

The Jak2 V617F mutation

Methodological approaches

Myeloproliferative Syndromes

- ▶ MPD are clonal hematological diseases
 - ▶ The major diseases included in this group are:
 1. CML (Philadelphia chromosome; bcr-abl FG)
 2. Vaquez disease
 3. Essential thrombocytaemia
 4. Idiopathic Myelofibrosis
- } ?

Physiopathology

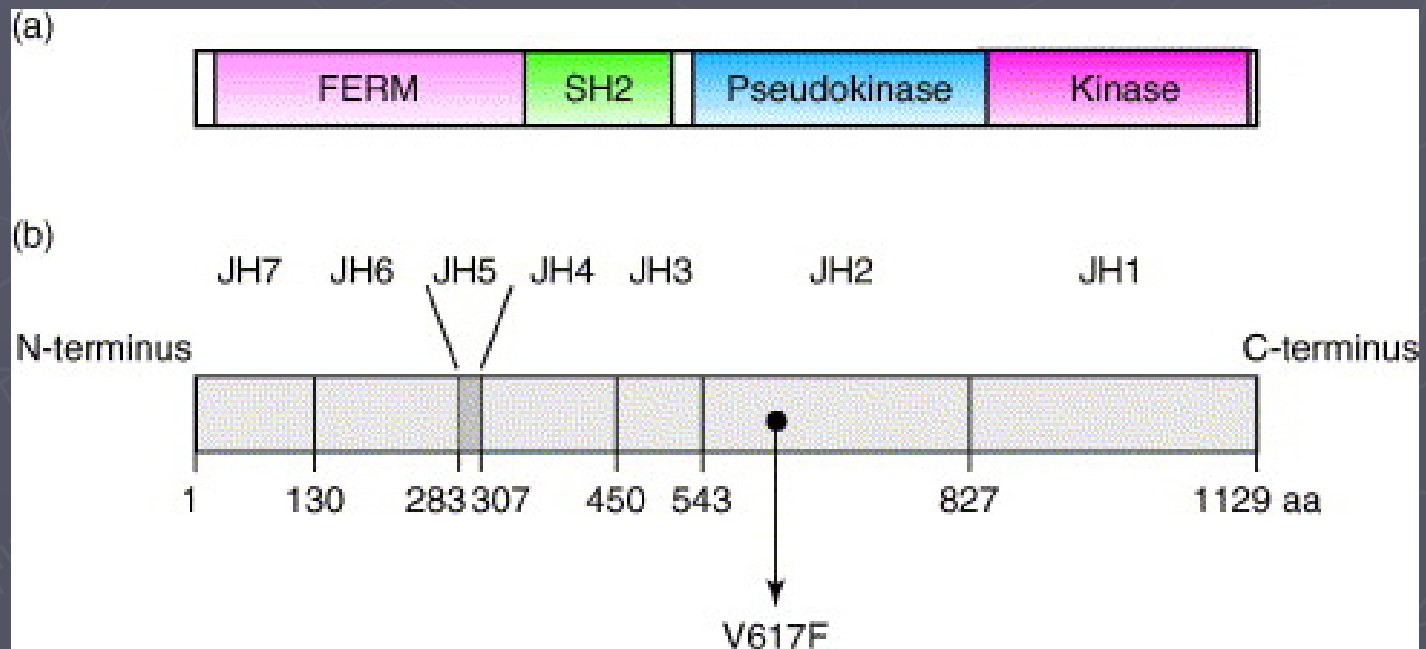
- ▶ A fraction of the progenitors among the Vaquez patients are EPO independent (or hypersensitive to) .
- ▶ Among the ET and IMF patients, megacaryocytic progenitors are hypersensitive to thrombopoietin.

