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REVIEW ARTICLE

Cladribine in the remission induction of adult acute myeloid leukemia: where do we stand?

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Abstract

The combination of cytarabine and an anthracycline has been the standard of care for the induction of remission in acute myeloid leukemia (AML). The response to treatment and survival of adult patients with AML are still variable and depend on multiple factors. Therefore, there have been many efforts to improve the response to treatment and survival rates by either increasing the cytarabine dose or adding a third agent to the standard induction chemotherapy regimen. Unfortunately, attempts to improve response and survival have been mostly unsuccessful. Recent clinical trials and retrospective studies explored the addition of cladribine to standard induction chemotherapy for AML. Some of these studies showed higher rates of complete remission, and one showed improved survival. In this review, we will discuss the antileukemic properties of cladribine and summarize the recent clinical data regarding its incorporation into the induction therapy for adult AML.

Keywords Cladribine · Acute myeloid leukemia · Remission · Induction · Chemotherapy

Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of clonal disorders that stem from the neoplastic transformation of myeloid precursors [1]. In 2017, there were an estimated 62,000 new cases of leukemia in the USA, with AML responsible for approximately one third of the cases [2]. The incidence of AML in both the USA and Europe is approximately three to five cases per 100,000 [2, 3]. The prognosis of AML has improved over the last few decades with an increase in overall survival [4].

Except for acute promyelocytic anemia, other types of AML are treated similarly. The treatment starts with induction chemotherapy, the goal of which is the rapid reduction of the disease burden and achievement of complete remission (CR). This is defined as normalization of peripheral blood counts (absolute neutrophil count > 1000/μL, platelet counts > 100×10^9/L, and absence of blood transfusion requirements), < 5% blasts in the bone marrow with a complete absence of Auer rods and absence of extramedullary disease [5, 6]. Response to treatment and prognosis are dependent on multiple factors including but not limited to age, comorbidities, de novo versus therapy-related AML, cytogenetics, and some

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molecular alterations such as *FLT3*-ITD, *NPM1*, and *KIT* [5, 7]. Attempts to improve response to induction are ongoing. Recent studies have explored the addition of cladribine to the standard induction chemotherapy protocol, with some showing promising results.

In this article, we will review the literature with regard to standard induction chemotherapy for AML as well as antileukemic properties of cladribine. Recent and ongoing trials incorporating cladribine in adult AML induction regimens will be discussed in details.

The standard induction chemotherapy for AML

The choice and dose of the anthracycline

The standard induction chemotherapy for AML is called 7 + 3 regimen, which is a combination of a 7-day infusion of cytarabine (ara-C) (100–200 mg/m^2/day) and an anthracycline given as bolus over 3 days [1, 5]. The most commonly used anthracycline is either daunorubicin or idarubicin [8–10]. In general, with conventional 7 + 3 regimens, approximately 60–80% of young adults and 40–60% of those 60 years of age or older achieve CR [1, 5, 11]. Various doses of the anthracyclines were used in clinical trials. In a recent meta-analysis, Gong et al. [9] compared low dose (45 mg/m^2/day) versus high dose (90 mg/m^2/day) of daunorubicin in combination with cytarabine. The authors also compared idarubicin (12 mg/m^2/day) versus high dose (50-80 mg/m^2/day) of daunorubicin combined with standard-dose cytarabine. Compared with the low dose, patients who received the high-dose daunorubicin had higher rates of CR, event-free survival (EFS), and overall survival (OS) and without increasing toxicity rates. However, young patients (< 65 years) or patients with unfavorable cytogenetic risk had the best benefit from the high dose. Furthermore, the meta-analysis revealed that there are no differences between the high dose of daunorubicin and idarubicin with regard to CR, disease-free survival (DFS), EFS, OS, and toxicity [9]. Another meta-analysis done in 2015 revealed that idarubicin increased CR rate, prolonged OS and DFS, and reduced the relapse rate when compared to daunorubicin. However, when the cumulative daunorubicin dose given over 3 days was ≥ 180 mg/m^2, the OS was no longer different. Furthermore, grade 3 and 4 cardiac toxicities were similar between the two groups. However, idarubicin increased the risk of death during induction (RR 1.18) and severe (grade 3 or 4) mucositis [12]. Based on these findings, the cumulative dose of induction daunorubicin administered over 3 days should be ≥ 180 mg/m^2. However, there is no clear consensus on the *optimal high dose*. A phase III multicenter randomized clinical trial (RCT) was done to compare daunorubicin 90 mg/m^2/day versus 60 mg/m^2/day for 3 days in AML induction. Both arms of daunorubicin doses had similar CR rate (73% vs. 75%; odds ratio (OR) = 1.07 [0.83–1.39]) and toxicity. The 60-day mortality was increased in the high-dose arm (10% vs. 5%; hazard ratio (HR) = 1.98 [1.30–3.02]). After a median follow-up of 14.8 months, the estimated 2-year survival was similar in both groups [13]. A subsequent analysis was performed with a median follow-up of 28 months which also showed similar OS between the two groups. Interestingly, patients who had *FLT3* internal tandem repeat (ITD) mutations had a lower relapse and an OS benefit (54% vs. 34%; HR = 0.65 [0.43–0.96]; $p = 0.03$), emerging after 12 months, with the 90 mg/m^2 dose [14].

Based on all the above-described results, it can be concluded that either idarubicin or daunorubicin can be used for induction of remission in AML. Daunorubicin might be preferred due to the slightly lower risk of death during induction and severe mucositis. If daunorubicin is used, the dose should be between 60 and 90 mg/m^2/day given improved outcomes with the higher doses.

The dose of cytarabine

Patients who do not clear blasts after induction chemotherapy have a poor outcome [15]. Therefore, multiple trials have been done in an attempt to increase response rate and survival by either increasing the cytarabine dose used in induction or adding a third agent. A recent meta-analysis of four studies which compared standard dose (200 mg/m^2) versus high doses of cytarabine (2000–3000 mg/m^2) in induction chemotherapy for AML showed no substantial differences in CR between both arms (HR = 1.01; 95% confidence interval (CI) = 0.93–1.09; $p = 0.88$). However, high-dose cytarabine (HiDAC) decreased the risk of relapse-free survival (RFS) compared with standard doses (HR = 0.57; 95% CI = 0.35–0.93; $p = 0.02$) [16]. Given the lack of significant benefit and potential for increased toxicity, HiDAC is not routinely suggested in induction regimens [5]. A more recent RCT (AML-12) randomized 1942 newly diagnosed patients with AML to either standard dose or HiDAC combined with daunorubicin and etoposide. The HiDAC group achieved higher CR rate (75.6% vs. 82.4%, respectively; $p = 0.01$) and OS (43.3% vs. 51.9%, respectively; $p = 0.009$) in patients younger than 46 years old. Nevertheless, the benefit seemed much less substantial in older age groups. Adverse events were similar in both arms except for an increase in the rate of grade 2–3 conjunctivitis in the HiDAC group. Interestingly, patients with secondary AML who received the HiDAC had a better CR rate across all age groups. Likewise, patients who had *FLT3*-ITD mutation and those with unfavorable cytogenetics had a better OS across all age groups [17]. These findings suggest that HiDAC can be considered in the induction therapy for AML for those who are healthy and younger than 45 years especially if they have poor cytogenetics or *FLT3*-ITD

mutation. Nonetheless, further research is needed for more evidence.

Liposomal cytarabine/daunorubicin (CPX-351)

CPX-351 is a liposomal formulation of cytarabine and daunorubicin in a fixed 5:1 M ratio. It was recently approved by the U.S. Food and Drug Administration (FDA) for adult patients with newly diagnosed therapy-related AML or AML with myelodysplasia-related changes. The approval was based on a multicenter phase III RCT which randomized older adults (aged 60–75) with newly diagnosed high-risk AML to either CPX-351 or standard therapy. In this study, the CPX-351 induction course consisted of 100 units/m^2 (100 mg/m^2 cytarabine and 44 mg/m^2 daunorubicin) administered as a 90-min infusion on days 1, 3, and 5. The control arm received standard 7 + 3 induction of daunorubicin and cytarabine. Consolidation therapy consisted of up to two courses of either CPX-351 (65 units/m^2 on days 1 and 3) or cytarabine and daunorubicin according to initial randomization. CPX-351 resulted in better response rates as well as a 31% reduction in the risk of death compared with standard induction (median OS = 9.56 months vs. 5.95 months; hazard ratio = 0.69; 95% CI = 0.52 to 0.90; one-sided $p = .003$). Toxicities were similar in both groups [18].

