TRAINING REGIONALE PER FARMACISTI OSPEDALIERI SU "LEUCEMIA MIELOIDE CRONICA (LMC): NUOVE TECNOLOGIE NUOVI APPROCCI"

Milano, Starhotels Echo

18 febbraio 2016

Il Next Generation Sequencing e la target Therapy

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Introduzione

[…] Con l'obiettivo di facilitare l'accesso del paziente ai trattamenti innovativi il seguente progetto formativo si propone di:

a) ***condividere il valore della terapia innovative*** in ematologia come ulteriore approccio terapeutico che genera speranza di concreti benefici in termini di sopravvivenza ai pazienti affetti da LMC e ALL Ph+;

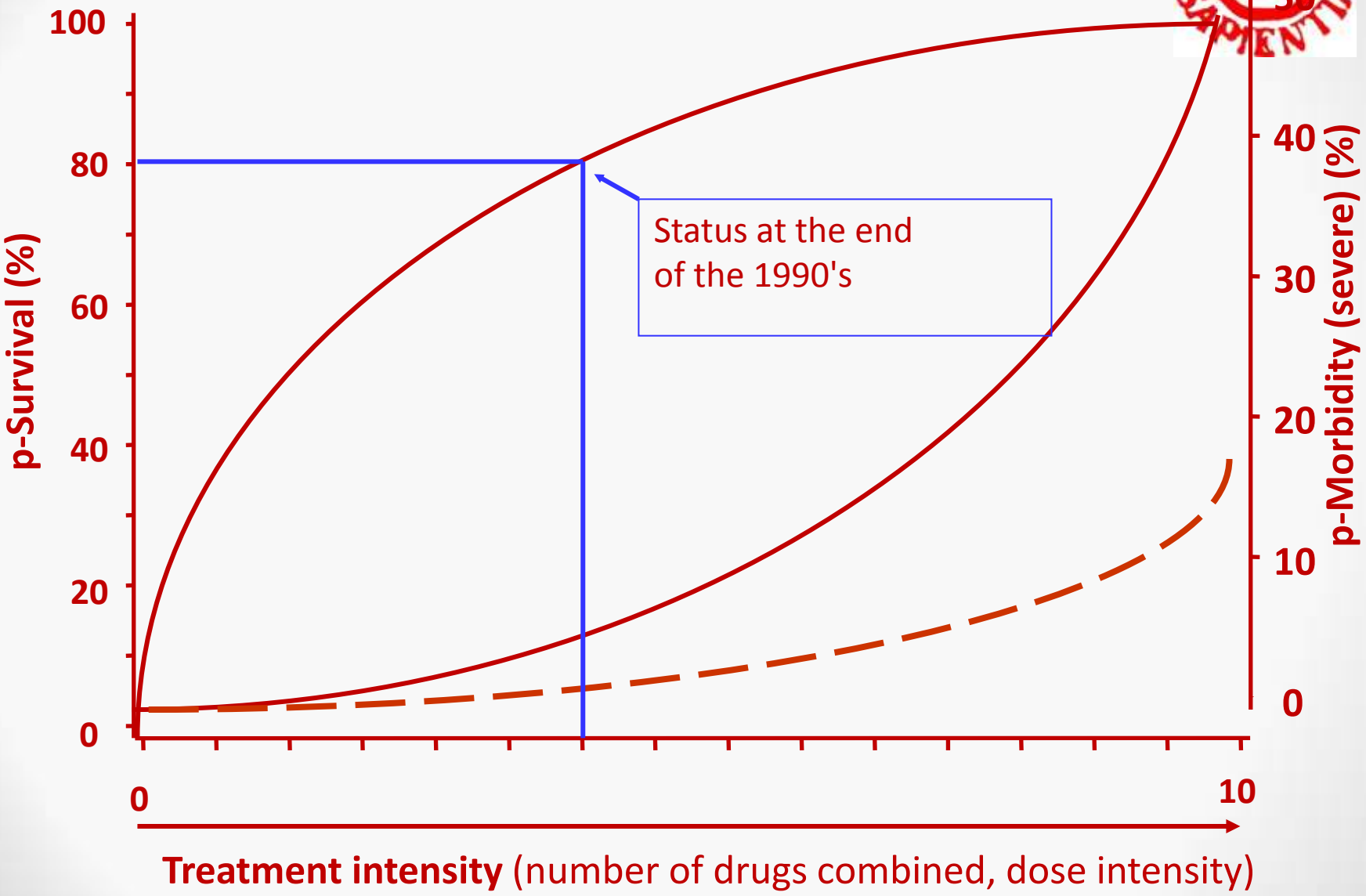
b) **promuovere la cultura delle terapie innovative** al fine di favorire, educare e coinvolgere i Farmacisti Ospedalieri, i Clinici e i Responsabili del controllo di gestione delle ASL/AO nel *corretto reperimento delle risorse e nell'organizzazione del percorso terapeutico in previsione della disponibilità di farmaci innovativi*.

Towards Personalized medicine

- Traditionally, doctors used:
  - Family history
  - Socioeconomic circumstances
  - Environmental factors

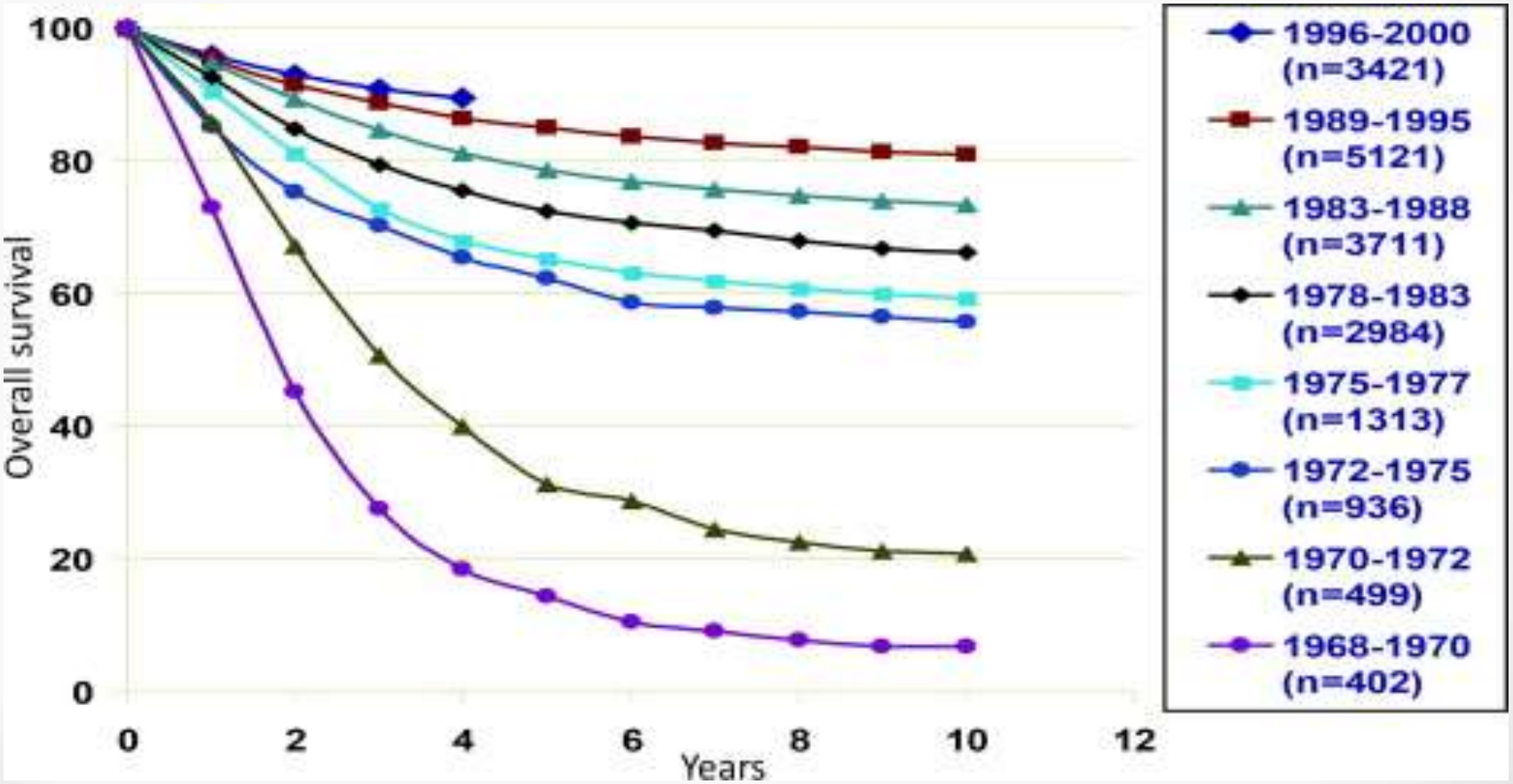
- Now:
  - genomic/genetic testing
  - proteomic profiling
  - metabolomic analysis (study metabolites)

Childhood ALL: The Treatment Dilemma



Courtesy of M.Schrappe

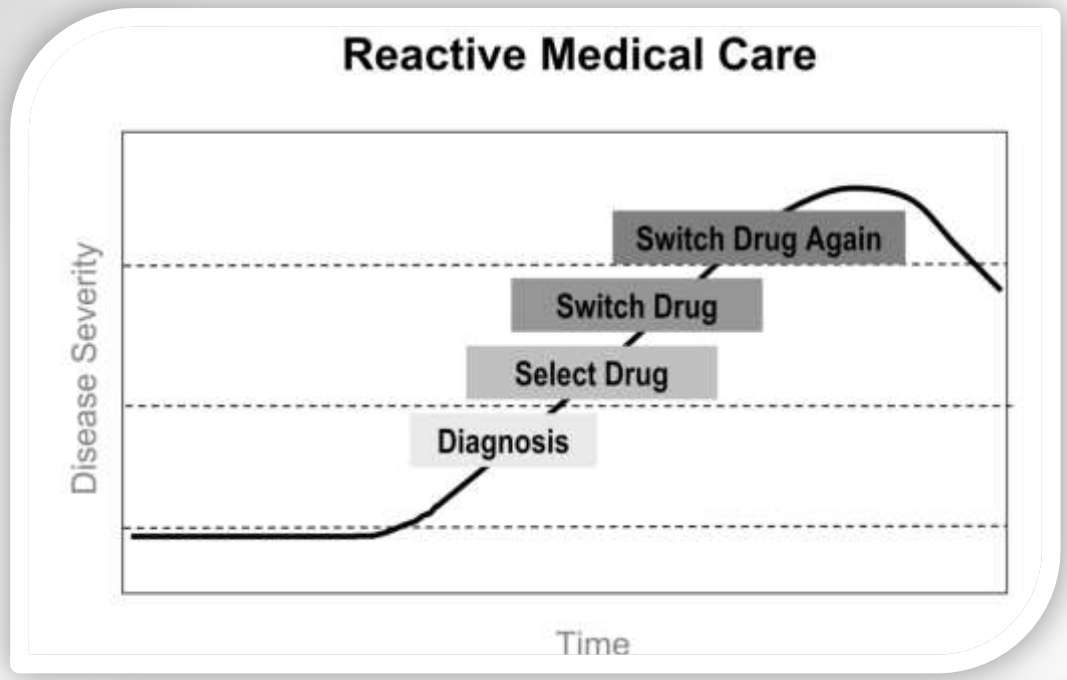
Improved overall survival in childhood acute lymphoblastic leukemia



