Array-based genomic screening at diagnosis and during follow-up in chronic lymphocytic leukemia

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Online Supplementary Design and Methods

IGHV *gene analysis*

Polymerase chain reaction (PCR) amplification of the *IGH* gene rearrangements was performed on genomic DNA from peripheral blood mononuclear cells using subgroup-specific *IGHV* leader- or framework region 1 primers together with one consensus *IGHJ* primer as previously described.[1,2] The PCR products were sequenced using the Big Dye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, CA, USA) and the ABI 3700 automated DNA sequencer (Applied Biosystems). The obtained IG sequences were submitted to IMGT[3] and GenBank/IgBlast, and aligned to the most homologous germline *IGHV*, *IGHD* and *IGHJ* genes. *IGHV* gene sequences with less than 98% identity with germline were defined as mutated.

Copy-number analysis

Copy-number analysis using the rank segmentation algorithm and group comparison was performed using BioDiscovery Nexus Copy Number 5.0 software applying the rank segmentation algorithm based on circular binary segmentation.[4] In the preceding interim analysis of a cohort of patients (n=203), we performed real-time quantitative PCR on genomic DNA for selected regions in order to validate gains and losses for adjustment of the settings in the copy-number analysis.[5] A high number of verified alterations (85%) was found for regions larger than 200 kbp, when applying a *P* value of $1x10^{-6}$ in the copy-number analysis. These criteria were, therefore, applied in the evaluation of copy-number aberrations in the present study. Moreover, the segmentation analysis was performed with a $\log_{2ratio}$ cut-off at ±0.15 or ±0.2 for regions greater than 500 kbp and less than 500 kbp, respectively, and a minimum of ten adjacent single nucleotide polymorphism probes to define a gain or loss. Copy number variations (CNV) are polymorphic regions which exist as genomic variants in the population.[6] Since we did not have access to matched normal DNA from the CLL patients for exclusion of CNV, we excluded these non-cancer specific events by matching the array data to CNV regions reported by Redon *et al.* and MacCarroll *et al.* and removed copy number aberrations that overlapped by more than 50% with these CNV from further analysis.[7,8] Examples of copy-number regions that overlapped with CNV and were removed are the centromeric region of chromosome 14q (gains and losses covering 19336854-20041951 bp), the telomere of chromosome 14q (losses covering 104578854-106368585 bp), the centromeric region of chromosome 15q (gains and losses covering 18427103-21350156 bp), chromosome 17 (gains covering 41358231-42176296 bp) and chromosome 22 (losses covering 20673195- 21601809 bp). The sex chromosomes were also excluded from analysis.

Copy-number neutral loss of heterozygosity analysis

Identification of copy-number neutral loss of heterozygosity (CNN-LOH) that are tumor cell-specific was performed using single nucleotide polymorphism-array and flow-cytometry data on the fraction of normal cells as described elsewhere.[9] Regions with CNN-LOH were visualized in Nexus Copy Number 5.0 software.

