MULTIPLEX DETECTION OF JAK2 AND MPL MUTATIONS IN MYELOPROLIFERATIVE NEOPLASMS

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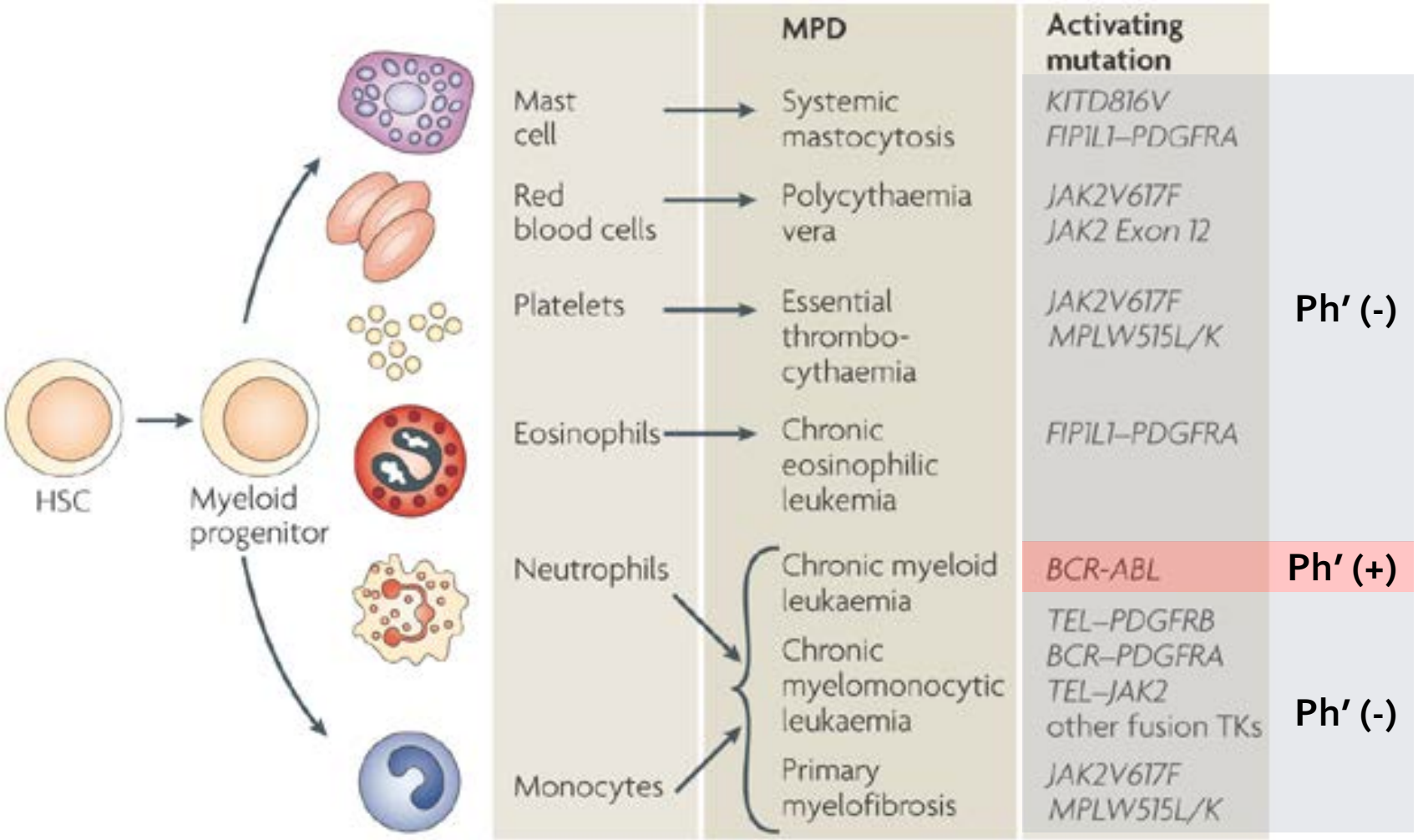
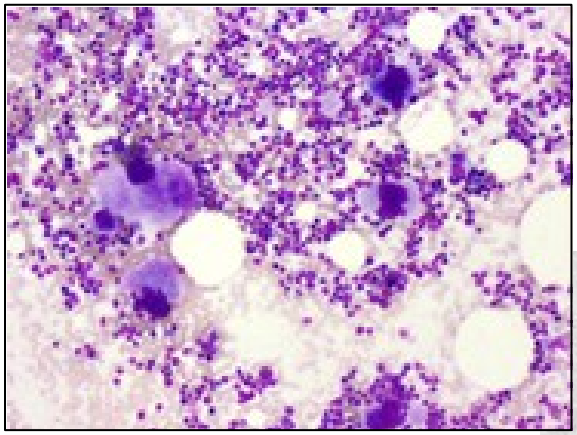
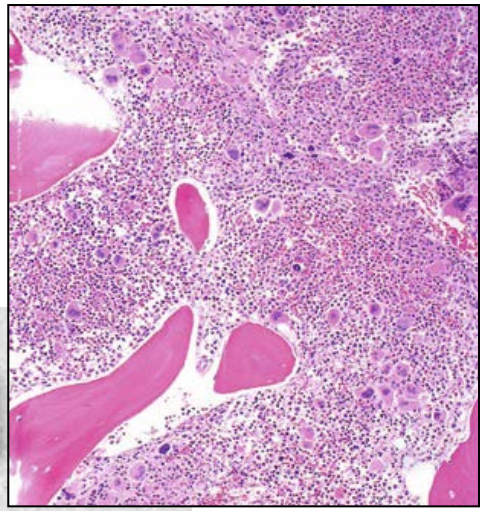
MYELOPROLIFERATIVE NEOPLASMS (MPNs) - DEFINITION

