Lomeguatrib, a Potent Inhibitor of O\textsuperscript{6}-Alkylguanine-DNA-Alkyltransferase: Phase I Safety, Pharmacodynamic, and Pharmacokinetic Trial and Evaluation in Combination with Temozolomide in Patients with Advanced Solid Tumors

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Abstract

Purpose: A major mechanism of resistance to temozolomide involves the DNA repair protein O\textsuperscript{6}-alkylguanine-DNA-alkyltransferase (ATase). The main aims of this phase I trial were to determine an ATase-depleting dose (ADD) of lomeguatrib, a potent pseudosubstrate inhibitor, and to define a suitable dose of temozolomide to be used in combination with lomeguatrib in patients with advanced cancer.

Experimental Design: Lomeguatrib was administered at dose levels of 10 to 40 mg/m\textsuperscript{2} days 1 to 5, as a single agent, and also in combination with temozolomide. Once the ADD of lomeguatrib was identified, the dose of temozolomide in combination was increased, in successive patient cohorts, from 50 to 175 mg/m\textsuperscript{2} on days 1 to 5 of a 28-day cycle to define the maximal tolerated dose and dose-limiting toxicity of the combination.

Results: Thirty-eight patients with advanced solid tumors were enrolled. More than 95% ATase depletion within 4 hours of the first dose was achieved in peripheral blood mononuclear cells at lomeguatrib doses of ≥10 mg/m\textsuperscript{2} i.v. or ≥20 mg/m\textsuperscript{2} orally, and tumor biopsies showed ≥92% ATase depletion. At the ADD of lomeguatrib i.v., the maximal tolerated dose of temozolomide in combination was 150 mg/m\textsuperscript{2} days 1 to 5. The dose limiting toxicity of the combination of lomeguatrib and temozolomide was myelosuppression. The toxicity of lomeguatrib alone was minimal. In 23 patients with measurable disease, one complete response was seen and 12 patients had stable disease for at least 3 months.

Conclusion: This first administration of lomeguatrib to man successfully established an oral ADD of lomeguatrib and identified a combination regimen with temozolomide suitable for future clinical evaluation.

Chloroethylnitrosoureas such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and methylating agents such as dacarbazine and temozolomide are cytotoxic through their ability to alkylate DNA, principally at the O\textsuperscript{6} position in guanine (1–3). Resistance to these agents can be conferred by the repair protein O\textsuperscript{6}-alkylguanine-DNA alkyltransferase (ATase), which removes the alkyl group in a stoichiometric auto-inactivating reaction (1, 4, 5). Tumor cells frequently express high levels of ATase (6, 7). ATase-mediated resistance to O\textsuperscript{6}-alkylating agents can be overcome by depleting cellular levels before drug administration (8, 9). Early approaches used methylating agents to deplete ATase before treatment with chloroethylating drugs but met with little success. The common toxicities of the drugs used severely limited the doses of chloroethylnitrosourea that could be given (10–13).

ATase can also be inactivated by low molecular weight pseudosubstrates (14–17). One such pseudosubstrate, O\textsuperscript{6}-benzylguanine, has undergone phase I and II trials in combination with BCNU and further trials are in progress (18–22). In combination with ATase inhibitory doses of O\textsuperscript{6}-benzylguanine, 20% to 25% of the single agent BCNU dose is deliverable (18, 20, 22) and this may limit the therapeutic effect of the O\textsuperscript{6}-benzylguanine/BCNU combination.

Lomeguatrib [6-(4-bromo-2-thienyl) methoxy[apurin-2-amine] is more potent than O\textsuperscript{6}-benzylguanine and has shown promising activity in sensitizing a variety of human tumor...
xenografts to the growth inhibitory effects of temozolomide and other O⁶-alkylating agents (16, 23). Tumor growth delay was observed in A375 (melanoma), HT144 (melanoma), MCF-7 (breast), and DU-145 (prostate) xenograft models (16, 23).

The efficacy of lomeguatrib and temozolomide given daily for 5 days was greater than that achievable at the maximal tolerated dose (MTD) of temozolomide alone, even with adjustment of scheduling to maximize antitumor efficacy (16). The additional antitumor activity was achievable at the expense of only limited additional toxicity and without major dose reduction of the O⁶-alkylating agent. Preclinical toxicologic assessment of lomeguatrib and temozolomide showed no additional toxicity to the dose limiting myelosuppression seen with temozolomide alone (Cancer Research UK data on file). Lomeguatrib was also effective in ATM inactivation and tumor growth inhibition after oral administration (Cancer Research UK data on file) thereby facilitating oral use and variations in dose schedule.

The objectives of this phase I trial were as follows:

(a) To determine the dose of lomeguatrib administered i.v. that inactivates ≥95% of ATM in peripheral blood mononuclear cells (PBMC) and tumor at 4 hours [i.e. the ATM-depleting dose (ADD)].

