Journal of Cancer Biomoleculars and Therapeutics

ETERNO PRESS

Review

STAT3 Inhibition in Cancer: A Review of Emerging Therapeutics

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Article history

Received: 10 February 2025
Revised: 27 March 2025
Accepted: 16 April 2025
Published online: 16 April 2025

Keywords

STAT3 inhibition
Cancer pathways
Small-molecule inhibitors
Targeted therapy

Abstract

Abnormal signal transducer and transcriptional activator (STAT3) activation occurs in many human tumors. Therefore, targeting STAT3 signaling has emerged as a promising therapeutic approach for treating many cancers. In addition, studies using genetic and pharmacological agents to alter the activity of STAT3 provide significant evidence for the role of STAT3 dysfunction. in malignant transformation and cancer, confirming STAT3 as a novel cancer target. In this review, we provide an outline of the importance of targeting the STAT3 signaling pathway, the mechanism of action of inhibitors, and the current development of STAT3-targeted drug therapy. We also summarize the efforts to provide new insights into the translation of STAT3 in cancer and may contribute to better treatments for these malignancies. The main purpose of this review is to provide a general framework for the development of STAT3 inhibitors and a discussion of the inhibition mechanisms developed.

1. Introduction

A family of latent cytoplasmic transcription factors known as signal transducers and activators of transcription (STAT) proteins were first found to pass cellular signaling to growth factors, cytokines, and other peptide ligands [1]. In mammals, STATs consist of seven proteins: STAT1, 2, 3, 4, 5a, 5b, and 6. Six unique patterns are shared by all family members. In addition, all members of this family have a key tyrosine (Tyr) residue at the C terminus (Tyr705 for STAT3) that is phosphorylated upon stimulation. Cytoplasmic kinases, including growth factor receptor tyrosine kinases, cytokine receptor-associated (JAK), and Src family kinases, are responsible for mediating STAT activation through phosphorylation. STAT formation is induced by phosphorylation: STAT dimers are formed by the interaction of the phosphor-Tyr (pTyr)-SH2 domain between two monomers [1,2]. Inactive STAT complex monomers have also been noticed previously [3,4]. STAT3 usually present in in the cytoplasm in inactive form and activates via phosphorylation by cytokines, hormones, and growth factors, which utilize STAT3 signaling to control a remarkable variety of biologic responses, including cell development, differentiation, proliferation, motility, and survival Activated STATs accumulate in the nucleus from the cytoplasm to stimulates gene transcription of specific DNA elements by binding to it [1].

Following phosphorylation, STAT3 homodimerization or heterodimerization, with addition to other members of STAT family, translocate to the nucleus, and induces transcription of several STAT3-dependent genes. For these reasons, the STAT3 signaling pathway is a potential target for tumor therapy [5]. Activation of STATs is a key event that regulates cellular and biological processes through cytokines and growth, including growth, differentiation, survival, growth spurts, and swelling.

Unusual Stat3 enhance abnormal gene expression, including cyclin D1, c-Myc, Bcl-xL, Mcl-1 and surviving genes, and which are play role in cancer promotion. According to recent research, Stat3 that is consistently activated both alters immune functions to favor tumor immune evasion and causes tumor angiogenesis by upregulating vascular endothelial growth factor induction. All things considered, research has confirmed Stat3 as a new target for cancer treatment, which has given rise to the development of low molecular weight Stat3 inhibitors [2].

the first discovery of the relationship between STAT3 activity and malignant transformation [6]. Since then, many studies have been conducted to confirm the effectiveness of STAT3 as a cancer drug target [7-10], and significant efforts have been devoted to the discovery of novel STAT3 inhibitors [11]. So far, many STAT3 inhibitors have been reported and documented in this article, demonstrating the progress in this field in recent years. Among these inhibitors, some effectively inhibit the biological activity of STAT3, associated cellular effects, and tumor growth inhibition in mice models of human malignancies. The majority of these inhibitors are not appropriate for clinical usage and are still in the experimental stage. The published research has no proof that the right STAT3 inhibitors are almost a cure. The delayed progress in finding STAT3 inhibitors suited for clinical and clinical trials will be ascribed to competition between protein-protein interactions since the majority of efforts in STAT3 drug development have

been devoted towards disrupting STAT3:STAT3 dimerization.

interactions, given the large space available. It is difficult to identify molecules small enough to interfere with the target. However, recent success in blocking the interaction between Bcl-xL and Bax [12,13] raises some hope that STAT3 inhibitors targeting dimerization events may be developed for early treatment. On the other hand, methods focusing on other characteristics of STAT3 and functions, such as DNA binding interfaces or oligonucleotide pathways, have not been very successful. One reason why significant progress has not been made in these other areas is that they have been given less attention than the effects of dimerization. Therefore, to achieve rapid progress, further efforts should be made to target other domains during STAT3 activation.

