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LES PUCES "SNP BEAD-ARRAYS" REVELENT UNE AMPLIFICATION DDX11 CHEZ LES PATIENTS ATTEINTS D'UNE LEUCEMIE LYMPHOÏDE CHRONIQUE AVEC TRISOMIE 12

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ABBREVIATIONS

2O patterns: 2 Orange patterns

3O patterns: 3 Orange patterns

AF: Allelic Frequency

CD: Cluster of Differentiation

CGH-a: Comparative Genomic Hybridization array

Chr12: Chromosome 12

CLL: Chronic Lymphocytic Leukemia

CNAs: Copy Number Alterations

CNV: Copy number variation

DAPI: 4,6 diamidino-2-phenylindole

DDX11: DEAD/H Box Helicase 11

DNA: Deoxyribonucleic acid

EDTA: Ethylene Diamine Tetraacetic Acid

I-FISH: Interphase Fluorescent in Situ Hybridization

IGHV: Variable region of the Immunoglobulin Heavy chain

LDH: Lactate Deshydrogenase

LDT: Lymphocyte Doubling Time

LOH: Loss Of Heterozigosity

OS: Overall Survival

s.d: Standard Deviations

SNP: Single Nucleotid Polymorphism

TFT: Time to First Treatment

UPN: Unique Patient Number

ZAP70: Zeta-chain-associated-protein-70

PLAN

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SNP BEAD-ARRAYS ILLUMINATE GAIN OF DDX11 IN CHRONIC LYMPHOCYTIC LEUKEMIA WITH TRISOMY 12

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SUMMARY

Background By interphase fluorescence in situ hybridization (I-FISH) studies, trisomy 12 accounts for ~ 16 % of chronic lymphocytic leukemia (CLL) cases, and is associated with an intermediate prognosis. By Single-Nucleotide Polymorphism (SNP) bead-arrays, we evaluated the prevalence of gains at chromosome 12 in CLL patients.

Methods A same primary blood sample from 100 patients with CLL at diagnosis was analysed simultaneously i) by interphase I-FISH and HumanOmniExpress BeadChips (Illumina) (study cohort, n = 60), ii) by I-FISH with different sets of probes (centromeric CEP®12 SpectrumOrangeTM and 12p11.21 probes) (validation cohort, n = 40).

Results i) Study cohort: gain of chromosome 12 was ascertained by a centromeric I-FISH probe in 9/60 cases, all of them detected by SNP-arrays, corresponding to trisomy 12 (n=7), isochromosome(12p) (n=1), or mosaicism (n=1). Bead-arrays allowed detection of subtle amplifications in 9 additional cases undetectable by the centromeric probe, located at 12p11.21 (encompassing DDX11). ii) Validation cohort: the 12p11.21 targeted FISH probe allowed detection of gain at chromosome 12 in 7/40 cases, negative with the centromeric I-FISH probe (used as a routine clinical test). The comparison of groups with (n=18) and without (n=42) abnormalities showed that patients with amplifications at chromosome 12 were significatively correlated with a shorter time to first treatment (TFT).

Conclusion In our study, ~ 30 % of CLL patients at diagnosis presented gains at chromosome 12, with a minimal amplified region involving DDX11, which could be responsible for genomic instability. SNP-assays should be of great value to define the prognosis of CLL patients with trisomy 12 more accurately.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most frequent type of leukemia in adult patients from Western countries (1). Clinical presentation, course and outcome are highly variable. Interphase fluorescent in situ hybridization (I-FISH) has been able to identify chromosomal abnormalities in about 80 % of CLL, including 13q14 deletions (55 %), 11q22-23 deletions (18 %), trisomy 12 (16 %), or 17p13 deletion (7 %) (2). Therefore, five prognostic categories were defined with a statistical model, showing the shortest median survival and treatment-free intervals in patients harboring 17p and 11q deletions, followed by trisomy 12 and a normal karyotype, whereas 13q deletion as the sole abnormality is associated with the best prognosis (2,3).

Various methods have been proposed to detect chromosomal aberrations in CLL patients. The use of new *in vitro* immunostimulants has improved a metaphase generation, enabling detection of chromosomal aberrations including trisomy 12 in respectively 415 (83 %) and 68 (13.6 %) of 500 CLL patients (4). However, conventional cytogenetics may fail to detect chromosome changes in some instances, corresponding to submicroscopic changes, low proliferative activity *in vitro*, or small clone size (5). Comparative genomic hybridization array (CGH-a) technologies have substantially improved the resolution, leading to more accurate delineation, and permitting detection of cryptic changes (e.g. small enough to be overlooked by I-FISH and conventional cytogenetics) (6,7). In contrast to other CGH-a, single nucleotide polymorphism (SNP) arrays provide independent measurements of two parameters [hybridization intensity and loss of heterozygosity (LOH)], increasing the precision of copy number determination, and permitting the detection of peculiar abnormalities such as copy number neutral LOH (8). However, small genomic alterations may be blurred in case of noise and/or if the number of tumor cells is low within samples analyzed (7,9).

In this report, we tested the high-density SNP bead-array technology (Illumina, using oligonucleotide probes immobilized onto beads, followed by hybridization by single-base enzymatic extension). In order to discriminate between delicate abnormal findings and

limits in the sensitivity of the method used (noise level) or copy number variations (CNV) on chromosome 12, we developed a strategy combining simultaneous and independent studies of SNP genotyping datas (log*R* ratio, LOH) and I-FISH using different targeted probes. By a direct comparison of methods, we attempted to assess whether bead-arrays can improve the detection of gains of chromosome 12.

