Advancing MPN Genomic Profiling with an Amplicon-Based Myeloid NGS Panel

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Learning Objectives:

1

Gain insight into the molecular profiling of MPN including classification and biomarkers implicated in the diagnosis, prognosis, and therapeutic selection for MPN patients.

2

Assess traditional sequential single genes testing via PCR in identifying driver genes.

3

Assess clinical scenarios where an amplicon-based myeloid NGS panel identified critical co-mutations of clinical significance.

4

Explore a proposed diagnostic algorithm that integrates both PCR and NGS for detecting very low variant allele frequencies (VAF) in driver genes.

Myeloproliferative Neoplasms (MPNs):

Hematological disorders, characterized by clonal overproduction of mature WBCs, platelets and/or erythrocytes in the bone marrow and blood

- No evidence of myelodysplasia or dysplastic features on morphology
- Insidious onset, asymptomatic, identified on routine blood count showing increased counts and/or organomegaly
- Progression to myeloid blast phase and secondary AML

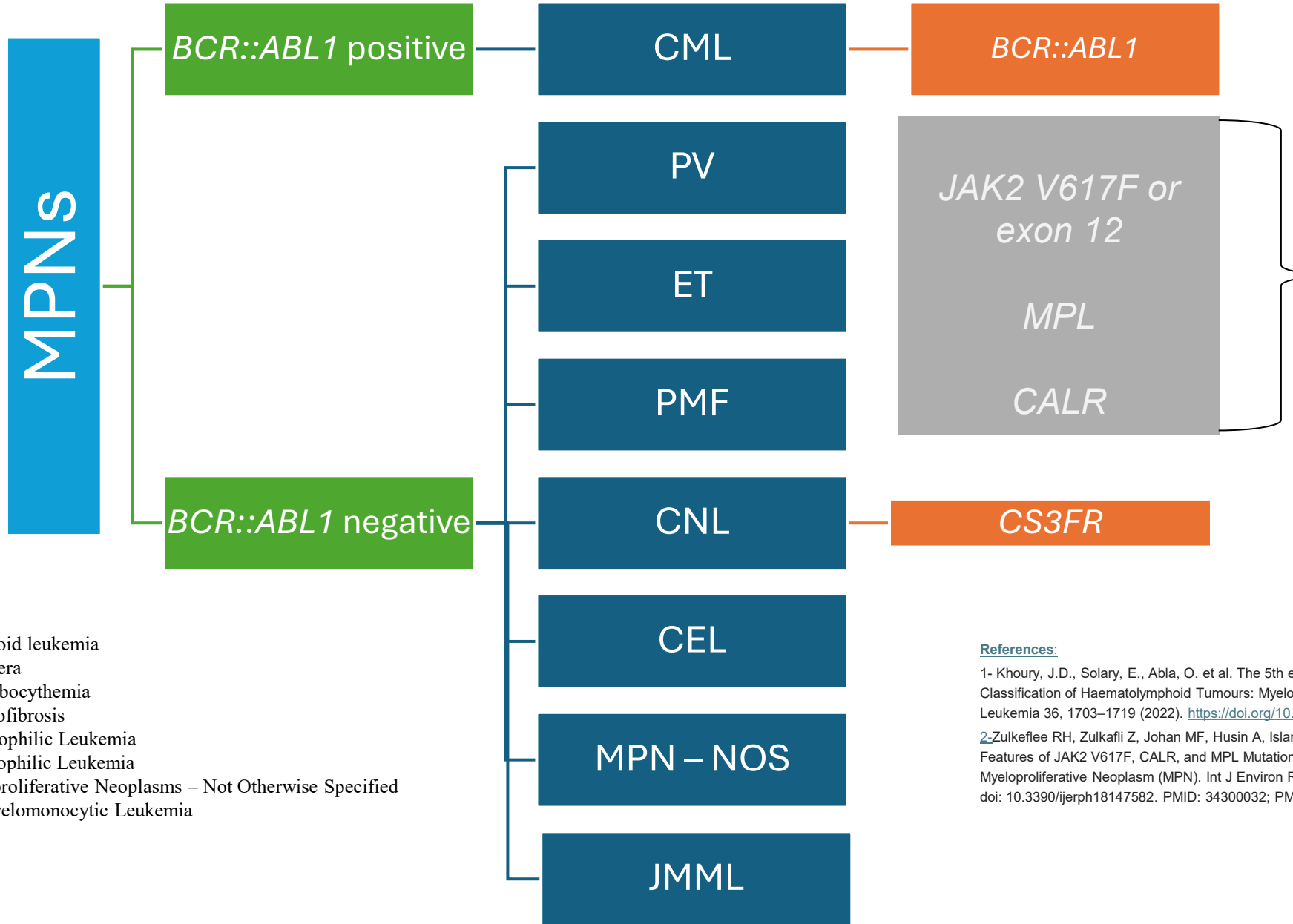
Myeloproliferative neoplasms

Myeloproliferative neoplasms

- Myeloproliferative neoplasms: Introduction
- Chronic myeloid leukaemia
- Chronic neutrophilic leukaemia
- Chronic eosinophilic leukaemia
- Polycythaemia vera
- Essential thrombocythaemia
- Primary myelofibrosis
- Juvenile myelomonocytic leukaemia
- Myeloproliferative neoplasm NOS (unclassifiable)

Khoury, J.D., Solary, E., Abla, O. et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia 36, 1703–1719 (2022). https://doi.org/10.1038/s41375-022-01613-1

WHO 2022 Classification of MPNs



Driver events[2]
Account for over 90% of MPN cases and are usually mutually exclusive to diagnosis

CML: Chronic myeloid leukemia
PV: Polycythemia Vera
ET: Essential Thrombocythemia
PMF: Primary Myelofibrosis
CNL: Chronic Neutrophilic Leukemia
CEL: Chronic Eosinophilic Leukemia
MPN- NOS: Myeloproliferative Neoplasms – Not Otherwise Specified
JMML: Juvenile Myelomonocytic Leukemia

References:

1- Khoury, J.D., Solary, E., Abla, O. et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia 36, 1703–1719 (2022). https://doi.org/10.1038/s41375-022-01613-1

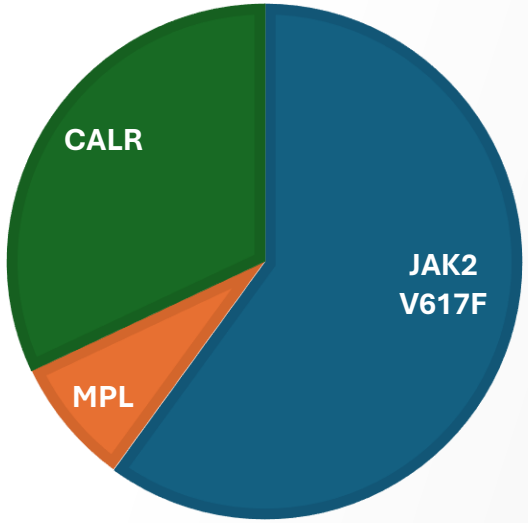
2-Zulkeflee RH, Zulkafli Z, Johan MF, Husin A, Islam MA, Hassan R. Clinical and Laboratory Features of JAK2 V617F, CALR, and MPL Mutations in Malaysian Patients with Classical Myeloproliferative Neoplasm (MPN). Int J Environ Res Public Health. 2021 Jul 16;18(14):7582. doi: 10.3390/ijerph18147582. PMID: 34300032; PMCID: PMC8307561.

Driver Events in MPNs

- ~90% of MPN cases harbor mutations in the three driver genes

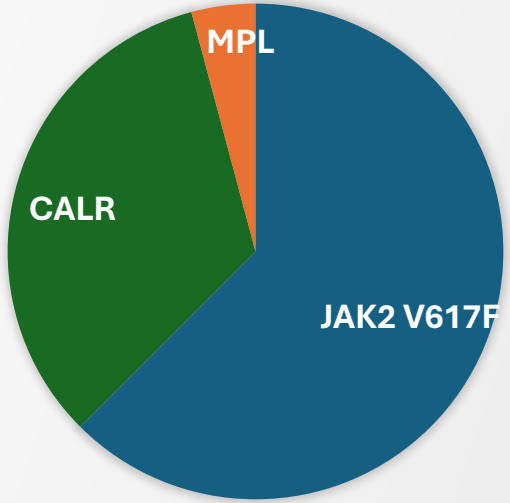
Polycythemia Vera (PV)

- ***JAK2* V617F** accounts for ~95% of PV cases
- ***JAK2* exon 12** rare insertions and deletions are found in 2% to 3% of patients with PV



Primary Myelofibrosis (PMF)

- ***JAK2* V617F** accounts for 60% of MF cases.
- ***CALR*** frameshift mutations in exon 9 are found in 20% to 30% of all patients with MF
- ***MPL* W515L/K** accounts for 7-10% of MF cases



Essential Thrombocythemia (ET)

- ***JAK2* V617F** accounts for 50-60% of ET cases.
- ***CALR*** frameshift mutations in exon 9 are found in 20% to 25% of all patients with ET
- ***MPL* W515L/K** accounts for 5-7% of ET cases

- ~10% of MPNs (ET/PMF) are considered "triple negative": *ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2, SF3B1* mutations

Reference:
NCCN Guidelines V1.2024 Myeloproliferative Neoplasms
Genetic basis and molecular profiling in myeloproliferative neoplasms | Blood | American Society of Hematology

MPN Workup

Diagnosis

Determine diagnosis & subclassification (PV, ET, PMF) via clinical features, blood smear, BM morphology, cytogenetics, and molecular testing (e.g., *JAK2, CALR, and MPL)*

PCR based sequential testing

Prognosis

Identify other MPN-associated genetic aberrations such as mutations in *ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2* and *SF3B1* for risk stratification and disease prognostication

Myeloid NGS panel

Therapy Selection

Select appropriate therapy based on diagnosis and risk category such as JAK2 inhibitors

Myeloid NGS panel

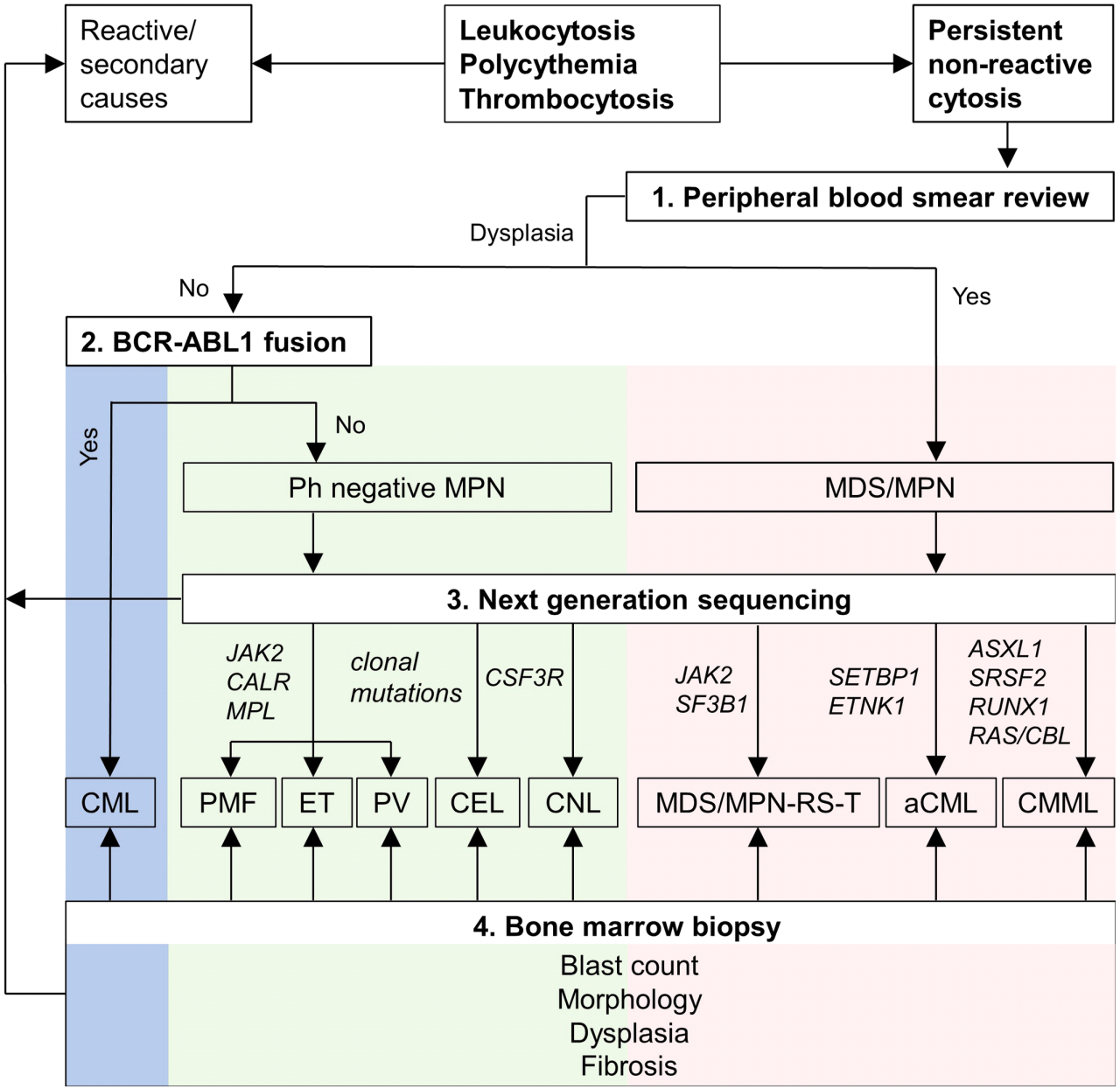
Disease Monitoring (MRD)

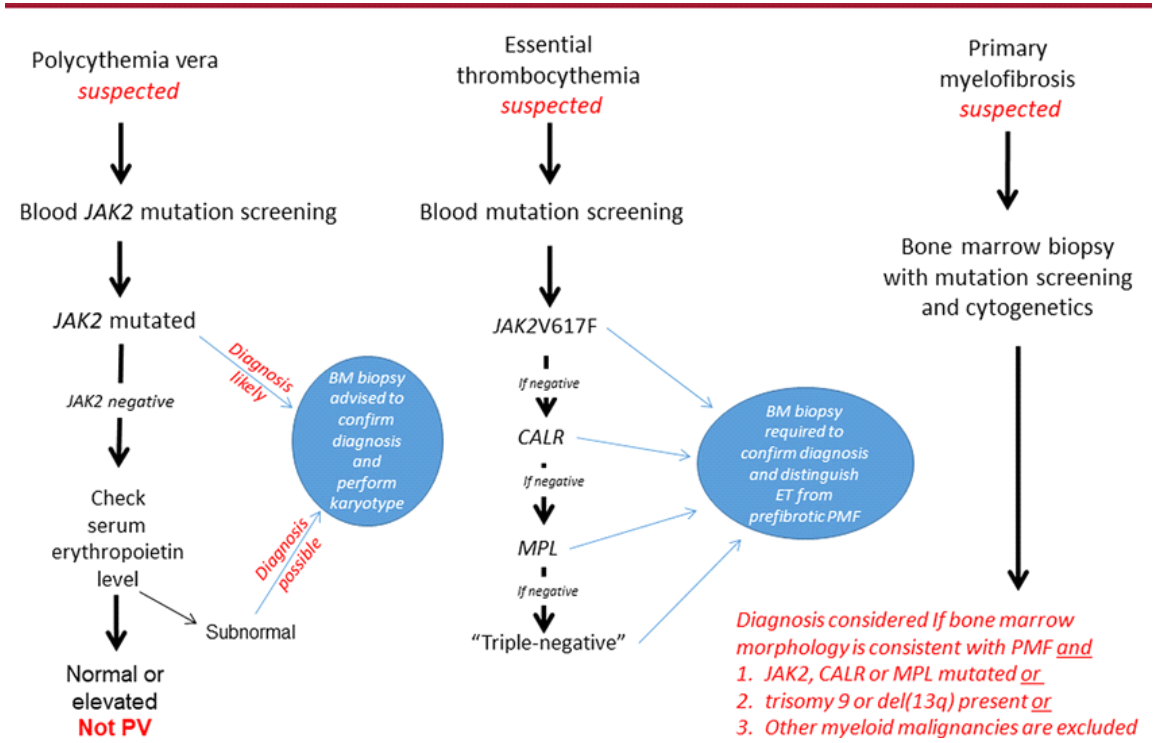
Acquired mutations in *JAK2, MPL, CALR.* Reduction of mutation allele burden are reported in many therapies such as JAK2 inhibitors, allogeneic stem cell transplant, and interferon.

qPCR

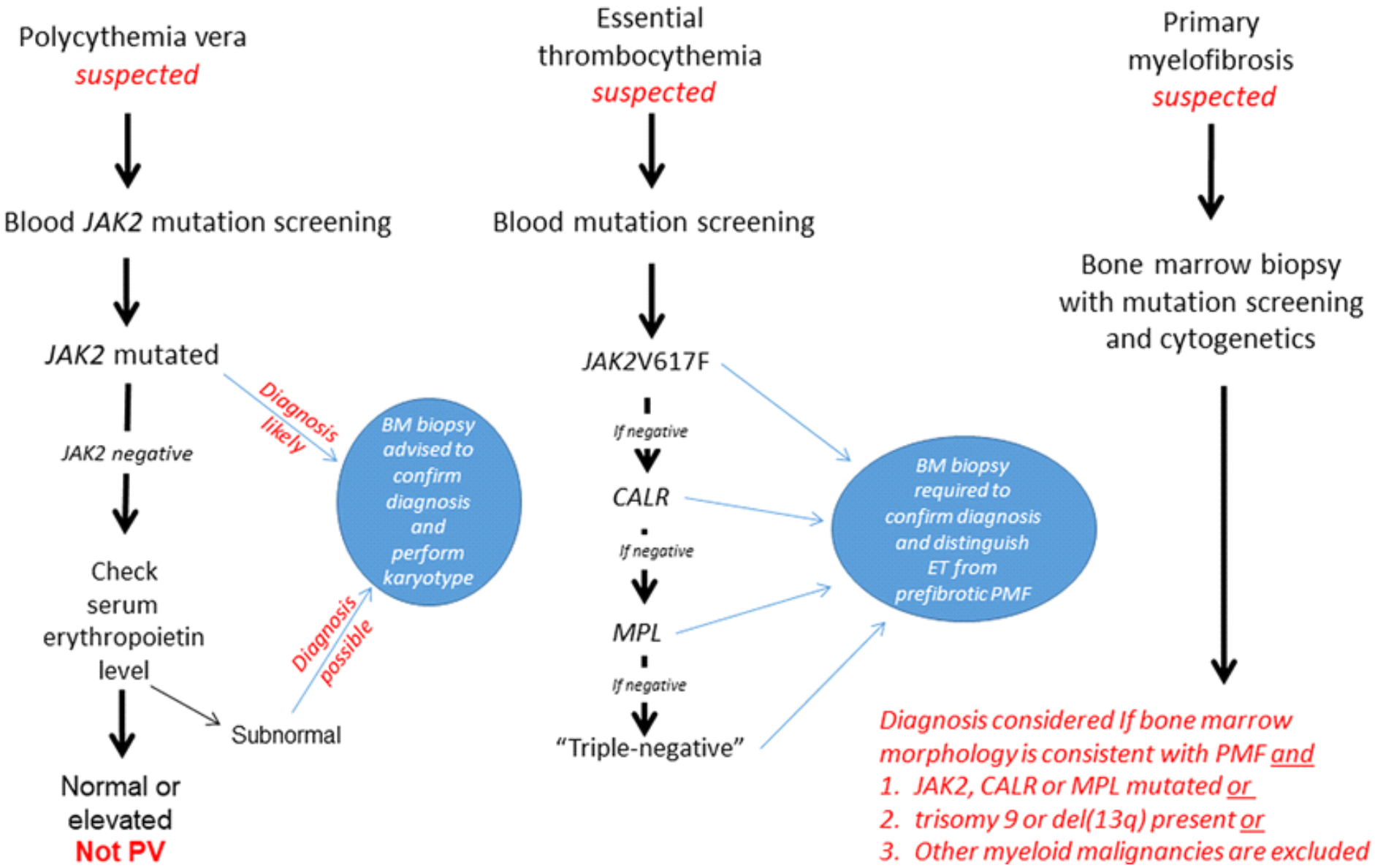
Tan, J., Chow, Y.P., Zainul Abidin, N. *et al.* Analysis of genetic variants in myeloproliferative neoplasms using a 22-gene next-generation sequencing panel. *BMC Med Genomics* **15**, 10 (2022).
Haslam K, Langabeer SE. Monitoring Minimal Residual Disease in the Myeloproliferative Neoplasms: Current Applications and Emerging Approaches. Biomed Res Int. 2016;2016:7241591.

