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The application of small molecule drug in clinical trial

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Abstract. In the most recent century, small molecule drugs have become prominent in treating different illnesses and diseases. These small molecule drugs, ranging between 0.1 and 1 kDa in size, have been specifically chemically synthesized not to trigger immune responses. In addition to their nonimmunogenic nature, small molecules tend to possess a low molecular weight and a well-defined structure. They are process-independent, stable, and completely characterizable.

Keywords: Small Molecule Drug, Clinical Trial, Kinases.

1. Introduction

Small molecule drugs often go through an extensive routine of processing [1, 2]. They are usually analyzed on their absorption, distribution, metabolism, excretion, and toxicity [3]. As for absorption, most small-molecule drugs are ingested orally, so there is a focus on intestinal absorption and permeability [3]. When analyzing absorption, the bioavailability of drugs is significant, so researchers have devised ways to predict it through self-organizing maps [4]. Distribution is also looked at as it helps assess the pharmacodynamics and toxicodynamics of a particular drug [3]. Metabolism, while hard to predict, is vital in helping determine bioavailability, toxicity, drug-to-drug interactions, enzyme inhibition, induction, and many other functionalities [5, 6]. Excretion is then looked at to determine half-lives and dosages [3]. Usually, small-molecule drugs are excreted through the kidney as urinary excretion or through the liver as biliary excretion [3]. Finally, toxicity, the most challenging aspect concerning the approval of small molecule drugs, is looked at on all levels since it could occur at the microscopic organelle level or on the systemic level [3].

The targets of small molecule drugs must also be determined, and it's done through three main methods: the ligand-based method, the structure-based method, or the phenotype-based method. The ligand-based method utilizes known ligands and is excellent for targets whose receptor sites are unknown [3]. The structure-based process uses ligands to bind to target proteins and is perfect for attaining important information on target proteins [3]. The phenotype-based method utilizes phenotypic information and is excellent when comparing whole genomes [3].

There have been many developments regarding small molecule drugs, especially their interactions with RNA. They are known to have the capabilities of regulating polymerase reactions, multilevel progression, viral infectivity, gene expression, and gene storage. Small molecules' ability to

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manipulate these various functions is essential in combating diseases associated [3]. On another note, there has been extensive research on small molecules and their potential to fight against cancer. Some novel small molecules like PAWI-2 can act as growth inhibitors against tumors [7]. Another small molecule called nicodamid has shown promise in treating cancer and other viral infections, for instance, Covid-19 [8]. The benefits of using small molecules don't just stop at their efficiency in treatment but also their viability in preserving healthy cells. This can be interpreted as a meeting point between chemotherapy and immunotherapy [3]. Small molecules have also been extensively used in treating and subduing inflammation. These small molecules are often called anti-inflammatory drugs and can be divided into two categories by their chemical structures. They can either be steroidal, like Glucocorticoids, or nonsteroidal agents, like JAK and PDE4 inhibitors [9]. These drugs often target chronic inflammation and autoimmune diseases such as multiple sclerosis, chronic obstructive pulmonary disease, inflammatory bowel diseases, psoriasis, and rheumatoid arthritis [6].

Small molecules, in general, are beneficial; however, there is an increasing challenge in selecting safe and effective ones. Some reasons some small molecules are rejected are their durability and molecular fit. As for draggability, some small molecules fail to engage in hydrophobic and polar interactions with their target [10]. Small molecules may also lack the structure to fit in ways that affect the target, such as getting into a cavity or pocket [11]. There are also general health risks that come with these small molecules. For instance, while large doses of Baricitinib are efficient in treating rheumatoid arthritis, there is a high chance of acquiring skin cancer and fatal strokes [12].

2. Kinases

2.1. Receptor tyrosine kinase inhibitors

2.2. FLT3 inhibitors

Fms-like tyrosine kinase 3 (FLT3) is a transmembrane protein of the proto-oncogene FLT3 coding, ubiquitous in progenitor and hematopoietic cells. FLT3 is a widely expressed protein in patients with acute myeloid leukemia (AML). The mutation of its gene will cause abnormal activation of its downstream pathway [13, 14].

