Forodesine (BCX-1777, Immucillin H) – A New Purine Nucleoside Analogue: Mechanism of Action and Potential Clinical Application

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Abstract: Recently a few new purine nucleoside analogues (PNA) have been synthesized and introduced into preclinical and clinical trials. The transition-state theory has led to the design of 9-deazanucleotide analogues that are purine nucleoside phosphorylase (PNP) inhibitors, termed immucillins. Among them the most promising results have been obtained with forodesine. Forodesine (BCX-1777, Immucillin H, 1-(9-deazahypoxanthin)-1,4-dideoxy-1,4-imino-D-ribitol) has carbon-carbon linkage between a cyclic amine moiety that replaces ribose and 9-deaza-hypixanthine. The drug is a novel T-cell selective immunosuppressive agent which in the presence of 2'-deoxyguanosine (dGuo) inhibits human lymphocyte proliferation activated by various agents such as interleukin-2 (IL-2), mixed lymphocyte reaction and phytohemagglutinin. In the mechanism of forodesine action two enzymes are involved: PNP and deoxycytidine kinase (dCK). PNP catalyzes the phosphorolysis of dGuo to guanine (Gu) and 2'-deoxyribose-1-phosphate, whereas dCK converts dGuo to deoxyguanosino-5'-monophosphate (dGMP) and finally to deoxyguanosino-5'-triphosphate (dGTP). The affinity of dGuo is higher for PNP than for dCK. Nevertheless, if PNP is blocked by forodesine, plasma dGuo is not cleaved to Gu, but instead it is intracellularly converted to dGTP by high dCK activity, which leads to inhibition of ribonucleotide reductase (RR), an enzyme required for DNA synthesis and cell replication, which eventually results in apoptosis. Forodesine is active in some experimental tumors in mice, however it could be used for the treatment of human T-cell proliferative disorders and it is undergoing phase II clinical trials for the treatment of T-cell non-Hodgkin's lymphoma, which includes cutaneous T-cell lymphoma (CTCL). Moreover, recent preclinical and clinical data showed activity of forodesine in B-cell acute lympholastic leukemia (ALL).

Key Words: Forodesine, BCX-1777, Immucillin H, purine nucleoside analogue, purine nucleoside phosphorylase inhibitor.

INTRODUCTION

Purine nucleoside analogues (PNA) compose a class of cytotoxic drugs that have played an important role in the treatment of hematological neoplasms, especially in lymphoid and myeloid malignancies and currently are also investigated in autoimmune disorders [1-4]. For the last few years three of them: 2-chlorodeoxyadenosine (2-CdA), fludarabine (FA) and deoxycoformycin (DCF) have been approved by FDA for the treatment of hematological disorders due to a great impact on patients overall survival and overall response. 2-CdA and DCF are drugs of choice in the treatment of hairy cell leukemia [5-7]. FA and 2-CdA have significant clinical activity in low-grade non-Hodgkin's lymphoma and chronic lymphocytic leukemia (CLL) [5, 8, 9]. 2-CdA exhibits also some activity in progressive multiple sclerosis and other autoimmune disorders [1,10]. More recently three novel PNA: clofarabine (CAFdA), nelarabine (ara-G) and forodesine (BCX-1777, immucillin H) have been synthesized and introduced into clinical trials [11, 12]. The chemical structure of all the drugs belonging to PNA class is similar to adenosine or guanosine. The metabolic properties as well as the mechanism of their action are different, however the induction of apoptosis plays the most important role [1, 11, 13, 14].

In the last few years among the drugs belonging to PNA, of great interest has become the family of agents chemically known as immucillins in which forodesine (7-(3,4-dihydroxy-5-hydroxymethylpyrrolidyn-2-yl)-3,5-dihydro-pyrrolo[3,2-d]pyrimidin-4-one) is best recognized (Fig. (**1**)) [15, 16]. Forodesine is a potent purine nucleoside phosphorylase (PNP) inhibitor which acts as a T-cell selective immunosuppressive agent [17]. It is active in some experimental tumors in animals such as mice, dogs, rats and monkeys, however it could be also used for the treatment of human T-cell proliferative disorders[1,2] [18]. Recently, preclinical and clinical data showed also activity of forodesine in B-cell acute lymphoblasic leukemia (ALL).

