C5. **IMPACT OF MOLECULAR BIOLOGY ON PROGNOSIS IN ACUTE PROMYELOCYTIC LEUKEMIA**

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The t(15;17), characteristc of acute promyelocytic leukemia (APL), results in the PML/RARα fusion transcript, which can have three major isoforms as a result of the occurrence of different breakpoints within the PML gene: intron 6, bcr-1 (L, long form); exon 6, bcr-2 (V, variable form) and intron 3, bcr-3 (S, short form). The influence of breakpoint locus on the clinical features and response to treatment are still controversial. In this paper we aimed to determine whether the three isoforms PML/RARα transcript is associated with differences in pre-treatment clinical parameters and are predictive of response of treatment.

The **material** is represented by 41 cases of acute promyelocytic leukemia analysis in Molecular Biology Laboratory of Hematology and Bone Marrow Transplantation Fundeni Clinical Institute in I 2008-VIII 2013. The material comes from three sources: 20 cases from Center of Hematology and Bone Marrow Transplantation Fundeni Clinical Institute, 6 cases from Fundeni Pediatric Clinic, 6 cases from Coltea Clinical Hospital. In 29 cases, the determination of PML/RARα fusion gene (L, V, S isoforms) was performed at diagnosis. 16 of 29 newly diagnosis cases were monitored in dynamic. 12 cases diagnosed prior 2008 which were monitored for minimal residual disease (BMR) and are in hematologic and molecular remission.

Method for determining of PML\/RARα gene fusion isoforms was Nested PCR.

Results: Of the 29 cases of APL from three clinics of Hematology, analyzed at diagnosis, 14 (48%) patients had been diagnosed with bcr-1 (L-form), 6 (20%) patients with bcr-2 (V-form) and 9 (31%) patients with bcr-3 (S-form). For the 20 cases admitted in the Center of Hematology and Bone Marrow Transplantation Fundeni Clinical Institute were able to analyse the clinical and Hematological pre-treatment data. Of these 20 cases: 11 (55%) cases were bcr-1 (L-form), 3 (15%) cases were bcr-2 (V-form) and 6 (30%) cases were bcr-3 (S-form). There were no differences regarding age, gender, basic status of performance, clinical signs of coagulopathy. But differences were notted between L and S isoforms on the following hematologic features at presentation:

a)3/6 (50%) of the cases with S isoform (bcr-3) had the WBC > 10,000\/µl to 2/11 (18%) of the cases with L isoform (bcr-1).

b)3/6 (50%) of the cases with S isoform (bcr-3) had the phenotype M3 v. All patients with L isoform (bcr-1) presented with classical hypergranular form of LAM3 . 1 patient with bcr-2 isoform presented with the microgranular form and increased number of leukocytes (56,000\/µl).

c)FLT3-ITD mutation was detected in 2/6 (44%) cases with S- isoform (bcr-3) and was not discovered in any of the 11 cases with L- isoform (bcr-1).

d)CD56, CD2, CD 34 antigens were not investigated systematically. CD 56 was found in a case of APL with S- isoform (bcr-3), increased number of leukocytes (64,000/µl) and M3 v.

e)Regarding to relapse risk Sanz score, 3/6 (50%) of patients with S isoform (bcr-3) were included in the high risk group compared to only 2/11 (18%) of cases with isoform L (bcr-1).

All 12 patients diagnosed and treated before the year 2008 which were followed in the dynamic of BMR using nested PCR remained in molecular remission. 20 patients treated in the center of Hematology and bone marrow transplantation during the period 2008-2013 received treatment with ATRA + chemotherapy (Fenaux and Sanz PETHEMA protocols). 2 patients with LAP-bcr1-isoform, from intermediary risk group died at induction due to differentiation of syndrome according to ATRA that couldn't be overcome by treatment with dexamethasone. Of 18 patients, 15 are in molecular remission, 1 patient is in hematologic remission, but PCR + and 2 patients relapsed: at 4 months (a 50 years old patient with M3-v, S-form, bcr-3, FLT3 +, CD56 +, high risk, L > 10,000\/µl) and at 48 months (a 43 years old patient with M3, L-form, bcr-1, intermediate risk).

Conclusions: the cases of PML\/RARα S form (bcr-3) were associated with negative prognostic indicators (increased number of leukocytes, M3 v variant; FLT3, CD 56) but the therapeutic strategy adapted for high risk factors was able to obtain complete hematologic and molecular remissions as long as L forms PML/RARα.

In the cases of APL with resistant disease or molecular relapse it is recommended emergency treatment in order to obtain a new complete hematologic and molecular remission that will need to be consolidated according by their status of PCR +/- with blood stem cell transplantation (autologous / allogenic) or ATO or innovative therapies.