LeukoStrat® Assays

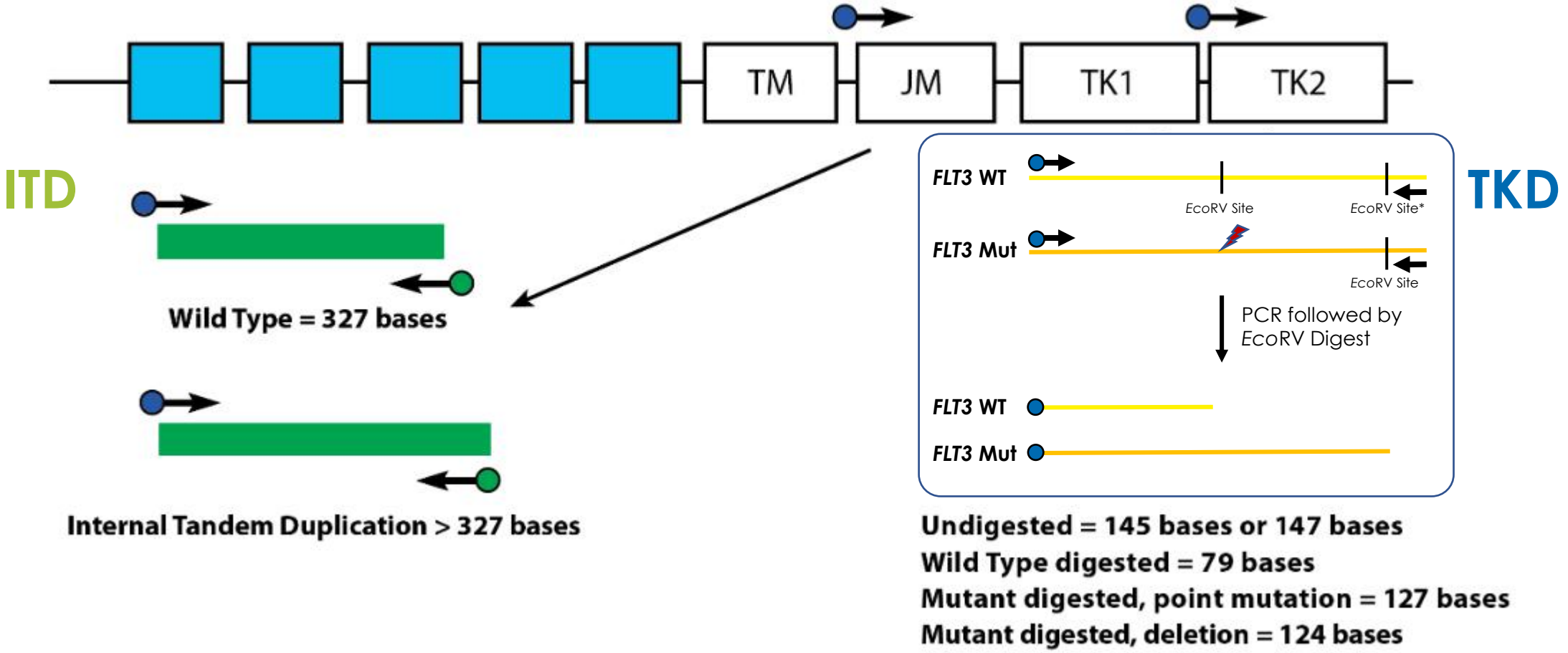
Invivoscribe *FLT3* Product Offerings

invivoscribe®
Improving Lives with Precision Diagnostics®

FLT3 ITD and TKD Mutations

LeukoStrat®

Overview of the *FLT3* Gene

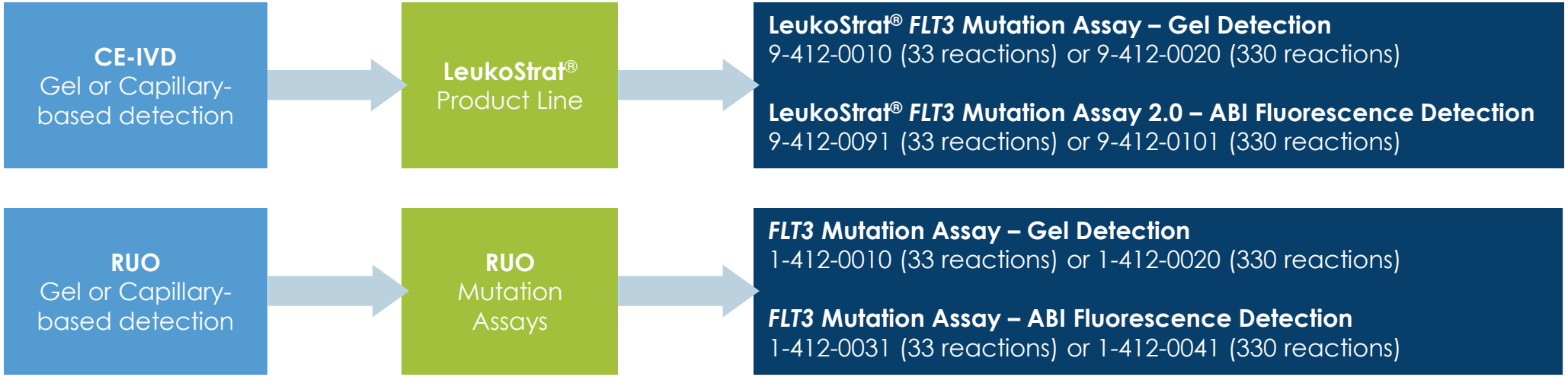


* This EcoRV site is engineered within the reverse primer.

LeukoStrat® *FLT3* Product Line Offerings

Proven Confidence in Detection of *FLT3* ITD and *FLT3* TKD Mutations

- Qualitative detection of *FLT3* mutations
- In formats that suit every laboratory's needs

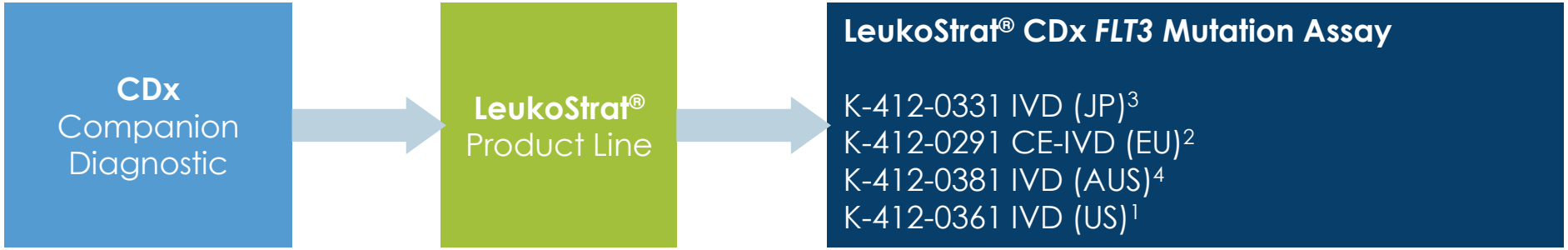


CE-IVD: This product is an *in vitro* diagnostic product; not available for sale or use within North America.
RUO: This product is for Research Use Only; not for use in diagnostic procedures.