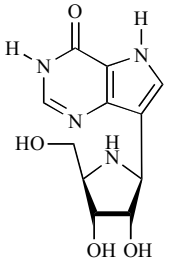

Fig. (1). Structure of forodesine (BCX-1777, immucillin H).

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[1] Thomas, D.A.; Wierda, W.; Faderl, S.; O'Brien, S.; Komblay, S.; Koller, C.; Bantia, S.; Kilpatrick, J.M.; Bennett, J.C.; Kartarjian, H.; Gandhi.V. *Blood* **2003**, 102, Abs 4772.

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This review will summarize current knowledge concerning the understanding of forodesine mechanism of action, and pharmacological properties. We will also present the results of *in vitro* studies on this agent and its potential clinical activity as a new drug available for clinical trials.

PURINE NUCLEOSIDE PHOSPHORYLASE (PNP)

Purine nucleoside phosphorylase (PNP) is an enzyme which is detected at micromolar concentrations in blood cells and plays a key role in the purine salvage metabolic pathway [19, 20]. It catalyzes the reversible phosphorolysis of N-ribosidic bonds of both purine nucleosides and deoxynucleosides, in the presence of inorganic ortophosphate as a second substrate generating purine base and α-ribose (or deoxyribose) -1-phosphate (Fig. (**2**)) [11, 20]. Mamalian PNP accepts only guanosine (Guo), dGuo or inosine as a substrate, but not adenosine (Ado) or 2'-deoxyadenosine (dAdo) [21]. This enzyme is specific for purine nucleosides in the β-configuration and converts inosine to hypoxanthine and Guo to Gu, providing an important source of nucleobases for hypoxanthine- xanthine- guanine phosphoribosyl transferase (HXGPRT) which converts guanylate nucleobases into nucleotides, including dGMP [22].

Generally, PNP may be grouped into two main families. Family 1 contains hexameric forms such as the *Escherichia coli* which are 26kDa. They are termed "high molecular mass" PNP because the average aggregate mass of the hexamer is near 150 kDa. PNA belonging to this family are active against both 6-oxopurines and 6-aminopurines (e.g. adenosine). Family 2 includes all mammalian PNP and represents trimeric forms such a human enzyme with typically 31kDa and prefer 6-oxopurines (e.g. inosine, guanosine) but not adenosine. They are formed of polypeptide chains with 284 amino acids residues. The members of this family are termed "low molecular mass" PNP because a mean aggregates mass is 90kDa [22, 23]. The third group of PNP do not fit into either family. *Plasmodium falciparum* PNP (*Pf* PNP) has been characterized in detail, and although its amino acid sequence is most similar to hexameric PNP, its substrate is distinct from either family [22, 23].

More than thirty years ago Giblett *et al*. [24] discovered that loss of functional PNP in child was associated with a relatively selective depletion of T-cells causing profound T-cell lymphopenia and leading to immunodeficiency, whereas B-cell immunity was normal. It is currently known that most of PNP-deficient children have undetectable level of PNP activity which is lower than 5% of normal activity in erythrocytes [25]. In parents of the PNP-deficient children the level of this enzyme activity is reduced in 30-50%. However, they are immunogically normal and their plasma dGuo level is not elevated like in their PNP-deficient children [25, 26].

It is currently known that the low activity of PNP in lymphocytes due to inhibition of this enzyme leads to an increase of plasma dGuo level resulting in its intracellular phosphorylation to dGTP, the accumulation of which culminates in apoptosis. This phenomenon became the base for the subsequent development of specific PNP inhibitors, which for the last few years have been investigated as a new class of antineoplastic agents with promising activity, in particular against T-cell –mediated disorders.

IMMUCILLINS

One of the methods used for the design of PNP inhibitors is transition state theory [27]. Transition state analysis indicates that perfect mimics of the transition state will bind tighter than substrate by the factors of enzymatic rate enhancement, typically 10^{10} to 10^{15} [28, 29].

The transition-state analysis using inosine as a substrate has led to the design of a few recently developed 9-deazanucleotide analogues, which are high affinity inhibitors of PNA and are termed immucillins. Immucillins have potential for the treatment of T-cell cancer, autoimmune disorders, tissue transplant rejection, psoriasis, cutaneous T-cell lymphoma (CTCL) and malaria [17, 18, 30].

