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CHRONIC MYELOID LEUKEMIA - BIOLOGY & TRANSLATIONAL RESEARCH

PUBLICATION ONLY ABSTRACTS
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(PB1933) DECISION TO STOP TYROSINE KINASE INHIBITORS (TKIS) WITH BONE MARROW LEUKEMIC STEM CELL LEVEL: DETECTION OF BCR-ABL IN PERIPHERAL BLOOD MAY NOT BE ENOUGH

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Background:

About 50% of the patients with CML achieving a major molecular remission (MMR) for a few yearscan discontinue TKI-therapy without leukemia, a conditioned named treatment free remission (TFR). A substantial proportion of participants in TFR trials experience molecular relapse even if they have achieved MMR. CML leukemic stem cells (LSCs), which persist in the bone marrow, may be responsible for these relapses.

Aims:

In this study (NCT04104035), we aimed to investigate the presence of the CML leukemic stem cell in peripheral blood and simultaneously in the bone marrow in CML patients particularly patients who achieved MMR.

Methods:

The study performed between February 2019 and February 2020. Peripheral blood samples were provided from all patients. Bone marrow aspiration and biopsy was performed in 32 (86.5%) patients. The demographic data, complete blood count, BCR-ABL IS level, kind and duration of TKI they received were recorded. Cells were obtained in the AÜTF Hematology Laboratory with Navios 2 device (3 laser-10 colors, Beckman Coulter). CML leukemic stem cell was detected using CD26-RPE, CD45-Krome Orange, CD34-PC7, CD38-APC-Alexa Fluor 750 kits and analyzed with Kaluza software (Beckman Coulter). Except for one patient with pancytopenia who was in blastic phase, 1.000.000 cells were counted in all samples. CD45 + / CD34 + / CD38− cells with positive CD26 expression were considered as CML leukemic stem cells (CD26 + LSC).

Results:

A total of 37 patients, of whom 23 (62.2%) were male and 14 (37.8%) females, were included. Median age was 52 years (26-87 years). Thirty-five (94.6%) patients were chronic phase CML and 2 (%5.4) were blastic phase CML. Six (16.2%) of 37 patients were newly diagnosed, 20 (%54.1) had BCR-ABL positive hematopoiesis under TKI treatment and 11 (29.7%) achieved MMR with TKIs. Mean BCR-ABL, PB LSC and BM LSC were 61.755 IS (37.405-83.414 IS), 267.9 LSC/µL (16-737.5 LSC/µL) and 805 LSC/106 cells (134,6-2470 LSC/106 cells) respectively, in newly diagnosed CML patients. Mean BCR-ABL, PB LSC and BM LSC were 35.995 IS (0.024-147.690 IS), 18.7 LSC/µL (248.7-0 LSC/µL) and 135.9 LSC/106 cells (9-455.2 LSC/106 cells) respectively, in patients with BCR-ABL positive hematopoiesis. Mean BCR-ABL and PB LSC were 61.73 IS (59.615 63.85 IS), 49.41 LSC/µL (0.12- 98.7 LSC/µL), respectively in patients who were in blastic phase. In patients who achieved MMR, no LSC was detected in the peripheral blood of any patient. BM LSC was 250.9 LSC/106 cells (3.1-574.3 LSC/106 cells) (Table 1). Median TKI duration was 12 months (1-185 months). First line and second line TKIs were given to 13 (41.9%) patients and 18 (58.1%) patients, respectively.

Summary/Conclusion:

In this study, we detected a high amount of CD26 leukemic stem cells in the peripheral blood of newly diagnosed and blastic phase CML patients. We also demonstrated that leukemic stem cells persist in the bone marrow in patients with MMR, whether there was no LSCs in the peripheral blood. In conclusion, although TKIs are effective in suppressing BCR-ABL positive hematopoiesis, they are insufficient in eradication of CML stem cells, particularly from the bone marrow. We suggest that CML LSCs must be investigated in bone marrow samples before TKI discontinuation to achieve and maintain TFR.

