Signal Transduction Therapies for Treatment of Chronic Leukemias

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Abstract: The term signal transduction includes the interaction of external signals that are driven by hormones, growth factors, chemokines, cytokines and small molecules such as ATP in order to receive a cellular response. These responses in turn effect gene transcription and translation, cell division, survival and death upon many signaling networks related with malignancies. Since almost all diseases exhibit dysfunctional aspects of the signaling pathways, drug discovery studies in means of signal transduction therapies have an accelerating importance including chronic leukemias.

Among chronic leukemias, chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML) are being investigated extensively for abnormalities of cellular signaling pathways. This review focuses on targeting B-cell antigen receptor (BCR) signaling and Wnt/β-Catenin/LEF-1 signaling pathways and their inhibitors that provided new opportunities for development of more effective therapies for CLL. Besides this, signaling network systems such as RAS/RAF/MAPK and JAK/STAT will be discussed that contribute high oncogenic activity of BCR-ABL1 oncoprotein in CML. Finally the molecular targets in treatment duration with clinical insights will be discussed.

Keywords: Chronic lymphocytic leukemia, Chronic myeloid leukemia, Signal transduction pathways, Treatment.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common type of lymphoid malignancy in adults aging between 65 -72 years and represents 11 % of all blood cancer types [1]. CLL is characterized by the clonal proliferation and accumulation of mature CD5+ B cells in the peripheral blood, bone marrow and lymphoid organs [2]. CLL is a heterogeneous disease and can be classified into two major groups according to the mutational status of Ig V: somatically unmutated Ig V genes (U-CLL) and mutated Ig V genes (M-CLL). U-CLL originates from naive B-cells in aim to compete against common pathogens. About 50% of CLL diagnosed patients' leukemic cells have somatic hyper mutation in rearranged immunoglobulin heavy-chain variable region genes; which in turn results in a more favorable clinical outcome. Immunoglobulin gene mutational status and prognosis of CLL can be associated due to median survival time: while median survival is determined as 8 years for patients with unmutated IgVH genes; approximately 25 years of survival is detected in patients carrying mutated IgVH genes [3].

The leukemic transformation starts by genetic modifications; especially deletion on 13th chromosome [del(13q14)] which in turn causes deletion of some specific microRNAs (miRNA) and strengthens B cells by avoiding apoptotic cell death [4-5]. Besides this, [del (11q)], [del (17p)] and trisomy 12 may occur later in the course of the disease and these alterations predict a worse clinical outcome such as short survival time and gain of resistance to conventional DNA-damaging chemotherapies such as alkylating agents, purine analogs and rituximab [6-7]. In addition, genome wide sequencing has revealed new somatic gene mutations occurring in CLL cells; affecting NOTCH1, MYD88, TP53, ATM, and SF3B1 genes which have prognostic impact [8]. Today, for early diagnosis of CLL in which patients suffer by an aggressive form of the disease; is guided by some different molecular markers such as mutation status of IgHV gene [9], the cell surface expression of CD38 [10] and CD49d [11] and intracellular presence of ZAP-70 (ζ -chain-associated protein kinase 70 kDa) that exhibits shorter survival. Since the mutational status of IgHV gene was an important prognostic factor in CLL, the expression of ZAP-70 in CLL cells and IgVH mutational status, disease progression and survival interaction was examined in order to find a correlation. The authors detected ZAP-70 expression in cells of T-cell lineage and in leukemic cells from 32 of 56 (57%) patients with CLL. Besides this, in all patients in whom at least 20 percent of the leukemic cells were positive for ZAP-70, IgVH was unmutated, whereas IgVH mutations were found in 21 of 24 patients (87.5%) in whom less than 20 percent of the leukemic cells were positive for ZAP-70. This refers that ZAP-70 expression was correlated with IgVH mutational status, disease progression, and survival [12].

Sufficient progress in molecular biology has resulted in a better characterization and understanding of the pathogenesis and prognosis of CLL. It is now clear that

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the origin, development and survival of CLL cells are dependent on microenvironment that includes T cells, macrophages or stromal follicular dendritic cells [13]. This microenvironment provides essential proteins such as cytokines, chemokines and angiogenic factors for activation of crucial survival and proliferative signaling pathways of transformed cells [14]. The mission of the signal transduction therapy is to turn the differences of transformed cell into normal cell or induce leukemic cell apoptosis. This review focuses on targeting signal transduction pathways such as B-cell antigen receptor (BCR) signaling and Wnt/β-Catenin/ LEF-1 signaling pathways and inhibitors such as Dasatinib, Fostamatinib, Idelalisib, Perifosine, Everolimus and Ibrutinib that provided new opportunities for development of more effective therapies in CLL.

Chronic myeloid leukemia (CML) is characterized by the presence of resiprocal translocation between chromosomes 9 and 22; referred as Philadelphia (Ph +). CML displays three phases of the disease by progressing from an initial chronic phase to accelerated and blast crisis phases. Also, increased BCR-ABL1 expression due to high tyrosine kinase activity is seen in the leukemic cells as a result of genomic instability in Ph (+) cells [15].

