Precision Medicine Test is Similar But Faster Than Conventional Cytogenetics Predicting Response in AML Patients and Provides Alternative Treatments

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INTRODUCTION & OBJECTIVE

- Cytogenetic analysis is still an important and mandatory component of Acute Myeloid Leukemia (AML) diagnosis and prognosis. Pretreatment cytogenetic and molecular genetic findings are one of the major independent prognostic markers in AML, and they determine chemotherapy response and outcome. However, cytogenetic does not provide alternative treatments when a patient have a high cytogenetic risk, and requires relatively long time until obtaining the results despite the treatment of these patients should begin as soon as possible.
- The aim of this study is providing data about the utility of a new AML Precision Medicine (PM) Test as a complementary tool to conventional cytogenetic to overcome the main obstacles this later has.

MATERIAL & METHODS

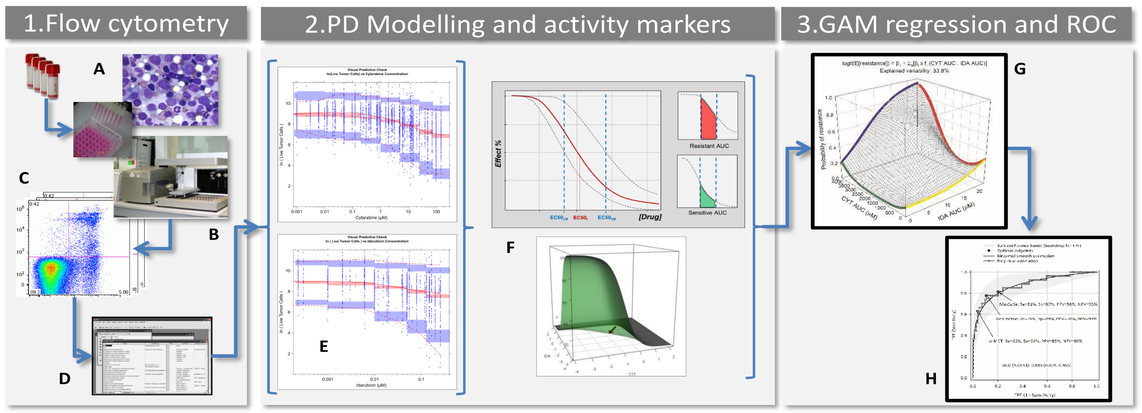


Fig 1. Sequential workflow of experimental (1) and analytical (2 & 3) methods applied in the study. Whole bone marrow samples [A] were incubated preserving the native microenvironment with drugs and drugs mixtures. Automated flow cytometry [B] followed by dot-plots analysis [C] allowed the counting after incubation of Live Pathologic Cells (LPC) at control wells and wells with increasing drugs concentrations. Data was uploaded into the LIMS system. Response vs drug concentration relationships were analyzed through non-linear mixed effect population modelling [E]. Predicted pharmacodynamic profiles were integrated between the 80% confidence interval of the individual estimate of EC_{50} in order to calculate the area under the curve (AUC) used as a single activity marker. Similarly, a double integration of the two-variables interaction surface function allows the calculation of the volume under the surface (VUS) that is effected by the sign (synergy or antagonism) of the interaction [F]. Correlation of activity markers with clinical output was analyzed by Generalized Additive Models (GAM) [G] and ROC curves [H]

RESULTS

Visual predictive check of the population pharmacokinetic models of cytarabine and idarubicin

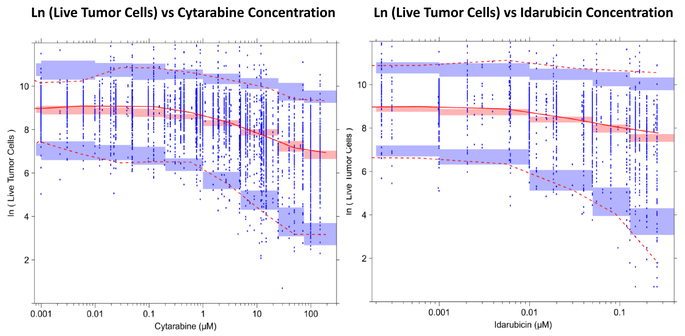


Fig 2. Open circles are the observed data points, the solid and dashed red lines are, respectively, the median and the 5-95th percentiles of the observed distribution of ln(cells), and the semitransparent red and blue bands represent, respectively, the simulation-based 95% confidence intervals for the median and 5-95th percentiles of the estimated population distribution of ln(cells).

