Application of Molecular Profiling by MassARRAY Techniques; Perspective for the management of common hematological malignancy

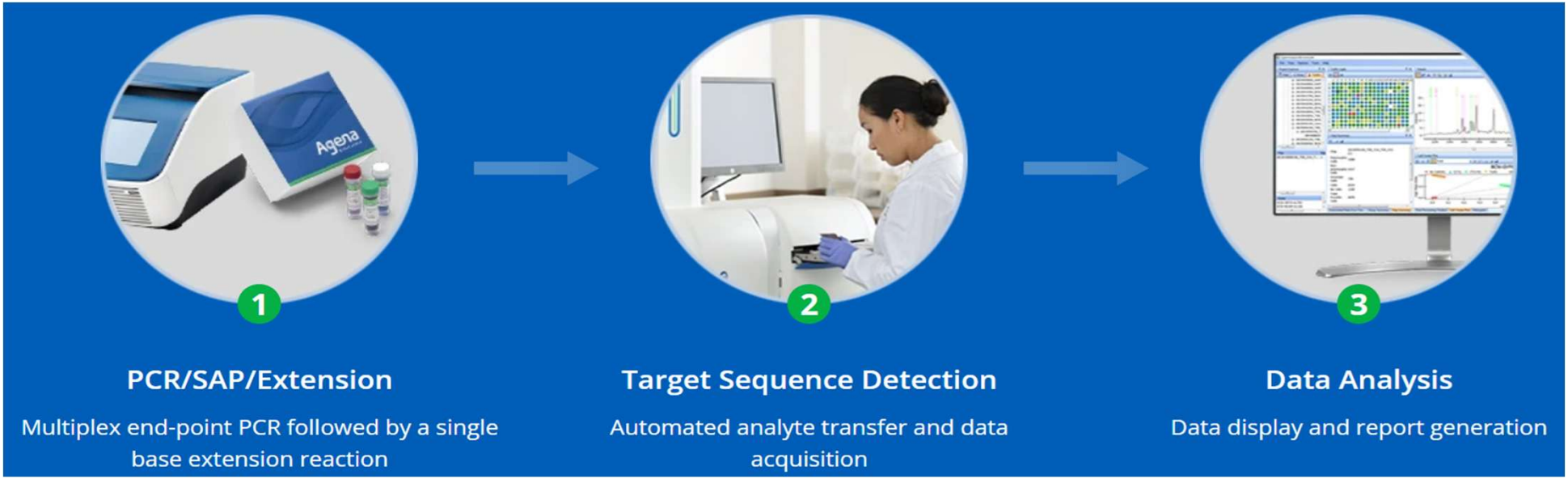
Teerapong Siriboonpiputtana, PhD (Cancer Studies Research)
Certificated of Genetic Counseling
MRes in Biomedical Sciences (Biology of Cancer)
MSc in Clinical Pathology
BSc in Medical Technology

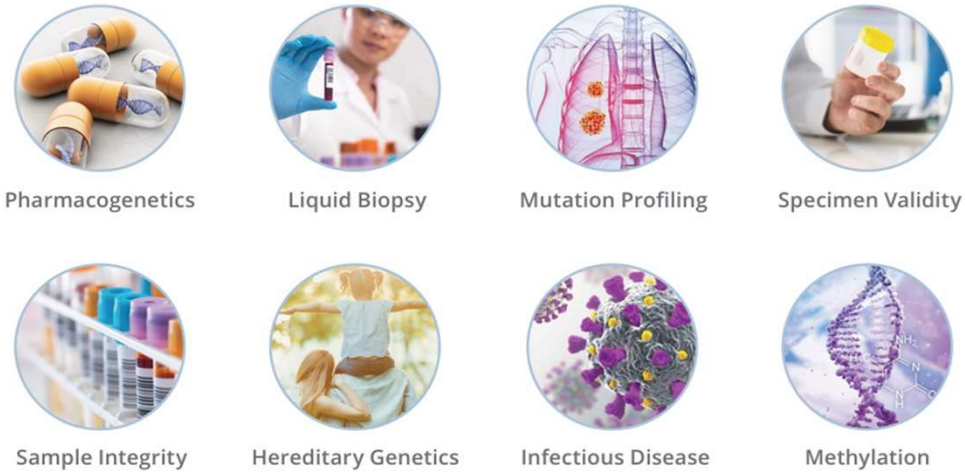
Disclosure

"I have NO financial disclosure or conflicts of interest with the presented material in this presentation."

Talk outline

- Concise to MassARRAY system
- Concept of leukemogenesis
- Application of MassARRAY for the management of hematologic malignancies
- Future and trends of genetic tests in cancer management
- Q &A





Customed panel

Services

Research

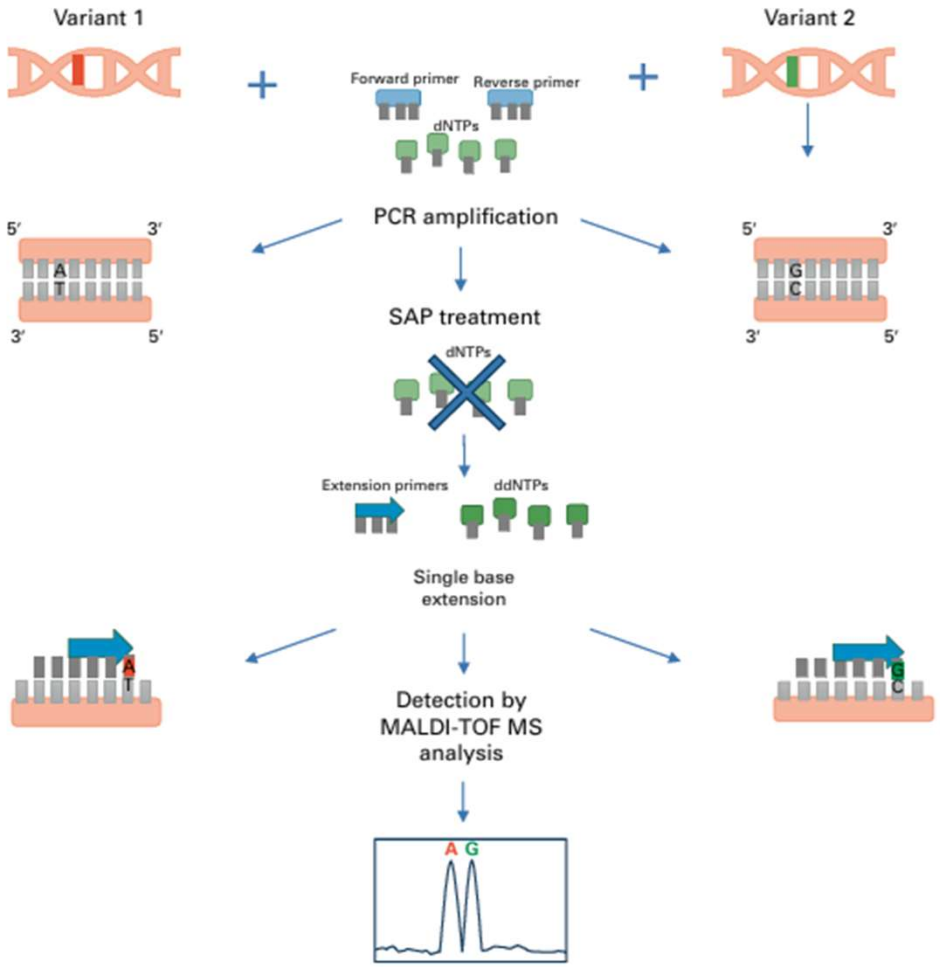
“Institution’s Intellectual”

1. Multiplex PCR

2. SAP treatment

3. SBE reaction

4. MS analysis



Stenuerwald et al., JCO Oncol Pract 20:1441-1451

Pros

- Up to 50 variants in one reaction
- Kits and Open-costumed
- DNA, cDNA
- SNPs, SNVs, deletions, insertions, translocations
- High specificity and sensitivity
- Need less samples
- Not complex bioinformatic lines
- Cost effective
- Short turnaround time (TAT)
- Mid-throughput technique
- Good for screening large sizes of the population

Limitations

- Potential for false-negative data (designed for known variants/hotspot mutations)

Blood/Hematologic Cancers

"Cancer that originate from blood-forming tissue, such as the bone marrow, or in the cells of the immune system (e.g., leukemia, lymphoma, and multiple myeloma)"

Very heterogeneity in both genetic and phenotypic backgrounds (largely depended on subtypes of leukemia)

Acute myeloid leukemia (AML)
Acute lymphoid leukemia (ALL)
Hairy cell leukemia
Lymphoma

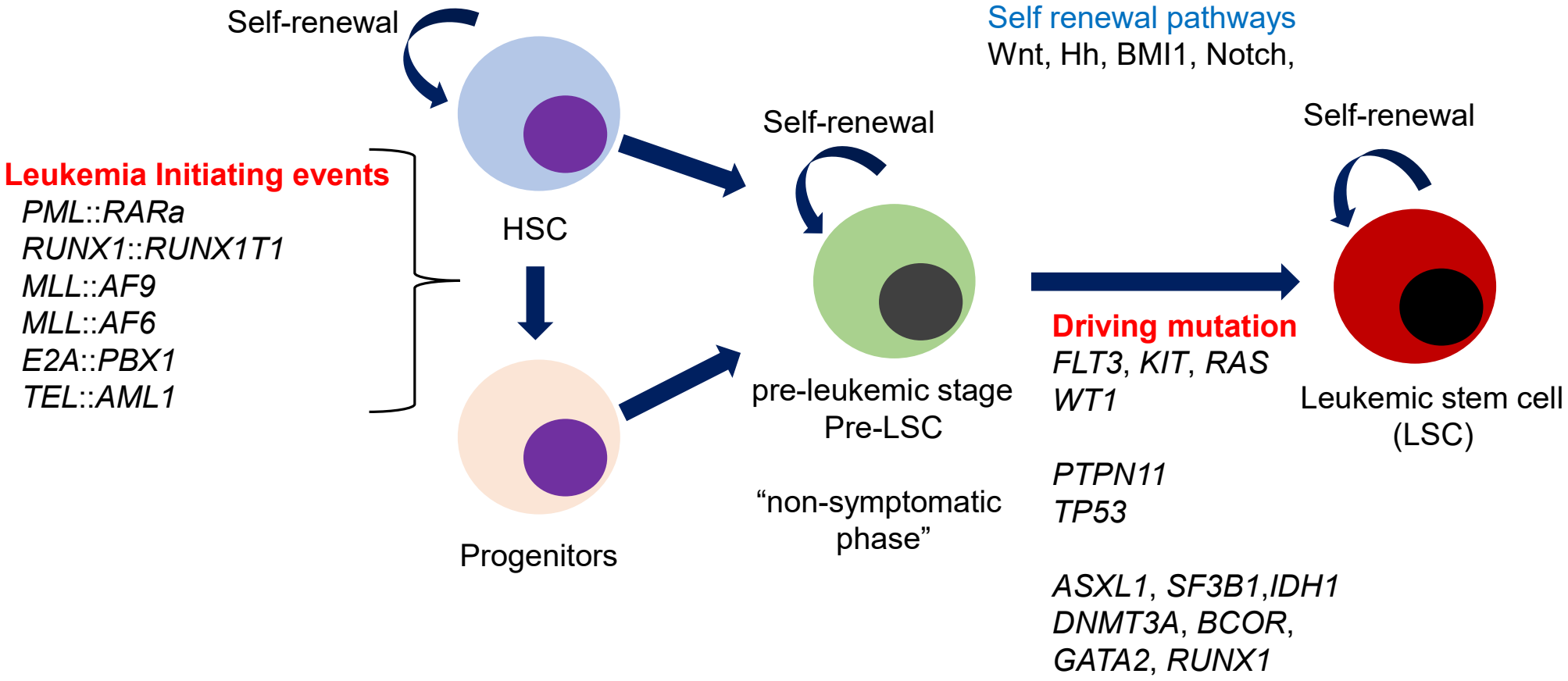
Multiple myeloma

Chronic myeloid leukemia (CML)
Chronic lymphoid leukemia (CLL)

Myelodysplastic syndrome (MDS)

Polycythemia vera (PV)
Essential thrombocytosis (ET)
Primary myelofibrosis (PMF)

Concept of leukemogenesis in AML and ALL



Common forms of blood cancers

Acute leukemia
- Acute myeloid leukemia (AML)
- Acute lymphoid leukemia (ALL)

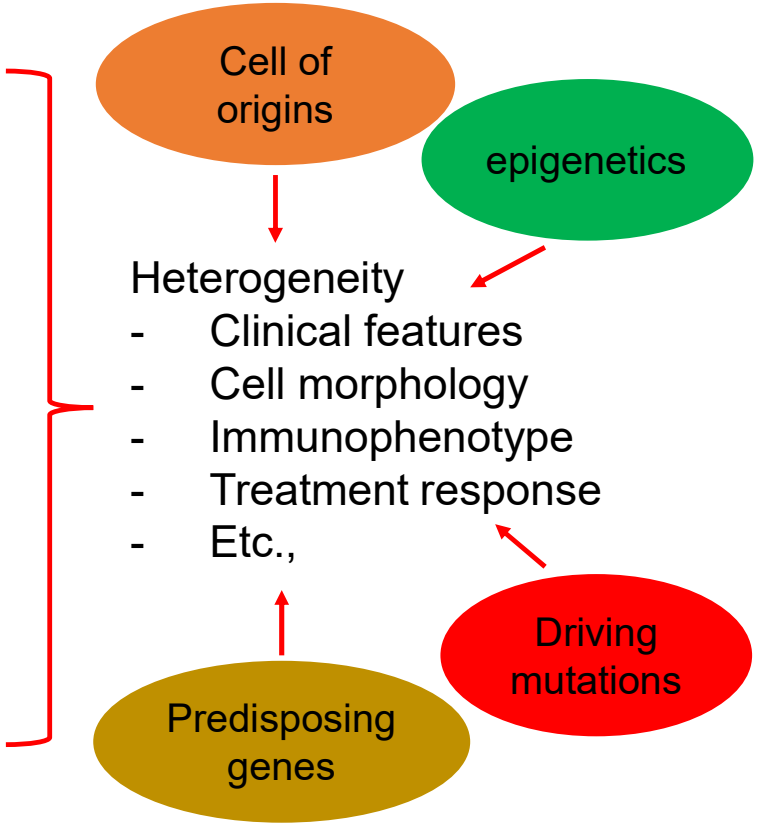
Chronic leukemia
- Chronic myeloid leukemia (CML)
- Chronic lymphoid leukemia (CLL)

Myelodysplastic syndrome (MDS)

