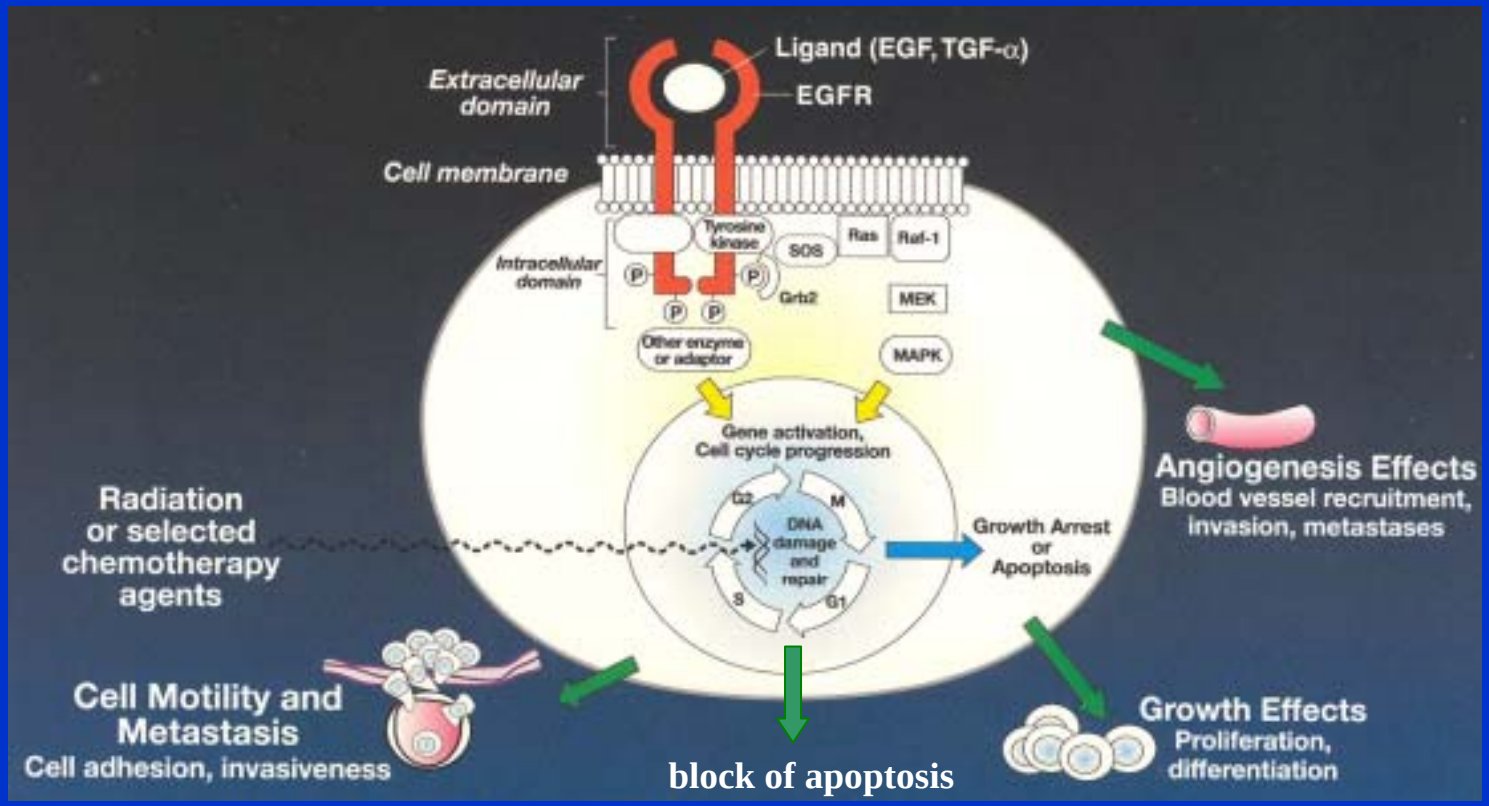
Activating mutations in Receptors tyrosine kinase (RTK)

