Pharmacokinetic Analysis and Phase 1 Study of MRX-1024 in Patients Treated with Radiation Therapy with or without Cisplatinum for Head and Neck Cancer

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Abstract

Purpose: A previous study reported radiation protection from mucosal injury with D-methionine (D-met) in preclinical evaluation; therefore, the pharmacokinetics, safety, and utility of D-met were evaluated clinically.

Experimental Design: The pharmacokinetics of D-met following oral administration of a bioavailable formulation (MRX-1024) was evaluated in normal volunteers. Subsequently, 25 patients were enrolled on a phase 1 study of MRX-1024 concurrent with radiation therapy (RT) with or without weekly cisplatinum. Toxicity and mucosal events were evaluated weekly.

Results: Oral MRX-1024 resulted in rapid and dose-dependent increases in plasma D-met concentrations with a half-life of 3 hours. When administered concurrent with RT without chemotherapy, it was associated with a modest increase in grade 2 (two of six patients) and grade 3 (one of six patients) emesis. In those treated with MRX-1024 along with RT and weekly cisplatinum, there was no appreciable increase in emesis. Overall, five patients withdrew from the study due to emesis (four grade 2 and one grade 3). Only one incidence of dose-limiting toxicity (grade 3 emesis) was identified in 25 patients (4%). Finally, in 18 evaluable patients treated with MRX-1024 at 100 mg/kg twice daily (BID), the incidence of severe (grade 3) oral mucositis was 6% (1 of 18) with no grade 4 mucositis.

Conclusions: There is a dose-dependent increase in D-met exposure following MRX-1024 administration at 50 and 100 mg/kg, and MRX-1024 is safe for use concurrent with combined radiation and chemotherapy. The observed rate of mucositis seems less than that for similar treatment regimens within the published literature.

In the United States, approximately 40,000 patients in 2009 will be newly diagnosed with cancer of the head and neck, and approximately 15,000 will die from this disease (1). Oral mucositis is a dose-limiting side effect of radiation therapy (RT) in which the cells of the oral mucosa undergo radiation-induced cell death, and ulcers are produced, often with secondary bacterial or fungal infections, which may cause increased risk of developing septicemia (2–4). Severe cases of oral mucositis are commonly observed in as many as 50% to 80% of patients during simultaneous radiation and chemotherapy for cancers of the head and neck (5). More than 100 published studies exist that document clinical investigations aimed at the palliation, prevention, or reduction of oral mucositis, with most targeting symptoms such as ulceration and infection. The range of treatments evaluated for mucositis after either chemotherapy or RT includes antimicrobials (6, 7), cytokines (8–11), keratinocyte growth factor (12), antiinflammatories (13–15), coating rinses (16), glutamine (17), cryotherapy (18), and laser treatment (19). However, to date, none of these has shown significant protection for head and neck cancer patients. A randomized trial for patients undergoing bone marrow transplantation for hematologic malignancies did show that keratinocyte growth factor offered significant protection from oral mucositis in this setting, which was the basis for the Food and Drug Administration approval of Kepivance (palifermin, Biovitrum AB; ref. 12). However, a recent randomized phase 2 trial of weekly bolus i.v. palifermin in patients being treated with concurrent chemotherapy and radiation did not
Translational Relevance

Mucositis is a common dose-limiting side effect of radiation therapy in head and neck cancer patients. To date, no clear treatment that mitigates this toxicity for this patient population has been identified. Previously, it was shown that D-methionine (D-met) could protect nontransformed human cells in culture from radiation-induced cell death while not similarly protecting tumor cell lines both in vitro and in vivo. In addition, i.p., i.v., and oral administration of D-met provided significant radiation protection in a murine model of radiation-induced mucositis. Therefore, clinical evaluation was undertaken and is presented here that an oral formulation of D-met (MRX-1024) provides rapid and dose-dependent uptake of D-met without significant toxicity and with an indication for mucosal protection in patients being treated for head and neck cancer.

show evidence of mucosal protection, although studies at higher doses or higher frequency are ongoing (20).

D-methionine (D-met) is the dextro isomer of the essential amino acid L-methionine (L-met), whereas MRX-1024 is a high-concentration (200 mg/mL) bioavailable suspension formulation of D-met, which is being evaluated as a radiation protector (Molecular Therapeutics, Inc.). Interest in D-met as a radioprotector was spurred by reports that, in animal models, it could effectively prevent the oxidative stress–induced ototoxicity and nephrotoxicity associated with the chemotherapeutic agent cisplatinum (21, 22). A recent report also documented radiation protection with D-met of primary nontransformed human cells (fibroblasts, keratinocytes, and endothelial cells) with a protective factor of 1.2 to 1.6 in clonogenic assays in vitro, whereas radiation protection was not observed in a panel of transformed human tumor cell lines in vitro or in vivo (23). Further, irradiation of the snouts of mice daily for 5 days resulted in mucositis compared with control animals, with a peak mucositis score of 3.5 ± 0.25 on a scale from 0 to 7 that was elevated compared with control animals (peak value, 0.4 ± 0.2; P < 0.001). Treatment of mice with D-met by i.v., i.p., or oral administration decreased both the peak value (all with peak <1.5 units) and the area under the toxicity versus time curve (AUC) compared with animals treated with radiation alone (P < 0.03 for each group compared with irradiated). In addition, there was also a dose-dependent increase in radiation protection when D-met doses of 200, 300, and 500 mg/kg were used, with protective factors of 1.6, 2.1, and 2.6, respectively (P < 0.0003; ref. 23).