This review sheds light on the serious function of abnormal STAT3 signaling in cancer's malignant transformation and evaluates STAT3 as a potential target for cancer.

2. Literature Review

2.1 Pathways

Although it is theoretically possible to target the STAT3 signaling pathway to influence malignant phenotypes in many ways, only a few of these strategies have been investigated. These are: i) directly to the STAT3 protein and N-terminal domain inhibitor via SH2 domain inhibitors or dimerization inhibitors (SDI, B domain), DNA binding domain inhibitors (DBDI, C domain Dot). (NDI, site D); ii) Indirect upstream pathway of STAT3 targeted (site A, tyrosine phosphorylation inhibitor, TPI). but in this review, we focus on direct inhibition.

2.2 Direct Inhibition Mechanism

This study discusses methods that directly target and inhibit STAT3. Additionally, completely blocking the STAT3 pathway requires more drugs than cell surface receptor or ligand inhibitors. Additionally, certain treatments, such as chemotherapy, or radiation can induce STAT3 expression and lead to resistance to various cancer treatments and drug therapy. Additionally, STAT3 inhibition may lead to feedback of other cancerrelated processes such as the RAS/RAF/MEK/ERK pathway [14]. Meanwhile, the development of STAT3 inhibitors was first reported in 2001 [15], and many other publications have followed, highlighting the importance of STAT3 as a research target to find an antidote. Figure 1 shows the STAT3 signaling pathway. The cytokine IL-6 causes the gp130 component of the IL6 receptor to dimerize and become active. In turn, JAK phosphorylation causes STAT3 phosphorylation, which dimerizes and moves into the nucleus to increase the expression of target genes linked to GSC and STE survival. Other ways to suppress the STAT3 pathway include blocking STAT3 dimeric and signaling with Stattic, STA-21, or S31-201 and targeting receptor signaling with WP1066 or bazedoxifene. STAT3 (signal transducer and activator of transcription 3), IL6R (phosphorylation of the IL6 receptor), and GP130 (glycoprotein 130).

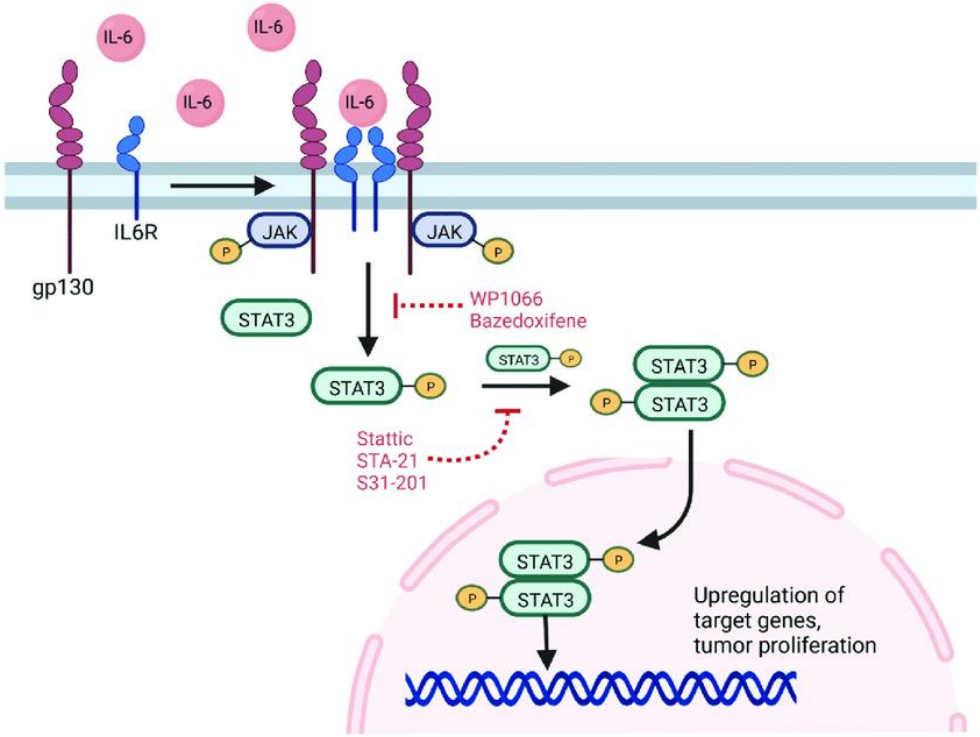

Figure 1. Signaling pathway of STAT3.