(b) To determine the toxicity profile of lomeguatrib and temozolomide after i.v. and oral administration.

(c) To determine the toxicity profile, dose limiting toxicity (DLT), and MTD of oral temozolomide in patients receiving lomeguatrib at the ADD over a 5-day schedule.

(d) To determine the pharmacokinetic profile of lomeguatrib.

(e) To identify a suitable regimen of lomeguatrib and temozolomide for phase II evaluation.

This phase I study was the first involving the administration of lomeguatrib to humans.

Patients and Methods

Inclusion and exclusion criteria. Patients with histologically proven cancer refractory to conventional treatment or for which no suitable conventional therapy existed were eligible. Patients were required to be ≥18 years of age with a Karnofsky performance status of ≥70 and an expected survival of ≥12 weeks. They needed adequate hematologic and biochemical function with hemoglobin ≥10.0 g/dL, WBC ≥3.0 x 10⁹/L, neutrophil count ≥1.5 x 10⁹/L, platelets ≥100 x 10⁹/L, serum creatinine ≤130 µmol/L, serum bilirubin ≤20 µmol/L, and serum aspartate aminotransferase or alanine aminotransferase ≤2 x upper limit of normal.

Patients were excluded if they had major thoracic or abdominal surgery, chemotherapeutic agents, or radiotherapy in the preceding 4 weeks; had mitomycin C or nitrosoureas within the 6 weeks; had persistent toxic manifestations of previous treatments (except alopecia); had active infection or significant nonmalignant intercurrent illness; were pregnant or lactating; with known central nervous system metastases; with history of seizures; or were receiving concurrent antacid medication. A negative serum pregnancy test was required in women with child-bearing potential and all patients were required to use medically approved contraceptive precautions during the study and for 4 weeks afterwards.

The study was conducted under the auspices of Cancer Research UK in accordance with the principles of the International Conference on Harmonisation of Good Clinical Practice guidelines and the Declaration of Helsinki. The trial was approved by the Cancer Research UK Independent Ethics Committee and the Local Research Ethics Committee of each trial center. All patients gave written informed consent.

Treatment. The i.v. formulation of lomeguatrib was supplied by Cancer Research UK Formulation Unit as a freeze-dried powder in glass vials containing 100 mg lomeguatrib and polyvinylpyrrolidone as a bulking agent. The vials were stored at 4°C. Lomeguatrib was reconstituted using 10 mL Pharmasolve (N-methylpyrrolidone) before diluting with 0.9% NaCl to a final concentration of 2 mg/mL. Intravenous administration was over 30 minutes via a peripheral venous cannula and commenced within 1 hour of reconstitution. The oral formulation of lomeguatrib was supplied by Cancer Research UK Formulation Unit as 10 mg enteric-coated capsules and stored at 4°C. Temozolomide was supplied by Schering Plough (Kenilworth, NJ) in capsule strengths of 5, 20, 100, and 250 mg. Temozolomide was stored at 2°C to 30°C in accordance with the instructions of the manufacturer.

When administered in combination with temozolomide, lomeguatrib was administered 2 hours before temozolomide, and for oral dosing, the patient fasted for 1 hour pre- and post-dosing.

The starting dose of lomeguatrib both i.v. and orally was 10 mg/m² on days 1 to 5. The starting dose level of 10 mg/m² was approximately a third of the lomeguatrib dose in the mouse and rat that produced ≥95% inactivation of ATM in liver at 4 hours following a single dose. The human ADD was predefined as the dose of lomeguatrib that produced ≥95% ATM depletion in PBMC at 4 hours in all patients at that dose level.

To assess the toxicity and ATM-depleting effects of lomeguatrib alone and to establish the bioavailability of oral lomeguatrib, patients initially received lomeguatrib alone. Lomeguatrib was administered on days 1 to 5 and days 15 to 19. The initial route of administration could be oral or i.v. with the alternative route of administration being used on days 15 to 19. Because the oral formulation of lomeguatrib was not available at the start of the trial, patients 1 to 6 received lomeguatrib only via the i.v. route before entering the combination phase. At day 28, patients received lomeguatrib i.v. and temozolomide on days 1 to 5 of a 4-weekly cycle for up to six cycles.

A minimum of three patients was treated at each dose level and any patient withdrawn before day 21 of the combination phase was replaced. The first patient at each dose level was observed for 3 weeks before the enrollment of subsequent patients at that dose level. All three patients at a dose level were required to complete one 28-day treatment cycle with the combination of lomeguatrib and temozolomide before a decision to dose escalate temozolomide could be made. If one of three patients at a dose level developed DLT, up to three additional patients were treated at that dose level. If one of the three additional patients developed a DLT, dose escalation ceased and a total of six patients were treated at the preceding dose level. This lower dose level was defined as the MTD unless ≥2 of 6 patients developed DLT. If a patient developed DLT but was felt to be benefiting from treatment, the patient was permitted to continue at the discretion of the investigator following temozolomide dose reduction.