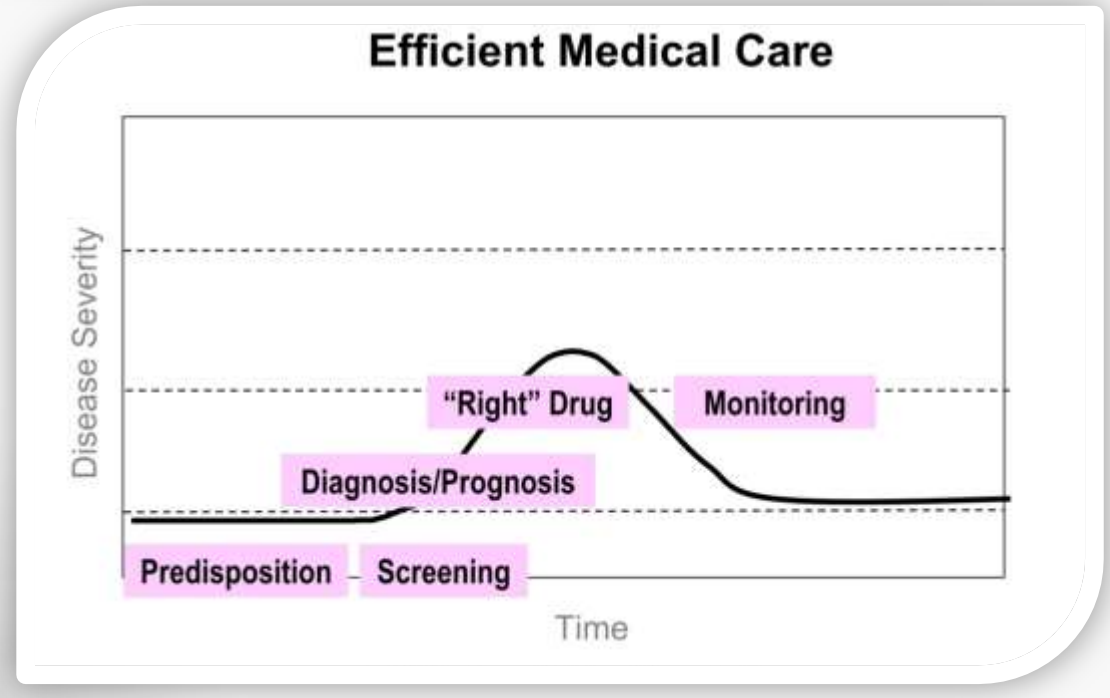
Hunger SP, Pediatr Blood Cancer 2005;45(7):876–80

Childhood ALL trials: criteria for risk group stratification

ALL Study (year)	70	76	79	81	83	86	90	95	2000	20
WBC		x	x					x		
Total leukemic cell mass (RF)				x	x	x	x			
Age		x	x					x		
Sex								x		
CNS		x	x			x	x			
Thymic tumor		x	x			x				
Immunophenotype (T vs non-T)		sP	sP			x	x	x		x
No CR to phase Ia				x	x	x	x	x	x	x
Prednisone response day 8						x	x	x	x	x
MRD									x	x
t(9;22) BCR-ABL							x	x	x	EsPhALL
t(4;11) MLL-AF4								x	x	x
t(12;21) ETV6-RUNX1										x
Ploidy										x

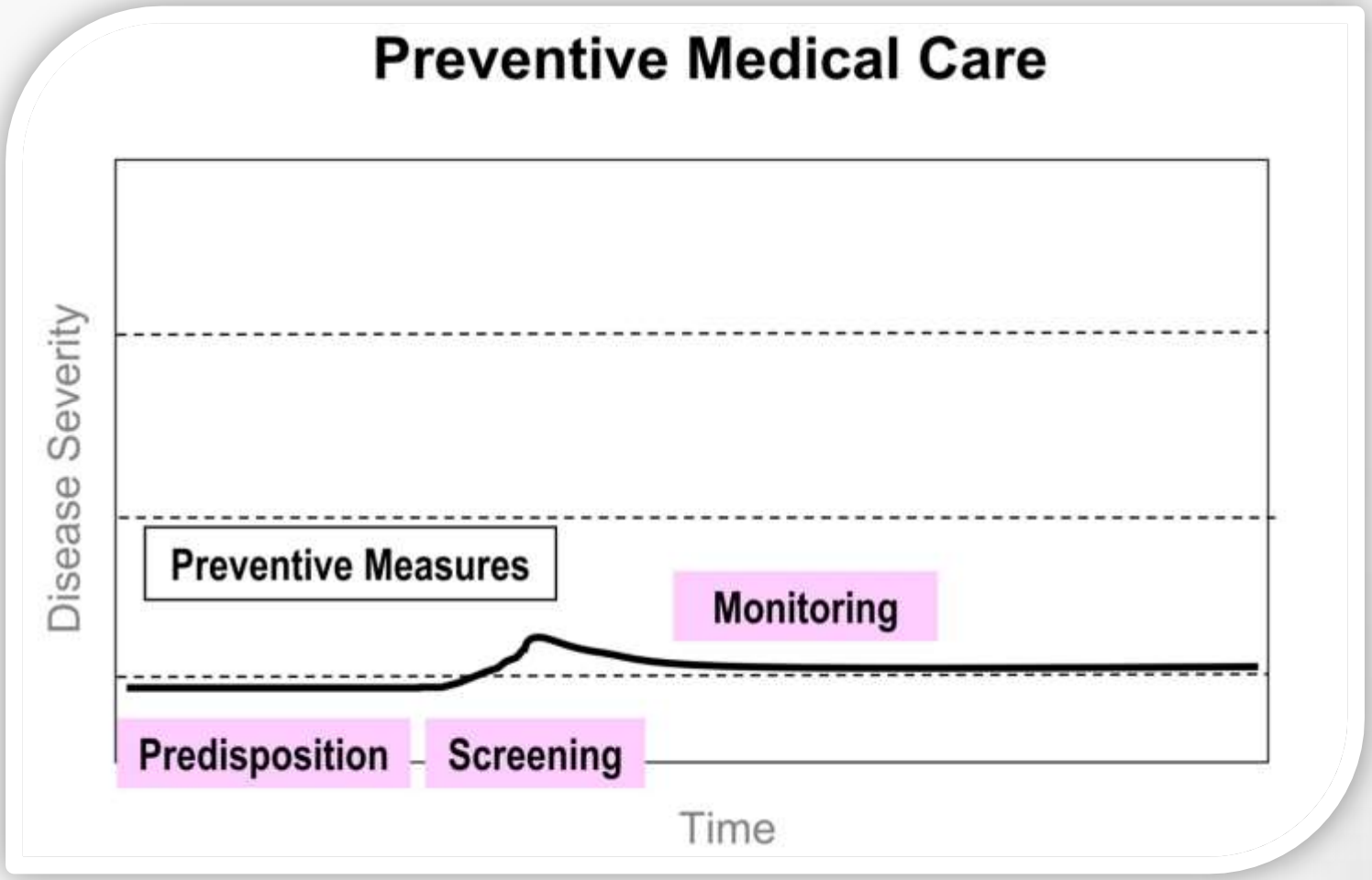


Old Paradigm



New Paradigm

Future Paradigm



Some Definitions

- Biological Marker (Biomarker): A characteristic that is measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. Biomarkers may relate to efficacy, safety, differentiation *etc*.
- Diagnostic: A biomarker that has applicability in clinical use or patient management (*e.g.* to identify a sub-population of patients who would benefit most from a drug).
- Surrogate Endpoint: A biomarker accepted by regulatory agencies as a substitute for a clinical endpoint.

Prognostic vs. Predictive Factors

Prognostic Factor: Any measurement that is associated with clinical outcome in the absence of therapy, or with the application of a standard therapy that all patients are likely to receive (a predictor of the natural history of the disease).

Predictive Factor: Any measurement associated with response or lack of response to a particular therapy, where response can be defined using any of the clinical endpoints commonly used in clinical trials.

Clark GM. Mol Oncol 2008, doi:10.1016/j.molonc.2007.12.001

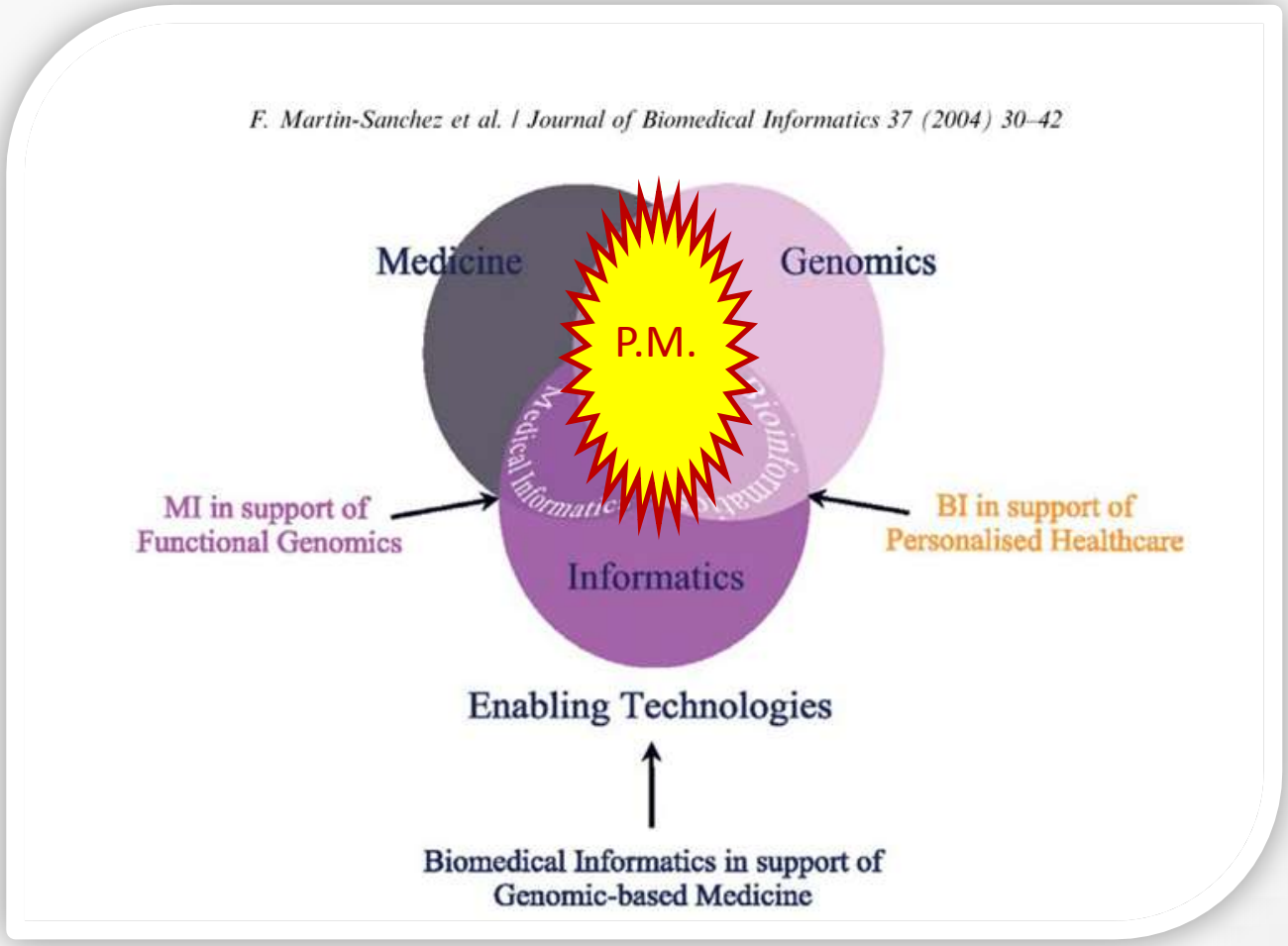
Personalized Medicine: Definition

"Personalized medicine is the use of diagnostic and screening methods to better manage the individual patient's disease or predisposition toward a disease….

