2020

Progetto
Ematologia
Romagna

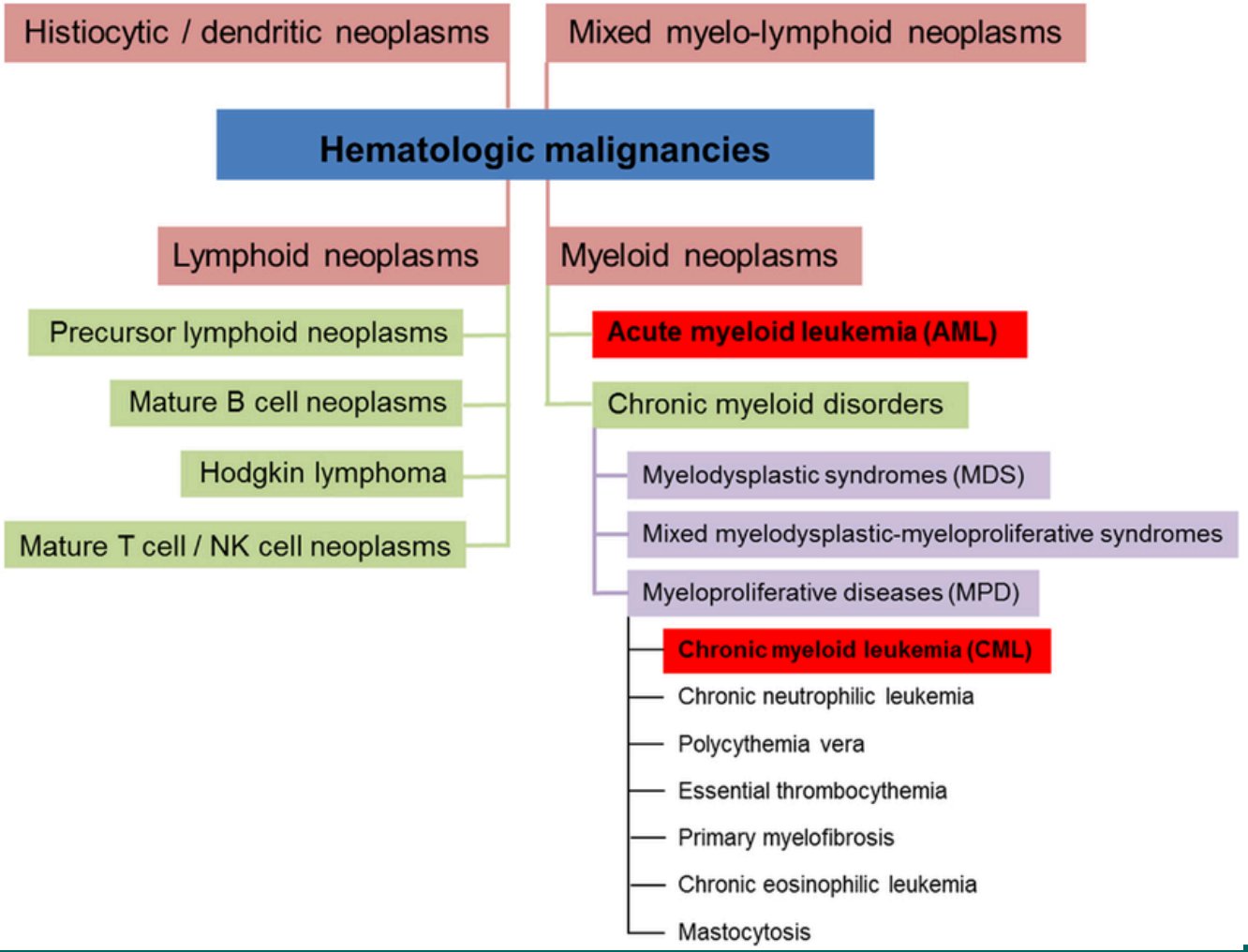
L'impatto delle nuove tecnologie nella diagnosi e nella terapia personalizzata delle leucemie

