A case of SRSF2 mutation in chronic lymphocytic leukemia

Eduardo Garza a,b,c, Giovanni Del Poeta b, Carmen Martínez-Losada a,d,e, Gianfranco Catalano b, Loredana Borgia a,b, Maria Liliana Piredda a,b, Emiliano Fabiani f, Valter Gatti g,h, Francesco Lo-Coco a,b, Nélida I. Noguera a,b,*

a Laboratory of Neuro-Oncosematology, Santa Lucia Foundation, Rome, Italy
b Department of Biomedicina e Prevenzione, “Tor Vergata” University, Rome, Italy
c Laboratory of Dr. Moreira, Monterrey, Mexico
d Alfonso Martin Escudero Foundation, Madrid, Spain
e Department of Hematology, IMIBIC, Hospital Universitario Reina Sofia, Universidad de Córdoba, Spain
f Department of Hematology, Università Cattolica S. Cuore, Rome, Italy
g Laboratory of Neuro-Oncoematology, Santa Lucia Foundation, Rome, Italy
h Clinical & Experimental Onco-Hematology Unit, Centro di Riferimento Oncologico, Istituto di Ricovero e Cura a Carattere Scientifico, Aviano, Italy

ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form 6 March 2016
Accepted 26 March 2016
Available online 23 June 2016

Keywords:
Genetic analysis
Chronic lymphocytic leukemia
Clinical aspect
SRSF2

A B S T R A C T

Chronic lymphocytic leukemia (CLL) is characterized by extremely variable clinical course indicating substantial differences in the biology of the disease. Molecular characterization provides new insights useful for treatment decision making. We report on a patient diagnosed with CLL, whose disease was characterized by episodes of rapid progression and disease stabilization, and in which a SRSF2 gene mutation was identified in the absence of other commonly known mutations of CLL. To the best of our knowledge this is the first case of SRSF2 gene mutation ever reported in CLL.

Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Chronic lymphocytic leukemia (CLL) is the most frequent hematologic neoplasm in Western countries. The clinical course of chronic lymphocytic leukemia ranges from a very indolent disorder with a normal lifespan, to a rapidly progressive disease leading to death. The variable clinical course of CLL is driven, at least in part, by the disease molecular heterogeneity. This makes it difficult to select treatment choice and estimate survival [1]. Most common chromosome abnormalities of CLL are deletions (6q, 11q, 13q, or 17p) and less frequently chromosomal gains (trisomy 12). Molecular markers with prognostic impact include inactivating mutations of TP53, NOTCH1, SF3B1 and baculoviral IAP repeat containing 3 (BIRC3) genes. Molecular genotyping of CLL enhances our understanding of the clinical heterogeneity of the disease and provides new insights useful for treatment decision making [2,3].

Splicing machinery dysfunctions have been associated to CLL, in particular SF3B1 gene mutations (splicing factor 3B subunit 1) which are present in about 15% of patients [4]. To our knowledge, no SRSF2 gene mutation has ever been detected in CLL. In order to broaden our knowledge on the frequency of SRSF2 splicing mutations in CLL we studied systematically peripheral blood samples with a DNA high-resolution melting analysis test [5] in a cohort of 115 patients diagnosed with CLL (Table 1) and found only 1 mutated case.

We report on a patient diagnosed with chronic lymphocytic leukemia (CLL) whose disease was characterized by marked clinical heterogeneity with episodes of rapid progression and disease stabilization and in which a SRSF2 gene mutation was identified in the absence of other commonly known mutations of CLL.

A female patient aged 55 was observed in December 1995 at Sant’ Eugenio hospital in Rome with lymphocytosis. The patient past medical records included: malaria 7 years before, bilateral oophorectomy and hysterectomy due to uterine fibroma, and hypothyroidism under medication with levothyroxine sodium. Laboratory tests disclosed 19.7 × 10^9/L leukocytes (15.5 × 10^9/L lymphocytes), hemoglobin 130 g/L, 218 × 10^3/L platelets and normal levels of β2-microglobulin and lactate dehydrogenase. The microscopic analysis of blood smear was consistent with a lymphoproliferative process with multiple mature-like small lymphocytes with scant cytoplasm, regular nucleus (dense chromatin without nucleoli) and presence of Gümprecht cells. Flow cytometry analysis showed positivity for: CD5, CD23, CD19 (LLCr score 5) CD38 and ZAP-70, with restriction to lambda light chain. The IGHV
status of the patient was unmutated. Karyotype studies showed del13q. Sequencing analysis of the following genes resulted negative for molecular alterations: NOTCH1 (Notch homolog 1, translocation-associated [Drosophila]) exon 34, NRAS (neuroblastoma RAS viral [v-ras] oncogene homolog) exons 1 and 2, KRAS (Kirsten sarcoma viral oncogene homolog) exons 1 and 2 and TP53 (tumor protein 53) exons 3, 4, 5, 6, 7 and 8 (exons 9–11 were excluded due to low mutation frequency). High resolution melting analysis screening for common mutations of IDH1/2 (isocitrate dehydrogenase 1 and 2 [NADP+]) and SF3B1 (exons 12–15) genes resulted as well negative. Clinically, the patient presented only one small cervical lymphadenopathy and no systemic symptoms. CLL was diagnosed as Rai stage I (Rai modified) and Binet stage A according to the International Working group CLL criteria.