"Personalized medicine will enable risk assessment, diagnosis, prevention, and therapy specifically tailored to the unique characteristics of the individual, thus enhancing the quality of life and public health."

– NHLBI Strategic Planning, Theme #10

Requirements of personalized medicine



Pharmacogenetics

- Study of genetic variation that gives rise to different responses to drugs
- It is estimated that genetics can account for 20 to 95 percent of variability in drug disposition and effects.
- Non-genetic factors include: age, organ function, concomitant therapy, drug interactions, and the nature of the disease.

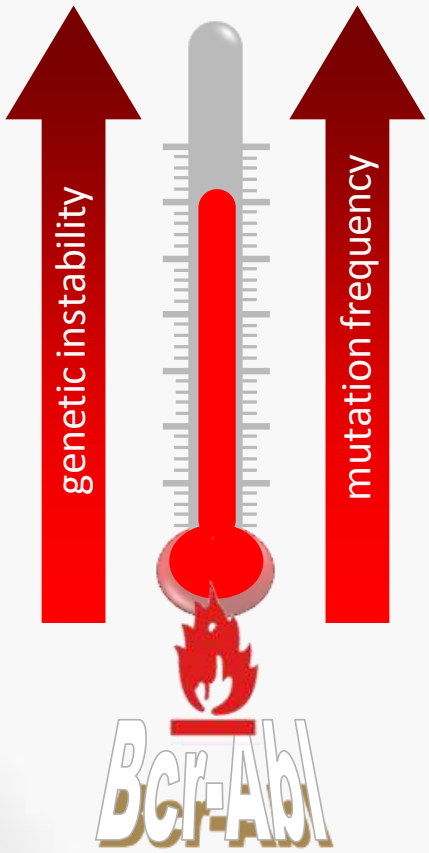
Targeted therapy in Ph+ leukemia

- Why mutation analysis
- When mutation analysis
- How to perform mutation analysis
  - Conventional Sanger sequencing
  - Next generation sequencing
- How to use mutation analysis results

Why BCR-ABL KD mutation analysis?

- Up to 25% of patients does not achieve a satisfactory **response**, and mutations are one of the most common reasons for TKI resistance
- The presence of a mutation (ANY mutation) is a FAILURE, hence it mandates a **change of therapy**
- Mutations are a sign of **genetic instability**, thus identify patients who need to be more carefully followed
- Five of the ten most frequent BCR-ABL KD mutations also confer **resistance to at least one 2G-TKI**

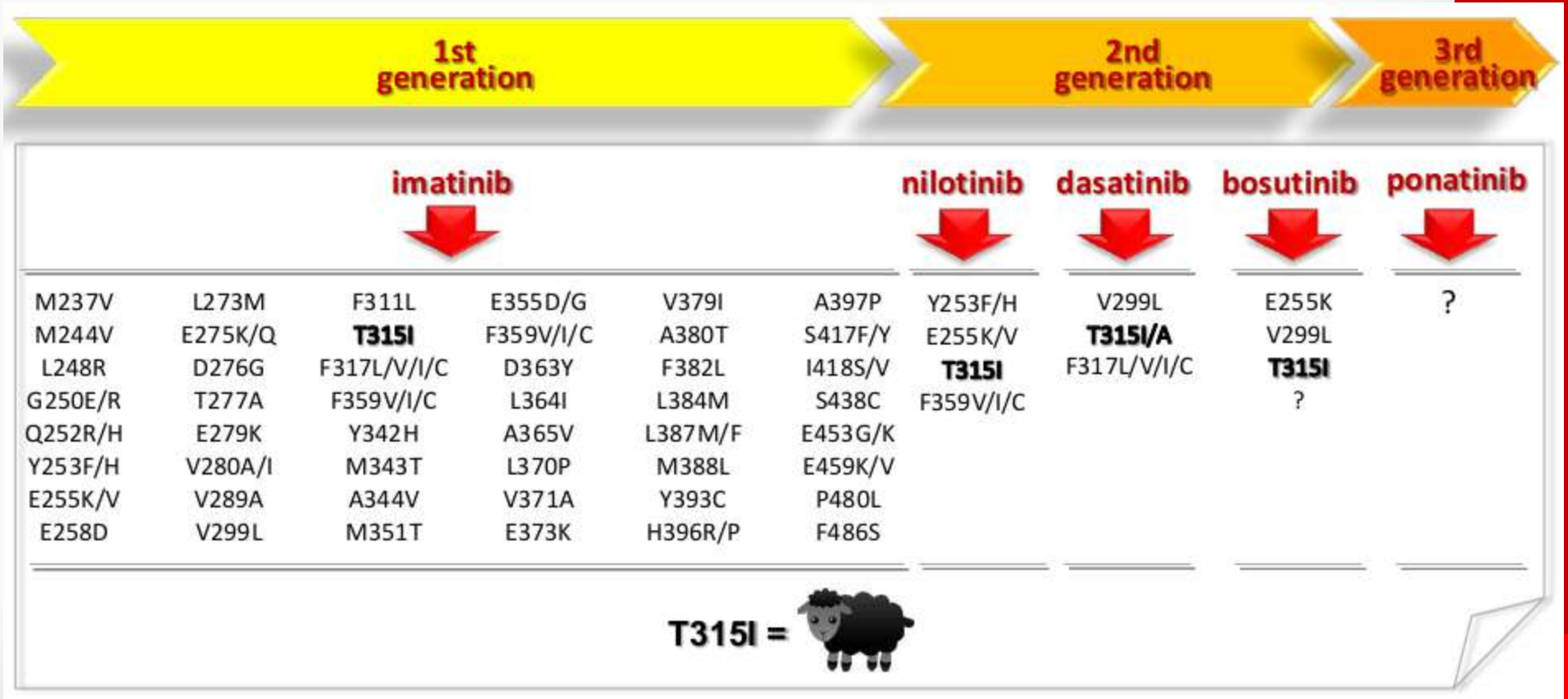
BCR-ABL KD mutations are a sign of genetic instability



- Mutations are not induced by TKI therapy
- TKI therapy simply results in **selection of mutations** independently arisen as a consequence of the genetic instability of the Ph+ clone
- Genetic instability is most probably fostered by Bcr-Abl itself
- The longer Bcr-Abl remains active, or the less efficiently it is inhibited, the higher the mutation rate
- Bcr-Abl is still the key target for therapeutic improvement

Jiang X, et al. J Natl Cancer Inst. 2007;99:680-693

Detection of specific mutations predicts for TKI inefficacy



Resistance to imatinib: the 'top-ten' most frequent mutations

	CP	myBC	lyBC/Ph+ ALL
1°	M351T	T315I	T315I
2°	M244V	M351T	E255K
3°	F359V	G250E	Y253H
4°	H396R	F359V	F359V
5°	G250E	Y253H	M244V
6°	E355G	M244V	M351T
7°	E255K	Q252H	F317L
8°	Y253H	E255K	F311L/I
9°	T315I	H396R	Q252H
10°	F317L	L384M	D276G

Resistant to NILOTINIB
Resistant to DASATINIB
Resistant to BOTH

Soverini et al, ASH 2011

Mutation analysis is thus a precious tool for:

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

When to perform BCR-ABL TKD mutation analysis

- Early detection of BCR-ABL mutations is very important for the therapeutic decisions.
- It is emphasized in the guidelines：mutation analysis should be done in the presence of poor response or treatment failure.

Guideline for CML Management in China 2013[1]	ELN 2013[2]	NCCN2014[3]
• In case of suboptimal response; • In case of treatment failure; • In case of loss of response to current treatments.	• In case of treatment failure in chronic phase; • In case of disease progression to accelerated or blast phases • When considering to switch to alternate TKI therapy.	1.Chronic phase • If there is inadequate initial response(failure to achieve BCR-ABL≤10% at 3 and 6 months） • Any sign of loss of response（defined as hematologic or cytogenetic relapse） • 1-log increase in BCR-ABL1 transcript levels and loss of MMR 2. Disease progression to accelerated or blast phase.

1..Chinese Society of Hematology. Chinese Journal of Hematology 2013;34(5): 464-470. 2.Baccarani M et al. Blood 2013;122(6): 872-884. 3.NCCN v2014.2.

How to perform mutation analysis

Conventional Sanger sequencing is still the recommended method

NCCN Guidelines

National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2014
Chronic Myelogenous Leukemia

- Chronic phase :
  - If there is inadequate initial response(failure to achieve PCyR or BCR-ABL≤10%[IS] at 3 and 6 months or CCyR at 12 and 18 months）
  - Any sign of loss of response（defined as hematologic or cytogenetic relapse）
  - 1-log increase in BCR-ABL1 transcript levels and loss of MMR
- Disease progression to accelerated or blast phases.

ELN Recommendations

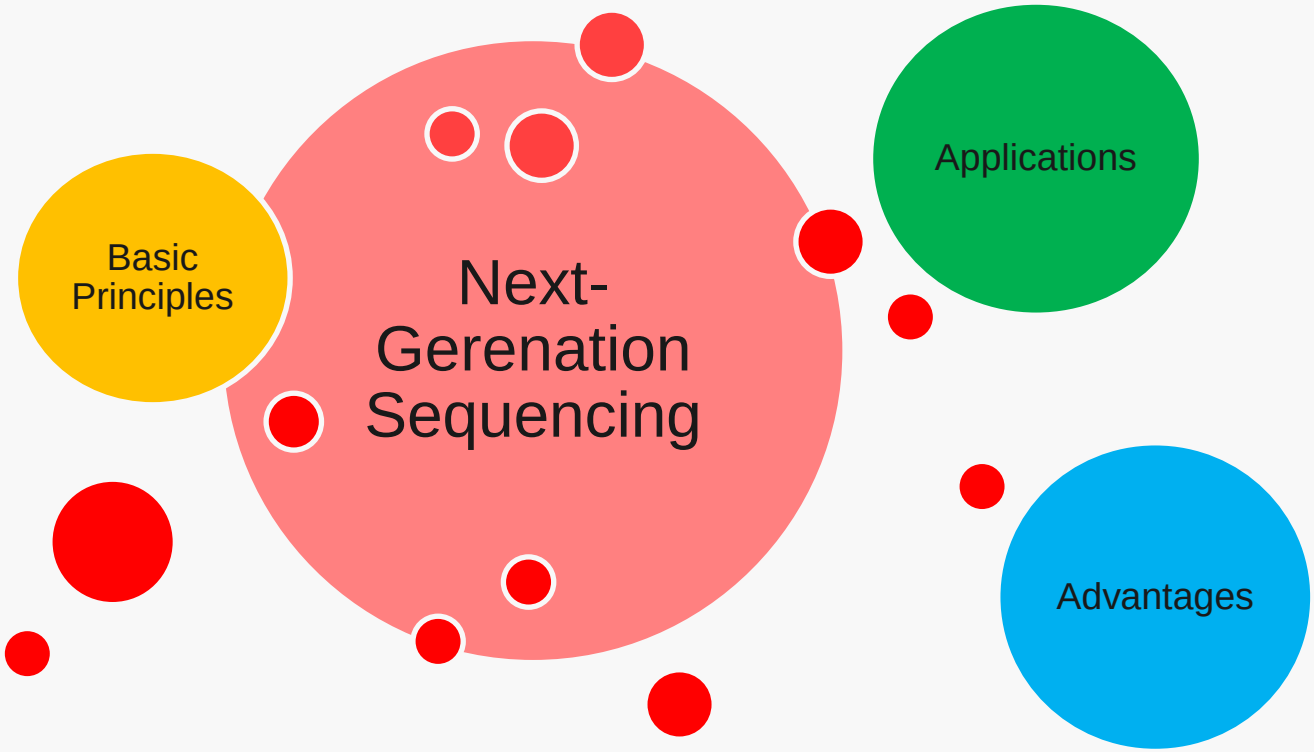
blood
Prepublished online June 26, 2013;
doi:10.1182/blood-2013-05-501569

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

It is recommended to perform BCR-ABL KD mutation analysis, or point mutation analysis (non ABL1 genetic polymorphism testing) using conventional Sanger sequencing in case of warning, treatment failure or disease progression to AP or BP.