Early CML treatment started with x-radiation applications and conventional chemotherapeutic drugs such as hydroxyurea or busulfan. Although these strategies helped to decelerate myeloid tissue enlargement and increase life expectancy in chronic phase, they caused no additional benefit in blastic duration of the illness. One of the most important improvements was achieved by allogeneic stem cell transplantation application. About half of the patients who were convenient for transplantation –especially under age of 40- became both Philadelphia and BCR-ABL1 negative and were finally cured [16]. The second climax of the therapy was the usage of interferon-alfa that provided a considerable survival length over conventional chemotherapy agents [17]. The most encouraging therapy strategy was developed by the discovery of the first signal transduction inhibitor; a class of small molecules referred as tyrosine kinase inhibitor (TKI): Imatinib Mesylate (Glivec or Gleevec, Novartis). It prevents the BCR-ABL1 oncoprotein from exerting its role in the oncogenic pathway; besides, directly inhibits the constitutive tyrosine kinase activity by blocking ATP binding site. This results in the modification of the function of various genes involved in cell cycle control, cell adhesion, cell proliferation and survival, cytoskeleton organization and finally in the apoptotic death of Ph (+) cells [18-19]. Next, other second generation TKIs were developed, namely Nilotinib (Tasigna, Novartis), Dasatinib (Sprycel, Bristol-Myers Squibb), Bosutinib (Busulif, Pfizer) and lastly Ponatinib (Iclusig, Ariad) [20-23]. However, there are still some limitations for the usage of these TKIs in clinic. For example, although imatinib is efficient in the cure of chronic phase, it is not curative enough in blastic crisis phase [24]. Besides, both first and second line TKIs might lack the efficient therapy either for developing required resistance to therapy through ABL1 gain of mutations or other mechanisms [25]. These common recurrent mutations in tyrosine kinases define the molecular biology of CML by activating signal transduction pathways that are critical for leukemic cell growth, proliferation and survival. The quest for identification of the specific signaling proteins involved in CML and understanding the biological importance of the signal transduction pathways has constructed the signal transduction therapies. Since BCR-ABL1 exerts its oncogenic activity through signaling network systems such as RAS/RAF/MAPK and JAK/STAT, these pathways will be discussed specifically.

1. CHRONIC LYMPHOCYTIC LEUKEMIA

1.1. BCR Signaling

Constitutively active BCR signaling is critical for chronic lymphocytic leukemia cell growth and survival. BCR pathway is aberrantly or excessively activated in CLL cells and therefore represents a promising target for therapeutic intervention. Normal B lymphocytes receive two types of signals from their BCRs. In normal functioning B lymphocytes, binding of BCR by an external antigen triggers a signaling pathway that controls proliferation, survival, differentiation, apoptosis, anergy and antibody production [26]. Activated BCR in the signaling pathway recruits spleen tyrosine kinase (SYK) and the SRC kinase LYN that phosphorylate immunoreceptor tyrosine-based activation (ITAM) motifs on the cytoplasmic domains of the Ig co-receptors CD79A and CD79B. Phosphorylation results in recruitment and activation of Bruton's tyrosine kinase (BTK) and PI3K, subsequently activating many downstream targets including AKT/mammalian target of rapamycin (mTOR), NF-κB, and ERK pathways. The basic interactions of signaling pathways involved in CLL are given in Figure **1**.

The second type of signal occurs in the absence of an external ligand and termed as "tonic BCR signal". The role of this signal has still not been fully understood in normal B cell biology; but, recent studies

suggest that it is essential for both survival of mature B cells and normal B cell development with correct maturation process. As aberrantly activated BCR signaling plays a key role in the pathogenesis of CLL by promoting leukemia cell survey in both ligand-induced and nonligand-dependent events, blocking BCR signaling via various components holds great therapeutic potentials in CLL [27].

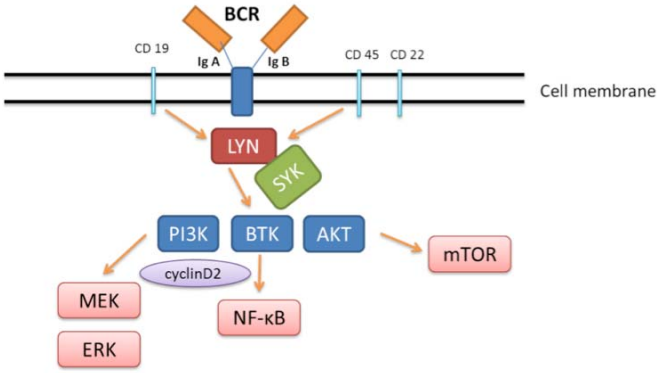

Figure 1: Basic signaling interactions in CLL.

1.2. Targeting BCR Signaling by Protein Tyrosine Kinases

The four proximal protein tyrosine kinases mediating BCR signaling LYN, SYK, PI3K, and BTK are all found to be over-expressed and constitutively activated in CLL cells compared to normal B lymphocytes. These upstream kinases can be targeted by small molecule inhibitors which are being studied in preclinical and clinical studies. These agents are discussed below.

