

A Novel Approach for Unique MRD Markers Identification in Acute Leukemia Patients

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Departments:

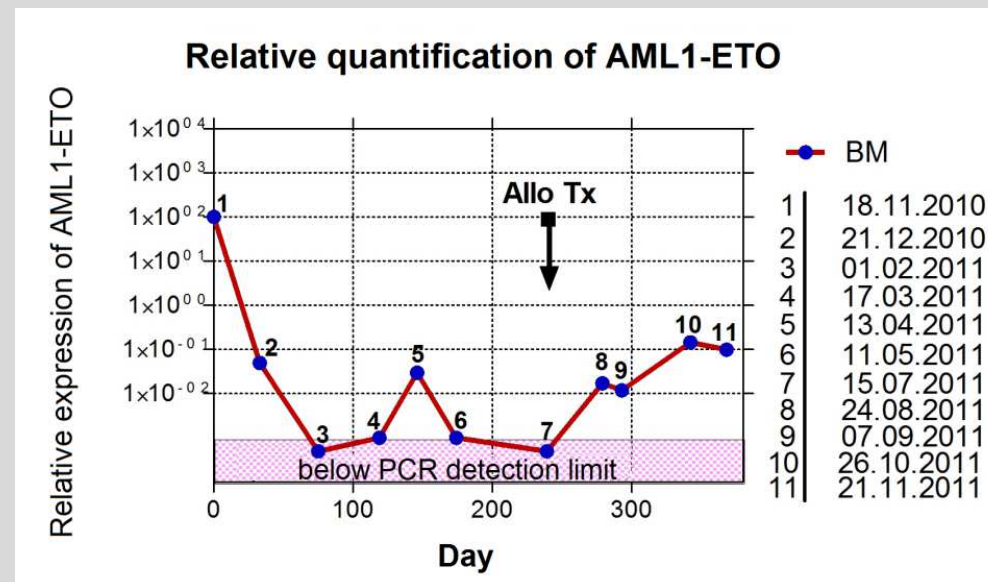
- Cytogenetics
- Molecular hematooncology
- Molecular detection of pathogens
- Molecular detection of rare genetic syndromes

Introduction – Acute Leukemia

- Acute leukemias (AL) – acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL)
- Different prognosis depending on many factors
- Sensitive minimal residual disease (MRD) monitoring:
 - strong prognostic factor
 - assessment of the quality of treatment response
 - prediction of individual risk of relapse
- Real-time PCR technique
 - sensitivity $\sim 10^{-5}$
 - molecular marker is necessary

Introduction – Molecular Markers

- adult ALL patients – in majority cases suitable marker is identified
 - IgH/TCR gene rearrangements
 - cytogenetic abnormalities = fusion transcripts (BCR-ABL, MLL-AF4...)
- adult AML patients – suitable molecular marker in 50 % only
 - cytogenetic abnormalities = fusion transcripts (PML-RARA, AML1-ETO...)
 - gene mutation (*NPM1*, *CEBPA*, *WT1*, *c-KIT*...)



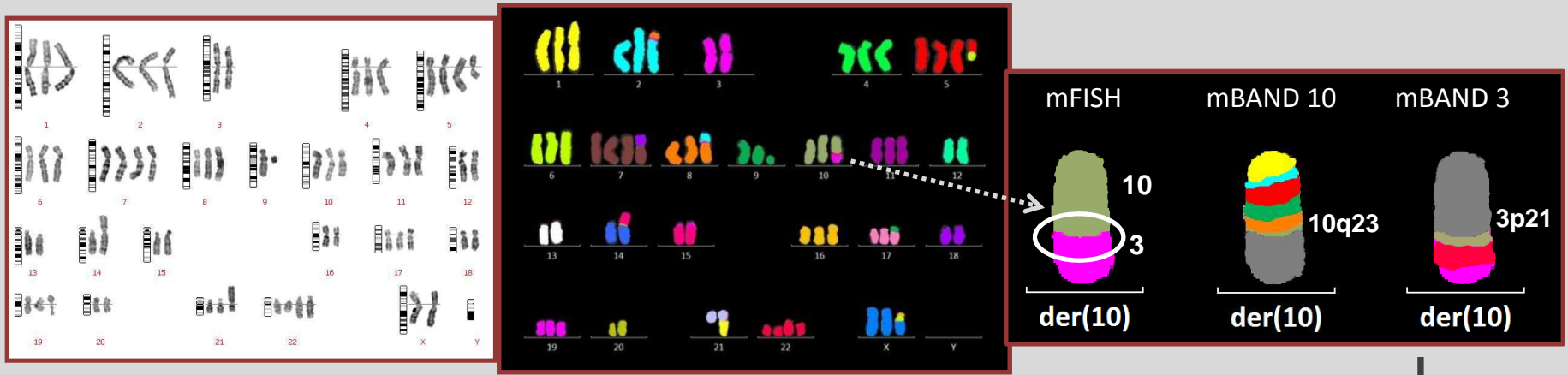
Our Aim

- To develop a flexible strategy for identification of unique molecular targets for sensitive MRD assessment in AL patients
 - mapping of cytogenetic abnormalities down to the single nucleotide level
 - design a specific real-time PCR assay

