CLLU1, DMD, GLO1, HCSL1, KIAA0977, LPL, MGC9913, PCDH9, PEG10, SEPT10, TCF7, TCL1, TP53, VIM, ZAP70, ZNF2) were determined by real-time quantitative RT-PCR (RQ-PCR) in CD19-purified as well as unpurified patients samples. Their predictive value regarding individual genetic subgroups, an integrated genetic risk model, treatment free (TFS) and overall survival times (OS) was investigated in 151 CD19 purified patients samples including multivariate analyses. In a subset of 55 cases ZAP-70 expression was determined by FACS for comparison with RQ-PCR data. *Results.* The strongest association with the VH mutation status was observed for LPL (correct prediction of 84% of cases) and ZAP70 (84%), followed by TCF7 (74%), a marker being overexpressed in VH mutated CLL. Additional significant associations were observed between the 11q- subgroup and ATM (downregulation), and between the 17p- subgroup and TCL1, TCF7, ZNF2, and ADAM29 (all downregulated). Patients at genetic risk (VH unmutated or V3-21 usage or 11q- or 17p-) were best assigned by ZAP70, whereby a determination by RQ-PCR yielded better results compared to the FACS method. When assessing a hierarchical risk model integrating the relevant genetic subgroups (risk 17p- > 11q- > VH unmutated or V3-21 usage > VH mutated) high misclassification rates occurred with any individual marker (42% for ZAP70), which was mainly due to the impossibility of separating cases with 11q- and 17p- from VH unmutated cases without these abnormalities. This was improved using a marker combination (misclassification rate 30% for a combination of LPL, TCF7, ZAP70, ZNF2, and ATM). Still, a discrimination of 11q- or 17p- from VH unmutated patients without these abnormalities was achieved in only approx. 50% of 11q- and 17p- cases. In multivariate analyses including all candidate genes, LPL was the strongest OS predictor, whereas TFS was best predicted by ZAP70 and TCF7. When genetic factors were included in multivariate OS and TFS analyses, the surrogate markers lost their independent prognostic significance. *Conclusions.* Screening for patients at genetic risk can be performed using the individual markers ZAP70 or LPL. Usage of a marker combination reduces misclassifications especially regarding the highest risk groups 11q- and 17p-. However, a reliable distinction of these risk groups is not achieved and the prognostic impact of the surrogate markers remains inferior compared to the established genetic factors.

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A DISTINCTIVE TRASCRIPTIONAL PROFILE CHARACTERIZES THE CHROMOSOME 17P LOSS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Distinct genetic abnormalities such as TP53 deletion at 17p13.1 have been identified as having an adverse prognostic relevance in B-cell chronic lymphocytic leukemia (B-CLL). Conventional cytogenetic studies have shown that TP53 deletion in B-CLL is associated predominantly with 17p loss (17p-) resulting from different complex chromosomal rearrangements such as unbalanced translocations or isochromosome 17q formation. *Aims*. The purpose of this study was to characterize a deletion-mapping of chromosome 17p in a subset of 17p- B-CLLs using genome-wide DNA profiling and Fluorescence in-situ hybridization (FISH) analyses. Additionally, gene expression profiling analysis was performed to identify specific transcriptional patterns or altered molecular pathways associated with 17p aberrations, which may have biological and clinical relevance in 17p- B-CLL. *Methods*. A panel of 71 untreated Binet A B-CLLs (18 of which carrying a TP53 monoallelic deletion) was characterized for the most recurrent genomic aberrations (trisomy 12 and deletions of 13q14, 11q22.3 and 17p13) and for the major prognostic markers. The genomic profile of chromosome 17p was investigated with GeneChip® Human Mapping 50K Xba 240 arrays in 12/18 17p- B-CLLs. Inferred copy numbers were derived from a Hidden Markov Model (HMM) based algorithm implemented in CNAT 4.0.1 software (Affymetrix). FISH probes covering a region of approximately 6 Mb in 17p11.2-p12 was selected to validate the array results. The transcriptional profiles of the 60 B-CLLs (7 carrying 17p-) have been generated on Affymetrix GeneChip® U133A arrays. The identified transcriptional fingerprints of the 17p- cases was validated on an independent dataset of 100 B-CLL cases (Haslinger *et al.*, 2004) using a Multi-class Prediction Analysis. Polymerase chain reaction was used to define the mutational status of the TP53. *Results*. Genome-wide DNA analysis of TP53 -deleted samples showed 17p loss in 11/12 cases, with distinct deletion patterns scattered along the 17p11.2-p12 region. FISH analysis confirmed these findings and revealed 17p loss in a small fraction of leukemic cells in the remaining TP53 -deleted case. In addition, FISH indicated 17p loss in the 6/18 cases not investigated by SNP. Mutations in exons 5 to 9 of the remaining TP53 allele were found in 9/12 deleted samples. Gene expression profiling of 60 B-CLLs, including 7 patients with 17p loss, identified 40 differentially expressed genes in 17p- *vs* 17p normal samples, 35 of which were down-regulated in 17p- tumors: the majority (30/35) of these transcripts, including putative tumor suppressor genes (GABARAP, GPS2 and OVCA1) mapped to 17p, indicating a remarkable gene dosage effect. *Conclusions*. Our study confirms and extends previous observations indicating that TP53 deletion in B-CLL patients is associated with the loss of chromosome 17p. Gene expression profiling showed that chromosome 17p loss is associated with significant down-regulation of genes located on 17p, indicating a gene-dosage effect. The potential coordinated loss of tumor suppressor genes on 17p other than TP53 , may represent an important mechanism for the negative clinical outcome associated with this lesion in B-CLL and warrants further investigation.

