

# The role of multidrug resistance polymorphisms in chronic lymphocytic essay

[Science](#), [Epidemiology](#)



Chronic lymphocytic leukemia ( CLL ) is characterised by an over-production of B cells ( lymph cells ) and is a extremely variable disease in footings of its clinical patterned advance.

Patients with CLL may show with disease which appears to be comparatively inactive and offers possible hope for intercession or the disease may attest as an highly aggressive malignant neoplastic disease, with a hapless result ( Hamblin, 2002 ) . It is the most common signifier of leukemia in the Western universe with about 2, 400 instances of CLL being diagnosed in the UK every twelvemonth ( MacMillan Cancer, 2010 ) . The induction of CLL is thought to be attributable to a individual familial defect of B-lymphocytes, which consequences in a peculiar form of reaction where the B-cell receptor is stimulated ( Hamblin, 2002 ) . CLL seldom affects kids and is more frequently associated with those over the age of 60, while it besides appears to be more associated with work forces instead than adult females.

Demuring root cell organ transplant, which is merely applicable for a little figure of patients, healing intervention has yet to be defined for the disease ( Schweighofer & A ; Wendtner, 2010 ) .

## **Signs and symptoms**

Like many other malignant neoplastic diseases, the oncoming of disease is frequently insidious and there may be no obvious marks or symptoms. In fact, diagnosing of disease is frequently secondary to a blood trial performed as portion of a everyday wellness cheque or prior to an operation. Typical symptomatology includes lassitude, weight loss, dark workout suits and frequent infections every bit good as swollen lymph nodes.

The frequent infections and enlarged lymph nodes exemplify the underlying job of CLL – sick persons over-produce immortalised but dysfunctional B lymph cells, which hinder an effectual immune response to occupying pathogens. Specifically, hypogammaglobulinaemia predisposes persons to infective complications and significantly contributes to the morbidity and mortality experienced by CLL patients ( Morrison, 2009 ) . Due to the more fresh methods of handling CLL ( e. g. purine parallels or monoclonal antibody interventions such as alemtuzumab ) , persons can be even more susceptible to infection during the intervention class to pathogens such as CMV, herpesvirus and unusual infections such as toxoplasmosis ( Abedalthagafi et al. , 2009 ) . Other noteworthy symptoms associated with CLL include autoimmune hemolytic anemia and idiopathic thrombocytopenia ( ITP ) ( Diehl & A ; Ketchum, 1998 ) .

## **Causes and Diagnosis**

Hazard factors for development of CLL include age, prolonged occupational exposure to benzene ( Khalade et al. , 2010 ) , gender-specific sensitivity in work forces who had diabetes ( Khan et al. , 2008 ) and other assorted familial venue which confer enhanced likeliness of developing CLL. These include six late elucidated low-penetrance hazard venue, which were identified as portion of a genome-wide survey ( Di Bernardo et al. , 2008 ) and a fresh chromosome part, 13q21. 33-q22.

2, which may hold immune functionality with any mutant in this venue ensuing in increased likeliness of developing familial CLL ( Ng et al. , 2007 ) .

The diagnosing of CLL has evolved well in recent old ages and involves

immunological analysis of bone marrow aspirate and biopsy tissue to to the full corroborate CLL and more crucially, whether the CLL is of the indolent or aggressive signifier.

Bone marrow analysis normally entails flow cytometric rating of B lymph cells with specific over-representation of cells which are monoclonal for the kappa/lambda visible radiation concatenation. Furthermore, immunological phenotyping of B cells confirms the presence of CD5, CD19 to CD24 inclusive, while more late the look of CD38 and intracellular zeta-associated protein ( ZAP-70 ) have become of import predictive markers for CLL disease ( Matrai, 2005 ; Wiestner et al. , 2003 ) . If possible, bone marrow tissue should be analysed by cytogenetics and a panel of chromosomal abnormalities should be screened utilizing fluorescence unmoved hybridization ( FISH ) ( Abbott, 2006 ) .

## **1. 4 Epidemiology**

In a recent UK epidemiological survey, the incidence and mortality from leukemias were investigated ( Bhayat et al. , 2009 ) . Specific aetiological facets of CLL included an incidence rate of 4.

2 per 100, 000 individual old ages ( compared with 0. 49 for acute lymphoblastic leukemia ) , females had a lower incidence compared with work forces and there was no association between socio-economic want position and disease incidence. In footings of endurance, adult females who developed CLL were much less likely to decease compared to work forces ( P

& It ; 0. 001 ) and this gender-specific difference in mortality was non observed amongst the other leukemia.

