

Overview of genome wide arrays in Haematological Malignancies

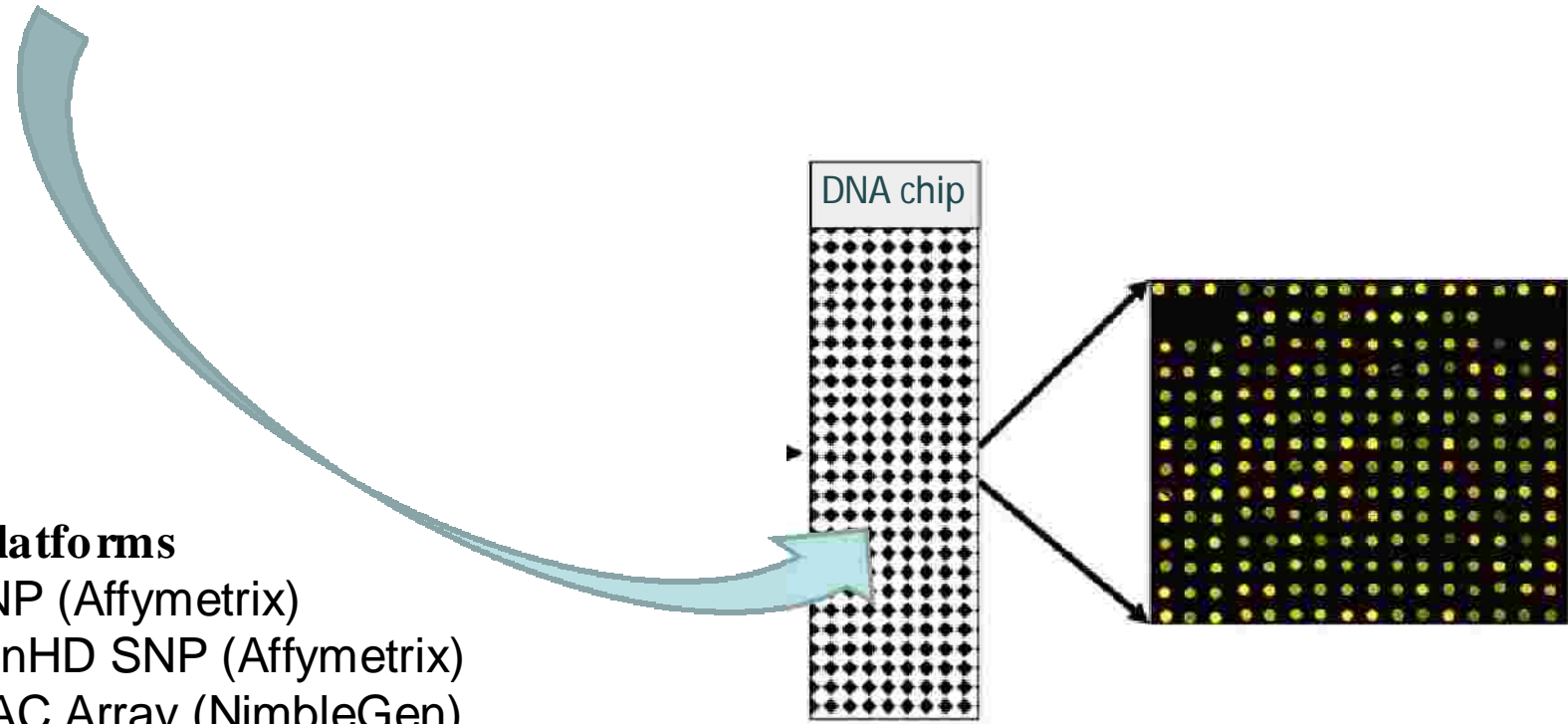
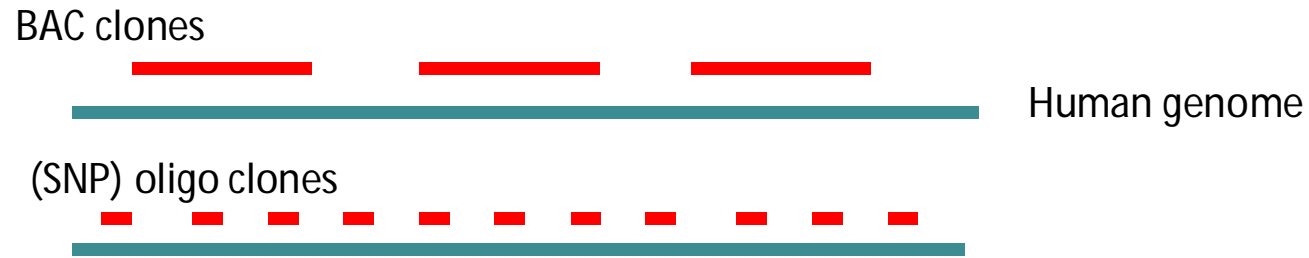
« Array-based or molecular karyotyping »

