patients) could be successfully identified either by RT-PCR or by direct genomic PCR. This controversial data provoked us to investigate 'MLL•AF4+/AF4•MLL-' leukemia patients in more detail by using a LDI-PCR based method. This allows to identify and characterize chromosomal aberrations of the human MLL gene in an unbiased fashion. 13 individual MLL•AF4+/AF4•MLL- leukemia patients out of 76 t(4;11) leukemia patients were indentified (63 were MLL•AF4+/AF4•MLL+ leukemia patients). The 13 MLL•AF4+/AF4•MLL- leukemia patients were analyzed for the presence of rearranged genomic MLL sequences. 10 patients displayed a complex rearrangement between chromosome 4 and 11 (and sometimes a third chromosome) that involved at least the MLL, the AF4 and a third partner. Funded by grant 2002.061.1 from the Wilhelm Sander Foundation to R.M., T.K. and T.D.

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FISH-MLL ABNORMALITIES IN PATIENTS WITH ACUTE MYELOBLASTIC LEUKEMIA AND ASSOCIATION WITH FLT3 AND MLL INTERNAL DUPLICATION

E. Giugliano,[1] G. Rege-Cambrin,[1] P. Scaravaglio,[1] D. Diverio,[2] A. Serra,[1] M. Mancini,[2] V. Brunetto,[1] G. Ferranti,[2] G. Mattioli,[1] C. Fava,[1] M. Fimognari,[1] S. Ulisciani,[1] F. Pane,[3] F. Lo Coco,[4] G. Saglio[1]

[1]University of Torino, ORBASSANO, TORINO, Italy; [2]La Sapienza University, ROMA, Italy; [3]University of Napoli, NAPOLI, Italy; [4]Tor Vergata University, ROMA, Italy

Background. The MLL gene on chromosome 11q23 is frequently involved in haematological malignances. It is possible to subdivide the MLL abnormalities in two groups: 1) rearrangements, usually as translocations or insertions, and partial tandem duplication (PTD); 2) amplification of the 11q23 region, leading to the presence of multiple copies of the MLL gene, located either intrachromosomally, as hsr and iso11q, or extrachromosomally in dmin and numerical abnormalities of chromosome 11. MLL/PTD is the in-frame fusion of a duplicated portion of the MLL gene. Internal tandem duplication (ITD) or mutations have been demonstrated as a activating mechanism also in another oncogene involved in AML, FLT3 gene, which encodes for a tyrosine kinase receptor widely expressed in hemopoietic lineage. The FLT3/ITD is observed in approximately 20% of unselected *de novo* adult AMLs, with a higher frequency around 30-40% reported for patients with normal cytogenetics. It is associated with poor prognosis in most series. It has been reported that FLT3/ITD is more common in patients with MLL/PTD than in cases with MLL translocations. Recently, a role for coduplication of MLL and FLT3 genes has been suggested in AML as possible marker of a common genotoxic stress. *Aim*. We investigated the incidence of MLL abnormalities in 207 patients with *de novo* acute myeloid leukemia, diagnosed following FAB criteria and treated according to the GIMEMA protocols. We used conventional cytogenetics and fluorescent *in situ* hybridization (FISH) analysis with a MLL probe. The patients were also tested for the presence of an internal duplication of the MLL and FLT3 gene and for the FLT3 D835 mutation. *Methods and Results.* Cytogenetic analysis on bone marrow was successful in 175 cases and showed abnormalities of chromosome 11 in 12 patients (6.9%). FISH analysis performed with MLL Dual-Color probe (Vysis) was available in 194 cases and demonstrated the MLL involvement in 25 cases (12.9%). Ten patients were rearranged (5.2%); 15 cases showed overrepresentation of MLL gene without evidence of rearrangement (7.7%). FLT3/ITD or D835 mutation were observed in 27.4% and MLL/PTD in 5.3% of the patients. FLT3 abnormalities were present in 20% (5/25) whereas MLL/PTD was observed in 18.7% (3/16) of the cases with involvement of MLL at FISH analysis. *Conclusions.* The FISH investigation of MLL contributes to the identification of multiple copies of the gene in marker chromosomes, rings, double minutes, hsr. The presence of MLL amplification is not rare in *de novo* AML and the FISH analysis allows to improve the characterization of MLL involvement when compared with conventional cytogenetics. The incidence of FLT3 alterations is similar in MLL abnormal patients (20%) when compared to the whole AML population (27.4%); on the contrary, MLL/PTD is confirmed to be more frequent in patients with abnormalities of chromosome 11 (18.7% vs 5.3% of unselected AML). The rate of MLL/PTD was superior in FLT3 positive (7.7%) than in FLT3 negative patients (4.4%). In this study the coduplication of FLT3 and MLL/PTD had a low incidence around 2.3% in all cases and did not correlate with cytogenetic MLL abnormalities.