Dose escalations. The initial study design comprised two dose escalations. First, the dose of lomeguatrib was escalated to define the ADD of lomeguatrib. During this phase, the temozolomide dose was fixed at 50 mg/m² days 1 to 5. Once an ADD of lomeguatrib had been identified in PBMC, six consecutive patients who had given consent for paired tumor biopsies were entered. Tumor biopsies pretreatment and at 4 hours post-dosing on day 1 were obtained to verify adequate ATM depletion.

Once an ADD had been defined, the lomeguatrib dose was fixed at the ADD. The dose of temozolomide was escalated by 25 mg/m²/d increments in successive patient cohorts to define the MTD and DLT of temozolomide when administered with the ADD of lomeguatrib (Table 1).

On the basis of results obtained during the course of the trial, two further patient cohorts were then studied. The purpose of these cohorts was to evaluate lomeguatrib given orally in combination with
temozolomide as this was the favored formulation for future studies. Variability in ATase depletion with oral lomeguatrib made it desirable to test the MTD of temozolomide at greater than the ADD of i.v. lomeguatrib so as to better inform the selection of a combination for phase II studies (dose levels 7 and 8, Table 1).

**Toxicity and response evaluation.** The pretreatment evaluation included a complete medical history and physical examination, full blood count, biochemical profile, urinalysis, and electrocardiogram. Females with child-bearing potential underwent a pregnancy test. Baseline chest radiographs and appropriate radiology to evaluate tumor sites were done. Safety and toxicity were evaluated at least weekly during the study period and toxicities were graded according to the National Cancer Institute Common Toxicity Criteria Version 2 (24).

DLT was defined as any of the following events: (a) grade 4 neutropenia lasting >5 days or if associated with infection/fever; (b) grade 4 thrombocytopenia for >5 days; (c) grade 3 or 4 nonhematologic toxicity (excluding grade 3 nausea and vomiting in patients who had not received optimal treatment with antiemetics); and (d) drug-related death. The MTD was defined as the dose level of temozolomide in combination with the ADD of lomeguatrib below that at which ≥30% of the patient population experienced DLT due to the drug combination. Patients were to be followed up until 4 weeks after the last drug administration. Tumor response was assessed using WHO response criteria (25) at baseline, after every two cycles, and after withdrawal. Treatment delay, dose modification, and treatment withdrawal. A treatment delay of up to 2 weeks was allowed for resolution of drug-related toxicity. Dose modification of temozolomide by one or two dose levels was permitted in the event of DLT in the preceding cycle. Patients could be withdrawn from the study for progressive disease, unacceptable toxicity, serious violation of the study drug protocol, or withdrawal of consent.

**Pharmacodynamics.** Samples for PBMC ATase activity were obtained before treatment and at 0.5, 0.75, 1, 2, 4, 6, and 12 hours on days 1 and 5, and at days 8, 11, 15, and 22 following lomeguatrib alone and following lomeguatrib and temozolomide. Five to ten milliliters of venous blood were collected into tubes containing 100 μL of 0.5 mol/L EDTA and stored on ice for a maximum of 4 hours before isolation of PBMC and analysis of ATase (26). Tumor biopsies were obtained by incision or core biopsy before and 4 hours after completion of the first dose of lomeguatrib. The biopsies were snap frozen on dry ice and stored at −80°C before determination of ATase activity (26).

**Pharmacokinetics.** Five-milliliter venous blood samples were drawn pre-dose and at 0.5, 0.75, 1, 2, 4, 6, and 12 hours post-dose for tumor lomeguatrib plasma levels on days 1 and 5 of administration. Samples were drawn into lithium heparin tubes and centrifuged immediately (if unable to centrifuge immediately, samples were placed on ice for <2 hours). Plasma was transferred to labeled tubes and frozen on dry ice before storage at −70°C. Lomeguatrib concentrations were determined by liquid chromatography-tandem mass spectrometry. Aliquots of plasma were added to deuterated lomeguatrib to give a concentration of 100 pg/μL as the internal standard. Patient plasma samples and standards were extracted by solid-phase extraction and analyzed within 30 minutes. Quality control sample and standard curve sample were each analyzed in triplicate.

**Data analysis.** Adverse events were graded according to National Cancer Institute Common Toxicity Criteria Version 2 (24). Tumor response was defined according to WHO criteria (25). Area under the concentration time curve (AUC) was calculated using the trapezoidal rule for the sum of AUC to the last measured time point. Each patient’s data were analyzed by nonlinear least squares regression to determine individual pharmacokinetic variables. ATase depletion data were summarized by dose level.