1. NCCN CML Guideline v2014.2
2. Baccarani M et al. Blood 2013;122(6): 872-884.

Next-Generation Sequencing



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

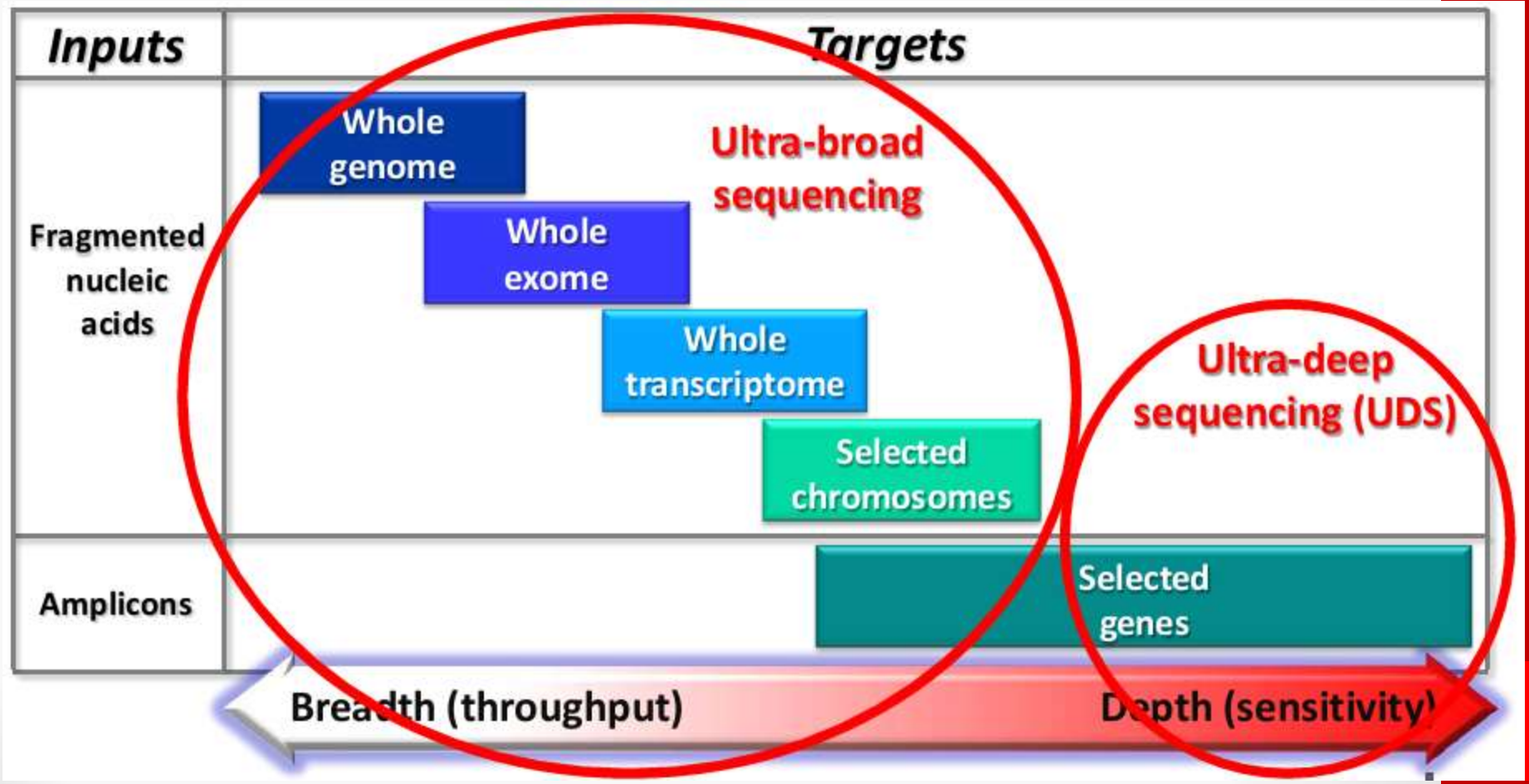
↓

Parallel sequencing of the clones

↓

Hundred thousands to millions sequence reads

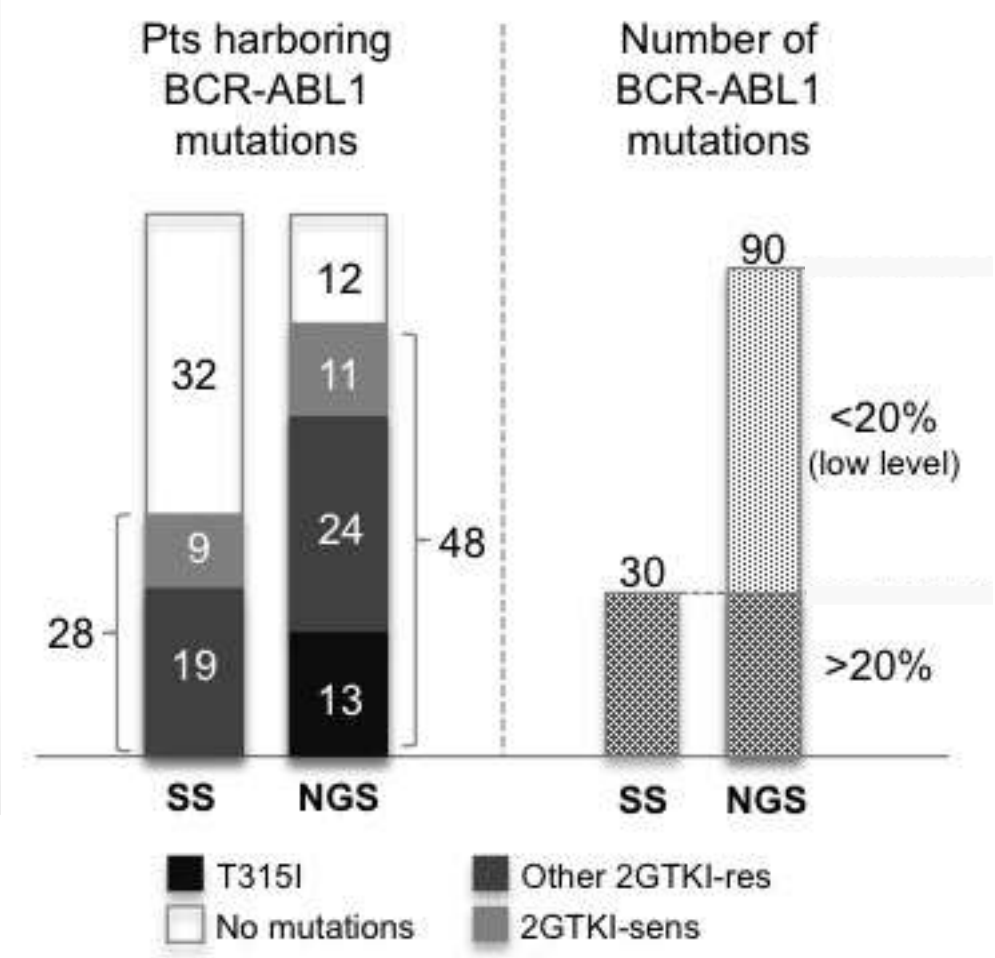
Next-Generation Sequencing: applications

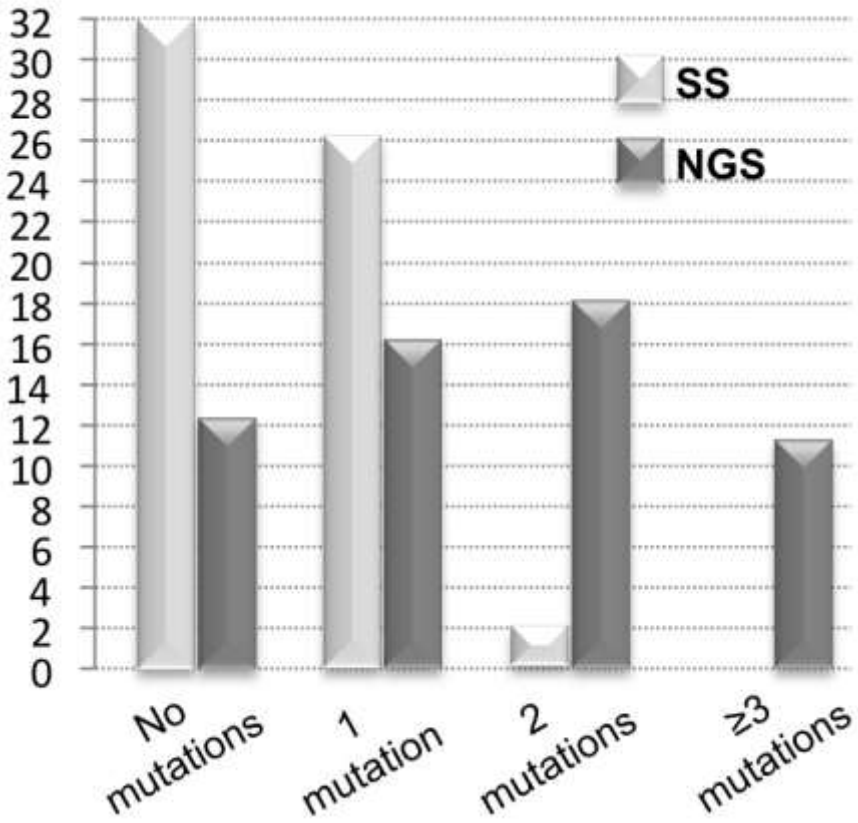


Next-Generation Sequencing: advantages

- Higher sensitivity
- Possibility to fully characterize the spectrum of minor mutated variants
- Clonal analysis and discrimination between compound and polyclonal mutations
- Quantitative analysis of the dynamics of mutant populations over time

