EX VIVO PHARMACOLOGICAL PROFILE OF 13 DRUGS IN 75+ ACUTE MYELOID LEUKEMIA PATIENTS USING WHOLE BONE MARROW SAMPLES ANALYZED BY AUTOMATED FLOW CYTOMETRY

Joan Ballesteros Nobell [1], Pau Montesinos[2], Federico Moscardó[2], David Martínez Cuadrón[2], Miguel A. Sanz[2], Joaquín Martínez López[3], Raimundo García Boyero[4], Jaime Pérez de Oteyza[5], Pascual Fernández[6], Josefina Serrano[7], Ángeles Fernández[8], Pilar Herrera[9], Arancha Alonso[10], Ataulfo Gonzalez[11], Concepcion Bethancourt[12], Esperanza Lavilla[13], Juan A. Vera[14], Begoña Navas [15], Gabriela Rodriguez [16], Juan A. López [17], Santiago Jimenez Bravo de Laguna[18], Adriana Simiele[19], Bernardo González[20], José A. Hernandez Rivas[21], Raúl Córdoba Mascuñano[22], Consolación Rayón[23], Carmen Burgaleta[24], Jordi Sierra[25], Daniel Primo[1], Pilar Hernández-Campo[1], Julian Gorrochategui[1], Belén Liébana[1], Teresa A. Bennett[1]

[1]Vivia Biotech, Madrid, [2]Haematology, Hospital Universitari i politecnic La Fe, Valencia, [3]Haematology, Hospital Universitario Doce de Octubre, Madrid, [4]Haematology, Hospital Universitario General de Castellón, Castellón, [5]Haematology, Hospital de Madrid Norte Sanchinarro, Madrid, [6]Haematology, Hospital General Universitario de Alicante, Alicante, [7]Haematology, Hospital Universitario Reina Sofía, Córdoba, [8]Haematology, Complejo Hospitalario Xeral Cíes de Vigo, Vigo, [9]Haematology, Hospital Universitario Ramón y Cajal, [10]Haematology, Hospital Quirón Madrid, [11]Haematology, Hospital Clínico San Carlos, Madrid, [12]Haematology, Hospital Regional Universitario Carlos Haya, Málaga,[13]Haematology, Hospital Universitario Lucus Augusti, Lugo, [14]Haematology, Hospital Universitario Virgen Macarena, Sevilla, [15]Haematology, Hospital Moncloa, Madrid, [16]Haematology, Hospital General Universitario Gregorio Marañón, Madrid, [17]Haematology, Complejo Hospitalario de Jaén, Jaén, [18]Haematology, Hospital Universitario de Gran Canaria Doctor Negrín, Gran Canaria, [19]Haematology, Hospital Povisa, Pontevedra, [20]Haematology, Hospital Universitario de Canarias, Tenerife, [21]Haematology, Hospital Universitario Infanta Leonor, Madrid, [22]Haematology, Hospital Infanta Sofía, Madrid, [23]Haematology, Hospital Universitario Central de Asturias, Oviedo, [24]Haematology, Hospital Universitario Príncipe de Asturias, Madrid, [25]Haematology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

ABSTRACT

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 a novel automated flow cytometry-based platform (ExviTech).

Aim: The purpose of this study is to examine the ex vivo pharmacology of single drugs used to treat AML against the malignant cell population in bone marrow samples from 80 AML patients.

