Pralatrexate Pharmacology and Clinical Development

Running Title: PRALATREXATE

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Conflict of Interest:

Dr. Owen O’Connor; Allos Therapeutics- Consultancy

Allos Therapeutics; previously marketed pralatrexate

Dr. Owen O’Connor; Millenium Pharmaceuticals-Membership on Board Director’s Advisory Committee.

All other authors have no conflicts of interest
Keywords
antifolate, pralatrexate, T-cell lymphoma; Lymphoma

Abstract
Folates are well known to be essential for many cellular processes, including cellular proliferation. As a consequence, antifolates, the fraudulent mimics of folic acid, have been shown to be potent therapeutic agents in many cancers. Over the past several decades, efforts to improve on this class of drugs have met with little success. Recently, one analog specifically designed to have high affinity for the reduced folate carrier, which efficiently internalizes natural folates and antifolates, has been shown to be very active in T-cell lymphoma. Pralatrexate, approved by the U.S. FDA in 2009, is highly active across many lymphoid malignancies, including chemotherapy resistant T-cell lymphoma. Emerging combination studies have now shown that pralatrexate is highly synergistic with gemcitabine, histone deacetylase inhibitors like romidepsin and bortezomib. These insights are leading to a number of novel Phase 1 and 2 combination studies which could challenge existing regimens like CHOP, and improve the outcome of patients with T-cell lymphoma.
Introduction

Mammalian cells lack the ability to synthesize folates. Consequently, these hydrophilic anionic molecules must be actively transported across the cellular membrane via sophisticated carrier-mediated transport systems, which include the reduced folate carrier (RFC), the folate receptors (FRs) and the recently discovered proton-coupled folate transporter (PCFT), or the soluble carrier 46A1 (SLC46A1) (1). Folate derivatives are essential one-carbon donors required for the synthesis of nucleic acid precursors and several amino acids, and are therefore critical to the de novo synthesis of DNA and proliferation of mammalian cells. After folates were discovered to be essential for many cellular processes, the development of fraudulent mimics of folic acid began to emerge, which initially included drugs like, aminopterin and methotrexate which were synthesized in the early 1940s (2). In 1948, aminopterin was the first drug shown to induce temporary remissions in childhood leukemia (2) (3). Soon thereafter, methotrexate became the more commonly employed antifolate in the treatment of many cancers, and is to this day still considered an important component of many chemotherapy regimens for solid tumors and hematologic malignancies, including: acute lymphoblastic leukemia (ALL), lymphoma, breast cancer, osteosarcoma, primary central nervous system, and head and neck cancer. A detailed understanding of the molecular pharmacology of antifolates has led to structural analogs with markedly improved activity, which includes the recently FDA approved agent, pralatrexate.
Pharmacology