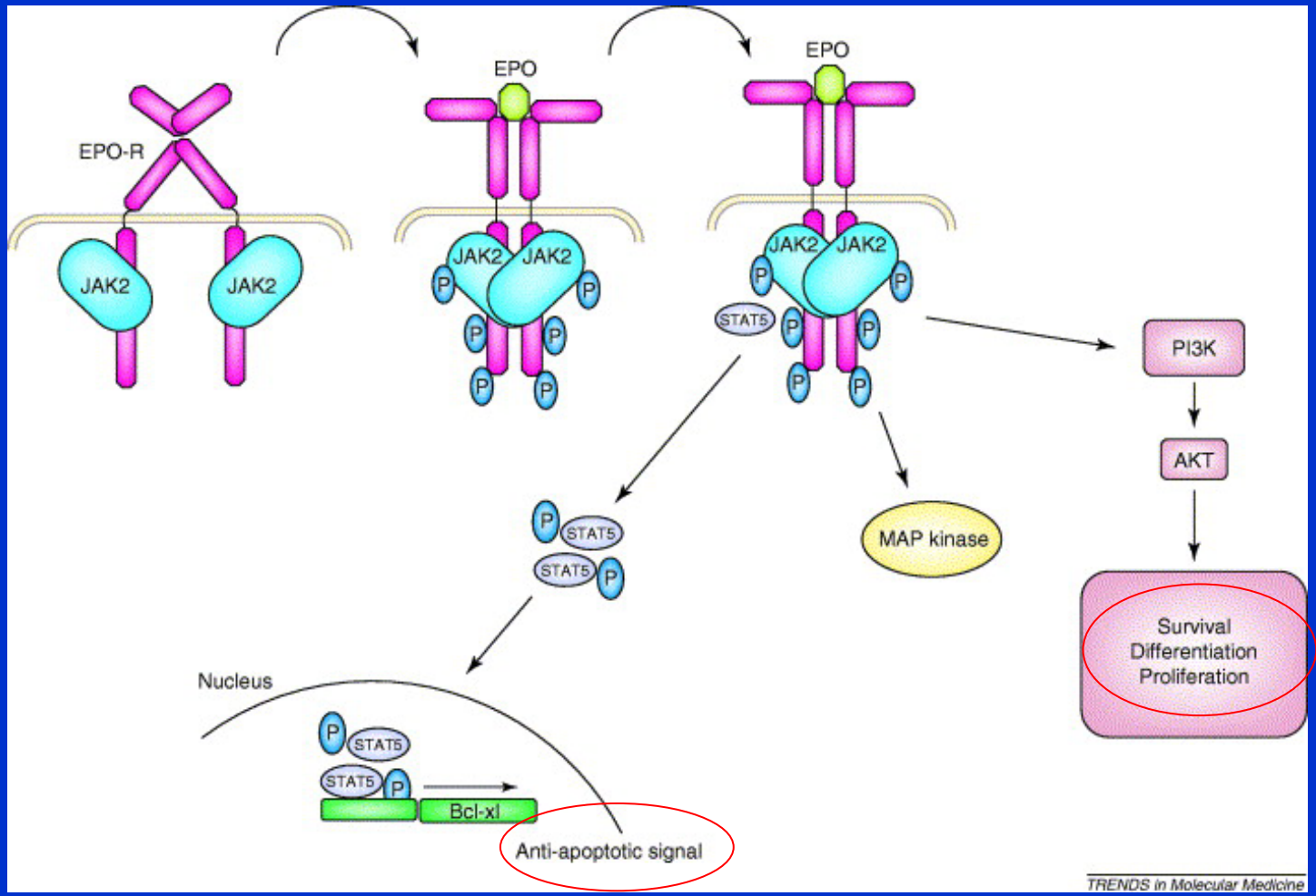
examples of JAK2 in MPD and EGFR in Lung Cancers

Receptors tyrosine kinases
how does it work?



