IL MONITORAGGIO DELLA MRD E LA DIGITAL PCR

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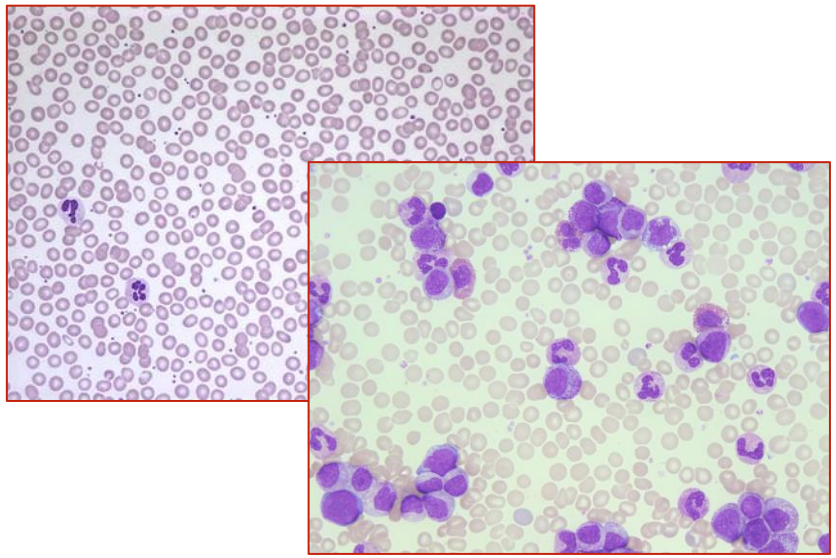
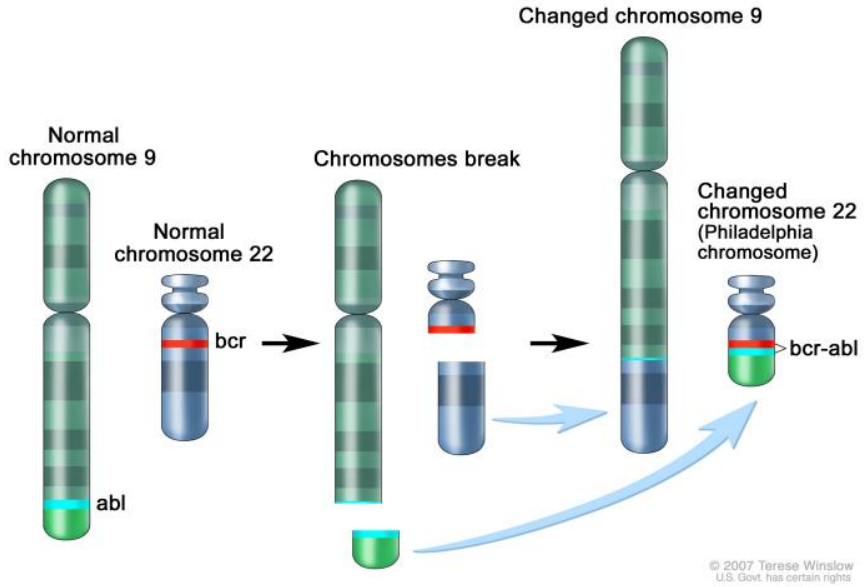
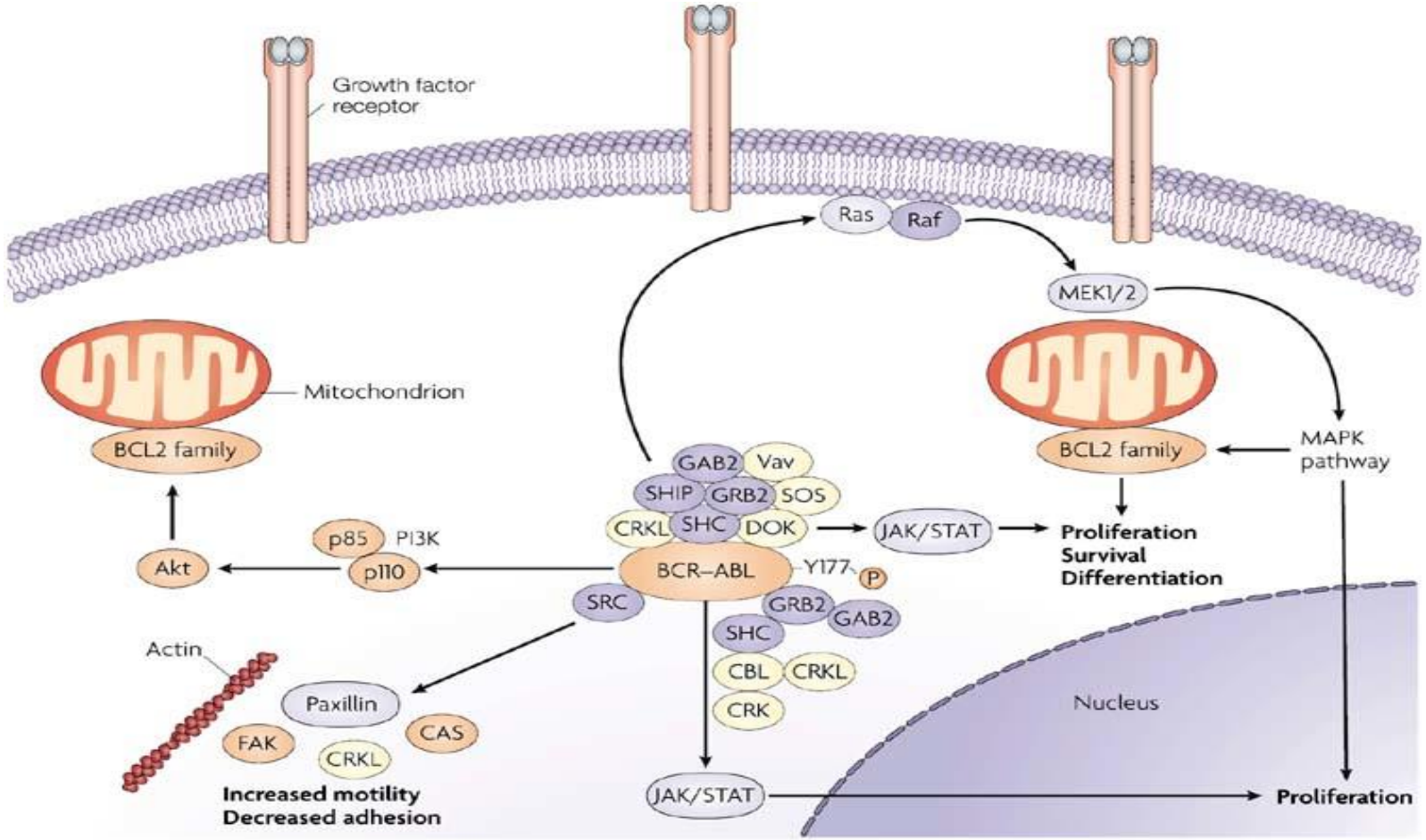
CHRONIC MYELOID LEUKEMIA: BACKGROUND