2.3 STAT3-targeted Drug Use as a Repurposing

Although no STAT3-targeted cancer therapy has yet received approval, the findings of several early-phase therapeutic studies have provided strong evidence in favor of clinical STAT3 targeting [16]. Several drug candidates have been repurposed successfully from non-cancer drugs into cancer therapy. These include the anti-

epileptic drug valproic acid for leukemia (NCT00530907), the arthritis treatment celecoxib for lung, colorectal, and breast cancers (NCT01695226), the diabetes treatment metformin for prostate, colorectal, and breast cancers (NCT00897884), and the angiotensin receptor blockers, like losartan used to treat hypertension, for pancreatic and breast cancer (NCT01821729).

thalidomide, is a drug designed for morning sickness therapy with distressing teratogenic impacts. Thalidomide derivatives are now being used to treat multiple myeloma (NCT00038090) with effectiveness, proving that medications with severe side effects may be utilized in well-controlled clinical settings. The logic for repurposing medications for novel indications is a valuable strategy for swiftly introducing new cancer therapies into the clinic, as demonstrated by several successful drug repurposing scenarios [17].

2.4 Inhibitors and Mechanism of Action in Inhibition

DNA Binding Domain Inhibitors that Target STAT3 Considering that the response gene's promoter region's consensus DNA binding domain and DNA binding sequence of STAT3 must physically interact for STAT3 to function, DNA interactions. The effect will inhibit STAT3-dependent gene transcription, thus blocking tumor activity. However, much less attention has been given to targeting the STAT3: DNA Binding interface in drug development. Some declarations have been made, but joint plans for the site have not yet been disclosed in documents.

DNA Binding activity of STAT3 can be block by platinum (IV) antibodies it has been reported. There are some IC50 values which is IS3 295, blocks STAT3 activity with an IC50 of 1.4 μM in some cases [18]. The exact mode of action of inhibition of STAT3 is unknown, but preliminary evidence suggests that it may bond with the STAT3 DNA binding domain and inhibit binding to STAT3-responsive DNA sequences [18]. Inhibition of intracellular STAT3 showed a decrease in pTyr705 STAT3 levels. In addition, IS3 295 inhibits the function of cyclin D1 and Bcl-xl which are STAT3-dependent gene modification, and blocks cell function, and inhibits NIH3T3/v-Src mouse fibroblasts with STAT3 activity and breast tissue from the same individuals. It induces apoptosis in cancer 10 μM [18].

Other such as CPA-1, CPA-7 as a platinum (IV) compounds and [7]. That can inhibit STAT3 DNA binding and reduce levels of pTyr705 STAT3 and STAT3-related activities like gene regulation, Growth inhibition and apoptosis in human breast, lung, and prostate cancer cells containing low micromolar levels of STAT3 activity. The level of activity of platinum (IV) complexes against STAT3 activation in vitro was better compared to peptide-based or non-peptide small molecules inhibitors.

The platinum (IV) compounds, only the in vivo activity of CPA-7 was evaluated. Experimentally few results indicates that In Initial stage in vivo studies shows that CPA-7 makes deterioration in the CT26 mouse cancer model at a dose of 5 mg/kg; this is an effect that should be sufficient to make an initial assessment of residual platinum (IV) complexes. Given the similar structure of CPA-7 and its drug class to the clinically approved drug cisplatin, further studies are needed to determine its therapeutic potential and provide appropriate treatment based on effective in vitro and in vivo effects. Efforts should be made to develop pharmacological and toxicity profiles for platinum (IV) complexes.

It is worth noting that platinum (IV) complexes exhibit different activity for STAT3 than cisplatin, a platinum (II) complex that has no effect on STAT3 activity [7,18]. In summary, all in vitro and in vivo activities of CPA-7 and platinum (IV) complexes are worth investigating based on their preclinical and clinical studies.