Samantha Bruno

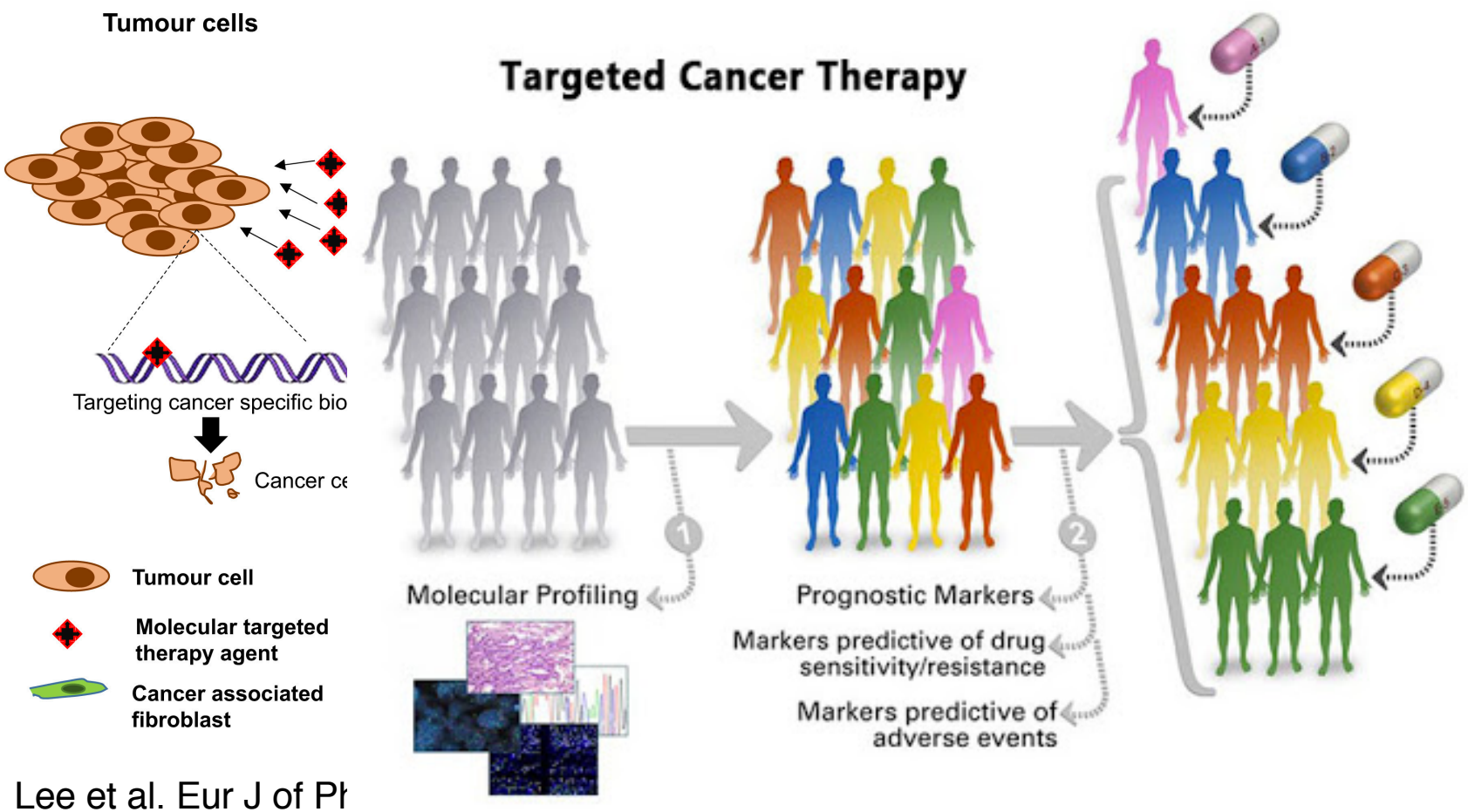
Disclosures

I have nothing to disclose

The heterogeneity of hematologic malignancies



Targeted drug delivery: a challenge to hit tumor cells



Lee et al. Eur J of Pl

ic alterations
ıtologic cancers:

t mutations &
ELs

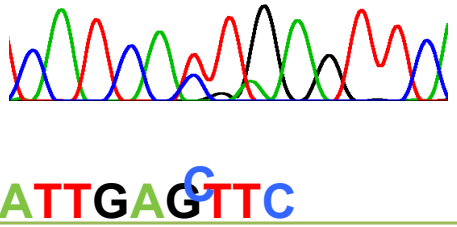
ctural variants

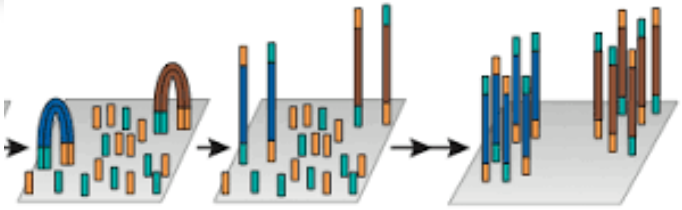
y number
ations

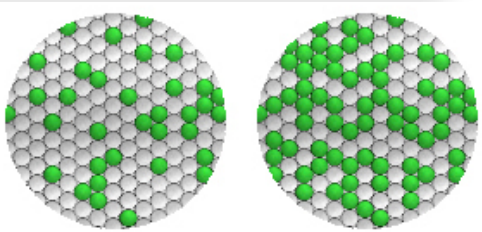
e expression

Sensitive mutations screening techniques for precision medicine

Sanger Seq | **NGS techniques** | **Digital PCR**







VAF (%)	**20**	**5-3**	**0.1-0.01**
COSTS	**900**	**700**	**36**
Exp time	**4gg**	**1-2 weeks**	**1gg**

The Next generation sequencing (NGS) technologies

Illumina

MiSeq NextSeq HiSeq 2500 HiSeq X Ten

Output	15 Gb	120 GB	1000 GB	1800 GB
Number of Reads	25 Million	400 Million	4 Billion	6 Billion
Read Length	2x300 bp	2x150 bp	2x125 bp (2x250 update mid-2014)	2x150 bp
Cost	$99K	$250K	$740K	$10M