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Principle of array-based karyotyping tools (1)



Array platforms

250k SNP (Affymetrix)

CytoscanHD SNP (Affymetrix)

720K BAC Array (NimbleGen)

...

Principle of array-based karyotyping tools (2) workflow

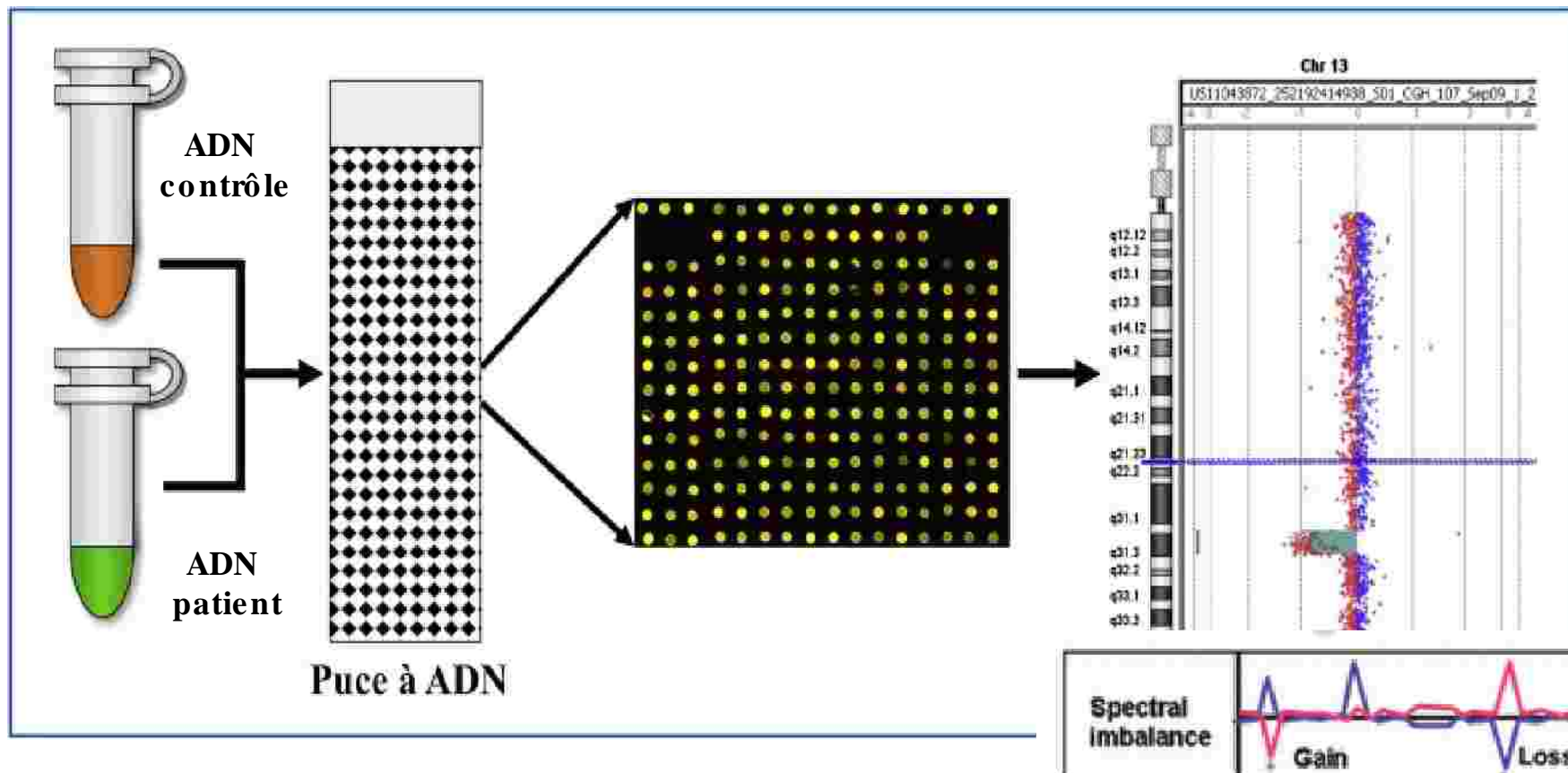
Differential labeling

Co-hybridization

scanning

Bioinformatic processing

Bioinformatic resolution of copy number imbalances



Arrays can detect only unbalanced DNA changes (ie chromosomal losses or gains) but are enable to detect balanced chromosomal translocation