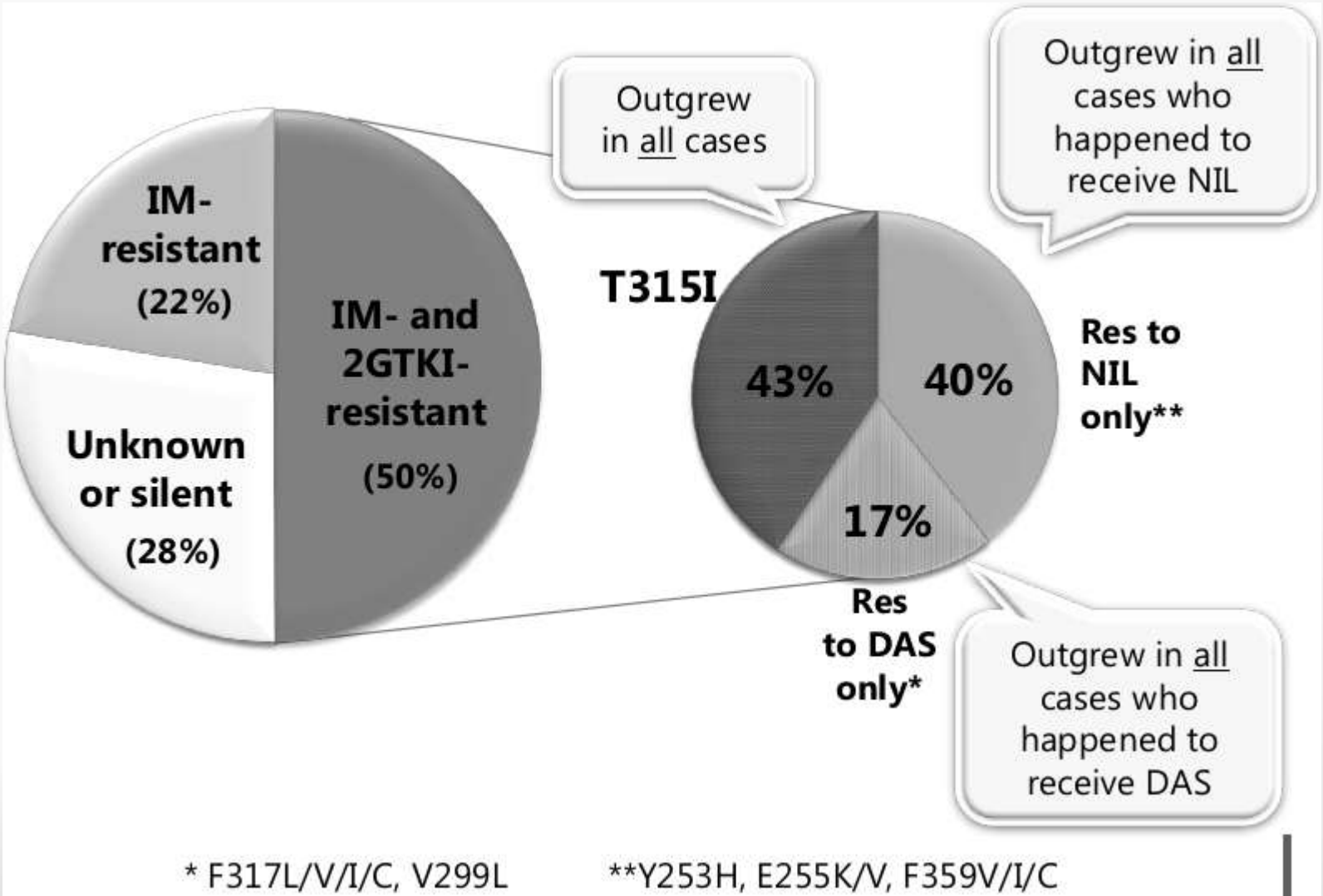
NGS at the time of switchover allows to detect 2G TKI-resistant mutations in more cases





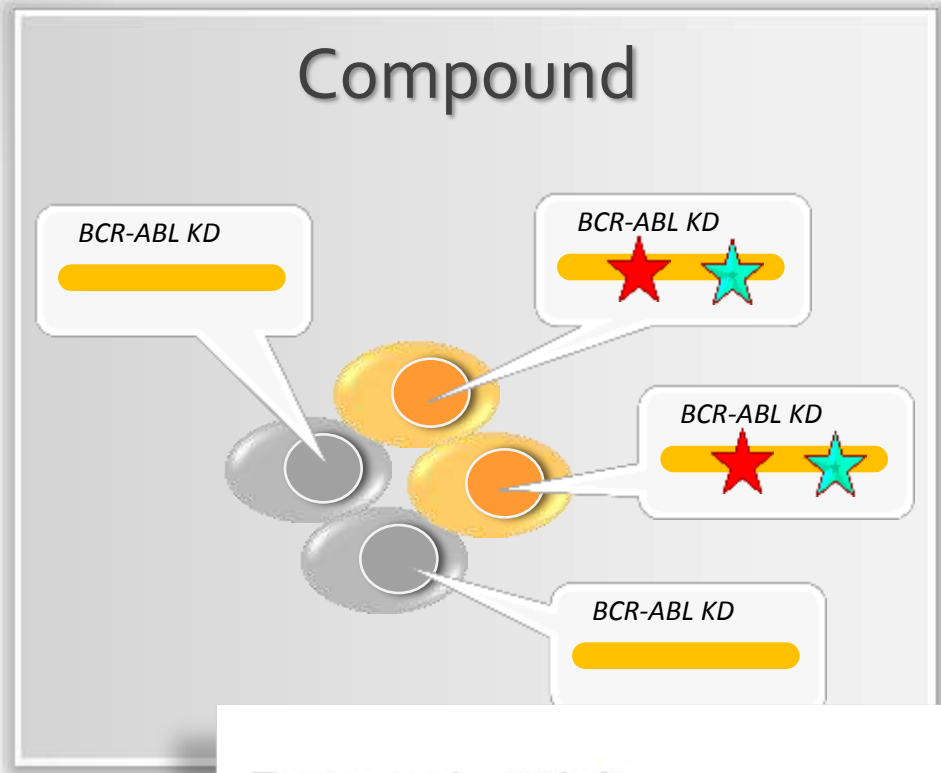
Soverini et al, Oncotarget 2016

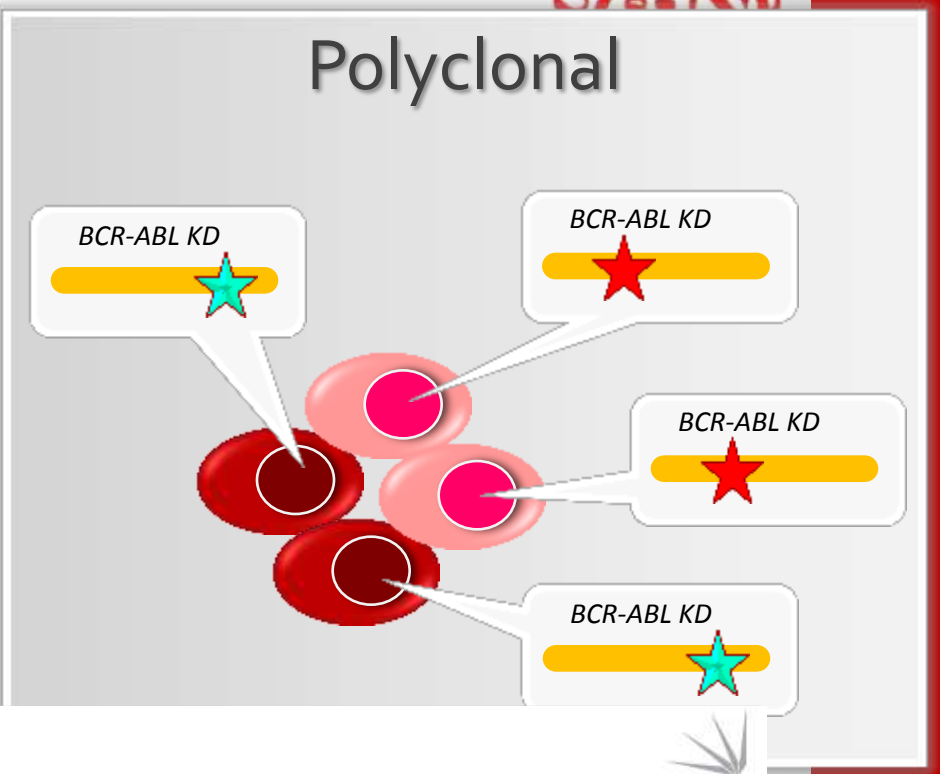
Resistance profile of the 60 low level mutations detected at switchover by NGS



Compound mutations
Unity is strength

Two biologically different scenarios





Research article

Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCR-ABL mutations with altered oncogenic potency

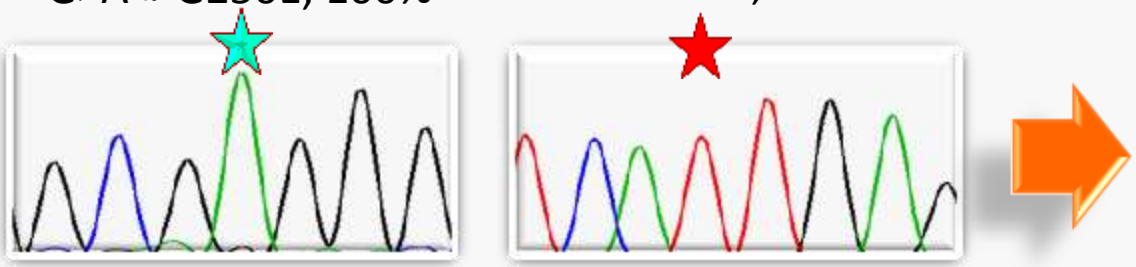
Neil P. Shah,[1] Brian J. Skaggs,[2] Susan Branford,[3] Timothy P. Hughes,[3] John M. Nicoll,[2] Ronald L. Paquette,[2] and Charles L. Sawyers[2,4]

The Journal of Clinical Investigation http://www.jci.org Volume 117 Number 9 September 2007

Compound mutants cannot easily be inferred by Sanger sequencing

G>A » G250E, 100%

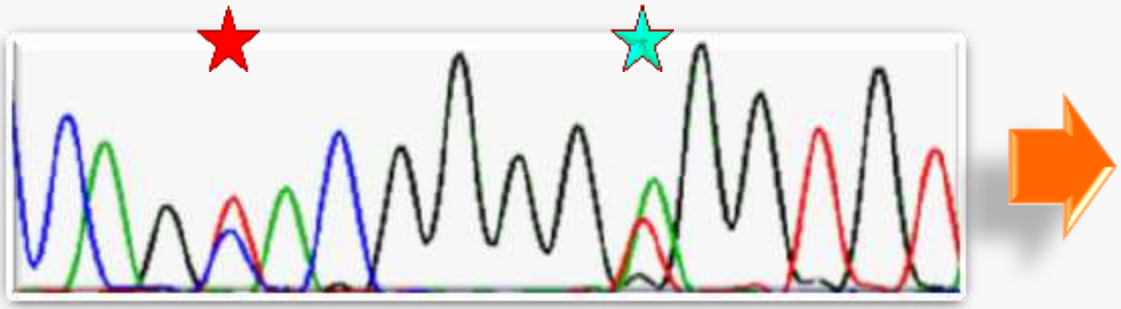
C>T » T315I, 100%



must be a single clone
G250E+T315I

T>C » Y253H, 40%

A>T » E255V, 40%



?

```
CACAAGCTGGGCGGGGGCCAGTACGGGGAGGTGTACGAGGGCGTGTGGAAGA
CACAAGCTGGGCGGGGGCCAGTACGGGGTGGTGTACGAGGGCGTGTGGAAGA
CACAAGCTGGGCGGGGGCCAGCACGGGGAGGTGTACGAGGGCGTGTGGAAGA
CACAAGCTGGGCGGGGGCCAGCACGGGGAGGTGTACGAGGGCGTGTGGAAGA
CACAAGCTGGGCGGGGGCCAGTACGGGGTGGTGTACGAGGGCGTGTGGAAGA
CACAAGCTGGGCGGGGGCCAGTACGGGGAGGTGTACGAGGGCGTGTGGAAGA
CACAAGCTGGGCGGGGGCCAGTACGGGGAGGTGTACGAGGGCGTGTGGAAGA
```