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(PB1934) GLOBAL GENE EXPRESSION AND SPECIFIC MRNA-MIRNA INTERACTIONS IN PATIENTS WITH CML PRIOR TO AND 3, 6, AND 12-MONTHS AFTER INTRODUCTION OF TKI THERAPY

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Background:

The role of short non-coding RNAs (microRNAs, miRNAs) in the pathophysiology and progression of chronic myeloid leukemia (CML) and response to tyrosine kinase inhibitors (TKIs) has been suggested.

Aims:

The aim of this study was to investigate global gene and miRNA expression in CML patients and to identify mRNA-miRNA interactions that might be influenced with TKI treatment initiation.

Methods:

We isolated total RNA from bone marrow mononuclear cells from patients with CML at diagnosis (n=3) and those treated with TKI for 3 (n=3), 6 (n=3) and 12 months (n=3). Then, RNA samples from the same time point were pooled in equal concentrations to perform microarray analysis with Affymetrix Human Gene 2.1 ST Array Strip and Affymetrix miRNA 4.1 Array Strip, according to manufacturer's protocol. All further bioinformatics analyses were performed using BioConductor software. For functional annotation and enrichment analysis, DAVID Bioinformatics Resources were used.

Results:

The largest difference in gene expression was observed between patients who were 3-months on TKI when compared with CML patients at diagnosis (1774 genes downregulated, 390 genes upregulated). Next, mRNA-miRNA interactions were annotated to specific biological processes and compared for each TKI treatment group with group at diagnosis. The most dysregulated processes were as follows: T cell receptor signalling pathway (3-month), T cell costimulation (6-month), DNA replication (12-month). Gene cluster analysis revealed that genes involved in several important processes that are dysregulated at 3-months after TKI treatment initiation (e.g. cell division, protein phosphorylation, DNA repair, T cell receptor signalling pathway,

regulation of immune response), at 12-months achieve expression levels that were observed at diagnosis.

Summary/Conclusion:

We found significantly dysregulated genes and miRNAs in CML patients undergoing TKI treatment for 3, 6, 12-months in comparison with newly diagnosed cases. Several groups of genes were dysregulated in 3-months TKI group, then their expression gradually normalized and at 12-month was comparable to the one observed at diagnosis. Significantly dysregulated mRNA-miRNA interactions in patients treated with TKI, affect crucial cellular and immune-related processes. Further studies on the role of specific mRNA–miRNA interactions are required to better understand mechanisms of TKI action in CML.

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(PB1935) MODELLING CHRONIC MYELOID LEUKEMIA IN ZEBRAFISH

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Background:

Zebrafish has proven to be a versatile experimental model to study human hematopoiesis and it is a reliable in vivo tool for modeling hematological malignancies. The powerful genome editing, genome-wide forward genetic screens and chemical screening tools generated models that recapitulate human malignant hematopoietic pathologies and unraveled cellular mechanisms involved in these diseases. Several Authors described zebrafish models of acute myeloid and lymphoid leukemia, myeloproliferative diseases and myelodysplastic syndromes. Moreover, hematologic malignancies are frequently characterized by dissemination and homing of cancer cells to the bone marrow. Because of his optical clarity, zebrafish is the perfect in vivo model to study and monitor cancer cell dissemination and homing processes.

Aims:

To model Philadelphia positive (Ph+) chronic myeloid leukemia, wegenerated a transgenic fish expressing the human cDNA (BCR-ABL1) encoding for the human fusion protein P210 by using the Gal4/UAS system. In addition, we investigated the dissemination and homing processes of leukemic cells using zebrafish animal model.

Methods:

We crossed the line otained with the Gal4/UAS system with the HSP70-Gal4 transgenic line and we obtained a new transgenic line named bcr/abl-pUAS-CFPY//HSP70-Gal4. Characterization of the transgenic fish was carried out using whole mount in situ hybridization and Real Time PCR for genes involved in hematopoiesis (gata1, scl, runx1, mpx, l-plastin). BrdU was used at 30 hours to label proliferating hematopoietic cells in the caudal hematopoietic tissue (CHT), the region where hematopoiesis occurs in the zebrafish embryo. Then, at 48 hours of the embryonic development of healthy zebrafishes we performed intracardiac injection of a human leukemia cell line and monitored their migration.