There is extensive clinical experience with orally administered methionine, which as an amino acid is a natural micronutrient with both the D- and L-isomers present in high concentrations in a normal diet. Methionine toxicity can occur from very high doses of racemic or L-met, particularly in developing animals fed a low protein diet (24); nevertheless, most human studies using L-met reported minimal to no side effects (25–27). Further, D-met itself is substantially safer than L-met, with some suggesting that D-met is not toxic unless it is converted to the L-isomer (28). In humans, due to minimal catabolism, D-met results in higher plasma levels than L-met, with >60% of D-met excreted without conversion to the L-isomer (24, 29–31). Clinically, L-met has been available for decades for treatment of dermatitis using an over-the-counter preparation with a recommended dose of 200 to 400 mg orally three to four times per day. The racemic mixture may also be used to treat acetaminophen overdose, where a total oral dose of 10 g is administered over 12 hours (32–36). Oral methionine was also previously evaluated for treatment of rheumatoid arthritis, where 24 patients were treated with 5 or 10 g of L-met daily for 16 or 8 weeks, respectively. Clinical tolerance was reported as good except for gastrointestinal distress in the majority of patients (37). In addition, recent reports documented patients with AIDS-related neuropathy who were administered oral L-met at a dose of 6 g/d for 6 months (38, 39). Again, the treatment was well tolerated except for gastrointestinal upset and nausea. In a follow-up study, 28 patients were randomized to placebo or the same dose of L-met (6 g/d) for 12 weeks also with no significant toxicity (40).

Therefore, given its potential role as a selective mucosal protector from radiation-induced injury and its apparent safe long-term use as an oral supplement, we report herein the oral pharmacokinetics of D-met when administered to normal human volunteers as a suspension formulation to identify the appropriate doses of the drug to be administered to match plasma level observed from preclinical evaluation where mucosal protection was observed (23). We also present a phase 1 study done to evaluate the safety of oral MRX-1024 administered to patients being treated with RT (with or without concurrent cisplatinum) for cancers of the head and neck and to provide an initial description of the rate of oral mucositis in patients receiving MRX-1024 concurrent with RT for head and neck cancer.

Materials and Methods

MRX-1024

The active pharmaceutical ingredient in MRX-1024, manufactured by stereo-specific chemical synthesis according to cGMP guidelines, is D-met (CAS Registry Number 348-67-4). The structural formula is CH₃SCH₂CH₂CH(NH₂)COOH and the molecular weight is 149.12. It is a dry white powder that is soluble in water. The manufactured purity is ≥98% and it is formulated for clinical use as a flavored suspension for oral administration. MRX-1024 was provided by Molecular Therapeutics at a concentration of 200 mg/mL in sealed brown glass bottles.

Pharmacokinetic evaluation of D-met

Subjects. The pharmacokinetics of D-met after oral administration of MRX-1024 was obtained from 12 normal
human volunteers who gave informed consent for the participation in this study (6 administered a single dose of MRX-1024 at 50 mg/kg and 6 administered a single dose of MRX-1024 at 100 mg/kg).

**Specimen collection, preparation, and analysis.** Plasma samples were collected by vein puncture using conventional techniques immediately before and then 0.5, 1, 2, 4, and 6 hours after administration of the indicated dose of MRX-1024. After collection, plasma samples were frozen, stored at −20°C, and analyzed within 100 days of collection. The samples were analyzed by high-performance liquid chromatography–UV by Vimta Labs Ltd., as previously described (23). Briefly, the frozen samples were thawed at room temperature, derivatized using Marfey's reagent (L-alaninamide), extracted with acetone, evaporated under N₂, and reconstituted in Tris buffer, and this final product was used for high-performance liquid chromatography. All samples were analyzed in batch over a 72-hour period. The assay was calibrated daily with standard concentrations of D-met over a range from 1.501 to 200.178 μg/mL. Clinical samples were serially diluted to within the calibrated level as necessary. Sample concentrations below 1.501 μg/mL were reported as "0."

**Phase 1 analysis of D-met in patients receiving RT**

A single-center, open-label, industry-sponsored nonrandomized phase 1 study was done at the Department of Radiation Oncology, Nizam's Institute of Medical Sciences. The protocol, consent documents, and amendments were approved by the institutional review board of Nizam's Institute of Medical Sciences. All patients were required to provide written informed consent before study enrollment. Given the long history of orally administered methionine, the primary end point of the phase 1 study was the identification of a safe and biologically relevant dose of oral MRX-1024 that would result in peak serum concentrations and total exposure to drug equivalent to that seen for radiation protection in preclinical models. Secondary end points included an initial assessment of acute mucosal injury for patients treated with radiation and MRX-1024 (with or without cisplatinum) to design subsequent phase 2 studies, an assessment of the utility of four different scales for mucosal assessment, and a comparison of mucosal injury to a previously treated cohort of patients from the same institution.

