

# Establishment of clinical recommendations for detection of mutations

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EUTOS for CML



*European Treatment and Outcome Study*

**What do we know about  
mutations in 2009?**

# Abl KD mutations are only one of many possible resistance mechanisms

## ■ IMATINIB BIOAVAILABILITY

- patient compliance
- interaction with other medications
- metabolism
  - Cytochrome P450 family
- influx
  - decreased hOCT-1 expression levels or activity
- efflux
  - increased ABCB1/ABCG2 expression levels or activity

## ■ INTERACTION WITH TARGET

- BCR-ABL gene amplification
- BCR-ABL kinase domain mutations

## ■ ACTIVATION OF ADDITIONAL OR ALTERNATIVE ONCOGENIC PATHWAYS

- overexpression/persistent activation of other tyrosine kinases
  - Lyn
  - Hck
  - ...
- alteration of oncogenes or tumor suppressor genes caused by additional cytogenetic abnormalities

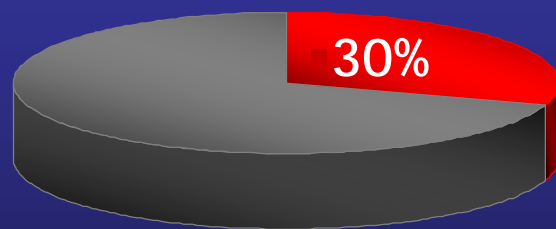
## ■ OTHER MECHANISM(S)?

- ..and actually they can be observed in some but not all resistant patients - with considerable differences in incidence depending on disease phase..

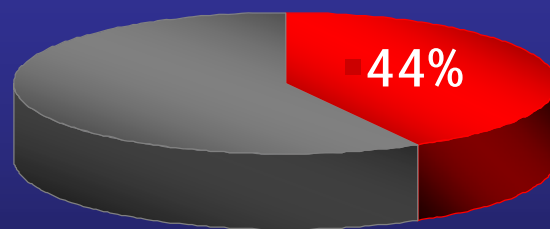
- Data of the GIMEMA WP on CML

- Phase at the time of IM failure:

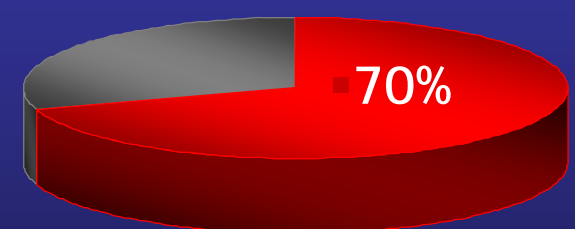
- CP (IM first-line)



- CP (after IFN failure)



- AP/BC

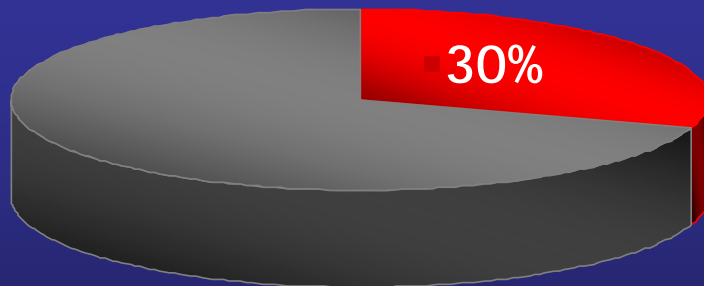


■ resistant pts harbouring Abl KD mutations

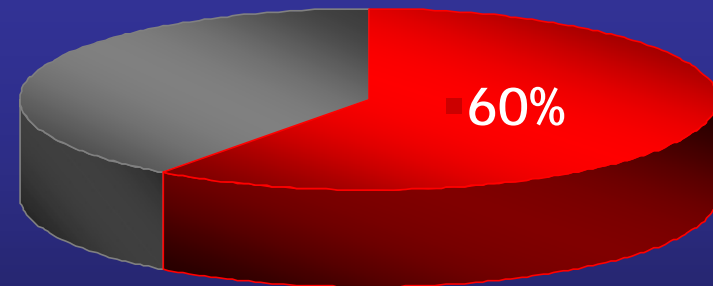
- ..and on type of resistance (primary or acquired)