2005: The discovery

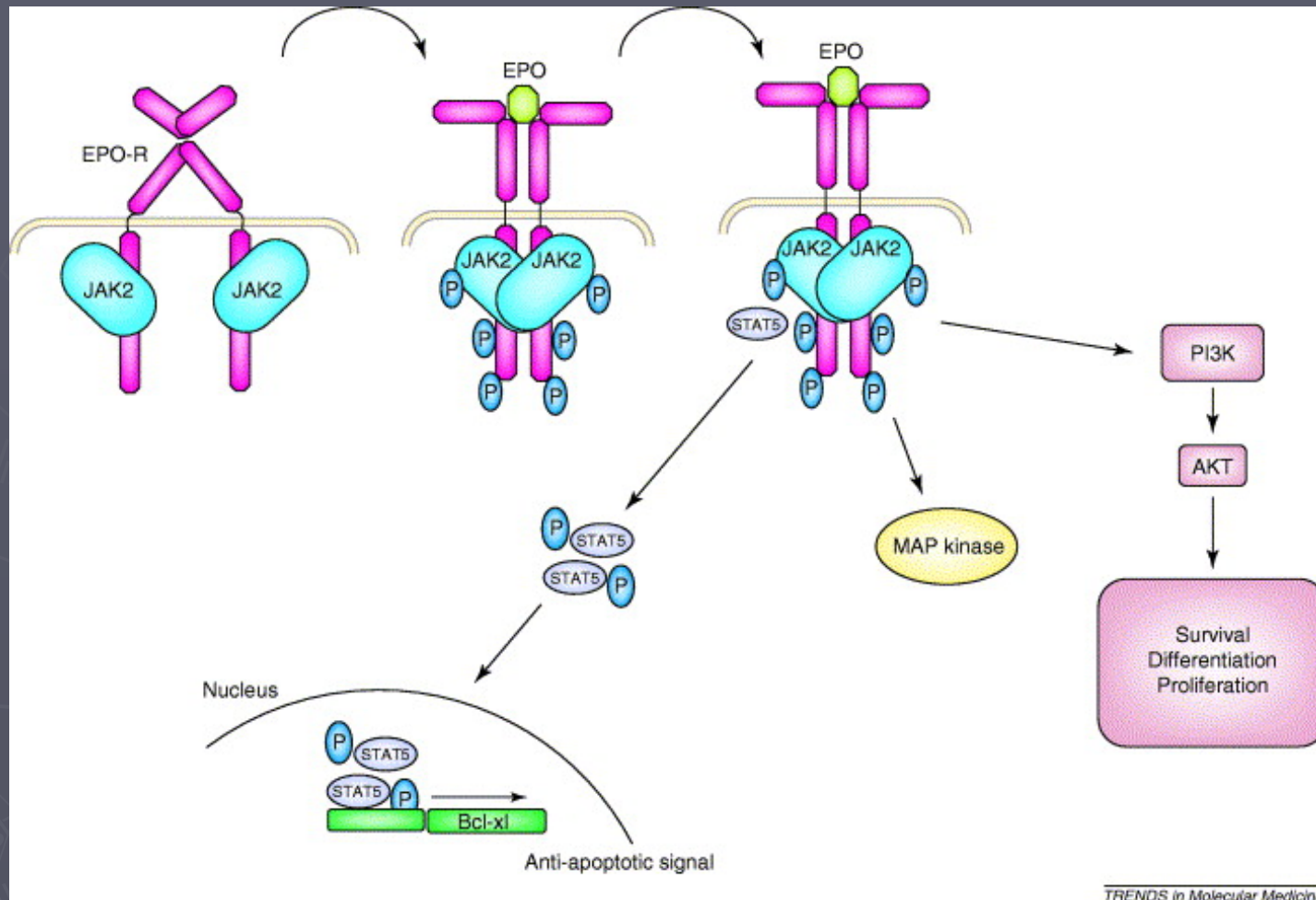
- ▶ 5 teams simultaneously discover a recurrent mutation in the JAK2 gene :

V617F

- ▶ Jak2: cytoplasmic tyrosine kinase playing a role in signal transduction mediated by hematopoietic growth factor receptors

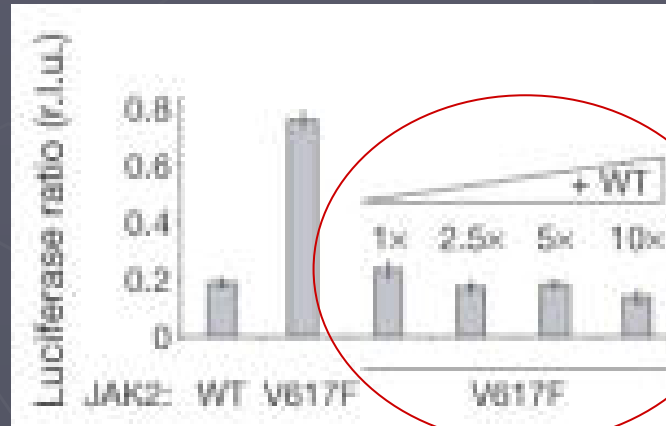


Transduction pathway



Molecular physiopathology of the mutation

Transcriptional activity of STAT-5



Dominant negative effect of the WT gene

Murine Model

Blood, 1 June 2006, Vol. 107, No. 11, pp. 4274-4281.

Prepublished online as a Blood First Edition Paper on February 14, 2006; DOI 10.1182/blood-2005-12-4824.

HEMATOPOIESIS

Expression of Jak2V617F causes a polycythemia vera–like disease with associated myelofibrosis in a murine bone marrow transplant model

Gerlinde Wernig, Thomas Mercher, Rachel Okabe, Ross L. Levine, Benjamin H. Lee, and D. Gary Gilliland

From the Division of Hematology, Department of Medicine, the Howard Hughes Medical Institute, and the Department of Pathology, Brigham and Women's Hospital, Boston; and the Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA.

- Differences observed between different strains of mice
- Could explain in human the pleiotropy of Jak2V617F-associated myeloproliferative disease