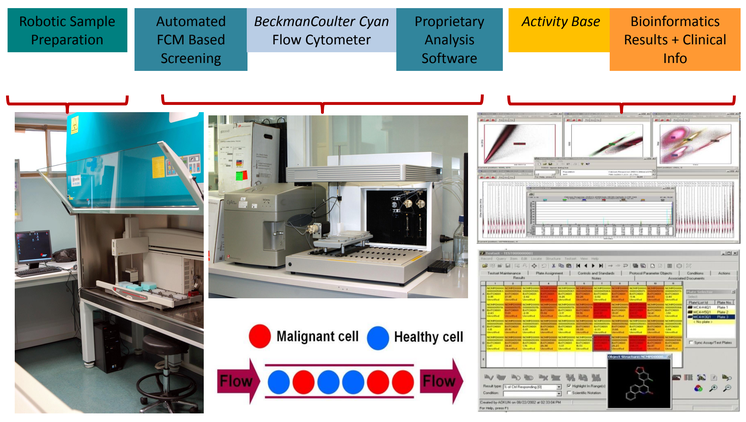
Patients and Methods: Bone-marrow samples from patients diagnosed with AML were sent to Vivia from 24 hospitals across Spain within 24 hrs. The whole sample was plated into 96-well assay plates containing 8 concentrations of each drug. The plates were incubated for 48-hours, and then prepared for analysis by our flow cytometry-based ExviTech© platform. All processes have been automated and multiple controls are used that greatly increase the accuracy of the analysis. The percentage of leukemic cell death was determined via labeling with monoclonal antibodies and AnnexinV-FITC. A survival index is computed for each drug, the lower the survival index, the more effective the drug. Dose-response curves of cytarabine, idarubicin, daunorubicine, etoposide, mitoxantrone, fludarabine, decitabine, 5-azacitidine, clofarabine, panobinostat, melphalan, cyclophosphamide and 6-thioguanine were measured in 64-99 patient samples.

Results: There is a large range of interpatient variability in the response to a single drug. These two results are depicted in **figure 1.** The colored lines are the average patient response to the drugs referenced above, demonstrating the range of effect of these drugs ex vivo, while the light grey lines are the individual results to fludarabine from 94 patients, representing wide interpatient variability. Interestingly, panobinostat (far left brown line), was the most potent and effective drug tested, suggesting that for a subset of patients it could potentially be a useful treatment. The anthracyclines, idarubicin, daunorubicin and mitoxantrone show a similar average response. Although anthracyclines are stronger drugs than fludarabine *on average*, certain fludarabine patient curves actually overlap with dauno and mito average curves. This means personalizing treatment may be as important as average drug strength. Clofarabine presented the widest variability of all of the drugs tested, with some patients responding very well while others were totally resistant.

Epigenetic drug 5-azacytidine, which clinically requires several cycles to work at low doses, shows depletion dose responses at 48 h similar to cytarabine. This likely reflects its cytotoxic mechanism at high doses, but still most sensitive patients identified here may also be sensitive for the hypomethylation mechanism. The related epigenetic drug decitabine acting on the same target is very inefficient in this assay (fig.2c).