The patient did not receive treatment until February 1997 (14 months later) when she rapidly progressed presenting splenomegaly and an increased number of lymphadenopathies (Rai II and Binet B), requiring treatment. A course of intravenous fludarabine 25 mg/Kg/day for 5 days every 28 days for a total of 5 cycles was prescribed. In June 1997, treatment outcome assessment showed only partial response (50% reduction of lymphadenopathy).

In December 1998, disease progression was observed with splenomegaly, increased number of lymphadenopathies and rapid increase of blood lymphocytes (lymphocyte doubling time < 12 months). A course of oral chlorambucil 10 mg/day with prednisone 25 mg/day for 10 days every month was given for a total of 9 cycles. Maintenance therapy was started with subcutaneous interferon α-2b using alternating doses of 1.5 MI and 3 MU 3 times / week and the patient remained in partial remission for the following 7 years.

A third disease progression occurred in March 2006 and a course of fludarabine 25 mg/Kg/day was given for a total of 6 cycles, followed by intravenous rituximab 375 mg/Kg every 28 days for a total of 4 cycles. Complete remission was achieved at the end of treatment. An additional cycle of rituximab was administered in June 2010. At present, the patient remains in complete remission with residual neutropenia and thrombocytopenia requiring treatment with low dose of prednisone.

SRSF2 mutation was confirmed by Sanger sequencing of different blood DNA samples from the patient during her last two follow up visits to the hospital in April and June 2014 (Fig. 1). Unfortunately, DNA from disease onset was not available, making it difficult to correlate the clinical behavior of the disease with the presence of SRSF2 mutation. The anomaly could have been acquired as a late event following different treatment lines or, alternatively, it might have been present at diagnosis in the bulk leukemic population or at sub-clonal level. At diagnosis the patient presented 13q– as a sole genetic abnormality (which has been associated to good prognosis) and overexpression of ZAP-70 which is known to be associated to poor prognosis.

In recent years, there has been growing interest regarding the role of spliceosome machinery in neoplastic diseases following evidence of the involvement of splicing factor mutations in diverse cancers [6]. SRSF2 (Serine-Arginine Splicing Factor 2) is a member of the serine/arginine (S/A) rich family pre-mRNA splicing factors. SRSF2 mutations are primarily detected in hematologic diseases, at a reported frequency of 5.5% in refractory anemia with ringed sideroblasts and refractory cytopenia with multilineage dysplasia with ringed sideroblasts, 11.6% in myelodysplastic syndrome (MDS) without ringed sideroblasts, 6.5% in acute myeloblastic leukemia secondary to MDS, 0.7% in de novo acute myeloid leukemia, 1.9% in myeloproliferative neoplasms, 18.8% in secondary acute myeloid leukemia evolving from myeloproliferative neoplasm and 28.4% in chronic myelomonocytic leukemia (CMML) [6,7]. In MDS and CMML, SRSF2 point mutations mostly occur in exon 1 of the SRSF2 gene, at amino acid position P95 (95% and 93% of cases respectively). In the remaining minority of cases insertions/deletions and frameshifts can be found around amino acid P95 and also point mutations in other positions such as P96, P84, P85 and P86 [7,8] have been reported. In MDS and CMML, SRSF2 mutated cases show higher transformation rate, shorter time to progression to acute myeloid leukemia and shorter overall survival compared to unmutated cases [7,9,10]. SRSF2 mutations are thought to interfere with cellular homeostasis via defective splicing of certain genes such as RUNX1 (runt-related transcription factor 1 gene) with a loss-of-function effect seen in MDS and CMML [8]. Recent publications have liked SRSF2 mutations with older male patients with MDS whose clinical course remains stable with no co-occurrence of other mutations [11].

The frequency of other splicing mutations in CLL besides SF3B1 such as SRSF2 has been studied recently and it is known that mutations in this gene are uncommon [12–14]. In our study, we found one case of SRSF2 mutation in a patient with CLL with a very variable clinical course and many relapses. Little can be assumed in this case due to the lack of similar reports in literature, however it is possible that the mutation could have contributed to the worsening of her disease.

Another assumption that arises from this case is that the presence of myelodysplasia secondary to treatment could explain the presence of SRSF2 mutation in this patient. This brings us to think on many relapsed CLL cases with residual cytopenias where no other molecular abnormalities or disease markers can explain poor clinical outcomes.

