SUPPLEMENTARY APPENDIX

IGHV-associated methylation signatures more accurately predict clinical outcomes of chronic lymphocytic leukemia patients than *IGHV* mutation load

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Supplementary information

Table of Contents

Supplementary Methods

Patient cohort

The patient cohort in our study was previously described in Kristensen et al. (1) where pre-treatment blood samples were collected at diagnosis between 2005-2011 at Odense University Hospital, Denmark. The Ethics Committee of Region of Southern Denmark approved the study (approval number: S-20100128). DNA was extracted from the samples using the MagNA Pure LC Instrument and MagNA Pure LC DNA Isolation Kit I (Roche Applied Sciences, Mannheim, Germany) (2). Patients were screened for standard CLL biomarkers including: mutations in *IGHV*, *TP53* and *NOTCH1*, FISH analysis of deletion 11q22.3, deletion 13q14.3, deletion 17p13.1 and trisomy 12, and flowcytometric analyses of ZAP70 and CD38 expression (1, 2). The clinicobiological characteristics of the patients included in our study are summarized in Table 1.

1st section: Selection procedure of the CpG sites with qualitative methylation changes

The sensitivity and performance of the quantitative methylation assessment in a clinical sample can be significantly affected by inherent contamination of the cancer specimen with DNA from normal tissue. Consequently, the methods used for quantification of methylation levels in clinical material in principle assess the level of methylation in a background of healthy cells, and only methylation changes with large methylation differences are most likely to overcome this measurement limitation in in vitro diagnostic settings. To at least partly overcome this limitation, we focused our analyses on CpG sites displaying bimodal distribution of beta-values defined as an interquartile range of minimum 0.80 (IQR: range between the 25-percentile and 75-percentile), see Figure 1B.

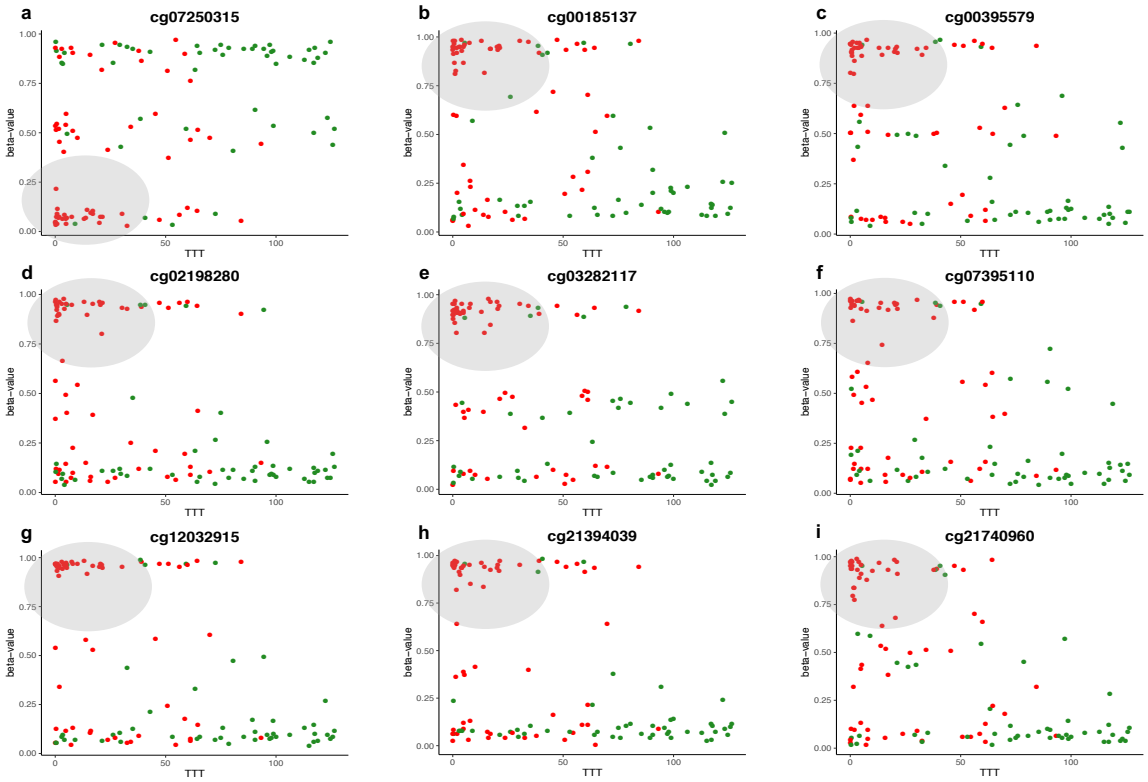
2nd section: Validation of EPIC microarray results with MS-HRM

To validate the methylation levels detected using the EPIC array, we used Methylation-Sensitive High-Resolution Melting (MS-HRM) (3). The MS-HRM technique is based on qPCR amplification of bisulfite-treated DNA followed by high-resolution melting of the resulting amplicon. Differences in methylation level can be identified as differences in melting profiles of the amplicons. Due to limited amounts of the clinical material, we optimized assays targeting 4 of the final CpG sites included in the methylation-based classification procedure. We validated the methylation levels in 18 patients from our cohort (9 patients with high beta-value and 9 patients with low beta-value for each of the CpG sites using the beta-value cutoff at 0.65). In that procedure 500 ng of patient DNA was subjected to sodium bisulfite modification (EZ-96 DNA Methylation-Gold™ kit, Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. MS-HRM assays were designed as described by Wojdacz et al. (3, 4). MS-HRM analyses were performed using the LightCycler480® platform (Roche, Mannheim, Germany) with a PCR reaction mixture consisting of 1x LightCycler® 480 High Resolution Melting Master (Roche, Mannheim, Germany), 3 mM $MgCl_2$, 500 nM of each primer, and 10 ng of bisulfite-modified DNA (theoretically calculated concentration) in a final volume of 10 μL. To evaluate the qualitative methylation level of patient samples, we included standard samples in each assay obtained from artificially methylated DNA (Universal Methylated Human DNA Standard, Zymo Research, Irvine, CA, USA) and artificially non-methylated DNA (EpiTect Control DNA, Qiagen, Hilden, Germany). The technical specifications for each assay are available on request.

3rd section: Development of a methylation-based classification procedure

To assess whether the combined information from all 9 CpGs selected in our analysis (see Results section: "Development of a methylation-based classification procedure") more accurately stratified patients into two groups with different TTT than information from the single CpG sites, we first needed to assess the prognostic power of methylation changes at each of the CpG sites and then develop a classification procedure that allowed to combine information from all 9 CpG sites.

To determine the prognostic power of methylation changes at single CpG sites, we first determined a beta-value cutoff that most accurately selected patients with the shortest TTT (aggressive disease) for each of the CpG sites. To find the optimal beta-value cutoff, we plotted beta-value (for individual CpGs) against TTT for each patients, and found that hypomethylation (low beta-values) of 1 CpG site (cg07250315) was associated with short TTT (Supplementary Fig. S1a), while hypermethylation (high beta-value) were associated with short TTT for 8 CpG sites (cg00185137, cg00395579, cg02198280, cg03282117, cg07395110, cg12032915, cg21394039, and cg21740960) (Supplementary Fig. S1b-i).

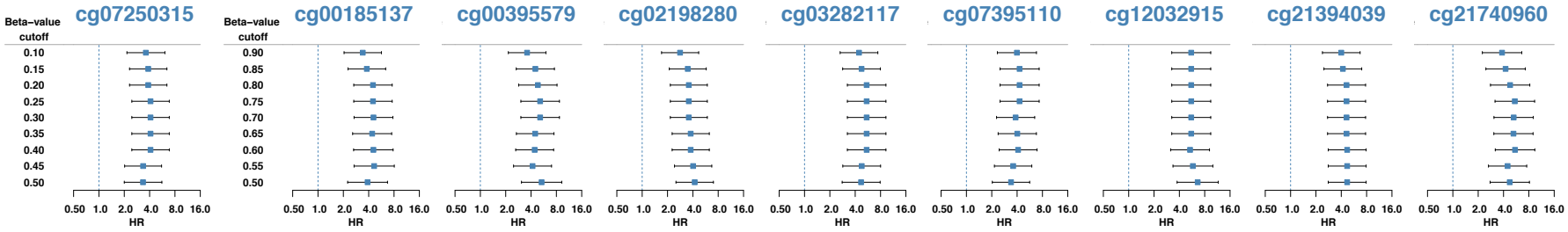


Supplementary Fig. S1. Scatterplot describing the association between methylation level and TTT for each of the CpG sites. a-i *Scatterplots for the individual CpG sites display the beta-values (y-axis) plotted against TTT (x-axis) for each patient. The individual patient is illustrated by a dot, and colored according to treatment status (red: treated; green: not treated).*

The grey areas indicate a concentration of the patients with short TTT, which for cg07250315 indicates that hypomethylation is associated with short TTT ***(a)****, and for cg00185137, cg00395579, cg02198280, cg03282117, cg07395110, cg12032915, cg21394039, and cg21740960 indicate that hypermethylation is associated with short TTT* ***(b-i)****.*

The above analysis also showed that for all CpG sites the direction of the association of TTT with the beta-value was not always the same, and thus, not uniformly informative for the outcome (e.g. beta value at cg07250315 (**a**) in most of the patients experiencing outcome was hypomethylated, but for small subset of the patients was hypermethylated). Thus, for each CpG site we choose a range of beta-values that we needed to test, to find the cutoff that most accurately selected patients with short TTT. Specifically, for cg07250315, for which TTT was associated with hypomethylation, we tested cutoffs in the low beta-value range (0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50). For all remaining CpG sites, for which TTT was associated with hypermethylation, we tested cutoffs in the high beta-value range (0.90, 0.85, 0.80, 0.75, 0.70, 0.65, 0.60, 0.55, and 0.50).

Then, to measure the accuracy of the classification at each beta-value cutoff, we stratified the patients using all the beta-value cutoffs listed above, and calculated HRs for "need of treatment" using Cox regression for all stratifications, and compared the HRs between the different cutoffs (Supplementary Fig. S2).