METHODS

ExviTech© Platform



Screening Setup and Workflow

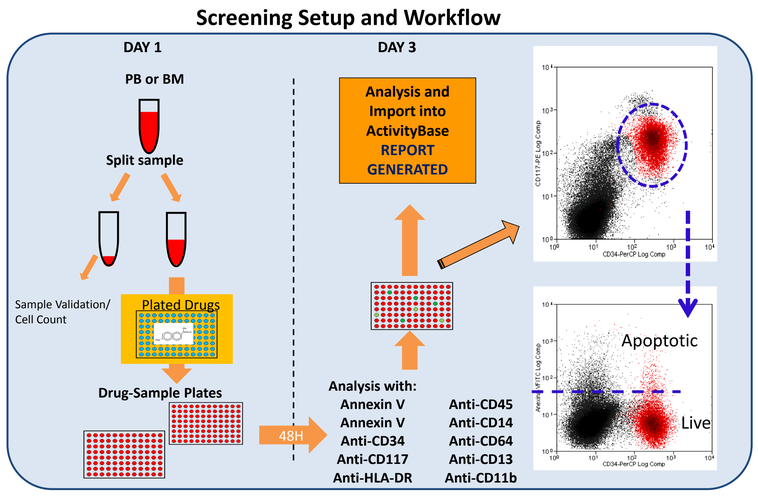
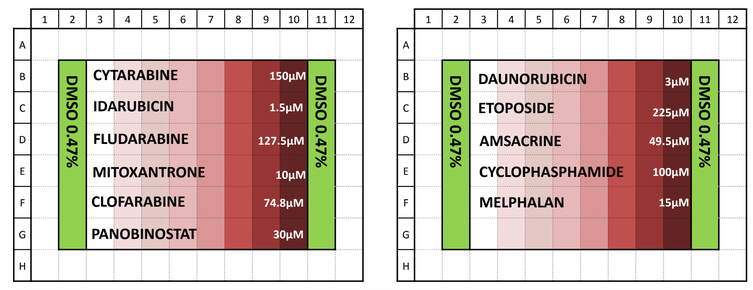
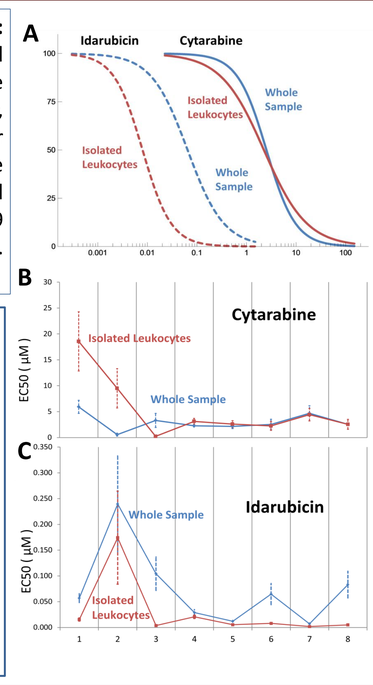


PLATE SETUP



Eight different concentrations of each drug or drug combination is run for the used treatment protocols. The max concentration used is listed

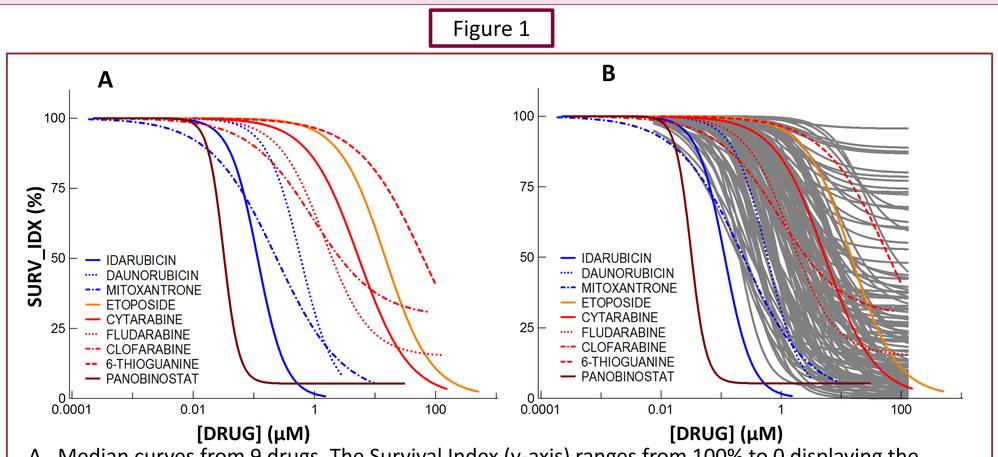
Whole sample vs. Isolated Leukocytes: A. Dose-response curves for IDA and CYT in isolated leukocytes and whole sample. Data, from sample 6 below, displays a log difference in the EC50s for IDA, but equal results for CYT. B. The EC50 (y-axis) of the whole sample and the isolated leukocyte fraction from 9 patient samples with cytarabine. C. EC50 of the same samples to idarubicin.



Data Analysis: performed using the population approach using NONMEM 7.2.: population PD modelling of the ex vivo response vs concentration data in monotherapy (fig.2), establishing for each patient the 95% prediction intervals (PI) of the isobologram from each individual parameter (fig.4) computation of the combination index using raw data descriptors from combination experiments. **Chou and Talalay. 2010. *Cancer Research* 70: 440-446.**

RESULTS

Figure 1



A. Median curves from 9 drugs. The Survival Index (y-axis) ranges from 100% to 0 displaying the selective AML cell depletion calculated with PKPD Population Models. B. The curves from A. overlaid on 125 individual dose-response curves to fludarabine.

