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IDENTIFICATION OF A COMMONLY USED CDR3 REGION OF LGLS TCR ALPHABETA+/CD4+ VB 13.1 PATIENTS

P. Garrido,[1] A. Orfao,[2] J. Canton,[1] J. Almeida[2] M. Lima,[3] P. Barcena,[2] M.A. Lopez-Nevot,[1] F. Garrido,[1] F. Ruiz-Cabello[1]

[1]*Virgen de las Nieves Hospital, GRANADA, Spain;* [2]*Cancer Investigation Center, SALAMANCA, Spain;* [3]*Santo Antonio General Hospital, PORTO, Portugal*

Background. Monoclonal TCRαβ+/CD4+/NKa+/CD8-/+dim T represent a subgroup of monoclonal LGL lymphoproliferative disorders different from both CD8+ T-LGL and NK-cell type LGL leukemias.The recently described TCRαβ+/CD4+ T-LGL leukemia/lymphocytosis has been shown to be associated in around one third of cases with a neoplasia other than the T-LGL, which prompted us to hypothesize that the TCRαβ+/CD4+ T-LGL may proliferate and expand as an effort of the immune system to control tumor growth, supporting in some way the antigen-driven selection model. We typed for HLA class I and II genes in patients with different TCR- Vβ expansions. TCR clonotypes and VDJ rearrangement structure were analyzed in a cohort of patients with CD4T-LGL expansions. *Aims.* Analyse the possible association between the TCR Vβ family expanded with HLA and CDR3 hypervariable region expressed in patients with Vβ expansions LGG TCR αβ+/CD4+ /CD8±d. *Methods.* A total of 36 individuals (19 males and 17 females; mean age of 64±11 years, ranging from 40 to 81 years) having a TCRαβ+/CD4+/NKa+/CD8-/+dim monoclonal T-LGL lymphoproliferative disorder were studied. For the immunophenotypic studies a panel of 24 monoclonal antibodies (MAb) directed against an identical number of members of 21 different TCR-Vβ families was used. A genotyping for HLA-ABC and both HLA-DRB1 and HLA-DQB1 were performed by SSPO-PCR. DNA were amplified and clonal products from the VH gene PCR were sequenced directly using the BigDye Terminator Cycle Sequencing Reaction Kit. *Results.* In all cases studied, expanded CD4+ LGL T-cells showed relatively high SSC features as compared to normal PB CD4+ T-lymphocytes and common phenotypic characteristics, consisting of TCRαβ+/CD4+/CD8-/+dim cells with a typical cytotoxic (granzyme B+, CD56+, CD57+, CD11b±) activated/memory T-cell immunophenotype ($CD2+^{bright}$, CD7-/+d, $CD11a+^{bright}$, CD28-, CD62L-, HLA-DR+). Flow cytometric analysis of the TCR-Vβ repertoire of CD4+/CD8-/+dim LGL T-cells was consistent with a (mono)clonal expansion in all cases studied, which accounted for 75%±26% of all PB CD4+ T-cells. In 27 cases the expanded TCR-Vβ family was identified with the panel of TCR-Vβ reagents used, corresponding to TCR-Vβ13.1 in 15 cases (42%), TCR-Vβ 2.1 in 2 (5.6%), TCR Vβ 3.1 in 2 (5.6%), TCR-Vβ 8.1 and Vβ 8.2 in 2 (5.6%), TCR-Vβ17.1 in 2 (5.6%), TCR-Vβ 22 in 2 (5.6%) and TCR-Vβ11 or TCR-Vβ14.1 in one case each (2.8%). In the remaining 9 patients, the expanded TCR Vβ family was not identified (25%) with the panel of MAb used. All 15 patients who showed expansions of TCRVβ 13.1+ CD4+ T cells were HLA-DRB*0701+.Comparison of CDR3 size distribution in clonal CD4+/CD8-/+dimT-cells from the same patients showed a highly restricted usage of VHDJH segments and shared CDR3 configurations. *Conclusions.* These findings suggest that the expansions were selected for this unique TCR structure. These results strongly suggest that Vβ13.1 CD4+ T cells with the described CDR3 motif may recognize a specific antigen presented by DR7 molecules, indicating the existence of a common associated antigen.