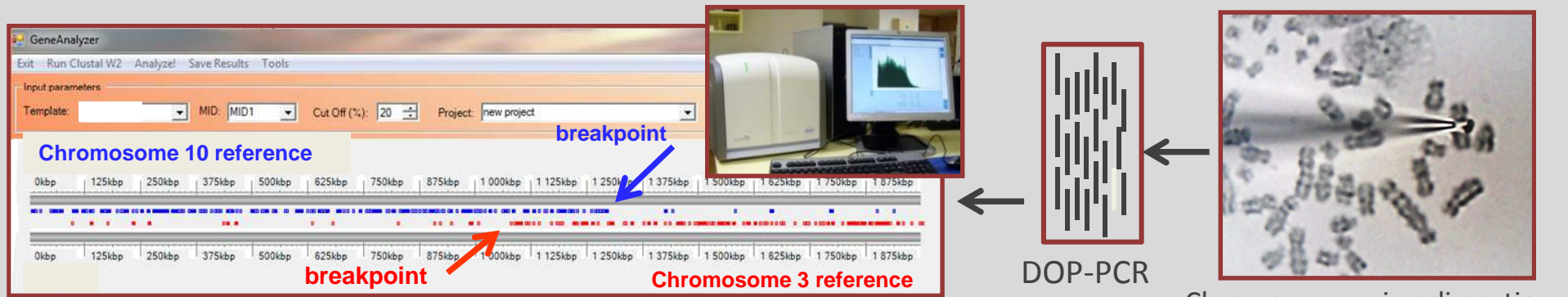
Study Design

- Pilot study - cell line K562
- Patients with acute leukemia

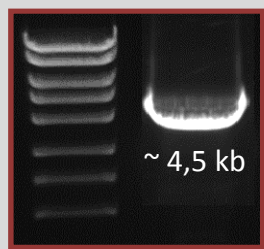
Our strategy – K562 cell line



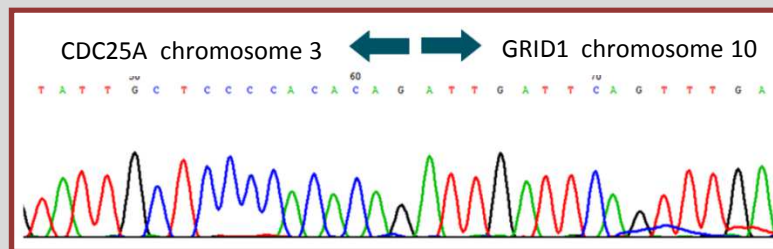
G-banding, mFISH a mBAND: der(10)t(3;10)(p21;q23)



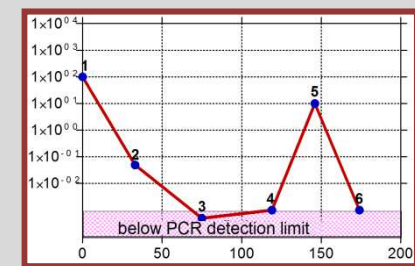
Next-generation sequencing and data analysis



