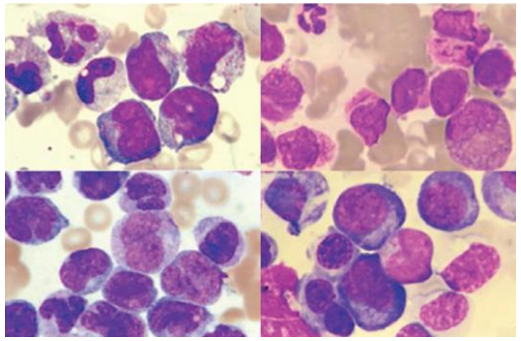

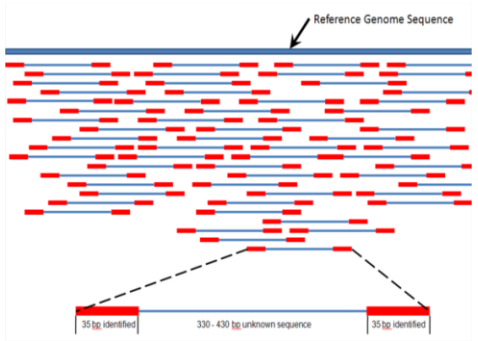

The impact of NGS molecular profiling in myeloid malignancies

Mónica López

Hematopathology Section

Pathology Department, Hospital Clínic

Barcelona

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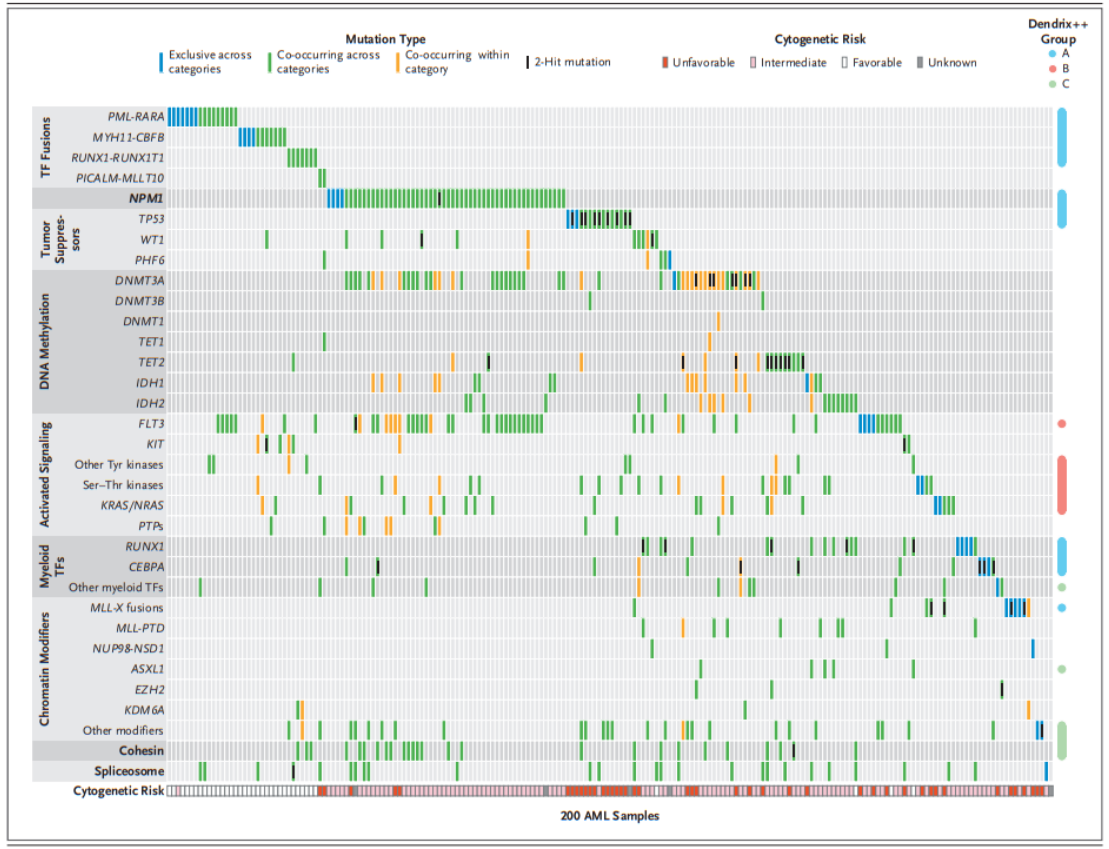
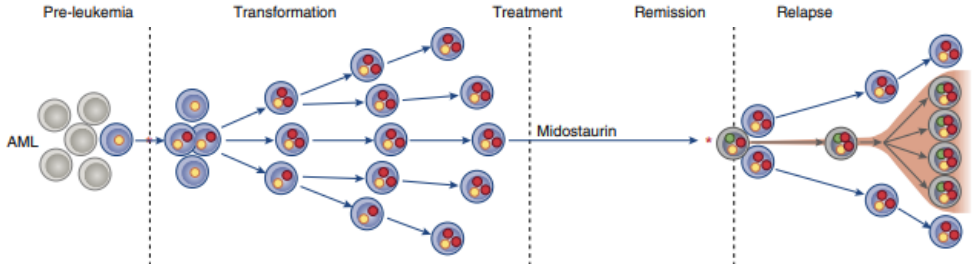
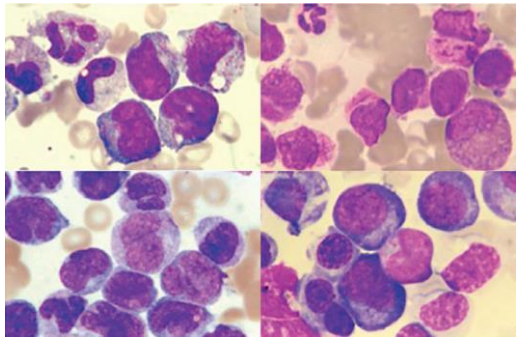
Acute myeloid leukemia (AML)

