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ABSTRACT BOOK

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Summary and Conclusions: High levels of 5hmC independently correlated with inferior overall survival in AML.

P190

FRONTLINE TREATMENT WITH INTENSIVE CHEMOTHERAPY, AZACITIDINE OR BEST SUPPORTIVE CARE IN OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA: A POPULATION-BASED ANALYSIS FROM A REGIONAL HEALTHCARE NETWORK

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Background: The efficacy of conventional treatments in older patients (pts) with acute myeloid leukemia (AML) remains unsatisfactory, with low remission rates and poor overall survival. Few pts above 60 years (yrs) really benefit from intensive chemotherapy (ICT). Azacitidine (AZA) has been recently approved for AML pts with 20-30% bone marrow (BM) blasts but little is known in pts with more than 30% BM blasts.

Aims: Taking advantage of our regional healthcare network, the first aim of this study was to describe the distribution of treatments, prognostic factors and outcome of all newly diagnosed non-M3 AML pts aged 60 years or older, selected in daily practice for ICT, AZA or best supportive care (BSC) over a 4-year period of time (2007-2010). The second aim was to compare the survival of pts treated with AZA vs respectively ICT and BSC using time-dependent analysis (Royston and Parmar model) and propensity score matching.

Results: Among the 385 AML pts aged 60 y or more, diagnosed between 2007 and 2010, 51 were excluded from the analysis because of early death (ED) occurring before therapeutic decision in 20 pts and missing information in 31 pts. Thus, 334 pts were evaluable (86.7%), including 115 pts treated with ICT (34.4%), 95 pts treated with AZA (28.4%), and 124 pts treated with BSC (37.1%). Median follow-up was 35 months. Overall median age was 75 yrs (ICT, 68 yrs; AZA, 76 yrs and BSC, 80.5 yrs; $p < 0.001$). Twenty percent of the entire population had prior history of myelodysplastic syndrome (ICT 6.7%, AZA 29.5%, and BSC 25%; $p < 0.01$ for AZA vs. ICT and $p = 0.017$ for AZA vs. BSC), median WBC was 4.25 G/L (ICT 9.5 G/L, AZA 2.3 G/L and BSC 5.3 G/L, $p < 0.001$) and cytogenetics (CG) were adverse in 28.4% (ICT 17.4%, AZA 45.3% and BSC 25.8%, $p < 0.001$ for AZA vs. ICT and $p = 1$ for AZA vs. BSC). ED (occurring within two months after diagnosis) was observed in 92 pts (27.5%) including 18 pts treated with ICT (15.7%), 11 pts in AZA group (11.6%) and 60 pts in BSC group (48.4%) (CTI vs AZA $p = 0.39$; AZA vs BSC $p < 0.0001$). We identified 56 pts in the AZA arm (59%) requiring hospitalization for infection (median number of hospitalization per patient, 1; range 1 to 7). Overall response (CR+CRi) was documented in 78 pts treated with ICT (67.8%), and in 18 pts treated with AZA (18.9%). Additionally 6 pts treated with AZA have obtained PR and 24 pts (25.3%) with stable disease were classified as major hematological improvement on at least one lineage according to MDS response criteria. Median overall survival (OS) in ICT, AZA and BSC were of 18.9, 11.3 and 1.8 months, respectively. In pts treated with AZA, OS was negatively impacted in multivariate analysis by higher age (95% Confidence Interval, 1.01-1.10, HR 1.05 for one year increase, $p = 0.010$), adverse CG (95% CI, 1.44-4.49, HR 2.55, $p = 0.001$), lymphocyte count < 0.5 G/L at diagnosis (95% CI, 1.19-5.26, HR 2.5, $p = 0.015$) and higher LDH level (95% CI, 1.0002-1.0012, HR 1.0007 for one unit increase, $p = 0.005$). We compared the survival of pts treated by AZA vs ICT and BSC using time-dependent analysis and propensity score matching (calculated on age, AML *de novo* or secondary status, BM blasts, platelets count and CG for ICT vs AZA and on age and WBC for AZA vs BSC comparison). Pts treated by ICT had a better overall survival compared to those treated with AZA from 6 months after diagnosis, whereas pts treated with AZA had a better overall survival compared to those treated with BSC from 1 day after diagnosis.