Array-based karyotyping can be done with two main different platforms

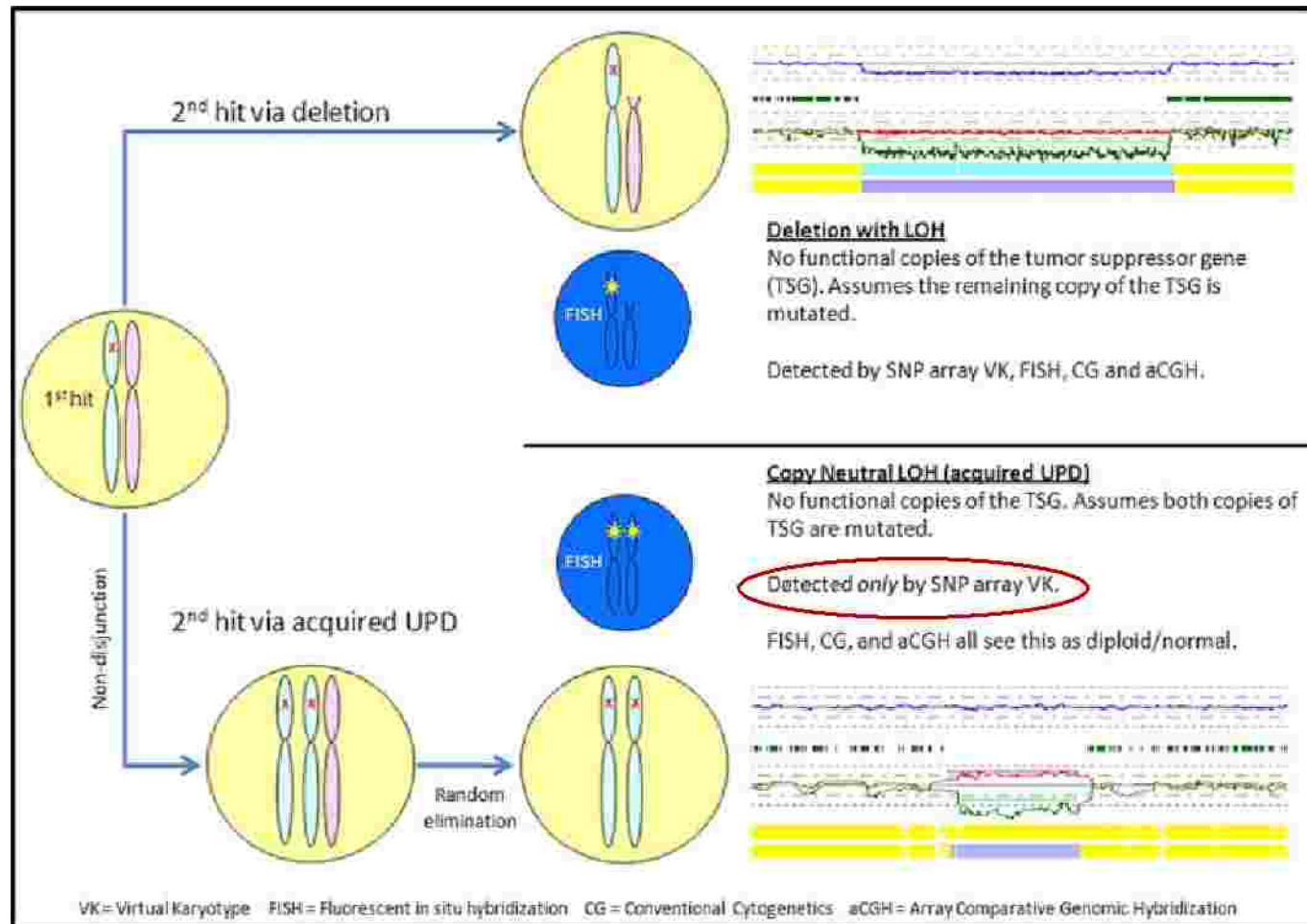
- Array comparative genomic hybridization (« array CGH »)
copy number changes only
- Single-nucleotide polymorphism microarrays (« SNP array »)
copy number changes **AND** copy-neutral alterations (or acquired uniparental disomy - aUPD)