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MDR1, MRP AND LRP EXPRESSION IN PATIENTS WITH UNTREATED ACUTE LEUKEMIA: CORRELATION WITH TC-99M MIBI BONE MARROW SCINTIGRAPHY

I. Ak, G. Demirel, Z. Gulbas

Eskisehir Osmangazi University, ESKISEHIR, Turkey

Background. Multidrug-resistance (MDR) phenotype concerns altered membrane transport that results in lower cell concentrations of cytotoxic drug in many cancer types, including leukemia and is related to the overexpression of a variety of proteins that act as ATP dependent extrusion pumps. Tc-99m Sestamibi (MIBI) is a transport substrate for Pgp pump. *Aim.*We assessed the bone marrow uptake of Tc-99m MIBI and its correlation with messenger RNA (mRNA) levels of MDR1, Multidrug-Resistance Associated protein (MRP) and Lung Resistance Protein (LRP) in acute leukaemia. *Methods.* A total of 26 patients with new diagnosed acute leukaemia (8 ALL and 18 ANLL) were included the in the study. The expression of MDR1, MRP, and LRP on mRNA levels were assessed by semi quantitative RT-PCR (Roche Light Cycler System, Metis Biotechnology primers and probes for MDR1, MRP and LRP) in the blast cells from the bone marrow samples. Planar images of the pelvis and thorax were acquired 20 min after injection of 740 MBq Tc-99m MIBI. The MIBI uptake in the bone marrow was evaluated using a quantitative scoring system with determination of the tumour-to-background ratios for the bone marrow in areas that included the proximal femur, anterior iliac crest and sternum. The correlation between the RT-PCR results and MIBI uptakes was analysed by using Spearman's rank correlation coefficients with two-tailed test of significance. *Results.* There was an inverse relationship between Tc-99m MIBI uptake of bone marrow and both mRNA levels of MDR1 and MRP (p=0.000, r= - 733 and p=0.001, r= - 610, respectively). No correlation was found between MIBI uptake and mRNA levels of LRP. Conclusion: Increased expression of MDR1 and MRP correlates with a low accumulation of Tc-99m MIBI in bone marrow areas in patients with acute leukaemia. As a functional imaging, Tc-99m MIBI bone marrow scintigraphy can identify the MDR1 and MRP phenotype, but not LRP, in patients with acute leukaemia.

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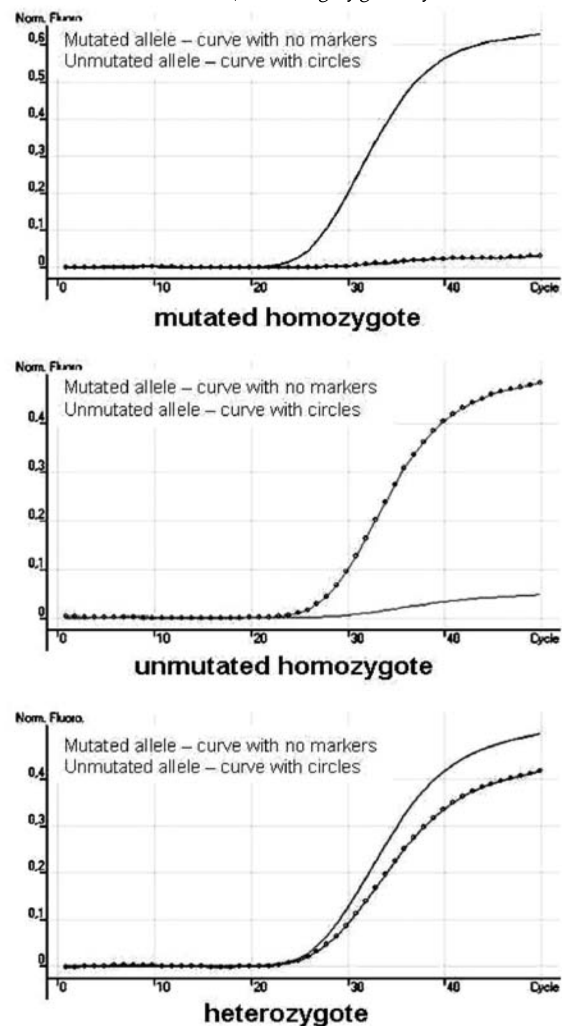
REAL-TIME RT-PCR ASSAY USING THE TAQMAN PROBES WITH LNA (LOCKED NUCLEIC ACID) MODIFICATION TO DETERMINE JAK2 GENE V617F MUTATIONS IN MYELOPROLIFERATIVE DISEASES (MPD)

J. Markovà,[1] J. Schwarz[2]

[1]*Institute of Hematology and Blood Transf, PRAGUE, Czech Republic;* [2]*Institute of Hematology & Blood Transfusion, PRAGUE* [2]*, Czech Republic*

Background. JAK2 V617F mutations are frequently found in MPDs. JAK2 mutations confirm clonality in MPD, and according to some authors, they may be relevant even prognostically. In the near future, therapy by JAK2 inhibitors may be foreseen. The *classical* method of detection of JAK2 V617F mutations published by Baxter *et al.*[1] takes advantage of mutation-specific primers in PCR and requires sequencing to distinguish between homo- and heterozygous mutations. *Aims.* We have developed a more straightforward, real-time RT-PCR method, to demonstrate JAK2 mutations, allowing zygosicity discrimination.