LeukoStrat® CDx *FLT3* Product Offerings

LeukoStrat® CDx *FLT3* Mutation Assay

Proven confidence in detection of *FLT3* ITD and *FLT3* TKD mutations



- Trusted in multiple clinical trials :
  - RATIFY (Midostaurin)
  - ADMIRAL (Gilteritinib)
  - QUANTUM-R (Quizartinib)
- Available as a regulated companion diagnostic product in most regions of the world

CE-IVD: This product is an in vitro diagnostic product; not available for sale or use within North America. CDx: In vitro diagnostic device available in select countries, please refer to specific product pages.
1,2,3,4 The LeukoStrat CDx FLT3 Mutation Assay is a PCR-based in vitro diagnostic test designed to detect internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations D835 and I836 in the FLT3 gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia [1,2 (AML)]. **1** The LeukoStrat CDx FLT3 Mutation Assay is used as an aid in the assessment of patients with AML for whom RYDAPT® (midostaurin) treatment is being considered. The LeukoStrat CDx FLT3 Mutation Assay is used as an aid in the assessment of patients with AML for whom XOSPATA® (gilteritinib) treatment is being considered. The test is for use on the 3500xL Dx Genetic Analyzer. **2** In regions where midostaurin is available, the LeukoStrat CDx FLT3 Mutation Assay is used as an aid in the assessment of patients with AML for whom RYDAPT® (midostaurin) treatment is being considered. In regions where gilteritinib fumarate is available, the LeukoStrat CDx FLT3 Mutation Assay is used as an aid in the assessment of patients with AML for whom XOSPATA® (gilteritinib fumarate) treatment is being considered. **3** The LeukoStrat CDx FLT3 Mutation Assay is used as an aid in the assessment of patients with AML for whom Gilteritinib Fumarate treatment is being considered. The LeukoStrat CDx FLT3 Mutation Assay is used as an aid in the assessment of patients with AML for whom Quizartinib Hydrochloride treatment is being considered. **4** In regions where midostaurin is available, the LeukoStrat® CDx FLT3 Mutation Assay is used as an aid in the selection of patients with AML for whom midostaurin treatment is being considered.

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LeukoStrat® CDx *FLT3* Mutation Assay

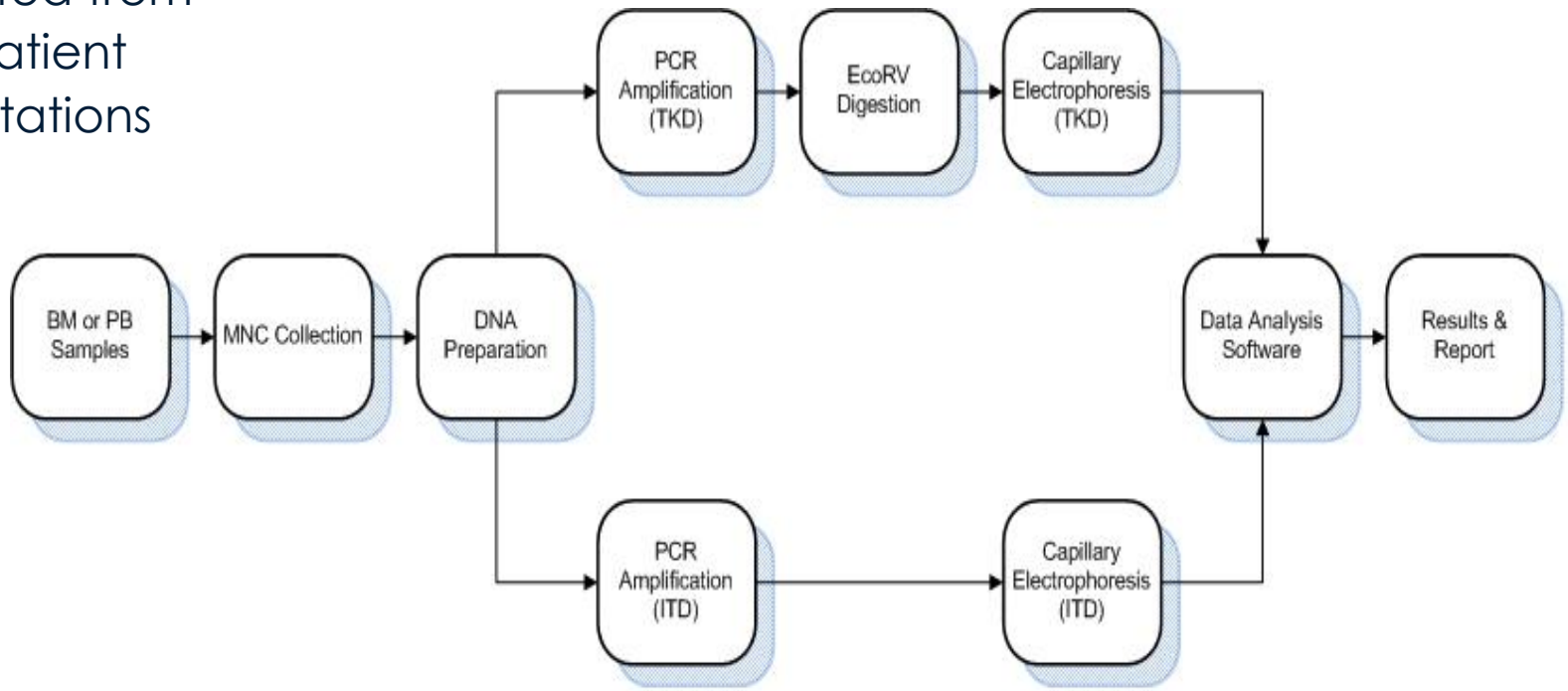
CDx Comprehensive Solution

LeukoStrat®
CDx *FLT3* Mutation Assay

Front-to-End

The LeukoStrat® CDx *FLT3* Mutation Assay includes controls, reagents, software and procedures for testing DNA extracted from mononuclear cells isolated from patient specimens to determine if *FLT3* mutations are present.

DNA is amplified via PCR and the amplicons are detected via capillary electrophoresis. The assay measures the ratio of signals from mutation against a background of signal from wild type. A *FLT3* mutation is detected if the mutant:wild type signal ratio meets or exceeds the clinical cutoff of **0.05**.