- 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



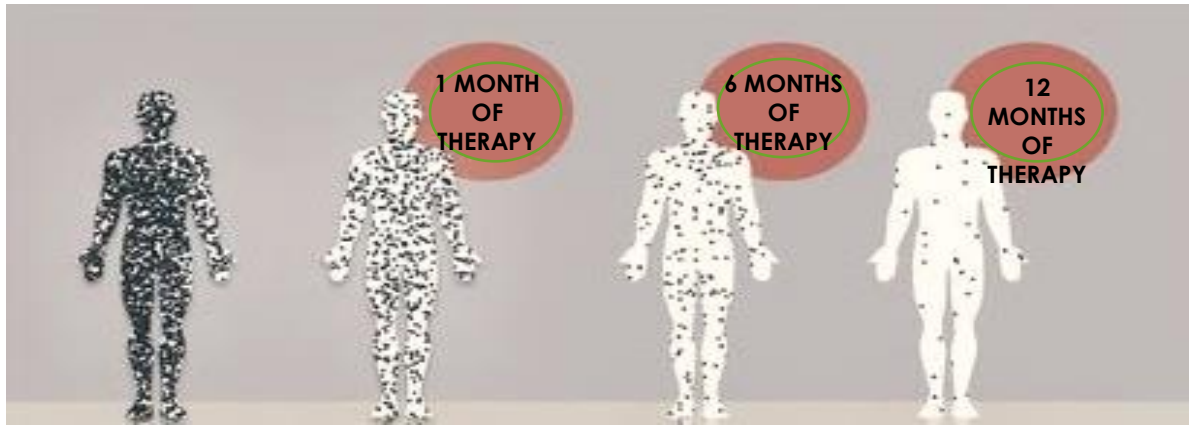
Nature Reviews | Cancer

Ph+ CML THERAPY

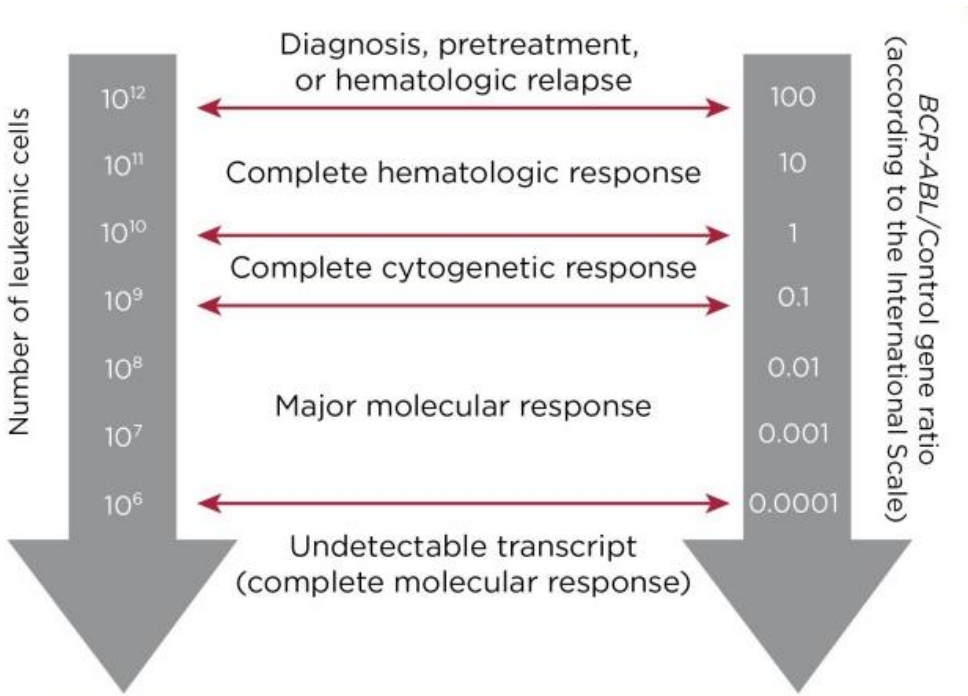
- Tyrosine-kinase inhibitors (TKIs) molecules selectively targeted against BCR-ABL1 protein have been developed about 20 YEARS AGO.

- 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%

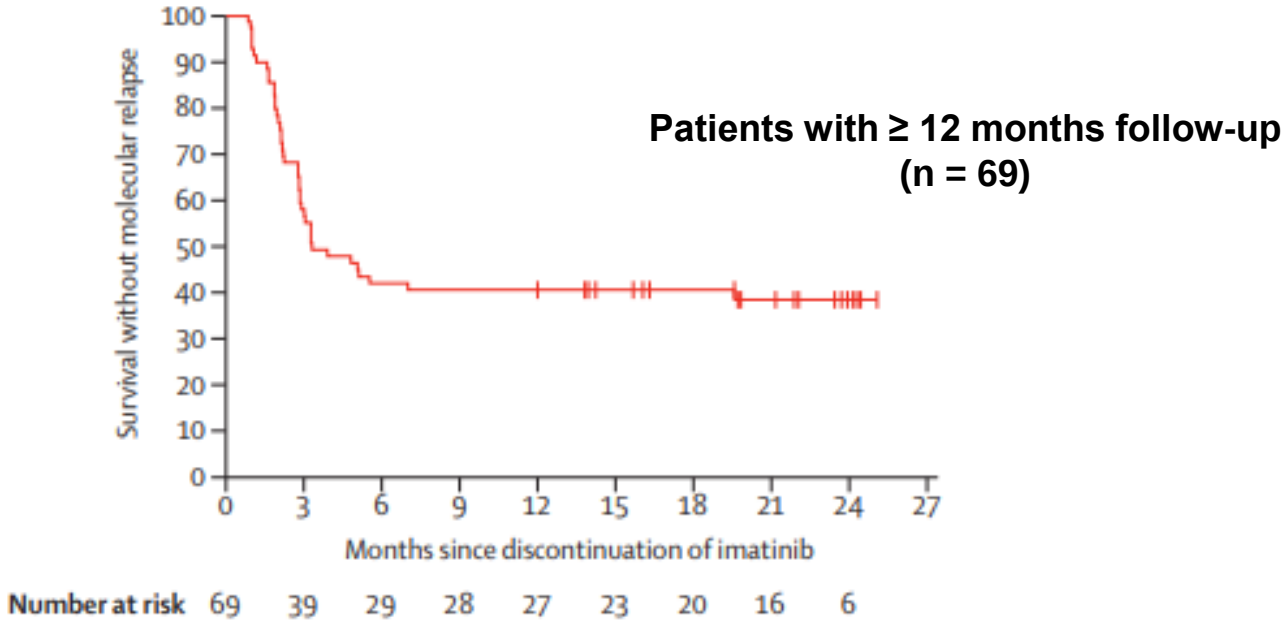
Current definition of MR classes following the last IS guide lines.