Summary and Conclusions: This study of "real life" practice shows that there is a room for low intensive therapies such as AZA in selected elderly AML patients.

P191

INCIDENCE AND MANAGEMENT OF VENOUS THROMBOSIS IN ACUTE LEUKEMIA: A MULTICENTER STUDY

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Background: Venous thrombosis (VT) frequently complicates the clinical

course of cancer. Available data on the incidence and management of VT in Acute Leukemia (AL) are scanty and quite discordant.

Aims: We have performed a multicenter study with the primary objective to evaluate the incidence of venous thrombotic complications in a population of patients with AL. Secondary objective was to evaluate the management of these complications in patients with AL.

Methods: Available clinical records of out and in-patients diagnosed with AL from January 2008 to June 2013 in 7 Regional Reference Hospitals were analyzed. Cases of venous thrombosis (VT), including thrombosis in atypical sites [Retinal occlusion (RO) and Cerebral Sinus Thrombosis (CST)], were reported. Available laboratory tests at diagnosis of VT included complete blood cells count (CBC), basal coagulation tests (PT, aPTT, fibrinogen), Antithrombin, anti-coagulant Protein S and C and D-dimer. Instrumental diagnosis of deep vein thrombosis (DVT), pulmonary embolism (PE) and RO and CST was performed according to ACCP guidelines. In the statistical analysis, logistic regression model was applied. Fisher's exact test was used to determine relationships between categorical variables.

Results: Over a population of 1487 patients with AL, 97 cases of VT were recorded: 71 cases were associated with Acute Myeloid Leukemia (AML) and 26 with Acute Lymphoblastic Leukemia (ALL). Fifty-four patients were males and 43 females (4 with AML, 10 with ALL), with a mean age of 52 ± 15 years; Twenty-four patients presented at least a concomitant chronic disease, 12 were receiving anticoagulant prophylactic treatment with low molecular weight heparin (LMWH). There were 72 cases of DVT of upper arms, 16 cases of proximal DVT of limbs (two complicated with PE), 4 cases of PE, 2 cases of RO, 2 of CST and 1 intracardiac clot. In 76/97 (78.6%) cases of VT, a central venous catheter (CVC) was placed; moreover, 68/72 DVT of upper arms were significantly associated with a CVC insertion ($p < 0.01$). VT occurred during chemotherapy (CHT) in 88/97 (90.7%) cases, 9 cases were diagnosed in concomitance with AL. In both subgroups with VT, there was no statistical significant difference between time at diagnosis of VT and time at diagnosis of AL. At CBC, thrombocytopenia was the most frequent abnormality. Severe thrombocytopenia (PLT < 30000 /mmc) was recorded in 25 cases. Coagulation tests were normal in all cases. Prothrombotic mutations were available only for 19/97 cases, 1 case was heterozygous for Factor V Leiden and 1 was homozygous for Factor II (G20210A) mutation. Most VT (83/97) were treated with LMWH at therapeutic doses for the first month with dose reduction in the following months, for severe thrombocytopenia after CHT; 1 case was treated with unfractionated heparin, 1 case with warfarin; 6 cases did not receive treatment due to severe thrombocytopenia. No cases of VT-related deaths nor fatal complications during treatment were recorded. Three cases of mild bleeding were reported. Treatments with LMWH lasted from 3 to 6 months. All patients clinically recovered from VT, only 2 late recurrences (PEs) were observed.

Summary and Conclusions: The incidence (6.6%) of VT in the analyzed cohort of patients with AL is almost similar to previous reports. Atypical sites VT must be suspected to be correctly diagnosed and treated. Anticoagulant treatment schedules and duration in patients with VT and AL seems safe and effective, even if it is influenced by many factors, mainly related to CHT and severe thrombocytopenia. The optimal management of VT in patients with AL requires further, prospective studies

P192

HIGH CORRELATION BETWEEN CLINICAL RESPONSES TO 1ST LINE AML PATIENTS TREATED WITH CYTARABINE AND IDARUBICIN AND THEIR PHARMACOLOGICAL PROFILES IN PATIENT SAMPLES MEASURED BY EXVITECH

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Background: Complete remission (CR) after induction therapy is the first treatment goal in acute myeloid leukemia (AML) patients.

