# PERFILES FARMACOLÓGICOS EX VIVO DE 11 FÁRMACOS EN MÁS DE 60 MUESTRAS DE MÉDULA ÓSEA DE PACIENTES CON LEUCEMIA MIELOIDE AGUDA

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## ABSTRACT

**Fundamentos**: para ayudar a identificar tratamientos efectivos en pacientes individuales, durante más de 20 años se han intentado desarrollar ensayos *ex vivo* que detectan muerte celular inducida por fármacos en enfermedades hematológicas malignas. Vivia Biotech ha desarrollado una novedosa plataforma basada en el análisis automatizado por citometría de flujo (ExviTech©) para este propósito.

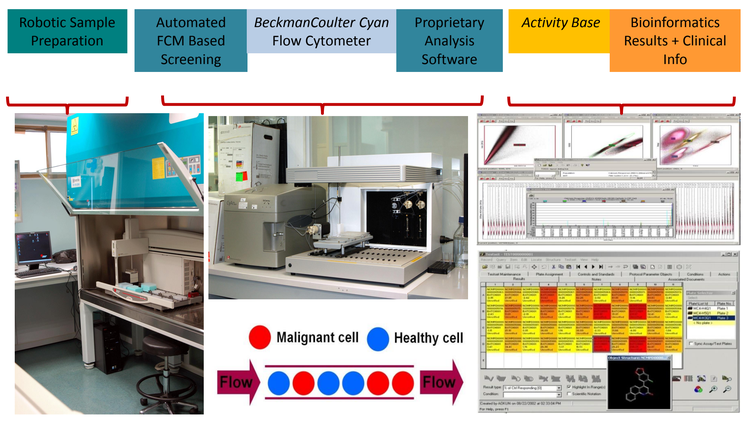
**Objetivo**: examinar la farmacología *ex vivo* de fármacos individuales empleados en el tratamiento de la leucemia mieloide aguda (LMA) en muestras de médula ósea en 125 pacientes con LMA.

**Métodos y pacientes**: 125 muestras de pacientes diagnosticados de LMA fueron enviadas a Vivia Biotech desde 26 hospitales españoles del grupo PETHEMA, en menos de 24 horas. La muestra completa fue alicuotada en placas de 96 pocillos con 8 concentraciones de cada fármaco. Tras 48h de incubación, se analizaron mediante la plataforma ExviTech©. El porcentaje de muerte celular de las células leucémicas se determinó marcándolas con anticuerpos monoclonales y Anexina V-FITC. El índice de supervivencia (IS) se calculó para cada fármaco; cuanto más bajo es su valor, más efectivo es un fármaco. Se calcularon curvas dosis respuesta para los siguientes fármacos: citarabina (ARAC), idarubicina (IDA), daunorubicina (DAU), etoposido, mitoxantrona (MIT), fludarabina (FLU), clofarabina (CLO), panobinostat (PAN), melfalán, 6-tioguanina y fueron medidas entre 84 y 125 muestras.

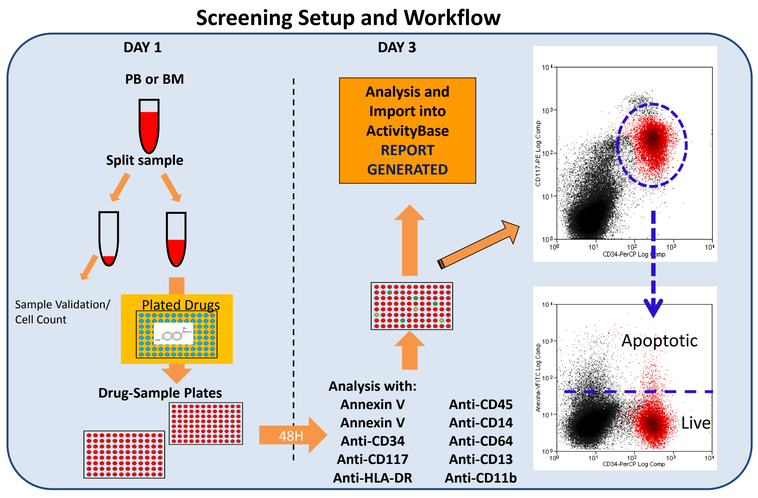
**Resultados**: hubo una gran variabilidad en la respuesta a los fármacos entre pacientes. Las curvas dosis respuesta media de todos los pacientes a los 11 fármacos ensayados muestran un amplio rango del efecto de estos fármacos *ex vivo*. Las antraciclicinas: IDA, DAU y MIT mostraron similar rango de respuesta. Aunque las antraciclinas mostraron más potencia que ARAC y FLU en media, en ciertos pacientes las curvas de FLU y ARAC se solapan con las de DAU y MIT. Esto significa que personalizar los tratamientos podría ser tan importante como la potencia media de ese fármaco. El rango de variabilidad de CLO fue el más amplio de todos; algunas células leucémicas responden muy bien, otras son totalmente resistentes.