Multiple myeloma

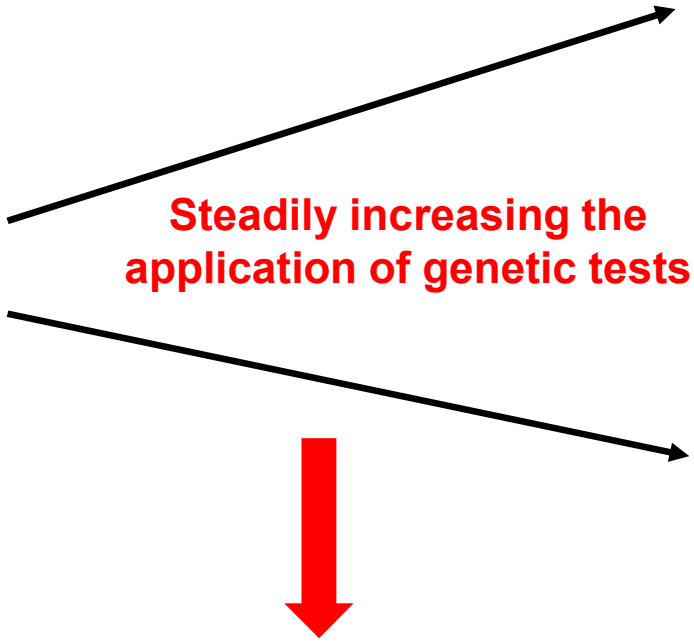
Lymphoma

Etc.,



Classification of tumors in myeloid and lymphoid tissues

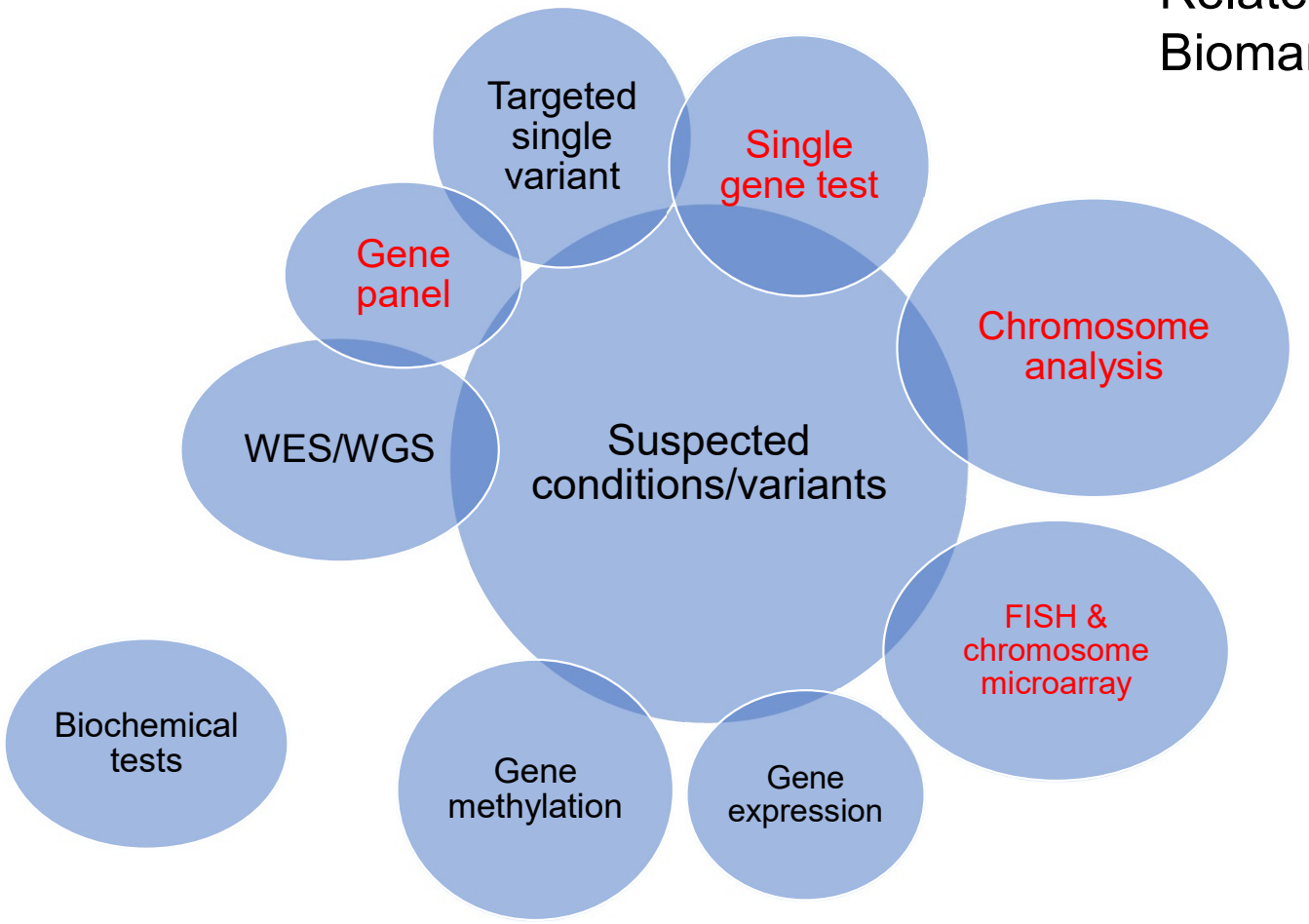
- WHO classification
- ELN guideline
- NCCN guideline
- and so on



- Novel leukemia genetic biomarkers
- Novel targeted therapies
- Promising clinical trials
- Precision medicine and genetic counseling

Improve diagnosis, classification, prognostication, and monitoring of disease response to treatment

Types of genetic tests



Related to leukemia/tumor
Biomarkers

Diagnosis, Prognosis,
Risk-assessment, Monitoring,
Measurable residual disease
(MRD)

Stepwise algorithms
Board-based testing

Myeloproliferative neoplasms (MPNs)

Chronic myeloid leukemia (CML)

Polycythemias vera (PV)

Essential thrombocythemia (ET)

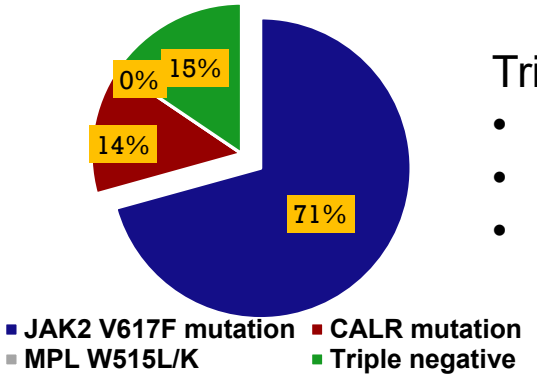
Primary myelofibrosis (PMF)

Chronic neutrophilic leukemia (CNL)

Chronic eosinophilic leukemia (CEL)

Juvenile myelomonocytic leukemia (JMML)

Myeloproliferative neoplasm, not otherwise specified



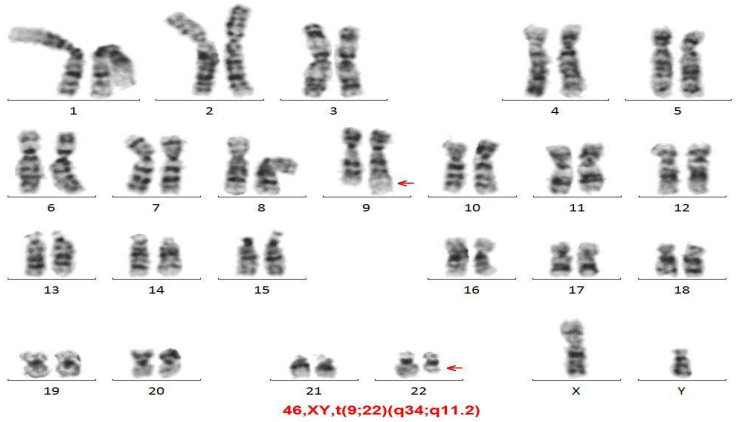
Triple Markers

- *JAK2*
- *MPL*
- *CALR*

Diagnosis:
Clinical features
Bone marrow biopsy
Molecular genetic profiling

Chronic phase → Transitional/ accelerated phase → Blast phase

Philadelphia Chromosome t(9;22)(q34;q11.2)



CML is positive for the Ph chromosome in > 95% of cases.

Myeloproliferative neoplasms (MPNs)

Recent = WHO 2017 (4th Edition)
Now = WHO 2022 (5th Edition) with minor changes in *BCR*::*ABL1* negative MPNs (PV, ET, PMF)

Chronic myeloid leukaemia (*BCR*::*ABL1* (Philadelphia) positive)

Polycythaemia vera
Essential thrombocythaemia
Primary myelofibrosis

Common Mut
JAK2 V617F
JAK2 exon 12
MPL W515L/K
CALR exon 9

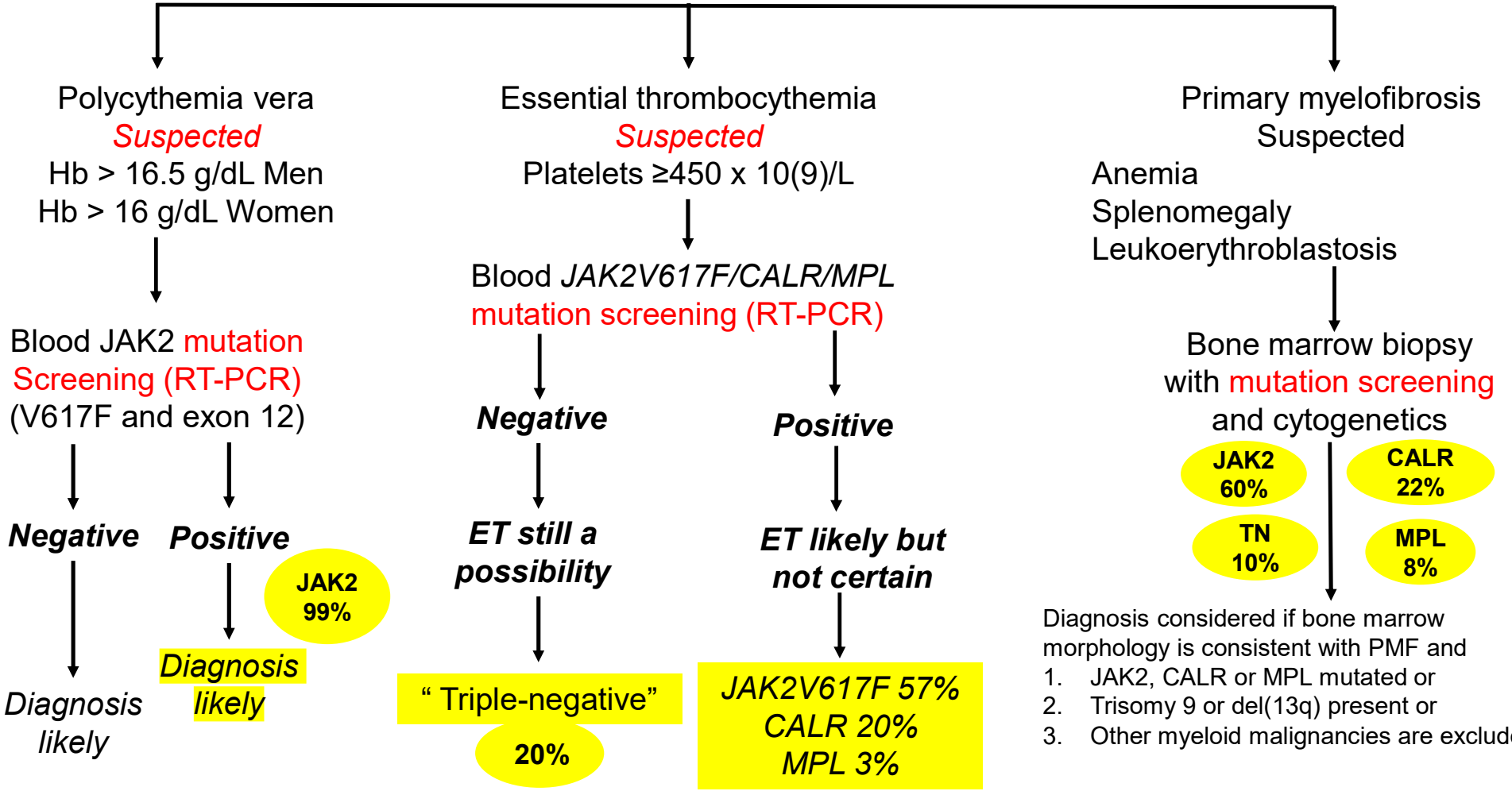
~ 50%
TET2
ASXL1
DNMT3A

Rare Mut
SRSF2,
SF3B1,
U2AF1,
ZRSR2

EZH2, *IDH1*, *IDH2*, *CBL*, *KRAS*, *NRAS*, *STAG2*, *TP53*

Chronic neutrophilic leukaemia
Chronic eosinophilic leukaemia
Juvenile myelomonocytic leukaemia
Myeloproliferative neoplasm, not otherwise specified

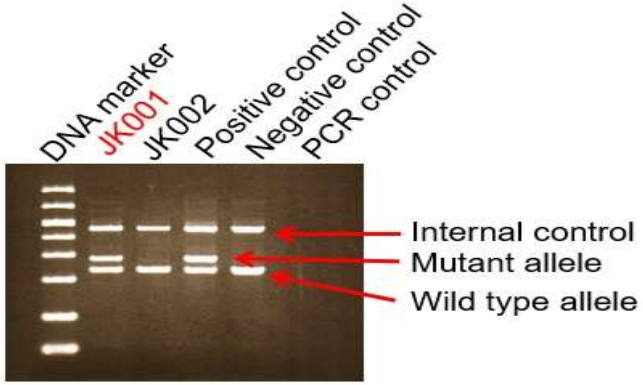
Diagnostic algorithm for myeloproliferative neoplasms

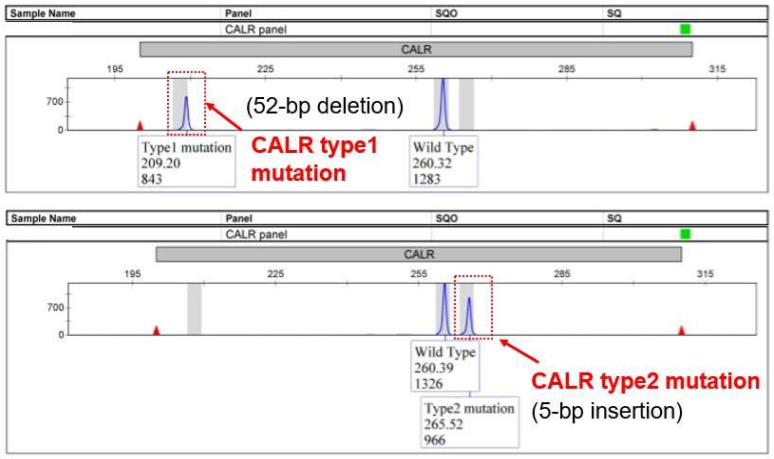


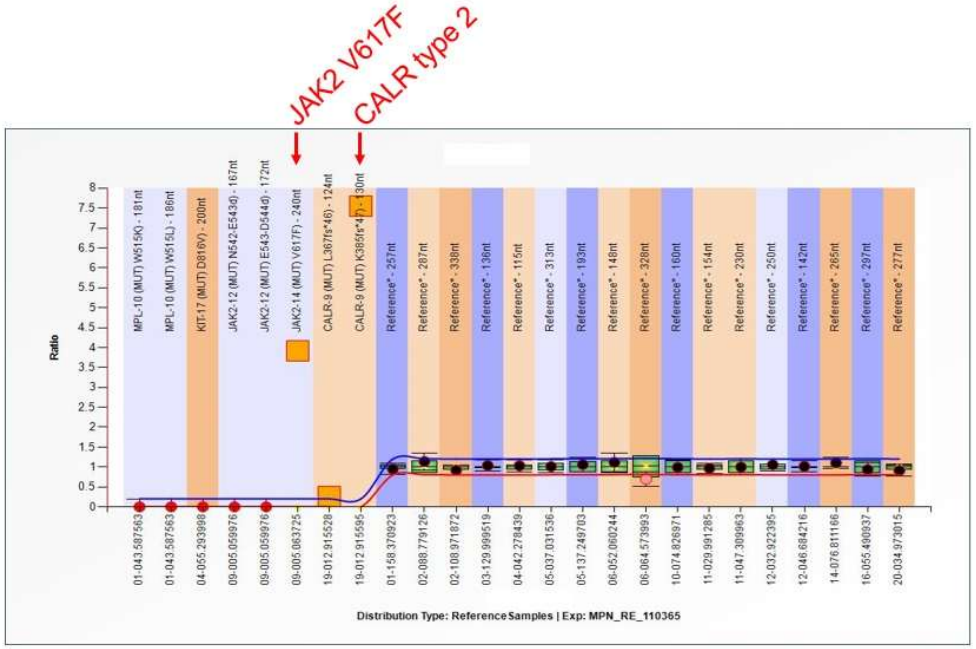
Tefferi and Barbui., Am J Hematol. 2023.