DESIGN AND METHODS

Blood samples

We performed a retrospective study on 100 CLL patients diagnosed at the University Hospital of Angers, as follows:

Study cohort (n = 60):
Peripheral blood samples from 60 consecutive unselected patients with CLL, at diagnosis or before treatment, were tested simultaneously by I-FISH and SNP bead-arrays. Diagnosis of CLL was performed according to the usual international criteria (1): blood count (> 5 x 10^9 B lymphocytes/ L), morphology, and demonstration of a monoclonal B-cell population by flow cytometry, positive for CD5 and CD23, weak CD22+, negative for FMC7, weak surface immunoglobulin (sIg), with a Royal Marsden Hospital (Matutes) score > 3 by immunophenotyping. Among these, 39 were men and 21 were women and their ages at diagnosis ranged from 40 to 87 years (median, 59; mean, 60). I-FISH was performed as a routine clinical test. SNP-arrays were performed using archived DNA (-80°C) extracted from peripheral blood samples obtained for routine clinical analysis, and we focused on chromosome 12. SNP-array based experiments were generated blind to the results of I-FISH. All patients provided written informed consent, and samples were anonymized.
Peripheral blood cells from 30 healthy donors were used as negative controls.

Validation cohort (n = 40):
To define the prevalence of chromosome 12 abnormalities on a larger cohort, we analyzed 40 other untreated CLL patients negative for trisomy 12 with the centromeric 12 FISH probe used as a routine clinical test. Among patients, 21 were men and 19 were women and their ages at diagnosis ranged from 50 to 97 years (median, 65; mean, 67).

Fluorescence in situ hybridization

To ascertain a gain copy number of chromosome 12, we used two independent available orange-labelled probes: the CEP® 12 SpectrumOrange™ DNA probe (Abbott Diagnostics, Wiesbaden, Germany) that hybridizes the AT rich alpha satellite sequences in the centromere region of chromosome 12 (12p11.1-q11); and the RP11-551L14 (Kreatech Diagnostics, Amsterdam, The Nederlands) that hybridizes a region located at 12p11.21 (31107356-31276128) encompassing the DDX11 (DEAD/ H box helicase 11) locus (annex 1).

Cells were fixed (methanol/ cold acetic acid, 3/ 1 volumes) and dropped on slides. Slides were denatured (45°C), hybridized and analyzed, as previously described (10). Chromosomes and nuclei are counterstained with the DNA specific stain DAPI (4,6 diamidino-2-phenylindole) that fluoresces blue. For each probes set in each sample, 200 non-overlapping nuclei were scored by three different observers, blind to the results of another I-FISH probes, and results were expressed as the percentage of nuclei displaying an abnormal signal pattern.

Cut-offs to ascertain a chromosomal abnormality by interphase I-FISH

The percent tri-signaled nuclei in normal specimens was used for the determination of a cut-off value for trisomy 12.

Using the centromeric 12 probe, a trisomy led to 3 separated orange compact signals (3O patterns). Depending on the level of condensation of DNA and on the cycle phase, a few normal nuclei (mean = 2.6) could exhibit diffuse or split signals of the same color: the threshold to ascertain a trisomy was defined over 5% (m + 3 standard deviations) of nuclei with three clearly separated compact signals, and was in accordance to the manufacturer protocol.

Using the RP11-551L14 Kreatech probe, a gain of 12p11.21 region led to 3 separated diffuse signals. So, unless the signals were clearly separated, the signal enumeration was more difficult than with the centromeric probe. As a mean of 10% normal nuclei could

exhibit superposed signals, the threshold to ascertain 12p11.21 trisomy was defined over 15% (m + 3 s.d.).

Single-nucleotide polymorphism array evaluation

Genomic DNA was extracted from peripheral mononuclear cells (Ficoll gradient centrifugation) using the NucleoSpin Tissue (Macherey-Nagel, Düren, Germany). The SNP mapping assay was performed according to the manufacturer's protocol (Illumina, Inc., San Diego, USA). Briefly, 200 ng DNA were fragmented and amplified, hybridized and labeled. Hybridizations were performed on the Illumina HumanOmniExpress BeadChips, which assess 730,525 tags SNPs with a median intermarker spacing of 2.1 kb (mean intermarker spacing 4.0 kb). The Illumina system scanner (iScann system) was used to scan the BeadChips and extract image intensities (primary data). GenomeStudio 2011.1 software and its Genotyping Module (1.9.4) were used to process primary data. Log *R* ratio (copy number value) and allelic frequency (AF) profiles were calculated and plotted against the physical position of the SNPs along the chromosome 12, to detect the gains and LOH regions. The log *R* ratio of signal intensities is shown as log2 (*R* subject/ *R* expected intensity).

Statistical analysis

Statistical analyses were perfomed using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, California). Comparisons of the distributions of clinical laboratory variables at the date of the genetic study were performed with the Mann Whitney test for unpaired samples. All tests were two-sided. An effect was considered statistically significant if the p value was 0.05 or lower. The endpoint of the study was Time to Fisrt Treatment (TFT). TFT was defined as the interval from initial diagnosis to the start of first-line treatment in previously untreated patients. TFT measured from the date of the diagnosis were plotted with the use of the Mantel-Cox test, and 95% confidence intervals for hazard ratios were computed. The proportional-hazards regression model of Cox with a limited backward-

selection procedure was used to identify differences with respect to TFT attributed to prognostic factors.

RESULTS

Correlation between I-FISH-positive nuclei and SNP bead-arrays signals

Centromeric I-FISH probe:

In a normal cell, the expected pattern for CEP 12 is two orange signal patterns (2O patterns).

Using the centromeric I-FISH probe (CEP® 12 SpectrumOrange™), 9 of the 60 (15%) tested CLL presented 3 separated orange signals (3 centromeres) in a number of cells over the cut-off value (median = 49%, 32% to 79%), and were thus considered to have an abnormal clone with gain of chromosome 12 (figure 1).

