EFFICIENT IDENTIFICATION OF RESPONSE TO CYTARABINE IDARUBICIN IN NEWLY DIAGNOSED AML BY A NEW INDIVIDUALIZED MEDICINE TEST BASED ON EX VIVO PHARMACOLOGY

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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 1stline 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 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 for both groups. Their separation was quantified as the area under the ROC curve and a cut point was selected using the Youden's criteria to optimize the classification probabilities (sensitivity, specificity). 95% confidence intervals for sampling errors were calculated for all these quantifiers.

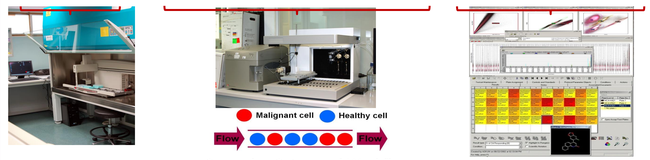
Results: 125patient samples were used to calculate the dose response curves for Ara-C alone, Ida alone, and synergism of the Ara-C plus Ida combination. For clinical correlation we used 64 patients with a median age of 55 years (range 31to72). Dose responses for Ara-C alone are shown in Figure 1.A; note that for many samples there is a significant number (>20%)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 2drugs. The generalized additive model identified an algebraic combination of these 2 variables that yielded the best marker to separate both groups of patients. The probability density functions had minimal overlap.The area under the corresponding ROC curve was 0.965 (0.928,1.000) and the classification probabilities for the optimal cut point (set at 0.414 for the marker)expressed as percentages, were 85% (62.1%to96.8%) and 86.4% (72.6%to94.8%) for sensitivity and specificity, respectively. Results are shown in Figure 1B; Forty-four patients(68.8%)achieved CR after Ida + Ara-C, and the remaining 20(31.3%)were resistant. Correlations of the PM test are shown in Figure 1B. Seventeen of the 20 (85%) patients who fail to achieve CR were predicted as resistance in the ex vivo test. Thirty-eight of the 44 patients(86.4%)who achieved CR showed good ex vivo sensitivity to Ida + Ara-C predicting for CR. When the ex vivo test predicted a patient as sensitive it was correct in 38/39 cases (93%) and when it predicted resistant it was correct 17/23 cases (74%). Overall, 45 patients (86%) had an accurate prediction of their response to treatment.

Conclusions: This study shows that this novel ex vivo pharmacological profile test is able to predict the clinical response to Ida + Ara-C induction. We are increasing the number of patients in this ongoing study, and we are planning a PM Test-adapted Clinical Trial.

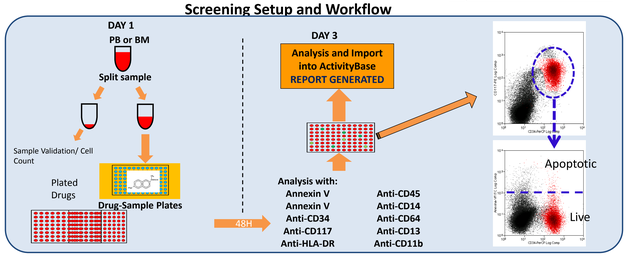
METHODS

ExviTech© Platform

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

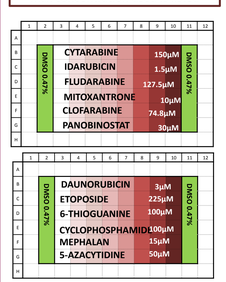


Screening Setup and Workflow

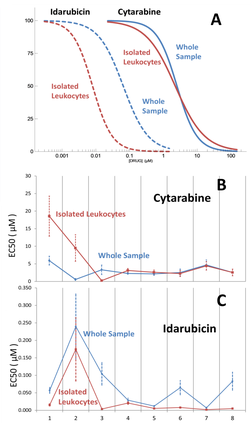


METHODS

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.1), 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 — 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 ex-vivo 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 group
- 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)

