Nucleoside Analogs in the Therapy of T-Cell Malignancies

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Introduction

The nucleoside analogs (NA) are one of the most active classes of drugs in patients with T-cell lymphoma. The analogs have developed based on the discovery metabolic syndromes associated with deficiency of purine catabolizing enzymes. The lack of adenosine deaminase (ADA) enzyme was associated with a variant of severe combined immunodeficiency syndrome where patient suffered with both B- and T-lymphocytopenia [1]. Deficiency of purine nucleoside phosphorylase on the other hand leads to T-lymphocytopenia in pediatric patients [2]. ADA and PNP enzymes are involved in metabolic clearance of dAdo and dGuo, respectively. As a result in these patients there is an accumulation of these nucleosides in the plasma and triphosphate (i.e., dATP and dGTP) in the cells. Inherently, T-cells have high levels of ADA, a key enzyme in the dAdo degradation pathway. Potent inhibitors of ADA and PNP were developed and two of these, deoxycoformycin and forodesine, made to the clinic and have been tested effectively in T-cell lymphoma. Among the pyrimidine nucleoside analogs, gemcitabine has shown efficacy in T-cell malignancies. For purine nucleoside agents, cladribine and fludarabine have shown activity in mature T-cell leukemias, with overall response rates between 35 and 41% [3, 4]. Nelarabine has shown selectivity and specificity for T-cell diseases that include leukemias and lymphomas. This chapter reviews and summarizes the activity of these agents in T-cell neoplasms.

Pentostatin

Structure and Synthesis

Pentostatin (2′-deoxycoformycin), a natural product, is a potent transition state inhibitor of adenosine [5, 6]. Structurally it resembles the ADA substrate dAdo but binds tightly with the enzyme resulting in its inhibition (Fig. 15.1; [7]).

Metabolism and Mechanisms of Action

There is very limited phosphorylation of deoxycoformycin itself [8]. The drug directly inhibits ADA enzyme. As this enzyme mediates the normal metabolic clearance of deoxyadenosine, inhibition of this enzyme cause deoxyadenosine levels to increase in plasma [9, 10] as do the dATP in erythrocytes and leukemia cells [11–14] mimicking ADA deficiency syndrome [15].

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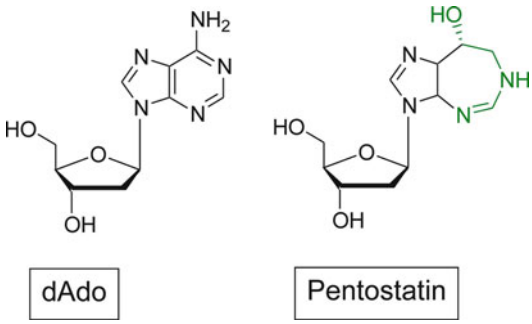

Fig. 15.1 Structures of deoxyadenosine and inhibitor of adenosine deaminase, pentostatin

Because deoxyadenosine uses deoxycytidine kinase for its phosphorylation, this enzyme is important for this drug also.

It is thought that the imbalance of deoxynucleotide pools caused by this greatly increased dATP concentration activates cell death mechanisms. More importantly, dATP is a global inactivator of the activity of ribonucleotide reductase and inhibits conversion of all four ribodiphosphates to their deoxy-forms [16]. Hence, this perturbation of dNTP pool results in inhibition of DNA synthesis and initiation of cell death. Additionally, dATP has been demonstrated to be part of apoptosomes; these are formed by cytochrome C, Apaf-1, procaspase 9, and dATP [17]. Binding of dATP triggers the reaction leading to cleavage of procaspase 9 to caspase 9 and downstream effect on the activation of caspase 3; the route which is known as intrinsic cell death pathway. Hence accumulation of increased dATP could further activate cell death pathway [18].

Pentostatin as a Single Agent in the Clinic

Pentostatin has been the most extensively studied in T-cell lymphoma and has shown variable response rates. However, many of the reports are limited to small single-center studies. Larger prospective randomized trials will be necessary to examine this therapy and to further explore combination regimens, which may result in increased responses. In the earliest clinical trials pentostatin was used in high doses to treat patients with T acute lymphoblastic leukemia (T-ALL) [19]. This was associated with treatment limiting toxicities. Since that time pentostatin in lower, well tolerated, doses has shown remarkable activity in patients with hairy-cell leukemia, chronic lymphocytic leukemia, low-grade B-cell malignancies as well as in T-cell lymphoma [20]. The commonly used schedule now is intravenous administration of pentostatin at 4 mg/m^2 given every 1–2 weeks. Other higher dose schedules have also been used in reported studies. Dose adjustments are required if renal function is impaired.

