The Novel Expanded Porphyrin, Motexafin Gadolinium, Combined with $^{90}\text{Y}$Ibritumomab Tiuxetan for Relapsed/Refractory Non-Hodgkin’s Lymphoma: Preclinical Findings and Results of a Phase I Trial

Andrew M. Evens,1 William G. Spies,2 Irene B. Helenowski,3 David Patton,1 Stewart Spies,2 Borko D. Jovanovic,3 Sarah Miyata,1 Elizabeth Hamilton,1 Daina Variakojis,4 Jun Chen,5 Louie Naumovski,5 Steven T. Rosen,1 Jane N. Winter,1 Richard A. Miller,5 and Leo I. Gordon1

Abstract

Purpose: Therapeutic strategies to enhance the efficacy of radioimmunotherapy have not been explored. Motexafin gadolinium is a novel anticancer agent that targets redox-dependent pathways and enhances sensitivity of tumor cells to ionizing radiation. Experimental Design: We did preclinical studies examining motexafin gadolinium combined with rituximab and/or radiation in lymphoma cells. We subsequently completed a phase I clinical trial combining escalating doses of motexafin gadolinium concurrently with standard $^{90}\text{Y}$Ibritumomab tiuxetan for patients with relapsed/refractory non-Hodgkin’s lymphoma.

Results: In HF1 lymphoma cells, motexafin gadolinium and rituximab resulted in synergistic cytotoxicity (combination index, 0.757) through a mitochondrial-mediated caspase-dependent pathway, whereas cell death in Ramos and SU-DHL4 cells was additive. Motexafin gadolinium/rituximab combined with radiation (1-3 Gy) resulted in additive apoptosis. Twenty-eight of 30 patients were evaluable on the phase I clinical trial. Median age was 65 years (47-87 years), and histologies were marginal-zone ($n=1$), mantle-cell ($n=3$), diffuse large cell ($n=6$), and follicular lymphoma ($n=18$). Of all patients, 86% were rituximab refractory. Therapy was well tolerated, and no dose-limiting toxicity was seen. Overall response rate was 57% (complete remission (CR), 43%), with median time-to-treatment failure of 10 months (1-48+ months) and median duration-of-response of 17 months. Of note, all responses were documented at 4 weeks. Furthermore, in rituximab-refractory follicular lymphoma ($n=14$), overall response rate was 86% (CR, 64%), with a median time-to-treatment failure of 14 months (2-48+ months).

Conclusions: This represents the first report of a novel agent to be combined safely concurrently with radioimmunotherapy. Furthermore, tumor responses with $^{90}\text{Y}$Ibritumomab tiuxetan/motexafin gadolinium were prompt with a high rate of CRs, especially in rituximab-refractory follicular lymphoma. (Clin Cancer Res 2009; 15(20):6462–71)

Authors' Affiliations: 1Lymphoma Program, Division of Hematology/Oncology, The Robert H. Lurie Comprehensive Cancer Center, Departments of 2Radiology/Nuclear Medicine, 3Preventive Medicine, and 4Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois; and 5Pharmacyclics, Inc., Sunnyvale, California

Received 4/9/09; revised 7/20/09; accepted 7/22/09; published OnlineFirst 10/13/09.

Grant support: National Cancer Institute K23 CA109613-A1 for translational research/clinical trial (A.M. Evens) and M01 RR00048 National Center for Research Resources, NIH (clinical trial).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

E-mail: a-evens@northwestern.edu.

© 2009 American Association for Cancer Research.

Requests for reprints: Andrew M. Evens, Division of Hematology/Oncology, The Robert H. Lurie Comprehensive Cancer Center, Suite 850, 676 North St. Clair Street, Chicago, IL, 60611. Phone: 312-695-4537; Fax: 1-312-695-6189; E-mail: a-evens@northwestern.edu.

Published OnlineFirst October 14, 2009; DOI: 10.1158/1078-0432.CCR-09-0905

Clin Cancer Res 2009;15(20) October 15, 2009 6462 www.aacrjournals.org

Downloaded from clincancerres.aacrjournals.org on July 17, 2017. © 2009 American Association for Cancer Research.
Clinical trials using single-agent [90Y]ibritumomab tiuxetan and [131I]tositumomab in relapsed and refractory non-Hodgkin's lymphoma are associated with overall response rates of 60% to 80% (complete remission (CR) rates of 20% to 50%) and median time-to-treatment failure or progression-free survival of 6 to 10 months (1-7). Two studies using [90Y]ibritumomab tiuxetan and [131I]tositumomab in rituximab-refractory follicular lymphoma showed overall response rates of 62% and 74%, with associated CR rates of 15% and 25%, respectively, and with associated median time-to-treatment failure of 7 and 9 months, respectively (5, 7). One potential approach to increase the efficacy of radioimmunotherapy is through the addition of concurrent non-cross-resistant therapy. No previous studies, however, have been reported combining concurrent therapy with radioimmunotherapy.