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A NEW CRYPTIC MOLECULAR LESION UNDERLIES 6P CHANGES IN SECONDARY ACUTE MYELOID LEUKEMIA/MYELODYSPLASTIC SYNDROME (AML/MDS)

R. La Starza,[1] A. Aventin,[2] C. Matteucci,[2] B. Crescenzi,[2] S. Romoli,[3] N. Testoni,[3] V. Pierini,[3] S. Ciolli,[4] C. Sambani,[5] A. Locasciulli,[6] M. Lafage-Pochitaloff,[7] M.F. Martelli,[3] P. Marynen,[8] C. Mecucci[3]

[1]Hematology, University of Perugia, PERUGIA, Italy; [2]Hospital Sant Pau, BARCELONA, Spain; [3]University of Perugia, PERUGIA, Italy; [4]University of Florence, FLORENCE, Italy; [5]Health Physics and Environmental Hygiene, NCSR 'DEMOKRITOS', ATHENS, Greece; [6]Hematology, S Camillo Hospital, ROME, Italy; [7]Lab de Biopathologie, Inserm U[119], MARSEILLE, France; [8]Center for Human Genetics and VIB, LEUVEN, Belgium

Background. Secondary AML/MDS are frequently associated with complex karyotypes involving chromosomes 3, 5, 7, 11, 12, 17, 18, and 21. Specific genetic pathways are related to physical and/or chemical toxics, such as -5/5q- to alkylating agents or 11q23 and 11p15 changes to topoisomerase II inhibitors. 6p aberrations are cytogenetically heterogeneous and often belong to complex karyotypes with del(5)(q)/-5 and/or del(7)(q)/-7. *Aim.* Molecular characterization of 6p rearrangements in secondary AML/MDS. *Methods.* We selected nine patients with secondary AML/MDS and one Fanconi Anemia patient with MDS with a rearrangement on the short arm of chromosome 6. Karyotypes of G-banded metaphases were described according to ISCN (1995). Metaphase FISH with a panel of 38 DNA clones for 6p12-p25 bands was performed in all cases. Multi-FISH, CGH and FISH with whole chromosome paints and/or centromeric probes were performed in selected cases. *Results.* 6p rearrangements were isolated in 4 patients and included in complex karyotypes in 6. Numerical or structural aberration typically associated with therapy-related AML/MDS, i.e. -5/5q-, -7/7q-, monosomy 18 were respectively found in four, three, and three patients. In three cases full or partial trisomy of the 6p arm was present: i(6)(q10) in one case and dup(6)(p) in two cases. The remaining 7 patients showed 6p unbalanced translocations with diverse chromosome partners or unidentified material. In 3 patients with unbalanced translocations, FISH detected cryptic duplications of a genomic region contiguous to the translocation breakpoints, at band p21, while in two patients a low copy gain with five copies of DNA clones mapping at band p21, were present on der(6) and/or inserted in other derivative chromosomes. In all cases a common over-represented 6p21 region was narrowed to a 5-6 megabase DNA segment extending from the TNF gene to ETV-7. Two patients did not show 6p21 gain. *Conclusion.* 6p21 gains, either as duplication/trisomy or low copy gain, emerged as a new recurrent genomic lesion in secondary AML/MDS with 6p abnormalities. Remarkably, they may be cryptic at conventional cytogenetics and underlie different types of chromosome changes. Putative candidate genes, such as the MHC complex, NOTCH-4, BAK, FANCE, ETV-7, HMGIY and FKBP51, map within the common over-represented 6p21 region. As duplications/low copy gains occurred in both treatment and environmentally induced AML/MDS as well as in the FA patient, toxic insults and congenital instability appear to share the same genetic pathway. *Acknowledgements.* BAC clones were kindly provided by Dr. M Rocchi, University of Bari, Italy.

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CYTOGENETIC AND FISH STUDY IN 203 B-CLL PATIENTS

M. Holzerova, H. Pospisilova, T. Papajik, R. Urbanova, Z. Pikalova, R. Plachy, K. Indrak, M. Jarosova

University Hospital, OLOMOUC, Czech Republic

Background. The progress in molecular genetic characterization of chronic lymphocytic leukemia (CLL) revealed the prognostic role of IgVH mutational status, of phenotypic changes involving expression of CD38 and ZAP-70, as well as, of chromosomal abnormalities defined by molecular cytogenetic *Methods.* Interphase fluorescence *in situ* hybridization (I-FISH) is able to detect the most common chromosomal abnormalities 13q, 11q, 17p deletions and trisomy 12. *Aims.* The aim of this study was to determine the chromosomal abnormalities in 203 CLL patients using cytogenetic and molecular cytogenetic methods, and to correlate the molecular cytogenetic findings with disease status (stable versus progressive), with immunoglobulin variable heavy chain (IgVH) mutational pattern, and with other clinical parameters. *Methods and patients*. 123 males and 80 females (median of age 62 years) were examined by con-