1.2.1. Targeting LYN and LYN Inhibitors with Clinical Experience

LYN initiates BCR signaling by phosphorylating ITAMs in the Igα and Igβ chains of the BCR. The phosphorylated ITAMs recruit SYK kinase, which then gains activation through SRC-family kinase-dependent phosphorylation at Y352 and trans-autophosphorylation at YY525/526. LYN not only phosphorylates and thereby activates SYK; but also activates phosphatases that in turn inhibit BCR signal transduction. Thus, LYN functions as both a positive and negative regulator of BCR signaling [28].

Targeting LYN with SRC-family kinase inhibitors such as PP2 and Dasatinib resulted in induced leukemic cell apoptosis [29-30]. But, it is still unclarified whether the cytotoxic effect of these agents is due to inhibition of LYN or inhibition of related kinases. In clinics, a phase 2 trial study (including 15 relapsed/refractory CLL patients), the cases were treated with 140 mg dasatinib which resulted with 20% overall response rate and progression-free survival length of 7.5 months [31].

1.2.2. Targeting SYK and SYK Inhibitors with Clinical Experience

Following antigen binding to BCR, LYN phosphorylates SYK that results in amplification in initial BCR signal and activates the downstream signaling cascade. Besides this, SYK is also involved in chemokine, integrin and Fc-receptor signaling [32]. SYK is constitutively phosphorylated on the Y352 residue of CLL cells; but, there is no correlation between the degree of SYK activation and clinical or biologic features of more aggressive disease [33].

Targeting SYK induces moderate levels of apoptosis in unstimulated CLL cells, further suggesting that the cell autonomous BCR signal increases leukemic cell survival [34]. SYK inhibitor Fostamatinib; R788, an oral pro-drug of the active metabolite R406 is an ATP-competitive kinase inhibitor that also inhibits a number of other kinases. Treatment of CLL cells with Fostamatinib results in blockage of transduction for extrinsic antigen-dependent BCR signals, inhibition in integrin signaling and also reduction in proliferation and survival of the malignant B cells with induced apoptosis [35]. In clinics, fostamatinib was tested in a phase 1/2 trial of relapsed or refractory non-Hodgkin lymphoma and CLL which in turn resulted with 55% achieved partial response that was considered as highest response rate in CLL patients [36]. Fostamatinib follow-up is not continuing today because the company is producing the agent as a rheumatoid arthritis treatment strategy. Although some clinical studies are going on for treatment of diffuse large B cell lymphoma, it is not clear whether fostamatinib can take place in CLL therapy. Nowadays two novel highly selective Syk inhibitors, PRT318 and P505-15 are being studied and expected to take place in clinics for CLL treatment [37].

1.2.3. Targeting PI3K/AKT/mTOR and Their Inhibitors with Clinical Experience

PI3K is a key downstream mediator of BCR signaling. CLL cells generally express high levels of active PI3K and consistent activation of this pathway increases their proliferative capacity and survival. Among several PI3K isoforms, PI3Kδ is the specifically expressed one in leukocytes and consists of two

components as regulatory p85 and catalytic p110δ subunits. When p85 binds to phosphotyrosine motifs on receptor tyrosine kinases or adaptor molecules, p110 becomes activated and phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to generate PIP3. PIP3 carries cytoplasmic kinases BTK and AKT to cellular membrane in order to form a functional signaling complex. AKT is responsible for effecting PI3K and after translocating onto cellular membrane, AKT membrane is activated by the mammalian target of rapamycin complex 2 (mTORC2). Activated AKT then phosphorylates a number of downstream targets that are essential for B-cell proliferation and survival [38].

Inhibition of PI3K activity by LY294002 inhibitor induced apoptosis of CLL cells; however it has been obstructed due to toxicity [39]. Idelalisib [(GS-1101) or (CAL-101)] is a highly selective inhibitor of the PI3Kδ which induces apoptosis in CLL cells *in vitro* and inhibits the assistive effect of many microenvironmental factors and inhibits survival signals generated by triggering of the BCR [40]. To date clinical trials CAL-101 of has not been finished but promising clinical activity was reported at the American Society of Hematology (ASH) 2010 scientific meeting as 33% partial response in CLL patients [41]. Updated data on 54 patients were presented at the American Society of Clinical Oncology (ASCO) annual meeting in 2011 as Phase I study; but overall response rate was reported as 26% [42]. Since then, combination studies accelerated and strong rational was provided for the combination of PI3K inhibitors and histone deacetylase inhibitor (HDI) in CLL clinic [43].

Since AKT is activated in CLL; it is reported that inhibition of this activation by selective inhibitor Akti-1/2 induced apoptosis of CLL cells in vitro [44]. Another study showed that AKT inhibitor AiX induced apoptosis preferentially in IgVH unmutated CLL cells [45]. Finally, Perifosine is currently being tested in a phase 2 trial in patients with relapsed/refractory CLL. They reported that Perifosine was cytotoxic to CLL cells *in vitro,* and largely induced stabilized disease *in vivo*, with an AKT-independent mechanism [46].

