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

ABSTRACT

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 of the PETHEMA group were included. Whole marrow samples were maintained at room temperature for 24 hours before preparation for ex vivo studies. The ex vivo sample was inoculated into 2 ml of a fibrin plug containing the patient’s own bone marrow. The suspension was then incubated for 72 hours at 37°C and 5% CO2 in pH 7.4. After incubation, the cell suspension was filtered through a 30 µm filter to remove the fibrin plug and the viability of the remaining viable cells was determined using the Trypan Blue exclusion method. The cell suspension was then analyzed by flow cytometry to assess the cytotoxic effect of different concentrations of drug combinations by evaluating the percentage of cells with 40% or lower viability.

RESULTS

390 patient samples were used to calculate the dose response (DR) curves for Ara-C alone, Ida alone, and their combination. 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 (R²=0.452, p<0.05) between these predictors and higher probabilities of post-induction remission. 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 obtains a high Specificity and Positive Predictive Value (95% and 83.3%) and a lower sensitivity (52.5%) with a general prediction of 72.5%. Interestingly, the 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 mismatch 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.

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 96.7% (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 as diagnosis at resistant or sensible.
• This novel test may be valuable information to guide first line patient treatment.

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FIGURE 1.
Plate setup. Eight different concentrations of each drug or drug combination is run for the used treatment protocols. The measurement units are shown in Figure 2.

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

FIGURE 3.
Drug combination clinical outcome. A total of 155 patients were included in the study (Ara-C alone n=40, Ida alone n=40, and Ara-C + Ida n=75). The cut off determined by the GAM models is shown in the lower right quadrant. The vertical line is the cut off of Ara-C alone and the horizontal line is the cut off of Ida alone. A high predictive capability was identified for dose-effect area under the curve variables. No statistical significance was observed when using the clinical outcome as the variability of the patient responses to chemotherapy. The dichotomized clinical response (resistant patient [PR or PD after induction] vs. sensitive patient [CR or CRi after induction] ).

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

Overall survival analysis

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

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