Exp Oncol 2011
33, 1, 47–51

Experimental ONCOLOGY

# MATURE B-CELL NEOPLASMS IN CHERNOBYL CLEAN-UP WORKERS OF 1986–1987: SUMMARY OF CYTOMORPHOLOGICAL AND IMMUNOCYTOCHEMICAL STUDY IN 25 YEARS AFTER CHERNOBYL ACCIDENT

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**The data on the verified cases of mature B-cell neoplasms (chronic lymphocytic leukemia – CLL, B-prolymphocytic leukemia, non-Hodgkin's lymphoma in leukemization phase and multiple myeloma – MM; 146 cases in total) in the consecutive group of Ukrainian clean-up workers within 10–25 years after Chernobyl accident are summarized. B-cell neoplasms represent the most prevalent group among all diagnosed neoplasms of hematopoietic and lymphoid tissues in clean-up worker patients under study (49.4%). MM percentage in the patients of Chernobyl clean-up worker group turned out to be significantly higher than in the patients of the general populations studied at the same period. While the percentage of B-CLL is similar in clean-up worker patients and patients of general population, the trend towards younger age of patients with mature B-cell neoplasms in clean-up worker group is evident. The current concepts on the possible association between mature B-cell neoplasms (mainly B-CLL) and radiation exposure are briefly outlined. Only the precise diagnosis of hematopoietic malignancies combining with large-scale analytical epidemiological studies with careful dose assessment and long-term follow-up may represent the basis for resolving the question whether mature B-cell neoplasms may be radiogenic.**
***Key Words:*** **Chernobyl, clean-up workers, leukemia, mature B-cell neoplasms.**

Chernobyl accident remains the worst in history of nuclear industry. Oncohematological consequences have been still very controversial, even in 25 years since the disaster.

The most intricate in this respect is the problem of the putative association between chronic lymphocytic leukemia (CLL) as well as other mature B-cell neoplasms and radiation exposure. CLL (and mature B-cell neoplasms in total) is one of the most prevalent forms of the tumors of hematopoietic and lymphoid tissues in the population of Europe and North America.

Chernobyl accident has resulted in the radiation exposure of the numerous group of population within the different dose range. Clean-up workers of 1986–1987 with the average dose estimate of approximately 100 mGy represent the most suffered group [1, 2].

Despite several research projects in Ukraine, Russian Federation and Belarus, the question whether the incidence of CLL among the exposed clean-up workers has been still a point of controversy. The lack of database of verified diagnoses in most research presented within 25 years after Chernobyl accident is still a problem preventing from accurate analysis of the data by various epidemiological approaches.

The aim of the study is to summarize the data on the verified cases of mature B-cell neoplasms (CLL, B-prolymphocytic leukemia, non-Hodgkin's lymphoma in leukemization phase and multiple myeloma) in the consecutive group of Ukrainian clean-up workers within 10–25 years after Chernobyl accident diagnosed in the Reference Laboratory representing the public service in RE Kavetsky Institute. The hematopoietic malignancies were diagnosed based on cytomorphology, cytochemistry and immunophenotyping in accordance with FAB, WHO, EGIL, ICD-10 and ICD-O-2 classifications.

Several aspects pertaining to the up-to-date views on the association between CLL and radiation exposure are also briefly outlined.

The research has been carried out within the framework of the collaboration with French-Ukrainian Center "Children of Chernobyl" set up in 1991 and Japanese-Ukrainian Leukemia/Lymphoma Study Group set up in 1998.

## PATIENTS AND METHODS

In all, 403 clean-up workers from Kyiv city and majority of the regional hospitals of Ukraine with suspected oncohematological disorders were examined. All the clean-up workers referred to the Reference Laboratory in 1996–2010 were examined consecutively without any previous selection of the cases. The radiation dose load of the clean-up workers under study varied from 75 to 250 mGy. The consecutive patients of general population aged over 30 (the total number of patients — 2697), mainly the residents of Kyiv city and district (hereinafter referred as "general population"), diagnosed in the Reference Laboratory at the same period comprised the group of comparison.