In all clinical studies of first-generation FLT3 inhibitors, midostaurin is the only monotherapy that continues. A randomized phase III trial (RATIFY study, NCT 00651261) showed that overall survival (OS) of FLT3-mutated AML patients is improved significantly through adding midostarin to cytarabine chemotherapy [15]. Gitlinib is the first approved second-generation Flt3 inhibitor, which has fewer side effects and more robust efficacy and specificity than conventional chemotherapy, and there are significant differences in average OS between the two groups ($p < 0.001$). Screened by KinomeScan technology, Quizartinib has improved affinity and specificity for FLT3 kinase and has strong selectivity and activity for FLT3-ITD [16], so it is approved for the relapse of refractory AML or FLT3-ITD mutation patients [17].

Crenolanib has inhibitory activity against both FLT3 D835 and FLT3-ITK mutations and is undergoing clinical trials focusing on assessing combination effects with conventional chemotherapy in terms of relapse and first-line treatment [18]. SKLB-1028 is conducting a phase I trial to evaluate its activity on FLTS secondary mutations such as FLT3-F691L and FLT3-D835Y [18,19]. Ponatinib is Bcr-Abl1 inhibitor as well as FLT3 inhibitor that is potent inhibition of FLT3-ITD [20]. It is ongoing phase I and II to evaluate the safety and efficacy of ponatinib in combination with cytarabine for AML patients with the FLT3-ITD mutation.

2.3. Non-receptor tyrosine kinase

2.4. 1Bcr-Abl1 inhibitors

Bcr-Abl1 inhibitors

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A Philadelphia (Ph) chromosome translocation results in the juxtaposition of the breakpoint cluster region (BCR) of chromosome 22 with the ABL1 molecule, resulting in an aberrant BCR-ABL fusion gene on chromosome 22 [21]. The gene is capable of autophosphorylation and constitutively activates downstream pathways, so it does not control the proliferation of leukemic cells in CML and ALL, so the Bcr-Abl1 fusion tyrosine kinase is considered a susceptibility target in some leukemias [22].

Imatinib was the first approved Bcr-Abl 1 inhibitor and the first approved small molecule TKI. A study of CML-CP patients treated with imatinib for 5 years showed an OS and PFS of 89% and 93%, respectively [23, 24]. Despite the exciting treatment result of imatinib on CML patients, it frequently emerged drug resistance that is caused by point mutations in the kinase domain of BCR-ABL [25-27]. There are second-generation inhibitors, such as dasatinib, nilotinib, bosutinib, redotinib, and so on, to improve the binding ability of Bcr-ABL [28-31]. Both dasatinib and bosutinib are oral dual Src/Abl1 kinase inhibitors, with the former approximately 300-fold more potent than imatinib. Nilotinib has better solubility and lipophilicity and about 30 times more potency than imatinib. Radotinib, clinically used as second-line therapy, can inhibit most BCR-ABL mutants [32]. Ponatinib acts similarly to imatinib by forming carbon-carbon triple bonds on its amino acids to enhance its affinity with T315l.

So far, there have been up to 13 inhibitors that have entered clinical trials against drug-resistant mutations. As a selective and potent allosteric Abl1 inhibitor, asciminib binds to the myristoyl pocket. Therefore, it reduces inactive kinase formation [33]. As another potent inhibitor against Bcr-Abl1, rebastinib induces kinase to the state of catalytically idle, which, however, is insufficient to supply the treatment of leukemia continuously [33]. To reduce side effects and expand the susceptibility, bafetinib was put in the second-line treatment of patients with CML and Ph^+ ALL and indicated potential clinical outcomes [34].

2.5. BTK inhibitors

Brunton's agammaglobulinemia tyrosine kinase (BTK) is a significant constituent of the B-cell receptor (BCR) pathway and is expressed in B-cell lymphomas and leukemias abundantly [35].