The structures of the agents belonging to this family are based on "nitrogen in the ring" D-ribofuranosyl C-glycosidic analogues of natural nucleosides [16]. Immucillins are modified at 2'-, 3'- or 5' positions of the azasuger moiety or at 6-, 7-, or 8- positions of the deazapurine [31]. For the last few years there have been studies carried out on three best recognized immucillins: immucillin H (ImmH), immucillin G and immucillin A [11,21]. In addition, more recently, PNP transition state structure studies have also led to development of DADMe –Immucillin transition state analogues which were synthesized as methyelene bridged derivatives [32, 33]. Between immucillins however, the most important role plays ImmH which has a carbon-carbon linkage between a cyclic amine moiety that replaces D-ribose and 9-deaza-hypoxanthine [11, 21]. ImmH was firstly found to be a 23 pM in-

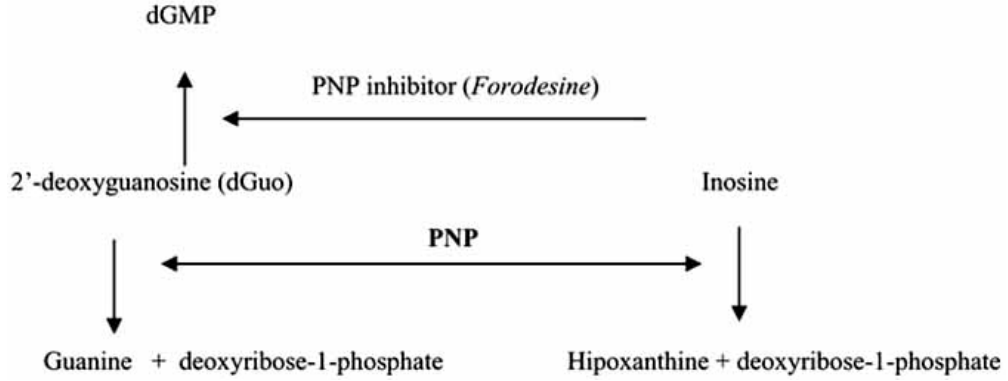

Fig. (2). Mechanism of PNP action.

Table 1. The Comparison of Some Characteristics of PNA [1, 11, 13]

	Cladribine (2-CdA)	Fludarabine (FA)	Pentostatin (DCF)	Clofarabine (CAFdA)	Nelarabine (ara-G)	Forodesine (BCX-1777)
Chemical structure	Deoxyadenosine analogue	Adenosine analogue	Coformycine analogue	Deoxyadenosine analogue	Guanosine analogue	9-deaza-hypoxanthine analogue
Active form	2-CdA triphosphate	FA-triphosphate	Nucleoside- DCF	CAFdA-triphosphate	Ara-G triphosphate	Nucleoside- Forodesine
Incorporation into RNA/DNA	Yes	Yes	No	Yes	Yes	No
Mechanism of action	Inhibition of RR and polymerase, inhibition of DNA repair, apoptosis	Inhibition of RR and polymerases, inhibition of DNA repair , apoptosis	ADA inhibition, dATP accumulation, apoptosis	Inhibition of RR and polymerases, inhibition of DNA repair , apoptosis	Inhibition of DNA synthesis and DNA repair, apoptosis	Highly selective PNP inhibitor, dGTP accumulation, apoptosis
Indication for treatment	HCL, CLL, B-and T-cells NHL, RA, SLE, MS, AIHA,AML	CLL, B-and T-cells NHL, AML	HCL	AML, ALL,CML-BC, ALL in children	T-cell disorders	T-cells NHL, CTCL, T-ALL, B-cell lymphoma/leukemia

RR-ribonucleotide reductase; HCL- hairy cell leukemia; CLL- chronic lymphocytic leukemia; NHL- non-Hodgkin's lymphoma; RA-rheumatoid arthritis; SLE- systemic lupus erythromatous; MS- multiple myeloma; AIHA- autoimmune hemolytic anemia; AML-acute myeloid leukemia; ALL-acute lymphoblastic leukemia; CML-BC- chronic myelogenous leukemia - blast phase.

hibitor of bovine PNP, nevertheless now it is also known as a potent human PNP inhibitor. In the group of ImmH two of the most potent members are recognized : D-ImmH and D-DADMe-ImmH, although their L-enantiomers were also synthesized and introduced into experimental studies [16]. These L-enantiomers bind to the PNP approximately 5 to 600- times less well than do D-compounds, but nevertheless remain powerful inhibitors with nanomolar dissociation constants [16]. Schramm has recently described that the most powerful PNP inhibitor reported for human is DADMe-ImmH [34].

