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 and aim: 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 first 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 155 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. Figure 4 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 83.3%) and a lower sensitivity (53.2%) with a general prediction of a 82.58%. 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 22/155 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.

METHODS

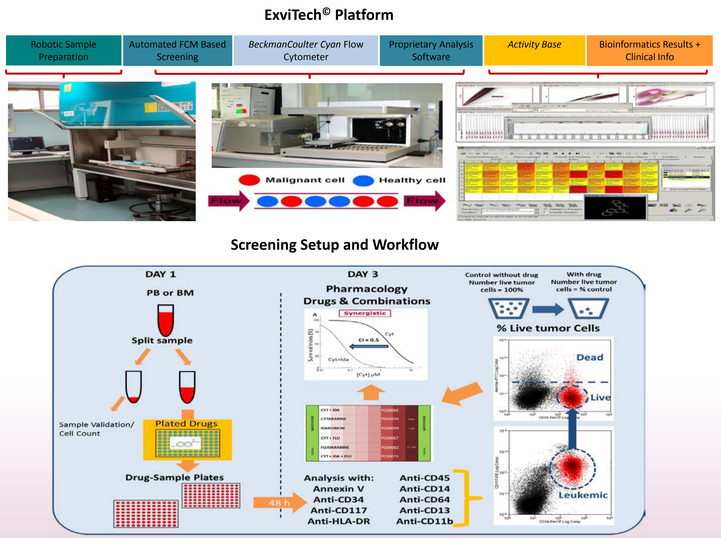
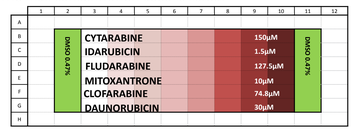
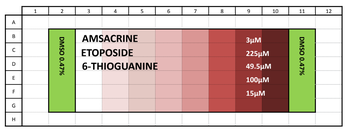


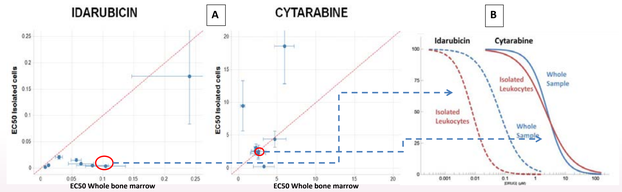
FIGURE 1.

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 for Cytarabine but very different for Idarubicin.

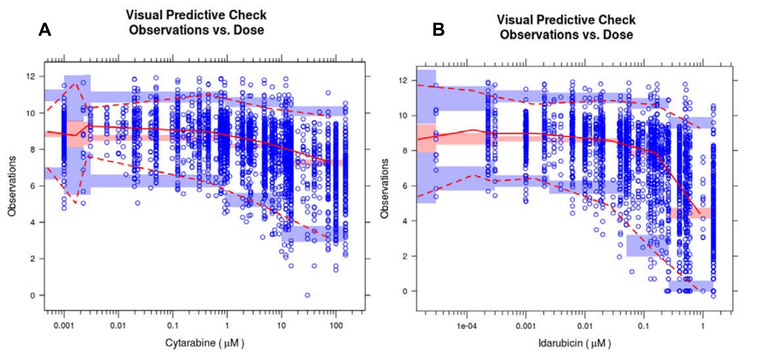


Data Analysis: performed using the population approach using NONMEM 7.2:

- 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

Pharmacological Population Parameters



Single Drugs

Parameter (units)	Cytarabine	Idarubicin
LPC_0 (cells)	6821 (0.05)	5992 (0.05)
I_{MAX} (unitless)	1 (0)	1 (0)
IC50 (µM)	7.42 (0.12)	0.16 (0.07)
γ(unitless)	0.747 (0.01)	2.619 (0)
IPV_LPC_0 [CV(%)]	108 (0.05)	111 (0.06)
IPV_I_{MAX} [CV(%)]	2 (0)	2 (0)
IPV_IC50 [CV(%)]	483 (0.09)	133 (0.07)
IPV_γ[CV(%)]	3 (0)	3 (0)
Residual Error [log(µM)]	0.258 (0.04)	0.439 (0.03)
IPV_Residual Error [CV(%)]	41 (0.05)	37 (0.06)

