PHARMACOLOGICAL PROFILE OF CYTARABINE AND IDARUBICIN IN PATIENT SAMPLES (EX VIVO) WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA IDENTIFIES RESPONDERS VS NON RESPONDERS Pau Montesinos¹, David Martinez¹, Joaquín Martínez-López², Raimundo Garcia³, Jaime Pérez de Oteyza⁴, Pascual Fernandez⁵, Josefina Serrano⁶, Ángeles Fernández⁷, Pilar Herrera⁸, Arancha Alonso⁹, Ataulfo Gonzalez¹⁰, Concepción Bethancourt¹¹, Esperanza Lavilla¹², Juan Antonio Vera¹³, Begoña Navas¹⁴, Gabriela Rodríguez-Macías¹⁵, Juan Antonio López¹⁶, Santiago Jiménez¹⁷, Adriana Simiele¹⁸, Bernardo

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ABSTRACT

Background and objectives: Complete remission (CR) after induction therapy is the first treatment goal in acute myeloid leukemia (AML) patients. The aim of this study is to determine the ability of the Vivia's novel ex vivo drug sensitivity platform Exvitech analyzing leukemic cell death to predict the CR rates after induction chemotherapy with cytarabine (Ara-C) and idarubicin (Ida) in 1st line AML.

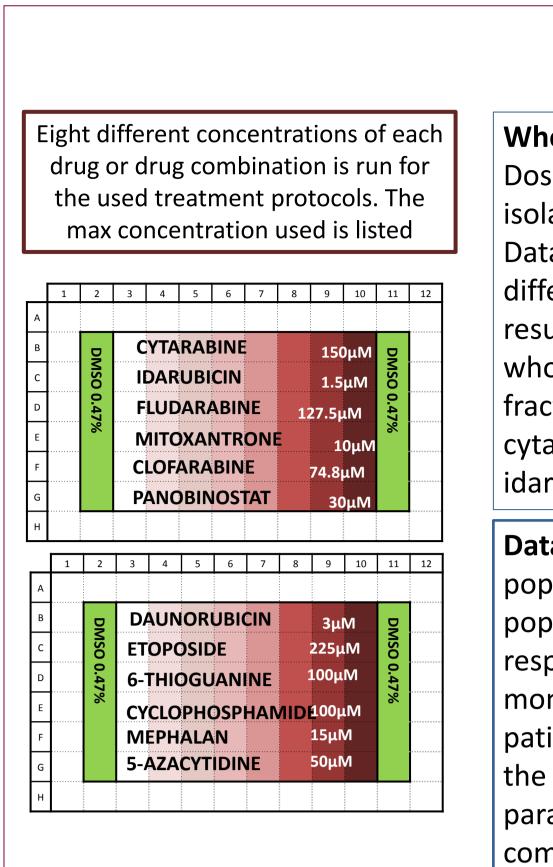
Patients and Methods: This non-interventional and prospective study included samples from patients over 18 years of age diagnosed with de novo AML in Spanish centers from the PETHEMA group. Marrow samples were collected at diagnosis, sent to the Vivia laboratories, and incubated for 48 hours in whole samples in well plates containing Ara-C, Ida, or the combination Ara-C + Ida, each at 8 different concentrations to calculate dose responses. Annexin V-FITC was used to quantify the drug-induced apoptosis. 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. The remaining patients were considered as resistant. Patients dying during induction response assessment were non-evaluable. The correlation between the pharmacologic parameters and the clinical response 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 of the model fitted values in the response scale for both groups. Their separation was quantified as the area under the ROC curve and a cut point was selected using the Youden's criterion to optimize the classification probabilities (sensitivity, specificity). 95% confidence intervals for sampling errors were calculated for all these quantifiers.