Long-range PCR



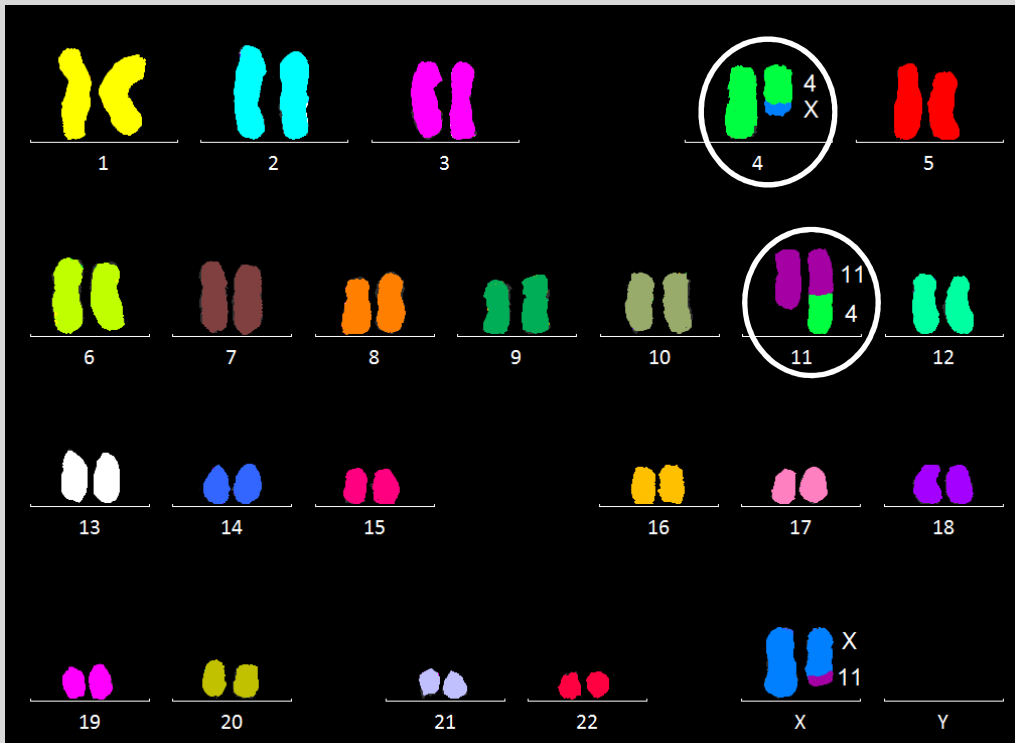
Sanger sequencing



Real-time PCR assay

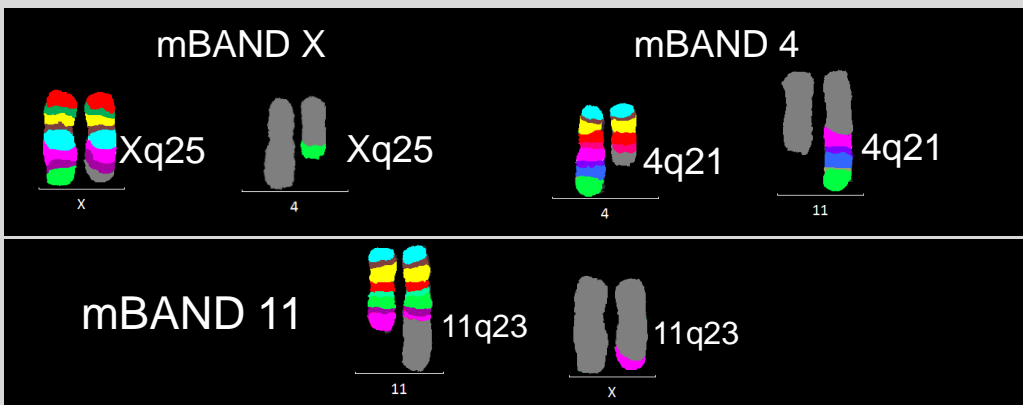
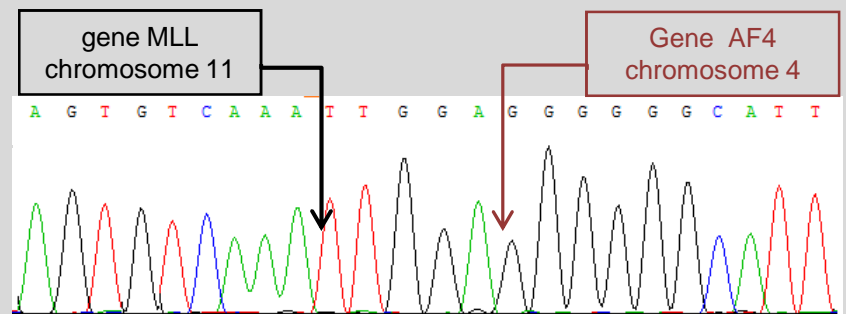
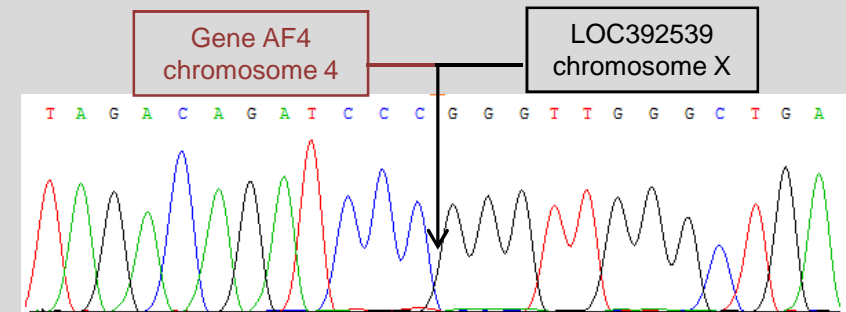
Acute Leukemia Patients

Patient 1



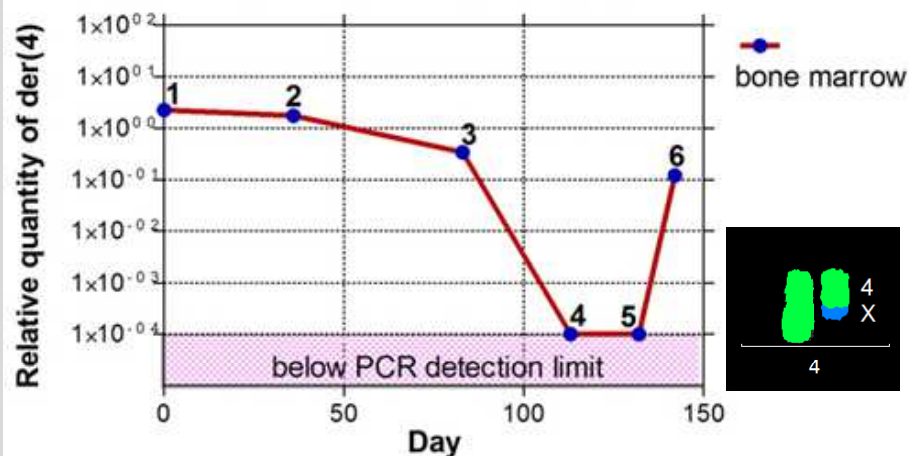
46,XX,t(X;4;11)(q25;q21;q23)[20]

- Fusion transcript MLL-AF4 = comparison of standardized target and newly characterized targets
- Dissection of der(4)
- Dissection of der(11) – with fusion gene MLL-AF4
- DNA sequences of chromosomal breakpoints