	IDA	DAU	MIT	ETO	CYT	FLU	CLO	THI	PAN
N	125	109	110	110	125	125	122	86	92
EC50	0.11	0.59	0.23	18.50	5.34	1.43	0.92	62.20	0.03

Figure 2

Dose-response analysis was completed for individual drug in 86-125 AML patient bone marrow samples. The Survival Index (y-axis) ranges from 100% to 0 displaying the selective AML cell depletion calculated with PKPD Population Models. The grey lines display each individual response with the median response shown in red.

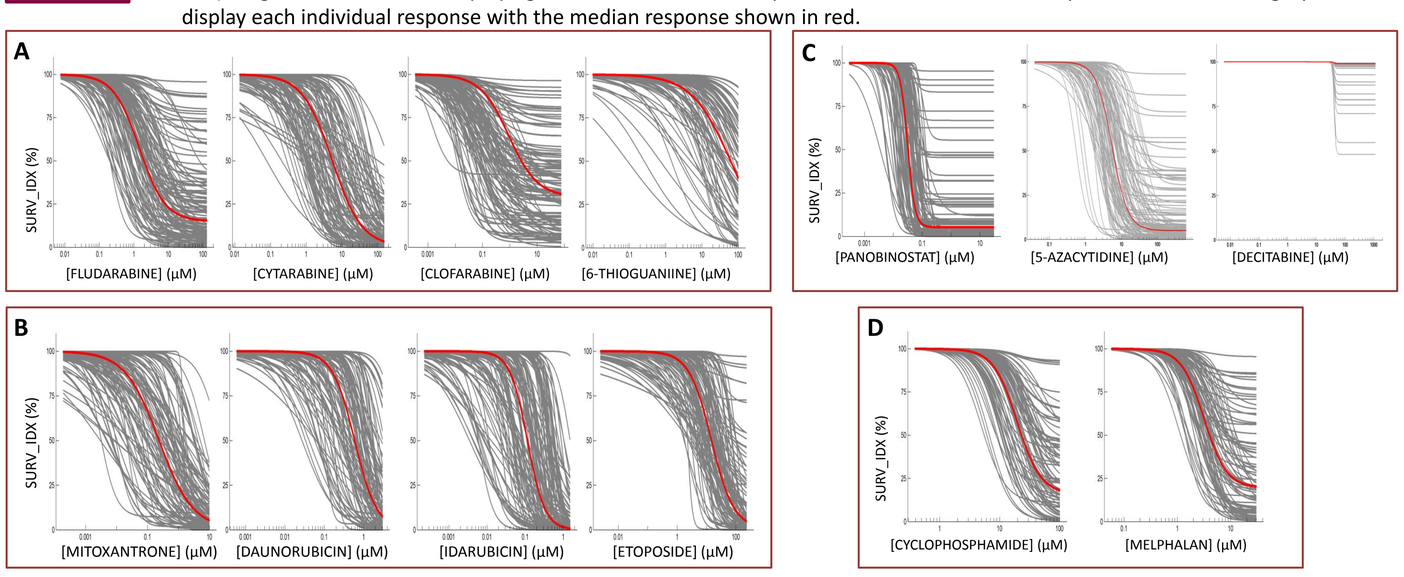


Figure 3

Conversion of dose-response analysis to a **Pharmacologic Profile** of ex vivo response to each drug.