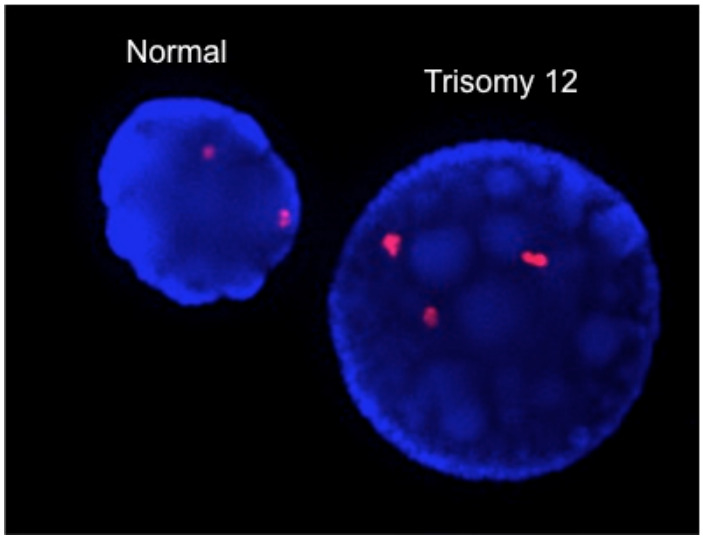

Figure 1. Trisomy 12 by centromeric I-FISH probe CEP® 12 SpectrumOrange™

CEP® 12 SpectrumOrange™ hybridized to an abnormal cell showing three orange signals indicating three copies of chromosome 12 (trisomy 12)

SNP-arrays:

In 7 of these 9 cases (UPN 1 to 7), a trisomy of the whole chromosome 12 was detected by SNP-arrays simultaneously by i) a positive deflection in the log *R* ratio, ii) a LOH in the AF plot (annex 2).

In the 2 other cases, SNP-arrays showed that the trisomy was limited to the short arm of chromosome 12 (12p) (UPN 8) and to the 12p11.21 region (UPN 9) (annex 3).

Conventional cytogenetic:

To explain abnormalities found in these 2 patients (UPN 8 and UPN 9), a conventional cytogenetic was performed on the same blood sample. It showed respectively an isochromosome (12)(p10) (UPN 8), and a coexistence of several abnormal clones in a few mitosis (UPN 9): 47, XX, +12, del18(q12) [5]/46,XX[20].

Additional abnormalities detected by SNP bead-arrays in CLL patients normal with the centromeric FISH probe

Study cohort (n = 60):

By SNP bead-arrays, nine other patients (UPN 10 to 18) showed cryptic amplifications (< 100 kb) located at 12p11.21 (encompassing *DDX11*). AF profiles (split of heterozygous with mirror signals on both sides from baseline) were more sensitive than log *R* ratio for detection of cryptic abnormalities. These gains of chromosome 12 were detected neither by the centromeric I-FISH probe, nor by conventional cytogenetics (when performed in 5 cases) on the same blood sample.

No abnormality was detected in 12q (long arm of chromosome 12).

Validation of 12p11.21 gain (DDX11) by a targeted FISH probe

The nine patients (9/60) with a gain of 12p11.21 (and negative with the centromeric probe: UPN 10 to 18) were studied with a I-FISH probe hybridizing specifically this locus (RP11-

551L14). 5/9 patients showed 3 separated signals into 17 to 30% (median = 21%) of nuclei, permetting to ascertain that bead-arrays signals were related to chromosomal gain, and not to background noise. A careful review of the 4 other cases showed a number of tri-signaled nuclei beyond the cut-off value (6 to 11 %), leading to difficult distinction between small subclones with abnormality, or negative cases.
All positive patients with centromeric probe were found positive with RP11-551L14 probe. However two of them have not been studied for lack of sampling.

Using different filters, the fluorescence microscope permitted simultaneous visualization of orange signals concentrated at the 12p11.21 region and blue counterstained nuclei. Tri-signaled nuclei were localized exclusively at mono- (and not poly-) nuclear cells, allowing to unambiguously distinguish a chromosomal gain from a CNV or a constitutional abnormality.

Prevalence of DDX11 in unselected CLL patients

Study cohort (n = 60):
Taken together, the prevalence of gains at chromosome 12 (e.g. whole or partial trisomy) on our cohort of unselected CLL patients was 30 % (18/60), with a minimal amplified region encompassing the 12p11.21 locus (DDX11).

Validation cohort (n = 40):
By using the RP11-551L14 targeted I-FISH probe, on a trisomy 12 negative cohort by using the centromeric FISH probe as a routine clinical test, 7 another patients (prevalence: 17.5 %) (UPN 19 to 25) showed 3 separated signals (> 15 %) of nuclei located at 12p11.21 (median = 20 %, 16 - 26 %).

Correlation between percentage of centromeric I-FISH-positive nuclei and percentage of tumor cells

Centromeric I-FISH probe (CEP® 12 SpectrumOrange™):

Flow cytometry using CD5 and CD19 permitted determination of percentage of CLL cells in blood samples. In 8 out these 9 cases with a gain of chromosome 12 ascertained by the centromeric FISH probe, a significant difference (> 20 %), between percentage of I-FISH-positive nuclei and B cell percentage in flow cytometry was observed (table I), indicating coexistence of different clones with or without trisomy (e.g. subclonal abnormality). In the other case, the trisomy was present in almost all the tumor cells (e.g. clonal abnormality).

Table I. Clonal vs subclonal abnormalities performed by centromeric I-FISH

UPN	Leucocytes (G/L)	Lymphocytes (G/L)	CD19+ (%)	CD19+CD5+ (%)	I-FISH centromeric probe (%)
1	25,82	18,33	82	100	58
2	20,77	19,52	85	99	62
3	18,09	14,83	60	97	32
4	20,6	17,51	83	99	64
5	17,53	11,92	70	92	42
6	80,15	73,74	89	99	79
7	11,25	6,08	63	98	34
8	33,08	29,11	88	94	49
9	27,12	23,05	86	97	32

RP11-551L14 targeted I-FISH probe:

The same analysis was performed to study the percentage of abnormalities (clonal vs subclonal) identified with the RP11-551L14 targeted I-FISH probe (table II).

Twelve patients showed tri signaled nuclei concentrated at the 12p11.21 region into 16 % to 30 % nuclei (median = 19.5 %). All of them presented subclonal abnormalities in 12p11.21.