Myeloproliferative Neoplasms





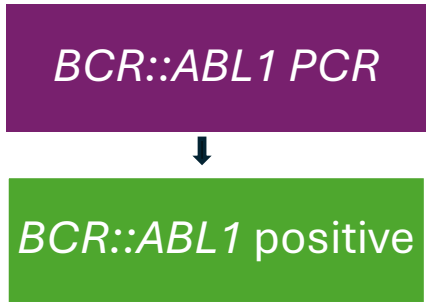
From: Wong WJ, Pozdnyakova O. Myeloproliferative neoplasms: Diagnostic workup of the cythemic patient. Int J Lab Hematol. 2019 May;41 Suppl 1:142-150. doi: 10.1111/ijlh.13005. PMID: 31069979.



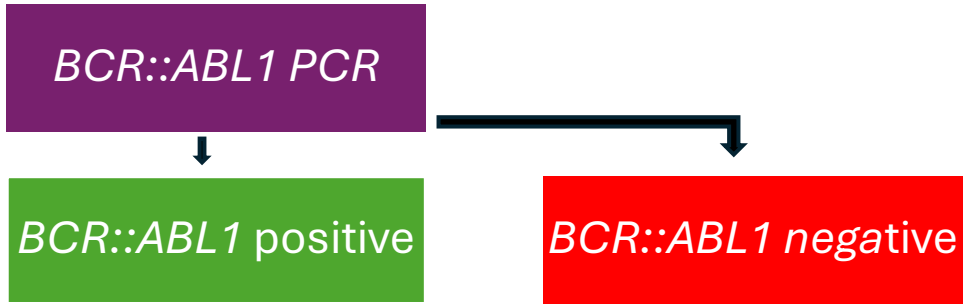
From: Wong WJ, Pozdnyakova O. Myeloproliferative neoplasms: Diagnostic workup of the cythemic patient. Int J Lab Hematol. 2019 May;41 Suppl 1:142-150. doi: 10.1111/ijlh.13005. PMID: 31069979.

BCR::ABL1 PCR

Sequential PCR based testing strategy



Sequential PCR based testing strategy



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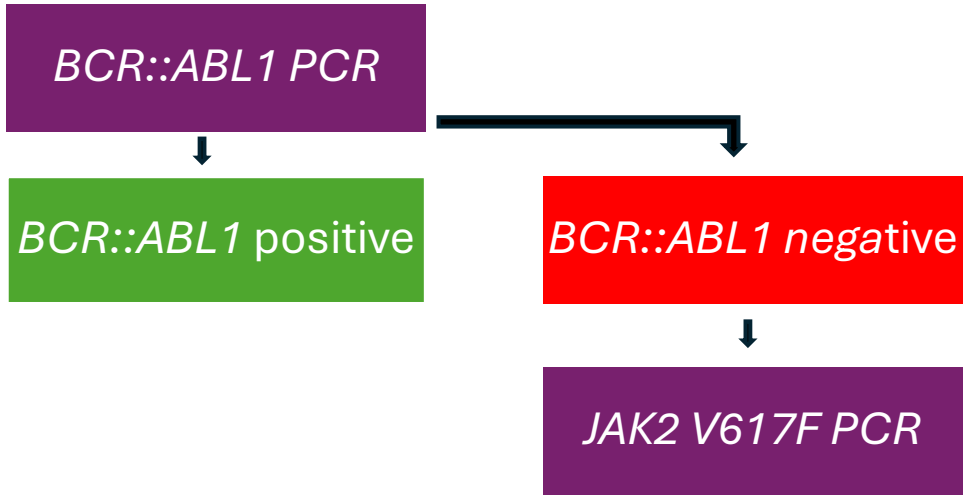

Sequential PCR based testing strategy

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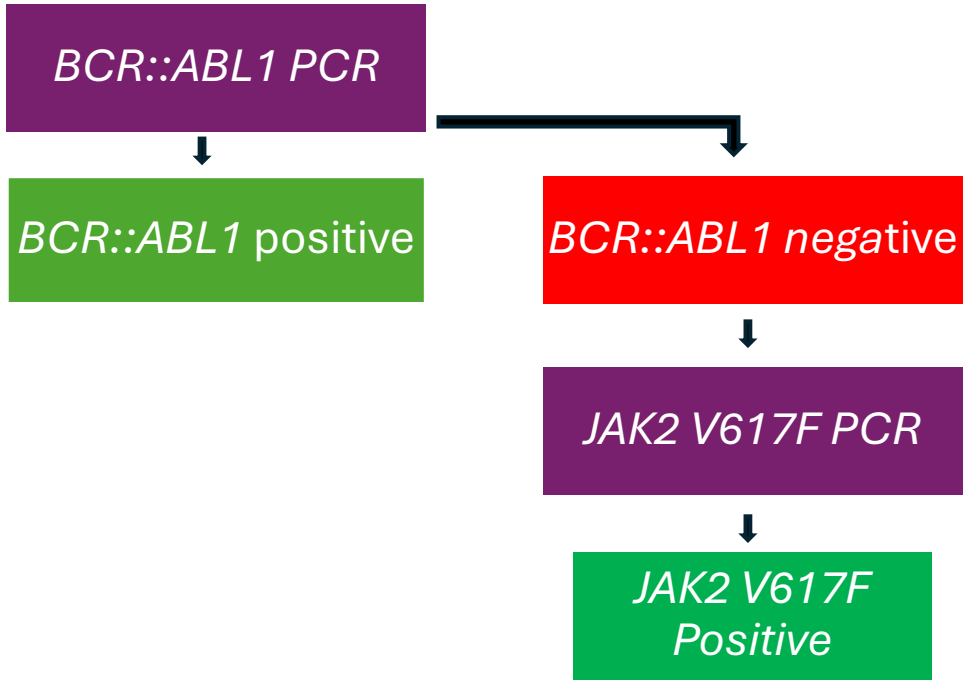

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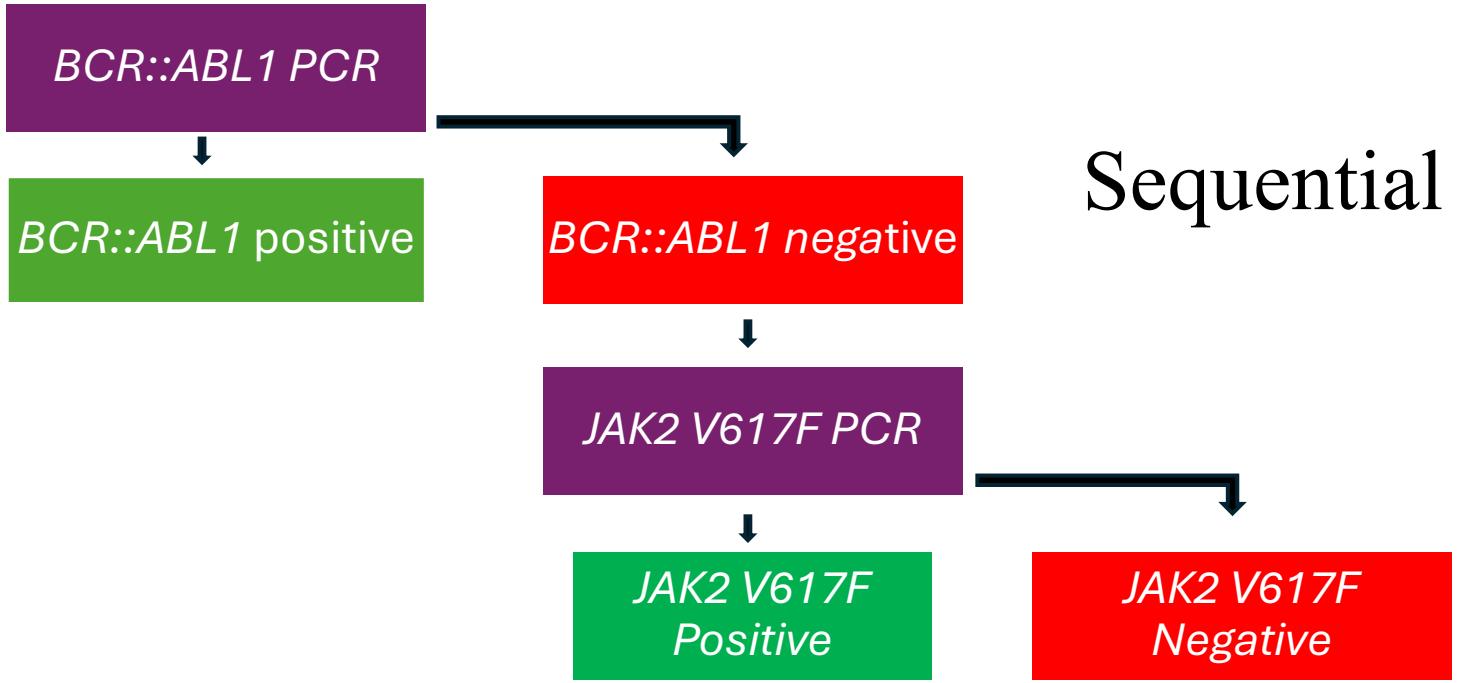

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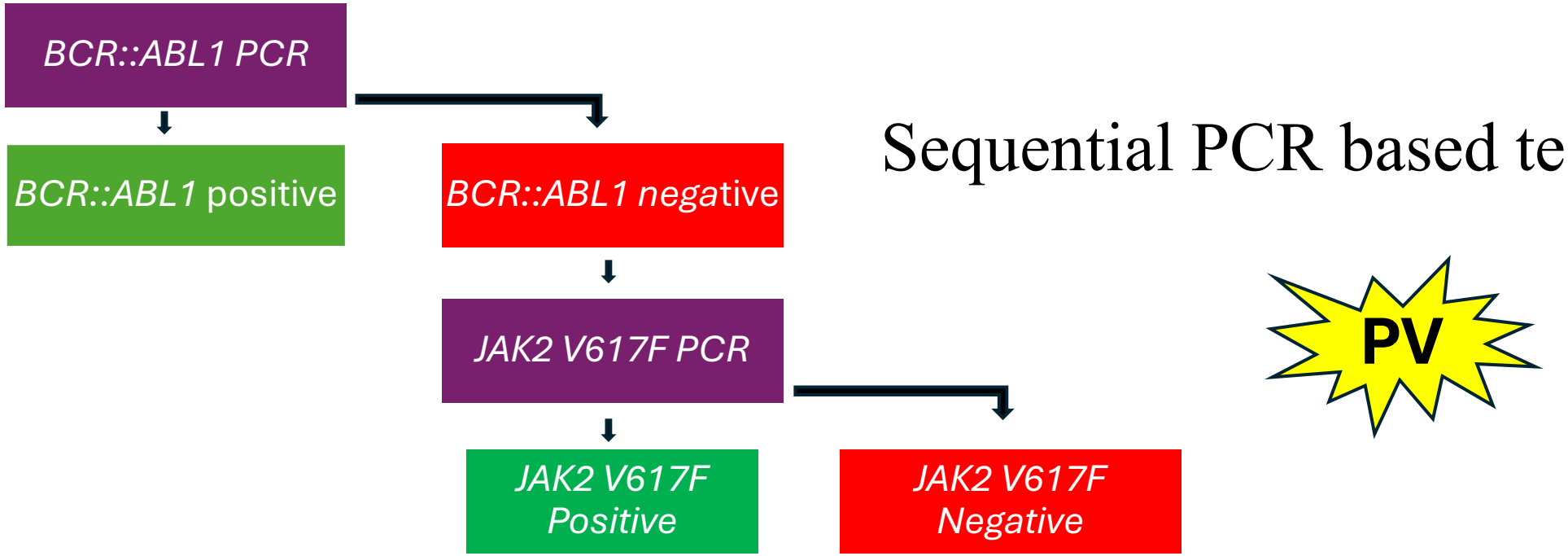

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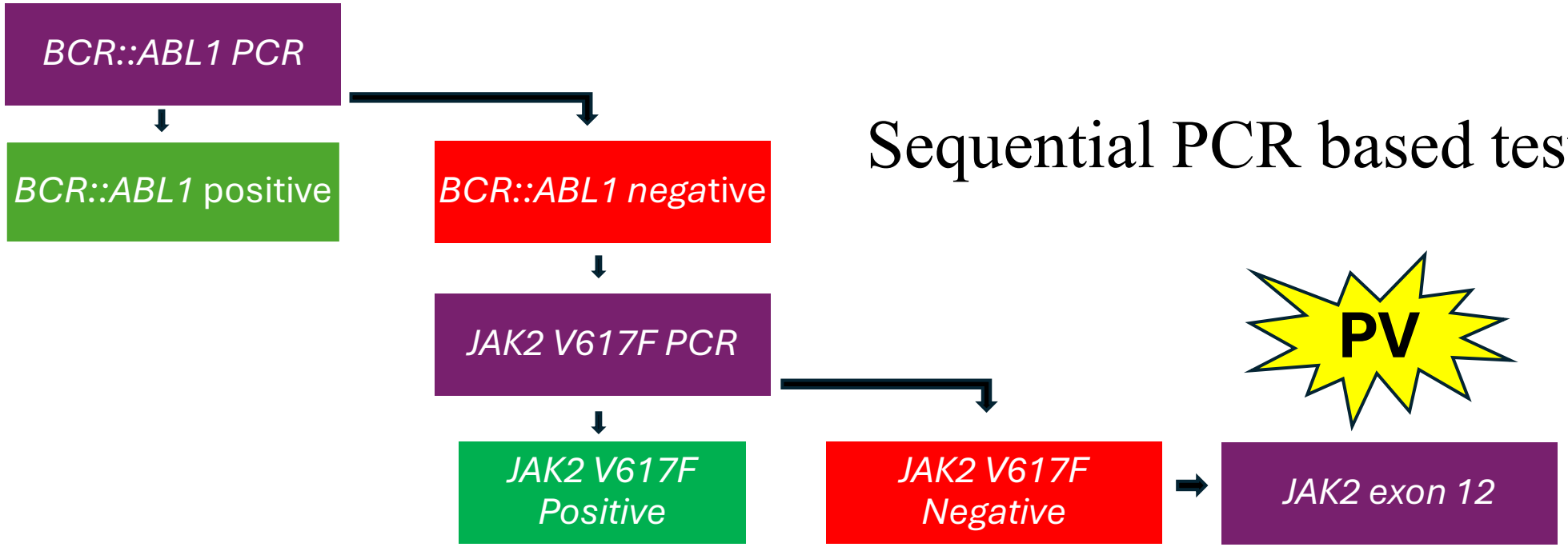