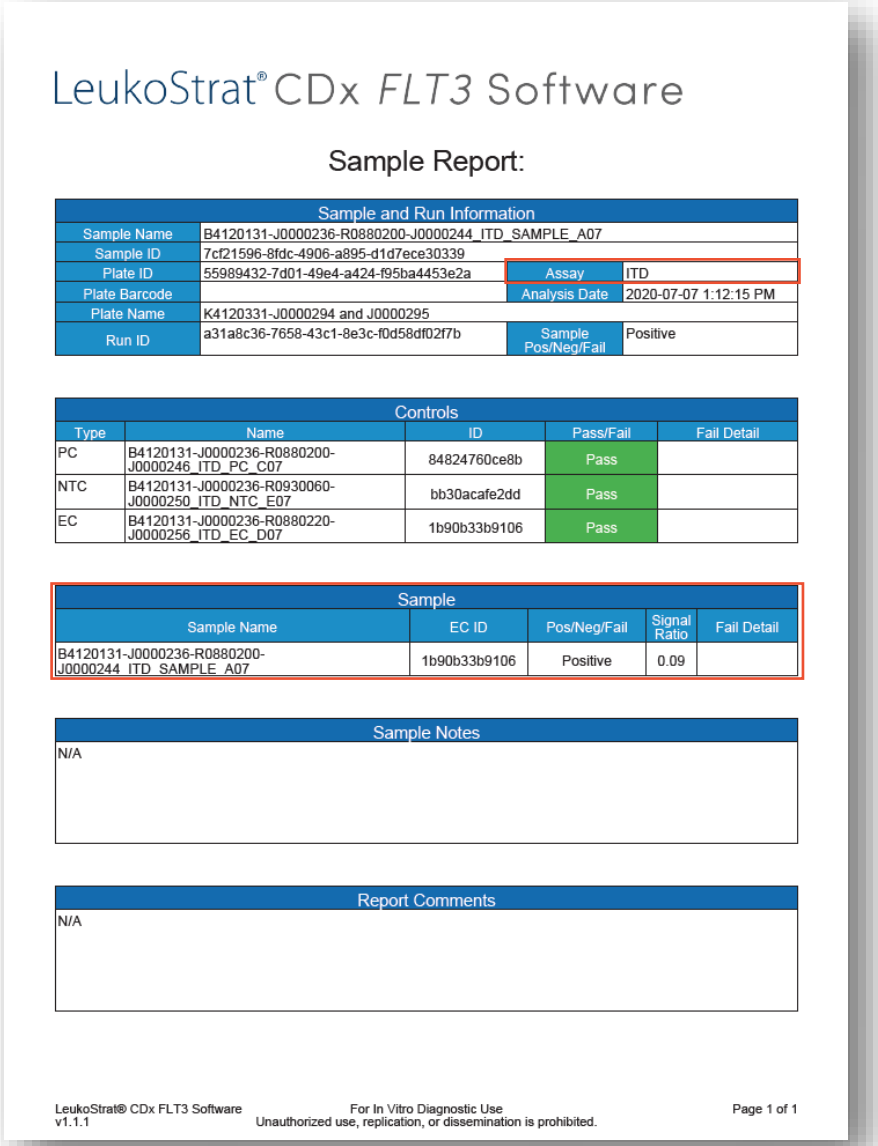


CDx Comprehensive Solution

LeukoStrat® CDx *FLT3* Mutation Assay

Automated results analysis

- Software included with the LeukoStrat® CDx *FLT3* Mutation Assay kit
- Easy-to-read sample reports
  - Control status is verified
  - Signal ratios are displayed
  - Sample status is determined
- Determine patient eligibility for treatment confidently



LeukoStrat® CDx *FLT3* Software

Sample Report:

Sample and Run Information			
Sample Name	B4120131-J0000236-R0880200-J0000244_ITD_SAMPLE_A07		
Sample ID	7cf21596-8fdc-4906-a895-d1d7ece30339		
Plate ID	55989432-7d01-49e4-a424-f95ba4453e2a	Assay	ITD
Plate Barcode		Analysis Date	2020-07-07 1:12:15 PM
Plate Name	K4120331-J0000294 and J0000295		
Run ID	a31a8c36-7658-43c1-8e3c-f0d58df02f7b	Sample Pos/Neg/Fail	Positive

Controls				
Type	Name	ID	Pass/Fail	Fail Detail
PC	B4120131-J0000236-R0880200-J0000246_ITD_PC_C07	84824760ce8b	Pass	
NTC	B4120131-J0000236-R0930060-J0000250_ITD_NTC_E07	bb30acafe2dd	Pass	
EC	B4120131-J0000236-R0880220-J0000256_ITD_EC_D07	1b90b33b9106	Pass	

Sample				
Sample Name	EC ID	Pos/Neg/Fail	Signal Ratio	Fail Detail
B4120131-J0000236-R0880200-J0000244_ITD_SAMPLE_A07	1b90b33b9106	Positive	0.09	

Sample Notes
N/A

Report Comments
N/A

LeukoStrat® CDx FLT3 Software v1.1.1 — For In Vitro Diagnostic Use — Unauthorized use, replication, or dissemination is prohibited. — Page 1 of 1

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LeukoStrat® CDx *FLT3* Software Interpretation