Results: 199 patient samples were used to calculate the dose response curves for Ara-C alone, Ida alone, and synergism of the Ara-C plus Ida combination. For clinical correlation we used 100 patients with a median age of 52 years (range 26 to 85). Dose responses for Ara-C alone are shown in Figure 3.A; note that for many samples there is a significant number (>XX%) 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 variables that included also the maximum percentage of cells depleted by Ara-C that yielded the best marker to separate both groups of patients. The overlap between the probability density functions of the fitted values was small (figure 5). The area under the corresponding ROC curve was 0.853 (0.773, 0.933) and the classification probabilities for the optimal cut point, expressed as percentages, were 81% (64% to 91%) and 80% (69% to 88%) for sensitivity and specificity, respectively. Results are shown in Figure 6; sixty-nine patients (69%) achieved CR after Ida + Ara-C, and the remaining 31 (31%) were resistant. When the ex vivo test predicted a patient as sensitive it was correct in 55/61 cases (90%) and when it predicted resistant it was correct 25/39 cases (64%). Overall, the clinical response of 80 patients (80%) was correctly anticipated.

Conclusions: This study shows that this novel ex vivo pharmacological profile test is able to predict the clinical response to Ida + Ara-C induction. Further efforts are in progress to refine the prediction model to remove as much as random variability as possible and to identify other sources of variability. A PM test-adapted Clinical Trial is planned to evaluate the impact of the PM test over clinical outcomes.

METHODS ExviTech[©] Platform Bioinformatics Results + Clinical Info Flow Flow **Screening Setup and Workflow Analysis and Import** into ActivityBase REPORT GENERATED Anti-CD14 Anti-CD64 Anti-CD34 Anti-CD13

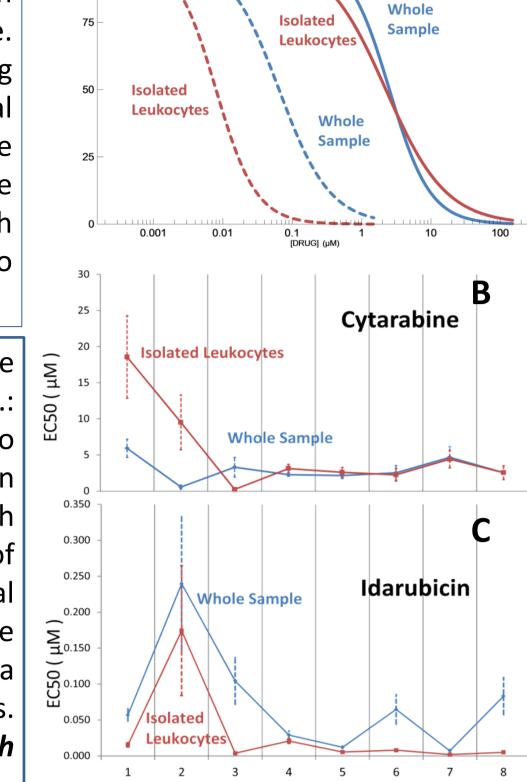
RESULTS



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.

METHODS

Data Analysis: performed using the population approach using NONMEM 7.2. population PD modelling of the ex vivo response vs concentration data monotherapy (fig.1), establishing for each patient the 95% prediction intervals (PI) of the isobologram from combination index using descriptors from combination experiments Chou and Talalay. 2010. Cancer Research 70: 440-446.