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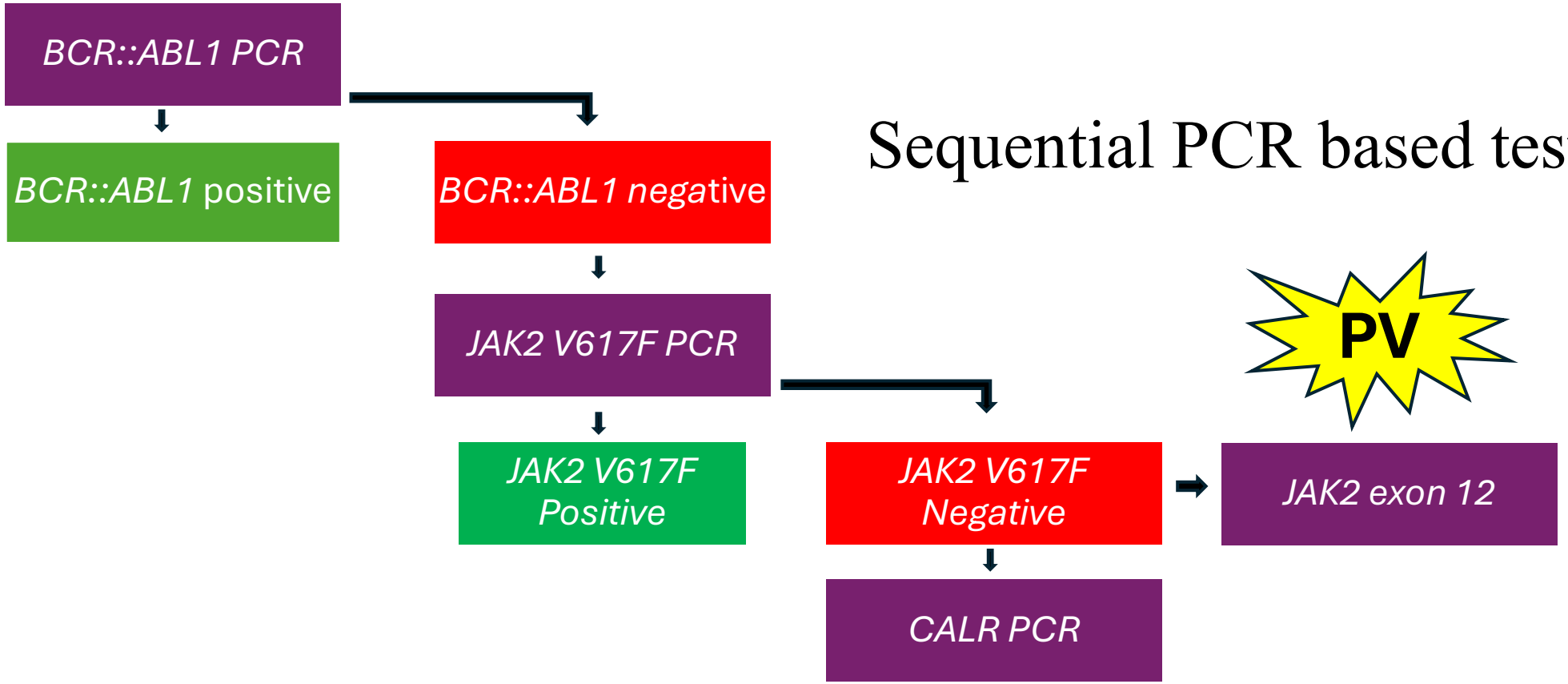

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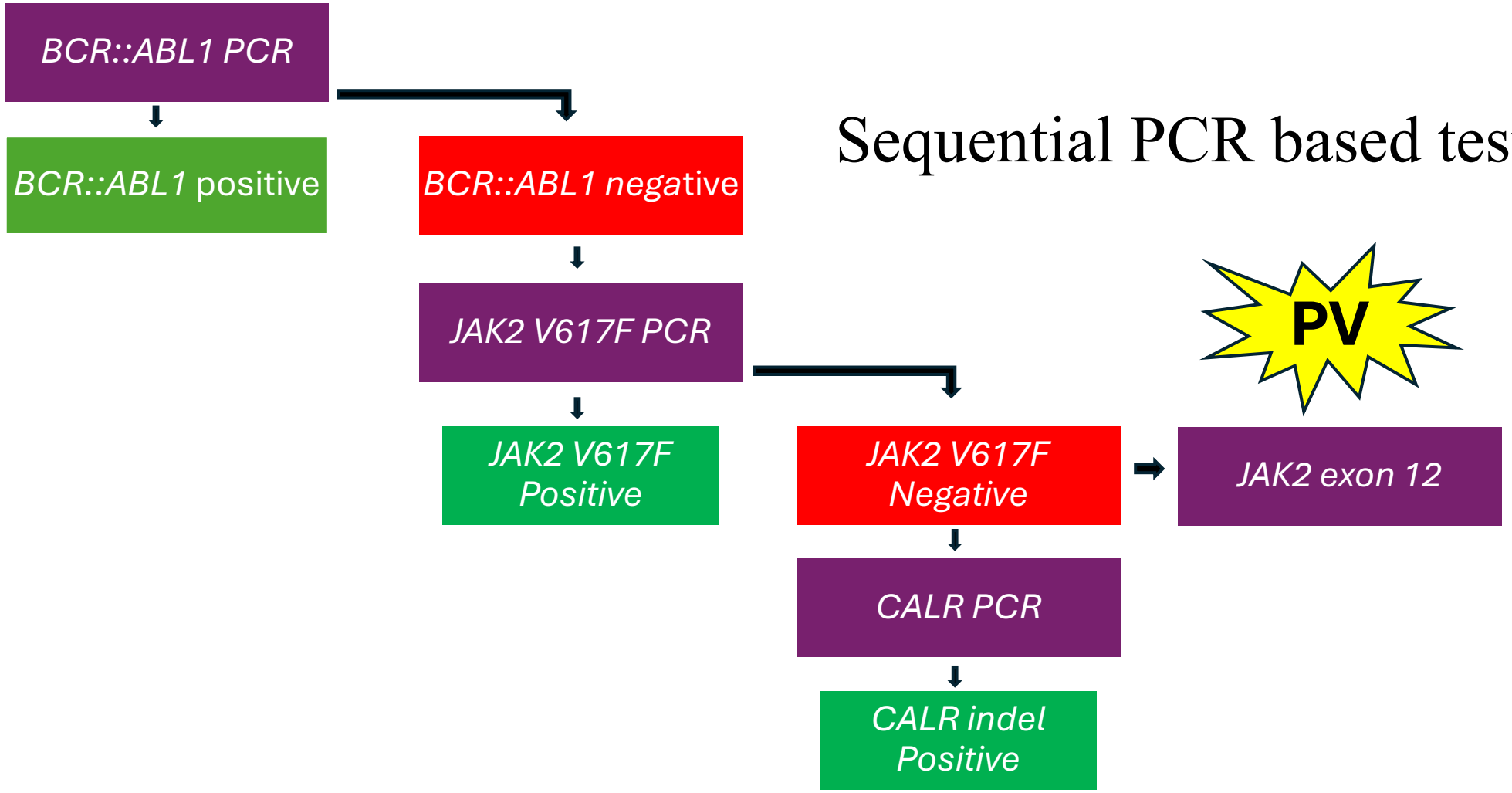

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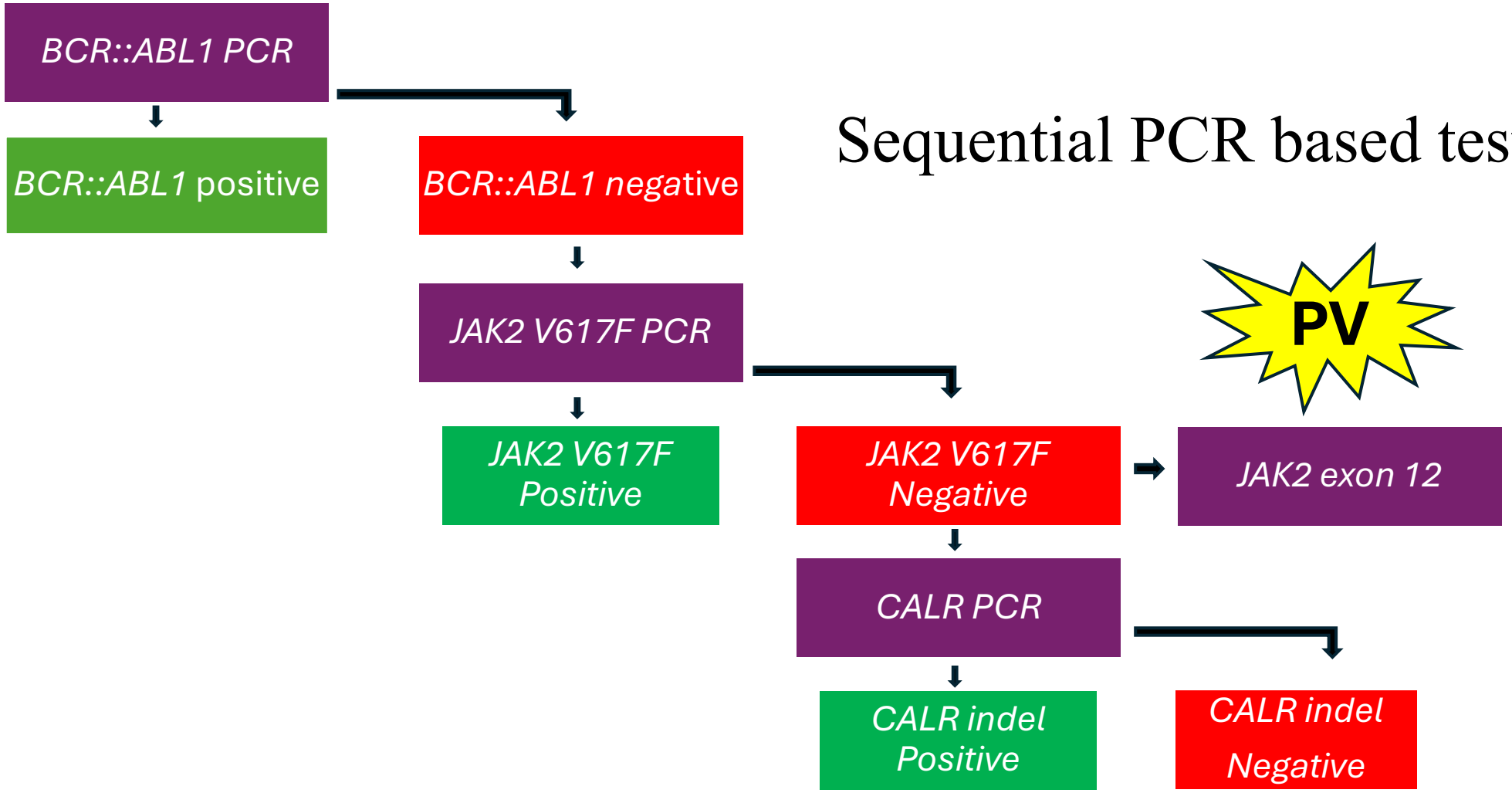

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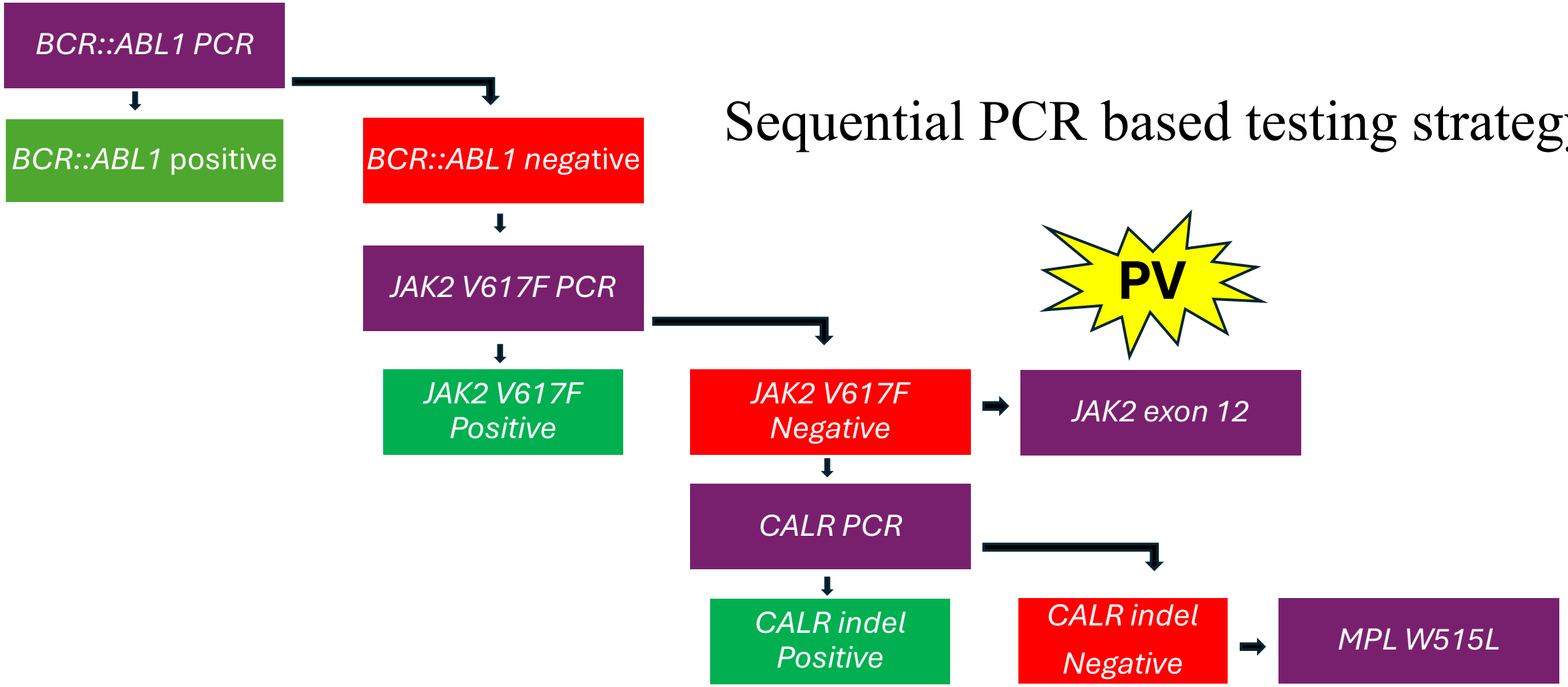

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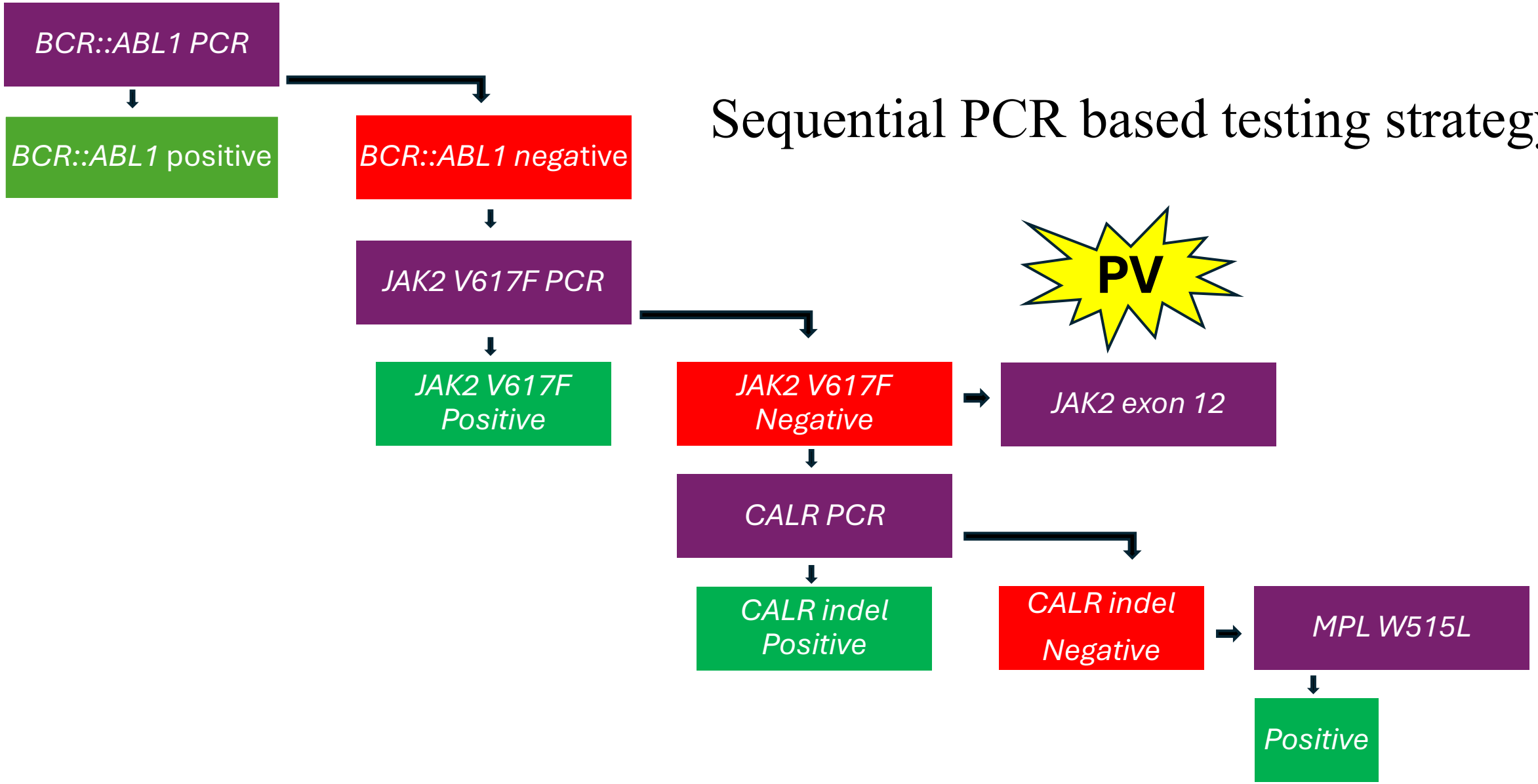

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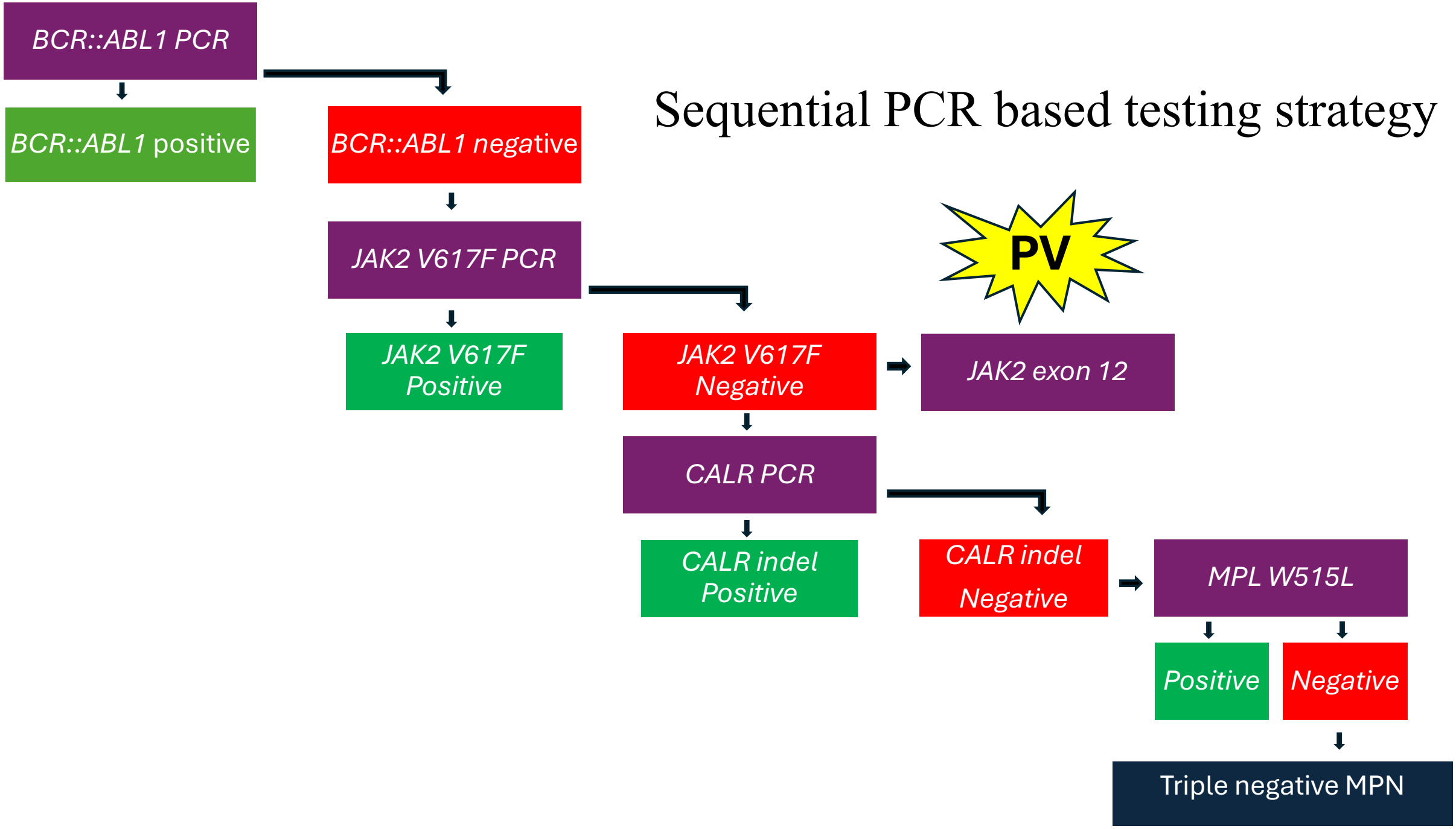