## METHODS

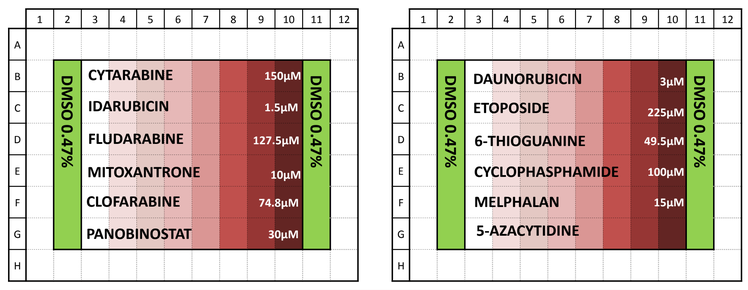
**ExviTech© Platform**




**Screening Setup and Workflow**



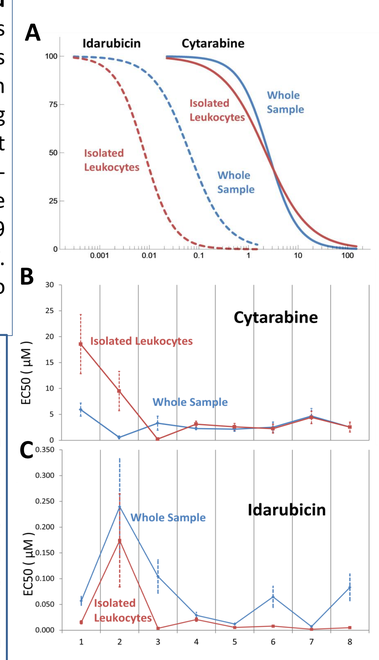

## PLATE SETUP




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

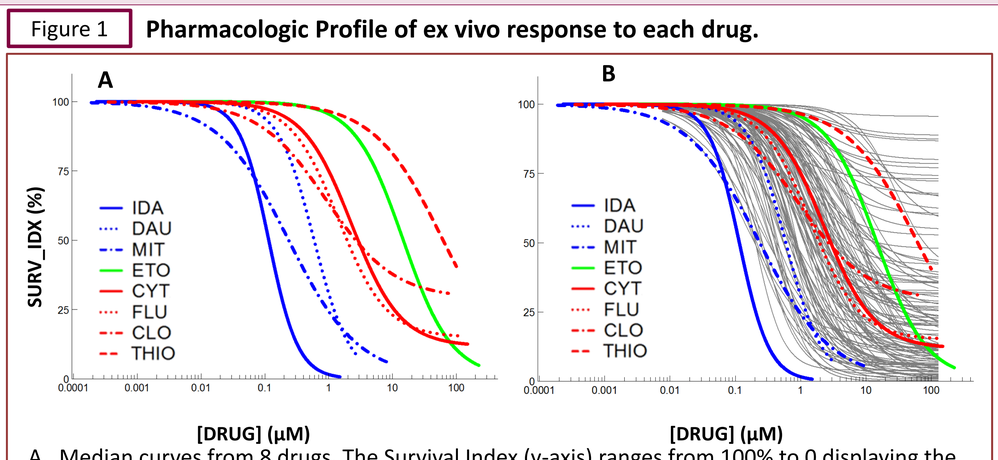
**Whole sample vs. Isolated Leukocytes:** A. Dose-response curves for IDA and CYT in isolated leukocytes and whole sample. Data, from sample 6 below, displays a log difference in the EC50s for IDA, but equal results for CYT. B. The EC50 (y-axis) of the whole sample and the isolated leukocyte fraction 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 **Pharmacologic Profile of ex vivo response to each drug.**




A. Median curves from 8 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 |
|---|---|---|---|---|---|---|---|---|
| N | 125 | 109 | 110 | 110 | 125 | 125 | 122 | 86 |
| EC50 | 0.1 | 0.6 | 0.2 | 14.6 | 2.3 | 1.4 | 0.9 | 62.2 |

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 average response shown in red.