Ascomycetes isolated derivatives Tetrahydro-isobenzofuranone have been block STAT3 DNA binding activity. Galiellactone helps to inhibits IL-6-induced STAT3 DBD and transcriptional activity but does not impact on phosphorylation of STAT3 in HepG2 hepatoma cells [19]. It is unclear if gallicolactone stops STAT3 dimers from binding to the DNA consensus sequence, despite the fact that this is assumed how the medicine works by interacting with the STAT3 DNA binding domain. This is a crucial question to have answered. In STAT3-overexpressing DU145 and PC-3 human breast cancer cells, inhibiting STAT3 activity with 25μM gallellactone effectively reduced STAT3-dependent gene expression and induced apoptosis. Furthermore, the medication has the ability to prevent the growth of human prostate tumor xenografts (DU145) when injected intraperitoneally once daily at a dose of 3 mg/kg in an *in vivo* assays [19].

Recently, a 20-mer peptide inhibitor was also found to interact exactly with the domain of STAT3, DNA binding, thereby inhibiting transcriptional activity and STAT3 DBD [20]. Random peptide aptamer sequences were generated as fusion proteins for functional and functional analysis. In the published study, a purified recombinant peptide aptamer was labeled with nine arginine protein modification motifs and combined with thioredoxin as a scaffold protein that is permeable to the cell and selectively induces cell growth inhibition and 50% growth inhibition by inducing apoptosis [21]. Human U266 multiple myeloma and glioblastoma cells and animal B16 cancer cells with active STAT3 at a absorption of 0.27 μM [21].

Based on the reported activity, peptide inhibitor represents a better way to inhibit STAT3 activity in vitro compared to other methods. Some main issues are suggested in which is the physical strength of the structure and the suitability of the method for various applications. Although these studies support the principle of using recombinant peptide as a STAT3 inhibitor, restrictions for in vivo use include the druglike properties, drug stability, and protein permeability.

Table 1: STAT3 inhibitors and its mechanism of actions.

S.No	Inhibitor Name	Mechanism of Action	Target Site
1	STA-21	Binding to the SH2 domain of STAT3, preventing its dimerization and activation. This inhibition blocks STAT3-mediated transcription of genes involved in cell survival, proliferation, and oncogenesis.	DNA binding domain
2	LLL-3	inhibits STAT3 activation by preventing its phosphorylation, dimerization, and DNA binding, thereby disrupting STAT3-mediated transcription.	
3	LLL-12	It works by blocking the phosphorylation and dimerization of STAT3, thereby preventing its translocation to the nucleus and subsequent transcriptional activation of target genes involved in cancer cell survival, proliferation, and immune evasion.	Phosphoryl tyrosine 705 (pTyr705)
4	S3I-201	It disrupts the STAT3 dimerization by binding to its SH2 domain, preventing its phosphorylation and activation. This inhibition blocks STAT3-mediated transcription, leading to reduced expression of genes involved in cell survival, proliferation, and immune evasion.	DNA binding
5	Stattic	It inhibits STAT3 activation by preventing its phosphorylation, dimerization, and DNA binding, thereby blocking its transcriptional activity. Stattic achieves this by interacting with the SH2 domain of STAT3, which is crucial for its activation and function.	STAT3 binding domain and phosphor-peptide GY*LPQTV-NH2
6	Napabucasin (BB1608)	Key regulator in cancer stem cells (CSCs). By inhibiting STAT3 activity, Napabucasin disrupts CSC survival, self-renewal, and tumor progression.	Transcription of target genes
7	InS3-54A18	It disrupts STAT3 dimerization and DNA binding, thereby preventing the transcription of STAT3-regulated genes involved in cancer cell survival, proliferation, and immune evasion.	DNA binding domain
8	OPB-31121	It blocks the phosphorylation and activation of STAT3, thereby inhibiting its downstream signaling pathways involved in cancer cell proliferation, survival, and immune evasion. By disrupting STAT3 activity, OPB-31121 induces apoptosis and suppresses tumor growth in various cancers.	SH2 domain
9	OPB-51602	It works by blocking STAT3 phosphorylation and activation, preventing its nuclear translocation and transcriptional activity. Since STAT3 plays a crucial role in tumor cell survival, proliferation, and immune evasion.	SH2 domain
10	ODNs	ODNs competitively inhibit the activation of TLR9 by blocking its interaction with stimulatory CpG DNA. This prevents downstream signaling pathways, reducing the activation of NF-κB and other pro-inflammatory transcription factors. As a result, the production of cytokines and immune responses mediated by TLR9, such as inflammation and adaptive immune activation, are suppressed.	SH2 domain

2.4.1 STA-21

STA-21 as a small molecule inhibitor, selectively inhibits the activity of DNA binding domain of STAT3 in vitro: that is, STA-21 dimerization STAT3 and inhibiting (Figure 2) . The gene is transcribed and inhibits the growth of cells and induce apoptosis. Stat3 activation also cause Caspase pathway activation in human breast cancer and rhabdomyosarcoma model cells [22,23]. Recently, STA-21 derivatives have been reported to have a similar inhibitory role as like STAT3. These are also effective in inhibiting the proliferation of prostate cancer cell lines [24].