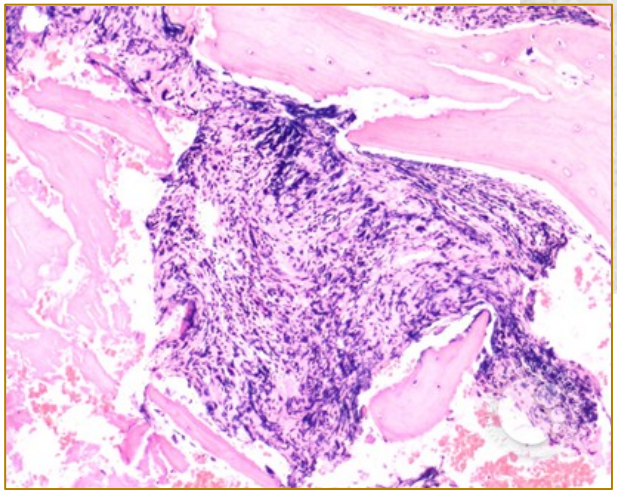


Levine RL et al. NRC (2007)

THE CLASSICAL VIEW OF Ph'(-) MPNS







Essential Thrombocytosis → Polycythemia vera

Thrombosis

Hemorrhage

Leukemic Transformation

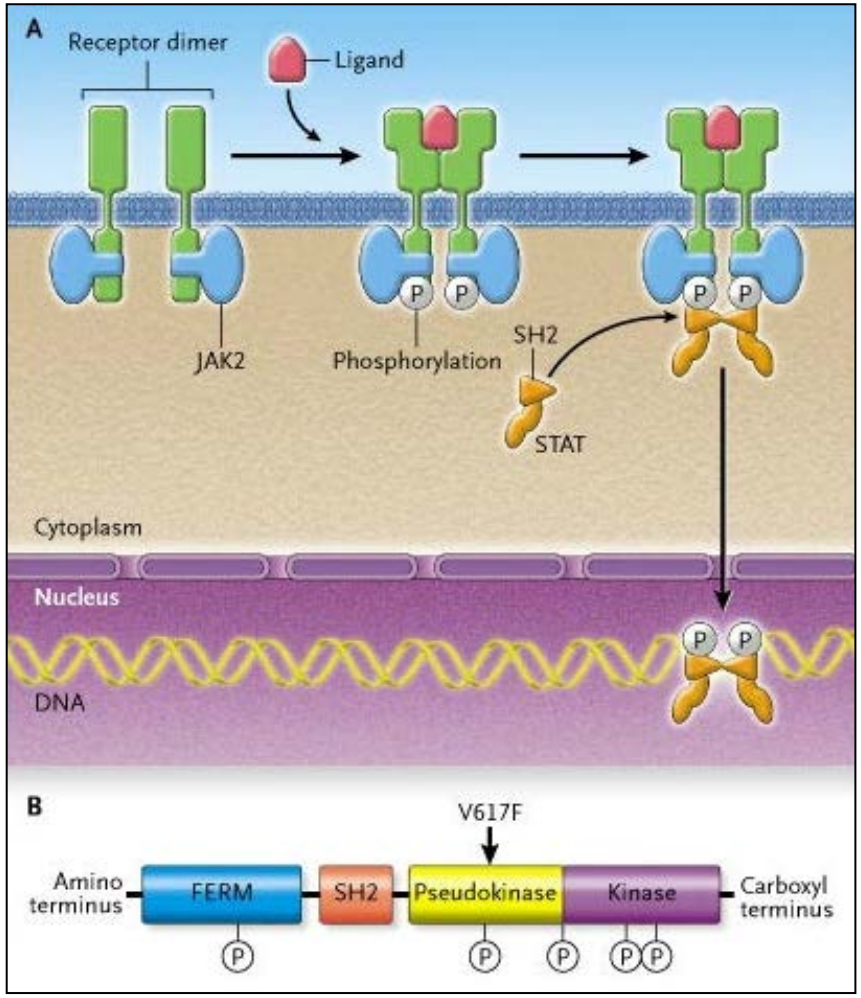
Myelofibrosis

William Dameshek

1951

Levine RL and Gilliland DG. Blood (2008)
ASH Image Bank

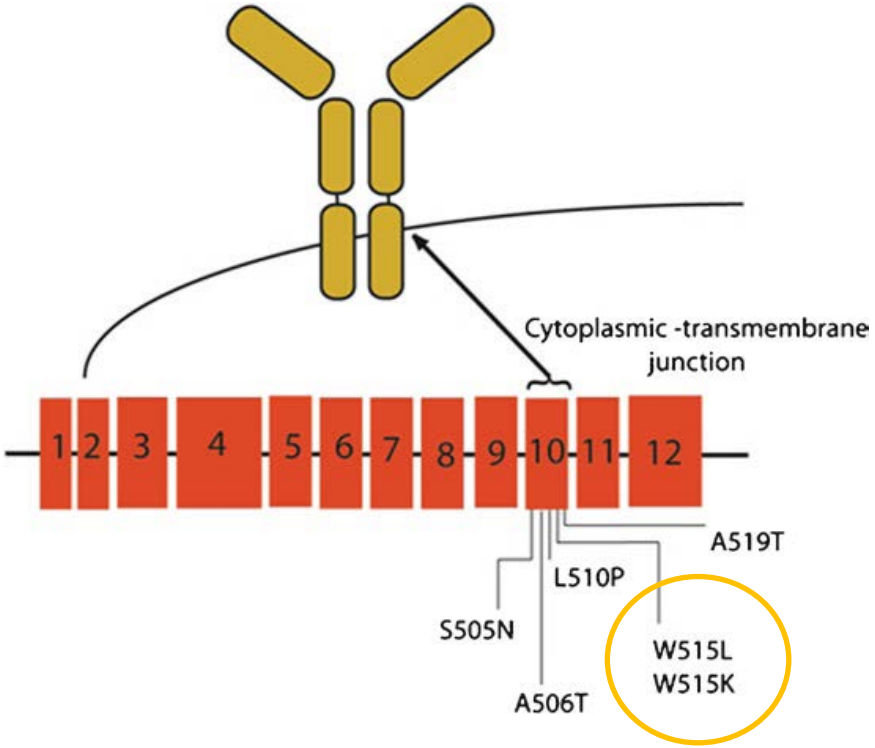
JAK2 V617F MUTATION FOR ALL Ph' (-) MPNS



Disease entity	*Frequency (%)*
Polycythemia vera	81–99
Essential thrombocythemia	41–72
Primary myelofibrosis	39–57

Goodman JM, NEJM (2005)

MPL EXON 10 MUTATIONS IN CONGENITAL THROMBOCYTOSIS, ET AND PMF



Disease entity	*Frequency (%)*
Polycythemia vera	0
Essential thrombocythemia	3–5
Primary myelofibrosis	8–10

Abdel-Wahab O and Levine RL (2010)

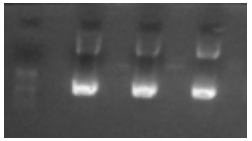
THE MISSION IMPOSSIBLE?

The MUSTs for the best method to detect the most common MPN mutations:

- ✓ Diagnose the vast majority of Ph'(-) MPN cases
- ✓ Be easy, rapid and reproducible
- ✓ Be sensitive and quantitative
- ✓ Allows multiplexing
- ✓ Be open for further development
- ✓ Be affordable

JAK2 V617F ANALYSIS BY PCR-SSOP USING LUMINEX® xMAP™ TECHNOLOGY

PCR of target sequences (345bp of exon 14) using 5` biotinylated primers



Hybridization with specific LNA modified capture probes conjugated via carbodiimide coupling to carboxylated beads

