Il Next Generation Sequencing e la Tailored Therapy

Simona Soverini, PhD
University of Bologna

2001:
first report of BCR-ABL mutations

NATURE | VOL 412 | 19 JULY 2001 | www.nature.com

news and views

New-age drug meets resistance

Frank McCormick

A new sort of anticancer drug, designed to suppress the wayward protein that causes a type of leukaemia, is under the spotlight. Biological detective work has now revealed how this cancer becomes resistant to the drug.

Gleevec is the anticancer drug that everybody has been talking about. Known during its development as STI-571, this drug was rushed to market so quickly that Novartis, the company that developed it, had no time to clear a new trade name. 'Gleevec' was available — the name had been cleared for a different type of drug that didn't make it to market — so this trade name was slapped on STI-571. In the United States, Gleevec was approved for the treatment of chronic myeloid leukaemia (CML) in record time because of its sensational success in clinical trials. In one study, 53 out of 54 patients suffering from the early, 'chronic' phase of the disease showed complete, lasting responses[1]. Patients suffering from the later stage, referred to as blast crisis, also responded well at first[2]. But most relapsed within a few months, despite continued treatment.

Now the cause of the relapse has been

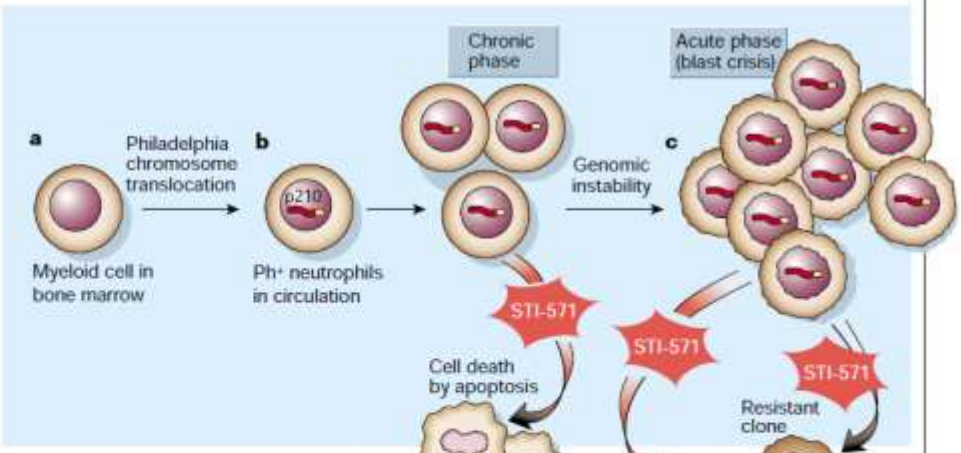
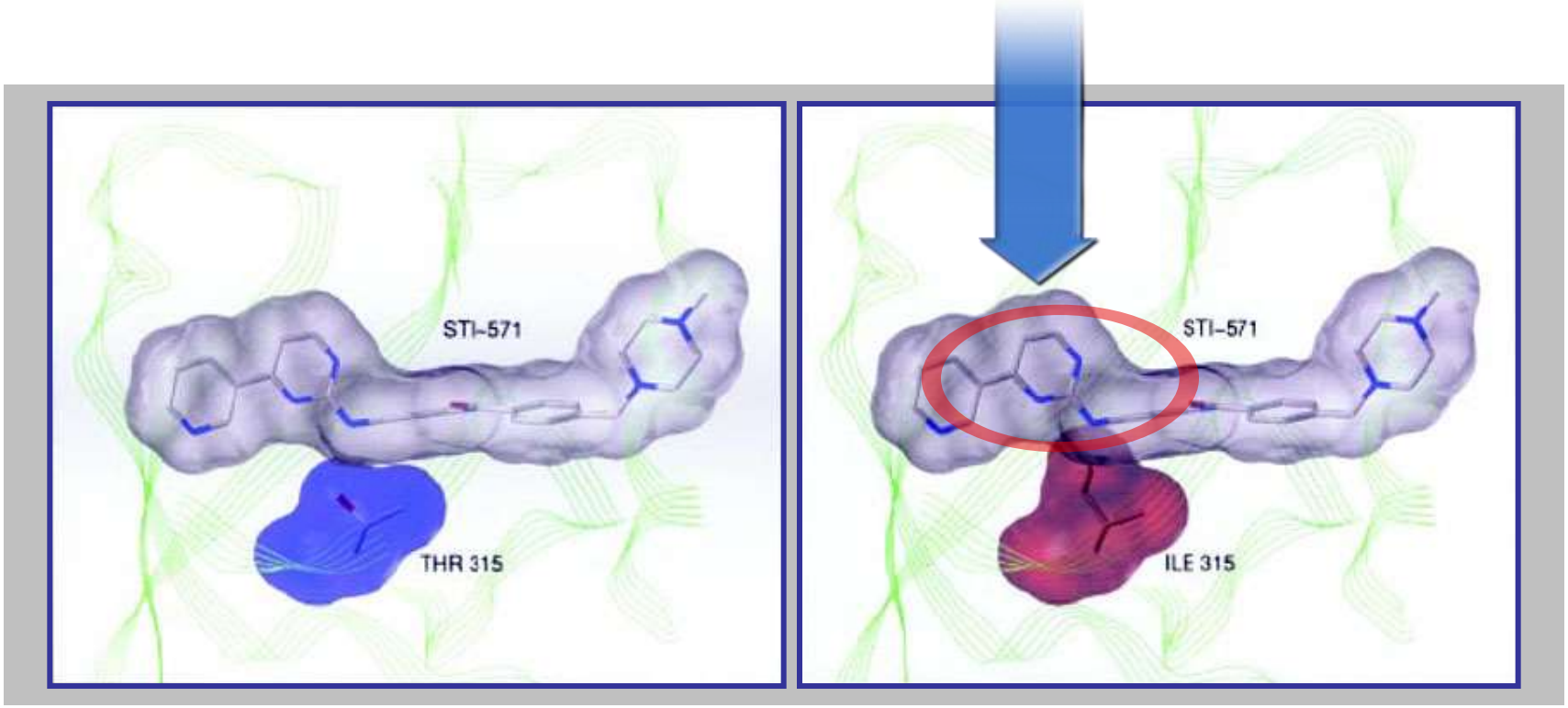


Figure 1 The progression of chronic myeloid leukaemia (CML). a, Myeloid cells in the bone marrow are precursors from which certain types of immune cell, including neutrophils, develop. b, The Philadelphia chromosomal translocation (Ph$^+$) marks the onset

Effetto della mutazione T315I sul legame imatinib-BCR-ABL

La catena laterale dell'isoleucina ha un ingombro sterico maggiore rispetto alla treonina e ostacola il legame di imatinib