Source: Illumina

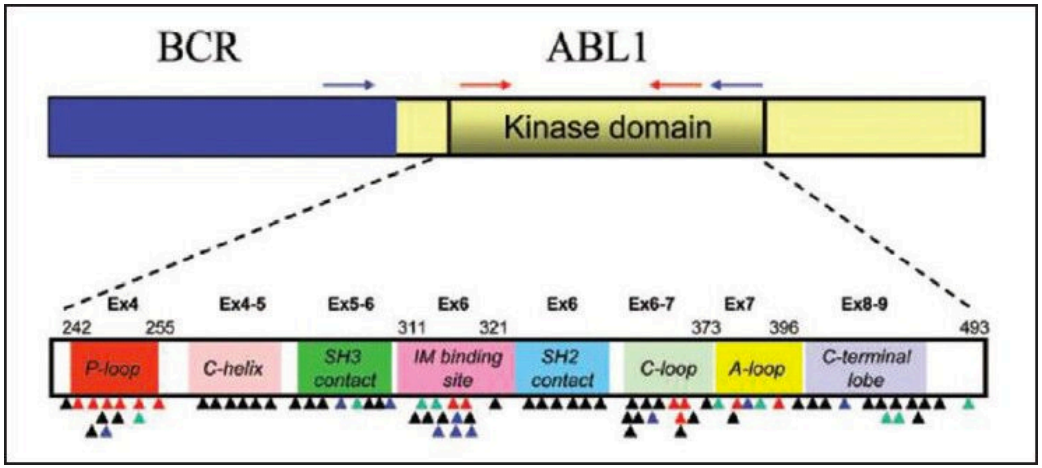
ion torrent

by life technologies

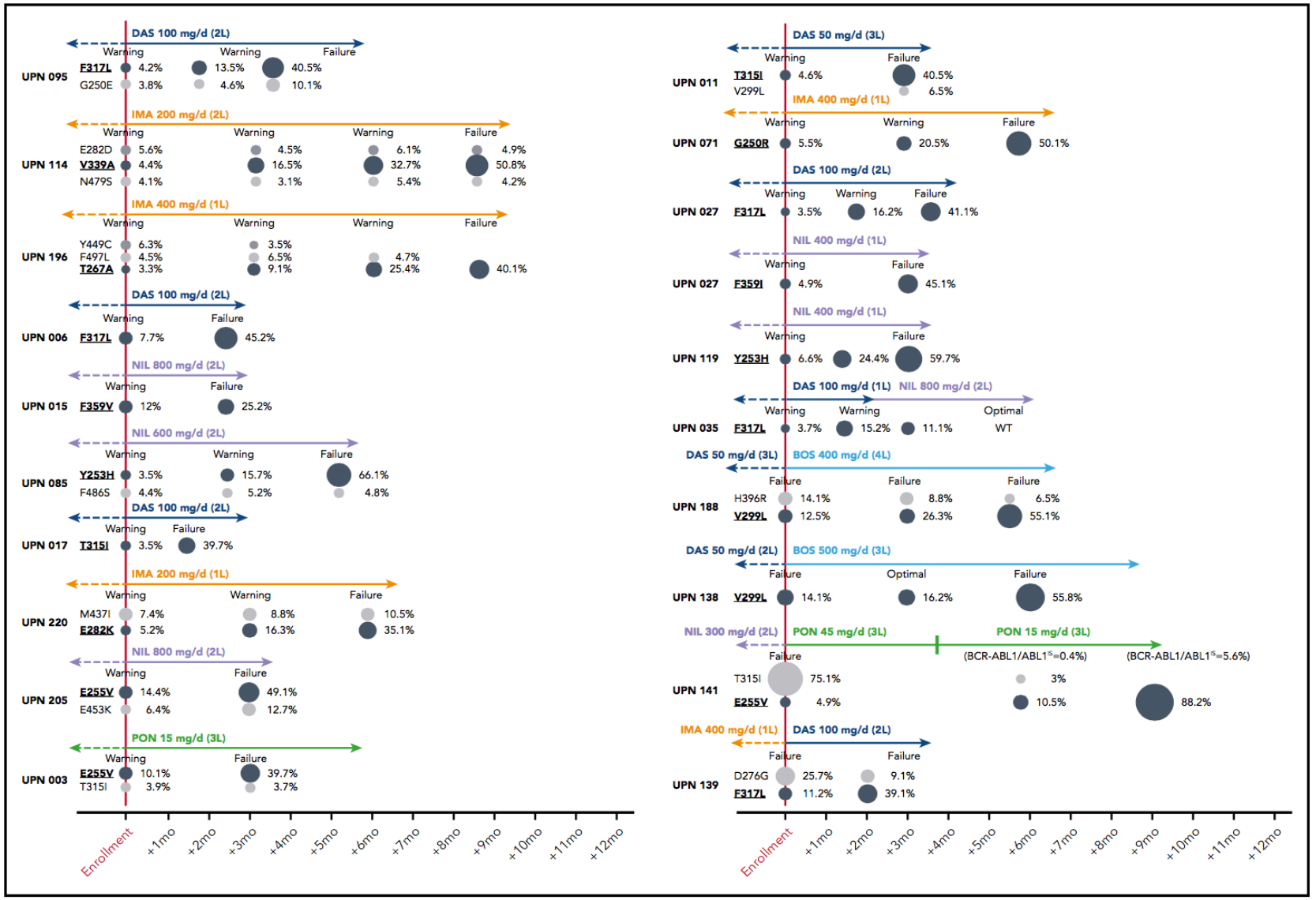
Ion PGM
- 3 types of chips
- 200 or 400 bp reads
- Up to 5.5 million reads / Ion 318 chip
- 4 – 7 h run time

Ion S5 System			Ion S5 XL System		
					
Simple workflow for panels, microbes, exomes, and transcriptomes			Simple, rapid workflow for panels, microbes, exomes, and transcriptomes		
Ion 520 Chip	Ion 530 Chip	Ion 540 Chip	Ion 520 Chip	Ion 530 Chip	Ion 540 Chip
Final Reads 3–5 million	Final Reads 15–20 million	Final Reads 60–80 million	Final Reads 3–5 million	Final Reads 15–20 million	Final Reads 60–80 million

NGS enables more sensitive detection of clinically actionable mutations in CML patients



Imatinib						Nilotinib	Dasatinib	Bosutinib	Ponatinib
M237V	L273M	F311L	E355D/G	V379I	A397P	Y253F/H*	V299L†	V299L†	?
M244V	E275K/Q	T315I‡	F359V/I/C*	A380T	S417F/Y	E255K/V*	T315I‡	T315I‡	
L248R	D276G	F317L/V/I/C†	D363Y	F382L	I418S/V	T315I‡	F317L/V/I/C†	?	
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				