**Trial design**

Initially, the trial was planned to enroll a maximum of 25 patients at one of three dose levels (25, 50, and 100 mg/kg) with dose escalation to be done using a conventional cohorts of three paradigms to identify the maximum tolerated dose (MTD; ref. 41). Per initial protocol design, once the MTD was established, an additional small cohort of patients was planned at the MTD to complete a total of 25 patients to serve as an initial assessment of mucositis in the setting of RT and MRX-1024 to aid in design and powering subsequent phase 2 studies.

**Definition of dose-limiting toxicity**

Patients were to receive two doses of the drug at the indicated level administered before and within 1 hour after RT during a planned 6-week course of RT. Toxicity data were collected on a weekly basis during treatment and scored by the treating physicians as definitely related to study drug, likely related to study drug, possibly related to study drug, or definitely not related to study drug. Dose-limiting toxicity (DLT) was defined as any Common Toxicity Criteria version 2 toxicity of grade ≥2, not including mucositis, which was definitely or likely related to study drug.

**Amendment of protocol design**

Following enrollment of the first patient, an amendment was subsequently approved by the local Institutional Review Board to treat further patients at the highest dose (100 mg/kg BID). If toxicity was not observed after three patients at 100 mg/kg BID, then an additional three patients would be treated to confirm this as a biologically relevant dose (due to pharmacokinetics indicating that this would achieve serum concentrations compatible with protection in preclinical models) without further dose escalation and without escalating to the MTD. If DLT was observed, then the dose would be deescalated. The reasons for this change in protocol were as follows: (a) the excellent tolerability of MRX-1024 during the normal human volunteer studies where six patients had received 50 mg/kg and six had received 100 mg/kg with no evidence for toxicity; (b) the previous 30-year experience using oral methionine for treatment of acetaminophen overdose (32–36); (c) the previous experience administering pharmacologic doses of L-met to patients for as long as 6 months without toxicity for the treatment of rheumatoid arthritis (37) or AIDS-related peripheral neuropathy (38, 40); (d) the previous data documenting toxicity associated with D-met only after conversion to L-met with >60% of D-met excreted unchanged in humans such that treatment on this study would result in lower exposure to L-met over a shorter period of time compared with accepted treatment schedules (24–28, 31, 38, 40); and (e) the previous dose-dependent mucosal protection observed in preclinical models (23) where maximal protection was observed with peak plasma concentrations analogous to those observed at the highest dose administered to patients in the pharmacokinetic portion of the study.

Therefore, given previous experience with orally administered methionine, it was felt to be acceptable to increase the dose of D-met to 100 mg/kg BID, and following the amended trial design, the data presented herein represent 24 patients treated at 100 mg/kg BID and 1 patient treated at 25 mg/kg BID.

**Patient selection for phase 1 study**

Inclusion criteria included patients ages 18 to 65 years, inclusive; a histologically confirmed malignancy of the head and neck region; Karnofsky performance status (KPS) of ≥60; and female reproductive status defined
as one of the following: surgically sterile, >12 months postmenopausal, or willing to use a double form of barrier birth control during and for 30 days after treatment, and the ability to provide written informed consent. Patients also needed to have a planned course of RT with our without concurrent cisplatinum chemotherapy, where at least 50% of the oropharynx would be included within the radiation fields.

Exclusion criteria included T1/T2 glottic tumor, previous exposure to pharmacologic doses of methionine, prior exposure to therapeutic radiation, inadequate liver or renal function (defined as bilirubin or creatinine >2 times the upper limit of normal; serum albumin <2.5 g/dL; or aspartate aminotransferase, alanine aminotransferase, or alkaline phosphatase <3 times the upper limit of normal), or inadequate hematologic parameters (defined as WBC <3.5 × 10^9/L, platelet count <100 × 10^9/L, hemoglobin <9 g/dL, and hematocrit <27%). Pregnant, lactating, or breast-feeding females were also excluded as were men planning on attempting to conceive a child during or within 30 days of the study period.

Treatment regimen
Patients received RT at the Department of Radiation Oncology, Nizam’s Institute of Medical Sciences. Treatment was planned via a clinical simulator with conventional two- or three-field head and neck treatment with a ^60^C source using a Telecobalt treatment unit (Theratronics, Atomic Energy of Canada Ltd.). Patients were to receive 1.8 to 2.0 Gy daily, 5 days a week, to a total dose of 59.4 to 60 Gy as defined at the iso-center. As determined by the treating physicians, patients could also be treated with up to five weekly doses of cisplatinum concurrent with RT. Before cisplatinum infusion, patients were pretreated with ondansetron (4-8 mg i.v. or orally) with or without domperidone (4 mg i.v. or orally). Cisplatinum (50 mg/m^2^) mixed in 500 mL normal saline was administered by i.v. infusion over 40 minutes. Following cisplatin infusion, an additional 500 mL of normal saline supplemented with 20 mEq of KCl and 50 mEq of MgSO_4_ were infused over 40 minutes.

Typical treatments for oral mucositis, such as oral narcotics, Diflucan, diphenhydramine, or viscous lidocaine, were allowed. Additional treatments for mucositis, such as amifostine, Carafate, or cryotherapy, were not allowed.