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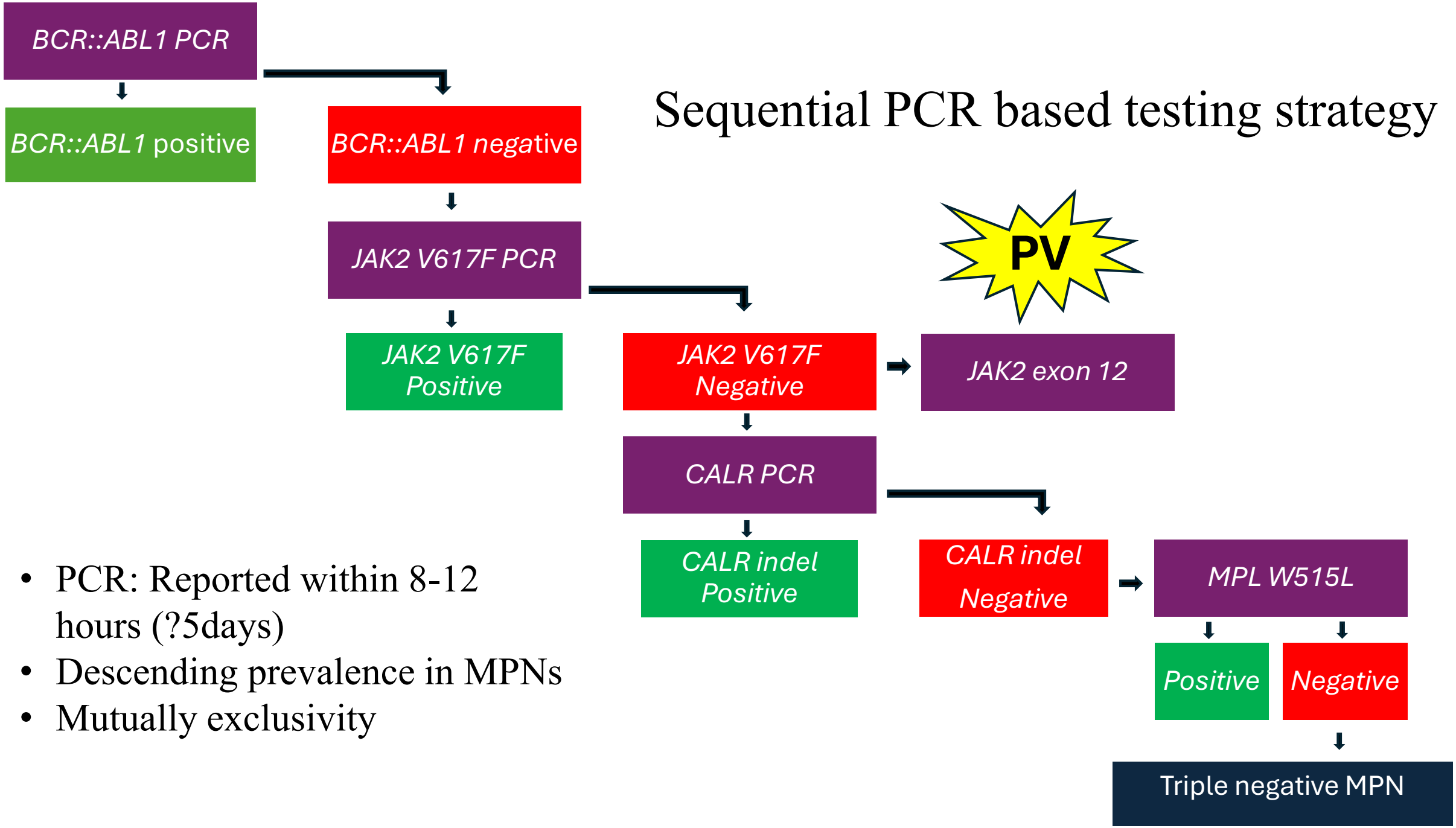


- PCR: Reported within 8-12 hours (?5days)
- Descending prevalence in MPNs
- Mutually exclusivity

Mutual exclusivity- Fact or Fiction?

> Sci Rep. 2025 Jul 18;15(1):26046. doi: 10.1038/s41598-025-11096-6.

Clinical, morphological and genetic characteristics of patients with concurrent presence of JAK2 V617F and BCR::ABL1

Nicole Naumann [1], Vito Dangelo [1], Johannes Lübke [1], Jakob Bresser [1], Volker Hagen [2], Jolanta Dengler [3], Georgia Metzgeroth [1], Sebastian Kreil [1], Tabea Hockenberger [1], Wolf-Karsten Hofmann [1], Alice Fabarius [1], Susanne Saussele [1], Nicholas C P Cross [4 5], Andreas Reiter [1], Juliana Schwaab [6 7]

Affiliations + expand

PMID: 40681596 PMCID: PMC12274285 DOI: 10.1038/s41598-025-11096-6

Case Reports > J Korean Med Sci. 2020 Jun 15;35(23):e168. doi: 10.3346/jkms.2020.35.e168.

A Rare Case of Essential Thrombocythemia with Coexisting *JAK2* and *MPL* Driver Mutations

Mi Ae Jang [1], Mi Yeon Seo [2], Kyoung Jin Choi [2], Dae Sik Hong [3]

Affiliations + expand

PMID: 32537949 PMCID: PMC7295601 DOI: 10.3346/jkms.2020.35.e168

Abstract

Philadelphia-negative (Ph-) classical myeloproliferative neoplasms (MPNs) include polycythemia vera, essential thrombocythemia (ET), and primary myelofibrosis. Somatic driver mutations in the *JAK2*, *CALR*, and *MPL* genes serve as major diagnostic criteria of the Ph- MPNs and these mutations occur in a mutually exclusive manner. In this report, we describe the first case of ET harboring double mutations in *JAK2* V617F and *MPL*. For *MPL*, the patient had multiple clones of *MPL* mutations: c.1543_1546delinsAGGG (p.Trp515_Gln516delinsArgGlu) and c.1546C>G (p.Gln516Glu). The *JAK2* V617F allele burden in our patient is very low (4%) compared to the relatively high (17%-78%) allele frequency of *MPL* mutations. The low *JAK2* mutant burden might be explained by preexisting clonal hematopoiesis before overt signs of MPNs, followed by the acquisition of a second oncogenic mutation of *CALR* or *MPL* leading to the MPN phenotype. This highlights that screening for a second driver mutation should be considered in patients with a low *JAK2* mutant burden by reporting a 57-year-old Korean man with ET.

Case Reports > Ann Hematol. 2015 May;94(5):865-7. doi: 10.1007/s00277-014-2248-0. Epub 2014 Nov 5.

A report on the co-occurrence of JAK2V617F and CALR mutations in myeloproliferative neoplasm patients

Na Xu [1], Li Ding, Changxin Yin, Xuan Zhou, Lin Li, Yulin Li, Qisi Lu, Xiao-li Liu

Affiliations + expand

PMID: 25366168 DOI: 10.1007/s00277-014-2248-0

> Oncotarget. 2016 Aug 30;7(35):57036-57049. doi: 10.18632/oncotarget.10958.

Coexistence of JAK2 and CALR mutations and their clinical implications in patients with essential thrombocythemia

Min-Gu Kang [1 2], Hyun-Woo Choi [1], Jun Hyung Lee [1], Yong Jun Choi [1], Hyun-Jung Choi [1], Jong-Hee Shin [1], Soon-Pal Suh [1], Michael Szardenings [3], Hye-Ran Kim [4], Myung-Geun Shin [1 2 5]

Affiliations + expand

PMID: 27486987 PMCID: PMC5302971 DOI: 10.18632/oncotarget.10958

Abstract

Janus kinase 2 (JAK2) and calreticulin (CALR) constitute the two most frequent mutations in essential thrombocythemia (ET), and both are reported to be mutually exclusive. Hence, we examined a cohort of 123 myeloproliferative neoplasm (MPN) patients without BCR-ABL1 rearrangement and additional ET patients (n=96) for coexistence of JAK2 and CALR mutations. The frequency of CALR mutations was 20.3% in 123 MPN patients; 31.1% in ET (n=74), 25% in primary myelofibrosis (n=4) and 2.2% in polycythemia vera (n=45). JAK2 and CALR mutations coexisted in 7 (4.2%) of 167 ET patients. Clinical characteristics, progression-free survival (PFS), and elapsed time to achieve partial remission across 4 groups (JAK2+/CALR+, JAK2+/CALR-, JAK2-/CALR+, JAK2-/CALR-) were reviewed. The JAK2+/CALR- group had higher leukocyte counts and hemoglobin levels and more frequent thrombotic events than JAK2-/CALR- group. JAK2 mutations have a greater effect on the disease phenotype and the clinical features of MPN patients rather than do CALR mutation. JAK2+ groups showed a tendency of poor PFS than JAK2- groups regardless of CALR mutation. CALR+ was a predictor of late response to the treatment. Our study also showed that thrombosis was more frequent in ET patients with type 2 CALR mutations than in those with type 1 CALR mutations.

> Am J Hematol. 2018 Aug;93(4):E84-E86. doi: 10.1002/ajh.25014. Epub 2018 Jan 12.

Clinical and biological characterization of MPN patients harboring two driver mutations, a French intergroup of myeloproliferative neoplasms (FIM) study

Olivier Mansier [1 2 3], Damien Luque Paz [4 5 6 7], Jean-Christophe Ianotto [7 8], Yannick Le Bris [7 9], Aurélie Chauveau [7 10 11 12], Françoise Boyer [7 13], Carole Conejero [14], Olivier Fitoussi [15], Jérémie Riou [16], Didier Adiko [17], Mohamed Touati [18], Jasmine Chauzeix [19 20], Jean-François Viallard [21], Marie C Béné [7 9], Stéphane Giraudier [14], Valérie Ugo [4 5 6 7], Eric Lippert [7 10 11 12]

Case Reports > Br J Haematol. 2014 Oct;167(2):276-8. doi: 10.1111/bjh.12969. Epub 2014 Jun 17.

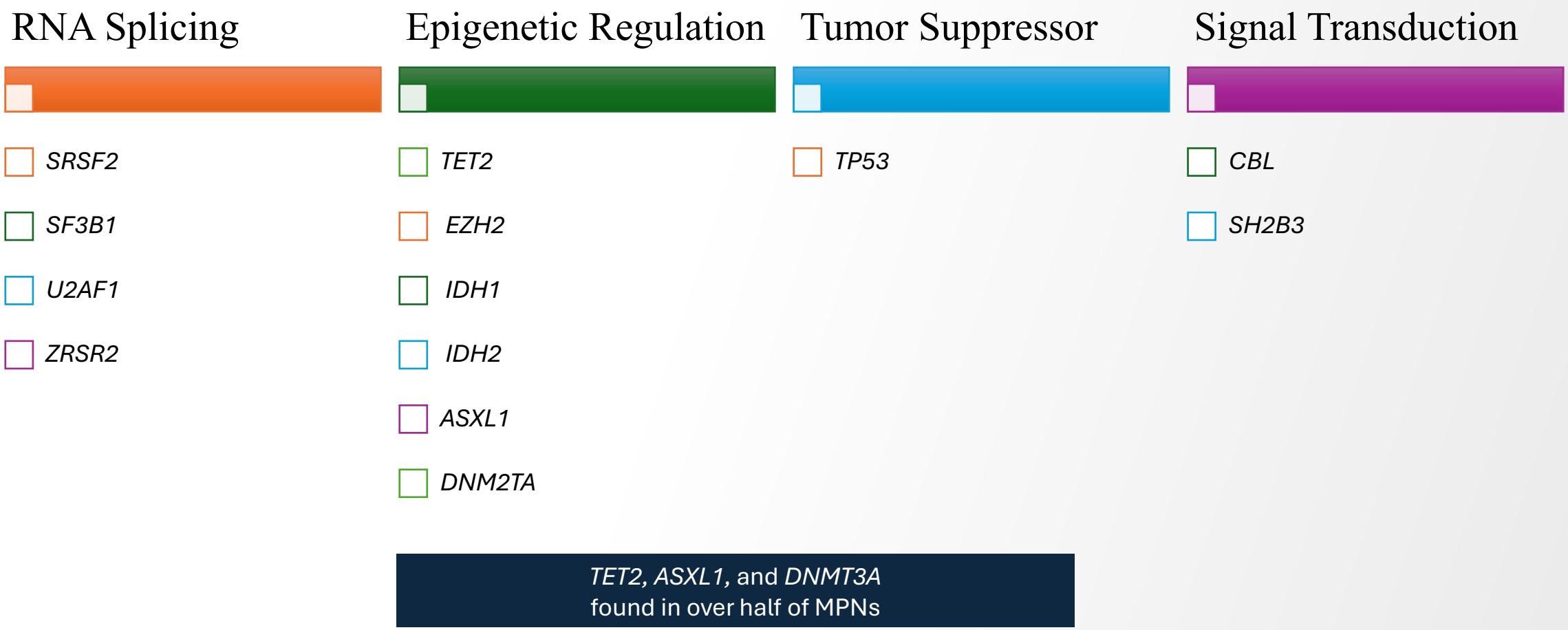
JAK2 V617F and CALR mutations are not mutually exclusive; findings from retrospective analysis of a small patient cohort

Gillian McGaffin [1], Kirsteen Harper, David Stirling, Lorna McLintock

Affiliations + expand

PMID: 24935260 DOI: 10.1111/bjh.12969

Other MPN- Related Mutations Recommended by NCCN Guidelines



Reference:

NCCN Guidelines V1.2025 Myeloproliferative Neoplasms (MPNs)

Khoury, J.D., Solary, E., Abla, O. et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia 36, 1703–1719 (2022). https://doi.org/10.1038/s41375-022-01613-1

NCCN: NGS- based panel recommended to assess prognosis and establishing clonality in TN-MPNs

Primary myelofibrosis (PMF)

- *ASXL1, EZH2, SRSF2, TP53, IDH1*, *IDH2, or U2AF1* mutations are considered as high-molecular-risk (HMR) mutations and are associated with significantly shorter OS and leukemia-free survival (LFS) in patients with PMF.