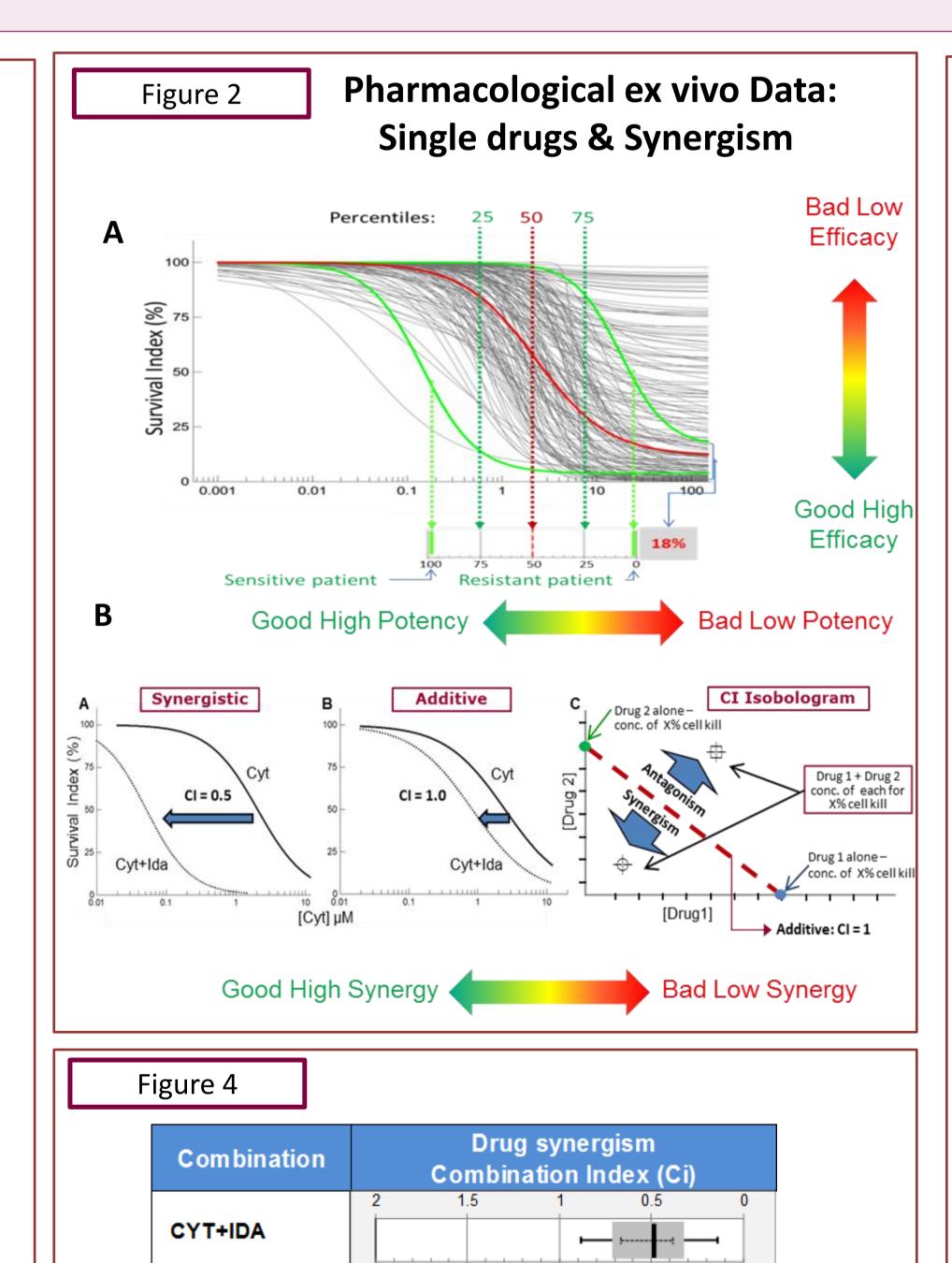
Objectives & Study Design

Background & Objectives

- Complete remission (CR) after induction is the first treatment goal in AML patients
- Response to chemotherapy is the main prognostic factor
- There is no test accurately predicting the response to specific drug schedules.
- The aim is to determine the ability of an exvivo drug sensitivity test to predict the clinical response to Ida+Ara-C (3+7) induction

Study Design

- Non-interventional and prospective study
- Samples from adult patients diagnosed with de novo AML in centers from the PETHEMA
- CR/CRi were classified as responders (vs. PR/resistance)
- Induction death non-evaluable
- 180 patient samples to calculate the dose response curves for Ara-C alone, Ida alone, and Ara-C plus Ida
- For clinical correlation, 63 patients (median age 54 years)