The majority of data evaluating pentostatin in T-cell lymphomas is based on trials in cutaneous T-cell lymphoma (CTCL). There are also reports that support the activity of these agents in other mature T-cell malignancies (Table 15.1). In the early 1980s, there were small reports describing the effectiveness of pentostatin in patients with T-cell leukemias, including patients refractory to other therapy [19, 20, 27–30]. The European Organization for Research and Treatment for Cancer Leukemia Cooperative Study Group (EORTC) conducted a Phase II trial that included 76 patients with advanced T-cell malignancies, including 25 patients with what was then termed "T chronic lymphocytic leukemia" (T-CLL) [21] (Table 15.1). The response rate with pentostatin was 8% with a median disease-free survival of 22 weeks. T-CLL has been reclassified by WHO as T prolymphocytic leukemia (T-PLL), which has been shown in other studies to be responsive to pentostatin. The first case study was published in 1986, showing that two patients achieved remission following pentostatin [31]. In the early 1990s, Matutes et al. [22] published a report of 78 patients with T-PLL describing the clinical and laboratory features of the disease. Of the 78 patients, 31 were treated with pentostatin. There were 15 responses (48%) including 3 complete responses (CR) and 12 partial responses (PR). Another phase Il study conducted by the EORTC treated 20 patients with T- or B-prolymphocytic leukemia with weekly pentostatin [23]. Of the 20 patients, 6 patients had T-PLL. There were nine overall responders in the entire study population

Table 15.1 Summary of ADA inhibitor, pentostatin, studies in peripheral T-cell lymphomas and other mature T-cell malignancies

Study	Dose	Total # patients	Patients subset	CR (%)	PR (%)	ORR (%)	Median overall survival
Ho et al. [21]	4 mg/m^2 Q week×3 then Q 2 week×6 then Q month×6	*N*=76 (T-cell NHL)	T-CLL=25	0	8	8	DFS=22 weeks
Matutes et al. [22]	4 mg/m^2 Q week	*N*=78 (T-PLL)	Pentostatin treated=31	9	39	48 (58)	16 months (responders) 10 months (nonresponders) 7 months (patients not treated with pentostatin)
Dohner et al. [23]	4 mg/m^2 Q week×3 then Q 2 week×3 if PR, Q month×6	*N*=20 (B & T-PLL)	T-PLL=6	0	33	33	N/A
Dearden et al. [24]	4 mg/m^2 Q week×4 then Q 2 week till optimal response	*N*=68 (T-cell NHL)	T-PLL=31 ATLL=20 LGL=4	9 10 25	39 5 0	48 (58) 15 (18) 25 (50)	T-PLL (10–16 months) ATLL (N/A) LGL (N/A)
Mercieca et al. [25]	4 mg/m^2 Q week×4 then Q 2 week till optimal response	*N*=145 (T-cell NHL)	T-PLL=55 LGL=5 ATLL=25	9 40 8	40 0 4	45 40 12	N/A
Tsimberidou et al. [26]	5 mg/m^2/day×3 days q 3 weeks	*N*=42	PTCL=4 ATLL=3 ALCL=1	50 0 0	50 33 0	100 33 0	Median 4 months (1–61)

(45%) including two (33%) of the patients with T-PLL. All of the responses were PR and the median duration of response was 9 months (range 2–30 months). The majority of patients (85%) enrolled in this study had received prior chemotherapy. By far the largest published experience with pentostatin in mature T-cell malignancies has been at the Royal Marsden Hospital in London [24, 25, 32] (Table 15.1). A total of 165 patients who had a range of relapsed/refractory post-thymic T-cell malignancies received pentostatin at a dose of 4 mg/m^2 weekly for 4 weeks and then every 2 weeks until maximal response. Responses were seen in 34% of patients with a median response duration of 6 months (range 3 months to 15 years). Some patients had durable remissions, with disease subtypes the main predictor of response; T-PLL and Sézary syndrome (SS) had the best response rates of 45% and 62%, respectively. Only a minority (<10%) of these responses were complete. Although some of the remissions have been prolonged (up to 15 years in an SS patient) most patients relapsed within 1 year [25]. Activity in ATLL was disappointing and this has been confirmed by a number of studies in Japan. Tsimberidou et al. [26] published the most recent report of pentostatin in peripheral T-cell lymphoma (PTCL), using a different dose schedule (5 mg/m^2 × 3 days).