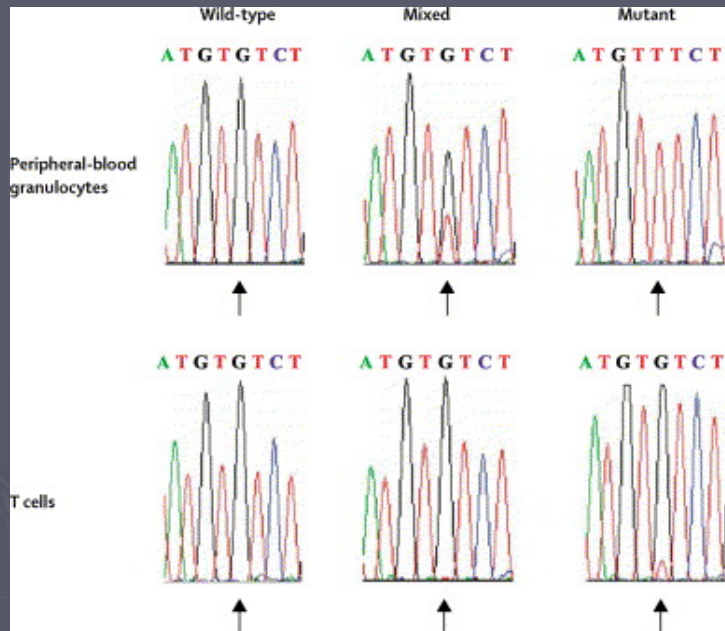
Disease	Frequency	%	Refs
Polycythemia vera	71/73	97	[11]
	40/45	89	[8]
	25/29	86	[60]
	58/72	81	[59]
	83/128	65	[9]
	121/164	74	[10]
	20/24	83	[12]
Essential thrombocythemia	29/51	23	[11]
	9/21	43	[8]
	3/10	30	[60]
	24/59	41	[59]
	21/93	23	[9]
	37/115	32	[10]
Idiopathic myelofibrosis	8/16	50	[11]
	3/7	50	[8]
	18/19	95	[60]
	15/35	43	[59]
	13/23	57	[9]
	16/46	35	[10]
Unclassified chronic myeloproliferative disorders	3/16	19	[60]
	30/152	20	[59]
Chronic myelomonocytic leukemia	7/52	13	[60]
	3/119	2	[58]
Myelodysplastic syndromes	1/68	1	[60]
	2/48	4	[56]
	5/101	5	[58]
Systemic mastocytosis	0/28	0	[59]
	2/8	20	[58]
Chronic neutrophilic leukemia	1/6	17	[58]
Hypereosinophilic syndromes	2/134	1	[59]
Chronic myeloid leukemia	0/99	0	[60]
	0/18	0	[59]
Acute myeloid leukemia (except megakaryocytic leukemia)	0/28	0	[60]
	0/17	0	[59]
Megakaryocytic leukemia (acute myeloid leukemia M7)	2/11	18	[60]
Acute myeloid leukemia and a history of chronic myeloproliferative disorders	12/22	55	[60]
B-lineage acute lymphoblastic leukemia	0/83	0	[56]
T-cell acute lymphoblastic leukemia	0/93	0	[56]
Chronic lymphocytic leukemia	0/45	0	[56]

Mutation frequency in human MPD

How to explain the differences in the frequency of the mutation

- ▶ Patient classification (WHO vs PVSG)
- ▶ Sensitivity of the different technical assays used

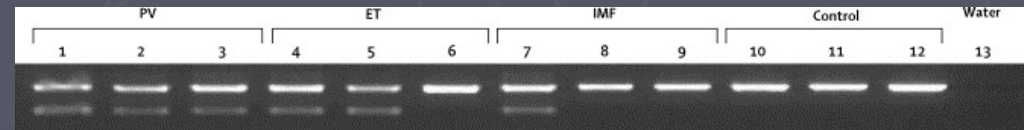
Methodological approaches (I): Sequencing and ASO PCR



Sensitivity: about 20%

Somatic mutation

Sensitivity: 3%

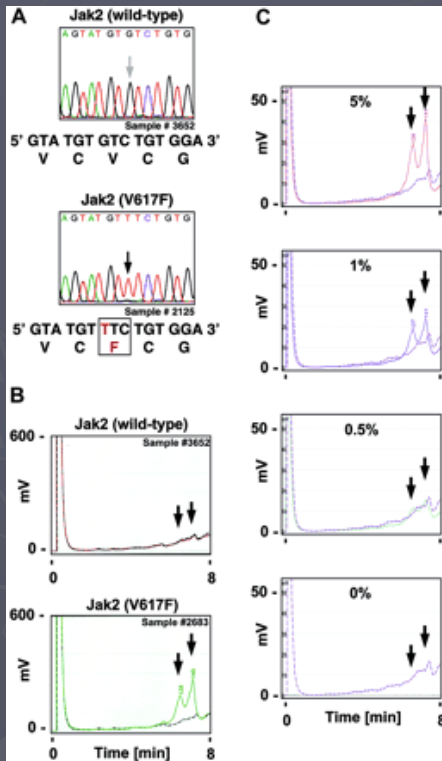


	Sequencing			Allele-specific PCR			Overall		
	Number tested	Mutant*	Mixed	Wild-type	Number tested	Mutant	Wild-type	Number	Proportion (95% CI)
Polycythaemia vera	73	19	34	20	20	18	2	71/73	97% (93-100)
Essential thrombocythemia	50	0	0	45	45	23	22	23/51	57% (43-70)
Idiopathic myelofibrosis	16	3	4	9	9	1	8	8/16	50% (26-74)
Controls	90	0	0	90	90	0	90	0/90	0%

Methodological approaches (II):

