We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

4

DNA Damage Response/Signaling and Genome (In)Stability as the New Reliable Biological Parameters Defining Clinical Feature of CLL

Jozo Delic[1], Jean-Brice Marteau[1], Karim Maloum[2], Florence Nguyen-Khac[2], Frédéric Davi[2], Zahia Azgui[2], Véronique Leblond[3], Jacques-Louis Binet[2], Sylvie Chevillard[1] and Hélène Merle-Béral

[1]Laboratoire de Cancérologie Expérimentale, Institut de Radiobiologie Cellulaire et Moléculaire, Commissariat à l'Energie Atomique et aux Energies Renouvelables (CEA),
[2]Services d'Hématologie Biologique, Groupe Hospitalier Pitié-Salpêtrière,
[3]Clinique, Groupe Hospitalier Pitié-Salpêtrièr,
France

1. Introduction

The commonest hematological malignancy chronic lymphocytic leukemia (CLL) is currently incurable with a high incidence of morbidity and mortality (Chiorazzi et al., 2005; Dighiero & Hamblin, 2008; Hallek & Pflug, 2011). Clinically, the disease is diagnosed in most cases accidentally as an indolent form of leukemia but subsequently it may turn rapidly into an aggressive form. Moreover, in a subset of patients, CLL is presented as high-risk progressive form at diagnosis. This heterogeneous clinical course of CLL relies on the variable expression of defined several biological factors which may affect susceptibility to apoptotic cell death upon treatment (Hamblin 2007; Kipps 2007;; Lanasa 2010; Caligaris-Cappio & Chiorazzi 2010; Zenz et al., 2011; Parker & Strout 2011; Fabris et al., 2011). Our understanding of the molecular alterations leading to the leukemogenesis of CLL, even if these appeared already complex, remains still far to be achieved. Current researches by performing new genomic approaches, allowed an identification of new genes recurrently mutated in CLL suggesting their oncogenic role of potential clinical relevance (Fabri et al., 2011; Puente et al., 2011). Two major biological features such as the usage of mutated or unmutated immunoglobulin heavy chain variable region genes (*IGHV*) and the number and the type of chromosomal aberrations, clearly distinguish distinct clinical patients' subgroups (Fais et al., 1998; Damle et al., 1999; Hamblin et al., 1999; Maloum et al., 2000; Zenz et al., 2007; Klein & Dalla-Favera, 2011). While the *IGHV* status may appear in some cases as a more complex and complicated prognostic marker (Ghiotto et al., 2011; Langerak et al., 2011), specific genomic aberrations appear as an accurate "drivers" of the disease and of its clinical characteristics (Zenz et al., 2011). In some high-risk CLL cases, there is an association between these two independent makers of poor prognosis such as the presence of 11q22 deletions in cells with unmutated *IGHV*.

Another biological hallmark of CLL cells, with an evident therapeutic impact, is the aberrantly increased B cell receptor (BCR) signaling. It consists of surface immunoglobulin associated with heterodimer CD79α and CD79β. This aberrant BCR signaling consequently activates the Src family protein tyrosine kinases Lyn and Syk which promotes an activation in cascade of downstream signaling pathways including phosphatidyl-3-kinase (PI3K, see below), which generates phosphatidylinositol-3-phosphate necessary for the activation of the kinase Akt. Simultaneously to PI3K activation, the phospholypase Cγ2 is also activated. This last enzyme is involved in protein kinase C (PKC) activation which is an essential cell surviving factor. Effectively, an activation of PKC leads to an activation of the transcriptional anti-apoptotic factor NF-κB (see later) and to activation of mitogen-activated kinases (MAPKs) such as MEK/ERK, JNK and p38 MAPK as well as mTorc1 inhibitor rapamycin and cyclin-dependent protein kinase. The final consequence of these cascades of events is an anti-apoptotic "attitude" of CLL cells that may present a major source of the identification of novel therapeutic targets (for review see Wickremasinghe et al., 2011 and references within).

Men are more frequently affected by an aggressive form of the disease and develop it at a younger age than women (Mauro et al., 1999; Cartwright et al., 2002). In addition, CLL cells in men more commonly display no mutations in *IGHV* genes that allow, according to gene expression profiling, putting in evidence that male patients may segregate in a distinct CLL subgroup (Haslinger et al., 2004). We have reported that the gene expression profiles may also be discriminating not only between apoptosis resistant and sensitive cells (Vallat et al., 2003), but also according to patients' gender (Marteau et al., 2011).

In addition to not yet fully defined defect in apoptotic death, the homeostatic balance or imbalance in a dynamic interplay between proliferation and cell death may underline the stable (indolent) or progressive forms of CLL, respectively (Messmer et al., 2005; Chiorazzi & Ferrarini, 2011). The mechanisms that induce a switch from indolent to more aggressive form of this malignancy remain unclear. Hence, clinical and biological heterogeneities may allow us to postulate two models of CLL cell origin; single- or multiple-cell origin model (Chiorazzi & Ferrarini, 2011). Although the microarray data suggested the same cell origin for two major subsets of CLL (i.e. CLLs with mutated and CLLs with unmutated *IGHV* genes, Klein et al., 2001; Rosenwald et al. 2001), according to B-cell receptors (BCRs) repertory and signaling capacity as well to the specific *IGHV* usages, a model of two-cell origin would be more appropriated to explain clonal cell expansion and thus an emergence of indolent and aggressive form of disease (Hamblin et al., 2000; Damle et al., 1999; Schroeder & Dighiero, 1994; Fais et al., 1998; Zupo et al., 1996; Lanham et al., 2003; Herve et al., 2005; Colombo et al., 2011). Both of these two models converge to an antigen-experienced lymphocyte(s) according to the membrane phenotype of CLL cells. Because of the possibility that CLL clones may develop and diversify its Ig receptor (with either mutated or unmutated *IGHV* genes), T-cell dependent, droved to the concept of the unique follicular marginal zone B cell origin. In spite of differences in poly- and auto-antigen-binding activities among CLL clones, the analyses of the amino acid sequences of B-cell receptor showed remarkably similarity in some but not all of these clones (Chiorazzi & Ferrarini, 2011), seeding thus a doubt of one-cell origin. However, if the normal B cell counterparts should absolutely be searched, we should consider also the arguments that human marginal zone B cell population is a separate population that develops and

diversifies Ig receptor outside T cell-dependent or –independent immune responses (Weill et al., 2009). In addition, considering the possibility of somatic diversification independent of antigen-driven responses and the existence of the subpopulation of circulating "memory" long-lived B cells harbouring a pre-diversified immunoglobulin repertoire in humans, then the concept of CLL cell origin may also radically differ from above hypotheses of two origin models (Weller et al., 2004; Weill & Reynaud, 2005; Weller et al., 2008). Alternatively, irrespectively to normal cellular counterparts, CLL cells may emerge from initially damaged cell in bone marrow which subsequently followed a development and immunoglobulin diversification according to the extend of its initial damage.

Although, the characterizations of several biological markers fit well with the appearance and/or maintenance of progressive disease, none of them are considered in a clinical decision regarding when and by applying which type of treatment the therapy should really start. The current front line therapies for CLL include drugs that directly or indirectly induce DNA damage which ultimately should result in apoptotic cell death.

2. Two classes of CLL cells according to their ability to activate or not DNA damage-induced apoptosis: Clinical relevance?

The aggressive form of disease resistant to front line treatment develops in approximately one third of patients who succumb rapidly due to the lack of effective therapies and/or a lack of prospective tools enabling the predicting treatment response including early relapse. Alkylating agents (i.e. chlorambucil) or purine nucleoside analogues such as fludarabine, mediate cell death of CLL cells through DNA damage, including double strand breaks (DSBs) and p53-dependent apoptosis (Rosenwald et al., 2004; Austen et al., 2007; Amrein et al, 2007; Döhner et al., 1995). Further, fludarabine treatment *in vivo* induces a gene expression response similar to that induced by the *in vitro* exposure of cells to ionizing irradiation (Rosenwald et al., 2004), suggesting the common mechanisms achievable by these two treatments. The loss of functional p53 or a defect in the ataxia telangiectasia protein (ATM) which acts upstream of p53, leads to a more rapid disease progression, is associated with resistance and shortened overall survival times as well as with an appearance of signs of disease complications (i.e. lymphadenopathy, Döhner et al. 1997).

We have reported that ~20% of patients harbor B cells resistant to DNA damage-induced apoptosis, irrespective of p53 status, while the remaining 80% of patients have p53wt-expressing cells sensitive to genotoxic agents (Figure 1). Although p53 deficiency (through point mutations or 17p13 deletions) defines poor disease outcome (Döhner et al., 1995; Grever et al., 2007; Catovsky et al., 2007; Mohr et al., 2011), we hypothesized that specific pathways independent of p53 and/or acting upstream of this tumor suppressor could operate in resistance mechanisms of CLL cells.

This last observation led us to perform a retrospective study to definitely establish: **i**) the relevance of whether an inherent resistance to DNA damage induced-apoptosis underlines poor disease outcome; **ii**) which dynamic biological alterations shepherd otherwise sensitive cells to become resistant and **iii**) whether these biological features of CLL cells should be considered by clinicians in a decision to apply or not the front line treatment (including DNA damaging drugs such as alkylants and base analogues) for patients harboring these cells.

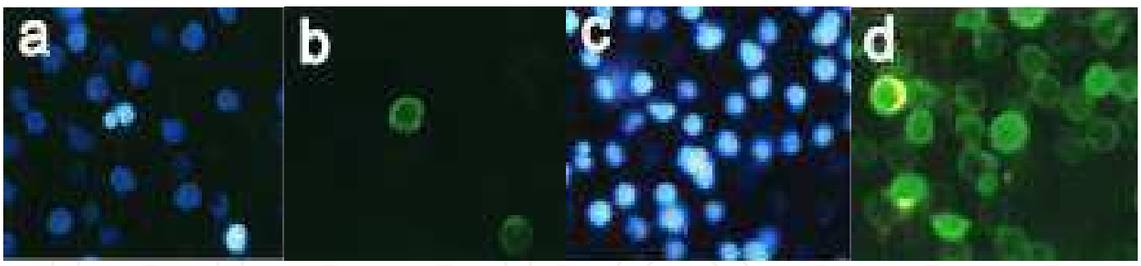


Fig. 1. Fluorescent labeling of apoptotic CLL cells.

Apoptotic cells are revealed by double staining of chromatin-DNA by Hoechst 33342 (a, c) and of phosphatydyl-serine externalization on membrane surface by annexin V-FITC (b, d). Resistant cells (a, b) are clearly distinguished from sensitive cells (c, d) by bright Hoechst staining (a) of annexin V positive cells (b).

A cohort of 308 CLL cases was examined for cell sensitivity/resistance to DNA damage-induced apoptosis and this biological parameter was correlated to the presence/expression of at least another bad prognostic factor described in literature. Together, these biological factors were correlated to the clinical features of each patient covering up to 25 years period.

As shown in Figure 2, 18,8% of CLL cell samples were resistant to DNA damage-induced apoptosis *in vitro* while remaining 82,2% were sensitive. Consistent with data in literature, in this cohort of CLL patients, men appear to be affected more frequently than women. Of note, this gender-dependent ratio appears also to be conserved for the subset of patients' samples resistant to DNA damage-induced apoptosis.

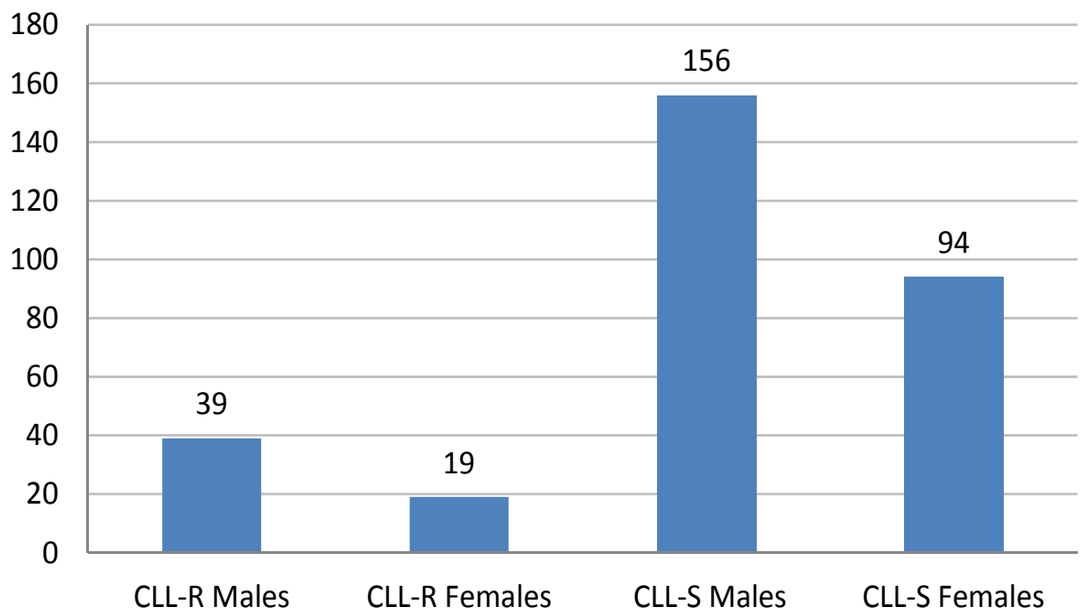
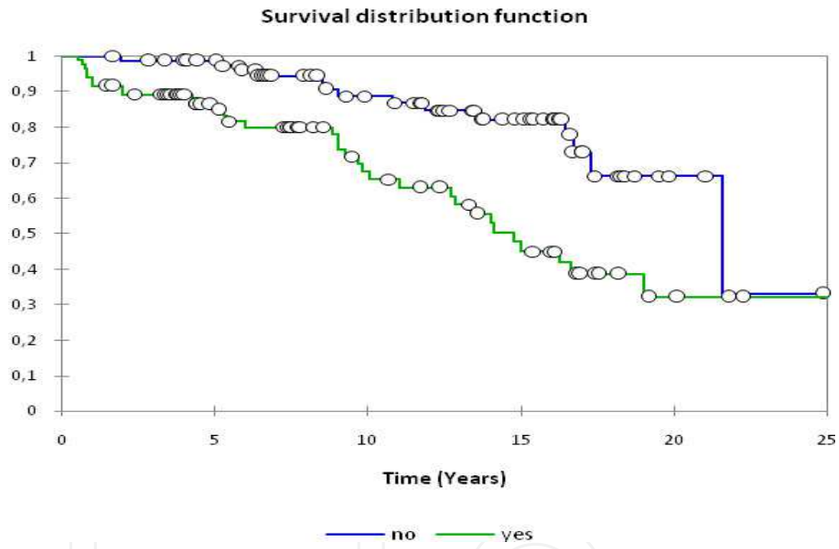


Fig. 2. Incidence of CLL cells resistant (CLL-R) or sensitive (CLL-S) to DNA damage-induced apoptosis *in vitro* in a cohort of 308 patients' samples according to patients' gender.