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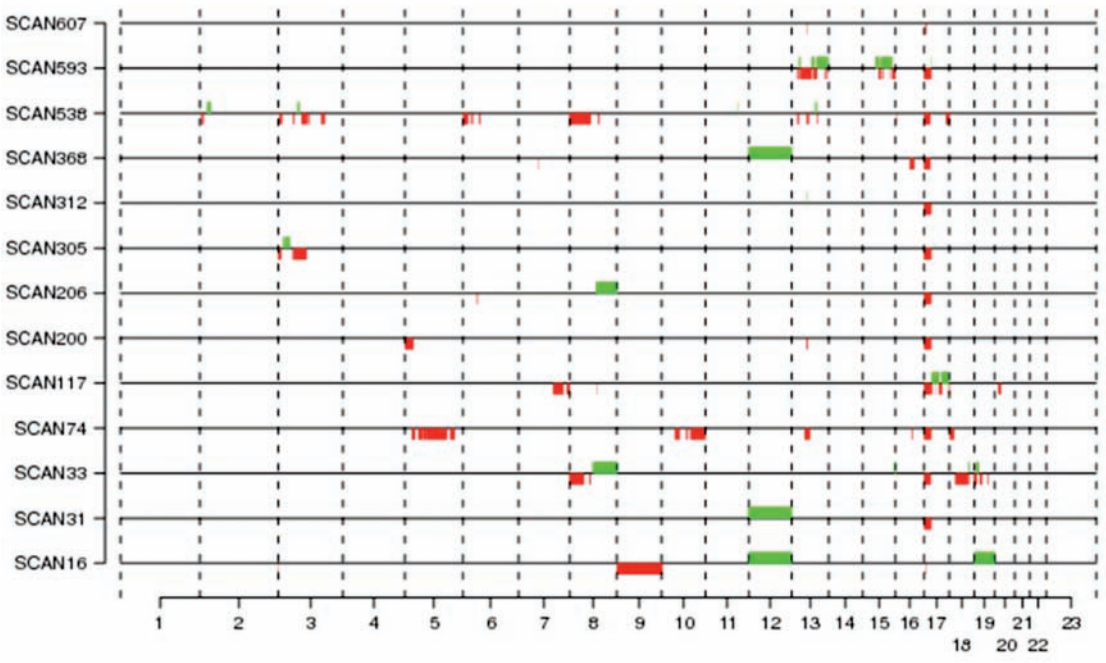
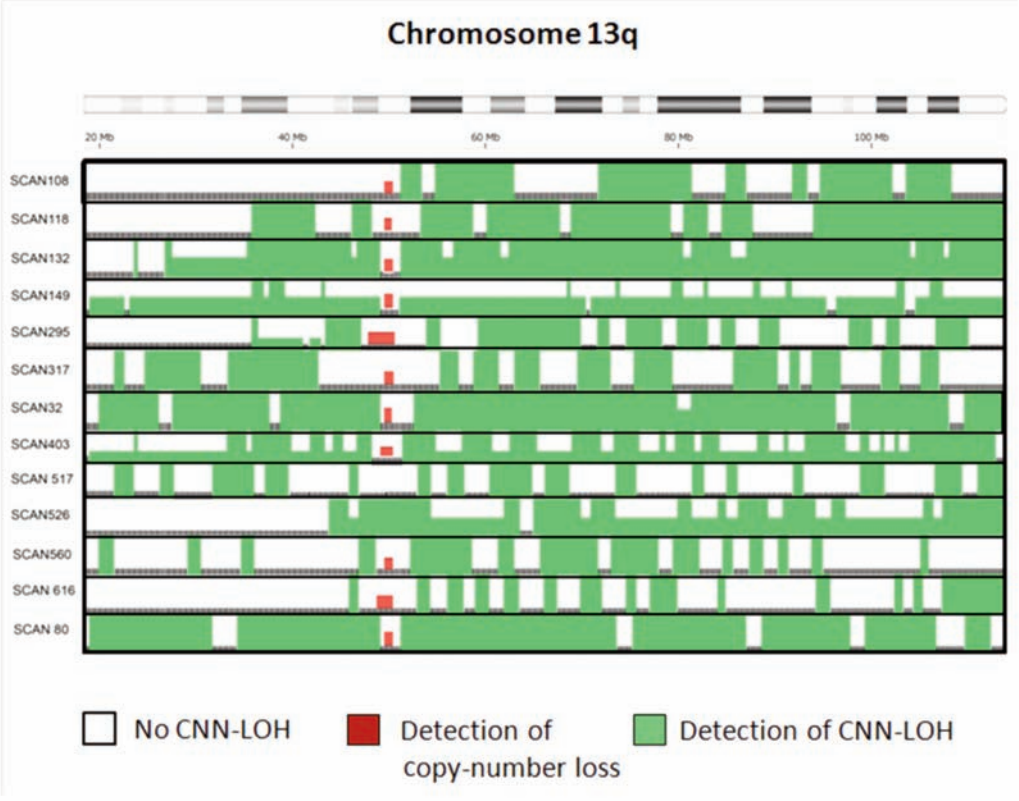
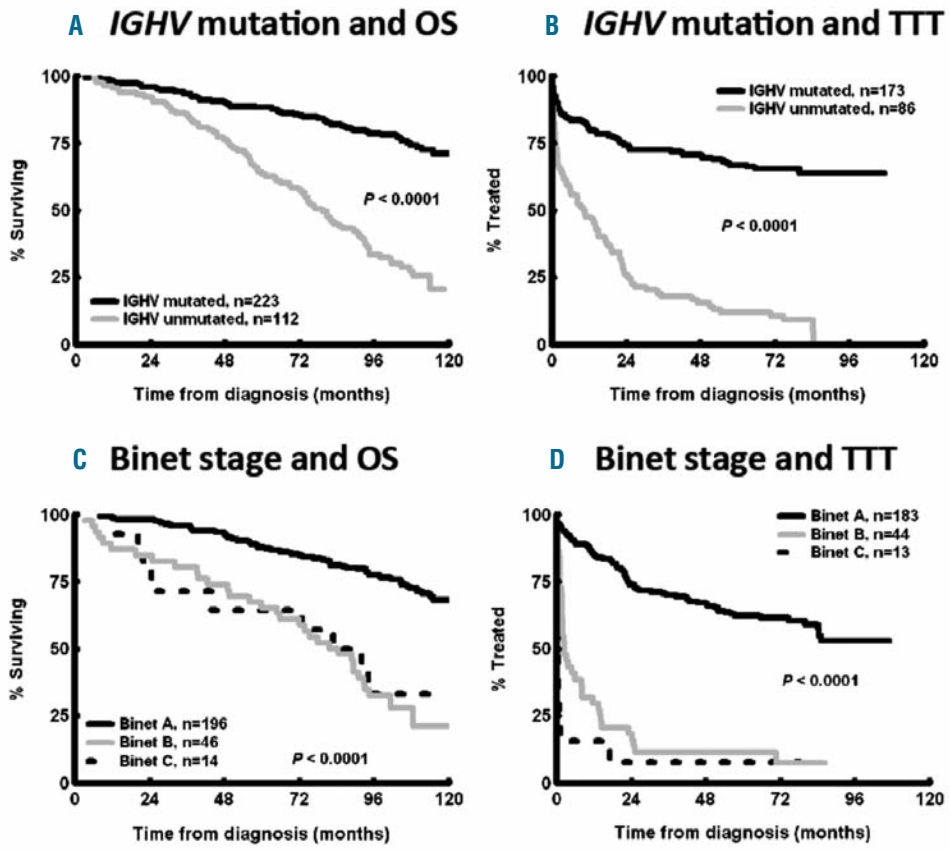
Online Supplementary Figure S1. Genomic display of 13 patients with del(17p) and additional genomic aberrations. Patients are indicated with SCAN-numbers and chromosomes 1-22 are shown on the x-axis. Red and green indicate losses and gains, respectively. The patients with del(17p) have a higher genomic complexity with an average of 9.2 CNA/sample and an average CNA size of 15.6 Mbp.



