Advances of signal transducer and activator of transcription 3 inhibitors in acute myeloid leukemia (Review)

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Received October 17, 2024; Accepted December 12, 2024

DOI: 10.3892/ol.2025.14881

Abstract. Signal transducer and activator of transcription 3 (STAT3), a crucial transcription factor, exerts a notable influence by hyperactivating or acquiring functional mutations in the occurrence and progression of cancers. Hyperactive STAT3 is also implicated in a range of hematopoietic malignancies, especially acute myeloid leukemia (AML). The function of STAT3 is associated with the phosphorylated parallel dimer structure, enabling them to stimulate the transcription of specific genes. AML is a highly heterogeneous hematological malignancy, which is challenging in terms of therapy. The current efficacy of chemotherapy and targeted therapy remains suboptimal. Targeted inhibition of STAT3 has the potential to enhance the efficacy of AML treatment, thereby possibly improving the prognosis of individuals suffering from AML. The present review summarizes the development of inhibitors against STAT3 and discusses their applicability as AML therapeutics, which could inspire new possibilities for enhancing AML treatment strategies.

Contents

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Key words: signal transducer and activator of transcription 3, function, acute myeloid leukemia, inhibitors, targeted therapy

1. Introduction

Acute myeloid leukemia (AML) is a diverse group of hematologic malignancies that are characterized by the abnormal growth and differentiation of precursor cells, and to clinical manifestations such as infection, anemia and bleeding (1). The clonal expansion results in the accumulation of immature myeloid precursors in the bone marrow, peripheral blood and/or other organs and tissues (2,3). AML usually occurs in older individuals and incidence increases with age, with the median age at diagnosis being 68 years (4). The development of genome sequencing technology has made it possible to deeply sequence AML samples to describe the mutation spectrum and understand the biological heterogeneity (5). Although increased understanding of the pathophysiology, next-generation sequencing and the recent approval of numerous treatment options, including BCL2, FMS-like tyrosine kinase 3 (FLT3) inhibitor, isocitrate Dehydrogenase 1/2 (IDH1/2) inhibitors and allogeneic hematopoietic stem cell transplantation have transformed the way AML is approached, the estimated 5-year survival rate is 62% for patients under the age of 50 years, 37% for patients aged 50-64 years old and only 9.4% for patients ≥65 years at diagnosis, leaving much room for improvement (6,7). The biological and clinical heterogeneity of the disease makes AML difficult to treat, and selective targeted inhibitors combined with chemotherapy may contribute towards improving efficacy.

Signal transducer and activator of transcription 3 (STAT3), a member of the STAT family, has an important function in controlling the proliferation of healthy and cancerous cells (8-10). STAT3 has four recognized subtypes, including STAT3α (92 kDa), STAT3β (83 kDa) and STAT3γ (72 kDa) and STAT3δ (64 kDa), of which STAT3α and STAT3β are produced by alternative splicing (11). The constitutive activation of STAT3α plays a key role in the activation of carcinogenic pathways and involves in the regulation of apoptosis, proliferation, differentiation and evolution of numerous neoplasms, while STAT3β is generally considered to be a dominant negative regulator of cancer (12). STAT3γ and STAT3δ are derived from proteolytic processing and have no transcriptional effect (13).

In hematopoietic cells, STAT3 facilitates aberrant signal transduction by enlisting receptor complexes that have undergone tyrosine phosphorylation. Upon being phosphorylated,

the STAT3 protein forms dimers and migrates to the nucleus, where it initiates transcription proteins, ultimately resulting in tumorigenesis (14). Since STAT3 is an important transcription factor in pathogenesis and chemotherapy resistance, a number of studies have been conducted targeting STAT3. Constitutive STAT3 activation is observed in ~ 50% of newly diagnosed AML. In addition, patients with leukemia cells demonstrating constitutive STAT3 activation have a shorter disease-free survival rate (15,16). The antitumor effects of blocking STAT3 activity have been demonstrated in both solid tumors (17-20) and AML (21,22). Therefore, STAT3 has been recognized as a promising protein target for the development of broad-spectrum therapeutic drugs (23).

