First-Line Treatment of Chronic Lymphocytic Leukaemia and the Impact of Genetic Aberrations in Chemotherapy

Molecular Medicine

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B cell chronic lymphocytic leukaemia has been historically thought of as a benign disease with no effect on the patients' livelihood. The exact cause of the disease is currently unknown as the molecular basis of the disease continues to be unveiled. The diseases occurs in people over 50 years. Interestingly, the incidence of the disease is mostly only found in Western countries. Research in the past decade has shown the variable clinical course of the disease with the identification of several important prognostic factors. Many of these factors include genetic aberrations observed and are used in determining which treatment strategy should be implemented. Of these, genetic aberrations involving the Ataxia Telangiectasia Mutated and p53 genes are most important in predicting the behaviour of chemotherapeutic agents used in treatment. Also, IGHV gene mutations are useful in identifying poor-prognosis patients. Chemotherapeutic agents currently under study are fludarabine-based, taken as a cocktail with cyclophosphamide (FC), or with cyclophosphamide and rituximab (FCR) which can be administered orally or intravenously. This review will discuss the behaviour of such chemotherapeutic agents and how specific genetic abnormalities affect the efficacy of such agents.

Introduction

B cell chronic lymphocytic leukaemia (CLL) is characterised by the monoclonal expansion of $CD19^+$, $CD5^+$, and $CD23^+$ B cells which do not produce effective antibodies (Chiorazzi *et al*. 2005). The rate of incidence is around 4.2/100000/year increasing to >30/100000/year at an age of >80 years, ranking this type of leukaemia as the most common in the Western world (Chiorazzi *et al*. 2005, Eichhorst *et al*. 2011). The median age at diagnosis is 72 years with only 10% of CLL patients reported to be younger than 55 years (Eichhorst *et al*. 2011). Discovery of the disease is usually through routine health check-ups by the elderly.

CLL is known to be a clinically heterogeneous disease that originates from B cells that may differ in activation, maturation state or cellular subgroup (Chiorazzi *et al*. 2005). Some patients with CLL survive for many years without the need for treatment and eventually succumb to an unrelated disease while others have a fatal disease despite undergoing treatment (Chiorazzi *et al*. 2005). The age of patients diagnosed with CLL is deemed to be an insignificant prognostic factor in determining the clinical course of the disease (Rai *et al*. 1975, Binet *et al*. 1977). Because of this, well-defined parameters for clinical staging are required.

There are two staging systems used in clinical settings today. Rai *et al.* (1975) proposed a staging system which is now commonly used in the United States. The Rai system was based on the fact that CLL is a disease of progressive accumulation of incompetent B lymphocytes: stage 0, peripheral and bone marrow lymphocytosis (>15,000/mm^3); stage I, lymphocytosis with enlarged lymph nodes; stage II, lymphocytosis with enlarged spleen (splenomegaly) or liver (hepatomegaly) or both (hepatosplenomegaly); stage III, lymphocytosis with anaemia (haemoglobin (Hb) <11 g/dL); and stage IV, lymphocytosis with thrombocytopenia (platelet count <100,000/mm^3) (Rai *et al*. 1975). From this, Binet *et al.* (1977, 1981) aimed to simplify the Rai system, describing the system to have too many defined stages. The Binet system is now widely used in Europe and was proposed after isolating important prognostic factors at diagnosis and defining three prognostic groups: group A (Binet A), no anaemia, no thrombocytopenia, less than three

involved areas (Rai stage 0, I and II); group B (Binet B), no anaemia, no thrombocytopenia, three or more involved areas; group C (Binet C), anaemia (Hb < 10 g) and/or thrombocytopenia (platelet count < 100,000/mm^3, Rai stage III and IV) (Binet *et al*. 1977, Binet *et al*. 1981).

At present, important cellular markers that are useful in predicting the aggressiveness of the disease have been identified. Most of these markers are genetic aberrations found in patients with the disease and predicts which treatment strategies serves to be most beneficial (Cheson 2011). It is important to realise that treatments readily available do not serve to cure the disease. Rather, they aim only to increase the period of remission for patients (Chiorazzi *et al*. 2005).

First-line Treatment

Treatment of CLL poses a challenge due to its heterogeneous clinical progression. Prognostic subgroups in CLL is defined by chromosomal abnormalities and this becomes a factor in choosing the effective treatment (Juliusson *et al*. 1990, Zenz *et al*. 2011).

The discovery of chlorambucil in the 1950s as the first-generation of alkylating agents remained the standard treatment for chronic lymphocytic leukaemia as well as other haematological malignancies, including chronic myeloid leukaemia and myeloma (Catovsky *et al*. 2011). However, the drug was replaced by fludarabine (a purine analogue) after several phase III trials found that the rate of complete remission (CR), the rate of response, the duration of the response and the progression-free survival (PFS) was significantly better among patients treated with fludarabine than with chlorambucil (Rai *et al*. 2000). Fludarabine showed better results to chlorambucil with regards to overall response (OR) rate in early stages Binet A and B but not in advanced Binet C. As such, fludarabine-based combination treatments have become the new standard in patients (Eichhorst *et al*. 2009).