AML mutational landscape



- ✓Genetically heterogeneous clonal disorder
- ✓Origin in hematopoietic progenitor cells
- ✓Increased proliferation and differentiation block
- ✓Disease evolution over time





Cancer Genome Atlas Research Network: Ley, TJ et al. N Engl J Med. 2013 May 30;368(22):2059-74.
Ferrando AA & Carlos López-Otín C . Nat Med . 2017 Oct 6;23(10):1135-1145.

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Risk stratification of AML by genetics

Table 6. 2022 European LeukemiaNet (ELN) risk classification by genetics at initial diagnosis[a]

Risk Category[b]	Genetic Abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*[b,c] • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*[b,c] • Mutated *NPM1*[b,d] without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*[e]
Intermediate	• Mutated *NPM1*[b,d] with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD • t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*[b,f] • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23;q34.1)/*DEK*::*NUP214* • t(v;11q23.3)/*KMT2A*-rearranged[g] • t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • t(8;16)(p11;p13)/*KAT6A*::*CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2*, *MECOM(EVI1)* • t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged • -5 or del(5q); -7; -17/abn(17p) • Complex karyotype,[h] monosomal karyotype[i] • Mutated *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*[j] • Mutated *TP53*[k]

Döhner H et al. Blood. 2022 Jul 7

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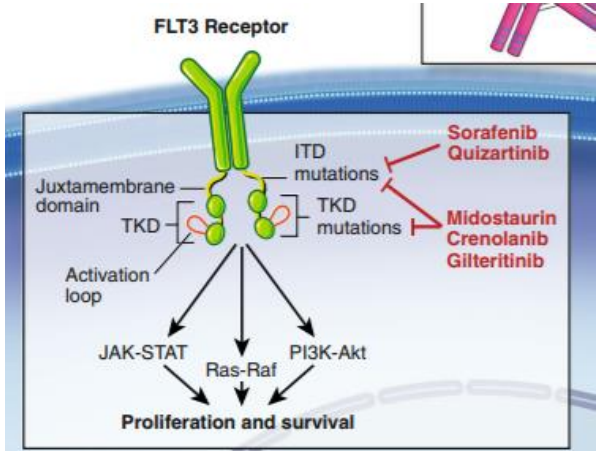
AML: targeted therapy

FLT3 mutations in AML

30% *FLT3*-ITD
7-10% *FLT3*-TKD

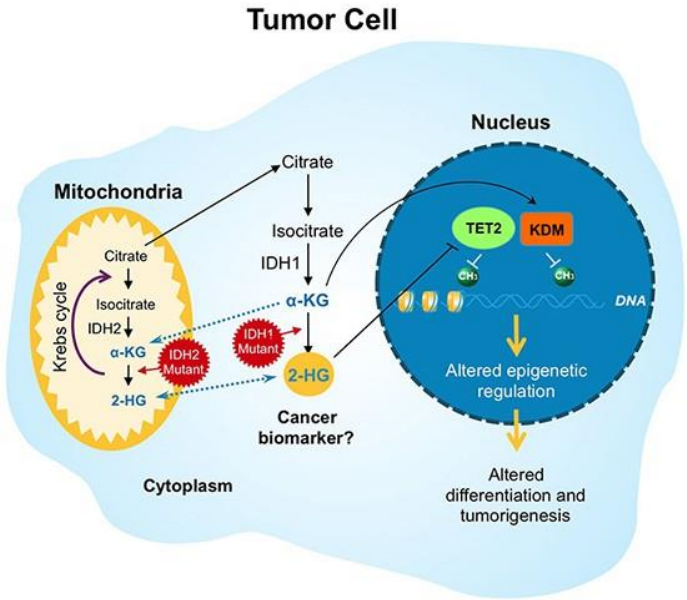


FLT3 inhibitors

Midostaurin
Quizartinib
Gilterinib
Sorafenib

IDH1/2 mutations in AML

6-16% *IDH1* mut
8-19% *IDH2* mut



IDH1/2 inhibitors

Enasidenib
Ivosidenib

Saygin C, et al. J Hematol Oncol. 2017 Apr 18;10(1):93

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Molecular diagnosis of AML: 2022 European Leukemia Net (ELN) recommendations

