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Therapeutic Approaches to Drug Targets in Leukemia

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Abstract

Non-communicable diseases like cancer are responsible for major social and health burden as millions of people are dying every year. Out of which, Leukemia is the leading cause of deaths worldwide. The ionizing radiation, chemotherapeutic agent and certain chemicals exposure are the risk factor for leukemia. Both genetic and environmental components are associated with the development of leukemia cells. Immune and inflammatory mediators have a complex role in the initiation and progression of leukemia. Understanding of all these processes will help to invent a range of new biomarkers and novel treatment modalities targeting various cellular events in acute and chronic inflammation that are accountable for leukemia. Several biochemical pathways, receptors and enzymes are involved in the development of leukemia that would be possible targets for improving strategies for disease diagnosis and management. However, the novel drug target like tyrosine kinase inhibitors, mcl-1inhibitor, jak-stat inhibitor, mTOR inhibitors, and mitogen-activated protein kinase (MEK) inhibitors, Heat shock protein inhibitors are more powerful to control the process of leukemia. Therefore, the review briefly focuses on different novel targets that act at starting stage in the formation of leukemia.

Keywords

Leukemia, Drug target, Heat shock protein, Jak-stat, Cycloxygenase-2

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1. INTRODUCTION

Leukemia is a disease of the blood or bone marrow, which is characterized by increased numbers of abnormal white blood cells. The abnormality of leukemic cells lies in their inhibited differentiation and increased proliferation rate. Leukemia is divided into acute and chronic, and further subdivided into lymphocytic and myeloid. In leukemias differentiation block occurs in early hematopoietic progenitors, and resulting malignant cells are named blast cells. Acute leukemias are diagnosed either on the basis of presence of over 20% of blasts in the blood or in bone marrow or on the basis of presence of specific cytogenetic or molecular abnormalities[1]. Leukemias are classified into 4 main categories, based on the type of white blood cell affected (lymphoid vs. myeloid) and characteristics of the disease (acute vs. chronic): Based on characteristics of disease classified as: Acute Leukemia, Chronic Leukemia and Based on types of WBCs affected classified as: Myelogenous Leukemia, Lymphocytic Leukemia[2]. Several biochemical pathways, receptors and enzymes are involved in the development of leukemia that would be possible targets for disease diagnosis and management.

2. NEW DRUG TARGETS OF LEUKEMIA

2.1. Heat shock protein inhibitors

Heat shock proteins (HSP) or molecular chaperone protein are a group of cytoplasmic proteins essential in maintaining cellular homeostasis by virtue of their role in transcriptional regulation, chromatin remodeling, and regulation of key signaling pathways such as Akt, Raf-1, and ERB-2. HSP also assist with the folding of mitochondrial proteins and regulate proteolytic degradation of misfolded protein in an ATP-dependent manner[3,4]. Hsp90 allows proliferation of cancer cells by keeping misfolded client proteins in their proper functional folded form and suppresses apoptotic pathways for cancer cell survival[5]. Based on their molecular weight, Hsps have been classified into five major classes. They are small Hsps, Hsp60, Hsp70, Hsp90, and Hsp100, which are localized in different cellular compartments such as cytosol, mitochondria, and endoplasmic reticulum. Hsps play significant roles in cellular homeostasis and cytoprotective processes during normal cell growth and for survival during and after various cellular stresses[5,6,7]. In several tumor models the selective inhibition of Hsp90

function causes a selective degradation of important signaling proteins that are involved in cell proliferation, cell cycle regulation, and apoptosis[8]. HSPs have been found to be overexpressed in a wide range of human carcinomas, including both solid tumours and haematological malignancies[6]. HSP90 is a ubiquitously expressed protein chaperone required for the stabilization of multiple oncogenic kinases, such as BCR-ABL, FLT3 and JAK2. Therefore, Hsp90 plays a critical role in the development of leukemia by regulating survival and proliferation of leukemia cells[9]. HSP90 client proteins, such as epidermal growth factor receptor, Bcr-Abl fusion proteins, mutant p53, hypoxia hypoxia inducible factor 1α and matrix metalloproteinase 2 are involved in various cancer signalling pathways[6]. Pharmacological targeting of HSP90 inhibitors, the most promising compounds currently undergoing Phase I and II trials are ansamycin antibiotics geldanamycin,17allyldimethoxygeldanamyc -in(17-AAG),17-dimethylaminoethylamino-17demethoxygeldanamycin (17-DMAG) and herbimycin A have demonstrated antileukemic activity. Ganetespib presently in phase II clinical trials showed potent activity against primary AML cells[3,6,7,10]. The exact mechanism of action of HSP is currently being explored in CLL but it has been suggested that this group exerts its effects possibly through depletion of Akt causing loss of survival signals, changes in p53 and p21, or depletion of ZAP-70 causing inhibition of prosurvival signals. In preclinical studies, the HSP inhibitor geldanamycin has shown induction of cell apoptosis irrespective of p53/ATM mutation status, suggesting a role in high-risk patients[3].