Common genetic tests for the management of MPNs

ASO-PCR for detection of ***JAK2* V671F**



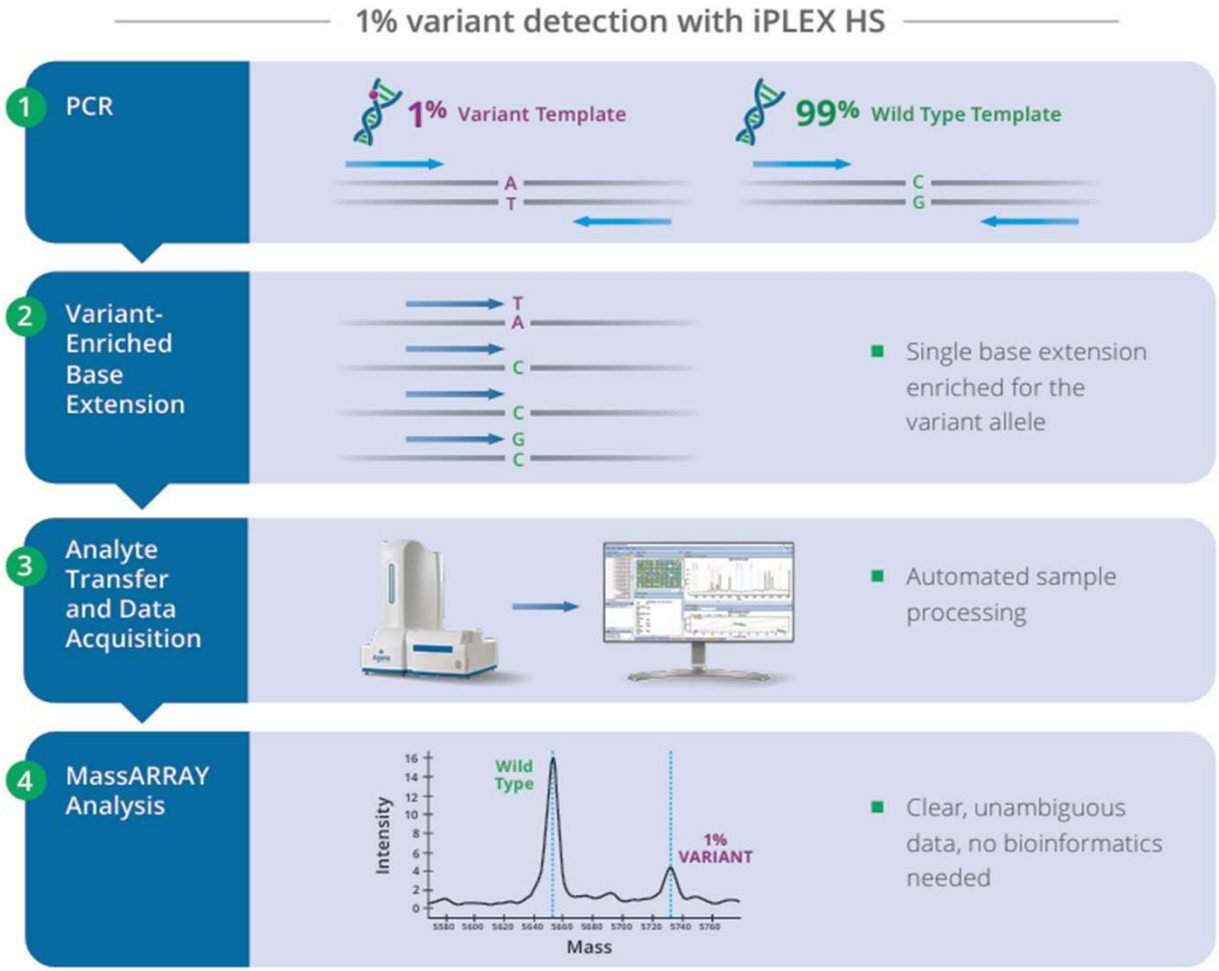




MLPA for detection of ***JAK2*, *CALR*, *MPL* and *c-kit* mutations**

CE-PCR for detection of ***CALR* mutations**

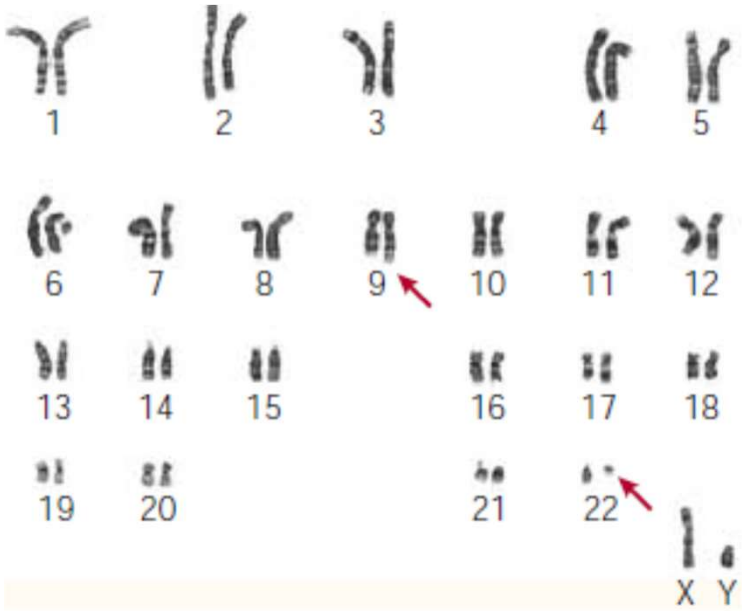
Internation consensus classification for diagnosis of PV



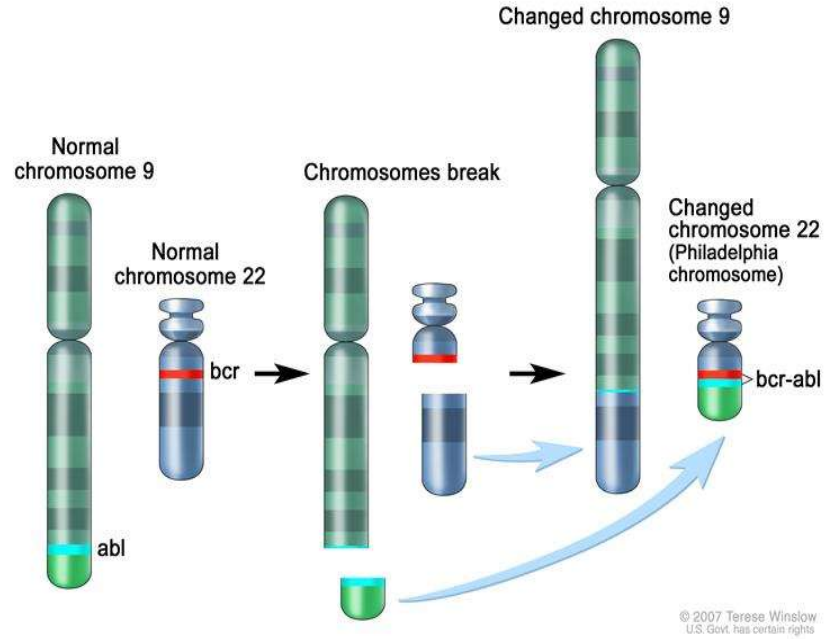
Recommended to use highly sensitive assay for JAK2 V617F (<1%) and CALR and MPL (1%-3%) in negative cases, consider searching non-canonical JAK2 mutations

Biology of chronic myeloid leukemia (CML)

- Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm with an incidence of 1–2 cases per 100,000 adults.
- It accounts for approximately 15% of newly diagnosed adult leukemia cases.
- In Thailand the incidence is about 1,000 new cases of CML ([1.5/100,000] x 66,052,615) in 2024.

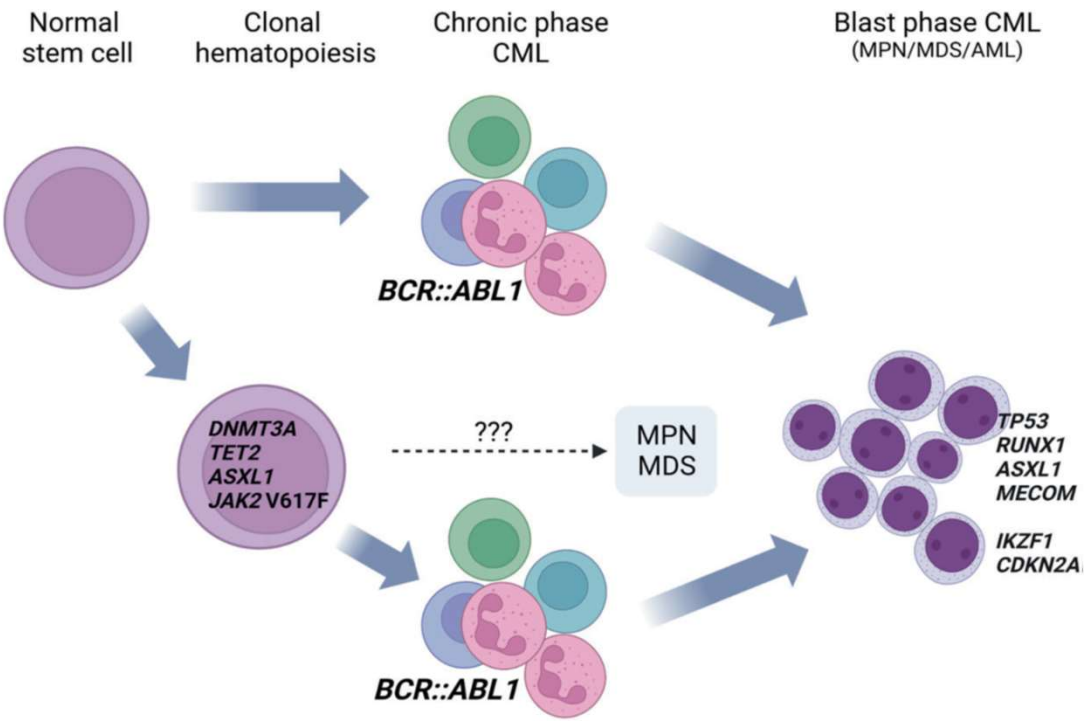


Knudson, Nat. Rev. Cancer, 2001.



http://www.cancer.gov/types/leukemia/patient/cml-treatment-pdq

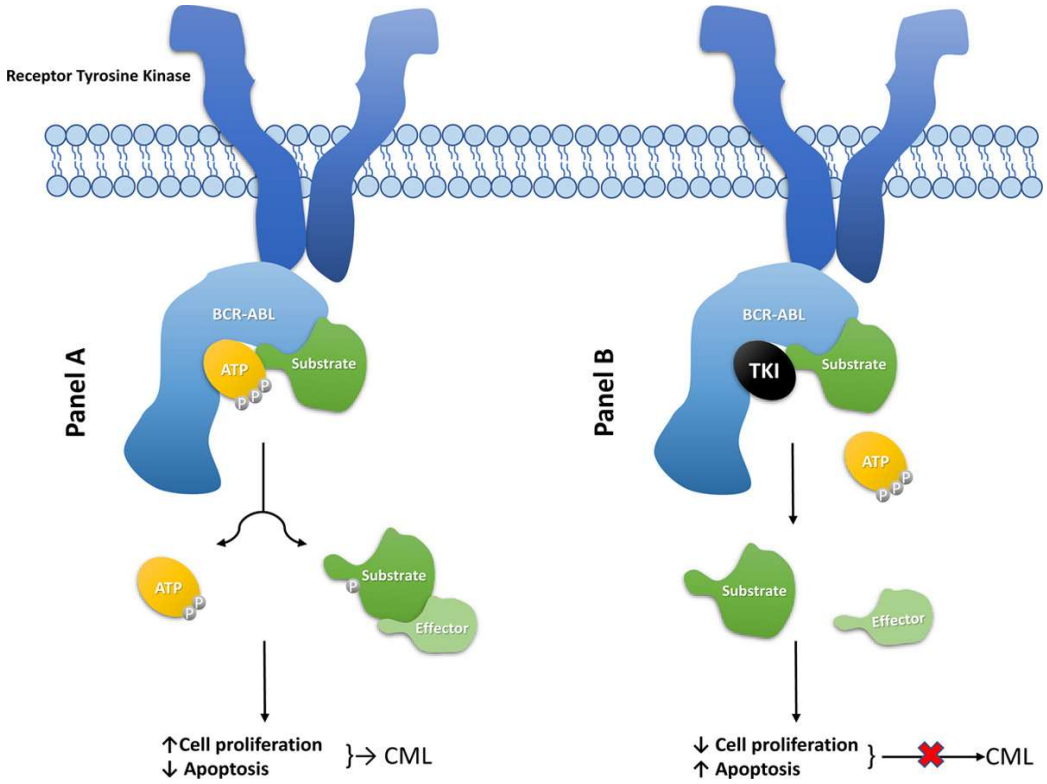
Model of two pathways to CML



- A normal hematopoietic stem cell acquires the BCR::ABL1 fusion leading to the development of CP CML
- commonly mutated genes at BP are indicated: **TP53, RUNX1, ASXL1 and MECOM** are associated with **myeloid BP**
- **IKZF1 and CDKN2A/B** are associated with **lymphoid BP**
- In some cases, BCR::ABL1 is acquired on a background of CH (either as a CH subclone or independently of the CH clone), for example, CH driven by mutations in DNMT3A, TET2, ASXL1, or JAK2
- The dotted line indicates a potential route to transformation from the CH clone (which may also develop ACAs) to a BCR::ABL1-negative myeloid neoplasm such as MPN or MDS

N.C.P. Cross et al., Leukemia. 2023.