Results:

Both by in situ hybridization and by Real Time PCR, at 24 and 48 hours of the

embryonic development we observed an increase of ematopoietic markers in transgenic fish compare to controls. All CHT cells were labeled in transgenic bcr/abl-pUAS-CFPY//HSP70-Gal4 larvae following exposure to BrdU, whereas fewer CHT cells were BrdU labeled in control larva (Figure 1A).

Thanks to the investigation of the behavior of the injected leukemia cells, we observed that during the embryo development they move across various anatomical districts, i.e. CHT, following the subsequent activation of the different hematopoietic tissues (Figure 1B).

Summary/Conclusion:

The presented transgenic and leukemia xenograft model could help to elucidate the mechanisms of Ph+ chronic myeloid leukemia progression and will probably allow high-throughput drug screening of putative targeted molecules with therapeutic effect by monitoring the down-regulation or the deactivation of BCR ABL1 p210 protein and the change in behavior of the leukemic injected cells.

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(PB1936) CLINICAL SIGNIFICANCE OF LARGE E3 UBIQUITIN LIGASE HERC1 IN CHRONIC MYELOGENOUS LEUKEMIA.

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Background:

The physiological relevance of the HERCs E3 ubiquitin ligases remains still poorly investigated. Accumulating evidence shows the HERC family proteins are key components of a wide range of cellular functions including pivotal roles in cancer related pathways. Recently, protein degradation by the ubiquitin proteasome system (UPS) has been shown to regulate hematopoietic stem cell (HSC) self-renewal, differentiation, and survival. The balance between HSC self-renewal and differentiation is critical to maintain hematopoiesis throughout the life of the organism and disruptions of this balance often result in malignancies, including chronic myeloid leukemia (CML). Chronic myeloid leukemia (CML) is a stem cell (SC) neoplasm sustained by a rare population of quiescent cells characterized by the BCR/ABL1 fusion onco-proteinthat activates a variety of downstream survival or proliferative pathways and inhibits cell differentiation depending on the cooperation between BCR/ABL and genes dysregulated during disease progression.

Aims:

Besides the Herc1 roles played in the nervous system of higher organisms like human beings, in the past few years it has emerged that few haematological neoplasms harbour somatic mutations affecting the HERC1 locus in different kind Leukaemia. However, the roles played by HERC1 in blood cells, under physiological and pathological conditions, remain at the moment unknown. Hence, we have recently started to assess whether HERC1 might be, or not, associated to a pathological and its role in physiological conditions.

Methods:

After written informed consent, specimens from CML patients were collected at diagnosis, remission and relapse. In addition, blood samples from healthy subjects were also collected. All the patients have been previously characterized at the cytogenetic level by conventional karyotyping, and screened by reverse transcriptase-PCR for the presence of the most frequent fusion transcripts. Differentiation was performed by incubation of HL60 and NB4, acute myeloid leukemia and K562 chronic myeloid leukemia cell lines for

48h with different concentrations of PMA and ATRA. Apoptosis and proliferation rate was investigated by Annexin V staining and MTT assay. Total RNA was extracted using TRIzol Reagent, according to the manufacturer's instruction. *Herc1* mRNA quantification was done by RT-qPCR. Immunofluorescence and western blots were performed to assess the protein expression. Flow cytometry was performed to assess cell surface differentiation markers.

Results:

Our results revealed that *HERC1* gene expression was significantly down-regulated in CML patients. Our data also indicate that, in CML, there is antagonistic interplay between *HERC1* and *Bcr-Abl* gene expression. Currently, the insights of this pattern is under investigation. By now, the evidence we collected, indicate that *Bcr-Abl* regulates *HERC1* gene expression. Howerer, how this occurs and which are downstream effectors implied in this process is under investigation. Differentiation of different leukemia cell lines revealed the upregulation of *HERC1* gene expression. In addition, we uncovered/identified HSP90 as novel interactor of large HERC1.