**Results**

**Patient and tumor characteristics.** Thirty-eight patients were enrolled and their baseline characteristics are shown in Table 2. All patients were evaluable for toxicity following lomeguatrib alone and 34 patients received lomeguatrib plus temozolomide and were evaluable for toxicity of the combination. Twenty-three patients had measurable disease and were evaluable for tumor response. The dose levels of lomeguatrib and temozolomide studied are shown in Table 1.

**Determination of the ADD of lomeguatrib.** Of the 38 patients entered, 37 patients were assessable for ATase depletion in PBMC following i.v. lomeguatrib administration (10 mg/m², 28 patients; 20 mg/m², 6 patients; 40 mg/m², 3 patients). PBMC samples were spoiled in one patient. Twenty-six patients were assessable for ATase depletion in PBMC following oral lomeguatrib (10 mg/m², 18 patients; 20 mg/m², 5 patients; 40 mg/m², 3 patients). In the cases not assessable, six patients were enrolled before oral lomeguatrib was available, three patients had undetectable ATase in the pretreatment sample, two samples were spoiled, and one patient was withdrawn before oral lomeguatrib dosing.

The determination of ADD was based on the percentage depletion observed 4 hours after the first dose of lomeguatrib. Depletion was rapid after i.v. dosing, with ATase usually undetectable at 30 minutes (end of lomeguatrib infusion),

<table>
<thead>
<tr>
<th>Dose level (mg/m²/d)</th>
<th>Lomeguatrib</th>
<th>Temozolomide (mg/m²/d)</th>
<th>No. patients assigned dose level</th>
<th>No. patients receiving combination</th>
<th>Total no. cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>50</td>
<td>6</td>
<td>6</td>
<td>12</td>
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<tr>
<td>2</td>
<td>10</td>
<td>75</td>
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<td>125</td>
<td>4</td>
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</tr>
<tr>
<td>8</td>
<td>40</td>
<td>125</td>
<td>3</td>
<td>3</td>
<td>9*</td>
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</table>

*Patient #29 was originally assigned dose level 6 but, because patients #27 and #28 had DLT, was treated at dose level 5.
*Escalated to 150 mg/m² in a total of four cycles.
*Three cycles at reduced temozolomide dose after toxicity.
Lomeguatrib was very well tolerated and produced mainly grade 1 toxicity, with the exception of nausea, which was often grade 2 when lomeguatrib was given in combination with temozolomide. There were no significant differences in the incidence of toxicity between patients receiving lomeguatrib alone or in combination with temozolomide. Partial recovery of ATase activity was observed in the patients who received lomeguatrib alone, with a median ATase recovery of 97% and 98% at 4 hours post-dosing. In the patients receiving lomeguatrib in combination with temozolomide, the median ATase recovery was 92.3%, 96.1%, and 96.6% at 4 hours post-dosing.

**Pharmacokinetics of lomeguatrib.** Table 4 summarizes the pharmacokinetics of lomeguatrib at each of the three dose levels studied. Following i.v. administration, plasma concentrations of lomeguatrib decreased rapidly and were biphasic, with a median half-life of 12 hours. Following oral dosing, lomeguatrib plasma levels were also biphasic, with a median half-life of 12 hours. Thereafter, lomeguatrib levels declined rapidly and were usually undetectable by 24 hours post-dosing. Paired pharmacokinetic profiles from 20 different patients were available to allow estimation of the bioavailability of the oral formulation and this was a mean of 27.6% (SD, 23.8) and 27.9% (SD, 22.0) on days 1 and 5, respectively.

**Tumor ATase depletion following lomeguatrib.** Tumor biopsies were obtained immediately before treatment and at 4 hours after 10 mg/m² lomeguatrib i.v. on day 1 in a total of 12 patients. In two cases, necrotic material was obtained and in one patient the pretreatment tumor sample had no detectable ATase. In six of the nine assessable patients, 100% ATase depletion was observed, and in the remaining three cases, depletion was 92.3%, 96.1%, and 96.6% at 4 hours.

**ATase depletion in PBMC in patients receiving lomeguatrib and temozolomide.** PBMC ATase depletion data were obtained in 33 patients treated with lomeguatrib and temozolomide. Four patients were withdrawn before combination therapy and one patient’s samples were unsuitable for analysis. In all but one case, ATase depletion was 100% at 4 hours and in the remaining patient, depletion was 96.3% and complete by the end of dosing. The pattern of ATase recovery was similar to that seen for lomeguatrib alone although in some patients the recovery was slightly slower.