2.4.2 LLL-3

LLL-3 and STA-21 inhibitors have a similar mode of inhibition; However, the inhibitions depend on the size of molecules LLL-3 (molecular weight of 266 g/mol compared to 306 g/mol of STA-21) would have improved cell capacity (Figure 3). LLL-3 inhibits the viability and induces apoptosis of these glioblastoma cell lines [25]. There are some experiments showing LLL-3 inhibition activity, but they are still unclear, while U87 glioblastoma tumor-bearing mice treated with LLL-3 showed extended [25]. LLL-3 should be further explored and using as a potential agent for other human carcinoma treatments [26].

2.4.3 LLL-12

A drug design approach that is structure-based led to the discovery of LLL12, a new STAT3 inhibitor [27]. By directly binding to the STAT3 monomer's phosphoryl tyrosine 705 (pTyr705) binding site, LLL12 prevents STAT3 phosphorylation and activity (Figure 4). Researchers discovered different types of cancers in human. LLL12 (5–10 μmol/L) downregulates STAT3 downstream targets, cyclin D1, Bcl-2, and surviving. This results in a suppression of cell viability and the activation of death. The most crucial finding is that LLL12 barely affects normal human cells in terms of apoptosis. These findings demonstrate the potential of LLL12 as a cancer therapy, and more research into its use as a possible treatment agent for malignancies that have constitutively active STAT3 is warranted [28].