Pralatrexate (10-propargyl-10-deazaaminopterin, PDX) is a novel antifolate belonging to a class of molecules known as 10-deazaaminopterins (4). Similar to other antifolates, pralatrexate inhibits the recycling of 5,10 methylene tetrahydrofolate which is required for the synthesis of thymidylate, by inhibiting the conversion of dihydrofolate (DHF) to tetrahydrofolate (THF) through the inhibition of dihydrofolate reductase (DHFR). DHFR converts dihydrofolate to tetrahydrofolate, a reduced form of folate, which is a co-factor required for the synthesis and catabolism of methionine, serine and glycine, as well as the synthesis of purines, mediating the methylation of nucleic acids and synthesis of thymidine monophosphate (TMP). The metabolic inhibition of DHFR by pralatrexate results in depletion of TMP and other precursors essential for DNA and RNA synthesis, resulting in cell cycle arrest and apoptosis (Figure 1). Pralatrexate was rationally designed to have high affinity for the reduced folate carrier (RFC) and folylpolyglutamate synthase (FPGS), leading to enhanced and selective intracellular internalization and retention in tumor cells (5). RFC is an oncofetal protein known to be highly expressed in embryonic and malignant tissues (6) (7). A number of oncogenes are known to upregulate the transporter, including c-myc and ras, making RFC an ideal target for cancer drug development. Interestingly, in one study, a punch biopsy of the skin from a patient with HTLV-1 ATLL demonstrated that pralatrexate only induced apoptosis in those T-cells marking positive for TAX (i.e. those positive for the HTLV-1 virus), and not in those surrounding normal cells that were not ATLL. This simple observation supports the contention that pralatrexate has selectivity not just for T-cells, but malignant T-cells in a patient (8). Like other folate derivatives and antifolates,
Pralatrexate enters cells via the RFC, after which it is polyglutamated by FPGS in the cytosol. The polyglutamylated forms of pralatrexate are then retained in the cytoplasm. The polyglutamylated derivatives of pralatrexate more potently inhibit DHFR. In virtually every enzymatic kinetic metric studied, consistently demonstrating that, pralatrexate is superior to methotrexate and other antifolates by at least one log. For example, the Km of pralatrexate and methotrexate for RFC are 0.3 and 4.8 mol/L respectively, whereas the Vmax/Km values (rate of intracellular transport) are 12.6 (pralatrexate) and 0.9 (MTX) (9). These data establish that the rate of pralatrexate influx is nearly 14-fold greater than that of methotrexate. Following a similar pattern, the Km of pralatrexate and methotrexate for FPGS are 5.9 and 32.3 mol/L respectively, whereas the Vmax/Km for folylpolyglutamate synthase is 23.2 (pralatrexate) and 2.2 (MTX) (Table 1) (9). These biochemical data similarly support a greater potential for pralatrexate to be polyglutamylated compared to other traditional antifolates. The favorable results from the enzyme kinetic experiments established the rationale for further study across malignant disease.

Preclinical Data

**Pralatrexate used as single agent.** Initial in vitro studies in the NCI cancer cell panel demonstrated that pralatrexate was potently cytotoxic across a broad panel of cancer cell types, including solid tumors and hematologic malignancies. The activity of pralatrexate was subsequently compared to methotrexate against five lymphoma cell lines including: RL (transformed follicular lymphoma), HT, SKI-DLBCL-1 (diffuse large B cell), Raji (Burkitt's), and Hs445 (Hodgkin's disease). Pralatrexate demonstrated more than 10-fold greater cytotoxicity than methotrexate in all cell lines as predicted by the
RFC binding assay results ($IC_{50}$ pralatrexate = 3-5 nM, $IC_{50}$ methotrexate = 30-50 nM). The activities of pralatrexate and methotrexate were also compared in vivo against three established NHL xenograft mouse models (HT, RL and SKI cells injected subcutaneously). Pralatrexate consistently exhibited statistically superior inhibition of tumor growth compared to methotrexate (10). Recently, the activity of pralatrexate has been investigated in multiple myeloma. Pralatrexate induced concentration-dependent apoptotic cell death in a subset of human myeloma cell lines (HMCLs) via induction of the intrinsic pathway, exhibiting a 10-fold greater potency compared to methotrexate. The sensitivity to pralatrexate correlated with higher relative levels of RFC mRNA expression in the sensitive HMCLs compared to resistant HMCLs. In addition, pralatrexate was also effective in vivo in an HMCL xenograft mouse model (11). From the in vitro assays to preclinical mouse models, the activity of pralatrexate has been noted to be consistently superior to all antifolates against which it was compared.