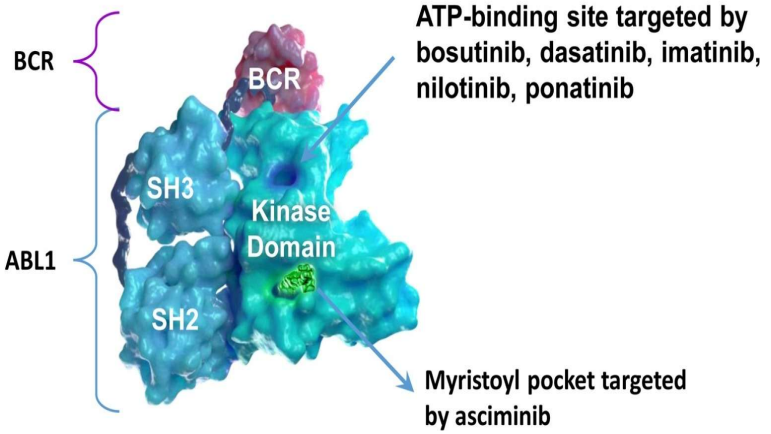
Classical and successful cancer-targeted therapy; tyrosine kinase inhibitor for CML



www.ahajournals.org/doi/10.1161/ATVBAHA.119.313353

- Imatinib (Gleevec)
- Dasatinib (Sprycel)
- Nilotinib (Tasigna)
- Ponatinib (Iclusig)
- Bosutinib (Bosulif)
- Asciminib

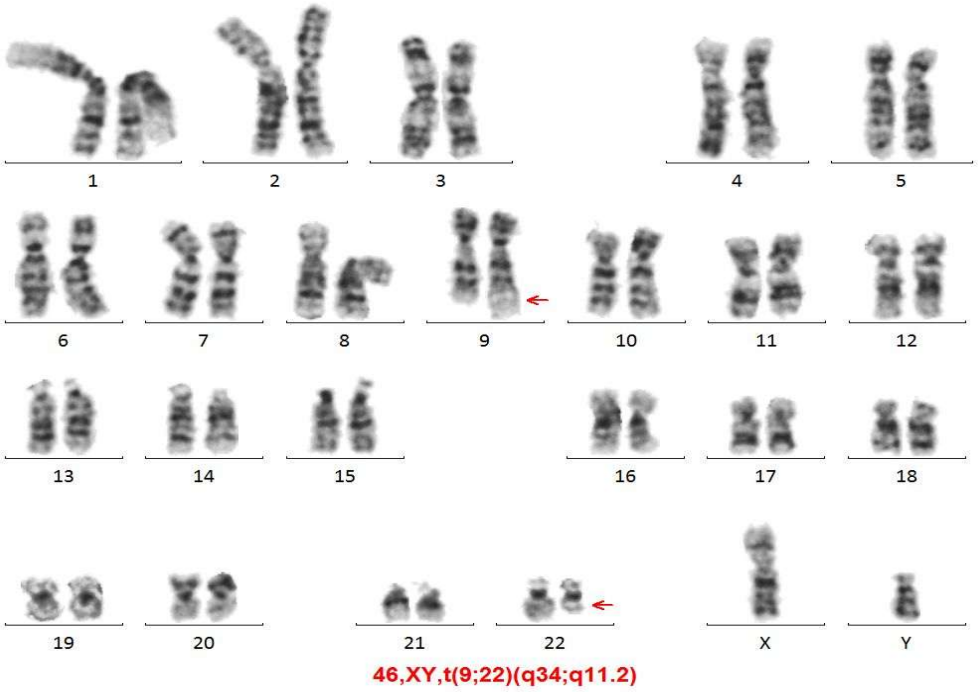
Assembled inactive conformation ABL1



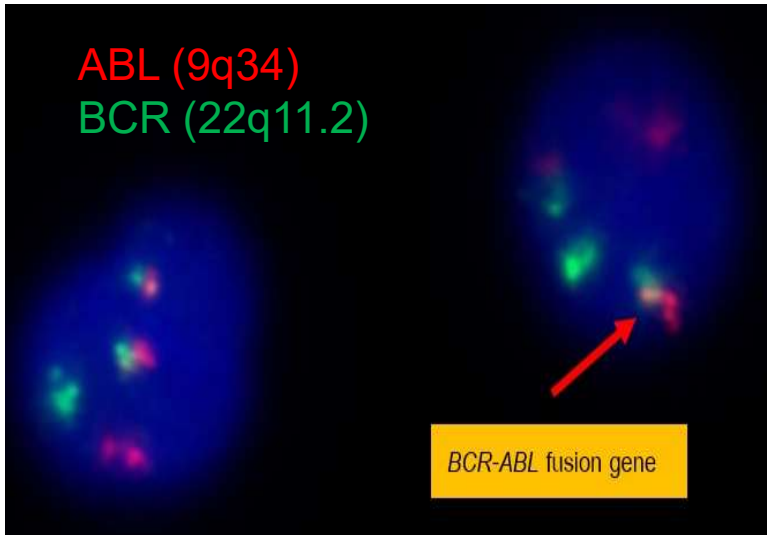
Manley et al., Leu Res. 2020

Philadelphia (Ph) chromosome

A gold standard method for CML diagnosis; positive > 95 % of CML



FISH is very helpful in a setting where it is not able to do chromosome analysis and in CML with atypical *BCR*::*ABL1*.



Human Genetic Lab, Pathology, Ramathibodi Hospital

CML; Minor changes in the WHO 5th edition

- Less considering a transition/accelerated phase (omit AP)
- Key attributes
  - Considering ABL1 kinase domain mutations
  - Additional cytogenetic abnormalities (ACAs)
- Emphasis on high-risk features associated with CP progression
- BP = ≥20% myeloid blasts in the blood or bone marrow; or the presence of an extramedullary proliferation of blasts; or the presence of increased lymphoblasts in peripheral blood or bone marrow.

Khoury et al., Leukemia (2022) 36:1703–1719

2023: ELN recommendations for the diagnosis and management of chronic myeloid leukemia

General laboratory recommendations:

- All tests for which the results are used for clinical management should be conducted in appropriately accredited laboratories, e.g., to **ISO15189.2022**, and fully validated before clinical use.
- Testing laboratories should participate in appropriate **external quality assurance (EQA)** schemes.

Recommendations:

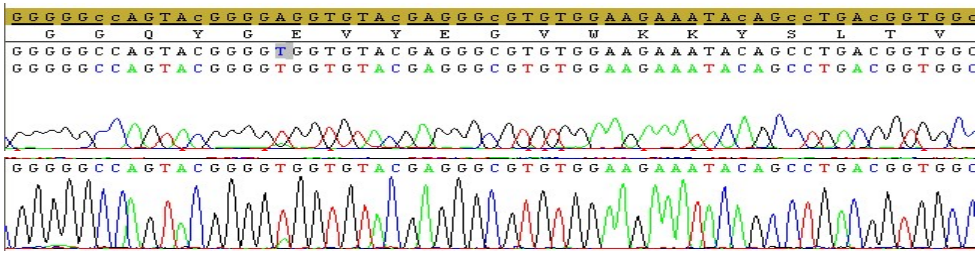
- Cytogenetics, along with FISH and/or RT-PCR, should be used in all cases to confirm a diagnosis of CML. The limitations of each approach as standalone tests need to be understood and, where appropriate, included in clinical reports.
- **Cytogenetic** testing should include a screen for **ACAs** at diagnosis.
- **BCR::ABL1 mRNA transcript** type should be determined for **all cases** prior to treatment to enable appropriate follow up.
- The possibility of a rare BCR::ABL1 variant should be excluded. If testing for rare variants is not available, the diagnostic report should clearly state that the presence of a BCR::ABL1 remains a possibility and that further testing in an appropriate reference laboratory should be performed.

N.C.P. Cross et al., Leukemia. 2023.

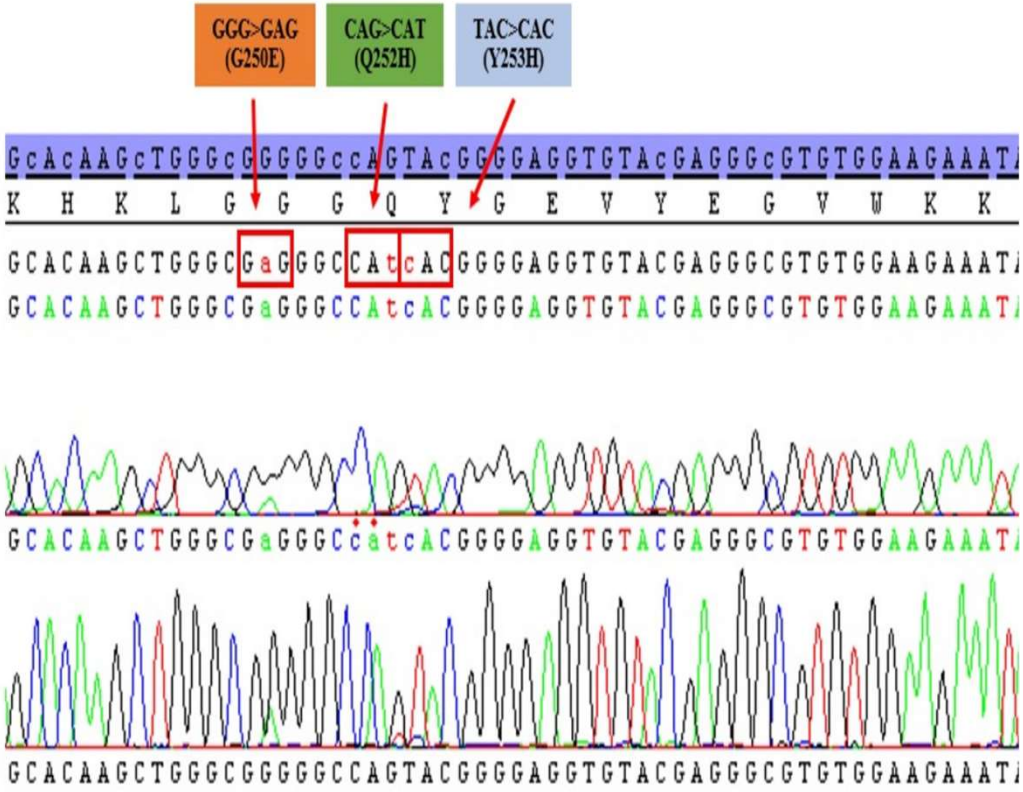
Direct sequencing of *BCR*::*ABL1* mutations

T315I (ACT>ATT)

E255V (GAG>GTG)

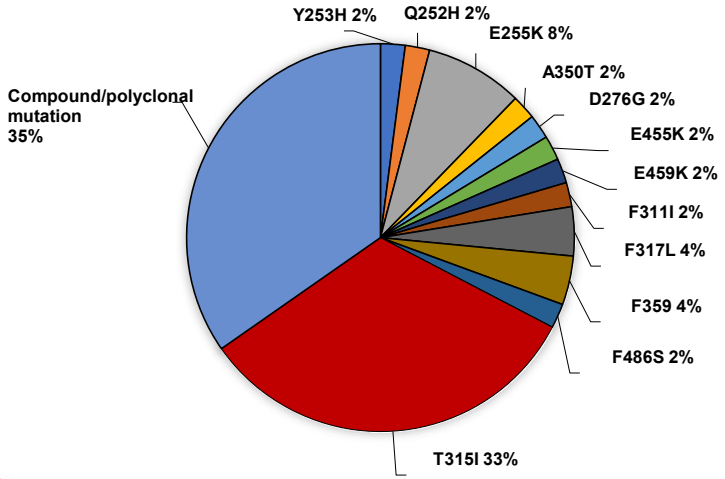


available from Human Genetic Lab, Pathology, Ramathibodi Hospital

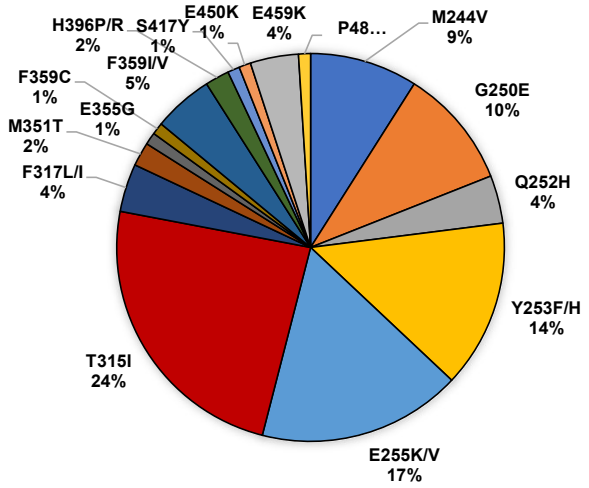


Electropherogram of *BCR-ABL1* tyrosine kinase domain sequencing profile from patient with G250E/Q252H/Y253H compound/polyclonal mutation.

