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## ABSTRACT BOOK

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which is constitutively secreted from the BM stroma and AML cells, is critical for the survival and retention of AML cells within the BM. CXCR4 expression is associated with poor prognosis in AML patients with or without a mutated FLT3 gene, and inhibition of CXCR4 was shown to sensitize AML blasts toward chemotherapy. It was found that FLT3-ITD mutation activate CXCR4 signaling and is associated with increased CXCR4 expression in primary AML cells.
**Aims:** In this work we studied the effect of the high affinity CXCR4 antagonist BL-8040 on the survival of AML cells with FLT3-ITD mutation alone or in combination with the FLT3 inhibitor AC220 (Quizartinib).
**Methods:** In this study, human AML MV4-11 cells *(FLT3-ITD*) were used. Cells were incubated in-vitro for 48 hrs in the presence of BL-8040 (20μM), AC220 (50nM) or their combination. Cells viability and the percentage of apoptotic events were evaluated by FACS analysis. In the in-vivo study an AML model of NOD scid gamma (NSG) mice engrafted with MV4-11 cells was used. Three weeks after the engraftment mice were treated daily for seven consecutive days with SC injections of BL-8040 (400ug/mouse) or with oral administration of AC220 (10mg/Kg) or their combination. The survival and apoptosis of AML cells were examined in the blood, BM and spleen of the engrafted mice.
**Results:** In-vitro, treatment of AML cells with BL-8040 directly inhibited cell growth by 35% and increased cell death by 40%. AC220 was found to induce cell death in 60% of the cells and the combination of BL-8040 with AC220 further increased the apoptotic effect achieving 97% reduction in cell viability and inducing cell death by 93% of the AML cells. In-vivo, BL-8040 was found to reduce the AML blasts in the blood from 13.5% in the control to 1.7%. Treatment with AC220 with or without BL-8040 reduced this level to 0.1%. Interestingly, the level of total mouse WBC following AC220 was significantly reduced in 65% compared to the control. This deep reduction in normal WBC was prevented when AC220 was combined with BL-8040. BL-8040 was found to decrease the number of AML cells in the BM to 2.6% compared to 12.6% in the control mice while AC220 reduced this level to 0.05%. The combination of AC220 with BL-8040 was found to further decrease this level to as low as 0.006% of AML cell in the BM. In 3/5 mice in this group the combination treatment completely eliminated the AML cells from the BM. Similar effect was observed in the spleen when BL-8040 reduced the level of AML cells from 21% in the control to 0.4% and AC220 reduced this level to 0.09%. The combination of AC220 with BL-8040 was further decreasing this level to 0.02%. The reduction in the number of AML cells in the blood, BM and spleen was accompanied with the induction of AML cells apoptosis.
**Summary and Conclusion:** The CXCR4 antagonist BL-8040 rapidly and efficiently induces cell death of AML cells both in-vitro and in-vivo. The combination of BL-8040 and AC220 was found to reduce the minimal residual disease of AML cells in this mice model. These results suggest potential therapeutic advantages of BL-8040 in AML patients with the FLT3-ITD-mutations by targeting not only AML anchorage in the BM but also AML survival. Furthermore, it could provide a rational basis for BL-8040 therapy in combination with the FLT3 inhibitor AC220 in this patient population.