Summary/Conclusion: In conclusion our findings suggest that the ubiquitin E3 ligase, *HERC1*, either act as a novel tumor suppressor or involved in the regulation of differentiation in blood cells.

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(PB1937) NOVEL BCR-ABL1 KINASE DOMAIN MUTATIONS IN RUSSIAN PATIENTS RESISTANT TO ALL GENERATIONS OF TYROSINE KINASE INHIBITORS

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Background:

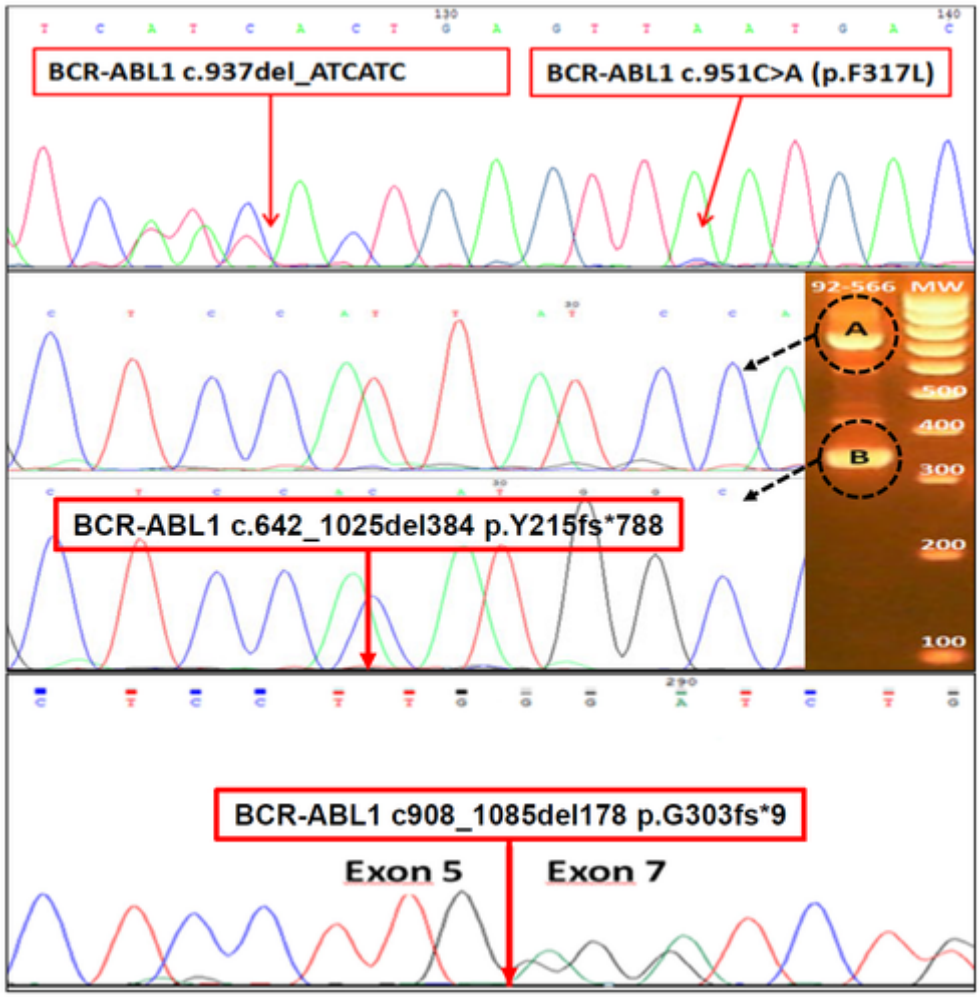
Mutations of the kinase domain (KD) of BCR-ABL1 are the main cause of resistance to tyrosine kinase inhibitors (TKI) therapy. Exact characterization of KD mutations is a clinically important task, since resistance to the first generation of TKI could be easily overcome by second or third generation drugs. However, clinical experience with different TKIs has proven the limitless ingenuity of nature: in response to the new generations of TKIs new BCR-ABL1 clones with previously undescribed KD mutations appear. **Aims:** Molecular characterization of tumor cells in chronic myeloid (CML) and acute lymphoblastic leukemia (ALL) patients resistant to all generations of TKIs.