**Table 2. Patient and tumor characteristics**

<table>
<thead>
<tr>
<th>No. patients</th>
<th>38</th>
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</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>18/20</td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>60 (18-74)</td>
</tr>
<tr>
<td>Performance status (Karnofsky)</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>Primary tumor site</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>11</td>
</tr>
<tr>
<td>Colorectal</td>
<td>9</td>
</tr>
<tr>
<td>Non – small cell lung</td>
<td>4</td>
</tr>
<tr>
<td>Renal</td>
<td>3</td>
</tr>
<tr>
<td>1° peritoneal carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Ovary</td>
<td>2</td>
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<tr>
<td>Other</td>
<td>7*</td>
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<td>Prior treatment</td>
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<td>Surgery</td>
<td>37</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>12</td>
</tr>
<tr>
<td>Hormonal/biological</td>
<td>11</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>32</td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Median no. prior regimens (range)</td>
<td>2 (1-6)</td>
</tr>
</tbody>
</table>

*One each for breast, mesothelioma, esophagus, soft tissue sarcoma, neuroectodermal, stomach, and thymus.

whereas after oral dosing, depletion occurred after a lag phase of 60 to 120 minutes. Complete depletion of ATase was observed in 26 of 28 assessable patients receiving 10 mg/m² lomeguatrib i.v., and in the remaining 2 patients, ATase depletion was 97.3% and 98.2%. Thus, 10 mg/m² i.v. was identified as the ADD for PBMC. Oral lomeguatrib at 10 mg/m² (in effect 20 mg daily dose due to 10 mg capsule size) resulted in 100% depletion in 10 of 18 patients at 4 hours post day 1 dosing. In the remaining 8 patients, depletion at 4 hours post day 1 dose ranged from 75.8 to 96.2%. Additional ATase depletion was seen at time points later than 4 hours in all patients. Partial recovery of ATase levels in PBMCs was observed by day 8 (3 days after the last dose of lomeguatrib) and levels continued to increase through days 11 to 15 (Table 3). The pattern of recovery was similar at all three dose levels and via both routes of administration.

**Table 3. ATase recovery in PBMCs**

<table>
<thead>
<tr>
<th>Lomeguatrib dose (mg/m²)</th>
<th>No. patients*</th>
<th>Route</th>
<th>Day 8 (mean % ± SE)</th>
<th>Day 11 (mean % ± SE)</th>
<th>Day 15 (mean % ± SE)</th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>Intra</td>
<td>18.8 ± 3.08</td>
<td>51.6 ± 8.36</td>
<td>117 ± 16.8</td>
</tr>
<tr>
<td>10</td>
<td>16</td>
<td>Oral</td>
<td>30.3 ± 7.37</td>
<td>85.9 ± 19.3</td>
<td>161 ± 42</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>Intra</td>
<td>17.1 ± 8.33</td>
<td>40.0</td>
<td>59.6 ± 17.6</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>Oral</td>
<td>21.1 ± 6.01</td>
<td>35.6 ± 11.1</td>
<td>91.3 ± 26.7</td>
</tr>
<tr>
<td>40</td>
<td>3</td>
<td>Intra</td>
<td>42.9 ± 7.77</td>
<td>67.0 ± 14.1</td>
<td>165 ± 45.6</td>
</tr>
<tr>
<td>40</td>
<td>3</td>
<td>Oral</td>
<td>51.5 ± 20.5</td>
<td>51.4 ± 27.0</td>
<td>82.4 ± 8.69</td>
</tr>
</tbody>
</table>

NOTE: Data expressed as percentage of pretreatment ATase activity.

*Number of patients for whom data were obtained at specified dose and route.

†Sample available from only one patient.
1 or 2 adverse effects (Table 5), the most significant adverse effect being local venous irritation with the i.v. formulation and short-lived (<1 hour) mild nausea (mainly at 40 mg/m²). Based on the chemical structures of lomeguatrib and the diluent N-methylpyrrolidone, it was considered likely that the local venous pain was diluent related rather than due to lomeguatrib. No significant toxicity was encountered with oral lomeguatrib alone.

The dose levels for lomeguatrib and temozolomide are shown in Table 1. The spectrum of adverse events was in keeping with those reported for temozolomide alone (27, 28) and the DLT of lomeguatrib with temozolomide was hematologic (Tables 5 and 6).

**Hematologic toxicity.** Hematologic toxicity is summarized by dose level in Table 6. Myelosuppression was an expected toxicity following temozolomide, and in combination with lomeguatrib the degree of myelosuppression was related to temozolomide dose. Grade 4 neutropenia was not encountered at or below dose level 4 but occurred in one of seven patients at level 5 and in two of two patients at level 6. The median day of neutropenia was day 24 (range, days 2-38) and median recovery was by day 30 (range, days 5-50). Grade 3 or 4 thrombocytopenia was observed at doses of temozolomide ≥100 mg/m²/d. The median nadir was day 22 (range, days 15-31) and mean recovery was by day 29 (range, days 25-54). Mild anemia was common although grade 3/4 anemia was seen in only three patients. Febrile neutropenia or infection with grade 3 or 4 neutropenia occurred in four patients. The two patients treated with lomeguatrib 10 mg/m²/d and temozolomide 175 mg/m²/d developed febrile neutropenia and single cases of febrile neutropenia were also seen at dose levels 7 and 8.