Table 15.2 summarizes the role of pentostatin in CTCL. Forty-two patients including 32 (76%) with mycosis fungoides (MF)/SS and 10 patients (24%) with other T-cell leukemias or lymphomas were enrolled. The overall response rate was 54.8% (CR = 14.3% and PR = 40.5%). Durable responses were observed mainly in patients with SS or PTCL. The median duration of response was 4.3 months (range 1–61 months). Several other smaller studies of pentostatin as a single agent in previously treated CTCL showed an overall response rate of approximately 50% (range 26–100%) [33–36]. The first report was by Grever et al. [33] in four patients with advanced refractory MF, with two achieving CR and two PR. Subsequent trials did not confirm these high response rates for MF with responses (mostly PR) seen in 0–57%. The distinction was not always clear between MF and SS in these studies but, where stated, the best responses were seen in erythrodermic CTCL/SS. Again, response rates appear to be dose-related. There have been numerous case reports of successful treatment of other, rarer, T-cell malignancies, including T-large granular lymphocyte (LGL) leukemia [38–40], hepatosplenic T-cell lymphoma [41–43], and granulomatous slack skin disease [44].

Pentostatin Combination Therapy

A study combining pentostatin with interferon-α for the treatment of refractory CTCL showed an increased response rate compared with historic data using pentostatin alone, with a response duration of 13 months [37]. Monoclonal antibody therapy is also emerging as a promising approach to treating PTCL. The anti-CD52 antibody alemtuzumab is an effective therapy in PTCL [45] and has produced durable responses in two-thirds of heavily pretreated patients with T-PLL [46]. There is some limited experience of the combination of pentostatin and alemtuzumab [47] and this deserves further exploration. However, the increased risk of infection must be considered.

There are other agents which have also shown single agent activity in small numbers of patients with PTCL, including anti-CD4 and anti-CD25 monoclonal antibodies, denileukin diftitox (ONTAK), bexarotene, and histone deacetylase (HDAC) inhibitors. Further study will be necessary to delineate the role of any of these agents in combination with purine analogs.

Forodesine

Structure and Synthesis

Several inhibitors of PNP that block dGuo degradation have been synthesized, developed, and tested in in vitro cultures and some were brought to animal in vivo systems (reviewed in [48]). None were potent enough to be effectively used in the clinic [49, 50]. A new strategy has subsequently been used to develop powerful inhibitor by identification of the transition-state structure

Table 15.2 Summary of trials of ADA inhibitor, pentostatin, in CTCL

Study	Pentostatin dose	Total # patients	CR (%)	PR (%)	ORR(%)	Duration of response
Ho et al. [21]	4 mg/m^2 Q week×3	21 SS	5	28	33	
	then Q 2 week×6 then Q month×6	22 MF	0	23	23	
Dearden et al. [24]	4 mg/m^2 week×4	7 SS	14	86	100	(not reported)
	then QOW	6 MF	0	0	0	
Mercieca et al. [25]	4 mg/m^2 Q week×4	16 SS	19	44	63	9 month for SS
	then Q 2 week till optional response	4 SL	0	50	50	
		13 MF	0	0	0	
Grever et al. [33]	4 mg/m^2×3 days then monthly	18	11	22	33	CR (7–10 month) PR (1–3 month)
Cummings et al. [34]	5 mg/m^2 x 3 days, Q 3 week	6	0	67	67	
Greiner et al. [35]	4 mg/m^2 QOW	18	11	28	39	CR (4 month–6 year) PR (1.5–6 month)
Kurzrock et al. [36]	3.75–5 mg/m^2 day× 3 days q 3 weeks	24	29	43	71	3.2 month for SS 2 month for MF
Foss et al. [37]	Alternating w/IFN	41	5	37	42	15.8 month

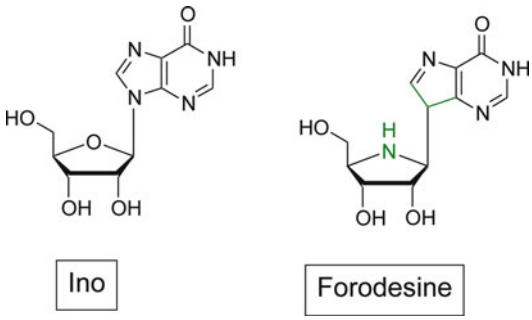

Fig. 15.2 Structures of inosine and inhibitor of purine nucleoside phosphorylase, forodesine

stabilized by the target enzyme. Using inosine as a substrate for transition-state analysis, a series of 9-deazanucleoside analogs, termed immucillins, was designed to mimic the transition-state (Fig. 15.2; [49]). The immucillins have a carbon–carbon linkage between a cyclic amine moiety that replaces ribose, and either 9-deaza-hypoxanthine (immucillin H, now forodesine, Fig. 15.2) and or 9-deaza-guanine (immucillin G), respectively. Chemically forodesine is [(1S)-1-(9-deazahypoxanthin-9-yl)-1,4-dideoxy-1,4-imino-D-ribitol]. These analogs inhibited PNP with high potency; the Ki values were in 20–80 pM range for human and bovine enzyme [51].