Single nucleotide polymorphism (SNP)



- SNP: single nucleotide variations between paired maternal and paternal chromosomes
 ➔ **heterozygosity**
- In SNP-arrays, oligo probes are specific to each allelic variants of selected SNP within a given locus
- Hybridization of genomic DNA to both probes variants indicates heterozygosity while a signal for only one allele is consistent with loss of heterozygosity (or homo/hemizyosity)

Deletion with LOH vs Copy neutral LOH (aUPD)



aUPD (or copy neutral LOH) refers to a chromosomal region in which both copies of that region are acquired from the same parent, resulting in a copy number of two but with LOH (thus **copy neutral LOH**)

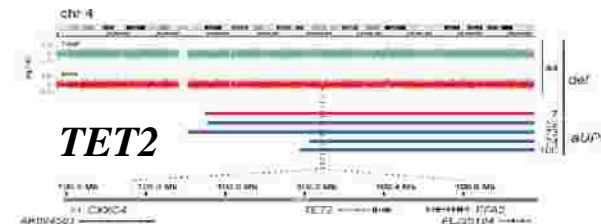
Copy neutral LOH or « acquired uniparental disomy (aUPD) in cancers

- constitute 20-80% of the LOH seen in both solid and liquid cancers [and generate homozygosity for mutated (inactivated) tumor suppressor genes or (activated) oncogenes involved in transformation)

- CNLOH in MDS

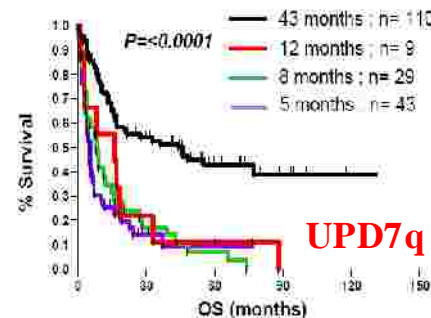
- *TET2*, *EZH2*,...

- Epigenetic deregulation



Langemeijer et al, Nat Genetics, 2009

- normal karyotype and aUPD 7q



Tiu et al *Blood* 117, 2011

Advantages

- High-resolution, genome-wide copy number assessment in **one** assay
 - sensitive whole global scanning of genomic imbalances
 - identification of very small copy-number aberrations (CNA)
- Does not require cell culture
 - performed on interphase cells (and archival samples)
- Simultaneous detections of CNA (« copy loss LOH ») and aUPD (« copy neutral LOH ») if using a SNP-based array.

Limitations

- **Inability to detect balanced translocations**
- Inability to assess regions of the genome not represented on the arrays
- **Decreased performance at low level of tumoral cells** (tumoral sub-clones undetectable by array-based genomic profiling when level < 20%)
 - « contamination » with normal cells can be problematic

Clinical applications of array-based karyotyping

Can arrays-based karyotyping serve as an alternative for conventional karyotyping and FISH in CNA detection?

Few studies on applications of whole genome arrays in ine
diagnostics

Clinical applications of array-based karyotyping

Diseases	Genetic aberrations
ALL	CNA, translocations
AML	translocations, gene mutations, CNA
MDS	CNA, gene mutations, translocations
MPN	gene mutations, CNA, translocations
MM	CNA, translocations
CLL	CNA, gene mutations, translocations