Genetic analyses	Results preferably available within
Cytogenetics[d]	• 5-7 days
Screening for gene mutations required for establishing the diagnosis and to identify actionable therapeutic targets[e]	
• *FLT3*,[f] *IDH1*, *IDH2*	• 3-5 days
• *NPM1*	• 3-5 days
• *CEBPA*,[g] *DDX41, TP53*; *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2*	• 1st cycle
Screening for gene rearrangements[h]	
• *PML*::*RARA*, *CBFB*::*MYH11*, *RUNX1*::*RUNX1T1*, *KMT2A* rearrangements, *BCR*::*ABL1*, other fusion genes (if available)	• 3-5 days
Additional genes recommended to test at diagnosis[i]	
• *ANKRD26*, *BCORL1*, *BRAF*, *CBL*, *CSF3R*, *DNMT3A*, *ETV6*, *GATA2*, *JAK2*, *KIT*, *KRAS*, *NRAS*, *NF1*, *PHF6*, *PPM1D*, *PTPN11*, *RAD21*, *SETBP1*, *TET2*, *WT1*	

→ **NGS offers a combined solution (DNA and RNA-fusion genes)**

→ **Some targets (*FLT3,NPM1, IDH1, IDH2*, fusion genes) require a fast turnaround time but NGS requires batching**

Döhner H et al. Blood. 2022 Jul 7

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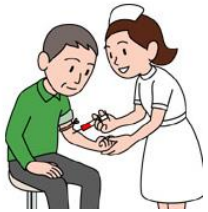
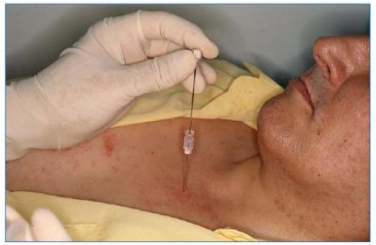
AML workflow

AML Protocol





Peripheral Blood
Bone marrow

- **MORPHOLOGY/CYTOCHEMISTRY**
- **FLOW CYTOMETRY**
- **CYTOGENETICS**
- **MOLECULAR BIOLOGY**

Cryopreservation
DNA and RNA extraction

3-5 days

"Fast" Molecular Diagnosis
Conventional PCR-based techniques

Fusion genes:
CBFB::MYH11
PML::RARa
RUNX1::RUNX1T1
BCR::ABL1

NPM1
FLT3-ITD
FLT3-TKD
IDH1
IDH2

2 weeks

Complete genetic characterization

NGS- Oncomine Myeloid Panel

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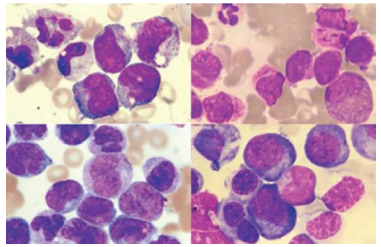
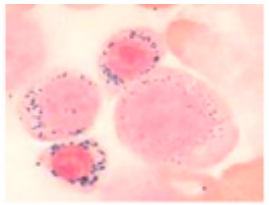
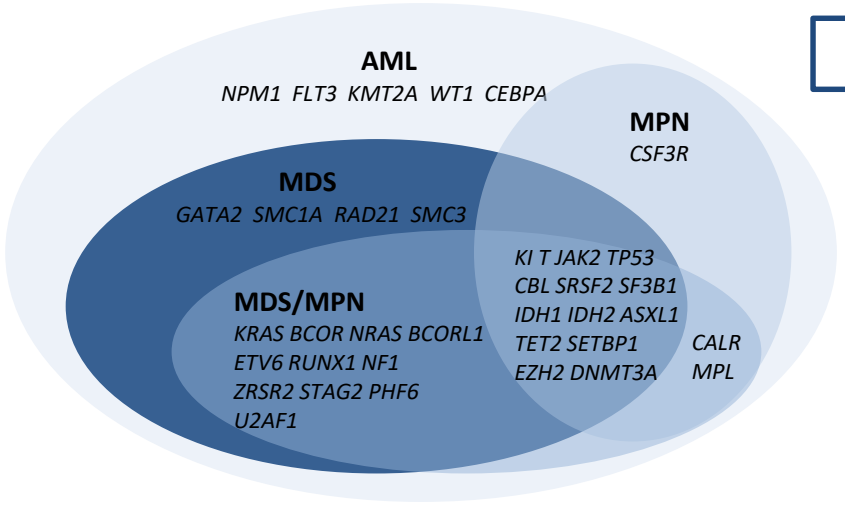
The molecular profiling influences the diagnosis, prognosis and treatment of myeloid neoplasms

Acute Myeloid Leukemia (AML)



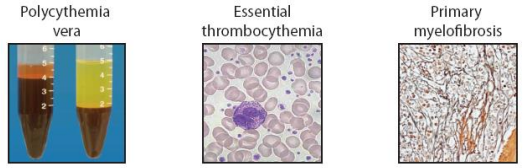
Myelodysplastic Neoplasms (MDS)



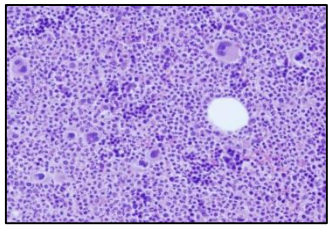


- ✓ **Clonal** markers
- ✓ **Diagnostic** markers
- ✓ **Prognostic** markers
- ✓ **Therapeutic** targets
- ✓ Potential **measurable residual disease** (MRD) targets
- ✓ Clonal evolution