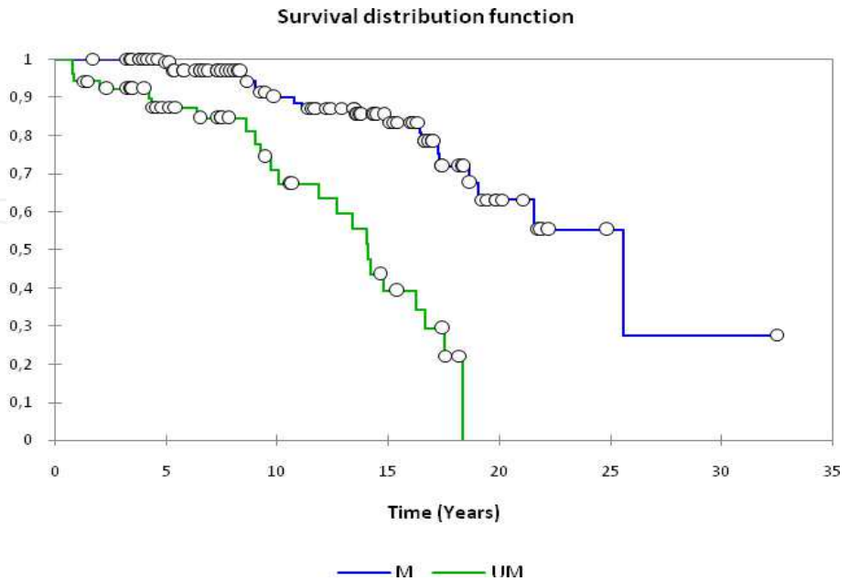
Percentage of apoptotic cells were determined by fluorescent labeling and microscopic counting at 24h of culture of CLL cells exposed *in vitro* to genotoxic stress (10Gy of γ-rays or 1µM of Neocarcinostatine). Y-axis: number of patients' samples.

After these first observations, our goal was to determine whether and how the presence of at least one biological factor (such as Zap70 and CD38 positivity, elevated level of sCD23, deletions/mutations of *TP53* and *ATM* and/or the presence of other multiple cytogenetic aberrations or complex karyotype), considered to be associated with poor disease outcome ("Bad prognostic factors" in graph 1), the *IGHV* status (graph 2), and the resistance or sensitivity to DNA damage-induced apoptosis (graph 3a, 3b and 3c) may influence overall time survival by comparing the survival curves of two well-defined groups on the basis of these phenotypes from this cohort of CLL patients.



Graph 1. Bad prognostic factors influence on survival time.

50 patients with at least one bad prognostic factor (**yes**) out of 84 have been censured (survival) *vs*. 66 of 80 without bad prognostic factor (**no**). We confirmed that the median survival time was significantly lower for the group of patients harboring malignant B cells with "unfavorable phenotype" than for its counterpart (15.200 ± 1.208 years vs. 18.529 ± 0.757 years). The difference between these two survivor functions is very significant (Log-rank, Wilcoxon and Tarone-ware -tests $p < 0.001$). Thus, the comparison of the two survival curves allows us to confirm that in our cohort, patients with one or more bad prognostic factors have significantly lower survival time than patients without the presence of these factors. These results are in agreement with other studies reported in literature.

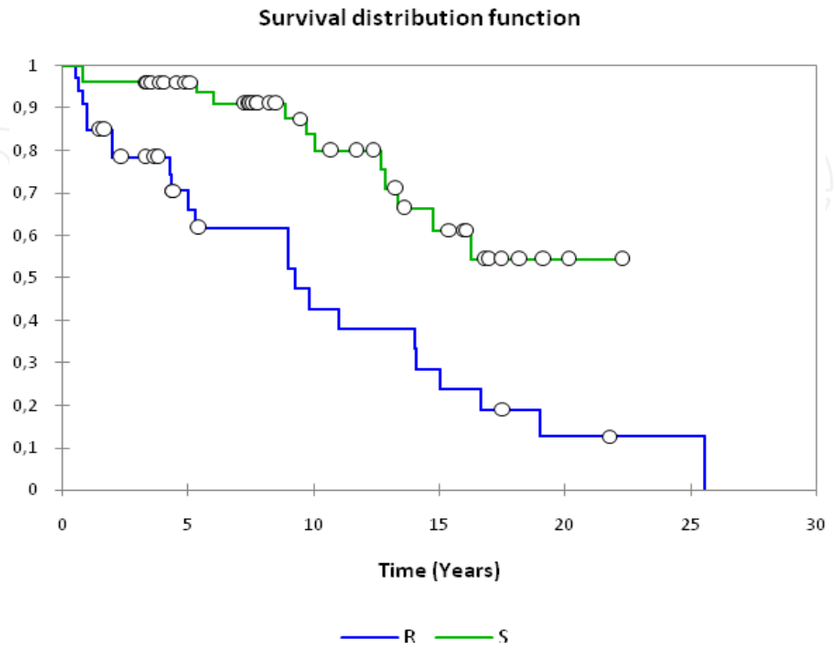


Graph 2. The status of variable regions of heavy chains of immunoglobulin genes (*IGHV*) influence on CLL patients' survival time.

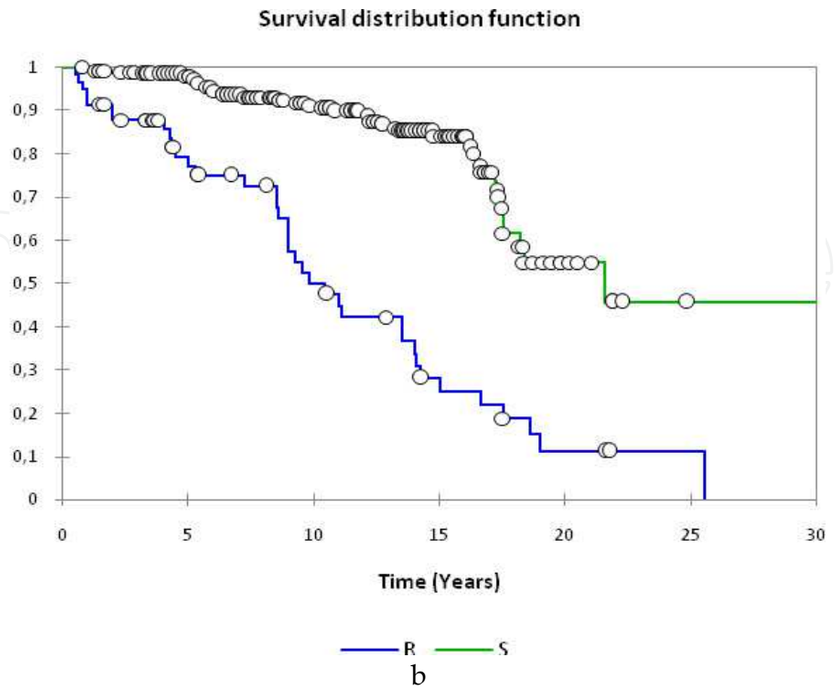
In patients harboring mutated (M) *IGHV* genes, 88 patients from 108 have been censured (survival) and 29 of 52 in the unmutated (UM) group. We notice that the median survival time is a lot lower for the *IGHV* unmutated group than for the *IGHV* mutated group (12.852 ± 0.879 years vs. 21.033 ± 0.876 years). The difference between the two survivor functions is very significant (Log-rank, Wilcoxon and Tarone-ware -tests $p < 0.0001$). The comparison of the two survival curves allows us to conclude and to confirm that the *IGHV* mutated status impacts significantly positively the survival time of patients.

We next addressed the question whether the resistance to DNA damage -induced apoptosis may be a new parameter that also may influence on overall survival of CLL patients and if yes, whether this influence was concomitant to that observed with other bad prognostic factors. For this purpose we designed three comparisons. Two groups of patients' cell samples were selected on the knowledge of their status according to the sensitivity or resistance to DNA damage-induced apoptosis (graph 3a) and then splitted according to the lack (graph 3b) or the presence (graph 3c) of at least one bad prognostic factor (i.e. Zap70, CD38 and sCD23 positivity, UM *IGHV*, *ATM* or *TP53* mutations or deletions or other cytogenetic abnormalities or aberrant karyotype).

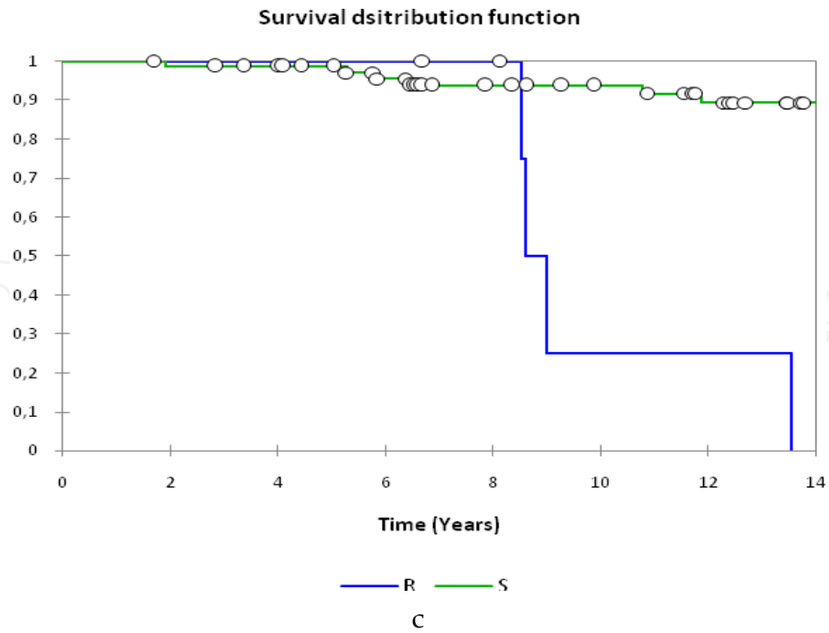
In the resistant sub-group, 21 patients out of 58 have been censured (survival) and 203 out of 245 in the sensitive group. We noticed that the median survival time was significantly lower for the resistant group of patients (R) than for the sensitive (S) group of patients (11.562 ± 1.097 years vs. 19.773 ± 0.672 years). The difference between the two survivor functions is very significant (Log-rank, Wilcoxon and Tarone-ware -tests $p < 0.0001$). The comparison of the two survival curves allows us to conclude that the resistance to DNA damage-induced apoptosis *in vitro* negatively impacts in a very significant manner on the survival time of CLL patients.


Survival distribution function
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
5
10
15
20
25
30
Time (Years)
R
S

a


Survival distribution function
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
5
10
15
20
25
30
Time (Years)
R
S

b



c

Graph 3. a) Survival time in sub-group of patients harboring CLL cells resistant (R) to DNA damage-induced apoptosis vs. sub-group harboring sensitive (S) cells. b) Influence of time survival between patients harboring resistant (R) or sensitive (S) CLL cells with at least one bad pronostic factor expression. c) Influence of time survival between R and S patients without any bad pronostic factor.

When patients have at least one bad pronostic factor (**graph 3b**), the same reduction of overall survival time was observed for the resistant group (11 censored out of 33) i.e. 10.593 ± 1.611 years in contrast to a higher survival time for the sensitive patients (39 censored out of 51) 15.946 ± 1.014 years (Log-rank, Wilcoxon and Tarone-ware –tests, $p < 0.0001$).

In the group of CLL cell samples resistant to radiation induced apoptosis, 2 patients out of 6 have been censured (survival) and 64 of 74 in the sensitive group. We noticed that the median survival time is a much lower for the resistant group (R) group than for the sensitive (S) group (9.92 ± 1.208 years vs. 19.773 ± 0.672 years). The difference between the two survivor functions is very significant (Log-rank, Wilcoxon and Tarone-ware - tests $p < 0.0001$). The comparison of the two survival curves allows us to conclude that the resistance to DNA damage-induced apoptosis negatively impacts the patients' survival time and, despite of the small number of patients without any bad pronostic factor, resistance to DNA damage-induced apoptosis can clearly be considered as a unique independent prognostic factor defining a subset of CLL with poor clinical outcome (**graph 3c**).

In our cohort seven patients (2,2%) clinically evolved during the study and their cells changed the apoptotic status. Effectively, initially sensitive cells became resistant to DNA damage-induced apoptosis *in vitro*. In five patients these changes occurred following front-line treatment because they expressed at least another bad prognostic factor (i.e. UM status of *IGHV*, delTP53, $CD38^+$ or $Zap70^+$ or presented two chromosomal aberrations). Disease evolved in two other patients who did not received chemotherapy and who did not

expressed another bad prognostic factor. Of note, concomitant to this change of the sensitivity toward resistance to activate apoptotic death pathway, increased activity of DNA repair through non-homologous end-joining as well as a shortening of telomeric sequences (see next two paragraphs), have been observed in these evolving cases. These observations let us to speculate that both front-line treatment, when inefficient, may contribute to an emergence of resistant cells and that in patients without an expression of another biological bad prognostic factor, the resistance to DNA damage-induced apoptosis may be a new independent bad prognostic factor for a subset of CLL patients.

Together, data clearly demonstrate that the resistance to DNA damage-induced apoptosis *in vitro* is a parameter reliable of resistant form of disease and that the switching from sensitive to resistant cell status *in vitro* is concomitant to disease progression from indolent to aggressive form. Acquisition of resistant phenotype, critical telomere shortening and NHEJ defect are proposed as events preceding disease switching according to other established parameters (i.e. TP53 and ATM status, chromosomal aberrations, $Zap70^+$, $CD38^+$).

Defining the molecular origin of the underlying mechanisms of cell resistance to DNA damage-induced apoptosis *in vitro* should open new perspectives of clinical use in CLL.

