

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Calin GA, Ferracin M, Cimmino A, et al. A microRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med* 2005;353:1793-801.

**Supplemental Table 1.** List of miRNAs associated with prognostic factors and disease progression in CLL patients selected by Prediction Analysis of Microarrays (PAM) and ANOVA analysis (GeneSpring) \*.

Nr. crt.	PAM signature	n- score	y+ score	map	GeneSpring signature	Anova p-value	map
1	<i>mir-222</i>	-0.022	0.0288	Xp11.2	<i>mir-34-prec</i>	0,048	1p36.22
2	<i>mir-24-2</i>	-0.0272	0.0355	19p13.12	<i>mir-192-2/3-prec</i>	0,0457	11q13
3	<i>mir-181a</i>	-0.0279	0.0364	1q32.1	<i>mir-15a-prec</i>	0,0353	13q14.3
4	<i>mir-15a</i>	-0.0372	0.0485	13q14.3	<i>mir-17</i>	0,0257	13q31
5	<i>mir-24-1</i>	-0.0427	0.0558	9q22.1	<i>mir-15a</i>	0,018	13q14.3
6	<i>mir-195</i>	-0.053	0.0692	17p13	<i>mir-195</i>	0,0175	17p13
7	<i>mir-23a</i>	-0.0748	0.0977	19p13.12	<i>mir-213-prec</i>	0,0153	1q31.3-q32.1
8	<i>mir-23b</i>	-0.0909	0.1187	9q22.1	<i>mir-221</i>	0,0105	Xp11.3
9	<i>mir-223</i>	0.1056	-0.1379	Xq12-13.3	<i>mir-023b</i>	0,00964	9q22.1
10	<i>mir-29a-2</i>	0.1139	-0.1487	7q32	<i>mir-155</i>	0,00959	21q21
11	<i>mir-155</i>	-0.1155	0.1508	21q21	<i>mir-223</i>	0,00774	Xq12-13.3
12	<i>mir-221</i>	-0.1157	0.1511	Xp11.3	<i>mir-132</i>	0,00461	17p13.3

13	<i>mir-16-1</i>	-0.1444	0.1886	13q14.3	<i>mir-029a-2</i>	0,00446	7q32
14	<i>mir-16-2</i>	-0.1619	0.2113	3q26.1	<i>mir-024-1</i>	0,00311	9q22.1
15	<i>mir-146</i>	-0.1803	0.2354	5q34	<i>mir-29b-2 (102)</i>	0,000778	1q32.2-32.3
16	<i>mir-29b-2 (102)</i>	0.2065	-0.2696	1q32.2-32.3	<i>mir-146</i>	0,000753	5q34
17	<i>mir-029c</i>	0.2174	-0.2839	1q32.2-32.3	<i>mir-016-1</i>	0,00042	13q14.3
18					<i>mir-016-2</i>	0,000327	3q26.1
19					<i>mir-029c</i>	0,000216	1q32.2-32.3

\* - the list of genes is in ascending order of significance, as represented by score or p value, respectively.

Supplemental Table 2. Predictions of ZAP-70 status and Immunoglobulin heavy chain variable gene status according to miRNA expression in CLL patients\*. The miRNA signature associated with prognostic factors was generated using panel 1 samples and then tested to cross validate the panel 1 and to predict the status of samples from panel 2.

CLL	True Value	Prediction	n margin	y margin
CLL01	Zap70<20 VhM	Zap70<20 V	1,278	-1,327
CLL02	Zap70<20 VhM	Zap70<20 V	1	-1
CLL03	Zap70<20 VhM	Zap70<20 V	1,247	-1,348
CLL04	Zap70<20 VhM	Zap70<20 V	1,16	-1,388
CLL05	Zap70<20 VhM	Zap70<20 V	1	-1
CLL06	Zap70<20 VhM	Zap70<20 V	1	-1
CLL07	Zap70<20 VhM	Zap70<20 V	1	-1,122
CLL08	Zap70<20 VhM	Zap70<20 V	1,391	-1,595
CLL09	Zap70<20 VhM	Zap70<20 V	0,953	-1,048
CLL10	Zap70<20 VhM	Zap70<20 V	1,059	-1,333
CLL11	Zap70<20 VhM	Zap70<20 V	1	-1
CLL12	Zap70<20 VhM	Zap70<20 V	0,997	-1,261
CLL13	Zap70<20 VhM	Zap70<20 V	1,488	-1,841
CLL14	Zap70<20 VhM	Zap70<20 V	2,171	-2,582
CLL15	Zap70<20 VhM	Zap70<20 V	1,252	-1,352
CLL16	Zap70<20 VhM	Zap70<20 V	1	-1,188
CLL17	Zap70<20 VhM	Zap70<20 V	1,19	-1,284
CLL18	Zap70<20 VhM	Zap70<20 V	1,747	-2,062
CLL19	Zap70<20 VhM	Zap70<20 V	1,503	-1,833
CLL20	Zap70<20 VhM	Zap70<20 V	1	-1
CLL21	Zap70<20 VhM	Zap70<20 V	1	-1
CLL22	Zap70<20 VhM	Zap70<20 V	1	-1
CLL23	Zap70<20 VhM	Zap70<20 V	2,047	-2,27
CLL24	Zap70<20 VhM	Zap70<20 V	1,464	-1,527
CLL25	Zap70<20 VhM	Zap70<20 V	1	-1
CLL26	Zap70<20 VhM	Zap70<20 V	1,034	-1,034
CLL27	Zap70<20 VhM	Zap70<20 V	1,479	-1,617
CLL28	Zap70<20 VhM	Zap70<20 V	2,355	-2,57
CLL29	Zap70<20 VhM	Zap70<20 V	1	-1
CLL30	Zap70<20 VhM	Zap70<20 V	1	-1
CLL31	Zap70<20 VhM	Zap70<20 V	1	-1
CLL32	Zap70<20 VhM	Zap70<20 V	1	-1
CLL33	Zap70<20 VhM	Zap70<20 V	2,229	-2,496