Methods. Peripheral blood granulocytes were separated from altogether 151 patients with already diagnosed or suspected Ph- MPD. Patients with polycythaemia vera (PV), secondary polyglobulia (SP), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF) and undifferentiated MPD (MPD-U) were included in the study. The cells were lyzed and RNA extracted using the Trizol reagent. Following reverse transcription, two methods were employed to detect JAK2 mutations. 1) The method according to Baxter *et al.*,[1] using two forward primers, one of them hybridizing to the mutated allele and a common reverse primer recognizing both the mutated and unmutated JAK2 alleles. Homo- and heterozygosicity of the mutated gene was discriminated by sequencing analysis. 2) The allelic discrimination real-time RT-PCR assay that uses one pair of primers and two dual labeled TaqMan probes with LNA modified nucleotides. The probes differ at the polymorphic site, one of them is complementary to the wild-type JAK2 allele and the other to the mutated one. The result is given by the curves arising from measured fluorescence of two different reporter dyes during the real-time PCR (FigURE 1).*Results.* Altogether 151 samples of patients with suspected Ph-MPD were analyzed using both of the above mentioned methods for JAK2 detection. In both of the assays, the same result was obtained, JAK2 mutation being found in the same 71 out of 151 patients (47.0%). Ten of the 71 JAK2 mutations (14.1%) were homozygous, half of which were found in PV patients. In ET, JAK2 mutations were demonstrated in 22/57 (38.6%) patients, none of them was homozygous. Of 43 patients with PV, 33 had mutations (76.7%), whereas only 1/10 patients with SP had the mutated allele of JAK2 gene. Six of 20 (30.0%) individuals with IMF had JAK2 mutations (3 were homozygous). In the remaining 21 MPD-U patients, 9 mutations (42.9%) were detected. *Conclusions.* The TaqMan allelic discrimination assay yields the same results as the method of Baxter *et al.*[1] In contrast to the latter, it is very simple and does not require sequencing to distinguish between homo- and heterozygotes. Thus it is less laborious and time-consuming and therefore also suitable for routine clinical laboratory testing.

References

1. Baxter EJ et al. Lancet 365, 1054-61, 2005.

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HIGH FREQUENCY OF AML1 MUTATIONS IN BOTH *DE NOVO* MYELODYSPLASTIC SYNDROME AND CHRONIC MYELOMONOCYTIC LEUKEMIA BUT WITH DIFFERENT MUTATION PATTERNS

L.Y. Shih,[1] D.C. Liang,[2] C.F. Huang,[1] J.H. Wu,[1] Y.S. Shih,[2] P.N. Wang,[1] M.C. Kuo,[1] P. Dunn,[1] T.L. Lin,[1] T.C. Tang,[1] H. Chang[1]

[1]*Chang Gung Memorial Hospital, TAIPEI, Taiwan;* [2]*Mackay Memorial Hospital, TAIPEI, Taiwan*

Background. Transcription factor AML1 is essential for normal hematopoiesis. AML1 mutations have been found in therapy-related myelodysplastic syndrome (MDS) but were rarely described in patients with *de novo* MDS or chronic myelomonocytic leukemia (CMML). *Aims.* We sought to determine the frequency and patterns of AML1 mutations in *de novo* MDS and CMML and to correlate the mutation status with the clinicohematologic features. *Methods.* Mutation analysis of AML1 was performed on bone marrow samples from 76 patients with MDS (11 RCMD, 31 RAEB1 and 34 RAEB2) and 67 patients with CMML by direct sequencing for all RT-PCR products amplified with 3 overlapping primer pairs which cover the coding sequences of AML1 gene from exon 3 through exon 8. *Results.* At initial presentation of MDS, 14 of 76 MDS patients (18.4%) had AML1 mutations; 3 mutations were located in Runt homologogy domain (RHD) (exons 3-5) whereas 11 mutations were located in the non-RHD region (exons 6-8). The 14 AML1 mutations included 6 missense mutations, 4 nonsense mutations, 2 frameshift mutations, and 2 silent mutations. AML1 mutations were detected in 27 of 67 CMML patients (40%) at initial diagnosis, 17 patients had 19 mutations located in RHD and 10 patients had mutations located in the non-RHD region; the patterns of 29 mutations consisted of 7 missense mutations, 5 nonsense mutations, 14 frameshift mutations and 3 silent mutations. One CMML patient had two missense mutations in RHD, another patient had two frameshift mutations in RHD. Cloning analysis showed that the two mutations were on different alleles in both patients. The frequency of AML1 mutations was significantly higher in patients with CMML than in MDS (p=0.005). Mutations in RHD occurred more frequently in CMML than in MDS (p=0.020). CMML patients had a higher frequency of frameshift mutations as compared with MDS patients (p=0.045). AML1$^+$ CMML patients had a significantly lower platelet count than AML1$^-$ patients (p=0.025). There were no differences in age, sex, hemoglobin level, WBC count, percentages of blasts in bone marrow and peripheral blood, morphologic subtype, and cytogenetic risk group between AML1$^+$ and AML1$^-$ patients in CMML or MDS. Eleven of 14 AML1$^+$ MDS patients (78.6%) progressed to AML compared with 39 of 62 AML1$^-$ patients (62.9%) (p=0.357). Eleven of 27 AML1$^+$ CMML patients (40.7%) progressed to AML compared to 13 of 40 AML1$^-$ patients (32.5%) (p=0.605). Time to AML transformation and overall survival of AML1$^+$ patients did not differ from AML1$^-$ patients in both MDS and CMML groups. *Conclusions.* Our study showed that AML1 mutations were frequently detected in *de novo* MDS and CMML, especially the latter. Patients with CMML were more frequently associated with mutations in RHD and frameshift patterns compared to patients with *de novo* MDS.