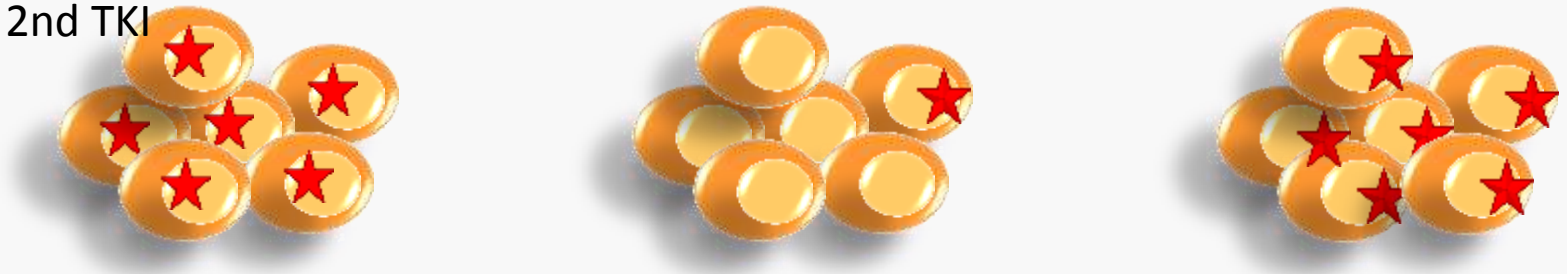
Two hits are most frequently the result of sequential TKI therapy

Relapse to 1st TKI → **Relapse to 2nd TKI**

1. Two mutations are sequentially acquired within a short timeframe

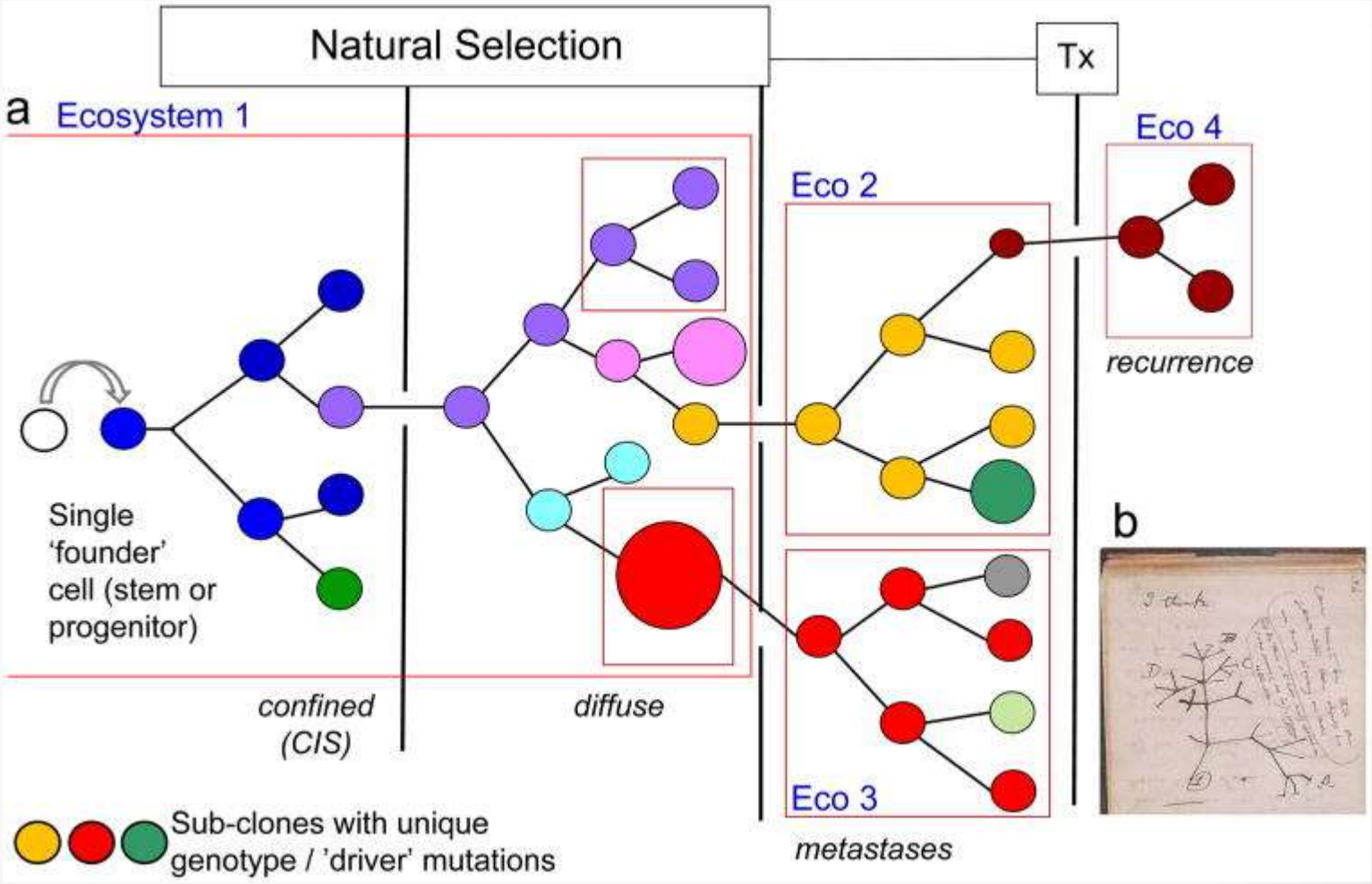
Very rare

2. The baseline mutant is eradicated and later replaced by another one conferring resistance to the 2nd TKI



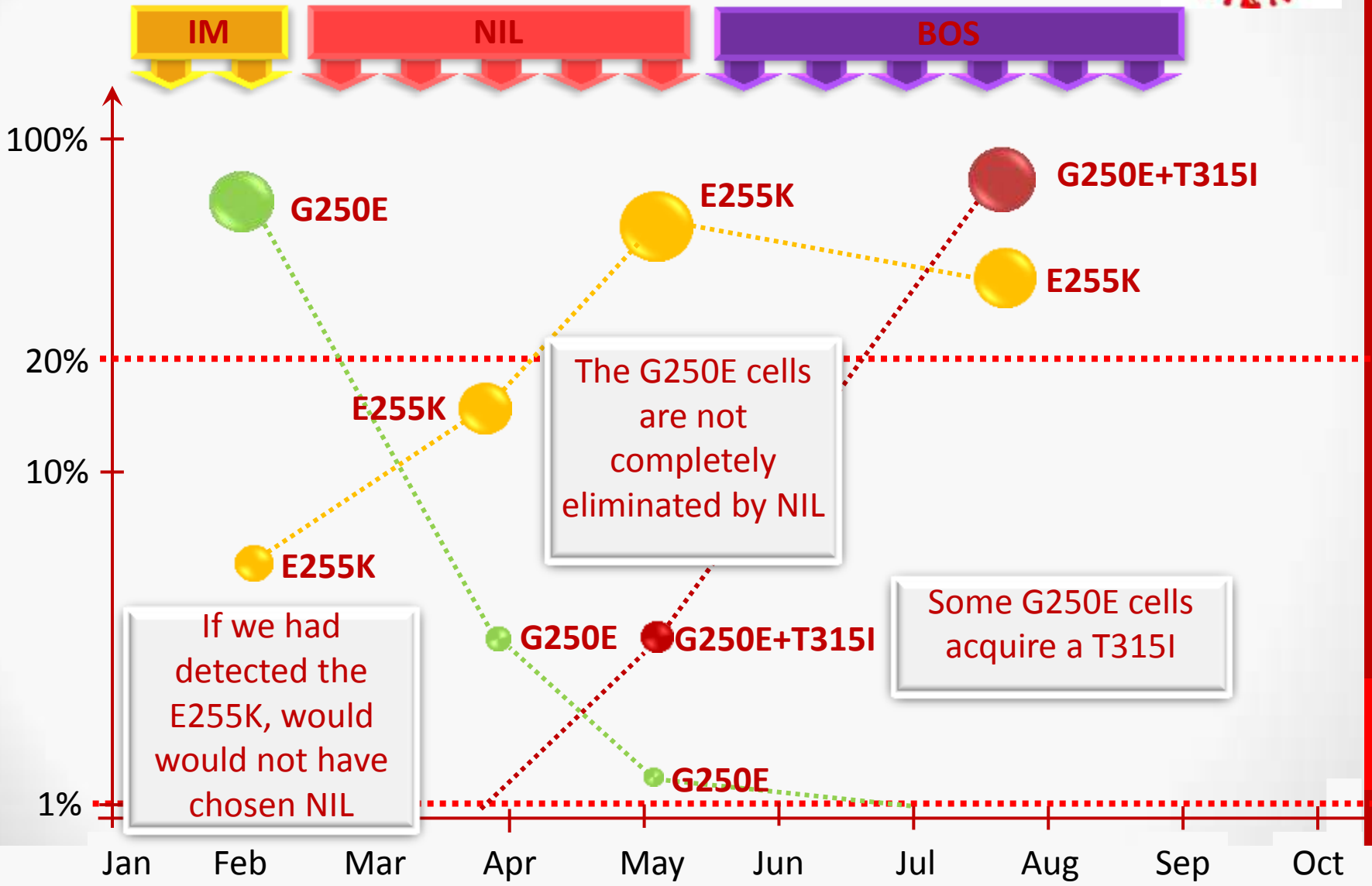
3. The baseline mutant persists and later acquires an additional mutation further increasing its fitness

Selective pressures on branching clonal architecture of clonal evolution in leukemia