Forodesine (D-ImmH) was initially developed as an intravenous formulation [35]. Nevertheless, it is currently known that forodesine administered orally shows also an immunosuppressive activity [17]. *In vitro* studies using cell lines have established that in the presence of dGuo, the drug selectively inhibited the growth of malignant T-cells and activated human lymphocytes, and its action was 10 to 100-fold more potent than other known PNP inhibitors like PD141955 and BCX-34 (peldesine) [17].

MECHANISM OF FORODESINE ACTION

In contrast to other PNA such as 2-CdA, FA, CAFdA or ara-G, for which the active form are nucleoside triphosphates, in case of forodesine active is nucleoside. The drug does not act *via* incorporation into DNA and inhibition of DNA synthesis but displays a highly selective PNP inhibitory action (Table **1**) [13, 36].

Under normal, physiologic conditions, dGuo present in plasma undergoes phosphorolysis by PNP to Gu and 2'-deoxyribose-1-phosphate. In case of either absence or inhibition of PNP in consequence of immucillins action (including forodesine), high level of plasma dGuo is achieved and instead of phosphorolysis, dGuo is intracellularly catalyzed first to dGMP and finally to dGTP (Fig. (**2**)) [25]. Administration of forodesine results in increases in plasma dGuo and intracellular dGTP level, which correlates with inhibition of T-cell proliferation[3].

Table 2. *In Vitro* Inhibition of T-Cells Proliferation Caused by Forodesine Added to the Culture Medium in the Presence of 3-10μM dGuo [17, 25, 39]

Human T-cells	IC_{50} (μM)
Immature T-cells from ALL patients	0.002
Normal peripheral lymphocyte	0.05 – 0.39
IL-2 stimulated lymphocyte	0.06
Phytohemagglutinin stimulated lymphocyte	0.39

In the mechanism of forodesine action, apart from PNP two other enzymes are involved: deoxycytidine kinase (dCK) or mitochondrial deoxyguanosine kinase (dGK). Normally the affinity of dGuo is higher for PNP than for dCK. Nevertheless, in the absence of PNP plasma dGuo reaches high level and is not cleaved to Gu, but instead it is intracellularly converted mainly by dCK or dGK to dGMP, and then by mono- and diphosphokinases to the active dGTP (Fig. (**3**)) [13,17].

However, similarly to other purine nucleoside, the first step of the cellular activity of dGuo is their uptake into the cells which occurs *via* one or more of nucleoside-specific membrane transporters (NT). In order to be biologically ac-

[3] Bantia, S.; Miller, P.; Parker, C.D.; Ananth, S.L.; Horn, L.L.; Babu, Y.; Sandhu, J. ICAAC 2002, 42, Abs. F2039.