3. Biological features of CLL cells resistant to DNA damage-induced apoptosis

3.1 DNA repair defect?

Our initial observation was that one CLL patient displayed malignant cells sensitive to ionizing irradiation-induced apoptosis *in vitro* while cells from a second patient were completely resistant. These first two cases were confirmed and validated in a large cohort of CLL samples thus allowing us to propose that CLL could be stratified into at least two new subgroups: resistant and sensitive groups. We next asked if this resistance to activate apoptotic death pathway could be due to DNA double strand breaks (DSBs) or to other effects induced by γ-rays. To answer this question we addressed comet assay to measure DNA damage directly in irradiated cells. This assay, performed in alkaline experimental conditions, allows assessing of resting single and double strand breaks directly in interphase nuclei. Surprisingly, an excess of resting DNA damage was established in sensitive rather than in resistant cells 20 min after radiation exposure (Blaise et al., 2001), emphasizing that resistant cells removed DNA damage more rapidly than sensitive cells. To further address DNA damage causality in apoptotic response, we next treated cells with radiomimetic drugs such as neocarcinostatin which is known to specifically induce DNA DSBs without other side effects in cell, or drugs currently used in cancer therapy (topoisomerase I and II inhibitors or fludarabine), also able to induce indirectly DSBs. In this way, we tested whether cell resistance to γ-rays-induced apoptosis would be validated by the same resistance induced by these drugs. Effectively, we reported (Deriano et al., 2005), that these cells were resistant to all tested DNA damaging agents concluding that the resistance to apoptosis should underscore a defect in DNA damage repair/signaling. DNA repair has already been postulated as the mechanism causing drug resistance in CLL (Panasci et al., 2001; rev. Guipaud et al., 2003). First observation of a defective nucleotide excision repair (NER, as the main pathway employed in modified DNA bases clearance after UV exposure or alkylating agent treatments during cancer therapy), occurring in CLL was first reported

in 1972 (Huang et al., 1972). Alkylation and interstrand cross-links produced by nitrogen mustards (i.e. chlorambucil) may activate recombinational DNA repair in CLL cells (Bramson et al., 1995). Non-homologous end-joining (NHEJ) was first suspected to play a role in CLL drug resistance through an increased activity of DNA-PK complex (including both, the DNA end-binding activity of heterodimer Ku70/Ku80 and the phosphorylation activity of DNA-PKcs; Muller and Salles, 1997). In consequence, use of wortmanin, an inhibitor of PI3-Kinases along with DNA-PKcs (which is PI3-K like kinase), was able to potentialize cytotoxic effect of chlorambucil in CLL cells (Christodoulopoulos et al., 1998). Also, a DNA-PKcs specific inhibitor Nu7441 combined with drugs inducing DNA DSBs has been pointed as a potential therapy for high risk CLL (Elliott et al., 2011). After genotoxic stress and first cell division, structural chromosomal aberrations (dicentric, acentric or ring chromosomes) occurred more frequently in resistant than in sensitive CLL cells (Blaise et al., 2001), suggesting an accelerated but certainly unfaithful DNA repair. We addressed an *in vitro* assay enabling us to measure the overall activity and fidelity of non-homologous end-joining (NHEJ) DNA repair and the activities of two essential components of NHEJ heterodimer Ku70/Ku80 and DNA-PKcs. Accelerated DNA repair, an increased activity of Ku DNA end-binding as well as an increased kinase activity of DNA-PKcs were observed in resistant cells (Deriano et al., 2005). Moreover, this upregulation of NHEJ was found to be error-prone and thus potentially mutagenic since large DNA deletions occurred at sites of repair (Deriano et al., 2006). The potential impact of such resistance upon the onset of malignancy is likely to be increased by the resulting block on apoptosis induction which may in consequence contribute to the emergence of additional resistant clones from a proliferative pool of mutant cells. Recent reports have shown that drug-induced DSBs in cells in culture *in vitro* (such as CsA or fludarabine) are repaired exclusively by NHEJ (O'Driscol & Jeggo 2009; De Campos-Nebel et al., 2009) which is the main cell cycle-independent repair pathway for this type of DNA damage in mammalian cells (Lieber 2008; Delacote and Lopez, 2008; Mari et al., 2006). According to protein components needed to achieve repair activity, two NHEJ pathways have been found operating in cells (for rev. see Mladenov and Iliakis, 2011); classical NHEJ depending on the activities of at least 7 identified factors (i.e. Ku70, Ku80, DNA-PKcs, Arthemis, XRCC4, Cernunos (also called XRCC4-like factor, XLF) and Ligase IV) and alternative NHEJ which depends on MRN trimmer complex but its repair activity is, obviously, independent of proteins needed for classical pathway (Corneo et al., 2007, Yan et al., 2007; Deriano et al., 2009; Lee-Theilen et al., 2011). This alternative NHEJ was demonstrated to be error-prone and consequently, mutagenic since it uses microhomology pairing and thus nucleotides loss. Whether this pathway may be really involved in initiation of malignant process in humans remain still to be elucidated. An upregulated classical NHEJ was reported to take place in Bloom's syndrome exhibiting high chromosomal instability and cancer susceptibility as well as in myeloid leukemia harboring multiple chromosomal aberrations (Rasool et al., 2003). Defect, also in classical NHEJ, due to ligase IV dysfunction, has been associated with the appearance of radiosensitive leukemia in patients exhibiting developmental delay and immunodeficiency (Riballo et al., 1999; O'Driscoll et al., 2001). ATM deficiency, occurring mainly through point mutations or 11q22 deletions, has been observed in high risk CLLs (Stankovic et al., 1999; Austen et al., 2005). This deficiency causes a defective DNA repair through homologous recombination and consequently, resistance to therapy. One of new concepts to overcome cancer resistance consists in a conversion of one form of DNA damage

into another form, that in a cell harboring defective gene involved in DNA damage response, cannot be repaired and inevitably leads to cell death (Helleday et al., 2008). Using this concept, inhibitors of poly (ADP-ribose) polymerase 1 (PARP), a component of the DNA single strand break (SSB) repair complex, may convert unrepaired SSB lesions of DNA into DSBs during DNA replication that require activation of HR repair proteins (i.e. BRCA1/2) for their resolution. If tumor cells defective in *BRCA1/2* were treated with PARP1 inhibitor, they accumulate extensive DNA DSBs and underwent cell death (Bryant et al., 2005; Farmer et al., 2005). Stankovic's group investigated whether this synthetic lethality resulting from inhibition of PARP would also be applicable to *ATM* mutant lymphoid tumors and consequently, may result in their specific killing. They demonstrated a differential *in vitro* and *in vivo* sensitivity of primary and transformed *ATM* mutant CLL and MCL tumor cells to a new clinically tested PARP inhibitor (olaparib) which may be a new promising therapy in high risk CLLs (Weston et al., 2010). Considering a functional overlapping between ATM and XLF (Cernunos) involved in classical NHEJ (Zha et al., 2011), then this strategy would be emphasized from homologous recombination to NHEJ in parallel. Another combined strategy to avoid the fludarabine-resistance of CLL cells uses simultaneously fludarabine and oxaliplatin treatment. In this case, synergistic killing of malignant cells was due to an inhibition of DNA repair by fludarabine that was incorporated into DNA at sites of nucleotide excision repair initiated by oxaliplatin-DNA adducts (Zecevic et al., 2011).

In conclusion, there are now several lines of evidences that aggressive form of CLL displays molecular characteristics of DNA repair defect (i.e. caused by p53 or ATM deficiency or by an upregulation of NHEJ). This new biological feature severely affects overall survival and therapy issues. Taking into account that defect in DNA damage repair and/or signaling contribute to the appearance of genome instability, the results obtained in CLL cells highly suggest that the defect in NHEJ should be a new reliable biological parameter critically impairing efficacy of DNA damaging agent therapies for this subgroup of patients. In consequence, particularly because of a possible mutagenic effect of this type of drugs, the front line treatment should be proscribed for these patients in which malignant cells apparently adhere to the creed of "better wrong than dead" with a deregulated NHEJ that help their illegitimate survival.

3.2 Telomere dysfunction

Telomeres are the capping structures of chromosome ends composed of repeated DNA sequences (~10kb in somatic cells) and a specific complex of associated proteins. Telomeric DNA contain two main domains: a double strand region composed of tandem TTAGGG repeats and a single strand G-rich 3' overhang (Henderson & Blackburn, 1989). A change in telomere function is one of the mechanisms developed by malignant cells enabling the evolution and maintenance of cancers (Blasco et al., 1997; Stewart & Weinberg, 2006; Cao et al., 2008; Ségal-Bendirdjian & Gilson 2008). The length of telomeric DNA is regulated during cell cycle and couples stress response to cell division and genome integrity (Blasco, 2007; Lansdorp, 2008; Aubert & Lansdorp, 2008). The regulation of telomere length results from the action of telomere lengthening mechanisms, such as the telomerase complex (hTERT, hTR and dyskerin), and of telomere shortening mechanisms, such as replication and recombination. Telomerase activity is regulated in *cis* by the shelterin hexa-protein complex (TRF1, TRF2, hRAP1, POT1, TIN2 and TPP1). Many other proteins involved in DNA

replication and repair are also associated in telomeric structure and function (De Lange 2005; Longhese, 2008; Horard & Gilson, 2008). The telomeric nucleoprotein complex ensures chromosome stability and protection. The shortening of telomere sequences upon cell divisions in most somatic cells results in irreversible cell growth arrest called senescence or in apoptosis. Telomere erosion may be critical in tumor suppression as it impairs cell proliferation. To circumvent this, cancer cells have developed molecular strategies to maintain their telomere length by reactivating expression and/or activity of telomerase or by alternative telomere lengthening (ALT) through homologous recombination (Stewart & Weinberg, 2006; Blasco, 2007; Collado et al., 2007; Gilson & Geli, 2007; Lansdorp, 2008). In CLL, telomeric DNA may shorten in a subset of patients in Binet B or C stage compared to patients in A stage. This correlation appears inversed for telomerase activity which increases in B and C stage and decreases for A stage derived cells (Bechter et al., 1998). CLL cells exhibiting unmutated IgVH genes display also short telomeres suggesting both, an increased proliferation history of these resistant cells (Damle et al., 2004) and short telomeres association with the disease of poor prognosis. Effectively, this association of short telomeric DNA sequences further fits with genetic complexity, high-risk genomic aberrations, and short survival in CLL (Roos et al., 2008).

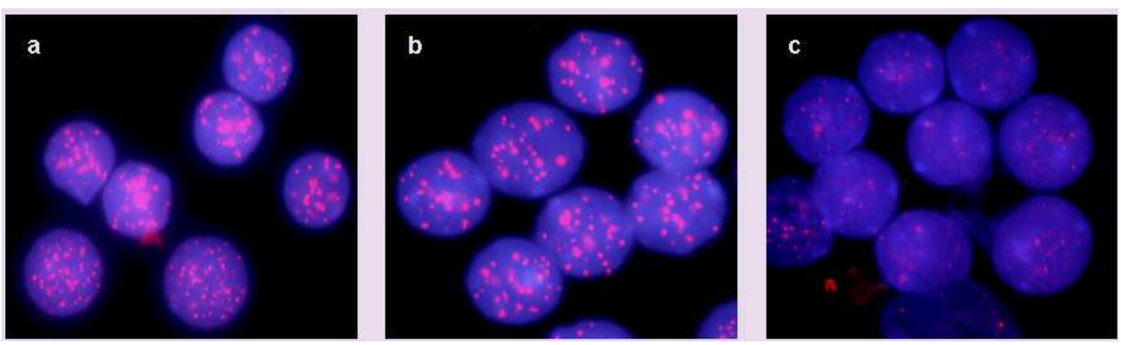


Fig. 3. Telomere labelling in interphase nuclei of CLL cells

Fluorescent *in situ* hybridization with peptide nucleic acid probe (FISH-PNA) was applied to reveal telomeric DNA sequences in interphase nuclei. Interphase nuclei (blue labelling by Hoechst H33342) of $CD19^+$ B cells from healthy donor (**a**), sensitive (**b**) and resistant (**c**) CLL cell samples. Telomere-specific (C3TA2)3 –PNA probe Cy3-labelled (red fluorescence) reveals subtle scattered labelling throughout the nucleus of telomeres in resistant cells (**c**) while sensitive cells (**b**) and normal B cells display very similar brighter and larger spots which may be indicative of longer telomeres and/or of telomeric associations.

Simple FISH-PNA labelling of telomeres in interphase nuclei (Figure 3) show very similar pattern in sensitive CLL cells and in B cells from healthy donors. This labelling in resistant cells was organised in more weaker and discreet spreader spots suggesting that in these cells telomeres are shortened. Effectively, by using Southern blot analysis, we found that CLL cells resistant to DNA damage-induced apoptosis have the mean telomere length below 4 Kb, whereas in sensitive cells telomeric sequences are longer than 6 Kb. Moreover, G-rich 3' single stranded overhangs that stabilize telomeric structure were found also shortened in resistant cells (Brugat et al., 2010a and 2010b). By chromatin immunoprecipitation assay we further showed that the telomeres of resistant cells are associated with increased levels of Ku70, an essential component of classical NHEJ, as

well as with histone H3 lysine 9 trimethylation (3met-H3K9), a hallmark of heterochromatic structures. No difference was observed in the expression of the shelterin components or the hTERT protein complex between resistant and sensitive cell. Together, these results define alterations in telomere structure in resistant forms of CLL that may result from aberrant epigenetic regulation. This altered telomeric structure in resistant cells may confer their recognition as DNA damage since both, DSBs signaling and repair proteins colocalize at these short telomeres (Brugat et al., 2010b). Effectively, in human cells, 5 Kb is considered to be of a critical length since it may induce the DNA damage response and cellular senescence (d'Adda di Fagagna et al., 2003). Thus, we evaluated whether altered telomeres in resistant cells could be revealed by assaying classical DNA damage double-strand break (DSB) signaling and testing for the induction of telomere dysfunction-induced foci (TIF). This hypothesis has been supported by our previous results showing that resistant cells were able to upregulate non-homologous end-joining and in particular, by evidencing an upregulation of the activity of Ku heterodimer DNA end-binding (Deriano et al., 2005). Both, Ku80 and Ku70 have been identified in telomeric complexes, thus emphasizing the deregulation of these factors also at the telomeres in resistant cells. We showed that resistant cells formed TIFs and displayed an increased telomeric concentration of two NHEJ factors Ku70 and phospho-S2056-DNA-PKcs (marker of DSBs). Moreover, these cells display telomeric deletions at one or two chromatids. It is noteworthy that the appearance of these telomeric anomalies in resistant cells is concomitant with the appearance of the multiple chromosomal aberrations and complex karyotypes which are the markers of a poor disease outcome. Thus, in addition to previously identified chromosomal aberrations (i.e. del13q14; del17p13; del11q22; del6q or trisomy12), telomeric deletions appear as new type of chromosomal aberration occurring in cells from patient having aggressive form of disease. It may be speculated that these deletions coincided with extremely short telomeres revealed by a single-molecule telomere length (STELA) method (Lin et al., 2010). This method allows the measurement of individual telomeres without bias in the detection of short telomeres unlike the determination of telomere length by conventional analysis by telomere restriction fragment (Baird et al., 2003). The authors from same group suggested that this critical telomere shortening could results in telomere fusions contributing to disease progression since their frequency increased with advanced disease. When fusion sequences were analyzed, then limited numbers of repeats, subtelomeric deletion, and microhomology (alternative NHEJ), were observed (Lin et al. 2010). Telomeric DNA damage signaling as detected by a recruitment of factors involved in DNA damage, at an early stage of CLL may also be correlated with a down-regulation of two protecting proteins of shelterin complex (TPP1 and TIN2), rather than with shortening of telomeric sequences (Augereau et al., 2011).