Other agents added to the induction regimen

Other agents such as thioguanine, topotecan, and etoposide have been investigated to be added to the induction chemotherapy regimens of AML; however, no outcome advantages have been reported [19–21]. Midostaurin was recently approved in combination with induction chemotherapy in patients with *FLT3*-mutated AML patients. This was based on the CALGB 10603/RATIFY (Alliance) RCT which showed that it significantly prolongs OS compared with chemotherapy alone (HR = 0.78 [0.63–0.96]) [22]. In this trial, induction chemotherapy consisted of 7-day continuous infusion of cytarabine (200 mg/m^2/day) and daunorubicin (60 mg/m^2/day) on days 1–3. Midostaurin (50 mg orally twice daily) or placebo was administered in a double-blind fashion, on day 8 through day 21. Patients with residual leukemia were given a second identical induction cycle. Four cycles of HiDAC consolidation were administered in patients who achieved CR. Midostaurin or placebo was given from days 8 to 21 of the cycles. Patients who remained in remission were given maintenance therapy with either midostaurin (50 mg twice daily) or placebo for 12 28-day cycles. Stem cell transplantation was performed at the discretion of the investigator. Median overall survival was 74.7 months (95% CI = 31.5 to not reached) in the midostaurin group and 25.6 months (95% CI = 18.6 to 42.9) in the placebo group (one-sided $p = 0.009$ by log-rank test). The survival advantage was consistent among all *FLT3* subtypes in both the primary analysis and after censoring the data for patients who underwent transplantation. Midostaurin increased the risk of skin rash compared with placebo, but all other adverse reactions were similar in the two arms. In addition, multiple clinical trials explored *FLT3* inhibitors such as sunitinib, lestaurtinib, quizartinib, and sorafenib either as monotherapy or in combination with sequential chemotherapy in *FLT3*-mutated AML patients [23].

Gemtuzumab ozogamicin (GO), a recombinant humanized anti-CD33 antibody linked to the cytotoxic agent calicheamicin, was also approved in September 2017 by the FDA for the treatment of adults with newly diagnosed $CD33^+$ acute myeloid leukemia [24]. GO is internalized by $CD33^+$ cells and subsequently leads to cell death. Approval was based on the ALFA-0701 phase III trial. Induction therapy consisted of up to two courses of cytarabine (200 mg/m^2/day on days 1 to 7) and daunorubicin (60 mg/m^2 on days 1 to 3) with or without GO (3 mg/m^2) on days 1, 4, and 7. Patients who achieved CR or CR with incomplete platelet recovery (CR_p) were given 2 cycles of consolidation with cytarabine and daunorubicin with or without GO. The results of the trial were originally published in 2012 and showed that the addition of GO to standard induction improved EFS but not OS [25]. Subsequently, an independent review of EFS, final OS, and additional safety results from the trial were also recently published. The results confirmed the EFS advantage with GO (13.6 months [95% CI = 9.0 to 19.2] in the GO arm and 8.5 months [95% CI = 7.5 to 12.0] in the control arm) (HR = 0.66; 95% CI = 0.49–0.89; $p = 0.006$). Median OS was 27.5 months in the GO arm and 21.8 months in the control arm, but the difference was not statistically significant. The main toxicity of GO was prolonged thrombocytopenia and increased risk of hemorrhage [26]. GO also carries the risk of infusion reactions and hepatic veno-occlusive disease [24]. Recent studies provided evidence that the hedgehog (Hh) pathway is upregulated in multidrug resistant (MRD) AML cell lines [27]. Therefore, it has been suggested that targeting the Hh pathway might be helpful in overcoming chemoresistance.

Glasdegib, or PF-04449913, is an oral inhibitor of smoothened (SMO), a transmembrane protein that is essential for activation of the Hh signaling pathway [28]. Glasdegib has shown activity and a good safety profile in phase I trials in both relapsed and newly diagnosed AML [29, 30]. In a phase II trial in patients with newly diagnosed AML or high-risk myelodysplastic syndromes (MDS) who were ineligible for intensive induction, combining glasdegib with low-dose ara-C improved OS in comparison to low-dose ara-C alone [31]. More recently, a phase II study evaluated glasdegib in combination with cytarabine and daunorubicin in patients with untreated AML and high-risk MDS. Glasdegib was administered continuously in 28-day cycles starting on day 3. Up to 2 cycles of induction with cytarabine and daunorubicin were given,

followed by 2 cycles to 4 cycles of consolidation therapy with HiDAC. After consolidation, up to 6 cycles of glasdegib maintenance were given. In this cohort of older adults (median age of 64), the CR rate was 46% with a median OS of 14.9 months [32]. A phase III trial evaluating glasdegib with intensive induction is ongoing (NCT03416179).

The addition of cladribine has been extensively investigated in induction regimens and will be discussed in details in the following sections.

Pharmacology of cladribine

Adenine nucleotide metabolism: the biochemical basis of cladribine

Cladribine (2-chloro-2′-deoxyadenosine (2-CdA), Fig. 1) is a synthetic deoxyadenosine analog which is metabolized by the purine nucleoside salvage pathways. In general, purines can be synthesized de novo or salvaged to the nucleotide forms [33, 34]. The de novo pathway is the main source of nucleotides in the dividing cells which are actively replicating DNA [35]. In the purine synthesis pathway, a molecule of ribose 5-phosphate is converted by a series of complex reactions to either adenosine monophosphate (AMP) or guanosine monophosphate (GMP) [33]. For DNA synthesis to occur, the ribose moiety of nucleotides should be reduced to 2′-dexoyribose. This reaction is catalyzed by ribonucleotide reductase during the S phase of the cell cycle [33, 34]. It catalyzes the reduction of both purine and pyrimidine nucleoside diphosphates to their respective deoxy forms (deoxyadenosine diphosphate (dADP), dGDP, dCDP, and dUDP) [33, 36]. dADP acts as a negative effector of all the reactions creating a balance [33, 34].

The purine salvage pathway provides another source for adenosine nucleotides in the cell. Both the free base adenine and adenosine can be salvaged [33]. In addition, deoxyadenosine can be salvaged by the enzyme deoxycytidine kinase (dCK). dCK phosphorylates deoxyribonucleosides—deoxycytidine (dC), deoxyguanosine (dG), and deoxyadenosine (dA)—into their respective mononucleotides [37, 38]. dCK is an important enzyme as it activates many antiviral and chemotherapeutic prodrugs including cladribine, cytarabine, clofarabine, fludarabine, gemcitabine, lamivudine, and zalcitabine among others [37, 39, 40]. 5′-Nucleotidases regulate intracellular nucleotide levels by hydrolyzing nucleotide monophosphates to their respective nucleosides [41, 42].

Another enzyme that is needed for adenosine turnover is adenosine deaminase (ADA). ADA deaminates adenosine and deoxyadenosine into inosine and deoxyinosine, respectively [33]. Both will be ultimately catabolized into uric acid [33]. A genetic deficiency of ADA causes approximately 10–15% of the cases of severe combined immunodeficiency (SCID) in humans [43]. Deficiency of ADA causes accumulation of adenosine and deoxyadenosine in the lymphocytes. Accumulated dADP inhibits ribonucleotide reductase by negative allosteric feedback leading to arrest in DNA synthesis [33, 34, 44]. Based on this principle, Carson et al. [45] postulated that an analog of deoxyadenosine that is resistant to deamination would mimic the ADA-deficient state. Multiple analogs were tested, and 2-chlorodeoxyadenosine proved to be toxic toward both human lymphoblasts and murine leukemic cell [45]. Subsequent clinical studies demonstrated activity in hairy cell and chronic lymphocytic leukemias as well as myeloid leukemia [46–49]. Figure 2 summarizes the important reactions in adenine nucleotide metabolism.