Supplementary Fig. S2. Forest Plots showing the HRs from Cox regression analyses performed for each of the 9 CpG sites at different beta-value cutoffs. *The left-side column indicates the specific beta-value cutoff used for patient stratification (in bold). The HR for each of the beta-value cutoffs tested is shown as a blue square with whiskers representing the 95% CI. The scale of beta-value cutoffs for cg07250315 is different from the remaining CpGs, as hypomethylation was associated with short TTT for this CpG site.*

The comparison of the calculated HRs showed that the individual CpGs at different beta-value cutoffs had almost identical HRs (Supplementary Fig. S2 for overview, and detailed analyses in the rightmost column of Supplementary Fig. S3a-i). Thus, this analysis did not identify an optimal beta-value cutoff for the individual CpGs sites, moreover showed that methylation changes at all CpGs identified patients with short TTT with similar accuracy.

a **cg07250315**

Cutoff	Stratification groups using beta-value cutoff				HR (95% CI)
	Beta-value interval	Median TTT (95% CI)	Beta-value interval	Median TTT (95% CI)	
0.10	0.00-0.10:	13.1 (4.8-30.4)	0.10-1.00:	70.0 (58.7-n.a.)	3.61 (2.15-6.08)
0.15	0.00-0.15:	14.4 (4.9-30.4)	0.15-1.00:	NR (58.7-n.a.)	3.85 (2.31-6.42)
0.20	0.00-0.20:	14.4 (4.9-30.4)	0.20-1.00:	NR (58.7-n.a.)	3.85 (2.31-6.42)
0.25	0.00-0.25:	13.9 (4.8-21.4)	0.25-1.00:	NR (61.2-n.a.)	4.10 (2.46-6.85)
0.30	0.00-0.30:	13.9 (4.8-21.4)	0.30-1.00:	NR (61.2-n.a.)	4.10 (2.46-6.85)
0.35	0.00-0.35:	13.9 (4.8-21.4)	0.35-1.00:	NR (61.2-n.a.)	4.10 (2.46-6.85)
0.40	0.00-0.40:	14.4 (4.9-30.4)	0.40-1.00:	NR (61.2-n.a.)	4.10 (2.46-6.85)
0.45	0.00-0.45:	17.1 (5.3-32.7)	0.45-1.00:	NR (61.2-n.a.)	3.35 (2.02-5.55)
0.50	0.00-0.50:	17.1 (10.1-47.2)	0.50-1.00:	NR (61.2-n.a.)	3.34 (2.00-5.59)

b **cg00185137**

Cutoff	Stratification groups using beta-value cutoff				HR (95% CI)
	Beta-value interval	Median TTT (95% CI)	Beta-value interval	Median TTT (95% CI)	
0.90	0.00-0.90:	NR (61.2-n.a.)	0.90-1.00:	17.1 (4.9-39.2)	3.40 (2.04-5.67)
0.85	0.00-0.85:	NR (61.2-n.a.)	0.85-1.00:	17.1 (4.9-34.3)	3.80 (2.27-6.36)
0.80	0.00-0.80:	NR (64.7-n.a.)	0.80-1.00:	14.4 (4.1-30.4)	4.50 (2.66-7.61)
0.75	0.00-0.75:	NR (64.7-n.a.)	0.75-1.00:	14.4 (4.1-30.4)	4.50 (2.66-7.61)
0.70	0.00-0.70:	NR (70.0-n.a.)	0.70-1.00:	17.1 (4.9-34.3)	4.56 (2.68-7.76)
0.65	0.00-0.65:	NR (70.0-n.a.)	0.65-1.00:	17.1 (5.0-39.2)	4.40 (2.58-7.50)
0.60	0.00-0.60:	NR (70.0-n.a.)	0.60-1.00:	17.1 (5.0-37.9)	4.55 (2.65-7.80)
0.55	0.00-0.55:	NR (93.3-n.a.)	0.55-1.00:	17.1 (5.0-39.2)	4.64 (2.68-8.05)
0.50	0.00-0.50:	NR (93.3-n.a.)	0.50-1.00:	20.5 (7.6-45.5)	3.88 (2.25-6.69)

c **cg00395579**

Cutoff	Stratification groups using beta-value cutoff				HR (95% CI)
	Beta-value interval	Median TTT (95% CI)	Beta-value interval	Median TTT (95% CI)	
0.90	0.00-0.90:	93.3 (58.7-n.a.)	0.90-1.00:	17.1 (4.1-34.3)	3.59 (2.15-6.01)
0.85	0.00-0.85:	NR (61.2-n.a.)	0.85-1.00:	13.1 (4.1-30.4)	4.50 (2.67-7.58)
0.80	0.00-0.80:	NR (61.2-n.a.)	0.80-1.00:	13.1 (4.1-30.4)	4.82 (2.85-8.14)
0.75	0.00-0.75:	NR (64.7-n.a.)	0.75-1.00:	7.6 (3.9-30.4)	5.13 (3.03-8.70)
0.70	0.00-0.70:	NR (64.7-n.a.)	0.70-1.00:	7.6 (3.9-30.4)	5.13 (3.03-8.70)
0.65	0.00-0.65:	NR (61.2-n.a.)	0.65-1.00:	13.1 (4.1-30.4)	4.48 (2.68-7.49)
0.60	0.00-0.60:	NR (64.7-n.a.)	0.60-1.00:	13.1 (4.1-30.4)	4.42 (2.63-7.42)
0.55	0.00-0.55:	NR (64.7-n.a.)	0.55-1.00:	13.1 (4.9-32.7)	4.17 (2.48-7.01)
0.50	0.00-0.50:	NR (93.3-n.a.)	0.50-1.00:	13.1 (4.9-32.7)	5.34 (3.08-9.27)

d **cg02198280**

Cutoff	Stratification groups using beta-value cutoff				HR (95% CI)
	Beta-value interval	Median TTT (95% CI)	Beta-value interval	Median TTT (95% CI)	
0.90	0.00-0.90:	93.3 (54.6-n.a.)	0.90-1.00:	20.1 (5.0-51.3)	2.82 (1.70-4.69)
0.85	0.00-0.85:	NR (58.7-n.a.)	0.85-1.00:	14.4 (3.9-39.2)	3.48 (2.10-5.77)
0.80	0.00-0.80:	NR (61.2-n.a.)	0.80-1.00:	17.1 (4.1-39.2)	3.58 (2.16-5.94)
0.75	0.00-0.75:	NR (61.2-n.a.)	0.75-1.00:	17.1 (4.1-39.2)	3.58 (2.16-5.94)
0.70	0.00-0.70:	NR (61.2-n.a.)	0.70-1.00:	17.1 (4.1-39.2)	3.58 (2.16-5.94)
0.65	0.00-0.65:	NR (61.2-n.a.)	0.65-1.00:	14.4 (3.9-32.7)	3.77 (2.27-6.27)
0.60	0.00-0.60:	NR (61.2-n.a.)	0.60-1.00:	14.4 (3.9-32.7)	3.77 (2.27-6.27)
0.55	0.00-0.55:	NR (61.2-n.a.)	0.55-1.00:	14.4 (3.9-32.7)	4.03 (2.42-6.71)
0.50	0.00-0.50:	NR (61.2-n.a.)	0.50-1.00:	13.1 (3.9-30.4)	4.21 (2.52-7.02)

e **cg03282117**

Cutoff	Stratification groups using beta-value cutoff				HR (95% CI)
	Beta-value interval	Median TTT (95% CI)	Beta-value interval	Median TTT (95% CI)	
0.90	0.00-0.90:	93.3 (59.9-n.a.)	0.90-1.00:	7.6 (3.4-21.1)	4.44 (2.65-7.43)
0.85	0.00-0.85:	NR (61.2-n.a.)	0.85-1.00:	7.6 (3.4-21.1)	4.76 (2.84-7.97)
0.80	0.00-0.80:	NR (64.7-n.a.)	0.80-1.00:	7.6 (3.4-20.5)	5.47 (3.23-9.27)
0.75	0.00-0.75:	NR (64.7-n.a.)	0.75-1.00:	7.6 (3.4-20.5)	5.47 (3.23-9.27)
0.70	0.00-0.70:	NR (64.7-n.a.)	0.70-1.00:	7.6 (3.4-20.5)	5.47 (3.23-9.27)
0.65	0.00-0.65:	NR (64.7-n.a.)	0.65-1.00:	7.6 (3.4-20.5)	5.47 (3.23-9.27)
0.60	0.00-0.60:	NR (64.7-n.a.)	0.60-1.00:	7.6 (3.4-20.5)	5.47 (3.23-9.27)
0.55	0.00-0.55:	NR (61.2-n.a.)	0.55-1.00:	8.0 (3.4-20.5)	4.78 (2.85-8.01)
0.50	0.00-0.50:	NR (64.7-n.a.)	0.50-1.00:	13.1 (3.9-21.1)	4.70 (2.80-7.91)

f **cg07395110**

Cutoff	Stratification groups using beta-value cutoff				HR (95% CI)
	Beta-value interval	Median TTT (95% CI)	Beta-value interval	Median TTT (95% CI)	
0.90	0.00-0.90:	93.3 (58.7-n.a.)	0.90-1.00:	13.9 (3.9-30.4)	3.98 (2.33-6.79)
0.85	0.00-0.85:	93.3 (61.2-n.a.)	0.85-1.00:	13.9 (3.9-30.4)	4.28 (2.51-7.31)
0.80	0.00-0.80:	93.3 (61.2-n.a.)	0.80-1.00:	13.9 (3.9-30.4)	4.28 (2.51-7.31)
0.75	0.00-0.75:	93.3 (61.2-n.a.)	0.75-1.00:	13.9 (3.9-30.4)	4.28 (2.51-7.31)
0.70	0.00-0.70:	93.3 (61.2-n.a.)	0.70-1.00:	14.4 (4.1-37.9)	3.84 (2.28-6.47)
0.65	0.00-0.65:	93.3 (61.2-n.a.)	0.65-1.00:	13.9 (4.1-30.4)	4.02 (2.38-6.78)
0.60	0.00-0.60:	NR (61.2-n.a.)	0.60-1.00:	13.9 (4.1-30.4)	4.11 (2.45-6.91)
0.55	0.00-0.55:	NR (61.2-n.a.)	0.55-1.00:	14.4 (5.0-39.2)	3.59 (2.15-5.98)
0.50	0.00-0.50:	NR (64.7-n.a.)	0.50-1.00:	17.1 (7.2-39.2)	3.39 (2.03-5.64)

g **cg12032915**

Cutoff	Stratification groups using beta-value cutoff				HR (95% CI)
	Beta-value interval	Median TTT (95% CI)	Beta-value interval	Median TTT (95% CI)	
0.90	0.00-0.90:	NR (70.0-n.a.)	0.90-1.00:	7.6 (4.1-21.1)	5.53 (3.25-9.43)
0.85	0.00-0.85:	NR (70.0-n.a.)	0.85-1.00:	7.6 (4.1-21.1)	5.53 (3.25-9.43)
0.80	0.00-0.80:	NR (70.0-n.a.)	0.80-1.00:	7.6 (4.1-21.1)	5.53 (3.25-9.43)
0.75	0.00-0.75:	NR (70.0-n.a.)	0.75-1.00:	7.6 (4.1-21.1)	5.53 (3.25-9.43)
0.70	0.00-0.70:	NR (70.0-n.a.)	0.70-1.00:	7.6 (4.1-21.1)	5.53 (3.25-9.43)
0.65	0.00-0.65:	NR (70.0-n.a.)	0.65-1.00:	7.6 (4.1-21.1)	5.53 (3.25-9.43)
0.60	0.00-0.60:	NR (64.7-n.a.)	0.60-1.00:	8.0 (4.1-21.1)	5.36 (3.15-9.12)
0.55	0.00-0.55:	NR (93.3-n.a.)	0.55-1.00:	10.1 (4.8-21.4)	5.79 (3.36-10.00)
0.50	0.00-0.50:	NR (93.3-n.a.)	0.50-1.00:	10.1 (4.8-21.1)	6.60 (3.76-11.58)