Bone marrow and peripheral blood smears stained by May-Grunwald-Giemsa were studied morphologically. Activities of myeloperoxidase, acid phosphatase (tartrate-sensitive and tartrate-resistant), non-specific esterase (sodium fluoride-sensitive), naphtol-AS-D-chloracetate esterase, alkaline phosphatase were analyzed cytochemically. Glycogen was assayed cytochemically by PAS-reaction. Immunocytochemi-

Received: February 25, 2011.
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*Abbreviations used*: ATM – ataxia teleangiectasia; CLL – chronic lymphocytic leukemia; MM – multiple myeloma; NHL – non-Hodgkin's lymphoma.

cal techniques (APAAP, LSAB-AP) and a broad panel of monoclonal antibodies (MoAbs) against lineage specific, differentiation and activation antigens of leukocytes were employed for immunophenotyping pathological cells in blood and bone marrow [3]. The main forms and cytological variants of hematological malignancies were diagnosed according to FAB-classification and REAL classification schemes. In 2001–2010 the diagnostic findings were revised in accordance with recently published new WHO classification [4].

## RESULTS

In 118 of 403 patients in clean-up worker group, the diagnosis of tumors of hematopoietic and lymphoid tissues has not been confirmed. In 285 Chernobyl clean-up workers various forms of malignant diseases of hematopoietic and lymphoid tissues were registered (see Table).

**Table.** Summary of tumors of hematopoietic and lymphoid tissues diagnosed in Chernobyl clean-up workers (1996–2010)

| Disease | Number of cases and percentage |
|---|---|
| **Mature B-cell neoplasms** | |
| Chronic lymphocytic leukemia | 77 (26.10 %) |
| B-cell prolymphocytic leukemia | 4 (1.36 %) |
| Hairy cell leukemia | 11 (3.73 %) |
| Non-Hodgkin's lymphoma in leukemization phase | 35 (11.79 %) |
| Multiple myeloma | 19 (6.44 %) |
| **Mature T-cell neoplasms** | |
| T-cell prolymphocytic leukemia | 2 (0.68 %) |
| T-cell large granular lymphocytic leukemia | 5 (1.69 %) |
| Sezary syndrome | 3 (1.02 %) |
| **Myeloproliferative neoplasms** | |
| Chronic myelogenous leukemia | 27 (9.01 %) |
| Polycythemia vera | 6 (2.03 %) |
| Primary myelofibrosis | 4 (1.36 %) |
| Essential thrombocythemia | 10 (3.39 %) |
| Chronic eosinophllic leukemia / eosinophilic syndrome | 3 (1.02 %) |
| **Myelodysplastic / myeloproliferative neoplasms** | |
| Chronic myelomonocytic leukemia | 10 (3.39 %) |
| Myelodysplastic syndromes | 16 (5.42 %) |
| **Acute leukemias** | |
| Acute myeloid leukemia | 46 (15.60 %) |
| Acute lymphoblastic leukemia | 17 (5.76 %) |

The mature B-cell neoplasms constitute in total about half of all cases of tumors of hematopoietic and lymphoid tissues in our group of clean-up workers, namely 49.42%, with B-CLL being predominant form of mature B-cell neoplasms (26.10%).

Immunophenotype of all B-CLL cases under study was quite typical (HLA-DR$^+$, CD19$^+$, CD20$^+$, CD22$^{low}$, CD5$^+$, CD23$^+$, CD79a$^+$, CD10$^-$, sIg$^{low}$).

B-cell prolymphocytic leukemia (B-PLL) was registered in 4 patients of clean-up worker group (1.36%) and 23 patients of general population group (0.85%).

The phenotype of hairy cell leukemia (HCL) was (HLA-DR$^+$, CD19$^+$, CD20$^+$, CD22$^+$, CD5$^-$, CD23$^-$, CD10$^-$, CD25$^+$, sIg$^{bright}$, $\kappa^+\lambda^+$) with relative frequency of 3.73% in clean-up workers and 4.73% in general population.