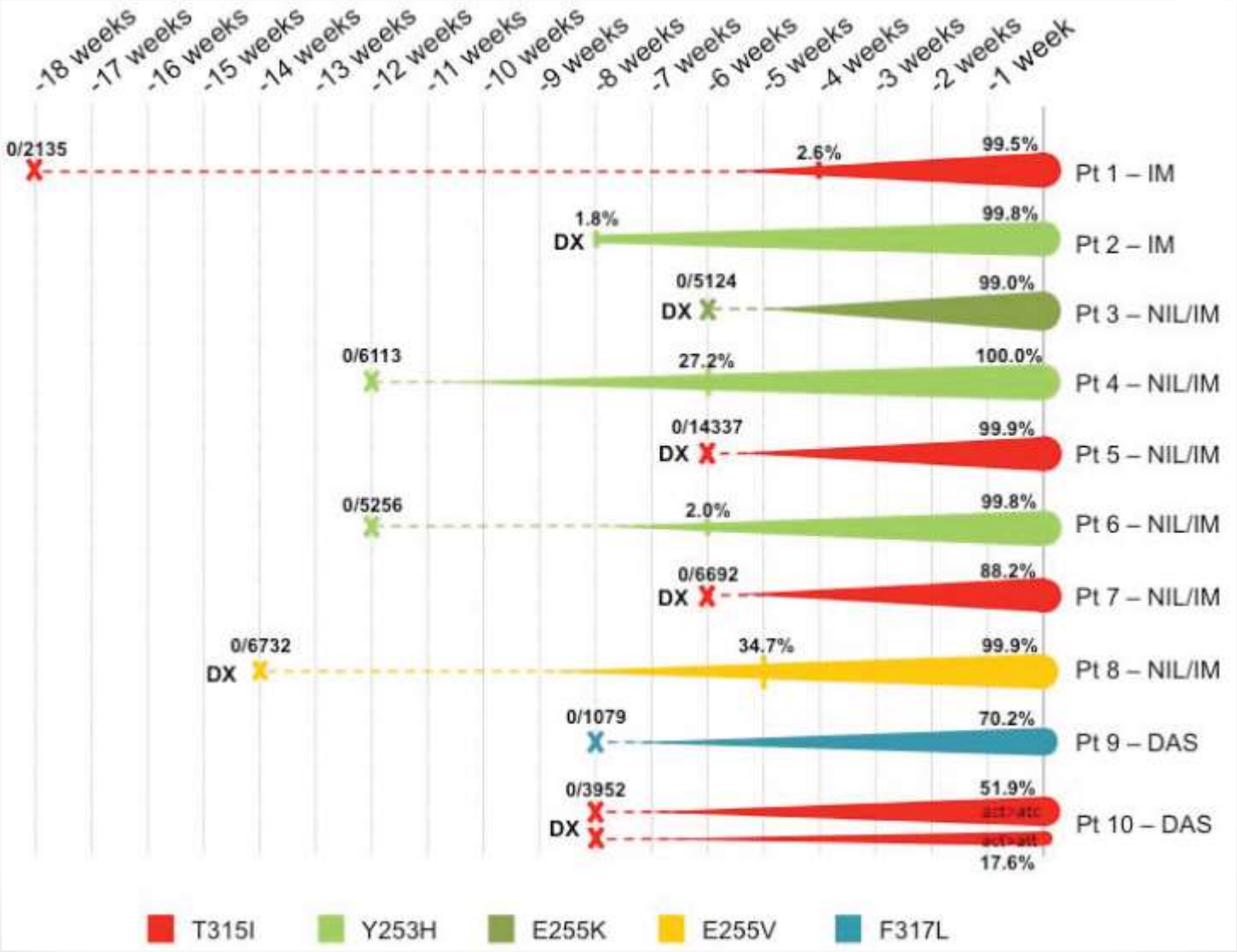


Greaves M, Nature 2012;481:306

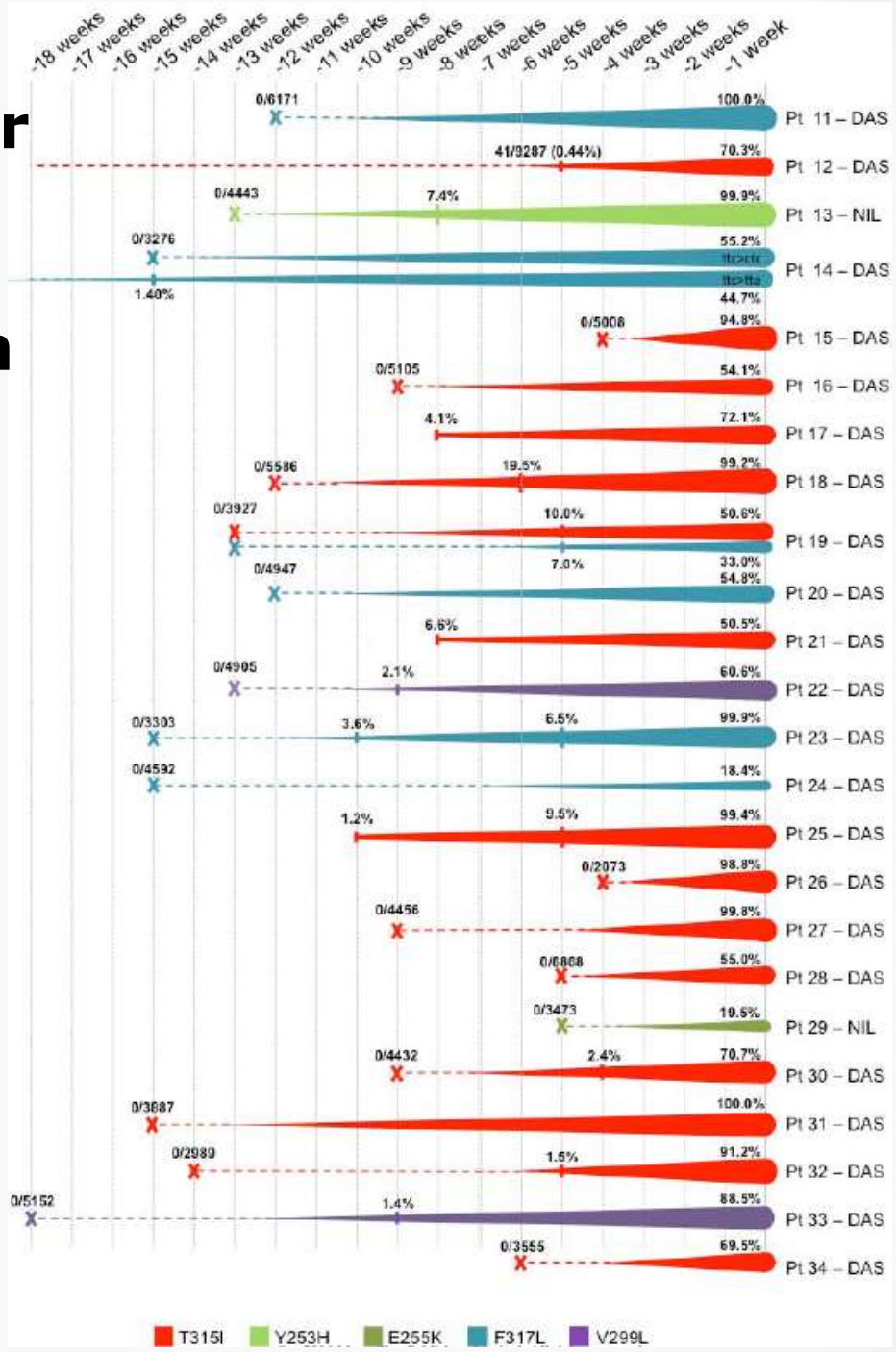
The complex and dynamic landscape of mutant populations can be best followed by NGS



Time course of resistance-driver mutations in ALL patients who relapsed on first-line TKI therapy



Time course of resistance-driver mutations in ALL patients who relapsed on second-line TKI therapy

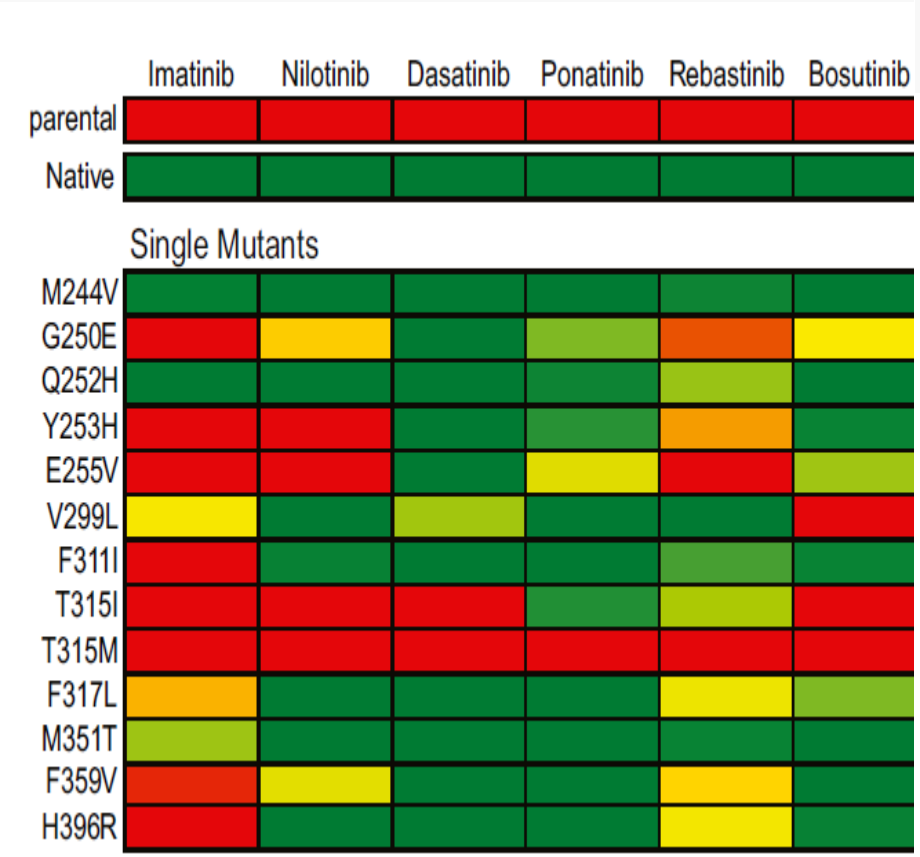


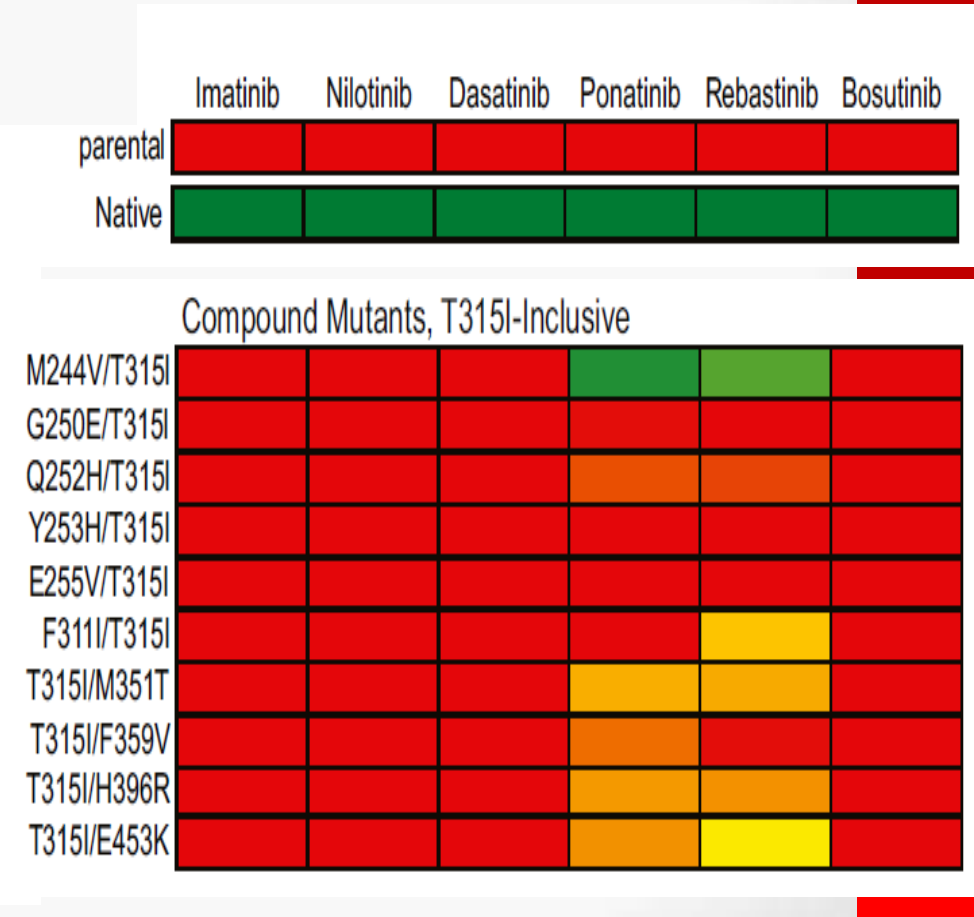
How to use mutation analysis results: the 2016 options