MRX-1024—method of administration
MRX-1024 was provided by the study sponsor as a suspension formulation of D-met (200 mg/mL), which was stored at controlled ambient room temperature. On the first day of treatment, patients were weighed and their individual dose and corresponding volume of suspension were calculated. For each dose, the suspension was measured out by study personnel using a 50 mL beaker, and the patients ingested the drug in their presence. Patients were not allowed to self-medicate with MRX-1024. No attempt to swish, swallow, or gargle the suspension was recommended or required. The drug was not taken on weekends, holidays, or other days when radiation was not delivered.

Patient evaluation
Potential study participants were screened against the eligibility criteria. Eligible and consenting patients completed a pretreatment evaluation that included a physical exam with a thorough head and neck evaluation, estimation of KPS, vital signs, blood and urine collection, electrocardiogram, and pregnancy test when indicated. Per protocol, patients were seen according to the following schedule: a screening visit (~21 to ~1 d before treatment), baseline (before first dose of drug on day 1), during treatment (at the end of the week for each of 6-7 planned weeks of radiation with the last appointment after the last dose of drug was taken), and then 1 week after the end of treatment. Patients had weekly complete blood count and comprehensive metabolic panel including liver function tests. Adverse events were documented at each study visit. Oral mucositis was assessed as indicated below. Posttreatment assessment included a physical examination, review of adverse events, measurement of vital signs, hematology and chemistry laboratory values, and final assessment of mucosal toxicity.

Mucosal evaluation
Per predefined study criteria, any patient who completed at least 20 d of RT with MRX-1024 was presumed to have completed study with regard to assessment of mucosal toxicity. The study was conducted using the following validated, standardized scoring systems (see Supplementary Materials and Methods), which were administered at baseline and at every scheduled visit thereafter: National Cancer Institute Common Toxicity Criteria version 2 for grading of stomatitis, WHO Index Scale, Radiation Therapy Oncology Group (RTOG) Oral Mucositis Grading System—Physician-based scoring, and RTOG Oral Mucositis Grading System—Patient-reported toxicity.

Statistical analysis
Kinetic analysis and AUCs were calculated using pharmacokinetic Functions for Microsoft Excel, a series of Add-in functions for Excel spreadsheets, designed and written by Joel I. Usansky, Atul Desai, and Diane Tang-Liu (Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA). Differences between groups were evaluated using a two-tailed t test, whereas trends across groups were assessed using the χ^2_ test. In each case, a P value of <0.05 was considered significant. As a phase 1 study, no direct statistical comparison of toxicity in those treated with MRX-1024 compared with the control group was done, although descriptive analyses are presented.

Results
Pharmacokinetics of D-met in normal human volunteers. Oral methionine has been used for the treatment of liver toxicity following acetaminophen overdose (32–36),
arthritis (37), or AIDS-related neuropathy (38, 40), but most typically, it has been administered as the L-isomer or racemic mixture. Therefore, given previously shown prolonged clearance and reduced toxicity when using the D-isomer in humans compared with the L-isomer, we set about evaluating the pharmacokinetics of D-met when administered as a high-concentration oral solution (MRX-1024; refs. 24, 25, 27). A total of 12 normal human volunteers gave informed consent to take a single oral dose of MRX-1024 at either 50 or 100 mg/kg, along with serial evaluation of plasma D-met concentrations for 6 hours. As depicted in Table 1 and Fig. 1, there was rapid absorption of D-met with a prolonged time to achieve maximal concentration (P < 0.02, two-tailed t test) at the higher dose, with peak plasma concentration observed on average within 35 to 70 minutes depending on the dose administered. Not unexpectedly, there was a statistically significant and dose-dependent 2-fold increase in C\text{max} at 101.1 ± 26.4 μg/mL and 192.7 ± 28.0 μg/mL for the 50 and 100 mg/kg doses, respectively (P < 0.0003, two-tailed t test). In addition, there was also a dose-dependent 2.0-fold increase in the total exposure to D-met, with AUC_{0-inf} of 382.3 ± 133.6 μg·h/mL and 774.8 ± 127.3 μg·h/mL for the 50 and 100 mg doses, respectively (P < 0.0003, two-tailed test). Clearance of D-met was described by a first-order exponential (R^2 > 0.97 for both concentrations), and despite differences in peak concentration and exposure, there was no difference in either clearance (P > 0.2) or half-life of D-met at higher doses (P > 0.2), with mean t_{1/2} of 3.2 ± 0.2 hours. There were no significant adverse events observed in any normal human volunteers who were administered D-met.

### Phase 1 study of MRX-1024 concurrent with RT
An industry-sponsored, open-label phase 1 study was done at the Department of Radiation Oncology, Nizam’s Institute of Medical Sciences. The trial was funded by Molecular Therapeutics. The first patient was enrolled on March 3, 2004 and the last patient on July 2, 2004. The clinical data for patients at enrollment are presented in Supplementary Data, whereas the treatment delivered for each patient and toxicities observed are provided in Table 2. A variety of head and neck sites were represented; however, 64% (16 of 25) entailed disease within the oral cavity or oropharynx. There were 15 males and 10 females with a median weight of 55 kg [interquartile range (IQR), 48-65]. Median age was 47 [IQR, 40-56] with excellent performance status (median KPS, 90; IQR, 80-90). Cisplatin was administered to the majority (78%) of patients. The status of all 25 enrolled patients is depicted in Fig. 2. Of 25 patients, 18 received at least 20 days of radiation and per protocol were considered to have completed the study for assessment of oral mucositis, whereas 7 withdrew before this point. Of those who withdrew, five were potentially related to toxicity of MRX-1024 and two for other reasons. The two patients who withdrew for reasons not related to MRX-1024 included the first enrolled patient who was treated with radiation, cisplatinum, and MRX-1024 at a dose of 25 mg/kg BID who developed grade 3 mucositis during the 3rd week of treatment and withdrew from the study. The second patient complained of weakness during the 2nd week of chemotherapy, radiation, and MRX-1024 and withdrew from study during the 3rd week. This patient had no significant mucositis or adverse events.