- Data of the GIMEMA WP on CML

- Upfront resistance



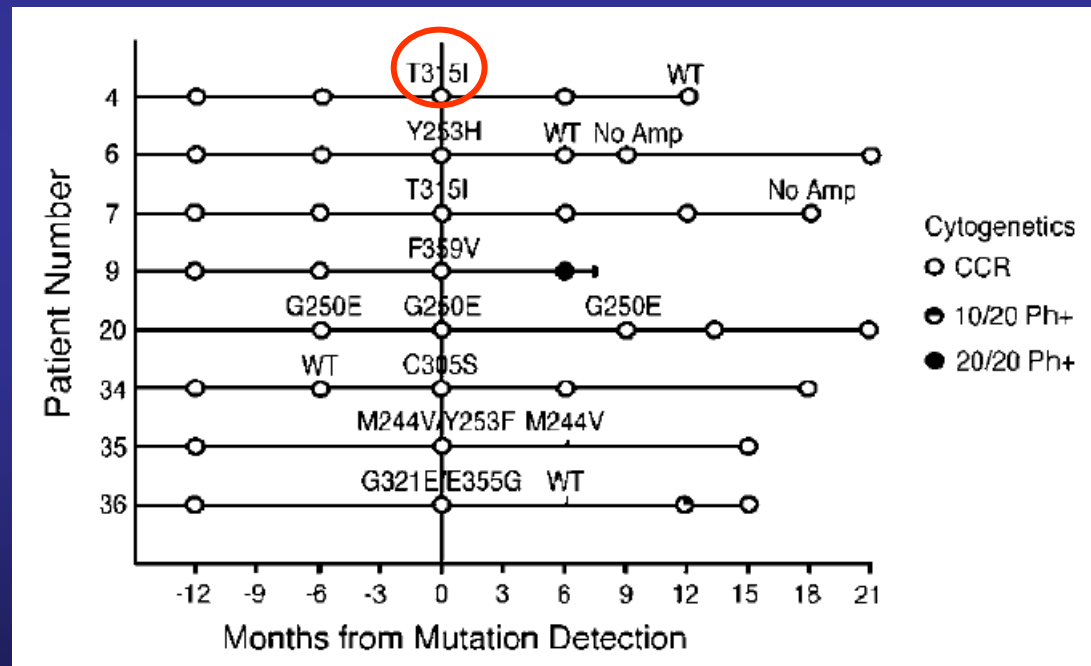
- Acquired resistance



- resistant pts harbouring Abl KD mutations

# .. and the detection of a KD domain mutation does not always imply drug resistance

- With very sensitive assays, small mutated clones may be detected in patients stable CCgR on imatinib, but they do not always expand



(from Sherbenou et al, Leukemia 2007)

■ **Mutations at low levels may be detected also in newly diagnosed CP patients before imatinib start - but they do not always outgrow and lead to treatment failure**

- We performed an unbiased scanning of the entire KD for sequence variations in samples collected at the time of diagnosis by cloning and sequencing 150-200 independent clones per patient

N	Disease	Sex	Age	Sokal	Status (time from the start of imatinib, mo)	Mutation at diagnosis (direct seq)	Mutation at relapse (direct seq)
1	CP CML	F	42	Low	MMR (48)	WT	N.A.
2	CP CML	M	70	Int	MMR (54)	WT	N.A.
3	CP CML	M	64	Int	MMR (48)	WT	N.A.
4	CP CML	F	60	Int	MMR (42)	WT	N.A.
5	CP CML	F	62	High	Progressed to BC (6)	WT	T315I
6	CP CML	F	43	High	Progressed to BC (13)	WT	E279K
7	CP CML	M	58	High	Progressed to BC (15)	WT	Y253H

▪ **Mutations at low levels may be detected also in newly diagnosed CP patients before imatinib start - but they do not always outgrow and lead to treatment failure**