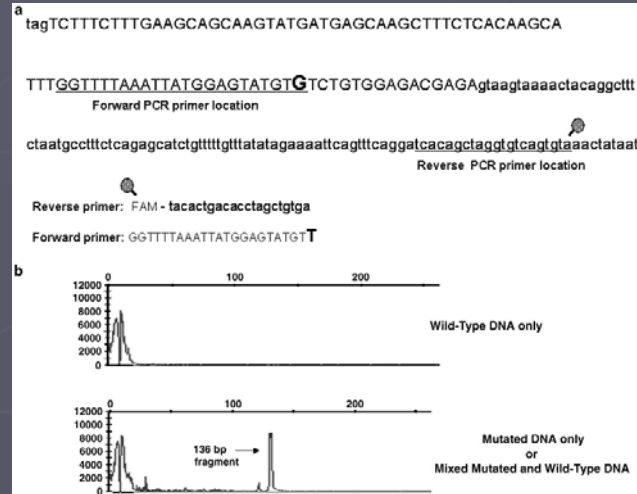
Multistep process

DHPLC



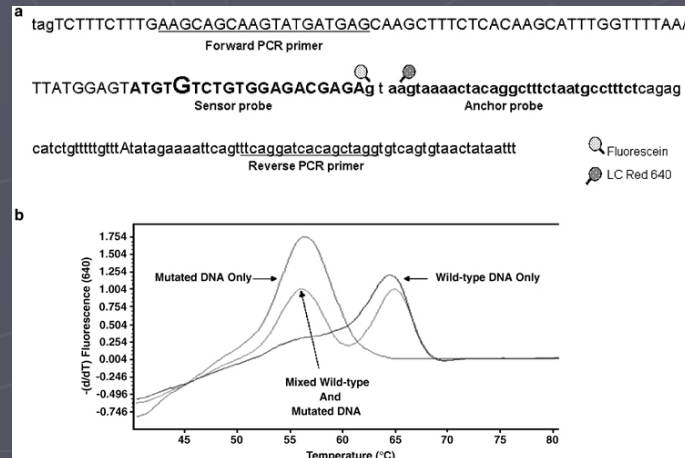
Sensitivity: about 1%

Sattler et al Blood 2006



Sensitivity: 0.1 to 0.01%

Fluorescent ASO PCR
(no internal control!)

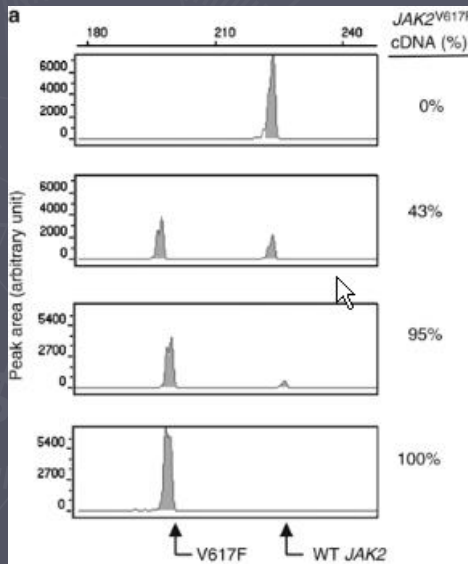
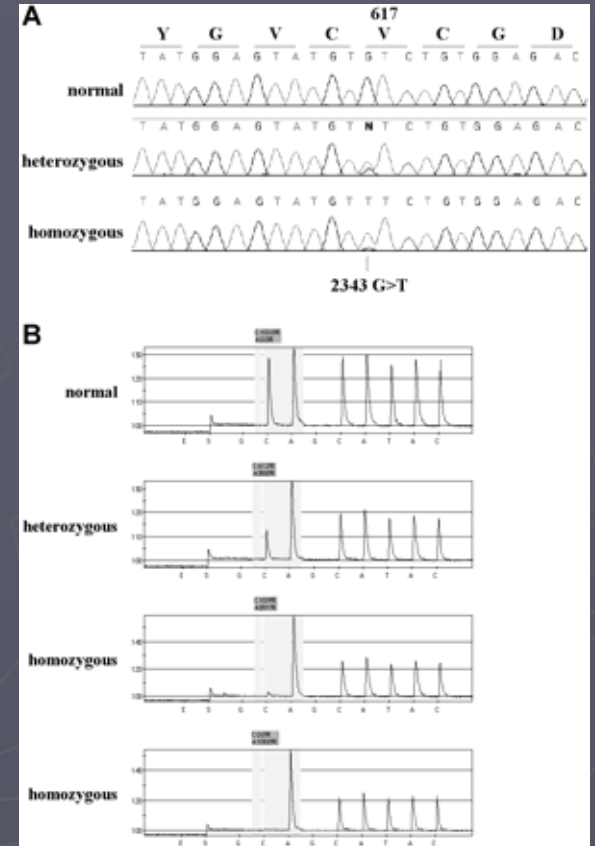
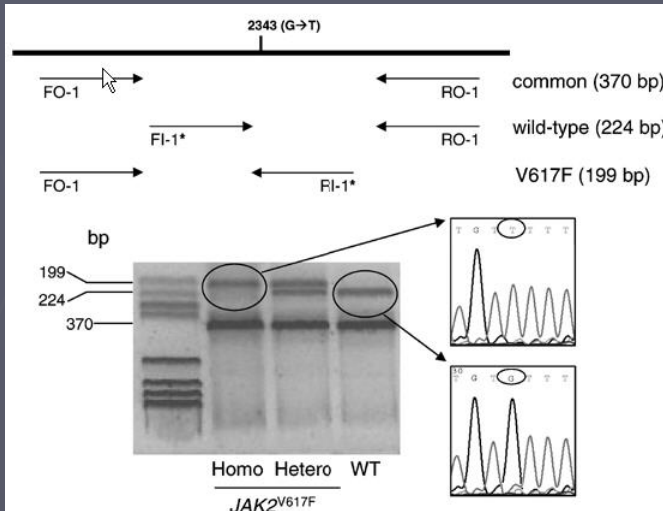


Sensitivity: 1-10 %

Melting curve

McClure et al ; Leukemia 2006

Methodological approaches (III):



