result of the fusion of bcr and c-abl genes. The correlation between the bcr-abl and prame (Preferentially Expressed Antigen in Melanoma) expression has previously been suggested, but this association is still unclear. In this study, our goal was to determine the possible correlation among prame expression, Bcr-Abl levels, CML progression, and response to imatinib (Gleevec®). Aim and methodology: For this purpose we evaluate prame expression in many cell lines, such as HL-60, HL-60.BcrAbl, HeLa, HeLa.BcrAbl, Jurkat, Jurkat.BcrAbl, K562, KBM5, KBM7, KCL22, LAMA-84, SKW.64, SKW.BcrAbl, THP1, and THP1.BcrAbl (with or without imatinib for 4 hours) and 22 CML patient samples in different phases, and in remission post-imatinib by real-time RT-PCR using taqman assays. *Results.* We only found a correlation between bcr-abl and prame in HL-60 X HL-60.BcrAbl in which prame expression was 48-times higher in HL-60.Bcr-Abl. Moreover, we did not detect any association between imatinib treatment and prame, which indicates that this is probably independent of the Bcr-Abl's tyrosine kinase activity. On the other hand, a higher prame expression was related to a disease progression, as we found 8-times more prame in accelerated than in chronic phase and 29-times more in blastic than in chronic phase and no prame expression was find in cytogenetic remission post-imatinib. *Conclusions.* Recently a function of prame was described as a dominant repressor of retinoic acid receptor (RAR) signaling. Signaling through RAR induce proliferation arrest, differentiation, and apoptosis in many cell types. Considering the function and our results, we can suggest that new therapeutical approaches can be developed, aiming to inhibit the function or expression of this gene, for the most delayed phase of the illness, in imatinib-refractory patients.

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GENETIC CHARACTERIZATION OF 203 *DE NOVO* CHRONIC MYELOID LEUKEMIA PATIENTS IN THE PORTUGUESE POPULATION

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Background. Philadelphia chromosome (Ph1) is the hallmark of almost all the cases of CML. The vast majority of patients express either the b2a2 (e13a2) or b3a2 (e14a2) BCR-ABL mRNA, characteristic of the p210BCR-ABL fusion protein. A very few patients express the e1a2 mRNA, characteristic of the p190BCR-ABL fusion protein and present in half of the adults who have BCR-ABL positive Acute Lymphoblastic Leukemia (ALL).[1] However, some patients have the protein p230BCR-ABL, originated from the e19a2 mRNA, and in some sporadic cases the BCR-ABL transcript is neither p210 nor p190, but another atypical product.[2,3] *Aims.* To identify the type and frequency of BCR-ABL fusion transcripts and Ph1 chromosomes in 203 Portuguese patients with *de novo* CML. Clinical diagnosis was confirmed by cytogenetics and/or molecular biology studies. *Methods.* Karyotypes were performed according to standard procedures. Molecular analyses were performed according to the BIOMED-1 Protocol.[4] 203 patients with *de novo* CML were studied (Table 1).

Table 1. Portoguese patients with the novo CML used in this study.

Patients	*Average age*	*Male*	*Female*	*Karyotype*	*Molecular Biology*
203	55.5	100 (49.3%)	103 (50.2%)	131 (64.5%)	180 (88.7%)

Results. Ph1 chromosome was found in 96.2% of patients; 3.8% were Ph1 negative BCR-ABL positive. In the Ph1 positive group 6.1% had variants [t(9;22;V), t(V;22) or t(9;V)] and 12.2% had additional anomalies, while the remaining (77.9%) presented the standard karyotype [46,XX,t(9;22)(q34;q11) or 46,XY,t(9;22)(q34;q11)]. 76% of CML patients expressed only BCR-ABL p210 transcripts, 21.2% co-expressed p210 and p190 transcripts while 2.8% expressed BCR-ABL p190 (1.7%) or b2a3 (e13a3) and e6a2, each one with a frequency of 0.56%. *Conclusions.* Our cytogenetics findings do not differ significantly from those described by other authors, except for the frequency of the Ph1 negative BCR-ABL positive cases, which is slightly below the one reported.[1,5] Based on molecular biology studies a discrepancy regarding BCR-ABL expression is shown. According to the literature more than 99% of patients express p210 transcripts, while the remaining express BCR-ABL p190 and other variants, considered rare.[1] In our population the frequency of non BCR-ABL p210 transcripts is higher than the one reported (1.7% for patients expressing p190 and 1.1% for atypical transcripts). Different transcripts may result from alternative splicing between BCR and ABL and within BCR itself. RNA splicing implies the recognition of consensus sequences, including 5' and 3' splice sites and a weakly conserved branchpoint in the intron upstream the 3' splice site. Polymorphisms affecting these sequences could activate cryptic branchpoints that are less efficiently used originating unusual products.[6] Being so, the reported frequency of atypical transcripts in our population might reflect a specific genetic *Background.* Nevertheless, the complete characterization of BCR-ABL transcripts, namely the uncommon ones, will ascertain correlations with different disease phenotypes and improve the outcome of single patients by individualizing therapeutic strategies.

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GENE EXPRESSION PROFILING IN CML PATIENTS RESISTANT TO TREATMENT SPECIFIC PROFILES IN NON-RESPONDERS WITH LOW BCR-ABL TRANSCRIPT LEVELS

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CML is characterised by a presence of fusion gene BCR-ABL. The level of BCR-ABL transcript characterises the disease status and BCR-ABL kinetics are an important prognostic factor. However, we found that among patient resistant to therapy there were those whose low BCR-ABL levels did not correlate with the disease status. Moreover, patients with non-correlating BCR-ABL levels had the worst clinical outcome. Our aim was to find gene expression differences underlying this discrepancy. To do this we turn to gene expression profiling using cDNA macroarrays. We analysed 28 samples of patients not responding to treatment. There were samples with BCR-ABL levels corresponding (n=21) and not corresponding (n=7) with the clinical state of disease. Hierarchical clustering (Euclidean distance, Average linkage) was used to cluster simultaneously both samples and genes. Hierarchical clustering showed that out of 28 samples of non-responding CML patients all 7 samples with BCR-ABL level not correlating with the disease status occupied a single cluster, clearly visible on the gene expression matrix. Among gene clusters our focus was kept on genes differentially expressed in non-correlating samples compared to the rest of the non-responders. We found clusters with genes up-regulated in non-correlating samples as well as clusters with genes down-regulated in these samples. Among up-regulated genes there were BAD, CDKN2A, O-6-metylguanin-DNA metyltransferase, Notch4, RhoC and VEGFR1. Clusters of down-regulated genes included e.g. Akt2, MAPK8, cyclins A, G1 and D3 and several caspases. In conclusion, we have found a group of CML patients not responding to the treatment whose BCR-ABL transcript levels were not correlating with the clinical disease status. This group was characterised to have clearly different gene expression profiles to the other non-responding patients. The genes differentially expressed in these samples are candidates for further investigations on mechanisms of both therapy resistance and possible lose of BCR-ABL dependency in CML. The BCR-ABL independency in these patients was further supported with our preliminary data on Western blot analyses and other kinase-inhibitor experiments.

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THE EXPRESSION OF PROTO-ONCOGENES IN THE COURSE OF CHRONIC MYELOID LEUKEMIA

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Background. The chronic phase (CP) of chronic myelogenous leukemia (CML) is characterised by the presence of chimeric BCR/ABL gene and a profligate growth of mature polymorphonuclears. The accelerated