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)

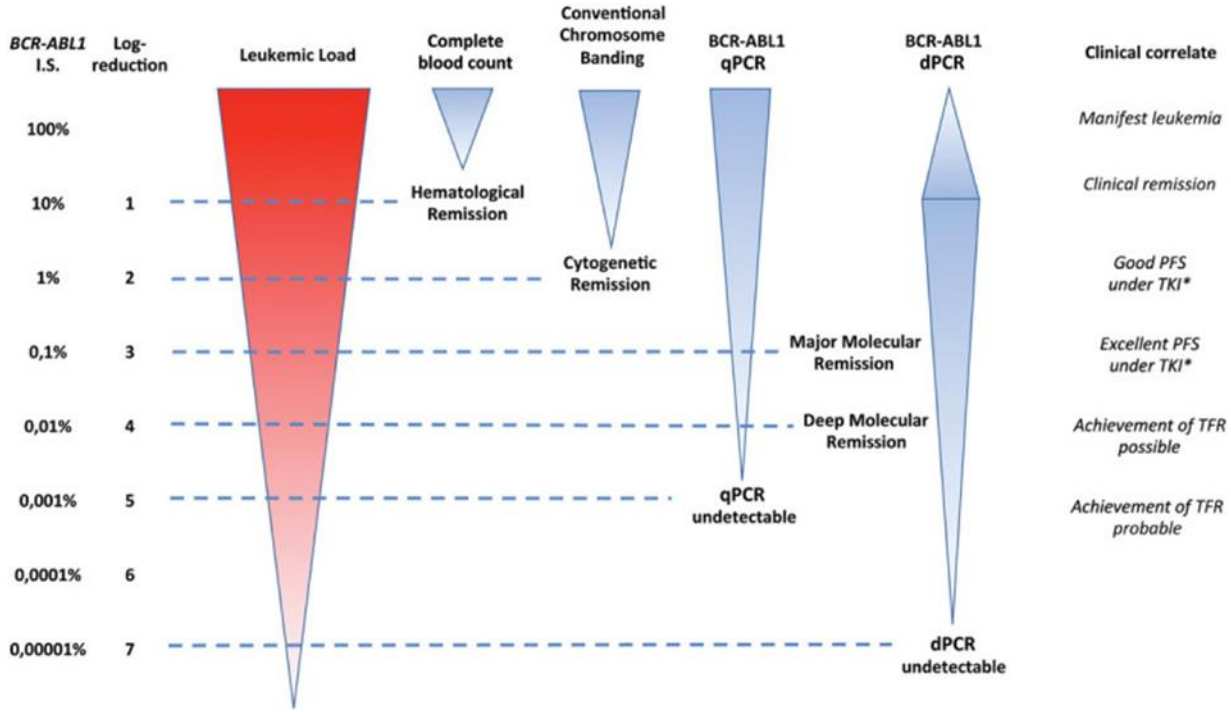
STIM study, Mahon et al, 2010

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



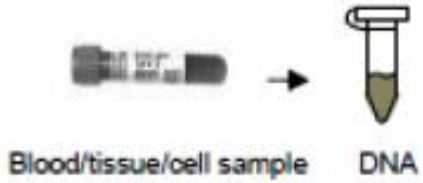
Kockerols et al, 2020

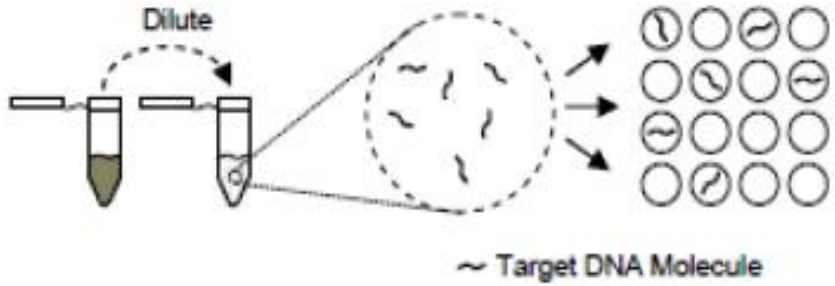
- Increased accuracy and precision
- Absolute quantification
- Reduced effect of PCR inhibitors
- Potentially improved sensitivity

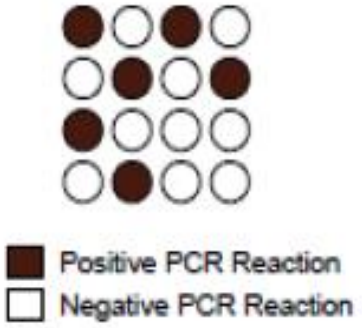
BCR-ABL1 ABSOLUTE QUANTIFICATION

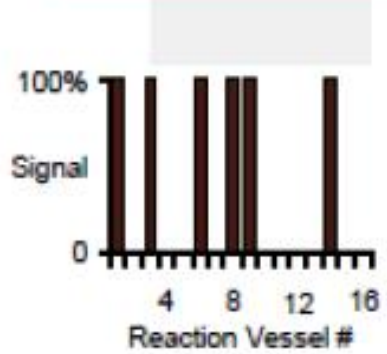
SAMPLE PREPARATION	DILUITION AT 50ng/µl	PARTITIONS by DROPLETS or CHIPS	REACTION OF AMPLIFICATION	DATA ANALYSIS

Illustration:







