**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 ViVA'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 PETHEM group were included. Whole marrow samples maintaining their Natural Environment were incubated for 4h 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=1.346-05), and the AUC of Ida (P=9.38-05). The GAM models revealed a significant relationship (R2=0.48-0.49) 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 lacks a High Specificity and Positive Predictive Value (58% and 86.77%) and a lower sensitivity (50%) with a general prediction of 8,169%. Interestingly, the 5 cases that the test classified 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. On the other hand, the test lacks a High Specificity and Positive Predictive Value (58% and 86.77%) and a lower sensitivity (50%) with a general prediction of 8,169%. Interestingly, the 5 cases that the test classified 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.

**RESULTS**

- A generalized binary logistic additive model was used to test the hypothesis that 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_B, E_C, IBD, and signature, idarubicin E_B, E_C, and signature, and the interaction parameter (s) 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 for both, idarubicin and, particularly, cytarabine which showed good predictive properties. In a lower magnitude, VUS values showed significant predictive ability.
- No significance though was observed for the interaction parameter.
- The variation of the cell viability in controls 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 equalizing the predictive values (FV- 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 reproducibility fashion.

**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 rates of 55.6%. The test was a significantly better than the 13/40 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 outcome.
- 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.

**METHODS**

ViVA Tech® Platform

**Plate setup.** Eight different concentrations of each drug or drug combination is run for the used treatment protocols. The max concentration used is listed.

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

- Population PD Hill-based modeling of the ex vivo response vs concentration data in monotherapy (Figs 1A, 1B). This combined the set of estimated parameters determined by bootstrapping over 1000 simulations.
- Surface interaction modeling and simulations to estimate the interaction parameter (s) as well as the corresponding confidence interval. A parameter is a measure of synergism (h0), additivity (0) or antagonism (-h0).

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