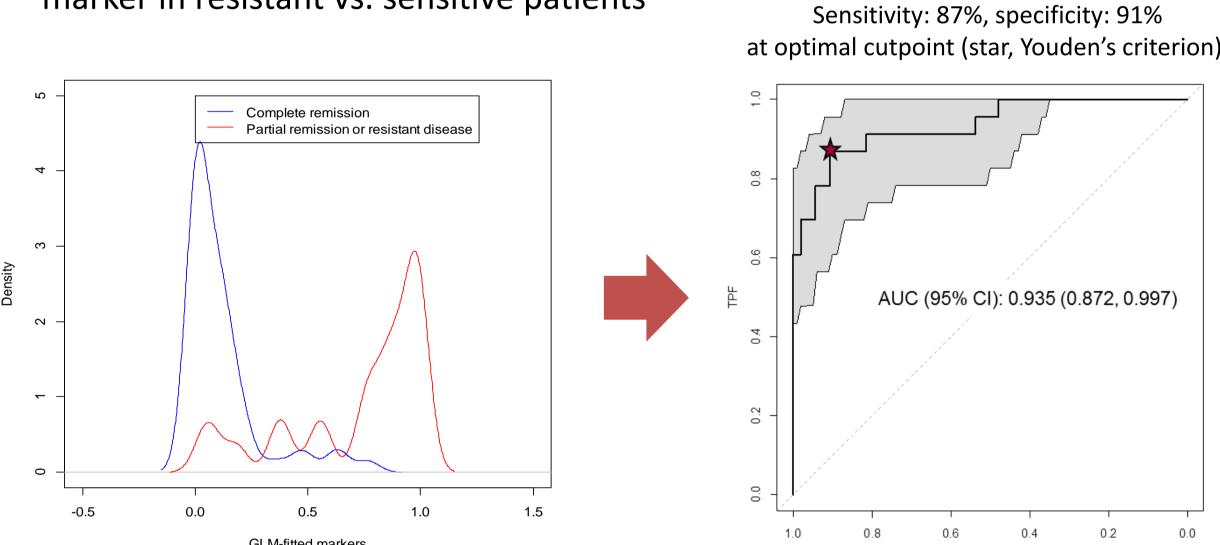
Individual Dose Response Curves Figure 3 [CYTARABINE] (µM) Dose-responses from 180 patient samples. The Survival Index (yaxis) ranges from 100% to 0 displaying the selective AML cell depletion calculated with PKPD Population Models. Median response shown in red. For CYT 40% patient samples have resistant cells left alive at 48 h. IDA eliminates all cells within this timeframe. SINGLE DRUG ex vivo PHARMACOLOGY CYT 125 11.8 4 2.28 0.13 32 0.21 105 0.25

Pharmacological Population Parameters

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

Logistic additive model of ex vivo CYT-IDA vs Clinical Outcome

ROC Curve Empirical probability distributions of the Statist. signif. low conf. limit AUC 0.872 > 0.5 marker in resistant vs. sensitive patients