**Pralatrexate used in combination.** The cytotoxicity of pralatrexate has been investigated in combination with classic chemotherapeutic agents in preclinical studies. It has been well established that methotrexate synergizes with cytarabine [1-beta-D-arabinofuranosylcytosine (cytarabine)] in a schedule-dependent manner. The activity of pralatrexate plus gemcitabine was compared to the standard combination of methotrexate plus cytarabine (12). In vitro and in vivo models demonstrated that the sequenced combination of pralatrexate followed by gemcitabine was superior to sequenced methotrexate followed by cytarabine. In addition, the sequenced pralatrexate-gemcitabine combination was significantly more potent at inducing apoptosis in diffuse large B-cell lymphoma (DLBCL). To further evaluate the activity of
pralatrexate in combination with other active drugs, our group investigated the effects of combining pralatrexate with the proteasome inhibitor, bortezomib (13) (14). In vitro, pralatrexate and bortezomib independently exhibited concentration and time dependent cytotoxicity against a broad panel of T-cell lymphoma cell lines. However, the combination of pralatrexate and bortezomib synergistically induced apoptosis and caspase activation across the panel of T-cell lymphoma lines studied. Studies on healthy donor peripheral blood mononuclear cells demonstrated that the combination of pralatrexate and bortezomib was not more toxic than the single agents, suggesting a highly favorable therapeutic index for the combination. Western blot assays for proteins involved in growth and survival pathways demonstrated that p27, NOXA, histone 3 (H3) and RFC were all significantly modulated by the combination. In a transformed cutaneous T-cell lymphoma (CTCL) mouse model, the cohort that received pralatrexate along with bortezomib exhibited a significantly greater reduction in tumor volume compared to cohorts that received either drug alone and the control. These data suggest that pralatrexate in combination with bortezomib represents a novel and potentially important platform for the treatment of T-cell malignancies. As a result of these preclinical studies, a phase I/II clinical trial is currently planned.

**Clinical Development**

As described above, pre-clinical data indicate that pralatrexate is significantly more potent than methotrexate in a wide array of tumor cell types, and especially across all lymphoid cell lines studied. These data led investigators to study the activity of pralatrexate in patients with lymphoma (Table 2). The initial phase 1 trial was opened using pralatrexate at a dose of 135 mg/m², which was the MTD previously defined in
lung cancer patients (15). Pralatrexate was given at this dose every other week (QOW) in patients with relapsed/ refractory Hodgkin’s and non-Hodgkin’s lymphoma (NHL). All 16 patients treated experienced stomatitis on this dose and schedule, with a majority of patients exhibiting grade 3 or higher (54%) toxicity. Analysis of various nutritional covariates and pharmacokinetic parameters revealed that the essentially of the stomatitis observed was associated with elevated levels of homocysteine (Hcy) and methylmalonic acid (MMA), or an elevated area under the curve of drug exposure. Correction of the elevated Hcy and MMA with the supplementation of folic acid and vitamin B12 prevented or substantially reduced the stomatitis/mucositis in most patients in the majority of patients (16). Due to the incidence of stomatitis along with laboratory data suggesting that lower more frequent dosing was associated with a more favorable treatment outcome, the trial was amended to a weekly phase I dose-escalation regimen (QW) accompanied with vitamin B12 and folic acid supplementation. A total of 17 patients with both NHL and Hodgkin’s lymphoma were enrolled in this weekly study, and the MTD was determined to be 30 mg/m² given weekly for 6 out of 7 weeks. This dosing schedule was examined further in another 24 patients in the phase II portion of the study, with a substantially improved tolerance and interesting clues into its activity. Overall, 48 patients were treated on this trial with a wide range of lymphoma diagnoses. The overall response rate (ORR) was 31% with a complete remission (CR) rate of 17% and a 95% confidence interval (CI) 18-46%. The majority of the responses were seen in patients with a T-cell lymphoma, including: PTCL NOS, anaplastic large cell lymphoma (ALCL), and angioimmunoblastic T-cell lymphoma (AITL). Of particular interest was the ORR seen in patients with T-cell lymphoma, which was 54% with a
95% CI of 33-74%, which when compared to the ORR of 5% observed among patients with B-cell lymphoma, suggested a possible selectivity of the T-cell malignancies. (17) Interestingly, 4 of the first 5 patients had T-cell lymphoma and achieved a CR within the first cycle of treatment. This trial established the specific activity of pralatrexate in T-cell lymphomas at the dose level of 30 mg/m² given as a single agent on a 6 out of 7-week schedule. (18) The success of this treatment schedule among the subset of patients with T-cell lymphoma led to an expanded, confirmatory phase II study.