CNA = copy number alterations

Array in CLL

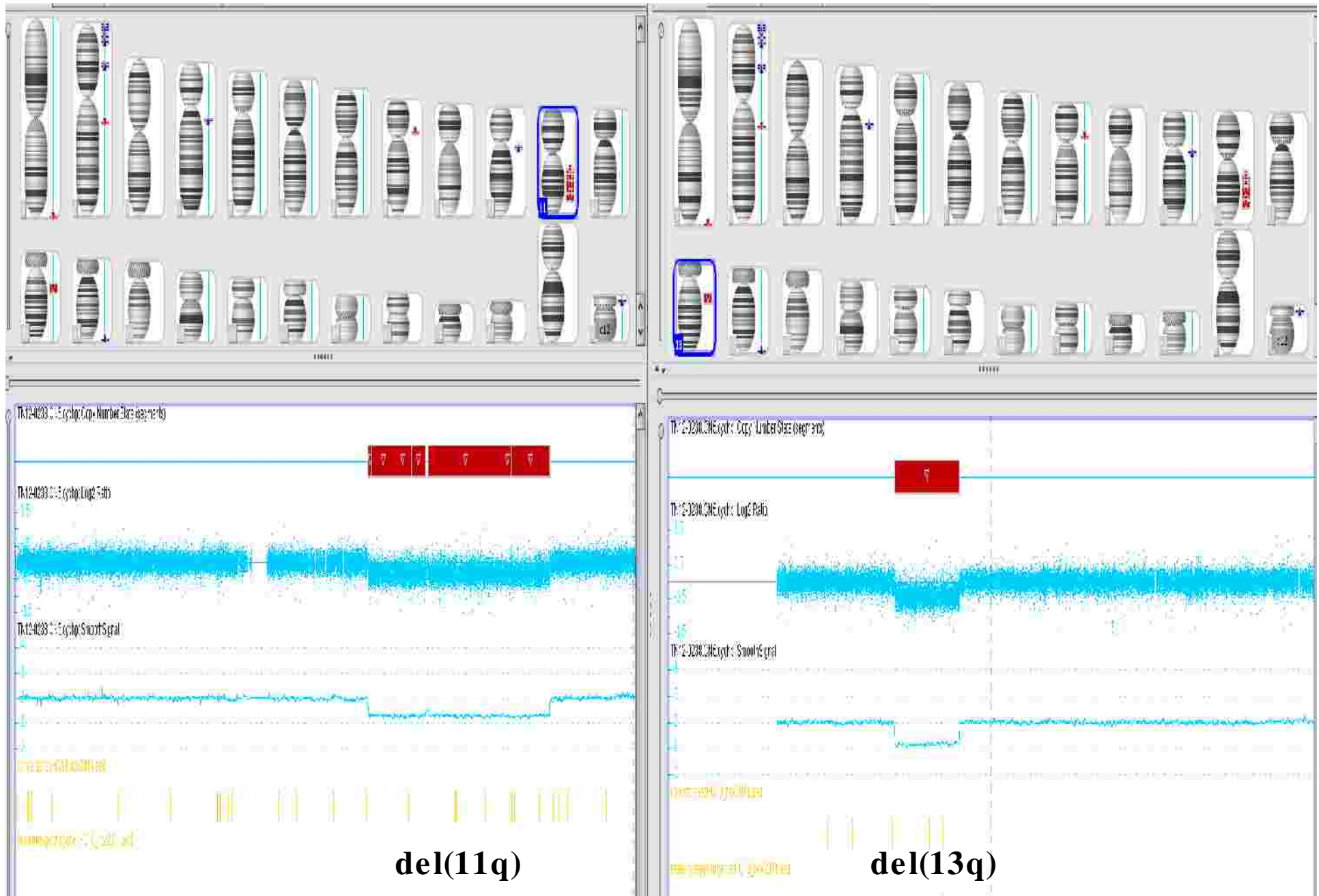
Genomic aberrations with prognostic relevance

Caryotype	% of cases, range	Prognosis	Known and/or putative involved genes
13q14.3 loss	14-40	Good	mir-16-1; mir 15a
trisomy 12	11-30	Intermediate	? <i>CLL1</i>
del(11q22-23)	10-32	poor	<i>ATM</i>
del(17p13.1)	3-27	poor	<i>TP53</i>
Translocation chromosomique	20-42	poor	<i>IGH</i>
Complex karyotype		poor	
del(6q)	2-9	Intermédiaire	?
adapted from Dal-Bo et al <i>J Trans Med</i> 2009			

Karyotype: low success rate (low mitotic index of the CLL cells)

Comparison FISH and array: **very good concordance**

- **Hagenkord *et al.*, *JMD* 2010:**
 - 134 CLL patients
 - 250K SNP-array (Affymetrix)
 - 98.5% concordance array and interphase FISH**
- **O'Malley *et al.*, *Int Jnl Lab Hem* 2011:**
 - 55 CLL patients
 - BAC array (Hemescan)
 - 93% concordance array and interphase FISH**
- **Unpublished studies in the Netherlands** [AMC Amsterdam & UMC St Radboud: Nijmegen] (data kindly provided by M. Stevens-Kroef Nijmegen)
 - 47 CLL patients compared array with FISH or MLPA
 - Agilent 180K oligo array or Affymetrix CytoScan HD array
 - 98% concordance array/FISH and 100% concordance array/MLPA**