The incidence above is comparable with another epidemiological survey in the Czech Republic where it was calculated at between 5. 8 and 6. 2 instances per 100, 000 for cohorts from 2006 and 2007 ( Panovska et al. , 2010 ) . This survey besides reinforces how hard it is to successfully pull off and handle CLL as a important figure were included in clinical tests ( presumptively because of intervention failures and/or progressive disease ) . Although dietetic factors have been studied, a recent survey did non happen any association of increased hazard of CLL and the function of diet still remains questionable ( Tsai et al. , 2010 ) .

## 1. 5 Phases

The phases of CLL have been graded in footings of disease patterned advance and are best described in table 1. Irrespective of the theatrical production system in table 1 ( Rai vs Binet ) , those patients with the most malignant phase merely have an expected endurance clip of 1-2 old ages, while patients with the most preliminary phase of disease have a average endurance clip of greater than 10 old ages. It is of import to observe that for instances who relapse, the presenting systems at a lower place have non been validated and depend on the initial site of disease engagement ( Abbott, 2006 ) . Table 1.

CLL theatrical production and hazard stratification ( adapted from Abbott, 2006 ) .

### **Staging at diagnosing ( Rai system )**

0. Lymphocytosis  
1. Lymph node expansion  
2. Expansion of lien  
3.

Haemoglobin & lt ; 11 g/dl  
4. Platelets & lt ; 100, 000/A $\mu$ l

### **Staging at diagnosing ( Binet system )**

Lymphocytosis  
Lymph node expansion in & gt ; 3 countries  
Cytopaenia:

hemoglobin & lt ; 10 g/dl or thrombocytes & lt ; 100, 000/A $\mu$ l

### **Bad characteristics for CLL**

CD38 look in & gt ; 30 % of lymph cells  
ZAP70 look in & gt ; 30 % of lymph cells  
Unmutated ( germline ) IgVH cistron  
Bad cytogenetic abnormalcies  
14q alterations  
11q alterations  
17p depletion  
Trisomy 12  
Rai phase 3 or 4 or Binet phase C  
Doubling clip of lymph cell count & lt ; 12 months  
Elevated beta-2 microglobulin  
Elevated serum thymidine kinase  
Presence of large-cell transmutation ( Richter ' s syndrome )

## **1. 6 Treatment and forecast**

One of the most of import and unsolved challenges in CLL is the early designation of instances, who may be conformable to curative intercession.

Apparently, intervention for a patient is dependent on the clinical manifestation of the disease but with CLL, the state of affairs is alone in that a diagnosing of CLL may be confirmed but there may be no clinical symptoms. At this point, persevering monitoring of the patient is all that is

required. However, the predicted likeliness of symptom oncoming and disease patterned advance can be inferred from a figure of molecular markers including CD38, ZAP-70 and serum beta-2-microglobulin ( table 1 ) . Not merely has CD38 look been associated with a more aggressive signifier of CLL but it has besides been discovered that there is a strong correlativity between its look and intervention failure with fludarabine ( Del Poeta et al. , 2001 ) . Although intracellular ZAP-70 look has been studied extensively and appears to be prognostic of metastatic disease and decease, its accurate quantification by flow cytometry remains debatable ( Wiestner et al.

, 2003 ) . Therefore, its usage a clinical predictive tool is still to be to the full justified. A figure of aforesaid symptoms and marks normally dictate the oncoming of intervention e. g. unexplained weight loss, declining anemia, episodes of thrombocytopaenia and/or progressive lymphadenopathy. Those patients who are younger or hold entered into the higher phases of disease with predictive markers of disease, as described above should be considered for aggressive signifiers of intervention.

Presently, merely allogeneic root cell organ transplant offers any hope of a long-run remedy although laparoscopic splenectomy has been used in a instance series with some success of long-run remedy ( Hill et al. , 2004 ) .

## **1. 6.**

### **1 Chemotherapy-based attacks**

Chemotherapeutic intervention of CLL, as with other malignances, is debatable due to the toxic effects of the drugs and the immune-deficient

province of the patient. Historically, a figure of rough anti-cancer drugs were used to handle CLL including Leukeran, cyclophosphamide and Oncovin. However, there appears to be no added clinical benefit when a combination of such drugs are used and in recent old ages, drug find focused on the household of drugs known as the purine nucleoside parallels. Such purine parallels have been shown to be really effectual in the intervention of CLL and include fludarabine, pentostatin and 2-chlorodeoxyadenosine and their action is thought to be due to their apoptotic effects on lymph cells, where they induce DNA atomization ( Robertson et al. , 1993 ) . By taking the immortalised B lymph cell population, the patient should in theory recover from the CLL and so, disease patterned advance and mortality rates have reduced since the clinical origin of these drugs, either singly or in combination with drugs such as Leukeran ( Rai et al. , 2000 ; Keating et al. , 1998 ) . Despite the evident clinical success of these drugs in handling CLL, their myelosuppressive effects on the immune system has resulted in timeserving infection with pathogens such as herpesvirus, Pneumocystis and other bacterial and fungous bugs ( Cheson, 1995 ; Anaisie et al. , 1998 ) .