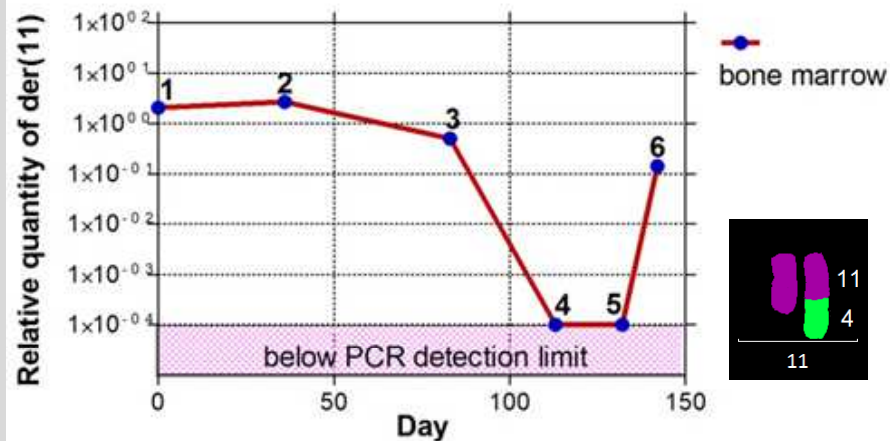


Patient 1 – Quantification Graphs

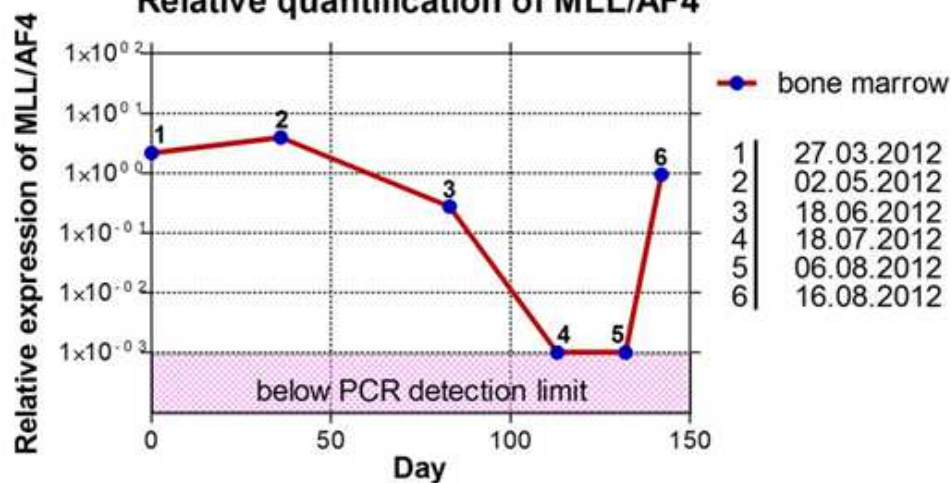
Relative quantity of derivative chromosome 4
(breakpoint 4q21;Xq25)



Relative quantity of derivative chromosome 11
(breakpoint 11q23;4q21)