mTOR is an ubiquitously expressed serine/ threonine kinase involved in cellular growth and proliferation and also functions as a downstream mediator of BCR signaling through PI3K/AKT pathway [47]. mTOR also regulates cell cycle transition from G1 to S phase [48]. Rapamycin (sirolimus) is an immunosuppressive agent and also exhibits growth inhibitory and proapoptotic effect upon lymphocytes [49]. Preclinical data showed that, while rapamycin treated cells lacking functional p53 underwent apoptosis; p53 wild type cells were arrested in G1 phase but remained viable. So, as a treatment option, CLL patients lacking p53 function [(del (17p) and p53 mutations] will show worse prognosis in clinics [50]. The importance of mTOR signaling in CLL has been demonstrated in Eμ-TCL1 transgenic mouse model; where rapamycin slowed leukemia growth and prolonged the survival of the treated animals [51]. In clinics, a pilot trial of Everolimus (RAD001) in patients with advanced B-CLL exhibited some degree of activity, but was stopped earlier because of toxicity concerns [52]. Later on, the second trial was done with a larger phase II study of everolimus and finally 18% of CLL patients achieved a partial response and showed signs of severe toxicity from immunosuppression and infectious complications [53]. In the third trial, the immunosuppressive effect of everolimus in CLL was substantiated also severe infectious complications were reported [54]. However, use of everolimus in combination therapy (idelalisib, alemtuzumab, panobinostat, bortezomib, lapitinib or sorafenib) for CLL will await further clinical trials in order to handle the immunosuppressive effect of everolimus in CLL [55].

1.2.4. Targeting BTK and BTK Inhibitors with Clinical Experience

BTK is a cytoplasmic tyrosine kinase belonging to Tec family and serves an essential role in B-cell development and BCR signaling. Loss of BTK causes X-linked agammaglobulinemia (Bruton's agammaglobulinemia), which is characterized by decreased immunoglobulin production and absence of B cells [56]. Two basic models have been proposed for activation of BTK in CLL. In the first model, BCR cross-linking promotes LYN activation, which in turn recruits BTK to the plasma membrane via PIP3 that is generated by PI3K. Then, BTK is phosphorylated by SRC family kinases at Y551 and autophosphorylated at Y223 residue of BTK [57]. In the second model, BTK is activated independent from PI3K. This activation leads to calcium mobilization (by activation of PLC-ɣ2); which in turn activate the transcription factor NF-κB. Active NF-κB induces antiapoptotic gene BCL-XL expression and increases cell survival by induction of cyclin D2 [58].

Since BTK is found to be significantly overexpressed in CLL as compared to normal B cells; targeting BTK is a promising therapeutic approach for CLL treatment. In preclinical studies, BTK inhibitor

Table 1: **Kinase Targeted Therapies in Clinical Trials for BCR Signaling in CLL**

Target Kinase	Family	Function	Inhibitor	Phase	In Clinic
LYN	Src	Activate and terminate BCR signaling	Dasatinib	II	Yes
SYK	Syk/ZAP70	Upstream amplification of BCR signaling	Fostamatinib	II	Yes
PI3Kδ	Lipid kinase	Intermediary in BCR signaling pathway	Idelalisib	II	Yes
AKT	Akt	Effect upon PI3K, phosphorylate downstream targets essential for B-cell proliferation	Perifosine	II	Yes
mTOR	Serine/threonine kinase	Downstream mediator of BCR signaling	Everolimus	I/II	Combination therapy
BTK	Tec	Downstream mediator of LYN/SYK in BCR signaling, roles in microenvironment interaction	Ibrutinib	II	Yes

Ibrutinib (PCI-32765) demonstrated its selectivity towards tumor B cells by inhibiting BTK phosphorylation (by covalently binding to its cysteine-481 residue), demolishing downstream survival molecules (ERK1/2, PI3K and NF-κB), inhibiting BCR and directly inducing modest levels of apoptosis in CLL cells [59]. In clinics, the initial phase I study with this agent examined in CLL patients resulted with 60% overall response rate, treatment was well tolerated and drug related grade 3/4 toxicity was reported in only 19% of the patients [60]. Combination therapies of ibrutinib with chemoimmunotherapy are being evaluated in relapsed/refractory CLL patients targeting CD20 and the BCR pathway. High overall response rates as 90% and 100% were recently reported for the combinations PCI-32765/bendamustine/rituximab and PCI-32765/ofatumumab, respectively [60-61]. Both combinations were well tolerated.

Another selective, irreversible and potent BTK inhibitor AVL-292-001 (CNX-774) has been tested and detected that, it inhibited and silenced BTK in preclinical studies and adapted to early clinical trials. A double-blind, placebo-controlled, single ascending dose study of AVL-292-001 in healthy volunteers demonstrated favorable safety, tolerability and pharmacokinetics of the drug [62]. A preliminary data from a phase Ib trial in CLL and B-NHL have been reported. In this trial, they reported that the drug was well tolerated and displayed the typical early effects of BCR inhibitors, including the initial rise in absolute lymphocyte counts and the simultaneous reduction in lymphadenopathy [63]. There are many other BTK inhibitors that are being preclinically tested such as GDC-0834, LFM-A13 and AVL-101 with no clinical studies yet [64].