ventinonal cytogenetic examination on TPA stimulated cells from peripheral blood (167), bone marrow (34) and/or lymph node (2), and by I-FISH on fixed cells. The locus specific and centromeric probes were used (ABBOT-VYSIS) for I-FISH. CGH and M-FISH were used to detect chromosomal changes in patients with complex karyotype. *Results.* Cytogenetic analysis was successful in 127 patients and abnormal karyotype was detected in 35 (28%) patients. Using I-FISH we detected trisomy 12 in 17 (9%) out of 193 analyzed cases, deletion of ATM in 41 (20%) and deletion of p53 in 18 (9%) out of 203 examined cases. Deletion of RB1 was found in 66 (43%) out of 153 analyzed cases and two abnormal clones were revealed in 8 (12%) of them ' one with deletion of only one copy of RB1 gene and the other with deletion of both copies. Two chromosomal abnormalities were detected in 19 patients (9%) – deletion of RB1 together with deletion of ATM gene in 11 of them, and – deletions of RB1 gene together with deletion of p53 in 8 patients in this group. Three abnormalities deletions of ATM, RB1 and trisomy 12 were detected in only one patient. *Summary*. FISH is rapid and sensitive method for determination of chromosomal aberrations of prognostic relevance in CLL patients. The deletion of 13q14 was the most frequent (43%) chromosomal aberration detected by I-FISH in our cohort of CLL patients. The correlation of molecular cytogenetic results with IgVH mutational pattern and with clinical data were analyzed and will be presented.

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A MOLECULAR CYTOGENETIC STUDY OF MANTLE CELL LYMPHOMA AT DIAGNOSIS AND FOLLOW-UP: EVIDENCE FOR A 'TEMPORALLY ORDERED' CYTOGENETIC EVOLUTION?

S.I. Papadhimitriou,[1] A. Davea,[2] I. Savvidou,[2] G. Paterakis,[2] E. Goumakou,[2] E. Griva-Kritikou,[2] G. Androutsos,[2] D. Pavlidis,[2] T. Marinakis,[2] N.I. Anagnostopoulos,[2] M. Georgiakaki[2]

[1]'G. Gennimatas' Athens Regional General, ATHENS, Greece; [2]'G. Gennimatas' Athens Regional General, ATHENS, Greece

Background. Apart from t(11;14)(q13;q32), MCL is also characterized by other nonrandom cytogenetic findings. These additional aberrations are well studied at diagnosis and believed to represent clonal evolution during lymphomagenesis, but little is known about karyotypic changes during the course of the disease. *Methods.* The study included 33 patients with MCL. In all cases, an interphase FISH assay was performed at diagnosis on lymphoma cells from peripheral blood, bone marrow or touch imprints of involved sites. Commercial probes were employed for the detection of t(11;14), +12, 13q-, and abnormalities of ATM, p53, p16, TEL, c-MYC and BCL6 genes. In 14 cases, the same FISH screening was repeated at least once (up to four times) during the course of the disease, at relapse or in the context of partial or no response. *Results.* The most frequent additional findings at diagnosis were ATM deletion in 15 cases (45.5%) and 13q- in 12 cases (36.4%), followed by p16 deletion (3 cases; 1 homozygous), p53 deletion (2 cases), and +12, duplication of the CCND1/IGH fusion gene and BCL6 triplication, in one case each. 11 of the 14 cases studied at follow-up showed karyotypic evolution, with acquisition of p16 deletion (6 cases; 4 homozygous), TEL deletion (5 cases; 2 on the basis of monosomy 12), duplication of the CCND1/IGH fusion (3 cases), p53 deletion (2 cases), and c-MYC amplification (1 case). There was no case with acquisition of ATM deletion, 13q- or +12, but in two cases with 13q- in a minor subclone at diagnosis the aberration was estimated to involve the total of the lymphoma cells at relapse. Interestingly, new BCL6 aberrations were seen in 3 cases (triplication in one and amplification in the other two, including the case with gene triplication at diagnosis) and were detected at the third or the fourth repetition of the screening. The longest survival after detection of these aberrations was 3 months. *Conclusions.* Our data suggest that in most cases of MCL clonal evolution also occurs during the course of the disease, with the acquisition of multiple additional chromosomal lesions. Despite the small number of patients in our series, it seems that some of the aberrations (like ATM deletion or 13q-) are most commonly already present at diagnosis, while others (such as monosomy 12 and/or TEL deletion) appear more often or even exclusively on follow-up. From the clinical point of view, we found that the most informative finding is the overrepresentation of the BCL6 gene, apparently associated with aggressive behavior and perhaps the terminal stage of MCL.