2.2. Aurora kinase inhibitors

The Aurora kinases are a family of serine threonine kinases involved in many cellular functions, including progression through mitosis, by regulating spindle formation, chromosome segregation and cytokinesis[11]. The aurora kinase regulates many processes during cell division[12]. Aurora kinases A, B, and C (AURKA, B, C) play important roles in centrosome/centromere function and spindle assembly during mitosis. AURKA is involved early in mitosis by regulating centrosome maturation and disjunction, thereby playing a role in establishing a bipolar mitotic spindle[13]. The overexpression of Aurora kinases has been reported in many human tumors, including acute leukemia cells. Aurora kinase function is mediated by

the phosphorylation of several substrates that play important roles in cell division, such as the proteins survivin, CENP-A and serine 10 on histone H3. The Aurora A isotype (also known as Aurora, Aurora-2) is widely expressed in proliferating normal tissues, with expression being cell-cycle-dependent and peaking at the G2/M point of the cell cycle. An overexpression of Aurora A causes an increase in centrosome numbers and aneuploidy. Human Aurora A has been proposed as a 'drugable' target in several tumors, including aggressive non-Hodgkin's lymphoma[11]. Both Aurora-A and Aurora-B inhibitors induce cell death. However, they induce apoptosis through distinct mechanisms. Aurora-A inhibition induces defects in mitotic spindle assembly, which causes a transient spindle checkpoint-dependent mitotic arrest. This cell cycle arrest is not maintained, and subsequently, Aurora-A inhibited cells exit from mitosis leading to apoptosis, either by induction of a G1 arrest, followed by apoptosis, or by a p53-independent mechanism. In contrast, inhibition of Aurora-B also interferes with normal chromosome alignment during mitosis and overrides the mitotic spindle checkpoint causing polyploidy, failure of cytokinesis and end reduplication followed by cell death[14]. The aurora kinase inhibitors AZD1152-HQPA and ZM447439 induced growth arrest and the accumulation of hyperploid cells in acute myeloid leukemia cell lines and primary acute myeloid leukemia cultures. Furthermore, both agents inhibited histone H3 phosphorylation and this preceded perturbations in cell cycle and the induction of apoptosis[12]. Danusertib (formerly PHA-739358) is a small ATP competitive molecule that inhibits aurora A, B and C kinases. Danusertib also inhibits several receptor tyrosine kinases such as Abl, Ret, FGFR-1 and TrkA. Danusertib was one of the first aurora kinase inhibitors to enter the clinical and has been studied in Phase I and II trials[15]. Barasertib AZD1152, Alisertib MLN8237 is a selective inhibitor of the Aurora-A AT9283 is a small-molecule multitargeted kinase inhibitor with potent Aurora kinase activity[14].

2.3. MEK inhibitors

Mitogen-activated protein kinase (MEK) is an important component of the RAS/RAF/MEK/ERK signaling pathway, which regulates cell growth[16]. MAPK pathways include at least four major signaling cascades: extracellular signal-related kinases (ERK) 1 and 2, c-JUN N-terminal kinase (JNK) 1, 2, and 3, p38

MAPK, and ERK5. Extensive studies from many groups have shown that molecular or pharmacological targeting of MAPK signaling cascades, alone or in combination with other drugs, results in enhanced anti-leukemic responses in AML. it has shown that the downstream effector of MAPK pathways, Mnk kinases, may be attractive targets for the treatment of AML[17]. Inhibition of this pathway inhibits growth factor–mediated cell signaling and tumor cell proliferation. Constitutive activation of this pathway has been implicated in many cancers. Potent small-molecule inhibitors of MEK effectively shut down the MEK/ERK pathway in a highly selective manner. Most preclinical and clinical information regarding MEK inhibitors to date has emerged mainly from solid-tumor studies. Recently, interest has arisen in the use of these molecules in hematologic malignancies including AML, chronic myeloid leukemia, myeloma, lymphomas, and ALL. Preclinical data demonstrating successful inhibition and cell death in lymphoma cell lines and lymphoma xenografts were reported at the 2009 meeting of the American Society of Hematology. Phase I/II trials of the MEK inhibitors AS703026 and GSK2230212 are currently enrolling participants at M.D. Anderson Cancer Center (Houston, TX)[16]. A recent comprehensive pre-clinical predictive biomarker analysis of 218 solid tumor and 81 haematological cancer cell lines revealed AML and CML cell lines as being particularly sensitive to the MEK inhibitor, GSK1120212. Amongst solid tumour cell lines, RAF/RAS mutations were predictive of sensitivity. In a panel of 12 AML cell lines, single-nanomolar IC50's were reported for all 6 RAS-mutant (N- or K-RAS) cell lines, as well as 2 RAS-wt cell lines. The paediatric cell line Kasumi-1 (RAS-wt) was resistant, although other paediatric cell lines, THP-1 (N-RAS mutant) and MV4-11 (FLT3-ITDþ, RAS-wt), were sensitive. A phase I trial of GSK1120212 was reported at the 2010 ASH meeting, with 12 of 14 patients treated having AML (including 2 transformed from MDS). Preliminary anti-leukaemic activity was reported, with one patient achieving CR. A phase I/II study of GSK1120212 in AML is ongoing (NCT00920140)[18].