2. Structure and function of STAT3

STAT3 is a signaling molecule that relays information from cell surface receptors to the nucleus. It is activated by a variety of soluble mediators, such as interleukins (IL-2, IL-3, IL-5, IL-6, IL-7, IL-9, IL-11), cytokines [granulocyte colony-stimulating factor (G-CSF), epidermal growth factor, platelet-derived growth factor] and hormones (growth hormone, prolactin, leptin) (24,25). Fig. 1 illustrates the various functional domains of the STAT3, including an N-terminal domain, a DNA-binding domain, a linker domain, an Src homology 2 (SH2) domain and a C-terminal transactivation domain (26).

The activation of STAT3 involves the interaction between specific cytokines and its receptors on the cell membrane, which triggers the activation of tyrosine kinase. The receptor-kinase complex undergoes phosphorylation and subsequently serves as a docking site for STAT3. The recruited STAT molecules are phosphorylated at Tyr705 and Ser727 of mitochondrial STAT3. Following activation, STAT3 is transferred to the nucleus where it acts as a transcription activator, stimulating the transcription of genes responsible for regulating cell proliferation, differentiation and apoptosis (Fig. 2) (27-31).

The constitutive STAT3 activity is often associated with adverse outcomes in human cancer, as it promotes tumor cell proliferation, survival and metastasis, while impairing antitumor immune responses (32,33). The phosphorylation of STAT3 can be activated in various cancerous cells, including multiple AML cell lines and primary cells obtained from AML patients (14,16).

3. STAT3 in AML

STAT3 is crucial for maintaining the balance of myeloid cells, as it can block myeloid differentiation and plays an important role in leukemogenesis (21,26,34). STAT3 regulates cell survival and proliferation through its target genes (MYC, cyclin D1, baculoviral IAP repeat-containing 5 and BCL2). The inhibition of STAT3 can induce cell apoptosis, and constitutive STAT3 activity is associated with adverse prognosis in patients with AML (16,35).

Genetic mutations or external factors can frequently disrupt the normal STAT3 signaling pathway (36-38). In 28-44% of patients with AML, constitutive STAT3 activation has been observed at the initial stage of diagnosis, whereas when patients experience a recurrence, constitutive activity is absent or decreased (15,16,38). The factors contributing to the increased activation of constitutive STAT3 in AML cells seem to differ among individuals. A potential factor is the continual activation of the IL-6 signaling pathway, and another factor contributing to this is the occurrence of activating mutations in the SH2 domain of STAT3, specifically between residues 585 and 688 (15,37).

Based on these findings, STAT3 has become a desirable focus for AML treatment. However, Lee *et al* (39) revealed that feedback activation of STAT3 in PC-9 NSCLC cells promotes drug resistance through the activation of multiple kinases, including EGFR, MET and KRAS. Therefore, the combination of STAT3 inhibitors with other chemotherapies (cytarabine + mitoxantrone/idarubicin/etoposide) may be effective against refractory or relapse AML.

4. STAT3-targeted inhibitors for AML

In previous years, multiple inhibitors targeting STAT3 for patients with AML have been studied (Table I). The present review will briefly review some of the most impressive developments regarding these inhibitors in the following paragraphs.

Small-molecule inhibitors

Stattic. Stattic is a small molecule compound screened by Schust *et al* (40) from Maybridge (https://maybridgechem.lookchem.com/). It can effectively inhibit STAT3 activation and dimerization by selectively acting on the STAT3 SH2 domain and induces the apoptosis of STAT3-dependent cancer cells. Luo *et al* (41) found that stattic can inhibit the proliferation of AML cell lines, promote apoptosis and arrest the cell cycle at G_0/G_1. The possible mechanism is that stattic can inhibit the function of DNA damage repair and block the repair of DNA double-strand breaks (DSBs), thus enhancing the sensitivity to chemotherapy drugs. Because most traditional chemotherapeutic drugs kill cancer cells by inducing DSBs (42). In addition, a study found that stattic can inhibit the homologous recombination pathway, which may also be the mechanism of static-induced apoptosis (41). Although stattic has shown good antitumor effects *in vitro* experiments, its specific mechanism and clinical trials still need to be further evaluated for future clinical applications.