The registration directed multicenter study PROPEL (Pralatrexate in Relapsed or Refractory Peripheral T-cell Lymphoma) enrolled 115 patients with T-cell lymphoma. In general, the patient population was heavily pretreated with a median of 3 prior treatment regimens (range of 1-12) including 18 patients with a prior autologous transplant (19). Interestingly, 20% of patients had received more than 5 lines of prior therapy. The majority of the patients exhibited aggressive, refractory disease (53%), while 25% of the patients never experienced a response to any therapy, consistent with primary refractory PTCL. Additionally, 10% of these patients were diagnosed with mycosis fungoides (MF), a rare and challenging sub-type of non-Hodgkin’s lymphoma typically from studies in T-cell lymphoma (20). The treatment schedule consisted of administering pralatrexate at 30 mg/m²/wk for 6 out of 7 weeks. All patients received folic acid and vitamin B12 supplementation as well. Based on an independent response review, an ORR of 29% was noted, with 9 patients (11%) achieving a CR or a CRu (unconfirmed). A 95% CI of 29-39 was also achieved during this study. Remarkably, the heavily pretreated group consisting of patients receiving two or more prior therapies including prior autologous stem cell transplantation experienced a favorable response.
rate of 30%. Four responding patients went on to definitive therapy with stem cell transplant. Also of interest was the investigator assessed response of 39%, which included a CR rate of 18%. At the time, this was the largest prospective study ever conducted in patients with relapsed or refractory PTCL. The PROPEL trial led to the FDA approval of pralatrexate for the treatment of relapsed and refractory T-cell lymphoma in October 2009.

Given the specific T-cell activity of pralatrexate, a phase 1 clinical dose-reduction trial was initiated for patients with relapsed or refractory CTCL(21). The primary objective of the study was to identify the optimal dose and schedule of pralatrexate for patients with this disease subtype. Due to the indolent nature of CTCL, a dose de-escalation study was designed with the intent of finding the least toxic effective dose for this population. In the dose-finding cohort, 31 CTCL patients received various dosages and schedules of pralatrexate. Similar to the previous trials, the most common treatment-related adverse event (all grades) was mucositis (58%) which was dose limiting (grade 2) in 8 patients (26%). A total of 11 responses were observed, including 2 CRs and 9 partial responses. Among the 18 patients who received pralatrexate at a dose intensity of at least 15 mg/m²/wk for 3 out of 4 weeks, the ORR was 61% (11/18 patients). The results of this trial demonstrated that pralatrexate has high activity with acceptable toxicity in patients with relapsed or refractory CTCL at the identified optimal dose and schedule of 15 mg/m² weekly for 3/4 weeks. The lack of significant hematologic toxicity or cumulative toxicity suggested that pralatrexate should be further evaluated as continuous or maintenance therapy for patients with CTCL. The positive response despite the dose reduction suggests that altering the schedule of pralatrexate
administration may allow for treatment in patient populations that would be otherwise restricted.

Advantages Over Other Agents

Pralatrexate, a compound rationally designed to be efficiently internalized in tumor cells, has been shown to be superior to methotrexate in preclinical models, and highly active in drug resistant PTCL. As discussed previously the increased affinity of pralatrexate for RFC and FPGS allows for rapid internalization and intracellular retention. It is believed the pharmacologic features of pralatrexate over other antimetabolites, coupled with its almost selective activity in PTCL, makes it worthy of future study with other T-cell lymphoma active drugs like romidepsin and bortezomib. Combination studies in both the preclinical and clinical setting have begun to establish that pralatrexate synergizes with a number of such agents including gemcitabine (12), bortezomib (14) and HDAC inhibitors in general (22). These observations are now being translated into phase I/II clinical trials, with the hope that the successful development of drugs with selective activity in PTCL will lead to new treatment platforms for this challenging disease.
References


Figure 1: PDX inhibits folate-mediated one-carbon metabolism.