DAS 100 mg/d (2L)
UPN 095 F317L 4.2% 13.5% 40.5%
G250E 3.8% 4.6% 10.1%
IMA 200 mg/d (2L)
UPN 114 E282D 5.6% 4.5% 6.1% 4.9%
V339A 4.4% 16.5% 32.7% 50.8%
N479S 4.1% 3.1% 5.4% 4.2%
IMA 400 mg/d (1L)
UPN 196 Y449C 6.3% 3.5%
F497L 4.5% 6.5% 4.7%
T267A 3.3% 9.1% 25.4% 40.1%
DAS 100 mg/d (2L)
UPN 006 F317L 7.7% 45.2%
NIL 800 mg/d (2L)
UPN 015 F359V 12% 25.2%
NIL 600 mg/d (2L)
UPN 085 Y253H 3.5% 15.7% 66.1%
F486S 4.4% 5.2% 4.8%
DAS 100 mg/d (2L)
UPN 017 T315I 3.5% 39.7%
IMA 200 mg/d (1L)
UPN 220 M437I 7.4% 8.8% 10.5%
E282K 5.2% 16.3% 35.1%
NIL 800 mg/d (2L)
UPN 205 E255V 14.4% 49.1%
E453K 6.4% 12.7%
PON 15 mg/d (3L)
UPN 003 E255V 10.1% 39.7%
T315I 3.9% 3.7%
Enrollment +1mo +2mo +3mo +4mo +5mo +6mo +7mo +8mo +9mo +10mo +11mo +12mo
DAS 50 mg/d (3L)
UPN 011 T315I 4.6% 40.5%
V299L 6.5%
IMA 400 mg/d (1L)
UPN 071 G250R 5.5% 20.5% 50.1%
DAS 100 mg/d (2L)
UPN 027 F317L 3.5% 16.2% 41.1%
NIL 400 mg/d (1L)
UPN 027 F359I 4.9% 45.1%
NIL 400 mg/d (1L)
UPN 119 Y253H 6.6% 24.4% 59.7%
DAS 100 mg/d (1L) NIL 800 mg/d (2L)
UPN 035 F317L 3.7% 15.2% 11.1% WT
DAS 50 mg/d (3L) BOS 400 mg/d (4L)
UPN 188 H396R 14.1% 8.8% 6.5%
V299L 12.5% 26.3% 55.1%
DAS 50 mg/d (2L) BOS 500 mg/d (3L)
UPN 138 V299L 14.1% 16.2% 55.8%
NIL 300 mg/d (2L) PON 45 mg/d (3L) PON 15 mg/d (3L)
(BCR-ABL1/ABL1IS=0.4%) (BCR-ABL1/ABL1IS=5.6%)
UPN 141 T315I 75.1% 3%
E255V 4.9% 10.5% 88.2%
IMA 400 mg/d (1L) DAS 100 mg/d (2L)
UPN 139 D276G 25.7% 9.1%
F317L 11.2% 39.1%
Enrollment +1mo +2mo +3mo +4mo +5mo +6mo +7mo +8mo +9mo +10mo +11mo +12mo

Myeloid Solution Panel provides sensitive identification of mutations in major myeloid disorders

AmpliSeq Myeloid Panel

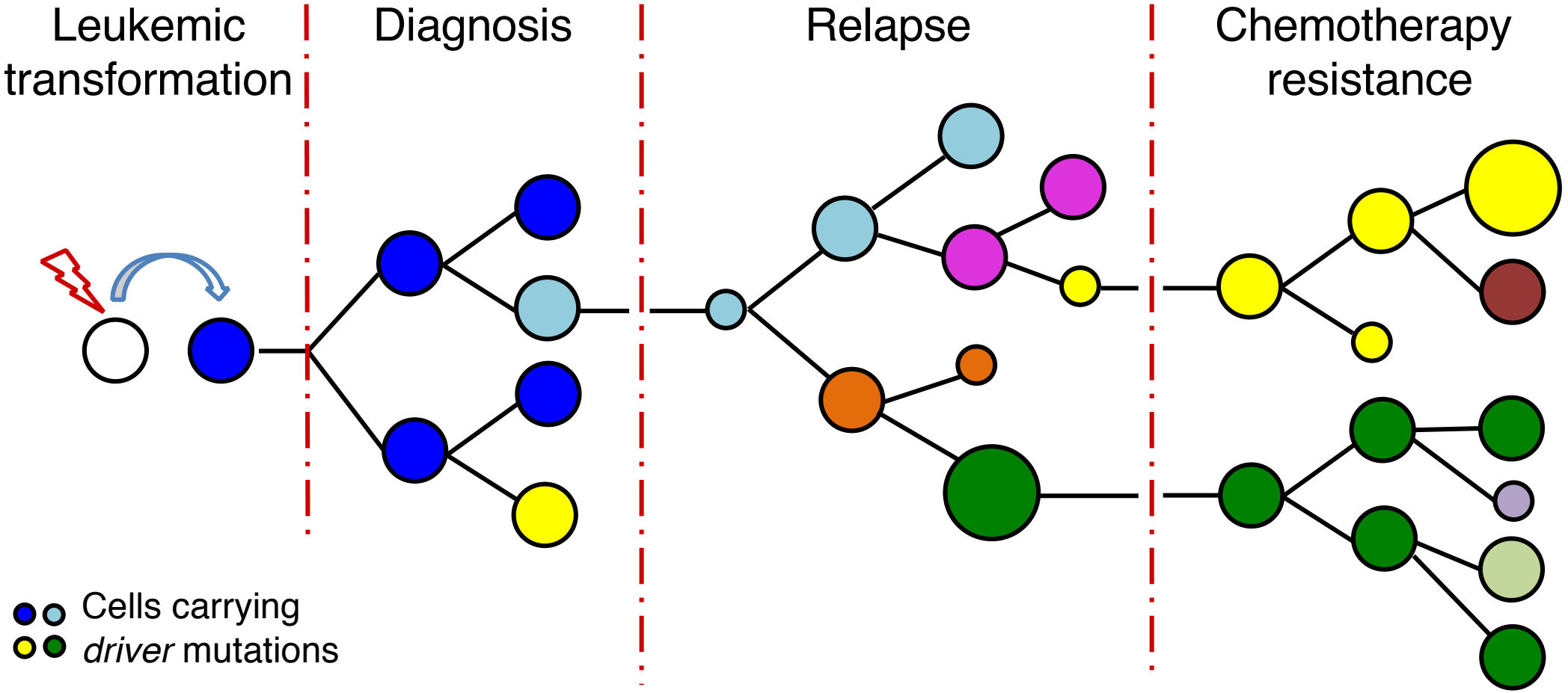
ABL1	**ASXL1**	BRAF	CALR	CBL	CEBPa
CSF3R	DNMT3A	ETV6	EZH2	**FLT3**	HRAS
IDH1	**IDH2**	JAK2	KIT	KRAS	MPL
NPM1	NRAS	PTPN11	**RUNX1**	SETBP1	SF3B1
SRSF2	TET2	**TP53**	U2AF1	WT1	ZRSR2