Polycythemia Vera (PV)

- In one report, a mutation in one of these genes *(ASXL1, SRSF2*, and *IDH2)* was associated with inferior OS and MF-free survival but it did not significantly affect the LFS in patients with PV

Essential Thrombocythemia (ET)

- *SH2B3, IDH2, U2AF1, SF3B1,SRSF2, EZH2, and TP53* mutations were identified as significant risk factors for inferior OS, MF-free survival, and LFS in patients with ET

NGS may also be useful to establish the clonality (e.g., triple-negative MPN with non-mutated *JAK2, MPL, and CALR*)

- NCCN Guidelines V2.2025 Myeloproliferative Neoplasms (MPNs)

Beyond The Three Driver Genes (ASH 2023 Publication)

- ❑ Recurrent somatic gene mutations in MPNs and secondary AML.
- ❑ Secondary AML is rare but devastating complications with a median survival of <6 months

Gene Name Location & Type of Mutation	PV %	ET %	PMF %	Post-MPN AML %	Clinical Implication
TET2 (all exons)	10%-20%	3%-10%	10%-20%	19%-25%	No prognostic impact reported
DNMT3A (R882 and all exons)	5%-10%	1%-5%	8%-12%	3%-17%	No prognostic impact reported
IDH1 (R312)	1%-2%	1%-2%	5%-6%	13%	High molecular risk (HMR) in PMF and adverse prognosis impact in all MPN subtype
IDH2 (R140 or R172)	1%-2%	1%-2%	5%-6%	7%-15%	HMR in PMF and adverse prognosis impact in all MPN subtype
ASXL1 (mostly nonsense/frameshift in the last exon)	2%-7%	5%-10%	15%-35%	17%-47%	HMR in PMF and adverse prognostic impact in all MPN subtypes
EZH2 (all exons)	1%-2%	1%-2%	7%-10%	7%-13%	HMR in PMF and adverse prognosis impact in all MPN subtype
NRAS (G12, G13, or Q61)	<2%	<2%	2%-4%	11%	Adverse prognostic impact in all MPN subtypes
KRAS (G12, G13, or Q61)	<2%	<2%	2%	4%-7%	Adverse prognostic impact in all MPN subtypes
SH2B3 (exon 2)	2%-9%	1%-3%	2%-4%	6%-11%	Rare mutations in JAK2 negative MPNs

Luque Paz, et al. Blood 2023; 141 (16): 1909–1921. doi: https://doi.org/10.1182/blood.2022017578

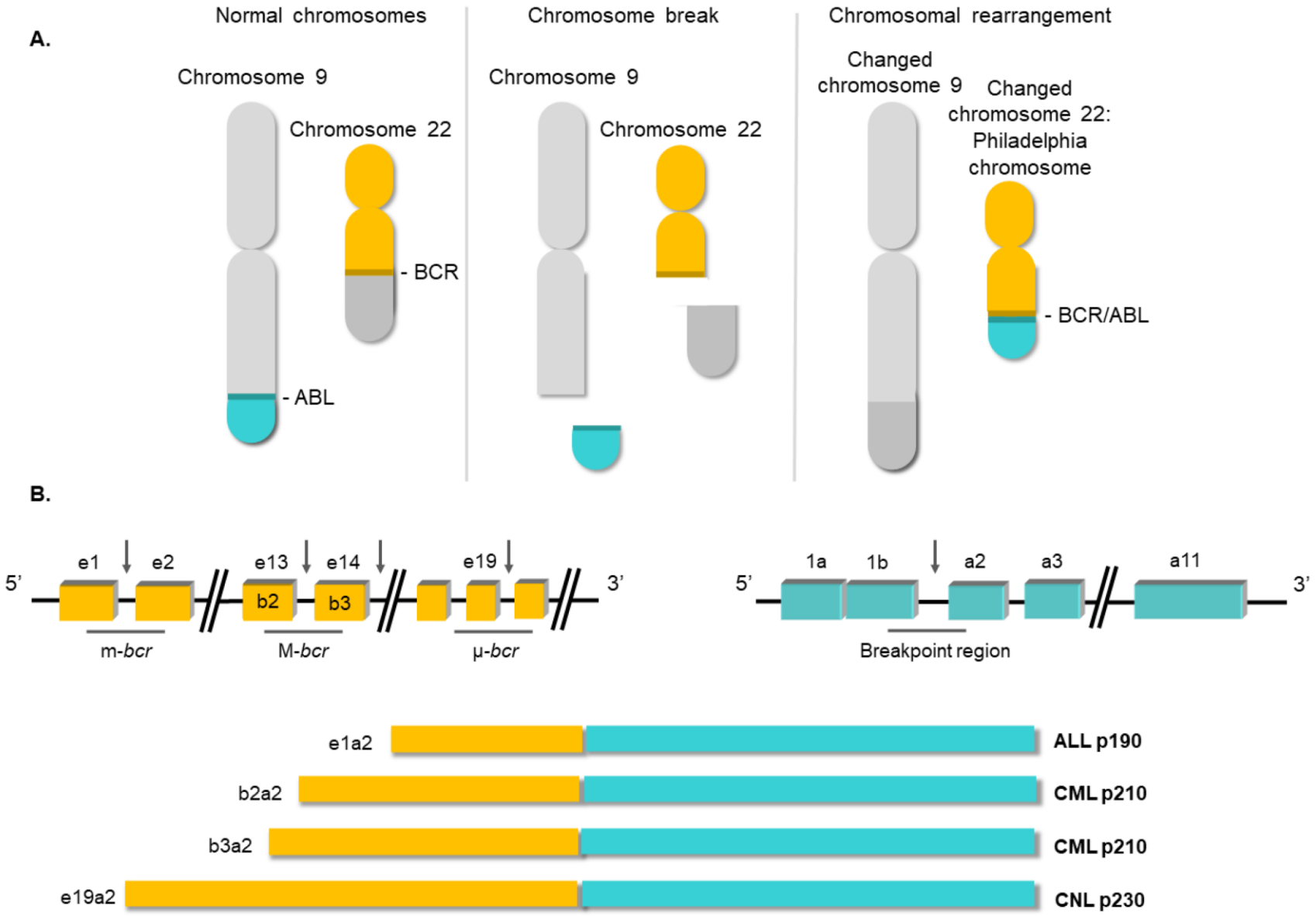
Beyond The Three Driver Genes (ASH 2023 Publication)

- ❑ Recurrent somatic gene mutations in MPNs and secondary AML.
- ❑ Secondary AML is rare but devastating complications with a median survival of <6 months

Gene Name Location & Type of Mutation	PV %	ET %	PMF %	Post-MPN AML %	Clinical Implication
CBL (exon 8 and 9)	<2%	<2%	4%	4%	Adverse prognostic impact in all MPN subtypes
SRSF2 (P95)	<2%	<2%	6%-14%	7%-22%	HMR in PMF and adverse prognostic impact in all MPN subtypes
*SF3B*1 (exon 14-16)	2%-3%	2%-5%	5%-7%	7%-11%	Adverse prognostic impact in ET
U2AF1 (S34 or q157)	<2%	<2%	7%-10%	5%-12%	Adverse prognostic impact in all MPN subtypes
NFE2 (all exons)	3%-6%	1%-7%	3%-5%	?	Increased risk of leukemic transformation
RUNX1 (all exons)	<2%	<2%	2%-3%	20%	Adverse prognostic impact in all MPN subtypes
TP53 (all exons)	<2%	<2%	4%-5%	16%-50%	Adverse prognostic impact in all MPN subtypes

Luque Paz, et al. Blood 2023; 141 (16): 1909–1921. doi: https://doi.org/10.1182/blood.2022017578

Chronic Myeloid Leukemia (CML):



From: Vuelta E, García-Tuñón I, Hernández-Carabias P, Méndez L, Sánchez-Martín M. Future Approaches for Treating Chronic Myeloid Leukemia: CRISPR Therapy. *Biology*. 2021; 10(2):118. https://doi.org/10.3390/biology10020118

Resistance Mutations:

BCR::ABL1 kinase domain mutations

T315I/A, M244V, G250E, Y253H, E255K/VV299L, F317L/V/I/C, A337T, F359V/I/C, P465S.

BCR::ABL1 kinase independent mutations

ASXL1, IKZF1, BCOR, TET1/2, IDH1/2, DNMT3A/3B, EZH2

NCCN Guidelines Version 1.2026 Chronic Myeloid Leukemia (CML).

AMPLICON-BASED MYELOID NGS PANEL

DNA panel: Hotspot genes (28)		DNA panel: Full genes (17)		RNA panel: Fusion driver genes (35)			RNA panel: Expression genes (5)	RNA panel: Expression control genes (5)
ANKRD26	*KRAS*	*ASXL1*	*PRPF8*	*ABL1*	*HMGA2*	*NUP98*	*BAALC*	*EIF2B1*
ABL1	*MPL*	*BCOR*	*RB1*	*ABL2*	*JAK2*	*NUP214*	*MECOM*	*FBXW2*
BRAF	*MYD88*	*CALR*	*RUNX1*	*BCL2*	*KAT6A (MOZ)*	*PAX5*	*MYC*	*PSMB2*
CBL	*NPM1*	*CEBPA*	*SH2B3*	*BRAF*	*KAT6B*	*PDGFRA*	*SMC1A*	*PUM1*
CSF3R	*NRAS*	*ETV6*	*STAG2*	*CCND1*	*KMT2A*	*PDGFRB*	*WT1*	*TRIM27*
DDX41	*PPM1D*	*EZH2*	*TET2*	*CREBBP*	*KMT2A PTDs*	*RARA*		
DNMT3A	*PTPN11*	*IKZF1*	*TP53*	*EGFR*	*MECOM*	*RUNX1*		
FLT3 (ITD,	*SMC1A*	*NF1*	*ZRSR2*	*ETV6*	*MET*	*TCF3*		
TKD)	*SMC3*	*PHF6*		*FGFR1*	*MLLT10*	*TFE3*		
GATA2	*SETBP1*			*FGFR2*	*MRTFA (MKL1)*	*ZNF384*		
HRAS	*SF3B1*			*FUS*	*MYBL1*			
IDH1	*SRSF2*				*MYH11*			
IDH2	*U2AF1*				*NTRK2*			
JAK2	*WT1*				*NTRK3*			
KIT								

AMPLICON-BASED MYELOID NGS PANEL

DNA panel: Hotspot genes (28)		DNA panel: Full genes (17)		RNA panel: Fusion driver genes (35)			RNA panel: Expression genes (5)	RNA panel: Expression control genes (5)
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JAK2	*WT1*				*NTRK3*			
KIT								

= MPN-related genes

AMPLICON-BASED MYELOID NGS PANEL

Missing : *NFE2*

DNA panel: Hotspot genes (28)		DNA panel: Full genes (17)		RNA panel: Fusion driver genes (35)			RNA panel: Expression genes (5)	RNA panel: Expression control genes (5)
ANKRD26	*KRAS*	*ASXL1*	*PRPF8*	*ABL1*	*HMGA2*	*NUP98*	*BAALC*	*EIF2B1*
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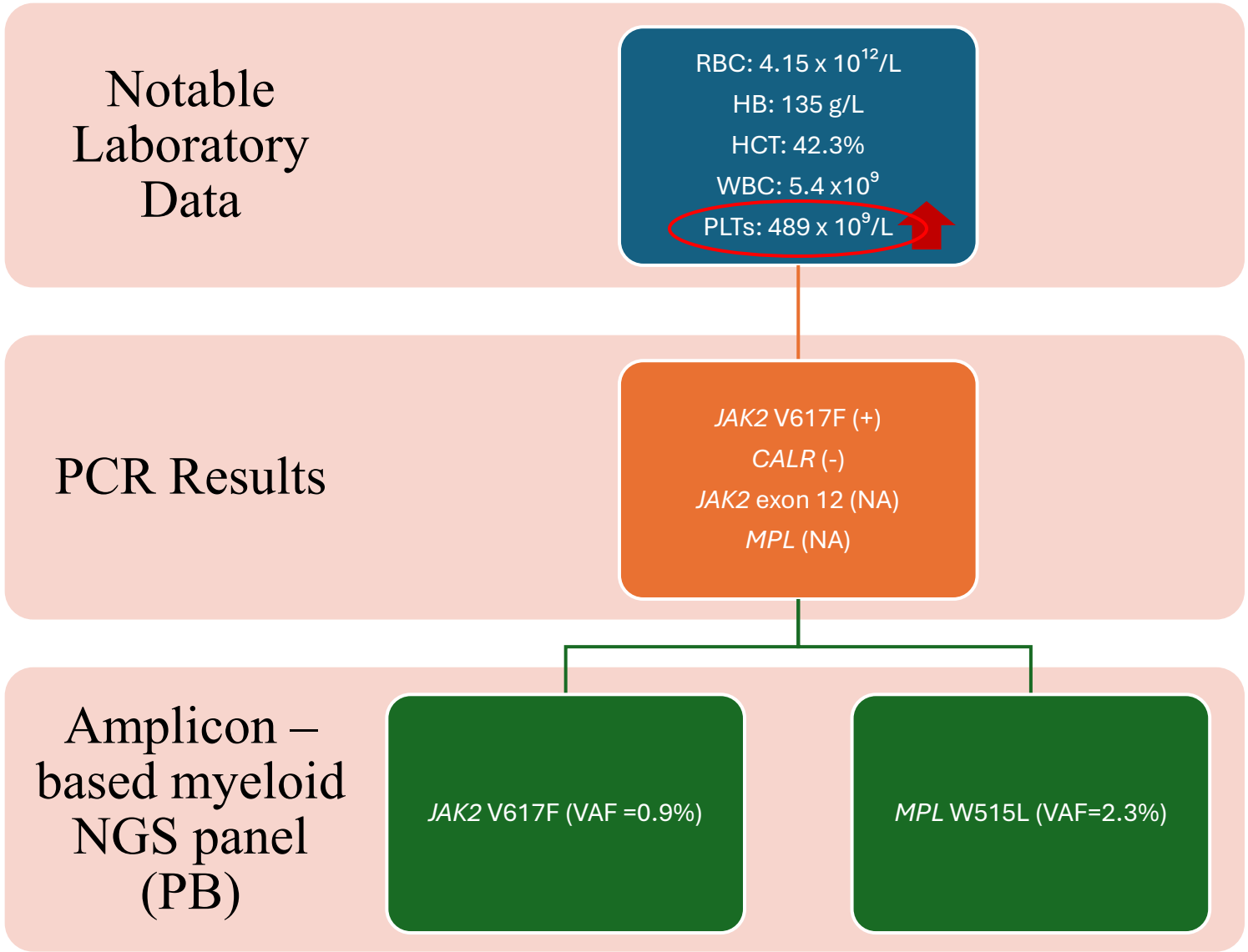
GATA2	*SETBP1*			*FGFR2*	*MRTFA (MKL1)*	*ZNF384*		
HRAS	*SF3B1*			*FUS*	*MYBL1*			
IDH1	*SRSF2*				*MYH11*			
IDH2	*U2AF1*				*NTRK2*			
JAK2	*WT1*				*NTRK3*			
KIT								

= MPN-related genes

Case	Clinical presentation	Notable laboratory data	*JAK2* V617F PCR	*CALR* exon 9 PCR	*JAK2* exon 12 test	*MPL* test	NGS result (blood)
Case 1	64-y-old man with persistent thrombocytosis for 2 y	RBC count: 4.15×10^{12}/L Hemoglobin: 135 g/L Hematocrit: 42.3% WBC count: 5.4×10^{9}/L Platelet count: 489×10^{9}/L	+	-	N/A	N/A	*JAK2* V617F (VAF = 0.9%)[a]; *MPL* W515L (VAF = 2.3%)
Case 2	65-y-old man with leukocytosis and thrombocytosis; hypersegmented neutrophils and giant platelets noted	RBC count: 3.76×10^{12}/L Hemoglobin: 111 g/L Hematocrit: 33.9% WBC count: 27.1×10^{9}/L Platelet count: 979×10^{9}/L	+	-	N/A	N/A	*JAK2* V617F (VAF = 46.1%); *IDH2* R140W (VAF = 2.2%)
Case 3	85-y-old man with thrombocytosis	RBC count: 3.43×10^{12}/L Hemoglobin: 105 g/L Hematocrit: 31.0% WBC count: 14.1×10^{9}/L Platelet count: 2553×10^{9}/L	+	-	N/A	N/A	*JAK2* V617F (VAF = 30.7%); *SF3B1* K666T (VAF = 35.5%)
Case 4	57-y-old woman with primary erythrocytosis	RBC count: 5.32×10^{12}/L Hemoglobin: 162 g/L Hematocrit: 49.7% WBC count: 6.4×10^{9}/L Platelet count: 248×10^{9}/L	-	-	-	N/A	*DNMT3A* R882H (VAF = 5.7%)
Case 5	63-y-old man with borderline erythrocytosis and thrombocytosis	RBC count: 5.67×10^{12}/L Hemoglobin: 166 g/L Hematocrit: 48.7% WBC count: 8.2×10^{9}/L Platelet count: 466×10^{9}/L	-	-	-	N/A	*DNMT3A* Q527* (VAF = 2.5%); *TET2* Q1852* (VAF = 1.9%)
Case 6	55-y-old woman with erythrocytosis	RBC count: 5.47×10^{12}/L Hemoglobin: 162 g/L Hematocrit: 49.5% WBC count: 7.3×10^{9}/L Platelet count: 308×10^{9}/L	-	-	-	-	*DNMT3A* N838D (VAF = 2.8%)

