MRD: INNOVAZIONI E IMPATTO DELLA CH

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03 Maggio 2024

Agenda:

- Definizione di MRD
- Le tecniche per monitorare l'MRD in oncoematologia
- I principali target
- Esempio di applicazione di una tecnica innovativa

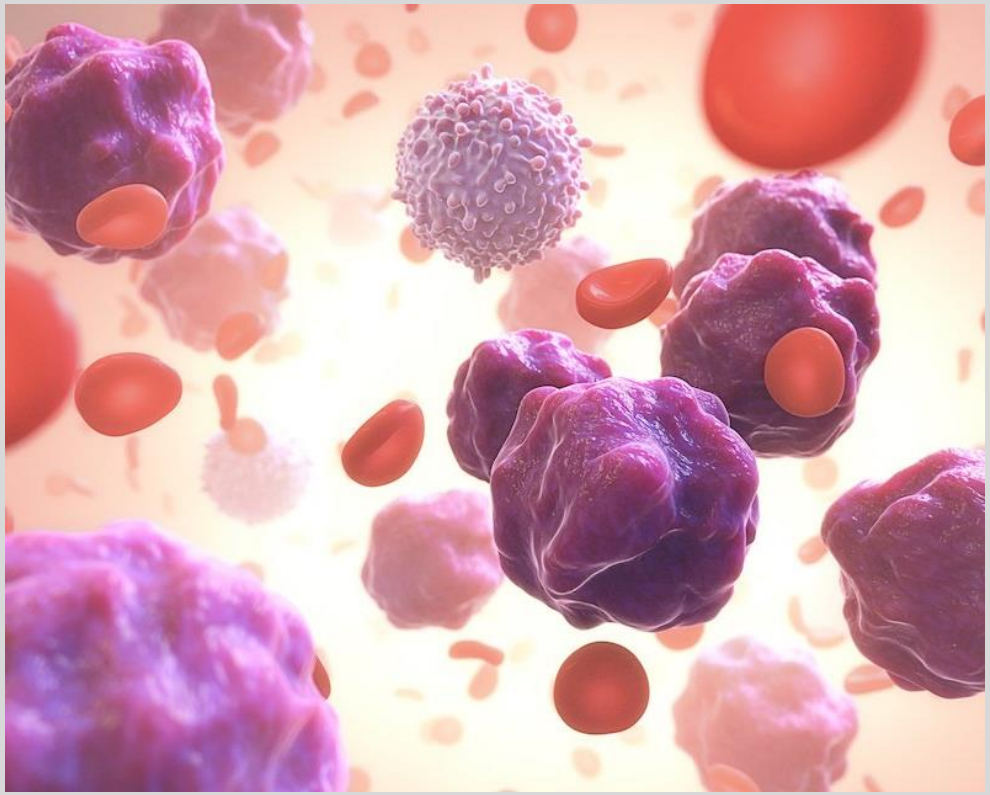
MRD: malattia residua minima o malattia residua misurabile

RIFLETTE LA RISPOSTA AL TRATTAMENTO E, QUINDI, DIMINUISCE CON IL PROGREDIRE DELLA RISPOSTA E AUMENTA IN CASO DI RECIDIVA O RESISTENZA AI FARMACI.

RAPPRESENTA UN NUMERO INFINITESIMALE DI CELLULE MALIGNE CHE PERMANGONO NEL PAZIENTE DICHIARATO IN REMISSIONE COMPLETA.

DEVE ESSERE MONITORATA RICERCANDO TARGET MALATTIA-SPECIFICI E CON UN TIMING BEN DEFINITO PER INTERCETTARE ALTERAZIONI E CINETICHE DI INTERESSE CLINICO.

MRD MONITORING: caratteristiche



- DISPONIBILITA' DI METODICHE ALTAMENTE SENSIBILI
- UTILITA' CLINICA
- DISPONIBILITA' DI MARCATORI MOLECOLARI VALIDI E SAGGIABILI CON TECNICHE SENSIBILI

MRD MONITORING: caratteristiche

- **DISPONIBILITA' DI METODICHE ALTAMENTE SENSIBILI**
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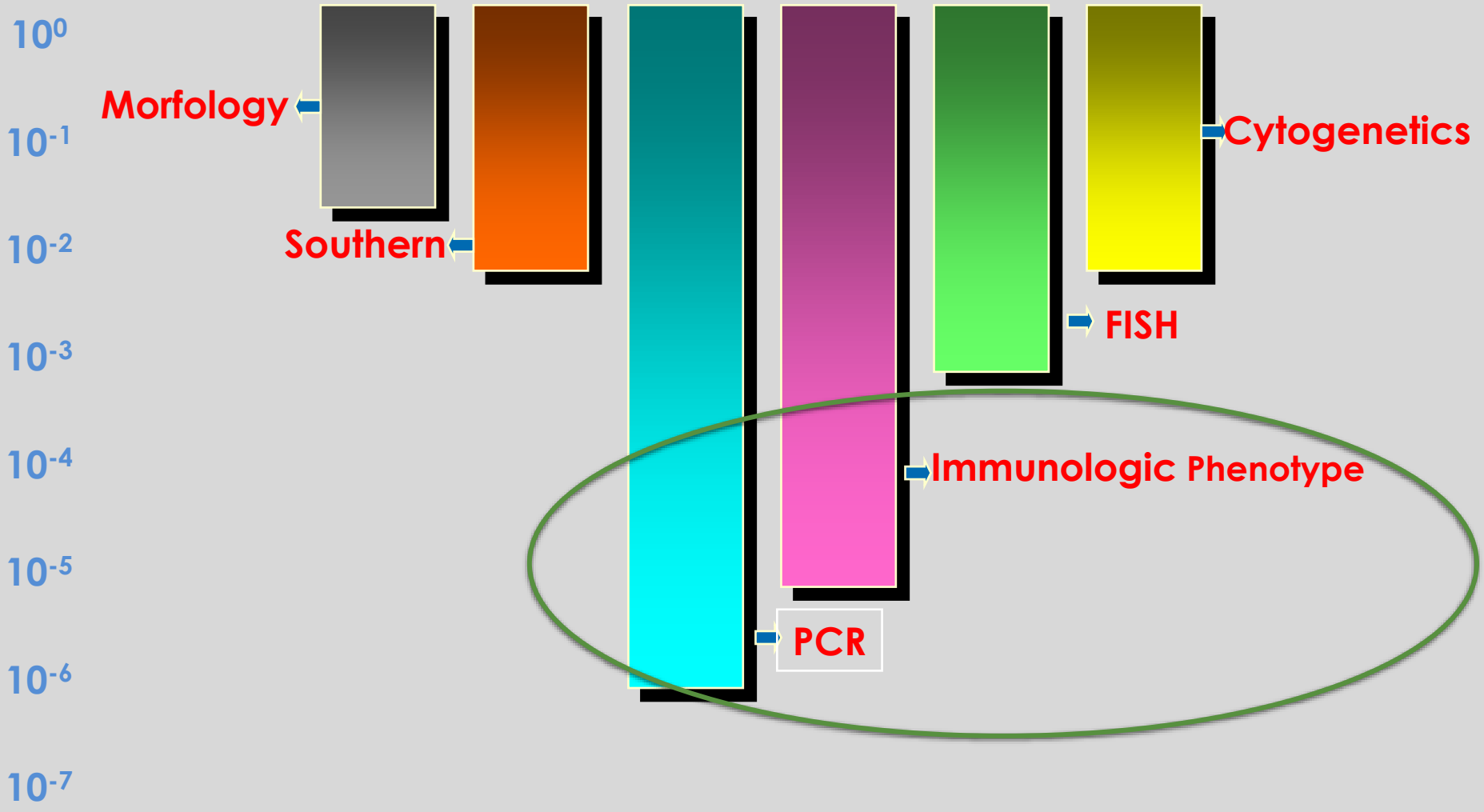
MRD: HOW?


10^0
10^{-1}
10^{-2}
10^{-3}
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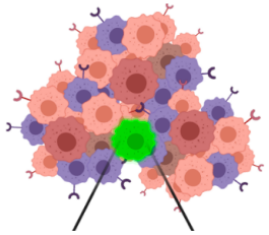
10^{-5}
10^{-6}
10^{-7}
Morfology
Southern
PCR
Immunologic Phenotype
FISH
Cytogenetics

MRD: HOW?



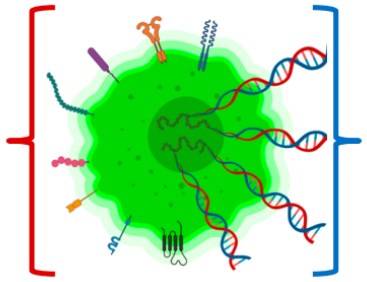
Measurable Residual Disease (MRD)

Detection of rare neoplastic cells (<1%) during post-treatment follow-up, by using complementary approaches:

Multiparametric Flow Cytometry (MFC)

Immunophenotypic analysis to detect abnormal expression of specific antigens

- sensitivity 10^{-3} – 10^{-4}
- applicability >95%



Molecular diagnostics (PCR, RT-qPCR)

Genetic analysis to detect specific DNA signatures

- sensitivity 10^{-3} – 10^{-6}
- applicability >90%

Next Generation Flow (NGF)

- sensitivity < 10^{-5} – 10^{-6}
- applicability >99%

Next Generation Sequencing (NGS), Digital PCR (ddPCR)

MRD MONITORING: caratteristiche

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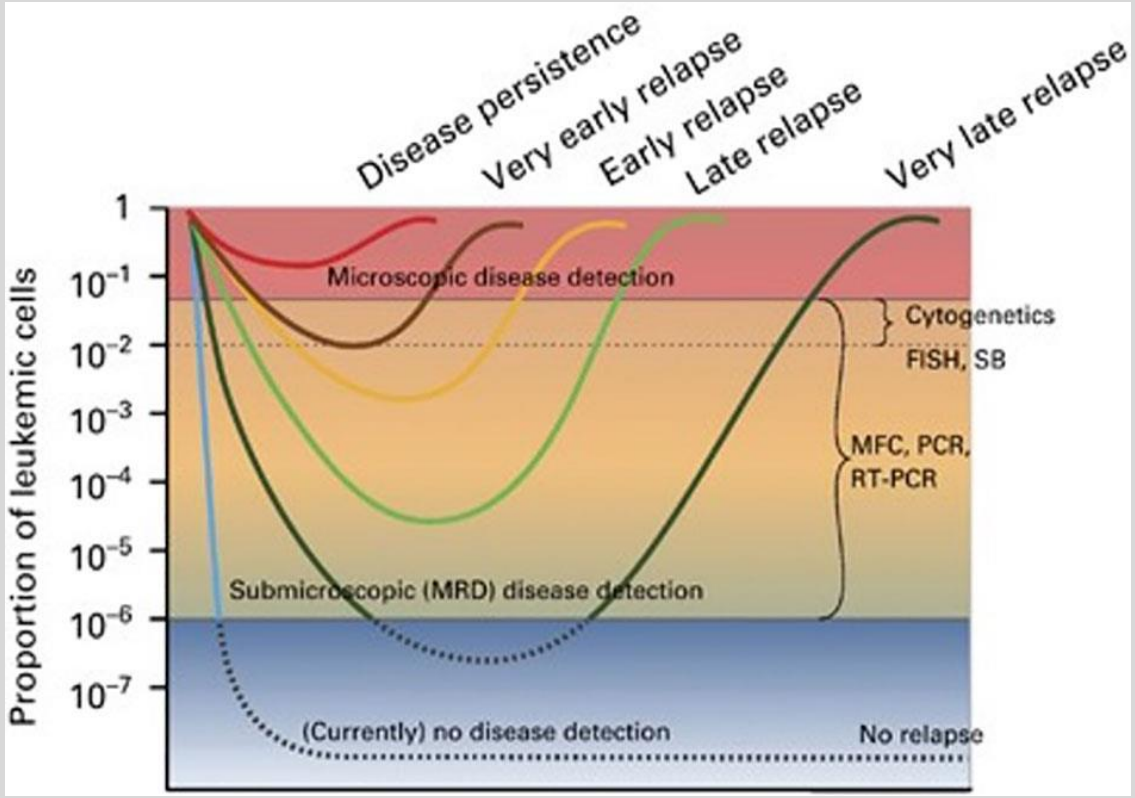
MRD: CLINICAL END-POINTS