Motexafin gadolinium, an expanded porphyrin containing the lanthanide cation gadolinium, is a novel anticancer agent that targets the redox-dependent machinery of tumor cells (8-12). Motexafin gadolinium is redox active as it catalyzes the intracellular oxidation of critical protein thiols and intracellular reducing metabolites that are protective to the tumor cell; depletion of these reducing metabolites leads to increased levels of reactive oxygen species (Fig. 1; refs. 8-11, 13, 14). Tipping the cellular redox balance within tumor cells through pharmacologic manipulation in favor of increasing intracellular reactive oxygen species and/or depleting reducing metabolites leads to oxidative stress and resultant induction of apoptosis. Clinically, motexafin gadolinium had been studied initially in combination with whole-brain radiation therapy to sensitize or increase the activity of ionizing radiation for patients with brain metastases from solid tumors (14-20).

Our previous preclinical studies examined the effects of motexafin gadolinium in multiple myeloma cells and showed dose-dependent apoptosis through redox-dependent pathways (8). Because motexafin gadolinium was known to enhance the sensitivity of tumor cells to ionizing radiation (14, 18, 21), we reasoned that motexafin gadolinium combined concurrently with the radioimmunoconjugate [90Y]ibritumomab tiuxetan would be additive or synergistic and provide higher response rates in relapsed/refractory lymphoma. We report here preclinical studies in lymphoma cell lines combining motexafin gadolinium with rituximab and/or radiation therapy. We subsequently completed a phase I trial using increasing doses of motexafin gadolinium, which was combined concurrently with fixed-dose [90Y]ibritumomab tiuxetan, for patients with relapsed or refractory non-Hodgkin's lymphoma.

Materials and Methods

Cell lines and experimental conditions. HF1, SUDHL4, and Ramos lymphoma cell lines were cultured in RPMI 1640 (Invitrogen) with 10% fetal bovine serum, l-glutamine, and penicillin/streptomycin. Cells were maintained at 37°C with 5% CO2, HF1, SUDHL4, and Ramos cells were treated with control vehicle solution (5% mannitol), increasing concentrations of motexafin gadolinium (30-100 μmol/L), and/or increasing concentrations of rituximab (20-200 ng/mL). Motexafin gadolinium was prepared as a 2 mmol/L (2.3 mg/mL) formulation in 5% aqueous mannitol. Cultures were irradiated using a 137Cs irradiator (model 40 γ cell; J. L. Shepherd and Associates) at a dose rate of 0.725 Gy/min and allowed to incubate for 72 h. Cells were harvested and washed twice with a solution of 0.9% bovine serum albumin in PBS.

Cell viability and apoptosis studies. Cell numbers were determined using the Model Z2 Coulter counter (Beckman-Coulter), and live cells were counted. Apoptosis was examined using FACS Calibur instrument (Becton-Dickinson) after staining the cells with Annexin V-fluorescein isothiocyanate and propidium iodide (Biosource-Invitrogen). In brief, 1 × 106 cells were washed with PBS and then labeled with Annexin V-FITC and propidium iodide in the binding buffer, according to the AnnexinV-FITC apoptosis detection kit instruction provided by the manufacturer. Fluorescent signals of FITC and propidium iodide were detected at FL1 and FL3 channels, respectively. Percent apoptosis was determined by positivity for Annexin V. Data are presented as mean of 3 independent experiments in triplicate ± SD. Measurement of mitochondrial membrane potential was measured by flow cytometry using JC-1 staining. Cells were washed with HBSS and incubated with 4 μg/mL JC-1 dye in HBSS for 15 min at 37°C in an incubator. Cells were washed with HBSS and immediately subjected to flow cytometric analysis. Caspase 3 activation was measured (EnzChek Caspase-3 Assay Kit 2, Molecular Probes), whereas Q-VD-OPh was used for pan-caspase inhibition (Calbiochem). For further examination of apoptosis, the cleavage of poly(ADP-ribose) polymerase (PARP) was detected with Western blots. For Western blotting, cells were lysed in triple-detergent lysis buffer. Equal amount of protein was run on SDS-PAGE (Bio-rad) and then transferred to PVDF membrane. Membrane was blotted with anti-PARP antibody (Cell Signaling) and image quantitated using an Odyssey IR Imaging System (LI-COR).