LeukoStrat® CDx *FLT3* Mutation Assay

Determining sample mutation status for *FLT3* inhibitor indication

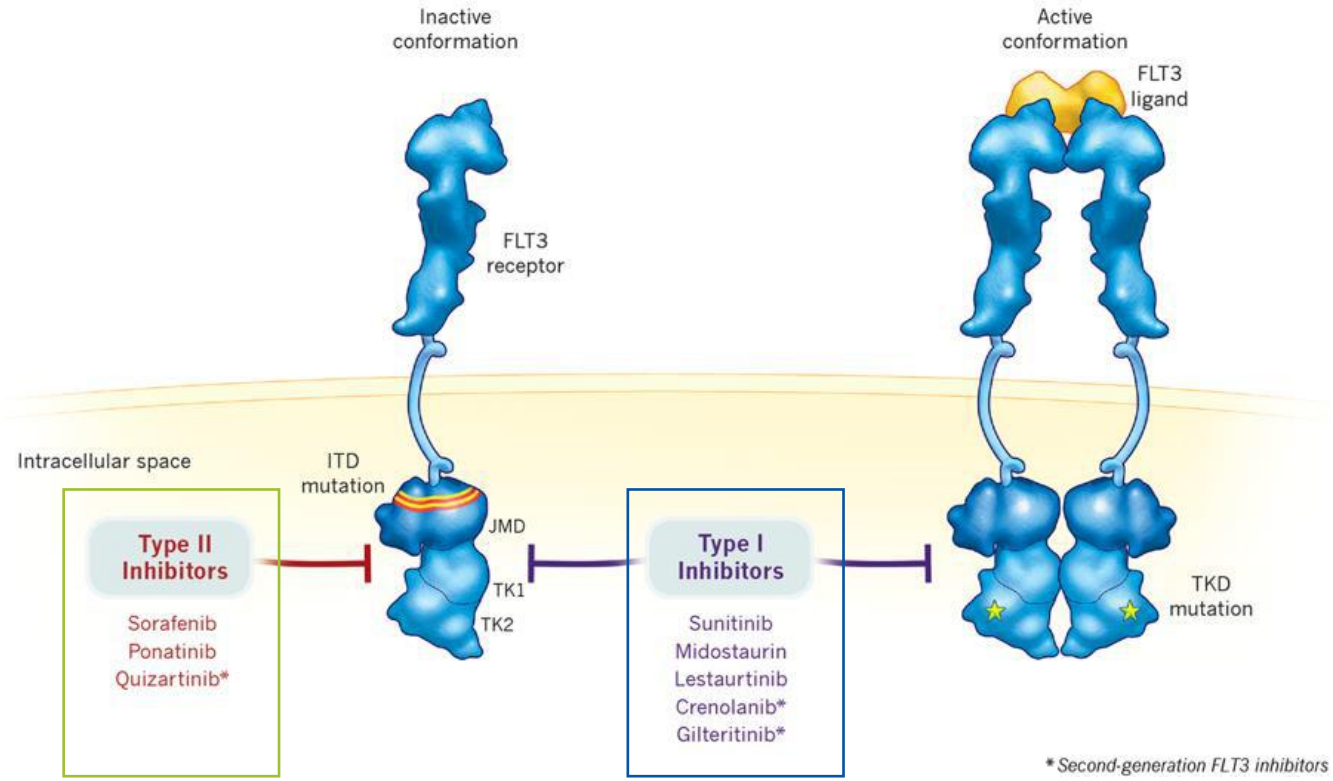
Scenario	ITD Result	TKD Result	Sample Mutation Status
1	Positive (SR ≥0.05)	Positive (SR ≥0.05)	Positive
2	Negative (SR <0.05)	Negative (SR <0.05)	Negative
3	Invalid	Invalid	Invalid
4	Positive (SR ≥0.05)	Negative (SR <0.05)	Positive
5	Negative (SR <0.05)	Positive (SR ≥0.05)	Positive
6	Positive (SR ≥0.05)	Invalid	Positive
7	Negative (SR <0.05)	Invalid	Invalid
8	Invalid	Positive (SR ≥0.05)	Positive
9	Invalid	Negative (SR <0.05)	Invalid

FLT3 Inhibitors

LeukoStrat® CDx *FLT3* Mutation Assay

Lots of promising new treatments

- **Type I** inhibitors are effective against both *FLT3* ITD and TKD mutations
- **Type II** inhibitors are effective against only *FLT3* ITD mutations



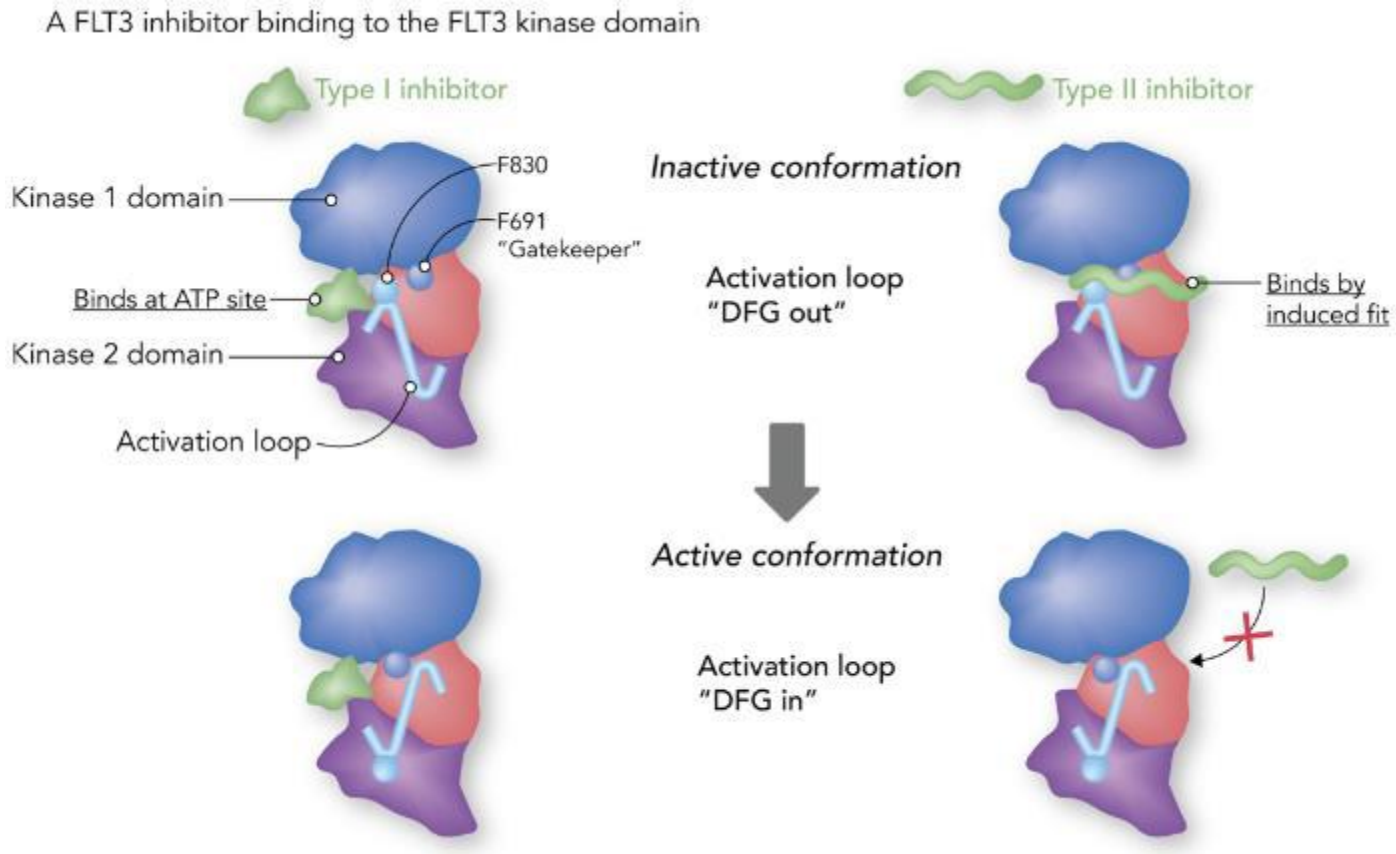
Leukemia (2019) 33:299–312

FLT3 Inhibitors

LeukoStrat®
CDx *FLT3* Mutation Assay

Inhibitor mechanism

- Closer look at the difference between type I and type II inhibitors



Lewis and Perl, DOI 10.1182/bloodadvances.2019000174

Clinical Utility

LeukoStrat® CDx *FLT3* Mutation Assay

Novartis	Astellas	Daiichi-Sankyo
FDA Approved (US) CE-Registered (EU) ARTG-Inclusion (AUS)	FDA Approved (US) CE-Registered (EU) MHLW Approved (Japan)	FDA Submitted (US) MHLW Approved (Japan)
RYDAPT® (Midostaurin)	XOSPATA® (Gilteritinib Fumarate)	VANFLYTA® (Quizartinib hydrocholoride)
Type 1 inhibitor	Type 1 inhibitor	Type 2 inhibitor

FLT3 Targeted Therapy revolutionized leukemia treatment with the first novel and targeted therapy for AML in over a decade.