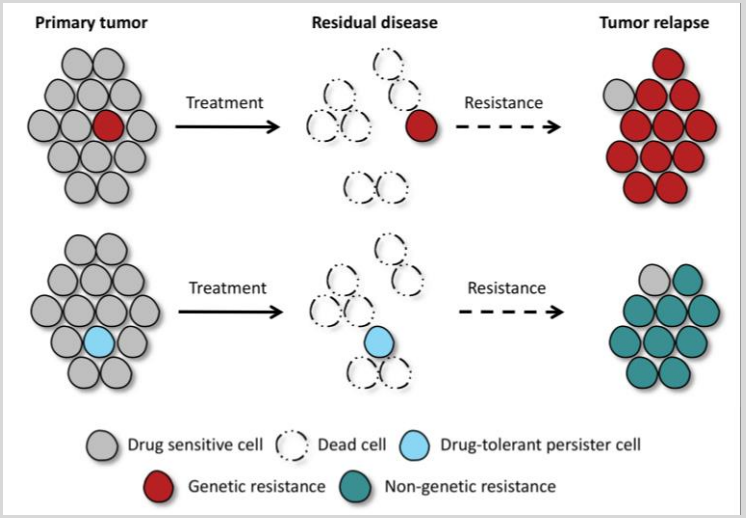
- Drug Sensitivity and grade of response for therapeutic strategy assessment
- Prognostic factor
- Predictive of Relapse

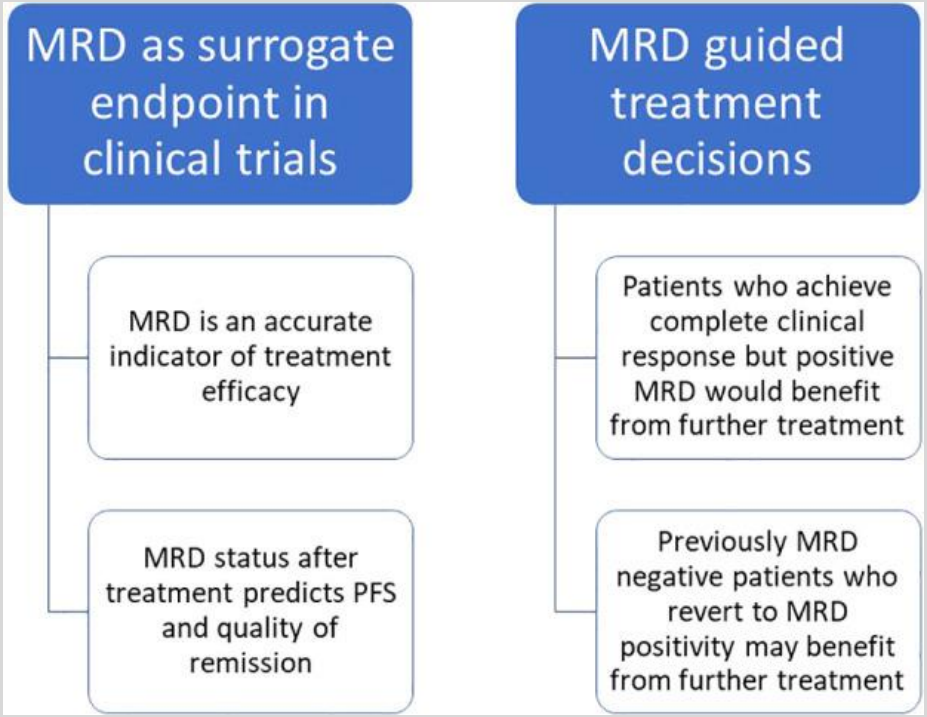


MRD: CLINICAL END-POINTS

- Drug sensitivity and grade of response for therapeutic strategy assessment

MRD in pazienti con CLL ha acquisito un nuovo significato grazie all'efficacia di nuovi trattamenti. MRD si basa su citofluorimetria e quantificazione molecolare del riarrangiamento specifico

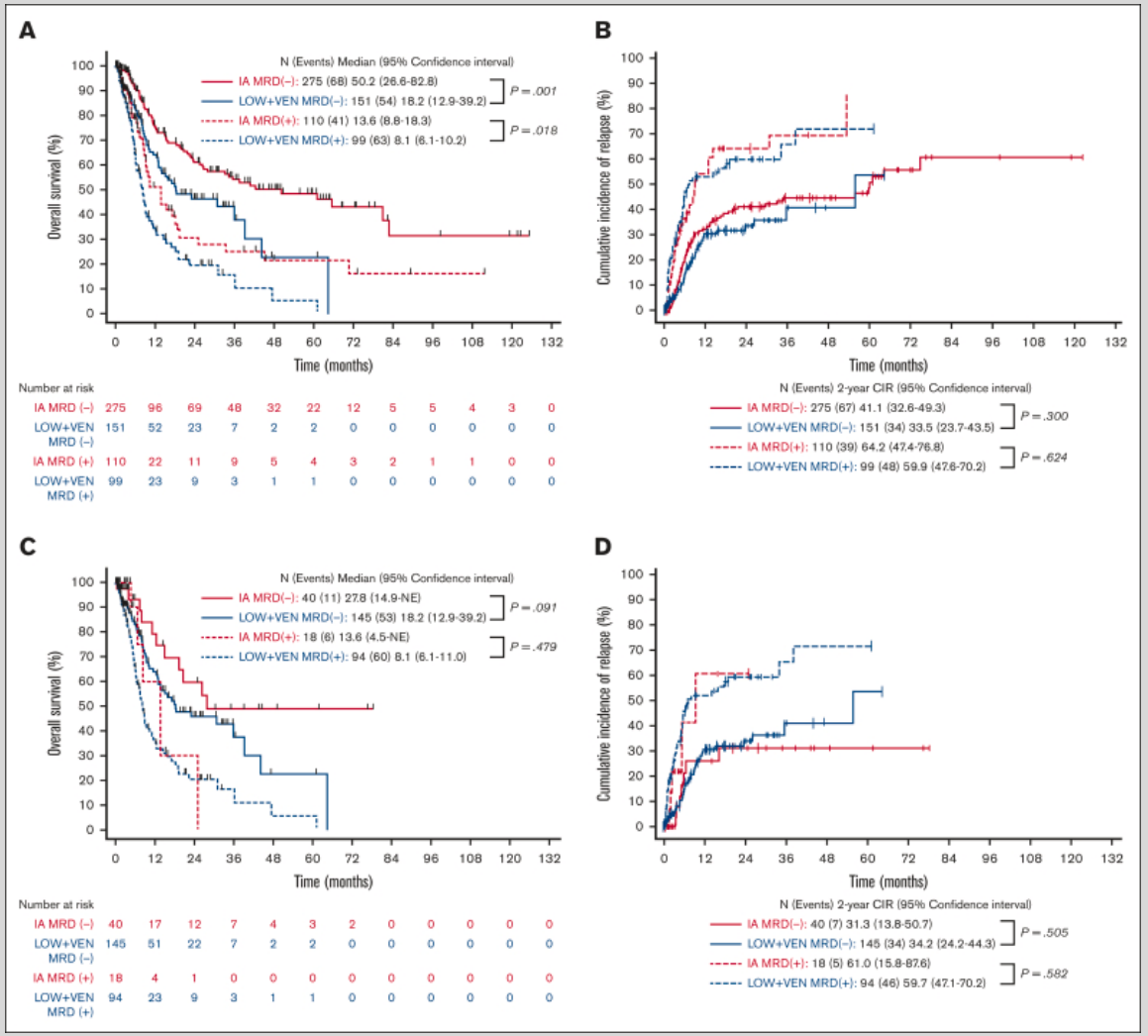




MRD: CLINICAL END-POINTS

- Prognostic factor

Monitoraggio della MRD nella LAM mediante citofluorimetria. MRD - quando sono presenti <20 cellule anomale. MRD è in grado di predire OS e CIR in modo indipendente dalla terapia e dall'età dei pazienti.

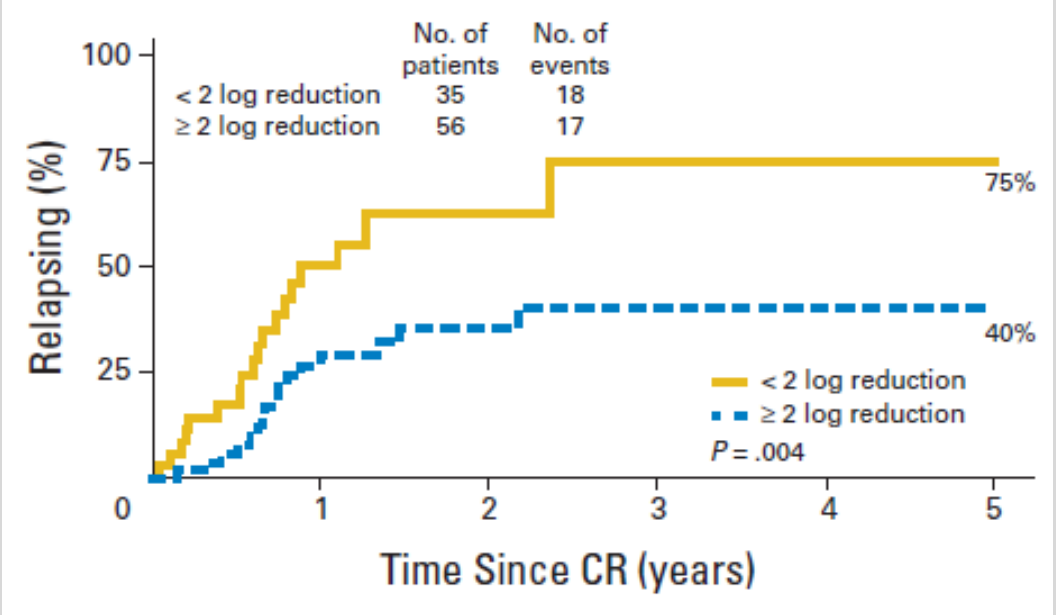


Bazinet A. Et al, Blood Adv 2023

MRD: CLINICAL END-POINTS

- Predictive of Relapse

WT1 è altamente espresso nelle cellule staminali e nei precursori. La maggior parte dei pazienti con LAM presenta un aumentata espressione (mRNA) di WT1 quantificato mediante tecniche PCR-based.