Different types of B-cell non-Hodgkin's lymphoma (NHL) in leukemization phase were diagnosed in 35 (11.79%) patients of Chernobyl clean-up worker group with 10.97% cases in general population. Taking into account the new WHO classification, follicular lymphoma was verified in 11 patients (CD19$^+$, CD20$^+$, CD22$^+$, CD79a$^+$, CD10$^+$, CD5$^-$, CD43$^-$), lymphoplasmacytic lymphoma — in 7 patients (CD19$^+$, CD20$^+$, CD22$^+$, CD79a$^+$, CD38$^+$, CD5$^-$, CD10$^-$, CD23$^-$), mantle cell lymphoma — in 3 patients (HLA-DR$^+$, CD19$^+$, CD20$^+$, CD22$^+$, CD5$^+$, cyclin D$^+$, CD23$^-$, CD10$^-$). Splenic marginal zone B-cell lymphoma characterized in some cases by the abundance of villous lymphocytes exhibiting high tartrate-sensitive acid phosphatase activity (HLA-DR$^+$, sIg$^{bright}$, CD19$^+$, CD20$^+$, CD22$^+$, CD5$^-$, CD23$^-$, CD10$^-$, CD43$^-$) was revealed in 5 patients. Diffuse large B-cell lymphoma (CD19$^+$ CD20$^+$ CD22$^+$ CD79a$^+$ CD5$^-$ CD22$^-$) was revealed in 6 patients and extranodal marginal zone B-cell lymphoma of MALT type (CD19$^+$ CD20$^+$ CD22$^+$ CD79a$^+$ CD23$^-$CD5$^-$ CD10$^-$CD43$^{+/-}$) — in 3 patients.

Multiple myeloma (MM) was diagnosed in 19 patients of clean-up worker group (6.44%). In 7 patients, the disease developed at the age below 50. According to the data of the available literature, the main peak of MM incidence could be registered at the age of 70–80. In our study, MM percentage in the patients of Chernobyl clean-up worker group turned out to be significantly higher than in the patients of the general populations studied at the same period (4.00%).

## DISCUSSION

The data provided above seem to represent one of the attempts to characterize in details the major forms and cytological variants of mature B-cell neoplasms in Chernobyl clean-up workers that became evident in 10–25 years after their exposure to radiation. The comparison of the relative distribution of the specified forms of mature B-cell neoplasms in the patients diagnosed among Chernobyl clean-up workers demonstrates the increasing multiple myeloma rate while no differences in the percentage of NHL (in leukemization phase) and B-CLL between clean-up workers and general population.

A study of a cohort of 71,870 Russian-resident males who were engaged in recovery operations within 30-km zone in 1986–1990 revealed a total of 58 morphologically verified leukemia cases diagnosed in this cohort between 1986 and 1998, of which 16 cases (27.6%) were CLL [5]. In multinational case-control study in group of clean-up workers (1986–1987) from Belarus, Russia and Baltic countries, 32 (27.4%) cases of CLL and 34 (29.0%) cases of NHL were recorded [6]. In Ukrainian-American nested case-control study of leukemia and related disorders based on a cohort of 110,645 clean-up workers (1986–1990), the follow-up (until 2000) has yielded 101 leukemia cases with 49 (48.9%) CLL and 8 (7.93%) MM [7].

According to the data of the scientists from the Research Center for Radiation Medicine of the National Academy of Medical Sciences of Ukraine, CLL in Chernobyl clean-up workers develops at younger age with more advanced symptoms and more aggressive course and resistance to standard therapy [8].

The absence of demonstrable association between CLL in Japanese A-bomb survivors (partly due to the rarity of CLL among Japanese in general) and the results of earlier epidemiological studies in patients treated with radiation [9, 10] have led to the long persisted conclusion that CLL is not associated with radiation exposure

[11, 12]. Nevertheless, recently these assumptions have been challenged. The epidemiological data based on studies in occupationally and medically exposed populations have required revising several aspects pertaining to CLL radiogenicity [11–15].

The clinical characteristics of CLL including its long latency and asymptomatic period, higher prevalence at older age, mild symptoms, and low rate of fatal outcomes resulted in underestimation of CLL judging by death certificates entries [13]. Many patients diagnosed with CLL often live for many years not requiring hospitalization and die from cases unrelated to their CLL (infectious or malignant diseases).

Although CLL as the clinical and hematological entity is recognized for more than hundred years, only in the middle of 70-s of the last century this entity was introduced into International Classification of Diseases (ICD-8) [12] allowing for separate accounting for CLL and ALL incidence (in Ukraine such amendments came into force only in 1989).

In research of the recent decade, new data on the origin and differentiation of B lymphocytes, the biology of leukemic lymphocytes, and peculiar clinical features of B-CLL have been obtained. The specific immunophenotypic and molecular genetic features of B-CLL have become evident. As a result, the novel hypotheses on the origin and the evolution of this disease have been put forward. In parallel, the methods for the laboratory diagnosis have been improved.