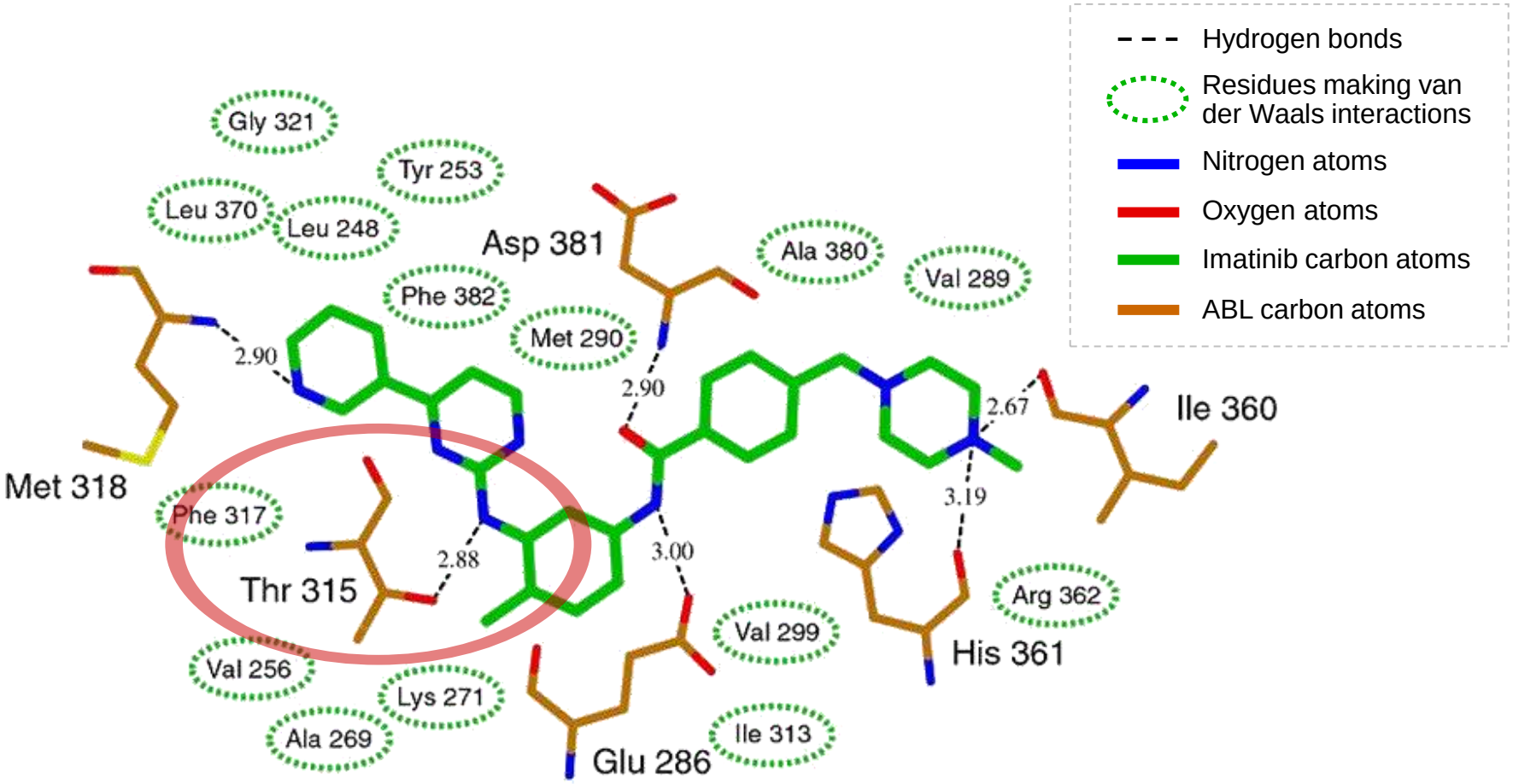


Wild-type

T3151 Mutant

Gorre et al., *Science*. 2001

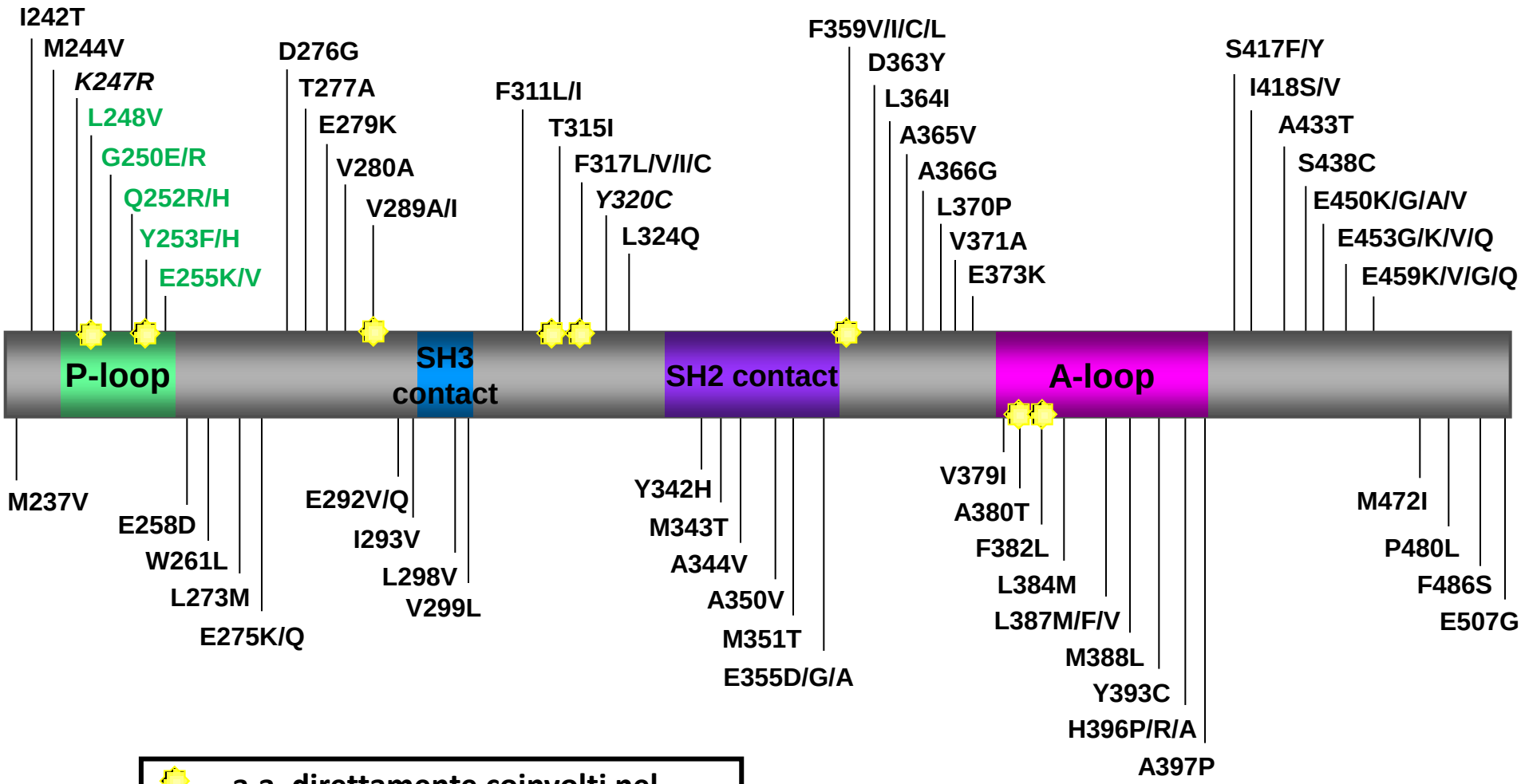
Il legame imatinib-BCR-ABL



Nagar et al., *Cancer Res*. 2002

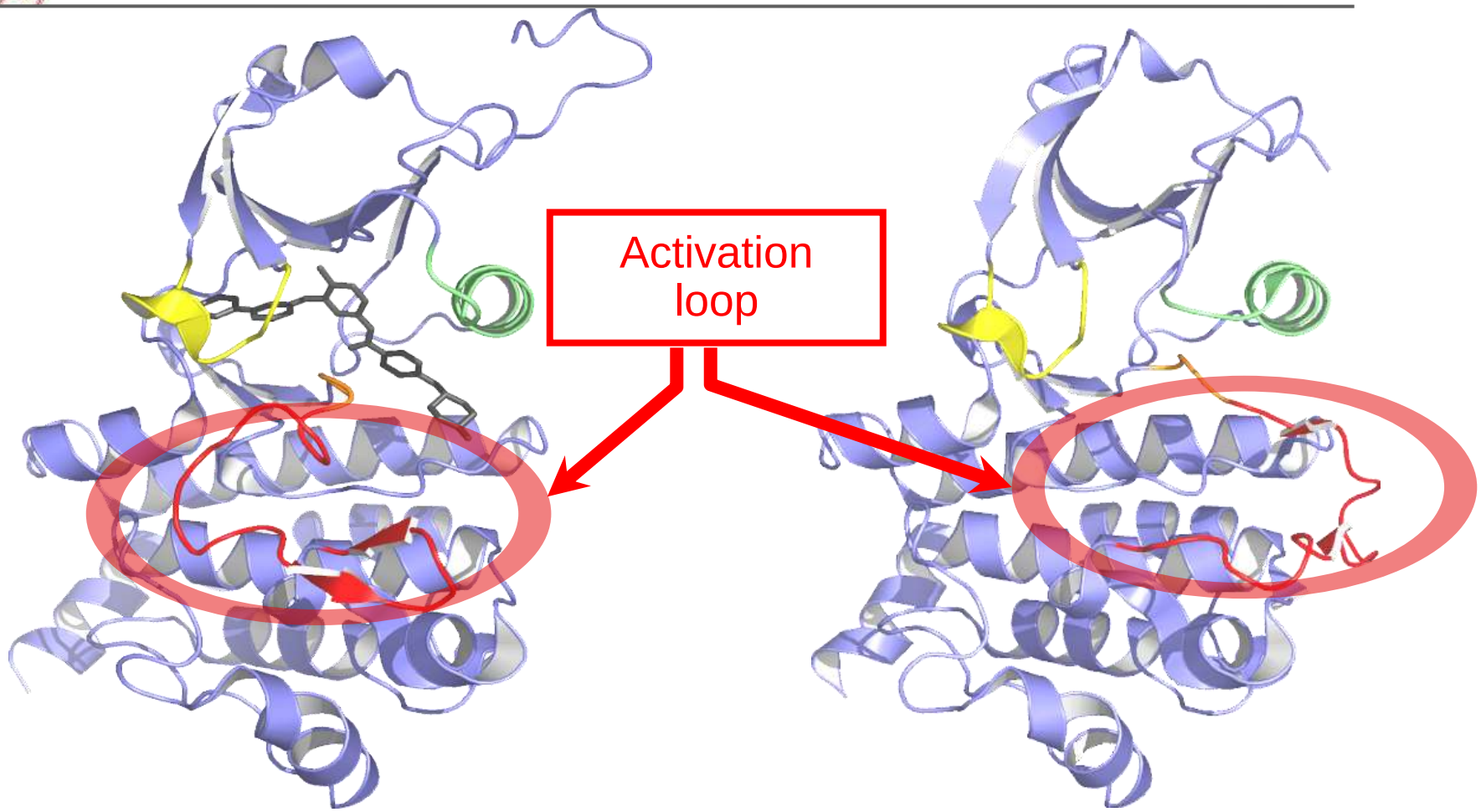
1. P-loop: a.a. coinvolti nel legame con il gruppo fosforico dell'ATP