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PROGNOSTIC SIGNIFICANCE OF COMPLEX CHROMOSOMAL REARRANGEMENTS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

L. Babicka,[1] Z. Zemanova,[1] L. Pavlistova,[1] J. Brezinova,[2] J. Moravcova,[2] H. Klamova,[2] K. Michalova[1]

[1]General Faculty Hospital and First *Med Fac, PRAGUE ,[2] Czech Republic; [2]Institute of Hematol. and Blood Transf., PRAGUE, Czech Republic*

Background. Ph chromosome i.e. transolaction t(9;22)(q34;q11) is specific chromosomal aberration in bone marrow cells of patients in chronic phase (CP) of CML. During progression of the disease from the chronic to the accelerated phase (AP) and/or blast crisis (BC), clonal evolution with non-random secondary numerical and structural aberrations is frequently observed. Complex chromosomal rearrangements (CCR) are rather rare and the significance and frequency of different anomalies are poorly understood. *Aims.* The aim of our study was a comprehensive analysis of complex chromosomal rearrangements found in bone marrow cells of 22 patients with CML by molecular cytogenetic methods, determination of chromosomes and chromosomal parts which are involved in CCR during progression of the disease and estimation of frequency of non-random changes if they exist. *Methods.* For the assessment of BCR/ABL fusion gene at the time of diagnosis RT-PCR and/or interphase FISH with locus-specific probe (Abbott-VysisTM) were used (200 interphase nuclei analyzed, cut-off level 2,5% tested on controls). In some patients further molecular analyses were performed by real-time RT-PCR according to EAC protocol using β-2-microglobuline as a control gene. Multicolor FISH (mFISH) was carried out using the '24XCyte' MetaSystems 24 color kit (MetaSystemsTM) to identify precisely complex chromosomal rearrangements in 22 patients. Most of the patients were in the CP at diagnosis. During the course of the disease clonal evolution with complex chromosomal rearrangements appeared in eight patients who remained in CP, two patients progressed to AP and the rest of them to BC. *Results.* The majority of the structural changes were unbalanced. Variant Ph translocations (involving chromosomes 9, 22 and one or more other chromosomes) were found in ten patients, the rest of the cohort had a classical Ph translocation associated with additional structural aberrations. The most frequent chromosomes involved into CCR were found to be Nos. 2 (6x), 7 and 17 (5x), 1, 3, 4 and 5 (4x). Chromosomal regions 1p, 2p, 5q, 7p and 17p were often involved in CCR and the bands repeatedly affected were 17p11.2 (3x) and 7p15 (2x). No one of complex translocation was seen more than once. *Conclusions.* The results of this study demonstrate the very high instability of the genome of malignant cells at the chromosomal level than was expected on the basis of classical cytogenetic analyses. We also proved that CCR are associated with rather poor prognosis and respond poorly to antileukemic treatment. Analysis of CCR by mFISH is important as we believe that such examinations of large cohorts of patients could confirm the significance and non-randomnes of this instability and to find out possible recurrent chromosomal aberrations specific for disease progression. Precise determination of breakpoints on chromosomes involved in CCR of bone marrow cells of CML patients can give new dimension to our understanding of genetic mechanisms which can play role in progression of malignant disease.

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TRANSLOCATION T(9;14)(P13;Q32) IN THREE CASES OF SPLENIC MARGINAL ZONE LYMPHOMA

C. Baró,[1] M. Salido,[1] B. Espinet,[1] A. Domingo,[2] I. Granada,[3] A. Carrió,[4] V. Romagosa,[2] F. Millà,[3] L.I. Colomo,[4] E. Campo,[4] S. Serrano,[1] F. Solé[1]

[1]Hospital del Mar, BARCELONA, Spain; [2]Hospital Universitari de Bellvitge, L'HOSPITALET DE LLOBREGAT, Spain; [3]Hospital Germans Trias i Pujol, BADALONA, Spain; [4]Hospital Clínic, BARCELONA, Spain

Background. Translocation t(9;14)(p13;q32) involving PAX-5 and IgH is an aberration that was first described in lymphoplasmacytic lymphoma (LPL). Nevertheless, new data suggest that t(9;14) is not restricted to a specific morphologic subtype and it is recurrent in other B-cell lymphomas (high-grade and low-grade). Moreover, chromosomal studies in splenic marginal zone lymphoma (SMZL) revealed a high incidence of deletions of 7q, gains of 3q and few incidence of translocations involving 14q32. Reviewing reported cases, only one SMZL patient with a complex translocation t(2;9;14)(p12;p13;q32) was previously referred. *Aims.* The aim of this study was to present the finding of t(9;14)(p13;q32) in SMZL patients (diagnosed by citology, immunophenotype and his-