AN EX VIVO NATIVE ENVIRONMENT PRECISION MEDICINE TEST SHOWS HIGH CLINICAL CORRELATION WITH RESPONSES TO 1ST LINE ACUTE MYELOID LEUKEMIA TREATMENT

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ABSTRACT

Background: We have overcome the limitations of 40 years of ex vivo testing. The aim of this study is to determine the ability of Vivia's novel test (based on studying the ex-vivo sensitivity to drugs) to predict the complete remission (CR) rates after induction chemotherapy with cytarabine (Ara-C) and idarubicin (Ida) in 1st line AML..

Material and Methods: This has been an observational clinical trial where bone marrow samples from adult patients diagnosed with de novo AML in Spanish centers from the PETHEMA group were included. Whole marrow samples maintaining their Native Environment were incubated for 48h in well plates containing Ara-C, Ida, or their combination. Pharmacological responses are calculated using population models. Induction response was assessed according to the Cheson criteria (2003). Patients attaining a CR/CRi were classified as responders and the remaining as resistant.

Results: 390 patient samples were used to calculate the dose response (DR) curves for Ara-C alone, Ida alone, and their synergism. For clinical correlation we used 142 patients with median 56 years. The strongest clinical predictors were the Area Under the Curve (AUC) of the DR of Ara-C (P=1.34E-05), and the AUC of IDA (P=3.9E-05). The GAM models revealed a significant relationship (RSquare=0.452 and deviance explained=45%) between these predictors and higher probabilities of post-induction resistance.

Fig 1A shows a table illustrating the correlation between clinical outcome (columns) and the test predictions (lines). Using the cut off determined by the GAM models. The test obtain a high Specificity and Positive Protective Value (95% and 80,77%) and a lower sensitivity (50%) with a general prediction of a 81,69%. Interestingly, the 5 cases that the test identify as resistant but were clinically sensitive have high level of minimal residual disease. On the other hand, the test does not properly identify 21/142 that are clinically resistant and the test predicts as sensitive (bottom left quadrant right panel). This mismatched subgroup mimics the problems from molecular markers where a resistant clone present in a minority of leukemic cells cannot be detected yet drives the patient response. Consistent with this analysis, adding the cytogenetic risk factor to the ex vivo results, identifying the high risk population by molecular markers that might be present in a minority of the cells, significantly improves the correlation; Fig. 1B shows the 90% overall correlation achieved in 117 patient samples adding the cytogenetic risk factor, with a major improvement in the sensitivity from 50% to 72%. Both approaches lead to substantial improvements in estimated overall survival.

A. Ex Vivo Test without clinical information

Exvivo response	Clinical outcome: RESISTANT	Clinical outcome: SENSITIVE	Positive predictive value %	Subtotal
RESISTANT	21 (14,8%)	5 (3,5%)	81	26 (18,3%)
SENSITIVE	21 (14,8%)	95 (66,9%)	Negative predictive value % 82	116 (81,7%)
	Sensitivity % 50	Specificity % 95	Correlation rate % 82	N 142 (100,0%)
Subtotal	42 (29,6%)	100 (70,4%)		

B. Ex Vivo Test with Cytogenetic Risk Factors

Exvivo response	Clinical outcome: RESISTANT	Clinical outcome: SENSITIVE	Positive predictive value %	Subtotal
RESISTANT	23 (19,7%)	3 (2,6%)	88	26 (22,2%)
SENSITIVE	9 (7,7%)	82 (70,1%)	Negative predictive value % 90	91 (77,8%)
	Sensitivity % 72	Specificity % 96	Correlation rate % 90	N 117 (100,0%)
Subtotal	32 (27,4%)	85 (72,6%)		