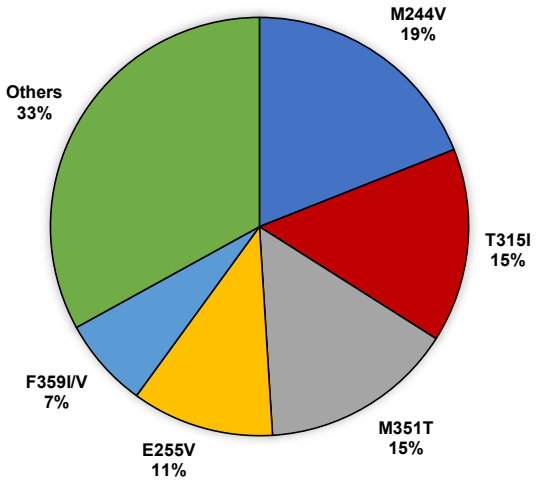
Ramathibodi, 2019



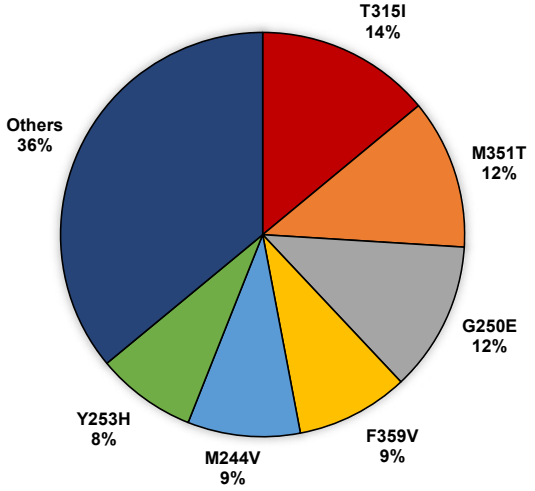
Korean (Kim So. 2009)



Hungarian (Meggyesi N. 2012)



Australian (Branford S. 2009)



Mass array panel for BCR-ABL1 and common genetic alterations in classical MPNs

Journal of Mass Spectrometry and Advances in the Clinical Lab 28 (2023) 122–132

Contents lists available at ScienceDirect

Journal of Mass Spectrometry and Advances in the Clinical Lab

journal homepage: www.sciencedirect.com/journal/journal-of-mass-spectrometry-and-advances-in-the-clinical-lab

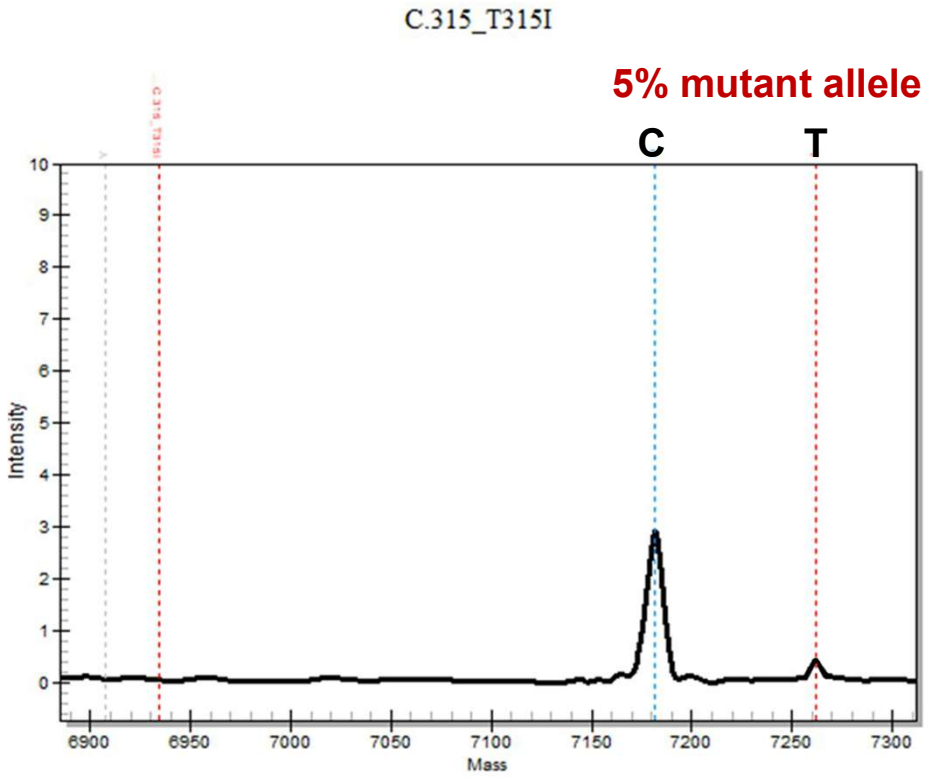
Research Article

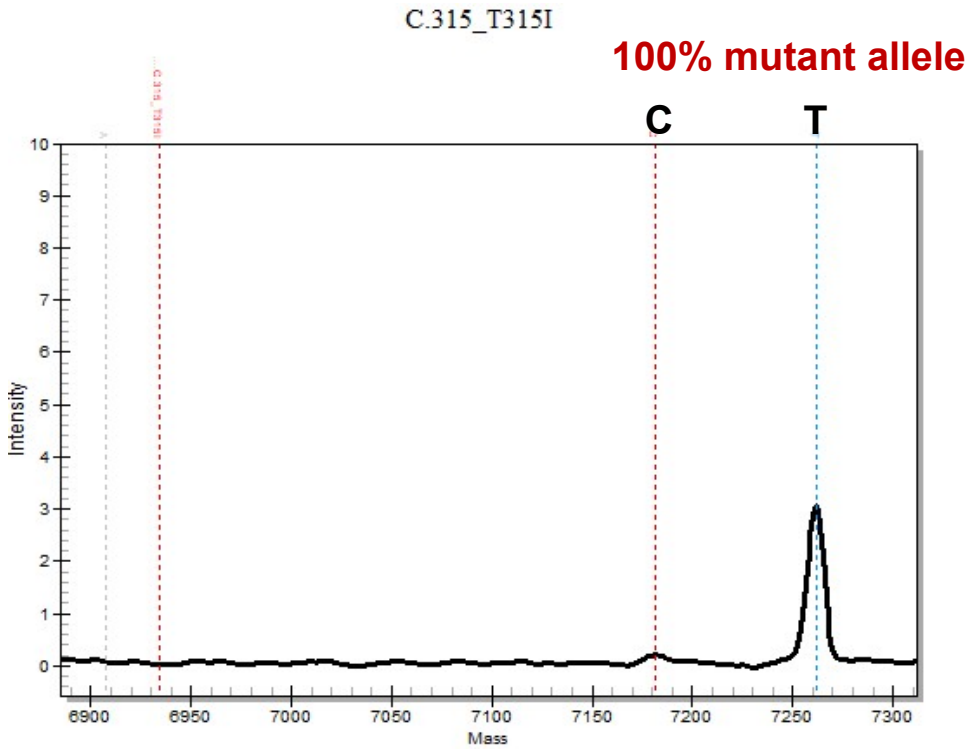
A customized mass array panel for *BCR::ABL1* tyrosine kinase domain mutation screening in chronic myeloid leukemia

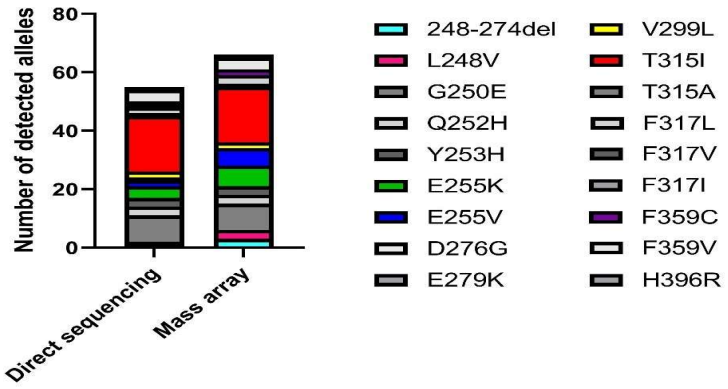
Nittaya Limsuwanachot [a], Budsaba Rerkamnuaychoke [a], Pimjai Niparuck [b], Roongrudee Singdong [a], Adcharee Kongruang [a], Piyapha Hirunpatrawong [c], Thanaporn Siriyakorn [c], Pa-thai Yenchitsomanus [d], Teerapong Siriboonpiputtana [a,*]

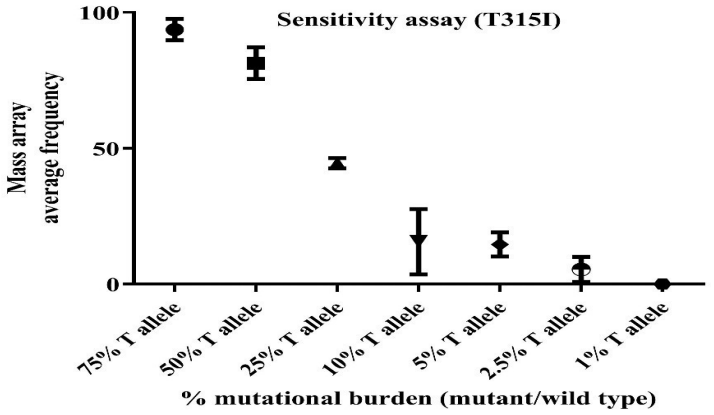
[a] *Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*
[b] *Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*
[c] *Lifomics Company Limited, Bangkok, Thailand*
[d] *Siriraj Center of Research Excellence for Cancer Immunotherapy (SiCORE-CIT), Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand*

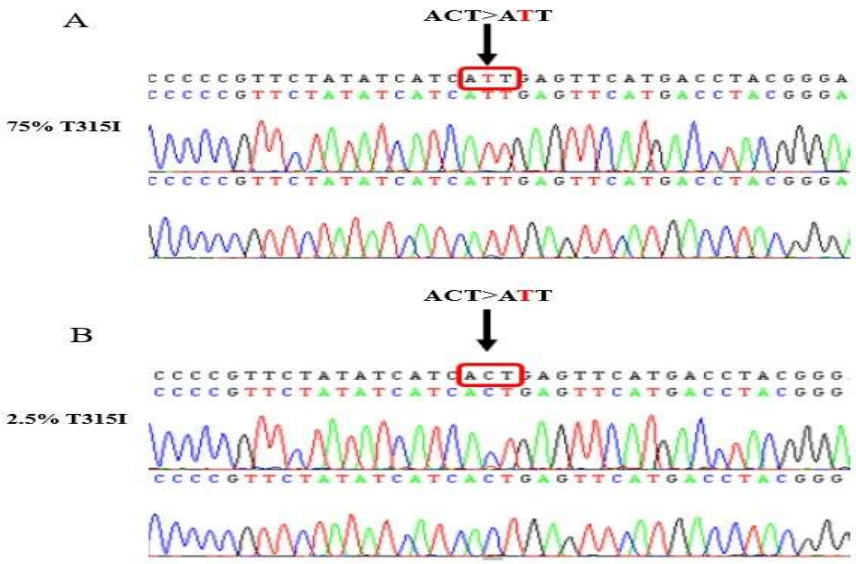
What is the mass array's data look like?

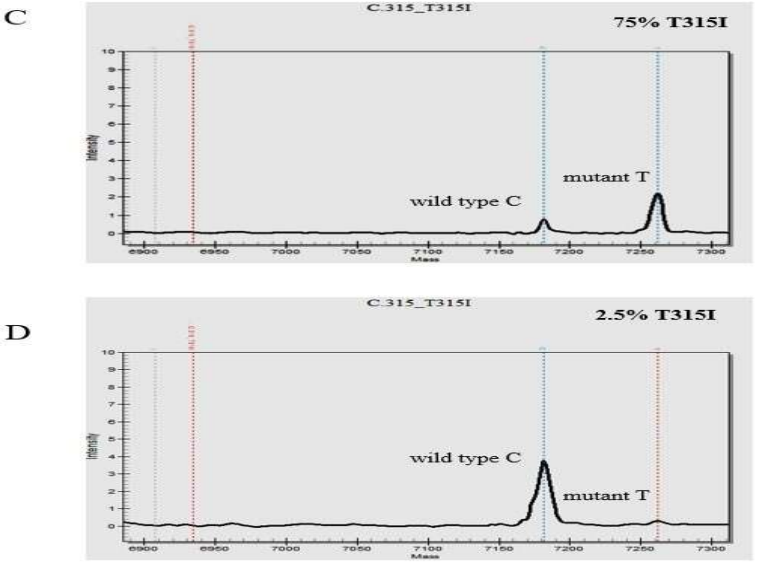












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Operational characteristics of the mass array and other comparable methods to detect *BCR*::*ABL1* mutations and recurrent genetic mutations in MPNs

Operational characteristics	**Mass array**	**Routine laboratory assays**			
		Direct sequencing for BCR::ABL1 TKD	AS-PCR for JAK2 V617F	CE-PCR for CALR	MLPA for JAK2, CALR, MPL, and c-Kit
Number of samples per run	1–96	1	1–10	1–4	1–4
Targets to detect per run	23	Whole of ABL1 TKD	1	Exon 9	8
Turnaround time	1-2 days	2-3 days	1 day	1 day	2-3 days
***Cost per sample**	~£28	£175	£51	£65	£121
****Overall operational complexity**	Intermediate	High	Low	Intermediate	Intermediate

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Comparison of currently available methods for analyzing *BCR*::*ABL* mutations.

Method	*BCR*::*ABL1* TKD target	Sensitivity	Advantage	Disadvantage	References
Direct sequencing	Whole of *BCR*::*ABL1* TKD	10%–25%	- Mutation identification - Semi-quantitative	- Low sensitivity - Cannot distinct compound/polyclonal mutations - High cost per sample - Labor-intensive - Long TAT	[37,60,64]
DHPLC	Whole of *BCR*::*ABL1* TKD	1%	- Screening test - High throughput - Reasonable cost	- Needs other downstream confirmatory assays - Requires normal DNA control	[64,67,86–89]
Pyrosequencing	Hotspot mutation	5%	- High sensitivity and specificity - Quantitative assay - Not too expensive	- Not suitable for screening test (requiring mutation data) - Labor-intensive - Short amplicon length of detection	[39,90–93]
AS-PCR	Hotspot mutation	0.01%–0.001%	- Easy to perform - High sensitivity and specificity - Quantitative assay	- Not suitable for screening test (requires mutation data) - False-positive and false-negative	[94–96]
Digital PCR (dPCR)	Hotspot mutation	0.01%–0.05%	- High specificity and sensitivity - Quantitative - Short turnaround time - Could be multiplexed - Not too expensive	- Not suitable for screening test (requiring mutation data)	[97–98]
Mass array	Hotspot mutation	0.05%–2.5%	- High specificity and sensitivity - Screening of hotspot *BCR*::*ABL1* TKD mutations - Quantitative - Short TAT - Not too expensive	- Cannot detect novel mutations or additional variants - Cannot distinguish compound/polyclonal mutations	This report, [62,99]
NGS	Whole of *BCR*::*ABL1* TKD	1%–3%	- Early identification and quantification - Able to distinguish compound/polyclonal mutations (subclonal identification)	- Expensive - Labor-intensive - Long TAT - Not well standardized and poor data implementation	[51,59]

Limsuwanachot et al. Journal of Mass Spectrometry and Advances in the Clinical Lab 28 (2023) 122–132

ตัวอย่างอนุสิทธิบัตร

วันที่สร้างเอกสาร 2 กรกฎาคม 2564

แบบ สป/สผ/อสป/001-ก
หน้า 1 ของจำนวน 4 หน้า

คำขอรับสิทธิบัตร/อนุสิทธิบัตร

☐ การประดิษฐ์
☐ การออกแบบผลิตภัณฑ์
☑ อนุสิทธิบัตร

ข้าพเจ้าผู้ลงลายมือชื่อในคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้
ขอรับสิทธิบัตร/อนุสิทธิบัตร ตามพระราชบัญญัติสิทธิบัตร พ.ศ.2522
แก้ไขเพิ่มเติมโดยพระราชบัญญัติสิทธิบัตร(ฉบับที่ 2) พ.ศ.2535
และพระราชบัญญัติสิทธิบัตร (ฉบับที่ 3) พ.ศ.2542