Together, it is now widely accepted that mean telomere length may be considered as a reliable prognostic marker for CLL. Moreover, telomere dysfunction appears to precede and/or to evolve in parallel with setting of progressive form of disease suggesting telomere shortening mechanisms to be involved in CLL leukemogenesis (Lin et al., 2010). In agreement with this, in our CLL cohort, 5% of sensitive cases developed the resistance to DNA damage-induced apoptosis *in vitro* and this resistance appeared simultaneously with clinical and biological phenomena such as disease progression, telomeric dysfunction (particularly characterized by TIF signaling and telomere shortening) and an acquisition of second chromosomal aberration (Brugat et al., 2011).

3.3 Epigenetic control and CLL cells resistance to DNA damage-induced apoptosis

The proper gene expression is subjected to epigenetic control through enzymatic modifications of chromatin at both DNA and histone levels. Thus, in addition to DNA code as genetic information, epigenetic modifications are another layer of heritable information controlling gene expression. The stepwise accumulation of genetic alterations and prominent epigenetic abnormalities are tightly coordinated in cancer initiation and maintenance. Effectively, DNA methylation of CpG islands in the promoter regions of specific cancer-relevant genes, which often occur concomitantly with covalent modifications of histones and/or with the appearance of their variants, establishes a direct epigenetic basis for cell transformation. In consequence, cancer cells display genetic lesions (mutations, deletions and translocations) and significant epigenetic changes that convey heritable gene expression profiles critical for tumorigenesis (Ting et al., 2006). With this regard, in addition to transcriptional changes defined by the microarray approach, it has become evident that epigenetic alterations should be integrated into approaches of genome activity in CLL cells. Indeed, molecular profiling in CLL has allowed the identification of new genes for which the expression is dependent on CpG island methylation (Plass et al., 2007). In parallel, global DNA hypomethylation have been reported to take place in CLL (Wahlfors et al., 1992). The evidences of down-regulation of the death-associated protein kinase 1 (DAPK1, involved in apoptotic cell death regulation) gene through promoter CpG methylation in CLL indicate that both genetic and epigenetic factors may define both the sporadic and inherited forms of this disease (Raval et al., 2007).

Thus, altered structural changes of telomeric chromatin regions due to an increased heterochromatinisation (i.e. through 3methylation of histone3-lysine9, Brugat et al., 2010; 2011)), appear to not be restricted to chromosome termini but rather may spread throughout euchromatin to. Effectively, non-coding repetitive DNA sequences (such as Alu sequences, long interspersed nuclear element-1 and satelit-α sequences), have been demonstrated as under-methylated and to be associated with 17p13 deletions in CLL. Moreover, a lower level of satelit-α sequence methylation has been proposed as a new independent prognostic marker associated with shorter therapy-free survival (Fabris et al., 2011).

By using microarray approach (Affymetrix technology), we have established that resistant cells display a specific subset of deregulated genes (Vallat et al., 2003). Intriguingly, we also showed that in male CLL cells resistant to DNA damage-induced apoptosis the global gene expression was down-regulated when compared to sensitive cells, whereas this was not the case in cells derived from female patients. This gene down-regulation was found to be associated with an overall gain of heterochromatin hallmarks (i.e. increase in trimethylated histone 3 lysine 9 (3met-H3K9) and 5-methylcytidine). This approach allowed us to identify *RELB* gene as a discriminatory candidate gene repressed in the male and upregulated in the female resistant cells. Epigenetic control was demonstrated to be involved in *RELB* silencing in male cells through an increase in 3met-H3K9 (Marteau et al., 2010). This finding may be of particular interest because RelB is one of five essential members of NF-κB family of transcriptional factors involved in cellular response to stress and inflammation as well as in cancer development and progression (Hayden and Ghosh, 2008). Another NF-κB member, RelA has already been involved in CLL aggressiveness (Hewamana et al., 2008) suggesting that an imbalance in both canonical and alternative NF-κB pathways may contribute to CLL progression. Considering that NF-κB pathway regulates both apoptosis (after its activation

by exogenous stress by reactive oxygen species or DNA damage or by death receptor activation) and early and late B cell differentiation (Mills et al., 2007; Goldmit et al., 2005), then an imbalance in expression of each member of this pathway should be crucial not only in cell response to therapy but also in course of early steps of cell transformation and leukemogenesis of CLL. In this regard, epigenetically up-regulated Aiolos, a member of Ikaros family of transcriptional factors involved in lymphocyte differentiation and lineage specification (rev. Mandel and Grosschedl, 2010; John and Ward, 2011), whose transcriptional regulation is under NF-κB control, may contribute to the resistance of CLL cells to activate apoptotic cell death (Billot et al. 2011).

More recently, emerging evidence imply epigenetic deregulation of microRNAs in carcinogenesis including CLL (Nicoloso et al., 2007). microRNAs are small (22nt) noncoding RNAs that regulate expression of downstream targets by messenger RNA (mRNA) destabilization and translational inhibition resulting in a specific profiling of gene expression. Thus, in cell, a large number of mRNAs are targeted each by multiple miRNAs. Also, a single miRNA can target several hundreds of mRNAs, making microRNAs extremely powerful and dynamic strategy of control of vital cell functions (rev. Subramanyam et al.,2011). Reports in cancer biology underlined general down-regulation of microRNAs. In CLL, microRNAs expression also profile disease prognostic and outcome. Effectively, Calin and co-workers (Calin et al., 2005) ,reported a unique microRNA signature enabling to differentiate the CLL cases with low versus high Zap-70 expression as well as the cases with unmutated from those with mutated IgV(H). Moreover, microRNAs are proposed to underlie the novel model of pathogenesis of indolent subset of CLL through a newly discovered regulation of TP53 (Fabbri et al., 2011). Moreover, microRNAs allowed putting in evidence a novel molecular link between critical chromosomes defects involved in CLL pathology such as interplay between 13q-17p and 17p-11q. In this model, miR-15a/miR-16-1 that regulate the expression level of TP53, are lost by 13q deletions resulting in increased levels of antiapoptotic proteins Bcl2 and Mcl1 and that of TP53. This last pathway remaining intact may explain relatively stable form of disease. Another microRNA, miR-181b, also involved in Mcl1 and Bcl2 regulation, have been associated with disease progression (Visone et al., 2011). In parallel to the loss of microRNAs due to chromosome deletions (at least those by 13q and 11q deletions), they are often down-regulated epigenetically. Effectively, overexpression of PLAG1, a putative oncogene in CLL due to a deregulated microRNAs, and an inactivation of miR-124-1 are another type of examples of epigenetic deregulations (Pallasch et al., 2009; Patz et al., 2010; Wong et al., 2011).

4. Future researches

Although genetic data teach us that CLL is a single disease, the main unsolved biological problem of CLL cells lays on not yet defined cell origin and/or differentiation step when transformation of B cell has been committed. This of course should not been so surprising because our understanding even of normal B cell differentiation remains still far from being complete. While in majority of CLL cases, the disease is preceded by a preleukemic monoclonal B cell lymphocytosis (MBL), the normal counterparts of both CLL and MBL remain unclear. Classical view of CLL resumes it as a mature B cell malignancy in which transformation of cells occurred after V(D)J recombination and germinal center reaction (Chiorazzi and Ferrarini, 2011). New concept of investigation of CLL-initiating cells was

open recently by Akashi's group reporting that self-renewing hematopoietic stem cells (HSCs) have already acquired necessary modifications enabling them to develop CLL-like phenotype after xenogeneic transplantation (Kikushige et al., 2011). Depicting the molecular events occurring in HSCs in CLL patients enabling their strict maturation into mono- or oligo-clonal CLL cells phenotype should certainly shed new insights into leukemogenesis of this type of mature B lymphomas. Further, although several biological abnormalities have been established to appear in cells specifying progressive or aggressive disease, none of them were clearly yet involved in causality of evolving of indolent form and/or of directly switching towards aggressive form of disease. Hence, whether DNA repair defect or telomere dysfunction resulting in telomere deletions and/or fusions should be a consequence or a cause of disease progression remain still elusive. This is of crucial importance since depicting causality should shed light on new potential targets in clinical trials and in impeding disease progression. Further, having insights into how the resistance has been developed, should also help our understanding of CLL cell origin. Actually, the refractoriness and/or relapse of front-line (i.e. fludarabine) treated CLL patients with complex karyotype and chromosomal aberrations known to confer poor outcome of disease, may be proved as a major obstacle without favorable therapeutic issues (Badoux et al., 2011). The fact that resistant cells are able to upregulate DNA damage error-prone repair allowed us to speculate that the upsetting of this event may be involved in observed chromosomal and telomeric abnormalities whose appearance in aggressive disease remain still murky. This hypothesis is further strengthened by the progressive feature of these two abnormalities in the course of disease. Our current research targets the molecular origin of how NHEJ could become upregulated in these cells allowing them to survive upon treatment. The molecular ways through which repair of chromatin DNA could be modified are multiples and relay on epigenetic and genetic control. Thus, DNA methylation and hydroxymethylation are not only associated with the control of gene expression (including genes involved in DNA repair), and differentiation but also conditioned the DNA repair; all of these functions which are controlled by the local and global presence of 5-methylcytosines may underlie malignant process (Schär and Fritsch, 2011). Effectively, CLL cells display both local DNA hypermethylation and global hypomethylation (Wahlfors et al., 1992; Plass et al., 2007; Raval et al. 2007; Marteau et al., 2010). While the consequences of global genome hypomethylation on DNA damage repair remain still to be established, local CpG island methylation controls the expression level of nearby genes (such as *DAPK1*, *RELB* or *Aiolos* works cited above). In addition to yet not identified target genes which could be directly or indirectly linked to DNA repair, the expression level of identified transcriptional factors was already suggested to define cell resistance to treatment.

Among other epigenetic modifications affecting vital cell functions, including DNA repair, are post-translational modifications of histones. Thus, following DSBs formation, in their vicinity, histones are modified (mainly through phosphorylation, methylation and acetylation) creating thus a dynamic platform for assembly of DNA repair protein complexes (Greenberg, 2011). The best defined histones modification, directly involved in the promotion of DNA repair is the ATM-dependent phosphorylation of histone variant H2AX at S139 in vicinity of DNA damage (rev. Dickey et al., 2009). This modification is the first and key step involved in the recruitment of other proteins in an ordered dynamic and strikingly hierarchical manner to form so-called DNA repair foci. This formation is achieved by an orchestration of protein-protein interactions which is triggered by a plethora of post-

translational modifications such as phosphorylation, acetylation, SUMOylation and ubiquitination. Thus, the formed foci involve protein complexes that should assure not only proper DNA repair but are also coupled with the relaxation of chromatin and the blockage of transcription. Interestingly, H2AX null mice exhibit reduced immunoglobulin class switching but not V(D)J recombination (involving NHEJ). However, in a p53 deficient background, these mice exhibit compromised genomic stability, an increased sensitivity to genotoxic stress and increased cancer susceptibility (Celeste et al., 2002, Celeste et al., 2003). Thus, the cellular level of γ-H2AX and foci formation have been proposed as an indicator of DNA DSBs which could be valuable in monitoring not only a detection of the genotoxic stress but also in monitoring cancer development and progression (rev. Dickey et al., 2009). Remarkably, resistant CLL cells as compared to sensitive, display an increased level of γ-H2AX foci which colocalized at telomeric sequences (Brugat et al., 2010).

Histone methylation involved in an epigenetic control of genome transcription activity also affects DNA repair function. Effectively, dimethyl histone H3 lysine 36 is generated as major event by DSBs induction. This histone modification has been demonstrated to be essential in recruitment of NBS1 and Ku70 to the site of DSB and is followed by an enhanced NHEJ DNA repair (Fnu et al., 2011). Cancer cells often display a plethora of covalent modifications of histones, called "histone onco-modifications" achieved by altered activity of modifying enzymes. These modifications are involved in both development and maintenance of malignant process and which may confer them the resistance to treatment (Füllgrabe et al., 2011). One could notice that the processing of DSBs may also be controlled by enzymes belonging to the family of histone acetyltransferases and deacetylases (HAT and HDAC) which acetylate/deacetylate DNA end-resection factors and participate in this way in DNA damage response and chromosome stability (Robert et al., 2011).

Another way to control the activity of NHEJ repair of DNA in the context of chromatin DNA in human cells involves interaction between Ku70 and ATP-dependent chromatin-remodeling factor (ACF1). This interaction is required for the accumulation of Ku heterodimer at DSBs (Lan et al., 2010).