Quantitative ARMS-PCR

Sensitivity: about 1%

Vannucchi et al Leukemia 2006

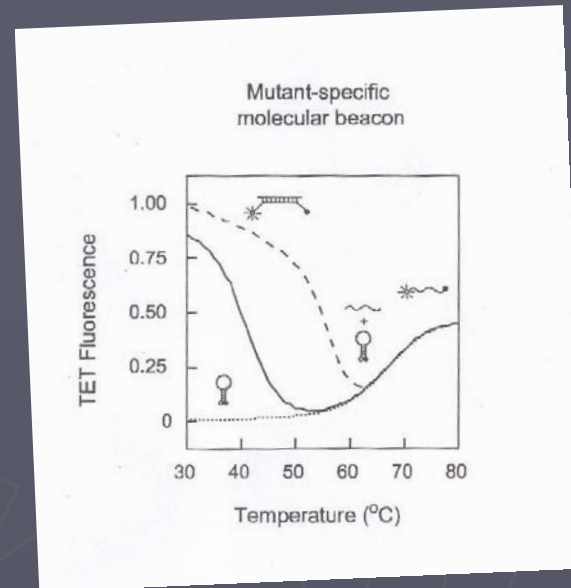
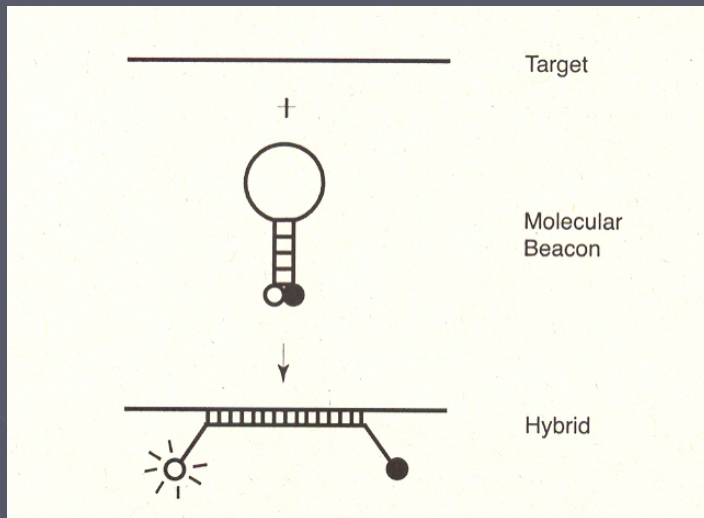
Sensitivity: about 5%