Methods:

The study included 22 patients with CML and 5 patients with ALL refractory to all generations of TKIs. BCR-ABL1 KD was amplified and Sanger sequenced using the following primers: 5′-TGGTTCATCATCATTCAACGG-3′ and 5′-GGACATGCCATAGGTAGCA-3′.

Results: In two CML patients, and in one patient with ALL who underwent long-term treatment with TKI drugs of all generations, clones with novel previously undescribed mutations were identified (see Fig.). Case 1: 64-year-old patient was diagnosed with CML in 2004. In 2013, after the development of resistance to Imatinib, a clone with the mutation BCR-ABL1c.951C>A (p.F317I) was revealed. For the next 6 years, the patient unsuccessfully received therapy with Nilotinib, Ponatinib, and Bosutinib. During this time, a new clone with a complex mutation BCR-ABL1c.951C>A (p.F317I) and BCR-ABL1c.937del_ATCATC developed from clone BCR-ABL1c.951C>A (p.F317I). Case 2: 56-year-old patient suffers from CML since 2008. Since the diagnosis was established, the patient was treated with Imatinib, Dasatinib and Ponatinib without substantial success due to the resistance possibly associated with novel BCR-ABL1 c.642_1025 del384 (p.Y215fs*788) mutation. Case 3: 30-year-old patient in 2016 was diagnosed with Ph+ ALL. Treatment with Dasatinib in combination with Blinotumab, Bosutinib, and Ponatinib was not effective. Clone with a

previously undescribed BCR-ABL1 c.908_1085del187 (p.G303fs*9) mutation was found in the patient.



Summary/Conclusion: Long-term use of TKIs including drugs of new generations can select TKI resistant leukemia clones. Here we report novel BCR-ABL1 mutations: c.937del_ATCATC, c.642_1025 del384 and c.908_1085del187. One should conclude that in all cases with TKI treatment failure that is not associated with resistance referred to known KD mutations complete sequencing of BCR-ABL1 KD may be essential for correct therapeutic decisions.

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(PB1938) EXPRESSION OF LEVEL MIR-17-92 GENES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN DIFFERENT PHASES OF DISEASE

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Background:

MicroRNAs (miRNAs) play an important role in the regulation of migration, survival, proliferation, and sensitivity to chemotherapy of tumor cells. Use of miRNAs in future can predict tumor development. Its outcomes are promising, but their prognostic value still needs to be studied. It has been shown in a literature, the high expression of miR-17-92 genes have crucial oncogenic activity in various types of tumor cells (glioblastomas, tumors of stomach, breast, and others), including chronic myeloid leukemia.

Aims:

Study expression of the miR-17-92 genes in clinical material in patients with chronic myeloid leukemia (CML) with different results of antitumor therapy: with major molecular response (MMR), complete cytogenetic response (CCR) and in progressive phases of the disease (chronic phase (CP) and acceleration phase (AP) CML in primary patients and patients resistant to specific therapy).

Methods:

It has been studied bone marrow, peripheral blood and extracellular microvesicles in 56 patients with CML from the City Hematology Center of Novosibirsk. All patients received therapy with tyrosine kinase inhibitors (imatinib, nilotinib, dasatinib) at recommended therapeutic doses. The patients were divided into 3 groups: group I - 28 people in CP, MMR (MO≥3, BCR/ABL<0.1%), group II - 15 people in CP, CCR (Ph + 0%, MO <3.0, BCR/ABL >0.1%); group III - 10 people - primary patients and resistant to therapy in CP without CCR and AP. Total RNA, including miRNA, was obtained using TRIzol reagent (Ambion, USA) according to the manufacturer's instructions. RNA was translated into complementary DNA using miRNA-specific primers of the hairpin structure and M-MuLV – RH reverse transcriptase (Biolabmix, Russia). The expression level of miR-17-92 genes was assessed using real-time PCR using the TaqMan principle on iCycler CFX96 equipment. Small nuclear RNA U6 was used as an internal standard. All reactions were carried out in a triplet. For data analysis, the 2-ΔCt method

was used. Statistical data processing was performed using the STATISTICA program (StatSoft, Inc., USA).