Clinical trial. This was a phase I clinical trial that enrolled 30 relapsed/refractory non-Hodgkin's lymphoma patients from October 2005 through October 2007. The protocol was approved by the Northwestern University Institutional Review Board. All patients had histologically confirmed relapsed or refractory B-cell non-Hodgkin’s lymphoma (all B-cell lymphoma histologies were allowed except Burkitt lymphoma). All nodal and bone marrow slides were reviewed by a single hematopathologist (D. Varjakos). Patients had <25% bone marrow involvement with lymphoma and an Eastern Cooperative Oncology Group performance status of ≤3, absolute neutrophil count of ≥1,500 cells/mm3, hemoglobin of ≥8.0 g/dL, platelet count of ≥100,000 cells/mm3, serum creatinine and total bilirubin of ≤2 mg/dL.
each, expected survival of 3 mo, and no antineoplastic therapy for 4 wk (6 wk if treated with nitrosourea and/or mitomycin). One patient with diffuse large B-cell lymphoma was removed from the study because of the development of acute renal failure due to a rapidly growing and obstructing perinephric mass that developed before (and precluded) the administration of [90Y]ibritumomab tiuxetan. Therefore, 29 patients received motexafin gadolinium/[90Y]ibritumomab tiuxetan therapy.

**Trial design and treatment.** Standard [90Y]ibritumomab tiuxetan was administered as described before (22). The [90Y]ibritumomab tiuxetan dose was 0.4 mCi/kg for patients with platelets of $\geq 150,000$ cells/mm$^3$, whereas 0.3 mCi/kg was given for 100,000 to 149,000 cells/mm$^3$. All patients, regardless of dose calculation, did not receive more than the maximum dose of [90Y]ibritumomab tiuxetan of 32 mCi. Doses for rituximab, [111In]zevalin, and [90Y]ibritumomab tiuxetan were based on adjusted ideal body weight. [90Y]ibritumomab tiuxetan was not to be given if the predicted delivered dose of radiation to any nontumor organ (liver, lung, kidneys, spleen, or heart) was $>20$ Gy or if the dose to the bone marrow was $>3$ Gy; no patient met this criterion. Patients received eight total doses of motexafin gadolinium (once daily), days 1 to 4 and 8 to 11, as shown in Supplementary Fig. S1A. Doses for motexafin gadolinium were based on actual body weight. Prophylactic antiemetics were given 30 min before motexafin gadolinium (e.g., compazine 5 mg). Day 1 and 8 rituximab infusions were given 1 h following the motexafin gadolinium infusion.

**Camera imaging with dosimetry** was done for all patients on days 1, 2, 4, and 7. Patients were treated in the Northwestern University General Clinical Research Center and had daily history and physical examinations from day +1 through day +11 of treatment, then weekly for the first month, and monthly for 6 mo. Complete blood count with differential was...
done weekly for the first 3 mo, then monthly for 1 y. Serum chemistries were done every 3 days from day +1 through day +12, then monthly for 6 mo. Response was assessed by an international workshop non-Hodgkin’s lymphoma response criteria (23). As a correlative analysis to study the timing of response, the initial restaging computerized tomodograms were done 4 wk following $^{[90]}\text{Y}$-ibritumomab tiuxetan treatment. Subsequent computerized tomodograms were completed every 3 mo following or sooner if clinically indicated.

**Phase 1 design.** An abbreviated dose escalation of motexafin gadolinium was planned with three dose cohorts: 2.5, 3.5, and 5.0 mg/kg as the phase 1 component of the trial. The standard dosing of motexafin gadolinium in previous clinical trials combined with whole-brain radiation therapy was 5 mg/kg/d given 2 to 5 h before each fraction (19, 20). Because of the known myelosuppressive toxicity of radioimmunoabation based on the amount of lymphoma marrow infiltration (24), the initial cohort accrued only patients with ≤5% bone marrow involvement (Supplementary Fig. S1B). Patients with 6% to 24% involvement began enrollment only after patients with ≤5% bone marrow involvement (Supplementary Fig. S1B). Patients with 6% to 24% marrow involvement, whereas accrual continued to the 6% to 24% marrow involvement for the expanded enrollment, a predicted overall response rate of 60% and a power of >84% to detect the difference for all patients and >74% power to detect the above difference for relapsed follicular lymphoma patients, using an exact one sample test for response rate (P).