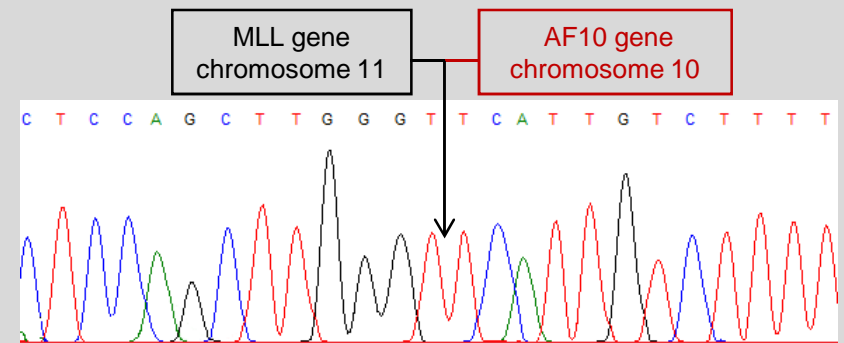
Relative quantification of MLL/AF4



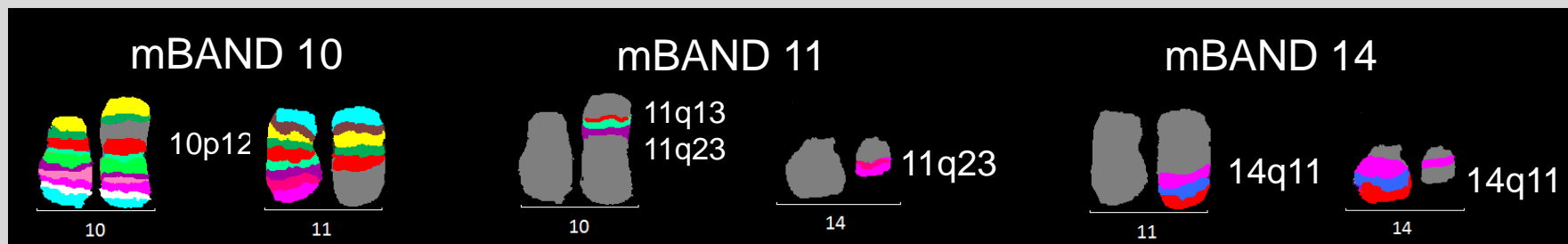
Patient 2



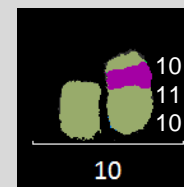
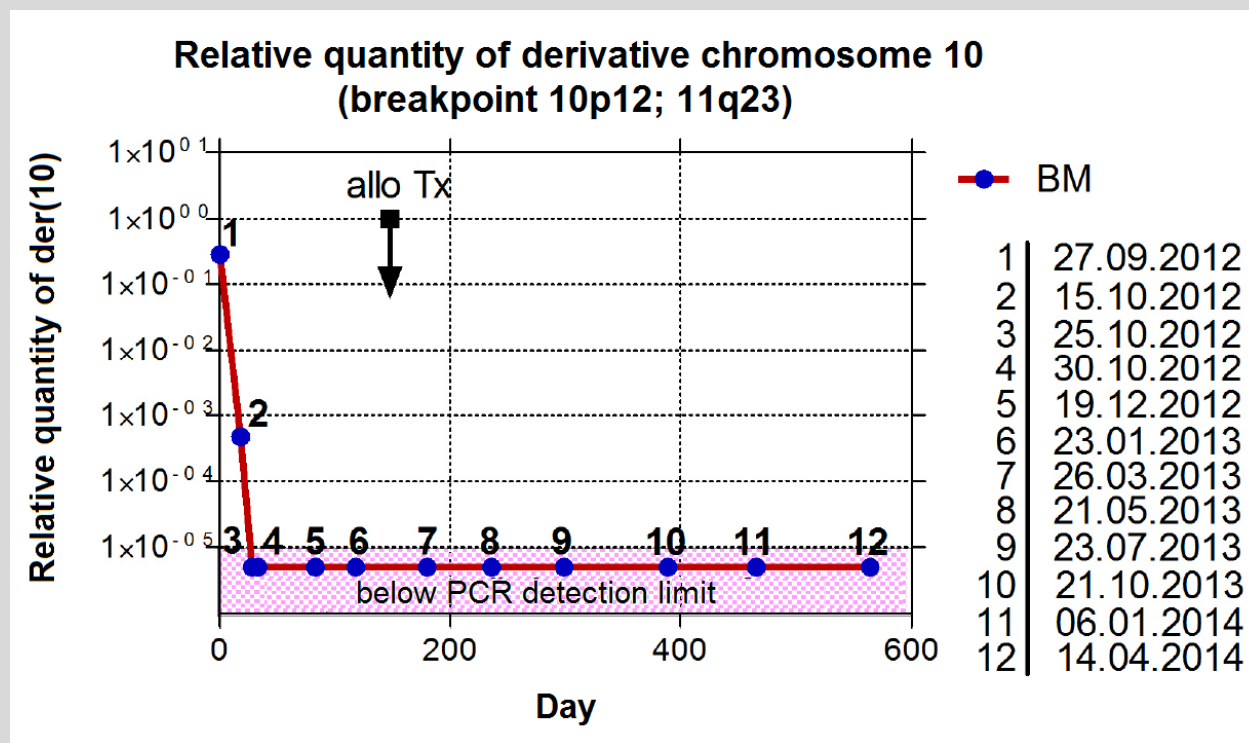
- Fusion transcript MLL-AF10 = low expression
- Need to quantify on DNA level
- Dissection of der(10)
- DNA sequence of MLL-AF10 fusion



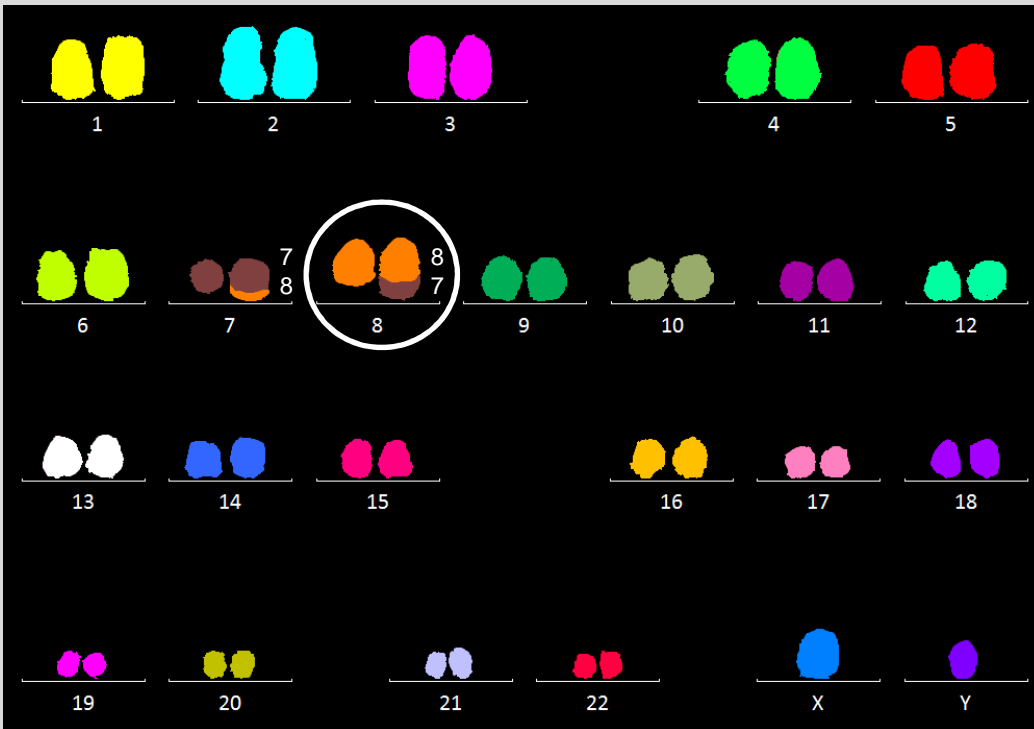
46,XY,ins(10;11)(p12;q13q23),t(11;14)(q13;q11)[20]