Pralatrexate (PDX) is actively transported across the cellular membrane through the reduced folate carrier (RFC) a member of the solute carrier transmembrane protein family. Retention in the cytoplasm depends upon polyglutamylation (PDX-E) of the antifolate compound, which is catalyzed by folylpolyglutamate synthase (FPGS). Conversely gamma-glutamyl hydrolase (GGH) removes glutamate groups from the antifolate causing the efflux of PDX into the extracellular space via multidrug resistance related protein (MRP)-like ATPase. Intracellular PDX competitively inhibits dihydrofolate reductase (DHFR). The reduction of dihydrofolate (DHF) molecules via DHFR into tetrahydrofolate (THF) is an essential prerequisite to folate-mediated one-carbon metabolism in the cell. THF and its family of cofactors (10-formyl-THF; 5,10 methylene-THF; 5-methyl-THF) contribute to the biosynthesis of nucleic acid precursors (purines, pyrimidines), amino acids (methionine, serine and glycine) and maintenance of methylated DNA and proteins (SAM). PDX disrupts several necessary metabolic cellular processes by targeting the upstream folate interconverting enzyme - DHFR.
Table 1: Vmax and Km of antifolates for RFC, FPGS, DHFR

<table>
<thead>
<tr>
<th>Antifolate</th>
<th>DHFR Inhibition Ki (pM)</th>
<th>Influx Km (mM)</th>
<th>Influx Vmax</th>
<th>Vmax/Km</th>
<th>FPGS Km (mM)</th>
<th>FPGS(Vmax)</th>
<th>Vmax/Km</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminopterin</td>
<td>4.9 ± 1</td>
<td>1.2 ± 0.2</td>
<td>3.6 ± 1.0</td>
<td>3</td>
<td>5.8 ± 1</td>
<td>117</td>
<td>20.2</td>
<td>Sirotnak et al., 1984</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>5.4 ± 2</td>
<td>4.8 ± 1.0</td>
<td>4.1 ± 1.2</td>
<td>0.9</td>
<td>32.2 ± 5</td>
<td>70</td>
<td>2.2</td>
<td>Sirotnak et al., 1984</td>
</tr>
<tr>
<td>Edatrexate</td>
<td>5.8 ± 1</td>
<td>1.1 ± 0.1</td>
<td>3.9 ± 0.9</td>
<td>3.5</td>
<td>6.3 ± 1</td>
<td>65</td>
<td>10.3</td>
<td>Sirotnak et al., 1984</td>
</tr>
<tr>
<td>Pralatrexate</td>
<td>13.4 ± 1</td>
<td>0.3 ± 0.1</td>
<td>3.8 ± 1.3</td>
<td>12.6</td>
<td>5.9 ± 1</td>
<td>137</td>
<td>23.2</td>
<td>Sirotnak et al., 1984</td>
</tr>
</tbody>
</table>
Table 2: Clinical trial results using PDX as a single agent in hematological malignancies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description</th>
<th>Patients (n)</th>
<th>ORR %</th>
<th>CR %</th>
<th>MDR (mo)</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>O'Connor et al., 2009</td>
<td>Ph II-I-II: non-Hodgkin's lymphomas</td>
<td>48</td>
<td>31</td>
<td>17</td>
<td>6</td>
<td>18-46</td>
</tr>
<tr>
<td></td>
<td>B-cell lymphoma subset</td>
<td>20</td>
<td>5</td>
<td>0</td>
<td>1.5</td>
<td>0.1-25</td>
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<tr>
<td></td>
<td>T-cell lymphoma subset</td>
<td>26</td>
<td>54</td>
<td>31</td>
<td>6.5</td>
<td>33-74</td>
</tr>
<tr>
<td>PROPEL study</td>
<td>Ph II: relapsed or refractory PTCL</td>
<td>109</td>
<td>29</td>
<td>11</td>
<td>10.1</td>
<td>21-39</td>
</tr>
<tr>
<td>Horwitz et al., 2012</td>
<td>Ph I: relapsed or refractory CTCL</td>
<td>54</td>
<td>41</td>
<td>5.5</td>
<td>N/A</td>
<td>27.6-55</td>
</tr>
</tbody>
</table>

a. ORR, overall response rate; CR, complete remission; MDR, median duration of response; mo, months; CI, confidence intervals; PTCL, peripheral T-cell lymphoma; CTCL, cutaneous T-cell lymphoma

b. This ORR is based upon rigorous independent response review. The investigator assess response rates was 39%