Metabolism and Mechanisms of Action

Forodesine itself is a poor substrate for phosphorylation and no metabolites were detected in cells when incubated with this compound [51]. Hence forodesine's actions are through PNP inhibition. Consistent with this statement, cells incubated with forodesine alone (without deoxyguanosine) did not have any cytotoxic effects. Because in the body PNP is responsible for metabolic clearance of deoxyguanosine; the levels of dGuo increases in the plasma when animals are infused with forodesine [52].

In vitro cytotoxicity to T-lineage leukemia cells requires deoxycytidine kinase and the presence of dGuo. This was associated with elevation of dGTP (154-fold), which was significantly higher than dGTP accumulation in normal lymphocytes (15-fold) [51, 53]. The proposed mechanism of action of forodesine is that the high accumulation of dGTP inhibits the ribonucleotide reductase. However, decrease in pyrimidine deoxynucleotides was not observed in leukemia blasts during therapy. Although the exact mechanism is not known, the nuclear consequence of the deregulated levels of deoxynucleotide pools inhibits DNA synthesis that eventually leads to cellular death [54].

Pharmacology studies demonstrated that cytotoxicity was associated with elevated plasma deoxyguanosine [54] and the pronounced intracellular accumulation of dGTP [54]. Models of this metabolic disease demonstrated that immature T lymphocytes and T-lymphoblastoid cells were selectively sensitive to treatment with deoxyguanosine, whereas lymphocytes of B cell lineage did not accumulate high levels of dGTP and were much less sensitive to deoxyguanosine [55–58]. Hence, for forodesine dGuo acts as a drug. Primary leukemia cells when incubated with forodesine and deoxyguanosine showed cytotoxicity which was directly correlated to levels of dGTP increase in leukemia cells [59] and resulted in increase in proapoptotic Bim protein [60].

Forodesine as a Single Agent in the Clinic

A phase I clinical trial, performed by Gandhi et al. [54], was designed to determine the maximum tolerated dose for forodesine and to correlate the drug pharmacodynamics to the administered dose. The patients with relapsed or refractory T-cell disorders were treated with forodesine at a dose of 40 mg/m^2 over 30 min of IV infusion on the first day, then the treatment was continued for days 2–5 at the same dose administered twice daily and repeated every 21–28 days. Recently, Furman et al. [61] have presented spectacular results of phase IIa, multicenter, open-label, single-arm, repeated dose, ongoing clinical trial in patients with advanced precursor T-ALL or T-PLL. Forodesine was administered intravenously, at the dose 40 mg/m^2 for 5 days weekly for a total of six cycles. In total

Table 15.3 Results of clinical trials with PNP inhibitor, forodesine

Drug	Phase	Disease	N	CR (%)	PR (%)	ORR (%)	Author
Forodesine	I	T-ALL T-PLL	5	0	0	0	Gandhi et al. [54]
	II	T-ALL T-PLL	34	21	11	32	Furman et al. [61]
	II	T-ALL	3	100	–	100	Stelljes et al. [62]
	I/II	CTCL	28	7	46	53	Duvic et al. [64]

34 pretreated patients, OR rate was 32.4% and CR was achieved in 20.6%. Time to progression for CR patients was 77–398 days and OS were 77–459 days. In the analyzed group only two patients died. In another report by Stelljes et al. [62], forodesine was given at 40 mg/m^2 for 5 days up to six cycles in three patients with refractory/relapsed T-ALL (two patients were prior to and one post-allogeneic hematopoietic stem cells transplantation; HSCT). Up to the publication of the study all three were alive and in CR with survival of 215+, 398+, and 180+ days, respectively. In authors' opinion, forodesine used in monotherapy can be effective before and after allogeneic HSCT with minimal toxicity and without affecting potential graft versus leukemia effect. Recently, Gore et al. [63] have shown that forodesine contributed to a primary antileukemic cytotoxic effect as well as a secondary immunologic effect by allowing the development of an ongoing graft-versus leukemia effect in T-ALL patients who relapsed following allogeneic HSCT and were treated with this drug.

Forodesine is clinically active also in CTCL. In a multicenter phase I/II, dose-escalation study, forodesine was administered IV at doses between 40 and 135 mg/m^2 [64]. In this study one CR (8%) and two PR (15%) were obtained. Recently, the same authors submitted results of phase I/II open-label a dose-escalation study, evaluating efficacy of oral forodesine administration at the doses from 40 to 320 mg/m^2 in 28 refractory CTCL patients. The OR rate was 53.6% (7.1% with CR and 46.4% with PR). Only lymphopenia of grade 3 or 4 was observed in two patients (5%) [65]. Results of clinical trials with forodesine used as monotherapy are presented in Table 15.3.