### Table 1

| Parameter | Category | Study population (n = 110) |
|-----------|----------|---------------------------|
| Gender    | Male (%) | 57 (51.8%)                |
| Age (years old), median (range) | 64 (39–83) | 37 (33.6%) |
| Rai stage | 0        | 73 (66.4%)                |
| WBC (× 10⁹/L), median (range) | 19.52 (7.64–270.38) | 13.70 (4.20–258.30) |
| Lymphocytes (× 10⁹/L), median (range) | 28 (25.5%) |                       |
| Beta-2-microglobulin | Abnormal high level (%) | 26 (23.6%) |
| Karyotype by fluorescence in situ hybridization (FISH) | High risk | 38 (34.5%) |
| Deletion 13p | 46 (41.8%) | 37 (33.6%) |
| Presence | 26 (23.6%) |                       |
| Lymphadenopathy/splenomegaly at diagnosis | Presence | 4 (3.6%) |
| Lymphocytes doubling time < 12 months | 36 (32.7%) |                       |
| Necessity of treatment | Yes | 81 (73.6%) |
| First time treatment | FC | 53 (48.1%) |
| RFC | 5 (4.54%) |                       |
| RB | 10 (9.09%) |                       |
| Leukeran + R | 3 (2.72%) |                       |
| Leukeran | 8 (7.27%) |                       |
| Days to start treatment, median (range) | 977 (7–3935) |                       |
| Infection grade IV | 19 (17.3%) |                       |
| Response to treatment | Complete remission | 50 (46.3%) |
| Partial remission | 24 (21.8%) |                       |
| Failure/progression | No valuable | 6 (5.41%) |
| Relapse in patients who achieved complete remission | 18 (36%) |                       |
| Days to relapse | 1258 (386–2725) |                       |

* FC – fludarabine + cyclophosphamide. RFC – rituximab + fludarabine + cyclophosphamide. RB – rituximab + bendamustine. R – rituximab.

* Median value of the days passed since complete remission after first line therapy until relapse occurred.

* All patients presenting infection grade IV received treatment (p = 0.01).
More research is needed to address the frequency and clinical significance of molecular abnormalities in CLL with myelodysplastic changes.

**Conflict of interest**

The authors report no potential conflicts of interest.

**Authors' contributions**

EG carried out and analyzed the experiments and co-wrote the manuscript; GDP, GC and VG recruited the patients and critically reviewed the manuscript; CM-L contributed with the laboratory work and co-wrote the manuscript, MLP, LB and EF contributed with the laboratory work; FL-C and NIN coordinated the research and co-wrote the manuscript.

**Acknowledgments**

We thank Dr. Felicitas Thol for providing us with control samples for specific splicing factor gene mutations. This work was partially funded by FIRB N. RBAP11TF7Z grant to Lo Coco Francesco.

**References**

[1] M. Hallek, B.D. Cheson, D. Catovsky, F. Caligaris-Cappio, G. Dighiero, H. Döhner, P. Hillmen, M.J. Keating, E. Montserrat, K.R. Rai, T.J. Kipps, Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute—Working Group 1996 guidelines, Blood 111 (2008) 5446–5456.

[2] P. Baliakas, M. Iskas, A. Gardiner, Z. Davis, K. Plevova, F. Nguyen-Khac, J. Malickova, A. Anagnostopoulous, S. Glode, S. Mould, K. Stepanovska, M. Brejcha, C. Belessi, F. Davi, S. Pospisilova, A. Athanasiadou, K. Stamatopoulos, D. Oscier, Chromosomal translocations and karyotype

---

**Fig. 1.** High resolution melting and sequencing analyses reports. Panel A: gene scanning difference plot showing patient’s (LLC-125) sample with a positive melting pattern. Wilde type (WT) samples are located at the baseline. Panel B: melting peak analysis showing patient’s sample with a different (early) melting temperature compared to WT samples. -d/dT: negative derivative of the change in fluorescence divided by the change in temperature in relation to the temperature. Panel C: sequencing analysis showing patient’s sample with SRSF2 P95L mutation compared to a WT control.
complexity in chronic lymphocytic leukemia: a systematic reappraisal of classic cytogenetic data, Am. J. Hematol. 89 (2014) 249–255.

[3] S.N. Malek, The biology and clinical significance of acquired genomic copy number aberrations and recurrent gene mutations in chronic lymphocytic leukemia, Oncogene 32 (2013) 2805–2817.