2. A-loop: a.a. che controllano lo stato di attivazione/ inattivazione della chinasi

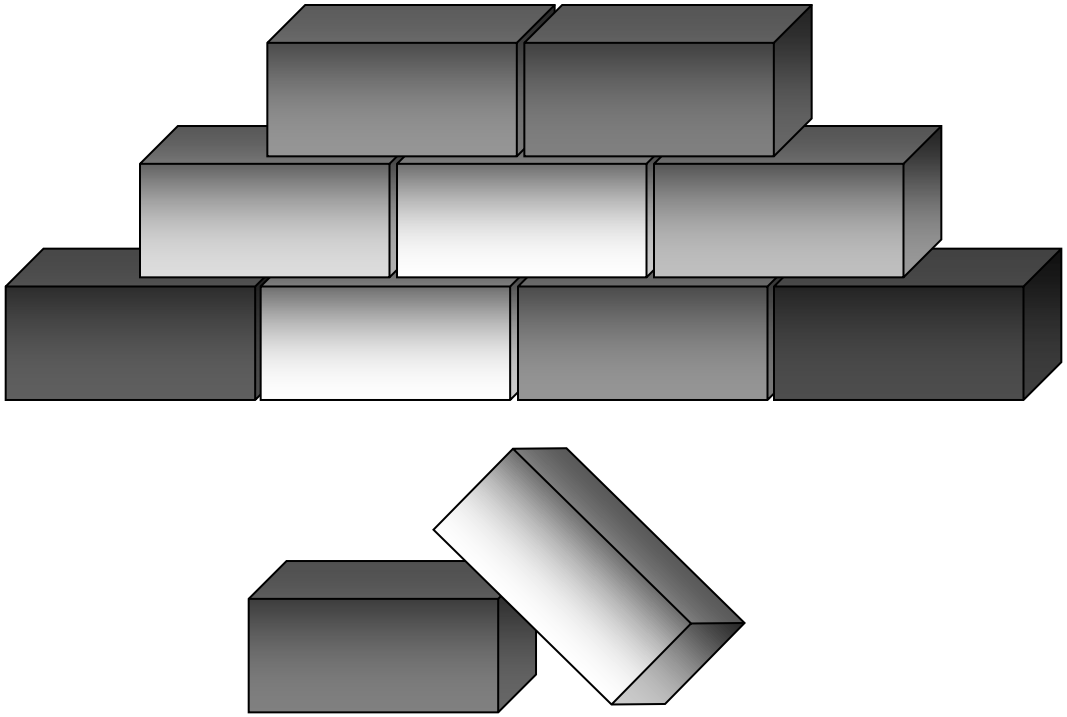


a.a. direttamente coinvolti nel legame con imatinib

Qualsiasi mutazione che sposti l'equilibrio verso la conformazione attiva è in grado di conferire resistenza a imatinib



TKI resistance



The same “resistant” phenotype may result from the assembly of different bricks

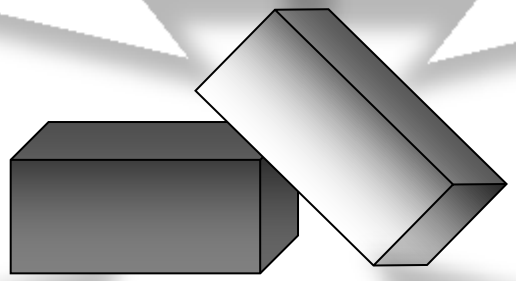
.. and these are the bricks

IMATINIB BIOAVAILABILITY

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

ADDITIONAL OR ALTERNATIVE ONCOGENIC PATHWAYS

- Other tyrosine kinases cross-talking with, or downstream of, Bcr-Abl
  - Src kinases
  - Jak2
  - …
- additional cytogenetic abnormalities



INTERACTION WITH TARGET

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

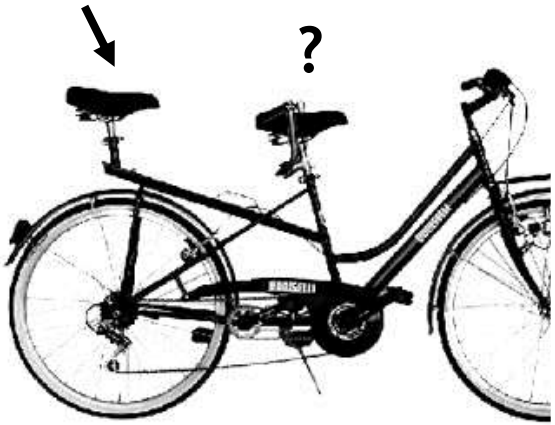
'STEM CELL RESISTANCE'

- Dependence from 'something' other than BCR-ABL kinase activity for survival
- Role of microenvironment

Although the contribution of mutations to resistance may not always be the same..

1. The ABL KD mutation drives resistance

2. The ABL KD mutation cooperates with other mechanisms



3. The ABL KD mutation is an innocent bystander

… knowledge of mutation status is important for optimal patient management

- Patients harboring mutations have a 'biologically advanced' disease
  (Radich et al, PNAS 2005)

- Patients harboring mutations have a higher likelihood of developing additional mutations leading to relapse on second-line TKI therapy
  (Soverini et al, Blood 2009; Hughes et al, J Clin Oncol 2009; Muller et al, Blood 2009)

- The type of mutation may be useful for second- or subsequent-line TKI selection

Chronic phase patients harboring mutations have expression profiles similar to advanced phase patients

PNAS

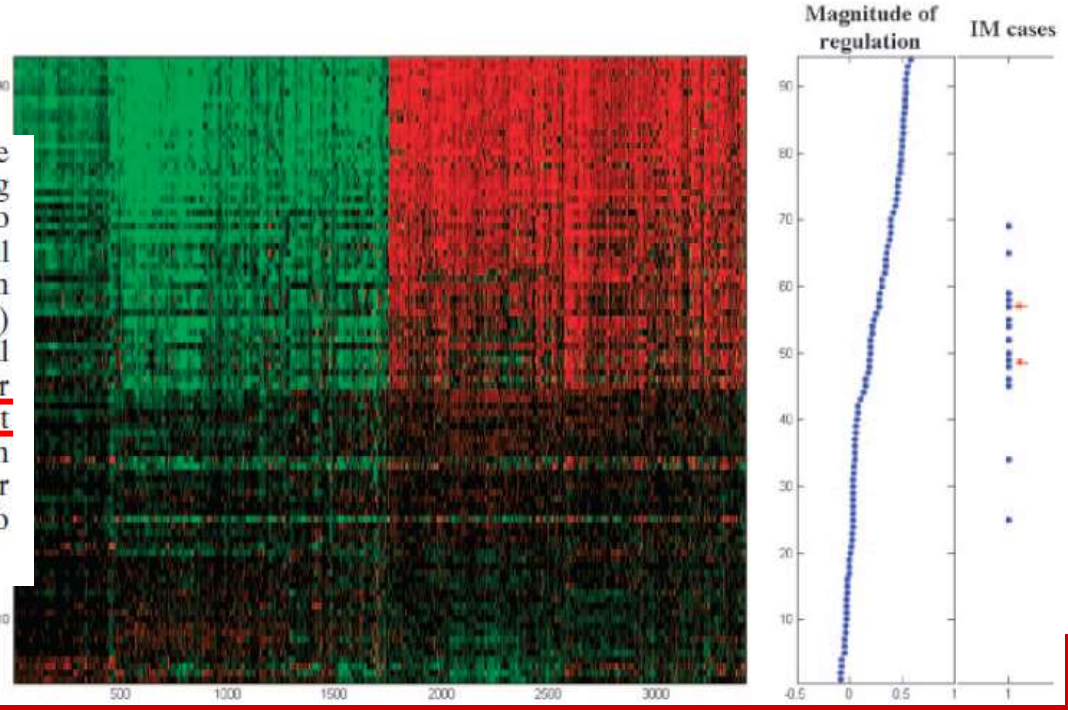
Gene expression changes associated with progression and response in chronic myeloid leukemia