Case #1:
64 y/o male with persistent thrombocytosis for two years



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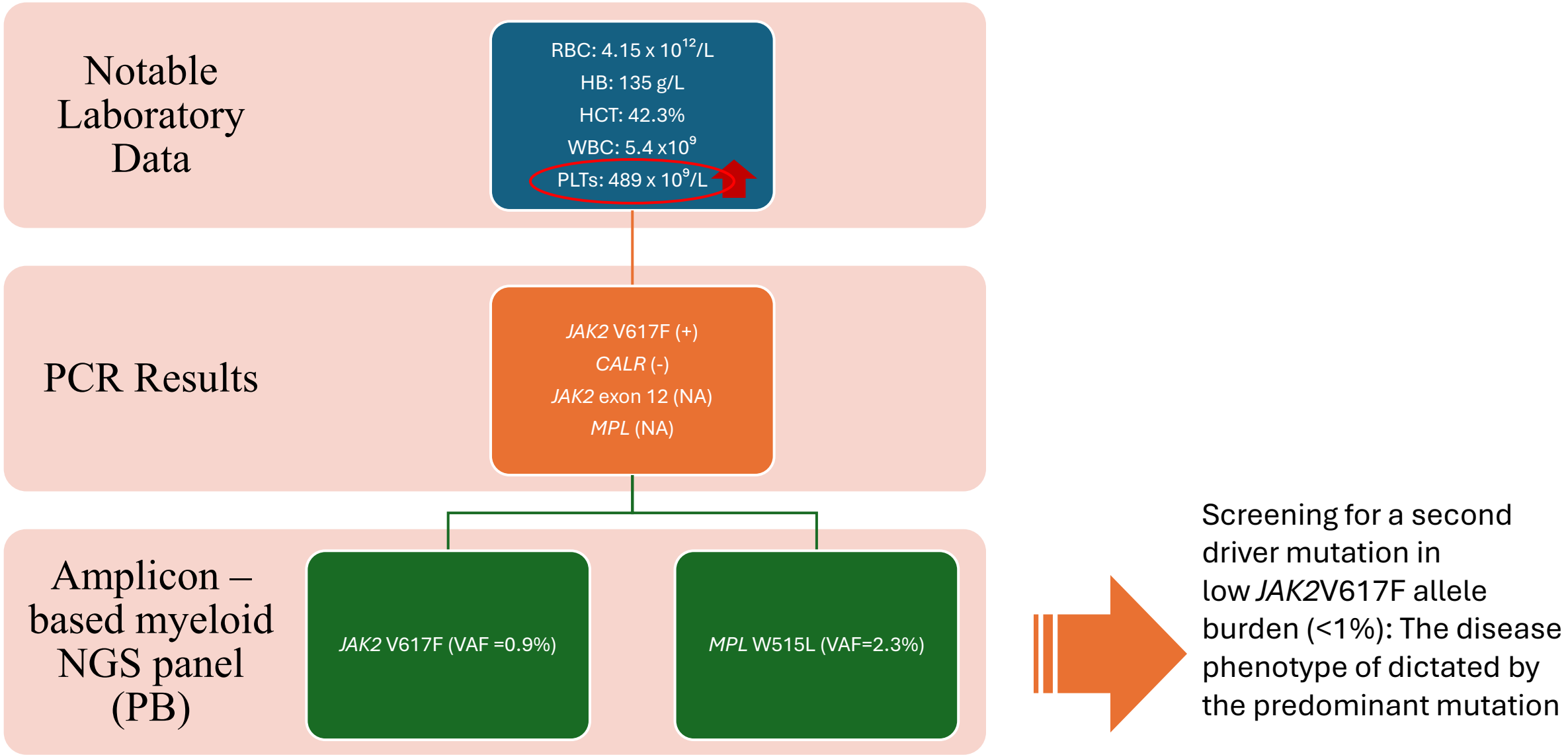
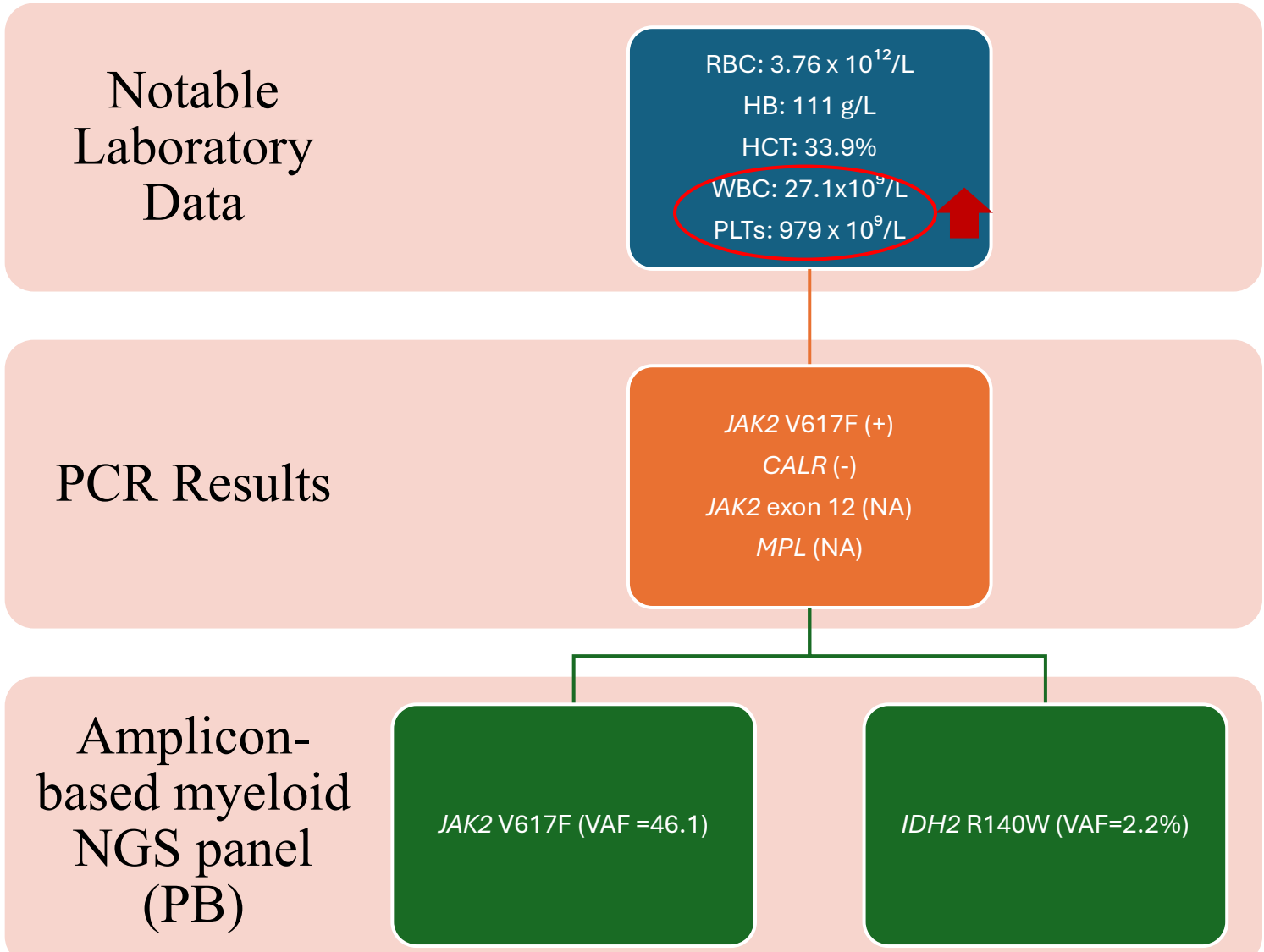
64 y/o male with persistent thrombocytosis for two years



Screening for a second driver mutation in low *JAK2*V617F allele burden (<1%): The disease phenotype of dictated by the predominant mutation

Case #2:

65 y/o male with leukocytosis & thrombocytosis; hypersegmented neutrophils and giant platelets



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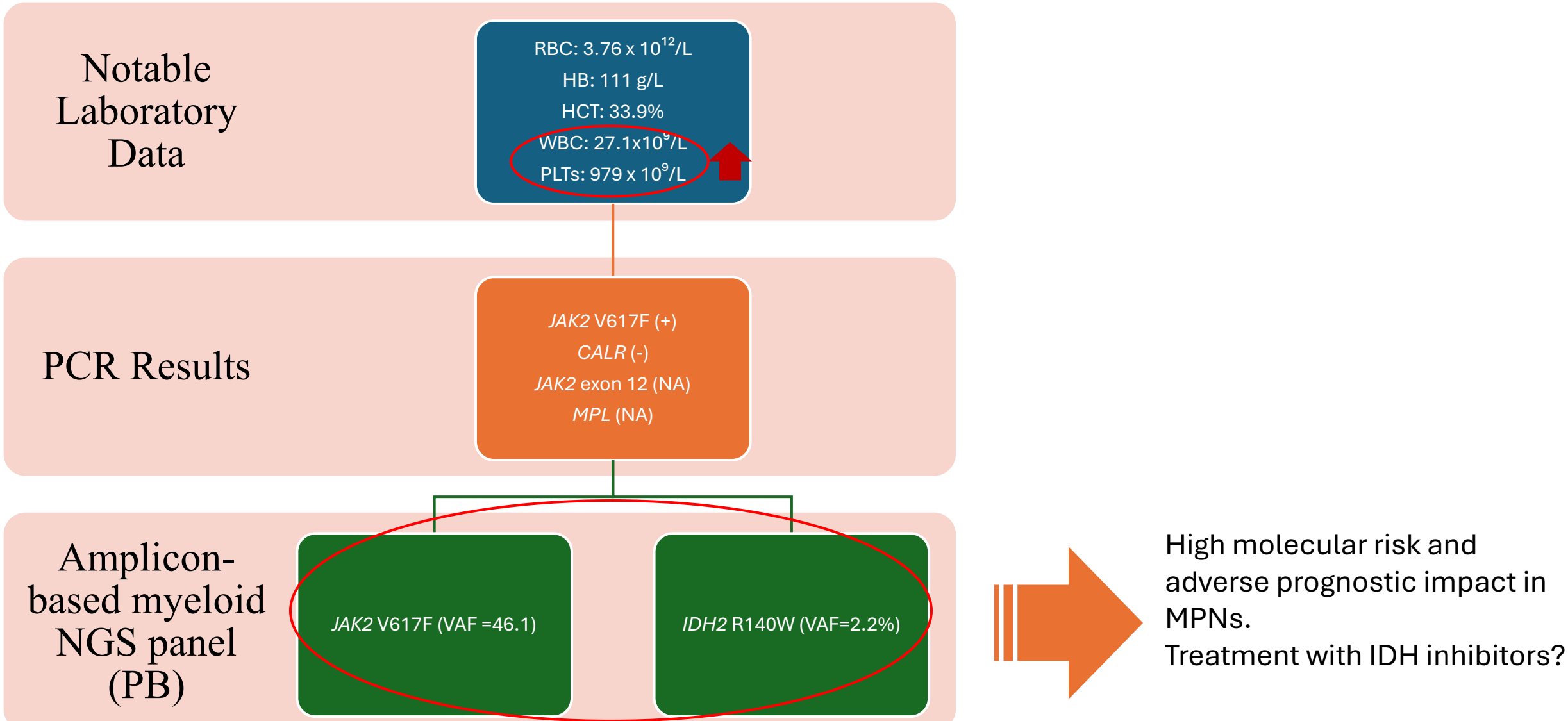
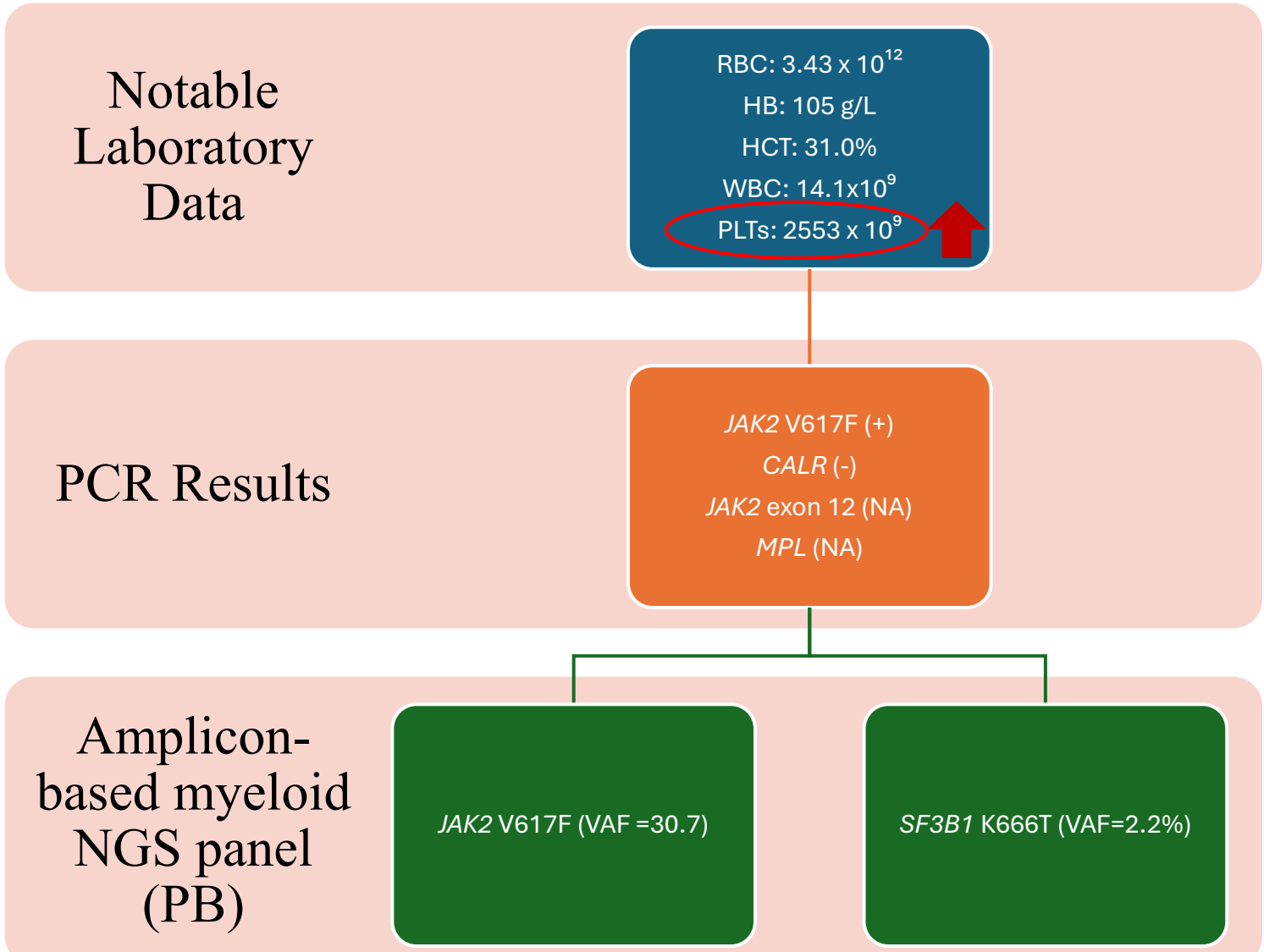
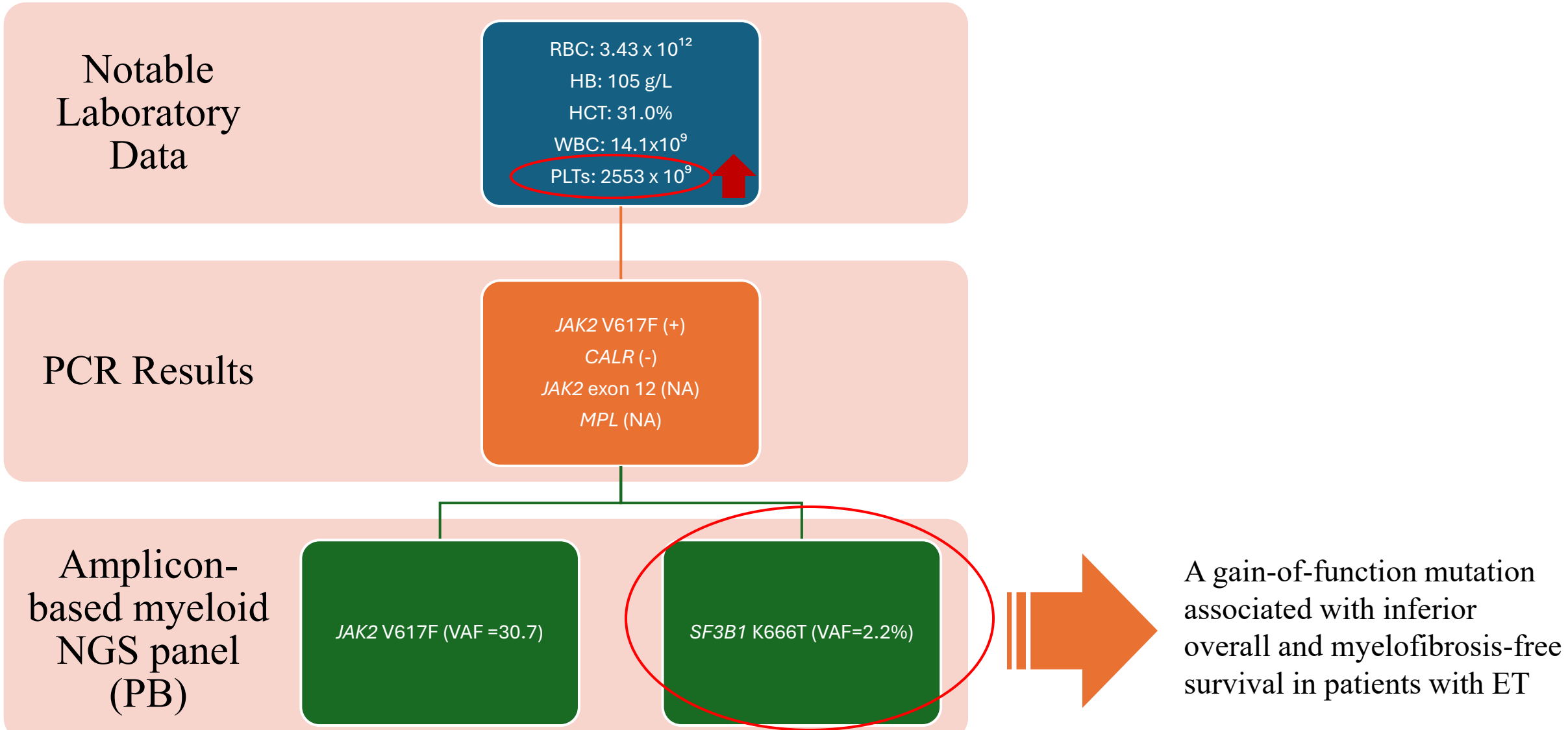


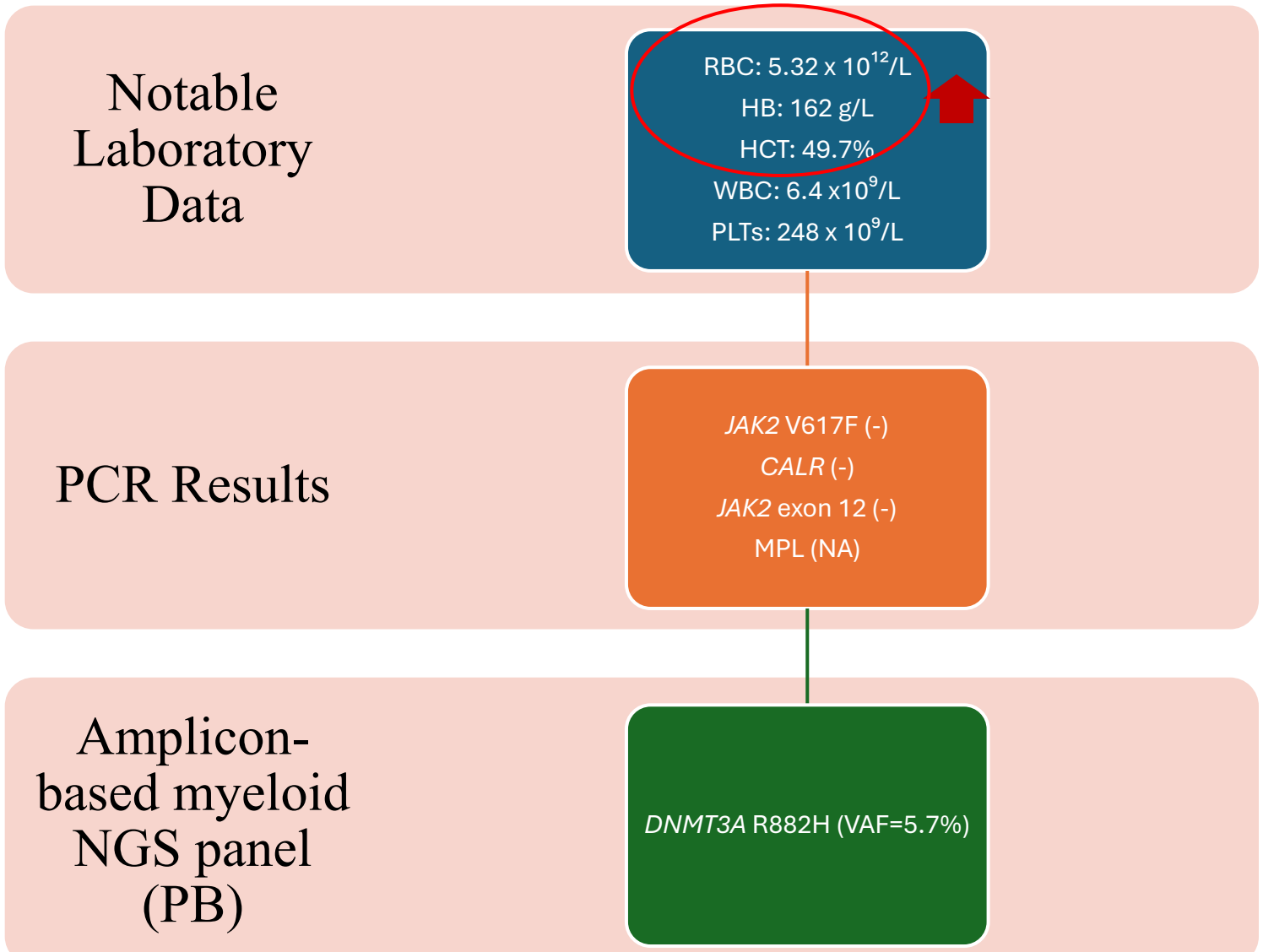
Case #3:
85 y/o male presented with thrombocytosis