Ibrutinib, as an irreversible inhibitor, can form a covalent bond with Cys-481 (ATP- BTK). Inbrutinib is an effective drug for the treatment of chronic lymphoblastic leukemia (CLL), lymphoma (SLL), lymphoma (SLL), lymphoma (MZL) and other lymphomas (MZL) [36-39]. At present, BTK inhibitors have been widely used in clinics. Acarabutinib interacts with Cys-481 (ATP- BTK) in BTK pocket [40], but its killing effect on TEC, ITK, EGFR, and other tumor cells is limited. Zanubutini had the same relationship with TEC, ITK and EGFR, and had a high anti-tumor effect on JAK3 and HER2 inhibitor Ibrutinib, but has a poor impact on ibrutinib [41].

To reduce drug resistance caused by BTK inhibitors covalently bonded to the sulfhydryl group of Cys-481, the active site of BTK, vecabrutinib, ARQ-521, fenebruntinib, and LOXO-305, which do not form a covalent bond with the sulfhydryl group, were developed.

3. Epigenetic Regulatory Proteins

3.1. HDAC inhibitors

Histone deacetylases (HDACs) are important epigenetic regulators, and it removes acetyl groups from N-acetylated lysin residues of histones and various non-histone substrates [42-44]. Among its four categories, hydroxamide HDAC inhibitors inhibit HDAC activity through coordination with Zn^{2+}, where a multicenter trial (NCT00741234) showed pracinostat was safe for patients with advanced hematological malignancies. So far, HDAC is applied clinically primarily to hematological malignancies, including leukemia, lymphomas, and MM [45].

3.2. IDH1/2 inhibitors

Isocitrate dehydrogenase (IDH) is the core of converting isocitrate to α- ketoglutaric acid (α-KG). It plays a vital role in tricarboxylic acid metabolism, which uses nicotinamide adenine dinucleotide phosphate (NADP+) or NAD+ as a cofactor in the tricarboxylic acid (TCA) cycle key enzyme [46].

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Mutated IDH1/2 loses its normal catalytic function and catalyzes the degradation of α-KG to the oncogenic metabolite 2-hydroxyglutarate (2-HG) [47, 48], which leads to transcriptional dysregulation and tumor formation, thereby affecting cellular normal differentiation [49].

Enasidenib (AG-221), an oral, selective multDH2 inhibitor, and its predecessor were used to find inhibitors of the IDH R140Q mutation [50,51]. Enasidenib can interact with IDH2R140Q protein, significantly inhibits IDH2R172K, and has a better therapeutic effect [52]. Ivosidenib (AG-120) is a reversible multDH1 inhibitor that blocks the formation of the catalytically active site primarily by binding to the cofactor (magnesium ion) of IDH1. The U.S. Food and Drug Administration (FDA) officially approved it in 2018 to treat refractory and relapsed acute myeloid leukemia (AML) in adults with MutlDH1.

In addition, targeting DNMT is also an anti-tumor method, and decitabine and azacytidine have been approved for treating MDS, AML, and other hematological malignancies [53-55]. For cytosine analogs, only the combination of dexamethasone, bortezomib, and panobinostat has been approved by the US Food and Drug Administration [56].

3.3. BCL-2 inhibitors

The B-cell lymphoma 2 (BCL-2) is an important molecule regulating the endogenous apoptosis pathway. [57]. Under different cellular stresses, multidomain pro-apoptotic effector proteins promote apoptosis [58]. The anti-apoptotic and pro-apoptotic proteins are the critical tumors in theBCL-2 family, which regulates apoptosis through interaction.

Early members of the anti-apoptotic BCL-2 family included the antisense oligonucleotide drug Oblimersen, the natural product gossypol, and its synthetic derivatives [59]. Based on BH3 analogs ABT-737 and Navitoclax, we found that ABT-199 (Ventoclax) has high specificity for BCL-2. In the single Resapy clinical study (NCT01889186), 79.4% CLL and 17 P gene knockout patients achieved an objective response of 12 months, and 107 patients completed the target efficacy of 8.2 months [60]. Following the expansion of indications to CLL or SLL patients, venetoclax, a standard chemotherapy anti-AML drug, has been widely used in treating AML in people aged 75 and over [61-62]. S55746 (also known as Servier-1 or BCL201) is another BHS mimic BCL-2 inhibitor under investigation in clinical trials, which is derived from a phenylpyrazole substituted with tetraquinoline amide and has a high effect on BCL-2. Selectivity has affinity [63]. AMG-176 is the first phase I study (NCT02675452) clinical trial of MCL-1 inhibitors to evaluate the efficacy, competitive activity, and tolerance of refractory/recurrent MM and AML [64]. MCL-1 Inhibitor S64315 (MILK665) has significant potential to induce multiple hematological malignancies in malignancies such as drug-resistant/relapsed AML, MDS (NCT02979366), and refractory/relapsed MM, lymphoma (NCT02992483) Apoptosis.