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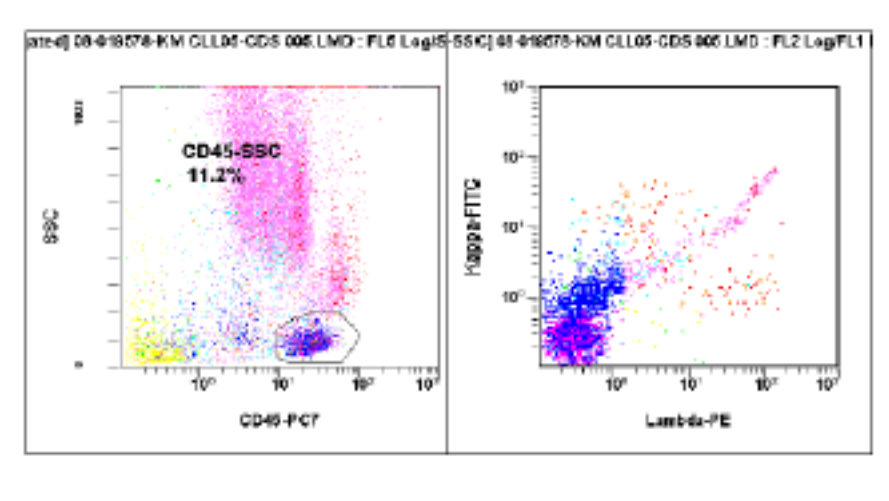
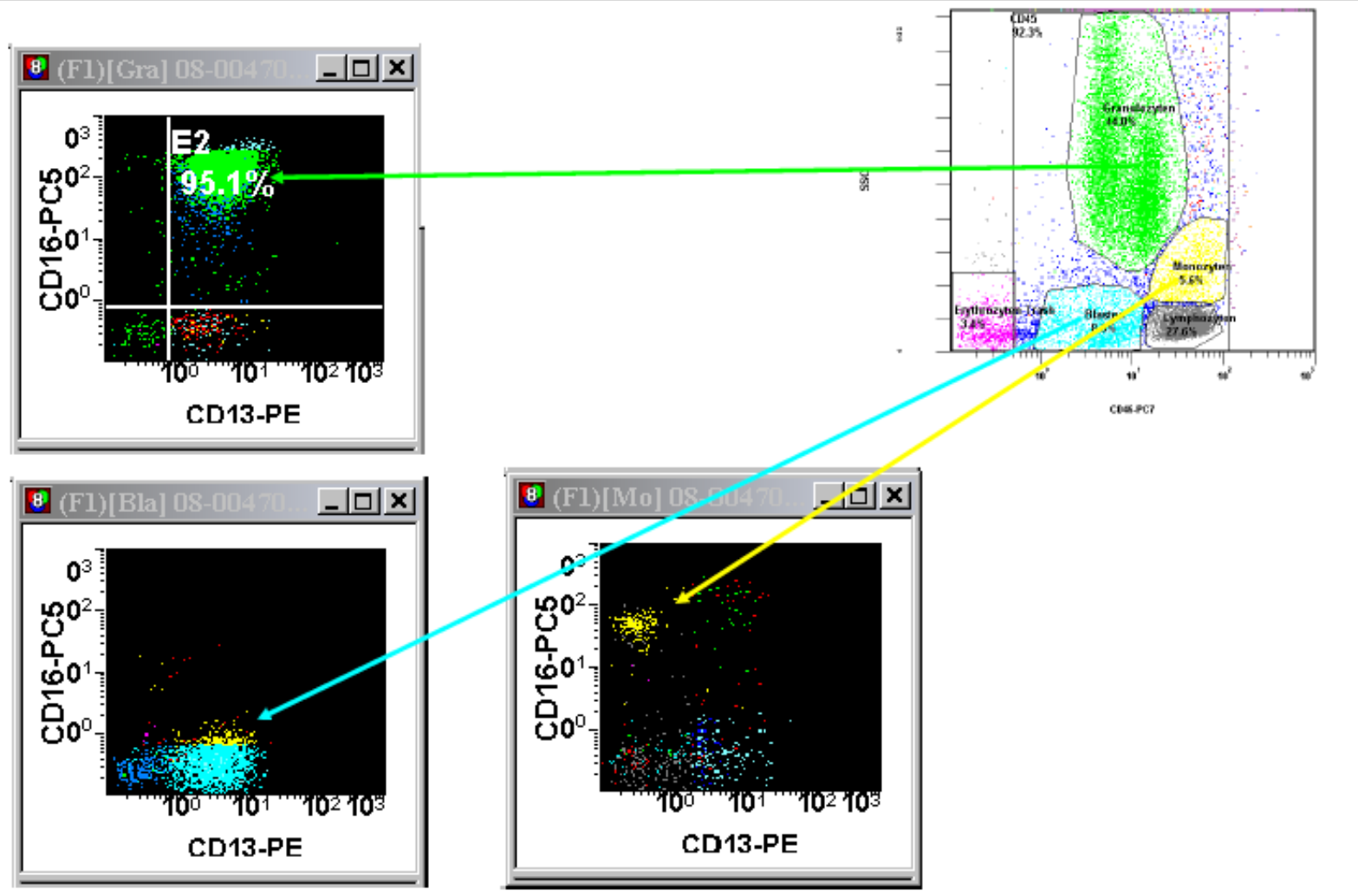
Analisi Immunofenotipica

INDICAZIONI

- SINTOMI CLINICI
- CITOPENIA
- LEUCOCITOSI
- PRESENZA DI CELLULE ATIPICHE/BLASTI
- PATOLOGIE DELLE PLASMACELLULE
- ORGANOMEGALIA O MASSE TISSUTALI
- MONITORAGGIO DELL'MRD

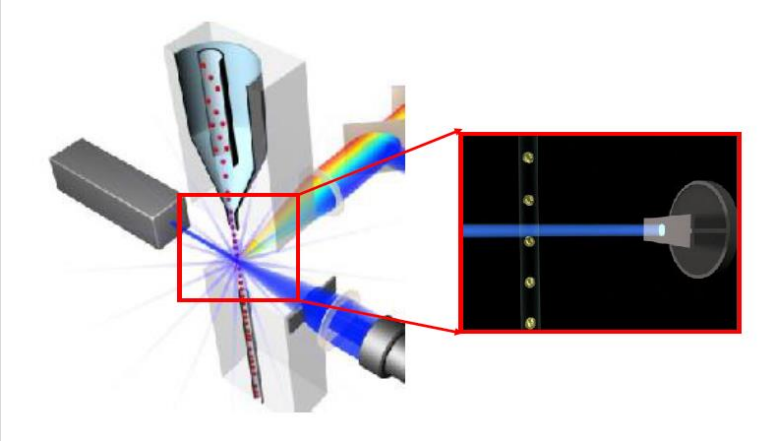


Citofluorimetro: il gating



Le strategie di gating sono in continua evoluzione grazie all'introduzione di target therapy e anticorpi specifici o bi-specifici che alterano l'espressione degli antigeni di superficie e, a volte, anche la popolazione sana

Immunofenotipi tipici: LAIP



HAIRY CELL LEUKEMIA:
SSC FORTE
CD103+
CD11c+
CD25+

CLL (90% dei casi):
CD5+
CD23+
FMC7-
sIgM(+)
aCD22(+) o CD79b(+)

LINFOMA FOLLICOLARE:
CD10+ DEBOLE
CD19+ DEBOLE

DLBCL:
CD10+
LIGHT CHAIN (Kappa/Lambda)

MIELOMA:
CD45-
CD38+-
CD138+
CD56+
CD19-CD20+

Immunofenotipo per MRD: le applicazioni

	AML	B-ALL	T-ALL	CLL	MM
Sensitivity	10^{-3}–10^{-5}	10^{-4}–10^{-5}	10^{-4}–10^{-5}	10^{-4}–10^{-5}	10^{-5}–10^{-6}
Sample origin	BM	BM	BM, PB	PB, BM	BM
N° of cells required	3×10^6	4×10^6	4×10^6	3×10^6	5–20 $\times 10^6$
Applicability (% of cases)	>97%	>99%	>99%	>95%	>99%
MRD "positivity" threshold	$\geq 10^{-3}$	$\geq 10^{-4}$	$\geq 10^{-4}$	$\geq 10^{-4}$	$\geq 10^{-5}$
Follow-up timepoints	Poorly standardized: usually performed early, after initial therapy (i.e., post-induction/consolidation treatments), then usually guided by clinical protocols (usually, every 3–6 months)				
Backbone panel	CD34, CD117, CD45, CD13, CD33, CD15, CD7	CD34, CD19, CD10, CD20, CD38, CD45	CD2, CD3, CD5, CD7, CD4, CD8, CD34, CD45, CD99, CD1a	CD19, CD20, CD5, CD79b, CD43, CD81	CD138, CD38, CD45, CD56, CD19, CD27, CD28, CD117, cy k/λ, CD81
Additional markers	CD14, CD64, HLA-DR, CD4, CD11b, CD123, CD133, CD38, CD90	CD22, CD81, CD66c, CD123, CD73, CD304	CD10, CD38, CD56, TdT	CD200, CD23, CD160, ROR1	CD33, CD54, CD200, CD229, CD307, CD319, CD150, VS38

MRD nelle LAM

- Combination of NGF and molecular monitoring based on AML-type
- Molecular monitoring is preferred in patients with molecularly defined group. E.g. APL, CBF-AML, NPM1+
- The use of PB and BM depends on the time of MRD monitoring
- The monitoring after 24mo is almost patient-specific

No.	Clinical MRD recommendation
D1	MRD should be assessed to refine relapse risk in patients who achieve morphologic remission, with full or partial hematologic recovery ($CR/CR_i/CR_p/CR_h$).
D2	For patients with mutant *NPM1*, CBF AML (*RUNX1-RUNX1T1* or *CBFB-MYH11*), or APL (*PML-RARA*), we recommend molecular MRD assessment by qPCR or dPCR.
D3	AML patients who are not included in the molecularly defined subgroups should be monitored for MRD by MFC.
D4	NGS-MRD monitoring is useful to refine prognosis in addition to MFC but, to date, there are insufficient data to recommend NGS-MRD as a stand-alone technique.
D5	In *NPM1*-mutated AML, MRD should be assessed preferentially in PB after 2 cycles of chemotherapy, in BM at the end of consolidation, and in BM every 3 mo for 24 mo after the end of consolidation. Alternatively, MRD may be assessed from PB every 4 to 6 wk during follow-up for 24 mo.
D6	In *RUNX1-RUNX1T1*, and *CBFB-MYH11* mutated AML MRD should be assessed preferentially in PB after 2 cycles of chemotherapy, in BM at the end of consolidation treatment, and in PB every 4 to 6 wk for 24 mo after the end of consolidation.
D7	In APL, the most important MRD end point is PCR negativity for *PML-RARA* at the end of consolidation.
D8	For patients with non–high-risk APL, MRD monitoring is recommended only after completion of consolidation and may be discontinued once BM MRD negativity is achieved.
D8a*	For high-risk APL MRD should be assessed by qPCR from BM every 3 mo for 24 months starting at the end of treatment. Alternatively, MRD may be assessed from PB every 4 to 6 wk during follow-up.
D9	Ongoing molecular MRD monitoring beyond 24 mo of follow-up should be based on individual clinical features.
D10	Patients who are followed-up with MFC-MRD should have BM assessment after 2 cycles of chemotherapy, at the end of consolidation, and prior to stem cell transplantation, if applicable.
D11	MFC-MRD test positivity is defined as ≥0.1% of CD45-expressing cells with the target immunophenotype.