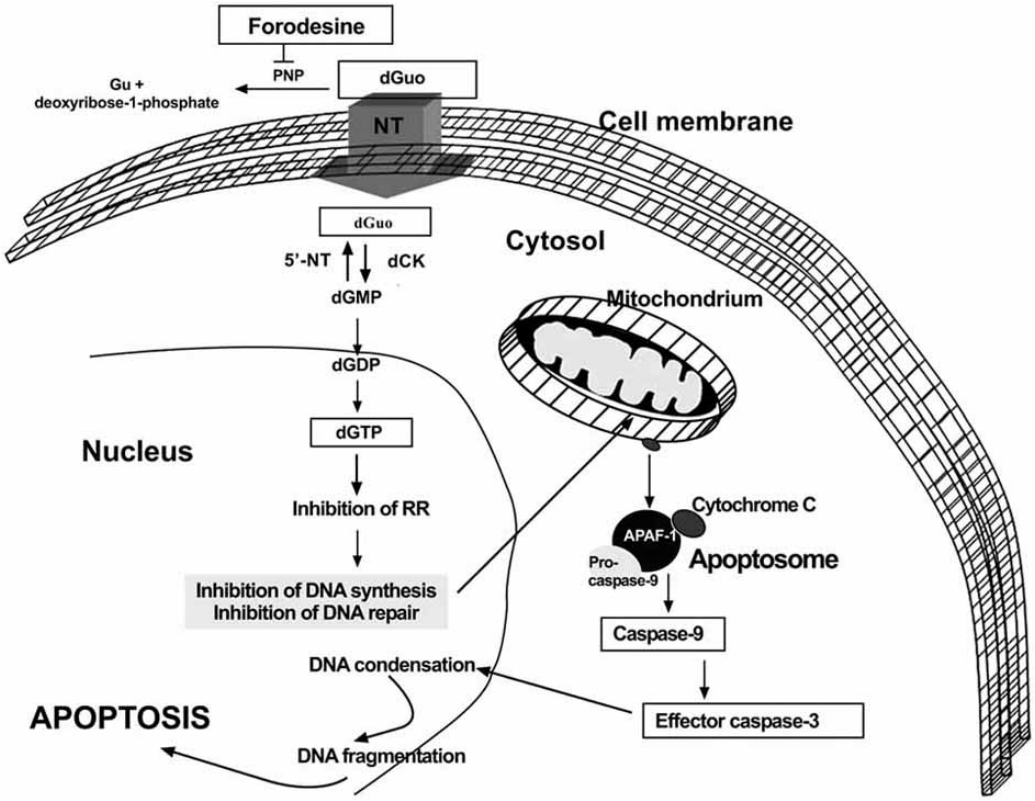

Fig. (3). Influence of forodesine on T-cells apoptosis.

tive, dGuo needs to be phosphorylated intracellularly, and its cytotoxicity depends on accumulation of dGMP in the cells.

In contrast to other antineoplastic drugs, PNA act cytotoxically both in mitotic and quiescent cell cycle phase [37]. The cytotoxicity of PNA triphosphate in the proliferating cells is mainly due to the inhibition of either DNA polymerases or ribonucleotide reductase (RR), leading to disequili-brium in deoxynucleotide triphosphates pool and *via* endonuclease activation results in DNA strand breaks [13]. Accumulation of dGTP as a result of forodesine action leads to RR inhibition, imbalance of deoxynucleotide triphosphate (dNTP) pool, inhibition of either DNA synthesis or DNA repair, which results in accumulation of DNA breaks [38-40]. DNA damage due to inhibition of DNA repair and accumulation of DNA breaks leads to the p53 expression which plays a key role in the control of apoptosis and cell cycle [13, 41]. It has been recently demonstrated that post-translational modification of p53 is required for its stability and activation following cell stress and is a determinant of p53-mediated apoptosis [36, 42]. Balakrishnan *et al*. [36] have recently shown that forodesine treatment also results in post-translational modification of p53, its stability, and p53 dependent p21 activation.

It is known that p53 influences Bax and other proapoptotic proteins activity leading to mitochondrial changes typical for apoptosis, and these result in secretion of cytochrome c from mitochondria to cytosol and its binding with APAF-1 (apoptotic protease activating factor-1) and procaspase-9. APAF-1, cytochrome c and procaspase-9 form a complex termed apoptosome which influences the activation of caspase-9 cascade and *via* DNA condensation and fragmentation leads to apoptosis (Fig. (**3**)) [43,44]. Cytochrome c release is a critical point during apoptosis, however the mechanism by which it exits mitochondria is not well understood.

On the other hand, similarly to other cells which were treated with PNA, in cells showing an accumulation of dGTP as a result of forodesine action, apart from intrinsic cell death pathways connected with DNA damage and p53 protein expression, a direct mechanism connected with mitochondrial permeability transition pore (MPT) and resulting in the release of proapoptotic proteins has been also described. Additionally, an extrinsic pathway *via* death receptor Fas/CD95 can be also important [13, 36].

PHARMACOKINETICS OF FORODESINE

Forodesine as a potent inhibitor of human and animals PNP shows IC_{50} values ranging between 0.48 nM for mice and 1.57nM for dogs [17]. Bantia *et al*. [17] have shown that IC_{50} for this drug against human PNP was 1.2nM. Above authors have also demonstrated that forodesine in the presence of dGuo inhibited *in vitro* proliferation of CEM-SS cells (immature T-cells derived from T-acute lymphoblastic leukemia patients) with an IC_{50} of 0.015μM (Table **2**) [25, 39]. In the presence of dGuo at the concentration of 3.0 to 10.0 μM forodesine also inhibited normal human lymphocyte proliferation activated by different agents such as interleukin-2 (Il-2) or phytohemagglutinin (PHA) with IC_{50} values from 0 to 0.39μM (Table **2**). In addition, $T_{1/2}$ of dGTP in T-ALL cells was 18 hours, whereas in normal lymphocytes it was 4 hours. These results suggest that the low nucleotide

levels and high dCK level in T-ALL cells make them more sensitive to forodesine inhibition than the normal human lymphocytes [17].

