Leukemia Research 39 (2015) 1312–1318

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Invited review

Clinical activity of alvocidib (flavopiridol) in acute myeloid leukemia

Joshua F. Zeidner[a,*], Judith E. Karp[b]

[a] University of North Carolina, Lineberger Comprehensive Cancer Center, Chapel Hill, NC, United States
[b] Johns Hopkins University School of Medicine, Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, United States

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 1 October 2015
Accepted 14 October 2015
Available online 19 October 2015

Keywords:
Alvocidib
Flavopiridol
FLAM
Acute myeloid leukemia
Cyclin dependent kinase
CDK inhibitors

ABSTRACT

There have been minimal therapeutic advancements in acute myeloid leukemia (AML) over the past 4 decades and outcomes remain unsatisfactory. Alvocidib (formerly flavopiridol) is a multi-serine threonine cyclin-dependent kinase inhibitor with demonstrable in vitro and clinical activity in AML when combined in a timed sequential chemotherapy regimen, FLAM (alvocidib followed by cytarabine continuous infusion and mitoxantrone). FLAM has been evaluated in sequential phase 1 and phase 2 studies in 149 and 256 relapsed/refractory and newly diagnosed non-favorable risk AML patients, respectively, with encouraging findings in both patient populations warranting further investigation. This review highlights the mechanism of action of alvocidib, pre-clinical studies of alvocidib in AML, and the clinical trials evaluating alvocidib alone and in combination with cytotoxic agents (FLAM) in AML.

Contents

1. Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy characterized by a clonal proliferation of immature myeloid precursor cells. Approximately 18,000 patients are diagnosed with AML each year in the United States and the majority of these patients

* *Corresponding author at: University of North Carolina, Lineberger Comprehensive Cancer Center, 170 Manning Drive, Physician's Office Building, 3rd floor, CB# 7305, Chapel Hill, NC 27599-7305, United States. Fax: +1 9199667748.
E-mail address: Joshua_Zeidner@med.unc.edu (J.F. Zeidner).

http://dx.doi.org/10.1016/j.leukres.2015.10.010
0145-2126/

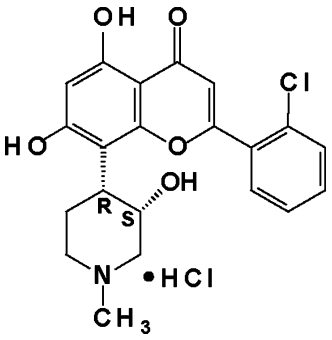

Fig. 1. Chemical structure of alvocidib.

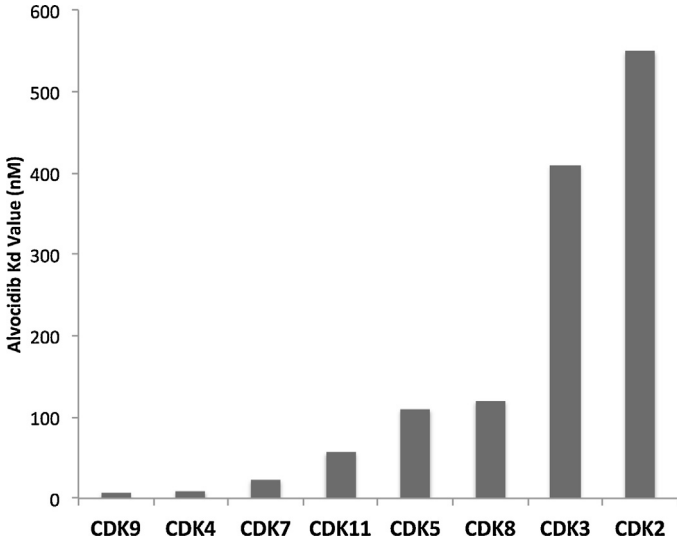

Fig. 2. Dissociation constants (Kd) for alvocidib against a panel of cyclin-dependent kinases (CDKs). Adapted from Karaman et al. [15].

will ultimately die of their disease [1]. Therapeutic advancements have been minimal in AML over the past 4 decades. "7 + 3," defined as 7 days of continuous infusion cytarabine (100–200 mg/m^2/day) and 3 days of an anthracycline (most typically daunorubicin 45–90 mg/m^2/day or idarubicin 12 mg/m^2/day), was originally studied in the 1970s by the Cancer and Leukemia Group B (CALGB) cooperative group [2–4]. Despite unsatisfactory outcomes, particularly for patients with non-favorable risk disease, 7 + 3 remains the most commonly used induction regimen in the United States for newly diagnosed AML patients who are fit for intensive therapy. Although 60–70% of patients will achieve a complete remission (CR) with 7 + 3, the majority of these patients will ultimately relapse [5,6]. Furthermore, patients with relapsed and refractory disease have a dismal overall outcome with 5-year overall survival rates <10% [7]. There is a lack of effective chemotherapeutic agents in patients with relapsed/refractory AML highlighting an area of a highly unmet need.