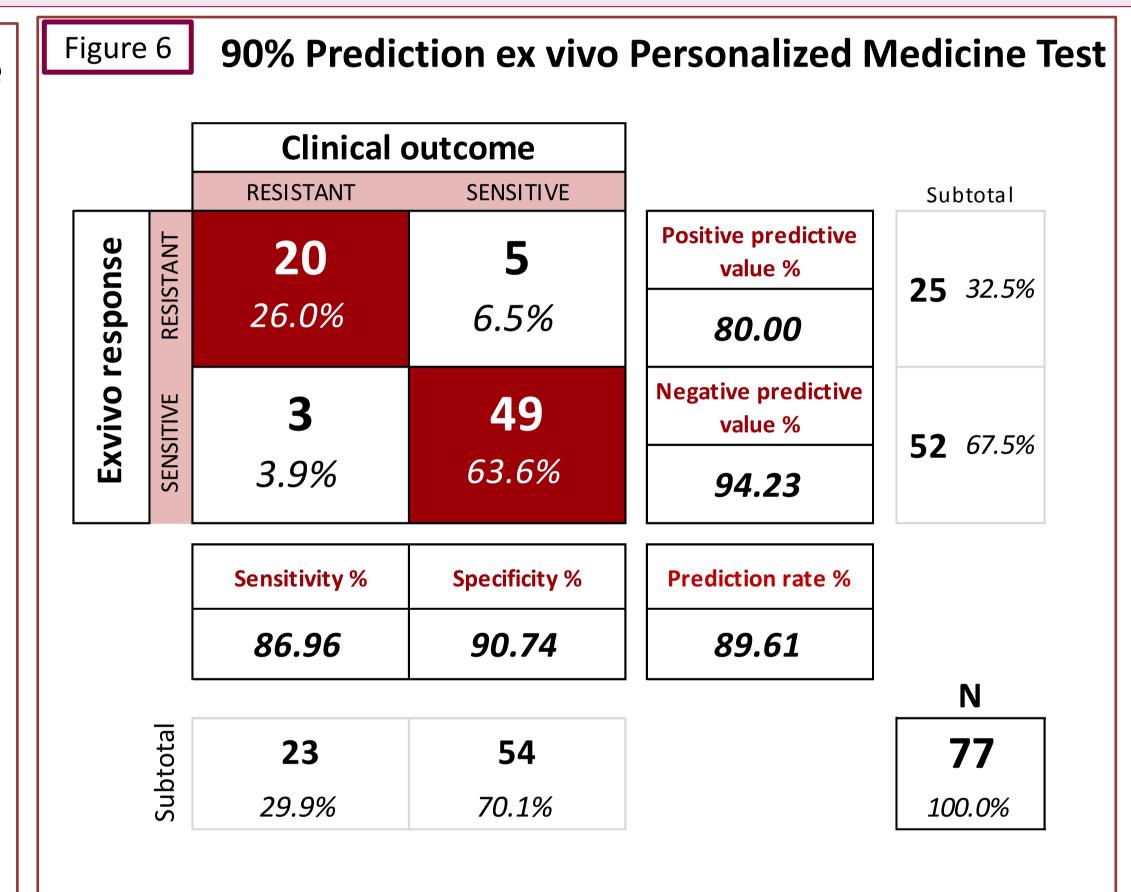


Polynomial function of CYT(EC50) & Synergism CYT-IDA(alpha) A generalized binary logistic additive model was used to explore nonparametric relationships between the fitted pharmacologic parameters and the dichotomized clinical

- response (resistant patient [PR or PD after induction] coded as 1 vs. sensitive patient [CR or CRi after induction] coded as 0). • Both linear dependence and nonlinear dependence structures were evaluated for available parameters (cytarabine E_0 , EC_{50} , I_{max} and sigmoidicity, idarubicin E_0 , EC_{50} and sigmoidicity, as
- well as an interaction index informing of the individual synergy/antagonism between these two drugs). Non-significant linear terms were discarded. Parameters without obvious nonlinearity in the smoothing component plots were discarded, as well • All linear terms were nonsignificant. Fifth order, quadratic and cubic polynomial dependences were found for cytarabine EC_{50} , the maximum number of depleted cells (I_{max})

and the interaction index, respectively. All model terms were significant, with the exception

of the smooth term of the interaction index. • The inclusion of the genetic group (favorable, intermediate, adverse) determined according to the standardized reporting criteria for reporting correlation of cytogenetic and molecular genetic data with clinical data (Döhner, 2010) somewhat improved the model predictive ability. However, since this variable was not informed in all cases, the available sample was



Key clinical indicators overall prediction 90% & NPV 94%

		Selected CI: 95%	
	Estimate	Lo	Hi
Sensitivity (Se):	87%	68%	95%
Specificity (Sp):	91%	80%	96%
Positive predictive value (PV+):	80%	63%	90%
Negative predictive value (PV-):	94%	85%	98%
Positive likelihood ratio (LR+):	9.39	4.01	21.97
Negative likelihood ratio (LR-):	0.14	0.05	0.41
Prevalence (res):	30%		

CONCLUSIONS

Distribution of CYT-IDA Synergism ex vivo across patient

population shown as Box-plots of calculated combination index

(Ci). This treatment as a tight distribution with high overall

- >This novel personalized medicine test may be able to predict the clinical response to Ida+Ara-C.
- >Potency(EC50) of CYT and synergism CYT-IDA are the predictive ex vivo variables in final algorithm. Though Efficacy
- (Emax) CYT also shows predictive value.
- > Validation cohort is ongoing and could achieve earliest validation by year end at N=100

synergism (0.5)

>Clinical trials demonstrating clinical benefits by using a personalized medicine test-adapted therapy are needed

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lower, thus reducing the model precision (data not shown).

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