Regression hyperplane of the predicted probability of resistance over the AUCs of cytarabine and idarubicin

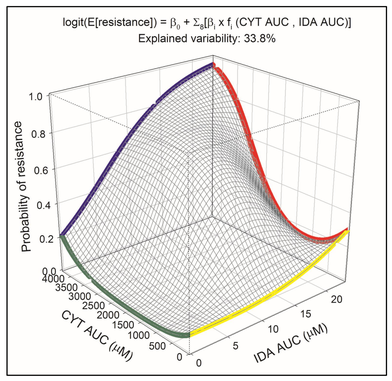
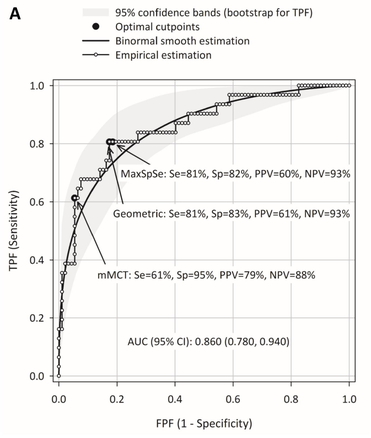


Fig 3. The AUCs are a summary of pharmacodynamic parameters such that the higher the AUC the lower the cytotoxic effect (efficacy or potency) of the drug. The regression hyperplane has been obtained using bi-dimensional smooth functions in a binary logistic GAM

RESULTS

Empirical and smoothed (binormal) ROC curves of the probability of resistance obtained in the binary logistic GAM



B

Prediction \ Clinical outcome	RESISTANT	SENSITIVE	Positive predictive value %	Subtotal
RESISTANT	25 20%	17 14%	60	42 34%
SENSITIVE	6 5%	75 61%	Negative predictive value % 93	81 66%
Sensitivity %	81	Specificity % 82	Correlation rate % 81	N 123 100%
Subtotal	31 25%	92 75%		

	Estimate	Selected CI: 95% Lo	Hi
Sensitivity (Se):	81%	64%	91%
Specificity (Sp):	82%	72%	88%
Positive predictive value (PV+):	60%	48%	70%
Negative predictive value (PV-):	93%	86%	96%
Positive likelihood ratio (LR+):	4.36	2.75	6.93
Negative likelihood ratio (LR-):	0.24	0.11	0.49
Kappa:	0.56	0.40	0.71

Fig 4. A) Open circles are the pairs of sensitivity and 1−specificity values at the estimated discrete individual values of the probability of resistance (used as a marker to classify the patients as responder or resistant), the solid large circles represent the pairs of sensitivity and 1−specificity values at the selected cut-points that were obtained with each of the three criteria: 'MaxSpSe' selects the point that maximizes both, the sensitivity and the specificity; 'Geometric' selects the closest point to the (1,0) coordinate (left upper corner of the [sensitivity,1−specificity] plane); and 'mMCT' selects the point that minimizes a misclassification cost term that assigned a greater cost to false positives than to false negatives (prioritizes specificity over sensitivity). B) Confusion matrix for MaxSpSe cutoff. AUC: area under the curve, CI: confidence interval, FPF: false positive fraction, NPV: negative predictive value, PPV: positive predictive value, Se: sensitivity, Sp: specificity, TPF: true positive fraction.

Kaplan-Meier plots of overall survival

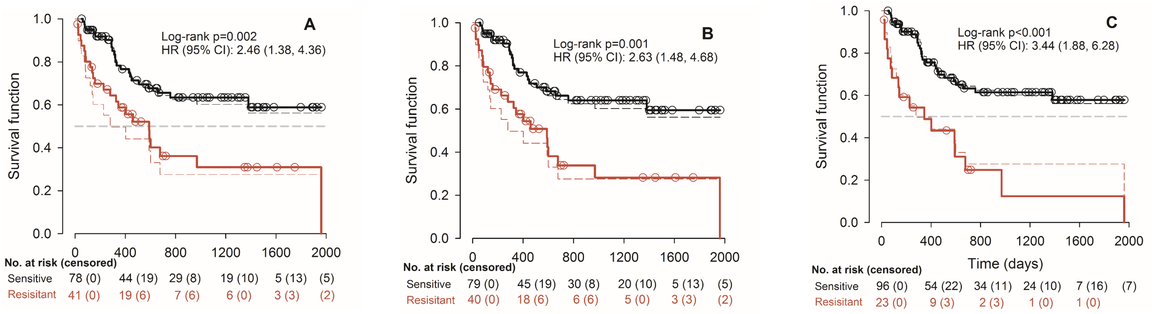
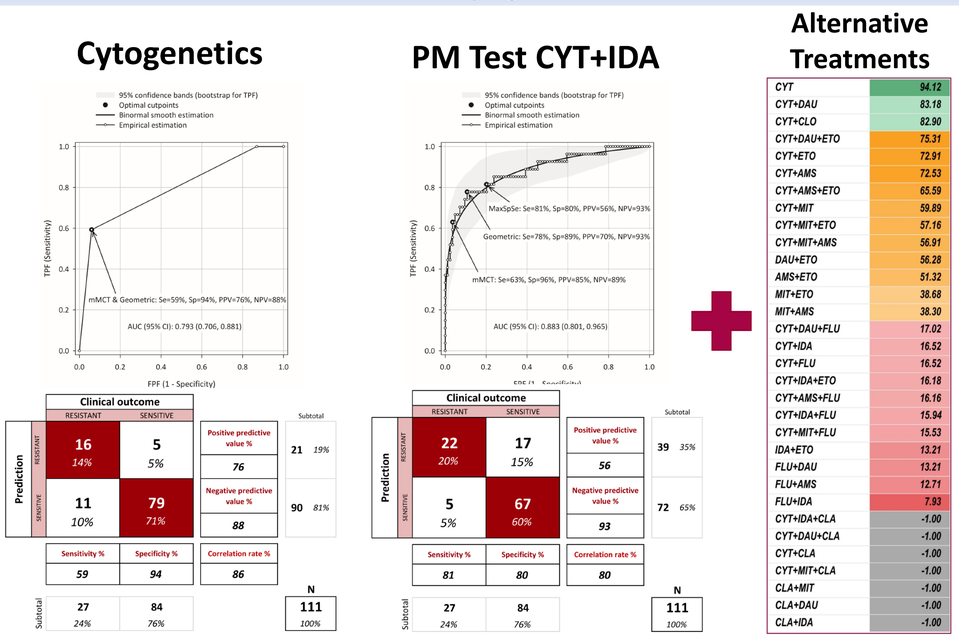