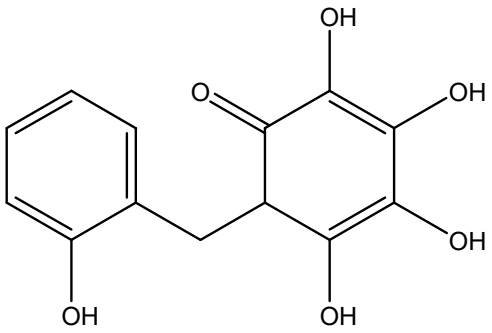

Figure 2. Structure of STA-21

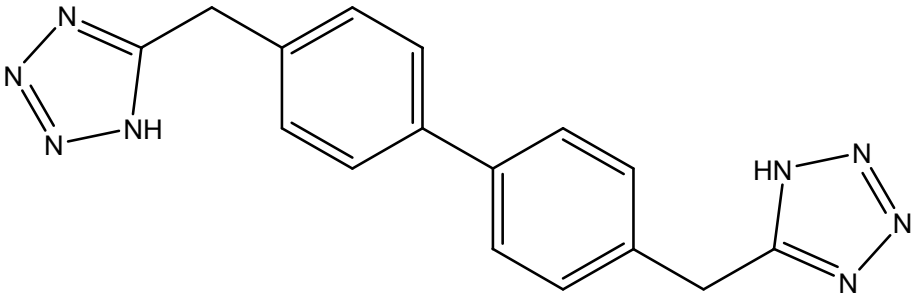

Figure 3. Structure of LLL-3

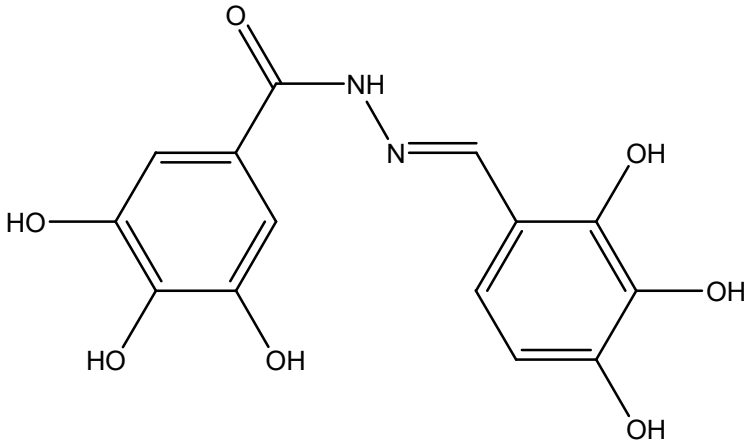

Figure 4. Structure of LLL-12

2.4.4 S3I-201

Another recently discovered S3I-201 (also known as NSC 74859) is a small molecule inhibitor [29]. This reagent selectively eliminates activity of DNA binding in vitro by blocking the formation of dimers that is STAT3: STAT3. The IC50 is 86 μM, and the selectivity of STAT3 is 3-fold greater than that of STAT1. At 100 μM, intracellular activity was realized, including the suppression of gene transcription that is dependent on STAT3 and the prevention of human breast cancer cells from proliferating and surviving due to STAT3 activity. In terms of the biological process, S3I-201 suppresses the expression and function. These cells express genes known as STAT3-regulated genes, including cyclin D1, surviving, and Bcl-xL. Furthermore, in MDA-MB-231 human breast tumor xenografts, S3I-201 had remarkable benefits, causing tumor regression in the nude mice model at a dosage of 5 mg/kg. These published studies [22,24,29] have highlighted some of the most promising STAT3-inhibiting chemical entities that show antitumor activity in xenografts and medium to high micromolar activity is required using chemical chemistry to provide analogues with improved activity, selectivity, and specificity to facilitate their preclinical and final treatments.

2.4.5 Stattic

Random screening of 17,298 chemicals produced the discovery of another non-peptide inhibitor of STAT3. With an IC50 of 5.1 μM, static (STAT tri-inhibitory compound) [30] inhibits the STAT3 binding domain and phosphor-peptide GY*LPQTV-NH2 in vitro. Furthermore, Static inhibitor overcome the STAT3 (Tyr705) phosphorylation in human carcinoma cell lines

and cause blockage of IL-6-induced STAT3 activation, nuclear growth, and DNA binding activity in human HepG2 liver cancer cells at 20 μM. MDA-MB-435 and MDA-MB-231 both caused cell death in the same manner. There are still many unanswered concerns, such as the molecular processes underlying STAT3 inhibition and the physiologic repercussions that follow stimulation. Furthermore, the effectiveness of Static medicines in human tumor xenografts in vivo is unknown. Such in vivo studies are still to be conducted and published and will provide the first assessment of the therapeutic potential of Static molecules. Recently, through modeling and virtual screening, compounds containing catechol (1, 2-dihydroxybenzene) [31] have been shown to be potential STAT3 inhibitors targeting the SH2 domain. Experimentally, these compounds inhibit STAT3 DBD domain on the bases of IC50 of few compounds [31]. No further evaluation of the effects of catechol has been reported.

2.4.6 Napabucasin (BB1608)

Too far, the most thoroughly studied STAT3-targeted drug against cancer has been napabucasin (BBI608). It is a novel inhibitor of cancer that prevents transcription of target genes downstream of STAT3. It has been demonstrated in preclinical settings to inhibit the survival and self-renewal of different cancer cells, including Hepato Cellular Carcinoma cells, in vitro, downregulated the expression of β-catenin, NANOG, genes and sex-determining zone Y-box protein 2 (Sox2), and prevent cancer deterioration and metastasis in vivo. Furthermore, it has been shown to make stomach cancer cells with high stemness more susceptible to the common chemotherapy drug paclitaxel. Among all STAT3-targeted medicines, napabucasin is the only one that has progressed into phase III studies [32].

2.5 Inhibitors Targeting the IL-6R/JAK/STAT3 Signaling Pathway

The IL6 neutralizing monoclonal antibody siltuximab, the JAK2 small molecule inhibitor OPB31121, and the JAK light inhibitor AZD1480 have undergone clinical trials and may be candidates for future EGFR-TKI trials. Ruxolitinib is currently the only JAK1/2 selective inhibitor that has received FDA approval to treat JAK2-dependent cancers like myelofibrosis. It was recently reported that microRNAs either control the activity state of STAT3 or are controlled by STAT3 activation in tumors. The mechanisms of cancer incursion, migration, and fighting against anti-cancer agents, including cytotoxic or targeted therapies, may be linked to the microRNA upregulation caused by STAT3 activation. Particularly, NSCLC cells were made more sensitive to paclitaxel by miR-337-3p's direct targeting of STAT3. MicroRNAs that down-regulate STAT3 may be used to treat cancer drugs [33].