Myeloproliferative Neoplasms (MPN)



Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN)



Cazzola M, et al, Blood. 2013; 122(25):4021-34

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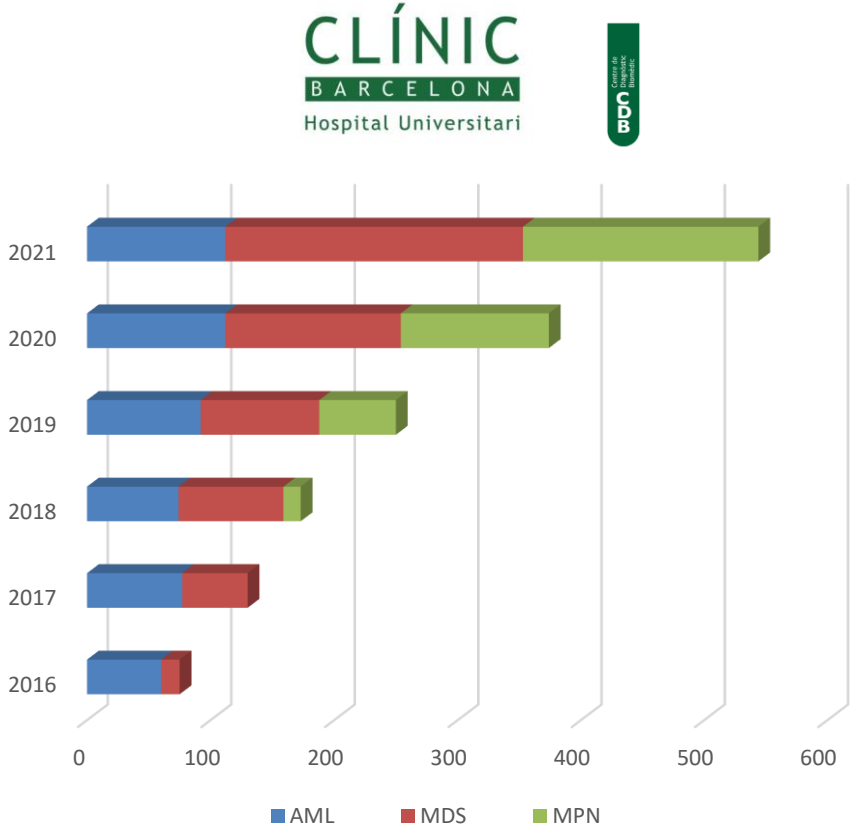
NGS activity in our institution

Ion PGM System

Ion GeneStudio™ S5 System
Ion Chef



Continuing growth of NGS studies means we need better solutions

2022

Genexus System

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For Research Use Only. Not for use in diagnostic procedures.

Research questions

- In the workflow of our routine laboratory, could the **Genexus system** achieve a **faster and easier turnaround time (TAT)** for the molecular characterization of myeloid neoplasm samples, in particular for AML?

- Could the **Oncomine Myeloid assay v2 GX** provide a fast and accurate result for ***FLT3*-ITD** in AML?

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Project objectives

<u>Project 1.</u> Comparison between the Oncomine Myeloid Assay GX, using the Ion Torrent Genexus System, with the standard laboratory workflows for myeloid neoplams samples (AML/MDS/MPN).

- Real time study measuring hands-on-time, staff training and TAT
- Fill the chip with retrospective RNA samples to look at some gene fusions

<u>Project 2.</u> Analysis of retrospective AML *FLT3*-ITD samples by the Oncomine Myeloid Assay GX V2 using the Ion Torrent Genexus System

- To compare the length and the allelic ratio of *FLT3*-ITD with capillary electrophoresis fragment analysis

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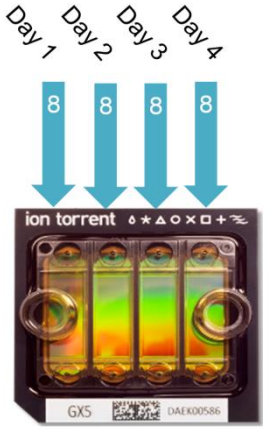
Project 1

Project 1. Comparison between the Oncomine Myeloid Assay GX, using the Ion Torrent Genexus System, with the standard laboratory workflows for myeloid neoplams samples (AML/MDS/MPN).

