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From cell biology to therapy: forodesine

Introduction and rationale

Forodesine [(BCX-1777; immucillin H; (1S)-1-(9-deazahypoxanthin-9-y-l)-1,4-dideoxy-1,4-imino-D-ribitol hydrochloride), BioCryst Pharmaceuticals, Birmingham, AL, USA] is a rationally designed, novel transition-state analog inhibitor of purine nucleoside phosphorylase (PNP)(Miles 1998; Bantia 2001; Bantia 2004). This designer small molecule has a molecular formula weight of 302.73 and the structure of a nucleoside analogue (such as fludarabine, 2-CDA, nelarabine, gemcitabine) that are active in cutaneous T-cell lymphoma (Korycka 2008) (Figure 1). However, as a transition state analogue, forodesine is unique in that it is not incorporated into DNA as are the other cytotoxic nucleoside analogues that are also active in T-cell malignancies (Galmarini 2008) (Figure 1). The rational for the clinical development of forodesine stems from the observation that children who have inherited a deficiency of purine nucleoside phosphorylase (PNP) have selective depletion of their T-lymphocytes. PNP deficiency alters the normal nucleoside pathway leading to accumunation of plasma 2'-deoxyguanosine (dGuo) and intra-cellular intracellular dGuo triphosphate (dGTP) that is toxic to lymphocytes resulting in their undergoing apoptosis(Gandhi 2007) (Figure 2). A nucleoside kinase, deoxycytidine kinase (dCK) found in activated T-lymphocytes, is required and lends specificity to the effect of PNP inhibition.

In vitro, forodesine inhibits the proliferation of activated human T lymphocytes (Gandhi 2007) (Kicska 2001; Bantia 2004) and acute lymphoblastic leukemic T cells(Bantia 2003). Nucleoside transporters (ENT1 and ENT2) facilitate the uptake of forodesine in leukemia cells, and cells with mutations in hypoxanthine-guanine phosphoribosyltransferase (HGPRT) had enhanced sensitivity to this agent (Huang 2008). The efficacy of forodesine was comparable to that of cyclosporine A in a mouse xenograph model of graft-versus-host disease (Bantia 2002). Almost complete depletion of of PNP is required to alter the purine pathway. Forodesine is 100-1000 times more potent than other PNP inhibitors and is able to cause almost complete deletion of PNP in animals, reversibly depleting circulating T-cells.

Phase I clinical trial of intravenous forodesine (BCX-1777) in CTCL

Based on the encouraging preclinical profile, Phase I and II clinical trials of forodesine were initiated in patients with refractory cutaneous T-cell lymphomas (CTCLs) as well as with other hematologic malignancies, including T-cell leukemias (Duvic 2004; Furman 2004; Isola 2004; Furman 2005). An IV formulation of 10 mg/mL under NDA 62,777 and 100 mg capsules under NDA 70,196 have been administered to patients participating in Phase I and then II clinical trials.

In a Phase I dose ranging, open label study, 13 refractory CTCL patients (mycosis fungoides or Sézary Syndrome) with a median age of 57 (range 33-74) were treated over five days with IV forodesine (10 mg/mL) (Duvic 2004). This study's objectives were to determine [1] the maximum tolerated dose (MTD) and [2] the peak and trough plasma concentrations of

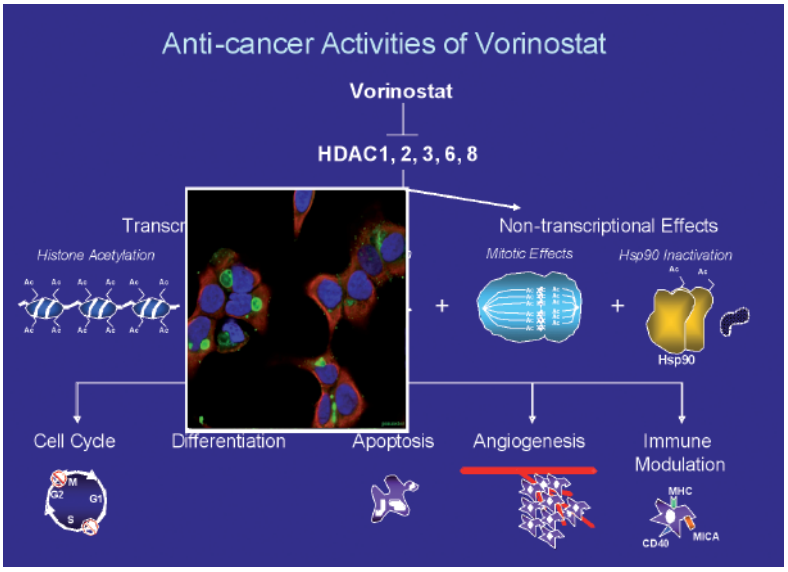

Figure 1. Chemical structure of forodesine and nucleoside analogues.