The bioavailability after oral administration of forodesine in mice was 63%. At a single dose of 10mg/kg the drug increased dGuo accumulation to approximately 5μM [17]. After increasing the oral administration of forodesine up to 10mg/kg no further increase in dGuo was observed. *In vivo* studies in primates revealed that oral and iv administration of forodesine induced a rapid elevation of plasma 2'-dGuo and that oral dosing at 8.8 and 17.6 mg/kg were at least equivalent to 4.4 mg/kg iv twice daily in effecting 2'-dGuo accumulation. Increasing the i.v. dose of immucillin-H did not increase dGuo accumulation, however plasma dGuo concentration remained elevated longer. In contrast, the increase of oral doses resulted in elevated plasma dGuo accumulation [45]. In human blood dGuo is degraded rapidly with a half-life ($T_{1/2}$) of 12 s and for achieving dGuo concentration needed to a significant reduction of T-cell function the continuous inhibition of PNP greater than 95% is required [17, 38].

Apart from forodesine, other PNP such as PD-141955 or BCX-34 (peldesine) have been also described [17, 35, 46, 47]. BCX-34 is an 2'-deoxy forodesine analogue, which with an IC_{50} of 30nM was evaluated *in vitro* for therapeutic efficacy for psoriasis and CTCL. Pharmacokinetic and pharmacodynamic properties of this agent in humans indicates however, that the agent has no significant clinical activity because its oral dosing could not achieve the dGuo level necessary for T-cell inhibition [18, 48].

PRECLINICAL STUDIES

Up to now a lot of *in vitro* studies concerning the mechanism of T-cell inhibition by forodesine have been published [17, 25, 38, 39]. Bantia *et al*. [17, 25] showed that inhibition of T cells proliferation in the presence of forodesine and dGuo was accompanied by dGTP accumulation resulting in apoptosis, however, the level of deoxyadenosine triphosphate (dATP) remained unchanged.

Kicska *et al*. [38] have reported that forodesine (up to 50μM) and dGuo had no direct toxic effect on unstimulated peripheral T-cells but markedly suppressed the proliferation of T-cell leukemia cell lines MOLT-4 and CCRF-CEM treated with the both agents for 36 hours. Lack of toxic effect when forodesine was added to cultures of variety of non-T-cell lines derived from various tissues was also described.

Bantia *et al*. [17] have also demonstrated that to achieve greater than 50% inhibition of human lymphocytes proliferation by forodesine used at the concentration of 1μM, the presence of dGuo at the concentration between 3 and 10 μM is required. The inhibition of proliferation correlated with dGTP levels in the cells. In the presence of forodesine and dGuo, a 154-fold higher accumulation of dGTP was observed in T-ALL cells compared with a 15-fold accumulation in human lymphocytes. In these cells 8-fold elevation of dATP was also described.

CLINICAL TRIALS

For the last few years there have been ongoing preclinical studies which led to clinical trials with forodesine in variety of T-cell mediated disorders, including CTCL as well as in B-cells lymphoma/leukemia [*6, *7, *9, *11]. Up to now, only few phase I or II clinical trials confirming an antileukemic activity of forodesine in patients with refractory or relapsed aggressive T-cell malignancies have been published[*4, *6-*11]. For the purpose of estimation of forodesine toxicity and its side effects the phase I studies in healthy individuals were performed, however the results have not been published yet[*5].