Earlier, CLL in majority of cases was considered to originate from naïve $CD5^+CD23^+IgM^+IgD^+$ B cells capable of recirculation and in some cases from $CD5^+$ $IgM^+$ subpopulation of memory B cells with mutation of IgHV genes. The results of the recent studies demonstrate that in CLL the initial genetic impairments occur in immature B cells of bone marrow. The subsequent repetitive antigenic stimulation with additional genetic lesions results in neoplastic transformation and leukemia development [16, 17]. Alternatively, the initiating events in CLL could occur in immature B cells circulating in peripheral blood, and in case of small lymphocytic lymphoma (SLL) — in similar B cells residing upon homing in lymph nodes or spleen.

One of the central points in pathogenesis of CLL is B cell receptor involved in transduction of signals associated with CLL cell microenvironment. The direct contact between B cells and accessory and stromal cells is also of high importance.

CLL is known to exhibit stronger familial tendency than any other malignancy. CLL risk is 3-6-fold increased in the relatives of CLL patients [12]. The role of hereditary factors in CLL is supported by the fact of strikingly low CLL incidence in China, Japan and Philippines. Such low CLL rates in Asians appear to be stable despite the migration of native population to western countries [17]. Adverse environmental exposure including the infectious agents, antigens, genotoxic chemicals and ionizing radiation may also contribute substantially to CLL development [12].

Prior to REAL classification (1994) and WHO classification of tumors of hematopoietic and lymphoid tissues (2004, 2008), the strict criteria for differentiating between B-CLL and other mature B cell neoplasms (B-cell prolymphocytic leukemia, hairy cell leukemia as well as nodular marginal zone lymphoma, follicular lymphoma and mantle cell lymphoma in leukemization phase) were lacking. Likewise, it was not possible to differentiate between B-CLL and rare T cell prolymphocytic leukemia, T-cell large granular lymphocytic leukemia, chronic lymphoproliferative disorder of NK cells. In this respect, monoclonal B-cell lymphocytosis (MBL) as the condition that may precede CLL deserves close attention [18]. In MBL, immunophenotype and the chromosomal abnormalities are similar to those of CLL. MBL is detected up to 5% of adult population depending on their age distribution. In this connection, the assay of phenotypic subpopulations of lymphocytes by flow cytometry seems to be of high importance in periodical laboratory examination of blood in clean-up workers.

We suppose that the integration of CLL and SLL into one nosological group referred to as CLL/SLL seems rather unwarranted since the term SLL is used mainly by hematopathologists for non-leukemic cases with histopathology and immunophenotype corresponding to that of CLL. This question may complicate the epidemiological studies involving such entity.

For many years, CLL cases have been underestimated. Even as late as in 1975, for diagnosing CLL lymphocyte count over $15 \times 10^9$/L was required. Therefore, many low count cases have gone unrecognized. At present, the diagnostic criteria for CLL is formulated as $\geqslant 5 \times 10^9$/L monoclonal lymphocytes with CLL phenotype in peripheral blood.

In 70–80s of the last century, the first reports describing specific chromosomal translocations in CLL begin to appear. At present, the broad spectrum of cytogenetic and molecular biological alterations are known to be associated with CLL development.

The heterogeneity of the clinical manifestations in CLL depends largely on the differences in the mutational status of variable regions of the genes of heavy chains of immunoglobulins (IgHV). Somatic mutations of IgHV genes are detected in more than 50–60% of CLL cases [16, 17]. The absence of the somatic mutations and presence of CD38 and ZAP-70 expression appear to be associated with more unfavorable course of the disease. Mutations in several other genes such as *BCL6, MYC, PAX5* and *RHOH* also modify the course of the disease in the patients with non-mutated immunoglobulin genes. In the stable course of the disease in patients with somatic mutations of IgHV genes, overexpression of *WNT3, CTLA4, ADAM29, TCF7* is evident [17].

Some of the somatic mutations in CLL may be a consequence of the environmental exposures [11]. The clonal chromosomal aberrations are revealed in 50–80% of CLL cases [16, 17]. About half of CLL patients with clonal chromosomal abnormalities are the carriers of one abnormality while there are at least two

genetic abnormalities in another half of the patients. The use of FISH technique allows for clarifying the frequencies of the most prevalent chromosomal aberrations in CLL. The most common are 13q14 deletion, trisomy 12 and 11q22-q23 deletion while 6q21 and 7p13 deletions are less prevalent [12, 13]. It is important that the most common 13q14 deletion involves the region covering two micro RNA genes, mir15 and mir16 [19]. These micro RNA may be important for regulating the functions of genes which may have relevance to cancer in general and CLL in particular [20]. The above mentioned micro RNA genes have been shown to be deleted or down-regulated in most cases of CLL [21]. It seems that this deletion confers a selective advantage possibly predisposing B-cell clones to undergo additional mutations via regulatory pathways involving key oncogenes [22]. Since mir15 and mir16 normally interact with BCL6, their absence in CLL may be important for preventing apoptosis.

