

# Kinase inhibitor therapies for Chronic lymphocytic leukaemia (CLL): SYK, BTK and PI3K inhibitors

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**Abstract.** Chronic lymphocytic leukaemia (CLL) is a prevalent tumor disease in developed countries, and related therapies have been designed. However, CLL is still incurable. Chemoimmunotherapy is effective in inhibiting the proliferation of CLL cells, but nonspecific treatment can affect the growth of other immune cells. Kinase inhibitors are considered to be effective treatments for CLL as their anti-proliferation effects, and currently, popular kinase inhibitor therapies include SYK, BTK, and PI3K inhibitor therapy. PI3K is characterized by high efficiency and low side effects compared with the other two kinase inhibitor therapies, for instance, idelalisib and duvelisib. This review compares the advantages of each kinase inhibitor therapy through relevant studies and concludes that duvelisib has significant advantages and promising prospects compared to other CLL drugs. Further research may focus on exploring the mechanism of the role of kinase inhibitors in CLL as well as the clinical trials of kinase inhibitors in CLL patients.

**Keywords:** Chronic lymphocytic leukaemia, SYK, BTK, PI3K, idelalisib, duvelisib

## 1. Introduction

Chronic lymphocytic leukaemia (CLL) is one of the highest prevalence which happens in adults of developed countries. The pathogenesis of CLL is the accumulation of B-cells which are defective for their functions in the body [1]. The accumulation of malignant lymphocytes is due partly to the activation of B-cell receptor (BCR), causing the increase in proliferation and inhibiting the apoptosis of malignant lymphocytes [2]. The key feature of CLL is that programmed cell death lost its normal function, although the reason why CLL can transfer normal B cells to be malignant is still unclear. Abnormalities in the apoptotic pathway appear to explain why the survival rate of mature malignant cells can be increased [3].

In recent years, therapies based on different mechanisms of action have been developed for CLL, including chemotherapy and immunotherapy. Clinical application in chemoimmunotherapy was first marked by steroids and alkylating agents, then nucleoside analogues which are mainly represented by fludarabine have been applied in the process of treatment of CLL and greatly promoted the progress of treatment research of CLL [4]. Nucleoside analogs intervene in DNA synthesis, repair and expression during cell division by entering into target cell DNA and RNA. However, this non-specific cell therapy will not only results in the reduction of target CLL cells, but also the proliferation process of other non-abnormal immune cells will be inhibited, resulting in lymphopenia, indicating that nucleoside analog therapy is not a risk-free approach [4]. Therefore, it is urgent to reduce the risk by combining fludarabine with other compounds, such as FC which is to combine fludarabine and cyclophosphamide together, and FCR which is to add rituximab to FC. However, results showed that poor prognosis still is the big problem with these methods [4]. Therefore, new therapeutic approaches based on different principles need to be developed and applied.

This review discusses currently available treatments for CLL based on kinase inhibitors, introduces the mechanisms of the therapies as well as their advantages and disadvantages, and focuses on phosphatidylinositol-3 kinase inhibition method.

## 2. Kinase inhibitors

Because of the potential adverse effects of chemoimmunotherapy, attempts have been made to target cell therapy by understanding the underlying mechanisms of CLL. Among the receptors of CLL cells, BCR has been considered to play a central role [5]. BCR, a receptor which is encoded by immunoglobulin genes and binds with the membrane of B-cell, is specific for B-cells and promising to be used to develop drugs that are highly specific for CLL cells. An increasing number of small pharmaceutical molecules targeting BCR kinases have been developed recently [6].

### 2.1. SYK inhibitors

Splenic tyrosine kinase (SYK), a kind of tyrosine kinase which does not bind with specific receptor, transmits signals from B-cells and has been considered an important adaptive immune receptor signal [7]. SYK is activated by (Ig)E and IgG which are Fc receptors linked to immunoglobulins, the activation occurs in the microenvironment of some patients and causes weak phagocytic response [7]. Therefore, SYK is critical in maturation and survival process of the entire lineage of B-cell [8]. Thus, the inhibition for SYK has been considered as a possible therapy of CLL.

Fostamatinib, a kind of SYK inhibitor, has been developed as oral drug for autoimmune disease such as CLL, by inhibiting the signal transmission process of BCR by SYK, fostamatinib can promote apoptosis of abnormal B cells [8].