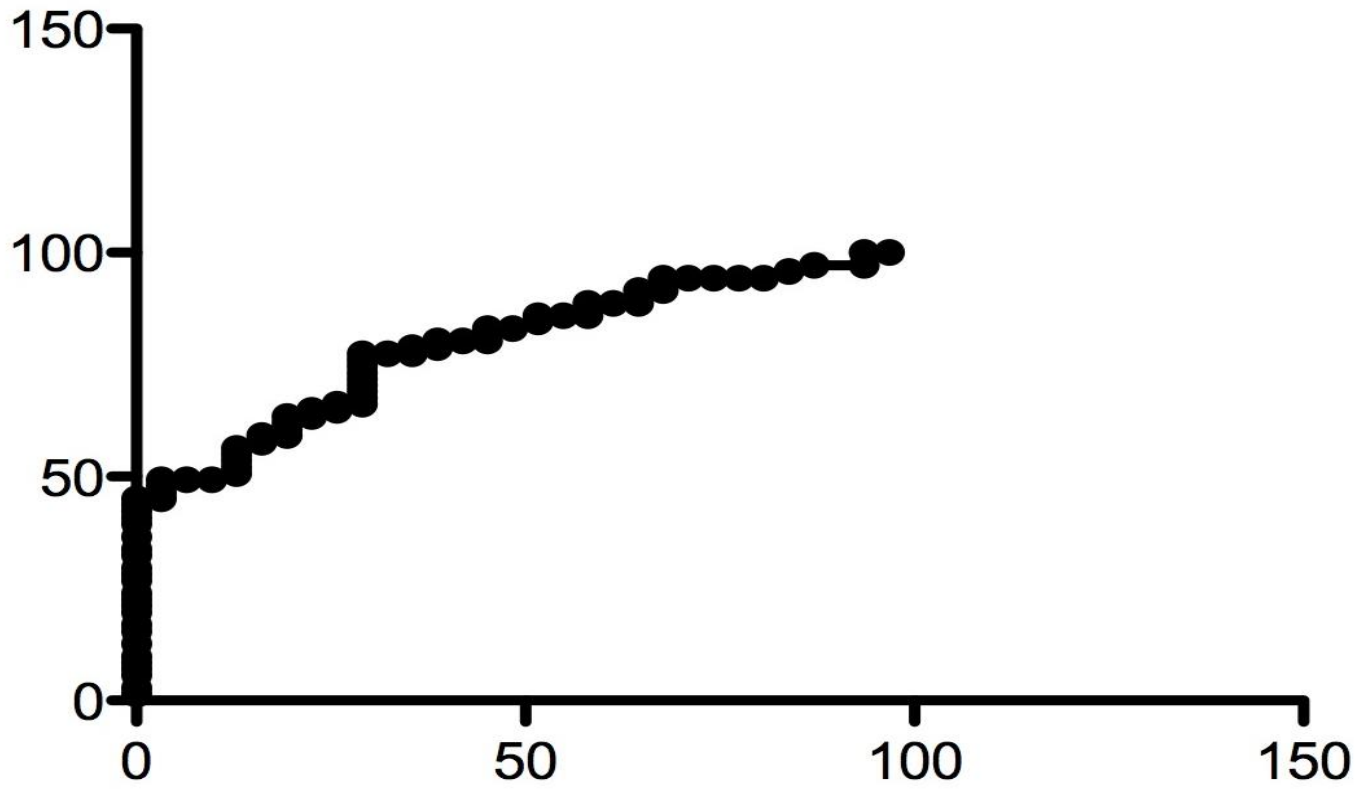


Description:

• 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

IS **DIGITAL PCR** SUITABLE FOR THE IDENTIFICATION OF CML PATIENTS WHO MAY SUCCESSFULLY ATTEMPT AN EARLY TKI DISCONTINUATION?

