# PERSONALIZING THERAPIES WITH EX VIVO PHARMACOLOGICAL RESPONSES MAY UNCOVER THE DIFFERENCES BETWEEN IDA-DNR-MIT AMONG EUROPEAN AML PROTOCOLS


Pau Montesinos[1], David Martínez [1], Raimundo García[2], Jaime Pérez de Oteyza[3] , Pascual Fernández[4], Josefina Serrano[5] , Ángeles Fernández[6], Pilar Herrrera[7] , Arancha Alonso[8], Ataulfo González[9], Concepción Bethancourt[10], Esperanza Lavilla[11], Juan Antonio Vera[12], Begoña Navas[13], Gabriela Rodríguez[14], Juan Antonio López[15], Santiago Jiménez Bravo de la Laguna[16], Adriana Simiele[17], Bernardo González[18], Jose Angel Hernández Rivas[19], Raúl Córdoba[20], Consolación Rayón[21], Carmen Burgaleta[22], Jorge Sierra[23], Iñaki F. Trocóniz[24], Ignacio Ortega[25], Andrew G. Bosanquet[26], Daniel Primo[27], Pilar Hernández-Campo[27], Julian Gorrochategui[27], Jose L. Rojas[27], Teresa A. Bennett[27] , Belén Liébana[27], Rocío López[27], Joan Ballesteros Nobell[27] , Federico Moscardó[1], Miguel Ángel Sanz[1], Joaquín Martínez López[28]

[1]Haematology, Hospital Universitari i politecnic La Fe, Valencia, Spain, [2]Haematology, Hospital Universitario General de Castellón, Castellón, Spain, [3]Haematology, Hospital de Madrid Norte Sanchinarro, Madrid, Spain, [4]Haematology, Hospital General Universitario de Alicante, Alicante, Spain, [5]Haematology, Hospital Universitario Reina Sofía, Córdoba, Spain, [6]Haematology, Complejo Hospitalario Xeral Cíes de Vigo, Vigo, Spain, [7]Haematology, Hospital Universitario Ramón y Cajal, Madrid, Spain, [8]Haematology, Hospital Quirón Madrid, Spain, [9]Haematology, Hospital Clínico San Carlos, Madrid, Spain, [10]Hospital Regional Universitario Carlos Haya, Málaga, Spain, [11]Hospital Universitario Lucus Augusti, Lugo, Spain, [12]Hospital Universitario Virgen de la Macarena, Sevilla, Spain, [13]Hospital Moncloa, Madrid, Spain, [14]Haematology, Hospital Universitario Gregorio Marañón, Madrid, Spain, [15]Haematology, Complejo Hospitalario de Jaén, Jaén, Spain, [16]Haematology, Hospital Universitario de Gran Canaria Doctor Negrín, Gran Canaria, Spain, [17]Haematology, Hospital Povisa, Vigo, Spain, [18]Haematology, Hospital Universitario de Canarias, Tenerife, Spain, [19]Haematology, Hospital Universitario Infanta Leonor, Madrid, Spain, [20]Haematology, Hospital Universitario Infanta Sofía, Madrid, Spain, [21]Haematology, Hospital Universitario Central de Asturias, Oviedo, Spain, [22]Haematology, Hospital Universitario Príncipe de Asturias, Madrid, Spain, [23]Haematology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [24]Universidad de Navarra, Pamplona, Spain, [25]Universidad del País Vasco, Bilbao, Spain, [26]Bath Cancer Research, Bath, England, [27]Vivia Biotech, Madrid, Spain, [28]Haematology, Hospital Universitario Doce de Octubre, Madrid, Spain


## ABSTRACT


**Background and objectives**: Protocols for acute myeloid leukemia (AML) 1st line patients are centered on the combination of Cytarabine and an anthracycline; Idarubicin (IDA), Daunorubicin (DNR),or Mitoxantrone (MIT).Patients may be treated with IDA, DNR, or MIT depending on the country of residence, because multiple clinical trials have not found significant differences among them. A new Personalized Medicine (PM) test developed by Vivia Biotech based on pharmacological responses in patient samples(ex vivo)is uncovering individual responses to these treatments. Our objective is to explore whether a significant % of individual patients may respond differently to IDA vs DNR vs MIT treatments, in spite that of their "on average" similar response shown by clinical trials.

**Patients and Methods**: Multicenter, prospective, non-interventional study of the PETHEMA group for treatment of AML. Bone Marrow (BM) samples were collected at diagnosis for 160 AML patients. Samples were incubated for 48 hours in 96well plates, each well containing different drugs or drug combinations, each at 8 different concentrations, enabling calculation of dose response curves for each single drug (CYT, IDA, DNR, MIT) and combination used in treatments(CYT-IDA, CYT-DNR, CYT-MIT). Drug response was evaluated as depletion of AML malignant cells in each well after 48 hours incubations. Annexin V-FITC was used to quantify the ability of the drugs to induce apoptosis. Malignant cells were identified with monoclonal antibodies and light scatter properties. 1)We use the whole bone marrow sample, retaining the erythrocyte population and serum proteins, during the entire incubation period; and after 48h leukocytes are isolated prior to evaluation by flow cytometry. 2)We have pioneered development of a proprietary automated flow cytometry platform called ExviTech. 3)Pharmacological responses are calculated using pharmacokinetic population models.