PANEL 1 - 83 correct predictions, 0 incorrect prediction

CLL34	Zap70<20 VhM	Zap70<20 V	2,683	-2,931
CLL35	Zap70<20 VhM	Zap70<20 V	1	-1
CLL36	Zap70<20 VhM	Zap70<20 V	2,578	-2,768
CLL37	Zap70<20 VhM	Zap70<20 V	2,079	-2,34
CLL38	Zap70<20 VhM	Zap70<20 V	1,745	-1,814
CLL39	Zap70<20 VhM	Zap70<20 V	1,559	-1,699
CLL40	Zap70<20 VhM	Zap70<20 V	2,608	-3,005
CLL41	Zap70<20 VhM	Zap70<20 V	2,357	-2,676
CLL42	Zap70<20 VhM	Zap70<20 V	1,102	-1,303
CLL43	Zap70<20 VhM	Zap70<20 V	1	-1
CLL44	Zap70<20 VhM	Zap70<20 V	2,464	-2,629
CLL45	Zap70<20 VhM	Zap70<20 V	1	-1
CLL46	Zap70<20 VhM	Zap70<20 V	1	-1
CLL47	Zap70<20 VhM	Zap70<20 V	2,074	-2,271
CLL48	Zap70>20 VhUM	Zap70>20 V	-1	1
CLL49	Zap70>20 VhUM	Zap70>20 V	-1,179	1,487
CLL50	Zap70>20 VhUM	Zap70>20 V	-1	0,88
CLL51	Zap70>20 VhUM	Zap70>20 V	-1	1
CLL52	Zap70>20 VhUM	Zap70>20 V	-1,836	2,405
CLL53	Zap70>20 VhUM	Zap70>20 V	-1	1
CLL54	Zap70>20 VhUM	Zap70>20 V	-1,334	1,649
CLL55	Zap70>20 VhUM	Zap70>20 V	-1	1,229
CLL56	Zap70>20 VhUM	Zap70>20 V	-1	1
CLL57	Zap70>20 VhUM	Zap70>20 V	-1	1
CLL58	Zap70>20 VhUM	Zap70>20 V	-1,171	1,566
CLL59	Zap70>20 VhUM	Zap70>20 V	-1	1
CLL60	Zap70>20 VhUM	Zap70>20 V	-1	1
CLL61	Zap70>20 VhUM	Zap70>20 V	-1,505	1,976
CLL62	Zap70>20 VhUM	Zap70>20 V	-1,095	1,46
CLL63	Zap70>20 VhUM	Zap70>20 V	-2,297	2,717
CLL64	Zap70>20 VhUM	Zap70>20 V	-1,187	1,381
CLL65	Zap70>20 VhUM	Zap70>20 V	-1	1
CLL66	Zap70>20 VhUM	Zap70>20 V	-1,344	1,479
CLL67	Zap70>20 VhUM	Zap70>20 V	-1,876	2,049
CLL68	Zap70>20 VhUM	Zap70>20 V	-1	1
CLL69	Zap70>20 VhUM	Zap70>20 V	-1,89	1,987
CLL70	Zap70>20 VhUM	Zap70>20 V	-2,658	2,938
CLL71	Zap70>20 VhUM	Zap70>20 V	-1,556	1,967
CLL72	Zap70>20 VhUM	Zap70>20 V	-2,574	2,81
CLL73	Zap70>20 VhUM	Zap70>20 V	-1	1