h **cg21394039**

Cutoff	Stratification groups using beta-value cutoff				HR (95% CI)
	Beta-value interval	Median TTT (95% CI)	Beta-value interval	Median TTT (95% CI)	
0.90	0.00-0.90:	NR (61.2-n.a.)	0.90-1.00:	14.4 (3.9-39.2)	4.00 (2.39-6.70)
0.85	0.00-0.85:	NR (61.2-n.a.)	0.85-1.00:	13.1 (3.9-30.4)	4.18 (2.49-7.00)
0.80	0.00-0.80:	NR (61.2-n.a.)	0.80-1.00:	13.1 (3.9-21.4)	4.62 (2.75-7.78)
0.75	0.00-0.75:	NR (61.2-n.a.)	0.75-1.00:	13.1 (3.9-21.4)	4.62 (2.75-7.78)
0.70	0.00-0.70:	NR (61.2-n.a.)	0.70-1.00:	13.1 (3.9-21.4)	4.62 (2.75-7.78)
0.65	0.00-0.65:	NR (61.2-n.a.)	0.65-1.00:	13.1 (3.9-21.4)	4.62 (2.75-7.78)
0.60	0.00-0.60:	NR (61.2-n.a.)	0.60-1.00:	13.1 (3.9-21.4)	4.70 (2.80-7.90)
0.55	0.00-0.55:	NR (61.2-n.a.)	0.55-1.00:	13.1 (3.9-21.4)	4.70 (2.80-7.90)
0.50	0.00-0.50:	NR (61.2-n.a.)	0.50-1.00:	13.1 (3.9-21.4)	4.70 (2.80-7.90)

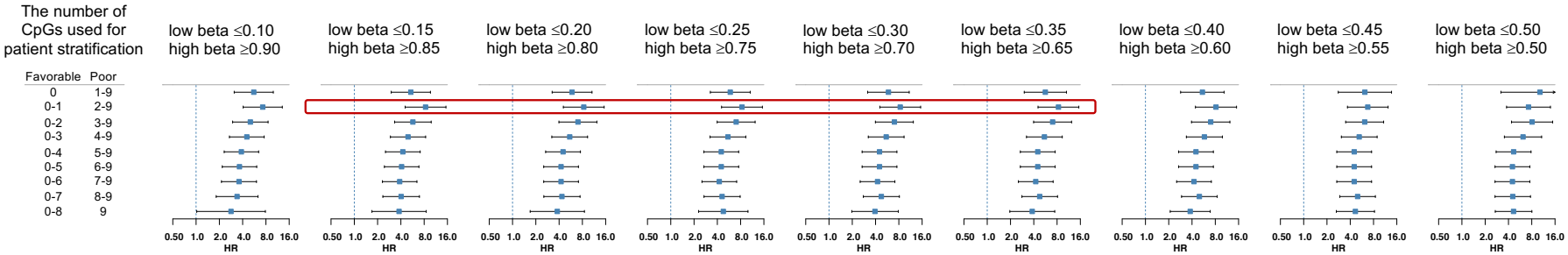
i **cg21740960**

Cutoff	Stratification groups using beta-value cutoff				HR (95% CI)
	Beta-value interval	Median TTT (95% CI)	Beta-value interval	Median TTT (95% CI)	
0.90	0.00-0.90:	84.3 (56.3-n.a.)	0.90-1.00:	10.1 (3.4-39.2)	3.84 (2.24-6.59)
0.85	0.00-0.85:	84.3 (58.7-n.a.)	0.85-1.00:	7.6 (3.9-37.9)	4.23 (2.47-7.25)
0.80	0.00-0.80:	93.3 (59.9-n.a.)	0.80-1.00:	6.3 (3.4-21.4)	4.79 (2.80-8.21)
0.75	0.00-0.75:	93.3 (61.2-n.a.)	0.75-1.00:	4.9 (3.0-21.1)	5.45 (3.17-9.36)
0.70	0.00-0.70:	NR (61.2-n.a.)	0.70-1.00:	5.0 (3.0-21.4)	5.28 (3.08-9.03)
0.65	0.00-0.65:	NR (61.2-n.a.)	0.65-1.00:	7.6 (3.4-21.4)	5.25 (3.07-8.97)
0.60	0.00-0.60:	NR (64.7-n.a.)	0.60-1.00:	7.6 (3.4-21.1)	5.48 (3.19-9.41)
0.55	0.00-0.55:	NR (61.2-n.a.)	0.55-1.00:	13.1 (4.1-21.4)	4.47 (2.65-7.54)
0.50	0.00-0.50:	NR (70.0-n.a.)	0.50-1.00:	14.4 (5.0-34.3)	4.74 (2.77-8.12)

Supplementary Fig. S3. Detailed results from Cox regression analyses performed for each of the 9 CpG sites at different beta-value cutoffs*. Specifically, in each table: the first column specifies the beta-value cutoff used for stratification. The range of cutoffs is different for hypomethylated cg07250315 (**a**) than other CpG sites for which hypermethylated was associated with short TTT CpGs (**b-i**). The second and third columns indicates the beta-value interval and median TTT (months) for each stratification group. The fourth column provides the HR (95% CI) calculated from the Cox regression, and indicates the hazard for requiring treatment for patients in the group with lowest median TTT. All regression analyses had a P <0.0001. NR: not reached; n.a.: not available.*

As the CpG sites in our study were selected to independently predict TTT, we next explored whether the combination of information from all 9 CpGs allowed to more accurately identify patients with short TTT.

To combine information from all CpG sites, we counted the number of CpGs for each patient with a beta-value predicting short TTT. Then, the patients were stratified into poor versus favorable prognosis, according to the number of CpGs predicting short TTT. We used Cox proportional hazard regression to test whether there was a specific number of CpGs that most accurately selected patients with poor prognosis. As we were not able to identify an optimal beta-value cutoff for the individual CpGs in analysis above (Supplementary Fig. S3a-i), we performed the regression analyses for all combinations of beta-value cutoffs (indicated on the top of the forest plots in Supplementary Fig. S4).



Supplementary Fig. S4. Forest Plots showing the HRs from Cox regression analyses performed for patients stratified according to different number of CpG sites predicting short TTT. *The number of the CpG sites indicating short TTT used for patient stratification in each Cox regression is shown on the left-side column. The range of the beta-values used for patient stratification in each analysis is shown on the top of the forest plot. Specifically, for each forest plot: the HR were calculated between two groups of patients displaying a specific number of CpGs predicting short TTT (indicated in left-side column that specifies the number of CpGs predicting short TTT for each stratification group). The HR is shown as a blue square with whiskers representing the 95% CI, and indicates the hazards for the group with poor prognosis. The red box highlights result of analyses, which resulted in identical classification of patients, and the highest HR with most narrow 95% CI and lowest p-value was obtained in these stratifications.*

Overall, this analysis showed that patients with 2 or more CpGs with methylation levels indicating short TTT, had the highest HR with most narrow 95% confidence intervals and lowest p-value (HR 8.34; 95% CI, 4.54-15.30; $P = 7.51 \times 10^{-12}$) (highlighted in red box in Supplementary

Fig. S4 below and for detailed analyses see Supplementary Fig. S5b-f) as opposed to patients with one or none CpG sites indicating short TTT.

Supplementary Fig. S5. (NEXT PAGE) Detailed results from Cox regression analyses performed for patients stratified according to different number of CpG sites predicting short TTT. *The range of the beta-values used for patient stratification in each analysis is shown on the top of the table. Specifically, in each table: the HR were calculated between two groups of patients displaying a specific number of CpGs predicting short TTT. The first and second column specifies the number of CpGs predicting short TTT and the median TTT (months) for each stratification group. The third column provides the HR (95% CI) calculated from the Cox regression, and indicates the hazards for the patients with lowest median TTT (poor prognosis). All regression analyses had a P <0 .0001, unless marked with (*) then P <0.05. The five stratifications highlighted in red (**b-f**) resulted in identical classification of patients, and the bolded stratification indicate the highest HR in the given table. The Cox regression in (**i**) [first row] was excluded despite a high HR due to a very wide 95% CI, and a high median TTT for the poor prognostic subgroup. NR: not reached; n.a.: not available.*

Interestingly, for the patients stratifications with most accurate HR (patients stratified between 1 and 2 CpGs predicting short TTT; red box in Supplementary Fig. S4), HRs were identical for all the beta-values cutoffs between 0.15 to 0.35 for cg07250315, and between 0.65 to 0.85 for the remaining CpGs (highlighted in red in Supplementary Fig. S4 and Supplementary Fig. S5b-f). Therefore, to simplify the patient classification procedure, we chose widest range of beta-values for the cutoff. Specifically, the CpG was scored as predicting short TTT (and assigned value of 1) if the beta-value was ≤0.35 for cg07250315 and if the beta value was ≥0.65 for cg00185137, cg00395579, cg02198280, cg03282117, cg07395110, cg12032915, cg21394039, and cg21740960. The final patient classification procedure is described in Fig. 2, and beta-values are provided in Supplementary File 1.