Real-time samples collected and run on S5 in parallel with GX

Phase 1: **Analysis of 48 samples with the Oncomine Myeloid Assay GX v1.**

- Runs a mixture of real-time samples and 'retrospective' RNA samples to maximise sequencing reagents
- 6 runs performed (8 DNA and 8 RNA samples).
- **13 AML** and 35 other myeloid neoplasm samples (MDS/MPN)



Phase 2: **Analysis of 64 samples with the Oncomine Myeloid Assay GX v2.**

- Runs DNA+RNA of real-time samples
- 8 runs performed (8 DNA and 8 RNA samples).
- **12 AML** and 52 other myeloid samples (MDS/MPN)

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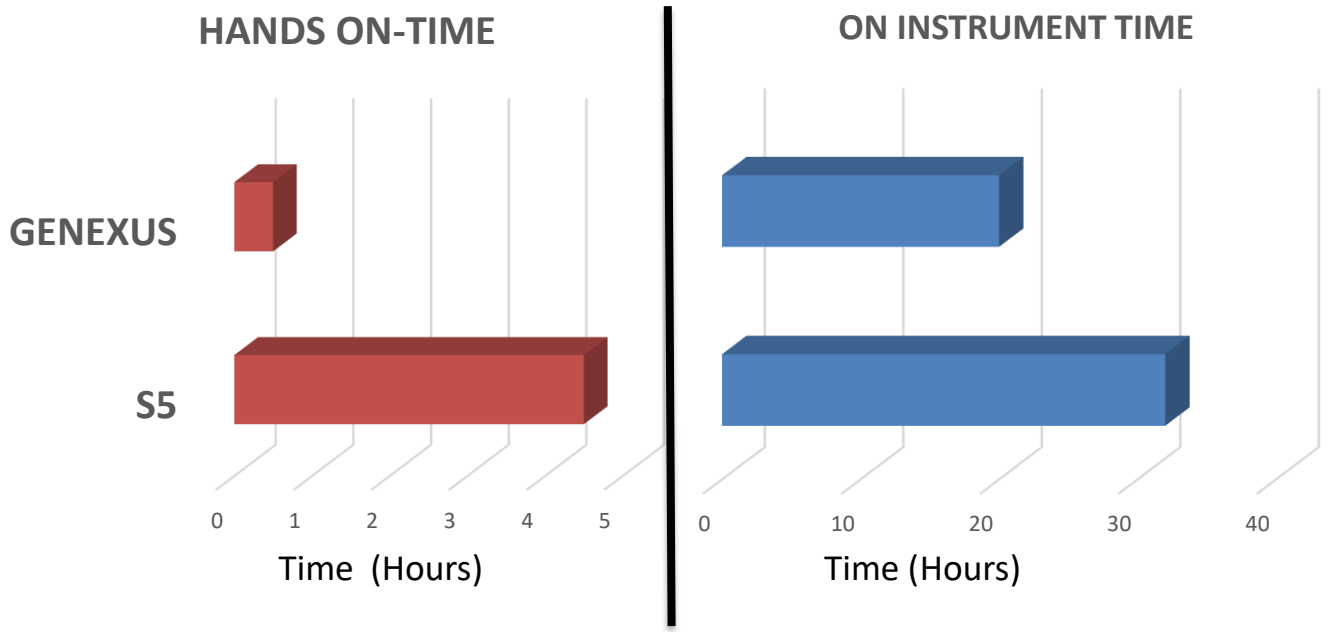
Results: Impact on lab resources



- ✓Increased automation
- ✓ Reduced technician hands
- ✓ Reduced staff training burden

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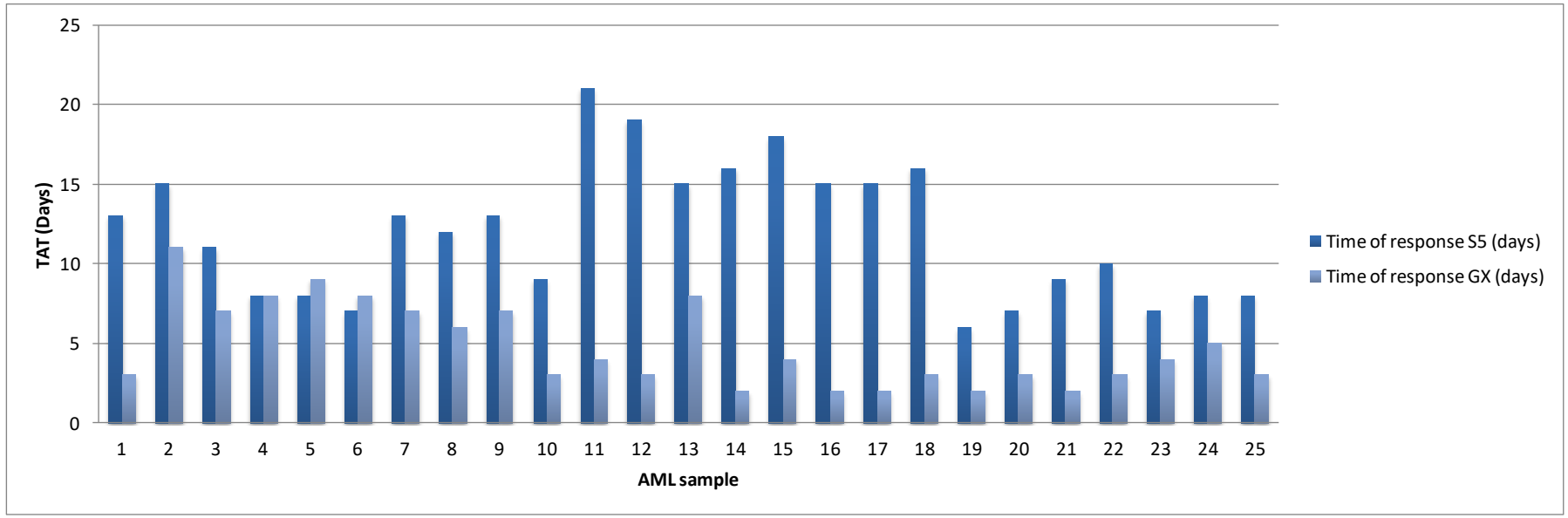
Results: Turnaround time for AML samples