Figure 2 — Pharmacological ex vivo Data: Single drugs & Synergism

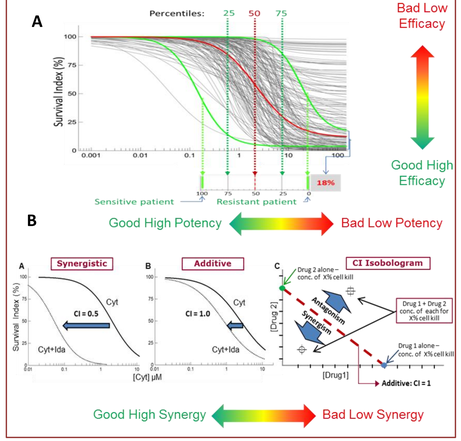
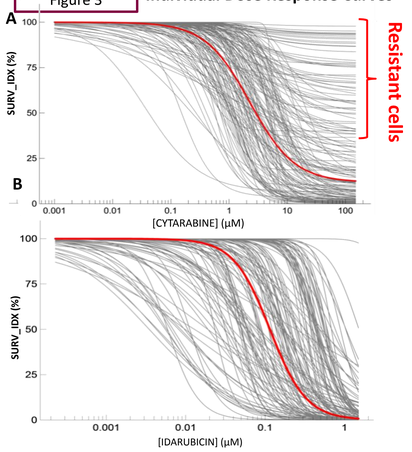


Figure 3 — Individual Dose Response Curves



Dose-responses from 180 patient samples. The Survival Index (y-axis) 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.

C Pharmacological Population Parameters

SINGLE DRUG ex vivo 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
CYT	125	**11.8**	4	**2.28**	0.13	**32**	0.21	**105**	0.25

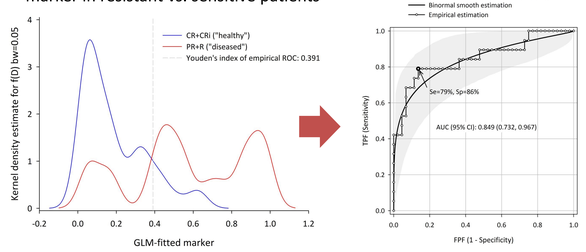
Individual drug typical and random error values (left). Inter-patient variability (IPV) expressed as CV(%); Synergism (right) using the CI. *, estimate not significantly different from 0; ne, not estimated

Figure 4

Combination	Drug synergism Combination Index (Ci)
CYT+IDA	2 1.5 1 0.5 0

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 synergism (0.5)

Figure 5 — Logistical Regression CYT-IDA *ex vivo* vs Clinical Outcome



Polynomial function of CYT(EC50) & Synergism CYT-IDA(CI)

- 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} and E_{max}, idarubicin E_0 and EC_{50}, and a combination 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. Quadratic and cubic polynomial dependences were found for cytarabine EC_{50} and the combination index, respectively. Both types of transformations were then modeled with a logistic regression to obtain a marker of response. Kernel density estimations were used to realize the empirical probability distributions of the marker in resistant vs. sensitive patients.
- The model classification performance was evaluated by calculating the area under the ROC curve of the classification probabilities (sensitivity, specificity) yielded by the marker. An optimal cutpoint was selected using the Youden's criterion, and the individual values of sensitivity and specificity were indicated with their 95% confidence intervals.

Figure 6 — 84% Prediction ex vivo Personalized Medicine Test

Ex vivo Prediction	Clinical Response: RESISTANT	Clinical Response: SENSITIVE	Subtotal
RESISTANT	**15** 23.8%	**6** 9.5%	Positive Predictive Value % **71.43** — **21** 33.3%
SENSITIVE	**4** 6.3%	**38** 60.3%	Negative Predictive Value % **90.48** — **42** 66.7%
Subtotal	**19** 30.2%	**44** 69.8%	N **63** 100.0%

Sensibility %	Specificity %	Correct Prediction %
78.95	86.36	84.13

Key clinical indicators (green) overall prediction 84% & NPV 91%

	Estimate	Selected CI: 95% Lo	Hi	
Sensitivity (Se):	79%	57%	91%	(Newcombe Stat Med 1998;17:857-72)
Specificity (Sp):	86%	73%	94%	(Newcombe Stat Med 1998;17:857-72)
Positive predictive value (PV+):	71%	53%	84%	
Negative predictive value (PV−):	90%	80%	96%	
Positive likelihood ratio (LR+):	5.79	2.66	12.62	(Simel y col. J Clin Epidemiol 1991;44:763-70)
Negative likelihood ratio (LR−):	0.24	0.10	0.59	(Simel y col. J Clin Epidemiol 1991;44:763-70)
Kappa:	0.63	0.43	0.84	(Fleiss y col. Statistical Methods for Rates and Proportions, third edition, pp. 598-608)
Prevalence (res):	30%			

Note about the results: a less parsimonious model that included linear terms some of which were nearly significant and a quadratic polynomial dependence for cytarabine E_{max} yielded slightly better results (area under the ROC curve 0.884). However, a detailed description of these results are not detailed because the predictive gain might be the result of model overparametrization, given that the available sample size was moderate (n = 63).

CONCLUSIONS

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

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