1.3. Wnt/β-Catenin/LEF-1 Signaling Pathway

There is enhancing evidence that Wnt signaling pathway that is already known to play critical roles in various types of cancer has also essential functions in B cell neoplasia; especially CLL. Wnt signaling indeed involves three different pathways. The classical Wnt/β-catenin pathway- also named as canonical Wnt pathway, the frizzled regulated planar cell polarity pathway (PCP) and finally the Wnt/Ca^{2+} pathway [65-66].

The classical canonical Wnt cascade plays critical roles in many developmental processes. It has also been included in development, regulation, proliferation and differentiation of T cell or B cell, [67-68] and also in the self-renewal of hematopoietic stem cells [69]. The transcription factors LEF/TCF (lymphoid enhancer binding factor/ T-cell factor) mediate a nuclear response to Wnt signals by interacting with β-catenin. Wnt stimulation upregulates β-catenin and by this approach β-catenin cooperates with LEF-1. Following a Wnt signal, β-catenin is stabilized and transported to the nucleus, where it binds to the LEF/TCF proteins to turn on Wnt target genes [70]. In the absence of a Wnt signal, although LEF/TCF proteins cannot activate target genes; a different type of β-catenin is post-translationally regulated by binding to the cytoplasmic tail of the E-cadherin receptor, independent from Wnt signaling [71].

As we focus on the factors that contribute constitutive Wnt signaling in CLL, Wnt ligands and receptors come forward besides β-Catenin and LEF-1. The Wnt gene family encodes secreted proteins that signal through cell surface receptors; which control development and responsible for malignant transformation in CLL [72]. A family member Wnt-3 is significantly overexpressed in CLL and has as the ability to induce proliferation of mouse pro B cells, which leads to transcriptional activation of LEF-1 [68]. Highly expressed Ror1 receptor binds to Wnt-5a and therefore activates NF-κB; leading to enhanced survival of CLL cells [73]. Besides these, Wnt-10a and

Wnt-16 were reported as the most abundant Wnt ligands expressed in CLL cells. However, Wnt-16 was also expressed in healthy B cells, but unlike Wnt-3, did not induce LEF-1-mediated transcription. In comparison to healthy B cells, CLL cells express higher levels of Wnt-3, Wnt-5b, Wnt-6, Wnt-10a and Wnt-14 ligands [74].

As for β-catenin; it takes place in cell-cell adhesion by interacting with the intracellular domain of E-cadherin, besides functions as a central molecule in the Wnt signaling pathway [75]. β-catenin based tumorigenesis in CLL comes out as a result of deregulation of β-catenin signaling properties and subsequent activation of its downstream targets. The stability of β-catenin is influenced by several posttranslational regulators; such as E-cadherin, GSK-3β, axin, conducting and APC [76].

LEF-1 is exclusively expressed transcription factor in transformed pre-B cell line; but its expression is rapidly downregulated in mature B cells [77]. LEF-1 knockout mice exhibited reduced proliferation, differentiation, and increased apoptosis of pro-B220+ cells; establishing the data that LEF-1 has significant importance in B cell development [68].

1.3.1. Targeting Wnt/β-Catenin/LEF-1 Signaling Pathway

Several therapeutic approaches have been detected in order to inhibit Wnt signaling such as DNA demethylating agents, small molecules and non-steroidal anti-inflammatory drugs. Negative regulator genes of the Wnt signaling pathway can be specified as secreted frizzled-related protein family members sFRP1, sFRP2, sFRP3, sFRP4, sFRP5, Wnt inhibitory factor-1 (WIF-1) and E-cadherin that undergo epigenetic silencing in CLL. The sFRPs are secreted glycoproteins that can competitively bind to Fzd (frizzeled related protein) receptors to form inactive receptor complexes; which in turn inhibit Wnt signaling [78]. WIF-1 competitively binds to Wnt ligands, rendering them incapable of binding to Fzd receptors and so acting as a negative modulator of the Wnt pathway [79]. E-cadherin is a receptor molecule involved in cell-cell adhesion and contains a β-catenin binding domain. E-cadherin sequesters β-catenin to the inner side of the cell membrane, thus inhibits its transactivation role in the nucleus. Loss of function mutations in the E-cadherin gene leads to cancer progression, invasion and metastasis [80]. The role of E-cadherin in hematologic malignancies was reported as that; E-cadherin expression was low or absent in CLL compared to healthy counterparts, due to hyper methylation at promoter region of E-cadherin [81]. Since epigenetic changes can be turned back by pharmacological agents; the demethylating compounds are promisingly used in order to inhibit endogenous methyl transferases to reactivate previously silenced genes. Thus usage of demethylating agents would be a promising strategy for treating CLL as an adjuvant to current drugs. The use of DNA demethylating agents named 5-azacytidine (AZA) and 5-aza-20-deoxycytidine (decitabine or DAC) has been established in the treatment of hematological malignancies [82]. There are two phase I clinical trials in progress using DAC agent [83] and AR-42 [84] in combination with chemotherapy in CLL.