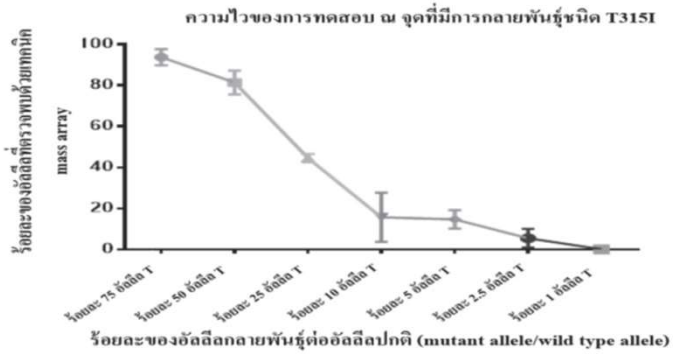
สำหรับเจ้าหน้าที่	
วันที่รับคำขอ 02/07/2564	เลขที่คำขอ 2103001903
วันที่ยื่นคำขอ 02/07/2564	
สัญลักษณ์จำแนกการประดิษฐ์ระหว่างประเทศ	
ใช้กับแบบผลิตภัณฑ์ ประเภทผลิตภัณฑ์	
วันประกาศโฆษณา	เลขที่ประกาศโฆษณา
วันออกสิทธิบัตร/อนุสิทธิบัตร	เลขที่สิทธิบัตร/อนุสิทธิบัตร
ลายมือชื่อเจ้าหน้าที่	

1. ชื่อที่แสดงถึงการประดิษฐ์/การออกแบบผลิตภัณฑ์ ชุดทดสอบสำหรับตรวจการกลายพันธุ์ที่เกี่ยวข้องกับมะเร็งเม็ดเลือดขาวกลุ่มมัยอีโลโพรลิเฟอเรทีฟ (Myelopolierative Neoplasm)

ตารางที่ 4 ผลการทดสอบชุดน้ำยาที่ประดิษฐ์ขึ้น (Mass array) เปรียบเทียบกับวิธีมาตรฐานที่ใช้ในงานทดสอบของห้องปฏิบัติการ (routine assay) ในผู้ป่วยซีเอ็มแอล (CML) จำนวน 28 ราย และผู้ป่วยเอ็มพีเอ็น(MPN) จำนวน 3 ราย

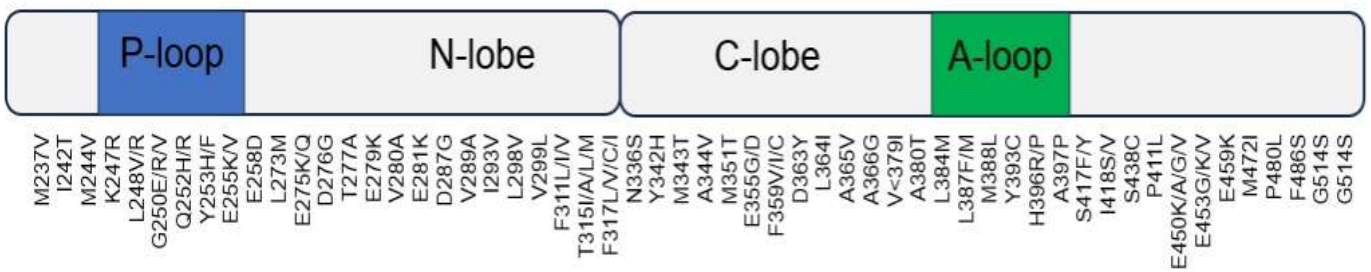
เลขที่	ชุดน้ำยามาตรฐาน (Routine assay)	ชุดน้ำยาตามการประดิษฐ์นี้ (Mass array)
CML001	E255K/T315I	E255K/T315I
CML002	G250E/Q252H /Y253H/T315I/F359V	G250E/Q252H/Y253H/T315I/F317L/F359V
CML003	T315I	T315I
CML004	E279K/H396R	H396R
CML005	G250E/T315A	G250E/T315A
CML006	L248V with del248-274/T315I	L248V with del248-274/T315I

วิธีใหม่



Our directions for Lab management of CML

- Revising Human Genetic Laboratory CML databases
  - Demographical data, patient's characteristics
  - Cytogenetic; additional chromosome abnormalities (ACA), Philadelphia variants
  - BCR::ABL1 transcriptional variants
- Digital PCR for BCR::ABL1 mRNA in ambiguous cases
- dPCR or ddPCR for TKD mutations
- NGS for BCR::ABL1 TKD mutations; operational/assay performances and economical characteristics
- Revision of Mass array panel to comply with recent ELN guidelines



Mass Array panel??

Mutations that have been consistently reported in the literature to confer resistance to 2G TKIs, ponatinib and asciminib	
Mutations conferring resistance to dasatinib	V299L, T315I/A, F317L/V/I/C
Mutations conferring resistance to nilotinib	Y253H, E255K/V, T315I, F359V/I/C
Mutations conferring resistance to bosutinib	E255K, V299L, T315I
Mutations conferring resistance to ponatinib	T315M/L
Mutations conferring resistance to asciminib	G109D, Y115N, M244V, V289I, A337V/T, E355G, F359V, E462K, G463D/S, P465S, V468F, S501R, I502L

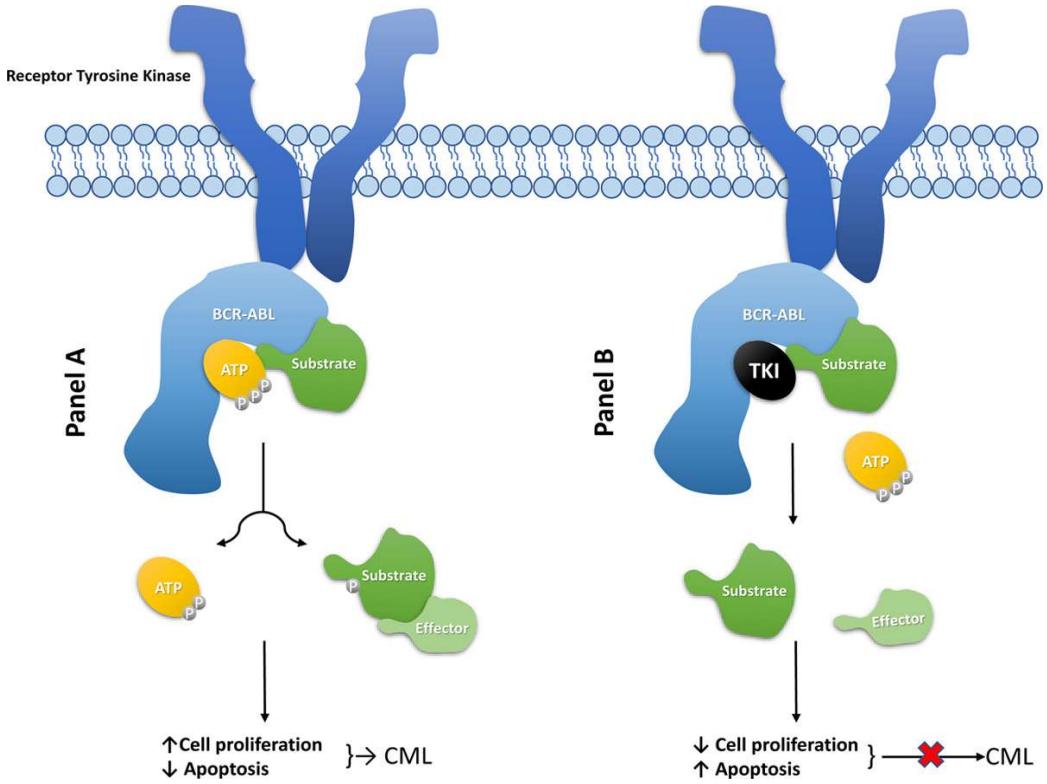
T315I-inclusive compound mutations

Ponatinib resistance; T315I/E255K, T315I/E255V,T315I/F359V, T315I/G250E; T315I/M351T

Asciminib resistance; T315I/E255K, T315I/F359I,T315I/E355G, T315I/M351T, T315I/E453Q

C.P. Cross. Et al., Leukemia (2023) 37:2150 – 2167.

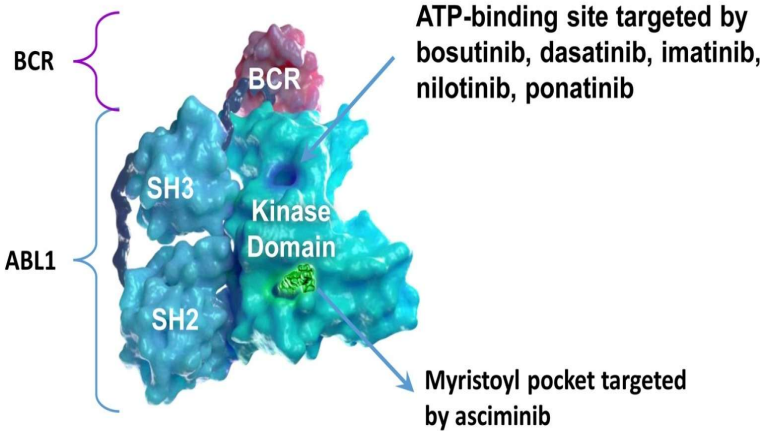
Classical and successful cancer-targeted therapy; tyrosine kinase inhibitor for CML



www.ahajournals.org/doi/10.1161/ATVBAHA.119.313353

- Imatinib (Gleevec)
- Dasatinib (Sprycel)
- Nilotinib (Tasigna)
- Ponatinib (Iclusig)
- Bosutinib (Bosulif)
- Asciminib

Assembled inactive conformation ABL1

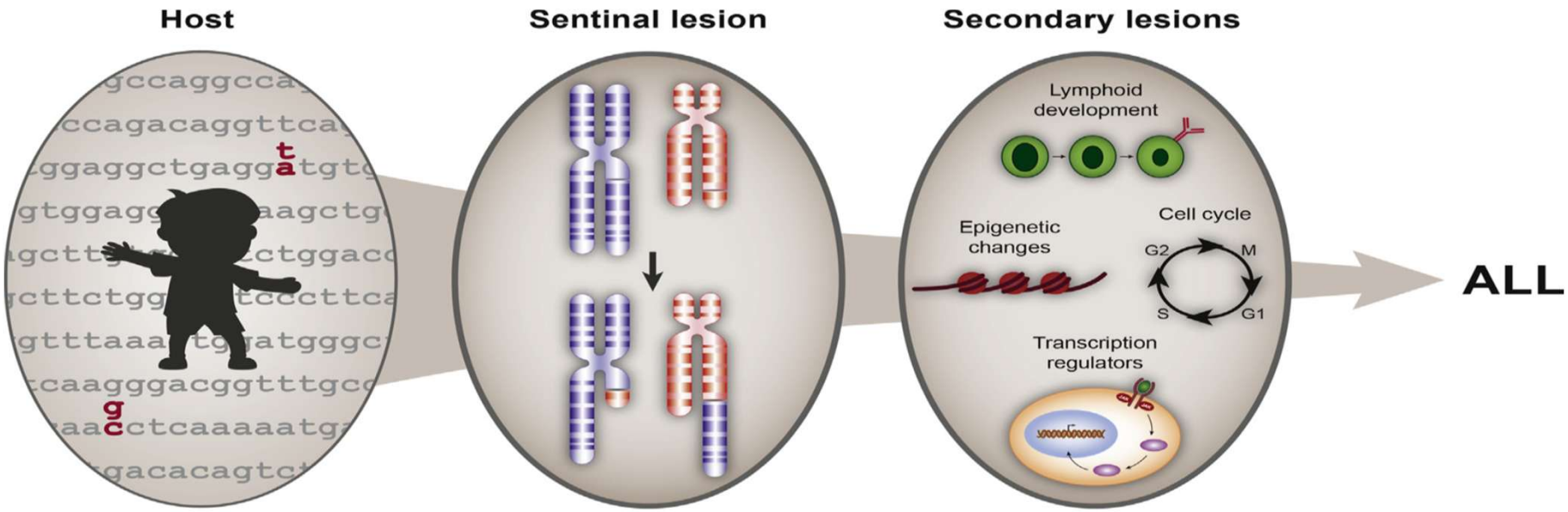


Manley et al., Leu Res. 2020

Acute leukemia

- Acute myeloid leukemia (AML)
  - Morphologic based = FAB classification M0-M7
  - Genetic based = WHO classification; e,g., t(8;21), t(15;17)
- Acute lymphoid leukemia (ALL)
  - B-cell ALL (~80%), T-cell ALL (~20%)
  - Morphologic based = FAB classification L1-L3
  - Genetic based = WHO classification; e,g., t(1;19), t(12;21), t(9;22), Ph-like ALL

Predisposing genes | Primary oncogenic events | Driving mutations



Examples

ARID5B
IKZF1
CDKN2A
CEBPE
PIP4K2A-BM1
GATA3
PAX5
TP53
TP63

Examples

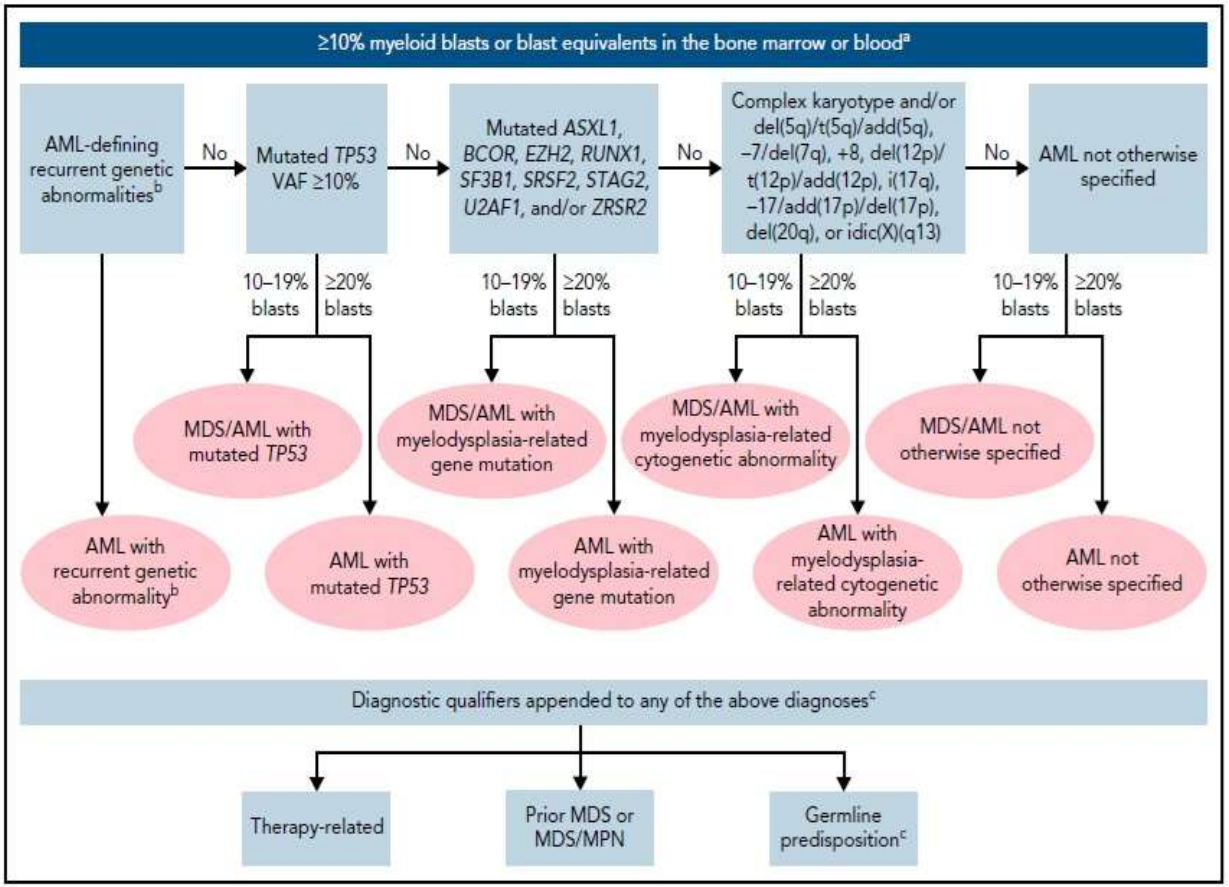
ETV6-RUNX1
BCR-ABL1
TCF3-PBX1
MLL rearrangements
CRLF2 rearrangements
Rearrangements associated with *BCR-ABL1*-like ALL (*EBF1-PDGFR* etc.)