**Nonhematologic toxicity.** No antiemetic prophylaxis was employed during lomeguatrib treatment alone. Nausea and vomiting were well-recognized adverse effects of oral temozolomide, and to ensure therapy compliance, all patients received prophylactic antiemetic therapy with ondansetron (8 mg twice a day orally for 5 days) during combined lomeguatrib and temozolomide. Nausea and vomiting was usually grade 1 or 2 although three patients experienced grade 3 or 4 fatigue. Six patients had venous irritation or pain due to i.v. lomeguatrib administration. This consisted of a burning sensation in the arm during drug administration. Slowing the infusion or the concurrent administration of 0.9% saline abolished the pain.

**DLT.** Both patients treated at a dose level of 10 mg/m² i.v. lomeguatrib and 175 mg/m² temozolomide experienced DLT (grade 4 thrombocytopenia and grade 4 neutropenia for >5 days). No further dose escalations of temozolomide were made. Dose levels 7 and 8 used 125 mg/m²/d temozolomide with 20 and 40 mg/m² of lomeguatrib (effectively 40 and 80 mg/d with the oral formulation). No significantly increased toxicity was encountered with these increased lomeguatrib doses compared with that seen at 10 mg/m² lomeguatrib with 125 mg/m² temozolomide. A temozolomide dose level of 125

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**Table 4. Pharmacokinetics of lomeguatrib**

<table>
<thead>
<tr>
<th>Vc (L)</th>
<th>CL (L/min)</th>
<th>T1/2a (h)</th>
<th>T1/2h (h)</th>
</tr>
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<tr>
<td>Day 1</td>
<td>Day 5</td>
<td>Day 1</td>
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<tr>
<td>-----------</td>
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<td>-----------</td>
</tr>
<tr>
<td>10 mg/m² i.v. (n = 24)</td>
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<tr>
<td>Mean 73.0</td>
<td>62.7</td>
<td>85.2</td>
<td>79.7</td>
</tr>
<tr>
<td>SD 42.3</td>
<td>34.5</td>
<td>36.9</td>
<td>46.8</td>
</tr>
<tr>
<td>20 mg/m² i.v. (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 90.9</td>
<td>59.5</td>
<td>91.6</td>
<td>64.9</td>
</tr>
<tr>
<td>SD 12.8</td>
<td>12.9</td>
<td>10.1</td>
<td>15.2</td>
</tr>
<tr>
<td>40 mg/m² i.v. (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean 99.6</td>
<td>117.7</td>
<td>131.8</td>
<td>102.8</td>
</tr>
<tr>
<td>SD 54.2</td>
<td>52.1</td>
<td>48.1</td>
<td>54.9</td>
</tr>
</tbody>
</table>

**AUC0-6 h following i.v. lomeguatrib**

<table>
<thead>
<tr>
<th>10 mg/m² (n = 24)</th>
<th>20 mg/m² (n = 3)</th>
<th>40 mg/m² (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Day 5</td>
<td>Day 1</td>
</tr>
<tr>
<td>Mean 259.9</td>
<td>278.4</td>
<td>440.5</td>
</tr>
<tr>
<td>SD 136.4</td>
<td>196.5</td>
<td>33.6</td>
</tr>
</tbody>
</table>

**AUC0-6 h following oral lomeguatrib**

<table>
<thead>
<tr>
<th>10 mg/m² (n = 12)</th>
<th>20 mg/m² (n = 3)</th>
<th>40 mg/m² (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Day 5</td>
<td>Day 1</td>
</tr>
<tr>
<td>Mean 52.7</td>
<td>70.6</td>
<td>115.7</td>
</tr>
<tr>
<td>SD 75.0</td>
<td>64.3</td>
<td>67.4</td>
</tr>
</tbody>
</table>

Abbreviations: Vc, central volume; CL, clearance; T1/2a, initial half life; T1/2h, terminal half life.
mg/m² days 1 to 5 was considered the recommended starting
dose for use with an ADD of lomeguatrib (10 mg/m² i.v. or 20-
40 mg/m² orally days 1-5). A total of 50 cycles were
administered to 16 patients at these dose levels and the
maximum grade of toxicity experienced during any cycle with
these dose levels was grade 4 thrombocytopenia
in 1 of 16 patients, grade 4 neutropenia in 6 patients, and
neutropenic fever in 3 patients. There seems to be no advantage
to surface area–calculated dosing for lomeguatrib and a
regimen of 40 or 80 mg/d orally for 5 days would be suitable
for investigation in phase II trials.

