with or without an optimal response was observed: in the cases without optimal response, we found as up-regulated: 1) FZD7, already known to be responsible for the protection of leukemic CML cell, proliferation and drug resistance in K562 cells; 2) WNT6, that predicts unfavorable survival in solid cancer and whose expression is inversely correlated to the response to ECF (Epi, cisplatin, 5-fluorouracil) chemotherapy in human gastric cancer cells; 3)WISP1, with anti-apoptotic activity, associated to poor prognosis and advanced stage in glioblastoma. On the other hand, the most frequently down-regulated gene was CSNK1A1, whose aploinsufficiency has been shown to result in a more probable transformation of MDS in AML and to induce proliferation, invasion and metastasis in MM, DLBCL and AML.

Conclusions:With this experiments of gene expression profiling we demonstrated, even if a small series of CML patients, that the beta-catenin /WNT pathway could be relevant in the conditioning the response to TKI. Obviously, the analysis of a larger number of patients will improve the biological suggestions coming from these preliminary data.

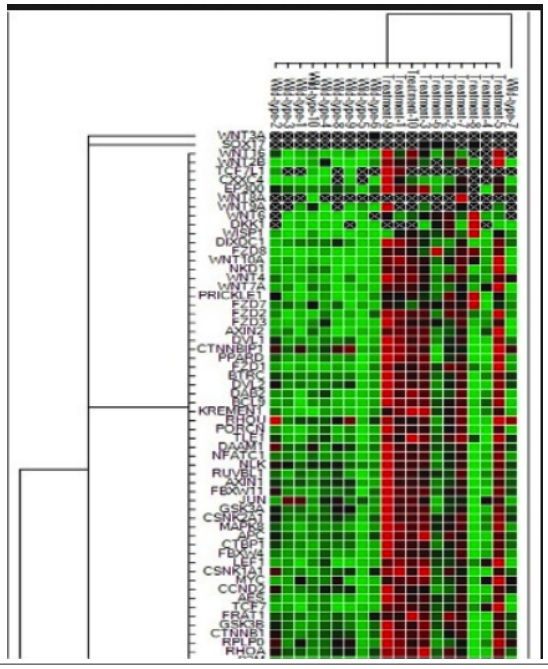

Figure 1.

PO115

BMI1 IS CO-EXPRESSED WITH BCR-ABL1: THE EXPERIENCE OF THE CONFOCAL MICROSCOPE

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Background: The introduction of Tyrosine kinase inhibitors (TKIs) improved overall survival in CML patients, today comparable to that of age- and sex-matched general population (Gunnarson et al. 2016). Nevertheless, a third of patients do not achieve deep molecular responses and has to suspend treatment (Hochhaus, 2009). This phenomenon can occur for the appearance of ABL1 mutations, but also numerous BCR/ABL1-independent mechanisms seem to be at the basis of resistance to TKIs. We previously demonstrated the correlation between some polycombs genes and prognosis, with the BMI1 oncogene high expression resulting to negatively condition the achievement of cytogenetic and molecular response (Crea, 2015). In the other hand, also the survival of leukemic stem cell (LSC) in the BM niche may cause resistance to TKIs. LSCs cells exhibit CD34+/CD38- phenotype, but recently the cytokine-targeting surface enzyme dipeptidyl- peptidase IV (CD26) has been added as phenotypic marker. We hypothesized that BMI1 could be expressed by immature cells and thereby able to cause a ABL1-independent resistance to TKIs. Therefore, we decide to use the laser scanning confocal microscopy to perform co-expression experiments.

Methods: Triple-immunofluorescence analysis was performed on buffy coat smears from 10 CML cases at diagnosis. Smears were treated with -MαBCR/ABL (1:300, Thermo Fisher, Rockford, IL, USA) , -RαBMI-1 (1:100, Thermo Fisher), and -GαCD26 (1:100, R&D system, Minneapolis, MN, USA) antibodies. Nuclei were stained with DAPI. The samples were observed at 20x, 43x or 63x magnification by a confocal laser scanning microscope (TC SSP8 Leica Microsystems, Mannheim, Germany) using a 488-nm, 561-nm and 642-nm excitation wavelength lasers. Negative controls for secondary antibodies were performed omitting primary antibodies. As further negative controls, the same reactions were performed on samples from patients affected by acute leukemia (AML), follicular lymphoma (FL), and essential thrombocytemia (ET).

Results: As shown in the figure, the analysis of the immunofluorescence pattern clearly showed the co-expression of BCR/ABL1 (green fluorescence), BMI1 (red) and CD26 (grey) proteins. Moreover, the tridimensional reconstruction documented for all these proteins a cytoplasmic localization. Moreover, we found the expression of BMI1 in AML, FL, and ET; nevertheless, as expected, this protein was not co-expressed with BCR-ABL1, nor with CD26, thus confirming the specificity of the finding.

Conclusion: Our work clearly demonstrated that the BMI1 protein co-localizes with BCR-ABL1 and CD26. This finding opens new interesting perspectives: in CML, BMI1 is an additional marker of the LSC and could be responsible for ABL1-independent resistance to TKIs. Thus, we can hypothesize that BMI1 could represent a new target for a patient-oriented treatment alternative to TKIs in CML resistant cases.

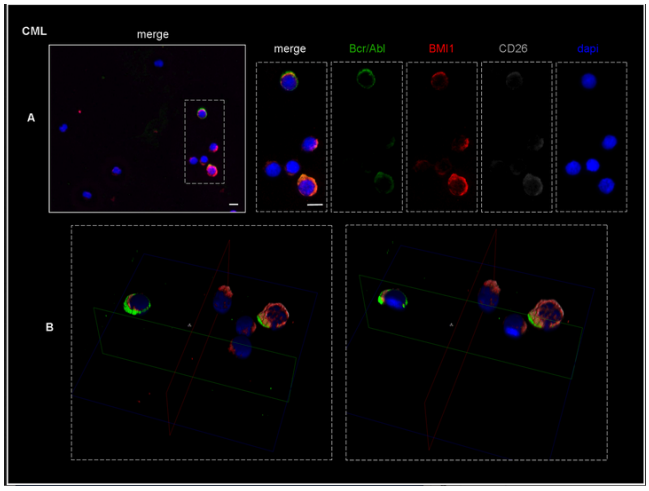

Figure 1.

PO116

A SIMPLE PCR-BASED TOOL FOR THE ASSESSMENT OF SOMATIC MUTATIONS IN ACUTE MYELOID LEUKEMIA AND ITS PROGNOSTIC POWER

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Background: Cytogenetic and molecular parameters are today relevant in the classification and prognostication of acute myeloid leukemia (AML). Among the molecular techniques, the NGS allows an accurate characterization, but optimization is often complicated and it is not avail-