Mode of action of cladribine

Cladribine is a prodrug and enters the target cells by uptake through the nucleoside transporters [50–52]. It is then phosphorylated into cladribine monophosphate (CdAMP) by cytosolic dCK [53]. This reaction leads to bioactivation of cladribine, and it is necessary for its action [38, 52, 53]. CdAMP is subsequently phosphorylated into the di- and triphosphate forms (CdADP, CdATP) by nucleoside phosphate kinases [52]. Cladribine has been shown to exhibit selective toxicity toward both dividing and resting lymphocytes [54]. In the dividing cells, CdATP inhibits the enzyme ribonucleotide reductase, which leads to deprivation of the cells of the deoxyribonucleotides [55]. This leads indirectly to decreased

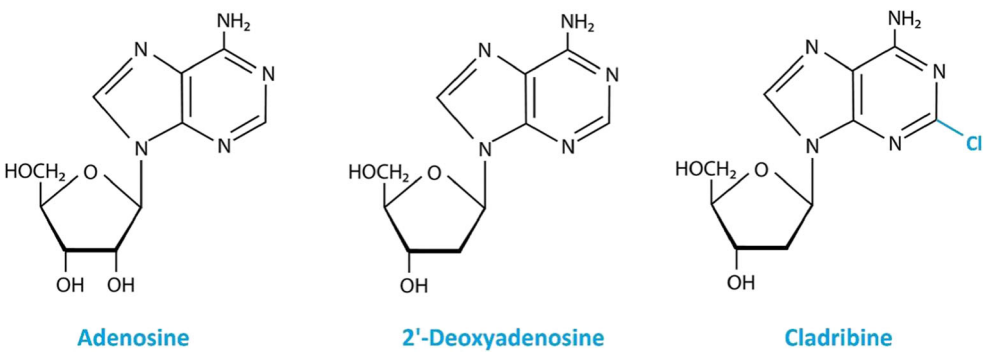

Fig. 1 The molecular structure of cladribine compared to its analogs, adenosine and 2′-deoxyadenosine

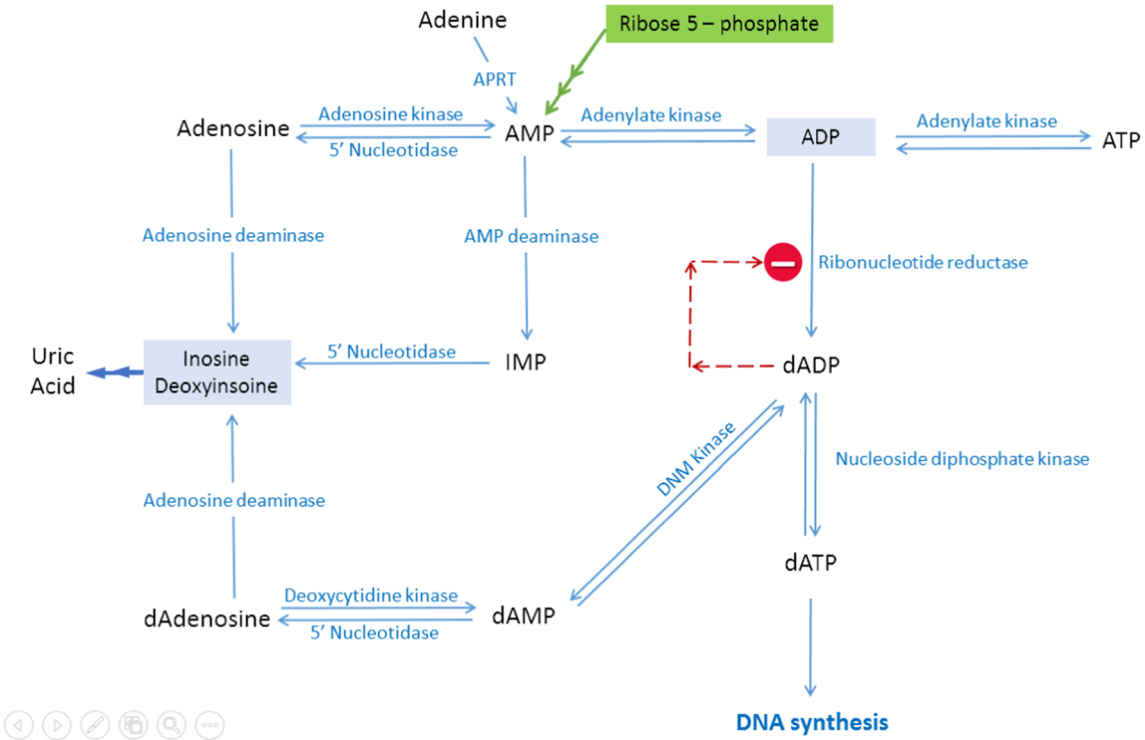

Fig. 2 Metabolism of adenine nucleotides. Ribose 5-phosphate is converted through complex reactions to AMP in the purine synthetic pathway. This pathway is the main source of adenine nucleotides in the dividing cells. Ribonucleotide reductase converts purine and pyrimidine nucleoside diphosphates to their respective deoxy forms needed for DNA synthesis. In addition, the purine salvage pathways provide another source for adenine nucleotides in the cells. Adenine, adenosine, and deoxyadenosine are salvaged by the enzymes APRT, adenosine kinase, and deoxyadenosine kinase (dCK), respectively. Adenosine and deoxyadenosine are catabolized by the enzyme adenosine deaminase. AMP adenosine monophosphate; ADP adenosine diphosphate; ATP adenosine triphosphate; APRT adenine phosphoribosyltransferase; d deoxy-; IMP inosine monophosphate

DNA synthesis. Furthermore, decreased cellular levels of deoxycytidine cause a loss of negative feedback on dCK, which leads to increased formation of cladribine nucleotides [56]. This phenomenon is termed "self-potentiation" [56, 57]. CdATP is also incorporated into DNA, which leads to chain elongation termination induced by DNA polymerases α and β [58–60]. In addition to its effects on nucleotide metabolism, cladribine acts as a hypomethylating agent by inhibiting *S*-adenosylhomocysteine hydrolase. This leads to a deficiency in *S*-adenosylmethionine, which is a methyl donor for DNA methylation reactions induced by DNA methyltransferase [61–63]. In the resting cells, cladribine induces DNA breaks, which leads to the release of the pro-apoptotic proteins [53, 64]. In addition, DNA breaks activate the DNA repair enzyme, poly (ADP-ribose) polymerase (PARP), which depletes intracellular pools of nicotinamide adenine dinucleotide (NAD) and ATP precipitating apoptosis [60, 65, 66].

Cladribine toxicity was initially thought to be specific to lymphocytes, based on the isolated lymphopenia noted in patients with SCID [56]. However, cladribine was also found to be toxic to myeloid cells. An early preclinical study showed that cladribine exhibited a dose-dependent inhibition of myeloid progenitor cells [67]. Furthermore, single-agent cladribine was shown to lead to at least 50% reduction in blasts in patients with refractory leukemias, including those previously treated with cytarabine [48]. These findings raised interest in the potential use of cladribine in myeloid malignancies.

Modulation of cytarabine by cladribine

Another important feature of cladribine is its influence on metabolism and action of cytarabine, which is referred to as biochemical modulation [68]. Similar to cladribine, the cytotoxicity of cytarabine depends on phosphorylation by dCK [39]. Cytotoxicity of cytarabine stems from DNA polymerase inhibition and from incorporation of cytarabine triphosphate (ara-CTP) into DNA, in competition with deoxycytidine triphosphate (dCTP) [60]. The activity of dCK is subject to inhibitory feedback regulation by dCTP [69]. Since dCTP inhibits dCK and, subsequently, the phosphorylation of cytarabine, giving an agent which inhibits the formation of deoxyribonucleotides can increase the phosphorylation of cytarabine [70]. Because cladribine inhibits ribonucleotide reductase and, subsequently, the formation of deoxycytidine, it increases the activity of dCK indirectly by releasing negative

feedback inhibition from dCTP [70, 71]. Pharmacologic studies showed that pretreatment with cladribine resulted in an increased intracellular accumulation of ara-CTP in AML blasts both in vitro and ex vivo [70–72]. Furthermore, a study conducted by Gandhi et al. [70, 71] showed that the DNA synthetic capacity of the circulating AML blasts was inhibited to a greater extent by administration of CdA and ara-C in combination than by either one alone.

Pharmacokinetic studies

Plasma pharmacokinetics of cladribine was determined using high-performance liquid chromatography and radioimmunoassay [73]. Pharmacokinetic studies have been evaluated for intravenous continuous and short infusions as well as subcutaneous and oral administrations [73]. The oral bioavailability after an oral administration of an IV saline solution of cladribine ranges between 37 and 51% [74–76]. The reason behind limited bioavailability is the instability of the N-glycosidic linkage in gastric pH leading to degradation to 2-chloroadenine [77]. Despite limited oral bioavailability, oral formulation of cladribine has been successfully used for multiple sclerosis following evidence from a placebo-controlled phase III RCT [78].

