Untreated chronic lymphocytic leukemia in Lebanese patients: an observational study using standard karyotyping and FISH

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Summary points

Background

- The chronic lymphocytic leukemia (CLL) pathogenesis shows a molecularly variable disease with particular cytogenetic characteristics that constitute nowadays the major prognostic factors.
- The epidemiology of CLL in the Middle Eastern population has not been fully studied.
- The modeling of the CLL epidemiology at the population level is required to personalize the treatment plan at the individual level.

Patients & methods

- All the newly diagnosed CLL patients who did not require treatment were eligible.
- Patients were investigated for demographic characteristics and CLL testing by cytogenetics and FISH.

Results

- Chromosomal aberrations on classical karyotype were found 53.6% among which 26.7% present complex rearrangements.
- The most frequent abnormality in all patients was del(13q14) [46.4%] occurring as a sole abnormality in ten cases.
- FISH studies identified ch14q32 rearrangement (25.0%), followed by trisomy 12 (14.3%), del(17p13) and del(11q22) in 7.1% each.

Discussion

- Similar to large studies from both Western and Eastern countries, del(13q14) was the most common abnormality in CLL patients.
- It is rational to encounter low incidence of poor prognosis FISH abnormalities in our watch and wait CLL patients.

Conclusion

- The higher prevalence of del(13q14) as a sole abnormality could be the primary event in inducing CLL.
- The del(17p13) and del(11q22) follow and could be drivers for CLL progression.

Aim: We aimed to understand the biology of chronic lymphocytic leukemia (CLL) patients in Lebanon. **Materials & methods:** We applied conventional cytogenetic and FISH studies on Lebanese patients diagnosed with CLL and undergoing a watch and wait approach. **Results:** Our study disclosed 53.6% of patients with aberrant karyotypes among which 26.7% were complex karyotypes. Genetic aberrations included del(13q14) 46.4%, 14q32 translocation in 25%, trisomy 12 in 14.3%, del(17p13) and del(11q22) in 7.1% each. The deletion of 6q21/6q23 was not found in any of our patients. **Conclusion:** The higher prevalence of del(13q14) as a sole abnormality could be the primary event in inducing CLL. The del(17p13) and del(11q22) followed as potential drivers for progression in CLL patients with a watch and wait approach.

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Chronic lymphocytic leukemia (CLL) is defined as a clonal accumulation of neoplastic B-lymphocytes in peripheral blood, bone marrow and lymph nodes. The indolent nature of the CLL and the recent advances in the treatment arsenal have led to the increased incidence of this disease [1]. The oldest prognostic factors in CLL were limited to the Rai and Binet staging systems that specify the subgroup of patients that would require a medical intervention instead of a watch and wait approach [2,3]. The CLL pathogenesis shows a molecularly variable disease with particular cytogenetic characteristics that constitute nowadays the major prognostic factors [4]. As such, recent cytogenetics and molecular analyses have demonstrated the presence of particular prognostic markers that would determine the aggressiveness of the disease and guide the treatment choice [5,6].

Major geographic differences in the epidemiology of CLL between Eastern and Western countries were shown in the literature and were initially attributed to the differences in the available medical resources. However, further analysis revealed that the prognostic factors, mainly the cytogenetic aberrations, found in CLL patients differed between geographic regions [7]. According to our review of the literature, the epidemiology of CLL in the Middle Eastern population has not been fully studied. Subsequently, the modeling of the CLL epidemiology at the population level is required to personalize the treatment plan at the individual level [8,9]. The only available leukemia reports in the Middle Eastern region and in Lebanon particularly are outdated and limited to small studies published before the regional civil wars [10]. Our team have already published several papers from Lebanon that showed particular trends that were closer to the Western demographics and characteristics [11–13]. In this regard, the Lebanese CLL epidemiology differed from the available literature in terms of age and stage at diagnosis [14]. In the same line, we conducted this study to prospectively evaluate the characteristics of our watch and wait CLL patients based on the recommended panel of cytogenetic studies. We also aimed to correlate these results to two newly prognostic factors, the neutrophil to lymphocyte and platelet to lymphocyte ratios (NLR and PLR, respectively), undergoing extensive evaluation in cancer patients [15,16].