2.4. Aminopeptidase inhibitors

Amino peptidases play a key role in removing amino acids from cellular peptides, a process required for protein regulation and recycling. Aminopeptidase

inhibition depletes intracellular amino acids required for new protein synthesis. AML cells have been shown to be sensitive to amino peptidase inhibition, undergoing apoptosis, whilst normal bone marrow cells are less susceptible. The amino peptidase inhibitor Tosedostat has shown promising activity in phase I/II trials of older adults with predominantly relapsed/refractory AML, with single-agent response rates of up to 27%. Tosedostat appears well tolerated with thrombocytopenia being the most common adverse event reported[18].

2.5. c-KIT inhibitors

The c-KIT gene (stem cell factor) encodes for a tyrosine kinase with a structure similar to platelet growth factor and is expressed in hematopoietic progenitor cells and AML blasts. Upon binding of the ligand stem cell factor to c-kit, phosphorylation of several cytoplasmic proteins occurs and pertinent downstream pathways get activated. Those include the JAK/ STAT pathway, the PI-3 kinase pathway and the MAP kinase pathway. Mutations in c-KIT receptor result in constitutive phosphorylation and activation of the receptor in absence of the ligand[19]. Targeting of the KIT-RTK is another promising approach because the c-KIT stem cell factor CD117 is expressed in more than 70% of all AML78 and KIT mutations occur in more than 40% of the CBF leukemias. Imatinib, which is known primarily because of its inhibitory function on the BCR-ABL tyrosine kinase, also inhibits KIT by prevention of autophosphorylation and by inhibition of downstream signaling via the MAPK and AKT pathways. However, the results of preclinical studies of imatinib in c-KIT+ AML were controversial in some studies there was little effect in KIT-mutated AML, whereas another study showed an in vitro dose-dependent increase in apoptosis in KIT-mutated cells. Several case studies demonstrated a potential benefit of imatinib treatment for KIT non-mutated but CD117-expressing AML[20]. Although c-KIT mutations confer an increased risk of relapse in adults with CBF AML, this does not appear to be the case in children. Murine models have demonstrated c-KIT mutations cooperate with AML1-ETO to cause aggressive AML in vivo, but are responsive to c-KIT inhibition with dasatinib. Dasatanib has also been shown to have single-agent activity against the paediatric cell line, Kasumi-1, which also harbours an N822K point mutation in the activation loop of c-KIT. Results of several ongoing clinical

trials in AML, particularly those enriched with CBF patients, are eagerly awaited[18].

2.6. Mcl-1

Mcl-1 (myeloid cell leukemia 1) is a member of the Bcl-2 family of proteins that when dysregulated prevents cancer cells from undergoing programmed cell death, a hallmark of cancer. By overexpression of the Mcl-1 protein or amplification of the Mcl-1 gene, a cancerous cell can avoid death, the normal fate for cells exhibiting abnormal and deregulated growth. Indeed, amplification of Mcl-1 is one of the most common genetic aberrations observed in human cancer, including lung, breast, prostate, pancreatic, ovarian, and cervical cancers, as well as melanoma and leukemia[21]. Under physiological conditions, Mcl-1 expression is tightly regulated at multiple levels, involving transcriptional, post-transcriptional and post-translational processes[22]. Mcl-1 is distinguished from other anti-apoptotic Bcl-2 proteins in that it has a very short half-life (only 2-4 h in most cells), making it dependent upon active transcription and translation for its maintenance. Mcl-1 also interacts with and sequesters the pro-apoptotic proteins Bim and truncated Bid. Noxa antagonizes Mcl-1 and promotes its proteasomal degradation, up-regulation of Mcl-1 represent important mechanism underlying leukemia cell. While up-regulation of Mcl-1 has been described at the time of AML relapse, in fms-like tyrosine kinase-internal and induplication (FLT3-ITD) AML stem cells, and in the regulation of stem cell self-renewal, the most compelling evidence to date in support of a critical role for Mcl-1 in the survival of human AML cells emanates from a recent study in which deletion of Mcl-1. Co-administration of roscovitine and ABT-737 untethered Bak from Mcl-1 and Bcl-XL, respectively, triggering Bak activation and Box translocation, with resultant induction of MOMP. Another mechanism of down regulating Mcl-1 involves inhibition of translation. It has been demonstrated that the multi-targeted tyrosine kinase inhibitors sorafenib mediates apoptotic cell death in human leukemia cells, atleast in part, through down-regulation of Mcl-1 via inhibition of translation. Accordingly, sorafenib synergizes with ABT-737 to induce apoptosis in AML cell lines. ABT-737 may induce activation of extracellular signal regulated kinase and up-regulation of Mcl-1 in AML cells, arguing for a combination strategy involving inhibitors of mitogen activated protein kinase (MEK),