Online Supplementary Figure S2. Samples with large CNN-LOH on chromosome 13q. The CNN-LOH analysis revealed that 13 samples (indicated by SCAN numbers) have a CNN-LOH on large parts of chromosome 13q. A single green line corresponds to a tumor cell fraction of 50-80% with CNN-LOH and a double green line indicates high-level CNN-LOH (>80%). Red indicates homozygous deletion of the 13q14-region which was observed in 11 of the 13 samples with CNN-LOH on 13q.





Online Supplementary Figure S3. Overall survival and time to treatment according to *IGHV* mutation status and Binet stage. (A-B) Patients with unmutated *IGHV* genes had an inferior survival ($P<0.0001$) and shorter time to initiation of treatment ($P<0.0001$) than patients with unmutated *IGHV* genes. (C) Survival according to Binet stage showed that stage A patients had a better survival; note the lack of difference between stages B and C ($P<0.0001$). (D) The treatment-free interval according to Binet stage shows that the majority of patients with Binet stage B and C at diagnosis were judged to be in need of therapy immediately ($P<0.0001$).

Online Supplementary Table S1. SEE PDF FILE.

Online Supplementary Table S2.

All 13q deletions (n=203)	<1 Mbp	1-5 Mbp	5-10 Mbp	10-20 Mbp	>20 Mbp	Coverage of *miR15/16*	Coverage of *RB*	Homozygous/ heterozygous deletions
Homozygous deletions (n=48)	25	21	1	0	1	40	5	NA
Frequency of homozygous deletions	52.1%	43.8%	2.1%	-	2.1%	83.3%	10%	NA
Heterozygous deletions encompassing homozygous regions (n=20)	1	10	1	3	5	20	12	NA
Frequency of heterozygous deletions encompassing homozygous regions	0.5%	50%	0.5%	15%	25%	100%	10.6%	NA
Heterozygous deletions (n=155)	33	87	11	11	13	147	59	NA
Frequency of heterozygous deletions	21.3%	56.1%	7.1%	7.1%	8.4%	94.8%	38%	NA
Deletion 13q according to the hierarchical model (n=175)	**<1 Mbp**	**1-5 Mbp**	**>5-10 Mbp**	**10-20 Mbp**	**>20 Mbp**	**Coverage of *miR15/16***	**Coverage of *RB***	**Homozygous/ Heterozygous deletions**
Small deletions according to ROC analysis (n=81)	48	33	NA	NA	NA	80	no	22/59
Frequency of small deletions according to ROC analysis	59.3%	40.7%	NA	NA	NA	98.8%	NA	27.2%/ 72.8%
Large deletions according to ROC analysis (n=94)	NA	65	9	10	10	85	56	21/73
Frequency of large deletions according to ROC analysis	NA	69.1%	9.6%	10.6%	10.6%	90.4%	59.6%	22.3%/ 77.7%