RTK serve as mediators of cell signalling by extra-cellular growth factors

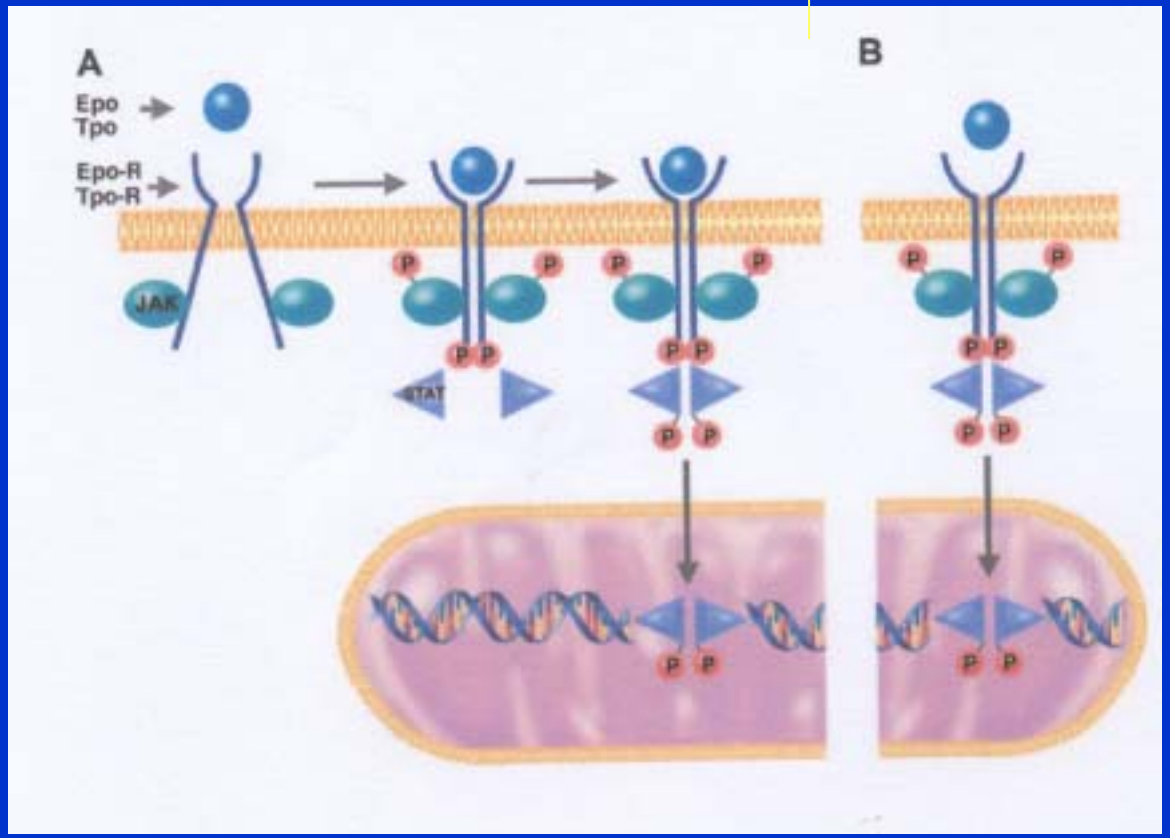
signalling pathway activation in normal cell: example of JAK2



James et al TIMM 2005

Signalling pathway

A. normal condition | **B. tumoral condition**



Schafer Blood 2006

***JAK2* V617F mutation as a diagnostic tool in MPD**

primary disease *vs* secondary lineage hyperplasia

***EGFR* mutation as molecular target for rationale and specific treatments**

high sensitivity to EGFR TKIs treatment (Gefitinib ou Erlotinib)

*JAK2*V617F mutation in myeloproliferative disorders

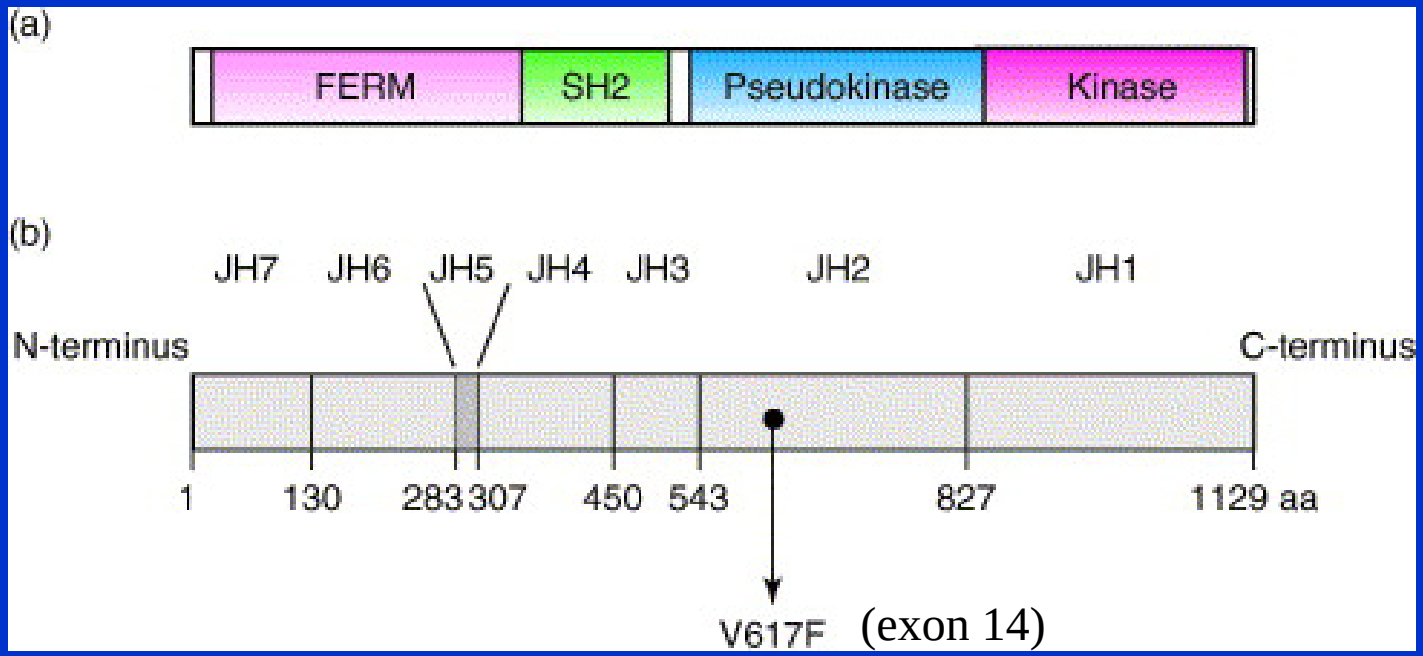
Myeloproliferative disorders

molecular signatures

CML	*BCR/ABL*
PV, ET, Myelofibrosis	*JAK2V617F*
Syst mastocytosis	*c-kit*
CEL	*FIP1L1-PDGFRA*