which have been used successfully to suppress Mcl-1[23].

2.7. Bcl-2 inhibitors

The Bcl-2 family comprises a group of proteins involved in the regulation of programmed cell death by modulating the mitochondrial membrane permeability in apoptosis. Functionally, members of the Bcl-2 family can be divided into the antiapoptotic (Bcl-2, Bcl-xl) and proapoptotic proteins (Bax, Bak, and the BH3) Increased Bcl-2 expression is observed in B cell malignancies including CLL and is associated with resistance to apoptosis. Several compounds are being investigated in clinical trials with the intent of inducing apoptosis by either activating the proapoptotic proteins or negating the antiapoptotic proteins. The earlier Bcl-2 inhibitors have demonstrated modest efficacy in cancer therapy, but the potential for newer pan Bcl-2 inhibitors appears promising due to improved target binding, bioavailability, and route of administration[3]. ABT-737 is a small molecule inhibitor of BCL-2, BCLXL, and BCL-w. ABT-737 showed in vitro activity against lymphoma and small cell carcinoma cells. Subsequent in vitro studies showed activity against myeloma, acute leukemia, and lymphoma. ABT-263 (navitoclax) is another potent small molecule inhibitor of BCL-2, BCL-XL, and BCL-w. It was tested on multiple cell lines in vitro and in xenograft models and shown to have significant activity against acute lymphoblastic leukemia (ALL) cell lines[24]. After the first published phase I study of navitoclax in non-Hodgkin's lymphoma, which had shown 22% partial responses (PRs), Roberts et. Al. reported encouraging data of a phase I trial in patients with relapsed or refractory CLL: 35% of PRs were recorded, albeit without complete responses (CRs) and with 18% of grade 4 thrombocytopenia[25]. ABT 199, a BH3 mimetic, is a targeted agent which promotes apoptosis by inhibiting BCL-2. Preliminary results on the activity of this agent report overall response rate of 84% in 56 patients with CLL. Treatment with ABT 199 was accompanied by a significant decrease in bone marrow infiltrate and lymphocyte count reduction[26]. Although the occurrence of tumor lysis syndrome has slowed clinical development, phase III trials combining ABT-199 with chemoimmunotherapy are now ongoing[25].

2.8. FLT3 inhibitors

FLT3 is a tyrosine kinase receptor involved in the differentiation and proliferation of

hematopoietic stem cells and is also expressed on AML blasts. two distinct types of activating mutations are internal tandem duplication (ITD) of the intracellular juxtamembrane region and point mutations in the tyrosine kinase domain (TKD). Both classes of mutation constitutively activate the FLT3 receptor. FLT3 ITD mutations activate STAT5, which is sufficient to cause Several receptor tyrosine kinase (RTK) inhibitors have been reported to inhibit the FLT3 receptor on AML or primary blast cell lines, including AG1295, AG1296 and herbimycin A[27]. Lestaurtinib (formerly CEP-701), one of the first FLT3 inhibitors was evaluated in a randomized, multicenter study comparing the drug combined with chemotherapy versus chemotherapy alone. Sorafenib, a multikinase inhibitor approved for renal cell and hepatocellular carcinomas also a potent FLT3[28]. Inhibition of FLT3 has also been described in several pre-clinical studies with other RTK inhibitors that suppressed tumor growth and showed an increased disease latency period in transplanted leukemia mice, including CEP-701 (Kyowa-Hakko Kogyo Co. Japan) PKC412 (Novartis), SU11248 (Sugen) and CT53518 (Millenium Pharmaceuticals). All four inhibitors are currently under evaluation in clinical trials cells to proliferate. FLT3 appears to be necessary for disease progression in AML and is therefore an excellent therapeutic target[27].