Cyclophosphamide is a drug that is commonly used in combination with fludarabine (FC) in the treatment of CLL. This combination of drugs yielded improved CR, OR and PFS in phase

III trials in patients with previously untreated CLL compared with those treated with fludarabine alone (Flinn *et al*. 2007). Similar results were observed by Eichhorst *et al.* in younger patients with combination therapy resulting in significantly higher CR and OR rate than treatment with fludarabine alone (Eichhorst *et al*. 2006).

Rituximab, a chimeric, monoclonal antibody, that targets the CD20 cell surface antigen, is further agent used in combination with fludarabine and cyclophosphamide. The agent binds CD20 on B cells, triggering natural killer (NK)-cell mediated antibody-dependent cellular cytotoxicity (ADCC). Laser scanning confocal microscopy shows that rituximab causes CD20 to cap at the B cell surface. Proteins, including intercellular adhesion molecule 1, and moesin are then recruited to the cap leading to the microtubule organising centre (MTOC) becoming polarised towards the cap. This polarisation increases NK cells' ADCC activity on B cells by 60% compared to unpolarised B cells (Rudnicka *et al*. 2013). In an international, multicentre, randomised trial of 552 patients with Binet stage A (1%), B (59%), or C (31%) disease, patients were randomly assigned to receive rituximab with FC (FCR) or FC alone. The FCR treatment significantly improved PFS in patients with previously treated CLL (median, 30.6 months for FCR versus 20.6 months for FC). The event-free survival, response rate, CR rate, duration of response, and time to new treatment or death were also improved. (Robak *et al.* 2010). Further tests were completed on relapsed CLL patients i.e. patients that achieve CR or partial remission but, after a period of 6 months or more, demonstrate the presence of disease progression and refractory CLL patients which include patients that had treatment failure (eg. nonreponse, stable disease or progressive disease) (Hallek *et al*. 2008a). The addition of rituximab to FC in this control group led to a observed response rate overall of 74% with 30% CR, indicating the addition improved the quality of response (Badoux *et al*. 2011).

Genetic Aberrations in Patients with CLL

The identification of specific gene aberrations and mutations in patients with CLL is crucial for distinguishing biological and clinical

subgroups of patients. Such aberrations act as prognostic markers that allow further understanding of the disease and ensure better management of patients with regards to treatment. Conventional cytogenetic analysis is only able to detect chromosomal aberrations in 40–50% of cases; due to the low mitotic activity of cells *in vitro* (Dohner *et al*. 2000). The most common aberrations found with this type of analysis are trisomy 12 and chromosome band 13q14 abnormalities (Juliusson *et al*. 1990). Fluorescence in situ hybridization (FISH) can detect these genomic aberrations in about 80% of CLL patients, not only in dividing cells, but also in interphase nuclei (interphase cytogenetics) (Dohner *et al*. 2000). The most frequent chromosomal aberrations in CLL include a deletion of 13q (55%), a deletion in 11q (18%), trisomy 12q (16%), a deletion in 17p (7%) and a deletion in 6q (6%) (Dohner *et al*. 2000). Defining subgroups of patients that show each genetic abnormality is important in predicting the disease progression as well as the effect of the treatment chosen. Of these chromosomal aberrations, 11q23 deletion, 17p13 deletion and p53 mutation have significant clinical consequences with regards to treatment (Table 1).

11q23 Deletion and Mutation

Deletions of the long arm of chromosome 11 (11q) are one of the most frequent chromosomal aberrations in various types of lymphoproliferative disorders. However in CLL, 11q deletions accounts for about 18% of patients chromosomal change, making it the second most genomic aberration present in CLL (Dohner *et al*. 2000). 11q deletions were seen to have a negative prognostic effect primarily in younger patients (Dohner *et al*. 2000).

Interphase cytogenetic study have identified a new clinical subset of CLL that is defined by the deletion of a genomic region in chromosome bands 11q22.3-q23.1 (Dohner *et al*. 1997). This chromosomal region includes the *Ataxia Telangiectasia Mutated (ATM)* gene in almost all cases. ATM is a serine/threonine protein kinase involved in DNA repair, recombination and the regulation of the cell cycle (Negrini *et al*. 2010). It is also the principal activator of the P53 protein (Austen *et al*. 2005). Based on the status of the

residual *ATM* allele, 11q deletion in CLL patients can be divided into two subgroups: those with wild-type residual *ATM* allele and those who have acquired mutated residual *ATM* allele in the progression of the disease. FC treatment *in vitro* leads to the phosphorylation of downstream ATM targets only when the wild-type *ATM* gene is retained. Therefore, complete loss of the *ATM* gene has defective responses to chemotherapy which in turn leads to a poorer clinical outcome. This poor outcome has also been associated with the mutated genes defective phosphorylation of downstream ATM targets (Austen *et al*. 2005, Austen *et al*. 2007).