Initial pharmacokinetic studies showed a rapid distribution of cladribine from plasma and undetectable levels 2 h after administration [45, 79]. Furthermore, in vitro data showed that adequate cell toxicity depended on a continuous exposure of cells to cladribine [45, 67]. Therefore, cladribine was used initially administered by continuous intravenous infusion [80]. However, studies by Liliemark and Juliusson [81] showed that the area under the curve (AUC) for plasma concentration of cladribine was similar for continuous intravenous and 2-h infusions. Additionally, further studies showed that intracellular drug concentrations are several hundred-fold higher than plasma concentrations [77]. Apart from that, cladribine nucleotides are retained in leukemic cells with an intracellular half-life of 9 h to 30 h [82, 83]. These studies supported the use of the intermittent infusion of cladribine [73]. However, the half-life of cladribine nucleotides in AML blasts appears to be lower as compared to chronic lymphocytic and hairy cell leukemias (9 h versus 12.9 h and 15.1 h, respectively) [82]. Therefore, twice daily administration of cladribine in AML might be advantageous and was implemented in one study which will be discussed later [73, 84].

Dosing

Phase I clinical trials determined the maximum tolerated dose (MTD) of cladribine delivered as a 7-day continuous infusion to be 0.1 mg/kg/day [48, 85]. The primary dose-limiting toxicity was myelosuppression. Subsequent phase II clinical trials confirmed this dosing regimen to produce long-lasting remissions in patients with hairy cell leukemia (HCL) after a single course [46, 86].

First reports of the clinical use of cladribine in AML come from pediatrics. In 1991, a phase I clinical trial studied cladribine monotherapy in children with relapsed or refractory AML. The MTD was found to be 8.9 mg/m^2/day given by continuous infusion over 5 days. Cladribine resulted in a 47% CR rate with a 59% overall response rate [49, 87]. The dose of cladribine was also examined in a phase I trial in adults with relapsed AML [88]. It was given by a 5-day continuous infusion course. The initial dose chosen was 5 mg/m^2/day, which approximated the MTD according to the hairy cell leukemia studies. However, bone marrow hypoplasia was seen only with doses of 15 mg/m^2/day or larger. Patients who received the higher doses of 19 mg/m^2/day and 21 mg/m^2/day also developed delayed sensorimotor peripheral neuropathy. Therefore, the MTD was established to be 17 mg/m^2/day for 5 days which is substantially higher than the effective dose described above in children. Only one patient developed CR at doses below the MTD. Another phase I/II trial in adult relapsed AML leads to disappointing results. The MTD for single-agent cladribine was found to be 10.8 mg/m^2/day for 7 days [72].

Clinical studies in remission induction of adult AML

The Polish Acute Leukemia Group experience: the DAC regimen

The first trial examining cladribine in the induction of remission of newly diagnosed AML patients was conducted by the Polish Acute Leukemia Group (PALG) in 2000. In this pilot study, 45 patients (age < 60 years) with newly diagnosed AML were recruited. Patients were classified into either standard-risk (SR) or high-risk (HR) groups. Induction regimen consisted of a 7-day continuous infusion of cytarabine (200 mg/m^2/day) and daunorubicin (60 mg/m^2/day) on days 1–3 along with cladribine (5 mg/m^2/day) (DAC-7 regimen). Fifty-eight percent achieved CR after one course and another 14% after two courses with a total CR rate of 72%. The rate was considerably higher in the SR group compared with the HR group (94% vs. 59%, $p < 0.05$). Nine patients (18%) died within a median period of 2 weeks after induction. All of the patients with these early deaths were within the HR group and had a higher median age compared to the whole group (52 years vs. 45 years of age). The main cause of early death was bacteremia. All patients developed grade 4 neutropenia, and 36% had grade 3/4 infections. Long-term results were not assessed in this pilot study [89].

The PALG then conducted a larger multicenter phase III trial. In this study, 400 patients with newly diagnosed AML (age < 60 years) were randomized 1:1 to receive either DAC-7 or daunorubicin and cytarabine (DA-7). The dosing and schedule for the three drugs in this trial were the same as in the previous PALG trial. Patients who achieved CR were entered into consolidation therapy. Those with *t*(8;21), inv(16), and who were not suitable for transplant were given maintenance therapy. All others were referred to either allogeneic or autologous transplant depending on the availability of a suitable donor. Cladribine was also incorporated in the consolidation and maintenance therapies in the DAC-7 arm. A single course of DAC-7 resulted in a higher CR compared with the DA-7 regimen (63.5% vs. 47%, $p = 0.0009$). Additionally, patients who had a white blood cell (WBC) count > 100×10^9 had a significantly higher overall CR with DAC-7 compared with DA-7 (71% vs. 43%, $p = 0.03$). Toxicities were comparable in both groups. It seemed that there was a trend toward a higher leukemia-free survival (LFS) rate for patients aged > 40 years receiving DAC-7 compared with DA-7 regimen (44% vs. 28%; 95% CIs = 38–50% vs. 21–35%; $p = 0.05$). The OS was similar between arms. However, it should be noted that this trial was not designed to analyze survival [90].

In a follow-up study, the PALG compared the relative efficacy and toxicity of the DAC and DA induction regimens, with OS being the primary study endpoint. The researchers also included a third study arm which included fludarabine with DA (DAF). A total of 652 patients were recruited. The dosing and schedule for cladribine, cytarabine, and daunorubicin were the same as described in the previous PALG trials. Patients with a partial response (PR) were given a second course of the same induction regimen. Patients who achieved CR were given consolidation therapy. Those with favorable cytogenetics were treated with maintenance therapy. On the other hand, patients with intermediate- and high-risk cytogenetics and those who needed 2 cycles of induction were referred to either allogeneic or autologous transplant depending on the availability of a suitable donor. In contrast to the earlier PALG trial, cladribine was not incorporated into consolidation and maintenance therapies. The CR rates after one and two induction courses were higher in the DAC arm compared with the DA arm (62% vs. 51% [$p = 0.02$] and 67.5% vs. 56% [$p = 0.01$], respectively). CR rates were similar between the DAF and DA arms. Toxicities were comparable among all three arms. OS was significantly improved for the DAC arm (3 years, 45% ± 4%; median, 24 months) compared with the DA arm (3 years, 33% ± 4%; median, 14 months; $p = 0.02$). The OS in the DAF arm did not differ from that of the DA arm. In subgroup analysis, an OS advantage for the DAC arm was also demonstrated for patients older than 50 years (40% ± 5% vs. 18% + 5%, $p = 0.005$) and those with an initial WBC count > 50×10^9/L (47% ± 8% vs. 18% ± 9%, $p = 0.03$) and unfavorable karyotype (36% ± 9% vs. 20% ± 7%, $p = 0.03$). The authors concluded from their results that the use of DAC regimen in remission induction of AML should be considered a new standard of care [91]. However, there are certain shortcomings in this trial that were criticized and should be considered [92]. The 56% CR rate in the DA control arm was lower than the reported CR rates among other studies which used the DA regimen in the remission induction of AML [13, 93]. It should be noted that in both PALG trials, bone marrow aspiration to assess remission status was not performed until the patient achieved peripheral blood count values required for CR, if blasts persisted or reappeared in the peripheral blood or if no hematopoietic recovery was noticed until day 40. It was argued that the delay in the assessment of bone marrows in the PALG trial resulted in delayed administration of a second induction cycle [56, 92]. In the PALG trial, only patients who achieved a PR were given a second induction cycle and the rest were classified as non-responders and disqualified from the trial. By comparison, in the JALSG AML201 study, all patients who did not achieve a CR after the first cycle were given a second induction course. The CR rate was 61% after 1 cycle and 77% after 2 cycles [93]. Only 5% was classified as PR after the first cycle of induction in the PALG trial and thus qualified for a second cycle. Almost all of these patients (33 out of 34) achieved a CR. Despite these issues, the PALG studies showed some benefits of the addition of cladribine to induction chemotherapy of AML patients younger than 60. Indeed, a comparison of different trials among different populations should be done carefully. The PALG trials did not include molecular results.

Libura et al. [94] retrospectively analyzed samples from 227 patients treated at PALG centers with newly diagnosed normal karyotype AML for *FLT3*-ITD and *NPM1* mutations. Although patients treated in the DAC group had higher CR compared with the DA group (81.6% vs. 73.4%, $p = 0.14$), the difference was not statistically significant. When stratified according to induction regimen, *FLT3*-ITD$^+$ patients treated with the DA protocol had lower CR rate when compared with *FLT3*-ITD$^-$ patients (64.5% vs. 82.5%, respectively; $p = 0.038$). In contrast, in the DAC arm, the CR rate was not statistically different between *FLT3*-ITD$^+$ and *FLT3*-ITD$^-$ subgroups of patients (86% vs. 80%, respectively; $p = 0.3$). For the whole study group, there was a non-statistically significant trend toward improved 4-year OS in the DAC when compared with the DA arm (41% vs. 30.4%, $p = 0.15$). However, an OS advantage could be demonstrated for *FLT3*-ITD$^+$ patients treated with DAC when compared with the DA arm (37% vs. 14%, respectively; $p = 0.05$). Regarding *NPM1* mutation, patients with *NPM1*$^+$/*FLT3*-ITD$^+$ who were treated with DAC had a significant OS advantage when compared to DA arm (38% vs. 8%, respectively; $p = 0.026$). Despite retrospective nature, this study demonstrated that cladribine might abolish the negative effect of *FLT3*-ITD on the CR of AML patients with normal karyotype and improve their OS [94].