2.9. Monoclonal antibodies

Monoclonal antibodies are designed to specifically bind to antigens present on the cell surface. In the treatment of leukemia, antigens are selected that can target leukemia cells in a relatively specific fashion. Monoclonal antibodies can exert their antitumor effects via a number of mechanisms. Once bound, they are able to invoke both antibody dependent cell-mediated toxicity and complement-dependent cytotoxicity, which ultimately leads to tumor cell death[29]. Gemtuzumab ozogamicin is an anti-CD33 monoclonal antibody conjugated to calicheamicin. It is being used in treating acute myeloid leukemia (AML) and acute promyelocytic leukemia (APML)[30]. The monoclonal antibody portion is directed against CD33, a cell surface marker expressed on myeloid cells. Once GO binds to CD33, it is internalized, where it releases a potent cytotoxin, calicheamicin, which causes cell death. This offered one of the first targeted approaches in AML. GO was withdrawn from the market in 2010 after preliminary

results of a randomized trial evaluating the drug as a component of frontline AML therapy showed that GO did not improve the outcome.There were also some concerns regarding toxicity, including early death[28]. Alemtuzumab is a humanized monoclonal antibody that targets CD52, a cell surface antigen expressed on the majority of B and T lymphocytes. It appears that, as a single agent, Alemtuzumab is not very effective for ALL. Epratuzumab is a humanized monoclonal antibody targeting CD22 that has recently been investigated as therapy for pediatric ALL following bone marrow relapse. Rituximab is a chimeric murine/human monoclonal antibody that targets CD20, a molecule present on the surface of B-cells. In a phase II window study in children with B-cell non-Hodgkin lymphoma, rituximab as a single agent resulted in a 41 % response rate, with response considered to be at least a 25 % decrease in tumor size. Inotuzumab ozogamicin is a humanized monoclonal antibody linked to calicheamicin that targets the CD22 antigen present on the majority of pre-B-ALLcells. As a single agent, Inotuzumab ozogamicin was able to induce overall response in57% of heavily pretreated adults and children with pre-B-ALL[29].

2.10. BCR-ABL inhibitors

Bcr-Abl-positive leukemias include chronic myelogenous leukemia (CML), both myeloid and lymphoid blast phase CML, and some cases of acute lymphoblastic leukemia. The chimeric *bcr-abl* gene codes for a tyrosine kinase that is constitutively activated in the leukemic cells and plays the central role in leukemogenesis[31]. Breakpoint cluster region (BCR)-ABL is the protein product caused by the translocation (also known as the Philadelphiachromosome) is most commonly found in CML. Expression of the translocation product is controlled by the BCR promoter, which is ubiquitously expressed throughout the body[30]. Hematologic malignancies, including Bcr-Abl-positive leukemias, also frequently have over activity of the Ras signaling pathway, leading to abnormal transduction of growth and survival signals. New and investigational therapeutic options that target these specific molecular defects of leukemic cells include the tyrosine kinase inhibitor imatinib mesylate (STI571) and farnesyltransferase inhibitors (R115777, SCH66336), which block localization of Ras proteins to the cell membrane. Therefore, the development of a multifaceted therapeutic approach to these leukemias is of

great interest. Arsenic trioxide (ATO), which has significant activity in patients with relapsed and refractory acute promyelocytic leukemia, is a potential addition to the therapeutic arsenal. While some of the molecular activities of ATO are specific to acute promyelocytic leukemia, arsenicals also have a broad variety of antineoplastic properties that may be useful in combination therapy with agents that target specific molecular defects of Bcr-Abl-positive leukemias[31]. Establishing new effective therapeutic approaches for chronic myeloid leukemia (CML) and Philadelphia chromosome - positive [Ph_+] acute lymphoblastic leukemia (ALL). Despite the emergence of imatinib mesylate resistant BCR–ABL mutations, the identification of second-generation kinase inhibitors such as nilotinib and dasatinib provided potent alternative approaches, with the notable exception of the T315I-BCR–ABL mutation. all clinically approved BCR–ABL kinase inhibitors (imatinib mesylate, nilotinib, dasatinib); approaches to target CML leukemic stem cells; and exploitation of other cellular functions abnormally regulated in BCR–ABL expressing cells, such as deregulation of the mRNA translation machinery and signaling pathways that control growth-promoting signals[32]. Currently, two classes of drug candidates are being evaluated as monotherapies for the treatment of CML. These are the second generation selective Bcr-Abl kinase inhibitor, AMN107 and the dual Abl-Src inhibitors, BMS-354825, AZD05340 and SKI-606[33].