a low beta ≤ 0.10 and high beta ≥ 0.90

Stratification groups according to number of CpGs predicting short TTT				HR (95% CI)
No. CpGs	Median TTT (95% CI)	No. CpGs	Median TTT (95% CI)	
0:	NR (93.3-n.a.)	1-9:	14.4 (5.3-30.4)	5.57 (3.11-9.96)
0-1:	**NR**	**2-9:**	**13.1 (4.9-21.1)**	**7.27 (4.06-13.02)**
0-2:	NR (64.7-n.a.)	3-9:	7.6 (4.1-30.4)	5.03 (2.97-8.53)
0-3:	NR (61.2-n.a.)	4-9:	7.6 (3.9-30.4)	4.53 (2.70-7.59)
0-4:	NR (58.7-n.a.)	5-9:	13.1 (3.9-39.2)	3.85 (2.31-6.44)
0-5:	93.3 (58.7-n.a.)	6-9:	17.1 (3.9-39.2)	3.64 (2.18-6.08)
0-6:	93.3 (58.7-n.a.)	7-9:	17.1 (4.1-39.2)	3.59 (2.13-6.04)
0-7:	61.2 (50.8-n.a.)	8-9:	10.3 (3.0-n.a.)	3.39 (1.82-6.32)*
0-8:	56.3 (34.3-93.3)	9:	16.6 (0.6-n.a.)	2.83 (1.02-7.87)*

b low beta ≤ 0.15 and high beta ≥ 0.85

Stratification groups according to number of CpGs predicting short TTT				HR (95% CI)
No. CpGs	Median TTT (95% CI)	No. CpGs	Median TTT (95% CI)	
0:	NR (93.3-n.a.)	1-9:	16.2 (5.3-32.7)	5.38 (3.00-9.64)
0-1:	**NR**	**2-9:**	**13.1 (4.9-21.1)**	**8.34 (4.54-15.30)**
0-2:	NR (70.0-n.a.)	3-9:	13.1 (4.8-21.4)	5.72 (3.32-9.86)
0-3:	NR (61.2-n.a.)	4-9:	7.6 (4.1-21.4)	4.95 (2.93-8.36)
0-4:	NR (61.2-n.a.)	5-9:	13.1 (3.4-30.4)	4.24 (2.53-7.11)
0-5:	NR (61.2-n.a.)	6-9:	13.1 (3.4-39.2)	4.09 (2.45-6.84)
0-6:	NR (58.7-n.a.)	7-9:	13.1 (3.9-39.2)	3.86 (2.31-6.45)
0-7:	70.0 (51.3-n.a.)	8-9:	7.6 (3.0-30.4)	4.03 (2.34-6.95)
0-8:	58.7 (37.9-n.a.)	9:	7.6 (1.0-n.a.)	3.79 (1.69-8.50)*

c low beta ≤ 0.20 and high beta ≥ 0.80

Stratification groups according to number of CpGs predicting short TTT				HR (95% CI)
No. CpGs	Median TTT (95% CI)	No. CpGs	Median TTT (95% CI)	
0:	NR	1-9:	14.4 (5.3-32.7)	5.88 (3.25-10.65)
0-1:	**NR**	**2-9:**	**13.1 (4.9-21.1)**	**8.34 (4.54-15.30)**
0-2:	NR (93.3-n.a.)	3-9:	8.0 (4.8-21.1)	7.01 (3.98-12.32)
0-3:	NR (64.7-n.a.)	4-9:	7.6 (4.1-21.1)	5.49 (3.22-9.34)
0-4:	NR (61.2-n.a.)	5-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-5:	NR (61.2-n.a.)	6-9:	13.1 (3.4-30.4)	4.24 (2.53-7.11)
0-6:	NR (61.2-n.a.)	7-9:	13.1 (3.4-30.4)	4.24 (2.53-7.11)
0-7:	84.3 (54.6-n.a.)	8-9:	7.6 (3.0-21.4)	4.35 (2.54-7.46)
0-8:	58.7 (37.9-n.a.)	9:	7.6 (1.0-n.a.)	3.79 (1.69-8.50)*

d low beta ≤ 0.25 and high beta ≥ 0.75

Stratification groups according to number of CpGs predicting short TTT				HR (95% CI)
No. CpGs	Median TTT (95% CI)	No. CpGs	Median TTT (95% CI)	
0:	NR	1-9:	14.4 (5.3-32.7)	5.88 (3.25-10.65)
0-1:	**NR**	**2-9:**	**13.1 (4.9-21.1)**	**8.34 (4.54-15.30)**
0-2:	NR (93.3-n.a.)	3-9:	8.0 (4.8-21.1)	7.01 (3.98-12.32)
0-3:	NR (64.7-n.a.)	4-9:	7.6 (4.1-21.1)	5.49 (3.22-9.34)
0-4:	NR (61.2-n.a.)	5-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-5:	NR (61.2-n.a.)	6-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-6:	NR (61.2-n.a.)	7-9:	13.1 (3.4-30.4)	4.24 (2.53-7.11)
0-7:	84.3 (58.7-n.a.)	8-9:	6.3 (1.9-21.4)	4.61 (2.69-7.89)
0-8:	59.9 (39.2-n.a.)	9:	4.1 (1.0-n.a.)	4.78 (2.29-9.96)

e low beta ≤ 0.30 and high beta ≥ 0.70

Stratification groups according to number of CpGs predicting short TTT				HR (95% CI)
No. CpGs	Median TTT (95% CI)	No. CpGs	Median TTT (95% CI)	
0:	NR	1-9:	17.1 (7.6-34.3)	5.87 (3.16-10.89)
0-1:	**NR**	**2-9:**	**13.1 (4.9-21.1)**	**8.34 (4.54-15.30)**
0-2:	NR (93.3-n.a.)	3-9:	8.0 (4.8-21.1)	7.01 (3.98-12.32)
0-3:	NR (64.7-n.a.)	4-9:	7.6 (4.1-21.1)	5.49 (3.22-9.34)
0-4:	NR (61.2-n.a.)	5-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-5:	NR (61.2-n.a.)	6-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-6:	NR (61.2-n.a.)	7-9:	13.1 (3.4-30.4)	4.24 (2.53-7.11)
0-7:	84.3 (58.7-n.a.)	8-9:	7.6 (3.0-21.1)	4.75 (2.78-8.13)
0-8:	61.2 (39.2-n.a.)	9:	5.9 (1.0-n.a.)	3.93 (1.97-7.87)*

f low beta ≤ 0.35 and high beta ≥ 0.65

Stratification groups according to number of CpGs predicting short TTT				HR (95% CI)
No. CpGs	Median TTT (95% CI)	No. CpGs	Median TTT (95% CI)	
0:	NR	1-9:	17.1 (8.0-37.9)	5.64 (3.01-10.58)
0-1:	**NR**	**2-9:**	**13.1 (4.9-21.1)**	**8.34 (4.54-15.30)**
0-2:	NR (93.3-n.a.)	3-9:	8.0 (4.8-21.1)	7.01 (3.98-12.32)
0-3:	NR (64.7-n.a.)	4-9:	7.6 (4.1-21.1)	5.49 (3.22-9.34)
0-4:	NR (61.2-n.a.)	5-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-5:	NR (61.2-n.a.)	6-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-6:	NR (61.2-n.a.)	7-9:	13.1 (3.4-30.4)	4.24 (2.53-7.11)
0-7:	93.3 (58.7-n.a.)	8-9:	7.6 (3.0-21.1)	4.77 (2.81-8.11)
0-8:	61.2 (39.2-n.a.)	9:	7.6 (1.1-n.a.)	3.82 (1.96-7.46)

g low beta ≤ 0.40 and high beta ≥ 0.60

Stratification groups according to number of CpGs predicting short TTT				HR (95% CI)
No. CpGs	Median TTT (95% CI)	No. CpGs	Median TTT (95% CI)	
0:	NR	1-9:	17.1 (8.0-39.2)	5.45 (2.85-10.39)
0-1:	**NR**	**2-9:**	**13.1 (4.9-21.1)**	**8.17 (4.43-15.06)**
0-2:	NR	3-9:	13.1 (4.9-21.4)	6.98 (3.94-12.35)
0-3:	NR (70.0-n.a.)	4-9:	7.6 (4.1-21.1)	5.78 (3.38-9.88)
0-4:	NR (61.2-n.a.)	5-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-5:	NR (61.2-n.a.)	6-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-6:	NR (61.2-n.a.)	7-9:	13.1 (3.4-30.4)	4.24 (2.53-7.11)
0-7:	NR (61.2-n.a.)	8-9:	7.6 (3.0-21.1)	4.98 (2.93-8.45)
0-8:	61.2 (45.5-n.a.)	9:	7.6 (1.8-47.2)	3.79 (2.09-6.88)

h low beta ≤ 0.45 and high beta ≥ 0.55

Stratification groups according to number of CpGs predicting short TTT				HR (95% CI)
No. CpGs	Median TTT (95% CI)	No. CpGs	Median TTT (95% CI)	
0:	NR	1-9:	21.1 (13.9-47.2)	6.18 (2.80-13.67)
0-1:	**NR**	**2-9:**	**14.4 (5.3-30.4)**	**6.74 (3.68-12.35)**
0-2:	NR	3-9:	13.9 (4.9-30.4)	6.15 (3.49-10.81)
0-3:	NR (64.7-n.a.)	4-9:	13.1 (4.8-21.1)	5.22 (3.07-8.90)
0-4:	NR (61.2-n.a.)	5-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-5:	NR (61.2-n.a.)	6-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-6:	NR (61.2-n.a.)	7-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-7:	NR (61.2-n.a.)	8-9:	7.6 (3.0-21.1)	4.98 (2.93-8.45)
0-8:	64.7 (50.8-n.a.)	9:	4.0 (1.1-20.5)	4.66 (2.64-8.23)

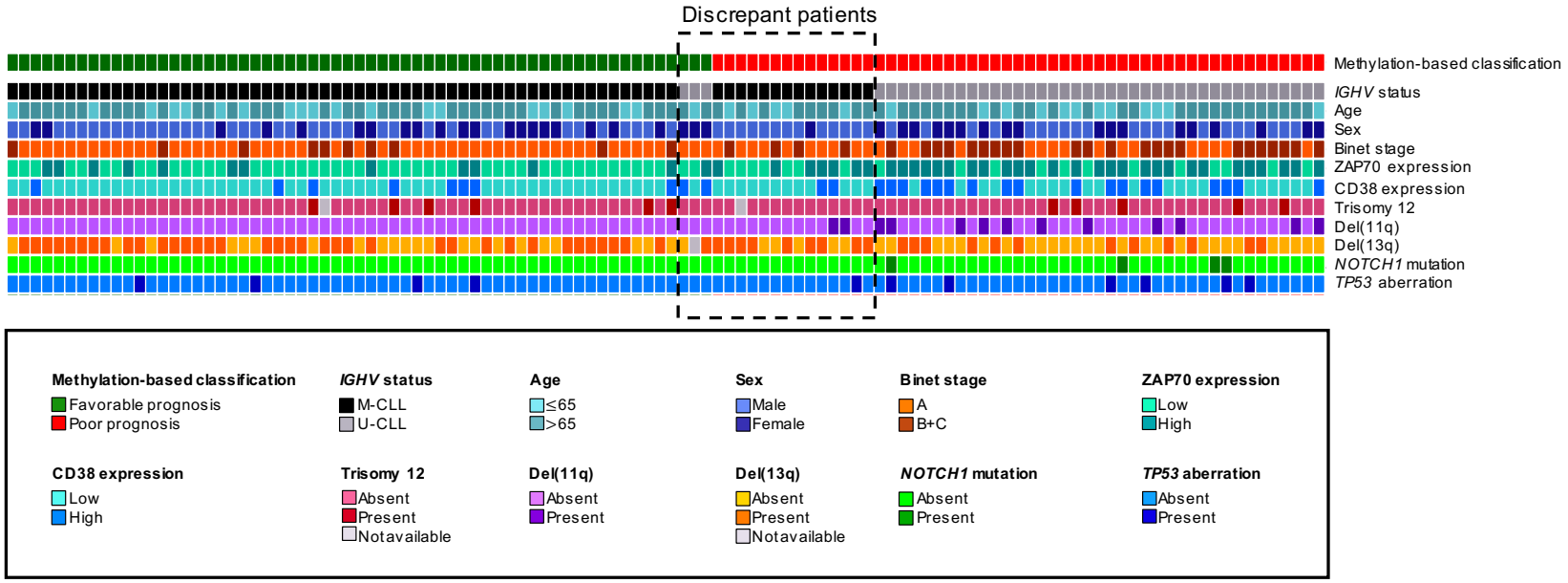
i low beta ≤ 0.50 and high beta ≥ 0.50

Stratification groups according to number of CpGs predicting short TTT				HR (95% CI)
No. CpGs	Median TTT (95% CI)	No. CpGs	Median TTT (95% CI)	
0:	**NR**	**1-9:**	**30.4 (16.2-56.3)**	**10.22 (3.19-32.72)**
0-1:	NR	2-9:	16.2 (8.0-34.3)	7.26 (3.78-13.95)
0-2:	NR	3-9:	13.9 (5.0-21.4)	8.08 (4.38-14.90)
0-3:	NR (93.3-n.a.)	4-9:	10.1 (4.9-21.1)	6.17 (3.54-10.73)
0-4:	NR (61.2-n.a.)	5-9:	13.9 (3.9-21.4)	4.66 (2.77-7.83)
0-5:	NR (61.2-n.a.)	6-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-6:	NR (61.2-n.a.)	7-9:	13.1 (3.4-30.4)	4.50 (2.68-7.55)
0-7:	NR (61.2-n.a.)	8-9:	13.1 (3.4-30.4)	4.54 (2.68-7.70)
0-8:	70.0 (51.3-n.a.)	9:	4.0 (1.8-20.5)	4.65 (2.70-8.03)