NEW CUT-OFF

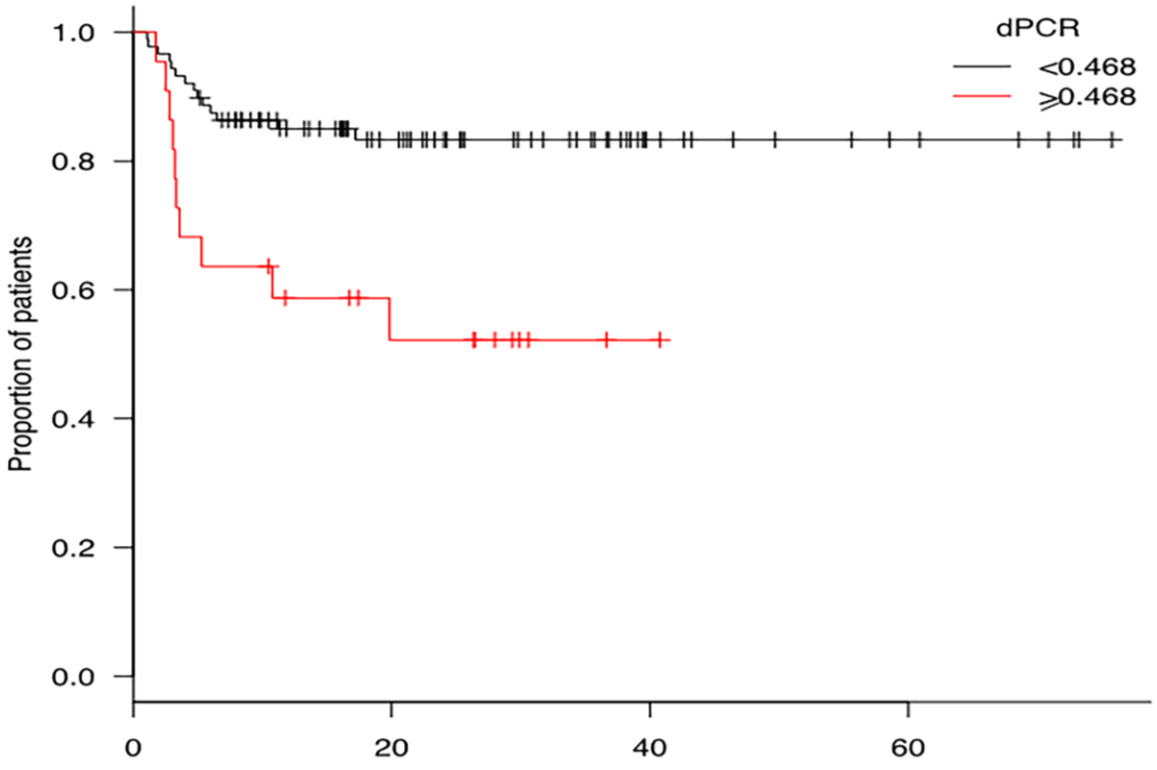


0,468 BCR-ABL1 copies/ul = 6 copies of transcript

(spec.= 71%, sens.= 78%, AUC=0,79)

Bernardi S. et al, J Mol Biom Diag, 2017

BCR-ABL1 QUANTIFIED by digital PCR QS3D



P=0.0017

Bernardi S. et al, 2019

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

First published: 19 July 2019 | https://doi.org/10.1002/hon.2650 | Citations: 12