2.5.1 InS3-54A18

Inhibitor not only binds to the DBD to inhibits the function and movement of STAT3 both by in vitro and invivo they also have some factors which inhibit the constitutive and interleukin-6-animated articulation of STAT3 downstream objective and qualities. InS3-54A18 is completely soluble in nature in an oral formulation and inhibits the growth and metastasis of lung xenograft tumors with little effect on animals. As a result, inS3-54A18 may be a good candidate for future development because anticancer therapies that target the domain of transcription factors and human STAT3 may not be "undruggable" as previously reported [34].

The resultant atom, DX10, inhibited STAT3 activation through bringing down the creation of IL-6. To upgrade the STAT3 inhibitory impact of DX10, we utilized WP (an industrially accessible STAT3 inhibitor) alongside DX10 [26].

2.5.2 OPB-31121 and OPB-51602

These are SH2 domain binding inhibitors which are binds to SH2 with high affinity. OPB-31121 and OPB-51602 inhibitions shows through cell culture assay this inhibited phosphorylation of two amino acids sites Tyr705 and Ser727 respectively. OPB-31121 is a high affinity STAT3 inhibitor that fights several human cancer cell lines e-I Liver. In addition, OPB-31121 has been shown to It inhibits the expression of anti-apoptotic proteins, induces apoptosis of gastric cancer cells, and reduces cell proliferation in gastric cells and xenograft models. In light of these results, OPB-31121 has entered clinical trials for cancers, including hematopoietic tumors and hepatocellular carcinoma (HCC). However, the antitumor activity of OPB-31121 against HCC and peripheral nerve-related toxicities is lacking. in a phase 1 pharmacologic trial [35]. Additionally, STAT3 inhibition may increase tumor cells' chemotherapy treatment like Cisplatin and Taxol.34, 35, 36, and 37 Future research ought to focus on the combination of STAT3 inhibitors and immune-checkpoint inhibitors (ICIs), as STAT3 inhibition may prevent adverse reactions caused by ICIs. In addition, by inhibiting STAT3 signaling, ICIs can enhance their anticancer effects.13, 38 AZD9150, an antisense oligonucleotide inhibitor of STAT3, has already demonstrated positive results when used in conjunction with durvalumab (ICI) in clinical studies (NCT02499328, NCT02983578, NCT03394144) [14].

2.6 Computer Added Drug Designing for STAT3

development of inhibitors with inhibitors of the SH2 domain responsible for the activation and dimerization of STAT3 bound to phosphotyrosine binding domains is a possible strategy to block both pathways. This study uses in silico site-directed FBDD, a novel fragment-based drug design (FBDD) strategy. Through fluorescence polarization studies, the novel target 5,8 dioxo-6-(pyridin-3-ylamino)- 5,8-di hydranathian sulfonamide (LY5) was confirmed to bind to STAT3 SH2 Additionally, four of the five selected compounds had IC50 values against U2OS becteria below 5μM. The IC50 range 8 in various cell lines is 0.5-1.4 μm. This study demonstrated the benefits of this approach and can often be used in other clinical settings [36]. STAT3 promotes tumor growth by increasing cell division and inhibiting apoptosis and is a potential target for cancer

therapy. To investigate direct inhibitors of STAT3, we performed binding experiments on previously used 1,2,5-oxadiazole derivatives. Specifically, the compound N-[4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl]-4-(trifluoromethyl) benzamide, MD77, binds to the STAT3-SH2 domain at a specific dose. Increases the ability to act in a binding-depended,manner (IC50=17.7). the data linking process has been studies using computer modeling. Additionally, compound MD77 has been shown to have anti-inflammatory effects on various tumor cell lines based on these considerations, MD77 was chosen for its potential for future development of direct STAT3 inhibitors [37].

Computationally designed STAT3 inhibitors from the SPECS library. MD simulations and binding energy calculations of the 28 tested compounds yielded the best results; This showed that the inhibitors had greater blocking potential. According to in silico analysis, 19 compounds showed in vitro anticancer activity against MDA-MB-231 and MCF-7 cell line according to the results two compounds showed significant activity compared to other compounds and apoptosis analysis was performed for these compounds. The morphological changes of the tested drugs are dose- and time-dependent so all the tested drugs have been shown to be effective against cancer [38].