2.11. JAK-STAT inhibitors

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway plays a critical role in the signaling of a wide array of cytokines and growth factors leading to various cellular functions, including proliferation, growth, Hematopoiesis, and immune response. JAK proteins consist a family of four non-receptor tyrosine kinases (JAK1, JAK2, JAK3 and Tyk2) that are closely associated with type I/II cytokine receptors. When activated via association to cell surface receptors they phosphorylate and translocate STATs to the nucleus to activate gene transcription. The JAK-STAT signalling pathway is responsible for a variety of different functions throughout development[34]. Cytokines are major mediators for cell maturation and multiplication. When cytokines bound to the receptor Jaks gets autophosphorylates and in

turn, phosphorylates STATs. Activated STAT is form dimer which is transported into nucleus where it shows gene regulatory effect. Due to mutations in JAK gene Janus kinase gets activated without Cytokines or other growth factors binding which leads to the antiapoptic activity[35]. There is an abundance of literature to suggest that this pathway is activated in CML LSC and family member JAK2 is a popular pharmacological target. Ruxolitinib, the first JAK inhibitor that recently received marketing authorization by FDA and EMA for the treatment of myelofibrosis, is now investigated in patients with relapsed or refractory acute leukemia[34].

2.12. RAS

Ras belongs to the family of small G proteins with intrinsic GTPases activity that governs various cellular signal transduction pathways. Activation of Ras GTPases results in a series of downstream signaling cascades, which initiate cell growth, differentiation, proliferation and cell survival[36]. RAS proto-oncogene belongs to the GTPase family and has 3 isoforms: N-Ras, K-Ras, and H-Ras. Mutant RAS isoforms are found in various types of tumors and leukemia[37]. The mitogen-activated protein kinase (MAPK) signaling cascade is constitutively activated in a high proportion of AML cases. Activation of the MAPK signaling cascade can be mediated by the RAS mutations. binding of the RAS proteins to the cell membrane requires structural modifications that are catalyzed by specific enzymes. Inhibition of these enzymes by farnesyltransferase inhibitors (FTI) was demonstrated to result in induction of apoptosis and to decrease proliferation[38].

2.13. Cyclin-dependent kinase inhibitors

Cyclin-dependent kinases (CDKs) are members of subfamily of serine/threonine kinases which are found in both unicellular organisms such as yeast and multicellular organisms such as plants, humans and other mammals[39]. a number of CDK inhibitors showed promise in the pre-clinical setting, flavopiridol and dinaciclib have been most extensively studied in clinical trials in CLL. Remarkably, both agents were active in high-risk CLL. The pan-CDK inhibitor flavopiridol (HMR-1275, alvocidib, Sanofi) led to cell cycle arrest and inhibited CDK9 in HeLa cells in vitro and induced responses in 53% of patients with relapsed/refractory CLL. Dinaciclib (SCH-727965, Merck) an inhibitor of CDK1/2/5/9, showed an overall response rate of 54% in the same group of

patients[40]. Cyclin-dependent kinases (CDK) are important regulators of the cell cycle that controls transcription in different haematological malignancies. CDK inhibitors including alvocidib and SNS-032 have shown activity in CLL. Alvocidib (NSC649890) is derived from a plant and has shown substantial cytotoxicity on CLL cells in vitro. Alvocidib inhibits the antiapoptotic proteins including the Mcl-1, X-linked inhibitor of apoptosis, additionally inhibits the transcription by abrogating the functions of CDK9 and CDK7. Dinaciclib has entered phase III clinical development for the treatment of chronic lymphocytic leukemia in 2012, therefore no data are available yet, but this study is being conducted to demonstrate the superiority of dinaciclib compared to of atumumab in chronic lymphocytic leukemia participants who are refractory to either fludarabine treatment or chemo immunotherapy. SNS032 is in phase I clinical trials for the treatment of chronic lymphoid leukemia along with multiple myeloma. TG02 was selected for phase I clinical trials and the results are expected in 2014 for the phase 1 study in patients with chronic lymphocytic leukemia and small symphocytic symphoma and in 2015for phase I study in patients with advanced hematological malignancies[41]. However, due to a high frequency of adverse events, including severe tumor lysis syndrome, novel CDK inhibitors are needed to improve therapeutic strategies in CLL[40].