Jak2 gene « Just another kinase » ou « Janus kinase » !

- JAK2 tyrosine kinase receptor: key role in the signalling pathway leading to cell growth. Activity mediated by growth factors (ligands) such as TPO or EPO
- point mutation in the auto-regulatory (pseudo-kinase) domain ⟹ **constitutive activation of JAK2 receptor**



James et al. Trends in Molecular Med 2005

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]

*JAK2*V617F mutation in MPD

97% of PV

(I^{ary} *vs* II^{ary} polyglobuly)

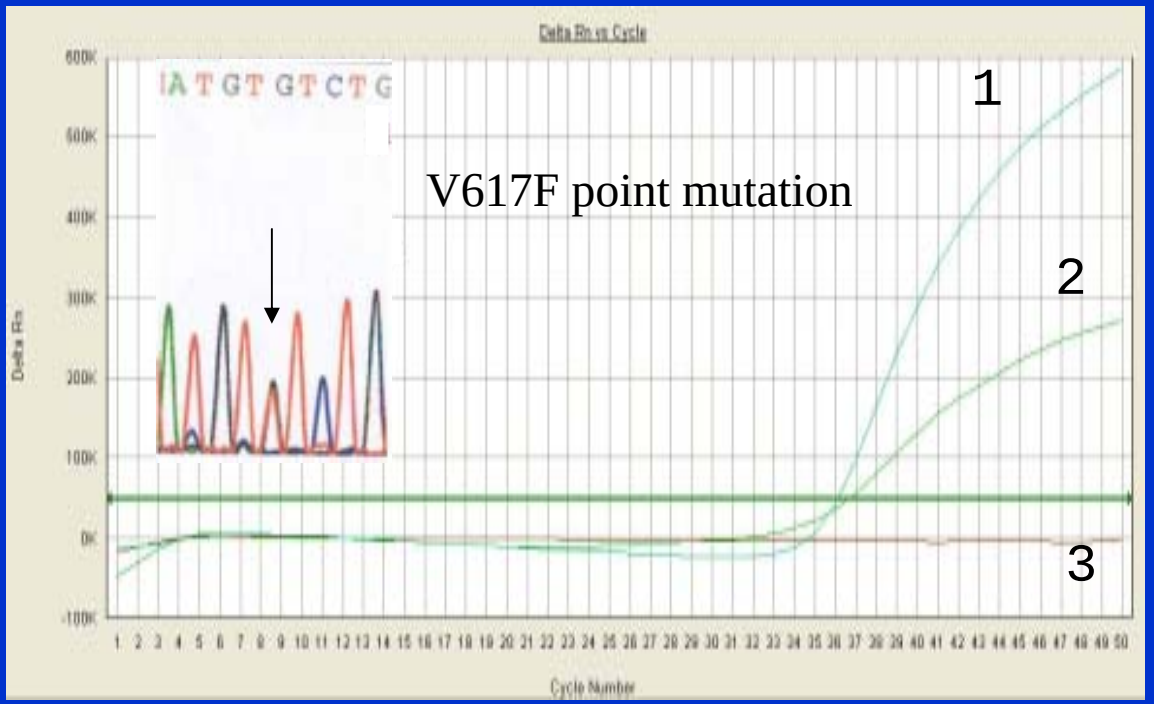
± 40% of ET

(I^{ary} *vs* II^{ary} thrombocytosis)

± 50% IM

NB: positive in some MDS
always negative in CML

*JAK2*V617F Mutation Illustration



*JAK2*V617F mutation and prognosis

- **PV**

  no difference in terms of prognosis between *JAK2*V617F mutation negative and positive cases

- **ET**

  higher risk of thrombosis in *JAK2*V617F mutation positive cases (Cheung et al, BJH,2006)

  less good responders to analegrid among *JAK2*V617F mutation positive cases (Campbell et al,The Lancet, 2005)