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META-ANALYSIS: the DESIGN by PRISMA-IPD guideline

1. Eligibility criteria: Adult CP-CML patients who discontinued TKI therapy, with a prior BCR-ABL1 digital PCR assessment

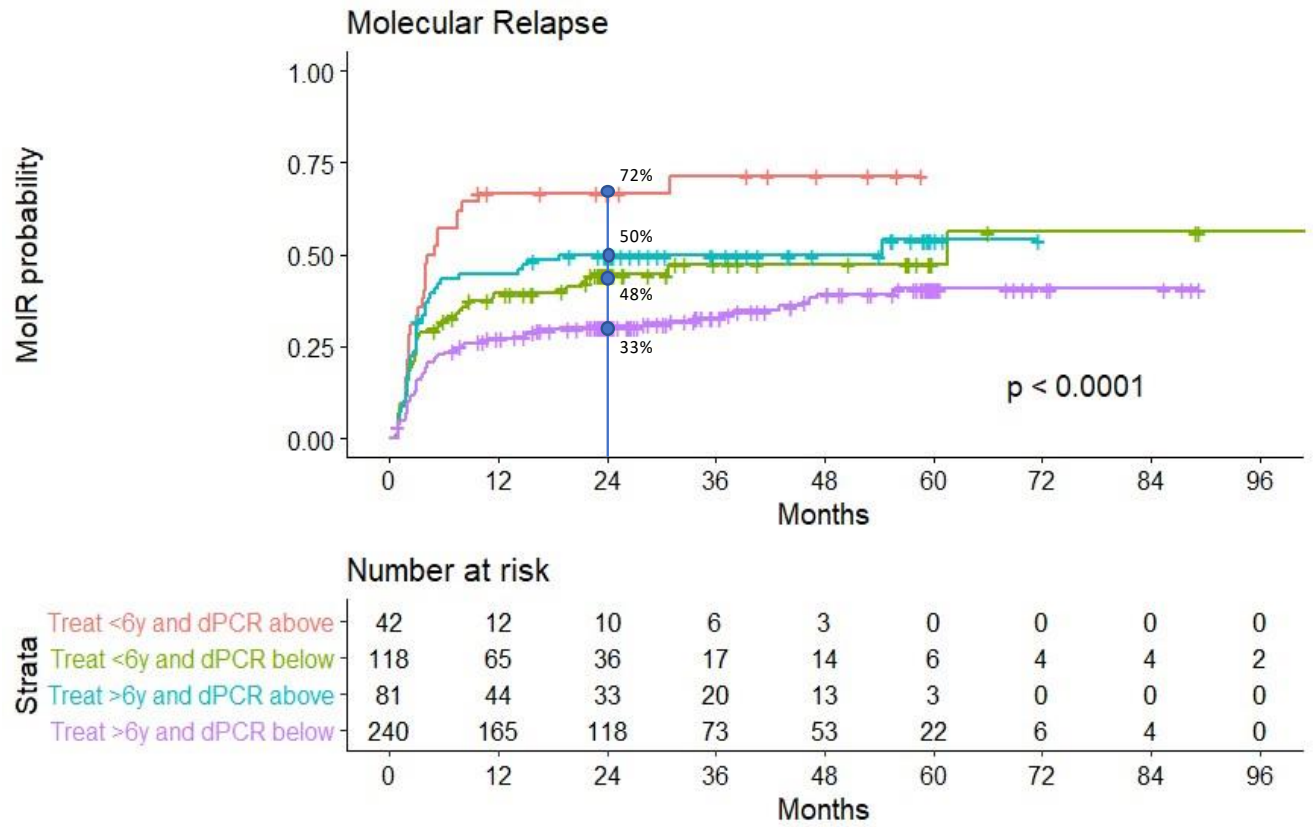
2. Identifying studies: Five published and one unpublished cohort