Currently available for:

- AML
- MDS
- MPN
- CML
- CMML & JMML

30 genes full
20 hotspots regions

NGS technologies allow to monitor the clonal evolution of leukemic stem cells



NGS technologies: from pre-clinical research to diagnostic routine

The Value of Next-Generation Sequencing in the Screening and Evaluation of Hematologic Neoplasms in Clinical Practice

Victoria Northrup, MSc,[1,2,3,*] Allison Maybank, MSc,[1] Nancy Carson, PhD,[2] and Tarek Rahmeh, MD[1,2]

REVIEW ARTICLE Blood Cancer Journal

Challenges in the introduction of next-generation sequencing (NGS) for diagnostics of myeloid malignancies into clinical routine use

Ulrike Bacher[1,2], Evgenii Shumilov[3], Johanna Flach[4], Naomi Porret[1], Raphael Joncourt[1], Gertrud Wiedemann[1], Martin Fiedler[2], Urban Novak[5], Ursula Amstutz[2] and Thomas Pabst[5]

cancers MDPI

Next Generation Sequencing in AML—On the Way to Becoming a New Standard for Treatment Initiation and/or Modulation?

Michael Leisch [1,2], Bettina Jansko [1,2,3], Nadja Zaborsky [1,2,3], Richard Greil [1,2,3] and Lisa Pleyer [1,2,3,*]

haematologica
Journal of the Ferrata Storti Foundation

Next-generation sequencing in the diagnosis and minimal residual disease assessment of acute myeloid leukemia