In some CLL patients, trisomy 12 may be detected in 25–72% of malignant cells [16, 17]. At present, the genes of chromosome 12 that may facilitate leukemic transformation upon increase in copy number have not been identified. The cases of CLL with the partial trisomy 12 limited by the long arm seem to be useful clue in identifying such genes since this chromosomal region contains several genes coding for growth factors which may well be the putative genes involved in the development of CLL [16, 17]. It is also of high importance that 12q22 region contains *CLLH1* gene that is the first gene recognized as CLL-specific.

11q22-q23 deletion is a characteristic feature of peculiar CLL variant [23, 24]. The detailed mapping of 11q22.3-q23.1 region revealed the minimal deletion area containing *NPAT*, *CUL5*, *PPP2RIB*, *DDXP* genes known to be involved in controlling cell cycle and apoptosis. The same region contains *ATM* gene considered as caretaker of genome and playing a role in DNA-damage recognition and signaling. *ATM* gene is responsible for inherited autosomal recessive disorder ataxia teleangiectasia considered as a marker for cancer predisposition [25] with neoplasms of the lymphoid system being predominant [26]. When *ATM* mutation affects both *ATM* alleles, a risk of leukemia is approximately 70 times higher than in general population [27]. The presence of somatic mutations of *ATM* gene in the cells of CLL patients suggests their possible role in CLL pathogenesis.

The first report of *ATM* involvement in sporadic B-CLL was published in 1999 [28]. Several other studies reported similar evidence for *ATM* role in CLL [29, 30]. 50% of ATM mutations affect phosphatidylinositol 3-kinase domain of ATM protein which is highly conserved among ATM-related proteins and crucial for protein kinase activity of ATM [25].

Sometimes, 17p13 deletion is detected in leukemic cells of CLL patients. This is the region wherein p53 gene is localized. The role of p53 for apoptosis induction in cells in response to their damage is well known. In this context, it is important that 10–20% cases of CLL with resistance to therapy and unfavorable prognosis demonstrate the abnormalities of p53 [23, 24].

6q deletion in CLL is considered as secondary one being associated with other chromosomal aberrations.

Recently, the epigenetic alterations including DNA methylation patterns have been also considered in the pathogenesis of CLL [31, 32].

To sum up, the current understanding of the pathogenesis of CLL has challenged the previous views on the absence of association between mature B-cell neoplasms and radiation exposure. Only the precise diagnosis of hematopoietic malignancies combining with large-scale epidemiological studies with careful dose assessment and long-term follow up may represent the basis for resolving the question whether CLL may be radiogenic as well.

## REFERENCES

1. **Boice JD.** Leakaemia, Chernobyl and epidemiology. J Radiat Prot 1997; **17**: 129–33.

2. **Howe GR.** Leukemia following the Chernobyl accident. Health Phys 2007; **93**: 512–5.

3. **Gluzman DF, Nadgornaya V, Machilo V, *et al.*** Malignant diseases of hematopoietic and lymphoid tissues in Chernobyl clean-up workers. Hematol J 2005; **5**: 565–71.

4. WHO classification of tumours of haematopoietic and lymphoid tissues. Ed. by Swerdlow SH, Campo E, Harris NL et al. Lyon, IARC 2008. 439 p.

5. **Ivanov VK, Tsyb AF, Gorsky AI, *et al.*** Elevated leukemia rates in Chernobyl accident liquidators. BMJ, Rapid Responses, April 15, 2003 (www.bmj.bmjjournals.com/cgi/eletters/319/7203/145/a).

6. **Kesminiene A, Evrard AS, Ivanov VK, *et al.*** Risk of hematological malignancies among Chernobyl liquidators. Radiat Res 2008; **170**: 721–35.

7. **Romanenko AY, Finch SC, Hatch M, *et al.*** The Ukrainian-American study of leukemia and related disorders among Chornobyl cleanup workers from Ukraine: III. Radiation risks. Radiat Res 2008; **170**: 711–20.