Table II. Clonal vs subclonal abnormalities performed by RP11-551L14 targeted I-FISH probe

UPN*	Leucocytes (G/L)	Lymphocytes (G/L)	CD19+ (%)	CD19CD5+ (%)	I-FISH 12p11.21 (%)
10	13,06	6,14	60	96	30
11	16,07	12,32	81	98	25
12	11,18	6,26	79	98	17
13	83	73,87	93	99	18
14	58,51	53,83	95	100	19
19	32	29	91	78	26
20	22	17	90	97	16
21	221	212	**	**	21
22	372	370	**	**	18
23	129	121	97	97	20
24	77	72	96	95	25
25	13	5,2	57	97	16

*UPN 15 to 18 were negative with the RP11-551L14 targeted I-FISH probe
** Missing data for UPN 21 and UPN 22

Different techniques for different genic lesions

SNP-arrays is currently the most sensitive technique to detect a complete or partial gain at chromosome 12, but fail to detect a complete trisomy 12 in one case, corresponding to coexistence of several clones (e.g. mosaicism).

I-FISH probes fail to detect aberrations beyond those defined by the probe used, or in case of small subclones with a percentage of positive nuclei below the cut-off defined for each probe. In our study, the targeted I-FISH probe encompassing DDX11 detect gains at chromosome 12 in more patients than the centromeric probe used as a routine clinical test (table III).

Table III. Chromosome 12 abnormality detected, according to the technique used (n = 60)

	SNP bead-array	CEP® 12 SpectrumOrange™ I-FISH probe (cut-off > 5 %)	RP11-551L14 I-FISH probe (cut-off > 15 %)
Total trisomy 12	**7/7**	**7/7**	**5/7***
isochromosome (12p)	**1/1**	**1/1**	**1/1**
cryptic gain at 12p11.21 (encompassing DDX11)	**9/9**	**0/9**	**6/9**

* 2/7 unrealized

Correlation with clinical and laboratory data

Study cohort (n = 60):
Taken together, 18/60 (30 %) of CLL patients at diagnosis presented with a gain (total or partial) of chromosome 12 abnormalities.

In Table IV, the baseline characteristics of patients with (n = 18) or without (n = 42) a gain at chromosome 12 are depicted separately for each group. Characteristics were not significantly different from haemoglobin, platelet count, lactate dehydrogenase (LDH), tumoral syndrome for each group.

Table IV. Comparison of clinical and laboratory data among patients with and without gain at chromosome 12

VARIABLE	Gain at Chr12 (DDX11)	Normal Chr12	p value
Nb of patients	18	42	
Median age (yr)	61	59	.155
Male sex (n)	12	27	.39
Disease stage at enrolment Binet*			.704
A	13	34	
B + C	4	8	
White-cell count (median, G/L)	21	23	.929
Haemoglobin (median, g/dl)	13.7	14.3	.845
Platelet count (median, G/L)	240	195	.101
Lymphocytes count (median, G/L)	19	18	.972
Lymph node*			.139
Lymph node ≥ 3	4	6	
Lymph node < 3	13	36	

* 1 missing data

On the basis of the regression analysis, we constructed a hierarchical model with two genetic subgroups: without (n = 42) or with (n = 18) chromosome 12 abnormalities as detected by SNP-arrays. The median TFT (time to fist treatment) of the subgroups were as follows: 31.5 months with gain of chromosome 12 vs 81 months without any chromosome 12 abnormality ($p < 0.05$). The Kaplan–Meier survival curve showed a significantly worse outcome in patients with a gain at chromosome 12 compared with those without change (Figure 2). Patients had significantly shorter median treatment-free survival with gain at chromosome 12.

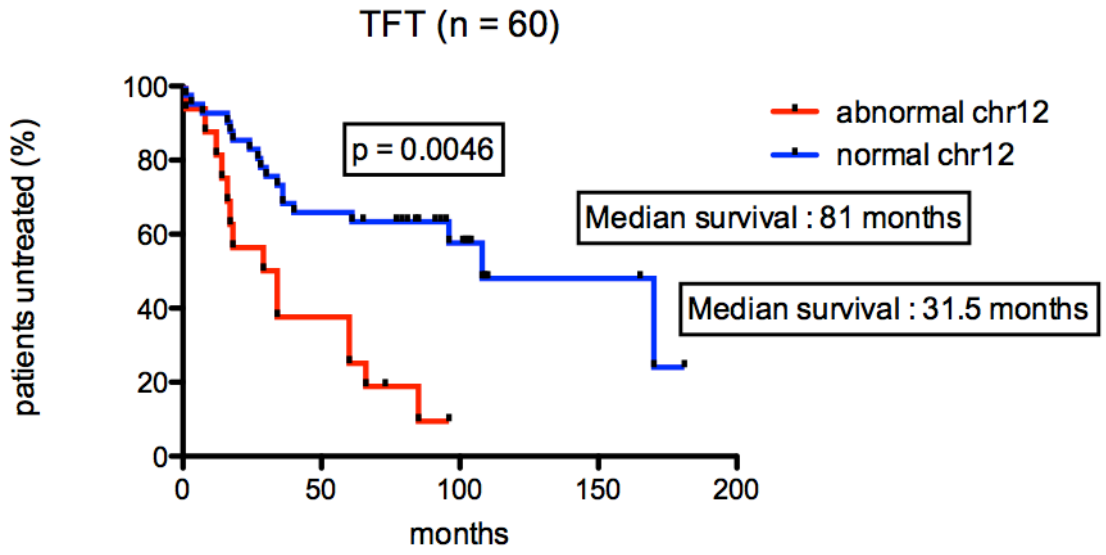

Figure 2. Time to first treatment (n = 60)

Probability of disease progression, as indicated by TFT. TFT of patients with abnormalities (n = 18) and without abnormalities (n = 42) detected by SNP.

We wanted to explain these results in relation to the TFT. So we studied lymphocyte doubling time (LDT), LDH count, B2 microglobulin count and percentage of ZAP70 (annex 4). We did not find any significative difference.

Validation cohort (n = 40):

The validation cohort confirmed that a targeted FISH probe detected a gain at chromosome 12 in more CLL patients than detected by the centromeric used as a routine test. Nevertheless, we cannot exclude that the RP11-551L14 probe is unable to detect gain DDX11 in small subclones.

Entire cohort (n = 100):

In the entire cohort, five patients died, 4 male and 1 female. Their average age at diagnosis was 62.8, median 59, range 54-86 years. Two of them had DDX11 amplification. Because of low number of patients, statistical overall survival (OS) analysis was not executable.