Fig 5. The three panels depict the pairs of survival functions for patients classified as responder (solid black lines) and resistant (solid red lines) according to the cut-points of the estimated probability of resistance that were obtained with each of the three criteria (panel A: 'MaxSpSe', panel B: 'Geometric', and panel C: 'mMCT'). The dashed lines represent the survival functions of clinical responders (black lines) and resistant patients (red lines). The hazard ratios of death were obtained from a Cox regression model that used the patients who were predicted to be responder as the reference category (patients predicted to be resistant over patients predicted to be responder). CI: confidence interval, HR: hazard ratio

Clinical correlation of cytogenetics vs PM Test



Prediction \ Clinical outcome	RESISTANT	SENSITIVE	Positive predictive value %	Subtotal
RESISTANT	16 14%	5 5%	76	21 19%
SENSITIVE	11 10%	79 71%	Negative predictive value % 88	90 81%
Sensitivity % 59		Specificity % 94	Correlation rate % 86	N 111 100%
Subtotal	27 24%	84 76%		

Prediction \ Clinical outcome	RESISTANT	SENSITIVE	Positive predictive value %	Subtotal
RESISTANT	22 20%	17 15%	56	39 35%
SENSITIVE	5 5%	67 60%	Negative predictive value % 93	72 65%
Sensitivity % 81		Specificity % 80	Correlation rate % 80	N 111 100%
Subtotal	27 24%	84 76%		

Alternative Treatments	
CYT	94.12
CYT+DAU	83.18
CYT+CLO	82.90
CYT+DAU+ETO	75.31
CYT+ETO	72.91
CYT+AMS	72.53
CYT+AMS+ETO	65.59
CYT+MIT	59.89
CYT+MIT+ETO	57.16
CYT+MIT+AMS	56.91
DAU+ETO	56.28
AMS+ETO	51.32
MIT+ETO	38.68
MIT+AMS	38.30
CYT+DAU+FLU	17.02
CYT+IDA	16.52
CYT+FLU	16.52
CYT+IDA+ETO	16.18
CYT+AMS+FLU	16.16
CYT+IDA+FLU	15.94
CYT+MIT+FLU	15.53
IDA+ETO	13.21
FLU+DAU	13.21
FLU+AMS	12.71
FLU+IDA	7.93
CYT+IDA+CLA	-1.00
CYT+DAU+CLA	-1.00
CYT+CLA	-1.00
CYT+MIT+CLA	-1.00
CLA+MIT	-1.00
CLA+DAU	-1.00
CLA+IDA	-1.00

Fig 6. Comparison between clinical correlation of cytogenetics (left) and PM Test (right), on a cohort of 111 patients sharing both results. ROC curves (top) and confusion matrices for MaxSpSe cutoff (down). Deviance explained is 29.4% for cytogenetics, and 40.9% for PM test. Figure 6 (right) also shows an example of the classification of AML treatments with the PharmaFlow PM Test in a patient sample according to a color scale from higher (green) to lower (red) ex vivo activity.

CONCLUSIONS

- PharmaFlow PM Test and cytogenetics provide similar information
- Results from this novel PM Test are available in 72h, prior to treatment, while results from cytogenetic risk are available typically in 10-14 days, and thus after patient treatment.
- This novel approach provides information to hematologist with higher predictive value than risk factor (deviance explained 40.8% vs 29.4%) and ahead of treatment.
- PharmaFlow PM Test represents valuable in-time information, prior to treatment decision making.
- In addition, PM Test can provide alternative treatments to AML patients based on their ex vivo activity

*CONFLICT OF INTEREST DISCLOSURE: JB, PHC, DP, JG and JLR are Vivia Biotech employees