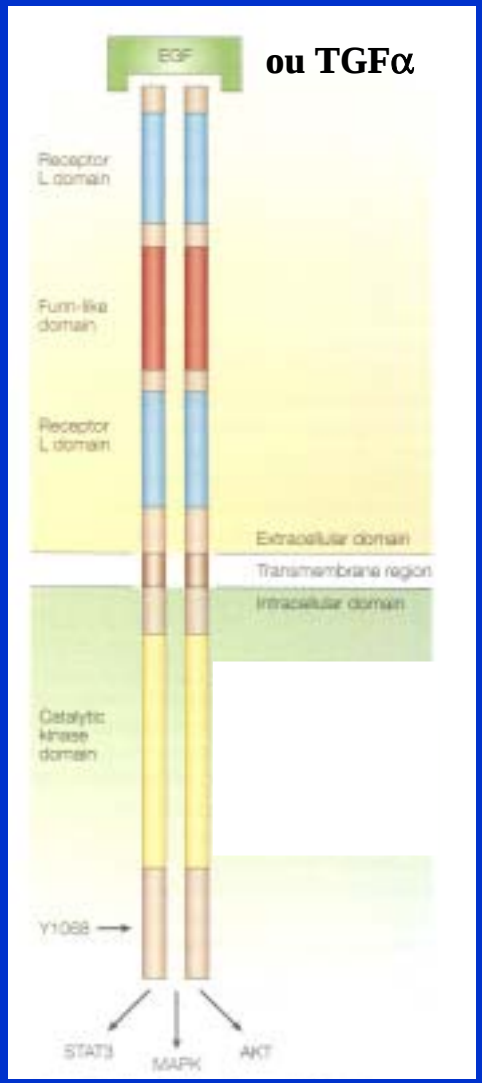
- **IM**

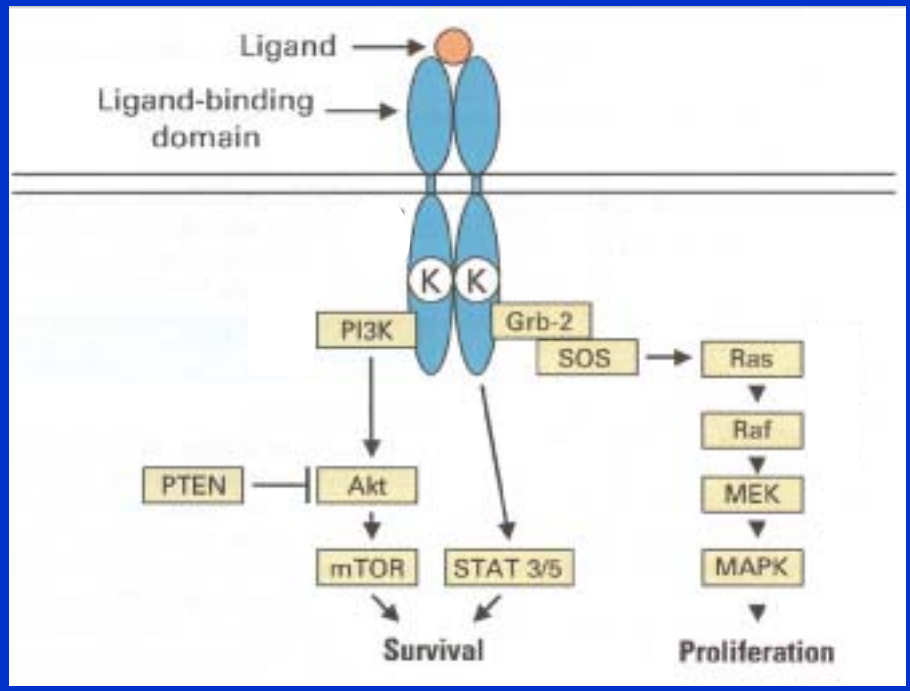
  lower overal survival among *JAK2*V617F mutation positive cases (Campbell et al,Blood, 2005)

EGFR mutation in NSCLC

NSCLC = « non small cell lung carcinoma »

EGFR


EGF
ou TGFα
Receptor L domain
Furin-like domain
Receptor L domain
Extracellular domain
Transmembrane region
Intracellular domain
Catalytic kinase domain
Y1068
STAT3
MAPK
AKT


Ligand
Ligand-binding domain
K
K
PI3K
Grb-2
SOS
Ras
Raf
MEK
MAPK
PTEN
Akt
mTOR
STAT 3/5
Survival
Proliferation

NSCLC

- USA: 170.000 new cases / year

- often advanced disease – short median survival

- high mortality

- treatment: conventional chemotherapy

EGFR

Involved in great number of epithelial cancers

overexpressed in about 50 % of « NSCLC » cases

correlated with poor prognosis

⇩

EGFR: prime candidate for targeted therapeutics

⇩

EGFR TKIs treatment (Gefinitib ou Erlonitib)

Clinical trials with EGFR TKIs treatment

(Gefinitib ou Erlonitib)