Over the last 10 years, alvocidib (formerly flavopiridol) has been studied alone or in combination with cytotoxic agents in AML with promising results. This review provides an overview of the pharmacologic properties, the pre-clinical development, and the results of clinical studies evaluating alvocidib in AML patients.

2. Mechanism of action of alvocidib

Alvocidib is a synthetic analog of a naturally occurring flavone derivative that was initially isolated from the stem bark of the Indian tree *Dysoxylum binectariferum* [8]. The chemical structure of alvocidib is shown in Fig. 1. Alvocidib is a potent growth inhibitor of diverse human tumor cell lines and induces apoptosis in hematopoietic cell lines derived from AML, B and T-cell lymphomas and multiple myeloma [9–11]. Mechanistically, alvocidib is a potent inhibitor of serine-threonine cyclin-dependent kinases (CDKs) with preferential activity against CDKs 9, 4, and 7 (Fig. 2). Alvocidib also has activity against CDK6, but exhibits its greatest inhibition against CDK9 (Kd = 6 nM) [12–16].

Historically, the mechanism of action attributed to alvocidib has been tied to its inhibition of the cell cycle at the G1 phase [17]. Although alvocidib treatment results in the inhibition of cell cycle progression through the targeting of CDK 4/6, it is now better understood that its primary mechanism of action is driven by its effects on transcriptional regulation through the inhibition of CDK9 and CDK7 [18,19]. CDK9 and CDK7 exist in a super enhancer complex that consists of many transcriptional regulatory proteins, including chromatin-modifying enzymes. Within this complex, CDK9 and CDK7 phosphorylate the c-terminal domain of RNA-polymerase 2, which relieves a transcriptional checkpoint, leading to transcriptional processivity and elongation (Fig. 3). Thus, alvocidib-induced apoptosis of tumor cells results, at least in part, from the inhibition of CDK9 and CDK7 leading to down-regulation of important transcripts that are critical for the survival and proliferation of tumor cells, such as cyclin D1, c-MYC, and MCL-1 [20]. Inhibition of CDK9 and CDK7, and the suppression of super enhancer transcriptional targets are now postulated to be the critical mechanism for the anti-tumor activity of alvocidib and is independent of its activity on the cell cycle [11,21,22].

Given alvocidib's effects on the cell cycle, it has been shown that alvocidib can antagonize the effects of S-phase-dependent cytotoxic agents when administered concomitantly [9]. In contrast, studies have shown that alvocidib's anti-tumor effects can be synergistic when given in sequential combination with other cell-cycle specific cytotoxic agents, such as cytarabine. In lung cancer cell lines, alvocidib-induced cytotoxicity is followed by recruitment and synchronization of residual tumor cells into cell cycle. The increase in the proportion of tumor cells entering S phase is observed 48–72 h after alvocidib washout, and persists for ≥3 days. Administration of cytarabine after alvocidib, timed during maximal proliferation of residual tumor cells, leads to synergistic growth inhibition and cytotoxicity in vitro [9,20]. These observations, coupled with the ability of alvocidib to kill non-cycling cells, suggest that alvocidib might be particularly effective when administered first, and then withdrawn, followed several days later by cytotoxic agents antagonizing the cell cycle.

3. Pre-clinical studies of alvocidib in AML

In this regard, alvocidib was investigated in combination with cytotoxic agents in models of primary human AML samples. An in vitro timed sequential therapy (TST) model was designed by Karp et al. to determine whether alvocidib can improve the activity of intensive chemotherapy in AML [20]. Timed sequential therapy (TST) refers to the opportune sequential timing of cytotoxic chemotherapy agents to exert maximal activity, particularly in the context of AML. TST relies on the premise that residual AML cells are recruited into cycle after administration of cell-cycle specific therapeutic agents, increasing the sensitivity of subsequent S-phase specific chemotherapy agents [23,24]. In this study, alvocidib was demonstrated to induce a mean 4.3-fold increase in apoptosis in primary human relapsed and refractory AML bone marrow populations in vitro. Furthermore, overall cytotoxicity was significantly higher after alvocidib pre-treatment followed by 72 h exposure to cytarabine, when compared with alvocidib or cytarabine alone. Importantly, the majority of the patients in this study had been exposed to cytarabine during their induction and consolidation