C188-9. The small molecule inhibitor known as C188-9 has demonstrated its ability to effectively induce apoptosis in AML cell lines and primary cells derived from patients with AML by inhibiting the STAT3 activation caused by G-CSF. The compound inhibits the binding sites between the phosphotyrosine peptide and the SH2 domain, consequently impeding the interaction and dimerization with tyrosine kinase. C188-9 exerts antileukemia effects by inhibiting ligand-induced STAT3 phosphorylation (21). However, whether C188-9 can improve the efficacy of chemotherapy for patients with AML still needs further confirmation through experiments *in vivo* and clinical trials.

OPB-51602. OPB-51602 is a novel small molecule compound that binds to the SH2 domain of STAT3 with high affinity and can effectively inhibit the proliferation of various cancer cells *in vitro* and *in vivo* by targeting the Tyr705 and

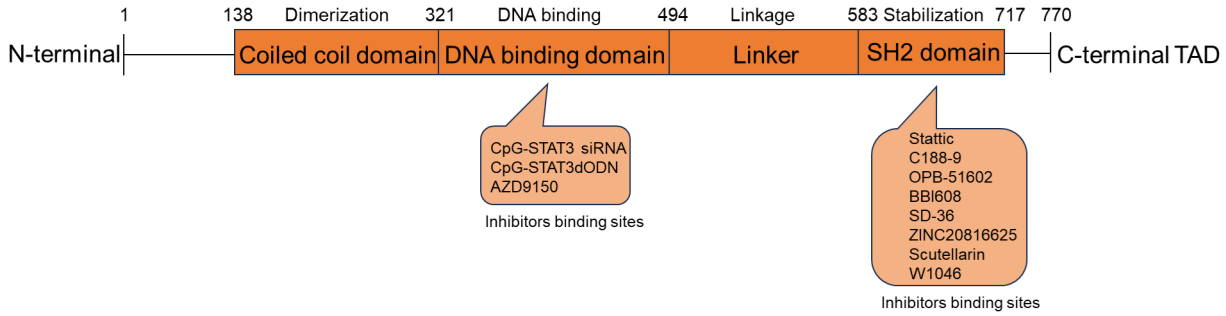

Figure 1. Functional domains of the STAT3 molecule and inhibitors binding sites. An N-terminal domain, a DNA-binding domain, a linker domain, a SH2 domain and a TAD. STAT3 targeted inhibitors mainly bind to DNA-binding domain and TAD domain. STAT3, signal transducer and activator of transcription 3; TAD, C-terminal transactivation domain; SH2, Src homology 2; CpG, cytosine-guanine dinucleotide.