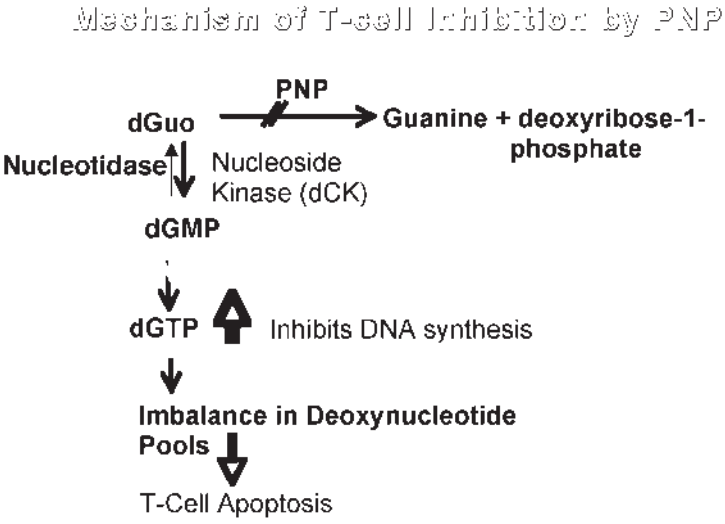

Figure 2. Mechanism of action of forodesine

Table 1. Common and severe adverse side effects in phase II dose ranging clinical trial of oral forodesine.

Frequent AEs (Gr ≤2)	*All AEs ≥Gr 3 (≥2 pts) ± cause*
Fatigue (38%)	Diarrhea
Peripheral edema (34%)	Acute RF (unrelated)
Nausea (34%)	Cellulitis
Pruritus (23%)	Rash (2 pts)
Pyrexia (21%)	
Headache (20%)	
Insomnia (20%)	

dGuo and forodesine. Secondary objectives included [1] correlating the toxicity profile and the dose levels of forodesine with the plasma concentrations of dGuo, and [2] correlatine plasma concentrations of dGuo with clinical response, and [3] determining the provisional optimal biologic dose (POBD). Ten patients with Sézary Sydrome, two with Mycosis fungoides, and one angioimmunoblastic T-cell lymphoma participated in this trial. Four cohorts of at least three patients each were sequentially treated and evaluated. Each patient received intravenous forodesine at doses of 40, 60, 90, or 135 mg/m^2 every 12 hours for nine doses over 5 days. There was a two-week rest period before the next infusion for 3 cycles.

Of the thirteen patients who began this study, 8 patients (62%) completed all three courses. Five patients (39%) discontinued treatment for progressive disease or logistical reasons. Nine of 13 patients showed some degree of skin improvement and/or a pharmacodynamic response, as measured by a decrease in the absolute Sézary cell numbers and/or CD4/CD8 ratio(Duvic 2004). All patients developed elevated plasma dGuo concentrations, and there were no dose-limiting toxicities. There were three complete responders, one partial response, and six with stable disease. One patient with existing hyperuricemia developed a high uric acid level treated with one dose of rasburicase. One patient with a CR unfortunately developed fatal staphylococcal line sepsis after the first course. A maximal tolerated dose (MTD) was not reached.

A further update of the clinical trial showed that forodesine administration reduced the involved body surface area from baseline screening, and produced a pronounced clinical improvement in erythroderma severity during and subsequent to forodesine therapy (Duvic 2006). The best cutaneous response to treatment by physician's global response was considered marked (n=2), moderate (n=4), or minimal improvement (n=3) in patients with refractory CTCL. None of the patients were graded as markedly worse at any time point, and there was only one patient whose condition was rated as probably worse. Importantly, median patient global assessment of disease status at screening for the 12 patients assessed indicated increasing improvement in disease status at each post-treatment visit. However, clinical assessments were at the beginning of each new course rather than at the end of the infusions when the patients' responses were optimal. Three patients continued prolonged treatment on compassionate use.

Phase I trials in other T-cell leukemia and other tumors

Three Phase I studies, with a similar dose-ranging schedules of IV forodesine, were performed in patients with more aggressive T-cell malignancies. In a pilot phase I study of T-cell-PLL (n=3) and T-cell ALL (n=2), four patients had clinical benefit with response based on a decrease in leukemia cells in blood or bone marrow. A second study of 15 patients with refractory T- or B-cell leukemias, AML, and lymphomas, one CR and one hematologic response was reported (Furman 2007). In a study of five patients with assorted tumor types, one patient had stable disease. Two

patients with relapsed T-cell acute lymphoblastic leukemia after allogeneic transplants achieved complete remission in the setting of graft-versus leukemia (Gore 2008).