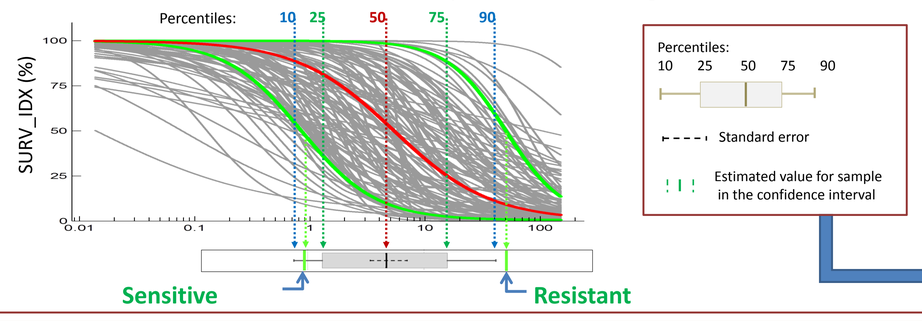


Figure 4

A. and B. Dose-response of 2 samples to Cyt alone (solid line) and to Cyt+Ida (dashed line). A displays synergism; B an additive response. C. The Combination Index (CI): Synergistic (CI<1), Additive (CI=1) or Antagonistic (CI>1).

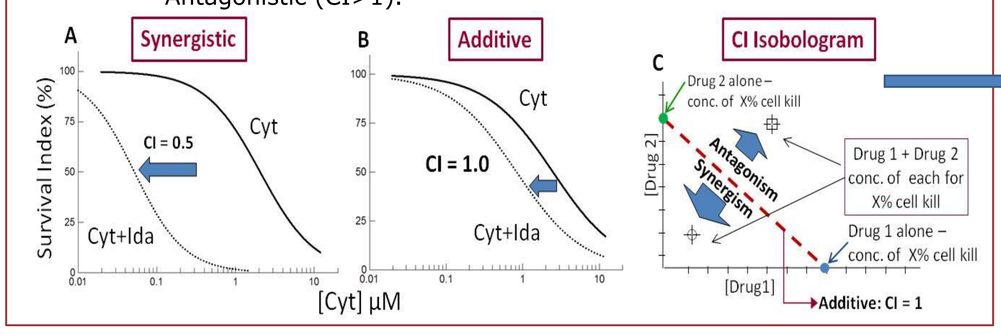
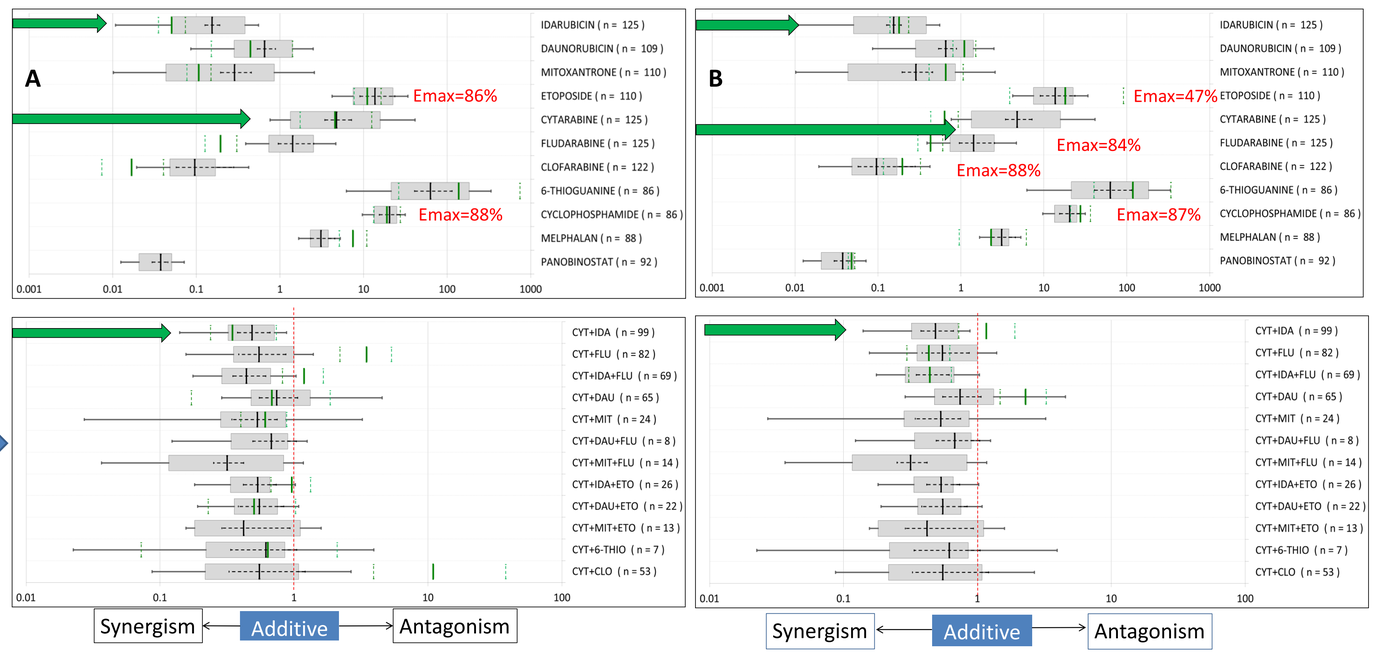