The LeukoStrat CDx *FLT3* Mutation Assay is the companion diagnostic to all approved Tyrosine Kinase Inhibitor Therapies.

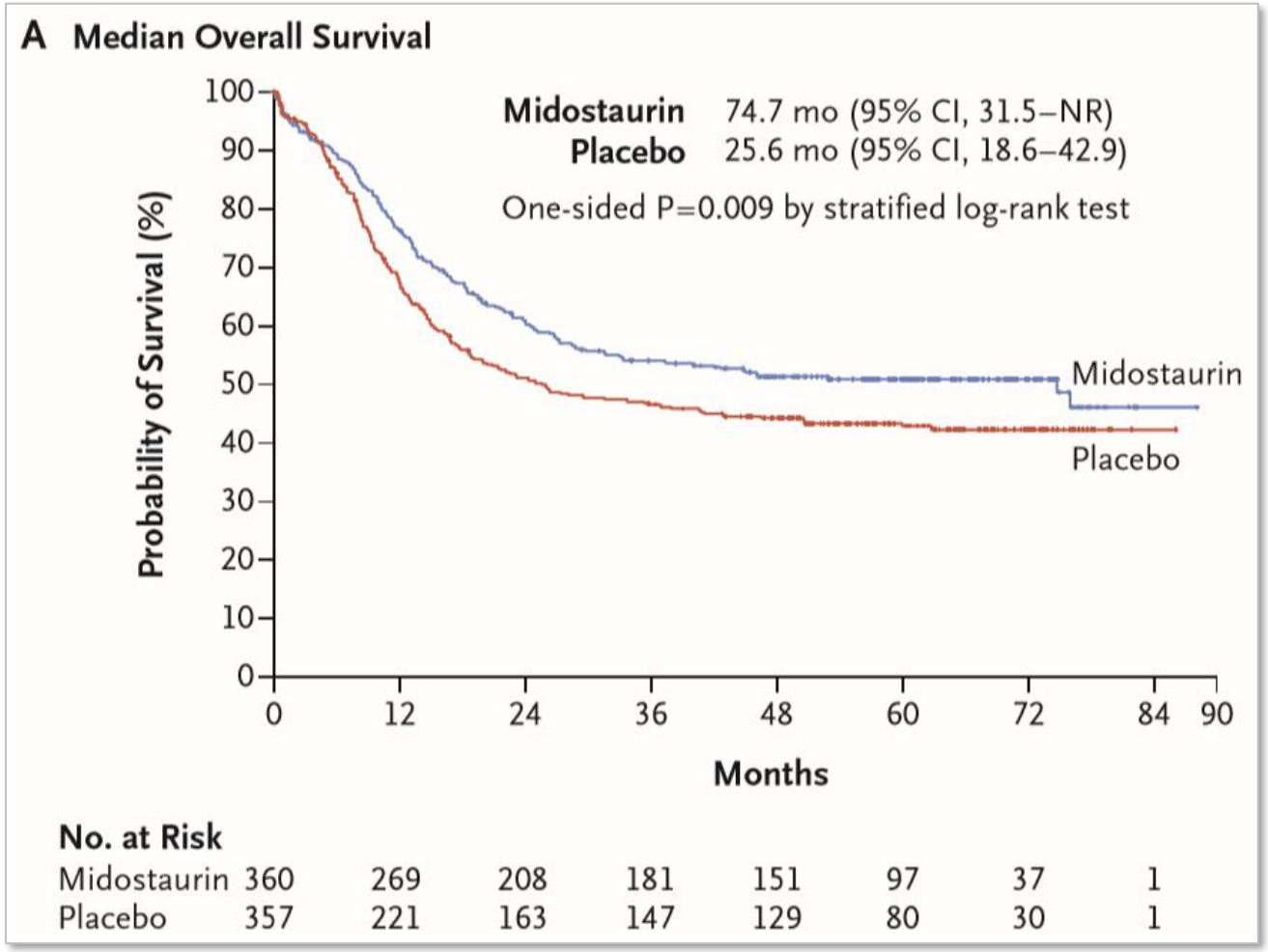
FLT3 Inhibitor – Midostaurin

LeukoStrat® CDx *FLT3* Mutation Assay

Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a *FLT3* Mutation

R.M. Stone, S.J. Mandrekar, B.L. Sanford, K. Laumann, S. Geyer, C.D. Bloomfield, C. Thiede, T.W. Prior, K. Döhner, G. Marcucci, F. Lo-Coco, R.B. Klisovic, A. Wei, J. Sierra, M.A. Sanz, J.M. Brandwein, T. de Witte, D. Niederwieser, F.R. Appelbaum, B.C. Medeiros, M.S. Tallman, J. Krauter, R.F. Schlenk, A. Ganser, H. Serve, G. Ehninger, S. Amadori, R.A. Larson, and H. Döhner