All five patients who withdrew for toxicity related to MRX-1024 did so because of nausea and emesis: one with

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### Table 1. Pharmacokinetics of D-met following oral administration of MRX-1024 in normal human volunteers

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>MRX-1024 (50 mg/kg)</th>
<th>MRX-1024 (100 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>0.5</td>
<td>97.0 (11.0)*</td>
<td>128.2 (25.6)</td>
</tr>
<tr>
<td>1</td>
<td>80.3 (10.0)*</td>
<td>189.0 (14.6)</td>
</tr>
<tr>
<td>2</td>
<td>50.1 (6.2)*</td>
<td>126.0 (13.1)</td>
</tr>
<tr>
<td>4</td>
<td>33.1 (5.6)*</td>
<td>69.9 (3.6)</td>
</tr>
<tr>
<td>6</td>
<td>21.9 (3.7)*</td>
<td>48.8 (4.3)</td>
</tr>
<tr>
<td>T\text{max} (h)</td>
<td>0.6 (0.1)*</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td>C\text{max} (μg/mL)</td>
<td>101.1 (11.8)*</td>
<td>192.7 (13.3)</td>
</tr>
<tr>
<td>AUC_{0-inf} (μg·h/mL)</td>
<td>382.3 (59.8)*</td>
<td>793.3 (54.8)</td>
</tr>
<tr>
<td>Elimination rate constant (1/h)</td>
<td>0.2 (0.0)</td>
<td>0.2 (0.0)</td>
</tr>
<tr>
<td>Half-life (t\text{1/2}, h)</td>
<td>3.3 (0.3)</td>
<td>3.0 (0.2)</td>
</tr>
</tbody>
</table>

NOTE: Plasma D-met concentration (μg/mL) and pharmacokinetic parameters obtained. Values represent the mean (SE) for six patients at each dose level. Values that are statistically different between the two administered dose levels of MRX-1024 are marked with an asterisk. *P < 0.05.

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### Fig. 1. Pharmacokinetics of D-met. Plasma values of D-met determined by high-performance liquid chromatography analysis following oral administration of MRX-1024 to normal human volunteers at 50 mg/kg (□) and 100 mg/kg (○). Points, mean of six patients at each point with the monoeponential function for clearance plotted starting with the time of peak plasma concentration (R^2 > 0.97 for both concentrations); bars, SE.
grade 3 nausea/emetis, which was considered a DLT as it was definitely or likely related to study drug, and four with grade 2 nausea/emetis, which were not considered DLTs (see Table 2). The one patient with grade 3 emesis likely related to study drug (patient 16) was being treated with RT alone and withdrew from the study at the end of the first week of treatment. The four additional patients who experienced grade 2 emesis (one without and three with concurrent cisplatinum) were felt to be definitely or likely related to study drug use because emesis occurred later in the week and on days that cisplatinum (and antiemetics) was not delivered. These patients were offered the option to continue on study with further antiemetic therapy; however, all declined and withdrew from the trial. Thus, per protocol, DLTs occurred in 1 of 24 patients (4%) at the 100 mg/kg BID dose level.

Per protocol, patients should have received a total of 30 days of MRX-1024, whereas only 36% (9 of 25) of patients actually completed all doses of drug, with a median number of days that drug was administered of 21 (IQR, 14-30). Four of the five patients that withdrew from study due to nausea and emesis did so within the first week of therapy. If compliance to MRX-1024 administration is evaluated excluding these four, then the median number of days that patients received the study drug was 28 (IQR, 21-30).

Aside from nausea and emesis, there were no other significant toxicities observed related to MRX-1024. However, given the nature of RT with or without cisplatinum, the baseline incidence of toxicity as depicted in Table 3 was high, with 85% (21 of 25) of patients having at least grade 2 toxicity during treatment and 20% (5 of 25) of patients having grade 3 toxicity (not including mucositis). Of note, grade 3 nausea/emetis did occur in five patients (20%); however, only one of these episodes due to timing in relation to study drug was felt to be a DLT, whereas the other four cases were felt by the treating physicians to be typical of and temporally related to cisplatinum treatment. Because mucositis was a primary end point of the study, it was addressed separately, and 20% (5 of 25) of patients