The upper panel describes the size distribution and coverage of miR15a/16-1 *and* RB *in homozygous deletions, heterozygous deletions encompassing the homozygous losses as well as in the continuous heterozygous deletion of 13q. The lower panel describes the size distribution, coverage of* miR15a/16-1 *and* RB *and frequency of homozygous and heterozygous deletions of small (<1.25 Mbp) and large sole 13q deletions (>1.25 Mbp) as defined by ROC analysis. The 33 small deletions that were between 1-5 Mbp ranged in size from 1 to 1.25 Mbp, whereas the 65 large deletions that were between 1-5 Mbp ranged in size from 1 to 25.5 Mbp).*

Online Supplementary Table S3. Evaluation of clonal evolution in follow-up samples.

Patient	Binet stage at diagnosis	First-line treatment	Second-line treatment	Time from 1st to 2nd sampling (months)	CNA at diagnosis	CNA at follow up, clonal evolution
				IGHV mutated and untreated (n=10)		
SCAN23	A	No	No	96	No	No CE
SCAN77	A	No	No	92	No	No CE
SCAN175	A	No	No	80	No	No CE
SCAN379	A	No	No	64	No	No CE
SCAN61	A	No	No	97	del(13)(q14.2q14.3)	Same, no CE
SCAN123	A	No	No	88	del(13)(q14.3)	Same, no CE
SCAN354	A	No	No	70	del(13)(q14.1q14.3)	Same, no CE
SCAN410	A	No	No	60	homozygous del(13)(q14)	Same, no CE
SCAN251	A	No	No	72	amp(3)(q26.2q29) del(13)(q14.2q14.3)	Same, no CE
SCAN299	A	No	No	73	del(4)(p15.2p16.3) amp(4)(p12p15.1) del(8)(p11.2p23.3)	Same, no CE
				IGHV mutated and treated (n=25)		
SCAN262	A	Alk+Ac	No	82	No	No CE
SCAN327	A	Alk+Ster	No	74	No	No CE
SCAN365	B	PA	No	72	No	No CE
SCAN389	A	Alk,	PA	92	No	No CE
SCAN589	C	PA	Alk	73	No	No CE
SCAN24	B	Alk	Alk	100	del(13)(q14.1q14.3)	Same, no CE
SCAN107	A	Alk	PA	108	del(13)(q14.3)	Same, no CE
SCAN132	A	PA+Alk	No	85	del(13)(q14.3)	Same, no CE
SCAN172	A	PA+Alk	No	87	del(13)(q14.3)	Same, no CE
SCAN396	C	Alk,	Alk	83	del(13)(q14.3)	Same, no CE
SCAN385	NA	Alk	Alk	93	del(13)(q14.1q21.3)	Same, no CE
SCAN624	A	Alk	No	88	del(13)(q14.3)	Same, no CE
SCAN158	A	Alk	No	87	homozygous del(13)(q14.3)	Same, no CE
SCAN616	A	PA +Alk		79	homozygous del(13)(q14.2q14.3)	Same, no CE
SCAN160	A	Alk+Ster	Alk+ster	90	heterozygous del(13)(q13.1q21.1) homozygous del(13)(q14.3)	Same, no CE
SCAN229	A	Alk	No	97	heterozygous del(13)(q14.2q33.3) homozygous del(13)(q14.3)	Same, no CE
SCAN341	A	Alk	No	73	trisomy 12	Same, no CE
SCAN362	A	Ster	No	69	amp(8)(p11.1p11.2)	Same, no CE
SCAN309	A	Alk	PA	77	del(9)(p24.1p24.3) del(13)(q14.1q14.3)	Same, no CE
SCAN44	A	Alk+Ster	Alk	93	del(5)(q21.1) amp(7)(q11.2) del(13)(q14.3)	Same, no CE
SCAN404	B	Alk	No	74	del(13)(q14.3) trisomy 12 trisomy 18 trisomy 19	Same, no CE
SCAN171	A	Alk	Alk	87	No	CE: del(13)(q14.3)
SCAN174	A	Alk	Alk	87	No	CE: del(13)(q14.2q14.3)
SCAN126	A	PA+Alk	Alk	91	del(13)(q14.1q14.3)	del(13)(q14.12q14.3) CE: del(17)(p13.3q11.1)
SCAN260	A	Alk	PA	82	del(13)(q14.3) del(11)(q22.3q24.1)	del(13)(q14.3) CE: normal chr 11
				IGHV unmutated and untreated (n=4)		
SCAN288	A	No	No	76	No	No CE
SCAN398	A	No	No	76	del(13)(q14.3) amp(18)(q12.1q23)	Same, no CE
SCAN162	A	No	No	77	del(13)(q14.2q14.3) del(11)(q13.5q24.1) del(6)(p22.2) del(6)(q25.2q25.3)	Same, no CE