DISCUSSION

I-FISH is considered as the gold standard to detect genomic changes in CLL patients (11). In CLL, I-FISH has been extensively used to ascertain genetic changes, demonstrating trisomy 12 in 53/325 (16 %) of patients tested, and that trisomy 12 as the sole abnormality was related to intermediate prognosis (2). However, I-FISH analysis has failed to define the extent, and to detect aberrations among those defined by the probe used. So far, studies attempting to compare I-FISH and/or CGH-arrays have been limited by technical considerations (background noise or copy number variations leading to low sensitivity and/or specificity for CGH-a), leading to misinterpret a part of genomic changes (12–14).

The SNP bead-array we used here as a screening test generated low noise levels and allowed to delineate a gain of chromosome 12 more accurately, heralding this change as a quite frequent abnormality, as it was observed in 18/60 (30 %) of CLL patients tested.

In this study, I-FISH has allowed unambiguous detection of trisomy 12 with all sets of probes in 9/60 (15 %) exclusively untreated patients, a result superimposable to the one found in literature data. The flow cytometry showed that trisomy 12 was mostly subclonal. Conventional cytogenetic or SNP-arrays showed that trisomy 12 detected by I-FISH were heterogeneous, displaying in some instances an isochromosome (12p), or a coexistence of several clones with various amplification sizes (e.g. mosaicism). SNP bead-arrays helped to delineate changes reported as trisomy 12 by I-FISH, and showed a range of deletion lengths (from submicroscopic to gain of the whole chromosome), encompassing the DDX11 locus in all our cases.

We observed one case with large (as ascertained by FISH) or localized gain (as ascertained by bead-arrays) of chromosome 12. As a conventional cytogenetic study detected a whole trisomy 12, this led to question about the sensitivity of bead-arrays. A careful review of I-FISH results showed tri-signals in different percentages of nuclei according to the probe

used, leading to hypothesize that distinct subclones were present at diagnosis, some of them undetectable whatever the technique used.

We also observed 9 cases with cryptic copy number alterations (CNAs) encompassing DDX11. These CNAs are too small to be detected by a centromeric I-FISH probe or conventional cytogenetics, and are difficult to discriminate from noise or CNV of genome. In this study, DDX11 probe allowed to detect more patients with a true gain at chromosome 12, but the threshold of this probe is very high. This specific RP11-551L14 I-FISH probe did not allow to detect small subclones (< 15 % of nuclei with abnormality) and showed only a targeted area. Our approach, combining in a single test sample whole genotyping parameters as a screening test followed by targeted I-FISH study permitted us a fine-grained single-feature view and a greater resolution.

The 2 cohorts (n=60 and n=100) cannot be compared because they were not analyzed exactly in the same way. The last 40 patients were not screened with SNP-array. However, the 100- patient cohort has shown the same tendency to a shorter TFT for the patients with an abnormality on the chromosome 12 as for those having no abnormality.

Other CGH-a were reported to study genomic changes in CLL. SNP-arrays [chip and bead-based] provide simultaneous measurements of signal intensity (log *R* ratio) and LOH. In some of our cases (corresponding to high contamination by normal cells leading to copy neutral-LOH, high degree of log *R* ratio baseline variation), only combination of these two parameters permitted to ascertain genomic changes. Gunnarsson et al. compared identical CLL samples with differently designed microarrays [bacterial artificial chromosome and oligonucleotide (Agilent) (e.g. non-SNP); Affymetrix and BeadChips Illumina (e.g. SNP-arrays)] (9). For log *R* ratio analysis, discordances between platforms concerned essentially small CNAs, leading to a difficult discrimination between true alterations or a false signature. For SNP-arrays, the LOH profile and lower noise-level led to higher abnormalities detection rate. Comparison of chip-based arrays and I-FISH showed high concordance, except in the case of small clone size and/or tumor burden, where array detection was limited irrespective of the deletion size. To circumvent that limitation, some authors proposed a B-cell enrichment procedure (negative selection or CD19+ cell sorting) before analysis, a proposal difficult to apply in routine conditions and failing to detect small clones in some instances (6).

A 12p rearrangement is observed in about rare CLL cases usually as part of a complex karyotype with multiple abnormalities and frequent disease progression (15). Aberrations of 12p in CLL are often addition to 12p. The breakpoint is commonly 12p13, 12p12, 12p11 and 12p10 (16).

In a preliminary study, we extended our gain detection strategy (bead-arrays followed by targeted I-FISH) to the whole chromosome 12 scanning. A comparison between groups gain of chromosome 12 (total or partial) versus no chromosome 12 abnormality showed a difference regarding clinical (TFT). Nevertheless, our study has to be confirmed with a multivariate analysis. Moreover, we do not have information on IGHV status.
Chromosomal abnormalities on chromosome 12 could be associated with others recurrent abnormalities (11q deletion, 17p deletion or 13q deletion).

In our study, we have found recurrent abnormality on DDX11. DDX11 was first isolated as the human homologue of the yeast CHL1 gene (17). It is a member of the DEAD/DEAH box family of DNA helicases, which comprises more than 40 members. DDX11 encodes a putative helix-turn-helix DNA binding motif. This gene is located on chromosome 12p11.21. The functions of DDX11 appear to be conserved throughout evolution. DDX11 is essential for the cohesion of chromosome arms and centromeres and when depleted, mitotic failure occurs due to replicated chromosomes failing to segregate after prometaphase arrest (18). Downregulation of DDX11 causes premature sister separation and delay in mitotic progression in human cells (18,19). The mechanism by which DDX11 preserves genomic instability is largely unknown. However a study that DDX11 depletion causes accumulation of DNA damage. Their results indicate that DDX11 plays an important role in efficient DNA repair during DNA replication (20).
Biochemical studies revealed that DDX11 possesses a ATPase domain vital to its enzymatic function (21,22). Another study shows that DDX11 is expressed at high levels in primary and metastatic melanoma, but not in melanocytes of normal skin (23). DDX11 is upregulated with progression from early to advanced melanoma. Besides they document that inhibiting DDX11 expression causes substantial chromosome segregation defects and telomere shortening, major inhibition of melanoma cell proliferation, and rapid and massive melanoma cell apoptosis. The first study, in a context of mouse mutants, shows that loss of DDX11 expression in a malignancy causes apoptosis, rapid and massive programmed cell death (19,24). DDX11 knockout mice show embryonic lethality and aneuploidy due to the loss of sister chromatid cohesion. They suggest that DDX11 is

required for normal mammalian development and the preservation of genomic instability. DDX11 gene plays an important role in maintaining a high fidelity of chromosome transmission during mitosis. DDX11 is emerging as an important gene for the prevention of human disease, cancer, and chromosomal instability.