Time-to-treatment failure was calculated from day 1 of treatment to treatment failure (relapse, secondary malignancy, or death from any cause). Overall survival was calculated from day 1 of treatment to the date of death from any cause or until the date of last known follow-up. Duration of response was estimated from the day of response assessment until relapse, progression, or death from any cause. Survival analyses were done using Kaplan-Meier curves (26). Prognostic factors were evaluated in univariate analyses using Cox proportional hazards regression (27) for indicators of response or survival.

**Results**

**Preclinical data.** We and others (28–30) previously reported single-agent cytotoxicity with motexafin gadolinium in B-cell lymphoma cell lines (Raji, SUDHL4, and HFI1). We examined here the cytotoxicity of motexafin gadolinium with and without rituximab in the lymphoma cell lines, HFI1, SUDHL4, and Ramos. In the follicular lymphoma cell line, HFI1, modest in vitro concentrations of motexafin gadolinium and rituximab resulted in additive apoptosis (30 μmol/L and 36 ng/mL, respectively, with a combination index of 1.013), whereas increasing concentrations resulted in synergistic cell death (40 μmol/L and 48 ng/mL, respectively, associated with a combination index of 0.855; 50 μmol/L and 60 ng/mL, respectively, with a combination index of 0.757; Fig. 1B).

Using similar concentrations, we found further evidence of apoptosis with higher levels of cleaved PARP (Fig. 2A) and >50% loss of mitochondrial membrane potential (Fig. 2B) with combined motexafin gadolinium and rituximab (versus either agent alone). Apoptosis studies were also completed in SUDHL4 and Ramos lymphoma cell lines wherein additive cell death was seen (data not shown). In HFI1 cells, caspase-3 activity was increased with motexafin gadolinium and, to a lesser extent, rituximab alone, whereas both agents combined resulted in additive increased caspase-3. Furthermore, the pan-caspase inhibitor Q-VD-OPH resulted in substantial blockade of motexafin gadolinium and/or rituximab-induced apoptosis, including abrogation of caspase-3 activation (Fig. 2C and D). We also studied cell death combining motexafin gadolinium and/or rituximab (with and without rituximab) in HFI1 cells (Fig. 2E). When motexafin gadolinium was combined with rituximab or radiation (1-3Gy), an additive decrease in cell proliferation and induction of apoptosis were seen. The highest level of cell death was seen when motexafin gadolinium, radiation, and rituximab were combined.

**Clinical trial patient characteristics.** As shown in Table 1, of 29 patients treated on trial had indolent lymphoma (18 follicular lymphoma). The remaining 10 patients had aggressive histologies. The median age for all patients was 64 years, whereas one third were >75 years. Most patients had advanced stage disease at study entry, whereas 55% had bulky disease ≥5 cm. Median number of previous regimens was 2 (range, 1–4). Of note, 86% of all patients entered on this clinical trial had rituximab-refractory disease, defined as previous treatment with rituximab with no response or time-to-treatment failure of <6 months. Seventy-eight percent (14 of 18) of follicular lymphoma patients had rituximab-refractory disease.

Among the group of rituximab-refractory follicular lymphoma, median age was 60 years (range, 47-85 years), median of previous regimens was 3 (range, 1–4). 79% had stage III/IV disease, 55% had bulky disease ≥5 cm, and 72% had intermediate or high Follicular Lymphoma International Prognostic Index (FLIPI) (≥3) at study entry. Twelve of 14 of these patients had received previous alkylator-based chemotherapy, the most common regimen being R-CVP (rituximab, cyclophosphamide, vincristine, and prednisone; 9 of 12). Among the 10 evaluable patients with relapsed/refractory aggressive lymphoma, median
Age was 77 years, and 70% were stage III/IV at study entry. Ninety percent of these patients had received frontline R-CHOP–like (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; anthracycline based) chemotherapy, whereas 90% of patients at time of study entry were rituximab refractory.