Aims: To determine the ability of the Vivia's novel *ex vivo* drug sensitivity platform Exvitech to predict the CR rates after induction chemotherapy with cytarabine (Ara-C) and idarubicin (Ida) in 1st line AML.

Methods: This non-interventional and prospective study included samples from adult patients diagnosed with *de novo* AML in Spanish centers from the PETHEMA group. Marrow samples were sent to Vivia laboratories and incubated for 48h in whole samples in well plates containing Ara-C, Ida, or their combination, each at 8 different concentrations. Depletion of leukemic cells was quantified by subtracting live cells in each well with drugs from the control wells without drugs. Pharmacological responses are calculated using pharmacokinetic population models. Induction response was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders and the remaining as resistant. Patients dying during induction response assessment were non-evaluable. The correlation was modeled using a generalized additive model with a logit link and a binomial distribution for residuals. Kernel density estimates were then used to plot empirical probability density functions for both groups. Their separation was quantified as the area under the ROC curve and a cut point was selected using the Youden's criteria to optimize the classification probabilities (sensitivity, specificity). 95% confidence intervals for sampling errors were calculated for all these quantifiers.

Results: 180 patient samples were used to calculate the dose response (DR) curves for Ara-C alone, Ida alone, and their synergism. For clinical correlation we used 77 patients with a median age of 55 years (range 31 to 73). DR for Ara-C alone are shown in Figure 1A; note that for many samples there is a significant number (>20%) of resistant cells to Ara-C (bracket). This is a strong clinical predictor of resistance because in the patient the drug will never be present at these high doses for 48h. The second variable that is a good predictor of response is the synergism between these 2 drugs. The generalized additive model identified an algebraic combination of these 2 variables that yielded the best marker to separate both groups of patients. The probability density functions had minimal overlap. The area under the corresponding ROC curve was 0.935 (0.872, 0.997), and the classification probabilities for the optimal cut point, were 87% (68% to 95%) and 91% (80% to 96%) for sensitivity and specificity, respectively. Results are shown in Figure 1B; 54 patients (70.1%) achieved CR after Ida+Ara-C, and the remaining 23 (29.9%) were resistant. Correlations of the PM test are shown in Figure 1B. 20 of the 23 (86.9%) patients who fail to achieve CR were predicted as resistance in the *ex vivo* test. 49 of the 54 patients (90.74%) who achieved CR showed good *ex vivo* sensitivity to Ida+Ara-C predicting for CR. When the *ex vivo* test predicted a patient as sensitive it was correct in 49/52 cases (94.23%), and when it predicted resistant it was correct 20/25 cases (80%). Overall, 69/77 patients (89.61%) had an accurate prediction of their response to treatment.

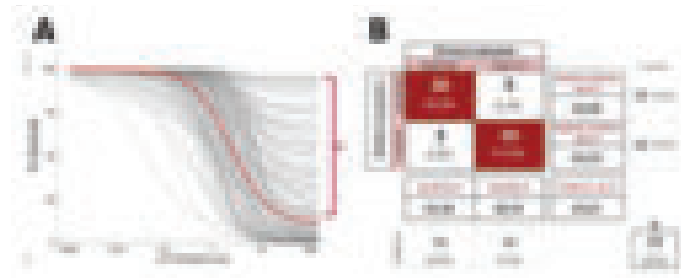


Figure 1. A) Dose response curves for Ara-C on 180 samples from AML patients in terms of the % survival of leukemic cells show the pharmacological profile of this drug. Note the 40% of samples with submaximal efficacy (bracket). **B)** Correlation with clinical outcome of the *ex vivo* pharmacological profiles in terms of sensitive vs resistant patients.

Summary and Conclusions: This study shows that this novel *ex vivo* pharmacological profile test is able to predict the clinical response to Ida+Ara-C induction. We are increasing the number of patients in this ongoing study, and we are planning a PM Test-adapted Clinical Trial.

Chronic lymphocytic leukemia and related disorders - Biology 1

P193

RUXOLITINIB INDUCES APOPTOSIS OF B-CELL RECEPTOR STIMULATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS

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Background: Stimulation of the B-cell receptor (BCR) protects chronic lymphocytic leukemia (CLL) cells from apoptosis and induces their proliferation. It is assumed that when CLL cells reside in the lymph-nodes extracellular signals activates the BCR and provides them with survival advantage. Whether the JAK-2/STAT3 pathway, known to elicit similar effects in various hematological and solid tumors is engaged in this process is unknown.