### Table 2. Treatment delivered as well as adverse events observed during treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Daily RT dose (Gy)</th>
<th>Total RT dose (Gy)</th>
<th>MRX-1024 dose (mg/kg)</th>
<th>Days MRX-1024</th>
<th>CDDP cycles</th>
<th>Status</th>
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<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>24</td>
<td>25</td>
<td>12</td>
<td>3</td>
<td>Withdrew due to mucositis</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>42</td>
<td>100</td>
<td>21</td>
<td>3</td>
<td>Completed*</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>60</td>
<td>100</td>
<td>30</td>
<td>5</td>
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<tr>
<td>4</td>
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<td>60</td>
<td>100</td>
<td>30</td>
<td>1</td>
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</tr>
<tr>
<td>5</td>
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<td>60</td>
<td>100</td>
<td>30</td>
<td>5</td>
<td>Completed*†</td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>6</td>
<td>100</td>
<td>3</td>
<td>0</td>
<td>Withdrew due to grade 2 nausea</td>
</tr>
<tr>
<td>7</td>
<td>2.0</td>
<td>60</td>
<td>100</td>
<td>30</td>
<td>4</td>
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</tr>
<tr>
<td>8</td>
<td>2.0</td>
<td>28</td>
<td>100</td>
<td>14</td>
<td>2</td>
<td>Withdrew due to grade 2 nausea</td>
</tr>
<tr>
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<td>5</td>
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<td>10</td>
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<td>11</td>
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NOTE: Because mucositis was the primary end point of the study, it was not considered an adverse event for determination of the phase 1 portion of the study.

*The 18 patients who received at least 20 days of RT and were considered to have completed treatment per predefined data analysis plan and included in the analysis of mucosal injury (Fig. 3A).

†The 10 patients treated with at least 50 Gy radiation and chemotherapy and included for an unplanned subset analysis (Fig. 3B).
had grade 2 mucositis and 8% (2 of 25) had grade 3 mucositis. There were no grade 4 or greater events of either mucosal or nonmucosal toxicity.

**Mucosal injury.** Patients were scored for mucosal injury at each study visit using three different physician-rated scoring systems (National Cancer Institute Common Toxicity Criteria version 2, WHO, and RTOG; see Supplementary Materials and Methods); in addition, patients also filled out an evaluation of mucosal injury (RTOG patient-reported score; see Supplementary Materials and Methods). There was no significant difference between the timing or magnitude of mucosal injury on any of these scales ($P > 0.1$, $\chi^2$ test), so the data are presented entirely based on the patient-reported RTOG scale. Figure 3A represents the worst mucosal injury score obtained during radiation treatment for the 18 of 25 patients in the study who by initial study design were considered eligible for evaluation because they received at least 20 daily fractions of radiation (median RT dose, 57 Gy; IQR, 42.5-60.0). Single-agent cisplatinum was administered to 78% (14 of 18) of patients in conjunction with their radiation (median cycle, 3.5; IQR, 3.0-4.75). The seven patients who withdrew from the study before the 20th fraction of radiation are not included in this analysis. In this group of 18 patients, 33% (6 of 18) had no significant mucosal injury throughout the course of the study, whereas 44% (8 of 18) had only grade 1 mucosal injury and 22% (4 of 18) had mucosal injury of grade $\geq 2$. Only one patient (6%) had grade 3 mucositis, which was observed in patient 22 who was treated with four cycles of cisplatinum and 60 Gy irradiation for a squamous cell carcinoma of the buccal mucosa and showed grade 3 mucosal injury at weeks 5 and 6 of radiation that had completely resolved by the final study visit. No mucosal injury of grade $\geq 4$ was observed in any patients treated with MRX-1024. As expected, there was a greater rate of mucositis in those treated with RT, cisplatinum, and MRX-1024 (gray columns), with 21% (3 of 14) of patients with no mucositis, 50% (7 of 14) grade 1 mucositis, 21% (3 of 14) grade 2 mucositis, and 7% (1 of 14) grade 3 mucositis, but with no grade $\geq 4$ mucositis. In those treated with RT and MRX-1024 without chemotherapy (white columns), 75% (three of four) of patients had no mucositis, whereas there was 25% (one of four) grade 1 mucositis, and no patients experienced mucositis of higher than grade 1.

Because a significant number of patients dropped out of the study for reasons other than DLT, this would limit the effect of RT on mucosal injury. Therefore, in an unplanned subset analysis, we present the data on patients who would be most at risk of mucosal injury due to being treated with chemotherapy and radiation (Fig. 3B). The white columns represent the 14 patients treated with RT, MRX-1024, and cisplatinum who received at least 60% of the planned radiation dose (median RT dose, 60 Gy; IQR, 45-60), whereas the black columns represent the 10 patients who received chemotherapy, MRX-1024, and RT to at least 50 Gy. The four patients who received a lower total dose of radiation discontinued after the 4th week of radiation because of one case of grade 2 dysphagia, one grade 2 fungal infection, and two grade 2 emesis. In this select group of 10 patients treated most aggressively, the median RT dose was 60 Gy (IQR, 60-60), and all 10 received at least one cycle of cisplatin (median cycle, 4; IQR, 3-5). Patients treated with concurrent cisplatinum and RT along with
Overall, 15% (5 of 33) of patients in the control group were treated with radiation in the historical control group. 61% (20 of 33), were treated with concurrent cisplatin-therapy and radiation, a similar number of patients, the MRX-1024 cohort who were treated with chemotherapy and radiation, were treated with concurrent cisplatin-therapy and radiation, a similar number of patients, between groups. Compared with the 78% of patients in the MRX-1024 cohort who were treated with chemotherapy and radiation, a similar number of patients, 61% (20 of 33), were treated with concurrent cisplatin-therapy and radiation in the historical control group. Overall, 15% (5 of 33) of patients in the control group had no significant mucosal injury throughout the course of 6 weeks of RT, with 85% (28 of 33) developing mucosal injury of grade ≥2. Finally, in this control group, 70% (23 of 33) of patients had severe (grade ≥2) mucositis, with 49% (16 of 33) exhibiting grade 3 mucosal injury and 21% (7 of 33) with grade 4 mucosal injury.