(illustrations kindly provided by M. Stevens-Kroef, Radboud University Nijmegen)

Array identifies additional CNA and CNLOH (1)

- Length Heterogeneity within the 13q14 deletions

two distinct subtypes: - type I and type II deletions (< 2 Mb and not including the *RB1* locus *vs* > 2Mb and including the *RB1* locus)

biological and prognostic distinct entities

(Ouillette et al, Clin Cancer research 2011)

- « Atypical deletions »:

11q22.3 deletions which do not involve the *ATM* gene

- Genomic complexity: additional abnormalities detected

21% of CLL cases have significant genome-wide aberrations at multiple loci not assessed by the standard FISH panels

SNP-Array identifies additional CNA and CNLOH

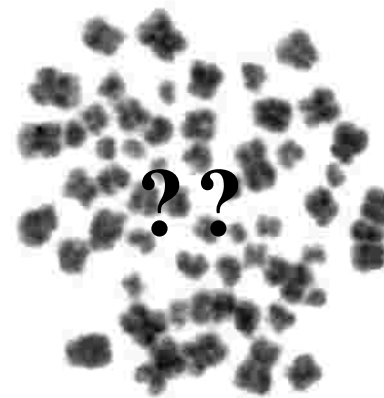
- CLL cases demonstrating UPD at regions with clinical relevances
 - acquired UPD at chromosomal region 17p13.1 involves the *TP53* locus and represents two inactivated mutated copies of *TP53* that current testing methods (karyotype and FISH) would not detect !

Conclusion: array on CLL samples

- Array can replace FISH or MLPA for CNA detection
- Can be implemented in a routine clinical diagnostic setting
- Provides additional genetic informations (but clinical relevances of « new » genetic alterations need to be determined)

ARRAYS in ALL

- Hyper/hypodiploidy, CNA, translocations
- Karyotype: low success rate (failures or normal)
- Yield and quality of chromosomes often poor
- Small recurrent CNA not detectable by karyotyping



Comparative study: conventional karyotyping vs microarray-based genomic profiling

Simons et al. Genes Chrom & Cancer, 2011

- Cohort of 60 childhood ALL

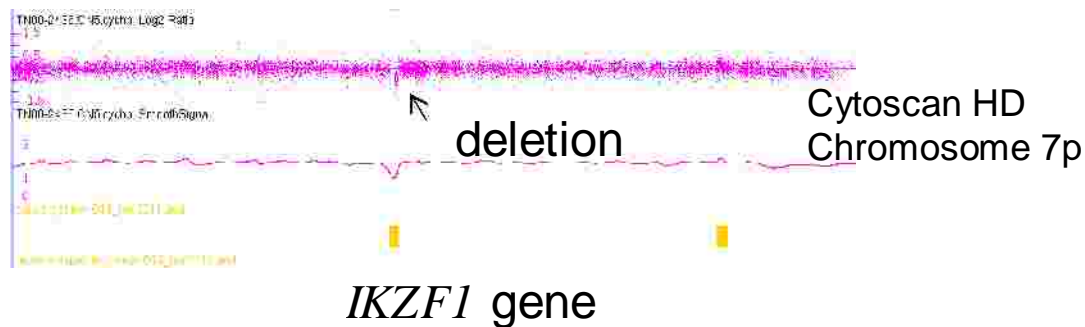
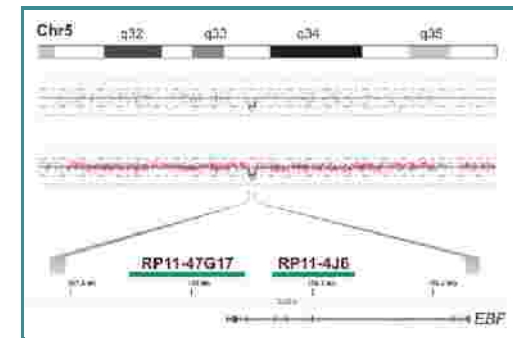
1 - Array has higher CNA detection rate than conventional karyotyping

- 61% CNAs detected by karyotyping vs 93% by array
- among nl karyotype cases, 88% showed CNAs