Patients & methods

Study design

Hotel Dieu de France University Hospital is one of the biggest tertiary care centers, serving a rural area in Beirut, Lebanon. The Lebanese population is constituted of four millions habitants that are characterized by high rates of consanguinity [17]. Between January 2016 and 2017, we reviewed at our institution the medical record of all the patients diagnosed with CLL or small lymphocytic lymphoma according to the following diagnostic criteria: presence of lymphocytosis above $5 \times 10^9/l$ with at least 50% B-lymphocytes and a flow cytometry signature characterized by CD5⁺, CD19⁺, CD23⁺, CD20 (dim) and surface immunoglobulin (dim). Small lymphocytic lymphoma patients did not require a lymphocytosis above $5 \times 10^9/l$ with at least 50% B-lymphocytes [18]. Among these patients, all the newly diagnosed patients who did not require treatment (absence of constitutional symptoms referable to CLL, progressive marrow failure, autoimmune anemia/thrombocytopenia poorly responsive to steroids, a splenomegaly above 6 cm and a lymphadenopathy above 10 cm) have been included in this study [19]. We excluded patients with foreign nationalities and those with missing data. The study was approved by the Institutional Board Review of Hotel Dieu de France University Hospital and Ethical Committee of Saint Joseph University – Faculty of Medicine.

Data source & variables

All eligible patients were investigated prospectively for demographic characteristics including age, gender, past personal and family medical history. Patients were evaluated for their Eastern Cooperative Oncology Group (ECOG) performance status scoring 0 to 4, the presence of B signs including unintentional weight loss of more than 10% over the previous 6 months, a fever greater than 38°C for more than 2 weeks without evidence of infectious etiologies, drenching night sweats and extreme fatigue defined by an ECOG of at least 2. All patients were evaluated clinically for the presence of cervical, inguinal and axillary lymph nodes, hepatomegaly and splenomegaly. The laboratory tests at diagnosis were collected including the hemoglobin level, the white blood cell count with the neutrophil and lymphocyte percentages and the platelet counts. We calculated NLR and PLR by dividing the absolute count of neutrophil and platelets by the lymphocyte counts, respectively. All patients were classified according to Binet and Rai staging systems [3,4]. Patients also underwent cytogenetic and FISH analysis as per the study protocol at Unité de Génétique Médicale, Saint Joseph University – Faculty of Medicine, Lebanon.

Conventional cytogenetics

Mononuclear cells in peripheral blood specimen were counted manually using Kovalslides (Hycor Biomedical Inc., CA, USA). We cultured 10^6 cells/ml in a milieu containing 10 ml culture medium (RPMI 1640, Gibco, USA), 10% fetal bovine serum (Gibco), 2% L-glutamine (Gibco) and 1% antibiotics (Gibco). One culture of 72 h with Premix AMPLI B mitogen (AmpliTech, Compiègne, France) was set up for each patient. We further harvested our cultures according to the standard cytogenetic methods (Hypotonic treatment and ethanol–acetic acid fix, Normapur, 3:1 ratio). R-banding technique was used and 20 metaphases were analyzed for each patient using IKAROS karyotyping software (Metasystems, Altlußheim, Germany), according to the International System for Human Cytogenetic nomenclature. Complex karyotype was defined by the presence of three or more chromosomal abnormalities [20].

Fluorescence in situ hybridization

Interphase FISH studies were performed on slides, taken from the 72 h culture with the Premix solution, using metasystems probes according to the manufacturer recommendations. All the samples were studied with our standard CLL FISH panel that includes probes for chromosome 12 centromere (CEP12), *DLEU* (13q14) and *LAMP1*(13q34), *ATM* (11q22) and *TP53* (17p13). In addition to the panel, the following regions were analyzed: chromosome 6 centromere (CEP6), *SEC63* (6q21), *MYB* (6q23) and translocations involving *IgH* at 14q32 using a dual color apart probe. In view of the rarity of *IgH* rearrangement in our series, our standard panel does not determine the *IGH* fusion partner thus will not be included in this analysis. Two technologists each independently analyzed 100 nuclei, for a total of 200 nuclei per probe set. The cutoff values for each probe were 4% for trisomy 12, 6% for 13q14 deletion and *ATM* deletion, 7% for *TP53* deletion and 6q21 deletion and 9% for *IgH* break. These cut-off values were established based on peripheral blood samples from normal individuals.