Ross L. Levine and Peter J.M. Valk

Conclusions

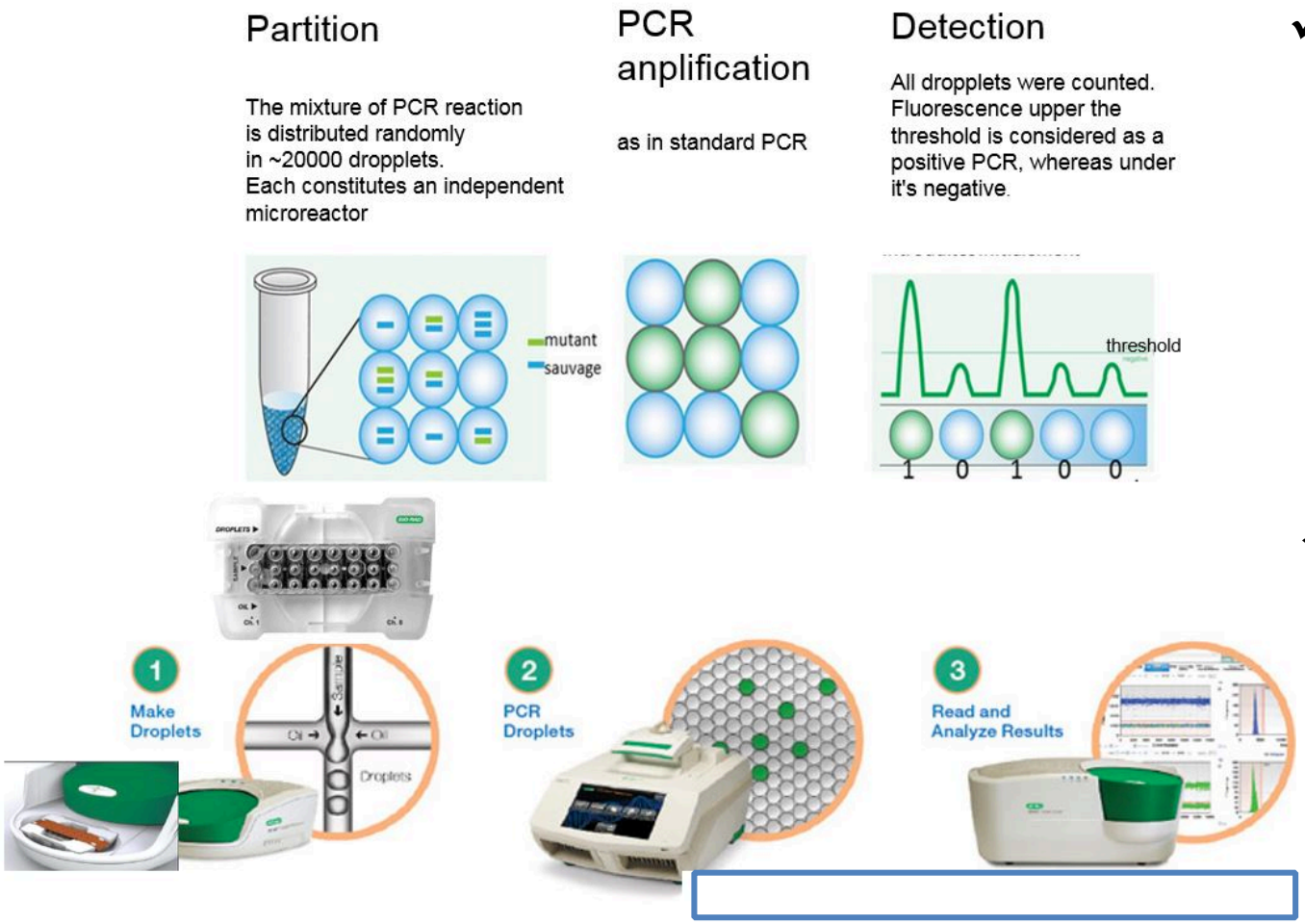
NGS for BCR-ABL KD mutations in CML pts

- ✓ Greater sensitivity and accuracy enable timely and rational TKI switch in the setting of Failure patients
- ✓ Mutations testing in the Warning setting may identify pts who need a change in therapy rather that a “watch and wait” approach.

NGS panel for myeloid malignancies

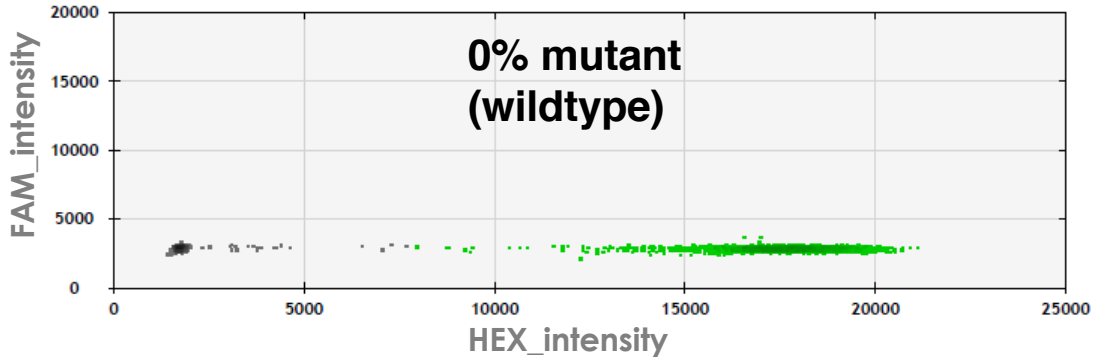
- ✓ Provides a “just one test” for all clinically relevant genetic mutations allowing prognostic stratification and therapy selection
- ✓ Mutation screening allows to monitor the clonal evolution of disease