JAK2 wt:	5′-CTCCACAGA**C**ACATACTCC
JAK2 V617F	5′-CTCCACAGA**A**ACATACTCC

Washing

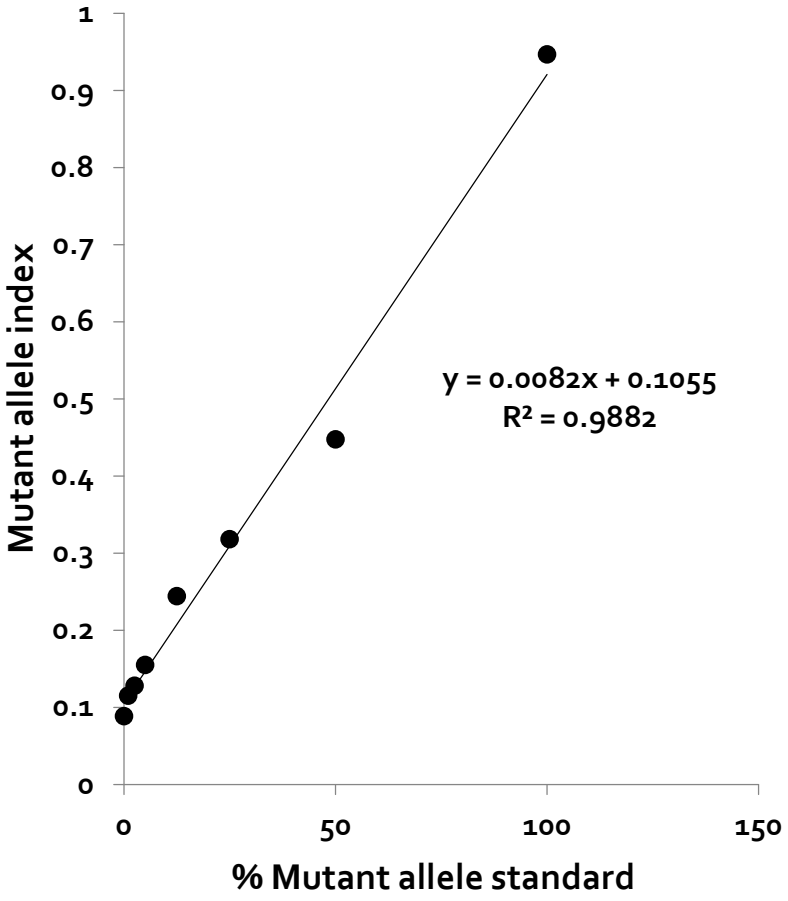
Data acquisition

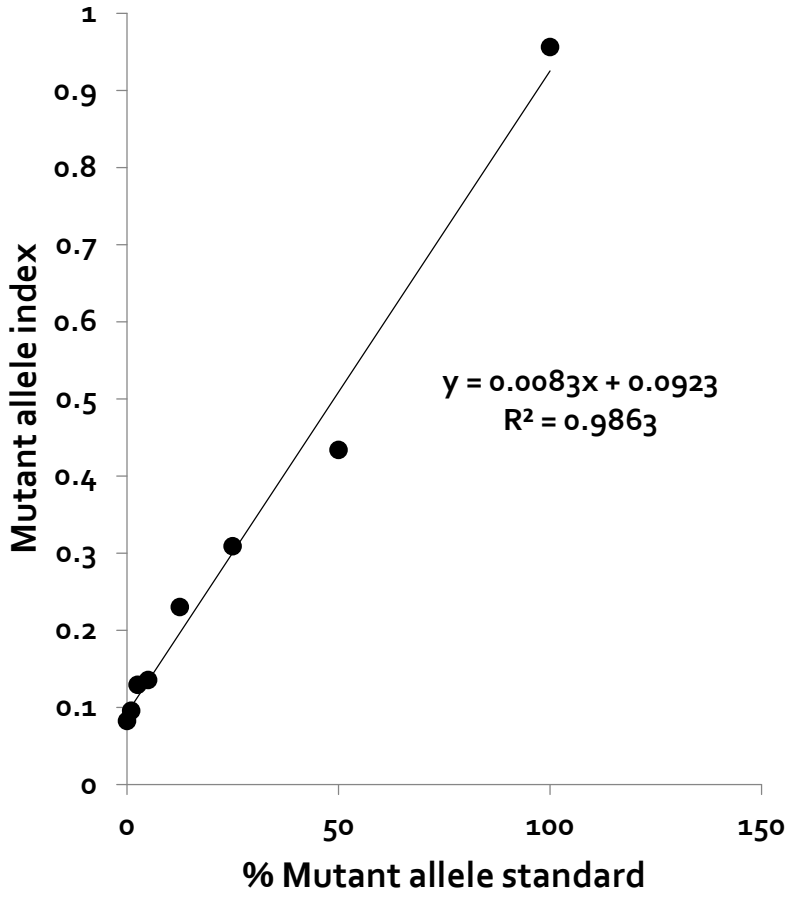
Background correction of MFI
Calculation of mutant/wild type allele index
Index (mutant allele) = [MFI (mutant allele)/[MFI (mut allele) + MFI (wt allele)]

Data analysis

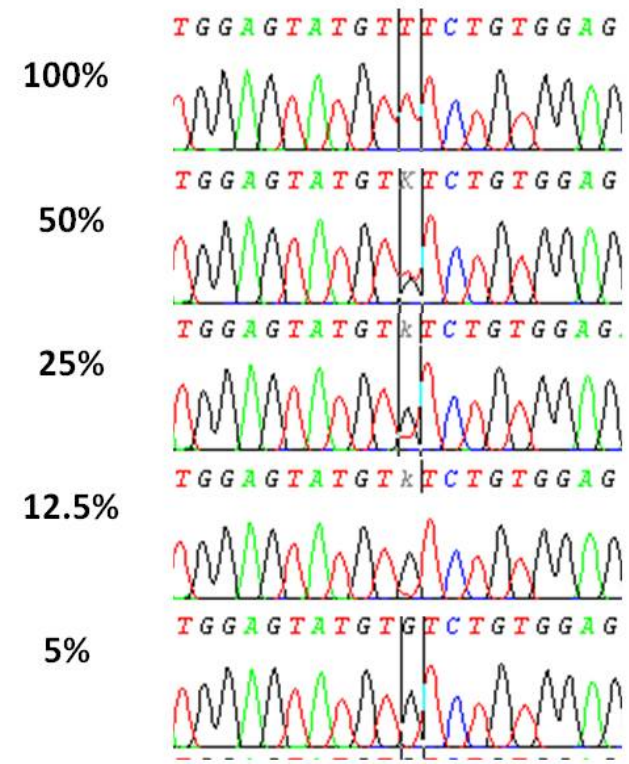
Shivarov et al. Leukemia and Lymphoma (2011)