However, the outcome of SYK inhibitor therapy is controversial due to the adverse reactions and excessive consumption of B lymphocytes after the drug. Although the adverse effects of SYK inhibitors are not often in the form of severe toxicity, neutropenia, nausea, diarrhoea and thrombocytopenia are common clinical manifestations [6]. In a study of 11 B-cell lymphoid malignancies patients, blood was obtained from patients after they took 200 or 250mg fostamatinib as treatment and used to analyze the influence of fostamatinib on B-cells in vitro [8]. The study have found that after SYK inhibitor treatment, although the amount of B-cells which are in mature and T3 transitional states in vivo will not be greatly affected, the growth of B-cells in T1/T2 transitional state is severely inhibited and that also leads to deficiency of B cells in peripheral lymphoid organs [8]. These results indicate that patients may face the problem of reduced immunity due to the lack of immune cells after receiving SYK treatment. Thus, SYK therapy is still not a perfect method, and further research is needed to eliminate adverse effects.

### 2.2. BTK inhibitors

Bruton's tyrosine kinase (BTK) has been considered as key regulator for receptors of B-cells which controls processes involved in all stages of B cell development [9]. The drugs which target the BCR pathway have received a lot of attention and research. Ibrutinib, a BTK inhibitor, has been developed as a therapeutic method. By binding with the C481 residue of BTK irreversibly, ibrutinib causes the inactivation of BTK, inactivation of the kinase further induces CLL cells to undergo apoptosis, blocks B-cell receptor signaling and inhibits proliferation. These remarkable properties of ibrutinib make it highly effective in inhibiting CLL cells [9]. In addition to their high inhibitory effect on cancer cells, some BTK inhibitors can also inhibit IL-2-inducible T-cell kinase (ITK) to modulate the function of T lymphocytes, which also gives ibrutinib the property of decreasing the toxicity of CAR-modified T lymphocytes and increase their efficacy [10]. Research has shown that ibrutinib can effectively inhibit BCR signaling in vivo and in vitro, while preventing lymphocyte stem cells from homing to central lymphoid organs and mature lymphocytes from homing to peripheral lymphoid organs, so the drug has gained the legal approval in European and U.S [6].

However, there are still problems with ibrutinib. In an in vitro study of ibrutinib, CD19+ ALL cell lines and CD33+ AML cell lines were exposed to different concentrations of ibrutinib with BiAbs at non-toxin concentration, the result showed that acute exposure of patients to BTK inhibitors has a high probability of impairing T lymphocyte activity and disrupting target cell lysis, the phenomenon is very common in malignant tumors for which BTK inhibitors cannot directly produce anticancer

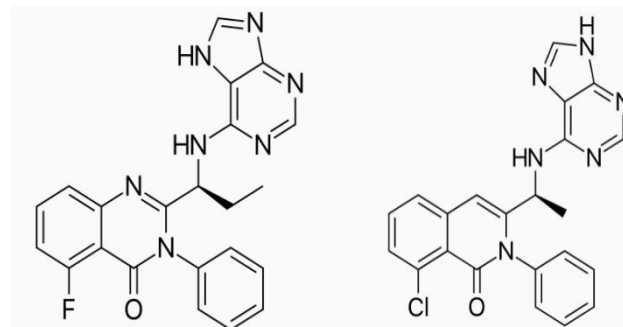
effects and reduces the overall treatment outcome [10]. On the other hand, since BTK inhibitors exist off-effects on other kinases such as JAK3, the adverse reaction of ibrutinib such as atrial fibrillation still be a problem [6].

### 2.3. PI3K inhibitors

Phosphatidylinositide-3-kinase (PI3K) is an important kinase for BCR-related pathways. There are three PI3K isoforms. The most important isoforms for CLL are p110 $\delta$  and p110 $\gamma$ , which have been considered key isoforms for CLL PI3K inhibiting therapies [11]. P110 $\delta$ , which is also called PI3K $\delta$ , is formed by a regulatory subunit and catalytic subunit [12]. As a class I PI3K, P110 $\delta$  can converse PIP2, which is short for phosphatidylinositol 4,5-bisphosphate, to PIP3, which is short for phosphatidylinositol 3,4,5-trisphosphate, the Akt that is a kind of signaling protein and relay signals to pathways to control and regulate the life activity of cells can be activated by PIP3 [13]. P110 $\delta$  mainly mediate the process of production of antibody, proliferation, antigen presentation and BCR signaling for B cells, the isoform is essential for activation and function for B and T cell, normal function for T-regulatory cells, mast cell Fc receptor signaling and Th1-Th2 differentiation [11]. The inhibition of P110 $\delta$  has been shown to inhibit the proliferation of various lymphoma cells and malignant B-cell while allowing normal immune cells to survive [13]. Although research showed that the B cell activation and function regulation of p110 $\gamma$  is less active than P110 $\delta$ , p110 $\gamma$  has a key role in leukocyte chemotaxis and the homing process for progenitor cells [11]. The inhibition of p110 $\gamma$  affects tumor cells in their normal differentiation and migration process and inhibits the proliferation of tumor cells [12]. That means the inhibition of both p110 $\gamma$  and p110 $\delta$  isoforms may provide an opportunity for new therapeutic treatment of B-cell malignancies [11-13].