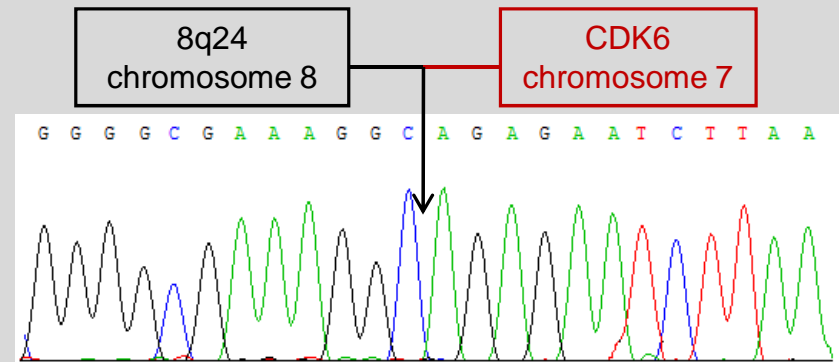
Patient 2 – Quantification Graph



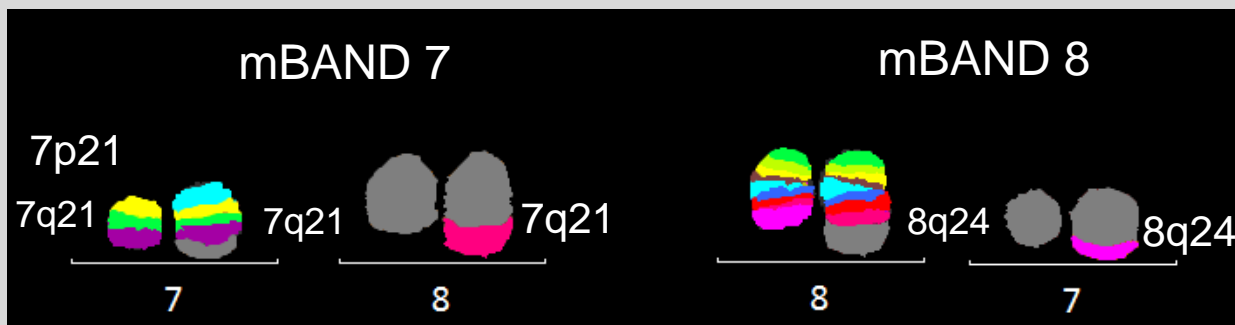
Patient 3



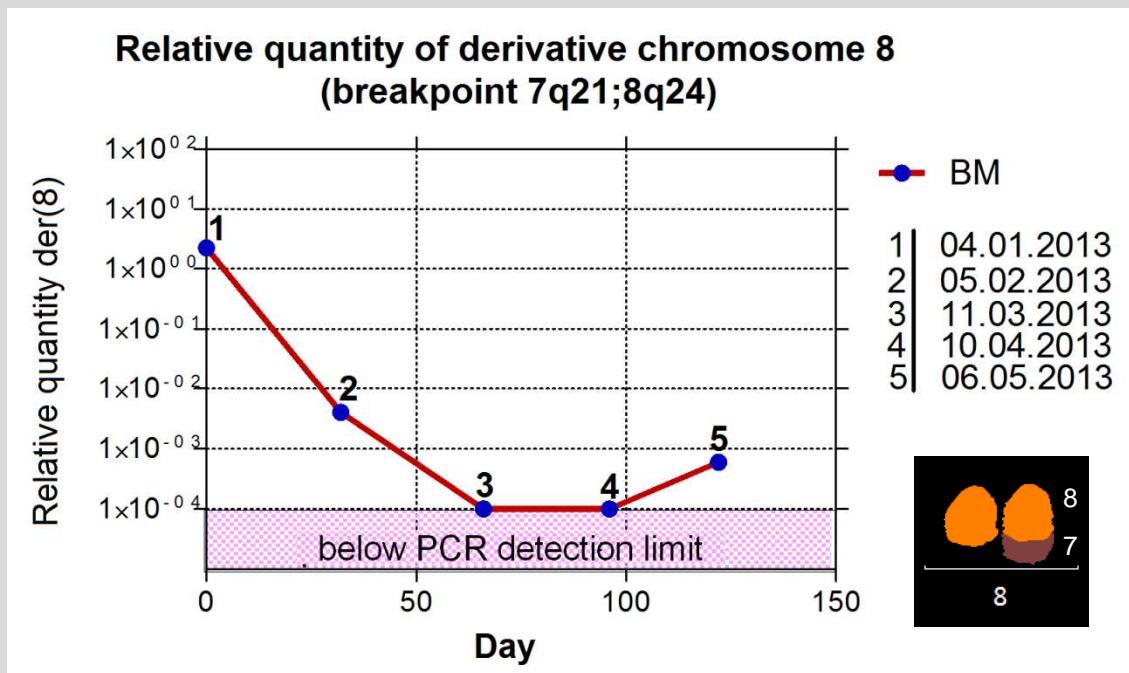
- Screening for MRD targets = negative
- Dissection of der(8)
- DNA sequence of der(8) breakpoint



46,XY,der(7)del(7)(p21)del(7)(q21),t(7;8)(q21;q24)[20]