Supplementary Fig. S5. (legend on previous page) Detailed results from Cox regression analyses performed for patients stratified according to different number of CpG sites predicting short TTT.

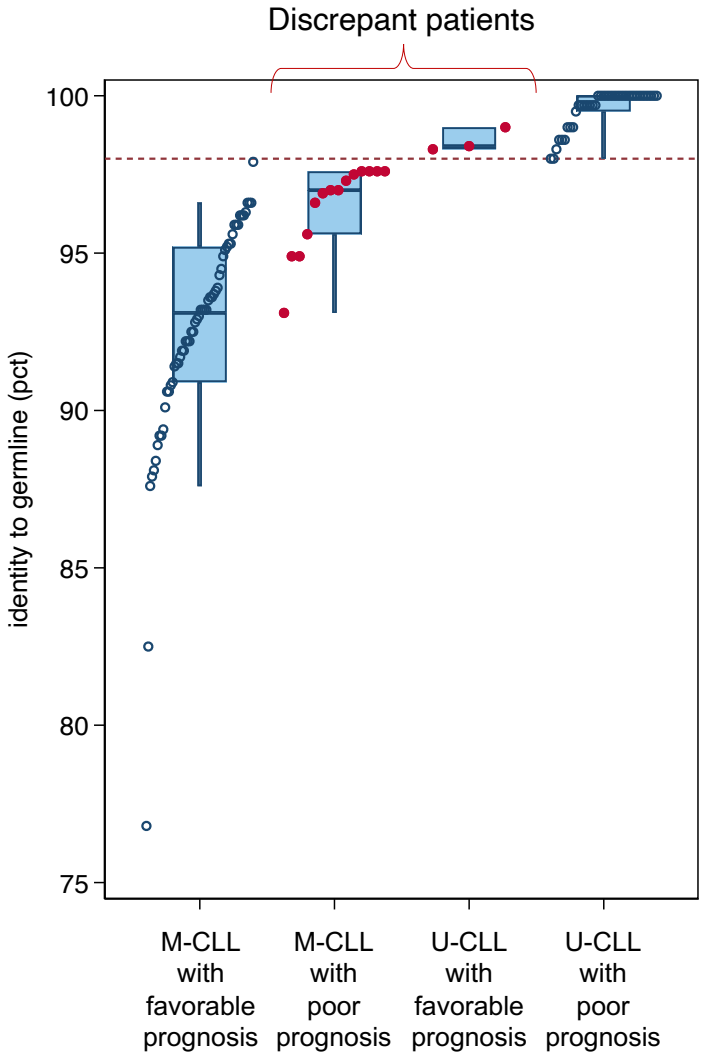
Additional Supplementary Figures

Supplementary Fig. S6. The frequency of the standard biomarkers used in CLL in patients classified with our procedure.



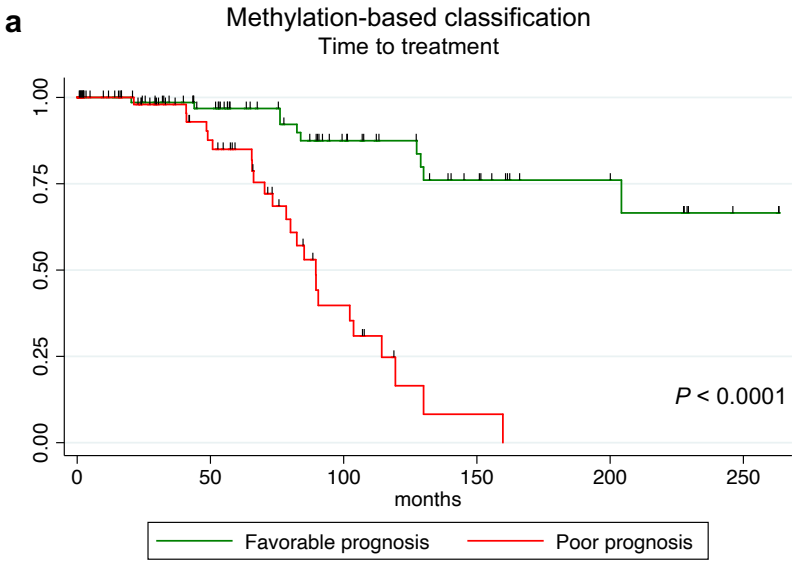
Supplementary Fig. S7. Boxplot describing IGHV mutation load in U-CLL and M-CLL patient groups subdivided into poor and favorable prognosis groups according to methylation signature.

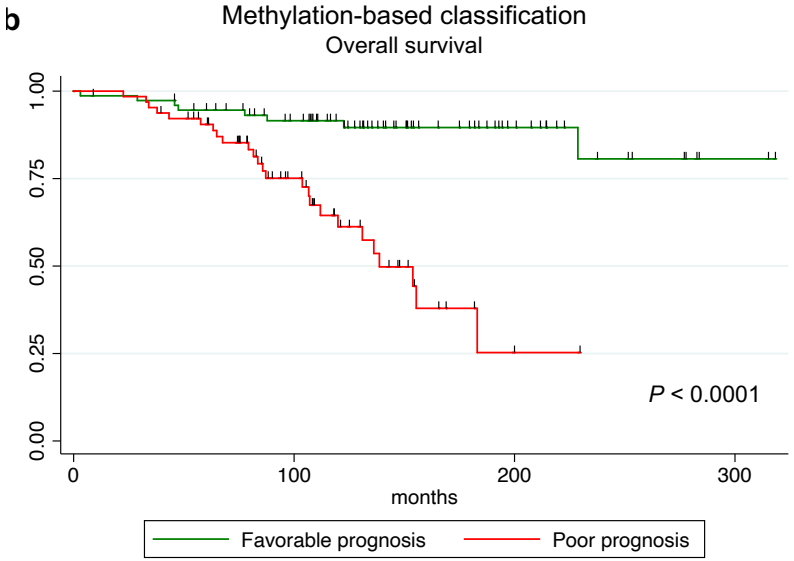
The boxplots display the mutation load of the IGHV gene (as %identify to germline sequence) in four patient groups, where the patients (dots) has been subdivided according to IGHV status (M-CLL or U-CLL) and methylation-based classification (favorable prognosis or poor prognosis). The boxes indicate the 25-, 50- and 75-percentiles, and whiskers indicate the 95% confidence intervals. The 98% identity cutoff used for IGHV status stratification is marked with a horizontal line. Patients discrepantly classified between the two stratification methods are highlighted with red dots.

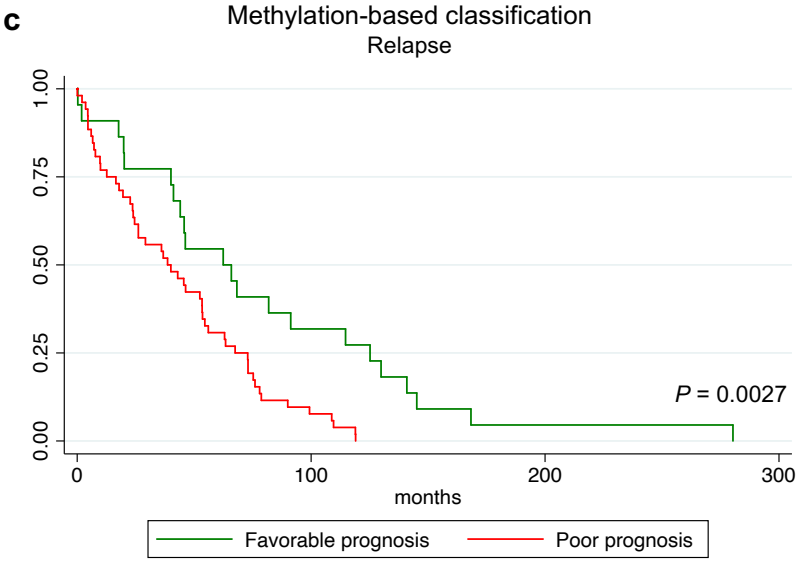


Supplementary Fig. S8. Kaplan-Meier curves of TTT, OS and relapse for methylation-based classification in the cohort for which 450K data were available.

a *Kaplan-Meier curves describing TTT,* ***b*** *Kaplan-Meier curves describing OS, and* ***c*** *Kaplan-Meier curves describing relapse for patient stratification using the methylation-based classification in independent patient cohort (450K data). The classification was based on 3 out of 9 discovered loci, which were available in the 450K dataset.*