## **Immunotherapy of CLL**

In the last decennary, one of the most important stairss in the field of immunology is the clinical application of immunomodulators to disease provinces. This is peculiarly relevant with chronic diseases such as arthritic arthritis ( rituximab ) and Crohn ' s disease ( Remicade ) . Unsurprisingly, the intervention of CLL has besides benefitted from these new monoclonal antibodies, which offer a safer option to some of the drugs listed supra.



Alemtuzumab is a monoclonal antibody ( mAb ) which targets CD52, a marker associated with lymphoma. Its usage has been approved for intervention of CLL and its activity appears to be efficacious particularly in patients who have relapsed, have refractory CLL and who are considered to be high hazard with a hapless forecast ( Schweighofer & A ; Wendtner, 2010 ) . The drug appears to be most successful in eliminating residuary disease from patients which confers longer-term endurance. However, CD52 is besides found on T lymphocytes therefore a side-effect of this drug regimen is an enhanced sensitivity to infection. To this terminal, the drug still remains on offer merely as portion of a clinical test regimen. Rituximab marks CD20, a marker antecedently described which appears to besides confabulate susceptibleness to CLL.

This mAb merely marks B cells and hence already has an advantage over alemtuzumab in that the susceptibleness to infection should be lessened. To that terminal, rituximab has been approved for clinical intervention and is normally offered in concurrence with fludarabine and cyclophosphamide ( FCR ) . This combination has been used in a figure of surveies and is considered the most active combination regimen for CLL sick persons with limited co-morbidity ( Johnson et al.

, 2009 ; Keating et al. , 2005 ) . The most modern mAb which has been approved for usage is ofatumumab, another antibody which targets the CD20 marker. In October 2009, this drug was approved by the US Food and Drug Administration ( FDA ) and is recommended for CLL patients who are stubborn to fludarabine and alemtuzumab ( Lemery et al. , 2010 ) .

This drug is highly efficient at lysing B lymph cells and does this via antibody and complement-mediated cytotoxicity. However, as with the other mAb discussed, infection is the most outstanding side-effect with a figure of patients yielding in the above survey. While the spreading field of immunotherapy undoubtedly promises new drugs for the future intervention of CLL, the job of susceptibility and sensitivity to infective agents remains an important issue, which requires further idea.

### **1. 6. 3 Novel targeted chemotherapeutic agents**

As described above, the quest to detect drugs which are more specific in their devastation of cells/mediators of import in CLL is really of import if the patient is to last timeserving infection and unwanted immunological side-effects such as neutropenia. While the agents of infection are to be feared, in recent old ages one of the most fresh chemotherapeutic drugs used to handle CLL patients is ironically a microbic toxin.

A diphtheria toxin has been fused with an interleukin-2 protein to bring forth DAB ( 389 ) -IL2 or denileukin diftotox. The protein binds to the IL-2 receptor on lymph cells and the endotoxin is so internalised into the cell whereby protein synthesis is stopped and the cell undergoes mortification. This drug has been used in a stage II clinical test and while at that place appeared to be some clinical benefit with moderate toxicity and side effects in CLL patients, there still needs to be further surveys done on this drug to heighten the response rate ( Frankel et al. , 2006 ) . Expression of Bcl-2 protein is associated with patterned advance of CLL and more significantly enhanced opposition to chemotherapy. Therefore, attempts to aim

production of this protein have resulted in the production of an anti-sense oligonucleotide, oblimersen ( Cheson, 2007 ) .

This drug maps by adhering to RNA which targets specific proteins for Bcl-2, interfering with the interlingual rendition procedure for this protein. This drug has been trialled at Phase 3 and like other drugs for CLL, its effects on tumor is modest when administered as a individual drug. However, in concurrence with fludarabine and cyclophosphamide, the clinical benefit challengers that of the intervention regimen presently being used for CLL ( O'Brien et al.

, 2005 ) . Furthermore, the side effects for CLL patients appear to be less terrible than for some of aforesaid drugs. While at that place look to be a figure of drugs ( pharmacological or immunological ) that provide intervention for CLL, the issue of multi-drug opposition remains a pertinent job for CLL patients.