Gemcitabine

Structure and Synthesis

Gemcitabine was synthesized at Eli Lilly by Larry Hertel [66] based on the fact that the atomic radius of hydrogen is similar to that of fluorine which suggested that metabolic enzymes may utilize a fluorine substituted nucleoside analog with high efficiency. This postulate was correct as gemcitabine is the best substrate known for deoxycytidine kinase with Km value of 1–10 μM [67, 68]. The positioning of geminal fluorine at the 2′-position inspired the generic name of the drug (Fig. 15.3).

Metabolism and Mechanisms of Action

Similar to cytarabine, gemcitabine is cleared rapidly by deamination [69], although because it is a much better substrate for phosphorylation by deoxycytidine kinase, high levels of gemcitabine triphosphate accumulate in different cell types during in vitro incubations [70, 71] and during therapy [72–74].

Gemcitabine has multiple mechanisms of actions and most of these actions are DNA-directed. The diphosphate of gemcitabine has been studied extensively regarding its biochemical actions on both subunits of ribonucleotide reductase to illustrate a mechanism-based irreversible inactivation of the enzyme [75, 76]. X-ray crystallographic studies with ribonucleotide reductase bound to gemcitabine diphosphate illustrates that the binding is different than the natural substrate CDP [77]. Unlike other known

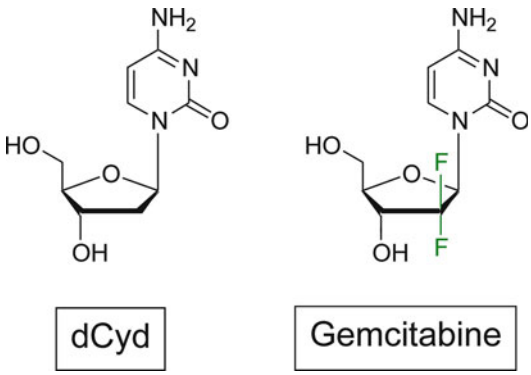

Fig. 15.3 Structures of deoxycytidine and its analog gemcitabine

ribonucleotide reductase inhibits, such as hydroxyurea, fludarabine, cladribine, and clofarabine triphosphate and dATP (accumulates with pentostatin), gemcitabine diphosphate is irreversible inhibitor. Hence the activity of the enzyme is restored only after new synthesis of the enzyme once the inhibitor is removed. Studies have demonstrated role of this action on cytotoxicity especially combination chemotherapy [78]. In addition, inhibition of ribonucleotide reductase results in a decrease in cellular dCTP pool which activates deoxycytidine kinase activity [67] and may facilitate incorporation of gemcitabine triphosphate into DNA [79, 80].

The activities of gemcitabine are DNA-directed in that the nucleoside and its nucleotides interact with numerous metabolic enzymes in the deoxycytidine salvage pathway acting as inhibitory alternative substrates for some. [70]. Gemcitabine is novel among nucleoside analogs as it is active against numerous solid tumors.

Incorporation of the analog in DNA is considered as the primary cytotoxic lesion leading to cell death [81]. Incorporation into DNA is followed by a DNA damage response and recent data suggest that the MRN complex (Mre11-Rad50-Nbs1 complex), which is involved in recognition and repair of double strand breaks is activated in response to gemcitabine-mediated stalled replication forks [82, 83]. Also role of ATM and DNA-PK as molecular sensors for replication halt has been identified [81, 82].

Gemcitabine as a Single Agent in the Clinic

In hematopoietic malignancies, gemcitabine has shown a high level of activity as a single agent in relapsed or refractory Hodgkin's disease and some degree of efficacy in aggressive and indolent non-Hodgkin's lymphoma. Among the several second-line and experimental drugs for PTCL and CTCL, gemcitabine should be considered among the most suitable options to date for pretreated PTCLU and MF patients. Gemcitabine has been demonstrated to be an effective monotherapy with a 60–70% overall response rate in patients with advanced, heavily pretreated patients [84–87]. In a phase II trial, Zinzani et al. [86] treated 44 consecutive, previously treated patients with MF (30 cases) and PTCLU (14 cases) with exclusive skin involvement. Gemcitabine was given to all patients on days 1,8, and 15 of a 28-day schedule at a dose of 1,200 mg/m for a total of three cycles. Of the 44 patients, 5 (11.5%) achieved CR, 26 (59%) PR, and the remaining 13 showed no benefit from the treatment. Two of the CRs were histologically confirmed. The complete and partial response rates were the same for patients with MF and those with PTCLU, respectively. No difference in terms of overall response rate was observed between relapsed and refractory patients. The median durations of CR and PR were 15 months and 10 months, respectively. Two other studies have shown good activity when using gemcitabine for the treatment of patients with refractory T-cell lymphoma. Sallah et al. [85] reported their experience in ten patients with refractory and relapsed T-celI malignancies treated with gemcitabine. Two patients had CTCL, two T-PLL, two nodal PTCL, two small lymphocytic lymphoma, one anaplastic, and one angiocentric lymphoma. The drug dose was the conventional 1,200 mg/m^2 on days 1, 8, and 15 of each 28-day cycle. Of the ten patients, two achieved a complete response (one T-PLL and one anaplastic) and four a partial response (two CTCL, one angiocentric, one PTCL) for an overall response rate of 60%. The median and mean duration of response was 13 months and 16 months, respectively. The second trial was conducted at the M.D. Anderson