Aims: 1) Determine whether stimulation of the BCR induces JAK-2/STAT3 mediated tyrosine pSTAT3 and protects CLL cells from apoptosis. 2) Determine whether the JAK1/2 inhibitor ruxolitinib inhibits this pathway in CLL cells

Methods: CLL cells, fractionated from the peripheral blood of 19 previously untreated patients were used in the different experiments performed in this study. Anti-IgM antibodies were used to stimulate the BCR, and stimulated or unstimulated cells were treated with the JAK1/2 inhibitor, ruxolitinib, the bcr-abl/lyn inhibitor, dasatinib or with U0126, a MAPK signaling pathway inhibitor. The protein levels of STAT3 and tyrosine pSTAT3 were assessed by western immunoblotting, and for immunoprecipitation studies we used JAK2, pJAK2, STAT3 and pSTAT3 antibodies. Nuclear and cytoplasmic extracts were prepared and the purity was confirmed by the absence of lamin B in the cytoplasmic extract and the absence of ribosomal S6 from the nuclear extracts. Localization of pSTAT3 to the nuclear and cytoplasmic extracts was confirmed by confocal microscopy. Apoptosis was assessed by Annexin V/PI, and levels of STAT3 target genes by real time PCR (RT-PCR) and quantitative reverse-transcription PCR (qRT-PCR).

Results: Tyrosine pSTAT3 was not detected in unstimulated CLL cells. Stimulation of the CLL-BCR using anti-IgM antibodies induced tyrosine phosphorylation of STAT3, which was transient. STAT3 remained phosphorylated for 48 h in the presence of anti-IgM antibodies. However, 2 h after anti-IgM antibodies were washed out of the culture media we could no longer detect tyrosine pSTAT3. When the BCR was stimulated, tyrosine pSTAT3 was found in the cytoplasmic and nuclear extracts. Confocal microscopy confirmed that following BCR stimulation tyrosine pSTAT3 was localized to the nucleus. By RT-PCR and qRT-PCR we show that following stimulation of the BCR and localization of tyrosine pSTAT3 in the nucleus, STAT3-regulated genes are upregulated, suggesting that BCR mediated induction of tyrosine pSTAT3 is biologically active and induce transcription. Immunoprecipitation studies revealed that pJAK2 and tyrosine pSTAT3 co-immunoprecipitated suggesting that STAT3 phosphorylation occurs through the JAK-2/STAT3 pathway. Because tyrosine pSTAT3 protects CLL from apoptosis we hypothesized that ruxolitinib, a JAK1/2 inhibitor, would prevent phosphorylation of STAT3 and induce apoptosis of BCR-stimulated CLL cells. Ruxolitinib, but not dasatinib or U0126 inhibited tyrosine phosphorylation of STAT3 in a time and dose dependent manner. Likewise, ruxolitinib, but not dasatinib or U0126 induced apoptosis of BCR-stimulated CLL cells.

Summary and Conclusions: Our findings suggest that stimulation of the BCR activates the JAK-2/STAT3 pathway and induces transient phosphorylation of STAT3. Ruxolitinib inhibited the phosphorylation of STAT3 and induced apoptosis of CLL cells. Whether treatment with ruxolitinib would benefit patients with CLL remains to be determined.

P194

VARIABLE REACTIVE OXYGEN SPECIES LEVELS ARE ASSOCIATED WITH ANERGY IN CLL

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Background: Reactive oxygen species (ROS) are important regulators of cell signalling, replication and survival, and are essential for normal cell physiology. If uncontrolled however, ROS are associated with cell damage and implicated in therapy resistance. Previous studies have demonstrated that chronic lymphocytic leukaemia (CLL) cells contain variable levels of ROS which are, on average, higher than normal B cells. However, the relationship between ROS levels and disease subsets has not been examined.

Aims: We have sought to investigate potential associations between ROS levels, IGHV mutation status and the extent of B-cell receptor (BCR) anergy, an important determinant of clinical behaviour.

Methods: Examine intracellular ROS levels in primary CLL cells stained with CM-H₂DCFDA using FACS.

Protein expression analysis of pErk-1/2 and p-Akt in cells treated with N-acetyl