Jerald P. Radich*†‡, Hongyue Dai§, Mao Mao§, Vivian Oehler*, Jan Schelter§, Brian Druker†¶, Charles Sawyers‖, Neil Shah‖, Wendy Stock**, Cheryl L. Willman†,††, Stephen Friend§, and Peter S. Linsley§

*Divisions of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; §Rosetta Inpharmatics, Seattle, WA 98109; ¶Oregon Health & Science University, University Center, Portland, OR 97239; ‖University of California, Los Angeles, CA 90095; **University of Chicago School of Medicine, Chicago, IL 60637; ††University of New Mexico Cancer Research and Treatment Center, Albuquerque, NM 87131; and †Southwest Oncology Group, Ann Arbor, MI 48106

Communicated by E. Donnall Thomas, Fred Hutchinson Cancer Research Center, Seattle, WA, December 13, 2005 (received for review June 24, 2005)

Fig. 3 shows the expression pattern of these 15 CML cases. We found that the cases that appeared in chronic phase after relapsing after an achievement of CCR had expression patterns similar to advanced disease. This can be demonstrated by segregating all CML cases by the correlation of gene expression signature between the boundaries of "most chronic" cases (bottom of the heat map) and "most advanced" gene expression (top of the heat map) for all 3,000 genes in the phase reporter gene set. The majority of the poor response patients have gene expression profiles more consistent with advanced disease rather than chronic phase. Both cases with T315I mutations, which have been shown to have especially poor prognosis (8, 15), have expression signatures more similar to advanced disease than chronic phase (red arrows).



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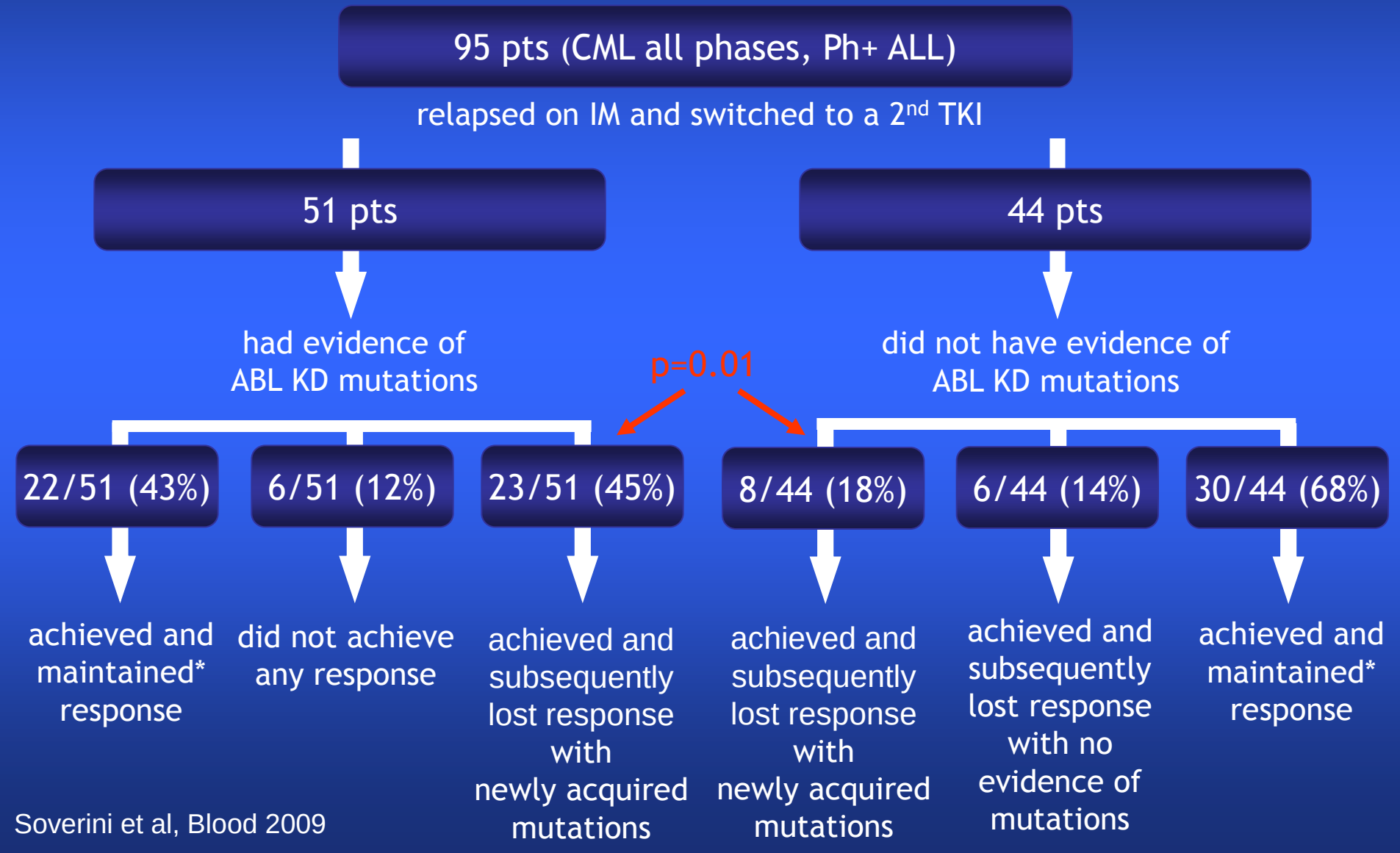
Imatinib-resistant pts already harbouring mutations have a higher likelihood of developing additional mutations



Soverini et al, Blood 2009

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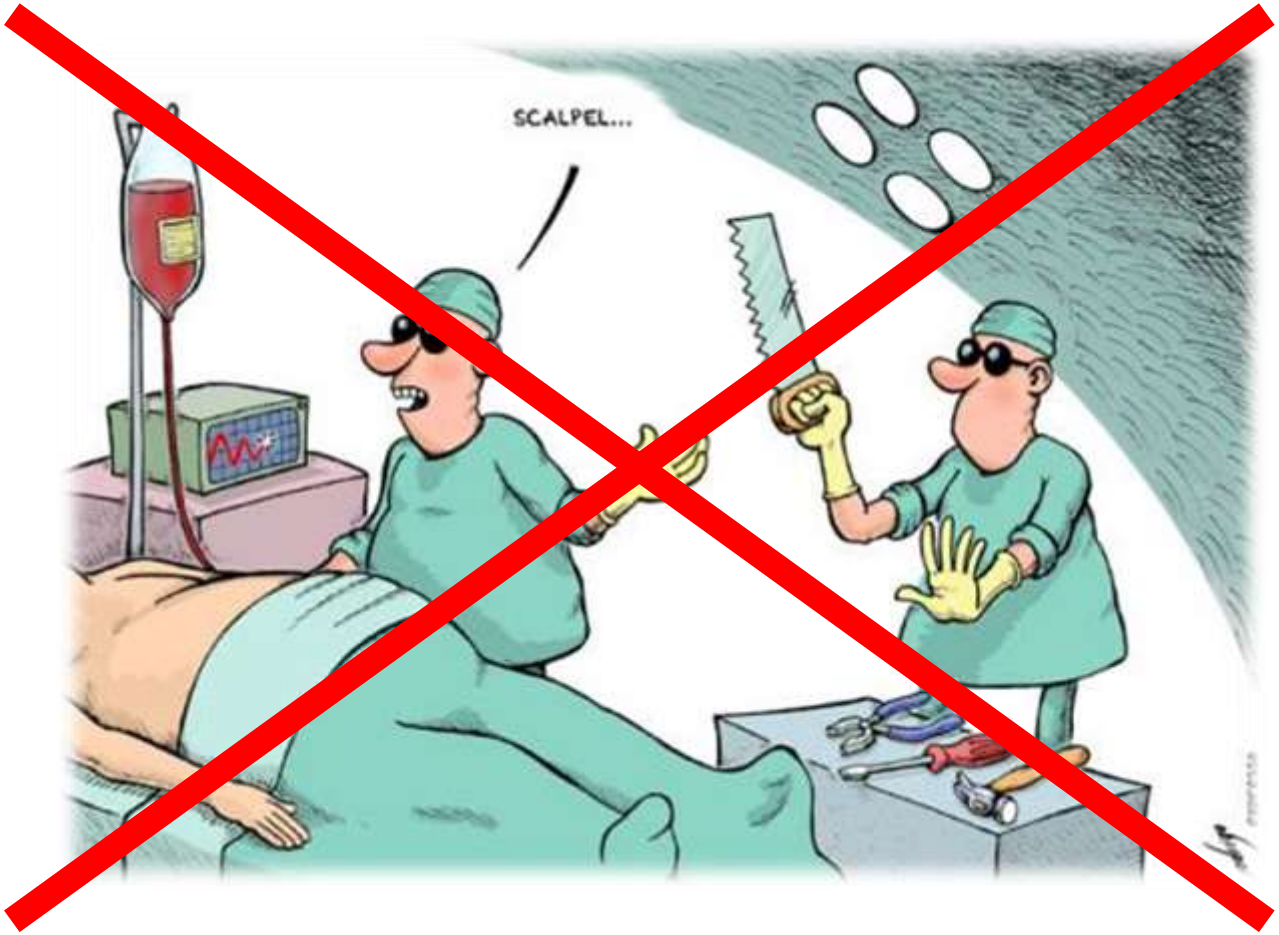
Detection of specific mutations predicts for TKI inefficacy