Examples

Lymphoid development:
PAX5, IKZF1, EBF1
Cell cycle:
CDKN2A, TP53
Transcription regulators:
JAK, RAS, ERG
Epigenetic changes:
CREBBP, NR3C1

Bhojwani et al. Pediatr Clin N Am. 2015.

Hierarchical classification of the International Consensus Classification of AML; ELN 2022 for AML/MDS



The blast threshold is changed to ≥ 10%

AML with recurrent genetic abnormalities (requiring ≥10% blasts in BM or PB)*

APL with t(15;17)(q24.1;q21.2)/PML::RARA
AML with t(8;21)(q22;q22.1)/RUNX1::RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11
AML with t(9;11)(p21.3;q23.3)/MLLT3::KMT2A
AML with t(6;9)(p22.3;q34.1)/DEK::NUP214
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/GATA2, MECOM(EVI1)
AML with other rare recurring translocations
AML with mutated NPM1
AML with in-frame bZIP mutated CEBPA
AML with t(9;22)(q34.1;q11.2)/BCR::ABL1

Considering TP53 status

Therapy-related AML

Germline predisposition

Dohner H., et al. Blood 2022

2022 ELN risk classification by genetics at initial diagnosis

Risk category	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/RUNX1::RUNX1T1 • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11 • Mutated NPM1, without FLT3-ITD • bZIP in-frame mutated CEBPA
Intermediate	• Mutated NPM1†,§ with FLT3-ITD • Wild-type NPM1 with FLT3-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/MLLT3::KMT2A • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/DEK::NUP214 • t(v;11q23.3)/KMT2A-rearranged • t(9;22)(q34.1;q11.2)/BCR::ABL1 • t(8;16)(p11.2;p13.3)/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); 27; 217/abn(17p) • Complex karyotype, monosomal karyotype • Mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2 • Mutated TP53

Dohner H., et al. Blood 2022

Tests and procedures at diagnosis for a patient with AML

Genetic tests	Results preferably available within	
Cytogenetics	7-10 days	Urgent karyotyping?
Screening for gene mutations required for establishing the diagnosis and identifying actionable therapeutic targets • FLT3, IDH1, IDH2 • NPM1 • CEBPA,# DDX41, TP53; ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2	 3-5 days 3-5 days 1st cycle	AS-PCR and/or NGS/massARRAY * Long FLT3-ITD may be missed by NGS
Screening for gene rearrangements • PML::RARA, CBFB::MYH11, RUNX1::RUNX1T1, KMT2A rearrangements, BCR::ABL1, other fusion genes (if available)	3-5 days	RT-PCR, RQ-PCR, NGS fusion, and FISH
Additional genes recommended to test at diagnosis • ANKRD26, BCORL1, BRAF, CBL, CSF3R, DNMT3A, ETV6, GATA2, JAK2, KIT, KRAS, NRAS, NF1, PHF6, PPM1D, PTPN11, RAD21, SETBP1, TET2, WT1		NGS

Dohner H., et al. Blood 2022

TP53 mutation in AML

p53 mutations occur in 5 to 10% of de novo AML patients

About 25% in patients >65 years

30–35% of cases with therapy-related AML and AML with myelodysplasia (MDS)-related changes

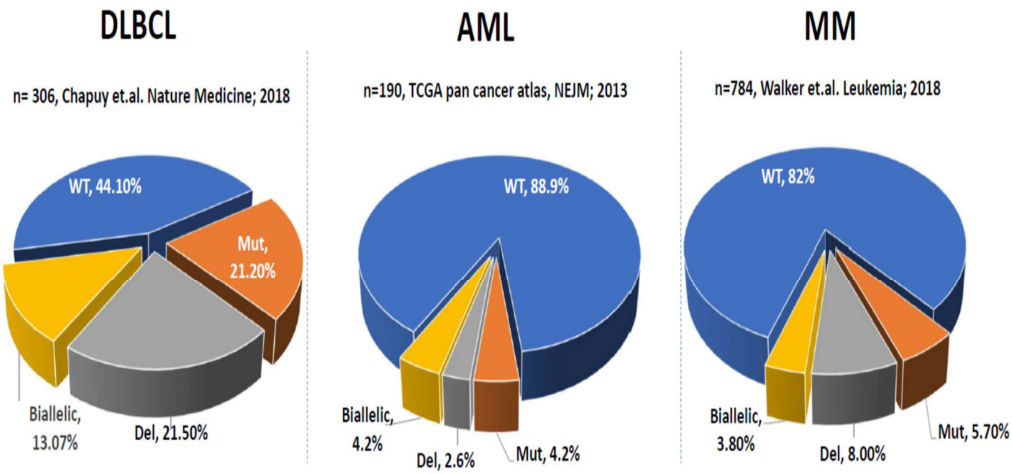
70% of cases with complex-karyotype AML

“hotspot mutations”, account for approximately 28% of all p53 mutations

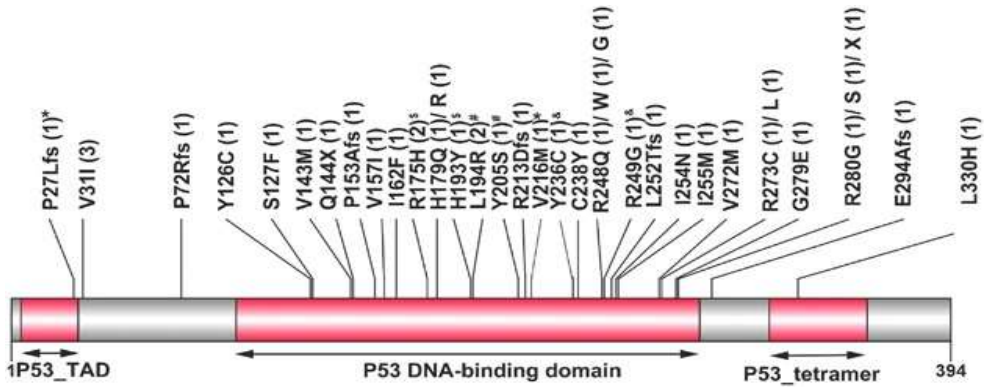
TP53 mutations in AML blast cells is widely associated with chemoresistance, especially in patients treated with anthracyclines and cytarabine

The European Leukemia Net (ELN) 2022
TP53 mutation defines the new entity AML with a very adverse prognosis

Zingarelli. Hemato. 2022.



Flynt et al. Cell. 2020.



Hou et al. Blood Cancer Journal. 2015.

The detection of TP53 mutations in AML using mass array

Niparuck *et al. Diagnostic Pathology* (2021) 16:100
https://doi.org/10.1186/s13000-021-01162-8

Diagnostic Pathology

RESEARCH Open Access

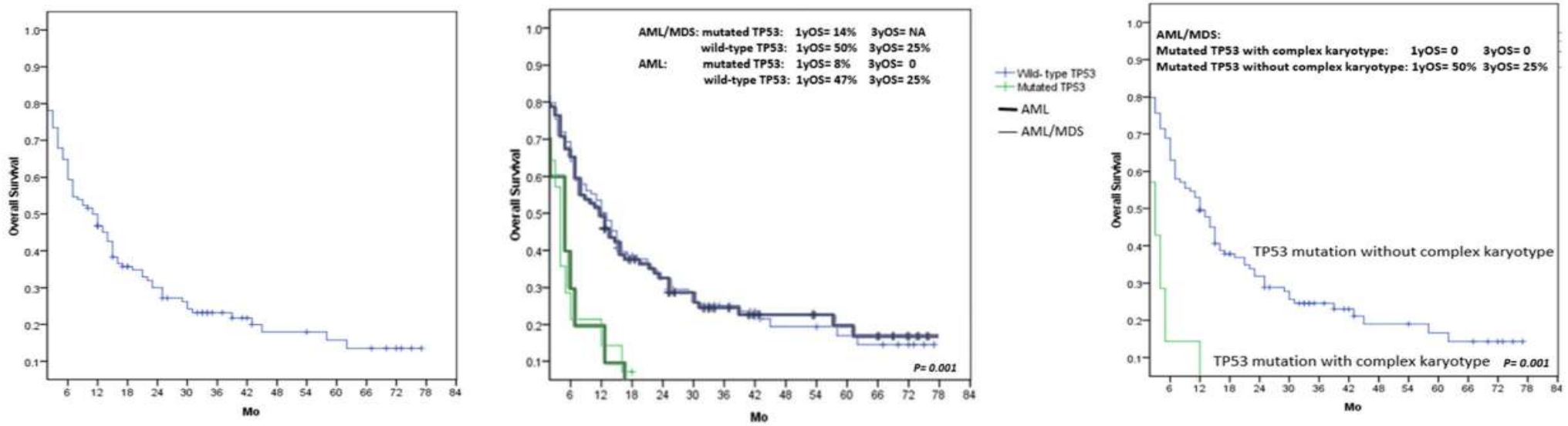
TP53 mutation in newly diagnosed acute myeloid leukemia and myelodysplastic syndrome

Pimjai Niparuck[1], Pornnapa Police[1], Phichchapha Noikongdee[1], Kanchana Siriputtanapong[1], Nittaya Limsuwanachot[2], Budsaba Rerkamnuaychoke[2], Suporn Chuncharunee[1] and Teerapong Siriboonpiputtana[2*]

Nipaluck et al. Diagnostic Pathology. 2021.

The prevalence of TP53 mutation in de novo AML and MDS-EB patients was low, but it impacted survival.

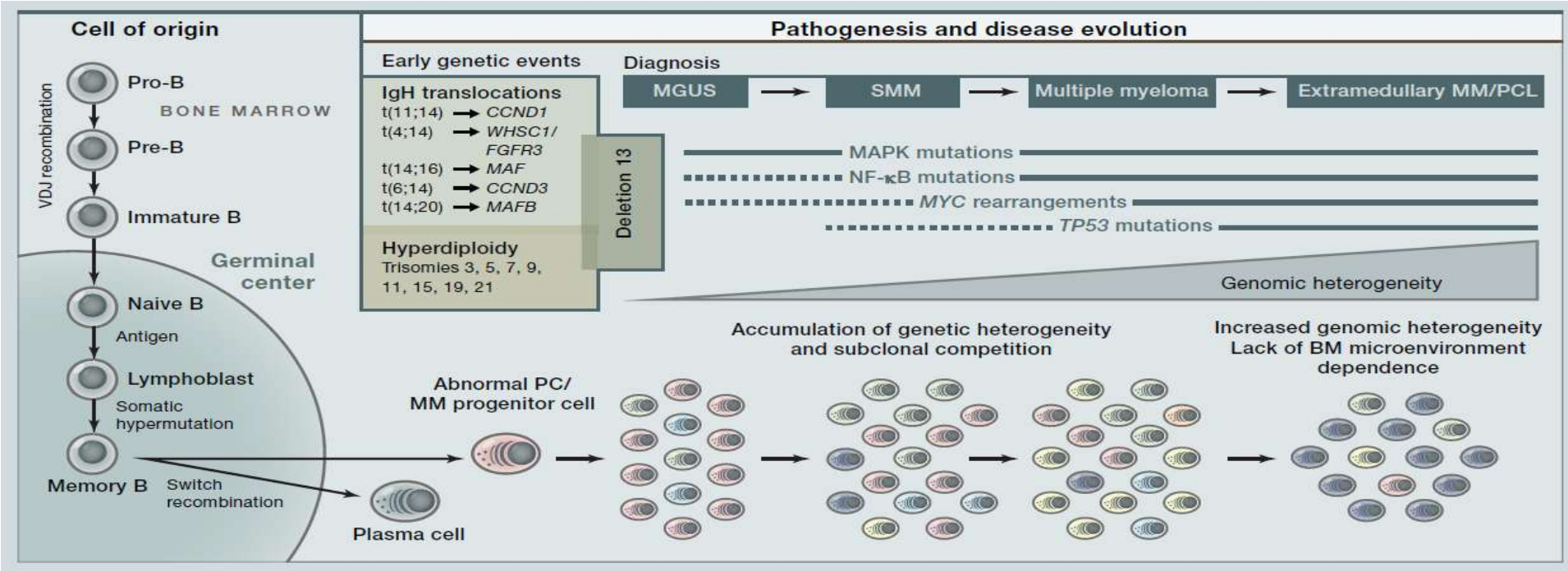
Patients with monosomy or complex karyotype had more frequent TP53 mutations.



A) OS in patients with AML/MDS B) OS in AML/MDS and AML patients with and without TP53 mutation C) OS in TP53mutated AML patients with and without complex karyotype

Nipaluck et al. Diagnostic Pathology. 2021.