Experience with regimens containing idarubicin: the IAC regimens

The largest data collection we have so far for the use of cladribine in AML induction stems from the PALG trials which used the DAC regimen. On the other hand, data regarding the use of cladribine with idarubicin is sparse. Moreover, the dose of cytarabine used by the PALG was the standard dose. However, the potential of cladribine to synergize with cytarabine might not be fully exploited when standard-dose ara-C is given [84]. In other respects, as stated previously, young patients (< 45 years) might benefit from HiDAC [17]. Boddu et al. [95] studied the addition of cladribine to idarubicin and HiDAC (CLIA) in newly diagnosed as well as relapsed or refractory patients (< 65 years) with AML. The phase II trial included 73 patients with de novo and 17 with secondary AML. Cladribine dose was 5 mg/m^2 on days 1–5, idarubicin was 10 mg/m^2 on days 1–3, while cytarabine was 1 g/m^2 from days 1 to 5. Sorafenib was also added for patients with *FLT3*-ITD$^+$. Consolidation consisted of up to 5 cycles of CLIA. The CR/CR$_p$ was 76% in the de novo group. The patients were also classified according to specific genomic subgroups. Interestingly, patients with *NPM1* and *FLT3*-ITD$^+$ had CR/CR$_p$ rates of 95% and 86%, respectively. Among patients with the de novo AML, the median OS was 21.9 months [95]. This study is small and lacks randomization. The results warrant validation in larger RCTs.

There are also several retrospective studies examining the efficacy of cladribine added to AML induction regimens which used idarubicin as the anthracycline of choice (IAC regimen). Grosicki et al. [96] retrospectively studied 52 adults (age < 60 years) with newly diagnosed AML at the Department of Hematology of City Hospital in Chorzow, Poland. The DAC-7 regimen was the standard induction regimen in this group. However, idarubicin 10 mg/m^2 was substituted for daunorubicin (60 mg/m^2) in case of daunorubicin unavailability. Among the 52 patients, 22 received idarubicin. Toxicities and all outcomes including CR, 3-year OS, and 2-year LFS were comparable in both arms [96].

Schoen et al. [97] also recently reported their experience with the IAC regimen at St. Louis University, Missouri, USA. In this retrospective study, 107 patients with de novo AML and high-risk MDS were given IAC for induction. Idarubicin dose was 12 mg/m^2. Fifty-three percent of the patients were 60 years of age or older. Aside from that, 80% had either intermediate- or high-risk cytogenetics. CR, overall response rate (ORR), and median OS were 70%, 79%, and 17.2 months, respectively, among the whole group. When stratified according to risk groups, patients with favorable risk had a 95% CR rate compared to 67% and 51% among intermediate- and high-risk groups, respectively. With regard to survival, 100%, 63%, and 35% were alive at 1 year among the favorable-, intermediate-, and high-risk groups, respectively. When stratified according to age, patients < 60 years had an 80% CR rate compared to 54% in patients 60 years or older. The 1-year survival rates were 72% and 47% among the younger and older age groups, respectively. Treatment-related mortality (TRM) rate was 11%, with 11 out of the 12 deaths occurring in the older age group [97]. This study showed a high CR rate with IAC regimen even among elderly patients.

Wiedower et al. [98] retrospectively studied 24 patients with AML who received IAC induction. Median age was 58 years, and 54% had at least one high-risk feature. This regimen, which included idarubicin at a dose of 12 mg/m^2, leads to CR in 79.2% of the subjects. The 30-day mortality was 8.3% with a 33-month OS, and the LFS rates were of 56% and 36%, respectively [98].

In another study, Shen et al. [99] retrospectively studied 27 patients with newly diagnosed AML who received IAC for induction. These patients were compared to two arms who received two IA regimens, the IA-10 which included an idarubicin dose of 10 mg/m^2 and the IA-12 which had an idarubicin dose of 12 mg/m^2. Subjects treated with the IAC regimen had a significantly higher CR rate compared with the IA-10 (77.8% vs. 37%, respectively; $p = 0.002$). Despite the seemingly lower CR rate in the IAC-12 arm (63%), the results were not statistically significant ($p = 0.23$). Toxicities did not differ among all arms of the study. This study also demonstrated the efficacy of the IAC regimen. The researchers used a low-dose idarubicin of 8 mg/m^2, with comparable results and toxicity to IA-12. The authors suggested that adding cladribine to IA regimens has the potential to reduce the dosage of idarubicin. This might be of benefit to elderly patients, those with prior exposure to anthracyclines, or those who have a risk of cardiomyopathy [99]. However, as described previously in the other studies, cladribine can also be safely combined with an idarubicin dose of 10–12 mg/m^2. Given the lack of RCTs, the optimal dose of idarubicin for combination with cladribine remains unknown.

In a more recent report, 37 patients with previously untreated AML were evaluated in a retrospective, propensity score–matched cohort study. Subjects were treated with either IAC or IA for induction. The rates of CR, toxicities, and OS were comparable between the two groups. However, after propensity score matching, the odds ratio of reaching CR in the IAC cohort was increased by 33% (OR = 1.33; 95% CI = 1.09–1.55; $p < 0.01$) compared with the IA cohort. Patients who received cladribine were also found to have a reduction in hospital length of stay. The dose of idarubicin was not mentioned in the study [100].

Experience with regimens containing HiDAC, granulocyte–colony-stimulating factor, and/or mitoxantrone

Cladribine also has been used in various combinations with high-dose cytarabine, granulocyte–colony-stimulating factor

(GCSF) or filgrastim, and/or mitoxantrone, including the CLAG and CLAG-M protocols. These regimens have been used successfully in relapsed AML [101, 102]. However, there is less experience in these regimens in the induction of de novo AML. Martin et al. [103] conducted a retrospective study of AML patients treated at the Washington University of St. Louis with a combination of cladribine and cytarabine. Fifteen patients with newly diagnosed AML were identified that were treated with CLAG and CLAG-M protocols. Seven out of the eight patients treated with CLAG-M achieved CR despite poor-risk features among all patients in this group. On the other hand, patients treated with CLAG did poorly with only 14% CR. However, the patients in this cohort were older and had a greater proportion of high-risk features [103].

In another study, Jaglal et al. [104] retrospectively compared CLAG-M with standard 7 + 3 induction (with either DA or IA) in patients with secondary AML who failed at least 1 cycle of azanucleoside. Patients who received CLAG-M ($n = 28$) had a higher CR and CR with incomplete count recovery (CR_i) in comparison with patients in the 7 + 3 cohort ($n = 24$) (64% vs. 29%, respectively; $p = 0.014$). Rates of early death and febrile neutropenia were similar. Furthermore, the CLAG-M improved median OS in comparison with the standard regimens (202 days vs. 86 days, respectively; 95% CIs = 37–367 days vs. 36–136 days, respectively; $p = 0.025$). The 1-year OS estimates were 54% versus 13% among the CLAG-M and 7 + 3, respectively ($p = 0.056$) [104].

The most promising prospective data come from a recent trial by Halpern et al. [105] at the University of Washington. In this phase I/II study, patients with newly diagnosed AML and high-risk MDS (> 10% blasts) were given G-CLAM for induction. In the phase I part, patients were assigned to escalated doses of mitoxantrone (12 mg/m^2/day to 18 mg/m^2/day, days 1–3, compared to a 10 mg/m^2/day standard dose used in CLAG-M). Other drugs given were GCSF (days 0–5), cladribine (5 mg/m^2/day, days 1–5), and cytarabine (2 g/m^2/day, days 1–5). The phase I part established the MTD of mitoxantrone to be 18 mg/m^2/day, along with G-CLA. In the phase II part of the study, 94 subjects were treated with G-CLAM at MTD. A second course of G-CLAM was given if CR was not achieved with cycle 1. Up to 4 cycles of consolidation with G-CLA were given if $CR/CR_{p/i}$ was achieved with 1–2 cycles of induction therapy. Among the 94 patients treated in phase II, six had received prior azanucleoside therapy. Among the 94 subjects, 67 patients achieved a negative minimal residual disease (MRD^{neg}) CR (71%). Seven patients achieved an MRD^{pos} CR and seven additional patients a CRi (five MRD^{neg} and two MRD^{pos}) for a CR/CR_i rate of 86%. Four-week mortality was 2%. Among the subjects treated in the phase II cohort, the median OS, 12-month LFS, and OS were 33.3 months, 65%, and 69%, respectively. The authors also compared their results to 245 patients treated with 7 + 3 on the SWOG S0106 trial as well as 100 treated at the University of Washington [106]. When compared to the patients who received 7 + 3 at the University of Washington cohort, G-CLAM was associated with an improved odds ratio of CR, CR/CR_i, and MRD^{neg} CR. However, survival estimates were similar between arms.