N	Sex	Age	Sokal	Status (time from the start of imatinib, mo)	Mutation at diagnosis (total no. of clones sequenced)	Mutation at relapse (direct seq)
1	F	42	Low	MMR (48)	T212M; D276G; V335A; N374D (150)	N.A.
2	M	70	Int	MMR (54)	Y226Y; G251D; Y264Y; V280A; E329G; E355G; H375Y; Y373H; G390E (200)	N.A.
3	F	47	Int	MMR (48)	I313I; L370P; V377A (150)	N.A.
4	F	45	Int	MMR (42)	Y257Y; L302L; I360I (150)	N.A.
5	F	62	High	Progressed to BC (6)	K274R; E316E; A366V; R386K (150)	T315I
6	F	43	High	Progressed to BC (13)	G249D; Q300stop; T345T; Y373C; L384L; H396Y (150)	E279K
7	M	58	High	Progressed to BC (15)	L248Q; Q252R; L273L; E279G; T315T (150)	Y253H

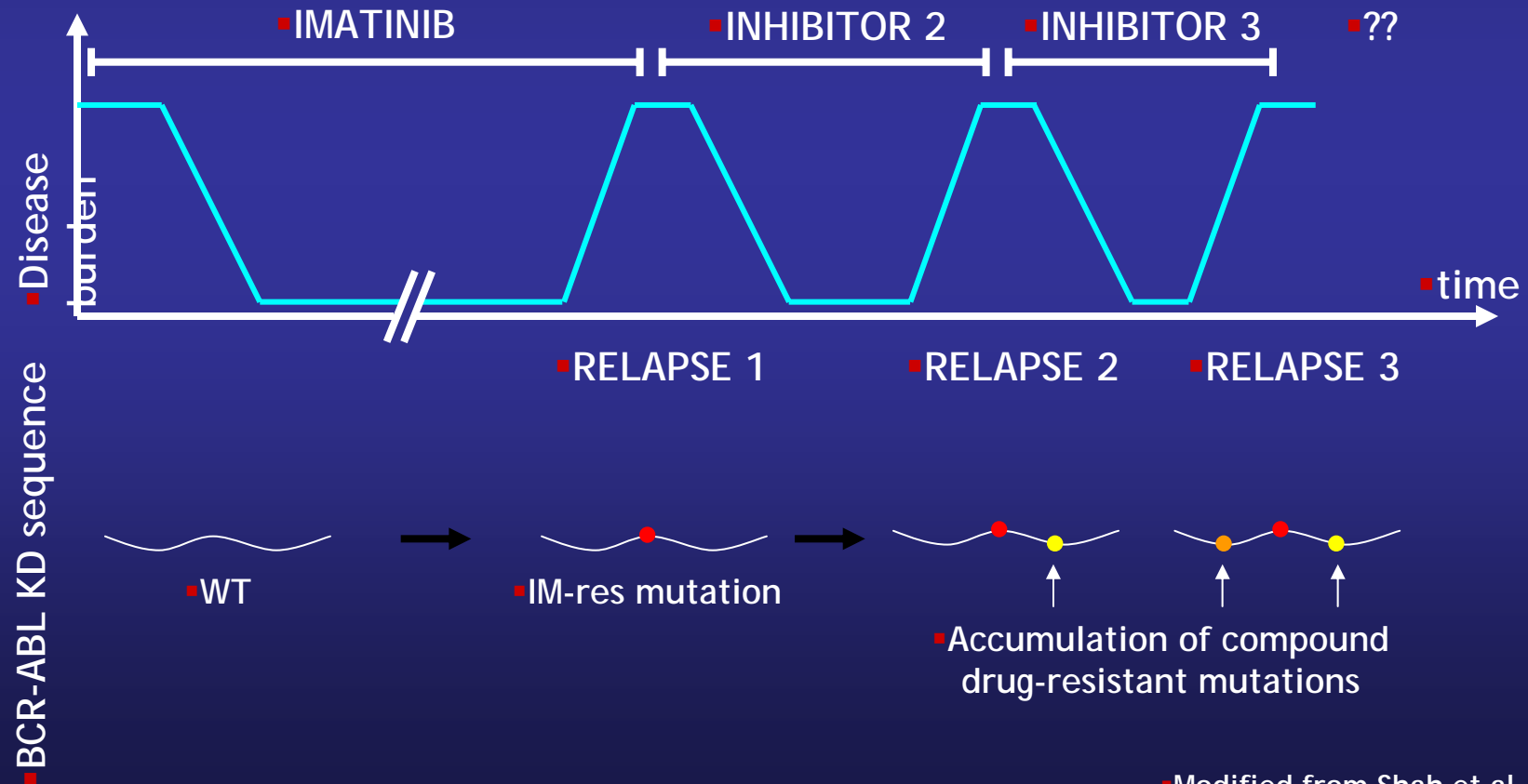
▪ \* Each mutation was detected in 2 to 4 clones out of 150-200 clones analyzed