Advanced NSCLC and refractory to conventional chemotherapy

+

EGFR TKIs

⇩

tumoral regression in 10 % of patients

Good responders to EGFR TKIs

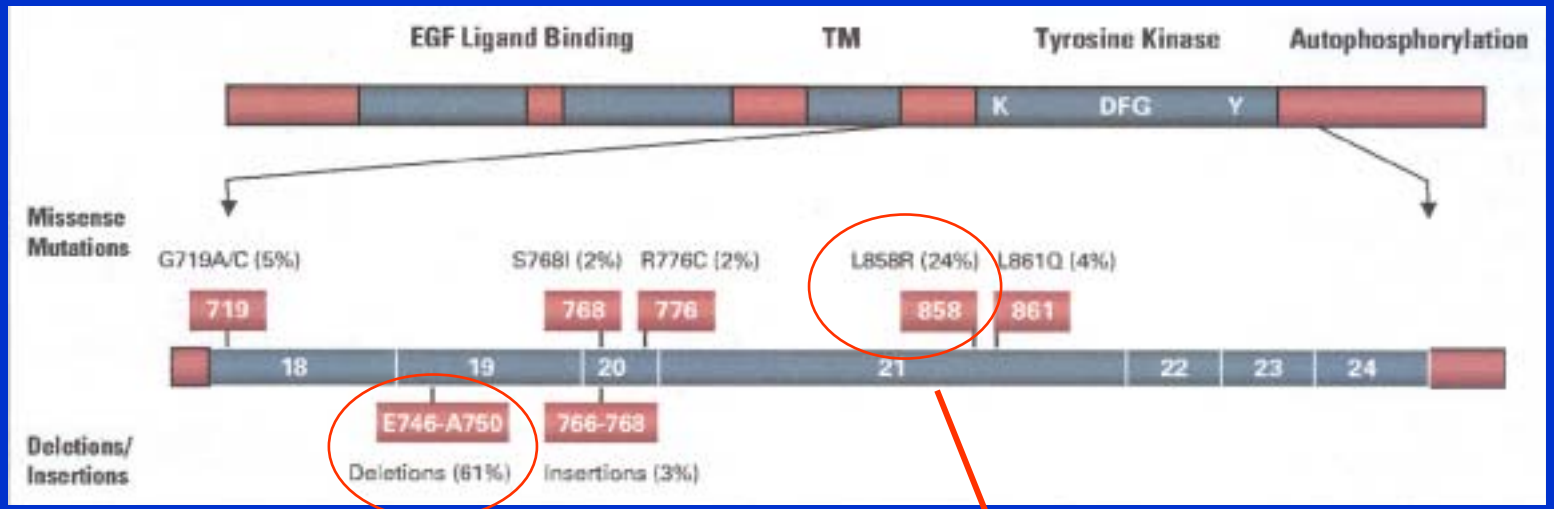
Specific subset of patients

- women
- non-smokers
- adénocarcinoma (mainly with BAC)
- East Asians

Good responders to EGFR TKIs
subjacent molecular events?

mutations within the EGFR TK domain in 82% of good responders and 0% of non-responders

exons 18 to 21



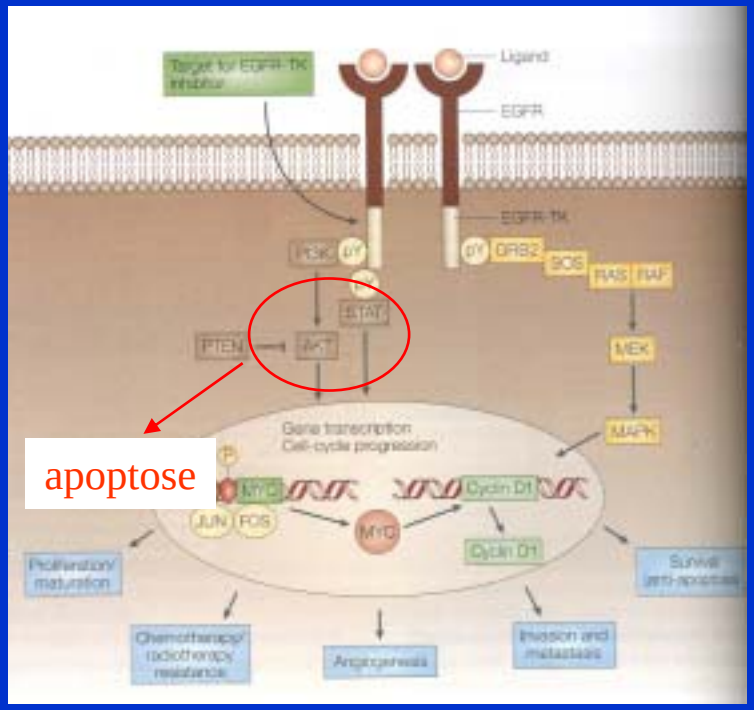
90 % of patients

Why such a sensitivity to EGFR TKIs?