## **2. MULTIDRUG RESISTANCE**

With respects to malignant neoplastic disease, there are several issues to see in the intervention of the patient. The patient is likely to hold a figure of tumors ; will necessitate cytotoxic intervention for those tumors ; will go even more susceptible to infection or other immune-mediated pathology and there is a opportunity that the drug of pick will non be effectual in tumour devastation. A portion of the latter job is attributable to tumour opposition to chemotherapeutic drugs.

Whilst there has been much advancement in the preparation of drugs needed to combat malignant neoplastic disease, for many old ages the job of

multi-drug opposition in malignant neoplastic disease has foiled successful remedy of patients and poses a clinical riddle which has yet to be solved.

## **2. 1 The construct of multidrug opposition**

Multi-drug opposition ( MDR ) is a term which has been used to depict the mechanism of opposition that cells ( procaryotic and eucaryotic ) have in response to chemotherapeutic drugs. Specifically, the construct implies that the mark cell of involvement may be exposed to a figure of chemotherapeutic agents but the cells will be able to pump these out before they accumulate in high adequate concentrations to hold a important consequence. In short, ' drug outflow ' underpins this procedure but in a recent survey, it appears that malignant neoplastic disease cells may besides be able to impact the bioavailability of certain anti-cancer drugs by ' drug inflow ' ( Burger et al.

, 2005 ) .

### **Multidrug efflux pumps: the mechanism of action**

The mechanism of how multidrug outflow pumps work is best summarised in figure 1.

#### **Figure 1.**

#### **Mechanism of action of outflow and inflow pumps ( from Gottesman et al. , 2002 ) .**

Figure 1 high spots a figure of mechanisms which are of import in driving multidrug opposition including increased drug outflow and activation of DNA fix. The procedure of drug ejection from a cell is mediated by ATP-dependent

pumps, which may themselves be over-expressed by malignant neoplastic disease cells. The pumps straddle the plasma membrane and due to an energy-driven gradient, drugs which have entered the cell are removed in an energy-efficient mode. If multidrug opposition can be circumvented, the endurance of the tumor cell is threatened and this offers an increased opportunity of host remedy.

## **Multidrug opposition in CLL**

The susceptibleness of tumors to anti-cancer drugs varies among the different signifiers of malignant neoplastic disease. Although CLL nowadays as a ' slow ' signifier of malignant neoplastic disease, the mortality rate is high and this is mostly due to the belongings of multidrug opposition which finally arise.

Host immunological factors are thought to be of import in commemorating B lymph cells and many of these signals are driven by bone-marrow primogenitor cells and their associated cytokines ( O'Hayre et al. , 2010 ) . One of these signals ( CXCL12 ) is besides thought to be partly responsible for MDR in CLL.

Because MDR is cardinal to CLL and host length of service, it is a really heavily-funded country of research. Cyclosporin A is a powerful immunomodulator and has been used in ablating immune responses to grafts therefore it has besides been used in CLL intervention. As with other immunomodulators, it has unwanted side-effects so there is much focal point

on derived functions of this agent as it has been shown to change by reversal MDR in CLL patient cell lines ( Jiang et al.

, 1995 ) . The manner of action of cyclosporin and its derived functions is thought to impact the outflow pumps, which are influenced by a P-glycoprotein and a multidrug opposition protein ( MRP ) . Other transporter proteins involved in MDR besides include lung opposition protein ( LRP ) .

### **2. 3. 1 ABCB1 ( P-glycoprotein )**

Probably the best understood mechanism of MDR action in CLL is over-production of the membrane P-glycoprotein ( Pgp ) or ABCB1. Pgp is encoded by the MDR-1 ( ABCB1 ) cistron and is a transmembrane transporter, which is portion of the adenosine triphosphate-binding cassette ( ABC ) superfamily ( Jamroziak et al. , 2006 ) .

This protein plays a important function in both ( a ) speed uping the outflow of cytotoxic drugs from the lymph cell and ( B ) contradicting the pro-apoptotic effects of such anti-cancer drugs ( Svoboda-Beusan et al. , 2000 ) .

It is apprehensible hence that Pgp itself has become the focal point of possible intervention intercession. Pgp is really expressed in normal host tissue and is of import in cut downing drug soaking up from the digestive piece of land but it is the over-expression of this protein in tumor cells which confers MDR to a wide scope of chemotherapeutic agents used for the intervention of CLL. The protein has been found to be present in some CLL surveies in up to 100 % of such patients and its importance in disease has

been shown by handling CLL patients with drugs which are acted upon by Pgp ( Robak et al. , 2001 ) .