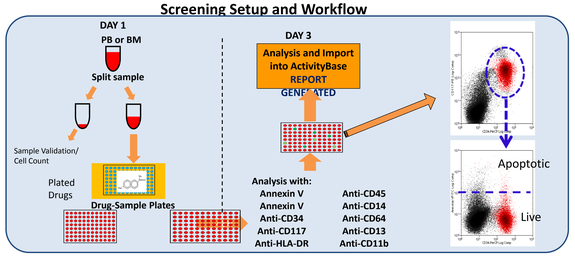
**Results**: Figure 3 shows dose responses for IDA (blue), DNR (red) and MIT (green) in 125AML patient samples. Although their average curves (Figure 2) are similar, the inter-patient variability of either drug is quite large. We hypothesized that some patients could show very differential sensitivities to these drugs, as illustrated in Figure 4 (panel A) where a patient sample is resistant to IDA (right shifted dose response curve) but sensitive to DNR (left shifted dose response curve). To identify these cases, Figure 5 panel A shows a comparison of the potency IDA vs DNR. Potency is represented by their EC50 (concentration that kills 50% of the cells). Most dots tend to line up, but red dots represent patient samples with a difference in potency between these drugs >30%. Repeating this exercise for IDA-MIT and DNR-MIT (panels B and C) to cover all alternatives among the 3 anthracyclines identifies 40% of patients samples with >30% different potency among IDA-DNR-MIT. Repeating this exercise with the combination treatments CYT-IDA, CYT-DNR, CYT-MIT (Figure 6) increases to 58% the population of patients whose samples have a differential sensitivity to these anthracyclines. A fraction of this 65% of patients may benefit in if treatment selection among these 3 treatments were to be aided by this ex vivo testing sensitivities. To identify which fraction would benefit we would need a trial specifically designed.


## METHODS

### ExviTech© Platform

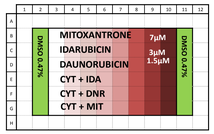
Robotic Sample Preparation | Automated FCM Based Screening | *BeckmanCoulter Cyan* Flow Cytometer | Proprietary Analysis Software | *Activity Base* | Bioinformatics Results + Clinical Info


### Screening Setup and Workflow




## PLATE SETUP

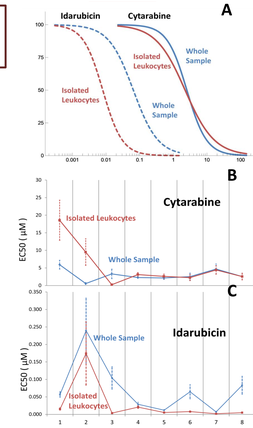
Eight different concentrations of each drug or drug combination is run for the used treatment protocols. The max concentration used is listed




**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.**

## WHOLE SAMPLE vs. ISOLATED LYMPHOCYTES

### CYT-IDA




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 CYT. C. EC50 of the same samples to IDA.

D-F. Dose response curves for IDA (D) DAU (E) MIT (F) for 2 samples (red, blue), in whole sample (continuous line) and isolated leukocytes (dotted lines). Blue sample shows small difference DAU vs large difference IDA & MIT. Red sample all more similar.

### IDA-DAU-MIT

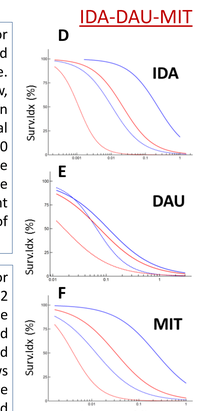
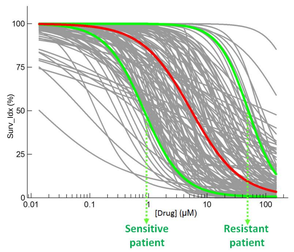
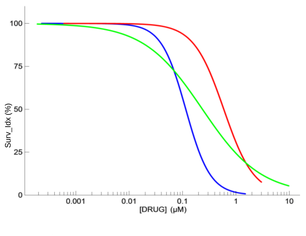



**Figure 1. Chemosensitivity test by dose-response modeling analysis**



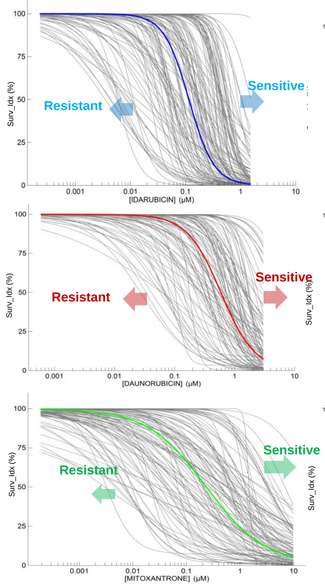

Shift of dose-response curves to the left (sensitive) or right (resistant) indicates differences of drug potency exvivo.