2.14. mTOR

mTOR can form two distinct multiprotein complexes: mTOR complex 1 (mTORC1) and 2 (mTORC2). Although the complexes include mTOR as their central common element, they have different purposes and functions. Activation of mTORC1 complexes controls mRNA translation of oncogenic proteins, cell cycle progression, autophagy, and cellular growth and metabolism, while mTORC2 complexes regulate cellular metabolism, and promotes malignant cell survival. Inhibition of mTORC1 pathways using rapamycin or other rapalogs alone or in combination with other anti-leukemic agents, including chemotherapeutic regimens, has shown potent anti-leukemic properties vitro and in vivo[42]. By inhibiting mTOR, rapamycin mimics growth factor withdrawal. This is a novel site of blockade not currently targeted by conventional cytotoxic agents[16]. This is important as such agents inhibit both mTORC1 and mTORC2 complexes and, potentially, other mTOR complexes that

may exist but have not yet been identified[42]. The prototype of classical mTOR inhibitors is sirolimus. The mechanism of action of sirolimus is rather complicated since it may inhibit mTORC1 or both the two mTOR complexes. In malignant B-cells, sirolimus may cause cell cycle arrest, reduce proliferation, and inhibit growth in culture or delay tumor progression in animal models. Currently, three of these chemical agents are available for clinical trials: temsirolimus (CCI-779, Torisel, Wyeth Pharmaceuticals), everolimus (RAD001, Affinitor, Novartis Pharmaceuticals), and ridaforolimus (AP23573, ARIAD Pharmaceuticals, formerly deforolimus). Similarly to rapamycin, rapalogs inhibit mTORC1, may downregulate mTORC2, and exert either excitatory or inhibitory effects on Akt protein, both *in vitro* and *in vivo*[43]. In addition, in May 2007, the FDA approved Torisel for the treatment of advanced renal cell carcinoma. Phase III clinical trials with Torisel in advanced breast cancer and mantle cell lymphoma and AP23573 in sarcomas, and phase II clinical studies with RAD001 in advanced renal cell cancer and hematological malignancies (Hodgkin/non-Hodgkin lymphoma Waldenstrom macroglobulinemia) are still ongoing[44].

2.15. NOTCH1

The Notch receptors are transmembrane ligand activated transcription factors whose activation results in gene expression changes in the nucleus. This pathway plays an essential role in T-cell development, and mice with deletion of Notch1 fail to develop T cells. Constitutive activation of the NOTCH1 pathway has been shown to be pathogenic in 60% of cases of T-ALL. Given the frequency of this abnormality, there is great interest in NOTCH1 as a molecular therapeutic target for the treatment of T-ALL. Gamma-secretase inhibitors (GSI), which inhibit the NOTCH pathway, have been developed for this purpose. Unfortunately initial experience demonstrated severe gastrointestinal toxicity and lack of clinical disease response. Therefore, combination therapies with other pathway inhibitors and corticosteroids, as well as alternate dosage schedules, are being explored[16]. Specific antibodies directed against individual NOTCH proteins can selectively block the activities of different NOTCH receptors. Anti-NOTCH1 antibodies recognizing the HD LNR repeat region of the receptor can effectively inhibit the activity of wild-type NOTCH1 and leukemia associated NOTCH1 mutants and

effectively block tumor growth *in vitro* and *in vivo*. Inhibition of ADAM10 may also facilitate effective inhibition of wild-type and mutant NOTCH receptors. An alternative strategy is to block NOTCH transcriptional complexes in the nucleus using chemically modified peptides. In this regard, the resolution of the NOTCH-RBPJ-MAML1 transcriptional complex was instrumental in developing SAHM1, a stapled peptide designed to displace MAML1 and block the transcriptional activity of NOTCH1 RBPJ complex[45]. NOTCH activity can be inhibited by γ-secretase inhibitors (GSI) or antibodies to NOTCH receptors or NOTCH ligands[46]. NOTCH inhibition is a promising therapy for various types of cancer such as T-ALL in which NOTCH plays oncogenic roles. Although GSI treatment suppresses *in vitro* growth and induces apoptosis of T-ALL cell lines successful clinical trials of GSI for T-ALL have not been reported[47].

2.16. SYK Inhibitors

Spleen tyrosine kinase SYK is a non-receptor cytoplasmic member of Syk family PTK. Pharmacologic inhibition of SYK in CLL primary cells led to the down regulation of pro-survival molecules such as ERK, AKT and MCL-1, signifying the direct regulatory role of SYK on these molecules[48]. SYK inhibition causes disruption of important signaling pathways such as BCR signaling pathway and mTOR pathway leading to apoptotic death in leukemia and lymphoma cell. SYK also has a BCR-independent anti-apoptotic function that is operative in human leukemic B-cell precursors corresponding to the earliest stages of human B-cell ontogeny. Most importantly, SYK inhibition causes apoptosis in primary leukemia cells from BPL patients that are resistant to chemotherapy[49]. Fostamatinib is a competitive inhibitor of SYK that has been shown in vitro to inhibit BCR signaling in CLL models. Similar to other inhibitors of the BCR, fostamatinib may also reduce the protective effect of stromal cells in the immune microenvironment. In aphase 1/2 study of fostamatinib in patients with relapsed hematologic malignancies, responses were observed in CLL (ORR 54%) as well as MCL, DLBCL, and FL. The most common toxicities reported were diarrhea, fatigue, cytopenias, hypertension, and nausea. Phase 2 studies are currently under way in aggressive B-cell NHL. Other SYK inhibitors such as GS-9973 are in development[50]. Here, the activity of two