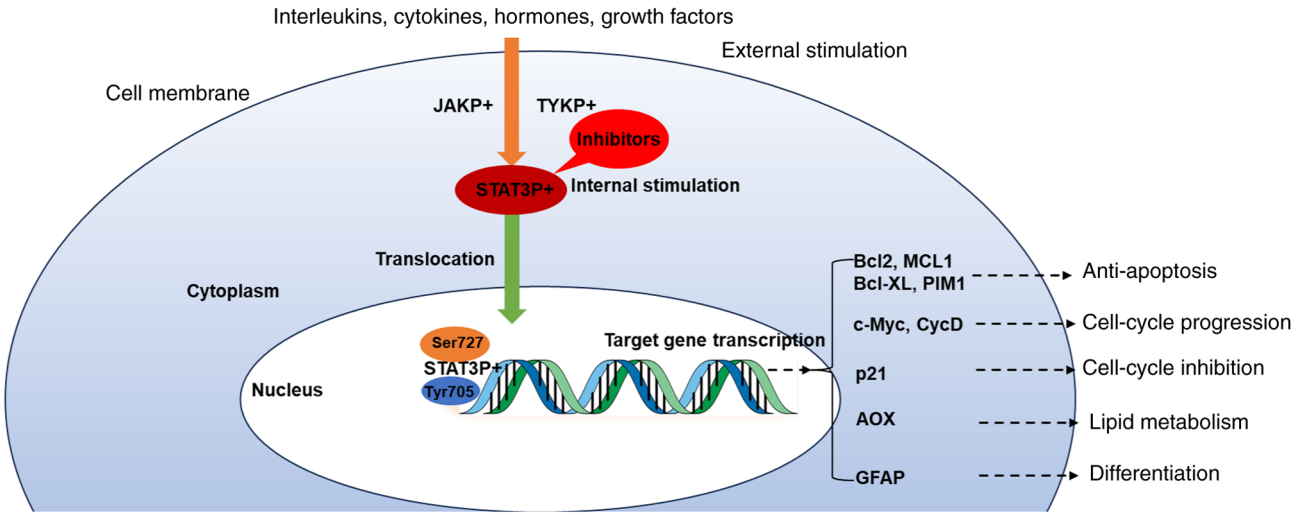

Figure 2. STAT3 signal transduction pathway. Ligands (interleukins, cytokines, hormones, growth factors) binding leads to conformational changes of the receptor and activates JAK and TYK2 by phosphorylation. This provides a docking site for the STAT3 protein and leads to its phosphorylation. The activated STAT3 is transported to the nucleus as homodimer or heterodimer and regulate gene transcription. P, phosphorylation; STAT3, signal transducer and activator of transcription 3; TYK2, tyrosine kinase 2; AOX, alternative oxidase; GFAP, glial fibrillary acidic protein.

Ser727 phosphorylation sites of mitochondrial STAT3 (43). The results of phase I trials demonstrated that 20 patients with recurrent or resistant hematological malignancies received OPB-51602 treatment on the basis of chemotherapy. Common side effects including nausea, peripheral sensory neuropathy and diarrhea were reported, and they were well tolerated and did not pose any safety concerns. Nevertheless, the patient cohort showed limited success in responding to the treatment, except for two individuals diagnosed with AML and one individual with multiple myeloma, both of whom exhibited persistent stable disease (44). In this diverse group of patients, the authors stated that determining the most effective dosage and frequency for long-term treatment is challenging.

BBI608. BBI608 (Napabucasin) is a compound that hinders the process of gene transcription mediated by the STAT3 protein. The characteristics of this compound have been verified when used alone and combined with paclitaxel during stage Ib/II and stage II trials in solid tumors (45). A previous study found that BBI608 demonstrates antileukemia properties in both the MOLM-13 cell line and primary cells obtained from patients with AML, and also had potent effects in immunodeficient mice xenograft models of AML *in vivo* (46). Meanwhile, the coadministration of BBI608 and venetoclax resulted in an increased cell death efficacy in Kasumi-1 cells that developed resistance to BBI608 (46).

Based on the available information, BBI608 shows promise as a potential treatment option for AML but needs to be further validated for its effectiveness in AML primary cells and clinical trials.

SD-36. SD-36 is protein hydrolysis targeted chimeric protein (PROTAC) that serves as a potent small molecule inhibitor of the STAT3 SH2 domain and exhibits the ability to selectively degrade STAT3 *in vitro* and *in vivo* (47). In contrast to traditional small molecule protein inhibitors, PROTAC degraders can effectively remove a protein target through degradation and block all functions related to the protein. In addition, a PROTAC degrader can achieve higher selectivity since the degrader needs to bind and recruit target proteins and E3 ligases to form a productive ternary complex for ubiquitination and degradation (48). Studies have provided evidence that the SD-36 compound demonstrates effective degradation of the

Table I. Inhibitors of STAT3 for AML.