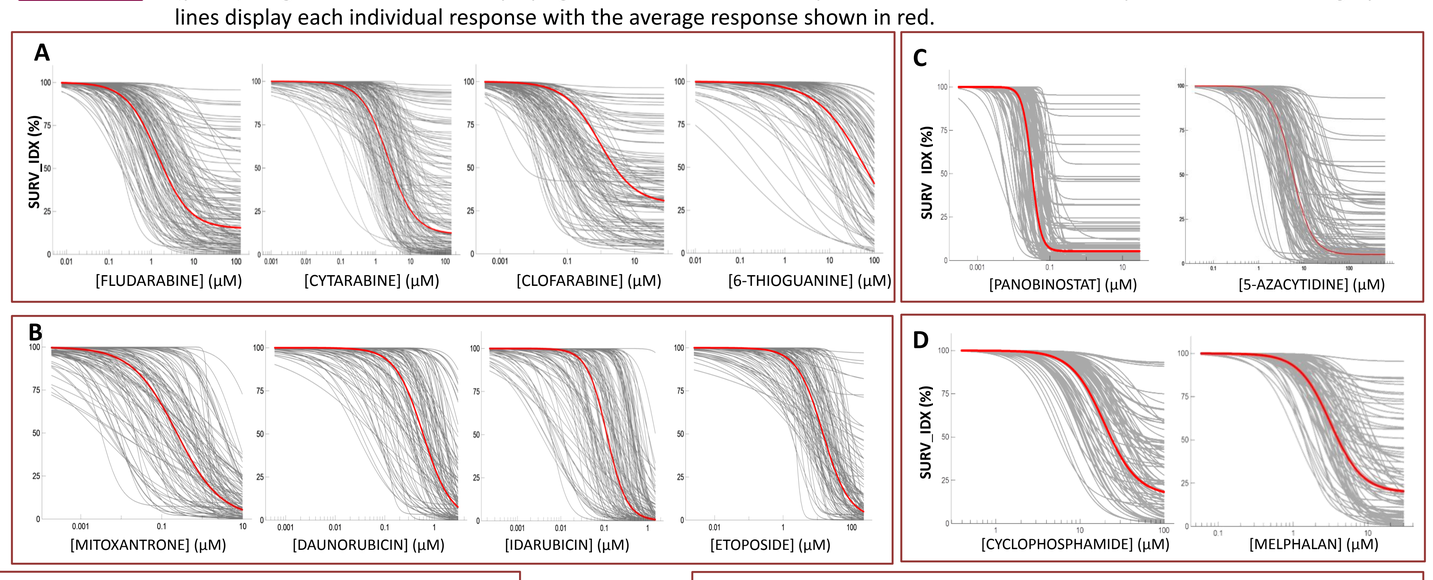
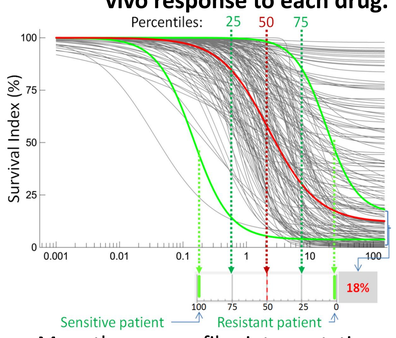
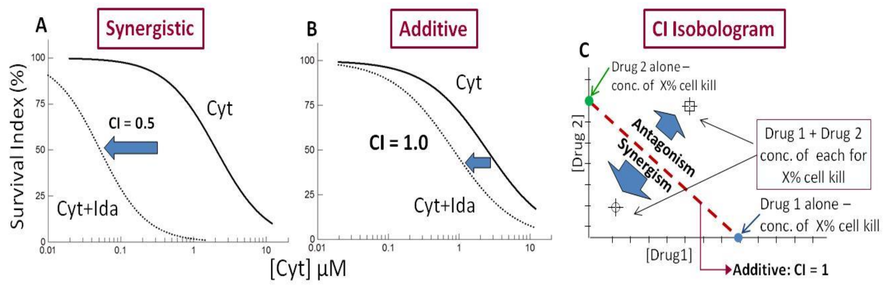



Figure 3 **Pharmacologic Profile of ex vivo response to each drug.**




Monotherapy profiles interpretation

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).