LA CH IN EMATOLOGIA

"Non-AML–related somatic genetic abnormality detectable after treatment, which may or may not have been detectable in the original diagnostic AML sample" Hasserjian et al, Blood 2020

- The Clonal Hematopoiesis (CH) is a phenomenon featured by the presence of a population of cells presenting a mutation that could be related with leukemias (3-10 fold in myeloid and increased in lymphoid).
- The cell population presenting the mutation does not present hyper-proliferation or neoplastic characteristics.
- The patients with CH does not present abnormal cell counting.

The significance of posttherapy persistence of genetic abnormalities commonly seen in AML

Genetic abnormality	Type	Techniques for detection	Usually cleared after successful therapy	therapy associated with adverse outcome
RUNX1-RUNX1T1, *CBFB-MYH11*, *PML-RARA*	AML-related	qPCR	Yes	Yes
NPM1	AML-related	qPCR	Yes	Yes
KMT2A rearrangement, *DEK-NUP214*, *BCR-ABL1*	AML-related	qPCR	Unknown	Unknown
NRAS/KRAS	AML-related	NGS	Yes	Yes
FLT3-ITD/FLT3-TKD	AML-related	NGS PCR	Yes (but may be lost at relapse or acquired at relapse of previously *FLT3* wild-type AML)	Unknown
KIT	AML-related	NGS PCR	Yes	Yes
PTPN11	AML-related	NGS	Yes	Yes
GATA2	Likely AML-related	NGS	Yes	Unknown
CEBPA	Likely AML-related	NGS	Yes	Unknown
WT1	Likely AML-related	NGS	Yes	Unknown
RUNX1	CH (potentially AML-related)	NGS	Variable	Yes
IDH1/IDH2	CH (potentially AML-related)	NGS ddPCR	Variable	Yes
DNMT3A	CH	NGS	Usually not	No
ASXL1	CH	NGS	Variable	No
TET2	CH	NGS	Usually not	No
SRSF2	CH	NGS	Variable	No
BCOR	CH	NGS	Variable	No
TP53	CH	NGS	Variable	Yes

LA CH IN EMATOLOGIA

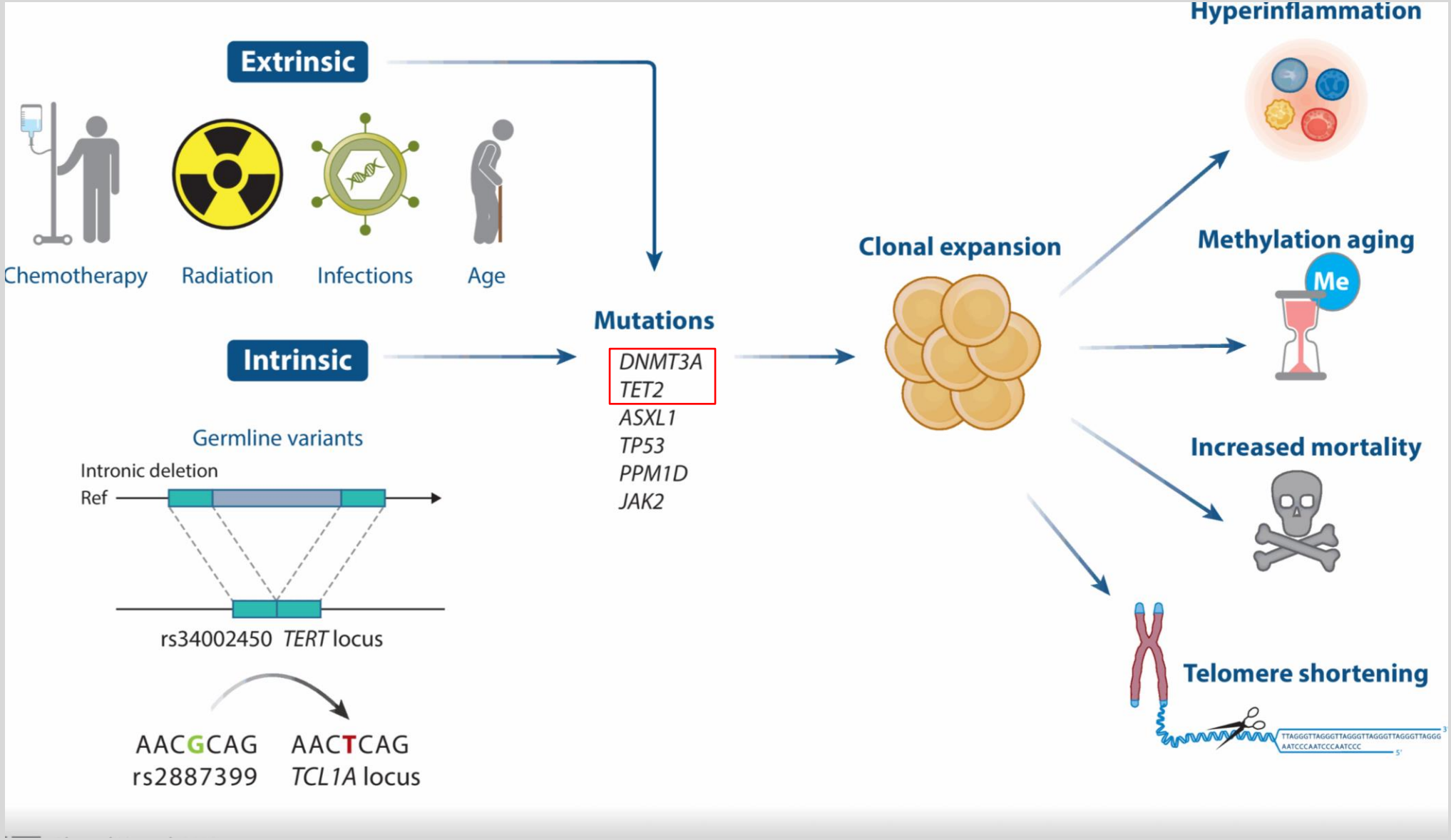
CH-related variants are not good markers for MRD monitoring!!

AML-related genetic abnormalities

- Often occur later in the mutation hierarchy; may be the sole detected genetic event
- Reduction in VAF or clearance associated with a reduction in the blast percentage after therapy
- Reappearance of genetic abnormality in relapsed disease
- Presence in CR associated with increased risk of relapse
- Eliminated following successful HCT

CH-type genetic abnormalities

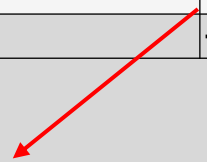
- Occur earlier in the mutation hierarchy, often at higher VAF in comparison to AML-related genetic abnormalities
- Often persist in CR, usually at similar VAF to the pretherapy disease
- Persistence in relapsed disease
- Presence in CR may not be associated with increased risk of relapse
- Eliminated following successful HCT


Extrinsic
Chemotherapy
Radiation
Infections
Age
Intrinsic
Germline variants
Intronic deletion
Ref
rs34002450 TERT locus
AACGCAG
rs2887399
AACTCAG
TCL1A locus
Mutations
DNMT3A
TET2
ASXL1
TP53
PPM1D
JAK2
Clonal expansion
Hyperinflammation
Methylation aging
Me
Increased mortality
Telomere shortening
TTAGGGTTAGGGTTAGGGTTAGGGTTAGGG
AATCCCAATCCCAATCCC

DIAGNOSI DIFFERENZIALE

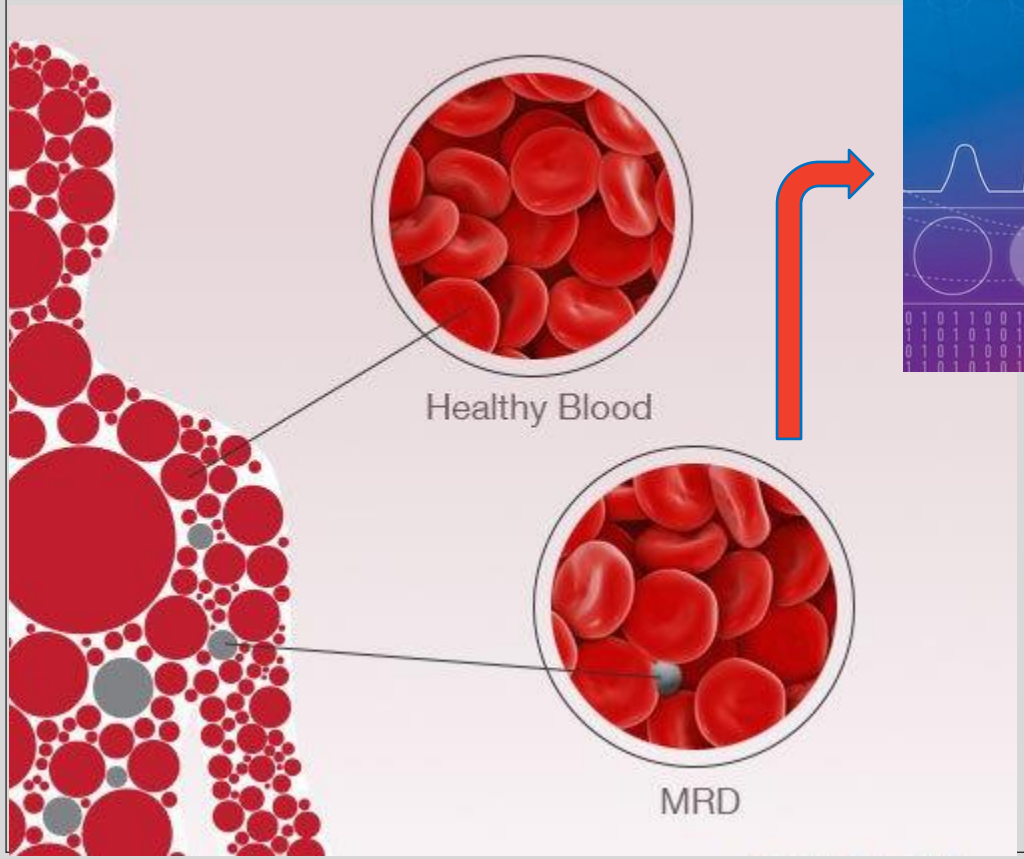
CHIP: Ematopoiesi clonale con potenziale indeterminato
ICUS: Citopenia ideopatica con significato indeterminato
CCUS: Citopenia clonale con significato indeterminato
CMUS: Monocitosi clonale con significato indeterminato
CCMUS: Citopenia e Monocitosi clonale con significato indeterminato