« oncogene addiction »

- **wild type cell lines** —Gefinitib→ **cell cycle arrest (G1/S)**
- **EGFR+ cell lines** —Gefinitib→ **apoptosis**

« oncogenic shock »

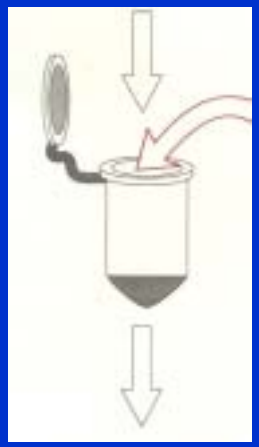


18% of good responders harbour no mutation??

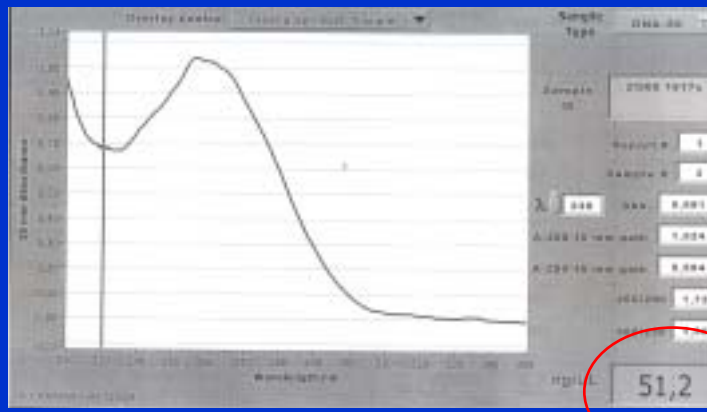
- false negative cases ?
- other molecular determinants ?
  HER2/neu ?
- other mechanisms of responses?

Methodology

« macrodissection »



DNA extraction



[ADN]
very low amount

Methodology

1. PCR amplification (4 exons)

Exons 19 and 21

Exons 18 and 20

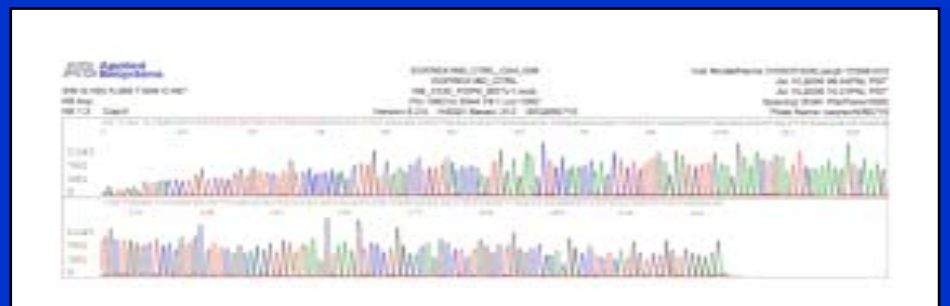
2. agarose gel:

PCR products

Bad sample

good sample

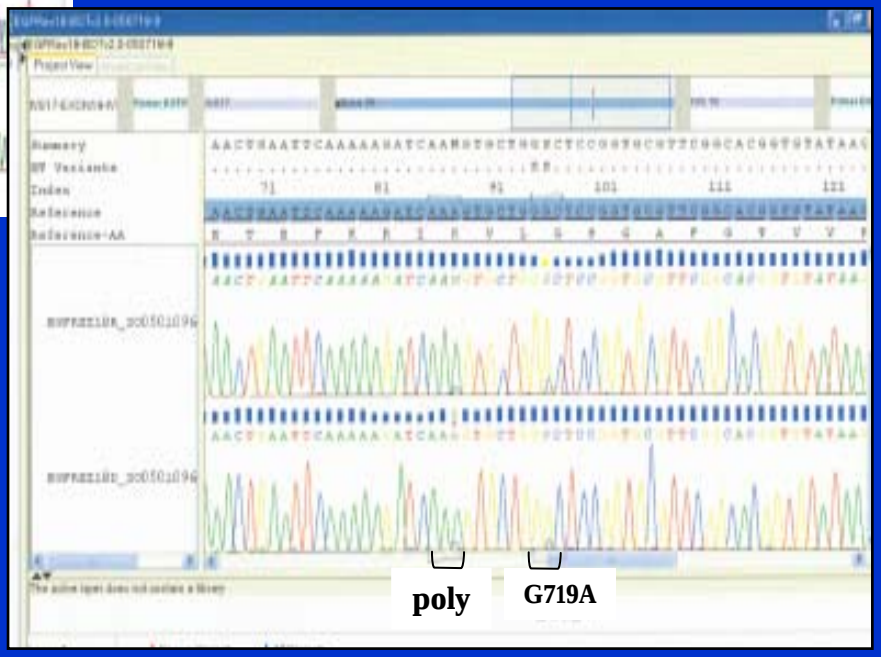
3. sequencing



Exon 18 sequence

Patient J. D.