• **T315I:**	
Ponatinib or HSCT	
• **T315A, F317L/V/I/C:**	
Consider nilotinib or bosutinib rather than dasatinib	
• **Y253H, F359V/C/I:**	
Consider dasatinib or bosutinib rather than nilotinib	
• **V299L:**	
Consider nilotinib rather than bosutinib or dasatinib	
• **E255K/V:**	
Consider dasatinib rather than bosutinib or nilotinib	
• **Any other mutation:**	
Consider dasatinib or nilotinib or bosutinib	

Ponatinib may also be an option if dasatinib and nilotinib already failed

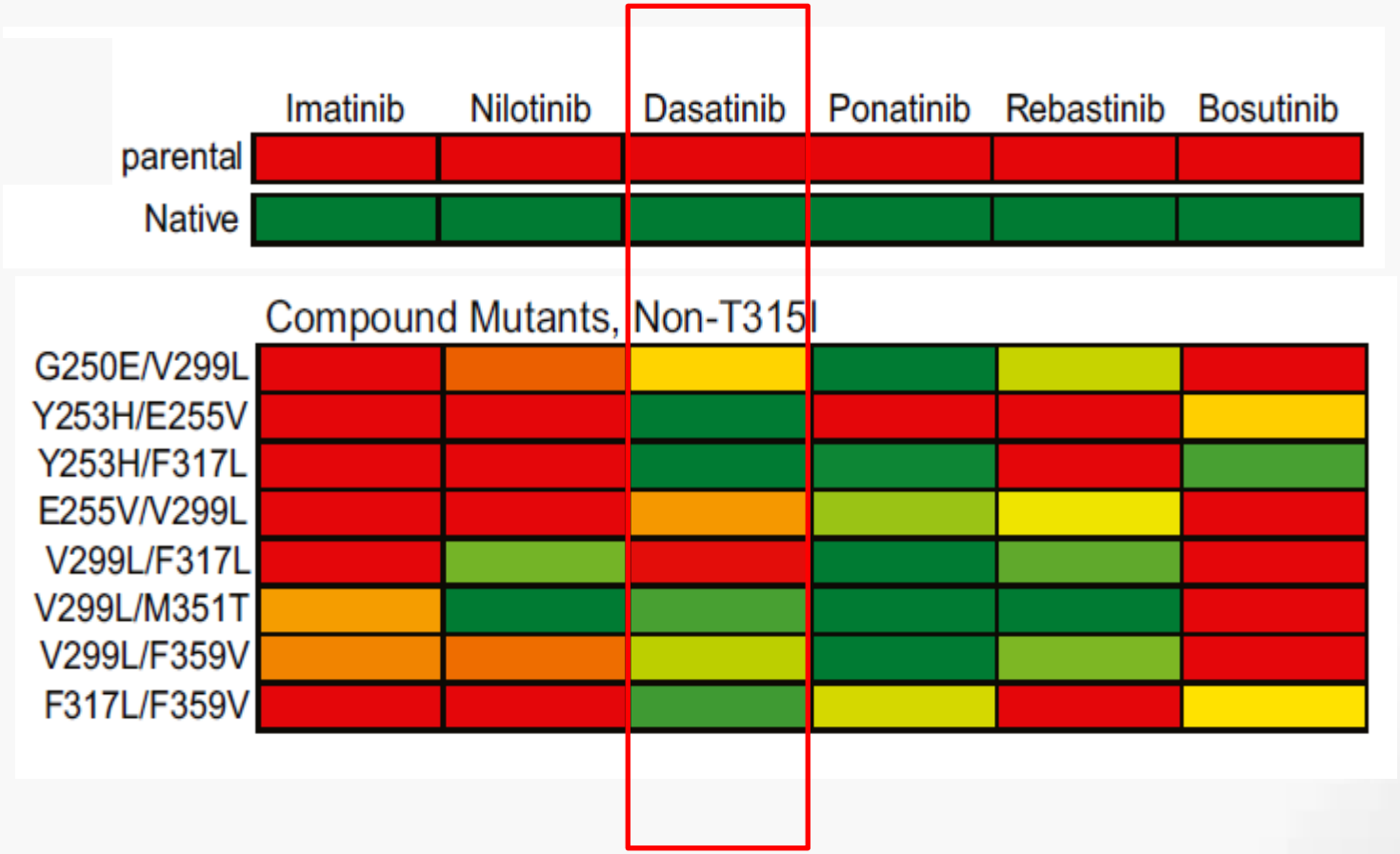
T315I-inclusive compound mutants confer resistance to all TKIs





Zabriskie M. S, et al. Cancer Cell. 2014,26, 428–442,

Nearly all non-T315I compound mutants are sensitive to Dasatinib



Zabriskie M. S, et al. Cancer Cell. 2014,26, 428–442,

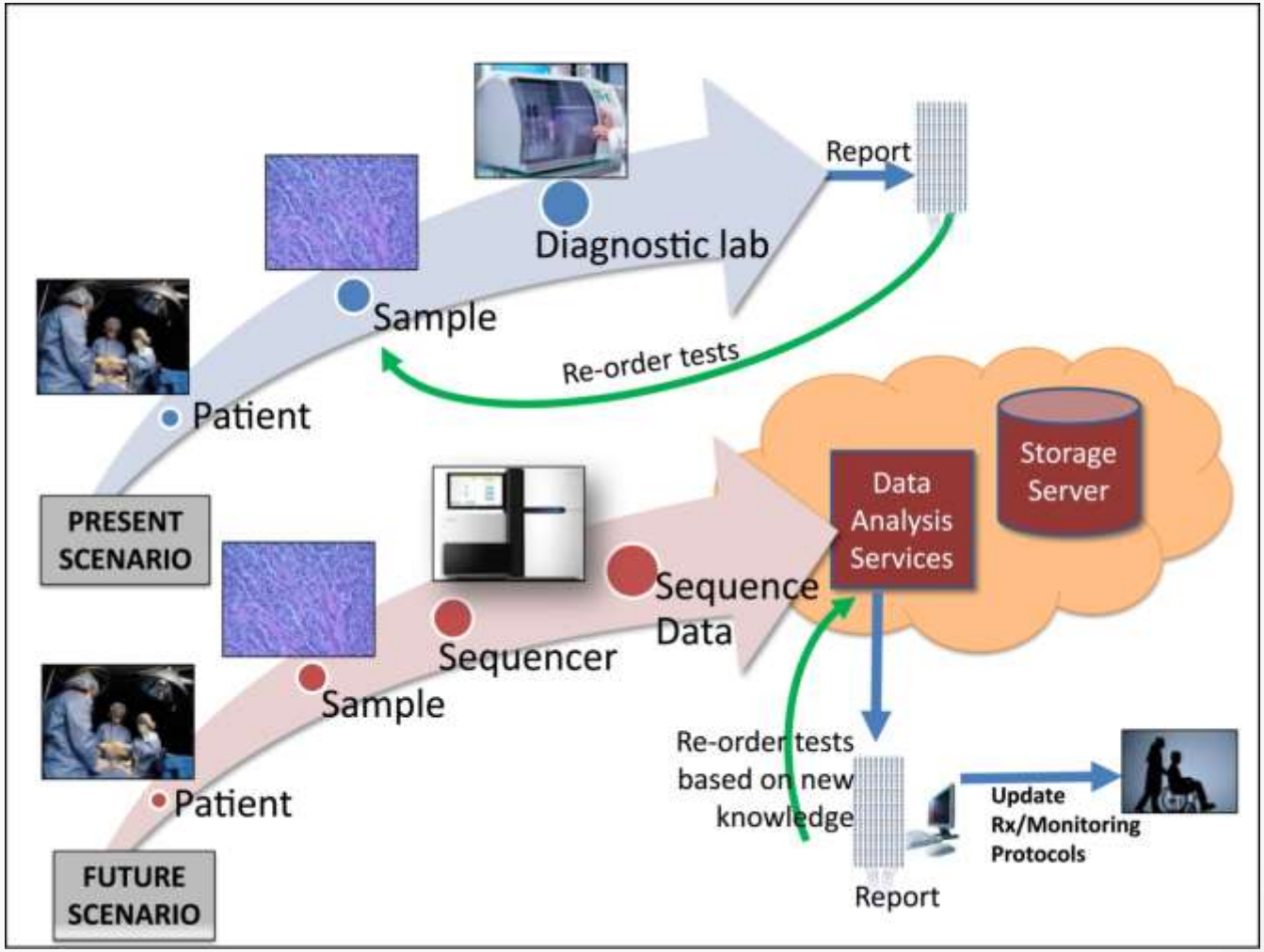
Conclusion /1

- To achieve optimal long-term outcomes, best use of molecular analysis tools and therapeutic opportunities is fundamental
- BCR-ABL mutation analysis is important since
  - precious information on the biology of the disease (genetic instability)
  - tailor 2GTKI treatment on the individual patient
- According to the guidelines, BCR-ABL KD mutation analysis should be performed once treatment response appears “unsatisfactory”

Conclusion /2

- Conventional Sanger sequencing is still the recommended method for BCR-ABL KD mutation screening
- NGS is a robust, powerful and versatile technology which is becoming accessible to a wider and wider number of diagnostic laboratories
- NGS has been shown to paint a more accurate picture of BCR-ABL KD mutation status, especially at the time of switch to 2G-TKIs, and is likely to replace conventional sequencing soon

A new scenario



NGS Application
Other Considerations

NGS- significant false positive rate → Mutation confirmation

Variable % tumor cells and variable % tumor cells with (presumably) secondary mutation →

- May overlap with NGS false positive rate
- Low level mutations- not easily confirmed by Sanger sequencing (limit 15-20%)
- Numerous heterogeneous aberrations- i.e. oncologic applications need algorithm development

Bioinformatics

- NGS diagnostics - shifted towards data analysis
- NGS infrastructures must consist of appropriate expertise and computational hardware
- Unprecedented amounts of medical data and various processing algorithms necessitate adequate tools for

  - Data management (alignment and assembly)

  - QC of image processing, base calling, filtering, alignment, SNP finding/application steps archiving

NGS Application
Other Considerations

NGS data density = frequently encountered variants of unknown significance

→

- Which variants are clinically actionable?
- Development of evidence-based scientific standards to evaluate
- utility in in different patient populations for accurate risk estimation
- Risk of over interpretation, unnecessary medical action, unwarranted psychological stress
- Careful selection of patients for genome sequencing and genetic counseling-crucial

Need for standardized guidelines and quality control

We look to a future in which medicine will be predictive, preventive, preemptive and *personalized*

Thanks

Simona Soverini

Federica Mottadelli

Lab.Tettamanti - Monza