**Dosimetry.**  
\[^{111}\text{In}]\text{ibritumomab tiuxetan imaging and dosimetry were done in 28 patients. The estimated radiation-absorbed doses were within acceptable levels, which allowed all patients to proceed with \[^{90}\text{Y}]\text{ibritumomab tiuxetan treatment. The estimated radiation-absorbed doses are shown in Table 2, which do not seem different compared with previous single-agent \[^{90}\text{Y}]\text{ibritumomab tiuxetan studies (5, 24).**}

**Phase 1 results.**  
Eighteen patients were enrolled and completed phase I motexafin gadolinium dose escalation (nine patients, motexafin gadolinium 2.5 mg/kg; six patients, motexafin gadolinium 3.5 mg/kg; and three patients, motexafin gadolinium 5 mg/kg). Of all 29 patients who received \[^{90}\text{Y}]\text{ibritumomab tiuxetan and motexafin gadolinium therapy, 25 had...**
Motexafin Gadolinium–Radioimmunotherapy for Lymphoma

Motexafin gadolinium dose escalation for the group of patients with >5% bone marrow involvement was not completed. No dose-limiting toxicities were seen for the 14 phase I patients with ≤5% bone marrow involvement or the four phase I patients with >5% marrow involvement. The remaining patients (n = 11) received motexafin gadolinium at 5 mg/kg.

**Efficacy.** Twenty-eight patients were evaluable for response. The nonevaluable patient was a 75-year-old man with relapsed/refractory diffuse large B-cell lymphoma who died 3 months following motexafin gadolinium/\(^{90}\)Yibritumomab tiuxetan therapy from accidental causes (before 3-month restaging studies; stable disease at 1 month). The overall response rate for all evaluable patients was 57% (16 of 28); most responses were complete (CR, 43%). Overall response rate by intent-to-treat for all patients (n = 30) was 53% (16 of 30), with 40% CR (12 of 30). The efficacy data in the subgroup of patients with rituximab-refractory follicular lymphoma (n = 14) showed an overall response rate of 86%, with a CR rate of 64%.

Among the 10 evaluable patients with relapsed/refractory aggressive lymphomas, the overall response rate was 20%. The two responses, however, were both CRs in rituximab-refractory diffuse large B-cell lymphoma patients (overall response rate in diffuse large B-cell lymphoma, 29%). One of the related CRs lasted 11 months, whereas the other CR is ongoing (25+ months). Both of these patients were elderly (72 and 87 years of age) with rituximab refractory–transformed diffuse large B-cell lymphoma with no response to their previous chemotherapeutic regimen.

Of note, all responses in this trial were documented at 4 weeks following combined motexafin gadolinium/\(^{90}\)Yibritumomab tiuxetan therapy. Six patients with partial remissions at 4 weeks improved to CR at subsequent (3 month) restaging. However, no “new” responders were identified after 4 weeks. Besides histology (i.e., indolent lymphoma versus aggressive; P < 0.01), there were no predictive factors of response on univariate analysis (e.g., bulk disease, age, performance status, lactate dehydrogenase, or rituximab-refractory versus rituximab-sensitive status).

At a median follow-up of 35 months, the median time to treatment progression (TTP) for all patients on intent-to-treat analysis (n = 30) was 10 months (range, 1-51+ months), and the median overall survival was not reached with 3-year overall survival of 77% (Fig. 3A and B). The median duration of response for all patients was 17 months (range, 2-51+ months). With a median follow-up of 37 months, the median TTP for patients with rituximab-refractory follicular lymphoma was 14 months (range, 2-51 months), and the median overall survival was not reached with 3-year overall survival of 80% (Fig. 3C and D). The median duration of response for this group was 18 months (range, 4-51+ months).