Mean TAT S5 (Days): 11.92 ±4.22

Mean TAT Genexus (Days): 4.76 ±2.63

- Shorter TAT for Genexus
- Genexus TAT was able to remain much **more stable** with limited staff (holidays, SARS-COV2…)

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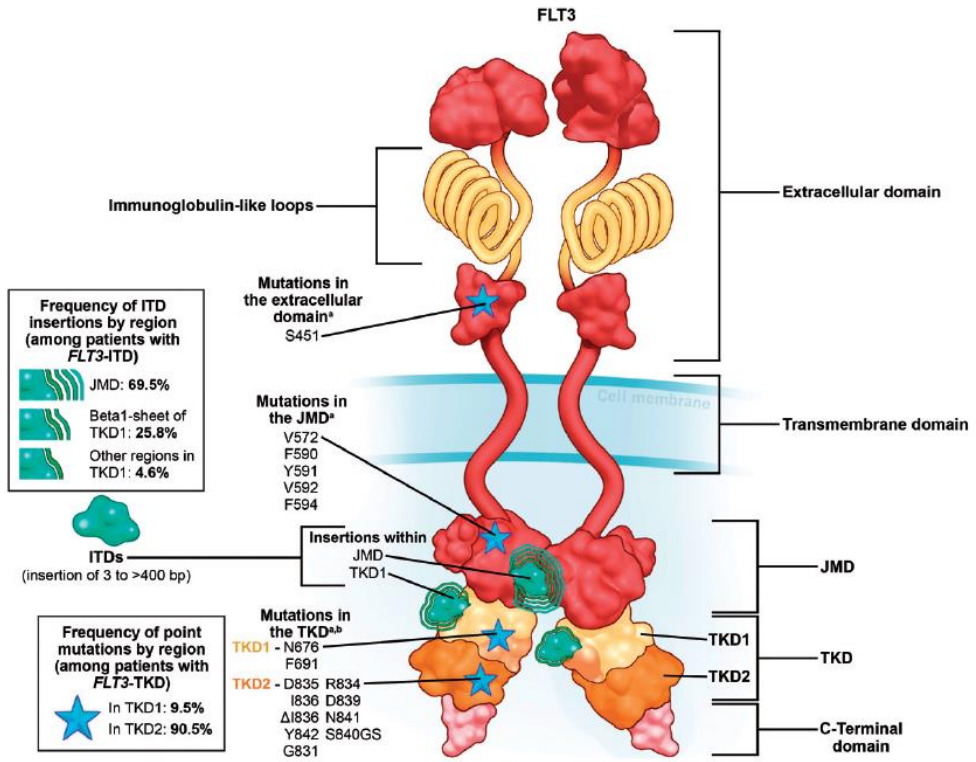
The fast NGS profiling optimizes the molecular characterization of AML



***Fast* NGS**: Detection of **subclonal** and **atypical** mutations in **targetable genes**

Case study

AML15 (Male, 57 yo; AML with *NPM1*mutation)

⬇ **TAT of 4 days**

Detection of **atypical FLT3 mutation** p.Val592Phe (exon 14, JM domain)

⬇

QT+Midostaurin

Patnaik MM. et al. Leuk Lymphoma. 2018 Oct;59(10):2273-2286

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The fast NGS profiling optimizes the molecular characterization of AML

Oncomine Myeloid Assay GX V2 Gene Content

DNA PANEL

Hotspot (28)

- *ABL1*
- *ANKRD26*
- *BRAF*
- *CBL*
- *CSF3R*
- *DDX41*
- *DNMT3A*
- *FLT3*
- *GATA2*
- *HRAS*
- *IDH1*
- *IDH2*
- *JAK2*
- *KIT*
- *KRAS*
- *WT1*
- *MPL*
- *MYD88*
- *NPM1*
- *NRAS*
- *PPM1D*
- *PTPN11*
- *SETBP1*
- *SF3B1*
- *SMC1A*
- *SMC3*
- *SRSF2*
- *U2AF1*

Full gene (17)

- *ASXL1*
- *BCOR*
- *CALR*
- *CEBPA*
- *ETV6*
- *EZH2*
- *IKZF1*
- *NF1*
- *PHF6*
- *PRPF8*
- *RB1*
- *RUNX1*
- *SH2B3*
- *STAG2*
- *TET2*
- *TP53*
- *ZRSR2*

RNA PANEL

Fusion (30) *

- *ABL1*
- *ALK*
- *BCL2*
- *BRAF*
- *CCND1*
- *CREBBP*
- *EGFR*
- *ETV6*
- *FGFR1*
- *FGFR2*
- *FUS*
- *HMGA2*
- *JAK2*
- *KMT2A (MLL-PTD)*
- *MECOM*
- *MET*
- *MLLT10*
- *MLLT3*
- *MYBL1*
- *MYH11*
- *NTRK3*
- *NUP214*
- *NUP98*
- *PDGFRA*
- *PDGFRB*
- *RARA*
- *RBM15*
- *RUNX1*
- *TCF3*
- *TFE3*