**Figure 2. Similar sensitivity IDA DAU MIT**



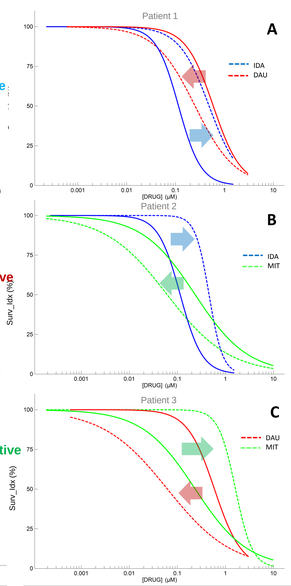

Each curve corresponds to the average exvivo effect of roughly 100 tests, showing similar overall drug sensitivity to Anthracylin drugs on AML patients.

**Figure 3. Inter-patient variability**



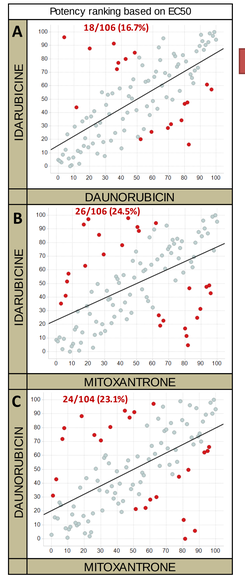

Wide Inter-patient variability shown on individual dose-response curves for the three Anthracylin drugs, much more higher than overall inter-drug variability.

**Figure 4. Examples of differential individual behavior**




Examples show how inter-patient variability may result in one anthracycline being more sensitive while another one being more resistant. Dotted lines correspond to the individual and straight lines show the population overall fit.

**Figure 5. Overall statistical differences among drugs**



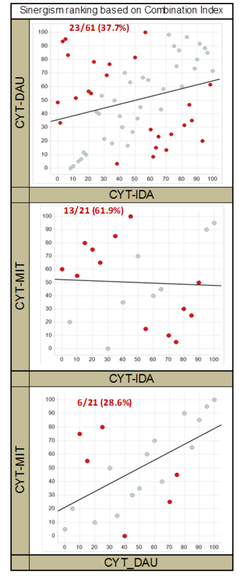

Red dots for patients with a response exvivo showing differences over 30% among rankings for drugs potency. The % of red dot patients for each drug pair (17%, 24%, 23%), represent 40% of total patients.

**40% of patients showed significant differences among potency of three drugs.**

**58% of patients showing any difference in synergy measurements among the three combinations**

**More than 65% of patients show differences either on drug potency or synergy measurements among CYT-IDA, CYT-DAU, CYT-MIT.**

**Figure 6. Overall statistical differences among combinations**




Red dots for patients and combinations indexes showing differences over 30% among rankings. Values in red indicate the proportion of those regarding the total, they represent 58% of total patients

**Figure 7. Drugs interaction in combination treatments**

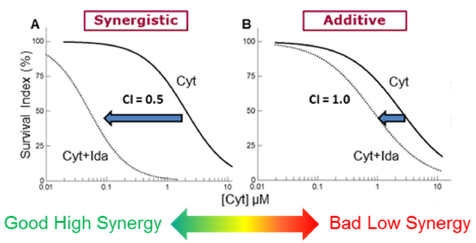
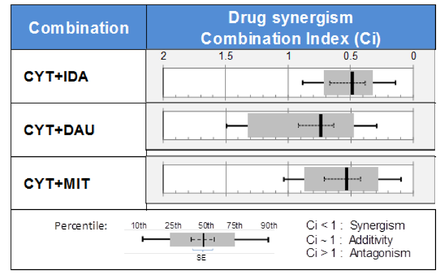



**Figure 7. Inter-patient variability on drug interaction measurement**




Box-plots showing population distribution of measured combination index (Ci) of combination treatments ex vivo for each Anthracylin drug together with Cytarabine. Although the three combinations show a trend for synergistic interactions, narrowest distribution is observed for CYT+IDA. A significant number of patients showed a poor drug interaction between Cytarabine and Daunorubicin.

## CONCLUSIONS

- This preliminary results show that Vivia's PM test seems able to identify a subset of AML patients who's ex vivo pharmacological response to anthracycline drugs is significantly different
- If these selective anthracycline ex vivo responses translate to clinical responses, a fraction of this 65% subpopulation could benefit significantly from receiving 1st or 2ndline treatments based on either IDA, DNR, MIT, and their combinations.
- This approach stands for European integration of treatment protocols, based on ex vivo individual responses data rather than nationality.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