Statistical methods

Our clinical data are expressed in mean $\pm$ standard deviation, or median, or percentage. SPSS Statistics version 20.0 (IBM Corporation, NY, USA) and XLStat version 2017.1 (Addinsoft, Paris, France) were used for statistical analysis. Univariate binary logistic and multinomial regressions were computed between clinical examination, genetic characteristics, demographics and blood test results. Multivariate stepwise binary logistic and multinomial regression models were subsequently computed with the variables found to be significant (or borderline significant) in the univariate analysis included. Effect sizes were reported as odds ratios (OR) for univariate tests and adjusted odds ratios (ORa) for multivariate tests, with their 95% confidence intervals. All tests were two-tailed and considered statistically significant for $p < 0.05$.

Results

During the year 2016, our center diagnosed 28 patients with CLL managed according to the watch and wait approach. The male to female ratio was 1.8 and the median age at diagnosis was 61 years (range 42–87 years). An ECOG performance status of 0 was noted in 96.4% of our patients. Incidental lymphocytosis was the most common presentation followed by palpable lymph nodes in 10.7%. None of the patients had B signs or a family history of other leukemias or lymphomas. The clinical exam of our patients was normal in the majority (75%), followed by detection of cervical lymph nodes (21.4%) and splenomegaly (3.6%). Two patients only had more than three lymph node site involvement. At the time of diagnosis, 20 patients (71.4%) had Rai stage 0, 7 patients (25.0%) had Rai stage I, one patient (3.6%) had Rai stage II. According to the Binet clinical staging system, 67 (96.4%) patients were in Binet A and 1 (3.6%) in Binet C. The findings of the peripheral blood count of our patients are reported in [Table 1](#).

Chromosomal aberrations on classical karyotype were found in 15 patients (53.6%) among which four patients (26.7%) present complex rearrangements ([Table 2](#)). The median number of abnormalities detected on FISH is one abnormality (range 0–3). The most frequent abnormality in all patients was del(13q14), which was detected in 13 cases (46.4%), including homozygous deletion (nullisomy, three cases), and as a sole abnormality in ten cases. FISH studies identified ch14q32 rearrangement in seven patients (25.0%) among which five patients with 5' deletions and two with separations, followed by trisomy 12 in four patients (14.3%), del(17p13) and del(11q22) in 7.1% each. The deletion of 6q21/6q23 was not found in the studied patients. Co-deletions were noted too, involving ch13q14 with ch17p13 in a patient and with ch11q22 in another patient. The percentage of cells with

Table 1. Characteristics of the peripheral blood count of our watch and wait chronic lymphocytic leukemia patients.

Hemoglobin (g/dl)	Median	13.90
	Range	9.5–17.4
White blood cell count (/mm ³)	Median	17,600.00
	Range	2000–73,000
Neutrophil count (/mm ³)	Median	4540.00
	Range	330–16,060
Lymphocyte count (/mm ³)	Median	13,760.00
	Range	2080–54,020
Platelet count (/mm ³)	Median	209,000.00
	Range	123,000–338,000
NLR	Median	0.29
	Range	0.02–0.92
PLR	Median	15.20
	Range	2.3–190.7
NLR: Neutrophil to lymphocyte ratio; PLR: Platelet to lymphocyte ratio.		

trisomy 12 ranged from 63 to 82.5%, and 40.5 to 93.5% for del(13q14), 49.5–87.5% for del(17p13), 85–88.5% for del(11q22) and 36.5–91.5% for rearrangement in 14q32, respectively.

A younger age was found to be significantly correlated to the presence of 14q32 rearrangement ($p = 0.036$; $OR = 0.88$ [0.78–0.99]). The presence of detectable lymph nodes on clinical examination was found to be significantly related to the presence of a 14q32 rearrangement ($p = 0.035$; $OR = 8.0$ [1.2–55.3]) and younger age ($p = 0.043$; $OR = 0.89$ [0.79–0.99]). In the multivariate analysis, the presence of a 14q32 rearrangement was found to be solely related to younger age ($p = 0.036$; $ORa = 0.88$ [0.78–0.99]).