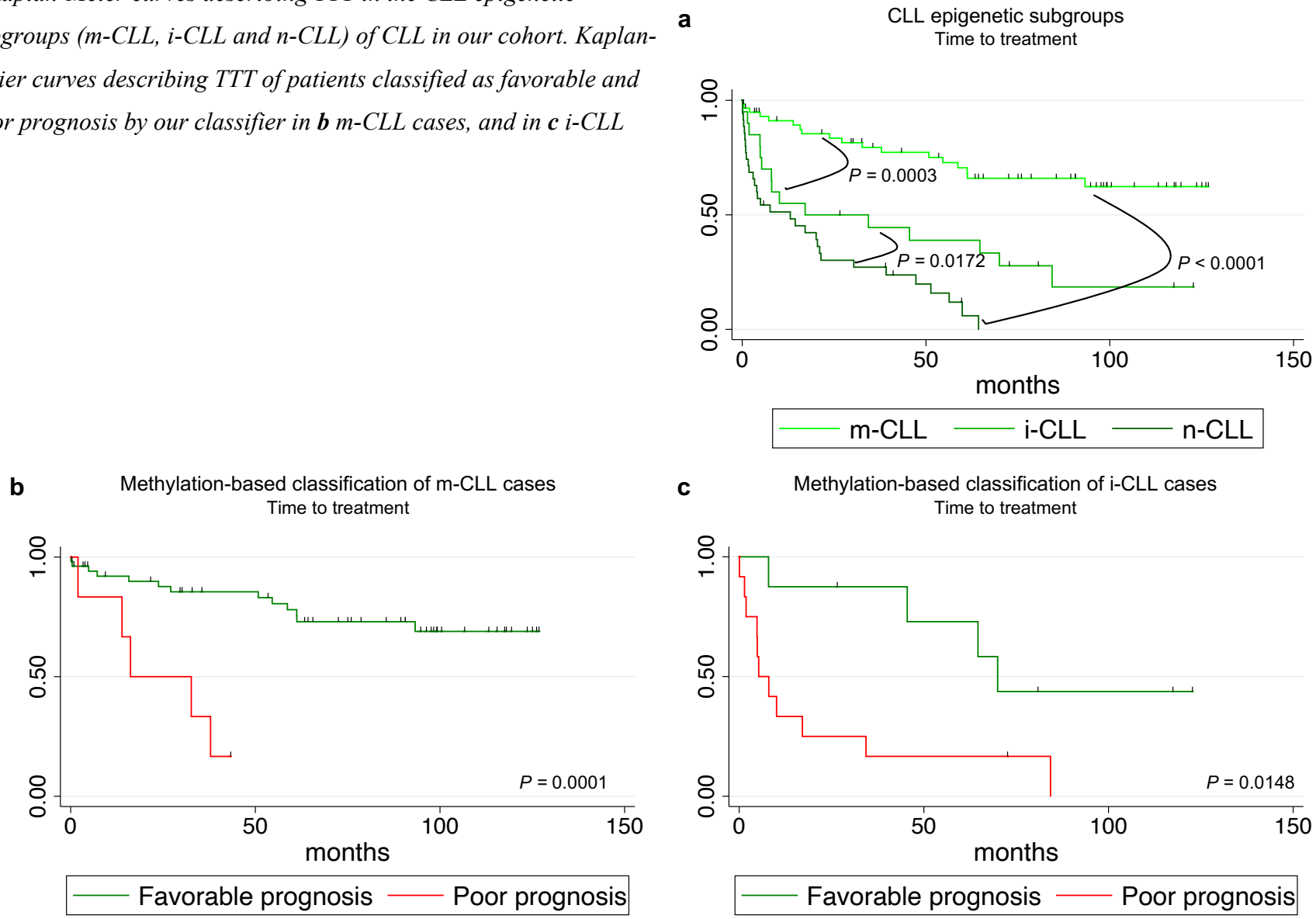






Supplementary Fig. S9. Kaplan-Meier curves of TTT in epigenetic subgroups of CLL in our cohort.

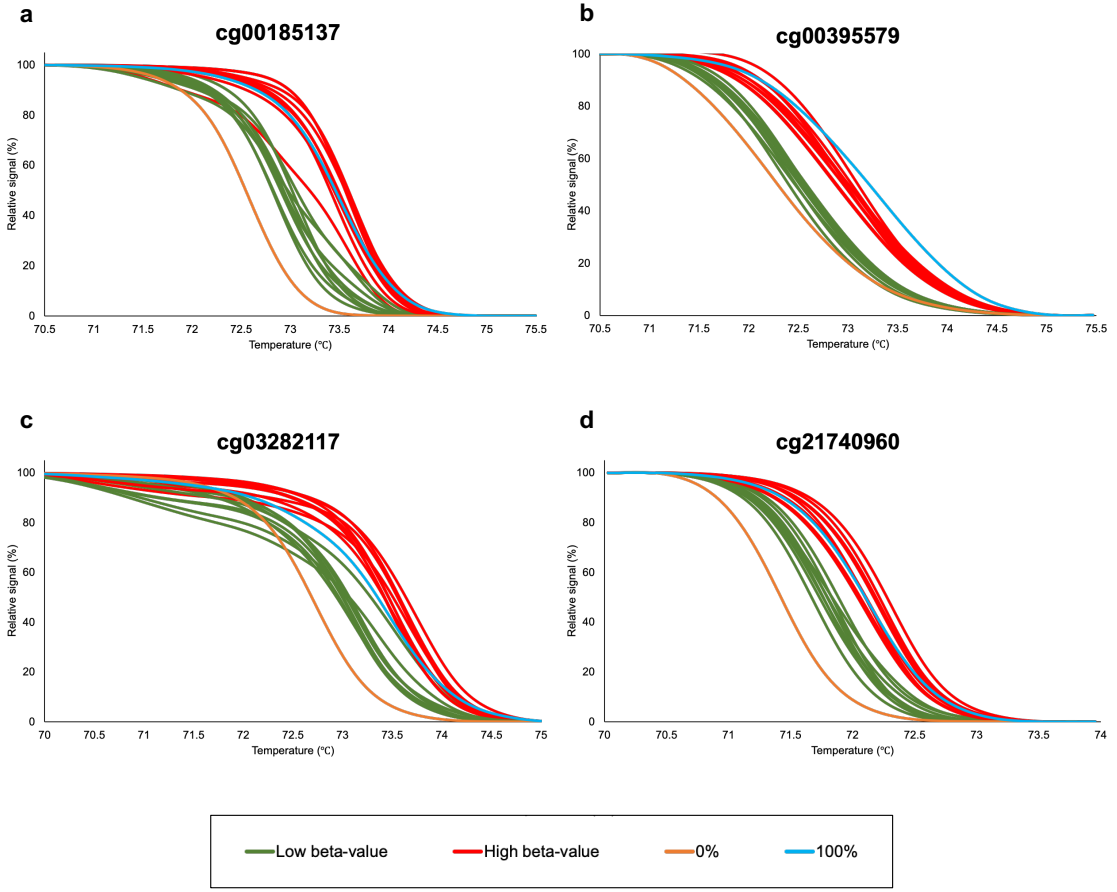
a *Kaplan-Meier curves describing TTT in the CLL epigenetic subgroups (m-CLL, i-CLL and n-CLL) of CLL in our cohort. Kaplan-Meier curves describing TTT of patients classified as favorable and poor prognosis by our classifier in* ***b*** *m-CLL cases, and in* ***c*** *i-CLL*



Supplementary Fig. S10. Validation of DNA methylation levels from EPIC array using MS-HRM. *The methylation levels of 4 out of the 9 CpG loci used in the methylation classification procedure were screened using MS-HRM. Each graph displays the relative fluorescence signal (%) after PCR amplification of the target region on the y-axis and the temperature (℃) on the x-axis. The melting curves illustrate the decrease in fluorescence as the amplicons dissociate during the melting process.*

The melting curves of the patients were colored according to the methylation level assessed from the EPIC array: low beta-value (green) and high beta-value (red). The methylation levels of the patient samples were qualitatively assessed in comparison to standard DNA samples of artificially methylated DNA (blue: 100%) and artificially non-methylated DNA (orange: 0%).

The melting curves observed for patients with high beta-value (red) followed the fully methylated standard DNA (blue), and the melting curves for patient with low beta-value (green) followed the non-methylated standard DNA (orange). Overall MS-HRM results confirmed the methylation status observed in microarray data.



Supplementary Tables

Supplementary Table S1. Association analyses between methylation-based classification and *IGHV* status with clinicobiological biomarkers.

		Methylation-based classification				***IGHV* status**			
		Poor prognosis	**Favorable prognosis**	**OR (95% CI)***	***P***	**U-CLL patients**	**M-CLL patients**	**OR (95% CI)***	***P***
Age	≤65 years	17	20			13	24		
	≥65 years	36	41	1.03 (0.47-2.27)	0.9355	29	48	1.12 (0.49-2.53)	0.7934
Binet stage	A	26	51			19	58		
	B+C	27	10	5.30 (2.23-12.59)	0.0001	23	14	5.02 (2.16-11.65)	0.0001
Sex	Male	34	38			21	51		
	Female	19	23	0.92 (0.43-1.98)	0.8377	21	21	2.43 (1.10-5.35)	0.0261
ZAP70 expression	Low	19	48			14	53		
	High	34	13	6.61 (2.88-15.17)	< 0.0001	28	19	5.58 (2.44-12.77)	< 0.0001
CD38 expression	Low	33	51			22	62		
	High	20	10	3.09 (1.29-7.42)	0.0098	20	10	5.64 (2.29-13.88)	0.0001
Trisomy 12	Absent	47	54			37	64		
	Present	5	6	0.96 (0.27-3.34)	0.9456	5	6	1.44 (0.41-5.05)	0.5661
Del(11q)	Absent	40	61			31	70		
	Present	13	0	n.a.	< 0.0001	11	2	12.42 (2.60-59.39)	0.0001
Del(13q)	Absent	32	23			28	27		
	Present	21	37	0.41 (0.19-0.87)	0.0193	13	45	0.28 (0.12-0.63)	0.0016
***NOTCH1* mutation**	Absent	49	61			38	72		
	Present	4	0	n.a.	0.0289	4	0	n.a.	0.0077
***TP53* aberration**	Absent	46	57			36	67		
	Present	7	4	2.17 (0.60-7.87)	0.2304	6	5	2.23 (0.64-7.83)	0.2003
***IGHV* status**	M-CLL	14	58						
	U-CLL	39	3	53.86 (14.51-199.87)	< 0.0001				
Methylation-based classification	Favorable					3	58		
	Poor					39	14	53.86 (14.51-199-87)	<0.0001

OR indicate the odds ratio for having poor prognosis or being U-CLL patient given the biomarker status in the specific row.

CI, confidence interval; M-CLL, mutated *IGHV*; n.a., not available; OR, Odds ratio; U-CLL, unmutated *IGHV*.

* OR by Woolf.

Supplementary Table S2. Distribution of clinicobiological biomarkers in patients classified according to *IGHV* status and methylation-based classification.