Finally, protein ubiquitination is another post-translational modification shown to be altered in CLL (Delic et al., 1998; Masdehors et al., 2000; Ma et al., 2008; Sampath et al., 2009), and to be involved in DNA repair (Daigaku et al., 2010; Shanbhag et al., 2010; Larsen et al., 2010; Weitzman et al., 2011; Ramadan and Meerang, 2011). This modification may be of particular interest since it may affect DNA repair through local structural alteration of chromatin (i.e. through histones H2A and H2B and/or chromatin-associating factors' ubiquitination), and directly, through an ubiquitination of the payers involved in DNA repair by NHEJ such as Ku70 or by homologous recombination such as BRCA1 (Gama et al., 2009; Ohta et al., 2011). Phospho-S473-AKT kinase which is activated in many types of human cancers including CLL (Shehata et al., 2010; Hofbauer et al., 2010; Wickremasinghe et al., 2011), is a DNA repair promoting factor through an activation of NHEJ. Moreover, this activity is dependent on the histone ubiquitin ligase RNF 168 (Fraser et al., 2011). Based on an induction of NHEJ in this way by exogenously produced DSBs (irradiation or drugs), it is highly suggestive that this pathway would be involved in resistance mechanisms developed by cancer cells. In agreement with this, in CLL cells decreased phosphorylation of Akt (and other PI3-K family kinases and tensin homolog detected on chromosome 10, PTEN) induces apoptosis in

response to fludarabine treatment. A combined inhibition of PI3-K/Akt and recovery of the activity of PTEN has been suggested as a novel concept for CLL therapy (Shehata et al., 2010). A prolonged effect of these kinases may be further strengthen by an over expression of SET oncoprotein which is documented as a potent physiological inhibitor of protein phosphatases 2A (Christensen et al., 2011).

The fact that CLL cells resistant to apoptosis exhibit a constitutive higher activity of Ku heterodimer to bind *in vitro* free ends of DNA (that mimics DSBs), suggest a post-translational modification of Ku70 and/or of Ku80 as well as a presence/absence of enzyme(s) involved in this modification. This hypothesis is supported by the fact that both cells derived from indolent or aggressive form of disease express Ku proteins at same level (protein and mRNA). Proteomic analysis of each subset of CLL cells should help to identify new factors which in turn, would shed light on a NHEJ defect expressed by resistant cells. This knowledge should indicate the new targeted strategies to be developed to improve clinical trials.

5. Conclusion

Biological defects we have identified in CLL cells resistant to DNA damage-induced apoptosis should functionally be interconnected (i.e. DNA repair defect may be impaired by epigenetic modifications; these modifications affect telomere chromatin structure which is also affected by components of DNA repair machinery). Whether and how these defects would be involved in a promotion of observed chromosomal aberrations occurring in majority of aggressive CLL cases, remain still to be demonstrated but their convergence highly suggest a common mechanisms.

Considered together, all biological data we have obtained with CLL cells led us to conclude that:

i. the resistant subset of CLL cells displays a defect in apoptotic pathway triggered by DNA damage *in vitro* and *in vivo*;
ii. resistant cells display a dysfunction of NHEJ DNA repair system (of yet unknown molecular origin) associated with heterochromatinisation of telomeric regions but, heterochromatinisation may also widespread throughout euchromatin regions affecting gene expression;
iii. in some CLL cases, sensitive cells may became resistant to apoptosis and then, telomeric dysfunction drive to an acquisition of new chromosomal abnormality which is associated with an appearance of an additional aberration characteristic of aggressive form of disease.

Since all of these features are hallmarks of cells resistant to DNA damage-induced apoptosis, then a simple and easy-to-perform test of cell susceptibility to activate or not apoptotic death pathway following genotoxic stress *in vitro*, should be useful and highly indicative of whether front line treatment would be appropriated or proscribed for CLL patients.

Future research in this domain should bring further insights into mechanisms of the origin of deregulated NHEJ in this particular subset of CLL. Knowing that during the course of disease progression, biological susceptibility to DNA damage apoptosis *in vitro*

simultaneously also evolve (i.e. otherwise sensitive cells become resistant), then this mechanistic knowledge should be certainly of new potential applications in clinic.

6. Acknowledgment

We are grateful to all leukemic benevolent blood donors allowing us to perform the research. We also would like to thank all benevolent healthy blood donors whose samples were used as control. We thank all PhD students and post-doctoral fellows who participated in works cited in main text. Finally, the entire technical staff of the hospital Pitié-Salpêtrière is also warmly thanked; we were enjoyed with the help and cooperation of Colette Barbarat et Isabelle Sevoz. Our work was supported by CEA, Association pour la Recherche sur le Cancer (ARC), Fondation de France (FdF) and Electricité de France (EDF).

7. References

Amrein, L., Loignon, M., Goulet, A.C., Dunn, M., Jean-Claude, B., Aloyz, R. & Panasci L. Chlorambucil cytotoxicity in malignant b lymphocytes is synergistically increased by 2-(morpholin-4-yl)-benzo[h]chomen-4-one (NU7026)-mediated inhibition of DNA double-strand break repair via inhibition of DNA-dependent protein kinase. *Journal of Pharmacology and Experimental Therapeutics,* Vol. 321, No.3, (June 2007), pp. 848-855, PMID: 17351105

Aubert, G. & Lansdorp, P.M. Telomeres and aging. *Physiological Reviews*, Vol. 88, No. 2, (April 2008), pp. 557-579, PMID: 18391173

Augereau, A., T'kint de Roodenbeke, C., Simonet, T., Bauwens, S., Horard, B., Callanan, M., Leroux, D., Jallades, L., Salles, G., Gilson, E. & Poncet, D. Telomeric damage in early stage of chronic lymphocytic leukemia correlates with shelterin dysregulation. *Blood,* 2011 Feb 25. [Epub ahead of print], PMID: 21355086

Austen, B., Skowronska, A., Baker, C., Powell, J.E., Gardiner, A., Oscier, D., Majid, A., Dyer, M., Siebert, R., Taylor, A.M., Moss, P.A. & Stankovic, T. Mutation status of the residual ATM allele is an important determinant of the cellular response to chemotherapy and survival in patients with chronic lymphocytic leukemia containing an 11q deletion. *Journal of Clinical Oncology*, Vol. 25, No. 34, (December 2007), pp. 5448-5457, PMID: 17968022

Austen, B., Powell, J.E., Alvi, A., Edwards, I., Hooper, L., Starczynski, J., Taylor, A.M., Fegan, C., Moss, P. & Stankovic, T. Mutations in the ATM gene lead to impaired overall and treatment-free survival that is independent of IGVH mutation status in patients with B-CLL. *Blood*, Vol. 106, No. 9, (November 2005), pp. 3175-3182, PMID: 16014569

Badoux, X.C., Keating, M.J., Wang, X., O'Brien, S.M., Ferrajoli, A., Faderl, S., Burger, J., Koller, C., Lerner, S., Kantarjian, H. & Wierda, W.G. Cyclophosphamide, fludarabine, rituximab and alemtuzumab (CFAR) as salvage therapy for heavily pre-treated patients with chronic lymphocytic leukemia. *Blood,* 2011 Jun 13. [Epub ahead of print], PMID : 21670470

Baird, D.M., Rowson, J., Wynford-Thomas, D. & Kipling, D. Extensive allelic variation and ultrashort telomeres in senescent human cells. *Nature Genetics*, Vol. 33, No. 2, (February 2003), pp. 203-207, PMID: 12539050

Bechter, O.E., Eisterer, W., Pall, G., Hilbe, W., Kühr, T. & Thaler, J. Telomere length and telomerase activity predict survival in patients with B cell chronic lymphocytic leukemia. *Cancer Research,* Vol.58, No. 21, (November 1998), pp. 4918-4922, PMID: 9810000

Billot, K., Soeur, J., Chereau, F., Arrouss, I., Merle-Béral, H., Huang, M.E., Mazier, D., Baud, V. & Rebollo, A. Deregulation of Aiolos expression in chronic lymphocytic leukemia is associated with epigenetic modifications. *Blood*, Vol. 117, No. 6, (February 2011), pp. 1917-1927, PMID: 21139082

Blaise, R., Alapetite, C., Masdehors, P., Merle-Beral, H., Roulin, C., Delic, J. & Sabatier, L. High levels of chromosome aberrations correlate with impaired in vitro radiation-induced apoptosis and DNA repair in human B-chronic lymphocytic leukaemia cells. *International Journal of Radiation Biology,* Vol. 78, No. 8, (August 2002), pp. 671-679, PMID: 12194750

Blasco, M.A., Lee, H.W., Hande, M.P., Samper, E., Lansdorp, P.M., DePinho, R.A.& Greider, C.W. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell*, Vol. 9, No. 1, (October 1997), pp. 25-34, PMID: 9335332

Blasco, M.A. The epigenetic regulation of mammalian telomeres. *Nature Reviews Genet*ics, Vol. 39, No. 4, (April 2007), pp. 299-309, PMID: 17363977

Bramson, J., McQuillan, A., Aubin, R., Alaoui-Jamali, M., Batist, G., Christodoulopoulos, G. & Panasci, L.C. Nitrogen mustard drug resistant B-cell chronic lymphocytic leukemia as an in vivo model for crosslinking agent resistance. *Mutation Research*, Vol. 336, No.3, (May 1995), pp. 269-278, PMID7739615

Brugat, T., Gault, N., Baccelli, I., Maës, J., Roborel de Climens, A., Nguyen-Khac, F., Davi, F., Merle-Béral, H., Gilson, E., Goodhardt, M. & Delic, J. Aberrant telomere structure is characteristic of resistant chronic lymphocytic leukaemia cells. *Leukemia*, Vol. 24, No. 1, (January 2010a), pp. 246-251, PMID: 19847201

Brugat, T., Nguyen-Khac, F., Grelier, A., Merle-Béral, H. & Delic, J. Telomere dysfunction-induced foci arise with the onset of telomeric deletions and complex chromosomal aberrations in resistant chronic lymphocytic leukemia cells. *Blood*, Vol. 116, No. 2, (July 2010b) pp. 239-249, PMID: 20424183

Brugat, T., Nguyen-Khac, F., Merle-Béral, H. & Delic, J. Concomitant telomere shortening, acquisition of multiple chromosomal aberrations and in vitro resistance to apoptosis in a single case of progressive CLL. *Leukemia Research*, Vol. 35, No. 5, (May 2011), pp.e37-40, PMID: 21176960

Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J. & Helleday,T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature*, Vol. 434, No. 7035, (April 2005), pp. 913-917, PMID: 15829966

Caligaris-Cappio, F. & Chiorazzi, N. Where is the biology of CLL leading us? *Seminars in Cancer Biol*ogy, Vol. 20, No. 6, (December 2010), pp. 361-362, PMID: 21130365

Calin, G.A., Ferracin, M., Cimmino, A., Di Leva, G., Shimizu, M., Wojcik, S.E., Iorio, M.V., Visone, R., Sever, N.I., Fabbri, M., Iuliano, R., Palumbo, T., Pichiorri, F., Roldo, C., Garzon, R., Sevignani, C., Rassenti, L., Alder, H., Volinia, S., Liu, C.G., Kipps, T.J., Negrini, M. & Croce, C.M. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *New England Journal of Medicine,* Vol. 353, No. 17, (October 2005) pp.1793-1801. Erratum in: *New England Journal of Medicine,* Vol. 355, No. 5, (August 2006), pp. 533, PMID: 16251535

Cao, Y., Bryan, T.M. & Reddel, R.R. Increased copy number of the TERT and TERC telomerase subunit genes in cancer cells. *Cancer Science,* Vol. 99, No. 6 (June 2008), pp. 1092-1099, PMID: 1848052

Cartwright, R.A., Gurney, K.A. & Moorman, A.V. Sex ratios and the risks of haematological malignancies. *British Journal of Haematology*, Vol. 118, No. 4 (September 2002), pp. 1071-1077, PMID: 12199787

Catovsky, D., Richards, S., Matutes, E., Oscier, D., Dyer, M.J., Bezares, R.F., Pettitt, A.R., Hamblin, T., Milligan, D.W., Child, J.A., Hamilton, M.S., Dearden, C.E., Smith, A.G., Bosanquet, A.G., Davis, Z., Brito-Babapulle, V., Else, M., Wade, R. & Hillmen, P. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. UK National Cancer Research Institute (NCRI) Haematological Oncology Clinical Studies Group; NCRI Chronic Lymphocytic Leukaemia Working Group. *Lancet*, Vol. 370, No. 9583, (July 2007), pp. 230-2399, PMID: 17658394

Celeste, A., Petersen, S., Romanienko, P.J., Fernandez-Capetillo, O., Chen, H.T., Sedelnikova, O.A., Reina-San-Martin, B., Coppola, V., Meffre, E., Difilippantonio, M.J., Redon, C., Pilch, D.R., Olaru, A., Eckhaus, M., Camerini-Otero, R.D., Tessarollo, L., Livak, F., Manova, K., Bonner, W.M., Nussenzweig, M.C. & Nussenzweig A. Genomic instability in mice lacking histone H2AX. *Science*, Vol. 296, No. 5569, (May 2002), pp. 922-927, PMID : 11934988

Celeste, A., Difilippantonio, S., Difilippantonio, M.J., Fernandez-Capetillo, O., Pilch, D.R., Sedelnikova, O.A., Eckhaus, M., Ried, T., Bonner, WM. & Nussenzweig, A. H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. *Cell*, Vol. 5, No. 7 (July 2003), pp. 675-679, PMID: 12792649

Chiorazzi, N., Rai, K.R. & Ferrarini, M. Chronic Lymphocitic Leukemia. *New England Journal of Medicine*, Vol. 352, No. 8, (February 2005), pp. 804-815, PMID: 15728813

Chiorazzi, N. & Ferrarini, M. Cellular origin(s) of chronic lymphocytic leukemia: cautionary notes and additional considerations and possibilities. *Blood,* Vol. 117, No. 6, (February 2011), pp. 1781-1791, PMID: 21148333

Christensen, D.J., Chen, Y., Oddo, J., Matta, K.M., Neil, J., Davis, E.D., Volkheimer, A.D., Lanasa, M.C., Friedman, D.R., Goodman, B.K., Gockerman, J.P., Diehl, L.F., de Castro, C.M., Moore, J.O., Vitek, M.P. & Weinberg, J.B. SET oncoprotein overexpression in B-cell chronic lymphocytic leukemia and non-Hodgkin's lymphoma: a predictor of aggressive disease and new treatment target. *Blood* 2011 Aug 15. [Epub ahead of print], PMID: 21844565

Christodoulopoulos, G., Muller, C., Salles, B., Kazmi, R. & Panasci, L. Potentiation of chlorambucil cytotoxicity in B-cell chronic lymphocytic leukemia by inhibition of

DNA-dependent protein kinase activity using wortmannin. *Cancer Research*, Vol. 58, No. 9, (May 1998), pp. 1789-1792, PMID: 9581813

Collado, M., Blasco, M.A. & Serrano, M. Cellular senescence in cancer and aging. *Cell*, Vol. 130, No. 2 (July 2007), pp. 223-233, PMID: 17662938

