Validation of the LeukoStrat® CDx *FLT3* Mutation Assay: Used to Detect both Internal Tandem Duplication (ITD) and Tyrosine Kinase Domain (TKD) Mutations and Response to Midostaurin in 1058 Patients with AML

H53

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Abstract

Introduction: Acute myeloid leukemia (AML) in general has a poor prognosis, but AML patients with mutations in the fms-related tyrosine kinase 3 (*FLT3*), have a particularly poor prognosis, as ITD and TKD *FLT3* mutations result in constitutive autophosphorylation and activation of *FLT3* - an important driver mutation in AML. The Invivoscribe® LeukoStrat® CDx *FLT3* Mutation Assay (CDx), recently approved by the FDA, targets regions of the *FLT3* gene to detect internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations to identify patients for whom midostaurin treatment might be beneficial. It is the only internationally standardized signal ratio test used to detect *FLT3* ITD and TKD mutations.

Methods: Mononuclear cells were isolated from peripheral blood and bone marrow aspirate by a proprietary method. DNA was extracted and amplified by PCR. TKD amplicon was enzymatically digested and along with the ITD amplicon were analyzed by capillary electrophoresis on serialized ABI 3500 xL instruments (Thermo Fisher). The assay measured the mutation:wild type signal using data from GeneMapper® analysis software. *FLT3* Mutation Analysis Software (FMAS) developed by Invivoscribe calculated the signal ratio (SR) for both ITD and TKD and reported "Positive" or "Negative" for valid sample results or "Fail" for invalid results. Invalid results were generated when runs did not meet validity criteria for analysis with FMAS. When *FLT3* mutations were detected, the results were reported as positive if the mutant:wild type signal ratio (SR) met or exceeded the clinical cut-off of 0.05. A validated Roche 454 Next Generation DNA Sequencing method (454 Sequencing) was used for comparison in which DNA was amplified, and the amplicons were inspected, purified, quantified, normalized, pooled and sequenced. Specimens tested were collected from AML patients during the ten year international RATIFY drug trial and from residual clinical samples tested at LabPMM in San Diego.

Results: The clinical performance of the CDx was evaluated using data from 1058 AML patient specimens from the RATIFY trial. The RATIFY trial used laboratory developed tests as clinical trial assays (CTAs), screening patients for enrollment at 8 different sites. The CTAs to CDx concordance for *FLT3* status was 97.2% for positive percent agreement (PPA), 97.3% negative percent agreement (NPA) and 97.3% for overall percent agreement (OPA). Further, the hazard ratio for the CTA+/CDx+ population was 0.67 versus 0.77 for patients stratified using the CTA+ assays. CDx agreement to 454 Sequencing (PPA, NPA and OPA) was 94.0%, 97.5% and 95.1%, respectively, based on 764 patient results. Peripheral blood and bone marrow concordance (PPA and NPA) was 98.4%, indicating both specimen types can be used for patient diagnosis (N=184 pairs). Additionally, clinical samples tested at 3 independent clinical LabPMM laboratory sites on 3 continents showed both 100% positive and negative percent agreement, and high concordance for signal ratios.

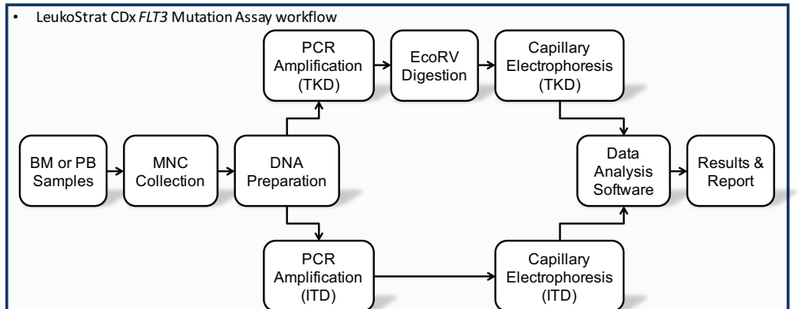
Conclusion: Overall, there was high concordance between the CDx, the RATIFY CTAs, and 454 Sequencing with respect to *FLT3* ITD and TKD gene mutation detection. In addition, analysis of patients stratified as positive by the CDx assay demonstrated superior prediction for response to midostaurin relative to the CTAs - 13% better drug efficacy compared with the CTAs used for patient enrollment (HR 0.67 vs 0.77). The high reproducibility between the 3 LabPMM clinical laboratories provides evidence that the CDx is a highly reproducible and reliable internationally harmonized test useful for patient enrollment on multiple continents. It is currently being used in more than a dozen large scale international clinical trials.

Materials

- Kit components for the LeukoStrat® CDx *FLT3* Mutation Assay were manufactured under cGMP and QC tested under a QSR compliant regulatory system prior to use in cross validation studies.
- DNA was extracted from CTA(+) and CTA(-) RATIFY study patient samples containing peripheral blood or bone marrow with a quantity of at least 50 ng at ≥10 ng/µL.
- Identical lots of cGMP reagents were used across all clinical enrollment sites.

Methods

- LeukoStrat CDx *FLT3* Mutation Assay workflow



- DNA extraction was conducted by Covance in Indianapolis, IN.
- Clinical testing with the CDx *FLT3* Mutation Assay was conducted by LabPMM in San Diego, CA.
- *FLT3* Mutation Detection by 454 Next Generation Sequencing was conducted by SeqWright in Houston, TX.
- The statistical program SAS was used to perform the statistical analysis.