**Discussion**

Definitive conclusions about the role of MRX-1024 in head and neck cancer treatment await further confirmatory studies. However, the pharmacokinetic data presented here show that following oral administration of MRX-1024 at 50 or 100 mg/kg, there is rapid and dose-dependent absorption of D-met, reaching a peak in ~1 hour with clearance similar to that observed for typical amino acids and as previously observed for methionine in humans (25, 27). Of note, the half-life at 3 hours is approximately six times longer than observed in rats following oral gavage, suggesting that if similar peak serum concentrations are obtained it is likely that patients will experience a much larger total exposure to the drug (23). For example, in rats administered 300 mg/kg D-met by oral gavage, peak plasma concentration was 175 μg/mL, with an AUC of 356 μg·h/mL, whereas in humans a dose of 100 mg/kg resulted in a similar peak plasma concentration of 193 mg/mL but an AUC twice as large at 775 μg·h/mL. Overall, this suggests that with regard to bioavailability, oral administration of MRX-1024 could be reasonable for prevention of mucositis from daily RT.

Previously, a dose-dependent increase in mucosal protection was observed in a mouse model of radiation-induced mucositis when daily doses of 200, 300, and 500 mg/kg were administered by oral gavage (23). In addition, D-met/L-met or strictly L-met has been reported in clinical use at doses up to 6 to 20 mg/d either acutely for acetaminophen toxicity (35) or for as long as 4 months in patients with rheumatoid arthritis (37) or 6 months for neuropathy from advanced HIV infection (38–40). Therefore, we deemed it reasonable to pursue a dose of MRX-1024 in phase 1 studies comparable with those previously used that would also achieve peak plasma D-met concentrations mirroring those providing maximal mucosal protection in preclinical models (23). As a result, following completion of pharmacokinetic studies, the phase 1 trial was altered from a dose-escalation study to a single-dose study at 100 mg/kg BID daily during radiation, and 24 of 25 patients were treated at this dose.

The only severe toxicity observed in this study that was attributable to MRX-1024 was nausea and emesis, which caused 5 of 25 patients to withdraw from the study. Per study protocol, only one of these five (as a grade 3 event) was considered a DLT. Although the other four caused the patients to withdraw from the study per protocol (as grade 2 events), they were not DLTs. In a cohort of three dose-escalation paradigm, two of six (33%) patients at a particular cohort would define the MTD, whereas one of six (17%) patients at a particular cohort would not define...
toxicity and would allow dose escalation (41). The observed rate of withdrawal due to DLT in this study was 1 of 24 (4%), and even if one includes the four additional patients who discontinued treatment due to grade 2 emesis without reaching a DLT, it was 5 of 24 (20.8%) that falls between the level considered above (33.3%) or below (16.7%) the rate of DLT in a cohort of three designs. As such, the MTD for D-met in this clinical setting has not been determined, and a dose of 100 mg/kg is considered a biologically relevant dose to continue within phase 2 analysis because it does not carry significant risk of toxicity and exposes patients to systemic doses of methionine that are shown to provide radiation protection in preclinical models.

Previously published studies can shed light on the rate and degree of nausea/emesis observed on this trial. Of the five patients who withdrew from study secondary to nausea, two of them were in patients who were receiving RT alone and as such were not being routinely premedicated with antiemetic medication, whereas three were in patients who were also receiving cisplatinum and were getting routine antiemetic medication (although all three patients withdrew on days in which they were not receiving either cisplatinum or antiemetics for cisplatinum therapy). The nausea and emesis seen for patients treated with MRX-1024 and RT alone are higher than expected; however, the rate of nausea and emesis observed in those receiving weekly cisplatinum and radiation in addition to MRX-1024 is not atypical compared with previously published studies. For instance, on RTOG 9501, 459 patients with high-risk head and neck cancers after surgical resection were randomized to 60 Gy RT alone versus 60 Gy RT with concurrent cisplatinum (42). Grade 2 toxicity was not presented in published form; however, there were no cases of grade ≥ 3 nausea/emesis (in 210 patients) for those being treated with RT alone. In comparison, in patients treated with MRX-1024 and radiation, one of six patients had nausea/emesis of grade 3. As expected, the rate of gastrointestinal disturbance was greater in those receiving cisplatinum as it is strongly emetic (43). On RTOG 9501, grade 3 to 4 nausea/emesis was observed in 19% (40 of 206) of patients during the period in which cisplatinum was administered, whereas in this phase 1 study grade 3 nausea/emesis occurred in 21% (4 of 19) of patients being treated with cisplatinum,