17p Deletion and Mutation of p53

17p deletion occurs in as much as 7% of the cases of CLL, frequently found in patients with a more advanced disease (Dohner *et al*. 2000, Oscier *et al*. 2002). Patients in the groups with 17p as well as 11q deletions exhibit rapid disease progression with a median time from diagnosis to first treatment of 9 and 13 months, respectively (Dohner *et al*. 2000). The deletion almost always includes the *P53* gene. P53 is a tumour suppressor protein involved in regulating cell growth and death and, as such, has been known to be the 'guardian of the genome' for its role in preventing the replication of altered DNA, which could eventually lead to tumourigenesis (Sionov & Haupt 1999). Around 80-90% of patients with 17p deletions that have *P53* mutations were identified in older patients (Zenz *et al*. 2010). Mutations involving *P53* is a characteristic present in almost all types of cancer. The residues most frequently mutated are at positions 175, 179, 248 and 273 of the amino acid sequence (Zenz *et al*. 2010). Rare tumour-derived mutations at residue 175, such as 175Ser, 175Leu and 175Pro, have shown loss of the protein's apoptotic function (Rowan *et al*. 1996).

Response to chemotherapy was significantly worse in patients with *P53* mutations or 17p deletion. Those with *P53* mutation showed a median PFS of 23.3 months compared to those without *P53* mutation where PFS median is 62.2 months (Zenz *et al*. 2010). Assessment of potential treatment effect was achieved by entering patients into a randomized CLL4 trial to compare F versus

FC as first-line treatment. The study suggested FC as the potential treatment for patients without *P53* mutations with CR found in 22.1% of patients compared to 5.6% CR rate in the F arm (Zenz *et al*. 2010). However, there was 0% CR in each treatment arm for patients with *P53* mutation and further research has yet to be conducted in this area (Zenz *et al*. 2010).

IGHV Mutations

Some CLL cells have somatically mutated immunoglobulin heavy chain V-III region VH26 (*IGHV*) genes, indicating that the cell of origin has passed through the germinal centre (Hamblin *et al*. 1999). *ATM* mutations have been shown to be associated with unmutated *IGVH* genes. However, the prognostic effect of an *ATM* mutation is independent of *IGVH* mutation status suggesting that mutations in the *ATM* gene alone lead to reduced overall and treatment-free survival periods (Austen *et al*. 2005). *P53* mutations were also shown to be associated with unmutated *IGHV* region genes (Zenz *et al*. 2010).

Patients with unmutated *IGHV* genes exhibit a more malignant disease and have much shorter survival than those with mutations, which is consistent with the fact that those with *ATM* and *P53* mutations also show a more malignant disease (Hamblin *et al*. 1999, Austen *et al*. 2005, Zenz *et al*. 2010). On the other hand, the mutated gene seems to be more favourable. In a multivariate analysis of prognostic factors in CLL, patients with mutated *IGHV* genes showed to have superior survival compared to those with unmutated *IGHV* genes (median survival of 310 and 119 months, respectively) (Oscier *et al*. 2002).

Table 1. Summary of genetic prognostic factors and respective potential treatment approach in patients with CLL and 1st line treatment indication. (Zenz et al. 2011).

	Risk factor	Incidence (1st line treatment)	Treatment approach
Ultra high risk (~10-15%)	17p deletion	~5-8%	Clinical trial with investigation agent acting independent of p53, allogeneic steam cell transplantation
	TP53 mutation	~4-5%	
	F-refractory CLL	~5%	
High risk (~70%)	Unmutated *IGHV*	~60%	FCR, maintenance trials, investigational agents + FCR
	V3-21 usage	<1%	
	High ß-2M (TK)	~20%	
	11q deletion		
Low risk (~20%)	Mutated *IGHV* (and none of the above)	~22%	FCR, De-escalation in clinical trials

Conclusions

Clinical treatment of CLL is a challenge due to the many genetic aberrations found in patients (Zenz *et al*. 2011). As previously mentioned, combination treatment with fludarabine, cyclophosphamide, and rituximab behaves differently from patient to patient depending on factors which include such genetic abnormalities but also, whether patients have stable, relapsed or refractory type of the disease (Austen *et al*. 2007, Zenz *et al*. 2010, Badoux *et al*. 2011).

Toxicity and other side-effects are major problems in treatment strategies. No excessive toxicity was found when FC combination treatment was implemented (Flinn *et al*. 2007). However, more thrombocytopenia and leukopenia was caused by FC treatment in younger patients (Eichhorst *et al*. 2006). FCR treatment was also well-tolerated with no significant detrimental effects found on quality of life in previously treated CLL patients (Robak *et al*. 2010). However,

in a study of the effects of FCR in previously untreated patients with advanced CLL, early death was observed due to toxicity and secondary malignancies caused by the treatment. Increased cases of neutropenia and leukopenia were also found. (Hallek *et al*. 2008b).

Learning from all of this, a pharmacogenomic approach to treatment would be more beneficial than going with the more generalised approach. Moreover, better correlation between chemotherapeutic and chemoimmunotherapeutic agents with the clinical history of patients and which agents would suit best for a given clinical history would prevent toxicity and genetic abnormality issues from arising in the care of CLL patients.

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