Patient 3 – Quantification Graph



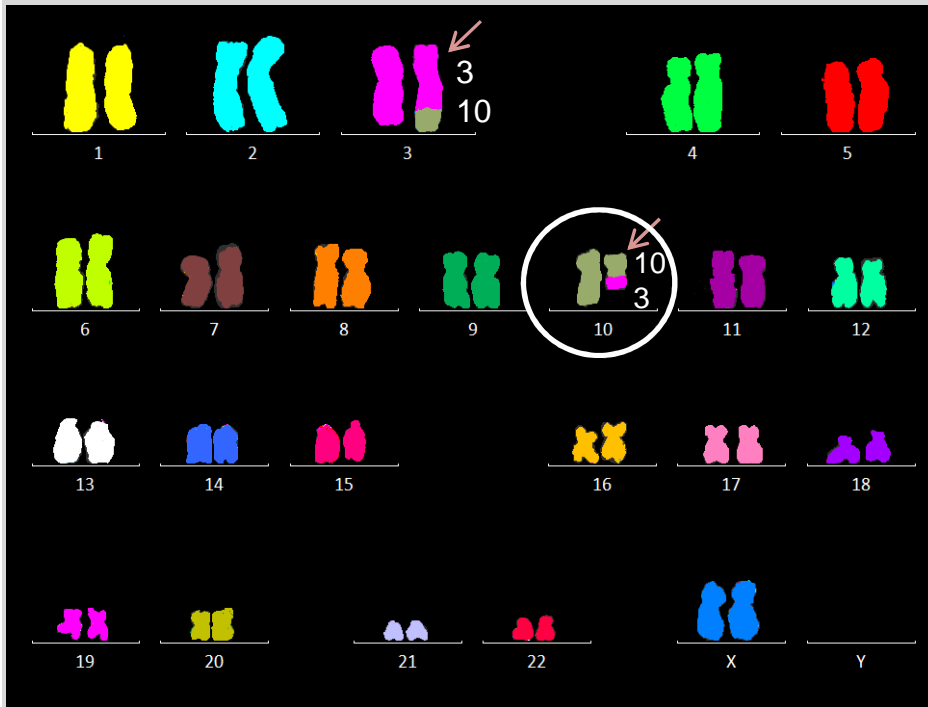
... and

- Beside characterization of unique markers for MRD monitoring
- Identification of unreported partner genes
- *MECOM* gene

MECOM gene

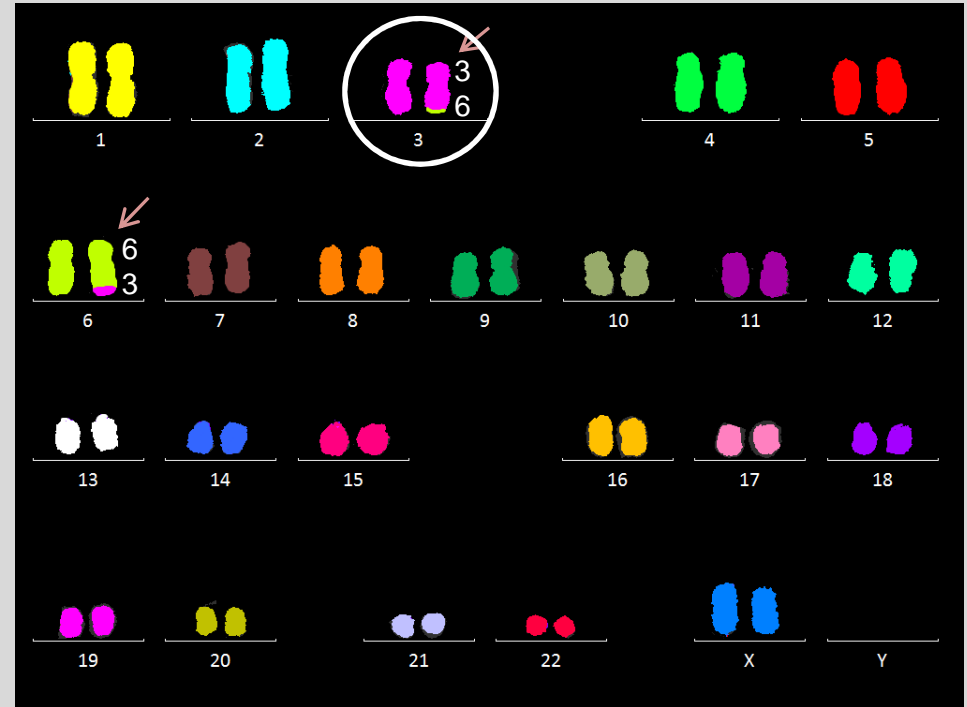
- MDS1 and EVI1 complex locus (*MECOM*)
- 3q26.2 region
- Fusion partners: 3q21 (*RPN1*), 7q21 (*CDK6*), 7q34 (*TCRB*), 12p13 (*ETV6*), 21q22 (*RUNX1*)...
- In healthy individuals - low EVI1 expression in PB and BM
- In AML patients - overexpression in BM/PB because of 3q26 rearrangements
- EVI1 overexpression - MRD target (low sensitivity = $\sim 10^{-2}$!)

Patient 4



46,XX,t(3;10)(q26;q21)[20]/46,XX[2]

Patient 5

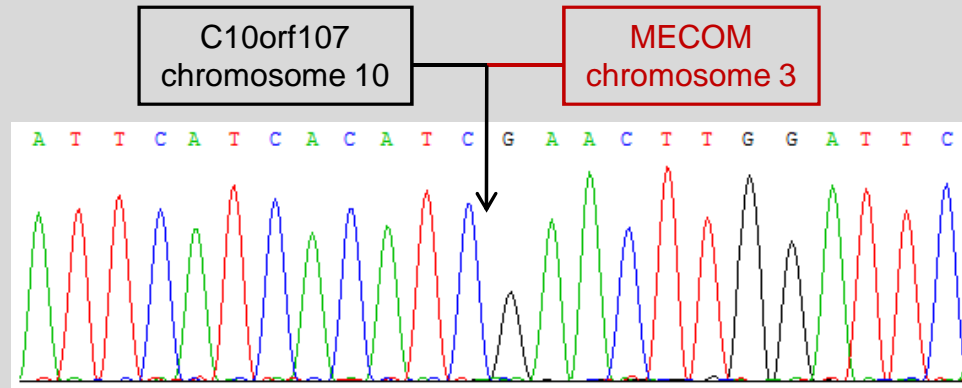


46,XX,t(3;6)(q26;q25)[20]