CONCLUSION

In our study, ~ 30 % of unselected CLL at diagnosis have presented gains at chromosome 12 (whole or partial trisomy), with a minimal amplified region involving DDX11, which could be responsible for a genomic instability. Our sensitive SNP-assays should be of great value to define more accurately the prognosis of CLL patients with trisomy 12. Our study has to be confirmed with a multivariate analysis.

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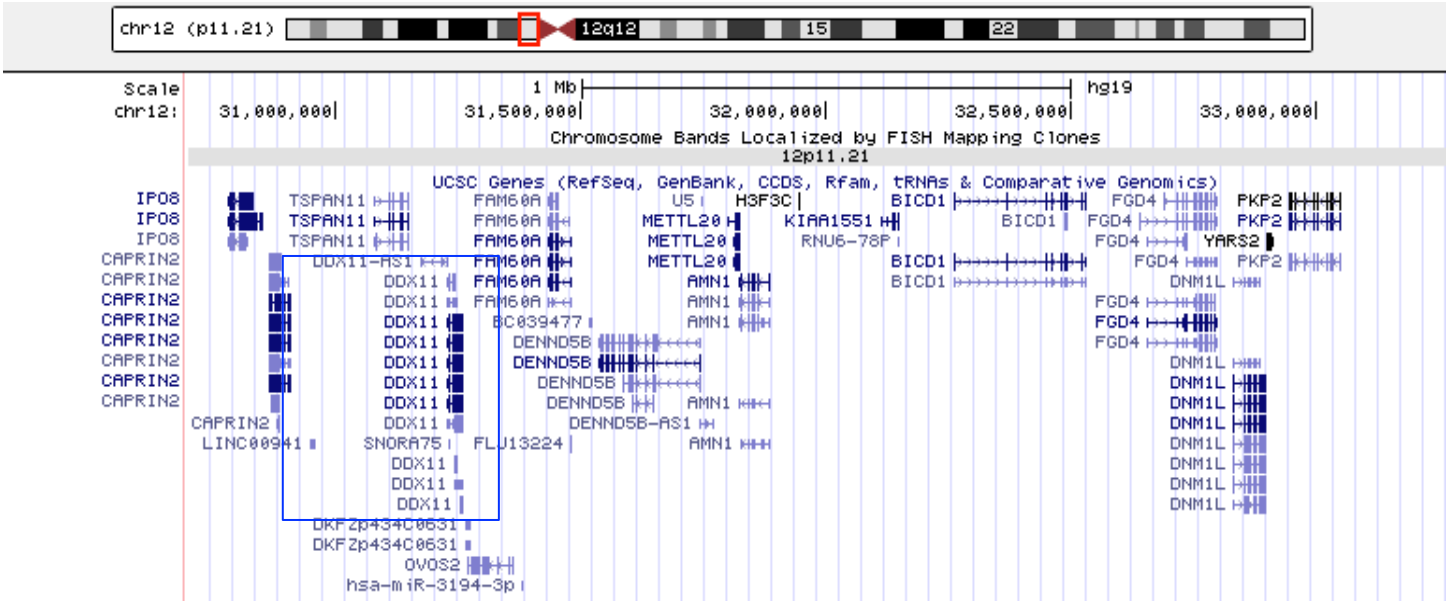
ANNEXIS

Annex 1: Probes used in I-FISH

12p11.21

RP11-551L14 Kreatech probe used in I-FISH

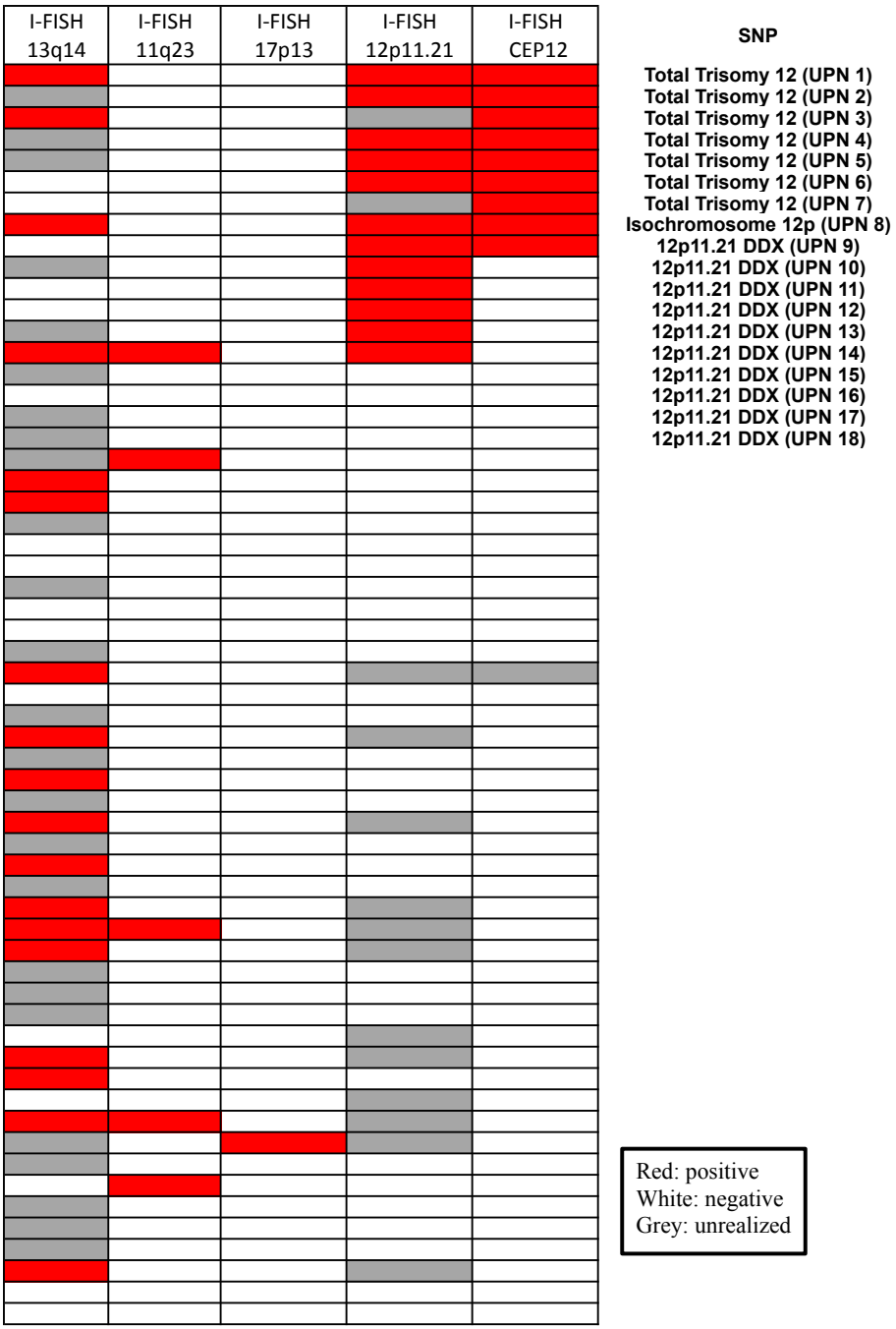
Chr12: 30,700,001-33,300,000 (Feb.2009GRCh37/hg19)