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SCAN392	A	No	No	81	heterozygous del(13)(q14.3)	homozygous del (13)(q14.3) CE: heterozygous del(13)(q13.3q14.3)
				***IGHV* unmutated and treated (n=20)**		
SCAN304	A	Alk	Alk	77	No	No CE
SCAN45	A	Alk	Alk, Alk+Ac	72	del(13)(q14.2q14.3)	Same, no CE
SCAN278	A	PA	No	70	del(13)(q14.3)	Same, no CE
SCAN321	C	PA	PA+Alk	77	del(13)(q14.3)	Same, no CE
SCAN568	A	PA+Alk	PA+Alk	84	del(13)(q14.3)	Same, no CE
SCAN224	NA	NA	NA	96	heterozygous del(13)(q13.3q21.3) homozygous del(13)(q14.3)	Same, no CE
SCAN32	A	Alk+Ac	NA	75	trisomy 7 del(13)(q14.3)	Same, no CE
SCAN600	B	Alk	PA+Alk	78	del (7)(q22.2 del (7)(q32.2) del (8)(p22p23.1)	Same, no CE
SCAN573	B	PA+Alk	PA+Alk+Ab	96	del(11)(q14.1q23.3) del(13)(q13.2q21.3)	Same, no CE
SCAN582	A	PA+Alk	PA+Alk	72	del(11)(q22.3q23.3) del(13)(q14.2q14.3)	Same, No CE
SCAN382	B	PA	Ac	97	del(11)(q13.5q23.2) del(13)(q14.1q21.3) del(10)(q21.1)	Same, No CE
SCAN406	B	PA	Alk+Ac+Ab	62	amp(2)(p11.2p25.3) del(8)(3p11.1p23.) amp(8)(q11.1q24.3) del(11)(q14.1q23.3)	Same, No CE
SCAN290	B	Alk	No	78	trisomy 12 del(14)(q23.3q32.3) amp(21)(q11.1q22.3)	Same, No CE
SCAN146	A	Alk	Alk+Ac	77	No	CE: del(11)(q21q24.2)
SCAN75	A	Alk	Alk	96	No	CE: del(6)(q16.1q22.3) CE: del(8)(p11.2p23.3) CE: amp(8)(q12.2) CE: del(9)(q31.1q32) CE: del(13)(q14.2q14.3) CE: del(17)(p12p13.3) CE: del(18)(p11.2p11.3)
SCAN3	A	Alk	Alk	86	heterozygous del(11)(q14.1q23.3) homozygous del(11)(q21q23.3)	CE: heterozygous del(11)(q14.1q21)
SCAN279	A	Alk	Alk	81	del(11)(q14.1q23.3)	del(11)(q14.1q23.3) CE: del(13)(q14.1q21.1)
SCAN182	A	Alk	Ab	84	del(15)(q15.1)	del(15)(q15.1) CE: del(10)(q23.3q26.1) CE: del(20)(q13.1)
SCAN204	C	Ac	PA	80	del(7)(p11.1p12.1) del(13)(q14.3)	del(7)(p11.1p12.1) del(13)(q14.3) CE: del(9)(p21.3)
SCAN6	A	Alk	Alk	83	amp(2)(p11.2p25.3) del(11)(q13.5q23.3) del(20)(p13q11.1)	amp(2)(p11.2p25.3) del(11)(q13.5q23.3) del(20)(p13q11.1) CE: del(13)(q14.1q21.3)

Patients are grouped according to IGHV *mutation/treatment status and listed according to the presence of CNA at diagnosis and during follow-up. Treatment indicates the type of treatment (Alk= alkylating agents, PA= purine analog, Ac= anthracycline, Ab=antibodies, Ster= steroids). Treatment is indicated for first and second lines of treatment. Acquired aberrations during follow-up are preceeded by clonal evolution (CE) as in clonal evolution.*