Results:

Expression level of the miR-17-92 genes was much lower ($\rho < 0.05$) in microvesicles and plasma in group I than in group III. When evaluating the results level of miR-17-92 genes in group II, significant differences were obtained between all studied substrates of group II (much lower) and group III (high), except for myelocytes from bone morrow of group II and plasma of group III. There were no significant differences between the groups that reached the MMR (group I) and those who reached the CCR (group II), which indicates the significant role of achieving a complete cytogenetic response. Also, significant direct correlation has obtained between levels expression of the miR-17-92 genes in bone morrow myelocytes, lymphocytes and plasma ($\rho < 0.05$) and severity of the CML phase.

The experiments were performed using equipment of the Institute of Molecular Biology and Biophysics (IMBB), center of «Proteomic analysis» (Novosibirsk, Russia).

Summary/Conclusion: The results demonstrate that the expression of miR-17-92 genes increases depending on the progression of the severity of hemoblastosis, which can lead to a decrease in tumor cell apoptosis, an increase in migration and resistance to antitumor therapy.

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(PB1939) DISCONTINUATION OF TYROSINE KINASE INHIBITORS IN CHRONIC MYELOID LEUKEMIA PATIENTS: A TUNISIAN EXPERIENCE.

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Background:

Patients with chronic myeloid leukemia (CML) treated with tyrosine kinase inhibitors (TKI) can have a life expectancy comparable to that of the general population. Due to the extended duration of TKI administration, treatment discontinuation has been increasingly sought. Moreover; various trials have recently demonstrated that a significant proportion of patients who achieved a stable and deep molecular response (DMR) can stop therapy without relapsing.

Aims:

Here we report the outcome of patient with CML who discontinued TKI therapy.

Methods:

Medical records of 8 patients with CML who discontinued their TKI outside clinical trials were reviewed. We investigated reasons for discontinuation, duration of TKI treatment before discontinuation, molecular response (MR) status at TKI discontinuation and treatment-free remission (TFR) duration.

Results:

TKI therapy was discontinued in 8 patients. The median treatment duration before discontinuation was 117 months. The response statuses at discontinuation were MR4 (n = 3), MR4.5 (n = 1), and ≥MR5 (n = 2).

The reasons were toxicity (n = 1); requests for prolonged deep molecular response (n=5), and desire to get pregnant, (n = 2).

After TKI discontinuation, a median follow-up of 9 months (range: 1-44 months), half of patients lost major molecular response (MMR). TFR was maintained in half of patients

All relapsed patients promptly resumed TKI therapy and regained at least major molecular response. The duration of TKI administration before discontinuation (≥ 134 months) was favored longer TFR durations.

Summary/Conclusion:

Our results suggest that TKI discontinuation is safe outside clinical trials and particularly effective in CML patients who are in sustained deep molecular response with longer TKI treatment duration.

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(PB1940) TARGETING AUTOPHAGY OVERCOMES IMATINIB RESISTANCE THROUGH THE INHIBITION OF HISTONE DEACETYLASES IN CHRONIC MYELOD LEUMEIC CELLS

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Background:

Despite the impressive results with imatinib mesylate (IM) in treatment of chronic myeloid leukemia (CML), the clinical resistance to IM in patients leads to a still serious clinical problems. Autophagy is an adaptation mechanism that is essential for cellular homeostasis in response to various stress environments. Many studies have linked alteration of autophagy with cancer initiation and progression and development of drug resistance. Imatinib inhibits the oncogenic BCR-ABL tyrosine kinase activity and also induce its autophagic proteolysis. However, the role of autophagy in the survival of CML cells remain ill-defined.