Cancer Center [84]. Thirty-three pretreated CTCL patients received gemcitabine at a lower dose of 1,000 mg/m^2 for six or more cycles. Thirty-one patients had MF; the overall response rate was 68% including three CR. These findings show that gemcitabine has substantial activity and acceptable toxicity in previously treated patients with MF and PTCL.

In addition, there are also interesting data in untreated patients [88] and few data describing the efficacy of gemcitabine combinations in patients with T-cell lymphoma [89–93]. For these reasons we ran a phase IIb multicenter study with gemcitabine as primary chemotherapy of patients with advanced CTCL (or pretreated only with PUVA or radiotherapy). The patients were recruited from the Italian Cutaneous Lymphoma Study Group. Thirty-two patients with untreated MF, PTCLU, and SS were treated with gemcitabine in seven Italian institutions. Twenty-six of 32 patients had a diagnosis of MF, 5 were diagnosed with PTCLU, and only 1 patient had SS. The median age of the patients was 58 years (range *25–77* year); 22 patients were male and 10 were female. Of the 32 patients studied, 4 had been previously treated with local radiotherapy, 10 had received previous PUVA therapy, and 8 had been treated previously with PUVA and radiotherapy, whereas 10 patients had not received any previous treatment. Gemcitabine was given to all patients on days 1, 8, and 15 of a 28-day schedule at a dose of 1,200 mg/m2 per day for a total of six cycles. The overall response rate (CR + PR) was 75% (24 of 32 patients). The CR were 22% (7 of 32 patients) and 53% (17 of 32 patients), respectively. Patients with MF had a CR rate of 23% (6 of 26 patients) and a PR rate of 50% (13 of 26 patients). Conversely, patients with PTCLU had a CR rate of 20% (1 of 5 patients) and a PR rate of 80% (4 of 5 patients). Of the seven patients who achieved a CR, three were still in disease remission after a median follow-up of 10 months (range 4–22). The median PFS and OS were, respectively, 10 months and 19 months.

Its modest toxicity profile and the easy schedule of administration make gemcitabine an ideal agent for consideration in the development of chemotherapy regimens. In particular, it would be interesting to evaluate the use of two different nucleoside analogs (fludarabine or pentostatin plus gemcitabine) in modulating the entry route into DNA and their action in terms of direct cytotoxicity and apoptosis, respectively. Earlier investigations demonstrated the possibility of potentiating fludarabine with low doses of gemcitabine. In addition, combinations of gemcitabine with other compounds are under investigation. For example pralatrexate, a 10-deazaaminopterin derivative is a novel antifolate designed to have high affinity for the reduced folate carrier type I [94]. The combination of pralatrexate with gemcitabine is currently being explored in a phase I/II clinical trial.

Hematologic toxicities: anemia of WHO grade III was observed in 5–10% of patients, neutropenia of WHO grades III and IV in 20% and 10% of patients, respectively, WHO grade III and IV thrombocytopenia in 20% and 10% of patients, respectively. Non-hematologic toxicity: transient elevations in liver transaminases were observed in 5–10% of patients. Renal and pulmonary toxicity was very rare; WHO grade III and IV less than 1%. Flu-like symptoms with headache, fever, myalgias, and fatigue occurred in up to 10% of patients. No alopecia usually occurs during gemcitabine therapy. Neurotoxicity in connection with gemcitabine is rare. Peripheral edema occurs in 10% of patients. These toxicities were usually mild and reversible after the end of therapy.