3. Collecting and pooling patient-level data
Digital PCR result dichotomized

4. Data-analysis: one-stage approach correcting for study heterogeneity including a frailty term in regression models

Total patient number: 483

INDIVIDUAL PATIENT DATA META-ANALYSIS: PRELIMINARY RESULTS



Kockerols C. et al, in submission

The STANDARDIZATION

diseases

MDPI

Article

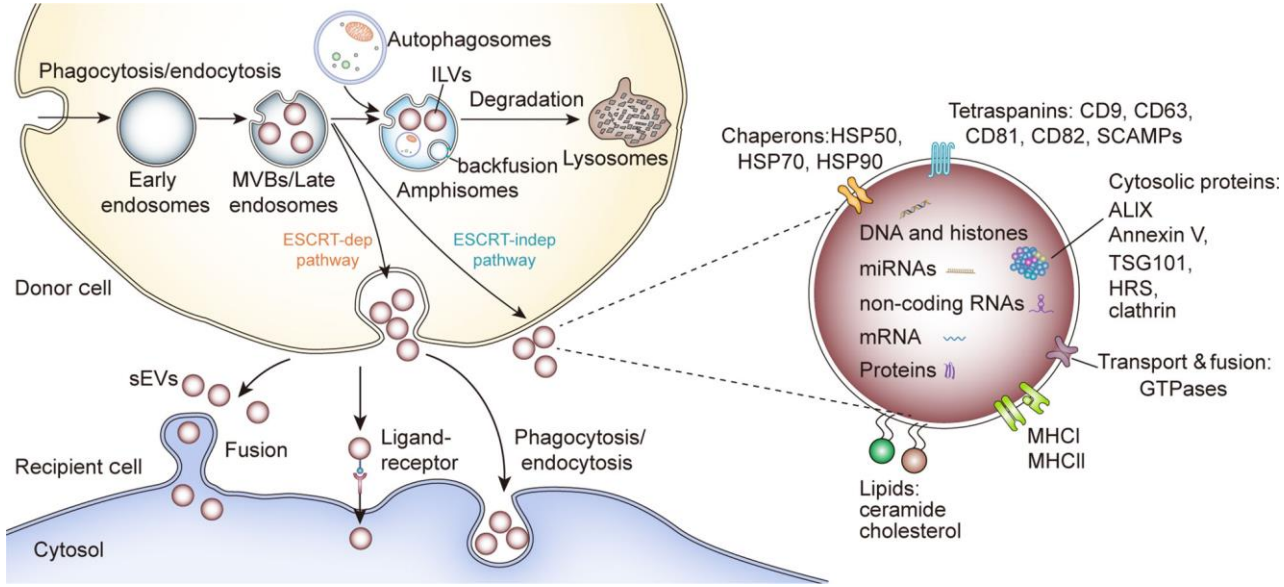
Alignment of Qx100/Qx200 Droplet Digital (Bio-Rad) and QuantStudio 3D (Thermofisher) Digital PCR for Quantification of BCR-ABL1 in Ph+ Chronic Myeloid Leukemia

Carmen Fava [1,*], Simona Bernardi [2], Enrico Marco Gottardi [3], Roberta Lorenzatti [3], Laura Galeotti [4], Francesco Ceccherini [4], Francesco Cordoni [4], Filomena Daraio [3], Emilia Giugliano [3], Aleksandar Jovanovski [1], Jessica Petiti [1], Marta Varotto [5], Davide Barberio [5], Giovanna Rege-Cambrin [3], Paola Berchialla [1], Veronica Sciannameo [6], Michele Malagola [2], Giuseppe Saglio [1] and Domenico Russo [2]

First study about the interlaboratory standardization of BCR-ABL1 transcript quantification by dPCR:

- chip-based and droplet-based platforms present compatible results
- An alignment factor is computable and helps in the improvement of the quantifications' comparability
- It opens to further studies for international standardization, considering also additional platforms