12p11.1-12q11

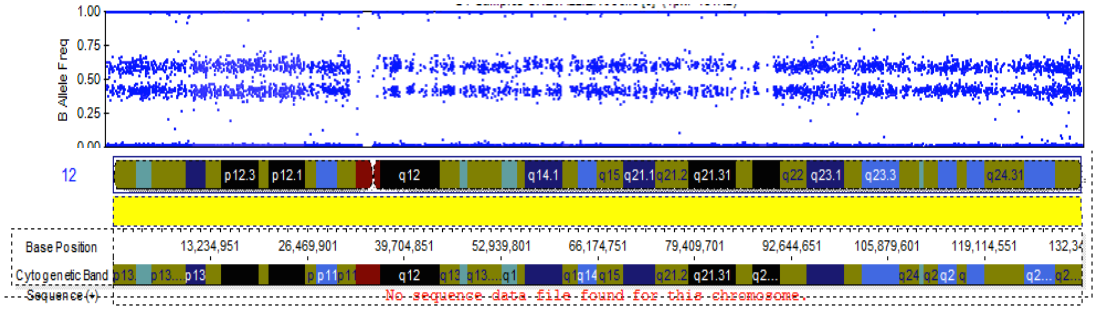
CEP12 Abbott probe used in I-FISH

Annex 2: Genomic lesion detected by I-FISH and SNP array (n = 60)

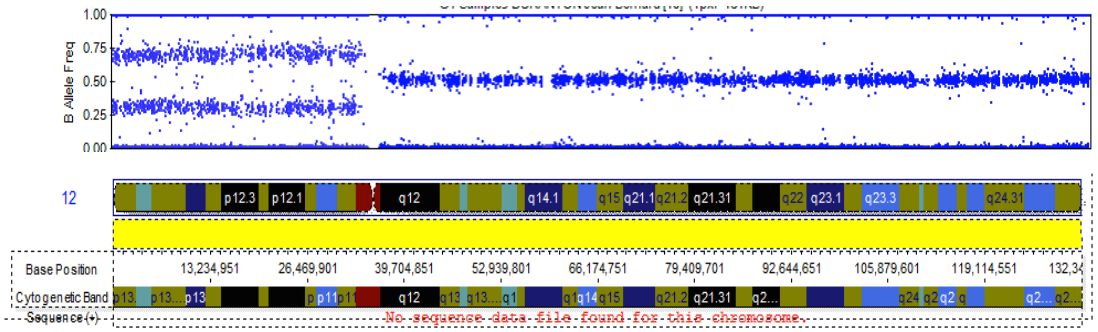


Annex 3: Examples of three genomic profiles with genomic aberrations detected by SNP array in CLL patients with abnormal centromeric FISH (n = 60)