Adult patients, ***Newly diagnosed AML***



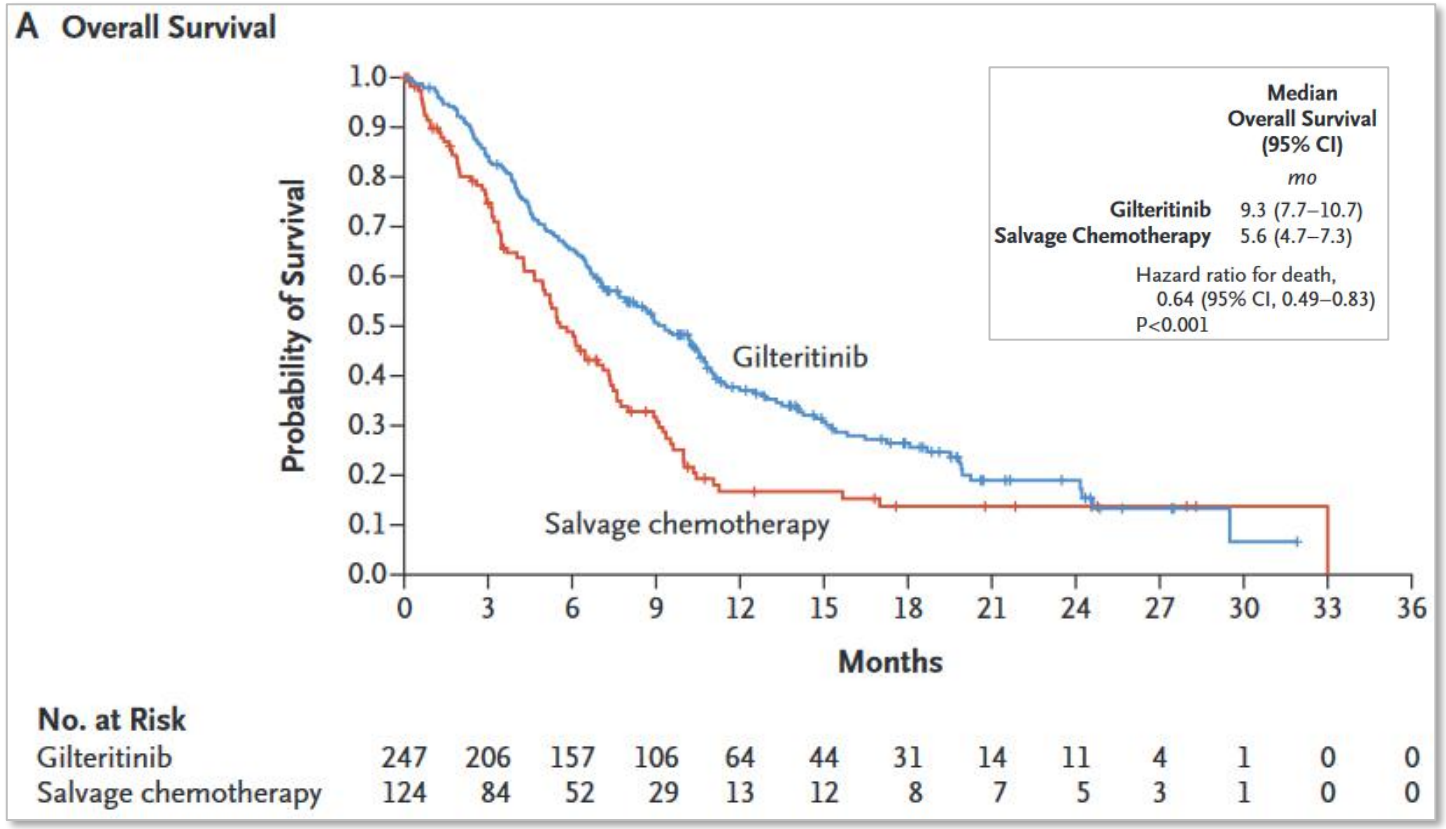
2017 NEJM Stone et al

FLT3 Inhibitor – Gilteritinib

LeukoStrat® CDx *FLT3* Mutation Assay

Gilteritinib or Chemotherapy for Relapsed or Refractory *FLT3*-Mutated AML

A.E. Perl, G. Martinelli, J.E. Cortes, A. Neubauer, E. Berman, S. Paolini, P. Montesinos, M.R. Baer, R.A. Larson, C. Ustun, F. Fabbiano, H.P. Erba, A. Di Stasi, R. Stuart, R. Olin, M. Kasner, F. Ciceri, W.-C. Chou, N. Podoltsev, C. Recher, H. Yokoyama, N. Hosono, S.-S. Yoon, J.-H. Lee, T. Pardee, A.T. Fathi, C. Liu, N. Hasabou, X. Liu, E. Bahceci, and M.J. Levis



DOI: 10.1056/NEJMoa1902688

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Laboratory Cross Validation Study

LeukoStrat® CDx *FLT3* Mutation Assay

*FLT3*mutation Assay Laboratory Cross Validation: Results from the CALGB 10603/Ratify Trial in Patients with Newly Diagnosed *FLT3*–Mutated Acute Myeloid Leukemia (AML)

Christian Thiede, Thomas W Prior, Serena Lavorgna, Jürgen Krauter, Eva Barragán, Josep Nomdedeu, Joop H. Jansen, Andrew H Wei, Weiqiang Zhao, Xiaohong Li, Celine Pallaud, Eva Tiecke, Richard A. Larson, Clara D. Bloomfield, Hartmut Döhner, Gerhard Ehninger, Richard M. Stone, and Konstanze Döhner

Blood 2018 132:2800; doi: https://doi.org/10.1182/blood-2018-99-112127

8 Rounds (every 6 months) | **50** Samples Prepared Centrally | **25** Samples were Analyzed

Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9
5 ITD wt	**5** ITD wt	**5** ITD wt	**5** ITD wt	**5** ITD wt	**5** ITD wt	**5** ITD wt	**5** ITD wt	**5** ITD wt
5 ITD AR 0.05	**5** ITD AR 0.05	**5** ITD AR 0.05	**5** ITD AR 0.05	**5** ITD AR 0.05	**5** ITD AR 0.05	**5** ITD AR 0.05	**5** ITD AR 0.05	**5** ITD AR 0.05
5 ITD AR 0.7	**5** ITD AR 0.7	**5** ITD AR 0.7	**5** ITD AR 0.7	**5** ITD AR 0.7	**5** ITD AR 0.7	**5** ITD AR 0.7	**5** ITD AR 0.7	**5** ITD AR 0.7
5 TKD wt	**5** TKD wt	**5** TKD wt	**5** TKD wt	**5** TKD wt	**5** TKD wt	**5** TKD wt	**5** TKD wt	**5** TKD wt
5 TKD AR 0.05	**5** TKD AR 0.05	**5** TKD AR 0.05	**5** TKD AR 0.05	**5** TKD AR 0.05	**5** TKD AR 0.05	**5** TKD AR 0.05	**5** TKD AR 0.05	**5** TKD AR 0.05

Thiede et.al "*FLT3mutation* Assay Laboratory Cross Validation: Results from the CALGB 10603 /Ratify Trial in Patients with Newly Diagnosed *FLT3-Mutaded* Acute Myeloid Leukemia (AML)", Blood (2018) 132:2800

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Laboratory Cross Validation Study

LeukoStrat® CDx *FLT3* Mutation Assay

FLT3 ITD Results

True AR = Median for each sample over all performed analyses

FLT3 ITD High, AR = 0.72 (0.61 – 0.93)

Median Error (ME) = 7.1 % (6.1 – 8.3)

FLT3 ITD LOD, AR = 0.06 (0 – 0.09)

ME = **13.4 %** (11.5 – 16.7)

Triplicate

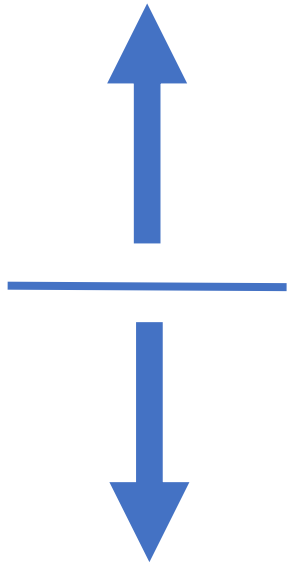
ME ITD AR 0.7 = 5.7% (4.8 – 6.6)

Thiede et.al "*FLT3mutation* Assay Laboratory Cross Validation: Results from the CALGB 10603 /Ratify Trial in Patients with Newly Diagnosed *FLT3-Mutaded* Acute Myeloid Leukemia (AML)", Blood (2018) 132:2800

Cross Laboratory Validation Study

LeukoStrat® CDx *FLT3* Mutation Assay

23%



AR = 0.7

89 of 379 values were wrongly assigned to be above or below the cut-off

Thiede et.al "*FLT3mutation* Assay Laboratory Cross Validation: Results from the CALGB 10603 /Ratify Trial in Patients with Newly Diagnosed *FLT3-Mutaded* Acute Myeloid Leukemia (AML)", Blood (2018) 132:2800

Cross Laboratory Validation Study

LeukoStrat® CDx *FLT3* Mutation Assay

Conclusion

- "This first report of round robin testing for *FLT3* mut shows that using a standardized protocol, the qualitative assessment of *FLT3* mut is feasible with high accuracy."