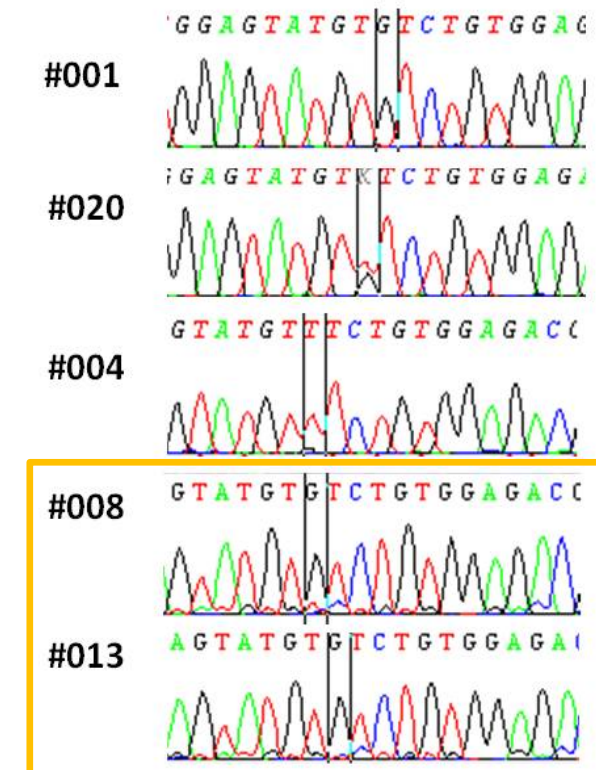
REPRODUCIBLE QUANTITAION OF V617F ALLELE ON PLASMID STANDARDS AT 1% SENSITIVITY LEVEL





BETTER SENSITIVITY ON CLINICAL SAMPLES THAN DIRECT DNA SEQUENCING





% based on bead-based assay

0%

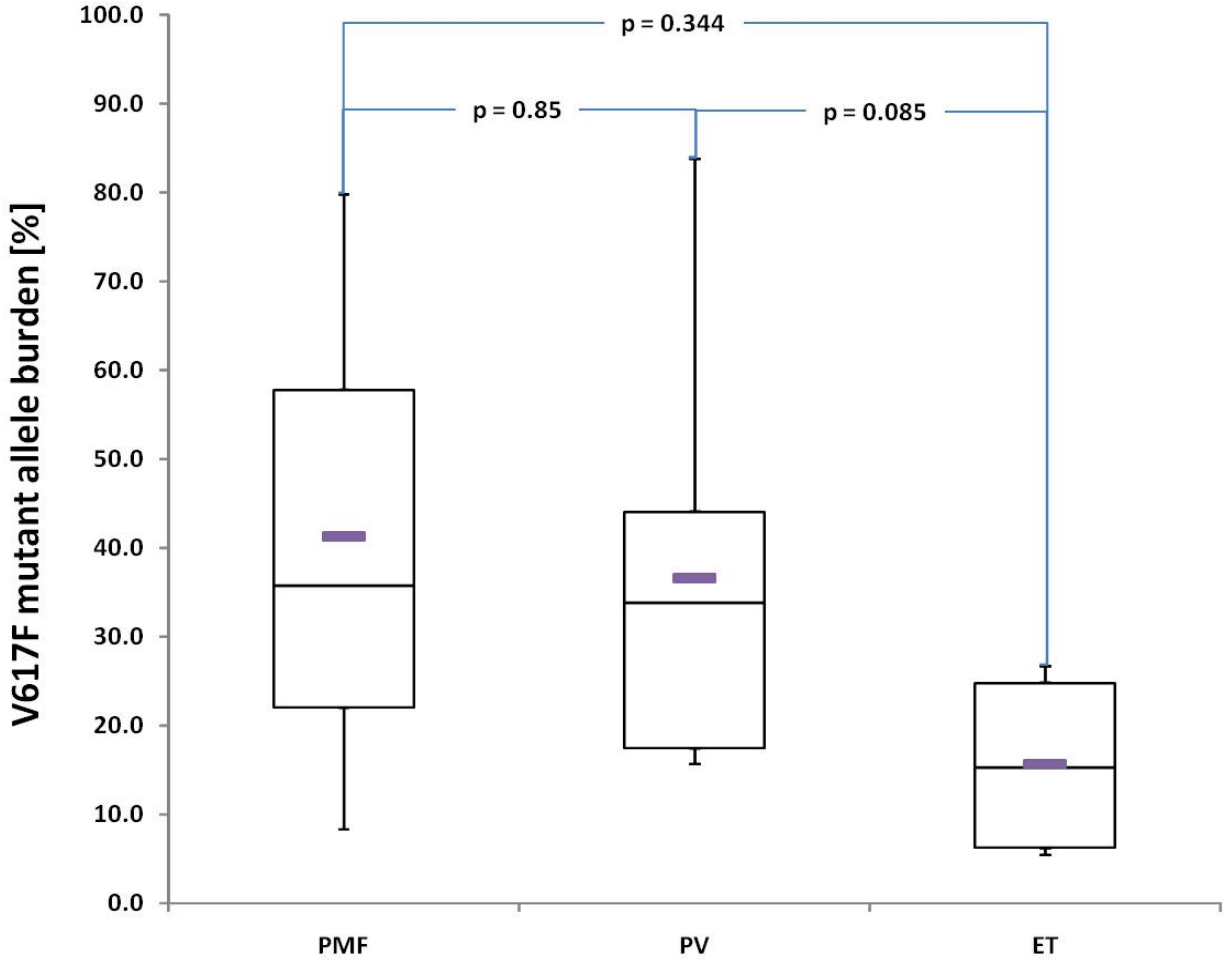
45.4%

83.8%

6.5%

8.3%

QUANTIATION OF THE V617F ALLELE BURDEN IN MPN PATIENTS



MPL EXON 10 MUTATIONS ANALYSIS BY PCR-SSOP USING LUMINEX® XMAP™ TECHNOLOGY

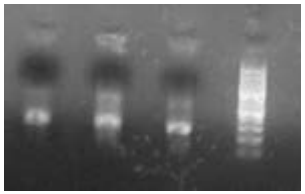
PCR of target sequences (exon 10) using 5` biotinylated primers