METHODS

ExviTech© Platform

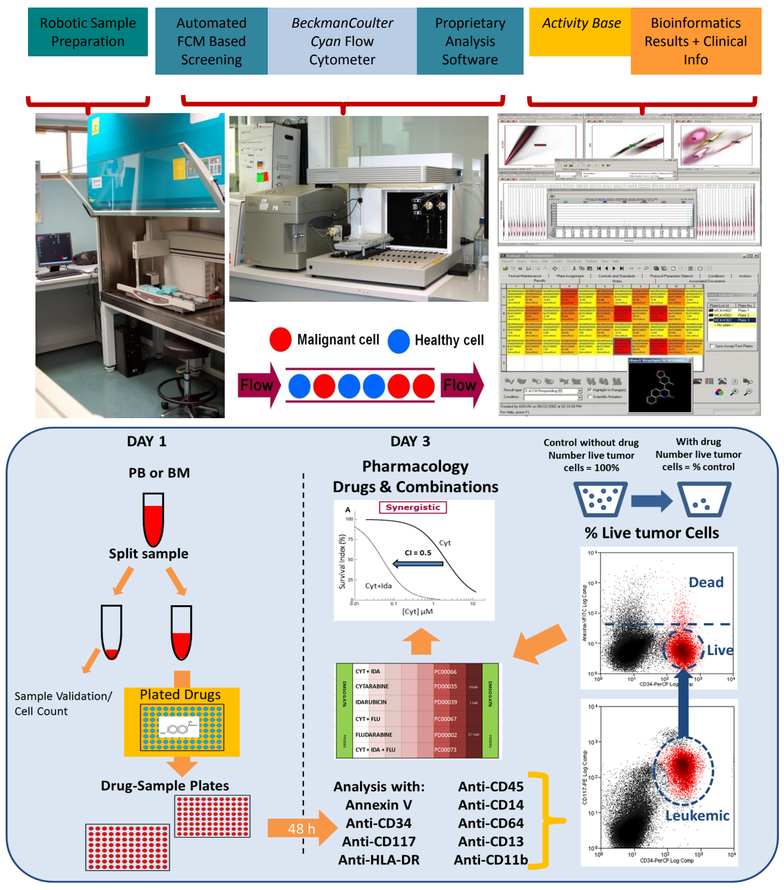
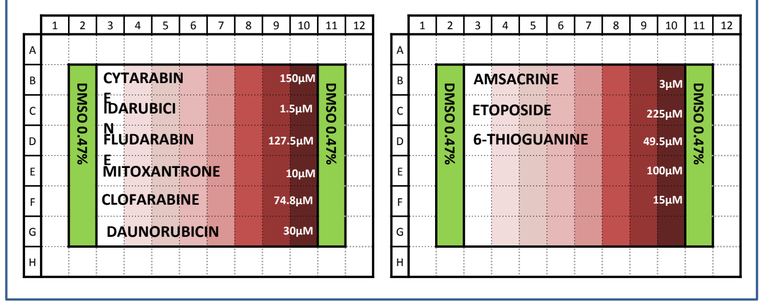


Figure 1

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. Correlation pairs showing differences among EC50 values from the same samples tested either as isolated leukocytes or whole sample. Error bars show the CI's of the estimated parameter. B. Dose-response curves for IDA and Cyta for the selected samples in both conditions, showing similar results form Cytarabine but very different for Idarubicin.