		Discrepant patients			
	***IGHV* status**	**M-CLL**		**U-CLL**	
	Methylation-based classification	**Favorable prognosis**	**Poor prognosis**	**Favorable prognosis**	**Poor prognosis**
Age	Median (range), years	71 (49-90)	74.5 (53-86)	70 (60-84)	70 (49-92)
	≤65 years	19	5	1	12
	65 years	39	9	2	27
Binet stage	A	48	10	3	16
	B	2	2	0	10
	C	8	2	0	13
Sex	Male	38	13	0	21
	Female	20	1	3	18
ZAP70 expression	Low	46	7	2	12
	High	12	7	1	27
CD38 expression	Low	50	12	1	21
	High	8	2	2	18
Trisomy 12	Absent	51	13	3	34
	Present	6	0	0	5
Del(11q)	Absent	58	12	3	28
	Present	0	2	0	11
Del(13q)	Absent	22	5	1	27
	Present	36	9	1	12
***NOTCH1* mutation**	Absent	58	14	3	35
	Present	0	0	0	4
***TP53* aberration**	Absent	54	13	3	33
	Present	4	1	0	6
***IGHV* status**	M-CLL	58	14	0	0
	U-CLL	0	0	3	39
Methylation-based classification	Favorable	58	0	3	0
	Poor	0	14	0	39

Supplementary Table S3. Univariate Cox regression analyses for TTT and OS according to different clinicobiological biomarkers.

		Time to treatment				Overall survival			
		Events/ Patients	**Median TTT (95%CI), months**	**HR (95% CI)**	***P***	**Events/ Patients**	**Median OS (95%CI), months**	**HR (95% CI)**	***P***
Age	≤65 years	20/37	54.6 (7.20-n.a.)			11/37	NR		
	>65 years	44/77	50.8 (30.4-70.0)	1.01 (0.59-1.72)	0.970	46/77	77.2 (62.0-97.7)	2.93 (1.50-5.69)	0.002
Binet stage	A	33/77	93.3 (59.9-n.a.)			35/77	106.3 (82.5-n.a.)		
	B+C	31/37	5.0 (1.6-13.1)	6.17 (3.63-10.47)	<0.001	22/37	78.4 (31-1-120.4)	1.53 (0.90-2.62)	0.117
Sex	Male	39/72	47.2 (17.1-n.a.)			38/72	97.7 (70.0-117.3)		
	Female	25/42	61.2 (27.1-93.3)	0.94 (0.57-1.56)	0.814	19/42	112.9 (73.2-n.a.)	0.73 (0.42-1.26)	0.254
ZAP70 expression	Low	28/67	NR			29/67	112.9 (88.8-n.a.)		
	High	36/47	17.1 (4.9-34.3)	3.30 (1.99-5.49)	<0.001	28/47	71.5 (47.1-120.4)	1.78 (1.05-2.98)	0.031
CD38 expression	Low	41/84	64.3 (39.2-n.a.)			38/84	112.9 (80.0-n.a.)		
	High	23/30	17.1 (4.1-47.2)	2.41 (1.43-4.04)	0.001	19/30	73.5 (34.9-98.2)	1.71 (0.99-2.98)	0.056
Trisomy 12	Absent	54/101	59.9 (30.4-93.3)			52/101	98.2 (77.2-117.3)		
	Present	8/11	15.8 (0.7-58.7)	2.04 (0.96-4.33)	0.062	5/11	73.5 (29.3-n.a.)	1.23 (0.49-3.12)	0.658
Del(11q)	Absent	52/101	58.7 (37.9-n.a.)			49/101	98.2 (77.2-n.a.)		
	Present	12/13	14.4 (1.1-20.1)	2.65 (1.40-5.01)	0.003	8/13	117.3 (18.5-n.a.)	1.10 (0.52-2.32)	0.811
Del(13q)	Absent	39/55	20.5 (8.0-56.3)			25/55	117.3 (73.5-n.a.)		
	Present	24/58	NR	0.44 (0.26-0.74)	0.002	32/58	92.0 (64.3-117.2)	1.34 (079-2.27)	0.274
***NOTCH1* mutation**	Absent	60/110	56.3 (32.7-84.3)			54/110	98.2 (77.2-n.a.)		
	Present	4/4	1.8 (1.1-n.a.)	3.4 (1.22-9.59)	0.019	3/4	97.7 (34.9-n.a.)	1.31 (0.41-420)	0.648
***TP53* aberration**	Absent	55/103	56.3 (30.4-93.3)			49/103	102.0 (77.2-n.a.)		
	Present	9/11	21.1 (0.6-61.2)	1.9 (0.92-3.8)	0.086	8/11	93.8 (538-102.4)	1.33 (0.63-2.81)	0.454
***IGHV* status**	M-CLL	27/72	NR			32/72	112.9 (73.5-n.a.)		
	U-CLL	37/42	10.1 (3.4-21.1)	4.35 (2.60-7.28)	<0.001	25/42	88.8 (59.3-117.3)	1.35 (0.80-2.28)	0.263
Methylation-based classification	Favorable	17/61	NR			23/61	117.2 (92.0-n.a.)		
	Poor	47/53	13.1 (4.1-20.1)	8.34 (4.54-15.30)	<0.001	34/53	77.2 (57.9-98.2)	1.82 (1.07-3.09)	0.027

HR indicates the likelihood of treatment (TTT) or death (OS) given the biomarker status in the specific row.

CI, confidence interval; HR, hazard ratio; M-CLL, mutated *IGHV*; n.a., not available; NR, not reached; U-CLL, unmutated *IGHV*.

Supplementary files

Supplementary File 1. Beta-values for 9 CpGs. Text file containing the normalized beta-values for the 9 CpG sites used for classification of 114 patients into favorable or poor prognosis according to the methylation-based classification procedure.

Patients	cg00185137	cg00395579	cg02198280	cg03282117	cg07250315	cg07395110	cg12032915	cg21394039	cg21740960
Case_1	0.947685055	0.952610803	0.951041686	0.906899109	0.925091309	0.965381604	0.978571923	0.915192356	0.988172034
Case_2	0.947208152	0.904845898	0.962009105	0.964395254	0.041990888	0.953711218	0.951379106	0.934440696	0.98589092
Case_3	0.944022243	0.927212622	0.944529448	0.931247186	0.10343189	0.600405294	0.981173558	0.937112609	0.982696088
Case_4	0.967196023	0.90428608	0.947468893	0.922398382	0.072700537	0.961885614	0.964234676	0.936852821	0.979059592
Case_5	0.986253267	0.928243136	0.97831153	0.908276017	0.404758772	0.970352229	0.962552295	0.932934661	0.974440996
Case_6	0.950287096	0.948926459	0.562840448	0.898250041	0.046750538	0.973336059	0.967811668	0.948741019	0.973985887
Case_7	0.982321937	0.915733507	0.949818244	0.857125019	0.040494272	0.962026192	0.952381977	0.974585555	0.97290071
Case_8	0.95229904	0.931504609	0.80279732	0.943924904	0.819949608	0.947077744	0.961427953	0.920629551	0.971459978
Case_9	0.981315941	0.928653534	0.962252414	0.911896267	0.061930667	0.930011571	0.982549585	0.962606506	0.969683127
Case_10	0.980579069	0.503447055	0.974332232	0.873886115	0.036159622	0.927596344	0.965066329	0.947593974	0.969309829
Case_11	0.94919377	0.078171663	0.054626746	0.39599334	0.038240827	0.12360906	0.971890694	0.119260833	0.957649992
Case_12	0.968907673	0.944003668	0.946403348	0.882275299	0.494768932	0.956401873	0.962499919	0.955932274	0.953552298
Case_13	0.935131814	0.945363075	0.96523436	0.952235373	0.535560406	0.963044598	0.970446857	0.961929394	0.953273069
Case_14	0.907702197	0.96735383	0.948165852	0.369752788	0.068212904	0.940192058	0.96240761	0.982020376	0.95280671
Case_15	0.984960616	0.939679899	0.956215474	0.942297896	0.060868215	0.959253562	0.970672677	0.967268611	0.952799688
Case_16	0.868507818	0.888267708	0.936232121	0.951799036	0.090597416	0.580218088	0.930533122	0.944995886	0.950870285
Case_17	0.91833495	0.501950668	0.938280666	0.902183033	0.865049958	0.940676571	0.976606907	0.97387126	0.938795664
Case_18	0.912946958	0.94113336	0.867038565	0.898463932	0.214104928	0.956791427	0.965995991	0.944750934	0.936156673
Case_19	0.946307862	0.902682776	0.920117034	0.910847423	0.546303998	0.944152049	0.96428782	0.955451218	0.934044942
Case_20	0.947317444	0.927957634	0.667366332	0.900311045	0.062459582	0.610028873	0.942035691	0.901111222	0.933916368
Case_21	0.936130136	0.935537613	0.931052657	0.07278037	0.373791513	0.959886663	0.970306124	0.940027641	0.933199326
Case_22	0.939000748	0.493238367	0.390837811	0.846134011	0.092221711	0.179035437	0.531065806	0.061505973	0.932894306
Case_23	0.955316786	0.956507901	0.94540401	0.931872402	0.570364714	0.950876692	0.990359977	0.913030297	0.932296148
Case_24	0.615111119	0.499992771	0.120927643	0.066280961	0.913846387	0.879171407	0.091764836	0.053472605	0.931449116
Case_25	0.111626376	0.070808563	0.542674014	0.076553919	0.474884559	0.465068458	0.966677488	0.414615535	0.925694665
Case_26	0.868786744	0.933098628	0.952479055	0.915465934	0.596160364	0.922169317	0.964181174	0.946212538	0.913038209
Case_27	0.944145834	0.90316311	0.956196855	0.466771172	0.072534834	0.922942842	0.945716423	0.974648302	0.90853167
Case_28	0.920018225	0.339034897	0.12057922	0.130093378	0.911713478	0.119794128	0.211806262	0.076610005	0.904094391
Case_29	0.931230025	0.952398124	0.928255147	0.951365552	0.083044104	0.962860913	0.969508389	0.934237934	0.892038387
Case_30	0.971804171	0.96497624	0.948356019	0.934425791	0.073603286	0.913286794	0.977094161	0.951589691	0.881017986
Case_31	0.59507121	0.797172166	0.904524497	0.805117886	0.52000036	0.494736075	0.908218275	0.817027914	0.837802325
Case_32	0.945052373	0.640037293	0.897200263	0.93297937	0.07684073	0.945091481	0.958201233	0.639561672	0.837397238
Case_33	0.810826302	0.954925969	0.893154221	0.968538318	0.113200597	0.860867624	0.969439455	0.949095527	0.795816621
Case_34	0.920697763	0.925117804	0.962493218	0.906165225	0.453188491	0.939958389	0.947326273	0.966269156	0.775035188
Case_35	0.963311171	0.961955001	0.959047255	0.898117769	0.08320315	0.91806314	0.95547236	0.957210239	0.700206806
Case_36	0.931677332	0.921142873	0.945264881	0.926010662	0.074998069	0.942361573	0.967387668	0.950534893	0.682588371
Case_37	0.935660322	0.946916213	0.964487843	0.505639216	0.118700592	0.956872376	0.964217503	0.91467138	0.660432321
Case_38	0.815374154	0.890445285	0.899285359	0.80576621	0.109980503	0.744833511	0.919750694	0.939334747	0.639426137
Case_39	0.118067778	0.43487754	0.093600085	0.091949629	0.847379444	0.213370485	0.094212859	0.076397464	0.595635985
Case_40	0.570832076	0.040018745	0.066135427	0.052939135	0.040042014	0.060909363	0.069815814	0.077636205	0.584734761
Case_41	0.098469872	0.076506142	0.090652781	0.063886762	0.943402195	0.075890986	0.073304573	0.060162769	0.569258612
Case_42	0.967702311	0.931108855	0.944322492	0.885142694	0.521056722	0.94933969	0.966803184	0.966221693	0.546851779
Case_43	0.0863591	0.086615663	0.151767152	0.396808521	0.070975923	0.952000177	0.581348425	0.837614146	0.535417571
Case_44	0.076363825	0.062632043	0.080881015	0.979231174	0.093711552	0.055458259	0.113692566	0.040663783	0.517822999
Case_45	0.977551831	0.928616859	0.250105472	0.944925061	0.52885441	0.370706091	0.05821441	0.396536423	0.514104471
Case_46	0.718095231	0.15279956	0.212569265	0.099534882	0.5938944	0.15633353	0.584023846	0.161783643	0.508683368
Case_47	0.06156422	0.051624941	0.075554202	0.476119813	0.955071033	0.079179982	0.079771277	0.067532462	0.498772039
Case_48	0.09720716	0.491175715	0.076195518	0.938955251	0.930724341	0.082110122	0.051820381	0.105768172	0.448343552
Case_49	0.165574442	0.495385292	0.108446775	0.065159092	0.943017615	0.117821131	0.062933765	0.05845109	0.44743843
Case_50	0.960863387	0.885542489	0.404041745	0.368373665	0.071427093	0.45421711	0.950132673	0.374760829	0.437099756
Case_51	0.082968598	0.487631152	0.091932791	0.061127452	0.429126212	0.08134229	0.060185015	0.047941591	0.435677382
Case_52	0.692707065	0.498850149	0.108692759	0.38529429	0.855637032	0.064198456	0.095209061	0.073377607	0.423492537
Case_53	0.091794996	0.93495798	0.494558255	0.908869148	0.065882471	0.05342467	0.951614248	0.388639415	0.411926139
Case_54	0.939745137	0.925244645	0.953129732	0.961116209	0.103146491	0.91921829	0.956630573	0.92641873	0.382084411
Case_55	0.982256272	0.937121437	0.902218349	0.916298144	0.051898318	0.087232253	0.977517641	0.939318096	0.322673264
Case_56	0.825945212	0.369685394	0.093525037	0.435461162	0.068125032	0.120208365	0.956330579	0.36402988	0.321840541
Case_57	0.140794704	0.101170135	0.097724161	0.075161165	0.931226102	0.128262123	0.102254537	0.10435995	0.284585372
Case_58	0.51339802	0.496527065	0.411226638	0.120334191	0.516501412	0.380219537	0.147650809	0.001900851	0.219970088
Case_59	0.378935826	0.278420259	0.210101298	0.246513495	0.818666649	0.234293615	0.330906174	0.215276276	0.206149451
Case_60	0.596441317	0.626136242	0.104561439	0.114182355	0.475296268	0.395291926	0.606610278	0.64097522	0.177605762
Case_61	0.226833755	0.167206717	0.134593489	0.127316633	0.914406044	0.522166804	0.166622891	0.133628336	0.144272513
Case_62	0.343048518	0.59571911	0.145258725	0.077894105	0.541375778	0.224881494	0.11689697	0.088516138	0.130438279
Case_63	0.701699776	0.12118217	0.131252646	0.503434908	0.762227246	0.158834389	0.072714534	0.111265725	0.126960544
Case_64	0.144070294	0.136114066	0.125168506	0.135962182	0.85357982	0.152675039	0.147663903	0.114635025	0.122617244
Case_65	0.594630101	0.096283335	0.267449148	0.082199425	0.088391081	0.574263756	0.97185648	0.375433116	0.115383336
Case_66	0.253255093	0.108379916	0.13052398	0.447393482	0.518231759	0.091195165	0.115457242	0.113621811	0.106095746
Case_67	0.535575182	0.112329905	0.107194119	0.062396441	0.92322208	0.556097458	0.17390932	0.04111512	0.101322467
Case_68	0.599890433	0.087561366	0.117482558	0.096322028	0.514622828	0.227748036	0.127260915	0.081115694	0.101072431
Case_69	0.318995798	0.114204833	0.129986477	0.073798099	0.617838293	0.723604496	0.069849805	0.102302828	0.09773345
Case_70	0.229607547	0.638646395	0.097411394	0.952502747	0.512248574	0.124766503	0.970818896	0.852959537	0.096570137