1st generation | 2nd generation | 3rd generation

imatinib						nilotinib	dasatinib	bosutinib	ponatinib
M237V	L273M	F311L	E355D/G	V379I	A397P	Y253F/H	V299L	E255K	?
M244V	E275K/Q	**T315I**	F359V/I/C	A380T	S417F/Y	E255K/V	**T315I/A**	V299L	
L248R	D276G	F317L/V/I/C	D363Y	F382L	I418S/V	**T315I**	F317L/V/I/C	**T315I**	
G250E/R	T277A	F359V/I/C	L364I	L384M	S438C	F359V/I/C		?	
Q252R/H	E279K	Y342H	A365V	L387M/F	E453G/K				
Y253F/H	V280A/I	M343T	L370P	M388L	E459K/V				
E255K/V	V289A	A344V	V371A	Y393C	P480L				
E258D	V299L	M351T	E373K	H396R/P	F486S				

T315I = 

The role of mutation analysis in 2015



When to perform BCR-ABL KD mutation analysis

BCR-ABL kinase domain mutation analysis in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors: recommendations from an expert panel on behalf of European LeukemiaNet

Simona Soverini,[1] Andreas Hochhaus,[2] Franck E. Nicolini,[3] Franz Gruber,[4] Thoralf Lange,[5] Giuseppe Saglio,[6] Fabrizio Pane,[7,8] Martin C. Müller,[9] Thomas Ernst,[2] Gianantonio Rosti,[1] Kimmo Porkka,[10] Michele Baccarani,[1] Nicholas C. P. Cross,[11,12] and Giovanni Martinelli[1]

BLOOD, 4 AUGUST 2011 • VOLUME 118, NUMBER 5

European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013

Michele Baccarani,[1] Michael W. Deininger,[2] Gianantonio Rosti,[3] Andreas Hochhaus,[4] Simona Soverini,[3] Jane F. Apperley,[5] Francisco Cervantes,[6] Richard E. Clark,[7] Jorge E. Cortes,[8] François Guilhot,[9] Henrik Hjorth-Hansen,[10] Timothy P. Hughes,[11] Hagop M. Kantarjian,[8] Dong-Wook Kim,[12] Richard A. Larson,[13] Jeffrey H. Lipton,[14] François-Xavier Mahon,[15] Giovanni Martinelli,[3] Jiri Mayer,[16] Martin C. Müller,[17] Dietger Niederwieser,[18] Fabrizio Pane,[19] Jerald P. Radich,[20] Philippe Rousselot,[21] Giuseppe Saglio,[22] Susanne Saußele,[17] Charles Schiffer,[23] Richard Silver,[24] Bengt Simonsson,[25] Juan-Luis Steegmann,[26] John M. Goldman,[27] and Rüdiger Hehlmann[17]

BLOOD, 8 AUGUST 2013 • VOLUME 122, NUMBER 6

- At diagnosis: only in pts who present in AP/BP
- During 1st line imatinib therapy: In case of FAILURE and WARNING (formerly SUBOPTIMAL RESPONSE)

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Table 5. Definition of the response to TKIs (any TKI) as first-line treatment

	Optimal	Warning	Failure
Baseline	NA	High risk Or CCA/Ph+, major route	NA
3 mo	BCR-ABL1 ≤10% and/or Ph+ ≤35%	BCR-ABL1 >10% and/or Ph+ 36-95%	Non-CHR and/or Ph+ >95%
6 mo	BCR-ABL1 <1% and/or Ph+ 0	BCR-ABL1 1-10% and/or Ph+ 1-35%	BCR-ABL1 >10% and/or Ph+ >35%
12 mo	BCR-ABL1 ≤0.1%	BCR-ABL1 >0.1-1%	BCR-ABL1 >1% and/or Ph+ >0
Then, and at any time	BCR-ABL1 ≤0.1%		Loss of CHR Loss of CCyR Confirmed loss of MMR* Mutations CCA/Ph+

Table 6. Definitions of the response to second-line therapy in case of failure of imatinib

	Optimal	Warning	Failure
Baseline	NA	No CHR or loss of CHR on imatinib or lack of CyR to first-line TKI or high risk	NA
3 mo	BCR-ABL1 ≤10% and/or Ph+ < 65%	BCR-ABL1 >10% and/or Ph+ 65-95%	No CHR or Ph+ >95% or new mutations
6 mo	BCR-ABL1 ≤10% and/or Ph+ < 35%	Ph+ 35-65%	BCR-ABL1 >10% and/or Ph+ >65% and/or new mutations
12 mo	BCR-ABL1 <1% and/or Ph+ 0	BCR-ABL1 1-10% and/or Ph+ 1-35%	BCR-ABL1 >10% and/or Ph+ >35% and/or new mutations
Then, and at any time	BCR-ABL1 ≤0.1%	CCA/Ph− (−7 or 7q−) or BCR-ABL1 >0.1%	Loss of CHR or loss of CCyR or PCyR Confirmed loss of MMR*

Warning implies that the characteristics of the disease and the response to treatment require more frequent monitoring to permit timely changes in therapy in case of treatment failure.

How to perform BCR-ABL KD mutation analysis

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- CONVENTIONAL (SANGER) SEQUENCING is the recommended method, but……..

Next-Generation Sequencing: basic principles

DNA molecules

(fragmented genomic DNA / exons or other target enrichment products / PCR amplicons)