Clinical studies in elderly patients

The initial PALG trials excluded elderly patients from their trials. However, there are other studies which examined the efficacy of cladribine in AML induction solely in this population. Juliusson et al. [84] studied cladribine in remission induction of elderly patients (age > 60 years) with newly diagnosed AML. Sixty-three patients were randomized 2:1 to receive either cladribine, intermediate-dose cytarabine (1 g/m^2 every 12 h for 4 days) and idarubicin (10 mg/m^2 for 2 days) (CCI regimen), or cytarabine and idarubicin. Cladribine was given at 5 mg/m^2 every 12 h for 4 days. As described before, pharmacokinetic studies showed that the half-life of cladribine in AML blasts is 9 h [82]. Therefore, it was given twice daily in this study. Apart from that, to exploit the maximum synergism between cladribine and cytarabine, the researchers increased the dose of the cytarabine. The patients were given multiple courses of treatment. The overall CR rate was 62%, with 51% after one course of triple-drug induction in comparison with 35% for the two-drug therapy ($p = 0.014$). There were no differences in toxicities. The 2-year survival was 30% without differences in the treatment arms [84].

The PALG recently published the results of their latest trial in the elderly population. This study recruited 117 elderly patients (aged 60–80 years) with de novo AML. The patients were randomized to receive either DA or low-dose DAC. Given the older age, daunorubicin was given at 45 mg/m^2 on days 1–3 while cytarabine was given at 100 mg/m^2/day for 7 days. Patients in the DAC arm had a non-statistically significant tendency toward higher CR compared to the DA arm (44% vs. 34%, $p = 0.19$). However, patients aged 60–65 had a significantly higher CR rate with the DAC arm compared to DA (51% vs. 29%, $p = 0.02$). Toxicities were comparable in both groups. In general, the OS was similar in both arms (8.6 months in the DAC group vs. 9.1 months in the DA group, $p = 0.64$). However, there was a tendency toward longer OS in the DAC arm in patients with good- and intermediate-risk but not in poor-risk karyotypes ($p = 0.057$). Moreover, this difference was significant in the patients < 65 years of age ($p = 0.024$), but not in patients > 65 years of age [107]. This study showed that the addition of cladribine to 7 + 3 induction might be of benefit to elderly population (aged 60–65) with favorable- or intermediate-risk groups. However, since the dose of daunorubicin the researchers used was low, it is difficult to extrapolate these results to most centers where the standard daunorubicin dose is 60–90 mg/m^2.

Kadia et al. [108, 109] designed a less intensive prolonged protocol but with promising results. A total of 118 patients with either de novo or secondary AML and high-risk MDS were included. Induction consisted of cladribine 5 g/m^2/day for 5 days along with cytarabine 20 mg subcutaneously (SQ) bid on days 1–10. This was followed by consolidation/maintenance which consisted of 2 cycles of cladribine for 3 days and low-dose cytarabine for 10 days alternating with 2 cycles of decitabine, for a maximum of 18 cycles. The median age was 69 (range, 49–85). The ORR was 68% with 58% CR. The median OS was 13.8 months, and the median CR duration was 21.1 months. The 1-year OS estimate was 64%. Interestingly, when response rates were stratified among individual molecular subgroups, the highest proportions of ORR were seen in patients with mutations in *NPM1* (100%), *FLT3*-ITD and *FLT3*-D835 (87%), and *DNMT3a* (85%). These mutations were also associated with improved survival, with 1-year survival ranging from 72 to 78% [108]. The study is still currently active and is recruiting subjects (NCT01515527) [110]. Table 1 summarizes all the studies described which used cladribine in the induction of AML. Table 2 describes the various cladribine-based induction regimens used in both adult and elderly AML.

Tolerability and toxicity of cladribine in AML induction

Cladribine toxicities can be extrapolated from studies in patients with hairy cell leukemia. Myelosuppression with thrombocytopenia, anemia, neutropenia, and monocytopenia in many studies was the major dose-limiting side effect of cladribine therapy [83]. According to one study, the rates of grade 3/4 neutropenia and grade 3/4 thrombocytopenia were 87% and 20%, respectively. Median time to recovery of blood counts after one cladribine course was 49 days (range, 9 to 378 days). The rate of febrile neutropenia was 42%, and 13% of patients had documented infections. Most common viral infections were herpes simplex and herpes zoster. CMV retinitis also occurred in one patient. Herpes zoster reactivation occurred up to 69 months from therapy. The most common bacterial infections were caused by *Staphylococci* [111]. In contrast to leukocytes, cladribine is less toxic to red blood cells. Therefore, the level of anemia can vary from one patient to another. This is explained by the fact that the late-stage erythroid progenitors and mature erythrocytes are resistant to the toxic effects of cladribine [67]. Cladribine treatment also results in prolonged lymphopenia, with most impressive suppression in CD4$^+$ lymphocytes with a median time for CD4 reconstitution of up to 40 months [112]. It should be kept in mind that generalization of these toxicities to AML might be problematic, given the small number of patients, lack of randomization, and single course of therapy in hairy cell leukemia trials. Cladribine toxicities can be also extrapolated from patients with multiple sclerosis [78, 113]. In the CLARITY trial, patients were initially randomized to two cumulative doses of cladribine (3.5 mg/kg vs. 5.25 mg/kg) or placebo given intermittently over 2 years [78]. In an extension trial, patients who received cladribine in the CLARITY trial were randomized to another 2 years of either cladribine (3.5 mg/kg) or placebo. On the other hand, patients who received placebo in the initial trial were given cladribine 3.5 mg/kg [113]. The most common overall adverse event was lymphopenia, which occurred in 28.4% of the patients. The two groups who received cladribine in both the initial and the extension trials (cumulative doses of 7 mg/kg and 8.75 mg/kg) had the highest incidence of grade 3–4 lymphopenia (40.9% and 53.2%, respectively) and longest median time to recovery to grade 0–1 lymphopenia (212 days and 168 days, respectively). The rate of infection was similar in all groups, with the exception of herpes zoster, which was higher in the 8.75 mg/kg group (4.8% vs. 1.1 to 2% in the other groups).

The main data regarding the toxicity of cladribine-based induction in AML can be extrapolated from the PALG trials. In their first phase III trial, all patients developed severe granulocytopenia and thrombocytopenia. Moreover, minimal platelet count and median time for recovery of platelet and neutrophil counts were comparable in both groups. However, granulocytopenia and lymphopenia were more profound in the DAC-7 treatment arm. Severe grade 3–4 infections occurred in about 38% of the patients, and incidence was similar in both arms. The transfusion of red blood concentrates or platelets, the duration of antibiotic therapy, and the administration of supportive treatment did not show any difference in both groups. Furthermore, patients assigned to DAC-7 stayed 7 days shorter in the hospital during the induction therapy due to more induction courses in the DA-7 arm patients. Non-hematological toxicities were infrequent and comparable in both study groups [90]. A follow-up analysis was conducted by the PALG to assess the frequency and spectrum of infections in patients who were enrolled in this study [90, 114]. The incidence of infection was the primary study endpoint; the secondary endpoints were spectrum of infection, absolute neutrophil and lymphocyte counts at the time of infection, duration of anti-infectious therapy, duration of supportive therapy with hematopoietic growth factors, and infection outcome. Prophylactic antibiotics were given to most of the patients on the first day of chemotherapy and were discontinued after the recovery of ANC $> 0.5 \times 10^9$/L. The use of supportive therapy was comparable in both arms. No difference in the occurrence of infections between the two arms was reported. The most common infection sites were oral cavity and upper respiratory tract. Positive blood cultures were reported in 21% of DAC arm and 31% of DA arm. The most common causative organisms were gram-positive

Table 1 Summary of all clinical trials and retrospective studies conducted for adding cladribine to induction chemotherapy of adults with AML