Aims:

In this study, we addressed these questions for targeting autophagy to modulate chemoresistance.

Methods:

To establish IM-resistant sublines, CML K562 cells were maintained and logarithmically growing cells were exposed to increasing concentrations of IM, starting with a concentration of 0.05 μM and increasing gradually by 0.1 μM increments. After the cells acquired the ability to grow in the presence of a specific concentration of IM, the level of resistance was determined. We analyzed the effects of oxidative stress on the cell growth, apoptosis, BCR/ABL expression by western blot analysis, intracellular ROS level, antioxidant enzymes and autophay-associated proteins in both IM-sensitive and IM-resistant K562 CML cells. We further investigated that suppression of autophagy using pharmacological inhibitors (3-MA) enhanced cell dealth induced by IM.

Results:

Those results have been implicated autophagy inhibitors may enhance the therapeutic effects of TKIs in the treatment of CML. Combining IM use with autophagy inhibition is a promising approach for overcoming drug resistance in Ph+ CML or Ph+ ALL patients especially for IM resistant.

Summary/Conclusion:

Those results have been implicated autophagy inhibitors may enhance the therapeutic effects of TKIs in the treatment of CML. Combining IM use with autophagy inhibition is a promising approach for overcoming drug resistance in Ph+ CML or Ph+ ALL patients especially for IM resistant.

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(PB1941) IMPACT OF THE MAJOR BCR-ABL1 TRANSCRIPT TYPE ON CLINICAL AND BIOLOGICAL PARAMETERS IN WESTERN ALGERIAN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background:

In chronic myeloid leukemia (CML), the impact of MBCR-ABL1 major transcript type on disease phenotype and response to treatment still controversial to date.

Aims:

This work aims to study the influence of Mb3a2 and Mb2a2 transcripts on clinico-biological parameters in Algerian patients with chronic phase chronic myeloid leukemia (CP-CML) treated with Imatinib (IM) as first-line therapy.

Methods:

This is six years prospective study started in March 1st, 2013. All CP-CML patients were treated by IM as first line therapy. Clinical and biological characteristics were collected for all patients. Molecular typing was performed by multiplex RT-PCR and quantification of transcripts by real-time quantitative PCR (qRT-PCR). The crosstabs and the t-Student test were employed for the comparison of two averages. The cumulative incidence of deep molecular response (CIMRD) was estimated by the Kaplan-Meier method and Log Rank test. A value of $p \leq 0.05$ was considered significant.

Results:

The comparative study involved 65 patients with major transcript with 46 years ± 13, 85 [19-78] mean age. There are 35 men (54%) and 30 women (46%) with a sex ratio H / F of 1,16. 63% of patients expressed b3a2 (n=41), 37% b2a2 (n=24). We don't found a significant statistical difference for Sokal (P = 0.26) and EUTOS (P = 0.087) prognostic scores as well as for the hematological parameters. The patients with b2a2 transcript had a higher average rate of leukocytes, platelet and hemoglobin (Hb) than those expressing the b3a2 transcript of 305.54 x 10^9 g/ L *vs* 170.72 x 10^9 g/ L respectively (P = 0.086), 506.91 x 10^9 g/L *vs* 457.92 x 10^9 g/ L (P = 0.61), and 10.64 *vs* 10.51 g / dL (P = 0.78).

The median follow-up was 36 months [6 to 72 months]. At diagnosis the median BCR-ABL1/ABL1 ratio was 59% [14 to 98%]. However, this ratio was significantly higher in the b2a2 type than b3a2 (70 *vs* 53%; P = 0.03). At

various follow-up points; the level of expression of the b3a2 type was lower than the b2a2 type, it had also a faster but insignificant decrease (*P* = 0.26).