### Table 1. Patient demographics and clinical characteristics (n = 29)

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (range)</td>
<td>65 (47-87)</td>
<td>100</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>52</td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td>48</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>18</td>
<td>62</td>
</tr>
<tr>
<td>Marginal zone</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>DLBCL</td>
<td>7*</td>
<td>25</td>
</tr>
<tr>
<td>Mantle cell</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Performance status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero</td>
<td>17</td>
<td>59</td>
</tr>
<tr>
<td>One</td>
<td>11</td>
<td>38</td>
</tr>
<tr>
<td>Two</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Stage (at study entry)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>III/IV</td>
<td>22</td>
<td>76</td>
</tr>
<tr>
<td>Bulky disease, cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>13</td>
<td>45</td>
</tr>
<tr>
<td>5-10</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td>&gt;10</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Bone marrow involvement, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>26</td>
<td>90</td>
</tr>
<tr>
<td>6-24</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>10</td>
<td>34</td>
</tr>
<tr>
<td>Previous treatments(^1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Two</td>
<td>11</td>
<td>38</td>
</tr>
<tr>
<td>Three</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td>Four</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Previous rituximab</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28</td>
<td>97</td>
</tr>
<tr>
<td>Rituximab refractory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25</td>
<td>86</td>
</tr>
<tr>
<td>Previous radiotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>21</td>
</tr>
</tbody>
</table>

Abbreviations: DLBCL, diffuse large B-cell lymphoma; LDH, lactate dehydrogenase.

*Four patients with transformed diffuse large B-cell lymphoma; three with de novo diffuse large B-cell lymphoma.

\(^1\)Postinduction (or maintenance) rituximab was not considered a separate/different treatment regimen.

### Table 2. Dosimetry

<table>
<thead>
<tr>
<th>Administered activity (MBq)</th>
<th>Radiation dose (Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney</td>
</tr>
<tr>
<td>Mean 974.9</td>
<td>4.9</td>
</tr>
<tr>
<td>SD 177.7</td>
<td>1.99</td>
</tr>
<tr>
<td>Minimum 703</td>
<td>1.37</td>
</tr>
<tr>
<td>Maximum 1,295</td>
<td>10.63</td>
</tr>
</tbody>
</table>

NOTE: Following \(^{111}\)Inibritumomab tiuxetan imaging and dosimetry (n = 29 patients).
Safety. Therapy was well tolerated. Most toxicity was hematologic, whereas the percentage of grade 3/4 hematologic adverse events and the median time-to-hematologic nadir levels seemed similar to single-agent [90Y]ibritumomab tiuxetan (Table 3). Grade 3/4 nonhematologic adverse events were uncommon (Table 3). Two grade 3 nonhematologic toxicities were seen (one infection and one hypokalemia). At a median follow-up of 35 months, no secondary MDS or leukemia has been seen. The most common nonhematologic adverse event was a temporary olive-green discoloration of the skin (80% of patients). The skin discoloration was due to the dark-green color of motexafin gadolinium. The discoloration developed gradually after repeated dosing of motexafin gadolinium and completely cleared within 4 to 5 days after the last dose. Some patients developed a transient pruritic rash on their fingertips ($n = 5$) that also disappeared 3 to 4 days after completion of motexafin gadolinium. Other adverse events seen, as shown in Table 3, included electrolyte abnormalities, liver enzyme abnormalities, and gastrointestinal symptoms, although most symptoms were mild and self-limiting.

Discussion

One strategy to increase the effectiveness of radioimmunotherapy is through combination with non-cross-resistant agents that may be additive or synergistic. Because of the activity of motexafin gadolinium combined with ionizing radiation in solid-tumor studies (16, 19, 20), the absence of hematologic
toxicity of motexafin gadolinium, our previous preclinical studies in hematologic malignancies (8, 28, 29), and the current preclinical studies in lymphoma, we reasoned that motexafin gadolinium might enhance the antitumor activity of [90Y]ibritumomab tiuxetan. To our knowledge, this report presents one of the first clinical trials combining a novel therapeutic agent concurrently with radioimmunotherapy for the treatment of lymphoma.

We and others (8, 28, 30–35) have shown that the production of oxidative stress through novel nonchemotherapeutic agents results in cell death in hematologic cancer cell lines. Motexafin gadolinium, also known as gadolinium texaphyrin, is a metalloporphyrin that had been developed as a radiation- and chemotherapy-enhancing agent (11, 14, 18). Motexafin gadolinium is known to have selective tumor biolocalization, similar to naturally occurring porphyrins (12, 14, 36). Animal studies using magnetic resonance scanning and radiolabeled drug documented rapid clearance of motexafin gadolinium from blood and normal tissues with delayed clearance from tumors (21, 37). Since this clinical study was initiated, motexafin gadolinium alone was shown to induce apoptosis in lymphoma cell lines (28–30). Chen et al. (29) showed that motexafin gadolinium triggered the mitochondrial apoptotic pathway and activated the caspase system in non-Hodgkin's lymphoma cells. In SUDHL4, Ramos, Raji, and HF1 cells, motexafin gadolinium was shown to induce oxidative stress as shown by oxidation of 2′,7′-dichlorofluorescin-diacetate to 2′,7′-dichlorofluorescein, whereas gene expression studies showed increased metal response element-binding transcription factor 1–regulated and hypoxia-inducible transcription factor 1–regulated genes (28). These genes were likely activated as an adaptive response to motexafin gadolinium–induced apoptosis. Ramos et al. (30)