## **2. 4 Single nucleotide polymorphism ( SNP ) in the MDR1 cistron**

Polymorphisms of the MDR1 cistron have late been reported and these have clinical deductions in that there may be a considerable impact on the pharmacological impact of anti-cancer drugs. To this terminal, the find of a figure of individual nucleotide polymorphisms ( SNPs ) and associated insertion/deletion mutants has resulted in an increasing cognition of how CLL MDR can originate and is invariably germinating ( Ishikawa et al. , 2004 ) . If single SNPs can be determined for specific instances of CLL, this may ensue in individually-tailored therapy for patients, which may heighten the opportunities of remedy and cut down unwanted side-effects.

The most of import SNPs associated with CLL disease patterned advance to day of the month include C3435T, G2677T/A and C1236T and these shall be discussed in the undermentioned subdivisions.

### **C3435T ( rs1045642 ) polymorphism**

The C3435T polymorphism has late been found to be associated with an increased susceptibleness to other diseases including Parkinson ' s disease, nephritic carcinoma and pediatric ague lymphoblastic leukemia ( Lee et al. , 2004 ; Jamroziak et al. , 2004 ; Siegsmund et al. , 2002 ) . It has been hypothesised from these and other surveies that the C3435T-mutated Pgp has reduced efflux belongingss and this contributes to toxin-mediated sensitivity to the above conditions. Conversely, with respects to CLL,

surveys have elucidated that there is an increased prevalence of this SNP in CLL patients hence it may be that cells in CLL patients are non able to transport toxins ( such as pesticides ) from cells every bit efficaciously as wild-type Pgp bearers ( Jamroziak et al.

, 2006 ) ensuing in an increased susceptibleness to Pgp-transported carcinogens. This may besides hold direct deductions for chemotherapy options in CLL patients.

### **G2677T/A ( rs2032582 ) polymorphism**

As with the C3435T polymorphism, the G2677T/A polymorphism is relevant in predisposing persons to a figure of other diseases including chronic myeloid leukemia.

G2677T/A has been evaluated and confirmed as a predictive marker for CLL but more significantly, the light grounds to day of the month suggests that this polymorphism may be associated with higher hemoglobin degrees and increased lymphocyte figure ( Penna et al. , 2009 ) . The clinical deductions of this polymorphism are yet to be to the full elucidated.

### **2. 4. 3 C1236T ( rs1128503 ) polymorphism**

Although an earlier survey found no association between C1236T and multidrug opposition in CLL patients ( Goreva et al. , 2004 ) , it is the combined effects of C1236T and the aforesaid SNPs that has been explored in greater item particularly with respect to organ transplant.



These SNPs have been appraised for their effects on cyclosporin ( antecedently discussed ) and tacrolimus, agents which are used to abrogate immune responses before and after organ transplant ( Statz et al. , 2010 ) . Therefore, it is likely that C1236T may besides play a function in how cyclosporin derived functions ( discussed in subdivision 2. 3 ) can aim MDR in CLL.

However, as with G2677T/A, the grounds is light at present.

### **3. Summary**

CLL is an insidious disease which chiefly affects the aged, peculiarly work forces and it is hence frequently another status which consequences in the decease of that patient. However, in some states the incidence of CLL is increasing and with omni-present jobs with MDR, the mentality may look bleak for patients who are diagnosed. However, there is optimism for increased remedy rates in this field. Molecular biological science and immunology are quickly progressing countries and in recent old ages, antecedently hard-to-treat conditions such as arthritic arthritis and Crohn ' s disease have benefitted from clinical application of immunomodulators such as rituximab and Remicade.

While the menace of timeserving infections still poses some jobs for CLL patients, by and large such infections are easier to handle than B-lymphocytes which get MDR over clip. The accent now appears to be centred on ablating minimum residuary disease and it truly appears to be rather assuring for patients who fall into this class. RNA-specific drugs offer a

tantalising penetration into the power of new drug find but their usage must be carefully monitored. While this therapy may assist to command CLL, it may unwittingly impact downstream production of proteins which may be indispensable in everyday cell map ensuing in aberrant cell behavior and this could do unanticipated disease provinces. Finally, MDR is a job which scientists, trefoils and clinicians have battled for old ages. Designation of SNPs may be one of the most of import findings of modern scientific discipline and may assist to understand MDR non merely in CLL but in a gamut of diseases, where drug opposition is a major issue.

It will take conjunct attempts from all those listed to clarify MDR forms and hopefully manner person-specific interventions, which result in a remedy for such conditions.

#### **4. AIMS OF STUDY**