**P800**

**PHARMACOLOGICAL PROFILES OF AML TREATMENTS IN PATIENT SAMPLES TO PERSONALIZE TREATMENT**

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**Background:** To aid in the identification of effective treatments for individual patients, *ex vivo* assays for detecting cell death inducible by drugs for hematological malignancies have been in development for over 20 years. We have developed an automated flow cytometry-based platform (ExviTech) that can address previous artifacts thus providing clinically predictive pharmacological data measuring leukemic cell depletion analyzed with pharmacodynamic population models.
**Aims:** The purpose of this study is to derive the *ex vivo* pharmacological profiles across the AML patient population of single drugs and combination treatments as a tool for individualized treatment selection.
**Methods:** Bone-marrow samples from 180 patients diagnosed with AML were sent to Vivia from 24 hospitals across Spain within 24 hrs. The plates were incubated for 48-hours prior to analysis with ExviTech. The percentage of leukemic cell death was determined via labeling with monoclonal antibodies and AnnexinV-FITC. Dose-response curves of cytarabine, idarubicin, daunorubicin, mitoxantrone, etoposide, fludarabine, clofarabine, and 6-thioguanine were measured in these patient samples. The added benefit of combining these drugs into 12 combination treatments was assessed by measuring their synergy in each individual patient.
**Results:** There was a large range of interpatient variability in the response to a single drug and even larger in the synergism between drugs. Alternative treatments are found even among CYT-(IDA/DAU/MIT). The Population Pharmacological Profiles for an individual patient is shown on the figure below. The relative drug potency in terms of their percentile ranking within the population is shown in the left panel from 0 (weakest) to 100 (most potent). Green lines represent the individual patient potency relative to the population ranking, with confidence intervals. Third column lists when a drug leaves a significant % of leukemic cells alive, potential resistant clones. The panel on the right side shows the synergism of the drug combinations treatments shown as box-plots at 10-25-75-90% to highlight their distribution. The synergism value for an individual patient in each combination is shown in green, with confidence interval as parallel dotted green lines. This representation of the Pharmacological Profile of an individual patient sample quickly identifies extreme values, when a drug or combination is very sensitive (rightward shift green lines, green boxes) or very resistant (leftward shift green lines, red boxes). This patient showed average sensitivities for most drugs though highly resistant to Clofarabine (red box) that leaves 45% alive. However this patient showed lack of synergism in multiple treatments (right, red boxes). CYT and IDA show average potencies but lack of synergism, suggesting CYT-DAU might be a more efficient treatment. These representations lead to clear guidelines in >90% samples.

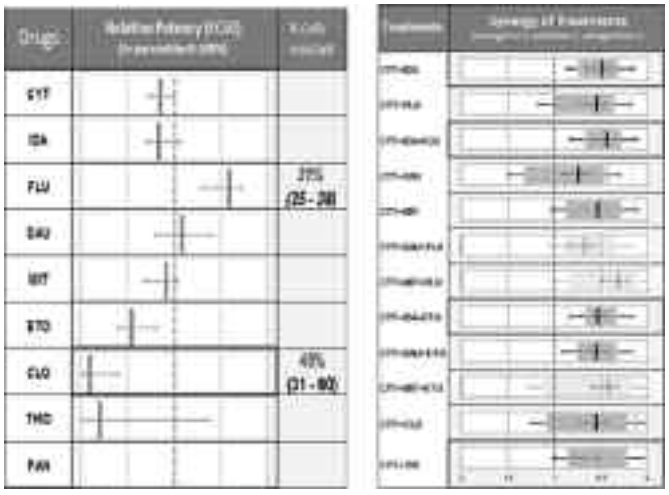


**Figure 1.**

**Summary and Conclusion:** We have developed an improved a methodology to measure the pharmacological activity of drugs and drug combinations in AML patient samples as well as modeling their pharmacological behavior. This information may be useful in selecting the optimal treatment for the individual patient, especially relapse/refractory patients in need of therapeutic alternatives. By testing the drugs used in the treatment protocols for AML directly on patient samples, a pharmacological based model has been developed to infer drug resistance or sensitivity, patient by patient.

**P801**

**XPO1 INHIBITION USING SELINEXOR RESTORES TOPOISOMERASE IIA (TOPO IIA) LOCALIZATION TO THE NUCLEUS AND SENSITIZE PIMARY REFRACTORY AND RELAPSED ACUTE MYELOID LEUKEMIA (AML) BLASTS TO CHEMOTHERAPY**

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