Jones A et al Blood 2005



Our « home made »
methodological approach

Molecular Beacon probe

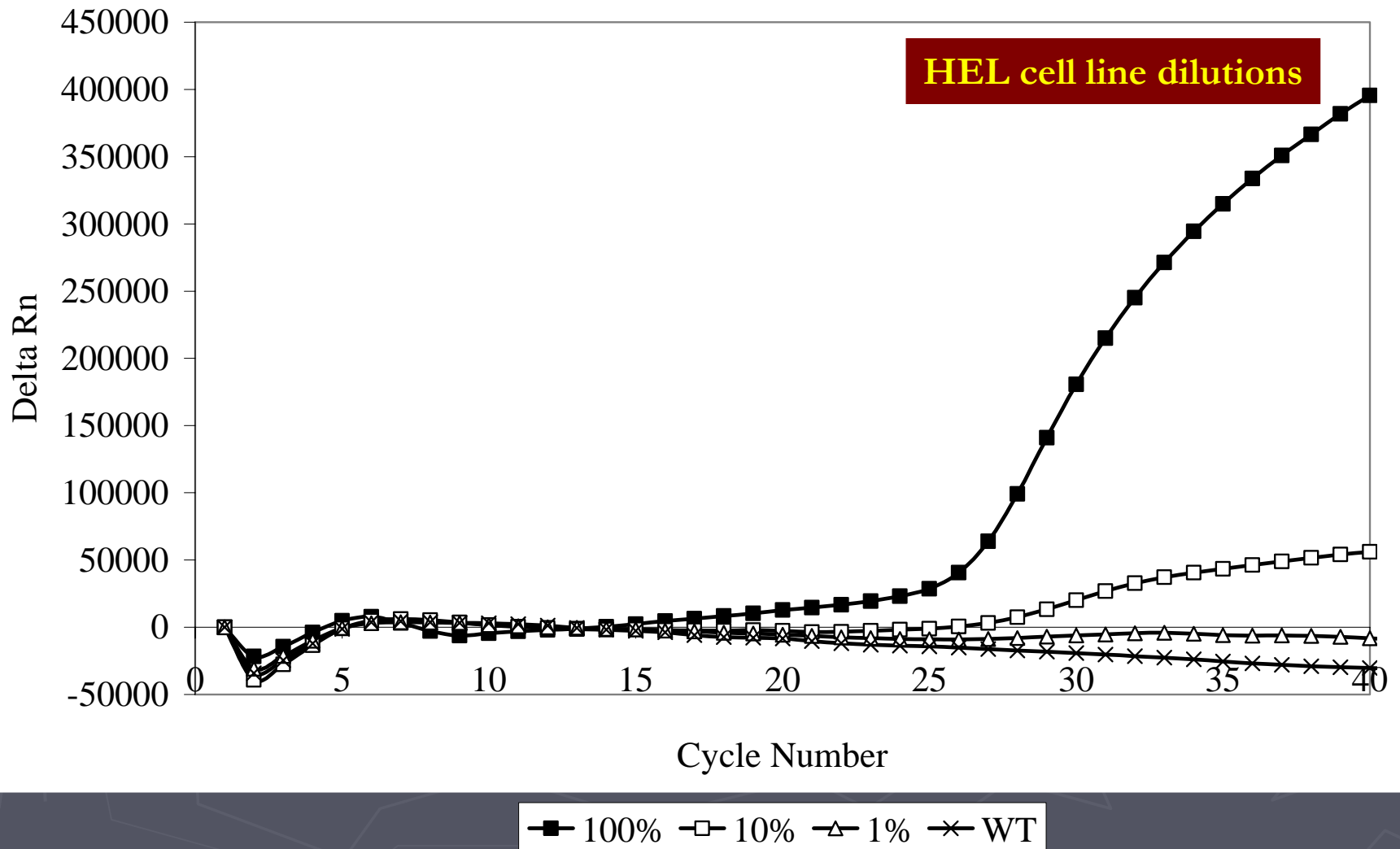


- at the annealing temperature, hybridization of the probe on the target
- the « free » probe stays in closed configuration (« no » fluorescence »)

Advantages :

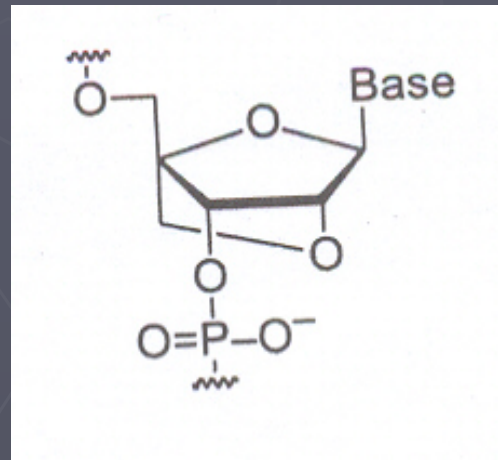
- Low background
- Very good discrimination between alleles differing by a single base

Results obtained with a Molecular Beacon Probe alone.



LNA (Locked Nucleic Acid)

Ribonucleotide modified with a 2' O - 4' C Methylene bridge

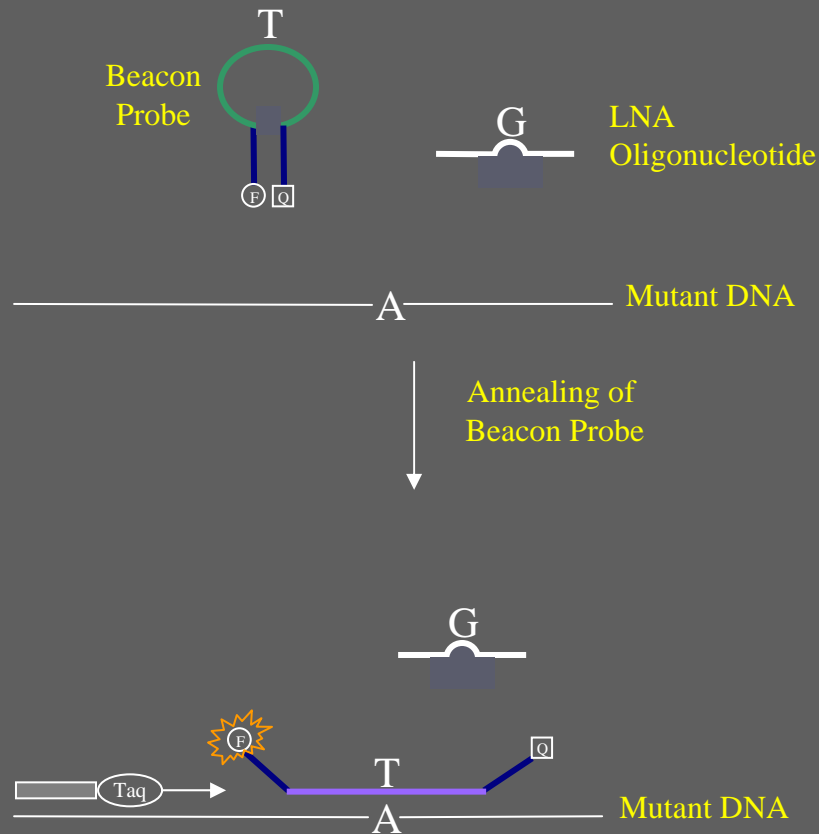


Conformation C3' -endo

- High melting temperature
- A single bp difference : T_m lowering of many degrees = **HIGHLY** specific