Fig. 3. Intent-to-treat progression-free survival and overall survival Kaplan-Meier curves for all patients (n = 30) and for rituximab-refractory follicular lymphoma patients (n = 14). A and B, the progression-free survival and overall survival for all patients enrolled on trial. At a median follow-up of 35 mo, the median progression-free survival was 10 mo and the median overall survival was not reached (progression-free survival and time-to-treatment failure were identical). C, the median progression-free survival for rituximab-refractory follicular lymphoma of 14 mo. This compares with data by Witzig et al. (5) using single-agent [90Y]ibritumomab tiuxetan therapy in rituximab-refractory follicular lymphoma with median TTP of 6.6 mo (5). D, median overall survival for rituximab refractory follicular lymphoma patients has not been reached.
reported that motexafin gadolinium increased Akt phosphorylation, while specific inhibitors of Akt were synergistic combined with motexafin gadolinium. We found in the current preclinical studies that motexafin gadolinium combined with rituximab led to synergistic apoptosis and that cell death seemed to occur through a mitochondrial-related pathway that was caspase dependent. Furthermore, motexafin gadolinium combined with radiation resulted in an increase in apoptosis that was additive.

Dosing of motexafin gadolinium in this study was conservative. When this trial was opened in 2003, motexafin gadolinium had only been used clinically before as a radiation-sensitizing agent in combination with whole-brain radiation therapy for the treatment of patients with metastatic brain disease. The maximum tolerated dose as determined by previous solid-tumor phase Ib/II studies with motexafin gadolinium (given daily for 10 days) with concurrent whole-brain radiation therapy was 6.3 mg/kg (17). Furthermore, plasma pharmacokinetics has shown no relationship between maximum concentration (Cmax) or area under the curve and motexafin gadolinium dose. In the phase III metastatic brain clinical trials, patients received 10 consecutive daily doses of motexafin gadolinium at 5 mg/kg/d (16, 19). Motexafin gadolinium here was administered for 4 days following [111In]ibritumomab tiuxetan and for 4 days immediately following [90Y]ibritumomab tiuxetan therapy. The rationale for administering motexafin gadolinium following [111In]ibritumomab tiuxetan was in part to determine if motexafin gadolinium altered the dosimetry of the radioimmunoconjugate. The rationale for continuing motexafin gadolinium 4 days following [90Y]ibritumomab tiuxetan was in part related to the known biological half-life of [90Y]ibritumomab tiuxetan of 28 hours and the observation that the maximal binding of the [90Y]ibritumomab tiuxetan antibody to the CD20 occurs at 96 to 144 hours (24). Continued treatment with motexafin gadolinium, beyond the 8 doses given here, was not done in part because the antilymphoma activity of motexafin gadolinium was not appreciated at the time this trial was planned.

The efficacy data from the phase I/II trial here using combined motexafin gadolinium/[90Y]ibritumomab tiuxetan compare favorably to previous single-agent radioimmunotherapy reports. In particular, there was a strong signal of clinical activity in the current clinical trial in the subset of rituximab-refractory follicular lymphoma. Witzig et al. (5), using [90Y]ibritumomab tiuxetan, reported an overall response rate of 74%, with associated 15% CR rate and median TTP of 6.8 months. In a similar patient population here, motexafin gadolinium/[90Y]ibritumomab tiuxetan was associated with a marked increase in CR rate (64%) and approximate doubling of time-to-treatment failure (14 months). In diffuse large B-cell lymphoma, the overall response rate here was 29% (CR, 29%), both responses in transformed diffuse large B-cell lymphoma. Morschhauser et al. (38) showed in relapsed/refractory diffuse large B-cell lymphoma (nontransformed) that the overall response rate in patients who received previous rituximab-based chemotherapy was 19% (CR, 8%).