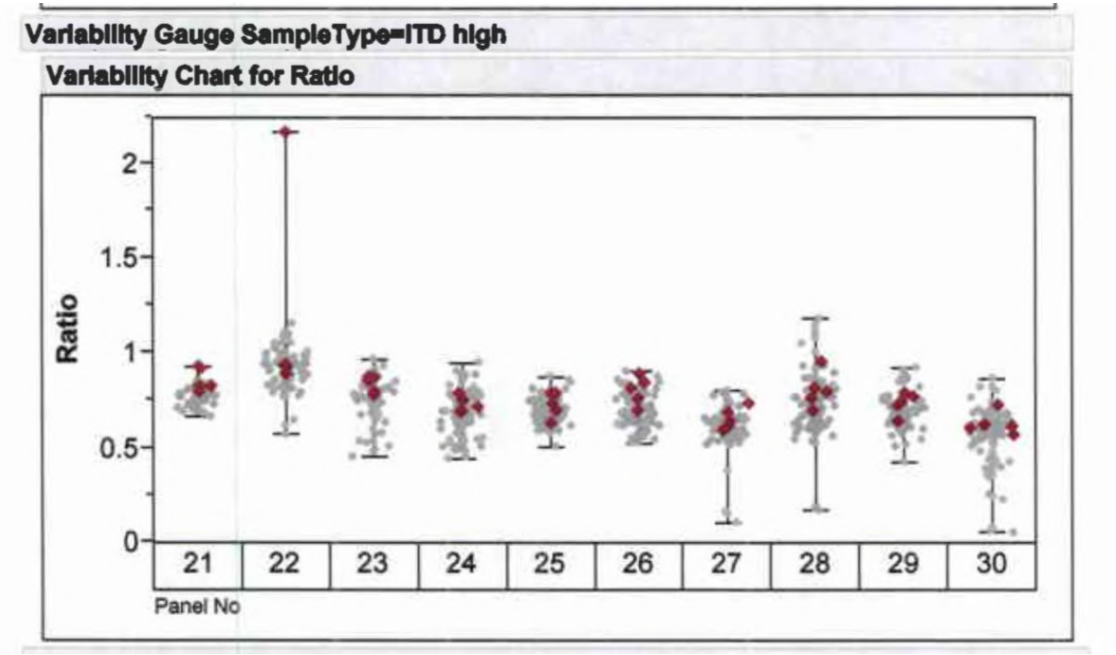
- "However the assessment of *FLT3*-AR clearly shows a considerable variability, which can be reduced by using a triplicate analysis, but still has to be taken into account, especially in patients evaluated for allogeneic stem cell transplantation. **Further standardization of *FLT3* testing appears highly warranted**."

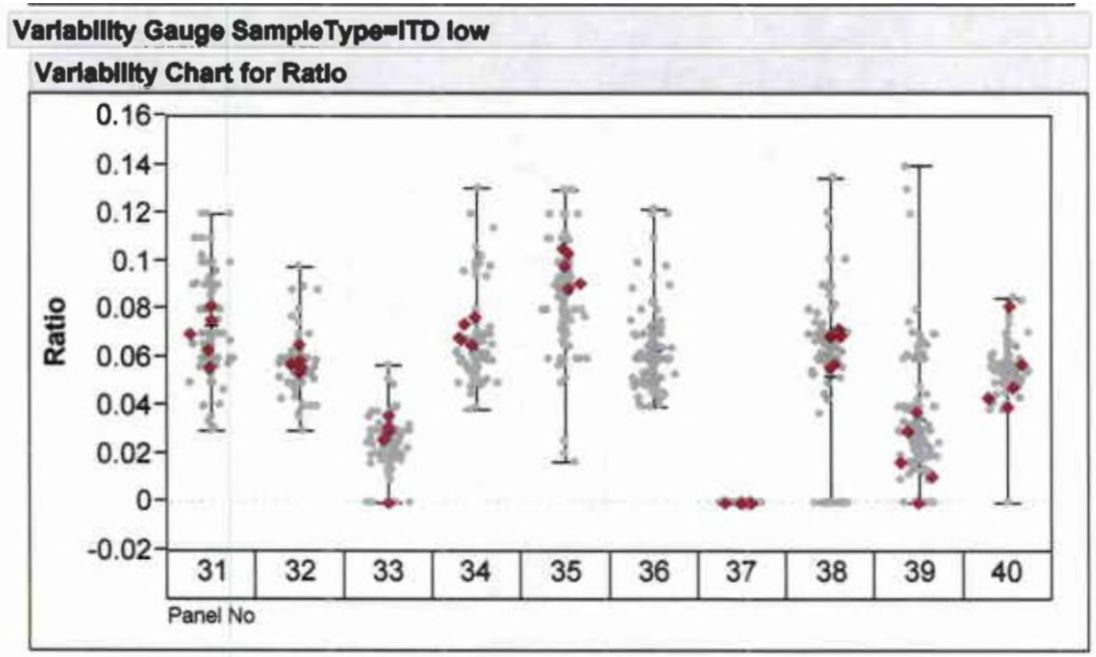
Thiede et.al "*FLT3mutation* Assay Laboratory Cross Validation: Results from the CALGB 10603 /Ratify Trial in Patients with Newly Diagnosed *FLT3-Mutaded* Acute Myeloid Leukemia (AML)", Blood (2018) 132:2800

Cross Laboratory Validation Study

LeukoStrat® CDx *FLT3* Mutation Assay

Signal Ratio variability of ITD clinical samples tested in the clinical sites (grey) and at Invivoscribe (red)





Thiede et.al "*FLT3mutation* Assay Laboratory Cross Validation: Results from the CALGB 10603 /Ratify Trial in Patients with Newly Diagnosed *FLT3-Mutaded* Acute Myeloid Leukemia (AML)", Blood (2018) 132:2800