3.4. Hedgehog pathway inhibitors

Hedgehog (HH) is an essential link in embryonic development and tissue regeneration due to its highly conserved signaling pathway.HH ligand releases and initiates activation of the canonical HH pathway, which can bind to and inhibit the 12-pass transmembrane receptor Patched-1 (PTCH1) [65], which then inhibits HH signaling by inhibiting the transmembrane transducer Smoothened (SMO). Smooth muscle protein (SMO) dissociates at the end of the cytoplasm and forms primary cilia. Active SMO can promote the transcriptional activation of tumor-related proteins, leading to the high expression of downstream target genes [66]. It has been found that HH signaling pathway is closely related to many kinds of tumors, such as vascular nature and malignant degree [66, 67].

In 2018, Glasgib therapy was approved with low-dose cytarabine chemotherapy for low-dose treatment of AML patients over 75 with chronic disease who cannot receive intensive treatment [68]. Arsenic trioxide (ATO), a small molecule inhibitor of GLI2, can effectively treat acute promyelocytic leukemia. The previous research found that ATO and itraconazole effectively treat SMO D477G gene variant myeloblastoma [69].

4. Small Molecules in Targeted Cancer Therapy Challenges and Future Perspectives

In summary, small molecule targeted anti-cancer drugs have succeeded and entered a rapid development stage. Despite the significant progress achieved, there are still some challenges that small molecule targeted anti-cancer drugs face.

Two significant challenges are drug resistance [70] and low efficiency. Almost all targeted anti-cancer drugs undergo resistance after the early clinical trial, where resistant mutant cells increase figure and show resistance after sensitive mutant cells are killed. Meanwhile, targeted anti-cancer drugs only work on limited patients due to the exclusive identification of inhibitors.

Many strategies have been applied to deal with these challenges, such as new-generation anti-cancer drugs against drug resistance mutations, multitarget drugs, combination therapy, and drugs targeting CSCs [71, 72]. Solutions mainly shed light on the combination between small-molecules targeted drugs and other medicines, including immunotherapy such as PD-1,[73, 74] antibody-drug conjugate drugs [75, 76], and PROTAC technology [77], which reduces the activity of target proteins by catalyzing the degradation of target proteins.

Small-molecule targeted drugs will continue to be the mainstream in cancer treatment because of their unique advantages despite the competition from macromolecule drugs. With the development of coupling technology, targeting, and effectiveness, combining variable drugs will be an unstoppable trend in anti-cancer therapy.

5. Conclusion

Often with cancer cases, surgery, radiotherapy, or biotherapy is used to treat the disease. Since its introduction, chemotherapy is often used to treat cancer by either killing or inhibiting the growth of cancerous cells. Recently, advancements have been made regarding small-molecule drugs and cancer treatment. Small molecule drugs have their benefits in specifically treating this kind of disease due to their characteristics, such as its storage and transportation; however, there are also some shortcomings, such as its low response rate and drug resistance. To expand on this, it’s often seen in clinical trials that opposition to the drug is developed after a period due to mechanisms such as gene mutation amplification, apoptosis, and autophagy. There is also a low response rate/efficiency, as observed in some trials where tiny groups of people are susceptible to minor molecule treatment. Small molecules are used in this area for various targets, such as RNA and miRNA. However, aside from these challenges, small molecules should continue to be tested and analyzed for their possible potential in treating and curing cancer. In the past twenty years, there has been significant progress concerning small molecules.

These small molecules often act as inhibitors to different protein kinase enzymes, tyrosine kinase receptors, and other structures such as proteasomes.

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