Inhibitors	Target sites	Types	Results	Clinical trial (ID no.)	Years	(Refs.)
Stattic	SH2	Small molecule	Inhibits cell proliferation and promotes apoptosis	No	2006, 2021	(40,41)
C188-9	SH2	Small molecule	Induces apoptosis	No	2011	(21)
OPB-51602	SH2	Small molecule	Suppresses cell proliferation	Stage I (NCT01344876)	2015	(43,44)
BBI608	SH2	Small molecule	Suppresses cancer stemness	Stage Ib/II and stage II (NCT02352558)	2019	(45,46)
SD-36	SH2	Small molecule	Promotes growth inhibition and induces apoptosis	No	2019	(47-50)
W1046	SH2	Small molecule	Inhibits cell proliferation and promotes cell apoptosis	No	2023	(53)
CpG-STAT3 siRNA	DBD	Nucleotide based	Increases immunogenicity of primary AML cells	No	2014	(55)
CpG-STAT3dODN	DBD	Nucleotide based	Eliminates leukemia stem/ progenitor cells	No	2016	(56)
AZD9150	DBD	Nucleotide based	Promotes hematopoietic differentiation	Stage I trials (NCT05986240)	2018, 2024	(57,58)
ZINC20816625	SH2	Natural compounds	Artificial intelligence screening, not yet validated	No	2020	(59)
Scutellarin	SH2	Natural compounds	Hinders the growth of AML cells, triggering cell cycle arrest and apoptosis	No	2020	(62-64)

AML, acute myeloid leukemia; STAT3, signal transducer and activator of transcription 3; SH2, Src homology 2; DBD, DNA binding domain.

STAT3 protein in different types of leukemia and lymphoma cells (49,50). SD-36 represents a novel PROTAC that specifically targets STAT3, indicating how advanced technology can enhance the identification of appropriate inhibitors. Although SD-36 is a selective small molecule inhibitor, interference with normal STAT3 protein function may be a challenge. The differential E3 ligase expression levels between tumor and normal cells should be utilized to develop STAT3 inhibitors with minimal side effects.

W1046. Currently, immune checkpoint blockades (ICBs) have also shown encouraging responses in a number of types of treatment for cancer. Mo *et al* (51) revealed that STAT3-regulated V-domain immunoglobulin suppressor of T cell activation (VISTA), a novel immune checkpoint that mediates immune escape primarily by blocking T cell activation, is highly expressed in AML (52). Therefore, a novel inhibitor W1046 was designed with a boronic acid pharmacophore targeting the STAT3 SH2 domain This inhibitor was identified as a highly effective inhibitor by replacing the carboxylic acid in the compound with boric acid, demonstrating higher binding affinity, better cellular efficacy, more favorable PK profile and higher *in vivo* anti-tumor activity (53). W1046 significantly inhibits proliferation and promotes apoptosis in both AML cells lines and primary AML with hyperactive STAT3 and has demonstrated anti-AML efficacy *in vivo* but lacks sensitivity in cell lines with low STAT3 activation and STAT3 deletion (51). The development of inhibitors and monoclonal antibodies that target the STAT3-VISTA axis may provide a promising therapeutic strategy for immunotherapy of AML.

Nucleotide-based inhibitors

Cytosine-guanine dinucleotide (CpG)-STAT3. Hossain *et al* (54) investigated the influence of CpG small interfering RNAs (siRNAs) that specifically targeted STAT3-silencing in Toll-like receptor 9 (TLR9)-positive hematopoietic cells. By conjugating the inhibitor with both the TLR9 ligand and the CpG, efficient targeting of the inhibitor to TLR9-positive antigen-presenting immune cells was achieved (54). TLR9 can mediate innate and adaptive immunity, making it an attractive strategy for enhancing anticancer therapies (54). The application of CpG-STAT3 siRNA in a mouse model that mimics human inv (16) AML leads to regression of the disease through a mechanism that relies on $CD8^+$ T cells. STAT3-silencing and TLR9 stimulation results in an increased immunogenicity of primary AML cells, as observed in a previous study (55). This finding suggests the potential to use targeted STAT3 inhibition/TLR9-triggering to break tumor tolerance and induce

immunity against AML and potentially other TLR9-positive hematological tumors. However, CpG-STAT3 siRNA has poor serum stability and needs to be optimized through chemical modification, binding with high molecular weight polymers or encapsulation to further improve its therapeutic effect.