8. **Kryachok I, Polyshchuk O, Dyagil I, *et al.*** Comparative analysis of CLL in persons who suffered after Chernobyl accident and in unexposed patients. Haematologica 2005; **90** (Suppl.): 454A.

9. **Finch SC, Hoshino T, Itoga T, *et al.*** Chronic lymphocytic leukemia in Hiroshima and Nagasaki, Japan. Blood 1969; **33**: 79–86.

10. **Preston DL, Kusumi S, Tomonaga M, *et al.*** Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950-1987. Radiat Res 1994; **137** (Suppl): S68–97.

11. **Richardson DB, Wing S, Schroeder J, *et al.*** Ionizing radiation and chronic lymphocytic leukemia. Environ Health Perspect 2005; **113**: 1–5.

12. **Linet MS, Schubauer-Berigan MK, Weisenburger DD, *et al.*** Chronic lymphocytic leukaemia: an overview of aetiology in light of recent developments in classification and pathogenesis. Br J Haematol 2007; **139**: 672–86.

13. **Vrijheid M, Cardis E, Blettner M, *et al.*** The 15-country collaborative study of cancer risk among radiation workers in the nuclear industry: design, epidemiological methods and descriptive results. Radiat Res 2007; **167**: 361–79.

14. **Hamblin TJ.** Have we been wrong about ionizing radiation and chronic lymphocytic leukemia? Leuk Res 2008; **32**: 523–5.

15. **Schubauer-Berigan MK, Daniels RD, *et al.*** Chronic lymphocytic leukaemia and radiation: findings among workers

at five US nuclear facilities and a review of the recent literature. Br J Haematol 2007; **139**: 799–808.

16. **Chiorazzi N, Rai KR, Ferrarini M.** Chronic lymphocytic leukemia. N Engl J Med 2005; **352**: 804–15.

17. **Dighiero G, Hamblin TJ.** Chronic lymphocytic leukaemia. Lancet 2008; **371**: 1017–29.

18. **Shim YK, Silver SR, Caporaso NE, *et al*.** B cells behaving badly. Br J Haematol 2007; **139**: 658–62.

19. **Calin GA, Dumitru CD, Shimizu M, *et al*.** Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 2002; **99**: 15524–9.

20. **Calin GA, Liu CG, Sevignani C, *et al*.** MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci USA 2004; **101**: 11755–60.

21. **Nicoloso MS, Kipps TJ, Croce CM, *et al* .** MicroRNAs in the pathogeny of chronic lymphocytic leukaemia. Br J Haematol 2007; **139**: 709–16.

22. **Caporaso N, Goldin L, Plass C, *et al*.** Chronic lymphocytic leukaemia genetics overview. Br J Haematol 2007; **139**: 630–4.

23. **Döhner H, Stilgenbauer S, Benner A, *et al*.** Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000; **343**: 1910–6.

24. **Stilgenbauer S, Lichter P, Döhner H.** Genetic features of B-cell chronic lymphocytic leukemia. Rev Clin Exp Hematol 2000; **4**: 48–72.

25. **Khanna KK.** Cancer risk and the ATM gene: a continuing debate. J Natl Cancer Inst 2000; **92**: 795–802.

26. **Taylor AM, Metcalfe JA, Thick J, *et al*.** Leukemia and lymphoma in ataxia telangiectasia. Blood 1996; **87**: 423–38.

27. **Morrell D, Cromartie E, Swift M.** Mortality and cancer incidence in 263 patients with ataxia-telangiectasia. J Natl Cancer Inst 1986; **77**: 89–92.

28. **Bullrich F, Rasio D, Kitada S, *et al*.** ATM mutations in B-cell chronic lymphocytic leukemia. Cancer Res 1999; **59**: 24–7.

29. **Schaffner C, Stilgenbauer S, Rappold GA, *et al*.** Somatic ATM mutations indicate a pathogenic role of ATM in B-cell chronic lymphocytic leukemia. Blood 1999; **94**: 748–53.

30. **Stankovic T, Weber P, Stewart G, *et al*.** Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukaemia. Lancet 1999; **353**: 26–9.

31. **Boultwood J, Wainscoat JS.** Gene silencing by DNA methylation in haematological malignancies. Br J Haematol 2007; **138**: 3–11.

32. **Plass C, Byrd JC, Raval A, *et al*.** Molecular profiling of chronic lymphocytic leukaemia: genetics meets epigenetics to identify predisposing genes. Br J Haematol 2007; **139**: 744–52.