Nelarabine

Structure and Synthesis

Nelarabine (Arranon®) resembles arabinosylguanine and acts as a prodrug of ara-G which is an analog of natural nucleoside deoxyguanosine (Fig. 15.2). Chemically it is 2-amino-9-β-D-arabinofuranosyl-6-methoxy-9H-purine and also known as compound-506U78, and GW506U. There were two reasons for an interest in ara-G prodrug. First, due to the success of cytarabine arabinosyl analogs were created with other bases

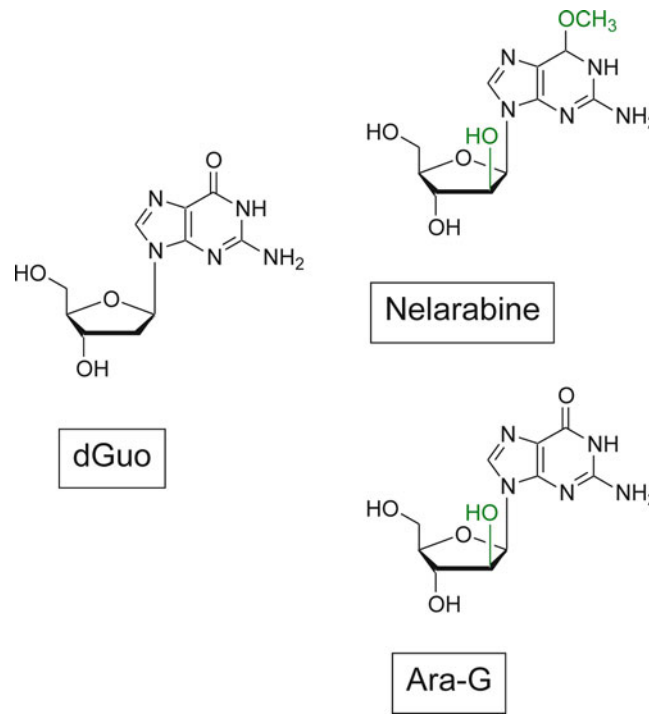

Fig. 15.4 Structures of deoxyguanosine and its analog nelarabine which acts as a prodrug for analog arabinosylguanine

such as adenine and guanine. Second, as mentioned earlier, the discovery that genetic deficiency of PNP results in a profound T-cell lymphopenia suggested that analog of dGuo which is resistant to PNP may show selectivity to T-cells.

Reist and Goodman [95] devised procedures for the chemical synthesis of 9-β-D-arabinosylguanine (ara-G). Despite this compelling evidence for its T-cell specificity and the pressing need for active agents in these diseases, ara-G had not been evaluated in clinical trials, probably due to the low solubility of the compound. The recent development of a more soluble 6-methoxy-prodrug (2-amino-9-B-D-arabinofuranosyl-6-methoxy-9H-purine, 506U, Compound-506U78, nelarabine; Fig. 15.4) of ara-G by Burroughs Wellcome Co. has now made such trials possible [96].

Metabolism and Mechanisms of Action

Nelarabine is a poor substrate for direct phosphorylation, which prohibits further anabolism of the parent drug [96]. When infused to patients, ara-G is liberated when ADA demethoxylates nelarabine. Although nelarabine is a poor substrate for this enzyme with a Km value of 170 μM (rel. Vmax = 2% of adenosine), the abundance of this enzyme in large body organs (including spleen and thymus) results in a rapid conversion. With respect to T-cell diseases, it is of importance to note that there are high levels of ADA in RBCs, which are probably responsible for the conversion in the circulation [97]. The generation of ara-G in the plasma of monkeys and humans infused with nelarabine was rapid [96, 97]. The t½ of nelarabine was 11 min in monkeys and between 14 and 17 min in humans with a concomitant peak of ara-G, reflecting the metabolic conversion to ara-G by ADA and a smaller element of renal clearance.

Ara-G is transported into T-lymphoblastoid cells via nucleoside transport system [98]. Despite the relatively low affinity of these systems, they have a generally high capacity that is not likely to limit cellular metabolism of ara-G but the initial phosphorylation of ara-G to its monophosphate appears to be the rate-limiting step in triphosphate formation. This step is catalyzed by two enzymes [99]; high affinity (Km dGuo, 7 μM; ara-G, 7 μM), low specific activity mitochondrial deoxyguanosine

Table 15.4 Results of clinical trials with nelarabine as monotherapy

Drug	Phase	Disease	N	CR (%)	PR (%)	ORR (%)	Author
Nelarabine	I	Pediatric T-ALL	26	27	15	42	Kurzberg et al. [116]
		Adult T-ALL	13	15	62	80	
		T-CLL/T-PLL	7	0	29	29	
		B-ALL/Pre-B-ALL	10	0	10	10	
		B-CLL/B-PLL	4	0	25	25	
		B-NHL	6	0	17	17	
		AML-CML-BC	8	13	0	13	
	II	T-ALL	106	26	8	32	Berg et al. [117]
	II	T-ALL	26	31	10	41	DeAngelo et al. [118]
		T-lymphoma	13				

kinase [100, 101] and low affinity (Km dCyd, <1 μM, ara-G, >100 μM) cytosolic deoxycytidine kinase [96, 101–103]. Subsequent phosphorylation steps are required to generate the triphosphate ara-GTP. As expected from experience with other nucleoside analogs, T-cell lines accumulated higher levels of ara-GTP [104–106], which was retained for a longer time [107]. Greater accumulation of analog triphosphate was also observed in primary leukemia cells [108, 109].