Multiple myeloma



Clinically and genetically heterogeneous plasma cell disorder
24000 new case and 1100 death annually in the US
Median age = 69 (different in ethnic background suggesting the persistent of genetic predisposing to MM)

Braggio E. Cancer Cell. 2015

Primary Molecular Cytogenetic Classification of Multiple Myeloma

Subtype	Gene(s)/chromosomes affected	Approximate Percentage of myeloma patients
Hyperdiploid multiple myeloma	Recurrent trisomies involving odd-numbered chromosomes with the exception of chromosomes 1, 13, and 21	**45**
IgH translocated multiple myeloma		**40**
t(11;14)(q13;q32)	CCND1 (cyclin D1)	20
t(6;14)(p21;q32)	CCND3 (cyclin D3)	5
t(4;14)(p16;q32)	NSD2	10
t(14;16)(q32;q23)	C-MAF	4
t(14;20)(q32;q11)	MAFB	<1
Other IgH translocations, other cytogenetic abnormalities, or normal		**5**

S. Vincent Rajkumar, Am J Hematol. 2022 August ; 97(8): 1086–1107

Multiple Myeloma: 2022 Update on Diagnosis, Risk-stratification, and Management

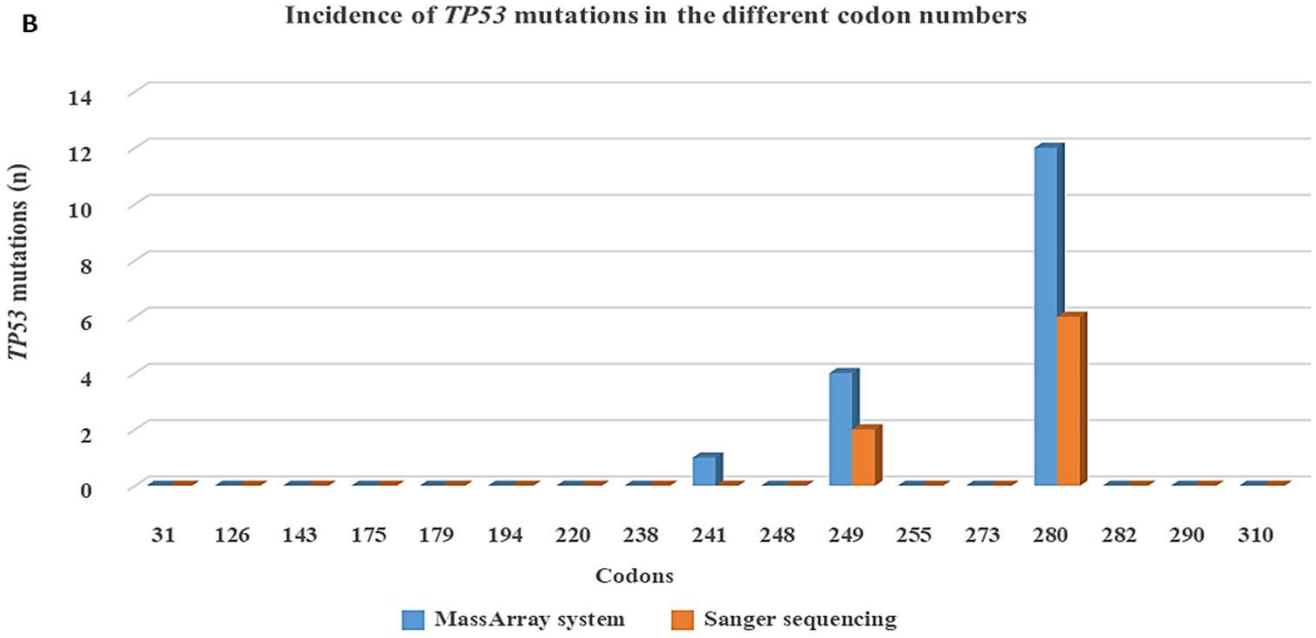
Mayo Clinic Risk Stratification for Multiple Myeloma (mSMART)

Risk group	Percentage of newly diagnosis patients with abnormality
Standard-risk Trisomies t(11;14) t(6;14)	60%
High-risk t(4;14) t(14;16) t(14;20) del(17p) gain(1q) double hit myeloma: any 2 high-risk factors triple hit myeloma: any 3 or more high-risk factors	40%

S. Vincent Rajkumar, Am J Hematol. 2022 August ; 97(8): 1086–1107

A customized Mass Array panel for mutational screening of *TP53*, *MYD88*, and *CXCR4* mutations in mature B-cell malignancies

Roongrudee Singdong[1], Budsaba Rerkarmnuaychoke[1], Takol Chareonsirisuthigul[1], Piyapha Hirunpatrawong[2], Teerapong Siriboonpiputtana[1]*

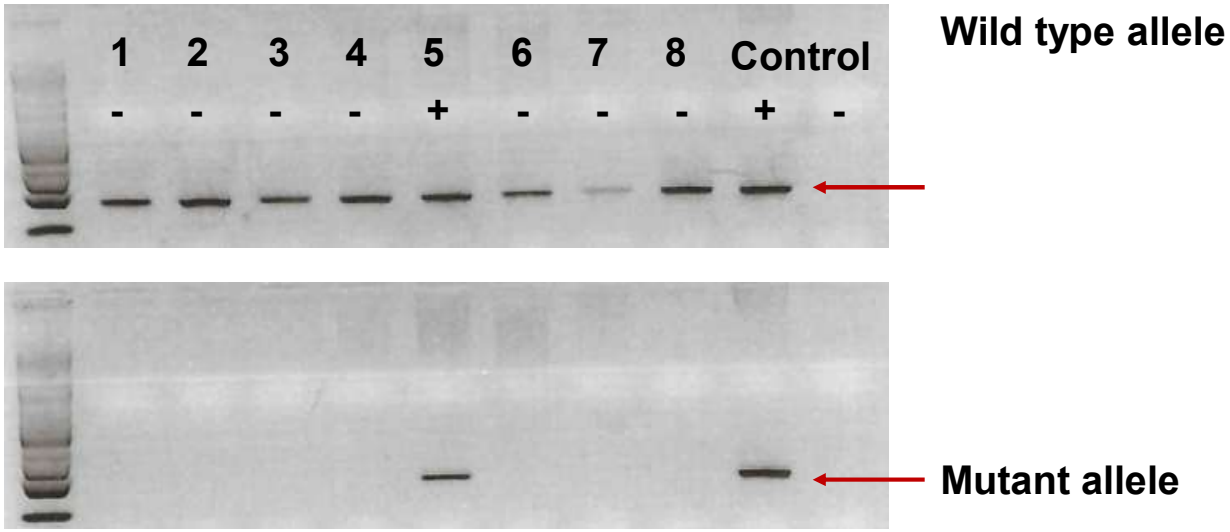


- 105 MM and 40 lymphoma
- TP53 mutations were positive in 7% (7/105) of MM and 25% (10/40) of lymphoma
- MYD88 (L265P) was positive in 5% (2/40) of lymphoma???????

Data in progress, 2025

MYD88 L265P mutation analysis

Increasing in demand of using MYD88 L265P mutation analysis in vitreous fluid and CNS samples for diagnosing primary central nervous system lymphoma (PCNSL)

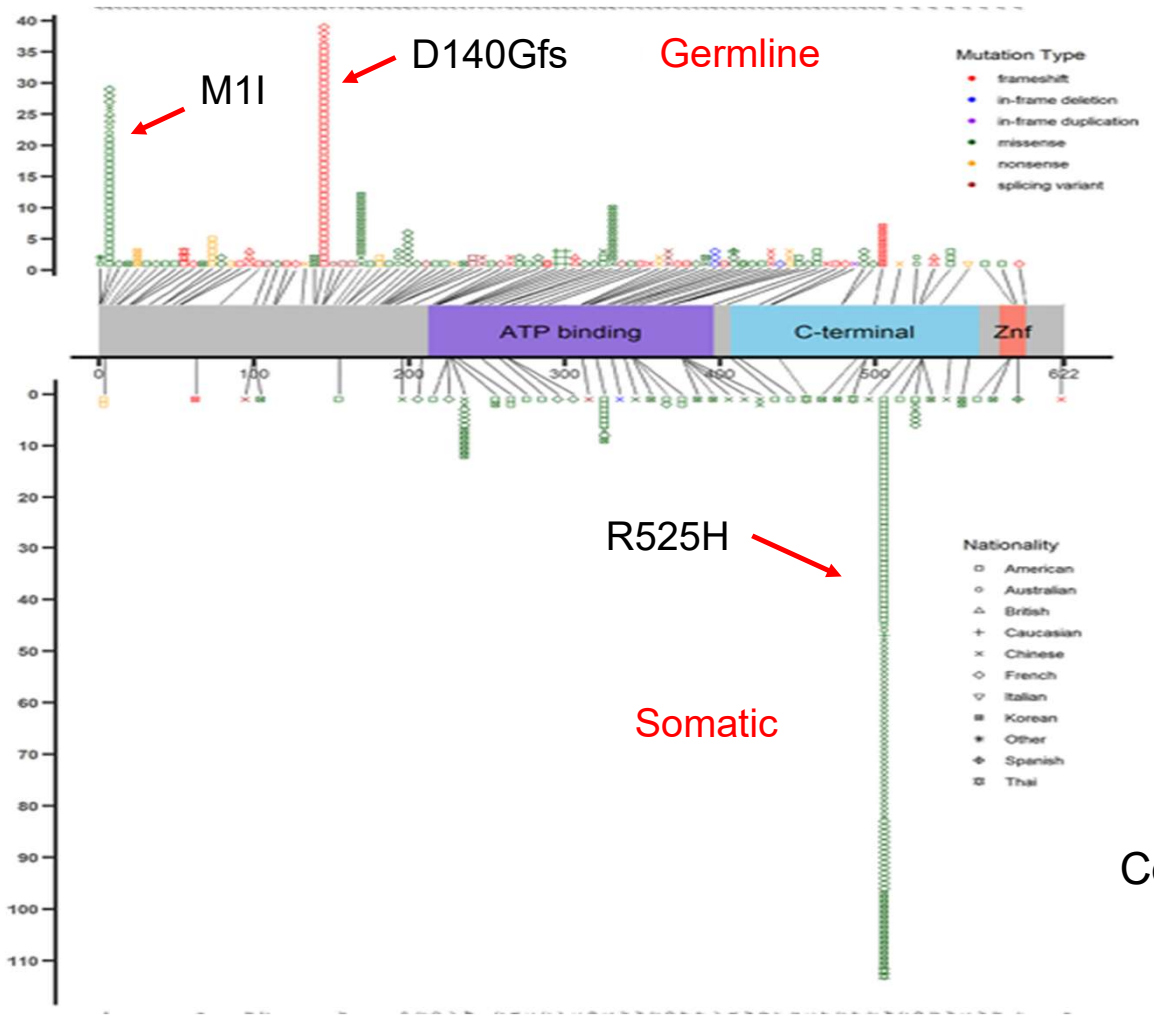


move to MassARRAY or digital PCR and combine with CXCR4 mutations????

Germline mutations in adult AML/MSD

- Germline predisposing to myeloid neoplasms identified in 5-15% of adult MDS and AML
- New entity of WHO 2016; “myeloid neoplasms with germline predisposition”
- DEAD-box RNA helicase-1 gene (*DDX41*) is located on the 5q35.3 and is identified in 1.5–3.8% of myeloid neoplasms
- DDX41 mutations cause double-strand break (genome instability) in HSC
- Cause idiopathic cytopenia of undetermined significance (ICUS)
- Surveillance and management of DDX41 mutation carriers
- Identified suitable donor for BMT

DDX41 MassARRAY or Real-time PCR or ASO-PCR???



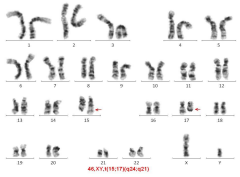
Somatic DDX41 mutation in adult myeloid leukemia/MDS

	Somatic mutations												
Germline DDX41 variant	DDX41	ASXL1	CDH26	DNMT3A	JAK2	LUC7L2	KRAS	NOTCH1	NRAS	PHF6	RUNX1	SETBP1	SMAD1	TP53
M1I	R525H													
		■		■										
Q52fs	A225D													
D140Gfs*2	R525H												■	
	R525H													
	R525H						■					■		
						■			■					
	R525H													
	R525H		■											■
	R525H										■			
F183I	R525H					■								
L237F/P238T	R525H													
I396T	R525H			■						■				
	R525H				■									
A500Cfs*9	R525H							■						

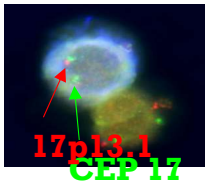
Common mutation predisposing to AML

Genetic aberrations in hematological malignancies

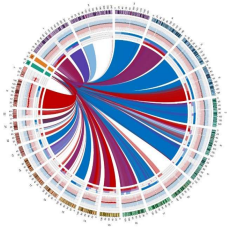
Present



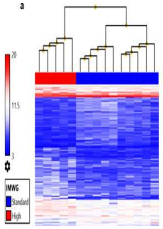
Chromosome



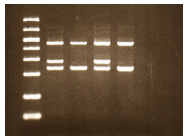
FISH



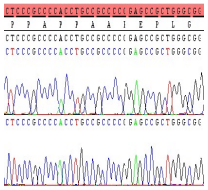
CMA



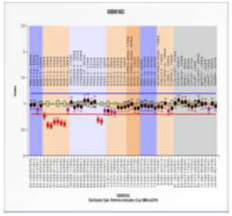
GEP



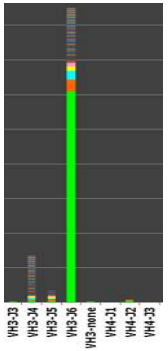
PCR-based



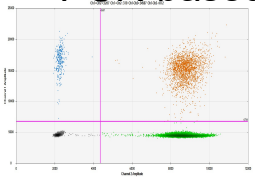
1st sequencing



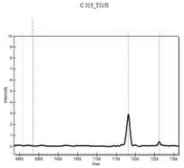
MLPA



NGS

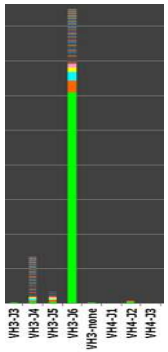


ddPCR

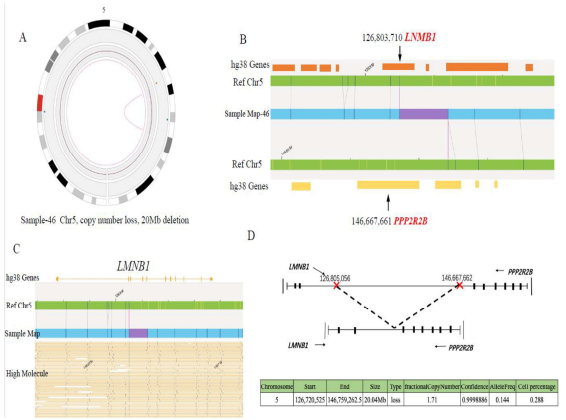


Mass array

Future



NGS



OGM

Acknowledgements

Assoc. Prof. Budsaba Rerkamnuaychoke

Leukaemia and Molecular oncology Group
Human Genetics Laboratory
Department of Pathology
Ramathibidi Hospital
Mahidol University
Bangkok, Thailand

Staffs

Research students

Current mentor

* The Royal Thai Government Scholarship
  Ministry of Science and Technology, Thailand
* Frontier Research & Discovery, Mahidol University
* TRF-MRG 2019 & TRG-RGJ 2018 Funds
* Ramathibodi research grant

Center for Medical Genomics, Faculty of Medicine Ramathibodi Hospital

Siriraj Center of Research Excellence for Cancer Immunotherapy (SiCORE-CIT)