and no mutations were identified in NPM1, CEBPA, and FLT3 genes. The capture-based NGS assay did not revealed any pathogenic Single Nucleotide Variant (SNV) or Insertion/deletion (Indel) in the 30 genes included in the panel, but suggested a few CNVs: a duplication of KIT and TET2 genes and a deletion of RUNX1 from exon 5 to 8 (Figure 1). CGH array confirmed a trisomy of chromosome 4, where KIT and TET2 genes are mapped, and a partial deletion of chromosome 21 containing a portion of the RUNX1 gene. In addition, a partial tetrasomy of chromosome 13 and a partial deletion of chromosome 17, regions not covered by the NGS panel, were identified by CGH array. The risk-category of the patient was revised following the ELN guidelines and the treatment was modified accordingly.

Conclusions: The laboratory evaluation of leukemia genetic profile is complex and has evolved significantly with the incorporation of advanced techniques. The combination of multiple innovative genetic approaches could help in identifying prognostic markers leading to a proper risk category stratification and a better patient management, especially in those cases in which standard approaches fail.

PO113

ALTERED EXPRESSION OF JAK-STAT PATHWAY AS POSSIBLE PREDICTIVE MARKER IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA RECEIVING TYROSIN KINASE INHIBITORS (TKIS)

S. Grassi, S. Palumbo, V. Mariotti, E. Ciabatti, F. Guerrini, F. Perutelli, C. Baratè, M. Petrini, S. Pellegrini, S. Galimberti

Dipartimento di Biotecnologie Mediche, Università di Siena; Dipartimento di Medicina Clinica e Sperimentale, Università di Pisa, Italy

The JAK-STAT pathway is involved in the transduction of signals mediated by cytokines, interferons and growth factors with consequent support of neoplastic cell growth and invasion in many types of cancer. Additional implications in inflammation and immunity in the tumor microenvironment have been recently recognized, because this pathway seems to sustain the stem cell maintenance. Moreover, persistent STAT3 activation would confer resistance to therapy with tyrosine kinase inhibitors in CML by controlling the leukemia stem cell self-renewal and favoring its hiding in the bone marrow niche (Groner, 2017). Inhibition of this pathway might represent a potential way to ameliorate the molecular response in warning or failed CML patients or to sustain deep responses in cases tempting the discontinuation of therapy. Our purpose was the evaluation of some BCR/ABL1-independent molecular predictive markers of response in CML patients. Thus, we analyzed the expression of 86 genes belonging to the JAK-STAT pathway in 10 cases assessed at diagnosis and after 6 months of therapy with TKIs. We quantitated the expression level of 86 JAK-STAT genes by RT-qPCR (PrimePCR SYBR® Green assay, Biorad©, Milan, Italy) at baseline and after 6 months of therapy. According to ELN guidelines, after six months of treatment 9 patients were in optimal response and 1 was in failure. Indeed, 79 genes resulted up-regulated, while only 7 were down-expressed. We correlated the gene expression results with the achievement of MR3. At 6 months of treatment, we identified correlation of MR3 with up-regulation of LRG1 (p=0.030), a gene belonging to the leucine-rich repeat (LRR) family that is overexpressed during the granulocyte differentiation, and down-regulation of IL2RA (p=0.030) and MPL (p=0.029).IL2RA is involved in the LSC growth and MPL is linked to persistent activation of JAK-STAT.Moreover, the up-regulation in responsive patients also involved the immunity signaling linked to interferon (IFN) receptors complex, and induced interferon-related factors with anti-proliferative and pro-apoptotic effects, such as CSF1R, IRF1, IRF9, and ISG15.The increased expression of GATA3, SOCS3, JAK3 is involved in NK and T cell recruitment as immunology protection in responsive patients. Finally, in responsive cases we observed a significant up-regulation of: OSM, involved in bone remodelling and reduction of fibrosis damaging the LSC survival in the niche and IFN receptor type 1 that is usually expressed in stromal cells as a protection factor against cancer progression. In this work, we demonstrated that the JAK-STAT pathway is really implicated in the resistance to TKIs and, on the other hand, that the de-regulation of some genes of this family might be related to the achievement of better molecular responses. This observation could have a practical clinical output, suggesting the effectiveness of the combination of JAK-STAT inhibitors (ruxolitinib or methotrexate) with TKIs in resistant CML patients.

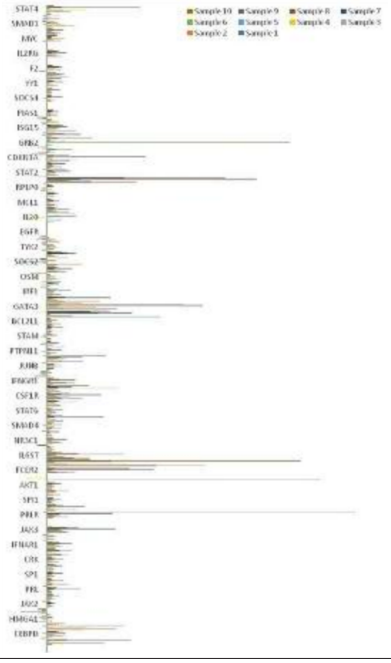

Figure 1.

PO114

WNT PATHWAY IS INVOLVED IN BCR-ABL1-INDEPENDENT RESISTANCE TO TKIS IN CHRONIC MYELOID LEUKEMIA PATIENTS

S. Grassi, S. Palumbo, V. Mariotti, E. Ciabatti, F. Guerrini, C. Baratè, M. Petrini, S. Pellegrini, S. Galimberti

Dipartimento di Biotecnologie Mediche, Università di Siena; Dipartimento di Medicina Clinica e Sperimentale, Università di Pisa, Italy

Background: After the introduction in the clinical practice of TKIs, the overall survival of CML patients is really improved, but several mechanisms of resistance have been reported. In addition to BCR-ABL1-related mechanisms, the persistence of the leukemic stem cell in the BM niche is a very relevant problem. The hypoxia and the presence of immunosuppressive cells in the microenvironment are well-known mechanisms that sustain the LSC; nevertheless, an increasing interest is today put also into the WNT/Beta-catenin pathway, that is necessary to self-renewal of normal cells also, but whose deregulation causes leukemogenesis and progression in several types of cancers (Zhao, 2007).

Aims:we decided to assess the expression of 86 genes of the beta-catenin/WNT pathway at diagnosis and after 6 months of treatment with TKIs in a cohort of 10 patients with different responses to treatment. Methods: Buffy coats obtained from peripheral blood samples of 10 patients (7 receiving imatinib, 2 nilotinib, and 1 dasatinib) have been used for the total RNA extraction. We used RQ-PCR for measuring the expression of 86 genes from the WNT pathway (PrimePCR pathway kit, Biorad, Milan, Italy) at diagnosis and after 6 months of therapy. Expression values were calculated by the Vandesompele method using four housekeeping genes. Data has been evaluated with "Gene Study" PrimePCR analysis software (Biorad).

Results: In our series of patients 5 achieved an optimal response and 5 were no responders, according to the ELN guidelines. Interestingly after 6 months of treatment, we observed a de-regulation of 36 genes. Down-expression occured in 14% of genes, while 79% of genes were up-regulated. When we compared the change of expression with the quality of response to TKIs, a differential expression between patients