Study	Year	Phase	Number	Median age (range), years	Induction regimen	CR (%)	OS (%)	TRM (%)
Studies incorporating DAC								
Holowiecki et al. [89]	2002	2	50	45 (18–59)	DAC	72	NR	18
Holowiecki et al. [90]	2004	3	200; 200	44 (16–60); 46 (17–60)	DAC; DA	72; 69	3 years, 34%; 3 years, 31%	15.5; 14
Holowiecki et al. [91]	2012	3	222; 211	48 (18–60); 47 (18–60)	DAC; DA	68; 56	3 years, 45%; 3 years, 33%	11; 10
Libura et al. [94]	2016	R	103; 124	49; 50	DAC; DA	82; 73	4 years, 41%; 4 years, 30%	NR; NR
Studies incorporating idarubicin								
Boddu et al. [95]	2017	2	73	54 (19–65)	CLIA	72	Median, 21.9 months	3
Grosicki et al. [96]	2012	R	30; 22	55 (20–72); 59 (38–70)	DAC; IAC-10	70; 59	3 years, 26%; 3 years, 23%	NR; NR
Schoen et al. [97]	2016	R	50; 57	<60; ≥ 60	IAC-12; IAC-12	84; 57	1 year, 72%; 1 year, 47%	2; 19
Wiedower et al. [98]	2015	R	24	58 (24–68)	IAC-12	79	33 months, 56%	8.3
Shen et al. [99]	2014	R	27; 27; 27	43 (19–53); 43 (18–60); 41 (18–53)	IAC-8; IA-10; IA-12	78; 37; 63	NR	NR
Seligson et al. [100]	2018	R	12; 25	58 (36–71); 61 (27–79)	IAC*; IA*	42; 34	73%**; 72%**	8; 8
Studies incorporating mitoxantrone and/or GCSF								
Halpern et al. [105]	2018	1/2	94	60 (21–81)	G-CLAM	79	1 year, 69%	2
Martin et al. [103]	2009	R	7; 8	70; 56	CLAG; CLAG-M	14; 88	NR; NR	43; 0
Jaglal et al. [104]	2014	R	28; 24	NR (79% > 60); NR (79% > 60)	CLAG-M; IA/DA	50; 21	1 year, 54%; 1 year, 13%	14; 8
Studies on elderly patients								
Juliusson et al. [84]	2003	2	43; 20	70 (64–75); 73 (65–77)	CCI; CI	51; 35	2 years, 30%; ND between arms	12; 10
Pluta et al. [107]	2017	3	80; 85	66 (60–79); 66 (60–79)	DAC; DA	44; 34	8.6 months; 9.1 months	23; 17
Kadia et al. [108]	2014	2	86	69 (49–85)	C-LDAC/D	58	1 year, 64%	1

The number of induction cycles given differ according to trials. Furthermore, the definition of CR and treatment-related mortality (TRM) might differ slightly in the trials. In general, most trials defined TRM as deaths occurring either 4 weeks or 1 month from induction chemotherapy

CR complete response; *OS* overall survival; *NR* not reported; *R* retrospective; *ND* no difference; *DAC* daunorubicin, ara-C, and cladribine; *DA* daunorubicin and ara-C; *CLIA* cladribine, idarubicin, and ara-C; *IAC* idarubicin, ara-C, and cladribine (IAC-8, IAC-10, and IAC-12 contain idarubicin 8 mg/m^2, 10 mg/m^2, and 12 mg/m^2, respectively); *IA* idarubicin and ara-C (IA-10 and IA-12 contain idarubicin 10 mg/m^2 and 12 mg/m^2, respectively); *G-CLAM* filgrastim (GCSF), cladribine, ara-C, and escalated-dose mitoxantrone; *CLAG* cladribine, ara-C, and GCSF; *CLAG-M* cladribine, ara-C, GCSF, and mitoxantrone; *CCI* cladribine, intermediate-dose ara-C, and idarubicin; *CI* intermediate-dose ara-C and idarubicin; *C-LDAC/D* cladribine and low-dose ara-C alternating with decitabine

*The dose of idarubicin was not mentioned

**The time point at these OS rates was not mentioned

bacteria. Other infections like gram-negative bacteria, polymicrobial infections, anaerobic bacteria, *Mycobacterium tuberculosis*, *Candida* species, *Aspergillus* species, varicella-zoster, and herpes viruses were reported. Complete recovery from infections was observed in the vast majority of patients in both arms (84% in the DAC-7 group vs. 84% in the DA-7 group), and the study concluded that adding cladribine to DA-7 therapy has no impact on the incidence and risk of infection in newly diagnosed AML patients. Among the second PALG phase III trial, all patients experienced grade 4 neutropenia and thrombocytopenia. The minimal neutrophil and platelet counts, duration of cytopenia, time to neutrophil and platelet recovery, the duration of hospital stay, the median number of RBCs and platelet transfusions, as well as the use of granulocyte–colony-stimulating factor were comparable among study groups. Alopecia, infections, mucositis, vomiting, and diarrhea were the most frequent grade 3 or 4 non-hematologic adverse events and were also similar among study groups [91]. The most recent PALG trial in elderly patients and the study by Juliusson et al. [84] also demonstrated similar results [107].

Antibacterial and antifungal prophylaxis should be given according to the routine guidelines. However, given prolonged lymphopenia induced by cladribine, prophylactic trimethoprim-sulfamethoxazole and acyclovir should be given to prevent pneumocystis infections and herpes reactivation,

Table 2 Various cladribine-based induction regimens used in adults and elderly

Adult induction regimens		
DAC	ara-C 200 mg/m^2/day CIVI for 7 days; daunorubicin 60 mg/m^2/day IV on days 1–3; cladribine 5 mg/m^2/day as 2-h IV infusions from days 1 to 5; G-CSF* was given only for those aged > 50 years without expression of respective surface receptor (CD114) on leukemic blasts	PALG trials [89–91]
IAC	ara-C 100–200 mg/m^2/day CIVI from days 1 to 7; idarubicin 8–12 mg/m^2/day IV on days 1–3; cladribine 5 mg/m^2/day as 2-h IV infusions from days 1 to 5	Grosicki et al. [96]; Schoen et al. [97]; Wiedower et al. [98]; Shen et al. [99]
CLIA	Cladribine 5 mg/m^2 IV over 30 min on days 1–5, followed by ara-C 1 g/m^2 IV on days 1–5; idarubicin 10 mg/m^2 IV on days 1–3; sorafenib 400 mg PO bid if *FLT3*-ITD$^+$	Boddu et al. [95]
CLAG/CLAG-M	Cladribine 5 mg/m^2 IV over 2 h on days 1–5; cytarabine 2 g/m^2 IV over 4 h on days 1–5; G-CSF 300 μg s.c. on days 0–5; and in case of CLAG-M, mitoxantrone 10 mg/m^2 IV on days 1–3	Martin et al. [103]
G-CLAM (with escalated-dose mitoxantrone)	Cladribine 5 mg/m^2 IV over 2 h on days 1–5; cytarabine 2 g/m^2 IV over 4 h on days 1–5; G-CSF 300 μg/day or 480 μg/day (for weight </≥ 76 kg) s.c. on days 0–5; mitoxantrone 18 mg/m^2 IV on days 1–3	Halpern et al. [105]
Elderly induction regimens		
CCI	Cladribine 5 mg/m^2 IV over 1 h twice daily 2 h before ara-C on days 1–4; ara-C 1 g/m^2 IV over 2 h twice daily on days 1–4; idarubicin 10 mg/m^2 IV over 1 h on days 1–2	Juliusson et al. [84]
Low-dose DAC	ara-C 200 mg/m^2/day CIVI for 7 days; daunorubicin 45 mg/m^2/day IV on days 1–3; cladribine 5 mg/m^2/day as 2-h IV infusions from days 1 to 5	Pluta et al. [107]
C-LDAC/H	Induction cycle: up to 2 cycles of cladribine 5 mg/m^2 IV over 1–2 h on days 1–5 combined with ara-C 20 mg s.c. twice daily on days 1–10, followed by consolidation cycles: cladribine 5 mg/m^2 IV over 1–2 h on days 1–3 combined with ara-C 20 mg s.c. twice daily on days 1–10, alternating with decitabine 20 mg/m^2 IV over 1–2 h on days 1–5. Up to a total of 18 cycles are allowed	Kadia et al. [108, 109]; U.S. National Library of Medicine [110]

ara-C cytosine arabinoside, *CIVI* continuous intravenous infusion, *G-CSF* granulocyte–colony-stimulating factor, *IV* intravenous, *PO* orally, *s.c.* subcutaneous

respectively [115]. Atovaquone, dapsone with or without pyrimethamine, pentamidine, or clindamycin plus primaquine can be considered in patients intolerant of trimethoprim-sulfamethoxazole [116]. Given that the duration of lymphopenia is variable, the duration of pneumocystis prophylaxis can be guided by CD4 count [56]. Moreover, patients receiving cladribine, who need blood transfusion, should receive irradiated blood components indefinitely to prevent transfusion-associated graft-versus-host disease [117].