**Antitumor effects.** Twenty-three patients were evaluable for
response. At dose level 7, one patient with colorectal cancer,
who had received three prior chemotherapy regimens, had a
complete response in multiple lung metastases but developed
brain metastases after 5 months of treatment. Two patients
(one ovarian, one colorectal) had declines of ~50% in serum
tumor markers during therapy with stable radiology over five
and six cycles, respectively. A further 10 patients had stable
disease of at least 3-month duration.

**Discussion**

The main objectives of this first phase I trial were to determine
a dose of lomeguatrib that depleted ATase in patients and to
define a suitable combination of lomeguatrib and temozolo-
mide to be used in future studies. The use of a pharmacodynamic
rather than toxicity end point to determine the dose of
lomeguatrib for further evaluation was based on the premise
that ATase depletion in clinical settings was likely to be
achievable at doses well below the MTD of lomeguatrib itself.
With the inherent low toxicity of lomeguatrib in preclinical
toxicology studies, a starting dose of one third of the ATase
inhibitory dose in rodents was chosen as the starting dose for
lomeguatrib.

Both PBMC and paired tumor samples were used to determine
the extent and the reliability of ATase depletion following
administration of lomeguatrib. At the first dose level of 10
mg/m²/d i.v., lomeguatrib was an effective ATase-inactivating
agent in human PBMC. It should be noted that lomeguatrib
dosing occurred over 5 consecutive days and that additional ATase
depletion was expected to occur beyond this early time point. An
early time point for the definition of ATase depletion is justified
because suppression of ATase activity throughout the duration of
temozolomide dosing was deemed important in maximizing the
potential for overcoming ATase-mediated resistance.

**Table 5. Nonhematologic adverse events related to lomeguatrib +/− temozolomide**

<table>
<thead>
<tr>
<th>Drug-related adverse event*</th>
<th>No. patients (%)</th>
<th>Maximum grade (90%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Laboratory adverse events:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lomeguatrib alone (n = 38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>4 (11)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>3 (8)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>3 (8)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Injection site reaction/pain</td>
<td>6 (16)</td>
<td>5 (13)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>5 (13)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>3 (8)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Laboratory adverse events:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lomeguatrib and temozolomide (n = 34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>5 (15)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>4 (12)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>4 (12)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>4 (12)</td>
<td>4 (12)</td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td>3 (9)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Non-laboratory adverse events:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lomeguatrib and temozolomide (n = 34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>16 (47)</td>
<td>9 (26)</td>
</tr>
<tr>
<td>Nausea</td>
<td>13 (38)</td>
<td>8 (24)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>8 (24)</td>
<td>5 (15)</td>
</tr>
<tr>
<td>Stomatitis/pharyngitis</td>
<td>5 (15)</td>
<td>5 (15)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>3 (9)</td>
<td>3 (9)</td>
</tr>
</tbody>
</table>

NOTE: N/A, not applicable.

*Adverse events occurring in ≥3 patients.
given orally at 10 mg/m², it took up to 6 hours to achieve maximal depletion and the slower onset of ATase depletion and the relatively low bioavailability probably accounted for the finding that only 10 of 18 patients had total ATase depletion in PBMC at 4 hours. However, at 40 and 80 mg/d orally, all patients had total ATase depletion by 4 hours after the first dose. Recovery of ATase in PBMC was evident within 3 days of completing dosing and ~ 50% recovery had occurred by 10 days following dosing. In contrast, ATase depletion with temozolomide alone is slow, usually incomplete (even after multiple doses), and ATase levels can recover during continued drug dosing (29, 30).

There is known to be substantial variation in ATase levels in different tumors even with the same histologic type (2) and pretreatment assessment of ATase activity was done to ensure that absent ATase activity reflected drug-induced depletion. In one patient, the pretreatment biopsy revealed undetectable ATase levels, and in two instances, tumor biopsies yielded necrotic material, suggesting that this paired biopsy approach was warranted. Based on tumor depletion data, the tumor ADD identified in this study was 10 mg/m² lomeguatrib i.v. days 1 to 5 (50 mg/m² total dose). This compares to a dose of 120 mg/m² i.v. for O₆-benzylguanine (31). The tumor ATase depletion data reported here are being extended in ongoing studies. With oral dosing, low toxicity, and a lack of effect of bovine serum albumin on lomeguatrrib pharmacokinetics, a fixed dose rather than body surface area–adjusted dosing can be recommended for future trials.

When administered with an ADD of lomeguatrib, the MTD for temozolomide was 150 mg/m²/d using a day 1 to 5 schedule. To permit ease of administration, an oral regimen is likely to be preferable and a regimen of oral lomeguatrib 40 mg/d with temozolomide 125 mg/m² days 1 to 5 is suggested for further evaluation. Although no DLT was observed in cycle one at this dose level, grade 3 and 4 hematologic toxicity was seen in later cycles in four of six patients, suggesting that an increase in the temozolomide dose to the MTD seen with lomeguatrib 10 mg/m² i.v. would not be sustainable.