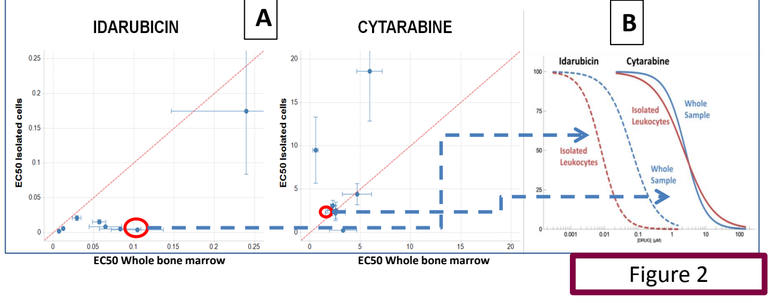


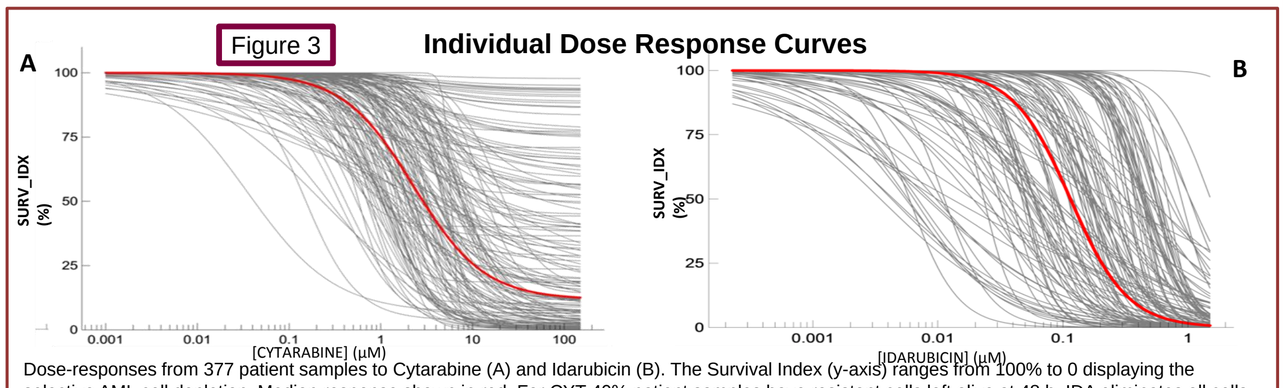
Figure 2

Data Analysis: performed using the population approach using NONMEM 7.2.:

- population PD Hill-based modelling of the ex vivo response vs concentration data in monotherapy (fig.2), 95% confidence interval of estimated parameters determined by bootstrapping over 1000 simulations.
- Surface interaction modelling and simulations to estimate the interaction parameter (α) as well as the corresponding confidence interval. α parameter is a measurement of synergism (>0), additivity (0) or antagonism (<0). ***Greco et al.1995.*** Pharmacol Rev June 1995 47:331-385

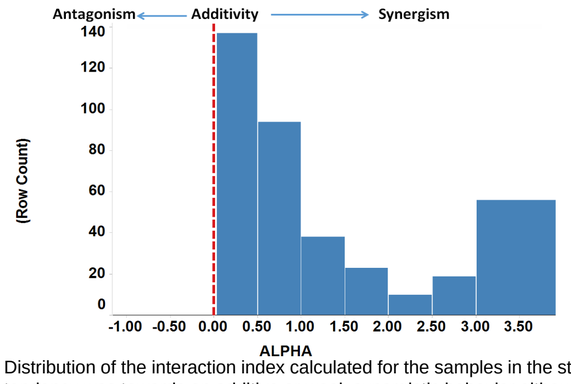
RESULTS

Figure 3 **Individual Dose Response Curves**



Dose-responses from 377 patient samples to Cytarabine (A) and Idarubicin (B). The Survival Index (y-axis) ranges from 100% to 0 displaying the selective AML cell depletion. 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.

Figure 4 **Interaction index**



Distribution of the interaction index calculated for the samples in the study. The central tendency was towards an additive or weak synergistic behavior although clear synergy occurred in many cases.

Figure 5 **Pharmacological Population Parameters**

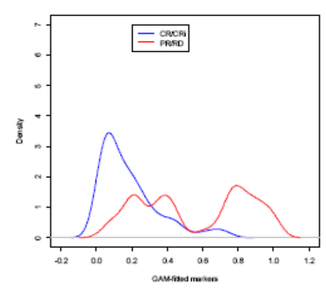
SINGLE DRUG *ex vivo* PHARMACOLOGY									
DRUG	N	Efficacy (E_{max}) % Survival		Potency (EC_{50}) µM		IPV- E_{max} %		IPV- EC_{50} %	
		Typical	RSE (%)	Typical	RSE (%)	Typical	RSE (%)	Typical	RSE (%)
CYT	377	**8.5**	1.3	**2.87**	8.1	**645**	9.1	**161**	8.4
IDA	377	**0**	-	**0.032**	9.4	ne		**207**	8.4

Individual drug typical and relative standard error values. Inter-patient variability (IPV) expressed as CV(%); ne, not estimated

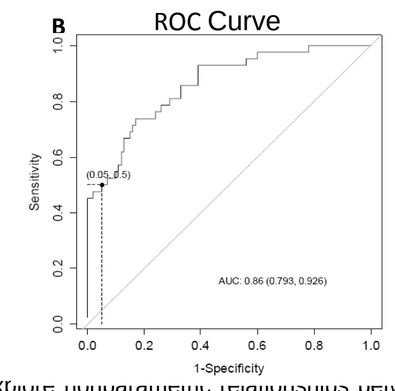
Figure 6 **Logistic additive model of *ex vivo CYT-IDA* vs Clinical Outcome**

A

Empirical probability distributions of the marker in resistant vs. sensitive patients