Hybridization with specific LNA modified capture probes conjugated via carbodiimide coupling to carboxylated beads

Washing

Data acquisition

Data analysis

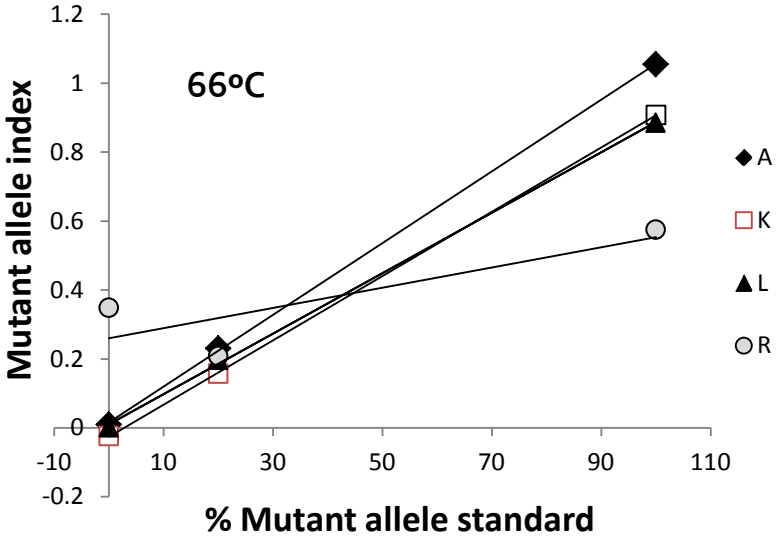


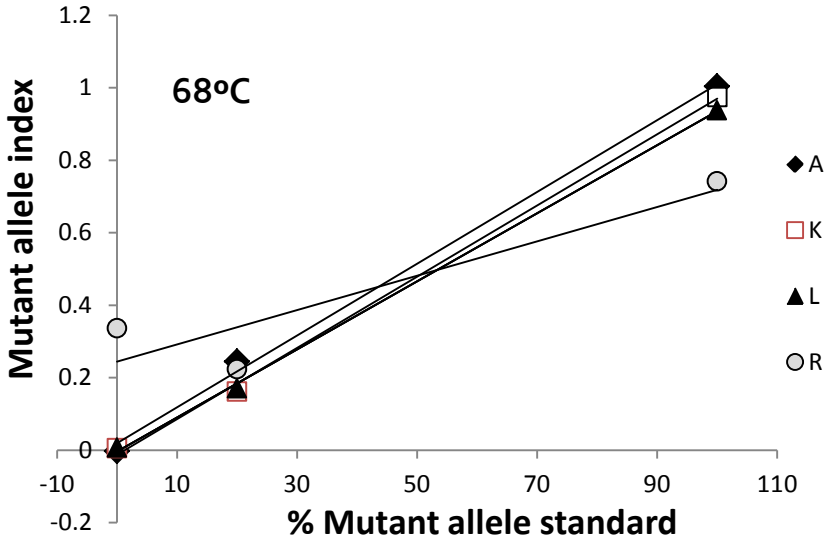
MPL wt:	5′-GAAACTGCCACCTCAGCA
W515A	5′-GAAACTGCGCCCTCAGCA
W515K	5′-GAAACTGCTTCCTCAGCAG
W515L	5′-GAAACTGCAACCTCAGCAG
W515R	5′-GAAACTGCCTCCTCAGCAG

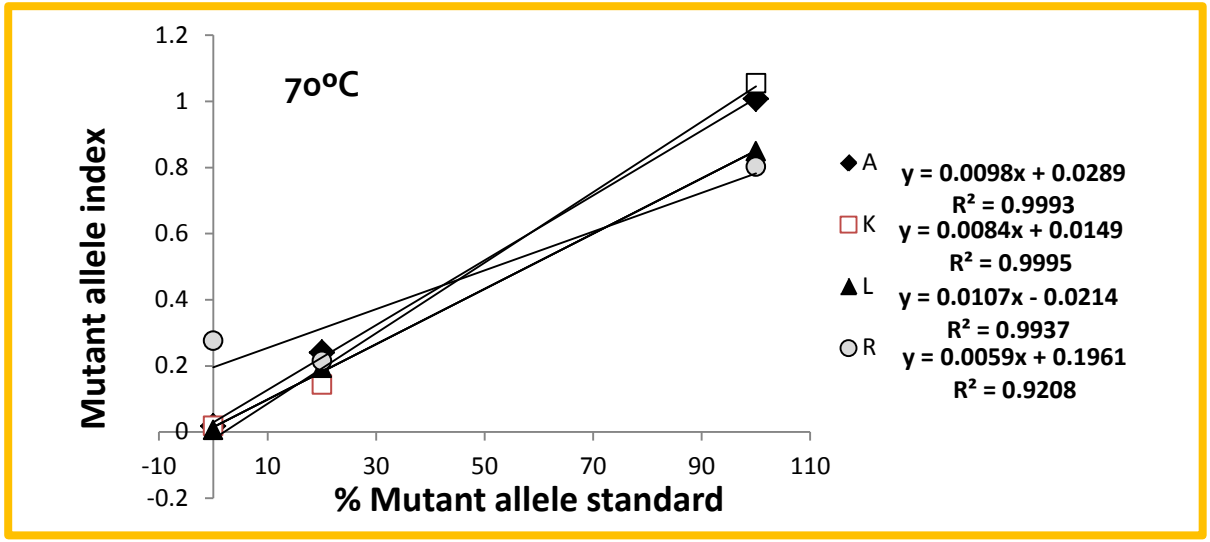
Background correction of MFI
Calculation of mutant/wild type allele index
Index (mutant allele) = [MFI (mutant allele)/[MFI (mut allele 1) +…+ MFI (mut allele 4) + MFI (wt allele)]