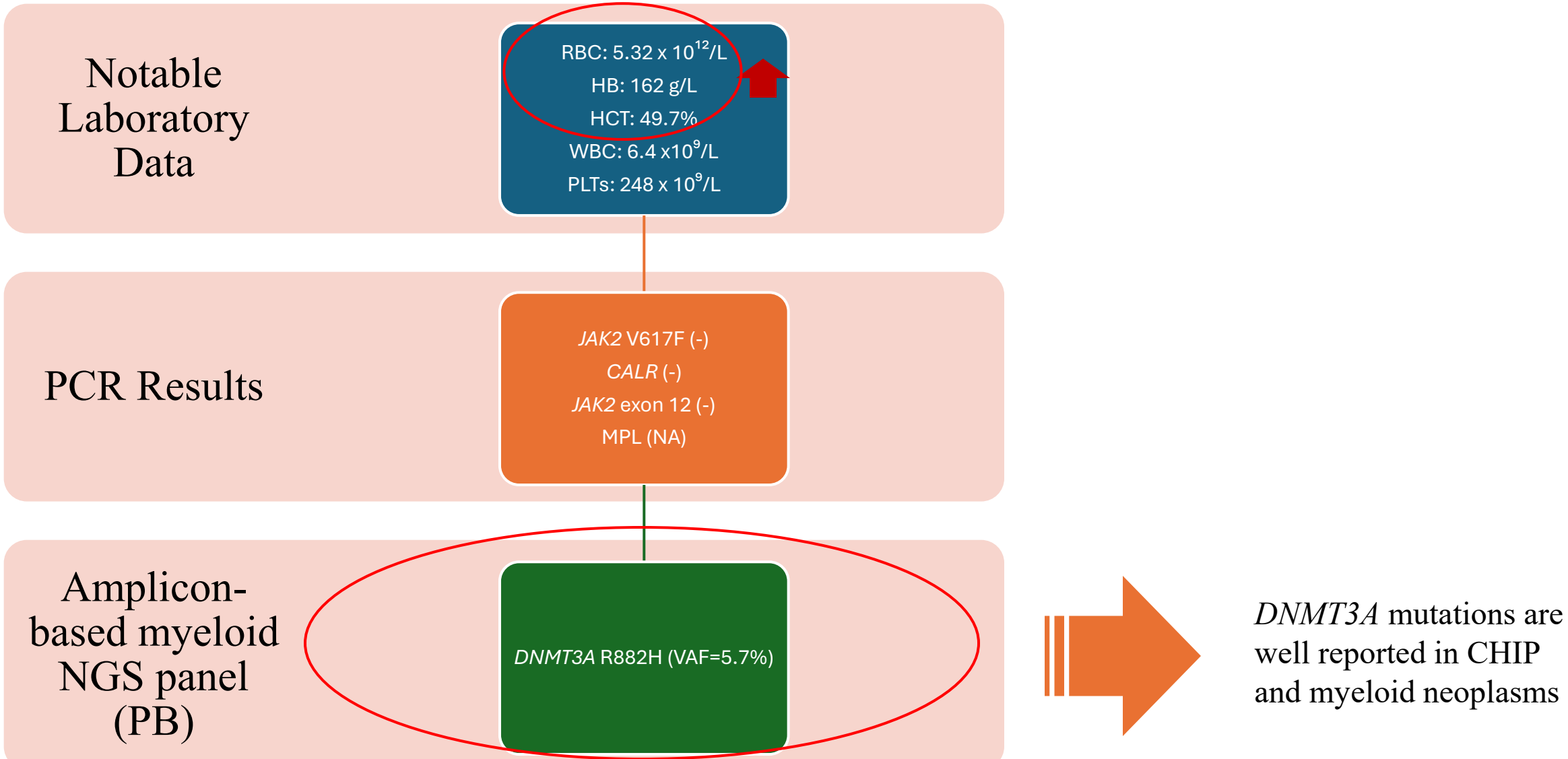
Case #3:
85 y/o male presented with thrombocytosis



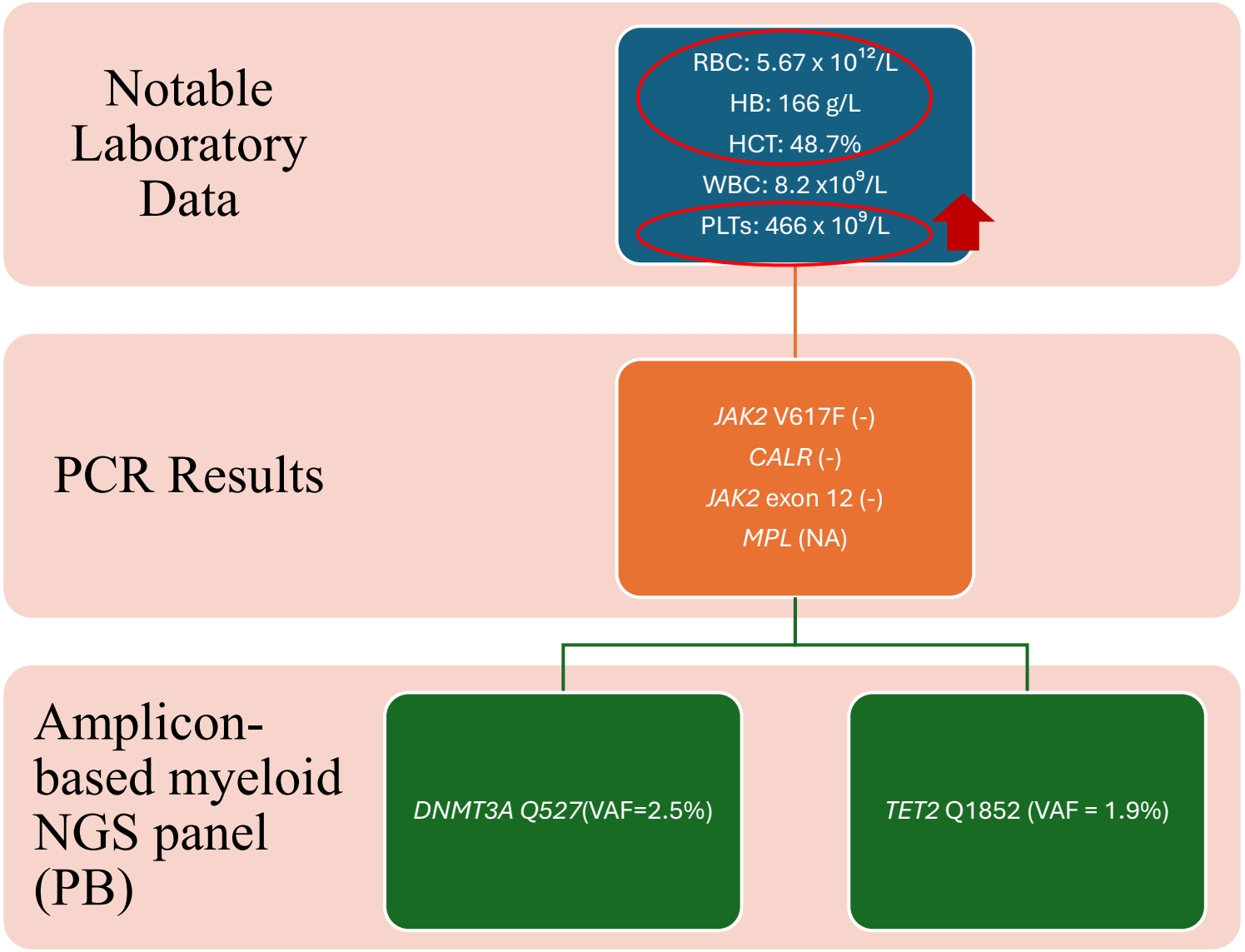
Case #4:
57 y/o female with primary erythrocytosis



Case #4:
57 y/o female with primary erythrocytosis



Case #5:
63 y/o male with borderline erythrocytosis and thrombocytosis



Case #5:
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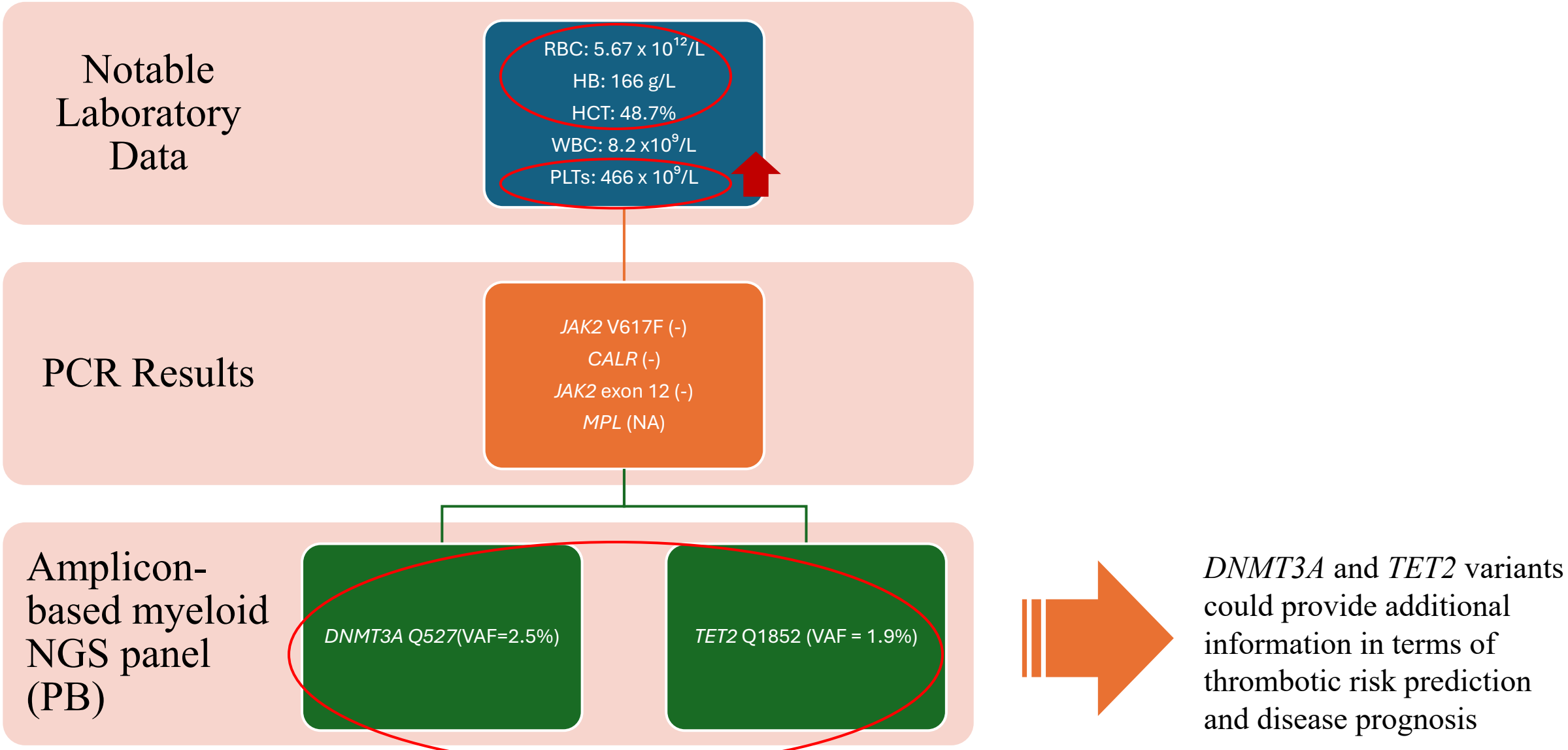
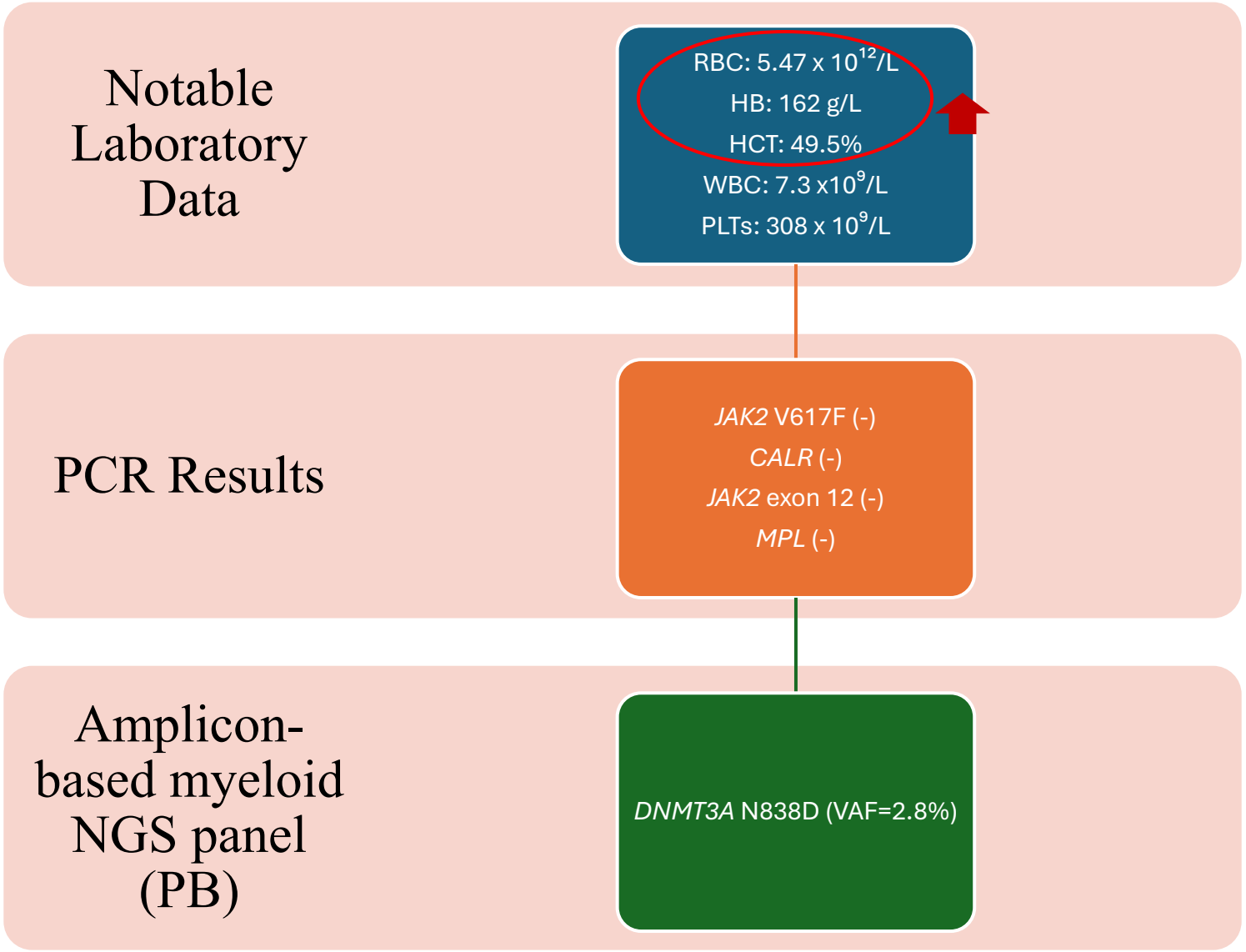


Case #6:
55 y/o female with erythrocytosis



Case #6:
55 y/o female with erythrocytosis

Notable Laboratory Data

RBC: 5.47 x 10^{12}/L
HB: 162 g/L
HCT: 49.5%
WBC: 7.3 x10^{9}/L
PLTs: 308 x 10^{9}/L

PCR Results

JAK2 V617F (-)
CALR (-)
JAK2 exon 12 (-)
MPL (-)

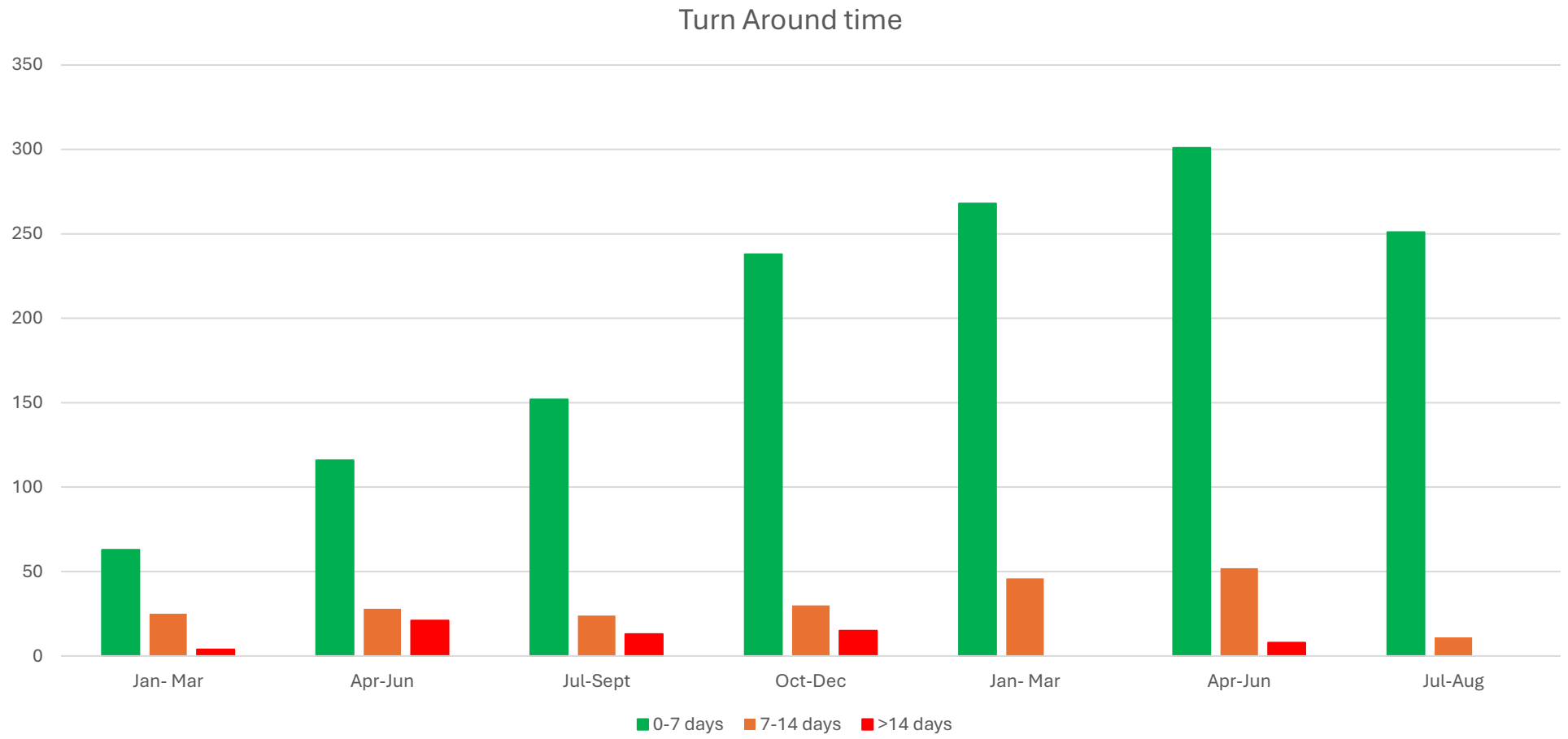
Amplicon-based myeloid NGS panel (PB)

DNMT3A N838D (VAF=2.8%)

DNMT3A mutations are well reported in CHIP and myeloid neoplasms

NGS is a Win Win?????