Colombo, M., Cutrona, G., Reverberi, D., Fabris, S., Neri, A., Fabbi, M., Quintana, G., Quarta, G., Ghiotto, F., Fais, F. & Ferrarini, M. Intraclonal cell expansion and selection driven by B cell receptor in Chronic Lymphocytic Leukemia. *Molecular Med*icine 2011 Apr 28. doi: 10.2119/molmed.2011.00047. [Epub ahead of print]

Corneo, B., Wendland, R.L., Deriano, L., Cui, X., Klein, I.A., Wong, S.Y., Arnal, S., Holub, A.J., Weller, G.R., Pancake, B.A., Shah, S., Brandt, V.L., Meek, K. & Roth DB. Rag mutations reveal robust alternative end joining. *Nature*, Vol. 449, No. 7161 (September 2007), pp. 483-486, PMID: 17898768

d'Adda di Fagagna, F., Reaper, P.M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., Saretzki, G., Carter, N.P. & Jackson, S.P.A. DNA damage checkpoint response in telomere-initiated senescence. *Nature*, Vol. 426, No. 6963, (November 2003), pp. 194-198, PMID: 14608368

Daigaku, Y., Davies, A.A. & Ulrich, H.D. Ubiquitin-dependent DNA damage bypass is separable from genome replication. *Nature*, Vol. 465, No. 7300 (June 2010), pp. 951-955, PMID: 20453836

Damle, R.N., Wasil, T., Fais, F., Ghiotto, F., Valetto, A., Allen, S.L., Buchbinder, A., Budman, D., Dittmar, K., Kolitz, J., Lichtman, S.M., Schulman, P., Vinciguerra, V.P., Rai, K.R., Ferrarini, M. & Chiorazzi N. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*, Vol. 94, No. 6, (September 1999), pp. 1840-1847, PMID: 10477712

Damle, R.N., Batliwalla, F.M., Ghiotto, F., Valetto, A., Albesiano, E., Sison, C., Allen, S.L., Kolitz, J., Vinciguerra, V.P., Kudalkar, P., Wasil, T., Rai, K.R., Ferrarini, M., Gregersen, P.K. & Chiorazzi, N. Telomere length and telomerase activity delineate distinctive replicative features of the B-CLL subgroups defined by immunoglobulin V gene mutations. *Blood*, Vol. 103, No. 2, (January 2004), pp. 375-382, PMID: 14504108

De Campos-Nebel, M., Larripa, I. & Gonzales-Cid, M. Non-homologous end joining is the responsible pathway for the repair of fludarabine-induced DNA double strand breaks in mammalian cells. *Mutation Research,* Vol. 646, No. 1-2 (November 2008), pp. 8-16, PMID: 18812179

Delacote, F. & Lopez, B.S. Importance of the cell cycle phases for the choice of the appropriate DSB repair pathway, for genome stability maintenance. *Cell Cycle,* Vol. 7, No. 1, (January 2008), pp. 33-38, PMID: 18196958

De Lange, T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes & Development*, Vol. 19, No. 18, (September 2005), pp. 2100-2110, PMID: 16166375

Delic, J., Masdehors, P., Omura, S., Cosset, J.M., Dumont, J., Binet, J.L. & Magdelénat H. The proteasome inhibitor lactacystin induces apoptosis and sensitizes chemo- and radioresistant human chronic lymphocytic leukaemia lymphocytes to TNF-alpha-

initiated apoptosis. *British Journal of Cancer,* Vol. 77, No. 7, (April 1998), pp. 1103-1107, PMID: 9569046

Deriano, L., Guipaud, O., Merle-Béral, H., Binet, J-L., Ricoul, M., Potocki-Veronese, G., Favaudon, V., Maciorowski, Z., Muller, C., Salles, B., Sabatier, L. & Delic, J. Human chronic lymphocytic leukemia B cells can escape DNA damage-induced apoptosis through the nonhomologous end-joining DNA repair pathway. *Blood*, Vol. 105, No. 12, (June 2005), pp. 4776-4783, PMID: 15718417

Deriano, L., Merle-Béral, H., Guipaud, O., Sabatier, L. & Delic, J. Mutagenicity of non-homologous end joining DNA repair in a resistant subset of human chronic lymphocytic leukaemia B cells. *British Journal of Haematology*, Vol. 133, No. 5 (June 2006), pp. 520-525, PMID: 16681639

Deriano, L., Stracker, T.H., Baker, A., Petrini, J.H. & Roth, D.B. Roles for NBS1 in alternative nonhomologous end-joining of V(D)J recombination intermediates. *Molecular Cell*, 2009, 34 :13-25.

Dickey, J.S., Redon, C.E., Nakamura, A.J., Baird, B.J., Sedelnikova, O.A. & Bonner, W.M. H2AX: functional roles and potential applications. *Chromosoma,* Vol. 118, No. 6, (December 2009), pp. 683-692, PMID: 19651821

Dighiero, G. & Hamblin, T.J. Chronic Lymphocytic Leukemia. *Lancet*, Vol. 371, No. 9617, (March 2008), pp. 1017-1029, PMID: 18358929

Döhner, H., Fischer, K., Bentz, M., Hansen, K., Benner, A., Cabot, G., Diehl, D., Schlenk, R., Coy, J., Stilgenbauer, S., Volkman, M., Galle, P.R., Poustka A.M., Hunstein, W. & Lichter, P. p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. *Blood*, Vol. 85, No. 6, (June 1995), pp. 1580-1589, PMID: 7888675

Döhner, H., Stilgenbauer, S., James, M.R., Benner, A., Weilguni, T., Bentz, M., Fischer, K., Hunstein, W. & Lichter, P. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. *Blood*, Vol. 89, No. 7, (1997 April 1997), pp. 2516-2522, PMID: 9116297

Elliott, S.L., Crawford, C., Mulligan, E., Summerfield, G., Newton, P., Wallis, J., Mainou-Fowler, T., Evans, P., Bedwell, C., Durkacz, B.W. & Willmore, E. Mitoxantrone in combination with an inhibitor of DNA-dependent protein kinase: a potential therapy for high risk B-cell chronic lymphocytic leukaemia. *British Journal Haematol*ogy, Vol. 152, No. 1, (January 2011), pp. 161-171, PMID: 21083655

Fabbri, M., Bottoni, A., Shimizu, M., Spizzo, R., Nicoloso, M.S., Rossi, S., Barbarotto, E., Cimmino, A., Adair, B., Wojcik, S.E., Valeri, N., Calore, F., Sampath, D., Fanini, F., Vannini, I., Musuraca, G., Dell'Aquila, M., Alder, H., Davuluri, R.V., Rassenti, L.Z., Negrini, M., Nakamura, T., Amadori, D., Kay, N.E., Rai, K.R., Keating, M.J., Kipps, T.J., Calin, G.A. & Croce, C.M. Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia. *The Journal of American Medical Association (JAMA)*, Vol. 305, No. 1, (January 2011), pp. 59-67, PMID: 21205967

Fabbri, G., Rasi, S., Rossi, D., Trifonov, V., Khiabanian, H., Ma, J., Grunn, A., Fangazio, M., Capello, D., Monti, S., Cresta, S., Gargiulo, E., Forconi, F., Guarini, A., Arcaini, L., Paulli, M., Laurenti, L., Larocca, L.M., Marasca, R., Gattei, V., Oscier, D., Bertoni, F.,

Mullighan, C.G., Foá, R., Pasqualucci, L., Rabadan, R., Dalla-Favera, R. & Gaidano, G. Analysis of the chronic lymphocytic leukemia coding genome: role of NOTCH1 mutational activation. *Journal of Experimental Medicine,* Vol. 208, No. 7, (July 2011), pp. 1389-1401, PMID: 21670202

Fabris, S., Scarciolla, O., Morabito, F., Cifarelli, R.A., Dininno, C., Cutrona, G., Matis, S., Recchia, A.G., Gentile, M., Ciceri, G., Ferrarini, M., Ciancio, A., Mannarella, C., Neri, A. & Fragasso, A. Multiplex ligation-dependent probe amplification and fluorescence in situ hybridization to detect chromosomal abnormalities in Chronic lymphocytic leukemia: A comparative study. *Genes Chromosomes and Cancer*, Vol. 50, No.5, (September 2011), pp. 726-734, PMID: 21638517

Fabris, S., Bollati, V., Agnelli, L., Morabito, F., Motta, V., Cutrona, G., Matis, S., Grazia Recchia, A., Gigliotti, V., Gentile, M., Deliliers, G.L., Bertazzi, P.A., Ferrarini, M., Neri, A. & Baccarelli A. Biological and clinical relevance of quantitative global methylation of repetitive DNA sequences in chronic lymphocytic leukemia. *Epigenetics*, Vol. 6, No. 2, (February 2011), pp. 188-894, PMID: 20930513

Fais, F., Ghiotto, F., Hashimoto, S., Sellars, B., Valetto, A., Allen, S.L., Schulman, P., Vinciguerra, V.P., Rai, K., Rassenti, L.Z., Kipps, T.J., Dighiero, G., Schroeder, H.W. Jr., Ferrarini, M. & Chiorazzi, N. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. *Journal of Clinical Investigation,* Vol. 102, No. 8, (October 1998), pp. 1515-1525, PMID: 9788964

Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N., Johnson. D.A., Richardson, T.B., Santarosa, M., Dillon, K.J., Hickson, I., Knights, C., Martin, N.M., Jackson, S.P., Smith, G.C. & Ashworth, A. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*, Vol. 434, No. 7035, (April 2005), pp. 917-921, PMID: 15829967

Fnu, S., Williamson, E.A., De Haro, L.P., Brenneman, M., Wray, J., Shaheen, M., Radhakrishnan, K., Lee, S.H., Nickoloff, J.A. & Hromas, R. Methylation of histone H3 lysine 36 enhances DNA repair by nonhomologous end-joining. *Proceedings of National Academy of Sciences, U S A,* Vol. 108, No. 2, (January 2011), pp. 540-545, PMID: 21187428

Fraser, M., Harding, S.M., Zhao, H., Coackley, C., Durocher, D. & Bristow, R.G. MRE11 promotes AKT phosphorylation in direct response to DNA double-strand breaks. *Cell Cycle*, 2011 Jul 1;10(13). [Epub ahead of print], PMID: 21623170

Füllgrabe, J., Kavanagh, E. & Joseph, B. Histones onco-modifications. *Oncogene*. 2011, April 25. [Epub ahead of print], PMID : 21516126

Gama, V., Gomez, J.A., Mayo, L.D., Jackson, M.W., Danielpour, D., Song, K., Haas, A.L., Laughlin, M.J. & Matsuyama S. Hdm2 is a ubiquitin ligase of Ku70-Akt promotes cell survival by inhibiting Hdm2-dependent Ku70 destabilization. *Cell Death and Differentiation*, Vol. 16, No. 5, (May 2009), pp. 758-769, PMID: 19247369

Ghiotto, F., Marcatili, P., Tenca, C., Calevo, M.G., Yan, X.J., Albesiano, E., Bagnara, D., Colombo, M., Cutrona, G., Chu, C.C., Morabito, F., Bruno, S., Ferrarini, M., Tramontano, A., Fais, F. & Chiorazzi, N. Mutation pattern of paired immunoglobulin heavy and light variable domains in chronic lymphocytic

leukemia B-cells. *Molecular Medicine*, (July 2011). doi: 10.2119/molmed.2011.00104. [Epub ahead of print

Gilson, E. & Géli, V. How telomeres are replicated. *Nature Reviews Molecular and Cellular Biology*, Vol. 8, No. 10 (October 2007), pp. 825-838, PMID: 17885666

Goldmit, M., Ji, Y., Skok, J., Roldan, E., Jung, S., Cedar, H. & Bergman, Y. Epigenetic ontogeny of the Igk locus during B cell development. *Nature Immunology*, Vol. 6, No. 2, (February 2005), pp. 198-203, PMID: 15619624

Greenberg, R.A. Histone tails: Directing the chromatin response to DNA damage. *FEBS Letter,* 2011 May 27. [Epub ahead of print], PMID: 21621538

Grever, M.R., Lucas, D.M., Dewald, G.W., Neuberg, D.S., Reed, J.C., Kitada, S., Flinn, I.W., Tallman, M.S., Appelbaum, F.R., Larson, R.A., Paietta, E., Jelinek, D.F., Gribben, J.G. & Byrd, J.C. Comprehensive assessment of genetic and molecular features predicting outcome in patients with chronic lymphocytic leukemia: results from the US Intergroup Phase III Trial E2997. *Journal of Clinical Oncology*, Vol. 25, No. 7, (March 2007), pp. 799-804, PMID: 17283363

Guipaud, O., Deriano, L., Salin, H., Vallat, L., Sabatier, L., Merle-Béral, H. & Delic, J. B-cell chronic lymphocytic leukaemia: a polymorphic family unified by genomic features. *Lancet Oncology*, Vol. 4, No. 8, (August 2003), pp. 505-514, PMID: 12901966

Hallek, M. & Pflug, N. State of the art treatment of chronic lymphocytic leukaemia. *Blood Reviews,* Vol. 25, No. 1 (January 2011), pp. 1-9, PMID21095047

Hamblin, T.J., Davis, Z., Gardiner, A., Oscier, D.G. & Stevenson, F.K. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*, Vol. 94, No. 6, (September 1999), pp. 1848-1854, PMID: 10477713

Hamblin, T.J., Orchard, J.A., Gardiner, A., Oscier, D.G., Davis, Z. & Stevenson FK. Immunoglobulin V genes and CD38 expression in CLL. *Blood*, Vol. 95, No7, (April 2000), pp. 2455-2457, PMID: 10787241

Hamblin, T.J. Prognostic markers in chronic lymphocytic leukaemia. *Best Practice & Research Clinical Haematology,* Vol. 20, No. 3, (September 2007), pp. 455-468, PMID: 17707833

Haslinger, C., Schweifer, N., Stilgenbauer, S., Döhner, H., Lichter, P., Kraut, N., Stratowa, C. & Abseher, R. Microarray gene expression profiling of B-cell chronic lymphocityc leukemia subgroups defined by genomic aberrations and VH mutation status. *Journal of Clinical Oncology,* Vol. 22, No. 19, (October 2004), pp. 3937-3949, PMID: 15459216

Hayden, M.S. & Ghosh, S. Shared principles in NF-kappaB signaling. *Cell*, Vol. 132, No. 3, (February 2008), pp. 344-362, PMID: 18267068