Discussion

We already reported on the CLL epidemiology in the Lebanese population. In comparison to the published literature, we noticed younger age and earlier stages at diagnosis as well as more aggressive diagnostic modalities including excessive use of bone marrow biopsy and aspiration. Yet, the adoption of a watch and wait strategy was lower when compared with Western, Eastern and even middle-eastern populations [14]. Subsequent to these findings, we developed our genetic testing for these patients to further understand the biology of the Lebanese CLL patients. We conducted the analysis in collaboration with a local referral center, the Unité de Génétique Médicale of Saint Joseph University – Faculty of Medicine, Lebanon. Overall our results were of particular interest despite the major limitations of the sample size. However, CLL is already a rare disease in Lebanon. The last data on the epidemiology of CLL in Lebanon go back to 2004 version where the National Cancer Registry reported only 46 CLL cases among 7197 cancer patient in 2004 (0.6%) [21].

The prognosis associated with del(13q14) does not differ from that of patients with normal cytogenetics as it has been correlated to an earlier stage of the disease and longer survival [22,23]. In large studies, FISH identified del(13q14) as the most frequent abnormality occurring in up to 50% of the cases requiring treatment with biallelic deletions in 15% in both Western and Eastern countries [22–24]. In our watch and wait CLL patients, del(13q14) was also the most common abnormality (46.4%) but biallelic deletion occurred in 23.1% [20].

The rearrangement in 14q32 is not rare in B-cell malignancies. It is known to be associate with a good prognosis in CLL patients but its incidence has been shown to vary between Western and Eastern populations (4 vs 29%) [22–25]. Our series showed a 25% incidence of *IgH* rearrangement that is closer to that of eastern population [24,25]. Interestingly, the presence of the 14q32 rearrangement correlated to younger age at diagnosis ($p = 0.036$; $ORa = 0.88$ [0.78–0.99]). The occurrence of the other aberrations (17p13, 11q22 and 6q21/6q23) was either rare or not found in any of our patients.

It is only rational to encounter low incidence of poor prognosis FISH abnormalities in our watch and wait CLL patients. Indeed, the prevalent mutations were those associated with good prognosis while the rare ones, including del(17p13), del(11q22) and unmutated *IgH*, are known to be poor prognostic factors. In line with these findings, we mention an interesting paper from MD Anderson Cancer Center showing *IgH* rearrangement in 65%, del(17p13) in 4% and del(11q22) in 9% of the watch and wait CLL patients. In this paper, Wierda *et al.* developed

Table 2. The karyotype and FISH of our watch and wait chronic lymphocytic leukemia patients.

No.	Sex/age	ISCN cytogenetics	FISH					
			TP53	ATM	c12	13q14	IGH	6q21/6q23
1	F/66	46,XX,del(11)(q14q23),del(13)(q13q22) (19)/46,XX (1)	N	D88.5%	N	D80%	D5'80.5%	N
2	M/55	46,XY,del(13)(q13q21) (8)/46,XY (12)	N	N	N	D63%	D5'83%	N
3	M/58	46,XY,del(13)(q13q21) (6)/46,XY (14)	N	N	N	D40.5%	N	N
4	F/71	46,XX (20)	N	N	N	N	D5'91.5%	N
5	M/68	46,XY (20)	N	N	N	D78.5%	N	N
6	F/73	46,XX (20)	N	N	N	N	N	N
7	M/51	46,XY,t(18;22)(q272;q172) (1)/46,idem,del(13)(q12q21) (17)/46,XY (2)	N	N	N	D85%	N	N
8	F/65	46,XX (20)	N	N	N	N	N	N
9	F/83	47,XX,+12 (18)/46,XX (2)	N	N	(+)63%	N	N	N
10	F/81	46,XX (20)	N	N	N	N	N	N
11	F/83	46,XX,inv(2)(p12q13) (15)/46,XX (5)	N	N	N	N	N	N
12	M/72	46,XY,del(11)(q173) (10)/46,XY (10)	N	D85%	N	D82.5%	N	N
13	M/71	46,Y,t(X;13)(q276;q13) (4)/46,XY,t(11;15)(q275;q21),del(13)(q12q22) (4)/46,XY,del(13)(q13q14) (3)/46,XY (19)	N	N	N	D35%	N	N
14	M/70	46,XY (20)	N	N	N	N	N	N
15	M/90	46,XY,dic(8;12)(p171;p171) (20)	N	N	(+)79.5%	N	N	N
16	M/60	46,XY (20)	N	N	N	N	N	N
17	F/61	46,XX,del(13)(q13q21),der(14)ins(7;14)(?;q21) (10)/46,XX (10)	N	N	N	D84%	N	N
18	M/83	46,XY (20)	N	N	N	DD93.5%	N	N
19	M/41	46,XY,t(1;6)(p374;p275) (15)/46,XY (5)	N	N	N	N	D5'42.4%	N
20	M/40	44,XY,add(3)(q279),add(7)(q376),add(8)(p27),-9,-11,tas(13;13)(q34;p13),-14,-15,-17,+4mar (7)/44,idem,del(10)(q272) (8)/46,XY (5)	D87.5%	N	N	D83.5%	S82%	N
21	M/70	45,XY,-13,der(18)t(13;18)(q21;q23) (17)/46,XY,add(13)(p13),-22,+mar (2)/46,XY (11)	N	N	N	D50.5%	N	N
22	M/44	46,XY (20)	D49.5%	N	N	N	S82%	N
23	F/62	46,XX (20)	N	N	N	DD70.5%	N	N
24	M/44	47,XY,+12 (18)/46,XX (2)	N	N	(+)78.5%	N	N	N
25	M/47	46,XY (20)	N	N	N	N	D5'36.5%	N
26	M/62	46,XY (20)	N	N	N	N	N	N
27	M/61	48,XY,+12,+mar (20)	N	N	(+)82.5%	DD82.5%	N	N
28	F/61	46,XX (20)	N	N	N	N	N	N