Expression (5)

- *BAALC*
- *MECOM*
- *MYC*
- *SMC1A*
- *WT1*

Control (5)

- *EIF2B1*
- *FBXW2*
- *PSMB2*
- *PUM1*
- *TRIM27*

New content

Case study

AML22 (Male, 74 yo): Detection of 2 *DDX41* variants (p.Arg525His and p.Asp140GlyfsTer2) → **TAT of 3 days** → AML with possible germline *DDX41* variant → **Germline study**

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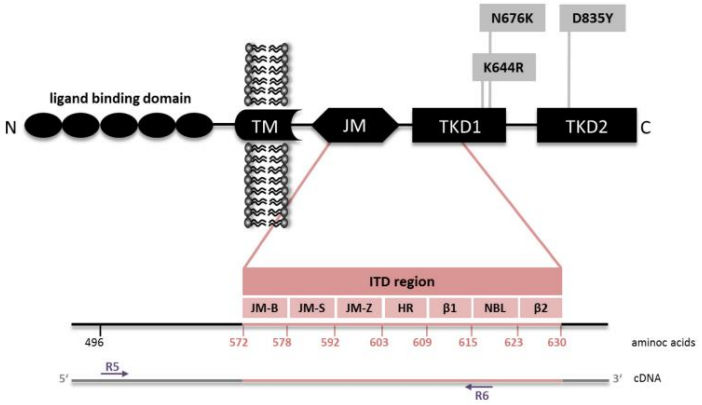
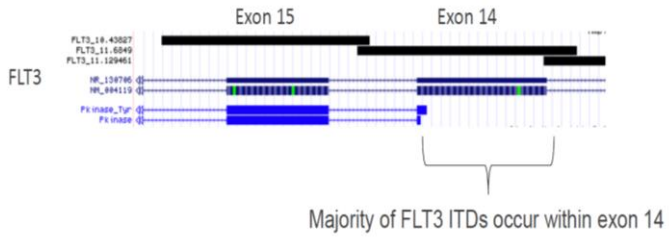
Project 2

Project 2. Analysis of retrospective AML *FLT3*-ITD samples by the Oncomine Myeloid Assay GX V2 using the Ion Torrent Genexus System

Analysis of 60 AML *FLT3*-ITD samples with the Oncomine Myeloid Assay GX v2.





FLT3-ITD as a prognostic and therapeutic marker in AML

- ✓ The most common type of *FLT3* mutation in AML (~25%)
- ✓ Constitutive activation of the receptor
- ✓ Confers a **poor prognosis** (allelic ratio mut/wt)
- ✓ **Therapeutic target** ➡ **Requires fast TAT**
- ✓Recommended technique still capillary electrophoresis
- ✓Lack of enough data generated by NGS

FLT3-ITDs occur in Exons 14 and 15 of the gene, and are covered by two amplicons that are anchored in the flanking introns to better accommodate any potential disruption of binding within the coding sequence by an ITD.

Schranz K, et al. Oncotarget. 2018 Jul 10;9(53):30128-30145

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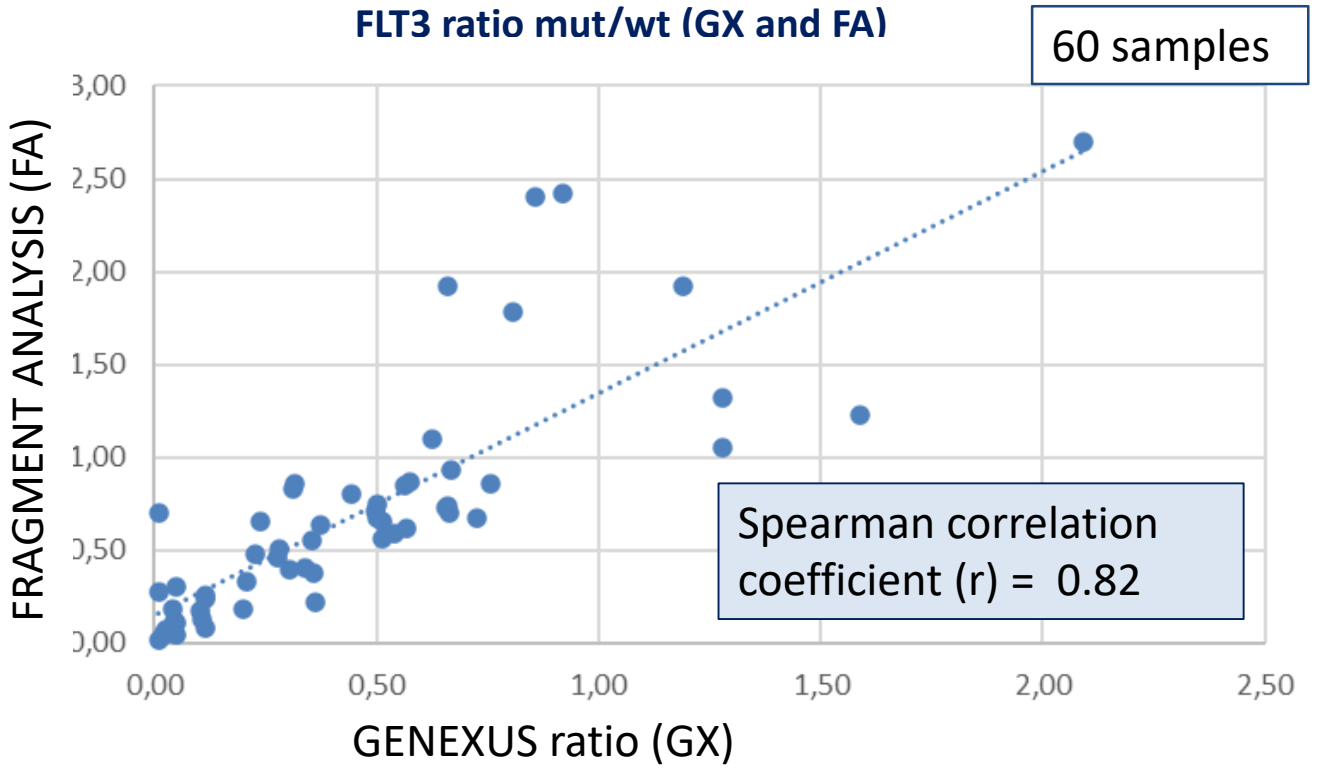
Results: The allelic FLT3 ratio detected by NGS highly correlates with the gold-standard technique