#### 2.3.1 Idelalisib

Idelalisib is a kind of small-molecule oral P110 $\delta$  inhibitor that is selective and potent. Idelalisib has been proved as an effective method to treat CLL within an acceptable range of toxicity in phase 1 studies [12]. Therefore, after the positive result was gotten from phase 3 trial, idelalisib has been approved to be used with rituximab which is a kind of anti-CD20 antibody by FDA in the US in 2014 [14].



**Figure 1.** The structure of idelalisib (left) and duvelisib (right)

In the research of the team of Susan started in September, 2010, they took a phase II clinical trial for the combination of idelalisib and rituximab in older adults who still had CLL or SLL (small lymphocytic leukemia) [14]. The whole research was performed as an open-label study that contains 64 untreated CLL or SLL older patients. The patients were treated with idelalisib 150mg twice daily and rituximab 375 mg/m<sup>2</sup> weekly for 48 weeks. The result showed that the overall response rate was 97% which contained 19% complete response, normal adverse effects were diarrhea, rash, pyrexia, ect. The overall results proved that the therapy of idelalisib and rituximab combination is effective and active to control the development of CLL and provided sufficient data for further research. Compared with the research which had been done with single-agent idelalisib, the combination of idelalisib and rituximab more significantly reduced the number of lymphocytes in patients, demonstrating that compared with single-agent, the therapy method which combine idelalisib and rituximab together is more promising [14].

A phase 3 clinical trial for idelalisib and rituximab was done by Sharman's team [15]. The research focused on the determination of different effects between single-agent rituximab and the combination of idelalisib and rituximab. The research was performed as a randomized and double-blind trial. The CLL patients who had previously been treated and had recurrent CLL randomly received either the combination of 150mg rituximab with placebo or the combination of 150mg rituximab with idelalisib twice daily. The results showed that the ORR of the therapy method which combine idelalisib and rituximab together was 83.6% which was a significant improvement compared to the ORR of the combination of placebo and rituximab therapy which was 15.5%, and the result has also been proved with longer follow-up. The most normal adverse effects in the research were pyrexia, fatigue, and diarrhea. Although the extent of diarrhea increased as the exposure to idelalisib and rituximab longer, the increase in the extent of diarrhea stopped with long-term exposure to the idelalisib and rituximab. The overall results confirmed that compared with single-agent rituximab, the therapy method which combine idelalisib and rituximab together is a more effective and safer method [15].

According to the results of the clinical trials, idelalisib has been evaluated as a safe, efficient treatment for CLL. At the same time, experiments have shown that the combination of idelalisib and rituximab can improve the therapeutic effect of idelalisib or rituximab, and this combination therapy achieves higher efficiency within the acceptable toxicity range.

### 2.3.2 Duvelisib

Duvelisib, an oral drug for CLL treatment, which is also called IPI-145, is the inhibitor of both p110 $\gamma$  and p110 $\delta$  [13]. Compared with idelalisib, which is highly selective to P110 $\delta$ , duvelisib, which is a dual PI3K $\gamma$  and PI3K $\delta$  inhibitor, shows greater activity in the process of CLL therapy [13]. Research have shown that by disrupting BCR signaling and cell chemotaxis process mediated by CXCR12, duvelisib is effective to induce the apoptosis process of CLL cells [16].

A phase I clinical trial of duvelisib was done to figure out the efficacy, safety, pharmacodynamics, pharmacokinetics and maximum tolerated dose of duvelisib to study the feasibility of the drug development [13]. The clinical trial was performed as an open-label study with increasing dose, patients were adults who were suffering advanced hematological malignancies and had a life expectancy of more than 3 months. At the same time, patients need to be intolerant to the established therapy, and patients who have previously been exposed to PI3K inhibitors or have other serious diseases such as HIV infection and renal insufficiency will be excluded from this study. Duvelisib was administered to patients in oral capsule form twice a day unless the patients had developed unexpected and intolerable disease development or drug toxicity. The normal cycle for the trial was 28 days. Plasma samples were obtained from the predose phase and day 1 of the first and second cycles to analyze the pharmacokinetics of duvelisib by using liquid chromatography and tandem mass spectrometry. Efficacy was determined by CR (complete response), which was determined by computed tomography scans, bone marrow biopsy, and positron emission tomography. According to the pharmacokinetics results, duvelisib could rapidly reduce the concentration PI3K signaling markers such as p-AKT, indicating that duvelisib has relatively high pharmacologic activity in vivo. The maximum p-AKT occurred when 25mg of duvelisib was taken twice a day, and the maximum tolerated dose of duvelisib was 75mg twice a day. The result showed that multiple-dose administration could increase the absorption of duvelisib. The results showed the acceptable safety profile of duvelisib for patients who have advanced malignancies, and adverse events of duvelisib were controllable by adjusting the amount of administration. Most adverse events were grade 1-2. Cytopenias and infections are common adverse events which only happened in heavily pretreated patients. The result showed that the duvelisib monotherapy has relatively high activity on CLL with acceptable safety profile. Further research is required to support the results [13].