B ROC Curve



- A generalized binary logistic additive model was used to explore nonparametric relationships between either the fitted pharmacologic parameters and processed response values and the dichotomized clinical response (resistant patient [PR or PD after induction] vs. sensitive patient [CR or CRi after induction]).
- Both linear dependence and nonlinear dependence structures were evaluated for available PD parameters (cytarabine E_0, EC_{50}, I_{max} and sigmoidicity, idarubicin E_0, EC_{50} and sigmoidicity, and the interaction parameter (α)) as well as integrated terms given by the calculation of the area under the curve (AUC) for both cytarabine and Idarubicin and the volume under the surface (VUS) from the interaction analysis from the combination.
- All linear terms were non-significant. Results using individual parameters were improved by the AUCs of the modelled effect-concentration curves of both, Idarubicin and, particularly, Cytarabine which showed good predictive properties. In a lower magnitude, VUS values also showed significant predictive ability. No significance though was observed for the interaction parameter.
- The variation of the cell viability in control wells before and after incubation provided additional predictive ability: the probability of response is higher for those patients for whom cell viability does not change or changes by a small amount (cell viability decreased by 40% or lower) during incubation.
- Using a criterion based on equalling the predictive values (PV+ and PV-) to set the cut point which defines positive and negative test results is a reasonable approach to prioritize specificity over sensitivity in an objective and reproducible fashion.

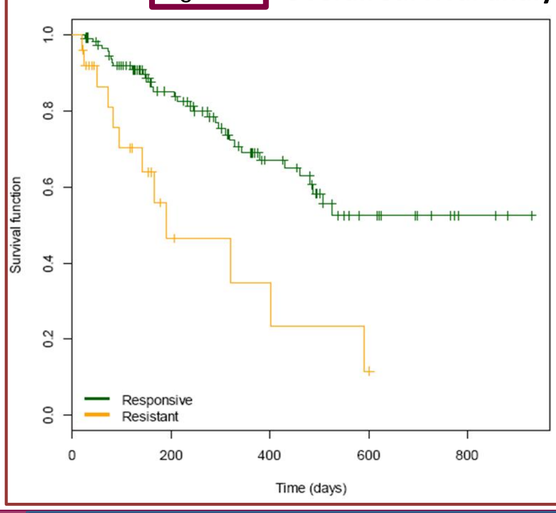
Figure 7 **Correlation results summary**

Exvivo response	Clinical outcome: RESISTANT	Clinical outcome: SENSITIVE	Positive predictive value %	Subtotal
RESISTANT	21 (14.8%)	5 (3.5%)	80.77	26 (18.3%)
SENSITIVE	21 (14.8%)	95 (66.9%)	Negative predictive value % 81.90	116 (81.7%)
	Sensitivity % 50.00	Specificity % 95.00	Prediction rate % 81.69	N 142 (100.0%)
Subtotal	42 (29.6%)	100 (70.4%)		

Key clinical indicators: overall prediction 81.7% & NPV 81.9%

	Estimate	Selected CI: 95% Lo	Hi
Sensitivity (Se):	50%	36%	64%
Specificity (Sp):	95%	89%	98%
Positive predictive value (PV+):	81%	63%	91%
Negative predictive value (PV−):	82%	77%	86%
Positive likelihood ratio (LR+):	10.00	4.04	24.75
Negative likelihood ratio (LR−):	0.53	0.39	0.71
Kappa:	0.51	0.35	0.67
Prevalence (res):	30%		

Figure 8 **Overall survival analysis.**



The survivor functions (Kaplan-Meier) of the overall survival (OS) of patients classified as responsive or resistant using the optimal cut point over the GAM-derived marker were clearly different. The OS was much shorter in patients classified as resistant than in patients classified as responsive.
This difference was highly significant (p=0.0002)

CONCLUSIONS

- This novel test is able to predict the clinical response to Ida+Ara-C induction with 82%, significantly higher than the current clinical response rate of 66.7%. The test did not properly identify 21/142 that were clinically resistant and the test predicted as sensitive. This mismatched subgroup mimics the problems from molecular markers where a resistant clone present in a minority of leukemic cells cannot be detected yet drives the patient response. However, this group mismatch does not prevent a good correlation with the test predicted outcomes.
- Good predictive capabilities were identified for dose-effect area under the curve variables.
- No statistical significance with the clinical outcome was found for the interaction index from the drugs combination analysis.
- Very significant separation was found in the overall survival analysis between the two branches of responsive and resistant cases according to the test results.

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