Separated signals; (+): three copies of CEP 12.

D: Monoallelic deletion; DD: Biallelic deletion; F: Female; ISCN: International System for Human Cytogenetic nomenclature; M: Male; N: Normal findings.

a nomogram that associated a shorter time to first treatment in patients with more than three involved lymph node sites, increased size of cervical lymph nodes, presence of 17p deletion or 11q deletion by FISH, increased serum lactate dehydrogenase and unmutated IgH rearrangement status [26]. Our patients seem to have a relatively good prognosis according to these findings.

The NLR and PLR concepts have evolved in this era with the recent understanding of the major role of the microenvironment in carcinogenesis [27]. The NLR and PLR, respectively, have undergone extensive evaluation in cancer patients and demonstrated to be potential prognostic factors [15,16]. Unfortunately, the NLR and PLR findings did not correlate to any of the already established prognostic markers in our series. This could be limited by the small sample size and would require evaluation in larger studies.

Conclusion

Our study supported the importance of cytogenetic and FISH testing in CLL patients with a watch and wait approach. Moreover, this paper is, to our knowledge, the first to report on the prevalence of complex FISH/karyotypes in the patients managed according to 'watch and wait.' The higher prevalence of del(13q14) as a sole abnormality could be the primary event in inducing CLL. The del(17p13) and del(11q22) could be seen as drivers for CLL progression. A detailed characterization of CLL cytogenetics with measurement of the clinical impacts on our patients is essential to ensure a better understanding of the CLL biology in the Middle East.

Future perspective

The discrepancies in the cytogenetic aberrations between the CLL patients of different regions has been well described however the cumulative accumulation of different cytogenetic aberrations that seems to dictate the natural history of the disease is common. The complete understanding of the CLL pathogenesis would reveal the causes of the variations in its epidemiology and would allow a personalized approach to its management in the watch and wait setting.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