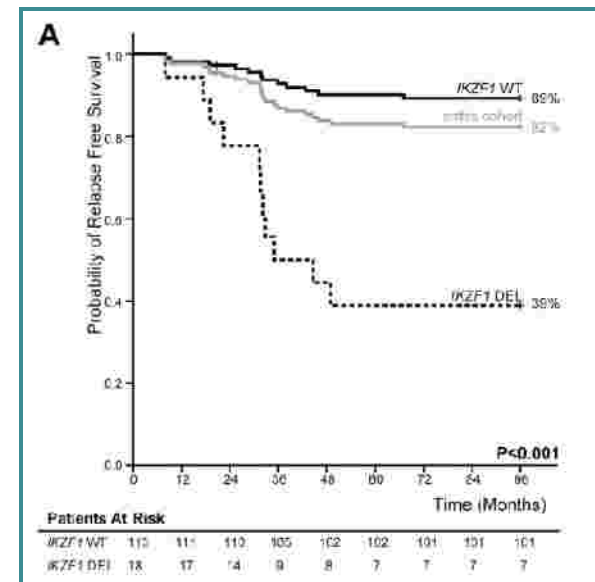
Karyotyping	array
61% CNAs	93% CNAs
4% balanced translocaton	
35% no aberrations detected (failure or normal karyotype)	7% no CNAs detected (3 normal profiles and 1 failure)

2- Recurrent small CNAs in ALL detected by genomic array technologies

- B-cell development (*EBF1*, *PAX5*, *IKZF1*)
- Cell cycle regulation (*RB1*, *CDKN2A*)
- Epigenetic factors (*BTG1*)
- Some deletions have clear prognostic impact
IKZF1 (*Ikaros*) deletions predict poor outcome



(illustration kindly provided by M. Stevens-Kroef, Radboud University, Nijmegen)



Conclusion: array on ALL samples

- High success rate (93%) in detecting CNAs; superior to karyotyping (65%)
- Provides additional genetic informations (clinically relevant) (e.g. *IKZF1* gene deletion)
- FISH tests remain necessary for clinically relevant balanced aberrations

Arrays in Multiple Myeloma

Genomic aberrations with prognostic relevance

Genetic abnormality	Genes involved	Incidence	Clinical impact
Abnormal karyotype (often complex)		30%	poor
t(4;14)(p16.3;q32)	<i>IGH-FGFR3</i>	10-15%	poor
t(14;16)(q32;q23)	<i>IGH-MAF</i>	~ 5%	poor
t(11;14)(q13;q32)	<i>IGH-CCND1</i>	~ 20%	intermediate
Hyperdiploidy (5,9,15)		45%	good
Loss 17p13	<i>TP53</i>	5-10%	poor
Loss 13q14	<i>RB1</i>	50%	good
1q21 gain	<i>CKS1B</i>	20%	poor
1p36 loss	?	20-30%	poor

Arrays in Multiple Myeloma

- Karyotype: low success rate (30-40% of cases)
- Cryptic genomic abnormalities [t(4,14), t(14;16)]
- Gold standard: interphase FISH on enriched plasma cells

Comparative study: interphase FISH vs microarray based genomic profiling

(Stevens-Kroef M et al. *Genes Chrom & Cancer*, 2012)

- on selected plasma cells from 13 patients
- **very good concordance** between FISH and arrays in the identification of CNAs

	FISH	ARRAY
Patients with aberrations	13/13	12/13
Additional	translocation	3 to 22 abnormalities per patient (including CNLOH) Tetraploid vs hyperdiploid karyotype
Missed		mosaic del(17p) (13% of the cells) <i>clinical relevance ?</i>

Conclusion: array on MM samples

- 92% concordance array and interphase FISH
- Allows discrimination between tetraploid and hyperdiploid karyotype
- FISH tests remain necessary for clinically relevant balanced translocations
- Identification of many additional lesions with array (clinical relevance to be determined)

Take-home messages

- **Array is becoming applicable in routine diagnostics for hematological neoplasms according to their specific profiles**

ALL: high success rate (93% vs 65%) and detection of clinically small deletions

CLL and MM: can replace interphase FISH/MLPA

- **Do not replace metaphase karyotyping: balanced abnormalities not detected**
- High resolution but **low sensitivity** (threshold: ~ 20%)
- identification of novel genomic abnormalities; clinical relevance has to be determined