- Is this because they arise in clones with limited self-renewal capacity?
- Is this because mutations are not (always) what really drives imatinib resistance?

▪ Soverini et al, ASH 2008

# How mutation relevance has changed in the era of 2<sup>nd</sup> TKI

- Novel TKIs retain one or some insensitive mutations and the Ph<sup>+</sup> clone may use these Achilles heels to escape inhibition



# Imatinib-resistant pts already harbouring mutations have a higher likelihood of developing additional mutations

■ 95 pts (CML all phases, Ph+ ALL)

■ relapsed on IM and switched to a 2<sup>nd</sup> TKI

■ 51 pts

■ had evidence of ABL KD mutations

■ 18/51 (35%)

■ achieved and maintained\* response

■ 14/51 (27%)

■ did not respond because of the mutation they had

■ 19/51 (38%)

■ relapsed with newly acquired mutations

■ 7/44 (16%)

■ relapsed with newly acquired mutations

■ 5/44 (11%)

■ relapsed with no evidence of mutations

■ 32/44 (73%)

■ achieved and maintained\* response

■ p=0.02

# How is it reliable to choose a 2<sup>nd</sup> TKI on the basis of the IC<sub>50</sub> values?

- Discrepancies (up to tenfold!) between IC<sub>50</sub> values reported in different studies for the same Bcr-Abl mutant
- Mutants frequently found in imatinib-resistant pts indicated as sensitive in some tables
- No correlation with PK data (C<sub>max</sub>, C<sub>min</sub>, AUC, etc)

Ba/F3 cellular proliferation IC<sub>50</sub> values

	imatinib (nM)	nilotinib (nM)	dasatinib (nM)
Native Bcr-Abl	260	13	0.8
244M>V	2000	38	1.3
250G>E	1350	48	1.8
252Q>H	1325	70	3.4
253Y>F	3475	125	1.4
253Y>H	>6400	450	1.3
255E>K	5200	200	5.6
255E>V	>6400	430	11
299V>L	540 <sup>†</sup>	nd	18 <sup>†</sup>
311F>L	480	23	1.3
315T>A	971	61	125 <sup>†</sup>
315T>I	>6400	>2000	>200
317F>L	1050	50	7.4
317F>V	350 <sup>†</sup>	nd	53 <sup>†</sup>
315M>T	880	15	1.1
355E>G	2300 <sup>†</sup>	nd	1.8 <sup>†</sup>
359F>V	1825	175	2.2
379V>I	1830	51	0.8
387L>M	1000	49	2
396H>P	850	41	0.6
396H>R	1750	41	1.3

■ Sensitive   
 ■ Intermediate sensitivity   
 ■ Insensitive

		IC <sub>50</sub> fold increase (WT = 1)			
		Bosutinib	Imatinib	Dasatinib	Nilotinib
	Parental	38.31	10.78	> 50	38.43
	WT	1	1	1	1
P-LOOP	L248V	2.97	3.54	5.11	2.80
	G250E	4.31	6.86	4.45	4.56
	Q252H	0.81	1.39	3.05	2.64
	Y253F	0.96	3.58	1.58	3.23
	E255K	9.47	6.02	5.61	6.69
	E255V	5.53	16.99	3.44	10.31
C-Helix	D276G	0.60	2.18	1.44	2.00
	E279K	0.95	3.55	1.64	2.05
ATP binding region (drug contact sites)	V299L	26.10	1.54	8.65	1.34
	T315I	45.42	17.50	75.03	39.41
	F317L	2.42	2.60	4.46	2.22
SH2-contact	M351T	0.70	1.76	0.88	0.44
Substrate binding region (drug contact sites)	F359V	0.93	2.86	1.49	5.16
A-LOOP	L384M	0.47	1.28	2.21	2.33
	H396P	0.43	2.43	1.07	2.41
	H396R	0.81	3.91	1.63	3.10
	G398R	1.16	0.35	0.69	0.49
C terminal lobe	F486S	2.31	8.10	3.04	1.85