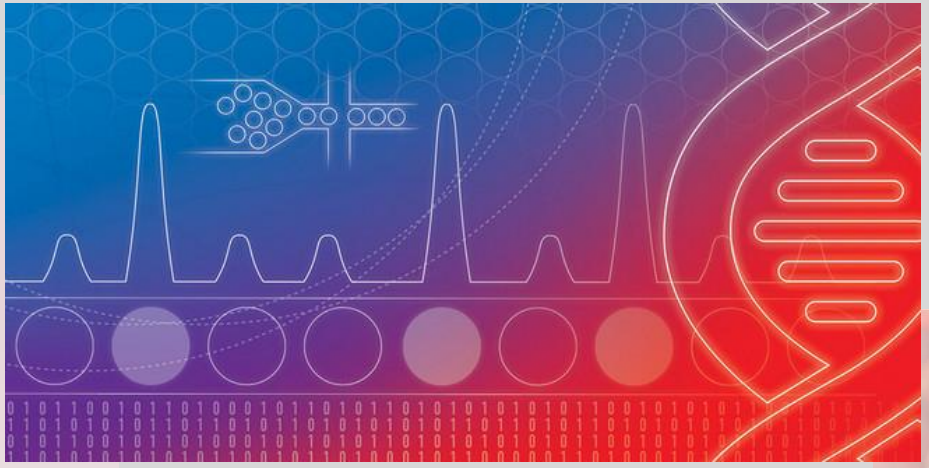
	CHIP	ICUS	CCUS	MDS
Cytopenia	**No**	**Yes**	**Yes**	**Yes**
Dysplasia (≥10%) in ≥1 lineages	**No**	**No**	**No**	**Yes**
Increased blasts (≥5% and <20%)	**No**	**No**	**No**	**Yes**
Somatic mutation	**Yes**	**No**	**Yes**	**Yes**
Cytogenetic abnormality	**+/−**	**No**	**+/−**	**Yes**

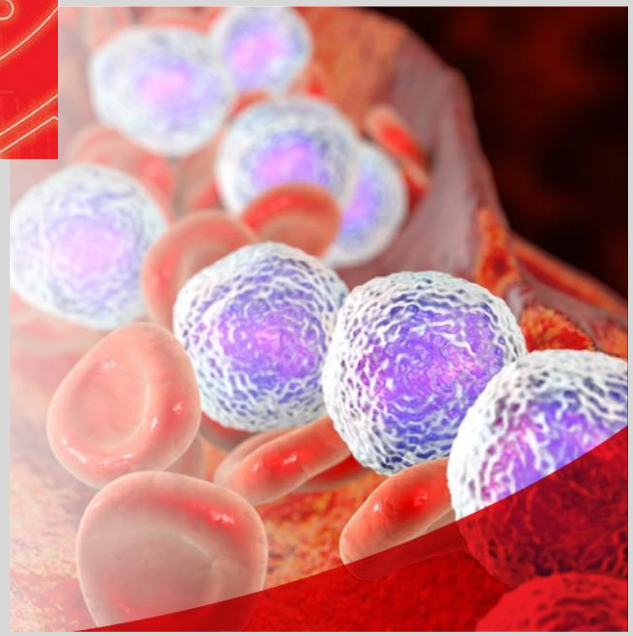


Not TP53 and MDS related

MRD: qualcosa di nuovo...

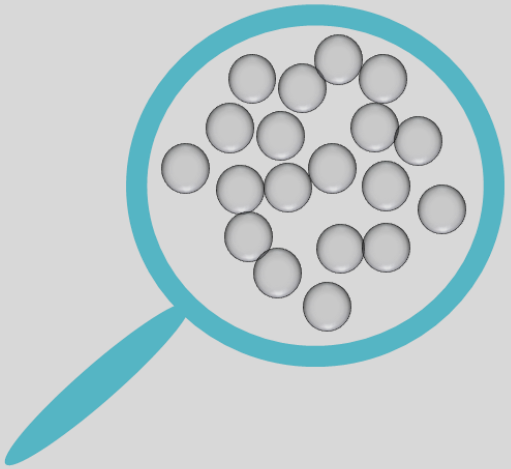






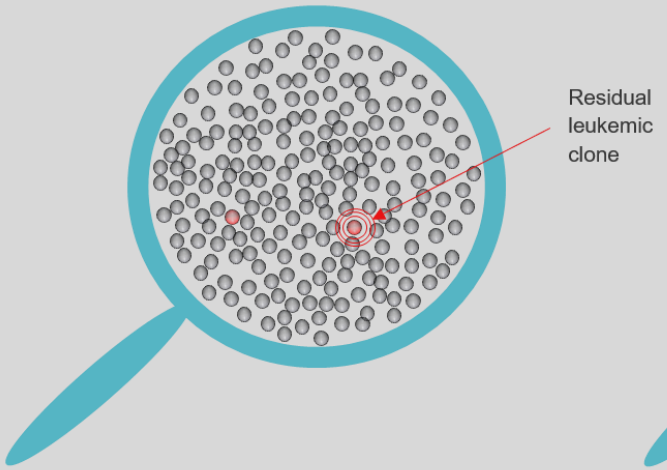
MRD nelle LAM: NGS come supporto

Morphology



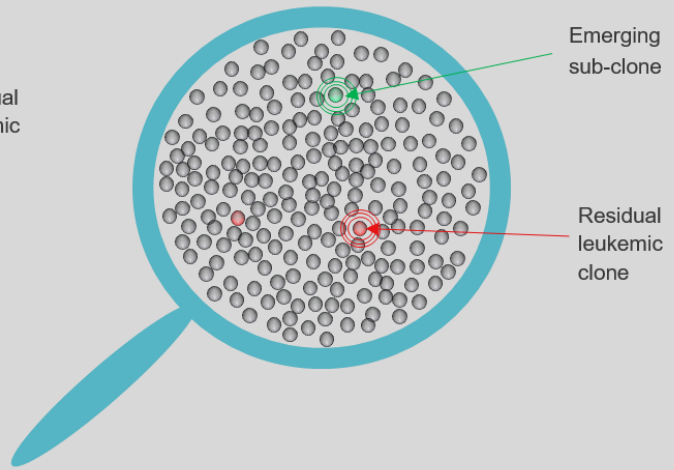
Traditional low-sensitivity methods, like morphological analysis, only evaluate a limited number of cells. They are generally unable to detect a rare residual leukemic cell, so they are not appropriate for MRD detection.

PCR/Flow Cytometry



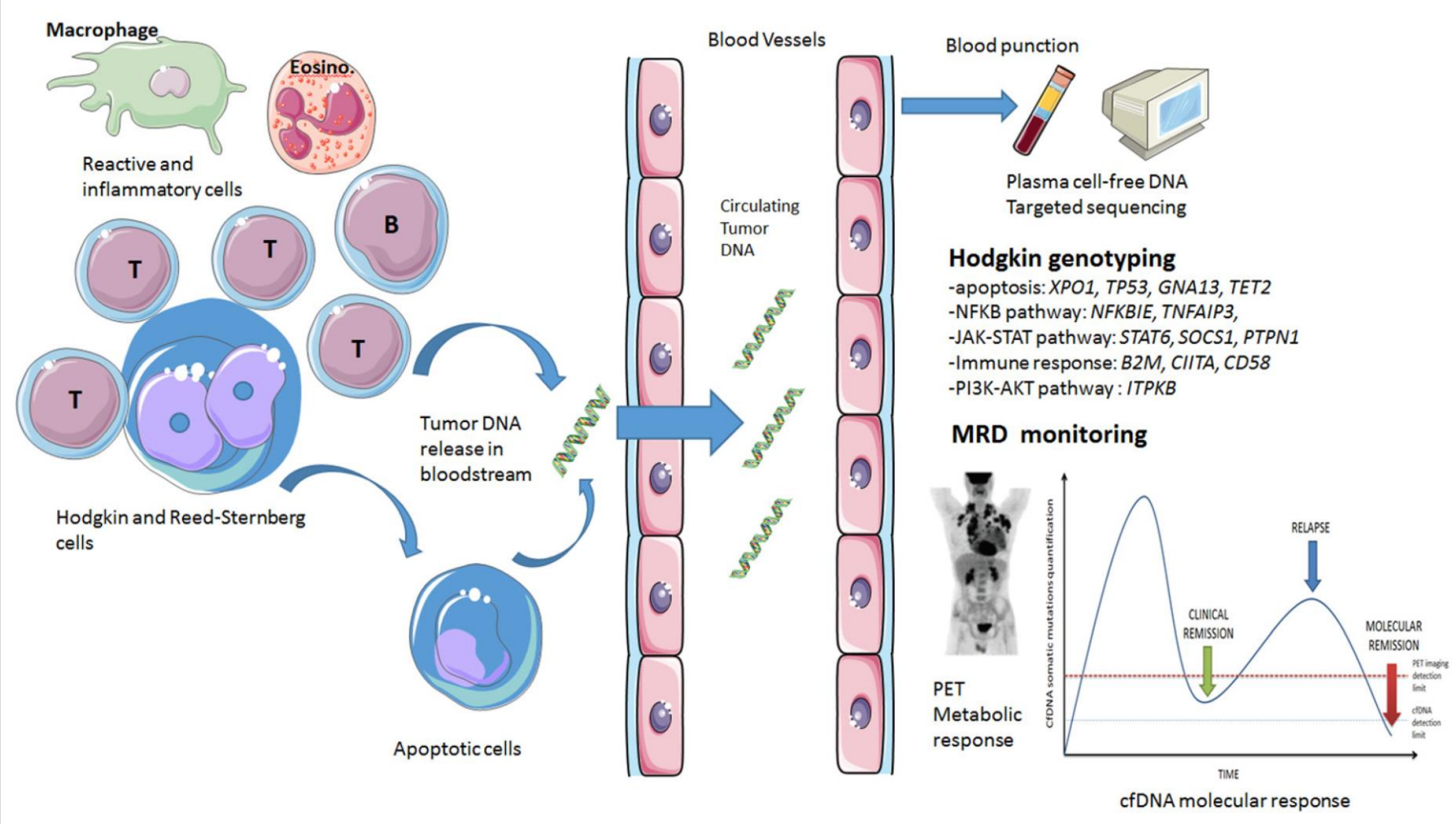
Highly sensitivity methods, like qPCR or flow cytometry, can evaluate a very large number of cells, so they can detect a rare residual leukemic cell. These techniques only evaluate a limited number of markers, so they can miss emerging sub-clones

NGS



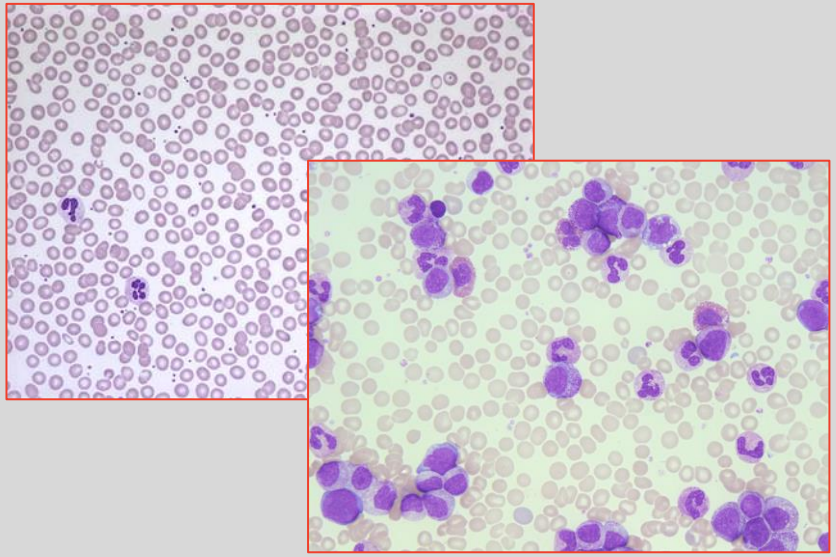
Next-generation sequencing provides high sensitivity and targets a wide range of genetic targets at once. This allows tracking of known targets, while allowing labs to identify emerging mutations, which can indicate the presence of a new sub clone.