ddPCR for deep investigation of specific mutations

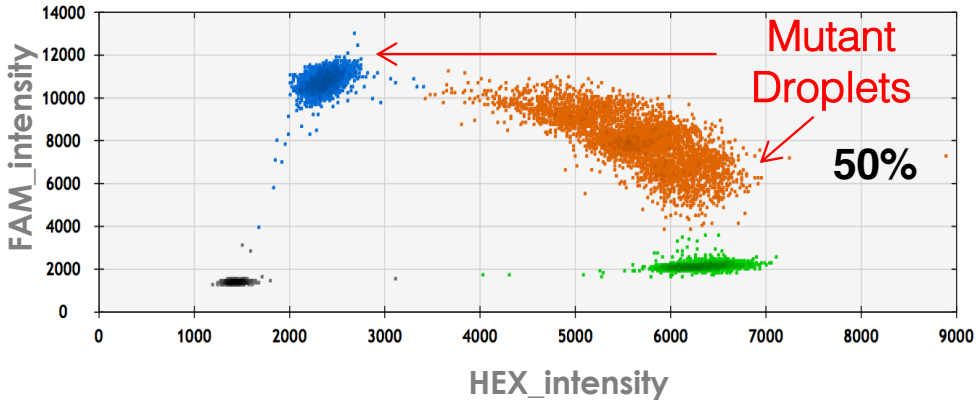


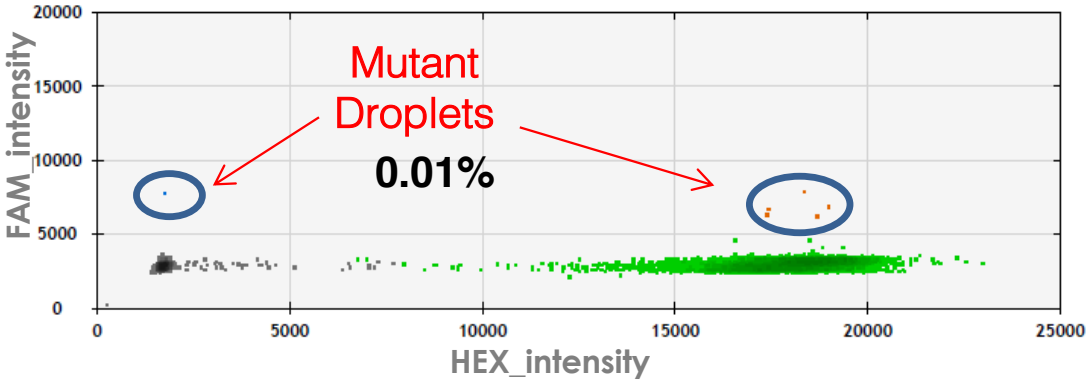
- ✓ dPCR performs a dilution of target nucleic acid across a large number of reactions (partitions) for accurate absolute quantification of DNA molecules.
- ✓ It has become a new standard for quantification of mutant alleles at low variant allele fraction (VAF ≥0.01%).

ddPCR allows the identification of specific hot-spot mutations at low frequency



- FAM negative, HEX positive
- FAM positive, HEX negative
- FAM positive, HEX positive (double-positive droplets)
- FAM negative, HEX negative (double-negative droplets)





ddPCR: a sensitive and precise method for KIT D816V quantification in mastocytosis

Prospective phase

179 BM samples from pts with suspected SM

35 [illegible] cell infiltration <0.01% by flow cytometry and low level positivity by ASO qPCR (or negative) were used for v[illegible]

WHO category	Pts n	Median VAF % (range)
ISM	55	0.16 (0.01-3.1)
SSM	4	5.82 (2.47-21.5)
AdvASM	23	15.23 (0.019-36.3)
All D816V-positive	**82**	**0.31**

Take home message

- ✓ The molecular landscape of genomic alterations involved in leukemic transformation and progression is enabling the implementation of personalised medicine
- ✓ Thanks to NGS techniques we are able to obtain a wide range of molecular information useful for diagnosis, prognosis, risk stratification and therapeutic choice
- ✓ dPCR is a simple and rapid technique for the identification of specific mutations with low VAF and represents the new gold standard for diagnosis and MRD monitoring

Thanks to:

Institute of Hematology "Lorenzo & Ariosto Seragnoli"

Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna

Prof. Sante Tura
Prof. Michele Cavo

Laboratory Unit:

Simona Soverini
Manuela Mancini
Emanuela Ottaviani
Sara De Santis
Cecilia Monaldi
Valentina Robustelli
Lorenza Baldini
Claudia Venturi

Clinical Research Unit:

Antonio Curti
Cristina Papayannidis
Stefania Paolini
Maria Chiara Abbenante
Chiara Sartor
Giovanni Marconi
Jacopo Nanni

Thank you for attention!