2.6.1 ODNs

STAT3 plays an important role as a canciuse and is activated in many human cells. Some genetic methods to knock out STAT3 activity stimulate the development of new drugs targeting STAT3 for cancer treatment by inhibiting cancer cells and increasing apoptosis. STAT3 DNA binding activity is inhibited in vitro by Gquadruplex oligodeoxynucleotide (ODN) with an IC50 of 7 μM for G-quadruplex ODN T40214. According to in silico docking experiments, G quadruplex ODN mainly interacts with the SH2 domain of STAT3 and can be docked onto the SH2 domains of DNA-bound STAT3 dimers. This Gquadruplex pattern is determined by intracellular but not extracellular K + ion concentrations. Therefore, Gquartet is a new drug that targets STAT3 in cancer cells [39]. In the discovery of STAT3 inhibitors through pharmacophore-based virtual screening and docking, molecular dynamic simulation additionally, 25 complexes were selected according to best s score and were further evaluated with similar drugs. Finally, five inhibitors that have druglike properties are selected as stable structures for drug discovery. It can be seen that the compound Sa32 has the ability as a novel potential STAT3 inhibitor and may provide information for the development of new STAT3 inhibitors. Sa32 was screened and filtered through pharmacophore-based screening and molecular docking against STAT3. The SA32 complex consistently shows the best molecular dynamic results for the STAT3 inhibitor as confirmed by RMSD and RMSF, RG analysis [40].

2.7 Drug Development from Discovery to Clinical Trial

A timeline or flowchart showing the stages of drug development from discovery to clinical trials. Figure 5 shows the drug discovery timeline and clinical stages towards FDA approval of a drug.

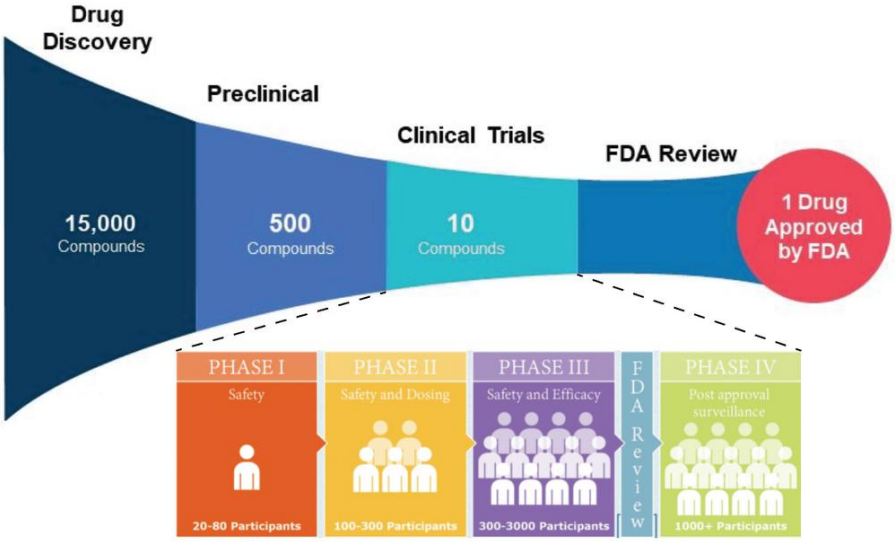

Figure 5. Schematic flowchart of drug discovery (https://blog.biodock.ai/image-based-profiling-drug-discovery/).

3. Future Perspective

A final question is how STAT3 inhibitors will respond to and prevent new antiinflammatory drugs. AntiSTAT3 drugs are available in treatment. Therefore, there is still a need to make suitable and effective STAT3 inhibitors to treat cancer patients and to test STAT3 protein in patients with malignancy. Clinically relevant STAT3 inhibitors will also provide cancer diagnostic tools for obtaining clinically relevant biomarkers with sensitivity profiles associated with blockade of abnormal STAT3 in human tumors. These biomarkers may play an important role in identifying patients who may benefit from antiSTAT3 therapy. It is hoped that these important drugs will contribute to the development of STAT3 small molecule inhibitors suitable for clinical use. Structural analysis tools (e.g., NMR analysis) combined with computational models should facilitate analysis of

important proteins (e.g., DNA binding interfaces and SH2 domains that bind small molecules) to understand the importance of this process. This is the basis for a new paradigm to create novel low molecular weight molecules that selectively inhibit STAT3, its dimerization and DNA binding activity and to develop new protein interaction models todevelop effective direct STAT3 inhibitors as new antiinflammatory drugs over time.

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