Small molecules are also being used in order to modulate Wnt/β-catenin signaling. Ethacrynic acid (EA) was previously shown to be cytotoxic upon CLL cells, but due to its diuretic side effects and lack of sufficient potency, it was not accepted as a novel therapeutic agent for CLL [85]. Afterwards, a research team produced 40 amide derivatives of ethacrynic acid in order to inhibit Wnt signaling and reduce CLL cell survival [86]. These agents achieved to alter Wnt reporter activity in functional *in vitro* studies but; driving mechanism has not been fully understood yet. Besides this, three compounds named "PKF115-584, CGP049090, and PKF222-815" were studied both in vitro and in vivo with promising results. These small molecules showed inhibition of LEF-1/β-catenin target gene expression and induced apoptosis in primary CLL cells. Also the group reported significant *in vivo* tumor inhibition in xenograft tumor model without gross systemic toxicity [87]. Another identified small molecule inhibitor is, ICG-001, which downregulated β-catenin/TCF/LEF signaling by binding to cyclic AMP response element binding protein (CBP). ICG-001 was demonstrated to selectively inhibit proliferation of transformed colon cancer cells *in vitro* and *in vivo* [88]. Recently it is found that ICG-001 leads to eradication of drug-resistant primary leukemia in combination with conventional therapy *in vitro* and significantly prolongs the survival of NOD/SCID mice engrafted with primary acute lymphoblastic leukemia (ALL). Therefore, specifically inhibiting CBP/catenin transcription represents a novel approach to overcome relapse in ALL [89]. But further investigations are needed to evaluate the efficiency of ICG-001 in a CLL disease model. Since small molecules have achieved Wnt signaling inhibition at sub-micromolar concentrations, such substances are potential candidates for monotherapy or in combination with standard chemotherapeutic agents currently used in CLL therapy.

Different non-steroidal anti-inflammatory drugs have been investigated in order to determine an agent capable of inhibiting the activity of β-catenin-dependent reporter genes in malignant cell lines, by repressing β-catenin. One of these agents R-enantiomer (R-etodolac, SDX-101) was shown to induce caspase-mediated apoptosis in CLL cells *in vitro*. But due to high concentration of the determined IC_{50} dose; it doesn't seem possible to take place in clinics because of high side effect risk [90]. Besides, etodolac-derivatives SDX-101, SDX-308 and SDX-309 were reported as potential candidates for combination treatment of CLL; especially, SDX-308 in combination with chlorambucil [91].

2. CHRONIC MYELOID LEUKEMIA

2.1. Effects of BCR/ABL1 Fusion Oncoprotein on Proliferation and Survival of Leukemic Cells

Hematopoietic CML cells that carry the Ph chromosome results in BCR/ABL fusion gene; encoding $p210^{BCR/ABL}$ oncoprotein. Unlike the normal p145 c-Abl, $p210^{BCR/ABL}$ has constitutive tyrosine kinase activity and is predominantly localized in the cytoplasm. The tyrosine kinase activity is essential for cellular transformation and the cytoplasmic localization of BCR/ABL allows the assembly of phosphorylated substrates in protein complexes that transduce mitogenic and anti-apoptotic signals. Ectopic expression of $p210^{BCR/ABL}$ results in growth factor independence and leukemic transformation of immortal hematopoietic cells; that indicate a direct and causal role of BCR/ABL in CML pathogenesis. BCR/ABL overexpression activates numerous signal transduction pathways responsible for self-renewal, increase in genomic instability, triggering blockage in differentiation and reduced susceptibility to apoptosis of these malignant cells. Specifically, BCR-ABL1 exerts its oncogenic activity through a complex network of pathways such as RAS/RAF/MAPK and JAK/STAT that promote proliferation and survival independent of the bone-marrow microenvironment [92]. BCR-ABL oncoprotein derived signaling interactions are summarized in Figure **2**.

2.2. RAS/RAF/MAPK Pathway and Treatment Strategies

Several interactions have been identified between BCR-ABL and Ras. Autophosphorylation of Tyrosine 177 within the BCR region of BCR-ABL1 provides a SH2-dependent docking site for the adapter molecule GRB-2 [93]. A GRB-2 effector molecule SOS (Son of Sevenless) is a guanine nucleotide exchange factor of RAS that mediates RAS activation [94]. When GRB-2 binds to SOS protein, it stabilizes RAS in its active GTP-bound form. Two other adapter molecules, Shc and Crkl can also activate Ras. Both are substrates of BCR-ABL and bind BCR-ABL through their SH2 (Shc) or SH3 (Crkl) domains [95]. Ras activation is important for the pathogenesis of CML and there are biochemical evidence suggesting that BCR-ABL1 activates ERK through the activation of the RAS/RAF/MEK/ERK pathway. Raf initiates the cascade through the serine–threonine kinases Mek1/Mek2 and Erk, which ultimately lead the activation of gene transcription [96]. The ERK1/2 pathway is constitutively activated in embryonic stem cells transformed by BCR-ABL1, and ERK2 activation may be involved in resistance to imatinib [97]. For treatment, inhibition of the RAS pathway by farnesyltransferase inhibitor BMS-214662 exhibits synergy with MEK/ERK inhibitor PD184352 in the aim of suppressing proliferation and survival in K562 CML cell model and in primary chronic phase CD34+ CML cells. Finally, CML cell death was achieved, addition of a MEK inhibitor improved ability of BMS-214662 to selectively target CML stem/progenitor cells and accelerated relapse rate of the disease [98].