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INTEGRATIVE GENOMIC ANALYSIS OF TRISOMY 12 ABNORMALITY IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. B-cell chronic lymphocytic leukemia (B-CLL) is a genetically heterogeneous disease with a high variable clinical course. Trisomy 12 (+12) is frequently associated with the disease (approximately 20%) and it has been reported to be correlated with an intermediate prognosis. Despite the recent remarkable progresses in the understanding the molecular pathogenesis of B-CLL, the biological significance of trisomy 12 remains to be fully elucidated. *Aims.* To identify signalling pathways and additional lesions associated with tumorigenesis in +12 B-CLLs. Methods. A panel of 80 Binet A untreated B-CLLs including 18 patients with trisomy 12 was investigated. This series was characterized for the most recurrent genomic aberrations (deletions of 13q14, 11q22.3 and 17p13) and for the major prognostic markers. Gene expression profiles were generated on Affymetrix GeneChip® U133A arrays. The identified transcriptional fingerprint of +12 was generated and then validated on an independent dataset of B-CLL cases (Haslinger *et al.*, 2004) by a Multi -class Prediction Analysis. Furthermore, genome-wide profiling data were generated by means of Affymetrix GeneChip® Human Mapping 250K Nsp SNP arrays in a subset of 45/80 B-CLLs including 9 patients with +12. Inferred copy numbers were derived from a Hidden Markov Model (HMM) based algorithm implemented in CNAT 4.0.1 software (Affymetrix). Results. The transcriptional analysis revealed 140 genes as the best classifier for the +12 B-CLLs. 92 out of the 118 genes up-regulated in +12 patients mapped on chromosome 12, most of which (86%) on the long arm. The remaining 26 genes and the 22 genes down-regulated in +12 samples showed different chromosomal localizations. The transcriptional fingerprint validation on an independent cohort of 100 B-CLL patients showed a global classification rate of 92.5%. A functional analysis of the deregulated genes revealed the involvement in transcriptional regulation, DNA, mRNA and protein metabolism. Many genes (ANAPC5, CCT2, CCT4, CDK2AP1, CDKN1B, CHFR, MCRS1, PCTK2) were also related to regulation of cell-cycle, cell death (ATXN1, CD63, DNM1L, DYRK2, HRK, OPTN, TEGT) and immune response (CKLF, CD58, CTLA4, HDAC9, IL21R) as well as to different cellular metabolic processes (AMPD3, CS, LDHB, NUDT4). Consistent with FISH data,

genome-wide DNA analysis showed that +12 is never found to be associated with the other most recurrent aberrations of B-CLL. Moreover, SNPs analysis identified, among the +12 patients, other recurrent copy number variations, such as gains of 14q32, 15q11, 17q21 and losses of 11p15, 14q11, 14q32 and 15q11: however, this correlation did not reach a statistically significant level. Conclusions. Trisomy 12 appears to affect gene expression in B-CLL not only by a dosage effect but also by influencing the expression of genes located on different chromosomes leading to the deregulation of multiple cellular functions. None of the copy number alterations identified by SNP genomic analysis appeared to be tightly correlated with +12.