The time-to-response with radioimmunotherapy has never been examined. One notion has been that the time-to-response may be delayed, in part given the delayed myelosuppression associated with radioimmunotherapy. We found in the current clinical trial that all responses occurred very promptly, all by 4 weeks, following motexafin gadolinium/[90Y]ibritumomab tiuxetan. It is important to note that it is not known to what degree motexafin gadolinium contributed to the clinical activity seen here. A randomized clinical trial of combined motexafin gadolinium/[90Y]ibritumomab tiuxetan versus [90Y]ibritumomab tiuxetan alone would be needed. Given the apparent single-agent cytotoxic activity of motexafin gadolinium in lymphoma seen in preclinical data, it would be rational in future trials to apply “extended dosing” of motexafin gadolinium.

Delayed thrombocytopenia and neutropenia have been the most common hematologic side effects documented with [90Y]ibritumomab tiuxetan therapy, with median days from baseline to nadir of 43 and 50 days, respectively (5, 39, 40). The hematologic toxicity seen in this trial (Table 3) seemed similar to previous single-agent [90Y]ibritumomab tiuxetan (median nadir counts with 0.4 mCi/kg: 50,000/mm³ for platelets, 1,100/mm³ for neutrophil count, and 9.9 g/dL for hemoglobin (24, 40). The most common nonhematologic adverse event seen with motexafin gadolinium was a temporary greenish discoloration of the skin, as described before. This has been seen in all previous motexafin gadolinium clinical trials that used >3 to 4 consecutive daily doses (16, 17, 36, 37), although the symptoms (including pruritis) are mostly mild and self-limited. Other nonhematologic adverse events seen here included several electrolyte changes, liver enzyme abnormalities, and gastrointestinal symptoms, although most symptoms were mild (grade 1) and transient. It should be noted that patients were followed very closely in this clinical trial, including daily history and physical examinations (during motexafin gadolinium dosing), whereas serum chemistry and hepatic function testing was done every 2 to 3 days initially. Other adverse events in the current trial did not seem to have increased compared with previous radioimmunotherapy studies.

In conclusion, novel therapeutic agents exist, such as motexafin gadolinium, which may be safely combined with radioimmunotherapy. We found that motexafin gadolinium, when given together with [90Y]ibritumomab tiuxetan, did not seem to increase hematologic or other toxicity. Furthermore, all responses here occurred promptly (within 4 weeks), although a high rate of CRs was seen, especially in rituximab-refractory follicular lymphoma patients. Continued preclinical and clinical studies combining other novel therapeutic agents, such as boratezomib (41) and CpG 7909 (42), together with radioimmunotherapy are warranted.

Disclosure of Potential Conflicts of Interest

J. Chen, L. Naumovski, R.A. Miller, employees, Pharmacycis.

Acknowledgments

We thank the AACR/American Society of Clinical Oncology Methods in Clinical Cancer Research Meeting (this clinical trial was developed/written at the 2002 Summer Workshop); the NIH/National Cancer Institute (this translational/clinical trial was the primary project of the K23 CA109613-A1 grant; A.M. Evens); the staff of the Northwestern University General Clinical Research Center for the treatment and care of all patients; Biogen Idec, succeeded by Cell Therapeutics, Inc., for supplying the [90Y]ibritumomab tiuxetan (Zevalin) for the clinical trial; and Pharmacia Inc., for supplying the motexafin gadolinium.
Motexafin Gadolinium—Radioimmunotherapy for Lymphoma

References

Correction: A First-in-Man Phase I and Pharmacokinetic Study on CHR-2797 (Tosedostat), an Inhibitor of M1 Aminopeptidases, in Patients with Advanced Solid Tumors

In this article (Clin Cancer Res 2009;15:4978–85), which was published in the August 1, 2009 issue of Clinical Cancer Research (1), the Acknowledgment section was incomplete. The correct statement is, as follows: The Royal Marsden NHS Foundation Trust, the Institute of Cancer Research, and the Oxford Radcliffe NHS Trust thank the NHS Biomedical Research Centre programme and Experimental Cancer Medicine Centre.

Reference

The Novel Expanded Porphyrin, Motexafin Gadolinium, Combined with [90Y]Ibritumomab Tiuxetan for Relapsed/Refractory Non-Hodgkin's Lymphoma: Preclinical Findings and Results of a Phase I Trial

Andrew M. Evens, William G. Spies, Irene B. Helenowski, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-09-0905

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2009/10/13/1078-0432.CCR-09-0905.DC1

Cited articles
This article cites 40 articles, 27 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/15/20/6462.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/15/20/6462.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.