Formulations of Intravenous versus oral forodesine

Due to the lack of dose-limiting toxicities, an optimal biological dose for IV forodesine was determined to be 40 mg/m^2. On a 12 hour continuous dosing schedule, IV forodesine at this dose completely inhibited PNP resulting in elevated plasma d-Guo levels. Increased dose levels did not cause further elevation in plasma d-Guo. Similar inhibition of PNP and elevations of d-Guo were observed at an oral dose of 160 mg/m^2 in a dose comparison study in normal volunteers. In healthy volunteers, adverse events included headache (94%), nausea (33%) and dizziness (28%) and the incidence of AEs was similar between IV and oral treatment groups. The bioavailability of forodesine was estimated to be 9-28% with a 13 to 30 hour half-life (Duvic 2006).

Phase II multi-center opened label dose ranging trial of oral forodesine capsules

The excellent safety and tolerability of intravenous forodesine in patients with refractory malignancies, justified further clinical development in patients with refractory CTCL (Duvic 2007). Since the IV formulation requires prolonged daily infusions, and indwelling lines greatly increase the risk of catheter sepsis in SS colonized with Staphylococcus aureus, an oral formulation was developed. Oral forodesine was evaluated in a multicenter, phase I/II, open-label, dose-escalation study for safety, pharmacokinetic profile, and efficacy in additional patients with refractory CTCL (Duvic 2006). The primary endpoint was objective response rate (OR) (OR = complete response [CR] + partial response [PR]), as measured by the severity-weighted assessment tool (SWAT) and Physician's Global Assessment of cutaneous response. Assessments included plasma d-Guo and red cell PNP levels, SWAT scores, pruritus VAS, Erythroderma VAS, patient and physician's global VAS, and flow cytometry assessed after 28 days of daily therapy.

Sequential cohorts of patients were given doses of 40, 80, 160 or 320 mg/m^2 once daily for 28 days. Again, an MTD could not be defined. Based on pharmacokinetic and pharmacodynamic results, an 80 mg/m^2 once-daily dose was identified as the optimal biologic dose and the cohort was expanded to 36 patients (Duvic 2004; Duvic 2006; Duvic 2006; Duvic 2007).

There were 56 CTC: patients enrolled with a median age of 62 years (28-82 yrs) and three prior therapies (range 0-8). The patients were distributed across stages ranging from IB n=11), IIA (n=1), III (n=17), and IVA. (n=8) and IVB (n=11). The overall response rate for all patients was 16 of 56 (42%). Patients treated with 80 mg/m^2 had an overall response rate of 39% (13/36) including two patients with CR (2%), 33% with partial responses, and 42% with stable disease. For all patients, secondary endpoints included time to response (38 days), median duration of response (127 days), time to progression (134 days) and time on treatment (102 days). The overall response using the erythroderma VAS score was 67% in erythrodermic MF and Sézary Syndrome. Four of the 8 patients with >1000 SS cells by flow had a >50% decrease in the $CD4^+CD26^-$ cell population. Decreases in the mean absolute numbers of $CD4^+$ and $CD8^+$ cells from baseline levels were measured by flow. T cell absolute numbers increased when the drug was discontinued.

Side effect profile of oral forodesine

The side effects seen among the 56 patients participating in the Phase II trial are shown in Figure 2 and are grouped by Grade. The most frequent complaints were fatigue, nausea, peripheral edema, headache and insomnia. Adverse events > grade 3 included one patient with unrelated renal failure, diarrhea, and cellulitis. There were no incidents of opportunistic infections or activation of cytomegalovirus, even though the drug reduced T-cell counts. Patients were prophylaxed with antibiotics or anti-viral agents while on the study.

Phase II multicenter trial of 200 mg of oral forodesine as third line therapy for CTCL patients

Based on the preliminary results of this trial, a second Phase II multi-center trial of forodesine monotherapy has been initiated to assess the safety and efficacy of oral forodesine at a dose of 200 mg daily. Patients with Stage IB – IVA CTCL at study entry will be eligible if they have had at least three prior systemic therapies, one of which was bexarotene if there are no contraindications. Cutaneous responses will be assessed by mSWAT, and responses will also require improvement in the lymph node tumor burdens, as evaluated by CT scans. There is currently no information from this trial.

Conclusion and future directions

Forodesine is a novel small molecule that selectively targets T-cells and potentially other cells with a nucleoside kinase. Unlike other therapies, its mechanism of action in CTCL is known. By inhibiting PNP it causes increased build up of d-Guo, changes nucleotide levels, increases d-GTP, and causes T-cell apoptosis. Phase I and II multicenter clinical trials support the safety and efficacy for treatment of patients with cutaneous T-cell lymphoma. The lack of severe adverse events including opportunistic infections is unexpected given its dramatic effects on absolute T-cell numbers making it an attractive and convenient way to deplete T-cells compared with other available agents. This agent should be of interest for treatment of other T-cell mediated skin and systemic diseases.

Conflict of Interest Disclosure; Dr Duvic is an investigator who has participated in the design and conduct of three forodesine clinical trials. She also has served on a medical scientific advisory board of Biocryst.

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