A phase I clinical trial, performed by Gandhi *et al*. [21], was designed to determine the maximum tolerated dose (MTD) for forodesine and to correlate the drug pharmacodynamics to the administered dose. Five patients with relapsed or refractory T-cell lymphoblastic lymphoma, acute leukemia and T-cell prolymphocytic leukemia were treated with forodesine at a dose of 40 mg/m^2 over 30 minutes of iv infusion on the first day and then the treatment was continued for days 2-5 at the same dose administered twice daily and cycles were repeated every 21-28 days. Median peak level of forodesine (5.4μM) was achieved at the end of the infusion. This concentration was sufficient to elevate plasma dGuo accumulation that reached a maximum concentration of 20μM at the end of the last infusion. The dGuo C_{max} values ranged from 2.6μM to 34μM for the 40 mg/m^2 of forodesine. The elimination of the drug was slow with a median $T_{1/2}$ of 11 hours. The intracellular dGTP levels increased by 2 to 40 times and were correlated with antileukemic activity of the drug in the patients studied. Forodesine is predominantly excreted by kidneys and approximately 54 to 73% of the administered dose is present in the urine. The excretion rate for dGuo was similar to that of forodesine. In this study objective responses were not observed (Table **3**) [21].

Recently, Furman *et al*.[*5] 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 40mg/m^2 for 5 days weekly for total of 6 cycles. In nonresponders, after 2 cycles the dose was escalated to 90 mg/m^2. At the last interim report, in total 34 pretreated patients (median 3 prior treatments) overall response (OR) rate was 32.4% and complete remission (CR) was achieved in 20.6%. Time to progression for CR patients was 77 to 398 days and overall survival (OS) was 77 to 459 days. In the analyzed group only 2 patients died. Authors concluded that forodesine used as a single agent in relapsed or refractory T-cell leukemia occurred effective with minimal toxicity. Additionally, they confirmed the dose 40 mg/m^2 for subsequent phase II development[*6].

In the consecutive report forodesine was given at 40mg/m^2 for 5 days up to 6 cycles in three patients with refractory/relapsed T-ALL (2 patients were prior and 1 post allogeneic hematopoietic stem cells transplantation; HSCT). Up to 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 tox-

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[6] Furman, R.R.; Gore L.; Ravandi F.; Hoelzer D., *Blood*, **2006**, 108, 11, Abs 1851.

Table 3. Results of Clinical Trials with Forodesine

Phase	Disease	Route	N	CR(%)	PR(%)	OR(%)	Authors
I	T-ALL T-PLL	iv	5	0	0	0	Ghandi *et al*. (21)
II	T-ALL T-PLL	iv	34	21	11	32	Furman *et al*.[*5,*6]
II	T-ALL	iv	3	100	-	100	Stelljes *et al*. [*7]
I/II	CTCL	iv	13	8	15	23	Duvic *et al*. [*8]
I/II	CTCL	po	28	7	46	53	Duvic *et al*. [*9]
I/II	B-ALL	iv	6	17	33	50	Furman *et al*. [*10]
II	B-ALL	iv	12	17	0	17	Ritchie *et al*. [*11]
I	CLL	po	2	?	?	?	Balakrishnan *et al*. [36]

T-ALL - T-cell acute lymphoblastic leukaemia; T-PLL- T-cell acute prolymphocytic leukemia ;
CTCL - cutaneous T-cell lymphoma; B-ALL - B-cell acute lymphoblastic leukemia; CLL- chronic lymphocytic leukemia
CR- complete remission; PR-partial remission; OR- overall response

icity and without affecting potential graft versus leukemia effect[*7].

According to the above studies, forodesine is safe and well tolerated, however published data did not report dose-limiting toxicities. To the most common drug related adverse events, without regard to grade belong: nausea, headache, thrombocytopenia, leukopenia, asthenia and anemia. Possible drug-related grade 3 or 4 adverse events are thrombocytopenia and leukopenia [*6]. In all published results only three severe adverse events (SAE) related to forodesine have been presented: cytomegalovirus pneumonitis in patient with biphenotypic leukemia, elevated transaminases in a Sezary's syndrome patient and recurrent neurologic symptoms in patient with a relapsed T-cell malignancy[*5] [21].

Forodesine is clinically active also in CTCL. In 13 patients with refractory CTCL, a multicenter phase I/II, dose escalation study, forodesine was administered iv at doses between 40 – 135 mg/m^2, [*8]. In this study one CR (8%) and two PR (15%) were obtained. Recently, the same authors submitted results of phase I/II open-label dose-escalation study, evaluating efficacy of oral forodesine administration at the doses from 40 to 320mg/m^2 in 28 refractory CTCL patients. The OR rate was 53.6% (7.1% with CR and 46.4% with PR). The only grade 3 or 4 adverse event was lymphopenia, which was observed in 2 patients (5%)[*9].