Drug Combination

Parameter (units)	Cytarabine+Idarubicin
α (unitless)	0.4 (0.25)
IPV_α [CV(%)]	387 (0.17)
Residual Error [log(µM)]	0.506 (0.09)
IPV_Residual Error [CV(%)]	56 (0.2)

FIGURE 2. Visual predictive check (VPC) from Cytarabine (A) and Idarubicin (B). Open circles are the observed data points; the solid red line represents the median observed Log10 (Cells) and the semi-transparent red field represents a simulation-based 95% confidence interval for the median. The observed 5% and 95% percentiles are presented with dashed red lines, and the 95% confidence intervals for the corresponding model predicted percentiles are shown as semi-transparent blue fields

TABLE 1. Population ex vivo Pharmacodynamic parameters estimates. Parameters typical and random (variability and residual error) are shown together with the corresponding relative standard error calculated as the ratio between the standard error provided by NONMEM and the estimate. Estimates of inter-patient variability (IPV) are expressed as coefficient of variation (%).

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

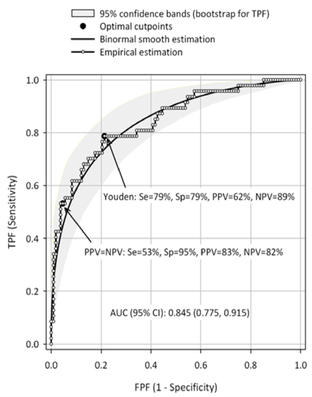


FIGURE 3. A generalized binary logistic additive model was used to explore nonparametric relationships between either the fitted pharmacologic parameters and processed response values and the dichotomized clinical response (resistant patient [PR or PD after induction] vs. sensitive patient [CR or CRi after induction]).

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. No significance though was observed for the interaction parameter. 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.

Correlation results summary

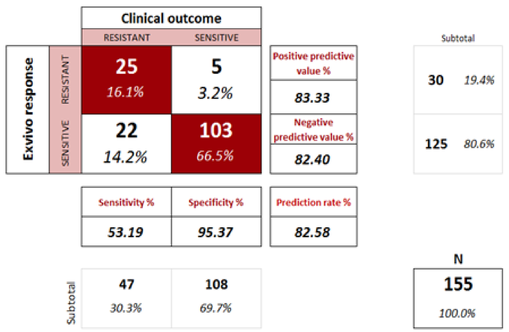


FIGURE 4. Correlation results summary from the AML patients included in the study.

Overall survival analysis

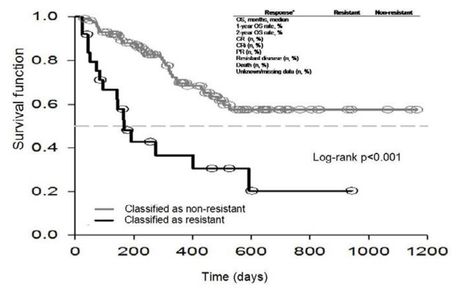


FIGURE 5. The survivor functions (Kaplan-Meier) of the overall survival (OS) of patients classified as responsive or resistant using the optimal cut point over the GAM-derived marker were clearly different. The OS was much shorter in patients classified as resistant than in patients classified as responsive. 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 overall correlation and predictive values of 82.5%, higher than ever achieved. Considering this result and current clinical response rate of 66.7% (66.5% in this study), clear benefits can be achieved with the use of the test.
- 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.
- The test predicts with a high significance (p=0.002) overall survival when patients are classified at diagnosis as resistant or sensible.
- This novel test may be valuable information to guide first line patient treatment.

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