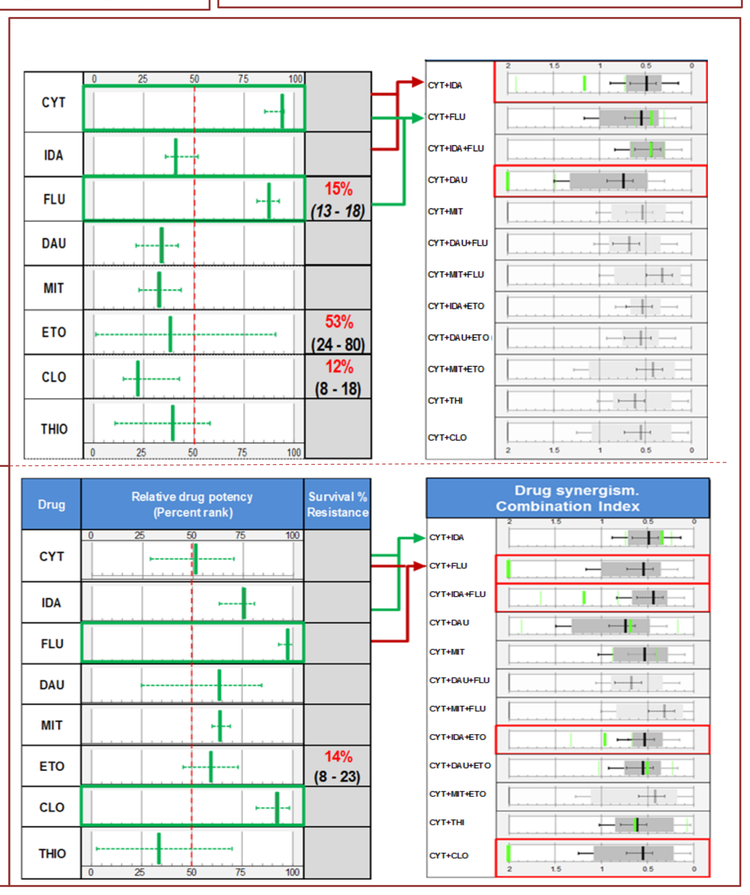



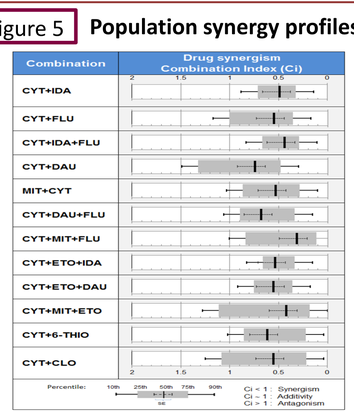

Table 1 **Pharmacological Population Parameters**

| SINGLE DRUG PHARMACOLOGY | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| DRUG | N | Efficacy ($E_{max}$) % Survival | | Potency ($EC_{50}$) µM | | IPV-$E_{max}$ | | IPV-$EC_{50}$ | |
| | | Typical | RE | Typical | RE | Typical | RE | Typical | RE |
| IDA | 125 | 0* | - | 0.106 | 0.016 | ne | | 157 | 0.15 |
| DAU | 109 | 0* | 0 | 0.592 | 0.13 | ne | | 135 | 0.17 |
| MIT | 110 | 0.5 | 0.2 | 0.233 | 0.22 | 1.2 | 0.69 | 221 | 0.14 |
| ETO | 110 | 0.1 | 0.1 | 18.5 | 0.13 | 0.3 | 0.41 | 141 | 0.17 |
| CYT | 125 | 11.8 | 4 | 2.28 | 0.13 | 32 | 0.21 | 105 | 0.25 |
| FLU | 125 | 15.0 | 3.2 | 1.43 | 0.19 | 25 | 0.24 | 113 | 0.41 |
| CLO | 122 | 29.0 | 5.1 | 0.92 | 0.019 | 36 | 0.2 | 142 | 0.25 |
| THIO | 86 | 0* | - | 62.2 | 0.23 | ne | | 204 | 0.27 |

| SYNERGY COMBINATION TREATMENT | | | |
|---|---|---|---|
| Combination | N | CI | |
| | | Median | SE |
| CYT+IDA | 99 | 0.486 | 0.14 |
| CYT+FLU | 82 | 0.548 | 0.24 |
| CYT+IDA+FLU | 69 | 0.441 | 0.13 |
| CYT+DAU | 65 | 0.743 | 0.26 |
| CYT+MIT | 24 | 0.533 | 0.20 |
| CYT+DAU+FLU | 8 | 0.677 | 0.27 |
| CYT+MIT+FLU | 14 | 0.317 | 0.09 |
| CYT+IDA+ETO | 26 | 0.535 | 0.16 |
| CYT+DAU+ETO | 22 | 0.551 | 0.23 |
| CYT+MIT+ETO | 13 | 0.421 | 0.32 |
| CYT+THIO | 7 | 0.617 | 0.35 |
| CYT+CLO | 53 | 0.552 | 0.44 |

Individual drug typical and random error values (left). Inter-patient variability (IPV) expressed as CV(%); Synergism (right) using the CI. *, estimate not significantly different from 0; ne, not estimated

Figure 5 **Population synergy profiles**




Synergy profiles of 12 drug combination treatments

Figure 6

**Pharmacological Profiles ex vivo of monotherapy (left) and treatment synergism (right) for 2 samples. Top**. Good sensitivities to CYT & IDA but no synergism → patient was resistant, while alternative CYT-FLU had all good parameters albeit 15% resistant cells. **Bottom**. Sample especially sensitive to FLU & CLO but treatments CYT-FLU and CYT-CLO have no synergy, while CYT-IDA parameter are all good (not best) → Patient was sensitive to CYT-IDA.

## 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

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