↓

Physical isolation of individual molecules in space

↓

Clonal amplification

↓

Parallel sequencing of the clones

↓

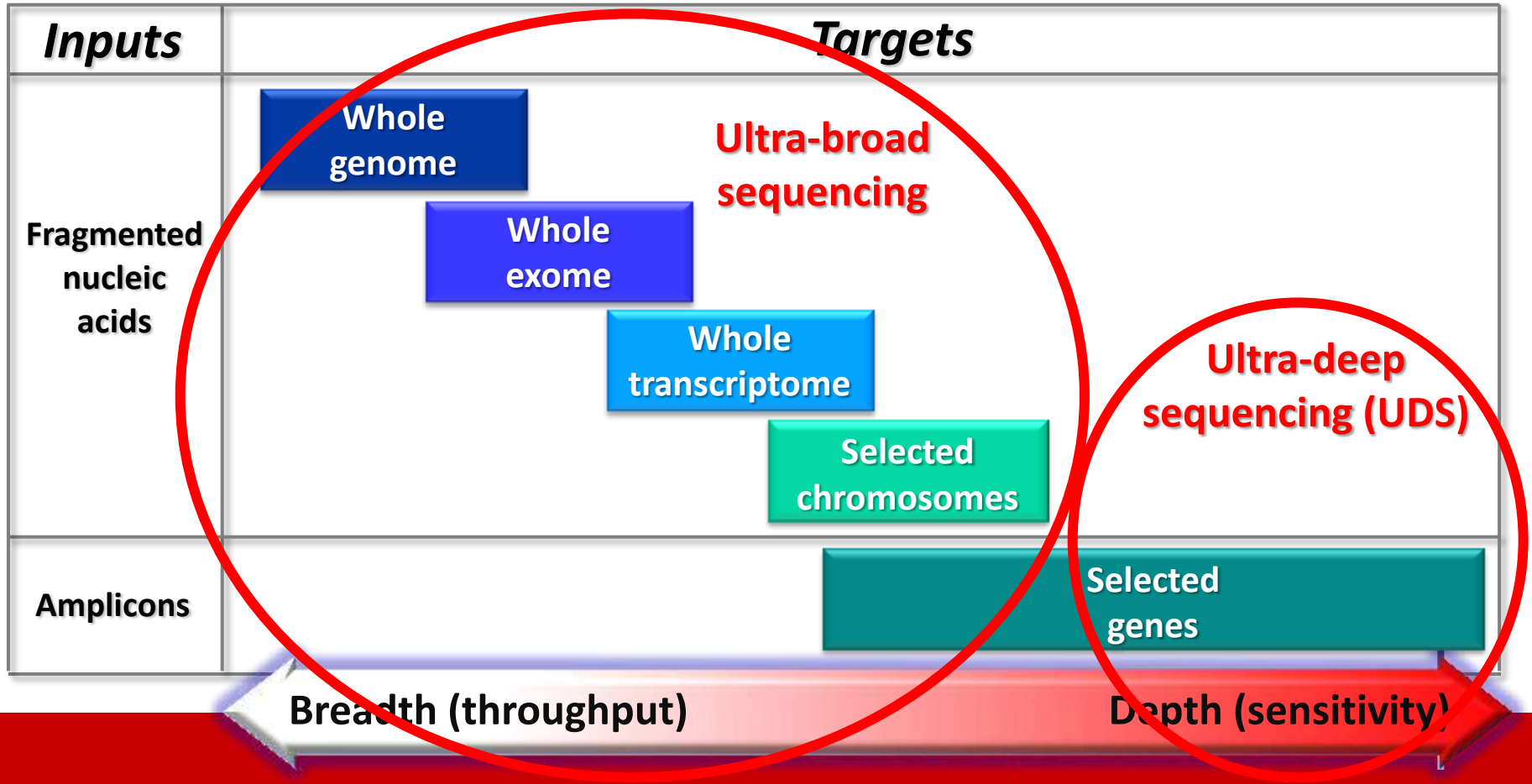
Hundred thousands to millions sequence reads

Next-Generation Sequencing: applications

Key features of NGS:

> millions of picoliter-scale sequencing reactions simultaneously→ high throughput

> one sequence read = one (clonally amplified) DNA molecule



Mutations detectable by conventional sequencing: the tip of the iceberg

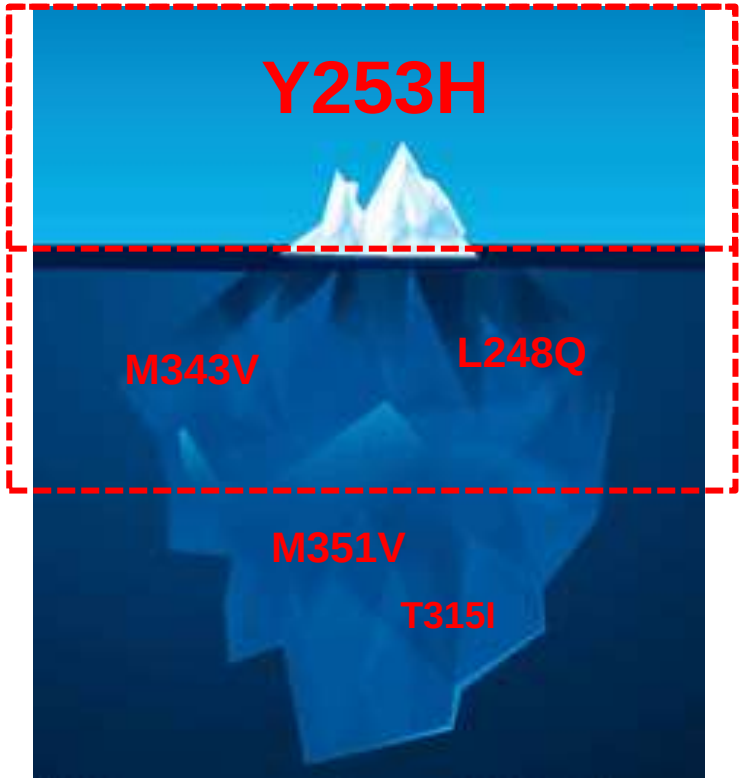
Conventional Sequencing

(15-20%)

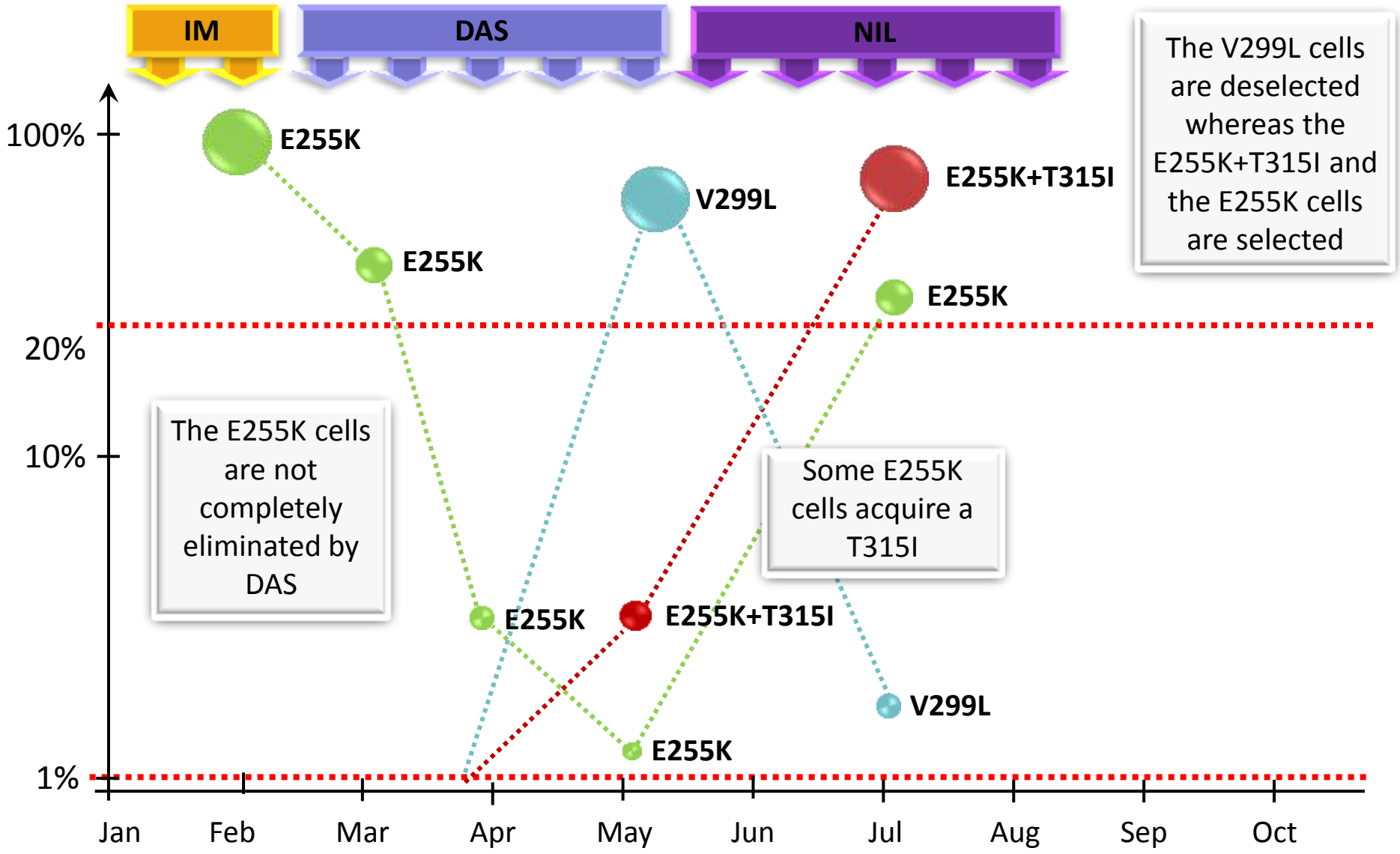
NGS

(1%)