Ivanova et al. Leukemia Research (2011)

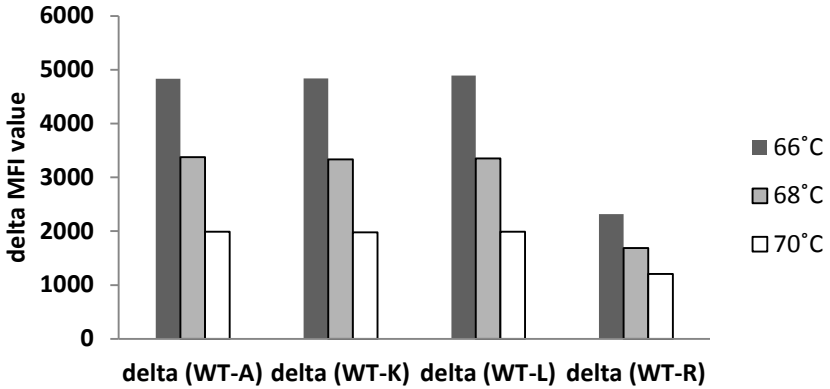
SELECTION OF THE OPTIMAL HYBRIDIZATION TEMPERATURE BASED ON STANDARD CURVES

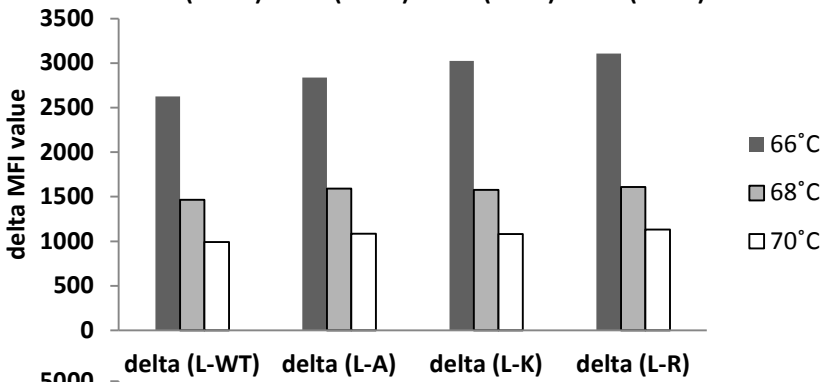


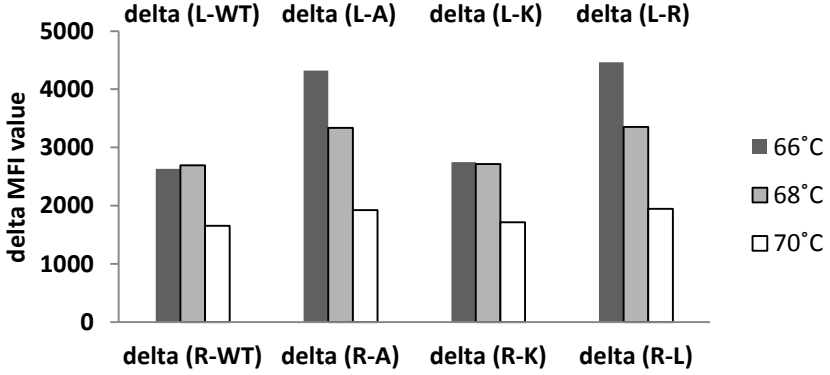


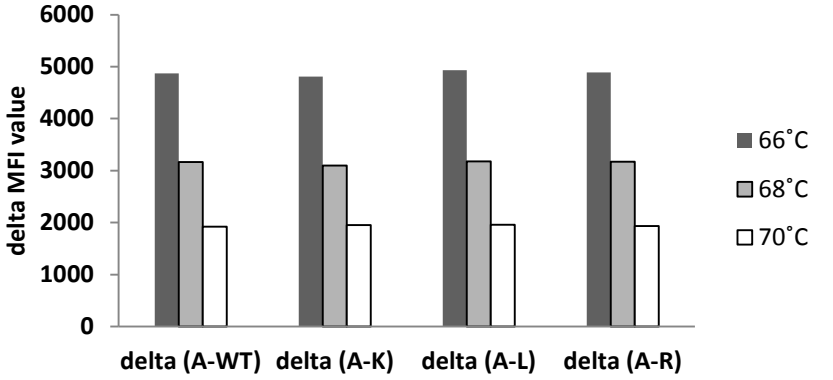


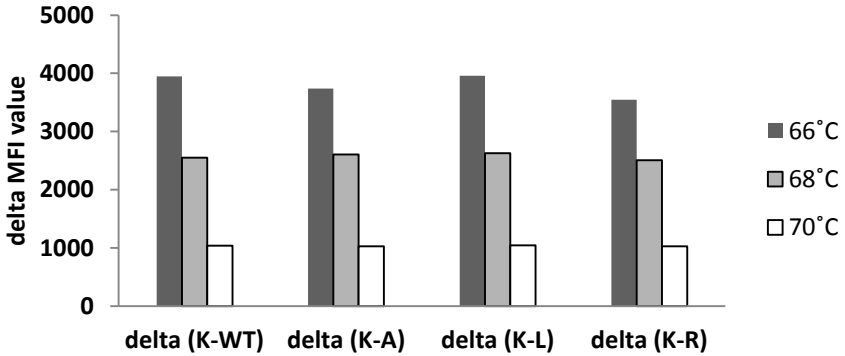
DETERMINATION OF THE OPTIMAL HYBRIDIZATION TEMPERATURE











Delta MFI values were above 1000 for temperatures tested

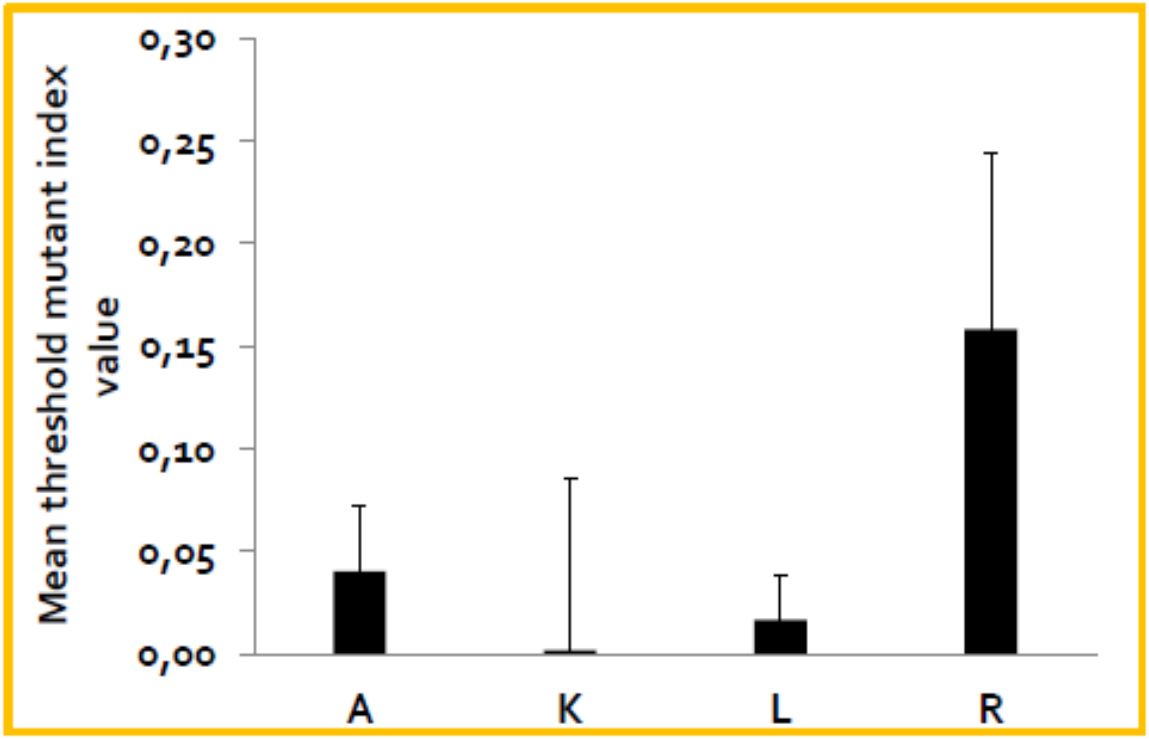
PROCEDURE FOR DETERMINATION OF THE POSITIVE CUT-OFF VALUES

100%
WT
W515A
W515L
W515K
W515R

3 repeats with all probes mix

Calculation of the mean of the maximum values for each mutant in the samples where it was not present

Cut-off value defined Mean + SD



ALL *MPL* MUTATIONS ARE DETECTED AT 1% SENSITIVITY

%	W515A	W515K	W515L	W515R
0	0.005	0.011	0.008	0.151
1	**0.088**	**0.100**	**0.094**	**0.203**
2.5	**0.145**	**0.200**	**0.160**	**0.247**
5	**0.300**	**0.240**	**0.163**	**0.264**
12.5	**0.334**	**0.444**	**0.200**	**0.373**
25	**0.488**	**0.545**	**0.366**	**0.429**

THE MISSION IS POSSIBLE!

Luminex based detection of JAK2 V617F and MPL W515 mutations:

- ✓ Diagnoses the vast majority of Ph'(-) MPN cases
- ✓ It's easy, rapid (4-5h) and reproducible
- ✓ It's sensitive (1%) and quantitative
- ✓ Allows mid-throughput sample processing
- ✓ It's open for further multiplex development
- ✓ It's affordable (Ask the local dealers for the reagents)

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