Case_71	0.199149847	0.862661911	0.112134539	0.919499407	0.88484829	0.148359734	0.341388234	0.061361387	0.094244517
Case_72	0.977664302	0.920820484	0.933176836	0.955362136	0.09170686	0.968953951	0.953050126	0.950216334	0.091360656
Case_73	0.090011836	0.075100555	0.068974712	0.088969339	0.869796404	0.094829825	0.128691457	0.068819061	0.086806523
Case_74	0.136908326	0.058961634	0.069499776	0.048724798	0.926184349	0.04072036	0.082938017	0.060535194	0.086706265
Case_75	0.125689352	0.108952889	0.196253969	0.083184243	0.436748577	0.146737773	0.08518916	0.101283187	0.084610684
Case_76	0.155114167	0.102900508	0.479559076	0.893602348	0.922350836	0.107063762	0.125487397	0.106424074	0.082744816
Case_77	0.233617266	0.081996657	0.121866067	0.438944989	0.883088747	0.103135711	0.096024013	0.07278051	0.081574698
Case_78	0.103064569	0.062441546	0.05166817	0.493643836	0.414715563	0.093733728	0.071322942	0.04273779	0.077489935
Case_79	0.216233826	0.530362613	0.197146906	0.482110011	0.901058756	0.123590767	0.175150778	0.107353862	0.076119323
Case_80	0.090226357	0.070749357	0.076683575	0.064022298	0.904064761	0.149035473	0.083455754	0.071676007	0.074029636
Case_81	0.080790774	0.082959806	0.109308281	0.041441518	0.877214517	0.448353827	0.064249142	0.070241891	0.072279776
Case_82	0.132506785	0.103734169	0.118657831	0.097461486	0.945641769	0.268922256	0.10497154	0.081239824	0.069767558
Case_83	0.105676983	0.13051951	0.093599524	0.069707126	0.910765227	0.094055747	0.080905346	0.070880716	0.067670351
Case_84	0.102852738	0.486728259	0.148082564	0.081413264	0.441807826	0.117827889	0.081517115	0.086193814	0.06658554
Case_85	0.088970424	0.556657838	0.037223084	0.445338901	0.904435538	0.112053085	0.06767162	0.079285857	0.06572787
Case_86	0.963423859	0.104238787	0.112183892	0.441986828	0.407987834	0.162182103	0.471019685	0.112733997	0.064085336
Case_87	0.116395936	0.077732001	0.922325659	0.416136136	0.926862306	0.070934531	0.49283446	0.310984549	0.06280057
Case_88	0.432900762	0.645202755	0.11409829	0.465926558	0.894363256	0.098931081	0.133035521	0.04257319	0.062661711
Case_89	0.195758496	0.196403977	0.078498689	0.029838283	0.812404472	0.558678354	0.242141051	0.031219319	0.061829977
Case_90	0.08059366	0.064454416	0.088486628	0.393579931	0.035150387	0.074604221	0.084762305	0.072392426	0.061526268
Case_91	0.200716685	0.124280792	0.07814333	0.052578193	0.849465848	0.044721224	0.08276915	0.139956275	0.061097874
Case_92	0.281697712	0.089658681	0.06638664	0.050345511	0.971370321	0.061990382	0.045938919	0.069769845	0.05994494
Case_93	0.16740664	0.110169705	0.403960506	0.420394007	0.943145171	0.058133625	0.080821844	0.091764729	0.056633816
Case_94	0.162647215	0.08037909	0.061243746	0.055997706	0.893173095	0.094599008	0.104118145	0.071899859	0.053682435
Case_95	0.203175489	0.069915427	0.059398149	0.05829827	0.942162095	0.076022189	0.108525392	0.056049261	0.053052023
Case_96	0.076236864	0.06204825	0.142779597	0.115819495	0.913376448	0.520139143	0.055784383	0.062283597	0.05219182
Case_97	0.101030487	0.685951773	0.255140207	0.100697267	0.891263258	0.196229966	0.097142838	0.093897468	0.052015198
Case_98	0.262853843	0.507498526	0.225367983	0.096202513	0.906450681	0.651356232	0.13049928	0.129124153	0.050331259
Case_99	0.212989594	0.120483514	0.089984407	0.490457595	0.537197136	0.087795296	0.099468812	0.040276532	0.048496509
Case_100	0.506653931	0.429865415	0.0759687	0.38635177	0.573320159	0.110711485	0.093399567	0.086175203	0.046310536
Case_101	0.063714133	0.503431769	0.371632485	0.917070133	0.044125072	0.069326784	0.54007372	0.062799193	0.044734983
Case_102	0.084730298	0.444297849	0.044632274	0.452276036	0.922275919	0.046961797	0.068815985	0.047211784	0.041804502
Case_103	0.257934534	0.551975592	0.116683034	0.555993578	0.905228359	0.142948835	0.269802432	0.243458877	0.040855257
Case_104	0.094174723	0.057143744	0.076109038	0.065686754	0.962671288	0.06102692	0.07318035	0.055357361	0.036767917
Case_105	0.068180791	0.8925879	0.926372406	0.314767373	0.028046943	0.109067757	0.054933401	0.041201602	0.036274765
Case_106	0.131642244	0.052213824	0.082640802	0.043977881	0.933441611	0.175515134	0.436804567	0.060479644	0.035721981
Case_107	0.121918705	0.05035104	0.05551697	0.024094944	0.499157986	0.068137062	0.059850259	0.030424171	0.034453868
Case_108	0.081758444	0.096476231	0.055250444	0.043950263	0.921409687	0.048774306	0.038825714	0.025772532	0.033403002
Case_109	0.309010358	0.06405291	0.090020267	0.460587863	0.465726318	0.540578332	0.065777743	0.214422109	0.033293703
Case_110	0.057646935	0.804155233	0.056153568	0.021453585	0.932415029	0.071291054	0.052019056	0.025722587	0.030161823
Case_111	0.152807931	0.115208518	0.067428533	0.071261354	0.855318515	0.090081373	0.085029765	0.077508974	0.023214933
Case_112	0.030590325	0.070650704	0.075232457	0.410462919	0.928436604	0.531189737	0.044203357	0.032036064	0.0190823
Case_113	0.12552732	0.160921823	0.055994236	0.07157141	0.938598915	0.09261307	0.076993225	0.056238675	0.016049912
Case_114	0.069249459	0.0788313	0.105126067	0.035101598	0.960286969	0.191302675	0.05333149	0.236930174	0.014934725

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