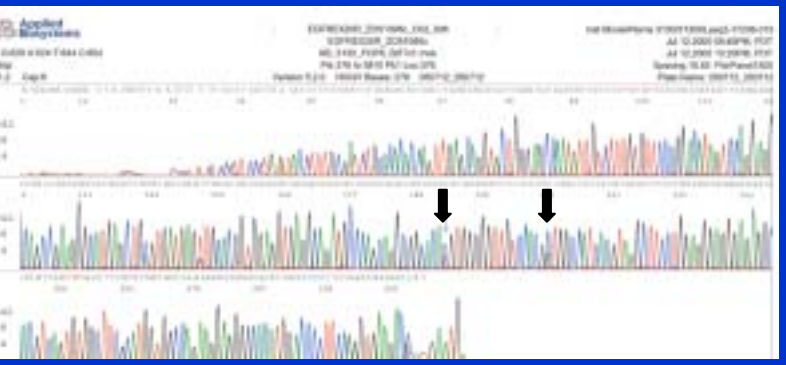
alignement program



mutation G719A in exon 18

Patient L. R.

exon 20 sequence



alignement program

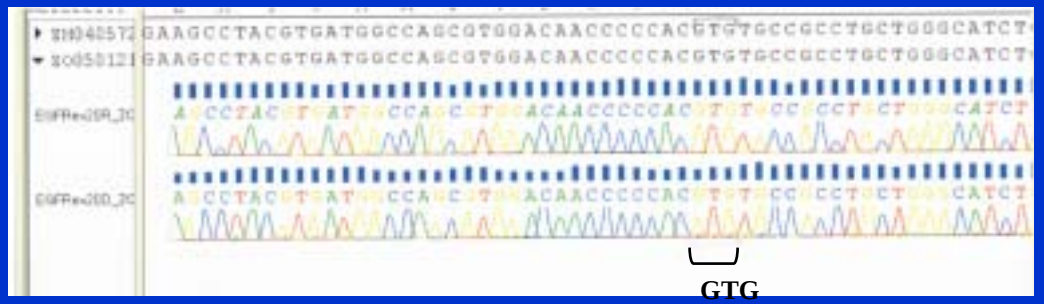
mutation S768I

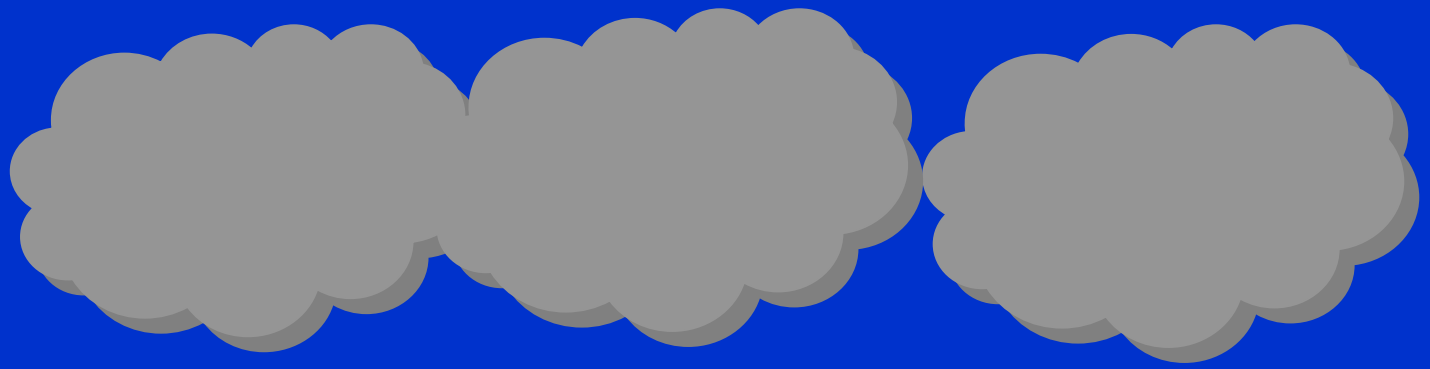
+

mutation? Polymorphism?

V774M

two mutations S768I et V774M in exon 20?





Acquired resistance in initially good responders

- mutation T790M in the catalytic domain of the kinase

cf mutation Bcr-Abl,c-kit…

- weakens the interaction of the inhibitor with its target

but … resistance can be now overcome *in vitro*

(CL-387787)