ASO-PCR
(0.001%; but it is mutation-specific)



The complex and dynamic landscape of mutant populations can be best followed by NGS



‘Benchtop’ Next-Generation Sequencers

Not powerful enough to cover a whole genome or exome, but ideal for candidate gene resequencing with high throughput or high sensitivity (UDS)

Company	Benchtop version	Read length	Expected output	Dimensions	Price
Roche	GS Junior	450	40 Mb	40x60x40 cm	125,000 $
Medium-scale diagnostic sequencing needs					
Illumina	MiSeq	2x250	15 Gb	68.6x56.5x52.3 cm	125,000 $
Large-scale diagnostic sequencing needs					
Life	Ion PGM	400	1-2 Gb	61x51x53 cm	55,000 $
Medium-scale diagnostic sequencing needs					
Coming soon..	?	Longer?	?	Smaller & smaller	Lower & lower

The IRON (Interlaboratory RObustness of Next-Generation Sequencing) study

- IRON I study (2010-2011): first evidence of technical feasibility of diagnostic NGS with the Roche 454 technology and concordance of results across multiple laboratories

Leukemia (2011) 25, 1840–1848

www.nature.com/leu

ORIGINAL ARTICLE

The Interlaboratory RObustness of Next-generation sequencing (IRON) study: a deep sequencing investigation of *TET2*, *CBL* and *KRAS* mutations by an international consortium involving 10 laboratories

A Kohlmann[1], H-U Klein[2], S Weissmann[1], S Bresolin[3], T Chaplin[4], H Cuppens[5], E Haschke-Becher[6], B Garicochea[7], V Grossmann[1], B Hanczaruk[8], K Hebestreit[2], C Gabriel[9], I Iacobucci[10], JH Jansen[11], G te Kronnie[3], L van de Locht[11], G Martinelli[10], K McGowan[8], MR Schweiger[12], B Timmermann[12], P Vandenberghe[5], BD Young[4], M Dugas[2] and T Haferlach[1]

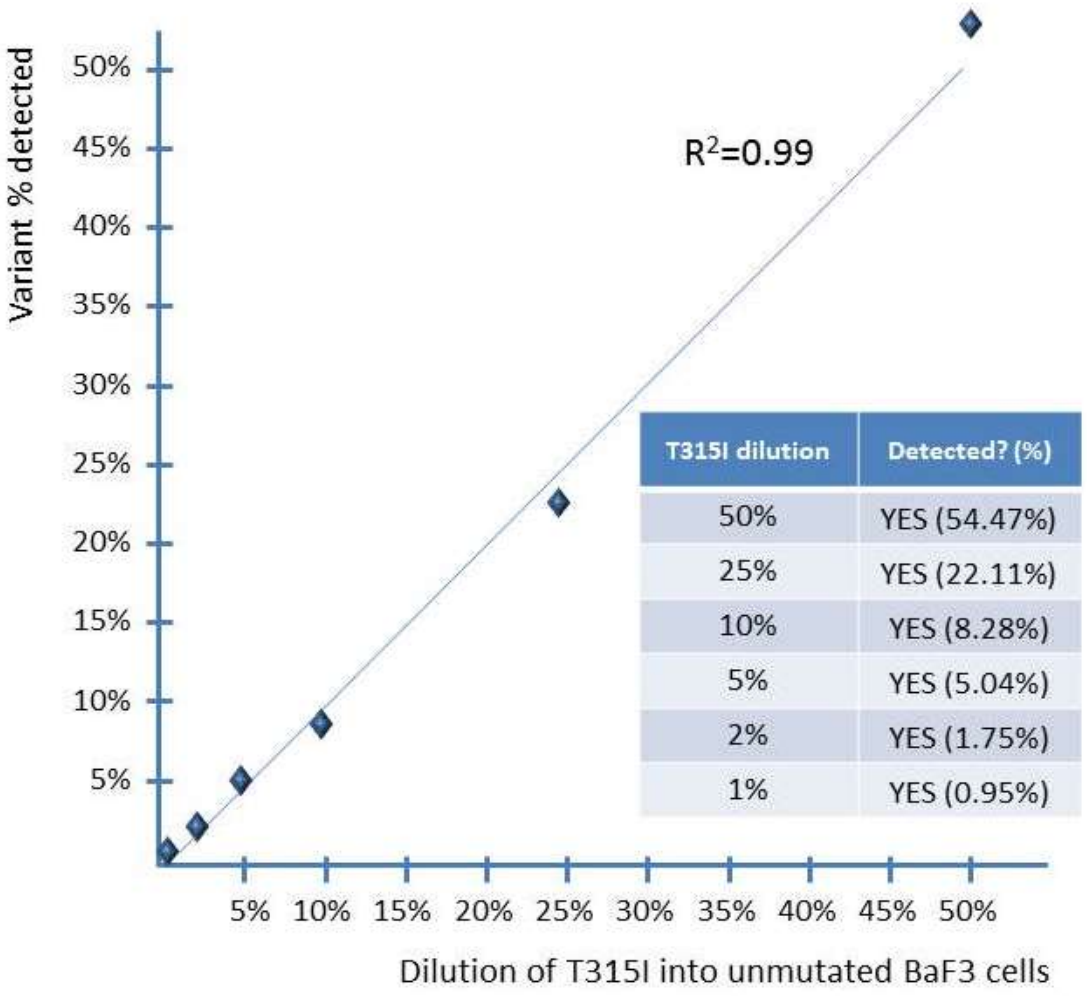
- IRON II study (2012-2014): set-up and validation of a wide menu of mutations screening assays (e.g., CEBPA, RUNX1, DNMT3A, TP53, TET2, NOTCH1, SF3B1, BIRC3, BCR-ABL..)

The IRON II study

International consortium of 10 laboratories from 8 countries engaged in the standardization and validation of a common UDS protocol for BCR-ABL KD mutation screening based on the Roche Titanium chemistry