Figure 5

Pharmacological Profile of 9 AML drugs for two patient samples. A. The sample from the first patient tested sensitive to IDA and at the median for CYT (top panel). Additionally, the Combination Index (CI) for CYT+IDA indicated a synergistic combination. This patient was subsequently treated with CYT+IDA and obtained complete response. B. The second sample tested sensitive to CYT and at the median for IDA (top panel), however the Combination Index (CI) for CYT+IDA indicated only and additive to antagonistic interaction. The patient also subsequently received CYT+IDA but experienced disease progression.



CONCLUSIONS

- By testing the drugs used in the treatment protocols for AML directly on patient samples, a pharmacological based model could be developed to infer drug resistance or sensitivity, patient by patient.
- Similarity, testing could be used as a companion diagnostic to identify subsets of patients for which specific cytotoxic drugs or targeted therapies would be effective.
- The Pharmacological Profiles could be used personalize treatment for individual patients.
- Correlation of this ex vivo sensitivity with the clinical efficacy is currently being performed in a study under the supervision of the PETHEMA group.

Grants supporting this work:
Programa PRIMER Castilla y Leon (04/09/AS/0028)
ADE Medicina Personalizada 2007 (04/06/SA/0009)
Programa Reindustrialización 2011 MITYC (REI-040000-2011-777)
Programa Torres Quevedo, MICINN
Programa Inncorpora, MICINN

ACKNOWLEDGEMENTS

Special Thanks to the Patients and Hospitals for Providing the Samples (listed alphabetically)

- Complejo Hospitalario de Jaén, JAEN
- Complejo Hospitario Xeral Cíes de Vigo, VIGO
- Hospital Carlos Haya, MÁLAGA
- Hospital Clínico San Carlos, MADRID
- Hospital de la Santa Creu i Sant Pau, BARCELONA
- Hospital de Madrid Norte Sanchinarro, MADRID
- Hospital Doce de Octubre, MADRID
- Hospital General Universitario de Alicante, ALICANTE
- Hospital Infanta Sofía, MADRID
- Hospital Moncloa, MADRID
- Hospital Povisa, PONTEVEDRA
- Hospital Quirón, MADRID
- Hospital Ramón y Cajal, MADRID
- Hospital Universitario Central de Asturias, OVIEDO
- Hospital Universitario de Canarias, TENERIFE
- Hospital Universitario de Gran Canaria Doctor Negrín, GRAN CANARIA
- Hospital Universitario General de Castellón, CASTELLÓN
- Hospital Universitario Gregorio Marañón, MADRID
- Hospital Universitari i Politecnic La Fe, VALENCIA
- Hospital Universitario Infanta Leonor, MADRID
- Hospital Universitario Lucus Augusti, LUGO
- Hospital Universitario Príncipe de Asturias, MADRID
- Hospital Universitario Reina Sofía, CÓRDOBA
- Hospital Universitario Virgen Macarena, SEVILLA