52.9	43.2	-	-	STABLE	no
10671	Unmutated	tris12	86	Complex karyotype (≥3 lesions)	*NOTCH1*	c.524G>A; pR175H	3.5	6.1	18.2	36.4	-	-	-	-	INCREASED	no
10714	Unmutated	del11q	na	na		c.584T>C; p.I195T	98.3	96.1	100.0	100.0	-	-	-	-	STABLE	no
10894	Mutated	del13q	99	na		c.536A>G; p.H179R	27.1	29.6	-	25.7	32.9	-	-	-	STABLE	no
10877	Unmutated	del17p	75	Complex karyotype (≥3 lesions)		c.961A>T; p.K321*	68.4	21.6	10.1	7.9	-	-	-	-	DECREASED	no
10796	Unmutated	del17p	41	Complex karyotype (≥3 lesions)		c.849_850insGC; p.T284Afs*62	63.8	-	24.6	32.2	-	-	-	-	DECREASED	no
						c.796G>A; p.G266R	10.3	23.0	30.8	13.6	32.9	18.2	WT	-	UNDETECTABLE	
						c.584T>C; p.I195T	26.4	6.6	13.5	17.5	25.6	12.1	10.6	-	DECREASED	
10025	Unmutated	del13q		1 or 2 lesions		c.1181_1181delG	53.0	33.5	63.6	39.3	60.6	33.8	32.8	-	DECREASED	no
						c.711G>A; p.M237I	2.3	WT	WT	WT	WT	WT	WT	-	UNDETECTABLE	
						c.469G>T; p.V157F	5.4	WT	WT	WT	WT	WT	WT	-	UNDETECTABLE	
						c.376-1G>A; p.?	5.6	12.1	7.4	11.8	-	-	-	-	INCREASED	
10875	Unmutated	normal		na		c.512_512delA; p.E171Gfs*3	22.5	55.8	30.1	28.8	-	-	-	-	STABLE	no
						c.745A>T; p.R249W	56.5	87.8	59.3	67.1	-	-	-	-	STABLE	

na=not available

Supplementary Table 2: Dynamics of major and minor *TP53* mutations in R/R patients, during ibrutinib treatment. In light grey the time points (TP) prior to ibrutinb treatment and in dark grey the time points post ibrutinib. Highlighted in bold the novel mutations emerged after ibrutinib.

Sample ID	IGVH	FISH	FISH lesion %	*TP53* Mutation	TP1	TP2	TP3	TP4	TP5	TP6	TREND of CCF	Ibrutinib discontinuation
				c.764_766del; p.I255del	4.9	3.9	4.9	2.9			DECREASED	
				c.742C>T; p.R248W	2.0	2.0	2.0	WT			UNDETECTABLE	
				c.731G>A; p.G244D	4.6	5.9	5.3	2.0			DECREASED	
				c.701A>G; p.Y234C	1.1	2.3	5.2	3.2			DECREASED	
				c.672+1G>T; p.?	4.0	5.3	7.7	5.8			STABLE	
8540	Mutated	del17p	23	c.400T>C; p.F134L	3.3	1.6	2.0	WT			UNDETECTABLE	no
				c.747G>T; p.R249S	WT	2.6	3.9	WT			UNDETECTABLE	
				c.526T>C; p.C176R	WT	2.8	4.5	WT			UNDETECTABLE	
				c.736A>T; p.M246L	WT	WT	1.4	WT			UNDETECTABLE	
				c.733G>A; p.G245S	WT	WT	1.1	WT			UNDETECTABLE	
				c.329G>T; p.R110L	WT	WT	1.1	WT			UNDETECTABLE	
8271	Unmutated	na	na	c.1010G>T; p.R337L	3.79	WT	45.5	WT			UNDETECTABLE	died
5708	Unmutated	tris12	75	c.488A>G; p.Y163C	WT	WT	3.83	22.3	13.8		DECREASED	yes (AE)
				:.336_350del; p.F113_G117de	WT	WT	4.95	24.7	16.4		DECREASED	
				c.743G>A; p.R248Q	WT	1.4	WT				UNDETECTABLE	
5717	Unmutated	tris12	51	c.551_554del; p.D184Afs*61	WT	38.6	16.6				DECREASED	no
				c.526T>C; p.C176R	WT	7.0	WT				UNDETECTABLE	
				c.720T>G; p.S240R	**WT**	**WT**	**2.7**				NOVEL	
3547	Mutated	del17p	55	:.439_456del; p.V147_P152de	WT	89	100.0	77.8			STABLE	no
8353	Mutated	del17p	34	c.817C>G; p.R273G	WT	11.4	26.2				INCREASED	yes (AE)
				c.663delG; p.E221Dfs*25	WT	1.1	1.4				STABLE	
				c.818G>A; p.R273H	WT	44.11	10.45	100	45.0	11.0	DECREASED	
				c.626_627del; p.R209Kfs*5	WT	4.69	WT	6.95	1.8	1.2	DECREASED	
3425	Mutated	del13q	86	c.733G>A; p.G245S	WT	0.89	WT	34.83	40.6	49.6	STABLE	yes (shift to venetoclax)
				c.745A>G; p.R249G	WT	WT	WT	WT	2.3	1.8	STABLE	
				c.272G>A; p.W91*	**WT**	**WT**	**WT**	**WT**	**WT**	**2.5**	NOVEL	
				c.736A>G; p.M246V	WT	2.8	4.1				INCREASED	
3546	Mutated	na		c.731G>T; p.G244V	WT	4.5	3.7				STABLE	died
				c.716A>G; p.N239S	WT	1.4	5.5				INCREASED	
9225	Mutated	na		c.743G>A; p.R248Q	36.6	31.7					STABLE	no
				c.375+1G>T; p.?	42.6	36.9					STABLE	
7458	Unmutated	del17p	80	327_830delCCTG; p.A276Vfs*	84.44	33.33	46.0	WT			UNDETECTABLE	yes (AE)
				c.830G>T; p.C277F	WT	WT	11.0	92.0			INCREASED	

na=not available