A randomized trial of lomeguatrib plus temozolomide versus temozolomide alone in patients with metastatic malignant melanoma is in progress and trials in other tumor types are planned. Work to define the ability of lomeguatrib to deplete ATase in a number of solid tumors is also under way. Because temozolomide has activity in central nervous system tumors, results from this group of patients will be of particular interest.

O₆-Benzylguanine, the only other ATase inactivator in clinical evaluation, has primarily been used in combination

| Table 6. Hematologic toxicity of lomeguatrib and temozolomide (worst grade per patient) |
|-----------------------------------------------|-----------------------------|------------------|------------------|------------------|------------------|
| Adverse event | Dose level number | Dose level Lo/TMZ (mg/m²/d) | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| Neutrophils | 1 | 10/50 | 0 | 0 | 0 | 0 |
| | 2 | 10/75 | 1 | 0 | 0 | 0 |
| | 3 | 10/100 | 0 | 1 | 0 | 0 |
| | 4 | 10/125 | 1 | 0 | 1 | 0 |
| | 5 | 10/150 | 1 | 2 | 2 | 1 |
| | 6 | 10/175 | 0 | 0 | 0 | 2 |
| | 7 | 20/125 | 0 | 0 | 0 | 4 |
| | 8 | 40/125 | 0 | 0 | 1 | 1 |
| Total | | | 2 | 3 | 3 | 8 |
| Platelets | 1 | 10/50 | 1 | 0 | 0 | 0 |
| | 2 | 10/75 | 1 | 0 | 0 | 0 |
| | 3 | 10/100 | 0 | 1 | 1 | 0 |
| | 4 | 10/125 | 0 | 0 | 2 | 0 |
| | 5 | 10/150 | 2 | 1 | 4 | 0 |
| | 6 | 10/175 | 0 | 0 | 1 | 1 |
| | 7 | 20/125 | 1 | 1 | 3 | 1 |
| | 8 | 40/125 | 0 | 0 | 2 | 0 |
| Total | | | 5 | 3 | 13 | 2 |
| Hemoglobin | 1 | 10/50 | 1 | 0 | 0 | 0 |
| | 2 | 10/75 | 0 | 1 | 0 | 0 |
| | 3 | 10/100 | 0 | 0 | 0 | 0 |
| | 4 | 10/125 | 2 | 1 | 0 | 0 |
| | 5 | 10/150 | 1 | 2 | 1 | 1 |
| | 6 | 10/175 | 1 | 1 | 0 | 0 |
| | 7 | 20/125 | 0 | 3 | 0 | 1 |
| | 8 | 40/125 | 0 | 2 | 0 | 0 |
| Total | | | 5 | 10 | 1 | 2 |

Abbreviation: Lo/TMZ, lomeguatrib/temozolomide.
*For dose levels, see Table 1. 
with BCNU. With ATase-inactivating doses of O6-benzylgua-
nine, only 20% to 25% of the standard dose of BCNU was
possible (22). In this study, at tumor ADDs of lomeguatri-
b, >60% of the standard single agent dose of temozolomide
could safely be administered over multiple cycles. A similar observa-
tion was made in preclinical studies (16), suggesting that the
context of ATase depletion and the choice of O6-alkylating
agent may be influential. One variable that has not been
extensively studied to date has been the optimal duration of
ATase inhibition during and following O6-alkylator therapy.
This may be important as relatively small amounts of newly
synthesized ATase may remove DNA adducts. Ultimately, the
clinical utility of depleting ATase in tumors to enhance the
therapeutic efficacy of O6-alkylating agents will need to be
evaluated in randomized phase III trials.

In conclusion, the preclinical development program was
highly successful in predicting suitable doses of lomeguatrifor
clinical evaluation. Detailed pharmacodynamic assessment of
both PBMCs and tumors successfully established ADDs of
lomeguatrit. Comparative pharmacokinetics using both i.v.
and oral formulations of lomeguatrit identified that whereas
bioavailability was low, the intrinsic potency and low toxicity
of lomeguatrit permit the further clinical evaluation of an oral
formulation. This phase I trial successfully identified a suitable
dose of temozolomide for use in combination with lomeguat-
tr for future studies.

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Lomeguatrib, a Potent Inhibitor of $O^6$-Alkylguanine-DNA-Alkyltransferase: Phase I Safety, Pharmacodynamic, and Pharmacokinetic Trial and Evaluation in Combination with Temozolomide in Patients with Advanced Solid Tumors

Malcolm Ranson, Mark R. Middleton, John Bridgewater, et al.


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