- Howlader N, Noone AM, Krapcho M (Eds). SEER Cancer Statistics Review, 1975–2008, National Cancer Institute. Bethesda, MD, USA (2011). http://seer.cancer.gov/csr/1975_2008
- Binet JL, Lepoprier M, Dighiero G *et al.* A clinical staging system for chronic lymphocytic leukemia: prognostic significance. *Cancer* 40(2), 855–864 (1977).
- Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. *Blood* 46(2), 219–234 (1975).
- Shahjahani M, Mohammadiasl J, Noroozi F *et al.* Molecular basis of chronic lymphocytic leukemia diagnosis and prognosis. *Cell. Oncol. (Dordr.)* 38(2), 93–109 (2015).
- Juliussan G, Oscier DG, Fitchett M *et al.* Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. *NEJM* 323(11), 720–724 (1990).
- Döhner H, Stilgenbauer S, Döhner K, Bentz M, Lichter P. Chromosome aberrations in B-cell chronic lymphocytic leukemia: reassessment based on molecular cytogenetic analysis. *J. Mol. Med. Berl. Ger.* 77(2), 266–281 (1999).
- Yang S-M, Li J-Y, Gale RP, Huang X-J. The mystery of chronic lymphocytic leukemia (CLL): why is it absent in Asians and what does this tell us about etiology, pathogenesis and biology? *Blood Rev.* 29(3), 205–213 (2015).
- Rozovski U, Hazan-Halevy I, Keating MJ, Estrov Z. Personalized medicine in CLL: current status and future perspectives. *Cancer Lett.* 352(1), 4–14 (2014).
- Troussard X, Herrera A, Cornet E, Solal-Céligny P. Modeling the epidemiology of chronic lymphocytic leukemia (CLL) in France. *Blood* 124(21), 5652–5652 (2014).
- Abou-Daoud KT. The incidence of leukemia in the lebanese population. *Int. J. Cancer* 1(3), 305–307 (1966).
- Chahine G, El Rassy E, Khazzaka A *et al.* Characteristics of incident female breast cancer in Lebanon, 1990–2013: descriptive study of 612 cases from a hospital tumor registry. *Cancer Epidemiol.* 39(3), 303–306 (2015).
- Assi T, El Rassy E, Khazzaka A *et al.* Characteristics of gastric cancer in Lebanon: a descriptive study from a single institutional experience. *J. Gastrointest. Cancer* (2016) (Epub ahead of print).
- Assi T, Rassy M, Nassereddine H *et al.* Distribution of testicular tumors in Lebanon: a single institution overview. *Asian Pac. J. Cancer Prev.* 16(8), 3443–3446 (2015).
- Lutfallah AA, Kourie HR, Eid R, Farhat F, Ghosn M, Kattan J. Watch and wait in Lebanese chronic lymphocytic leukemia patients: how relevant is it? *Asian Pac. J. Cancer Prev.* 17(1), 215–217 (2016).

- 15 Templeton AJ, McNamara MG, Šeruga B *et al.* Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. *J. Natl Cancer Inst.* 106(6), dju124 (2014).
- 16 Zhou X, Du Y, Huang Z *et al.* Prognostic value of PLR in various cancers: a meta-analysis. *PLoS ONE* 9(6), e101119 (2014).
- 17 Barbour B, Salameh P. Consanguinity in Lebanon: prevalence, distribution and determinants. *J. Biosoc. Sci.* 41(4), 505–517 (2009).
- 18 Cheson BD, Bennett JM, Grever M *et al.* National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood* 87(12), 4990–4997 (1996).
- 19 Eichhorst B, Hallek M. Revision of the guidelines for diagnosis and therapy of chronic lymphocytic leukemia (CLL). *Best Pract. Res. Clin. Haematol.* 20(3), 469–477 (2007).
- 20 Puiggros A, Collado R, Calasanz MJ *et al.* Patients with chronic lymphocytic leukemia and complex karyotype show an adverse outcome even in absence of TP53/ATM FISH deletions. *Oncotarget* (2017) (Epub ahead of print).
- 21 National Cancer Registry 2004. Cited 15 May 2017. www.moph.gov.lb
- 22 Dewald GW, Brockman SR, Paternoster SF *et al.* Chromosome anomalies detected by interphase fluorescence in situ hybridization: correlation with significant biological features of B-cell chronic lymphocytic leukaemia. *Br. J. Haematol.* 121(2), 287–295 (2003).
- 23 Döhner H, Stilgenbauer S, Benner A *et al.* Genomic aberrations and survival in chronic lymphocytic leukemia. *NEJM* 343(26), 1910–1916 (2000).
- 24 Qiu H-X, Xu W, Cao X-S *et al.* Cytogenetic characterisation in Chinese patients with chronic lymphocytic leukemia: a prospective, multicenter study on 143 cases analysed with interphase fluorescence in situ hybridisation. *Leuk. Lymphoma* 49(10), 1887–1892 (2008).
- 25 Amare PSK, Gadage V, Jain H *et al.* Clinico-pathological impact of cytogenetic subgroups in B-cell chronic lymphocytic leukemia: experience from India. *Indian J. Cancer* 50(3), 261–267 (2013).
- 26 Wierda WG, O'Brien S, Wang X *et al.* Multivariable model for time to first treatment in patients with chronic lymphocytic leukemia. *J. Clin. Oncol.* 29(31), 4088–4095 (2011).
- 27 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 144(5), 646–674 (2011).