(MRD) in FLT3/ITD AML could guide decisions on transplant or maintenance therapy. To date, polymerase chain reaction (PCR) based assays for MRD in FLT3/ITD AML have been hampered by competition from the wild type allele, limiting the overall sensitivity to approximately 1 cell in 100. While several groups have reported the development of MRD assays for this disease, none of these diagnostics have been developed in concert with bioinformatics software under a quality system with the intent of being submitted to regulatory authorities as a harmonized assay available to the international community. We have developed a sensitive and specific MRD assay for FLT3/ITD mutations using next-generation sequencing (NGS).

Aims: We report here the development of an assay for FLT3/ITD mutations using a next-generation sequencing (NGS) platform.

Methods: Exons 14 and 15 are amplified by PCR and the products were detected by a refined NGS technique developed at Invivoscribe, Inc. Initial validation was carried out by spiking in fixed amounts of mutant DNA into wild type DNA to establish a sensitivity equivalent to detection of at least one ITD-containing cell out of 10,000 with a minimum input of 100,000 cell equivalents of DNA.

Results: We tested a series of 15 bone marrow aspirate samples from patients previously diagnosed with FLT3/ITD AML. All patients gave informed consent according to the Declaration of Helsinki. The investigator conducting the MRD assay was blind to the clinical information about the sample- no information was provided beforehand regarding the presence or absence of FLT3/ITD mutation, its length, or the mutant-to-wild type allelic ratio. All patients tested were in clinical remission by IWG criteria (J. Clin Oncol 2003; 24:4642) and all samples were derived from the first pull aspirate material used for the clinical confirmation of that remission. In all samples, both the standard CLIA-certified assay for the FLT3/ITD mutation (J Mol Diagn 2003; 5:96) as well as standard multi-parameter flow cytometry (for a leukemia-associated phenotype) were negative for detectable FLT3 mutations or cells with a leukemia phenotype. The first 4 samples were from patients who were newly-diagnosed, had just completed induction therapy, had achieved first CR, and were awaiting consolidation therapy. In all 4 cases, the FLT3/ITD mutation detected at diagnosis was detected in these remission samples, with mutation levels ranging from 1.35E-05 to 1.74E-04. Three samples were from patients who had relapsed and had responded to salvage therapy, achieving a CR2. In these 3 cases, the original FLT3/ITD length mutation was detected, with mutation levels ranging from 1.38E-06 to 1.11E-04 mutant ITD reads/total reads. Six samples were from patients who had undergone allogeneic transplant in remission. The samples were collected during routine post-transplant surveillance, 2-5 years after transplant. No mutation was detected in any of these patients, all 6 of whom are alive and disease free 2.5-5.5 years after transplant. Finally, 2 samples were from patients who had undergone allogeneic transplant for FLT3/ITD AML in first CR. At 2 and 6 months post-transplant, respectively, bone marrow aspirates from these 2 patients confirmed ongoing morphologic remission, with 100% donor chimerism in both the marrow and the T-cell compartment, and a negative standard assay for FLT3/ITD mutations. Using DNA from these same time points, the MRD assay detected FLT3/ITD mutations at levels of 3.67E-03 and 1.04E-04 mutant ITD reads/total reads, respectively. Both of these patients relapsed with AML carrying the detected FLT3/ITD mutation within 6 months.

Summary/Conclusions: This novel MRD assay is specific, and is 2 orders of magnitude more sensitive than current commercially available assays for FLT3/ITD mutations. We anticipate that this assay will be broadly available to the public soon, and will have a significant impact in the clinical management of this disease.

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

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Background: We have overcome the limitations of 40 years of *ex vivo* testing

Aims: 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.

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. Figure 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; Figure 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.

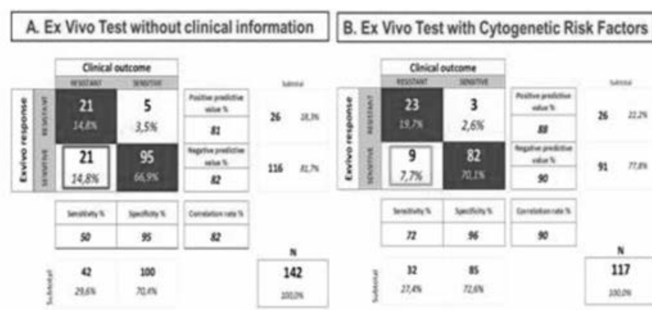

Figure 1. Clinical correlation of the *ex vivo* test alone (A, left) or adding the cytogenetic risk factor (B, right).

Summary/Conclusions: This novel test is able to predict the clinical response to Ida+Ara-C induction with overall correlation and predictive values of 80%, higher than ever achieved. Considering this result and current clinical response rate of 66.7% (70% in this study), clear clinical benefits can be achieved with the use of the test. Adding the cytogenetic risk profile further increase the correlation to 90%. Thus this novel test may be valuable information to guide 1st line patient therapy

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RECURSIVE PARTITIONING ANALYSIS FOR GENETIC STRATIFICATION AND PROGNOSTICATION OF ACUTE MYELOID LEUKAEMIA

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