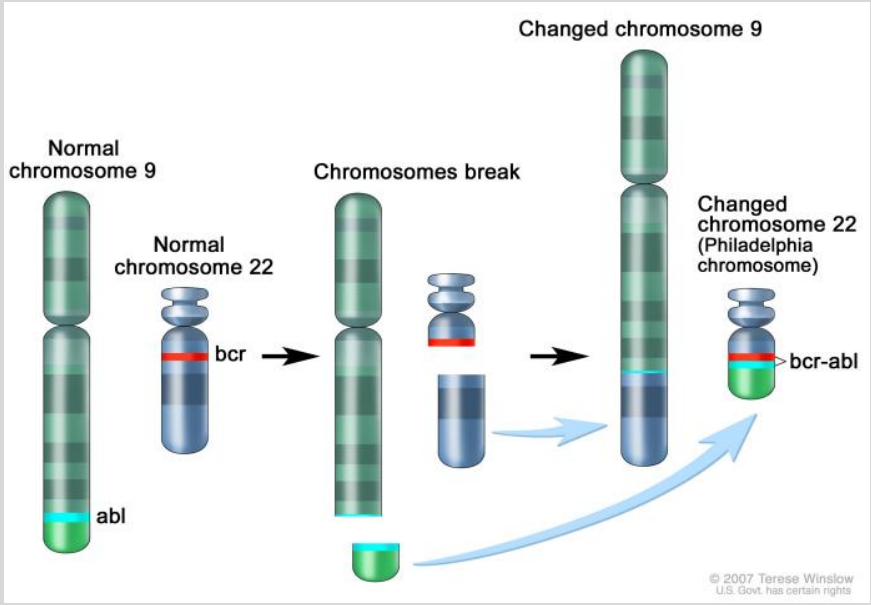
MRD nel cHL: LB come innovazione



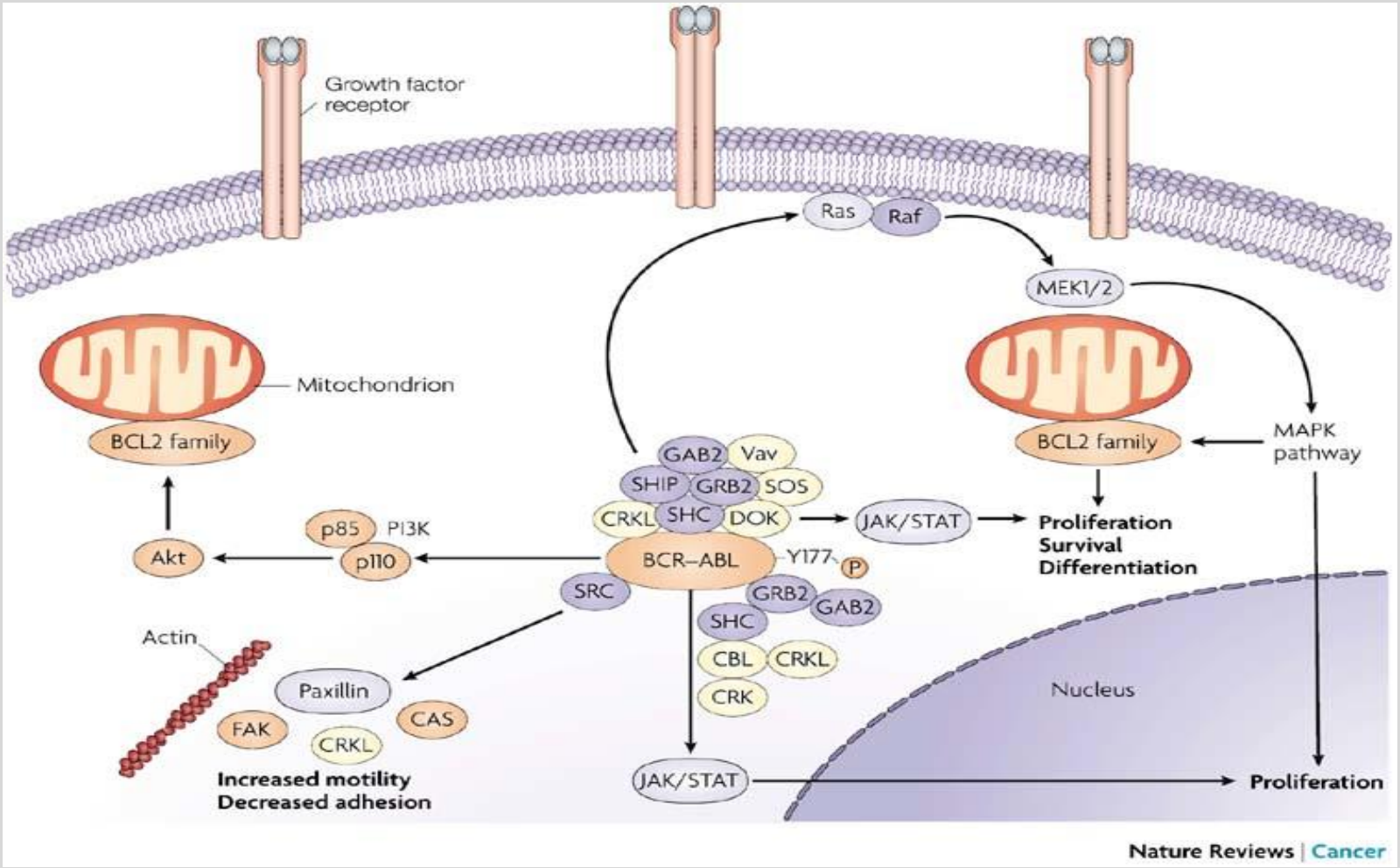
CHRONIC MYELOID LEUKEMIA: BACKGROUND

- Philadelphia+ Chronic Myeloid Leukemia (Ph+ CML) is an hematologic malignancy arising from the chromosomal alteration t(9;22).
- The fusion gene BCR-ABL1 is generated by this translocation and it is the hallmark of Ph+ CML.





BCR-ABL1

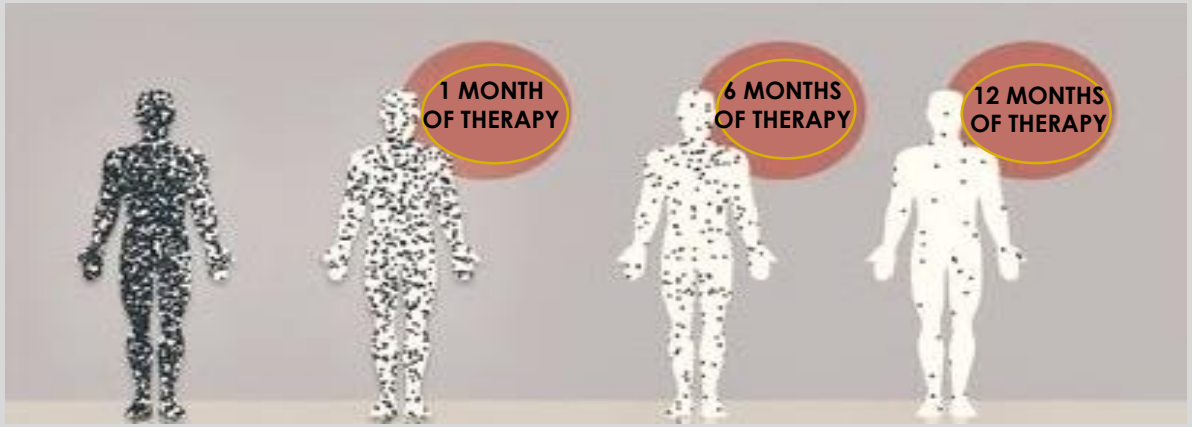

Growth factor receptor
Ras
Raf
MEK1/2
Mitochondrion
BCL2 family
BCL2 family
MAPK pathway
GAB2
Vav
SHIP
GRB2
SOS
CRKL
SHC
DOK
JAK/STAT
Proliferation
Survival
Differentiation
p85
PI3K
p110
Akt
BCR–ABL
Y177
P
SRC
GRB2
GAB2
SHC
CBL
CRKL
CRK
Actin
Paxillin
CAS
FAK
CRKL
Increased motility
Decreased adhesion
Nucleus
JAK/STAT
Proliferation
Nature Reviews | Cancer

Ph+ CML THERAPY

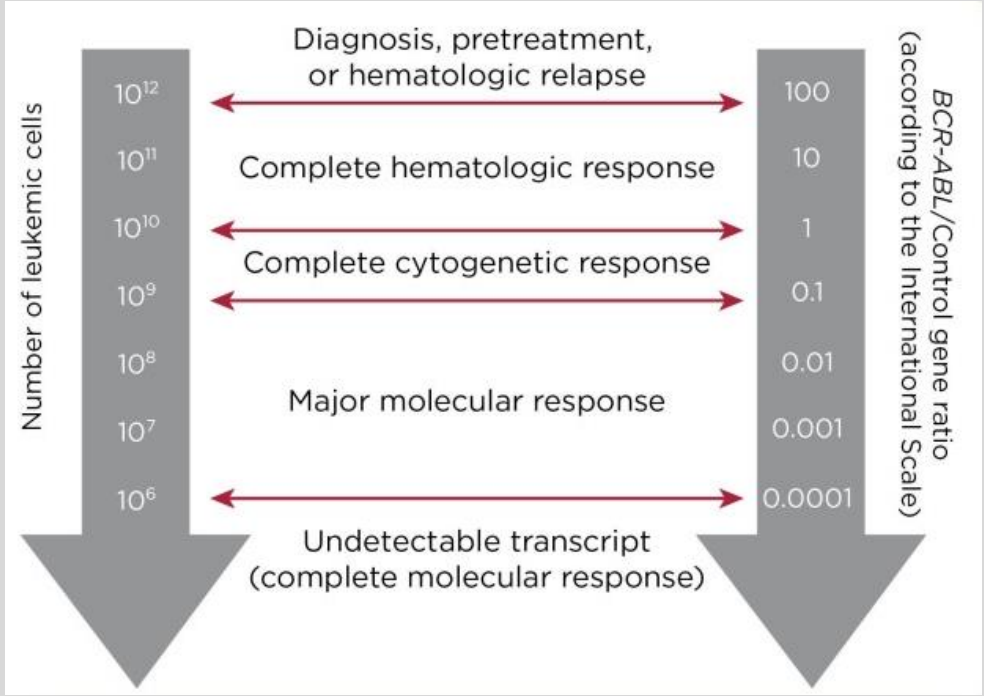
- Tyrosine-kinase inhibitors (TKIs) molecules selectively targeted against BCR-ABL1 protein have been developed about 20 YEARS AGO.

- TKIs transformed Ph+ CML to a real chronic disease.

- The key goal of the TKIs treatment is to achieve a Minimal Residual Disease so low that CML may be clinically "cured".