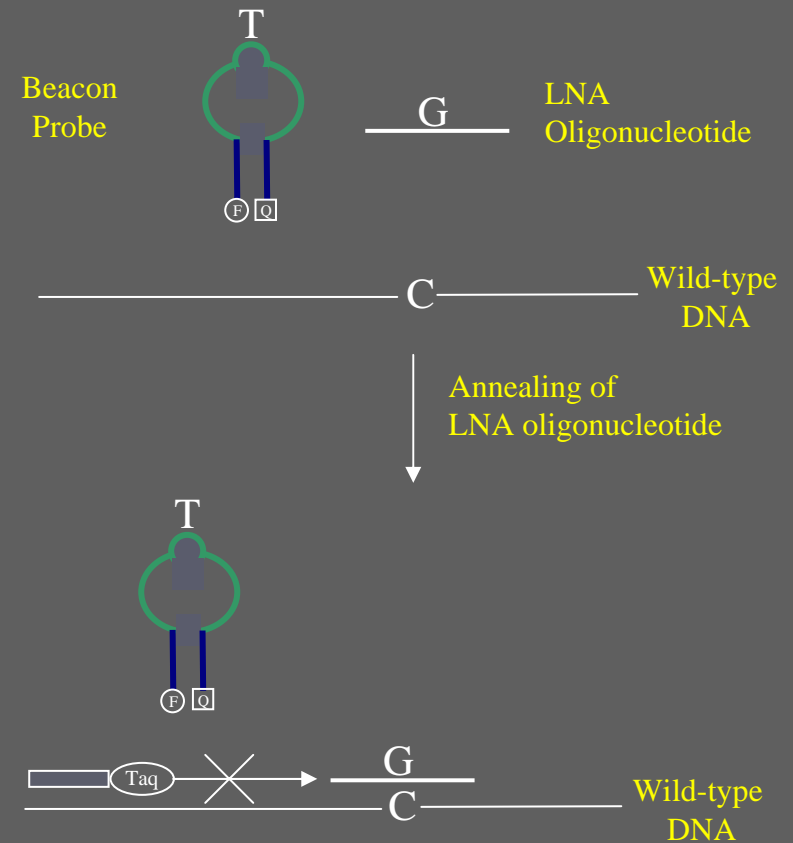
Principle of the approach

A. Mutant DNA



Amplification AND Signal detected

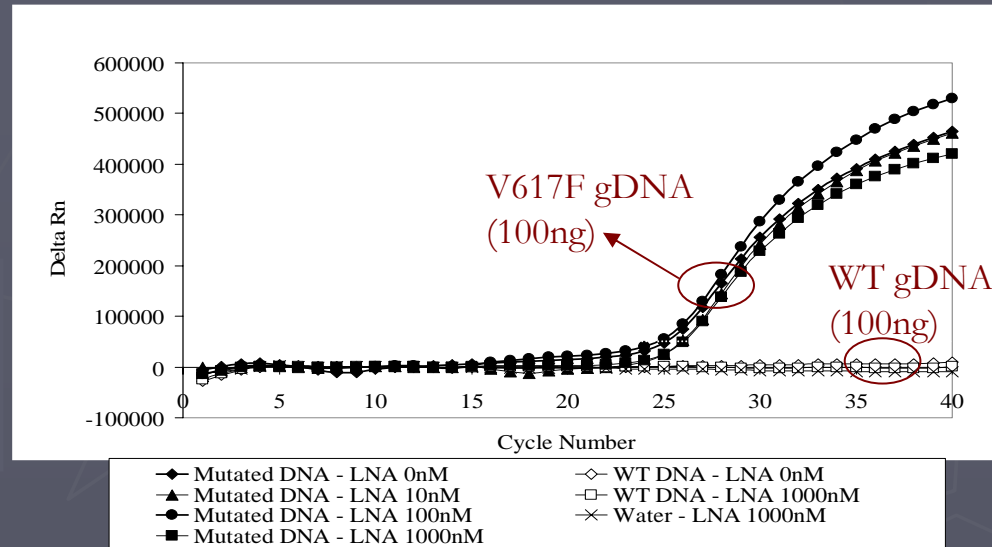
B. Wild-type DNA



Weak Amplification without any signal detected

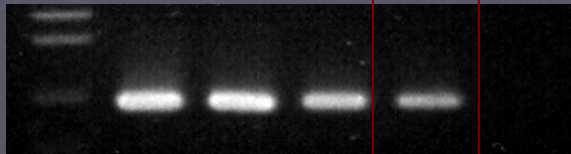
Results obtained

SPECIFICITY OF THE BEACON

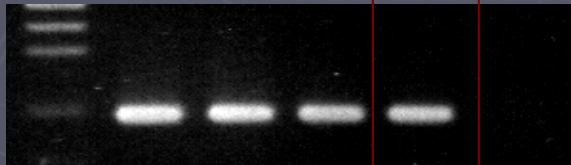


LNA concentration (nM) 0 10 100 1000 H₂O

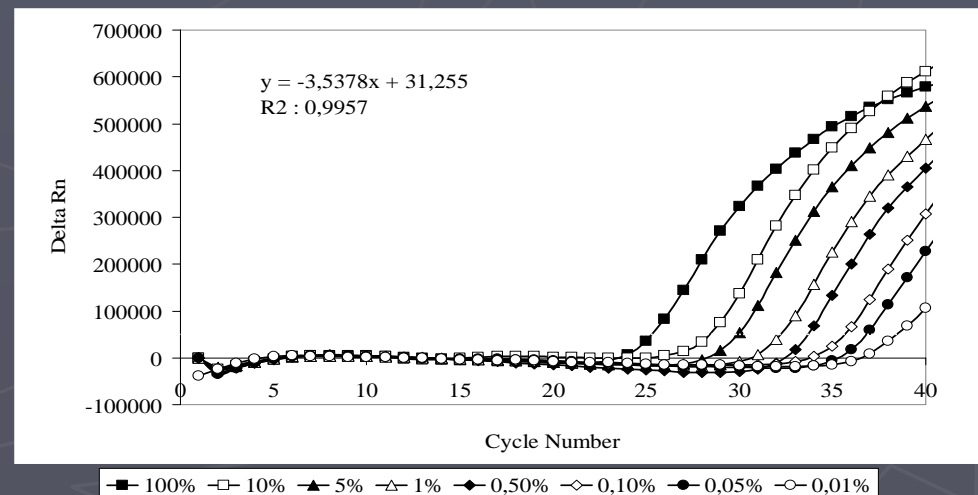
WT DNA



Mutated DNA



SPECIFICITY OF THE LNA



Detection limit based on HEL cell line dilutions

0.01% (for 100ng gDNA)

1 diploid cell \sim > \sim 6pg of DNA

In 100ng of DNA \sim > 17.000 genomes \sim > 34.000 alleles.