For its mechanism of actions, the triphosphate of ara-G behaves similar to other nucleoside analogs. It is the proximal active metabolite which competes with natural substrate dGTP for incorporation into DNA. Because dGTP levels are low in cells, the analog triphosphate incorporation is favored. Following analog incorporation, the modified DNA is resistant to further deoxynucleotide addition in DNA replication and repair reactions [110]. Using in vitro DNA primer extension assays, it has been demonstrated that the molecular basis for ara-GTP-induced inhibition of DNA synthesis is due in part to incorporation of the nucleoside analog [111, 112]. T-cell selective cytotoxicity investigations illustrated that in addition to higher accumulation of ara-GTP, induction of Fas-mediated cell death pathway further enhanced the cell killing in T-cell types [113].

Nelarabine as a Single Agent in the Clinic

Nelarabine is a potent agent for the treatment of hematologic malignancies with major efficacy in T-cell disorders [114–116]. Kurtzberg et al. [116] reported the clinical outcome of pediatric and adult patients with refractory hematological malignancies treated with nelarabine. The OR rate was 31%, however this rate was 54% in the subgroups of patients with T-ALL who achieved a complete or PR after one or two cycles of nelarabine (Table 15.4). The efficacy of intravenous nelarabine in patients with refractory or relapsed T-ALL or T-lymphoblastic lymphoma (T-LBL) was evaluated in phase II, noncomparative, open-label, multicentre trials in children and young adults aged <21 years when first diagnosed [117], and adolescents and adults aged ≥16 years [118], and in the GMALL (German Multicentre Study Group for Adult ALL) trial in adults aged ≥19 years [119]. The pediatrics [114] and adult [118] trials recruited 153 and 40 patients; of these, 39 [117] and 28 [118] had not responded to, or had relapsed following treatment with, at least two prior chemotherapy regimens. In the initial group of pediatric patients, nelarabine 1.2 g/m^2/day was infused over 1 h for 5 consecutive days every 3 weeks, but was reduced to 650 mg/m^2/day for 5 days in subsequent patients. In adult patients, the treatment regimen for the first three patients was nelarabine 2,200 mg/m^2/day infused over 2 h on days 1, 3, and 5, but for subsequent patients, the dose was reduced to 1,500 mg/m^2. In the GMALL trial, of 49 evaluable patients, 34 were in first relapse (of these, 32 were refractory to at least one salvage chemotherapy regimen), 7 were in second relapse, 7 were in second relapse after stem cell transplantation and 2 patients never reached a CR. Patients

received nelarabine 1,500 mg/m^2/day on days 1, 3, and 5 (number of cycles not stated) [119].

Nelarabine treatment induced CR or CR-incomplete (CRi; it was defined as CR without full hematological recovery) in approximately one-fifth of pediatric and adult patients who had not responded to, or had relapsed following treatment with two or more prior chemotherapy regimens. The CR rate in pediatric patients was 13% and a further 10% had a CRi, for a total of CR/CRi response rate of 23%. In the adult trial, 18% had a CR and a further 3% had a CRi, for a total CR/CRi response rate of 21% (Table 15.4). For responders as a group, the duration of CR ranged from 4.7 to 36.4 weeks in pediatric patients and from 15.1 through >195.4 weeks in adult patients. In the GMALL trial, 25 patients (51%) achieved a CR and 19 patients went on to receive stem cell transplantation. Neurological adverse events are the most likely adverse events to limit treatment with nelarabine.

The drug is approved for the treatment of pediatric and adult patients with T-ALL and T-LBL whose disease has not responded to, or has relapsed after treatment with at least two prior chemotherapy regimens. The recommended dose of the drug in adults is 1,500 mg/m^2/day infused undiluted over 2 h on day 1, 3, and 5, and repeated every 21 days. In pediatric patients the recommended dose is 650 mg/m^2/day infused intravenously undiluted over 1 h for 5 consecutive days, repeated every 21 days. The efficacy of nelarabine in combination chemotherapy for newly diagnosed T-ALL is currently being investigated in a large, multinational, phase III trial in patients aged 1–30 years.

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