[4] L. Wang, M.S. Lawrence, Y. Wan, A.M. Jankowska, S. Abu Kar, A. Jerez, L. Werner, A. Sivachenko, D.S. DeLuca, L. Zhang, W. Zhang, A.R. Vartanov, S. M. Fernandes, N.R. Goldstein, E.G. Polco, K. Cubblitskis, B. Tesar, Q.L. Sievers, E. Sheffer, S. Gabriel, N. Hacohen, R. Reed, M. Meyerson, T.R. Golub, E.S. Lander, D. Neuberg, J.R. Brown, G. Getz, C.J. Wu, SF3B1 and other novel cancer genes in chronic lymphocytic leukemia, N. Engl. J. Med. 365 (2011) 2497–2506.

[5] E. Garza, E. Fabiani, N. Noguera, P. Panetta, M.L. Piredda, L. Borgia, L. Maurillo, G. Catalano, M.T. Voso, F. Lo-Coco, Development of a high-resolution melting curve analysis screening test for SRSF2 splicing factor gene mutations in myelodysplastic syndromes, J. Mol. Diagn. 17 (2015) 85–89.

[6] M. Gruber, C.J. Wu, Evolving understanding of the CLL genome, Semin. Hematol. 51 (2014) 177–187.

[7] K. Yoshida, M. Sanada, Y. Shiroma, D. Nowak, Y. Nagata, R. Yamamoto, Y. Sato, A. Sato-Otsudo, A. Kon, M. Nagasaki, G. Chalkidis, Y. Suzuki, M. Shiosaka, R. Kawahata, T. Yamaguchi, M. Otsu, T. Yamaguchi, M. Otsu, N. Obara, M. Sakata-Yanagimoto, K. Ishiyama, H. Mori, F. Nolte, W.K. Hofmann, F. Nolte, W.K. Hofmann, B. Schlegelberger, M. Heuser, Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndrome, Blood 119 (2012) 3578–3584.

[10] M.M. Patnaik, E.A. Wassie, E. Padron, F. Onida, R. Itzykson, T.L. Lasho, O. Kosmider, C.M. Finke, C.A. Hanson, R.P. Ketterling, R. Komrokj, A. Tefferi, E. Solary, Chronic myelomonocytic leukemia in younger patients: molecular and cytogenetic predictors of survival and treatment outcome, Blood Cancer J. 5 (2015) e270.

[11] S.J. Wu, Y.Y. Kuo, H.A. Hou, L.Y. Li, M.H. Tseng, C.F. Huang, F.Y. Lee, M.C. Liu, C. W. Liu, C.T. Lin, C.Y. Chen, W.C. Chou, M. Yau, S.Y. Huang, B.S. Ko, J.L. Tang, W. Tsay, H.F. Tien, The clinical implication of SRSF2 mutation in patients with myelodysplastic syndrome and its stability during disease evolution, Blood 120 (15) (2012) 3106–3111.

[12] H. Makishima, V. Visconte, H. Sakaguchi, A.M. Jankowska, S. Abu Kar, A. Jerez, B. Przychodzen, M. Bujalowski, K. Gintis, M.A. Sekeres, R.A. Padgett, R.V. Tiu, J.P. Maciejewski, Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis, Blood 119 (2012) 3203–3210.

[13] D.A. Landau, S.L. Carter, P. Stojanov, A. McKenna, K. Stevenson, M.S. Lawrence, C. Sougnez, C. Stewart, A. Sivachenko, L. Wang, Y. Wan, W. Zhang, S.A. Shukla, A. Vartanov, S.M. Fernandes, G. Saksena, K. Cubblitskis, B. Tesar, S. Gabriel, N. Hacohen, M. Meyerson, E.S. Lander, D. Neuberg, J.R. Brown, G. Getz, C.J. Wu, Evolution and impact of subclonal mutations in chronic lymphocytic leukemia, Cell 152 (4) (2013) 714–726.

[14] X.S. Puetze, S. Böe, R. Valdés-Más, N. Villamor, J. Gutiérrez-Abril, J.I. Martín-Subero, M. Munar, C. Rubio-Pérez, P. Jares, M. Aymerich, T. Baumann, R. Beekman, L. Belver, A. Carrió, G. Castellano, G. Clot, E. Colado, D. Colomer, D. Costa, J. Delgado, A. Enjuanes, X. Estivill, A.A. Ferrando, J.L. Gelpí, B. González, S. González, M. Gutiérrez-Guerra, D. Martín-García, A. Navarro, P. Nicolás, M. Orozco, Á.R. Payer, M. Pinyol, D.G. Pisano, D.A. Puente, A.C. Queiroz, V. Quesada, C.M. Romeo-Casabona, C. Royo, R. Royo, M. Rozman, N. Rusiñol, I. Salaverria, K. Stamatopoulos, H.G. Strunzenberg, D. Tamborero, M.J. Terol, A.J. Valencia, N. López-Bigas, D. Torrents, I. Gut, A. López-Guillermo, C. López-Otín, E. Campo, Non-coding recurrent mutations in chronic lymphocytic leukaemia, Nature 526 (7574) (2015) 519–524.