Patients with a b3a2 transcript have a better MMR that those with b2a2 At 18 months (71 vs 42%; *P*=0,20) (Figure4), and a better MRD at 24 (50 *vs* 32%; *P*= 0,20) and 36 months (75*vs*70%; *P*= 0,54). The cumulative probability of achieving MRD at 5 years was higher in patients with type b3a2: CIMRD (80 vs. 68%, *P* =0.17)

Summary/Conclusion:

Patients with b3a2 transcript may be associated with a better response to imatinib therapy. Our study confirms the literature data; it found that patients expressing the b3a2 transcript obtained higher and faster molecular response rates to MI than those with the b2a2 type.

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(PB1942) IN VITRO INVESTIGATION OF THE IMPACT OF BCR-ABL TYROSINE KINASE INHIBITORS ON ENDOTHELIAL CELLS

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Background: New-generation BCR-ABL tyrosine kinase inhibitors (TKIs) used in chronic myeloid leukemia (CML) induce cardiovascular events. However, the mechanism(s) by which these treatments induce these events is currently not clear. First in vitro investigations indicate that they influence the viability of endothelial cells, potentially explaining the occurrence of cardiovascular events. New-generation BCR-ABL tyrosine kinase inhibitors (TKIs) used in chronic myeloid leukemia (CML) induce cardiovascular events. However, the mechanism(s) by which these treatments induce these events is currently not clear. First in vitro investigations indicate that they influence the viability of endothelial cells, potentially explaining the occurrence of cardiovascular events. New-generation BCR-ABL tyrosine kinase inhibitors (TKIs) used in chronic myeloid leukemia (CML) induce cardiovascular events. However, the mechanism(s) by which these treatments induce these events is currently not clear. First in vitro investigations indicate that they influence the viability of endothelial cells, potentially explaining the occurrence of cardiovascular events.

Aims:

This research aims to specify the mechanism(s) by which BCR-ABL TKIs affect endothelial cell viability.

Methods:

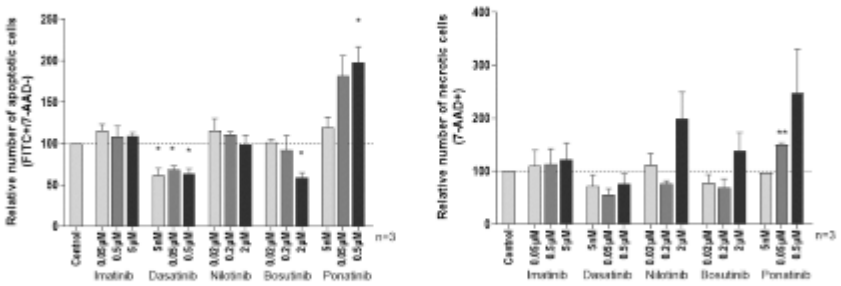
Three concentration of the five BCR-ABL TKIs have been tested on Human Umbilical Venous Endothelial Cells (HUVECs). All experiments have been performed in media with 10% dialyzed FBS in order to minimize the effect of plasma protein binding. MTS and LDH assays were performed to assess the effect of TKI on cell viability. Rate of apoptotic and necrotic cells have been measured using Annexin V staining, and BrdU assay assessed the effect on cell proliferation. Generation of reactive oxygen species (ROS) was evaluated using a specific fluorescent probe (CM-H2DCFDA) by flow cytometry.

Results:

All BCR-ABL TKIs affect endothelial cell viability after 72h of treatment. However, their impact on HUVECs are different according to the TKI. Ponatinib induces apoptosis and necrosis of endothelial cells (Figure). Nilotinib induces necrosis but at high dose only (2µM). Dasatinib increases the generation of ROS by HUVEC. Finally, all BCR-ABL TKIs dose-dependently influence

HUVEC proliferation as early as 24h of treatment (decrease of number of cells in S-phase and increase of HUVEC in G0-G1 phase). However, this impact is significant only at the highest concentration for imatinib, nilotinib and bosutinib.

Figure: Apoptosis and necrosis of HUVEC after treatment with BCR-ABL TKIs.



Summary/Conclusion:

Cell death within the arterial wall has already been recognized in atherosclerosis, and it appears plausible that dasatinib, nilotinib and ponatinib facilitate atherosclerosis development through alteration of endothelial cell viability.