CpG-STAT3dODN. The decoy oligodeoxynucleotide (dODN) inhibitor CpG-STAT3dODN, which acts as a DNA decoy molecule and binds STAT3 within the cytoplasm, effectively blocking STAT3 transcriptional activity by using this immunostimulatory approach. This study specifically delivered STAT3dODN to myeloid cells by connecting STAT3dODN to the TLR9 ligand and CpG. The CpG-STAT3dODN conjugates are quickly internalized by human and mouse TLR9$^+$ immune cells and AML primary cells. Following their uptake, CpG-STAT3dODNs are released from endosomes, which bind and isolate cytoplasmic STAT3, thereby inhibiting downstream gene expression. In an experiment using xenografts from patients with AML, CpG-STAT3dODN achieve immune-mediated AML cell eradication in mice by CD8+/CD4+T cells. However, the direct cytotoxic effects of STAT3 inhibition were limited by alternative survival signaling in AML cells, and the rapid degradation of decoy oligonucleotides also pose a major therapeutic challenge (56). This study mainly provides evidence for further development of the dual-function CpG-STAT3dODN for the treatment of AML.

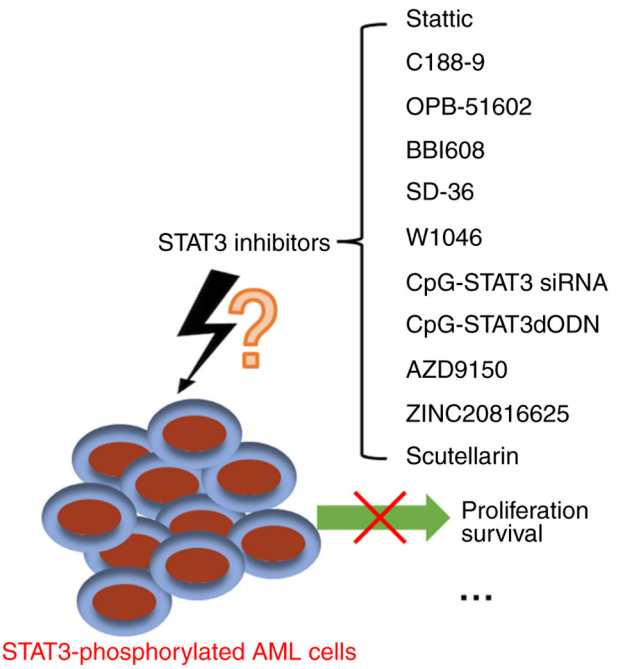

Figure 3. Targeted STAT3 inhibitors for AML. OPB-51602, AZD9150 and BBI608 have entered phase I clinical trials. Stattic, C188-9, SD-36, CpG-STAT3, CpG-STAT3dODN, ZINC20816625, scutellarin and W1046 are in preclinical studies. STAT3, signal transducer and activator of transcription 3; dODN, decoy oligodeoxynucleotide; siRNA, small interfering RNA; AML, acute myeloid leukemia.

AZD9150. Due to the swift advancements in next-generation sequencing technology, an antisense oligonucleotide (ASO) inhibitor, designed using the genetic sequence of the target gene, has been arisen. AZD9150 (danvatirsen) is a highly effective inhibitor of STAT3 ASO that has shown promising results in preclinical trials involving patients with lymphoma and non-small cell lung cancer, with adverse events that include transaminase abnormalities, fatigue, thrombocytopenia, nausea and anemia (57). A previous study also verified that AZD9150 promotes hematopoietic differentiation in myelodysplastic syndrome (MDS) and AML (58). Currently, a Stage I clinical trial is underway to investigate the safety and efficacy of danvatirsen as a monotherapy followed by combination with venetoclax in patients with relapsed/refractory MDS/AML (NCT05986240). Based on the aforementioned studies, the results of this clinical trial on relapsed/refractory MDS/AML will be notable.

Natural compounds

ZINC20816625. The swift advancement of artificial intelligence technology enables its extensive application in various facets of pharmaceutical research and development. Chen *et al* (59) have discovered promising candidates for STAT3 inhibitors (ZINC20816625) through the utilization of artificial intelligence models. The nitro group of ZINC20816625 interacts with the GLU638 and PRO725 of STAT3 through two hydrogen bonds. Based on *in vitro* experiments, researchers have determined that ZINC20816625 exhibits potential as an efficacious medication for treating AML (59). However, the mechanism and effectiveness of ZINC20816625 still need to be further verified by basic experimental methods.