novel, highly selective Syk inhibitors, PRT318 and P505-15, in assays that model CLLinteractions with the microenvironment. PRT318 and P505-15 effectively antagonize CLL cell survival after BCR triggering and in nurse-like cell-co-cultures. Moreover, they inhibit BCR-dependent secretion of the chemokines CCL3 and CCL4 by CLL cells, and leukemia cell migration toward the tissue homing chemokines CXCL12, CXCL13, and beneath stromal cells. PRT318 and P505-15furthermore inhibit Syk and extracellular signal-regulated kinase phosphorylation after BCR triggering. These findings demonstrate that the selective Syk inhibitors PRT318 and P505-15 are highly effective for inhibition of CLL survival and tissue homing circuits, and support the therapeutic development of these agents in patients with CLL, other B-cell malignancies and autoimmune disorders[51].

2.17. Cycloxygenase-2 (COX-2) Inhibitors

Cyclooxygenase-2 (COX-2), the rate controlling enzyme that catalyzes the conversion of arachidonic acid (AA) to different endogenous prostaglandins (PG), is involved in several physiological and pathological pathways, such as inflammation, fever, bleeding and blotting[52]. Prostaglandins (PG) are known to play important roles in the proliferation and differentiation of leukemia cells. The effect of the inhibitors of cyclooxygenase-2 (COX-2), a rate-limiting enzyme for the synthesis of PG, on the proliferation and differentiation of leukaemia cell lines was investigated.COX-2 inhibitors, NS-398 and nabumetone, suppressed the proliferation of U-937 and ML-1 cells by inducing a G0/G1 cell-cycle arrest. Cell-cycle arrest induced by these COX-2 inhibitors was not associated with an up regulation of the cyclin-dependent kinase inhibitors.COX-2 inhibitors also inhibited the differentiation of these cells induced by interferon-g (IFN-g), tumour necrosis factor-a (TNF-a) and retinoic acid (RA). Treatment with NS-398 did not suppress the levels of PGs produced by these cells. Although COX-2 antisense oligonucleotide showed a similar inhibitory effect on these cells, its inhibitory effect was smaller than that of NS-398. These results suggest that COX-2 inhibitors may suppress the proliferation and differentiation of leukaemia cells both via COX-2-dependent and independent pathways[53].It has been discovered that the action of COX-2 involved in tumorienesis include: (a) stimulating the proliferation through PGI2; (b) inhibiting tumor cells apoptosis. This

process is associated with an anti-apoptotic, anti-oxidation protein, Bcl-2; (c) Stimulating angiogenesis of tumor cells; (d) The PGE2 produced inhibit the proliferation of T or B-lymphocytes and cytotoxic reaction of NK cells. Currently, a number of COX-2 inhibitors have been evaluated in all kinds of clinical trials for the treatment of cancer, including Celecoxib, Rofecoxib, NS-398, among which is combined with Isotreinoin for the treatment of recurrent and deteriorated malignant neuroglioma in a phase II clinical trial[52]. There is much interest in the potential use of Cox-2 selective inhibitors in combination with other cancer therapeutics. Malignancies of hematopoietic and non-hematopoietic origin often have increased expression of cyclooxygenase-2, a key modulator of inflammation. For example, hematological malignancies such as chronic lymphocytic leukemia, chronic myeloid leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma and multiple myeloma often highly express Cox-2, which correlates with poor patient prognosis. Expression of Cox-2 enhances survival and proliferation of malignant cells,while negatively influencing anti-tumor immunity. Hematological malignancies expressing elevated levels of Cox-2 potentially avoid immune responses by producing factors that enhance angiogenesis and metastases. Cellular immune responses regulated by natural killer cells, cytotoxic T lymphocytes, and T regulatory cells are also influenced by Cox-2 expression. Therefore, Cox-2 selective inhibitors have promising therapeutic potential in patients suffering from certain hematological malignancies[54.]

3. CONCLUSION

Leukemia is disorder related to the blood cell and its development. During the developmental stage of blood cells due to genetic or other reasons growth of blood cells arrest in immature state, where it is unable to work their functions properly as well as has tendency to multiply rapidly. These cells are called blast cells, which are responsible for the development of leukemia. The drug target like Jak-stat inhibitor, Sky inhibitor, mitogen-activated protein kinase inhibitors, Heat shock protein inhibitors and Cox inhibitors will be the new therapeutic drug target in the management of leukemia. Hence, the present review mainly gives the idea about the recent receptor and signaling pathway that take part in the starting stage of leukemia cells formation.

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