50 correct predictions, 0 incorrect predictions	CLL74	Zap70>20 VhUM	Zap70>20 V	-1	1
	CLL75	Zap70>20 VhUM	Zap70>20 V	-1	1
	CLL76	Zap70>20 VhUM	Zap70>20 V	-2,671	3,041
	CLL77	Zap70>20 VhUM	Zap70>20 V	-1	1,376
	CLL78	Zap70>20 VhUM	Zap70>20 V	-1	1
	CLL79	Zap70>20 VhUM	Zap70>20 V	-1,678	1,914
	CLL80	Zap70>20 VhUM	Zap70>20 V	-2,416	2,953
	CLL81	Zap70>20 VhUM	Zap70>20 V	-1	1
	CLL82	Zap70>20 VhUM	Zap70>20 V	-1,782	1,846
	CLL83	Zap70>20 VhUM	Zap70>20 V	-2,307	2,716
	CLL95	Zap70<20	Zap70<20	8,494	-8,494
	CLL96	Zap70<20	Zap70<20	1	-1
	CLL97	Zap70<20	Zap70<20	0,763	-0,763
CLL98	Zap70<20	Zap70<20	11,19	-11,19	
CLL99	Zap70<20	Zap70<20	7,561	-7,561	
CLL100	Zap70<20	Zap70<20	14,51	-14,51	
CLL101	Zap70<20	Zap70<20	5,585	-5,585	
CLL102	Zap70<20	Zap70<20	1	-1	
CLL103	Zap70<20	Zap70<20	10,09	-10,09	
CLL104	Zap70<20	Zap70<20	5,521	-5,521	
CLL105	Zap70<20	Zap70<20	7,33	-7,33	
CLL106	Zap70<20	Zap70<20	3,264	-3,264	
CLL107	Zap70<20	Zap70<20	7,774	-7,774	
CLL108	Zap70<20	Zap70<20	5,3	-5,3	
CLL109	Zap70<20	Zap70<20	4,34	-4,34	
CLL110	Zap70<20	Zap70<20	1,822	-1,822	
CLL111	Zap70<20	Zap70<20	3,879	-3,879	
CLL112	Zap70<20	Zap70<20	8,514	-8,514	
CLL113	Zap70<20	Zap70<20	5,866	-5,866	
CLL114	Zap70<20	Zap70<20	10,69	-10,69	
CLL115	Zap70<20	Zap70<20	4,141	-4,141	
CLL116	Zap70<20	Zap70<20	1	-1	
CLL117	Zap70<20	Zap70<20	1	-1	
CLL118	Zap70<20	Zap70<20	1	-1	
CLL119	Zap70<20	Zap70<20	10,11	-10,11	
CLL120	Zap70>20	Zap70>20	-3,109	3,109	
CLL121	Zap70>20	Zap70>20	-4,722	4,722	
CLL122	Zap70>20	Zap70>20	-5,166	5,166	
CLL123	Zap70>20	Zap70>20	-7,828	7,828	
CLL124	Zap70>20	Zap70>20	-7,468	7,468	

PANEL 2 -	CLL125	Zap70>20	Zap70>20	-11,44	11,44
	CLL126	Zap70>20	Zap70>20	-1	1
	CLL127	Zap70>20	Zap70>20	-6,617	6,617
	CLL128	Zap70>20	Zap70>20	-7,011	7,011
	CLL129	Zap70>20	Zap70>20	-7,479	7,479
	CLL130	Zap70>20	Zap70>20	-9,568	9,568
	CLL131	Zap70>20	Zap70>20	-5,286	5,286
	CLL132	Zap70>20	Zap70>20	-5,045	5,045
	CLL133	Zap70>20	Zap70>20	-1	1
	CLL134	Zap70>20	Zap70>20	-1	1
	CLL135	Zap70>20	Zap70>20	-1,324	1,324
	CLL136	Zap70>20	Zap70>20	-1	1
	CLL137	Zap70>20	Zap70>20	-1	1
	CLL138	Zap70>20	Zap70>20	-9,649	9,649
	CLL139	Zap70>20	Zap70>20	-9,264	9,264
	CLL140	Zap70>20	Zap70>20	-7,13	7,13
CLL141	Zap70>20	Zap70>20	-11,77	11,77	
CLL142	Zap70>20	Zap70>20	-2,986	2,986	
CLL143	Zap70>20	Zap70>20	-1	1	
CLL144	Zap70>20	Zap70>20	-1	1	

Note \* - Prediction for 83 CLLs, from groups 1 and 4 (see text). Classification was generated by the 'Support Vector Machines' algorithm (Kernel Function used: Polynomial Dot Product (Order 2). Diagonal Scaling Factor: 0). 13 miRNA from the signature were used. VhM = mutated; VhUM = unmutated (see text for details).

Supplemental Table 3. **PAM score for each of the components of microRNA signature associated with the time from diagnosis to initial therapy.**

	<b>1 score</b>	<b>2 score</b>
hsa-mir-181a	<b>0.1862</b>	<b>-0.0603</b>
hsa-mir-155	<b>0.1409</b>	<b>-0.0456</b>
hsa-mir-146	<b>0.07</b>	<b>-0.0227</b>
hsa-mir-024-2	<b>0.0696</b>	<b>-0.0225</b>
hsa-mir-023b	<b>0.0643</b>	<b>-0.0208</b>
hsa-mir-023a	<b>0.0587</b>	<b>-0.019</b>
hsa-mir-222	<b>0.0458</b>	<b>-0.0148</b>
hsa-mir-221	<b>0.0343</b>	<b>-0.0111</b>
hsa-mir-029c	<b>-0.0221</b>	<b>0.0072</b>

Note: Score 1 characterize the short time and score 2 the long time from diagnosis to initial therapy in a panel of 94 CLL patients.