1. Bologna
2. Munich
3. Jena
4. London
5. Madrid
6. Salamanca
7. Brno
8. Prague
9. Vienna
10. Istanbul

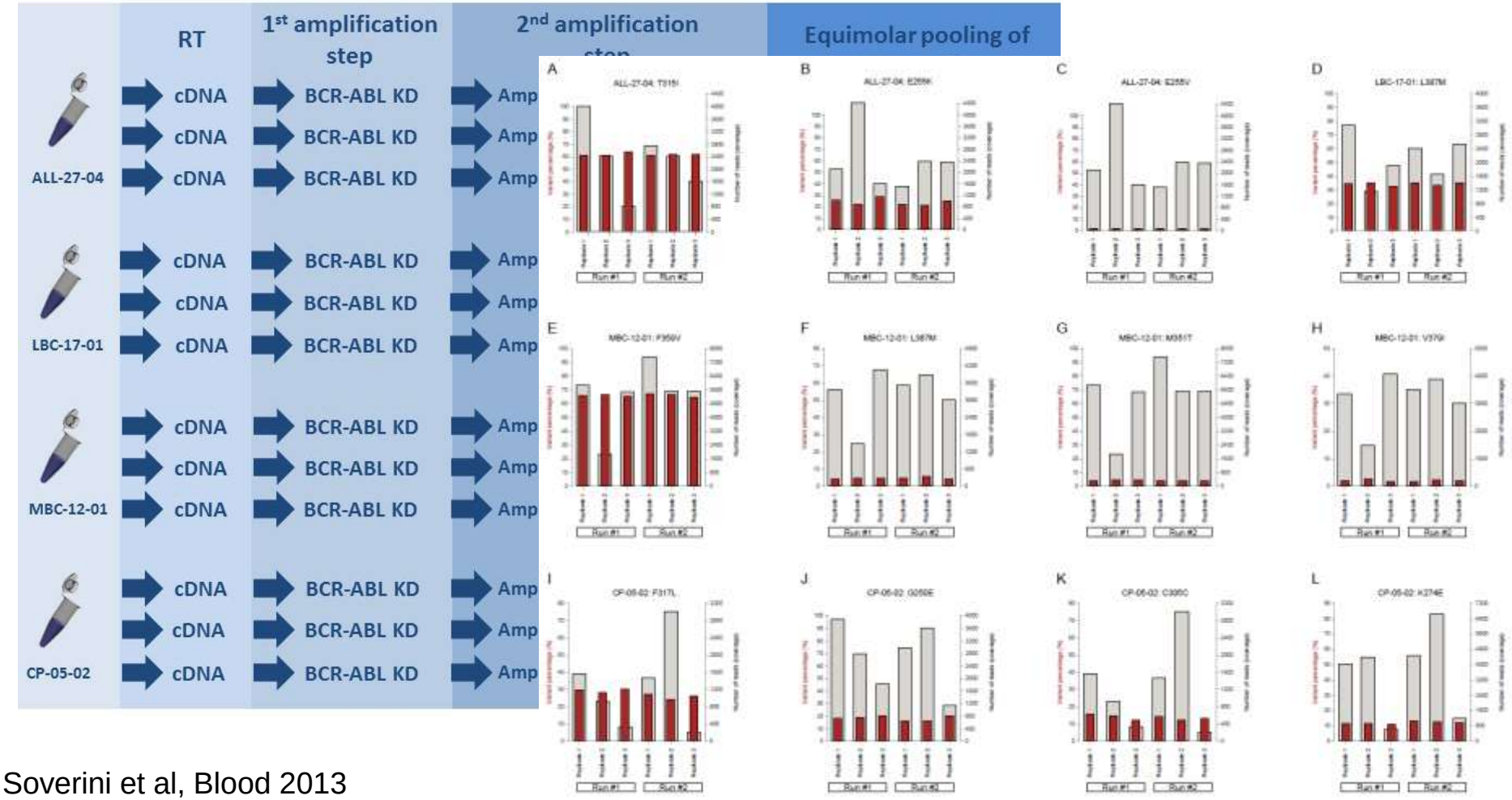
1. Sensitivity



T315I dilution	Detected? (%)
50%	YES (54.47%)
25%	YES (22.11%)
10%	YES (8.28%)
5%	YES (5.04%)
2%	YES (1.75%)
1%	YES (0.95%)

Soverini et al, Blood 2013

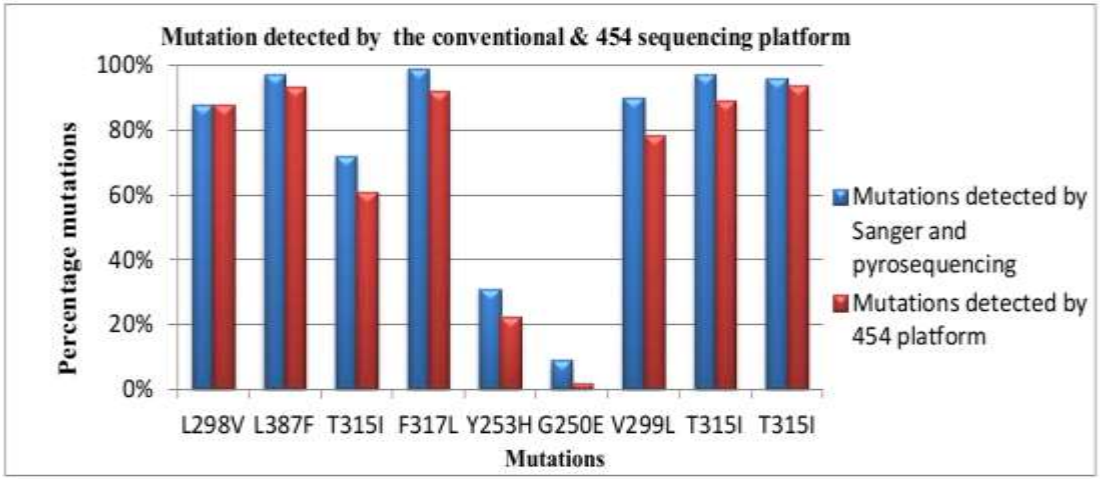
2. Repeatability



Soverini et al, Blood 2013

3. Accuracy

- ✓ 554 CML samples analyzed, including:
  - 517 clinical samples analyzed in parallel by UDS and SS;
  - 30 clinical samples analyzed in parallel by UDS, SS and conventional pyrosequencing
- ✓ 394/398 (99%) variants detected by SS were also detected by UDS
- ✓ Very good concordance in the estimation of variant abundance between UDS, SS and conventional pyrosequencing



Soverini et al, ASH 2013

4. Inter-laboratory reproducibility

- ✓ identical aliquots of 22 plasmids containing wild-type or mutated BCR-ABL distributed and analyzed in parallel by the 10 laboratories

	Lab 1 (Prague)	Lab 2 (Bologna)
1	No mutations	No mutations
2	E255V (36.9%) F311L (60%)	E255V (33.7%) F311L (56.8%)
3	M244V (33.1%) E282K (14.7%) T315I (18.3%)	M244V (36.5%) E282K (14.5%) T315I (16.3%)
4	No mutations	No mutations
5	M244V (99.8%) E282K (41.4%)	M244V (100%) E282K (40.8%)
6	H396P (11.5%)	H396P (12.7%)
7	No mutations	No mutations
8	No mutations	No mutations
9	No mutations	No mutations
10	F311L (2.1%)	F311L (1.8%)
11	L387M (4.3%)	L387M (4.9%)
12	No mutations	No mutations
13	E255V (3.3%)	E255V (2.7%)
14	M351T (18.2%)	M351T (18.5%)
15	No mutations	No mutations
16	E255K (7.2%)	E255K (8.6%)
17	M351T (45.1%)	M351T (45.5%)
18	E255K (68.5%) K357E (16.5%) S385S (6.3%)	E255K (67.8%) K357E (12.4%) S385S (4.6%)
19	T315I (99.7%)	T315I (100%)
20	M244V (64.4%) E282K (35.4%)	M244V (61.8%) E282K (32.9%)
21	Y253F (17.9%)	Y253F (19.8%)
22	M351T (81.1%)	M351T (81.8%)

Soverini and Machova Polakova, unpublished 2014

The Italian 'NEXT-IN-CML' study

STUDY TITLE:

"NEXT-GENERATION SEQUENCING FOR BCR-ABL KD MUTATION SCREENING IN PHILADELPHIA CHROMOSOME-POSITIVE LEUKEMIAS"

STUDY ACRONYM: "NEXT-IN-CML"

Prospective Investigational Multi-Center Tissue Study

Study Sponsor:
Unità Operativa di Ematologia – Azienda Ospedaliero-Universitaria Sant'Orsola-Malpighi - Bologna

Creazione di un network di 5 Laboratori di riferimento per l'analisi mutazionale in NGS:

- **Orbassano (TO)**
- **Monza**
- **Bologna**
- **Napoli**
- **Catania**

The Italian 'NEXT-IN-CML' study

PHASE A **PHASE B**

Phase A (technical validation phase)

- distribute the NGS protocol and fine tune its performances across the 5 Labs
- verify inter-laboratory reproducibility of results on common set of samples with known mutation status and mutation load

The Italian 'NEXT-IN-CML' study

PHASE A | PHASE B

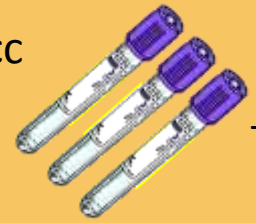
Phase B (clinical validation phase)

- prospectively assess the frequency and clinical relevance of minor mutated clones and compound mutants in patients with failure or warning to TKI therapies
- correlate NGS data with:
  1) baseline disease features, therapy and level of response at the time of sampling
  2) response to subsequent therapy(ies) and 12-month outcome

The Italian 'NEXT-IN-CML' study

CML pts with **failure** or **warning**

PB, 20 cc



+

CRF

Ph+ ALL pts with hematological or molecular **relapse**

BM, 5 cc

+

CRF

Reply and therapeutic decision

Reference Lab

(=geographically closest)

Correlation with clinical data and outcome

CONVENTIONAL SEQUENCING

NGS

Take-Home Messages (I)

BCR-ABL mutation analysis is a precious tool for:

- ✓ **timely selecting those patients who will benefit from a change in the therapeutic strategy**
- ✓ **identifying 'higher risk' patients who will need a more careful monitoring**
- ✓ **tailoring 2GTKI treatment on the individual patient, thus aiming to the best possible outcome**

Take-Home Messages (II)

NGS is being evaluated as a candidate alternative to conventional sequencing for BCR-ABL KD mutation screening

- ✓ **Because we are ready to go**
- ✓ **Because we can afford it**
- ✓ **Because NGS delivers more information – and it's time to assess whether this is clinically useful**

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