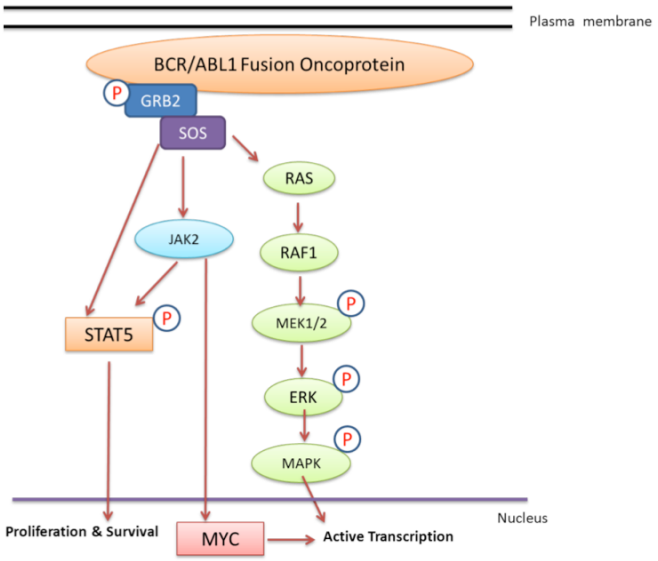

Figure 2: BCR-ABL oncoprotein derived signaling interactions in CML.

Besides this MEK/ERK pathway, JNK pathway in the pathogenesis of BCR-ABL1-induced leukemogenesis needs some further investigation in order to determine the exact role. Some researchers suggest that activation of the JNK pathway promotes pro-apoptotic signals in BCR-ABL1-expressing cells in

response to various agents (eg; arsenic trioxide and ceramide). While constitutive BCR-ABL1 kinase activity suppresses JNK activation; TKIs like imatinib redintegrate JNK phosphorylation which results in induced apoptosis [99]. However some other groups reported that activation of the JNK pathway by BCR-ABL1 was required for malignant transformation and induced leukemogenesis [100]. Thus, inhibition of JNK ortholog Mapk8 (also known as JNK1) prevented BCR-ABL1-mediated transformation both *in vitro* and *in vivo* [101]. In another study the authors reported that resveratrol promoted autophagic cell death in CML cells via JNK-mediated p62/SQSTM1 expression and JNK inhibition or p62 knockdown reversed resveratrol-mediated autophagy and antileukemic effects [102]. In a recent study, effects of Icaritin (from Chinese herb medicine) was studied on CML cell model and concluded that; icaritin inhibited proliferation, induced apoptosis and promoted the erythroid differentiation of K562 cells. Besides, growth of Imatinib-resistant cells was suppressed and icaritin treated NOD-SCID nude mice lifespan was prolonged without suppression of bone marrow. Since icaritin up-regulate phospho-JNK or phospho-C-Jun and down-regulate phospho-ERK, the action of mechanism was attributed to regulation of BCR/ABL downstream signaling [103]. In a very recent study, Wnt5a was reported as imatinib efficacy enhancer through JNK/β-catenin/Survivin pathway and when JNK activity was inhibited; influence of Wnt5a upon Imatinib effects was attenuated [104]. Together, these data suggest that the JNK pathway promotes diverse effects in BCR-ABL1 leukomogenesis and should be further studied.

2.3. JAK/STAT Pathway and Treatment Strategies

Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) pathway is one of the most important signaling pathways in the regulation of cell proliferation, survival and apoptosis which are activated by BCR/ABL. JAK/STAT signaling pathway includes JAK tyrosine kinases, STATs and suppressers of cytokine signaling (CIS/SOCS) families. Cytokines, hormones and growth factors are the ligands of JAK/STAT pathway and this pathway transduces signals into the nucleus driven from cytokine receptor. JAKs are stimulated by activation of a cytokine receptor which in turn activates STATs as transcription factors [105]. The first evidence for involvement of the JAK-STAT pathway in CML pathogenesis came from studies in v-abl–transformed B cells [106]. Constitutive phosphorylation and activation of STAT transcription factors have been reported in BCR-ABL positive cell lines and in primary CML cells [107-108]. STAT proteins that take part in the activation or suppression of malignant transformations are STAT1 and STAT5. While STAT1 exerts an apoptosis inducer gene function, STAT5 is constitutively activated in various types of hematooncologic malignancies in which triggers the formation of leukemia named p210- and p190-transformed leukemic cells [109]. Overexpression of STATs, especially STAT5A provides an efficient mechanism to transduce an extracellular signal which results in posttranscriptional responses in hematological diseases [110]. Thus, STATs play important roles in anti-apoptotic activity mediated by Bcr-Abl [111]. Recent molecular biologic studies have revealed that in mice models, loss of STAT5 abolished CML-like leukemia induced by BCR-ABL1; pointing STAT5A/5B and the genes they activate as therapeutic targets for CML [112-113]. Inhibition of STAT5 phosphorylation has been shown to be an interesting target for eliminating leukemic stem cells [114].