Results

Agreement Table Between CDx and CTAs Based on CDx Results

Measure of Agreement	Without CDx Invalid		With CDx Invalid	
	Percent Agreement (N)	95% CI [(1)]	Percent Agreement (N)	95% CI [(1)]
PPA	98.2% (489/498)	(96.6%, 99.2%)	97.2% (489/503)	(95.4%, 98.5%)
NPA	98.5% (540/548)	(97.1%, 99.4%)	97.3% (540/555)	(95.6%, 98.5%)
OPA	98.4% (1029/1046)	(97.4%, 99.1%)	97.3% (1029/1058)	(96.1%, 98.2%)

[(1)] The 95% CI was calculated using the Exact (Clopper-Pearson) method
- Invalid means that a sample was tested in the CDx assay but failed to return a valid result.

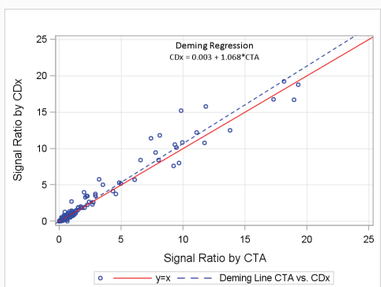
Agreement Table Between CDx and 454 Sequencing

Measure of Agreement	Without CDx Invalid		With CDx Invalid	
	Percent Agreement (N)	95% CI [(1)]	Percent Agreement (N)	95% CI [(1)]
PPA	94.0% (487/518)	(91.6%, 95.9%)	92.8% (487/525)	(90.2%, 94.8%)
NPA	97.5% (230/236)	(94.5%, 99.1%)	96.2% (230/239)	(93.0%, 98.3%)
OPA	95.1% (717/754)	(93.3%, 96.5%)	93.8% (717/764)	(91.9%, 95.4%)

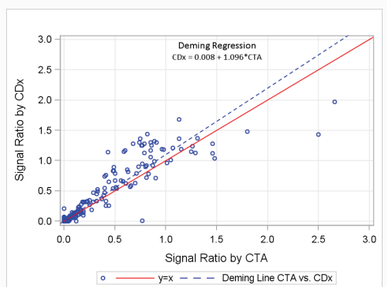
[(1)] The 95% CI was calculated using the Exact (Clopper-Pearson) method
- Invalid means that a sample was tested in the CDx assay but failed to return a valid result.

Deming Regression Analysis of ITD and TKD Signal Ratios Between CTAs and CDx in the Overall Population

(Samples with extremely high signal ratios were not displayed in order to focus on the spread of values around the Deming line.)



ITD



TKD

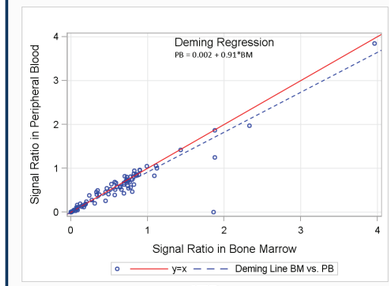
Agreement Table Between Peripheral Blood and Bone Marrow in 93 *FLT3* Positive Patients and 91 *FLT3* Negative Patients

Measure of Agreement	Percent Agreement	95% CI [(1)]
APA	98.4%	(96.2%, 100.0%)
ANA	98.4%	(96.2%, 100.0%)

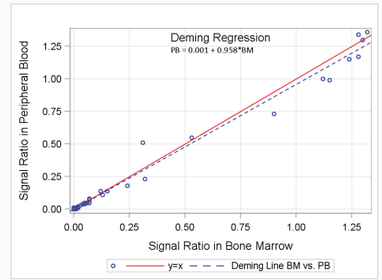
[(1)] The 95% CI was calculated using a non-parametric bootstrapping method

Deming Regression Analysis of ITD and TKD Signal Ratios Measured by the CDx Assay Between Peripheral Blood and Bone Marrow in the Overall Population

(Samples with extremely high signal ratios were not displayed in order to focus on the spread of values around the Deming line.)

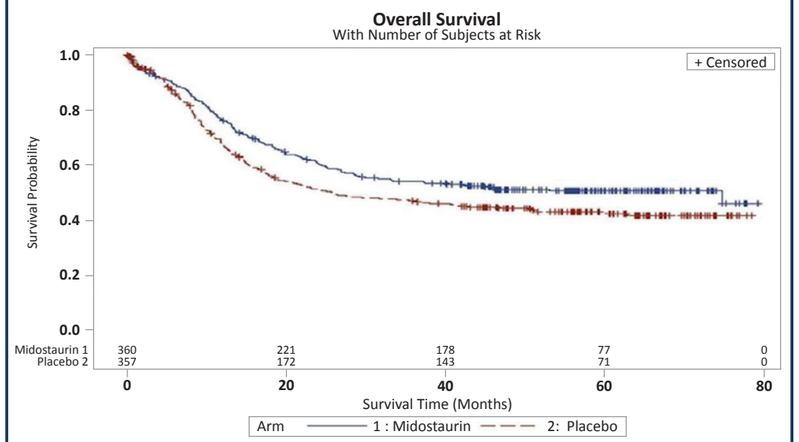


ITD



TKD

Kaplan-Meier Curve for Overall Survival in the (CTA+, CDx+) Population – Model 2, Non-censored for Stem Cell Transplantation



Conclusions

The study showed a high degree of concordance between the CDx and the clinical trial assay with superior prediction for midostaurin response by (CDx+, CTA+) patients. This assay is the only internationally harmonized *FLT3* signal ratio assay whose performance has been defined and is being used in more than a dozen large scale international clinical trials.

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