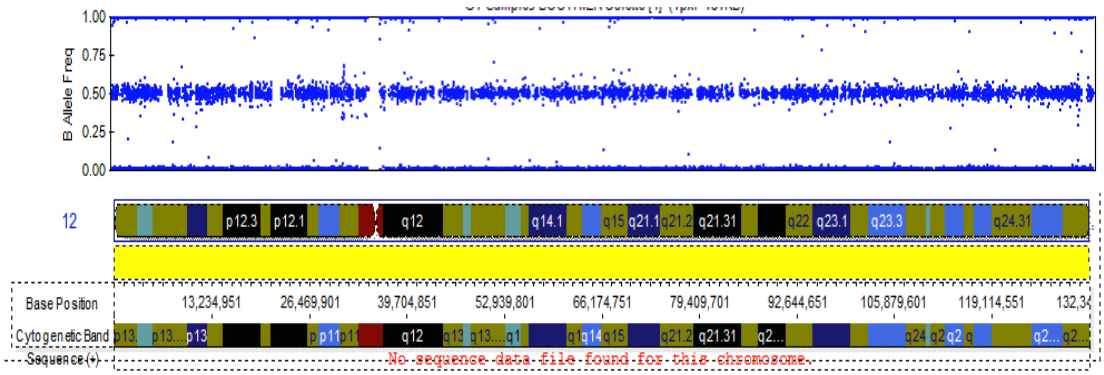
UPN 1 to 7 : total trisomy 12



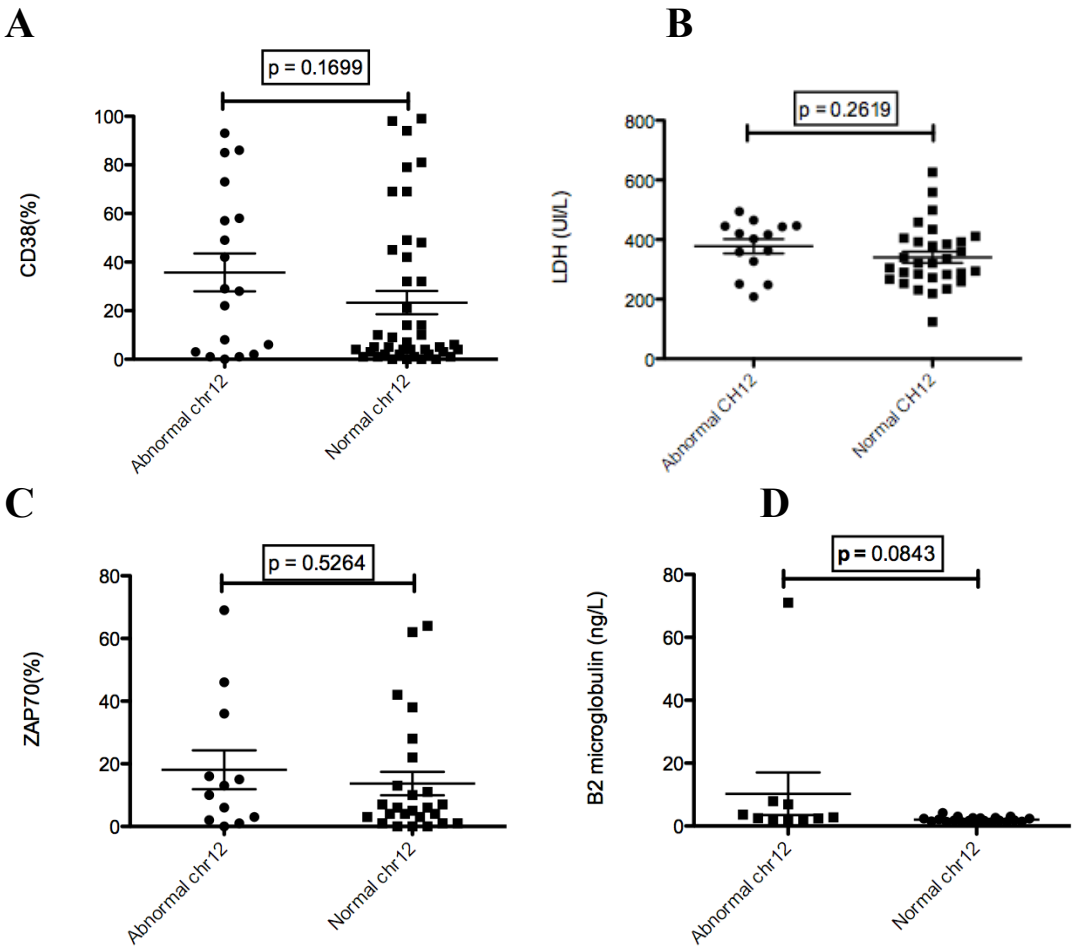
UPN 8 : isochromosome(12p)



UPN 9: gain at 12p11.21



Annex 4: Link between chromosome 12 abnormality and biological characteristics (n = 60)



A. Link between percentage of CD38 and chromosome 12 genomic lesion
B. Link between rate of LDH and chromosome 12 genomic lesion
C. Link between percentage of ZAP70 and chromosome 12 genomic lesion
D. Link between rate of B2microglobulin and chromosome 12 genomic lesion

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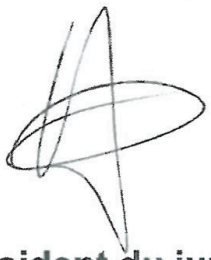

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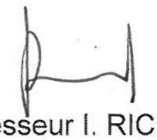

Professeur I. RICHARD

Vu et permis d'imprimer

LE GUYADER Maïlys

Les puces "SNP array" révèlent une amplification DDX11 chez les patients atteints d'une leucémie lymphoïde chronique avec trisomie 12

RESUME

Introduction Par hybridation in situ en fluorescence interphasique (I-FISH), une trisomie 12 est détectée dans ~ 16 % des cas de leucémie lymphoïde chronique (LLC), et est associée à un pronostic intermédiaire. Nous avons évalué par SNP array la prévalence des gains sur le chromosome 12 dans la LLC.

Méthodes Un échantillon sanguin de 100 patients avec une LLC au diagnostic a été analysé simultanément i) par I-FISH et HumanOmniExpress BeadChips (Illumina®) (n=60), ii) par différentes sondes FISH : RP11-551L14 (Kreatech Diagnostics) ; et sonde centromérique CEP® 12 SpectrumOrangeTM (Abbott Diagnostics) (n=40, cohorte de validation).

Résultats i) la sonde CEP12 a révélé une "trisomie 12" chez 9/60 patients, tous détectés par SNP-arrays, correspondant à : trisomie 12 totale (n=7), isochromosome (12p) (n=1), mosaïcisme (n=1). Les puces "SNP" ont détecté une amplification dans 9 cas supplémentaires indétectables par la sonde CEP12, localisée en 12p11.21 (incluant le gène DDX11). ii) Cohorte de validation : une sonde FISH ciblant la région 12p11.21 a permis la détection de 7 cas avec une amplification, non détectée par la sonde CEP12. La comparaison des groupes avec (n=18) et sans (n=42) anomalies montre qu'une amplification sur le chromosome 12 est significativement corrélée à un temps avant premier traitement (TFT) plus court.

Conclusion Au total, 18/60 (30 %) des patients avec un gain sur le chromosome 12 présentent une région minimale amplifiée (locus DDX11), qui pourrait être responsable d'instabilité génomique. Par rapport aux techniques conventionnelles, les puces "SNP" pourraient permettre une meilleure définition des anomalies pronostiques.

MOTS-CLES

Leucémie Lymphoïde Chronique — trisomie 12

SNP — DDX11

FORMAT

☐ **Mémoire**

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