![Fig. 3. A. worst mucosal injury for all patients completing treatment. The worst mucosal injury by patient-reported score on the RTOG patient-reported toxicity scale during the course of RT (with or without chemotherapy) for 18 patients treated with MRX-1024 during radiation: overall (black columns), for 4 patients treated with radiation and MRX-1024 (white columns), and for 14 patients treated with radiation, cisplatinum, and MRX-1024 (gray columns). B, worst mucosal injury for patients treated most aggressively. The worst mucosal injury by patient-reported score on the RTOG patient-reported toxicity scale during the course of RT for the 14 patients treated with cisplatinum, MRX-1024, and RT (white columns) as well as the 10 patients treated with cisplatinum, MRX-1024, and at least 50 Gy of RT (black columns).](https://cancerres.aacrjournals.org/content/canres/16/9/2674/F3.large.jpg)
radiation, and MRX-1024. An increase in nausea with pharmacologic doses of methionine is similar to that observed by Di Rocco et al. [38–40] who noted the most common side effects of abdominal discomfort, dyspepsia, and nausea when evaluating L-met for prevention of AIDS-related peripheral neuropathy. Therefore, for any future evaluations of MRX-1024 for mucosal protection, it is suggested that routine antiemetic therapy be provided as necessary.

This phase 1 study was not designed or powered to make conclusions about the efficacy of D-met in decreasing mucosal injury but simply to describe the initial clinical experience and safety of this agent. The overall incidence of severe (defined as grade ≥3) mucositis in this cohort of 18 patients based on either physician- or patient-rated scoring was 6% (1 of 18), which was all grade 3, and no patients exhibited grade 4 mucositis. Even if limited to the 14 patients who received cisplatinum and RT, the rate of severe mucosal injury was still very low at 7% (1 of 14), with again no cases of grade 4 mucositis. In comparison, on RTOG 9501, patients treated with RT alone had an 18% (37 of 206) chance of severe mucositis (17% grade 3 and 1% grade 4), and those treated with concurrent cisplatinum and radiation had a 30% (62 of 206) incidence of severe mucositis (27% grade 3 and 3% grade 4 [ref. 42]). Although patients in RTOG 9501 were all treated postoperatively, the planned dose of 60 Gy in 6 weeks was identical to the planned dose on this study. The rate of severe mucosal injury in the historical control group evaluated as part of this study at 70% was somewhat higher than expected, which may be related to the high incidence of patients with oral cavity or oropharynx lesions as well as the use of 131I treatment. However, other studies of concurrent chemotherapy and RT for head and neck cancer have also recorded such high rates of mucositis [5]. Adelstein et al. reported a phase 3 trial in which 100 patients were randomized to radiation alone versus radiation with concurrent chemotherapy in locally advanced head and neck cancer and noted severe mucosal injury in 24% of patients randomized to radiation alone compared with 84% in those treated with concurrent chemotherapy and radiation [44]. Because radiation dose, fraction size, field size, and technique, along with dose, schedule, and type of chemotherapy, can all influence mucositis (2–5), it is difficult to make strong conclusions about the rates of mucosal injury in the current phase 1 study, except to note that they seem compelling enough to warrant further evaluation in phase 2 studies.

Finally, an assessment of tumor protection with concurrent RT and MRX-1024 is also beyond the power of the current phase 1 study. However, in preclinical evaluation, it was shown that unlike conventional free radical scavengers, D-met (which contains a sulfur atom but not a free sulphydryl group to act as a free radical scavenger) did not alter the initial DNA-damaging event following ionizing radiation and did not protect tumor cells from radiation both in vitro and in vivo [23]. This was in contrast to the free radical scavengers N-acetylcysteine and glutathione that both inhibited DNA damage and protected tumor cells from radiation. These results would support that D-met is not affecting either the initial DNA-damaging event or DNA-damaging sensing but instead is altering subsequent cellular responses to this damage and is doing so differently in transformed and nontransformed cells. Nevertheless, the precise cellular mechanism by which D-met seems to provide selective protection to nontransformed cells remains to be elucidated. The lack of free radical scavenging by D-met is important because a randomized trial of vitamin E and/or β-carotene administered concurrently with radiation treatment for head and neck cancer resulted in a decrease in acute toxicity [45], but with an apparent increase in tumor recurrence [45], and a decline in both cause-specific and overall survival [46].

In conclusion, oral administration of MRX-1024 resulted in a rapid dose-dependent increase in plasma D-met concentrations. In addition, when administered at a dose of 100 mg/kg BID before and after RT (with or without concurrent chemotherapy) for patients being treated for cancers of the head and neck, MRX-1024 did not result in significant acute toxicity aside from a modest increase in nausea and emesis while appearing to provide a decline in the expected rate of oral mucositis. Evaluation of MRX-1024 for mucosal protection in head and neck cancer patients in phase 2 studies is planned.

Disclose of Potential Conflicts of Interest

P. Sunkara, B.D. Ross, and A. Rehemtulla: financial interest in Molecular Therapeutics; A. Eisbruch: consultant, Molecular Therapeutics; G.V. Ramana and M.I.I.R. Naidu: commercial research grant, Molecular Therapeutics; K.C.M. Campbell and Southern Illinois University hold a patent on the use of D-met for normal tissue protection; K.C.M. Campbell: unpaid consultant to Molecular Therapeutics. All data were available to D.A. Hamstra, who was solely responsible for data analysis and preparation of this manuscript as well as for the final revision of the manuscript.

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References

# Clinical Cancer Research

## Pharmacokinetic Analysis and Phase 1 Study of MRX-1024 in Patients Treated with Radiation Therapy with or without Cisplatinum for Head and Neck Cancer


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