(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 PCR based assays for FLT3/ITD mutations, there has been no validated clinical assay for the detection of this disease. New studies have been conducted 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) platform.

Methods: 14 and 15 amplified PCR 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 1 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; 21:4842) and all samples were derived from the first post induction aspirate material used for the clinical confirmation of the MRD negativity. In both cases a standard CLIA-certified assay was used for detection of 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 levels of FLT3/ITD positive cells with a leukemia-phenotype. The first 4 samples were from patients who were newly-diagnosed, had just completed induction chemotherapy and a negative standard assay for FLT3/ITD mutation (J Mol Diagn 2003; 5:96) as well as standard multi-parameter flow cytometry (for a leukemia-associated phenotype) was performed. The remaining 11 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 chimera 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. 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 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 chimera 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 detect- ed FLT3/ITD mutation within 6 months.

Summary/Conclusions: This novel MRD assay is specific, and is 2 orders of magnitude more sensitive than currently 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.

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 of 66.7% (70% in this study), clear clinical benefits can be achieved with the use of the test. Additional studies are needed to confirm the results and of the test in clinical practice.

P570 RECURSIVE PARTITIONING ANALYSIS FOR GENETIC STRATIFICATION AND PROGNOSTICATION OF ACUTE MYELOID LEUKAEMIA

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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 response rates. This is done by performing chemotherapeutics with cytarabine (Ara-C) and idarubicin (Ida) in further work.

Methods: This has been an observational clinical trial where bone marrow samples from adult patients diagnosed with de novo AML in Spanish centers 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 combination. For clinical comparison, 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 cases 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 improve- ment in the sensitivity from 50% to 72%. Both approaches lead to substantial improvements in estimated overall survival.