To date, several curative strategies have been developed for CML, but the usage of first (imatinib), second (nilotinib, dasatinib) or third line (ponatinib: responsive to T315I mutation) tyrosine kinase inhibitors was a new generation therapy [115] till gain of resistance. Since then, specifically gene silencing studies have come out such as antisense oligonucleotides (ODN) or siRNA (small interfering RNA, RNAi) applications. Also our group has demonstrated a number studies aimed to induce leukemic cell apoptosis due to RNAi-mediated gene-specific silencing of STAT5 that function as silent transcription factors but when activated, overexpressed and triggered leukemia development. In these studies we concluded that due to induced CML cell apoptosis following silencing of STAT5A; RNAi technology provided utility *in vitro* and will have contribution to clinics for both imatinib sensitive and resistant CML cells [116-118].

Besides these, in another study, effects of the BCR/ABL kinase inhibitors STI571 and adaphostin (NSC 680410) were determined on CML cells *in vitro* and finally the agents caused a synergistic effect since STAT5 phosphorylation was inhibited and apoptosis was induced [119]. In a more recent study, CML patient samples and a model cell line were treated with STAT5 inhibitor sorafenib with nanomedicine applications. As a result, pSTAT5 and antiapoptotic protein MCL-1 expressions were inhibited. This study revealed that, combining molecular diagnosis and personalized nanomedicines could have therapeutic functionality to endogenous proteins to overcome clinically important

Table 2: The Inhibitors Used in Clinic for CML

Inhibitor	Related Pathway	Function
Farnesyltransferase inhibitor BMS-214662 + MEK/ERK inhibitor PD184352	RAS pathway + MEK/ERK pathway	These two inhibitors exhibit synergy and suppresses proliferation and survival of CML cells *in vitro* and *in vivo*
TKI - Imatinib	JNK pathway	Redintegrate JNK phosphorylation thus induces apoptosis
Mapk8 (JNK1)	JNK pathway	prevents BCR-ABL1-mediated transformation both *in vitro* and *in vivo*
First (Imatinib) - Second (nilotinib, dasatinib) – Third (ponatinib) class TKIs	JAK/STAT and Wnt-β-catenin pathways	Inhibit BCR-ABL kinase activity in CML, responsive to various mutations in the binding domain of BCR-ABL.
STAT5 inhibitor Sorafenib	JAK/STAT pathway	Inhibits active pSTAT5 and anti-apoptotic protein MCL-1 expressions
JAK2 inhibitor AG490	JAK/STAT pathway	Reduces BCR-ABL induced oncogenicity and inhibits cell survival of imatinib-sensitive and resistant CML cells also in patients
JAK2 inhibitors TG101209 and HBC	JAK/STAT pathway	Have clinical impact upon CML cell lines and combination therapy of imatinib + HBC induces apoptosis of CML cells
JAK2 inhibitor ON044580	JAK/STAT pathway	A potent inhibitor for both JAK2 and ABL kinases and targets both imatinib-sensitive and resistant CML cells

challenges like molecular drug resistance [120]. All these studies point out the importance of STAT5 from JAK/STAT pathway in CML treatment.

Besides STAT5, JAK2 is also activated in CML, but its direct role is not fully understood. It is well known that inhibition of JAK2 signalling reduces BCR-ABL and other downstream oncogenic signaling pathways [121]. Several inhibitors of JAK2 have been developed since the discovery that its inhibition overcame imatinib resistance by inducing apoptosis in imatinib-resistant cell lines. AG490, a potent and specific JAK2 inhibitor reduced BCR-ABL-induced oncogenicity and inhibited cell survival of imatinib-sensitive CML cell lines. AG490 induced apoptosis also in imatinib-resistant CML cell [122] and also in CML diagnosed patients [123]. Other JAK2 inhibitors like TG101209 and HBC were shown to have clinical efficacy against CML cell lines and besides combination therapy of imatinib + HBC significantly induced apoptosis in CML cells [123]. A new dual kinase inhibitor for JAK2 and ABL kinases called ON044580 was recently discovered and was shown to target both imatinib-sensitive and resistant K562 CML cells [124]. By contrast, it has been shown in a recent study that JAK2 is dispensable for CML cell survival and maintenance *in vitro* and *in vivo* [125]. Since there are controversial findings about the impact of JAK2 upon CML, further investigation is still needed to confirm its access as a therapeutic target.

3. CONCLUSIONS AND FUTURE DIRECTIONS

The chronic leukemia is a holding promise for targeted therapies in cancer, in order to make a new era in cellular signaling pathways responsible for proliferation, survival, and self-renewal. Discovery of molecularly targeted drugs have dramatically enhanced the bad ending story of the disease. Besides traditional therapies, new and alternative mechanisms and agents are also being constantly investigated. While the strategy is to inhibit BCR signaling in CLL, the designed therapies are based on inhibiting BCR-ABL signaling in CML and inducing apoptosis for both. Till now, many successful treatments were achieved especially in CLL because of reflecting into clinic. Besides TKIs; many other promising agents are being investigated for CML. The identification of driving pathway in CML stem cells that can be targeted could solve the problem of minimal residual disease and potentially cure CML suffering patients. With the development of more efficiently signaling pathways targeted agents and mouse modeling studies, it may become possible to make progress towards individualized therapy with longer remissions and even cures in chronic leukemias.

CONFLICT OF INTEREST

None declared.

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