Ph+ CML MONITORING



Baccarani et al, 2006

	MMR	DMR		
	MR$^{3.0}$	MR$^{4.0}$	MR$^{4.5}$	MR$^{5.0}$
Minimum sum of ABL1 transcripts irrespective of whether BCR-ABL1 is detected or not	-	10.000 ABL1 copies	32.000 ABL1 copies	100.000 ABL1 copies
BCR-ABL1 IS levels for positive samples	≤ 0.1%	≤ 0.01%	≤ 0.0032%	≤ 0.001%

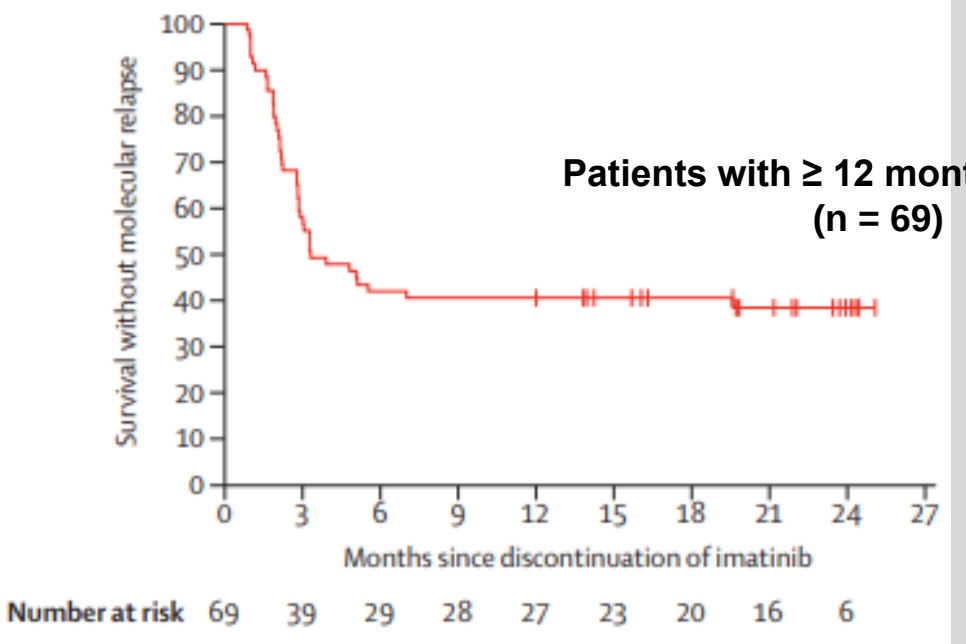
Current definition of MR classes following the last IS guide lines.

THE AVAILABILITY OF POWERFUL NEW GENERATION TKIs INCREASED THE ACHIEVEMENT OF DURABLE UNDETECTABLE DMR IN MANY PATIENTS.

↓

…MIGHT THEY BE REALLY CURED?

THE *TREAMENT FREE REMISSION (TFR)*



Patients with ≥ 12 months follow-up (n = 69)

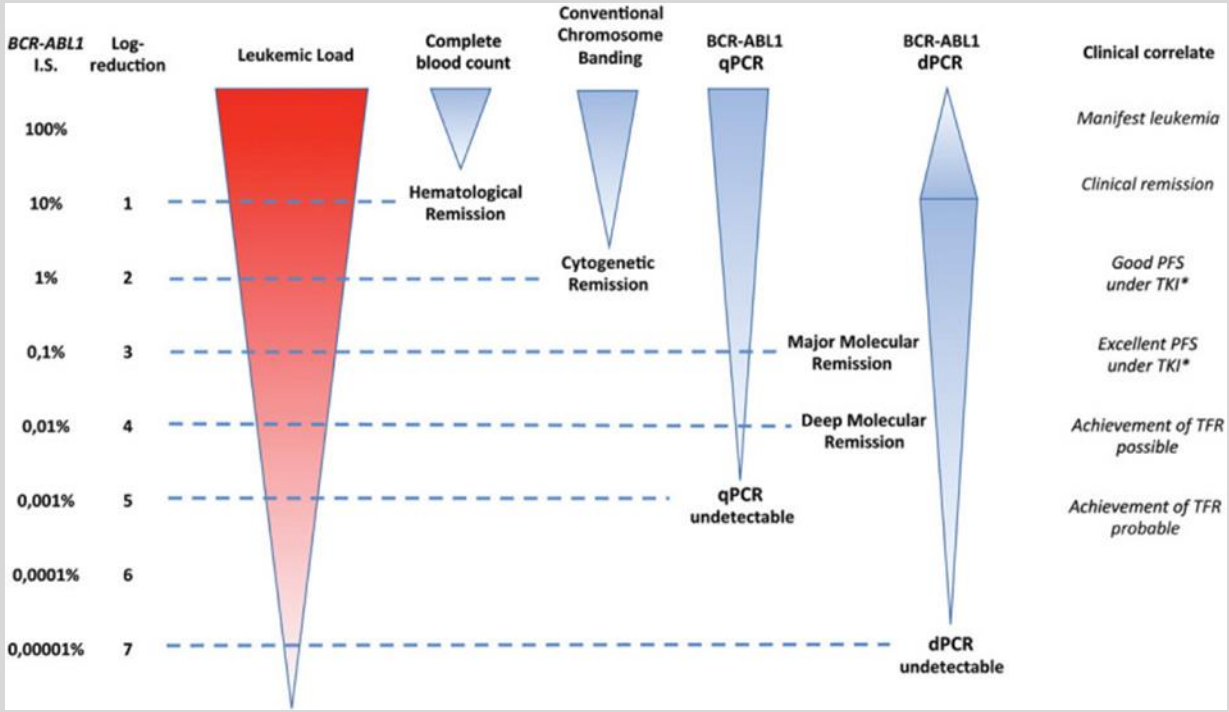
STIM study, Mahon et al, 2010

DEFINITION OF MOLECULAR RELAPSE:
loss of DMR or 1Log increased BLR-ABL1 transcript ratio for 2 consecutive quantification

TKI discontinuation has been conventionally conducted **IN THE REAL LIFE** for about 3 years

TKI discontinuation **IS NOT A TOTALLY SAFE POLICY**

NEW TOOLS FOR THE MRD: THE DIGITAL PCR



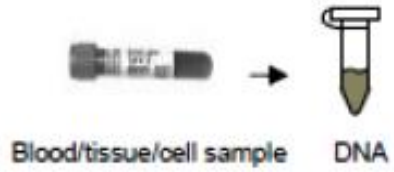
Kockerols et al, 2020

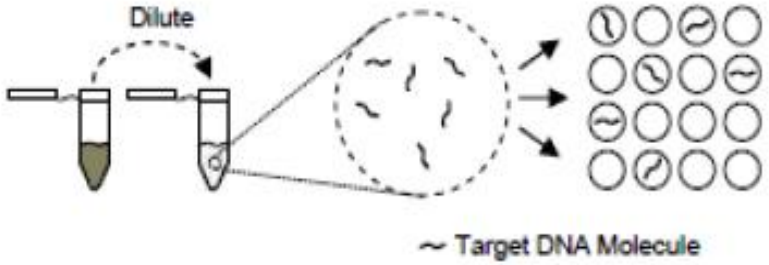
- Increased accuracy and precision
- Absolute quantification
- Reduced effect of PCR inhibitors
- Potentially improved sensitivity

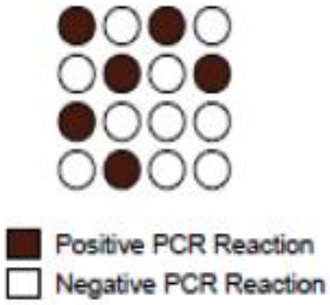
BCR-ABL1 ABSOLUTE QUANTIFICATION

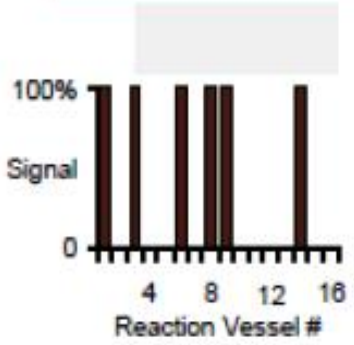
SAMPLE PREPARATION	DILUITION AT 50ng/μl	PARTITIONS by DROPLETS or CHIPS	REACTION OF AMPLIFICATION	DATA ANALYSIS

Illustration:









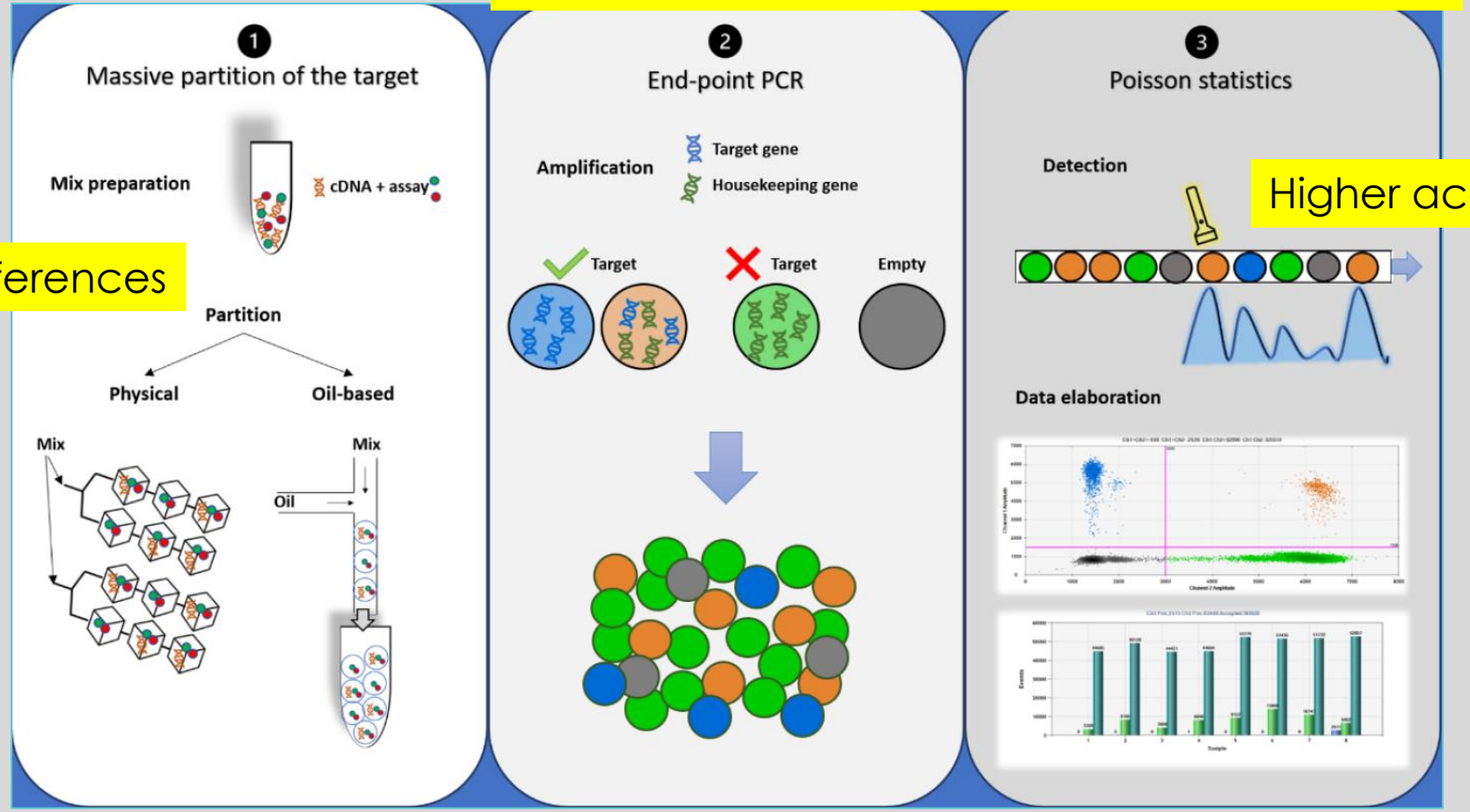
Description:

• Isolate nucleic acid starting material for analysis	• Dilute DNA to achieve a single copy of template per reaction once distributed	• Distribute DNA into multiple reaction vessels	• Perform PCR reactions to amplify single template molecules	• Determine the number of template molecules present

QUANTIFICATION by dPCR

< dependence from amplification efficiency

No external references

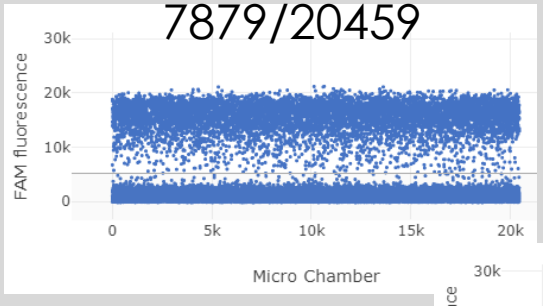


Higher accuracy

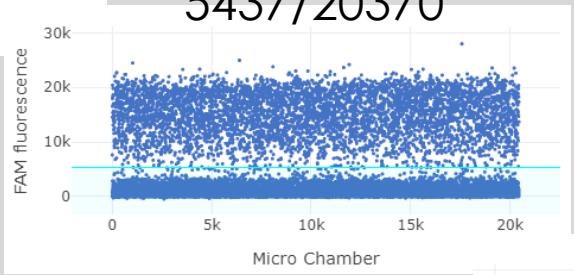
CML PATIENTS MONITORING BY dPCR

NUMBER OF LEUKEMIC CELLS

7879/20459



5437/20370



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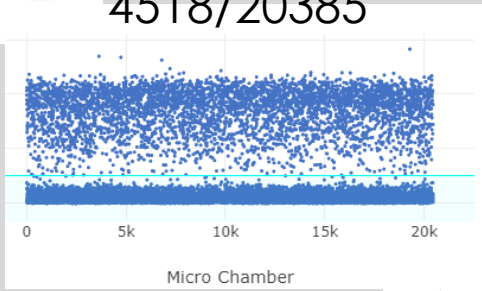

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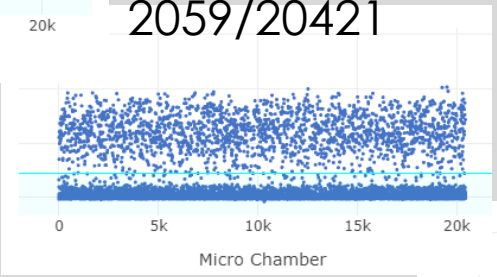
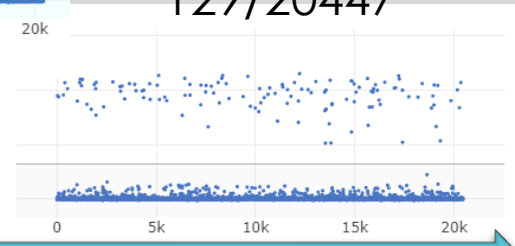


129/20447



ONGOING TREATMENT BY TKI

BCR-ABL1 QUANTIFIABLE by both CHIP- and DROPLET-BASED dPCR

Clinical Trials: Targeted Therapy

Clinical Cancer Research

Evaluation of Residual Disease and TKI Duration Are Critical Predictive Factors for Molecular Recurrence after Stopping Imatinib First-line in Chronic Phase CML Patients

Franck Emmanuel Nicolini[1,2,3], Stéphanie Dulucq[3,4], Lisa Boureau[4], Pascale Cony-Makhoul[3,5], Aude Charbonnier[3,6], Martine Escoffre-Barbe[3,7], Françoise Rigal-Huguet[3,8], Valérie Coiteux[3,9], Bruno Varet[3,10], Viviane Dubruille[3,11], Pascal Lenain[3,12], Philippe Rousselot[3,13], Delphine Rea[3,14], Agnès Guerci-Bresler[3,15], Laurence Legros[3,16], Jixing Liu[3,17], Martine Gardembas[3,18], Jean-Christophe Ianotto[3,19], Pascal Turlure[3,20], Hyacinthe Johnson-Ansah[3,21], Juliana Martiniuc[22], Henry Jardel[23], Bertrand Joly[24], Patricia Zunic[3,25], Tawfiq Henni[26], Bruno Villemagne[27], Marc G. Berger[3,28], Emilie Cayssials[3,29], François Guilhot[3,29], Fabrice Larosa[3,30], Joëlle Guilhot[3,29], Gabriel Etienne[3,31], and François-Xavier Mahon[3,31]

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ORIGINAL RESEARCH

WILEY Cancer Medicine

Digital PCR improves the quantitation of DMR and the selection of CML candidates to TKIs discontinuation

Simona Bernardi[1,2] | Michele Malagola[1] | Camilla Zanaglio[1,2] | Nicola Polverelli[1] | Elif Dereli Eke[1,2] | Mariella D'Adda[3] | Mirko Farina[3] | Cristina Bucelli[4] | Luigi Scaffidi[5] | Eleonora Toffoletti[6] | Clara Deambrogi[7] | Fabio Stagno[8] | Micaela Bergamaschi[9] | Luca Franceschini[10] | Elisabetta Abruzzese[11] | Maria Domenica Divona[10] | Marco Gobbi[9] | Francesco Di Raimondo[8] | Gianluca Gaidano[7] | Mario Tiribelli[6] | Massimiliano Bonifacio[5] | Chiara Cattaneo[3] | Alessandra Iurlo[4] | Domenico Russo[1]

RESEARCH ARTICLE

AJH

Age and dPCR can predict relapse in CML patients who discontinued imatinib: The ISAV study

Silvia Mori,[1] Elisabetta Vagge,[1†] Philipp le Coutre,[2] Elisabetta Abruzzese,[3] Bruno Martino,[4] Ester Pungolino,[5] Chiara Elena,[6] Ivana Pierri,[7] Sarit Assouline,[8] Anna D'Emilio,[9] Antonella Gozzini,[10] Pilar Giraldo,[11] Fabio Stagno,[12] Alessandra Iurlo,[13] Michela Luciani,[1] Giulia De Riso,[1] Sara Redaelli,[1] Dong-Wook Kim,[14] Alessandra Pirola,[1] Caterina Mezzatesta,[1] Anna Petroccione,[15] Agnese Lodolo D'Oria,[15] Patrizia Crivori,[15] Rocco Piazza,[1] and Carlo Gambacorti-Passerini[1,16*]

Hematological ONCOLOGY

LETTER TO THE EDITOR

Digital droplet PCR at the time of TKI discontinuation in chronic-phase chronic myeloid leukemia patients is predictive of treatment-free remission outcome

Gioia Colafigli, Emilia Scalzulli, Marika Porrazzo, Daniela Diverio, Maria Giovanna Loglisci, Roberto Latagliata, Anna Guarini, Robin Foà, Massimo Breccia

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Peer Review:
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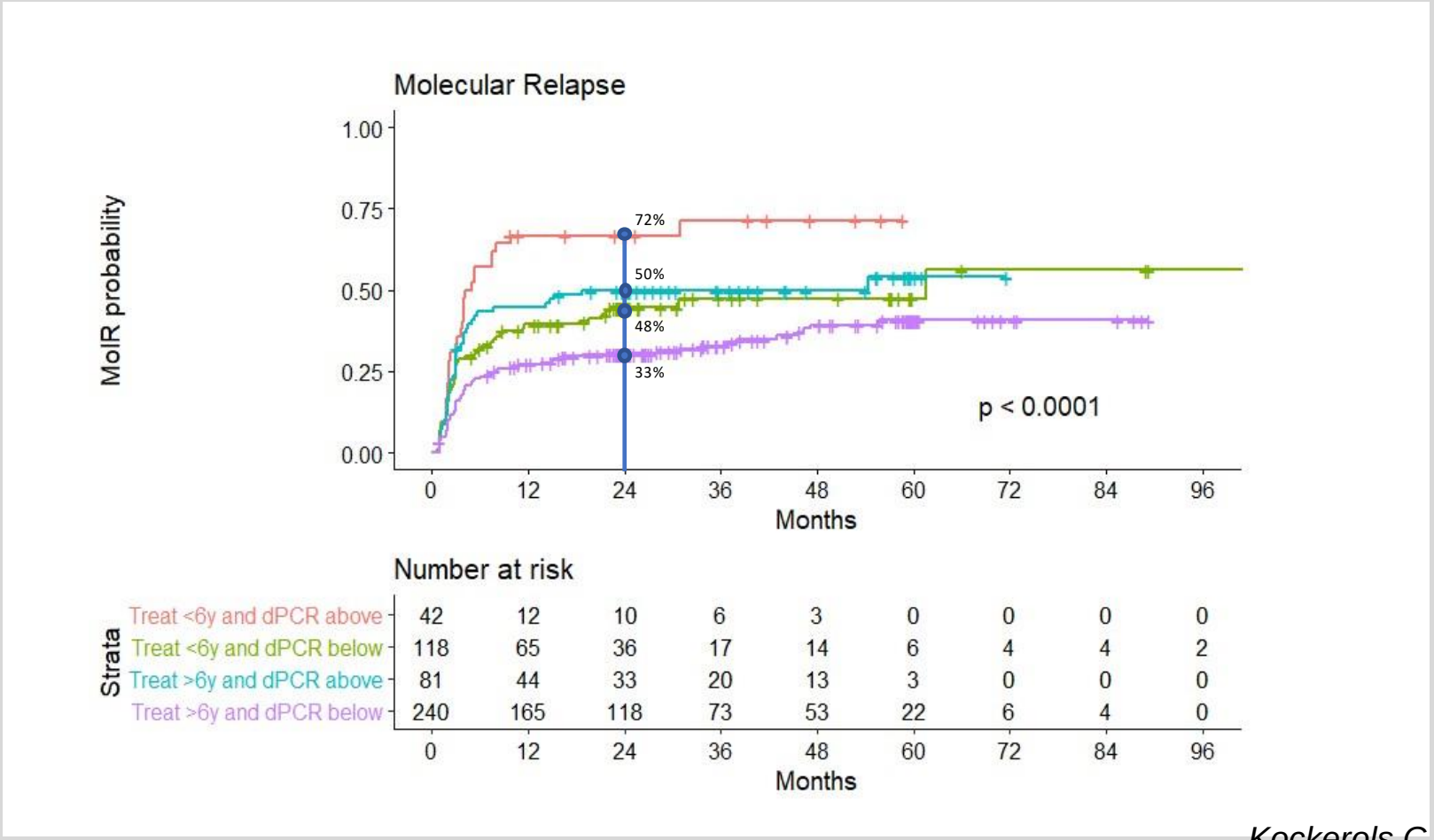
INDIVIDUAL PATIENT DATA META-ANALYSIS: PRELIMINARY RESULTS



Kockerols C. et al, in submission

dPCR ONLY FOR CML?

DISEASE	TARGET
ALL	IgH; BCR-ABL1
HCL	B-RAF
CLL	TP53; NOTCH1
WM	MYD88
HL	STAT6
FL	EZH2; BCL2/JH
MCL	BCL1/JH
MM	IgH

THANK YOU…