0535

ANALYSIS OF TP53 MUTATION IN A LARGE COHORT OF CLL PATIENTS BEFORE FIRST-LINE TREATMENT: ANALYSIS OF THE GENETIC PROFILE WITHIN THE CLL4 TRIAL (F *vs* FC) OF THE GCLLSG

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CLL patients with 17p deletion have a dismal prognosis after treatment. The exact prognostic role of TP53 mutations in the absence of 17p deletion and any differential impact of the mutation in cases with 17p deletion (*vs* sole deletion) is currently unclear. *Aims.* To assess the incidence, profile and prognostic impact of TP53 mutations as assessed by sensitive DHPLC followed by direct sequencing in a well characterized and homogeneous patient population from a prospective trial (CLL4 trial GCLLSG). *Methods.* We studied 342 of 375 CLL4 trial patients with available material. The population is well characterized and detailed genetic characteristics are available (FISH, VH-Status). We used DHPLC to detect TP53 mutations in the coding exons (2-11). Aberrant profiles were confirmed by sequencing including the use of fragment collection in cases with low grade mutations. *Results.* We found an overall incidence of TP53 mutations of 8.2 % (28/342 patients). The mutations were exclusively located in the DNA binding domain. We observed 2 splice site mutations, 3 deletions, 2 insertions but the majority of mutations were missense mutations in exons 5-8. Two patients had 2 different mutations. Fourteen of 16 patients with 17p deletions also had a TP53 mutation (87.5 %). Interestingly, both patients with a 17p deletion where no TP53 mutation was identified, showed a low proportion of 17p- cells (19-21%) suggesting that detection limits of the technique might explain this finding. We found TP53 mutations in the absence of 17p deletions in 4.3% (14/326). The proportion of mutated allele in cases without 17p deletion ranged from 10 to 90 percent. In two patients with follow-up samples we found evidence of clonal evolution at the time of relapse. The genetic profile of cases with TP53 mutation showed a high incidence of 17p deletions (13/28 cases), but also included cases with 11q deletion 3/28, 13 deletion as the sole abnormality (5/28) and normal karyotype (4/28). The majority of cases with TP53 mutations had an unmutated VH status (n=21)(75%), while 6 of 28 had a mutated VH status (V3-21: n=1). Correlation with baseline parameters and clinical outcome is currently being performed and will be presented. *Conclusions*. In this first line treatment trial population, TP53 mutations without 17p deletion occurred in 4.3% (14/326) of patients and the majority of cases with 17p deletion also have TP53 mutations. The demonstration of clonal evolution in cases with TP53 mutation without 17p deletion after F-based therapy points to the biological and clinical significance of TP53 mutations in CLL.

0536

SCREENING FOR COPY-NUMBER ALTERATIONS AND LOSS-OF-HETEROZYGOSITY IN CHRONIC LYMPHOCYTIC LEUKEMIA - A COMPARATIVE STUDY OF FOUR DIFFERENTLY DESIGNED, HIGH RESOLUTION MICROARRAY PLATFORMS

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Background and Aims. Screening for gene copy-number alterations (CNAs) has improved by applying genome-wide microarrays, where SNP-arrays also allow analysis of loss-of-heterozygozity (LOH). Few comparative studies of high-resolution platforms have thus far been performed and there is a current need to compare platforms in order to understand the pros and cons with the differently designed arrays. Here we investigated chronic lymphocytic leukemia (CLL) samples by applying four differently designed microarrays for evaluation of: 1) baseline variation and ratio response to FISH-validated genomic aberrations, 2) detection of known recurrent and novel CNAs and concordance between platforms, and 3) detection of LOH with the Illumina and Affymetrix SNP-arrays. *Materials and Methods*. We screened 10 CLL samples using four different high-resolution platforms: BAC-arrays (32K), oligonucleotide-arrays (185K, Agilent), and two SNP-arrays (250K, Affymetrix and 317K, Illumina). Baseline variation and copy-number ratio response was calculated on normalized array-data. Analysis of CNAs and LOH was performed applying the Bio Array Software Environment (BASE) and dChip software, respectively. *Results*. Evaluation of baseline variation and copy-number ratio response showed the best performance for the Agilent platform and confirmed the robustness of BAC-arrays. Accordingly, these platforms demonstrated a higher degree of platform-specific CNAs. The SNP-arrays displayed higher technical variation, although this was compensated by high density of elements. Cross-platform comparison revealed 29 concordantly detected CNAs, including FISH validated known recurrent alterations, which confirmed that all platforms are powerful tools when screening for large aberrations. However, detection of 32 additional regions present in 2-3 platforms illustrated a discrepancy in detection of small CNAs, which often involved reported copy-number variations. LOH-analysis using dChip revealed concordance of mainly large regions, but showed numerous, small non-overlapping regions and LOH escaping detection. Affymetrix detected a higher degree of CNAs compared to Illumina, while the latter showed a lower noise level and higher detection rate in the LOH analysis. *Conclusions*. Usage of high resolution microarrays will improve the possibility to detect new recurrent microevents in CLL leading to identification of new important subgroups potentially refining the prognostic hierarchy established by FISH. If only copy-number data is preferred, oligonucleotide-arrays such as Agilent provide a high sensitivity of this type of analysis. If LOH-analysis is desirable, SNP-arrays are the preferred choice but, parallel improvement of analysis tools is required for an in-depth analysis of allelic imbalances.