Turn around times for NGS



MMP198250004_MM_09711720 QC ✓ Key Findings **Variants** Plugins Reports ⋯

All | SNVs/Indels | Fusions

[redacted]

[redacted] Filter Chain Applied
2 of 2,599 Variants

Edit Filters | Export | Columns

User Classification	Variant ID	Variant Name	Key Variant	[redacted]	Locus	[redacted]
Classification	.	DNMT3A C557W	No	No	chr2:25467204	
Classification	KMT2A-KMT2A.K9K2	KMT2A PTD	Yes	No	chr11:118355029 - chr11:118339490	RNAExonVariant Gain-of-Funct

MMP198250004_MM_09711720 QC ✓ Key Findings **Variants** Plugins Reports ⋯

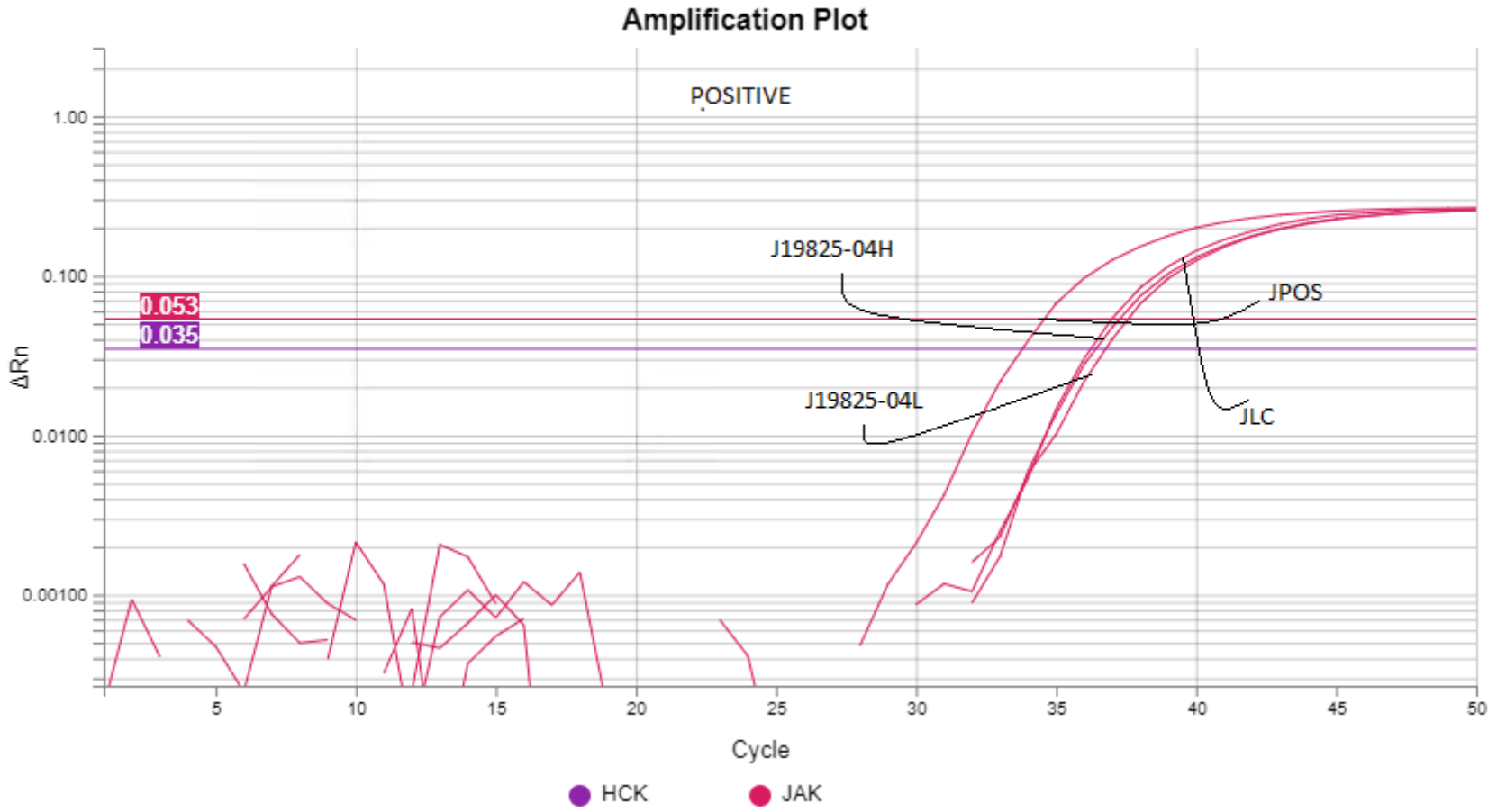
All | SNVs/Indels | Fusions

) Filter Chain Applied
2 of 2,599 Variants

Edit Filters | Export | Columns

User Classification	Variant ID	Variant Name	Key Variant		Locus		
Classification	.	DNMT3A C557W	No	No	chr2:25467204		
Classification	KMT2A-KMT2A.K9K2	KMT2A PTD	Yes	No	chr11:118355029 - chr11:118339490	RNAExonVariant	Gain-of-Funct

Discrepant JAK2 V617F PCR?



MMP198250004_MM_09711720 QC ✓ Key Findings **Variants** Plugins Reports

All | SNVs/Indels | Fusions

No Filter Chain Applied
1,741 of 1,741 SNVs/Indels

Edit Filters

Variant ID	Variant Name	Locus	Gene	AA Change	Call	Effective Read Depth	Nuc Change	Allele Frequency (%)	Allele read Count
COSM12600	JAK2 V617F	chr9:5073770	JAK2	p.Val617Phe	NO CALL	1987	c.1849G>T	1.31	

MMP198250004_MM_09711720 QC ✓ Key Findings **Variants** Plugins Reports

** More than one gene is annotated for this variant. Click on the variant row to view the annotation details.

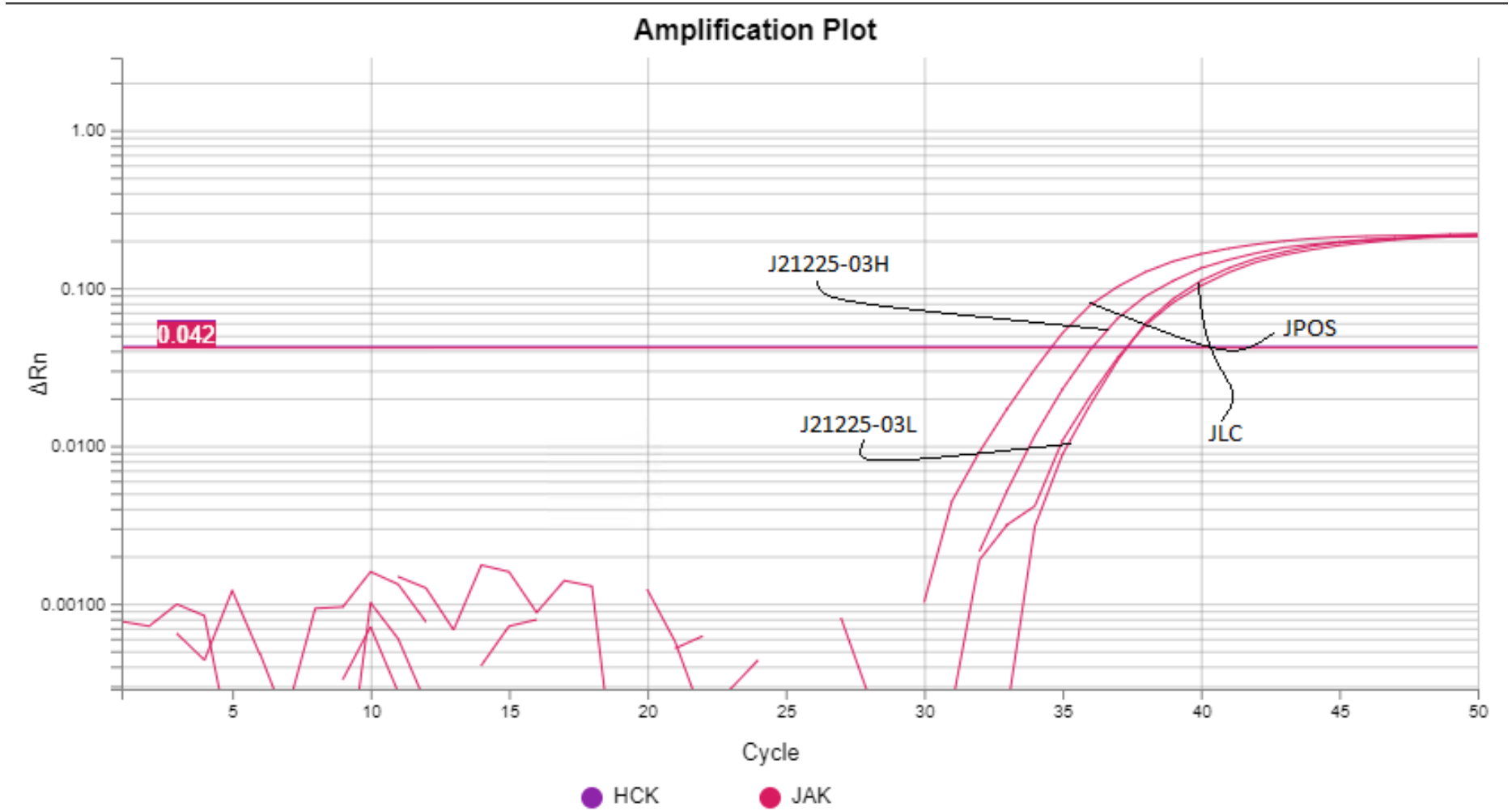
Pileup Annotations

Gene Browser 40 bp

View in IGV

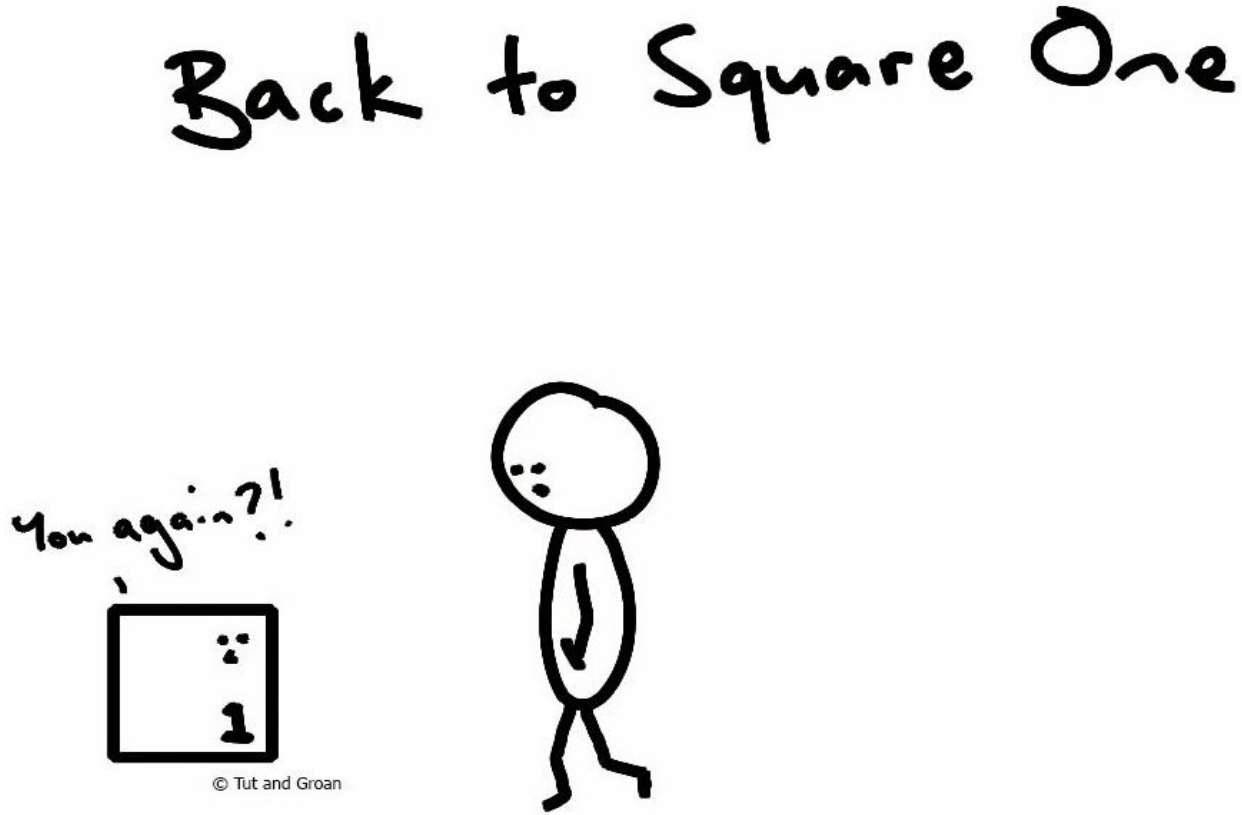
Cursor Guide | Center Line | Track Labels

Follow up PCR 6 weeks later

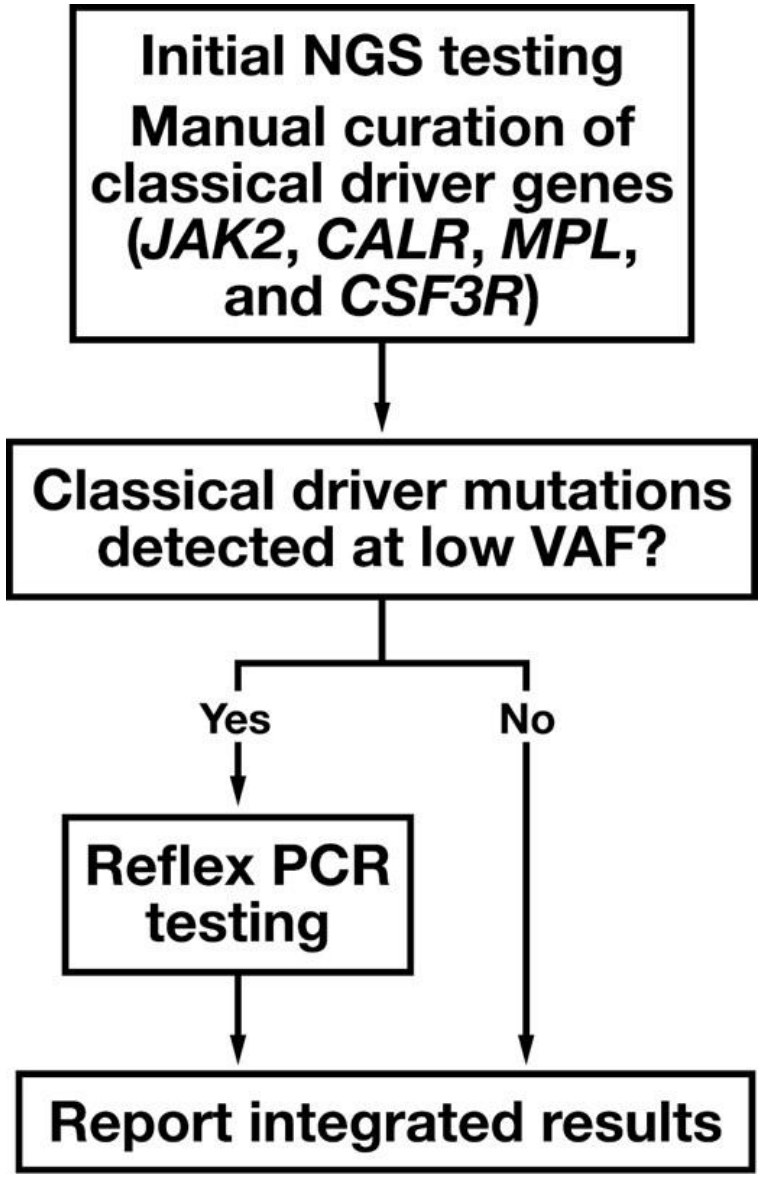


- Sensitivity of NGS for detecting low allele-frequency variants is limited by the proportional representation of these variants within the amplified product

- PCR-based testing and targeted amplification ensures that even low frequency variants are preferentially amplified.



INCORPORATING THE ADVANTAGES OF NGS-BASED AND PCR-BASED TESTING



MPN Workup

Diagnosis	Proposed integrated algorithm
Prognosis	Proposed integrated algorithm
Therapy Selection	Proposed integrated algorithm
Disease Monitoring (MRD)	Proposed integrated algorithm

THANK YOU

- Dr Goldstein
- Dr Naeem
- Dr KH Ramesh
- Dr Xiaowei Liu
- Juan Du
- Kang, Darya, Saif, Emily, Guimin.
- All our wonderful technologists,

LAB HELPERS, SECRETARIES AND TEAM!