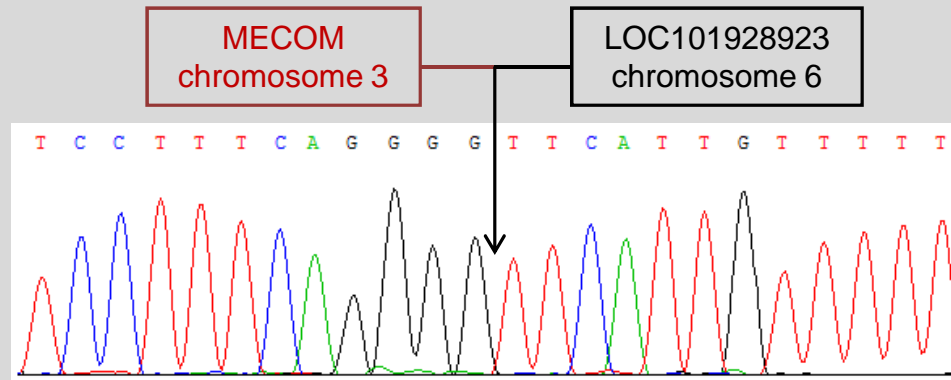
- MECOM locus involved in both translocation
- Dissection of der(10) and der(3)
- Identification of MRD targets and MECOM's fusion partners

DNA sequences of breakpoints

Patient 4



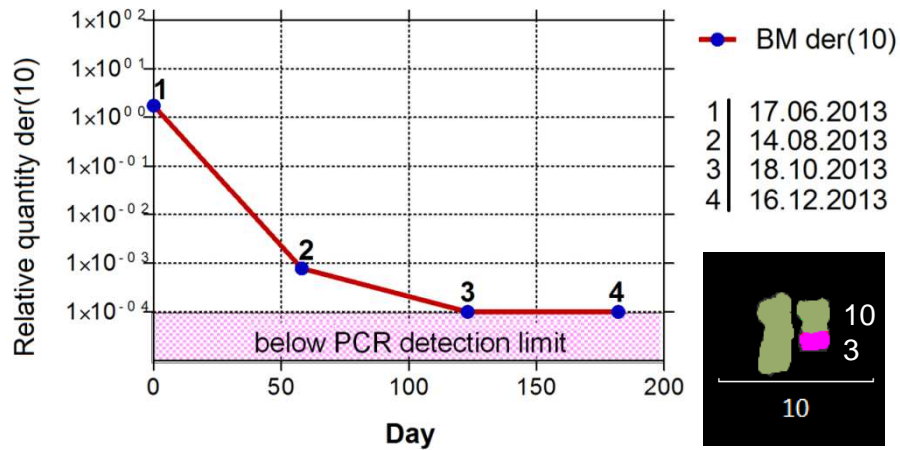
Patient 5



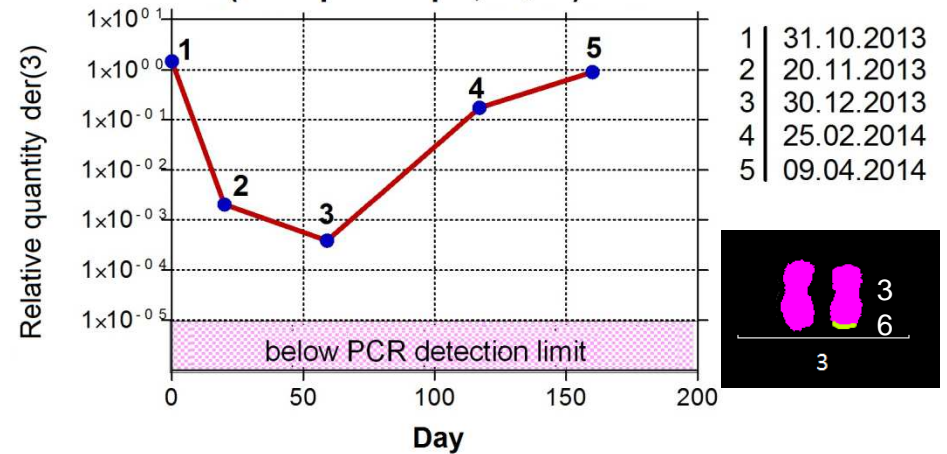
Patient 4

Patient 5

Relative quantity of derivative chromosome 10
(breakpoint 3q26; 10q21)



Relative quantity of derivative chromosome 3
(breakpoint 3q26; 6q25)



Conclusions

- Techniques combination – from chromosomal level to single nucleotide level
- Identification of unique clone-specific marker of leukemic blasts
- Design of patient-specific molecular real-time PCR assay for MRD assessment
- New fusion partners of *MECOM*
 - *C10orf107* chromosome 10q21
 - *LOC101928923* chromosome 6q25
- Personalized medicine – „tailor-made“
- Also suitable for characterizing unique chromosomal translocations in other fields (e.g. human genetics)



**Brains
and
Hands**





Thank you for your attention