Helleday, T., Petermann, E., Lundin, C., Hodgson, B. & Sharma, R.A. DNA repair pathways as targets for cancer therapy. *Nature Reviews Cancer,* Vol. 8, No. 3, (March 2008), pp. 193-204*, PMID: 18284484*

Hervé, M., Xu, K., Ng, Y.S., Wardemann, H., Albesiano, E., Messmer, B.T., Chiorazzi, N. & Meffre, E. Unmutated and mutated chronic lymphocytic leukemias derive from self-reactive B cell precursors despite expressing different antibody reactivity. *Journal of Clinical Investigation,* Vol. 115, No. 6, (June 2005), pp. 1636-1643, PMID: 15902303

Henderson, E.R. & Blackburn, E.H. An overhanging 3' terminus is a conserved feature of telomeres. *Molecular and Cellular Biology*, Vol. 9, No. 1, (January 1989), pp. 345-348, PMID: 2927395

Hewamana, S., Alghazal, S., Lin, T.T., Clement, M., Jenkins, C., Guzman, M.L., Jordan, C.T., Neelakantan, S., Crooks, P.A., Burnett, A.K., Pratt, G., Fegan, C., Rowntree, C., Brennan, P. & Pepper C. The NF-kappaB subunit Rel A is associated with in vitro survival and clinical disease progression in chronic lymphocytic leukemia and represents a promising therapeutic target. *Blood*, Vol. 111, No. 9, (May 2008), pp. 4681-4689, PMID: 18227347

Hofbauer, S.W., Piñón, J.D., Brachtl, G., Haginger, L., Wang, W., Jöhrer, K., Tinhofer, I., Hartmann, T.N. & Greil, R. Modifying akt signaling in B-cell chronic lymphocytic leukemia cells. *Cancer Res*earch, Vol. 70, No. 18, (September 2010), pp. 7336-7344, PMID: 20823161

Horard, B. & Gilson, E. Telomeric RNA enters the game. *Nature Cell Biology*, Vol. 10, No. 2, (February 2008), pp. 113-115, PMID: 18246034

Huang, A.T., Kremer, W.B., Laszlo, J. & Setlow, R.B. DNA repair in human leukaemic lymphocytes. *Nature New Biology,* Vol. 240, No. 99, (November 1972), pp. 114-116, PMID: 4509023

John, L.B. & Ward, A.C. The Ikaros gene family: Transcriptional regulators of hematopoiesis and immunity. *Molecular Immunology,* Vol. 48, No. 9-10, (May 2011), pp. 1272-1278, PMID: 21477865

Kikushige, Y., Ishikawa, F., Miyamoto, T., Shima, T., Urata, S., Yoshimoto, G., Mori, Y., Iino, T., Yamauchi, T., Eto, T., Niiro, H., Iwasaki, H., Takenaka, K. & Akashi, K. Self-renewing hematopoietic stem cell is the primary target in pathogenesis of human chronic lymphocytic leukemia. *Cancer Cell*, Vol. 20, No. 2, (August 2011), pp. 246-259, PMID: 21840488

Kipps, T.J. The B-cell receptor and ZAP-70 in chronic lymphocytic leukemia. *Best Practice & Research Clinical Haem*atology, Vol. 20, No. 3 (September 2007), pp. 415-424, PMID: 17707830

Klein, U., Tu, Y., Stolovitzky, G.A., Mattioli, M., Cattoretti, G., Husson, H., Freedman, A., Inghirami, G., Cro, L., Baldini, L., Neri, A., Califano, A. & Dalla-Favera R. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *Journal of Experimental Medicine,* Vol. 194, No. 11, (December 2001), pp. 1625-1638, PMID: 11733577

Klein, U. & Dalla-Favera R. New insights into the pathogenesis of chronic lymphocytic leukemia. *Seminars in Cancer Bio*logy, Vol. 20, No. 6, (December 2010), pp. 377-383, PMID: 21029776

Lan, L., Ui, A.A., Nakajima, S., Hatakeyama, K., Hoshi, M., Watanabe, R., Janicki, S., Ogiwaraa, H., Kohno, T., Kanno, S-I. & Yasui, A. The ACF1 complex is required for DNA double-strand break repair in human cells. *Molecular Cell*, Vol. 40, No. 6, (December 2010), pp. 976-987, PMID: 21172662

Lanasa, M.C. Novel insights into the biology of CLL. *Hematology/ American Society of Hematology Education Program*, 2010; 2010:70-76, PMID: 21239773

Langerak, A.W., Davi, F., Ghia, P., Hadzidimitriou, A., Murray, F., Potter, K.N., Rosenquist, R., Stamatopoulos, K. & Belessi, C. Immunoglobulin sequence analysis and prognostication in CLL: guidelines from the ERIC review board for reliable interpretation of problematic cases. *Leukemia*, Vol., 25, No. 6 (June 2011), pp. 979-984, PMID: 21455216

Lanham S, Hamblin T, Oscier D, Ibbotson R, Stevenson F, Packham G. Differential signaling via surface IgM is associated with VH gene mutational status and CD38 expression in chronic lymphocytic leukemia. *Blood*, Vol. 101, No. 3, (February 2003), pp. 1087-1093, PMID: 12393552

Lansdorp, P.M. Telomeres, stem cells, and haematology. *Blood*, Vol. 111, No. 4, (February 2008), pp. 1759-1766, PMID: 18263784

Larsen, D.H., Poinsignon, C., Gudjonsson, T., Dinant, C., Payne, M.R., Hari, F.J., Danielsen, J.M., Menard, P., Sand, J.C., Stucki, M., Lukas, C., Bartek, J., Andersen, J.S. & Lukas, J. The chromatin-remodeling factor CHD4 coordinates signaling and repair after DNA damage. *Journal of Cell Biology, Vol*. 190, No. 5, (September 2010), pp. 731-740, PMID: 20805324

Lee-Theilen, M., Matthews, A.J., Kelly, D., Zheng, S., Chaudhuri, J. CtIP promotes microhomology-mediated alternative end joining during class-switch recombination. *Nature Structural & Molecular Biology*, Vol. 18, No. 1, (January 2011), pp. 75-79, PMID: 21131982

Lieber, M.R. The mechanism of human nonhomologous DNA end joining. *Journal of Biological Chemistry*, Vol. 283, No. 1, (January 2008), pp. 1-5, PMID: 17999957

Lin, T.T., Letsolo, B.T., Jones, R.E., Rowson, J., Pratt, G., Hewamana, S., Fegan, C., Pepper, C. & Baird, D.M. Telomere dysfunction and fusion during the progression of chronic lymphocytic leukemia: evidence for a telomere crisis. *Blood*, Vol. 116, No. 11, (September 2010), pp. 1899-1907, PMID: 20538793

Longhese, M.P. DNA damage response at functional and dysfunctional telomeres. *Genes & Development*, Vol. 22, No. 2, (January 2008), pp. 125-140, PMID: 18198332

Ma, W., Kantarjian, H., O'Brien, S., Jilani, I., Zhang, X., Estrov, Z., Ferrajoli, A., Keating, M., Giles, F. & Albitar, M. Enzymatic activity of circulating proteasomes correlates with clinical behavior in patients with chronic lymphocytic leukemia. *Cancer*, Vol. 112, No. 6, (March 2008), pp. 1306-1312, PMID: 18224667

Mandel, E.M. & Grosschedl, R. Transcription control of early B cell differentiation. *Current Opinion in Immunology,* Vol. 22, No. 2, (April 2010), pp. 161-167, PMID: 20144854

Mari, P.O., Florea, B.I., Persengiev, S.P., Verkaik, N.S., Brüggenwirth, H.T., Modesti, M., Giglia-Mari, G., Bezstarosti, K., Demmers, J.A., Luider, T.M., Houtsmuller, A.B. & van Gent, D.C. (2006) Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. *Proceedings of National Academy of Sciences, USA*, Vol. 103, No. 49, (December 2006), pp. 18597-18602, PMID: 17124166

Marteau, J-B., Rigaud, O., Brugat, T., Gault, N., Vallat, L., Kruhoffer, M., Orntoft, T.F., Nguyen-Khac, F., Chevillard, S., Merle-Beral, H. & Delic, J. Concomitant heterochromatinisation and down-regulation of gene expression unveils epigenetic silencing of *RELB* in an aggressive subset of chronic lymphocytic leukemia in males. *BMC Med Genomics*, Vol. 3 (November 2010), 3:53, PMID: 21062507

Masdehors, P., Merle-Béral, H., Maloum, K., Omura, S., Magdelénat, H. & Delic J. Deregulation of the ubiquitin system and p53 proteolysis modify the apoptotic response in B-CLL lymphocytes. *Blood*, Vol.96, No. 1, (July 2000), pp. 269-274, PMID: 10891461

Mauro, F.R., Foa, R., Giannarelli, D., Cordone, I., Crescenzi, S., Pescarmona, E., Salla, R., Cerretti, R. & Mandelli, F. Clinical characteristics and outcome of young chronic lymphocytic leukemia patients: a single institution study of 204 cases. *Blood*, Vol. 94, No. 2, (July 1999), pp. 448-454, PMID: 10397712

Messmer, B.T., Messmer, D., Allen, S.L., Kolitz, J.E., Kudalkar, P., Cesar, D., Murphy, E.J., Koduru, P., Ferrarini, M., Zupo, S., Cutrona, G., Damle, R.N., Wasil, T., Rai, K.R., Hellerstein, M.K. & Chiorazzi, N. *In vivo* measurements document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. *Journal of Clinical Investigation*, Vol. 115, No. 3, (March 2005), pp. 755-764, PMID: 15711642

Mills, D.M., Bonizzi, G. & Karin, M. Rickert RC. Regulation of late B cell differentiation by intrinsic IKKalpha-dependent signals. *Proceedings of National Academy of Sciences, U S A*, Vol. 104, No. 15, (April 2007), pp. 6359-6364, PMID: 17404218

Mladenov, E. & Iliakis, G. Induction and repair of DNA double strand breaks: The increasing spectrum of non-homologous end joining pathway. *Mutation Research,* Vol. 711, No. 1-2, (June 2011), pp. 61-72, PMID: 21329706

Mohr, J., Helfrich, H., Fuge, M., Eldering, E., Bühler, A., Winkler, D., Volden, M., Kater, A.P., Mertens, D., Te Raa, D., Döhner, H., Stilgenbauer, S. & Zenz, T. DNA damage-induced transcriptional program in CLL: biological and diagnostic implications for functional p53 testing. *Blood*, Vol. 117, No. 5, (February 2011), pp. 1622-1632, PMID: 21115975

Muller, C. & Salles, B. Regulation of DNA-dependent protein kinase activity in leukemic cells. *Oncogene*, Vol. 15, No. 19, (November 1997), pp. 2343-2348, PMID: 9393878

Nicoloso, M.S., Kipps, T.J., Croce, C.M. & Calin, G.A. MicroRNAs in the pathogeny of chronic lymphocytic leukaemia. *British Journal of Haematology,* Vol. 139, No. 5, (December 2007), pp. 709-716, PMID: 18021085

O'Driscoll, M., Cerosaletti, K.M., Girard, P.M., Dai, Y., Stumm, M., Kysela, B., Hirsch, B., Gennery, A., Palmer, S.E., Seidel, J., Gatti, R.A., Varon, R., Oettinger, M.A., Neitzel, H., Jeggo, P.A. & Concannon, P. DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Molecular Cell,* Vol.8, No. 6, (December 2001), pp. 1175-1185, PMID: 11779494

O'Driscoll, M. & Jeggo, P.A. CsA can induce DNA double-strand breaks: implications for BMT regimens particularly for individuals with defective DNA repair. *Bonne Marrow Transplantation*, Vol. 41, No. 11, (June 2008), pp. 983-989, PMID: 18278071

Ohta, T., Sato, K. & Wu, W. The BRCA1 ubiquitin ligase and homologous recombination repair. *FEBS Lett*er, 2011 May 9. [Epub ahead of print], PMID: 21570976

Pallasch, C.P., Patz, M., Park, Y.J., Hagist, S., Eggle, D., Claus, R., Debey-Pascher, S., Schulz, A., Frenzel, L.P., Claasen, J., Kutsch, N., Krause, G., Mayr, C., Rosenwald, A., Plass, C., Schultze, J.L., Hallek, M. & Wendtner, C.M. miRNA deregulation by epigenetic silencing disrupts suppression of the oncogene PLAG1 in chronic

lymphocytic leukemia. *Blood*, Vol. 114, No. 15, (October 2009), pp. 3255-3264, PMID: 19692702

Panasci, L. Chlorambucil drug resistance in chronic lymphocytic leukemia: the emerging role of DNA repair. *Clinical Cancer Research*, Vol. 7, No. 3, (March 2001), pp. 454-461, PMID: 11297233

Parker, T.L. & Strout, M.P. Chronic lymphocytic leukemia: prognostic factors and impact on treatment. *Discovery Medicine*, Vol. 11, No. 57, (February 2011), pp. 115-123, PMID: 21356166

Patz, M., Pallasch, C.P.& Wendtner, C.M. Critical role of microRNAs in chronic lymphocytic leukemia: overexpression of the oncogene PLAG1 by deregulated miRNAs. *Leukemia & Lymphoma*, Vol. 51, No. 8, (August 2010), pp. 1379-1381, PMID: 20687796

Plass, C., Byrd, J.C., Raval, A., Tanner, S.M. & de la Chapelle, A. Molecular profiling of chronic lymphocytic leukaemia: genetics meets epigenetics to identify predisposing genes. *British Journal of Haematology,* Vol. 139, No. 5, (December 2007), pp. 744-752, PMID: 18021078

Puente, X.S., Pinyol, M., Quesada, V., Conde, L., Ordóñez, G.R., Villamor, N., Escaramis, G., Jares, P., Beà, S., González-Díaz, M., Bassaganyas, L., Baumann, T., Juan, M., López-Guerra, M., Colomer, D., Tubío, J.M., López, C., Navarro, A., Tornador, C., Aymerich, M., Rozman, M., Hernández, J.M., Puente, D.A., Freije, J.M., Velasco, G., Gutiérrez-Fernández, A., Costa, D., Carrió, A., Guijarro, S., Enjuanes, A., Hernández, L., Yagüe, J., Nicolás, P., Romeo-Casabona, C.M., Himmelbauer, H., Castillo, E., Dohm, JC., de Sanjosé, S., Piris, M.A., de Alava, E., Miguel, J.S., Royo, R., Gelpí, J.L., Torrents, D., Orozco, M., Pisano, D.G., Valencia, A., Guigó, R., Bayés, M., Heath, S., Gut, M., Klatt, P., Marshall, J., Raine, K., Stebbings, L.A., Futreal, P.A., Stratton, M.R., Campbell, P.J., Gut, I., López-Guillermo, A., Estivill, X., Montserrat, E., López-Otín, C. & Campo, E. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature*, Vol. 475, No. 7354, (June 2011), pp. 101-105, PMID: 21642962