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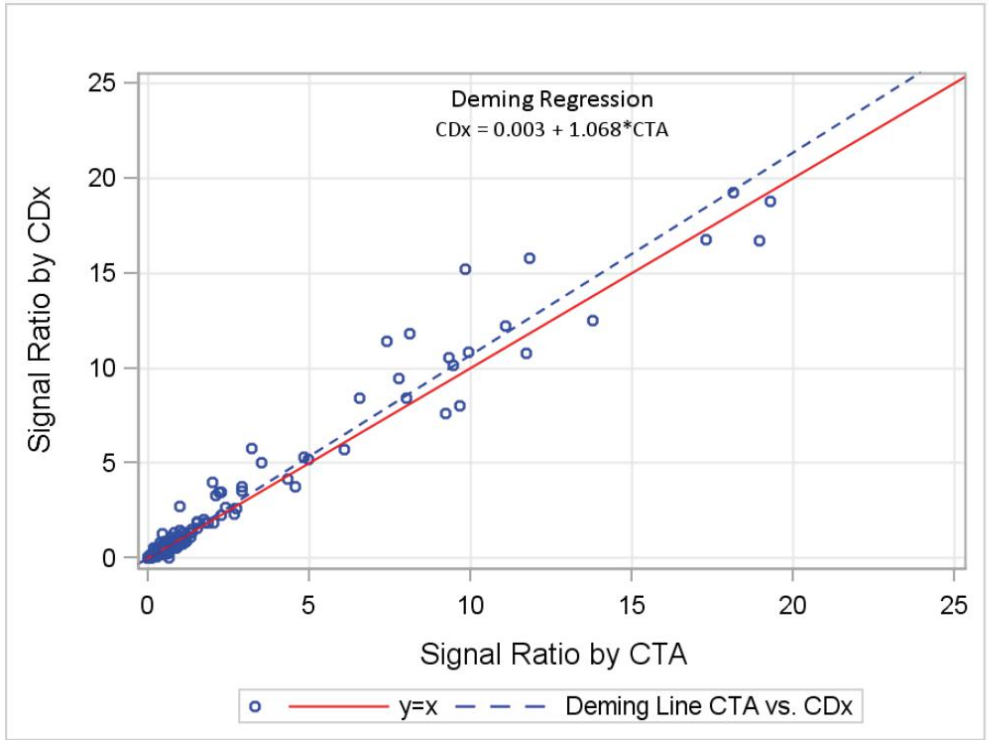
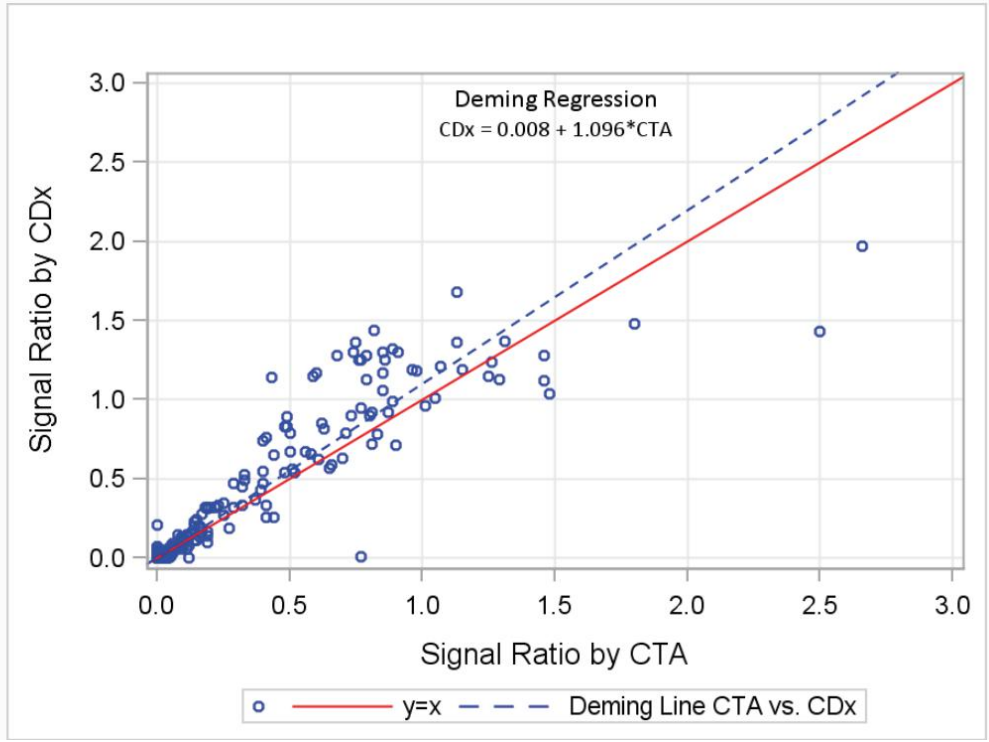
Cross Laboratory Validation Study

LeukoStrat® CDx *FLT3* Mutation Assay

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



ITD



TKD

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

Signal Ratio vs. Allelic Ratio

What is the difference?

- Signal Ratio refers to the comparison of the amount of mutant signal / wild-type signal (area under the curve)
- Allelic ratio in the literature often refers to the same thing
- Measuring the true number of alleles is very challenging which is why a comparison of signals is used

Take Home Message

LeukoStrat® CDx *FLT3* Mutation Assay

- Determining the *FLT3* mutation status is very important in determining appropriate treatment options for AML patients
- The LeukoStrat® CDx *FLT3* Mutation Assay is a companion diagnostic assay for exciting new therapies
- Using an internationally standardized test such at the LeukoStrat® CDx *FLT3* Mutation Assay is the best way to help meet your patients' needs

Trusted choice for *FLT3* testing

LeukoStrat
CDx *FLT3* Mutation Assay

Available Globally
Allowing patients to get the best standard of care

Regulated IVD
Available in Australia, Japan, the EU and USA

Accessible
Available for capillary analysis

Complete
Controls, Reagents and Software

Standardized
Validated to give consistent performance

Proven
Used in clinical trials and published studies

invivoscribe®
The most trusted partner for *FLT3* Testing

Quiz

invivoscribe®

Quiz

Which of the following is the clinical cut off value of the signal ratio from the LeukoStrat® CDx *FLT3* Mutation Assay?

1. 0.01
2. 0.05
3. 0.5
4. 0.7

Quiz

Which sequencer model can I run the CE-IVD LeukoStrat® CDx *FLT3* Mutation Assay on?

1. ABI3130 xl
2. ABI3500 xl
3. ABI3730 xl

Quiz

How long does it take to run the LeukoStrat® CDx *FLT3* Mutation Assay at minimum?

1. 2 days
2. 3 days
3. 4 days

Quiz

For which *FLT3* mutation(s) are AML treatments approved in the EU?

1. *FLT3* ITD
2. *FLT3* TKD
3. All of the above

Quiz

Which EU approved treatment(s) is associated with newly diagnosed *FLT3*mut+ AML?

1. RYDAPT® (midostaurin)
2. XOSPATA® (gilteritinib)
3. All of the above

Quiz

Which EU approved treatment(s) is associated with Relapse/Refractory *FLT3*mut+ AML?

1. RYDAPT® (midostaurin)
2. XOSPATA® (gilteritinib)
3. All of the above