Cutaneous reactions can be precipitated by cladribine therapy, especially in patients treated with allopurinol, trimethoprim-sulfamethoxazole, and penicillins. This might be due to an increased rate of drug hypersensitivity, possibly due to T cell imbalance induced by cladribine [118]. GI symptoms ranging from mucositis, nausea, vomiting, and loss of appetite to diarrhea were seen in some patients [84, 89, 107]. Nephrotoxicity was observed when cladribine was used in the higher doses. In the phase I trial by Kornblau et al. [72], serum creatinine increased to > 2 mg/dL in six out of 25 patients, and two required dialysis when they were given cladribine at doses of 9 m^2/day and 13 m^2/day for 7 days. However, both patients were also on other nephrotoxic agents. Likewise, during the phase II trial when cladribine was used at a dose of 12 mg/m^2/day for 5 days, six out of 17 patients had serum creatinine greater than 1.5 mg/dL, but none required dialysis [72]. Therefore, close monitoring and avoiding other nephrotoxic medications should be considered while using high doses of cladribine.

One of the major side effects of cladribine is neurotoxicity, but it seems to be a dose-dependent consequence. In the phase I study by Vahdat et al. [88], neurotoxicity developed in six patients: four of them at the 21 mg/m^2/day dose and two patients at the 19 mg/m^2/day dose. All six patients developed severe leg weakness and inability to walk in 4 weeks to 7 weeks. These patients also reported sensory symptoms like paresthesia, dysarthria, and numbness. Interestingly, postmortem pathology in one of the patients showed chronic peripheral neuropathy with active axonal degeneration. Reactivation of EBV was reported in one of the patients who received a 15 mg/m^2/day dose [88]. Of note, life-threatening and fatal neurotoxicity was also reported with other purine analogs, such as fludarabine and pentostatin, when used at higher than recommended doses, whereas at the recommended doses, the neurotoxicity was seen in about 15% of the cases and usually was mild and reversible [119].

Finally, there has been some concern about the development of second malignancies in patients treated with cladribine including MDS [66]. In one study, comparison of cladribine-treated patients to an age-adjusted population from the Surveillance, Epidemiology, and End Results (SEER)

database found a slight increase in observed-to-expected secondary malignancies [111]. This might be related to cladribine's DNA-damaging effects [66]. However, whether this stands true for patients with AML remains largely unknown. The possible increased risk of cancer in patients treated with cladribine was also studied in patients with multiple sclerosis. In the CLARITY trial, there were four cases of cancer reported in the cladribine arms (melanoma, carcinomas of the pancreas and ovary, and choriocarcinoma) compared to none in the placebo group [78]. A subsequent meta-analysis compared the cancer risk of cladribine and other disease-modifying drugs in placebo-controlled phase III trials of patients with relapsing multiple sclerosis. The cancer rate in the treatment group of the CLARITY trial was not different from all other treatment groups of placebo-controlled trials (0.34% vs. 0.6%, respectively; $p = 0.46$). In addition, although there were no cancers reported in the placebo group in the CLARITY trial, the combined cancer rate of all other placebo groups was 1.19%. Based on these results, the authors concluded that there is no evidence for a higher risk of cancer in patients with multiple sclerosis treated with cladribine [120].

Conclusions and future directions

In summary, the combination of cytarabine and an anthracycline has been the standard of care for remission induction of AML for many years. The addition of other agents such as etoposide or 6-thioguanine did not improve outcomes. The addition of cladribine to standard induction has shown some promising results. It has unique antileukemic properties, and it is active against both dividing and quiescent cells. Some of these important properties are inhibition of ribonucleotide reductase and DNA polymerases, induction of DNA breaks, and inhibition of DNA methylation. Furthermore, cladribine is synergistic with cytarabine. Multiple studies were conducted to examine the efficacy of cladribine in the remission induction of adult AML. The PALG trials showed that the addition of cladribine to daunorubicin 60 mg/m^2/day and ara-C (DAC) regimen improved CR rate and OS [90, 91]. However, the trial was criticized because of its design as well as the low response in the control arm compared with historical data [92]. In addition to the DAC regimen, multiple retrospective and phase I/II trials showed promising results in combination with either HiDAC, mitoxantrone, or idarubicin. However, no RCTs were conducted. Results from a recent phase II trial at the University of Washington utilizing G-CSF, HiDAC, cladribine, and high-dose mitoxantrone (G-CLAM) showed a high MRD-negative CR rate [105]. When the authors compared their results to historical date for patients who were treated at the same institution with 7 + 3, G-CALM resulted in better MRD-negative CR rates. However, keeping in mind the limitations in generalizing from historically controlled studies, this did not translate to an OS advantage. Another phase II trials also showed durable responses with other regimens such as CLIA and low-dose ara-C/cladribine alternating with decitabine [95, 108]. Confirmation of these findings in larger RCTs is warranted to see if such regimens would lead to a survival advantage.

It might be worthwhile to mention that results from the phase II trial conducted by Boddu et al. [95] showed high remission rates among patients with *NPM1* and *FLT3*-ITD$^+$ mutations. Also, preliminary results from the ongoing phase II trial conducted by Kadia et al. [108, 109] showed high response rates among elderly patients with either *NPM1* or *FLT3*-ITD$^+$ mutations. These mutations were also associated

Table 3 Ongoing clinical trials of adding cladribine to induction chemotherapy of AML

Clinical trial identifier	Phase	Summary	Estimated completion date
NCT02921061 [121]	1	Studies MTD of either concurrent or sequential decitabine with G-CLAM in patients with newly diagnosed or relapsed/refractory AML or high-risk MDS	December 2018
NCT02728050 [122]	1/2	Studies MTD and side effects of sorafenib with CLAG-M in patients with newly diagnosed AML independent of *FLT3*-ITD status	December 2018
NCT02115295 [123]	2	Examines cladribine, HiDAC 1.5–2 g/m^2/day, and idarubicin (ARAC) in adults with AML, high-risk MDS, and CML with blast crisis	May 2019
NCT02096055 [124]	2	Studies a SGI-110, a new hypomethylating agent in induction of elderly patients with AML. The study has 4 arms: single-agent SGI-110 for 5 days (Arm A) or 10 days (Arm B) or combined with idarubicin (Arm C) or cladribine (Arm D)	April 2026
NCT01515527 [110]	2	Examines cladribine combined with low-dose cytarabine alternating with decitabine in elderly patients with AML or high-risk MDS. Please see study by Kadia et al. [109]	February 2021
NCT03012672 [125]	3	Compares higher and lower doses of CLAG-M in induction of less-fit adult patients with AML	July 2020
NCT02323022 [126]	3	This is a follow-up phase III trial to the retrospective study by Shen et al. [99]. It compares IAC-8 with IA-10 and IA-12 in the induction of newly diagnosed AML	December 2020
NCT03257241 [127]	3	This a phase III trial conducted by the PALG. It compares the DAC regimen with DA. The dose of daunorubicin in the DA regimen is 90 mg/m^2 as compared to 60 mg/m^2 in the old PALG trials	December 2020

with improved survival. Interestingly, as mentioned previously, a retrospective analysis done by the PALG showed improved CR and OS among normal-karyotype *FLT3*-ITD$^+$ AML patients who were treated with DAC in comparison with DA [94]. Even though we cannot draw conclusions from these studies due to the very limited number of patients, the lack of randomization, and the retrospective nature of the third mentioned study, however, the results might make an entry-level hypothesis to investigate in future studies. Midostaurin was recently approved in newly diagnosed *FLT3*-ITD$^+$ AML, and other agents are under investigation. It might be useful to investigate if combinations of cladribine-based regimens and *FLT3* inhibitors improve the outcome of *FLT3*-ITD$^+$ AML.

Finally, it might also be worthwhile to mention that almost all adult trials, may be except the one by Juliusson et al. [84], used the same dose of cladribine, which was 5 mg/m^2 daily for 5 days. Based on the pharmacokinetic studies that showed a shorter half-life of cladribine in AML blasts, Liliemark and Juliusson [82] and Juliusson et al. [84] decided to give cladribine twice daily in their trial. Furthermore, as explained before, previous phase I clinical trials showed that adult relapsed AML needs a much higher dose to produce an antileukemic activity. Whether using a higher dose or twice daily dosing produces stronger antileukemic effects is unknown and would be useful to know. Table 3 summarizes the ongoing clinical trials that incorporate cladribine in remission induction of AML.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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