A detection limit of 0.01 % means about **4 copies**.

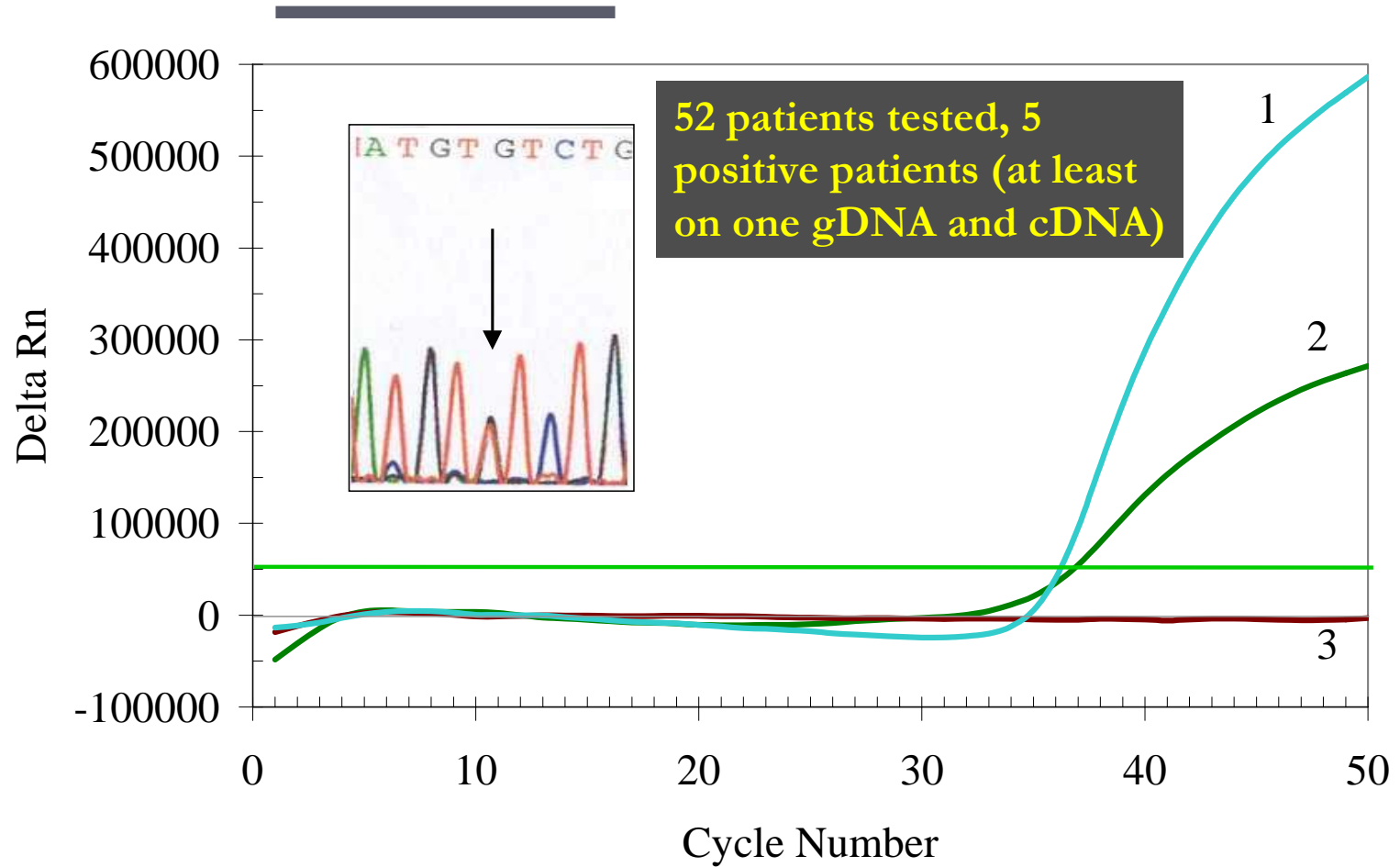
Validation

- ▶ 54 patients tested by our method compared to the published ARMS-PCR approach.
- ▶ 100% concordance
- ▶ For 2 cases, ARMS-PCR results were borderline but frankly positive by our approach

Conclusions

- ▶ 3 components to add in a tube (aliquoted premix of primers, LNA and Beacon probe; Master Mix and sample)
- ▶ Closed tube approach
- ▶ 40 minutes (Fast PCR)
- ▶ Probe highly specific for the mutant allele
- ▶ Quantitative
- ▶ Work on DNA and cDNA

Healthy patients



Controversial hypothesis

- ▶ « Acquisition of the V617F mutation of Jak2 is a late genetic event in a subset of patients with myeloproliferative disorders » Kralovics et al. 2006
- ▶ « The Jak2 V617F mutation occurs in hematopoietic stem cells in PV and predisposes toward erythroid differentiation » Jamieson et al 2006

 Is it a primary or secondary event?

To be continued...