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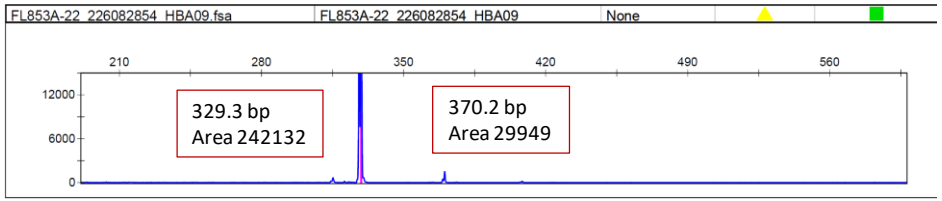
Results: Accurate detection of low allele frequency FLT3-ITDs

Case study	AML53, AML with *NPM1* mutation

CE

Mut/wt Ratio	Length
0.074	42bp



NGS

Protein	cDNA	Allele Frequency	Mut/wt Ratio	Length
p.Tyr597_Leu610dup	c.1787_1787delins	2.3 %	0.024	42 bp

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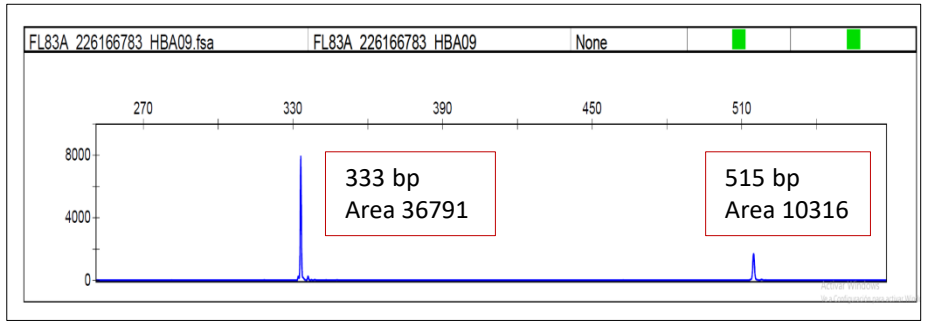
Results: Accurate detection of long FLT3-ITDs

Ranging size: 12 to 180 bp (60 samples)

Case study	AML57, AML with *NPM1* mutation

CE

Mut/wt Ratio	Length
0.28	180bp



NGS

Protein	cDNA	Allele Frequency	Mut/wt Ratio	Length
p.Lys614_Val615ins	c.1773_1774ins	0.3 %	0.3	180 bp

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Conclusions

PROJECT 1

- Rapid method for testing 8 samples
- Fast way to see the results and download files
- Reduced lab training, hands on time and turnaround time compared to current worflow
- More stable TAT in the face of staff absences

PROJECT 2

- High correlation in *FLT3*-ITD detection between NGS and capillary electrophoresis
- Detection of low allele frequency and long *FLT3*-ITDs
- Regarding *FLT3*-ITD annotation, some issues to solve

Next steps

- Optimization of filters for *FLT3*-ITD detection
- Optimization of *FLT3*-ITD annotation
- Analysis of the prognostic value of the allelic *FLT3*-ITD ratio and the VAF detected by NGS

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Hematopathology Section, Pathology Department

Dolors Colomer, PhD
Senior Consultant, Chief Hematopathology Section
Mònica Lopez-Guerra, PhD,
Senior Specialist

Molecular Core Facility

Pedro Jares, PhD
Consultant

Irene Dapia , MSc, PhD
Clinical Application Consultant, Spain, Portugal

Katelyn Gilmour, PhD
Senior Medical Science Liaison | EMEA Medical Affairs Team
Clinical NGS and Oncology Division | Speciality Diagnostics Group

Thanks!