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NPM1 AND FLT3 MUTATIONS, DUPLICATIONS OF MLL AND EXPRESSION OF GENES WT1, EVI AND BAALC AS PROGNOSTIC FACTORS IN PATIENTS WITH *DE NOVO* ACUTE MYELOID LEUKEMIA

S. Ballester,[1] E. Barragán,[1] J. Cervera,[1] P. Bolufer,[1] E. Afan de Ribera,[1] P. Fernandez,[2] R. Andreu,[3] R. Collado,[2] G. Martìn,[1] P. Montesinos,[1] J.C. Pajuello,[1] M. Collado,[1] M. Maiques,[2] C. Martin,[3] M.A. Sanz[1]

[1]*Hospital La Fe, VALENCIA, Spain;* [2]*Hospital General Universitario, ALICANTE, Spain;* [3]*Hospital Dr. Peset, VALENCIA, Spain*

The cytogenetic analysis allows to classify the AML in different risk groups, nevertheless in about 50% of AML patients carry normal caryotype by conventional cytogeneticts and they lack of prognostic markers. Recently, several molecular alterations have been related to AML. This study analyze the prognostic impact of FLT3 mutations (ITD and D835 mutations) NPM1 mutations, partial tandem duplications (PTD) of MLL as well as WT1, EVI1 and BAALC gene expression in a group of 100 adult patients (48 female and 52 male; median age 62 years, range 22-94) with AML *de novo*. Screening for NPM1 was performed using a melting curve assay based Lightcycler (Schnittger S *et al.* Blood 2005) and confirmed by direct sequencing in ABI 310. The presence of FLT3 ITD was detected according to the method of Nakao M *et al.* (Leukemia 1997) and D835 using a melting curve based Lightcycler assay designed by Tib Molbiol (Berlin, Germany). MLL PTD was analyzed according to the method of Caligiuri MA et at. (Cancer research 1996). Gene expression quantification for WT1, EVI1 and BAALC was performed by real-time PCR ABI Prism using β-glucuronidase (GUS) as control gene and TaqManTM probes technology. Frequency of mutations: NPM1 mutations were found in 25/82 patients (30.5%) and four different mutations were detected: type A (72%), B (8%), D (12%), Km (8%), FLT3 mutations were present in 16/97patients (16.5%) (12 ITD and 4 D835) and the incidence of mutation for MLL PTD was of 4/74 patients (5.4%). Gene Expression: WT1, EVI1 and BAALC showed a median gene expression ratio: 0.28 (range 0-7.03), 0.013 (range 0-3.35) and 0.01 (range 0-18.90) respectively. Overexpression criteria was defined according to the median expression ratio Clinical characteristics: FLT3 and NPM1 mutations were significantly associated with a high white blood cell count (WBC) (p=0.003 and p=0.002 respectively). In addition, NPM1 mutated cases were significantly associated with FLT3 mutations (p< 0.0001), normal karyotype (p=0.024), and the monocytic lineage (FAB M4/M5, p=0,036). Prognostic impact: The response to induction showed no relation with any of the molecular markers. The disease -free survival (DFS) was significant influenced by overexpression of WT1 (p=0.045), FLT3 mutations (p=0.037), a high WBC (p= 0.047) and the cytogenetic risk group (p= 0.0034). In conclusion, our data show that the analysis of WT1 expression accompanying the FLT3 of mutations may be useful to predict prognosis beside cytogenetic findings. This study partially has been supported by grant FIS03/0400.