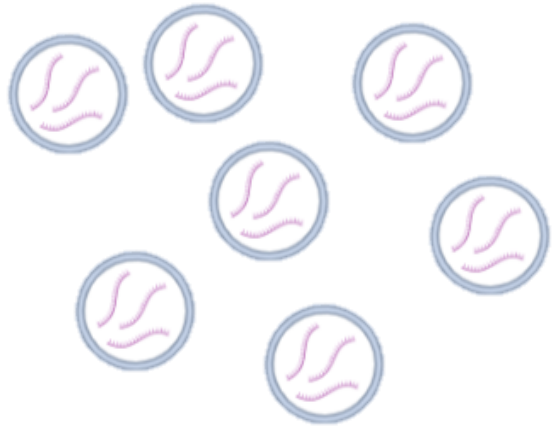
Small EXTRACELLULAR VESICLES (sEVs)



sEVs in Ph+ CML:

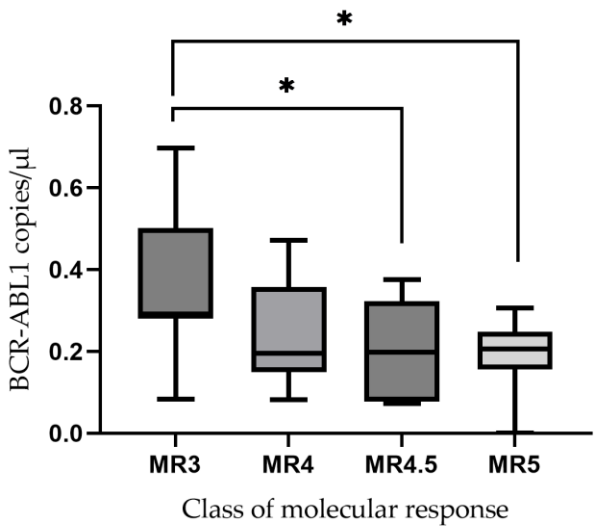
- LEUKEMIC CELLS PROLIFERATION
- PRO-LEUKEMIC MICROENVIRONMENT
- ANGIOGENESIS

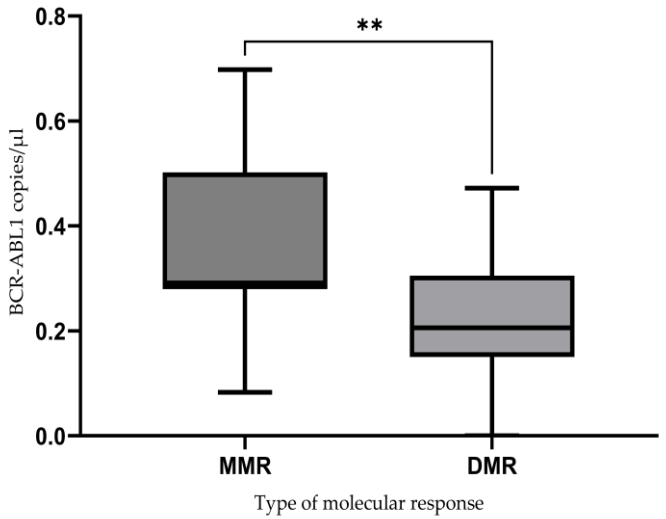
What about *BCR-ABL1*+ sEVs?

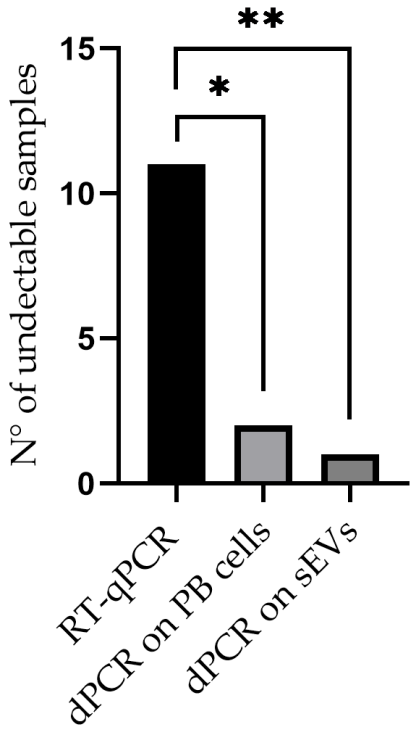


BCR-ABL1+ vesicles

BCR-ABL1+ sEVs: PRELIMINARY DATA







CONCLUSIONS

- MRD monitoring in Ph+ CML patients MUST BE IMPROVED
- dPCR IS A VALUABLE TOOL FOR MRD MONITORING IN ADULT PATIENTS AFFECTED BY Ph+ CML
- dPCR HELPS IN THE SELECTION OF PATIENTS FOR TERAPY DISCONTINUATION
- dPCR STANDARDIZATION IS POSSIBLE AND OPENS TO A WIDE CLINICAL APPLICATION
- WOULD NEW BIOLOGICAL MATRIXES IMPROVE THE DETECTION OF BCR-ABL1+ LEUKEMIC CELLS?