Scutellarin. Traditional Chinese medicine (TCM) has been employed as a significant therapeutic approach to treat AML for over two millennia in China (60). TCMs are known for their intricate system, which consists of several components, diverse targets and multiple pathways of action (61). Scutellarin, an ingredient in TCM, was discovered to exhibit cytotoxic effects on diverse types of tumor cells. This compound exhibits anticancer properties by activating various pathways such as inducing apoptosis, inhibiting cell proliferation and blocking cell invasion, demonstrating beneficial therapeutic outcomes and minimal toxic side effects (62-64).

In a recent study, it was found that scutellarin has potential in hindering the proliferation of AML cells, triggering cell cycle arrest and apoptosis in a concentration- and time-dependent manner. This effect is thought to be associated with the regulation of the JAK2/STAT3 signaling pathway, because the activation of this pathway contributes to the formation of tumor inflammatory microenvironment and is closely related to tumorigenesis and progression (65). Based on the aforementioned studies, it is suggested that scutellarin may be a promising candidate for new natural inhibitor and deserves special attention in further studies.

5. Conclusions and perspectives

AML is a challenging type of hematological neoplasm characterized by a diverse range of genetic and cytogenetic markers, as highlighted in the most recent classification systems issued by the World Health Organization and International Consensus Classification (66,67). The use of personalized treatments based on the specific molecular data of patients is markedly increasing

in the field of oncology, allowing for targeted therapies aligned with the underlying pathobiology of the disease (68).

STAT3 is an important regulator in normal hematopoiesis, and constitutive activation of STAT3 is associated with the occurrence and prognosis of AML. Currently, targeted STAT3 inhibitors are being tried in the treatment of several cancers, including AML. Due to STAT3 and its associated upstream JAKs that play a crucial role in AML, inhibitors targeting this pathway are an important direction for improving AML efficacy. Selective targeting of the JAK/STAT pathway has shown promising results both *in vitro* and *in vivo* models (26). Targeted IL-6/JAK/STAT3 inhibitors have also been revealed to be beneficial for treating ovarian, prostate and myeloproliferative neoplasms, with potential to inhibit tumor growth (14). Furthermore, the proliferation of cancer cells can be driven by a diverse range of activated kinases, including EGFR, HER2, ALK and MET. Specifically, inhibition of enzymes can lead to autocrine activation of STAT3, which suppresses tumor cells through the FGF/JAK/STAT3 feedback loop (39). Therefore, these comprehensive summaries may provide new strategies and insights for targeted STAT3 therapy in AML. Some notable STAT3-targeting inhibitors in hematological malignancies, such as BBI608 (trial no. NCT02352558) and AZD9150 (NCT05986240), have been identified and have undergone clinical trials and achieved good therapeutic effects. However, despite extensive research and ongoing clinical trials, the efficacy of STAT3 inhibitors in clinical studies remains inconclusive (Fig. 3). The present review mainly focused on various inhibitors targeting STAT3 and systematically elucidated the mechanism of various STAT3 inhibitors in AML, current research status and existing challenges, which is expected to contribute to future research.

In order to enhance our understanding of STAT3 in AML, it is crucial to gain deeper insights into the biological traits and roles of STAT3 within primary AML cells. The investigation of STAT3 regulatory effects on the proliferation, apoptosis and cell cycle of AML primary cells, especially in AML cells with high STAT3 phosphorylation, may be of great use. Incorporating STAT3 inhibitors into chemotherapy has the potential to revolutionize clinical practice and improve treatment outcomes for patients with AML due to numerous ongoing studies in this field.

Acknowledgements

Not applicable.

Funding

This work was supported by the Clinical Research Innovation Team Project (grant no. CTCCR-2019B03).

Availability of data and materials

Not applicable.

Authors' contributions

HC and LC drafted the manuscript and created the figures. CH edited the manuscript. All authors have read and approved the final manuscript, took responsibility for the content and approved the publication. Data authentication is not required.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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