Following positive results from phase I and II clinical trials, a phase III clinical trial was done by Flinn's team [17]. The open-label and randomized trial characterized the pharmacodynamic profile of duvelisib monotherapy compared with ofatumumab monotherapy which is a kind of anti-CD20 antibody which is used in US to treat CLL. Patients would be excluded from the study if they had the experience of treatment with other kinase inhibitors such as BTK and PI3K- $\delta$  inhibitors, were

refractory to prior ofatumumab treatment, etc. With the exception of the first cycle, which was 21 days, patients were randomly assigned to 25 mg duvelisib to self-administer twice daily for consecutive cycles which were 28 days to complement the dosing of ofatumumab infusions. 18 cycles of administration of duvelisib were allowed until patients had disease progression or unacceptable toxicity. As the first occurrence of progressive disease was determined by IRC or death because of any case, the key end point was determined and used for assessments. In addition to primary end point, some essential secondary end points such as ORR and OS were also determined. The results showed that duvelisib can effectively reduce the target lymph nodes about 85% for most patients who had taken the administration, which was larger than the reduction by the treatment of ofatumumab which was about 16%. As the results showed before, most of the adverse events of duvelisib were 1-2 grade events such as cytopenias and constitutional symptoms. Grade 3 adverse reactions such as colitis that were common in clinical studies of idelalisib were also rarely seen with duvelisib. All results showed high efficacy and acceptable safety profile of duvelisib against CLL [17].

**Table 1.** The major CLL drugs on the market and the corresponding adverse reactions

	Category	Common adverse reaction
Fludarabine	nucleoside analogues	Lymphopenia, arm, back, or jaw pain, constipation, chest pain or discomfort
Fostamatinib	SYK inhibitor	Neutropenia, nausea, diarrhoea and thrombocytopenia
Ibrutinib	BTK inhibitor	Diarrhea, bleeding, upper respiratory tract infection, fatigue and cardiac side effects
Idelalisib	P110 $\delta$ inhibitor	Anemia, pneumonia, fever, chills, nausea
Duvelisib	P110 $\delta$ +P110 $\gamma$ inhibitor	Infection, Decreased Kidney function, common cold, low Energy

### 3. Conclusion

SYK inhibitor therapy inhibits B-cell maturation and survival by inhibiting the signaling kinase SYK of B-cells. However, because of adverse reactions and excessive consumption of B lymphocytes, SYK inhibitor therapy has gotten a lot of skepticism. Some studies also showed that SYK inhibitor therapy could lead deficiency of B cells in peripheral lymphoid organs and cause reduced immunity in patients. BTK inhibitor therapy is highly effective in inhibiting CLL cells by inactivating BTK to induce apoptosis of CLL cells. BTK inhibitor such as ibrutinib can also modulate the function of T lymphocytes and decrease the toxicity of CAR-modified T lymphocytes and increase their efficacy. However, target cell lysis can be disrupted, and T lymphocyte activity can be impaired if acute exposure of patients to BTK inhibitors and the off-effect property of BTK inhibitors can inhibit such as JAK3 and cause side effects. Compared with the above treatments, PI3K inhibitor therapy demonstrates superiority. By inhibiting the key isoforms of CLL phosphatidylinositol-3-kinase which are p110 $\delta$  and p110 $\gamma$ , the proliferation and growth of CLL cells were significantly inhibited. As a P110 $\delta$  inhibitor, idelalisib can reduce the proliferation of various lymphoma cells and malignant B-cell while allowing normal immune cells to survive. Multiple clinical trials have shown that idelalisib has high efficiency within the acceptable toxicity range. The therapy method which combines idelalisib and rituximab together is a more effective and safer method for CLL therapy. Compared with idelalisib, duvelisib can inhibit both PI3K $\gamma$  and PI3K $\delta$ , and experimental data showed that duvelisib has fewer adverse effects than idelalisib, which make duvelisib a better method for CLL therapy. At the current stage of CLL therapy, duvelisib exhibits greater developability, and more relevant experiments can be completed to explore relevant feasible combination therapy of duvelisib to achieve higher efficacy and lower toxicity.

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