Preclinical data showed also activity of forodesine in B-cell ALL, which led to a phase I/II study. Up to now, only the results of two trials are available[*10]. First of these presents dose-escalation study (starting dose: 40mg/m^2, maximal dose: 135mg/m^2) in 6 patients with B-ALL. Hematological benefit, defined as a decrease of tumor burden, was demonstrated in 5 patients. One of the patients, treated with a high dose of forodesine had CR and 2 patients PR[*10]. In the second study, 12 patients with refractory/relapsed B-ALL were treated with forodesine at the dose 80 mg/m^2. Two of patients achieved CR and there were no patients with PR. The authors emphasize, that forodesine was well-tolerated with preliminary evidence of activity as a single agent in B-ALL[*11].

Recently, the first studies concerning forodesine efficacy in the treatment of patients with advanced and refractory CLL have been also initiated, however only preliminary pharmacokinetic data have been presented [36].

CONCLUSIONS

Forodesine similarly to 2-CdA, FA, DCF, CAFdA and ara-G belongs to the PNA group, however its chemical structure, active form, metabolic properties and mechanism of action are different from other PNA. Forodesine which represents immucillins class is a novel T-cell selective immunosuppressive agent, which in the presence of dGuo inhibits human lymphocyte proliferation. The most important role in the mechanism of forodesine action plays its inhibition of PNP, an enzyme involved in catalyzing plasma dGuo to Gu and 2'-deoxyribose- 1-phosphate, resulting in conversion of dGuo to dGMP and leading to a high intracellular dGTP accumulation and finally to apoptosis of T-cells. The agent has proved active both in *in vitro* studies and in some preclinical experimental tumors in mice. Recently it has also been undergoing clinical trials phase I and II for the treatment of T-cell non-Hodgkin's lymphoma, including CTCL, T-cell ALL as well as B-cell lymphoma. Great hopes are also set on the use of forodesine in chronic lymphocytic leukemia (CLL)

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[10] Furman, R.R.; Gandhi, V.V.; Bennett, J.C.; Bantia, S.; Kilpatrick, J.M., *Blood*, **2004**, 104, Abs 2743.

[11] Ritchie, E.; Gore, L.; Roboz, G.J.; Feldman, E.; Ravandi, F.; Furman, R., *Blood*, **2006**, 108, 11, Abs 1881.

treatment, however up to now only the first pilot results of all clinical trials have been approachable.

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ABBREVIATIONS

2-CdA = 2-chloro-2'-deoxyadenosine; cladribine

5'-NT = 5'-nucleotidase

ADA = Adenine deaminase

Ado = Adenosine

AIHA = Autoimmune hemolytic anemia

ALL = Acute lymphoblastic leukemia

AML = Acute myeloid leukemia

APAF-1 = Apoptotic protease activating factor

Ara-G = 9-β-D-arabinosylguanine

AUC = Area under the concentration-time curve

BCX-1777 = Immucillin H; forodesine

CAFdA = 2-chloro-2'-fluoro-2'-deoxyadenosine; clofarabine

CLL = Chronic lymphocytic leukemia

C_{max} = Maximum plasma concentration

CML-BP = Chronic myelogenous leukemia blast phase

CR = Complete remission

CTCL = Cutaneous T-cell lymphoma

dAdo = Deoxyadenosine

DCF = 2'-deoxyocoformycin; pentostatin

dCK = Deoxycytidine kinase

dGK = Deoxyguanine kinase

dGuo = 2'-deoxyguanosine

DLT = Dose limiting toxicity

FA = 2-fluoroadenosine; fludarabine

Gu = Guanine

Guo = Guanosine

HCL = Hairy cell leukaemia

HSCT = Hematopoietic stem cells transplantation

NT = Human nucleoside transporter

i.v. = Intravenous

IC_{50} = Concentration that due to 50% inhibition of cell proliferation

NHL = Non-Hodgkin's lymphoma

MPT = Mitochondrial permeability transition

MS = Multiple sclerosis

MTD = Maximum tolerated dose

OR = Overall response

OS = Overall survival

PNA = Purine nucleoside analogues

PNP = Purine nucleoside phosphorylase

PR = Partial remission

RA = Rheumatoid arthritis

RR = Ribonucleotide reductase

SAE = Severe adverse event

s.c. = Subcutaneously

SLE = Systemic lupus erythematous

$T_{1/2}$ = Half life time

V_d = Value of distribution

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