Sensitive	≤ 2
Moderately resistant	2.01-4
Resistant	4.01-10
Highly resistant	> 10

# Practical implications for mutation monitoring of CML patients

# ■ Practical relevance of Abl KD mutation analysis

- The knowledge of the mutation status of a patient who fails\* or has a suboptimal response\* to imatinib offers a precious piece of information that, when integrated within the clinical context, may help in best tailoring subsequent therapeutic strategy:
- For this reason, mutation analysis must enter the panel of routine molecular assessments
  - imatinib dose escalation (400 > 800 mg/d)
- This allows to conjugate the most effective therapy for our CML patients and the optimization of healthcare costs (tyrosine kinase inhibitors are expensive!)
  - dasatinib or nilotinib → for mutations except T315I
  - allogeneic transplant → if feasible, for T315I+ patients
  - investigational drugs → e.g., aurora kinase inhibitors, for T315I+ patients

\* For definitions, see European LeukemiaNet recommendations (Baccarani et al, Blood 2006)

- Practical relevance of Abl KD mutation analysis

- This does not mean we have to routinely perform mutation analyses on ALL our patients!!

▪ **Aim:**  
**to concile patient benefit with cost/time-effectiveness**

- When is it worth (→ clinically useful) looking for mutations?
  - When is it useless?
  - When is it misleading?
- No recommendations are available at present..
  - Based on what we currently know about mutations, should EUTOS work on suggestions about this topic?

■ Some issues that could be discussed and addressed by such recommendations

■ 1) how early should we wish to detect a KD mutation?

Technology	Sensitivity	Bias*?	Quantitative?	Availability	Cost
Direct sequencing	20-30%	No	Semi-	++	+++
D-HPLC	0.1-10%	No	No	+	++
Pyrosequencing	5%	Yes	Yes	+	++
DGGE	5%	No	No	++	+
RFLP	5%	Yes	No	+++	+
SSCP	2-10%	No	No	++	+
PNA clamping and fluorescent PCR	0.2%	Yes	Yes	+	+++
Tagged allele discrimination (aPCR/MUT)	0.1-1%	Yes	Yes	+	+++
ASO-PCR	10 <sup>-2</sup> -10 <sup>-5</sup>	Yes	Semi-	+++	+

Can we conclude from presently available data that direct sequencing, D-HPLC, pyrosequencing, RFLP, DGGE, SSCP etc (lower detection limit >1%) may be used for routine mutation monitoring of patients since we may be pretty confident that the mutations we identify are clinically relevant?

Can we conclude that, in contrast, higher-sensitivity methods should be avoided for routine assessment of patients - although they are important to further investigate the significance of low level mutations

\* Detects only specific mutations

## Some issues that could be discussed addressed by such recommendations

- 2) When?
- 3) How regularly?

▪ Should I perform a mutation analysis?

▪ YES

- IN CASE OF FAILURE/SUBOPTIMAL RESPONSE TO IMATINIB\*
- IN PATIENTS RESPONDING TO IMATINIB BUT SHOWING AN INCREASE OF BCR-ABL TRANSCRIPT LEVELS (but to what extent?)
- IN IMATINIB-RESISTANT PATIENTS RECEIVING DASATINIB OR NILOTINIB (but how frequently?)

▪ NO

- IN CP CML PATIENTS AT DIAGNOSIS, BEFORE IMATINIB START
- IN PATIENTS WITH STABLE CCgR ON IMATINIB

▪\* ELN recommendations - Baccarani et al, Blood