Ramadan, K. & Meerang, M. Degradation-linked ubiquitin signal and proteasome are integral components of DNA double strand break repair: New perspectives for anti-cancer therapy. *FEBS Letter*, 2011, Apr 28. [Epub ahead of print], PMID: 21536036

Rassool, F.V. DNA double strand breaks (DSB) and non-homologous end joining (NHEJ) pathways in human leukemia. *Cancer Letter, Vol*. 193, No. 1, (April 2003) pp. 1-9, PMID: 12691817

Raval, A., Tanner, S.M., Byrd, J.C., Angerman, E.B., Perko, J.D., Chen, S.S., Hackanson, B., Grever, M.R., Lucas, D.M., Matkovic, J.J., Lin, T.S., Kipps, T.J., Murray, F., Weisenburger, D., Sanger, W., Lynch, J., Watson, P., Jansen, M., Yoshinaga, Y., Rosenquist, R., de Jong, P.J., Coggill, P., Beck, S., Lynch, H., de la Chapelle, A. & Plass C: Downregulation of death-associated protein kinase 1 (DAPK1) in chronic lymphocytic leukemia. *Cell*, Vol. 129, No. 5, (June 2007), pp. 879-890, PMID: 17540169

Riballo, E., Critchlow, S.E., Teo, S.H., Doherty, A.J., Priestley, A., Broughton, B., Kysela, B., Beamish, H., Plowman, N., Arlett, C.F., Lehmann, A.R., Jackson, S.P. & Jeggo, P.A. Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Current Biology*, Vol. 9, No. 13, (July 1999), pp. 699-702, PMID: 10395545

Robert, T., Vanoli, F., Chiolo, I., Shubassi, G., Bernstein, K.A., Rothstein, R., Botrugno, O.A., Parazzoli, D., Oldani, A., Minucci, S. & Foiani, M. HDACs link the DNA damage response, processing of double-strand breaks and autophagy. *Nature*, Vol. 471, No. 7336, (March 2011), pp. 74-79, PMID: 21368826

Roos, G., Kröber, A., Grabowski, P., Kienle, D., Bühler, A., Döhner, H., Rosenquist, R. & Stilgenbauer, S. Short telomeres are associated with genetic complexity, high-risk genomic aberrations, and short survival in chronic lymphocytic leukemia. *Blood*, Vol. 111, No. 4, (February 2008), pp. 2246-2252, PMID: 18045969

Rosenwald, A., Alizadeh, A.A., Widhopf, G., Simon, R., Davis, R.E., Yu, X., Yang, L., Pickeral, O.K., Rassenti, L.Z., Powell, J., Botstein, D., Byrd, J.C., Grever, M.R., Cheson, B.D., Chiorazzi, N., Wilson, W.H., Kipps, T.J., Brown, P.O. & Staudt, LM. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *Journal of Experimental Medicine*, Vol. 194, No. 2001, 194:1639-1647, PMID: 11733578

Rosenwald. A., Chuang, E.Y., Davis, R.E., Wiestner, A., Alizadeh, A.A., Arthur, D.C., Mitchell, J.B., Marti, G.E., Fowler, D.H., Wilson, W.H. & Staudt, L.M. Fludarabine treatment of patients with chronic lymphocytic leukemia induces a p53-dependent gene expression response. *Blood, Vol*. 104, No. 5, (September 2004), pp. 1428-1434, PMID: 15138159

Sampath, D., Calin, G.A., Puduvalli, V.K., Gopisetty, G., Taccioli, C., Liu, C.G., Ewald, B., Liu, C., Keating, M.J. & Plunkett W. Specific activation of microRNA106b enables the p73 apoptotic response in chronic lymphocytic leukemia by targeting the ubiquitin ligase Itch for degradation. *Blood*, Vol. 113, No. 16, (April 2009), pp. 3744-3753, PMID: 19096009

Schär, P. & Fritsch, O. DNA repair and the control of DNA methylation. *Progress in Drug* Research Vol. 67, 2011, pp. 51-68, PMID: 21141724

Schroeder, H.W .Jr., & Dighiero, G. The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire. *Immunology Today*, Vol. 15, No. 6, (June 1994), pp. 288-294, PMID: 7520700

Ségal-Bendirdjian, E. & Gilson, E. Telomeres and telomerase: from basic research to clinical applications. *Biochimie*, Vol. 90, No. 4, (April 2008), pp. 1-4, PMID: 20096746

Shanbhag, N.M., Rafalska-Metcalf, I.U., Balane-Bolivar, C., Janicki, S.M. & Greenberg, R.A. ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. *Cell*, Vol. 141, No. 6, (June 2010), pp. 970-981, PMID: 20550933

Shehata, M., Schnabl, S., Demirtas, D., Hilgarth, M., Hubmann, R., Ponath, E., Badrnya, S., Lehner, C., Hoelbl, A., Duechler, M., Gaiger, A., Zielinski, C., Schwarzmeier, J.D. & Jaeger U. Reconstitution of PTEN activity by CK2 inhibitors and interference with the PI3-K/Akt cascade counteract the antiapoptotic effect of human stromal cells in

chronic lymphocytic leukemia. *Blood,* Vol. 116, No. 14, (October 2010), pp. 2513-2521, PMID: 20576813

Stankovic, T., Weber, P., Stewart, G., Bedenham, T., Murray, J., Byrd, P.J., Moss, P.A. & Taylor, A.M. Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukaemia. *Lancet*, Vol. 353, No. 9146, (January 1999), pp. 26-29, PMID: 10023947

Stewart, S.A. & Weinberg, R.A. Telomeres: cancer to human aging. Annual Reviews Cell and Developmental Biology, Vol. 22, 2006, pp. 531-557, PMID: 16824017

Subramanyam, D. & Blelloch, R. From microRNAs to targets: pathway discovery in cell fate transitions. *Current Opinion in Genetics and Development, Vol.* 2011 May 31. [Epub ahead of print], PMID: 21636265

Ting, A.H., McGarvey, K.M. & Baylin, S.B. The cancer epigenome--components and functional correlates. *Genes & Development*, Vol. 20, No. 23, (December 2006), pp. 3215-3231, PMID: 17158741

Vallat, L., Magdelénat, H., Merle-Béral, H., Masdehors, P., Potocki de Montalk, G., Davi, F., Kruhoffer, M., Sabatier, L., Orntoft, T.F. & Delic J. The resistance of B-CLL cells to DNA damage-induced apoptosis defined by DNA microarrays. *Blood*, Vol. 101, No. 11, (June 2003), pp. 4598-4606, PMID: 12586635

Visone, R., Veronese, A., Rassenti, L.Z., Balatti, V., Pearl, D.K., Acunzo, M., Volinia, S., Taccioli, C., Kipps, T.J.& Croce CM. MiR-181b is a biomarker of disease progression in chronic lymphocytic leukemia. *Blood*, 2011 Jun 2. [Epub ahead of print], PMID: 21636858

Wahlfors, J., Hiltunen, H., Heinonen, K., Hämäläinen, E., Alhonen, L. & Jänne, J. Genomic hypomethylation in human chronic lymphocytic leukemia. *Blood*, Vol. 80, No. 8, (October 1992), pp. 2074-2080, PMID: 138719

Weill, JC. & Reynaud, CA. Do developing B cells need antigen? *Journal of Experimental Medicine*, Vol. 201, No. 1, (January 2005), pp. 7-9, PMID: 15630132

Weill, JC., Weller, S. & Reynaud CA. Human Marginal Zone B Cells. *Annual Review of Immunology*, Vol., 27 (2009), pp. 267-285, PMID: 19302041

Weitzman, M.D., Lilley, C.E. & Chaurushiya MS. Changing the ubiquitin landscape during viral manipulation of the DNA damage response. *FEBS Letter,* 2011 May 5. [Epub ahead of print], PMID: 21549706

Weller, S., Mamani-Matsuda, M., Picard, C., Cordier, C., Lecoeuche, D., Gauthier, F., Weill, JC. & Reynaud, C.A. Somatic diversification in the absence of antigen-driven responses is the hallmark of the IgM+ IgD+ CD27+ B cell repertoire in infants. *Journal of Experimental Medicine*, Vol. 205, No. 6, (June 2008), pp. 1331-1342, PMID: 18519648

Weller, S., Braun, M.C., Tan, B.K., Rosenwald, A., Cordier, C., Conley, M.E., Plebani, A., Kumararatne, D.S., Bonnet, D., Tournilhac, O., Tchernia, G., Steiniger, B., Staudt, L.M., Casanova, J.L., Reynaud, C.A. & Weill, JC. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. *Blood*, Vol. 104, No. 12, (December 2004), pp. 3647-3654, PMID: 15191950

Weston, V.J., Oldreive, C.E., Skowronska, A., Oscier, D.G., Pratt, G., Dyer, M.J., Smith, G., Powell, J.E., Rudzki, Z., Kearns, P., Moss, P.A., Taylor, A.M., & Stankovic, T. The PARP inhibitor olaparib induces significant killing of ATM-deficient lymphoid tumor cells in vitro and in vivo. *Blood*, Vol. 116, No. 22, (November 2010), pp. 4578-4587, PMID: 20739657

Wickremasinghe, R., Prentice, A.G. & Steele AJ. Aberrantly activated anti-apoptotic signalling mechanisms in chronic lymphocytic leukaemia cells: clues to the identification of novel therapeutic targets. *British Journal of Haematology,* Vol. 153, No. 5, (June 2011), pp. 545-556, PMID: 21501136

Wong, K.Y., So, C.C., Loong, F., Chung, L.P., Lam, W.W., Liang, R., Li, G.K., Jin, D.Y.& Chim, C.S. Epigenetic Inactivation of the miR-124-1 in Haematological Malignancies. *PLoS One*. April 2011, 6(4):e19027, PMID: 21544199

Yan, C.T., Boboila, C., Souza, E.K., Franco, S., Hickernell, T.R., Murphy, M., Gumaste, S., Geyer, M., Zarrin, A.A., Manis, J.P., Rajewsky, K. & Alt, F.W. IgH class switching and translocations use a robust non-classical end-joining pathway. *Nature*, Vol. 449, No. 7161, (September 2007), pp. 478-482, PMID: 17713479

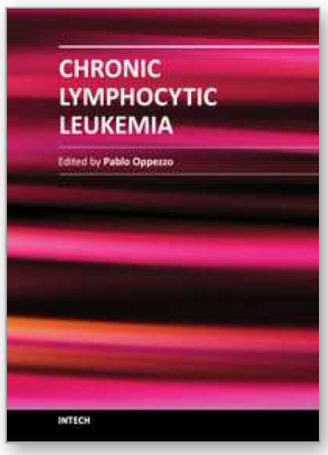
Zecevic, A., Sampath, D., Ewald, B., Chen, R., Wierda, W. & Pliunkett, W. Killing of chronic lymphocytic leukemia by the combination of fludarabine and oxaliplatin is dependent on the activity of XPF endonuclease. *Clinical Cancer Research*, Vol. 17, No. 14, (July 2011), pp. 4731-4741, PMID: 21632856

Zenz, T., Döhner, H. & Stilgenbauer, S. Genetics and risk-stratified approach to therapy in chronic lymphocytic leukemia. *Best Practice & Research Clinical Haematology,* Vol. 20, No. 3, (September 2007), pp. 439-453, PMID: 17707832

Zenz, T., Mertens, D., Döhner, H. & Stilgenbauer, S. Importance of genetics in chronic lymphocytic leukemia. *Blood Reviews*, Vol. 25, No. 3, (May 2011), pp. 131-137, PMID: 21435757

Zha, S., Guo, C., Boboila, C., Oksenych, V., Cheng, H.L., Zhang, Y., Wesemann, D.R., Yuen, G., Patel, H., Goff, P.H., Dubois, R.L. & Alt FW. ATM damage response and XLF repair factor are functionally redundant in joining DNA breaks. *Nature*, Vol. 469, No. 7329, (Jaanuary 2011), pp. 250-254, PMID: 21160472

Zupo, S., Isnardi, L., Megna, M., Massara, R., Malavasi, F., Dono, M., Cosulich, E.& Ferrarini, M. CD38 expression distinguishes two groups of B-cell chronic lymphocytic leukemias with different responses to anti-IgM antibodies and propensity to apoptosis. *Blood*, Vol. 88, No. 4, (August 1996), pp. 1365-1374, PMID : 8695855



Chronic Lymphocytic Leukemia
Edited by Dr. Pablo Oppezzo

ISBN 978-953-307-881-6
Hard cover, 448 pages
Publisher InTech
Published online 10, February, 2012
Published in print edition February, 2012

B-cell chronic lymphocytic leukemia (CLL) is considered a single disease with extremely variable course, and survival rates ranging from months to decades. It is clear that clinical heterogeneity reflects biologic diversity with at least two major subtypes in terms of cellular proliferation, clinical aggressiveness and prognosis. As CLL progresses, abnormal hematopoiesis results in pancitopenia and decreased immunoglobulin production, followed by nonspecific symptoms such as fatigue or malaise. A cure is usually not possible, and delayed treatment (until symptoms develop) is aimed at lengthening life and decreasing symptoms. Researchers are playing a lead role in investigating CLL's cause and the role of genetics in the pathogenesis of this disorder. Research programs are dedicated towards understanding the basic mechanisms underlying CLL with the hope of improving treatment options.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jozo Delic, Jean-Brice Marteau, Karim Maloum, Florence Nguyen-Khac, Frédéric Davi, Zahia Azgui, Véronique Leblond, Jacques-Louis Binet, Sylvie Chevillard and Hélène Merle-Béral (2012). DNA Damage Response/Signaling and Genome (In)Stability as the New Reliable Biological Parameters Defining Clinical Feature of CLL, Chronic Lymphocytic Leukemia, Dr. Pablo Oppezzo (Ed.), ISBN: 978-953-307-881-6, InTech, Available from: http://www.intechopen.com/books/chronic-lymphocytic-leukemia/dna-damage-response-signaling-and-genome-in-stability-as-the-new-reliable-biological-parameters-defi

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen