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Double-blind placebo-controlled multicenter phase II trial to evaluate D-methionine in preventing/reducing oral mucositis induced by radiation and chemotherapy for head and neck cancer

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Abstract

Background: The purpose of this study was to test if oral D-methionine (D-met) reduced mucositis during chemoradiotherapy.

Methods: We conducted a placebo-controlled double-blind randomized phase II trial of D-met (100 mg/kg p.o. b.i.d.) testing the rate of severe (grades 3-4) mucositis.

Results: Sixty patients were randomized. Grade 2 + oral pain was higher with placebo (79% vs 45%; $P = .0165$), whereas grade 2 + body odor was greater with D-met (3% vs 41%; $P = .0015$). Mucositis was decreased with D-met by the physician (World Health Organization [WHO], $P = .007$; Radiation Therapy Oncology Group [RTOG], $P = .009$) and patient functional scales (RTOG, $P = .0023$). The primary end point of grades 3 to 4 mucositis on the composite scale demonstrated a decrease with D-met (48% vs 24%; $P = .058$), which was borderline in significance. A planned secondary analysis of a semiquantitative scoring system noted decreased oral ulceration (2.2 vs 1.5; $P = .023$) and erythema (1.6 vs 1.1; $P = .048$) with D-met.

Conclusion: Although not meeting the primary end point, results of multiple assessments suggest that D-met decreased mucositis.

KEYWORDS
clinical trial, mucositis, radioprotector, radiotherapy, toxicity

1 | INTRODUCTION

1.1 | Statement of translational relevance

Mucositis is a common dose-limiting side-effect of radiotherapy (RT) in patients with head and neck cancer. To date, no clear treatment that mitigates this toxicity for this patient population has been routinely adopted. Previously, it was demonstrated that D-methionine (D-met) could protect nontransformed human cells in culture from radiation-induced cell death while not similarly protecting tumors cells. In addition, a phase I trial demonstrated the safety and bioavailability of oral D-met with a suggestion of decreased mucositis compared with historical controls. Here, we demonstrate in a multi-institutional randomized placebo-controlled phase II trial that D-met had no significant increased toxicity but was associated with decreased oral mouth pain and mucositis for patients treated with concurrent RT and cisplatin for squamous cell carcinoma of the head and neck (SCCHN).

In the United States, approximately 49 670 patients in 2017 will be newly diagnosed with cancers of the head and neck, and approximately 9700 will die from this disease.[1] The combination of chemotherapy and radiotherapy (CRT) is commonly utilized in patients with SCCHN. Oral mucositis (OM) is a dose-limiting side-effect of CRT, which is characterized by mucosal erythema and ulceration, often with secondary bacterial or fungal infections with severe OM occurring in 40%-80% of patients.[2] A wide range of different therapies have been evaluated for OM, including: antimicrobials,[3,4] cytokines,[5–8] keratinocyte growth factor,[9] anti-inflammatories,[10–12] coating rinses,[13] honey,[14–17] glutamine,[18] cryotherapy,[19] and laser treatment.[20] The microbial makeup of the oral cavity has also been noted to influence the development of mucositis with the flora within the oral cavity or the cytokine response prognostic for OM.[21,22]

The D-met is the dextro isomer of the essential amino acid, L-methionine; whereas MRX-1024 is a high-concentration (200 mg/mL) bioavailable suspension formulation of D-met (Molecular Therapeutics, Ann Arbor, MI). The D-met is a natural micronutrient with both the D-isomers and L-isomers present in high-concentrations in a normal diet. Due to minimal human catabolism, D-met results in higher plasma levels than L-met with $>60\%$ of D-met excreted without conversion.[23–26] Clinically, L-met has been available for decades for treatment of dermatitis (200-400 mg p.o. t.i.d.-q.i.d.), whereas the racemic mixture has been used to treat acetaminophen overdose (10 g p.o. over 12 hours).[27–31] The most common side effect of oral methionine is nausea.

The D-met was previously demonstrated in animal models to protect against oxidative stress-associated ototoxicity and nephrotoxicity from cisplatin, aminoglycosides, or noise-related injury.[32,33] The D-met also protected nontransformed human cells (fibroblasts, keratinocytes, and endothelial cells) from RT-associated cell death with a protective factor in clonogenic assays of 1.2 to 1.6. Notably, radiation protection was not observed in transformed human tumor cell lines in vitro or in vivo.[34] Fractionated irradiation of mouse oral mucosa for 5 days resulted in higher peak mucositis in control animals compared with animals pretreated with D-met with a dose-dependent increase in radiation protection from 200, 300, and 500 mg/kg yielding protective factors of 1.6, 2.1, and 2.6, respectively ($P < .003$).[34] More recently, others demonstrated protection from radiation injury with D-met in mouse and zebrafish models.[35,36]

The long clinical use of D-met plus the preclinical data showing protection from mucosal injury led to a previously reported phase I clinical trial in which 25 patients with SCCHN were treated with fractioned RT (with 78% also receiving cisplatin).[37] Pharmacokinetic analysis revealed that when administered orally at 100 mg/kg, peak and area under the curve (AUC) levels of D-met were comparable to the levels previously associated with mucosal protection in rodents. There was a modest increase in nausea/vomiting after D-met, with 5 patients withdrawing from the study due to nausea and emesis, but only 1 (1/25; 4%) incidence of dose limiting toxicity (grade 3 emesis). Only 1 in 18 patients (6%) had grade 3 mucositis with no grade 4 mucositis.

We report here a randomized controlled phase II trial of orally administered D-met along with concurrent weekly cisplatin and RT for SCCHN involving the oral cavity and/or oral pharynx.

2 | MATERIALS AND METHODS

2.1 | Trial design

After local institutional review board approval, a double-blind placebo-controlled clinical trial was performed at 4 institutions in India (See Figure 1, Supporting Information Table S1). Patients were to have newly diagnosed cancers of the head and neck with a plan to receive concurrent cisplatin and RT (minimum 60 Gy in conventional fractions) to at least 50% of the oral cavity, oral pharynx, or both. After informed consent and enrollment patients were randomly assigned to 1 of 2 treatments (D-met or placebo) in a 1:1 ratio using a computer-generated algorithm stratified by center using a fixed block size.

2.2 | Radiotherapy

Treatment was with either a ^{60}Co teletherapy unit or linear accelerator ($\geq$4 MeV) using either 2D or 3D-based CT-planning. No intensity-modulated radiotherapy (IMRT) was used. Portal margins were shaped using cerrobend blocks or a multileaf collimator. Compensators or wedges were used to assure dose homogeneity that was $\pm 5\%$ of the midplane

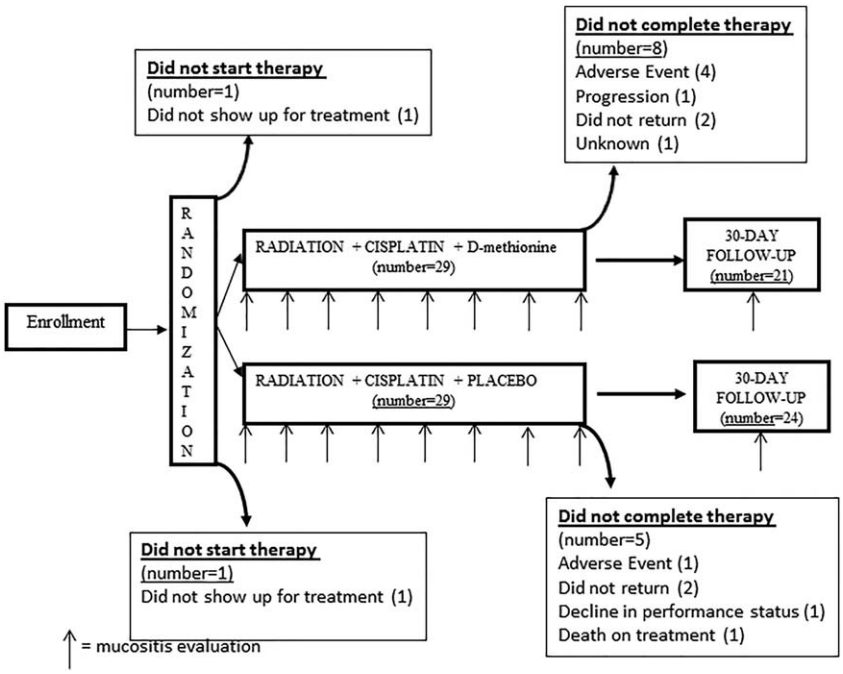

FIGURE 1 Consort diagram

central axis dose. Opposed photon portals were used while wedge pair techniques that spare mucosa on one side were excluded, except when used to boost the primary tumor after delivery of a minimum dose of 60 Gy. The administration of radiation was such that the oropharyngeal mucosa was planned to receive a central axis midplane dose of 60 to 70 Gy over 6 to 7 weeks, 1.8 to 2.0 Gy once a day.

2.3 | Cisplatin

All patients entering the study were medically appropriate to receive cisplatin, which was administered intravenously (50 mg per week) after the patient received the RT scheduled for that day. This was, on average, 28 mg/m^2 and reflected the common practice. Patients were hydrated with normal saline administered intravenously (500-1500 mL over 3-4 hours). All patients receiving cisplatin were to receive an antiemetic regimen sufficient to ameliorate this expected adverse event (AE) with 4 to 16 mg of ondansetron plus 5 to 20 mg of dexamethasone recommended; variation was allowed by institution.

2.4 | Study drug

The active pharmaceutical ingredient in MRX-1024, manufactured by stereo-specific chemical synthesis according to cyclic guanosine monophosphate guidelines, is D-methionine (CAS Registry Number 348-67-4, manufactured by Natco Pharma, Banjara Hills, Hyderabad, India).

Supplies of D-met or placebo were provided in identical amber bottles with the same labels, buffered solution, and flavoring. Patients, physicians, or study personnel responsible for preparing individual doses or for evaluating patient outcomes were unable to distinguish D-met from placebo.

2.5 | D-methionine: Method of administration

The D-met (200 mg/mL) or placebo was stored at controlled ambient room temperature. The amount to be administered was based upon the patient's body weight in the preceding week at a dose of 100 mg/kg b.i.d. The suspension was measured out by study personnel and the patients ingested the drug in their presence. No attempt to swish, swallow, or gargle the suspension was recommended or required. Patients were not allowed to self-medicate. Based upon preclinical data, the first dose was to be taken 30 to 60 minutes before RT and the second 30 to 60 minutes post-RT daily.[34] The drug was not taken on days when radiation was not delivered. Patients should not have consumed anything by mouth (other than water and scheduled medications) for 1 hour before receiving the study drug.

2.6 | Study assessments, visit schedule

Potential study participants were screened versus the inclusion and exclusion eligibility criteria, which are provided in Supporting Information Table S1. All patients had to have head and neck cancer with a plan to deliver concurrent cisplatin and RT. Eligible and consenting patients completed a baseline evaluation that included a physical examination with an oral examination, medical history, vital signs, blood collection for specified laboratory tests, and, when appropriate, a serum pregnancy test.

Patients were seen according to the following schedule: a screening visit (−21 to −1 day before treatment), baseline (before the first dose of the drug on day 1), during treatment (at the end of the week for each of 6-7 planned weeks of CRT with the last appointment after the last dose of drug was taken), and then 30 days after the end of treatment. Patients had weekly complete blood count and comprehensive metabolic panel. Toxicities were evaluated by Common Terminology Criteria for Adverse Events version 3.0 at each planned visit.

The AEs were documented at each study visit. Oral mucositis was assessed as indicted below. All patients who received at least 1 dose of the study drug and 1 fraction of RT were considered evaluable and included for analysis. The last follow-up per protocol was 30 days posttreatment with no extended follow-up planned.

Initially, an analysis of patient-reported outcomes with the Functional Assessment Cancer Therapy-Head & Neck instrument was planned; however, due to a lack of validated instruments in several of the local dialects, this aim was discontinued.

2.7 | Adverse events

Investigators, blinded to the assigned study medication being received by each patient, evaluated each reported AE for the likelihood that the event was attributable to the study medication (D-met/placebo). The investigators judged the AE as being definitely, probably, possibly, not likely, or unrelated to the study medication.

Serious adverse events (SAEs) were reported to the local institutional review boards and were defined as an AE that met any of the following: death; life-threatening; persistent or significant disability and/or incapacity; required inpatient hospitalization; and other medically significant event that may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

2.8 | Purpose

The primary purpose was to determine the efficacy of orally administered D-met in reducing the percentage of patients who develop serious (grade 3 or 4) oral mucositis.

2.9 | Planned secondary purpose included

Other purposes of this study were to determine if patients receiving D-met experience fewer complications normally associated with the development of oral mucositis compared with patients receiving placebo, specifically fewer hospitalizations for infection, less weight loss during treatment, less opioid analgesic consumption, and fewer days receiving parenteral nutrition; to determine if patients receiving D-met were able to complete their radiation and chemotherapy treatment sooner than patients receiving placebo; and to determine if patients receiving D-met obtained a similar antitumor response to radiation and chemotherapy as patients receiving placebo.

2.10 | Oral mucositis assessments

Study personnel at each site were trained in standardized mucosal evaluations before opening the study. At each visit (see Figure 1), study personnel examined the oral cavity and recorded results using each of 4 methods for assessing oral mucositis. These included the World Health Organization (WHO) grading scale for mucositis (Supporting Information Table S2), The Radiation Therapy Oncology Group (RTOG) Oral Mucositis Grading System: Gross Physician Rating (Supporting Information Table S3), the RTOG Functional Patient Rating (Supporting Information Table S3), and the Objective Scoring System for Site Assessment (Supporting Information Table S4).[38]

2.11 | Assessment of tumor response

Each patient had a CT scan of the head and neck performed within 15 days before the beginning of treatment and again 30 days after receiving their last dose of RT. The CT scans were reviewed by an independent radiologist (B.P.) at the completion of the study who was blinded to treatment allocation. The mass lesions from the baseline and follow-up CT scans were recorded and their measurements were used to stratify patients by Response Evaluation Criteria in Solid Tumors criteria.

2.12 | Sample size and statistical plan

Patients with SCCHN receiving treatment with CRT were anticipated to have 70% incidence of severe (grade 3 or grade 4) oral mucositis. Based on the phase I trial of MRX-1024, this was estimated at 10% in the experimental arm. Using a power of 0.9 and a significance level of 0.01, required a total of 40 evaluable patients; 20 per arm. Historical data within India suggested that a higher number of patients should be enrolled to account for noncompleting patients due to economic, social, cultural, or other reasons. For this reason, a sample size of 60 patients, 30 patients per arm, was selected in order to achieve 40 evaluable subjects. The study was powered for the primary but not for the secondary purposes.

The statistical analysis plan, determined before unmasking of the randomization code, established the primary end point as the proportion of patients experiencing grade 3 or greater OM using a composite of the highest score noted during treatment using the WHO and the 2 RTOG scales. Secondary analyses were planned per protocol, whereas unplanned secondary analyses were performed as indicated in a post hoc manner.

The protective effect of D-met was measured based upon cumulative mucositis and peak mucositis measurements using the area under the time mucositis curve, which was calculated using PK 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). All other statistical analyses were performed with MedCalc Statistical Software version 17.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017). All P values of $< .05$ were considered statistically significant without correction for multiple comparisons.

3 | RESULTS

3.1 | Treatment plan

The first patient was enrolled on July 29, 2005, and the last on March 17, 2006. All patients have completed their participation on this protocol. Eligible and consenting patients were randomized 1:1 to receive either MRX-1024 (an oral suspension of D-met) or a placebo. Treatment with the combination of radiation, cisplatin, and D-met/placebo continued until a total of 60 to 70 Gy of radiation was administered over 6 to 7 weeks, or until the patient terminated treatment for any reason.

3.2 | Demographics

There were no differences between treatment arms in any clinical or demographic criteria (Table 1). All patients were of Indian ancestry with 76% men, median age of 51 years, a median Karnofsky Performance Scale of 90, and >95% with squamous cell carcinoma (with 3 cases of poorly differentiated carcinoma) with involvement of the oral cavity (90%) and/or oropharynx (10%). Forty-five percent of patients had stage group III/IV disease with 15.5% with positive lymph nodes.

3.3 | Treatment

The treatment delivered is outlined in Table 2. The median number of radiation fractions delivered was 31 with no difference between arms with a median total dose 62 Gy in 1.8 to 2.0 Gy fractions. There was no difference in the type of radiation equipment utilized (Linac vs ^{60}Co; $P > .5$). Patients on the placebo arm did take longer to complete all treatment (median 48 vs 42 days; $P = .05$). On both arms, 86% of patients received at least 1 dose of cisplatin with the median number of weekly cycles on each arm being 4. The median doses of study drug delivered was 62, which was slightly higher for placebo (64) as compared to the control (60; $P = .096$).

3.4 | Adverse events, patient withdrawals, and deviations

Overall 30 patients were randomized to each arm (n = 60 total) with 29 patients on each arm initiating treatment. A similar proportion of patients did not complete treatment and follow-up on the D-met arm (8/29; 28%) as compared to the placebo arm (5/29; 17%; $P = .6$). On the experimental arm, four of eight patients not completing treatment were because of AEs, all 4 of which were from nausea and/or vomiting. There was 1 case of grade 1, 2 of grade 2, and 1 of grade 3. On the control arm, 1 patient had neutropenic fever and sepsis and subsequently died on day 32 of the study. This was not felt to be related to the study drug (placebo).

The AEs by maximum intensity for those reported in >10% of all patients are listed in Table 3. All patients experienced at least 1 AE of grade 1 or greater. The proportion of patient experiencing grade 2 + AEs (27/29; 93% control vs 28/29; 97% D-met; $P = .7$) or grade 3 + AEs (12/29; 41% control vs 10/29; 34% D-met; $P = .8$) were also not different between treatment arms There was greater nausea with D-met as compared to placebo (55% vs 17%; $P = .005$) but the majority (11/16) was grade 1. There was no difference in grade 2 + nausea between arms (17% vs 10%; $P = .7$). For grade 2 or greater AEs, only pain in the oral cavity (grade 2+: 23/29 [79%] placebo vs 13/29 [45%]; D-met; $P = .0165$) and body odor (grade 2+: 1/29 [3%] placebo vs 12/29 [41%] D-met; $P = .0015$) were different between arms. There were no differences in adverse laboratory assessments (Supporting Information Tables S5 and S6).

The SAEs, per protocol, are provided (Supporting Information Table S7) with all SAEs deemed not related to the study medication and no differences in the rate of SAEs per arm (placebo: 13; D-met: 11; $P > .5$). There were also no differences in significant protocol violations between arms (Supporting Information Table S8). One notable violation is that 11 of 29 patients (38%) on D-met and 12 of 29 patients (41%) on placebo received 5-fluorouracil (5-FU) in addition to cisplatin during CRT, which was not part of the protocol treatment.

3.5 | Mucositis evaluations

Patients were evaluated by the treating team at the start of treatment, weekly during RT, and then posttreatment day 30 (see Figure 1). Three mucositis scales were evaluated: the WHO physician-scored scale (Figure 2A), the RTOG Gross Physician Rating (Figure 2B), and the RTOG Functional Patient Rating (Figure 2C). A composite scale was also utilized that was the highest score on each of the 3 scales (Figure 2D). For both physician-scored scales (WHO and RTOG), there was a greater rate of mucositis (on a 0-4 scale) with placebo as compared to D-met ($P = .007$ WHO; $P = .0009$ RTOG) as well as a higher rate of grade 3 to 4

TABLE 1 Demographic and clinical characteristics

	D-met (n = 29)	Placebo (n = 29)	*P* value
Age, years			
Mean (SD)	50.2 (11.4)	47.7 (9.4)	.4[a]
Median (min, max)	52 (23, 64)	50 (28, 64)	
Sex, no. (%)			
Male	22 (75.9)	22 (75.9)	>0.9[b]
Female	7 (24.1)	7 (24.1)	
Ethnicity, no. (%)			
Indian	29 (100)	29 (100)	>0.9[b]
Weight, Kg			
Mean (SD)	56.3 (10.4)	53.3 (11.0)	.3[a]
Median (min, max)	56 (35, 80)	49 (30, 73)	
Body mass index, kg/m^2			
Mean (SD)	21.6 (3.5)	20.9 (4.7)	.5[a]
Median (min, max)	22.0 (15.1, 30)	19.9 (11.7, 29.9)	
Karnofsky Performance Scale			
Mean (SD)	88.2 (4.7)	87.2 (4.6)	
Median (min, max)	90 (70, 90)	90 (80, 90)	.8[a]
70, no. (%)	1 (3.4)	0	
80, no. (%)	3 (10.3)	8 (27.6)	
90, no. (%)	25 (86.2)	21 (72.4)	
Time from diagnosis to randomization			
Mean days (SD)	47.8 (92.9)	34.5 (59.9)	.5[a]
Histology/pathology, no. (%)			
Squamous cell	28 (96.6)	27 (93.1)	>0.9[b]
Other (poorly differentiated carcinoma)	1 (3.4)	2 (6.9)	
Site of primary tumor, no. (%)[c]			
Oral cavity	27 (93.1)	25 (86.2)	
Oropharynx	3 (10.3)	3 (10.3)	.7[d]
Hypopharyngeal	1 (3.4)	1 (3.4)	
Salivary gland	1 (3.4)	0	
Nasopharyngeal	0	1 (3.4)	
Nasal cavity and paranasal sinuses	0	1 (3.4)	
Stage, no. (%)			
I	6 (20.7)	3 (10.3)	.16[d overall]
II	6 (20.7)	14 (48.3)	
III	12 (41.4)	7 (24.1)	
IV	4 (13.8)	5 (17.2)	
Stage III/IV	16 (55.2)	12 (41.3)	.43[b Stage III/IV]
Not done	1 (3.4)	0	

(Continues)

mucositis (41% vs 17% WHO, $P = .045$; 48% vs 21% RTOG, $P = .0285$). For the RTOG Functional Patient Rating, there was a lower rate of mucositis overall with D-met ($P = .0023$) but the difference in grade 3 to 4 mucositis favoring D-met was not statistically significant (41% vs 24%; $P = .16$).

The primary end point, which was predetermined before analysis, was a reduction in the rate of grade 3 to 4 mucositis

TABLE 1 (Continued)

	D-met (n = 29)	Placebo (n = 29)	*P* value
Sites of metastases, no. (%)[c]			
Any	6 (20.7)	3 (10.3)	.5[b]
Lymph nodes, neck	3 (10.3)	1 (3.4)	
Cervical	1 (3.4)	2 (6.9)	
Submandibular	2 (6.9)	0	
Thyroid	1 (3.4)	0	

Abbreviation: D-met, D-methionine.
[a]*T* test.
[b]Fisher's Exact Test.
[c]The total exceeds 100% because some patients had multiple sites of primary tumor and multiple sites of nodal metastases reported.
[d]Chi-square test.

using the composite scale (Figure 2D). This was twice as likely with placebo (14/29; 48%) as compared to D-met (7/29; 24%), but this difference was not statistically significant ($P = .058$). However, the overall mucositis score (range 0-4) was lower with the use of D-met ($P = .0018$). On the composite scale, 31% of D-met patients (9/29) had grade 0 to 1 mucositis,

TABLE 2 Summary of study treatment

Treatment	D-met (n = 29)	Placebo (n = 29)	*P* value
RT			
No. of fractions received per patient			
Mean (SD)	26.6 (9.0)	29.7 (5.7)	.4[a]
Median (min, max)	30 (1, 35)	32 (10, 34)	
Total Gy administered per patient			
Mean (SD)	52.2 (18.6)	57.8 (12.1)	.2[a]
Median (min, max)	60 (1.8, 70)	64 (18, 68)	
Time to complete RT per patient, days			
Mean (SD)	39.6 (14.6)	47.1 (12.2)	0.05[a]
Median (min, max)	42 (1, 65)	48 (13, 75)	
No. of treatment device (%)			
Cobalt	10 (34)	10 (34)	1.0[b]
Linear accelerator (≥4 MV)	19 (66)	19 (66)	
Cisplatin			
No. of patients receiving ≥1 (%)			
Dose of cisplatin	25 (86.2)	25 (86.2)	1.0[b]
No. of cisplatin doses per patient			
Mean (SD)	3.2 (1.9)	3.2 (1.8)	0.98[a]
Median (min, max)	4 (0, 6)	4 (0, 7)	
Study drug			
No. of doses administered per patient			
Mean (SD)	52.7 (18.3)	59.5 (11.5)	0.096[a]
Median (min, max)	60 (2, 69)	64 (20, 69)	
No. of days dosed per patient			
Mean (SD)	26.7 (9.1)	29.7 (5.8)	0.6[a]
Median (min, max)	30 (1, 35)	32 (10, 34)	
Dose drug/RT treatment			1.0[b]
Mean/mean	2.0	2.0	
Median/median	2.0	2.0	

Abbreviations: D-met, D-methionine; RT, radiotherapy.
[a]*T* test.
[b]Fisher's exact test.

TABLE 3 Summary of most frequently reported (>10%) all-cause adverse events by body system, maximum intensity, and treatment group

	D-met (n = 29)					Placebo (Number = 29)				
Body system[a]	Total (%)	Grade 1	Grade 2	Grade 3	Grade 4	Total (%)	Grade 1	Grade 2	Grade 3	Grade 4
Total patients	29 (100)	1	18	10	0	29 (100)	2	15	11	1 (Gr. 5[c])
Digestive/gastrointestinal	29 (100)	5	16	8	0	28 (96.6)	1	15	12	0
Vomiting	16 (55.2)	8	7	1	0	13 (44.8)	3	9	1	0
Nausea	16 (55.2)	11	4	1	0	5 (17.2)	2	3	0	0
Pain in oral cavity	16 (55.2)	3	9	4	0	25 (86.2)	2	15	8	0
Constipation	12 (41.4)	9	3	0	0	6 (20.7)	5	1	0	0
Anorexia	6 (20.7)	1	2	3	0	8 (27.6)	0	3	5	0
Dysphagia	5 (17.2)	2	2	1	0	6 (20.7)	2	2	2	0
Diarrhea	4 (13.8)	4	0	0	0	3 (10.4)	2	1	0	0
Dyspepsia/heartburn	3 (10.3)	1	2	0	0	1 (3.4)	1	0	0	0
Xerostomia	1 (3.4)	1	0	0	0	3 (10.4)	2	0	1	0
Infection in oral cavity	1 (3.4)	0	1	0	0	3 (10.4)	0	3	0	0
Constitutional symptoms	22 (75.9)	2	18	2	0	18 (62.1)	6	11	1	0
Odor (body, breath, or urine)	15 (51.7)	3	11	1	0	2 (6.9)	1	1	0	0
Fatigue	10 (34.5)	2	7	1	0	11 (37.9)	2	8	1	0
Fever	5 (17.2)	2	3	0	0	7 (24.1)	5	2	0	0
Insomnia	4 (13.8)	1	3	0	0	5 (17.2)	2	3	0	0
Musculoskeletal system	12 (41.4)	4	7	1	0	9 (31.0)	4	4	1	0
Pain in jaw	7 (24.1)	3	3	1	0	6 (20.7)	1	4	1	0
Pain in ear	4 (13.8)	1	3	0	0	2 (6.9)	1	1	0	0
Pulmonary	10 (34.5)	6	4	0	0	11 (37.9)	5	4	1	1
Cough	8 (27.6)	5	3	0	0	9 (31.0)	5	4	0	0
Pain, sore throat	3 (10.3)	1	2	0	0	1 (3.4)	0	1	0	0
Dermatologic conditions	9 (31.0)	6	3	0	0	9 (31.0)	7	2	0	0
Rash	8 (27.6)	6	2	0	0	8 (27.6)	6	2	0	0
Body as a whole	4 (13.8)	0	2	2	0	3 (10.3)	1	2	0	0
Infection	3 (10.3)	6	2	0	0	1 (3.4)	0	1	0	0
Blood/bone marrow	4 (13.8)	0	2	2	0	3 (10.3)	1	1	0	0
Leukopenia	1 (3.4)	0	0	1	0	3 (10.3)	1	1	1	0
Anemia	3 (10.3)	0	2	1	0	1 (3.4)	0	0	0	1
Neurologic system	4 (13.8)	1	2	1	0	4 (13.8)	0	3	1	0
Headache	2 (6.9)	0	2	0	0	4 (13.8)	0	3	1	0

[a]Totals for each body system count each patient once, using the highest grade of adverse event reported within that body system.
[b]Totals for individual adverse events count each patient once. If multiple occurrences of the same adverse event were reported, the patient was counted once under the highest intensity of that event.
[c]One patient in the placebo treatment group developed grade 4 adverse events of anemia, hypotension, and dyspnea resulting in his death (grade 5).

although this was only 3% (1/29) on the placebo arm ($P = .008$). In addition, if grades 3 and 4 mucositis were considered separately (where there were 2 cases [7%] grade 4 on the control arm as compared to zero cases on the experimental arm) there was also a difference favoring D-met ($P = .033$). Finally, 1 patient on the placebo arm died of sepsis after developing grade 4 mucositis (by the WHO and the RTOG patient scale with grade 3 mucositis by the RTOG physician scale) after 38 Gy in 2 Gy fractions and 4 weekly doses of cisplatin, although there were no deaths on the experimental arm.

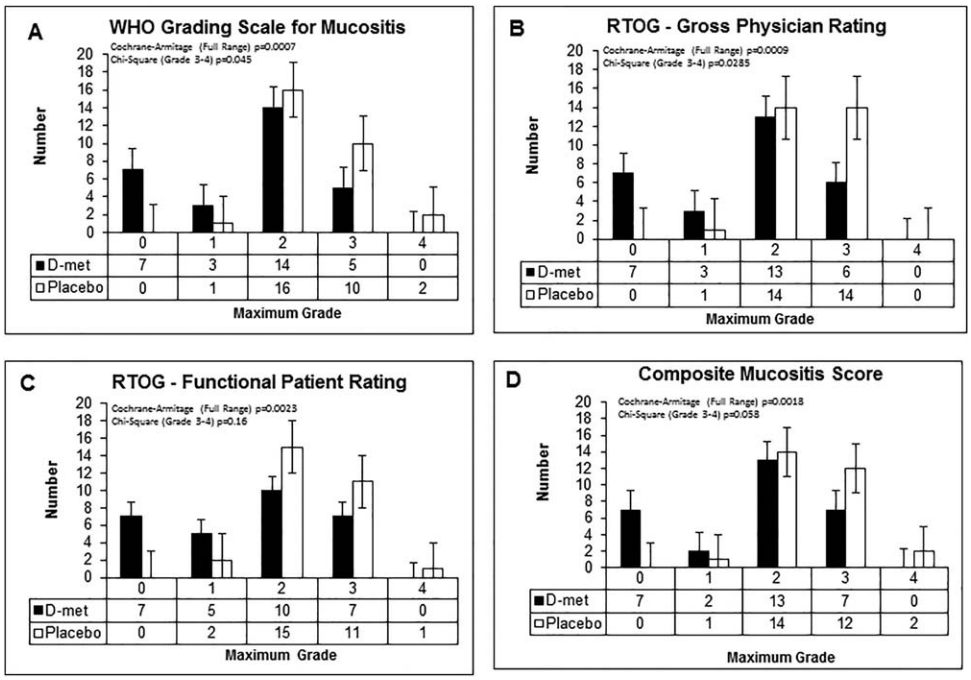

FIGURE 2 Maximum mucositis score (and SE) observed for the World Health Organization (WHO) A, Radiation Therapy Oncology Group (RTOG) Physician B, RTOG Functional Patient C, or Composite Scale D. D-met, D-methionine

For those who developed grade 3 to 4 mucositis using the composite scale (14 placebo and 7 control) this occurred, on average, 24 (SD 13) days from starting treatment on the placebo arm and 30 (SD 8) days on the D-met arm ($P > .2$).

3.6 | Secondary end points

3.6.1 | Planned secondary end points

An additional scoring system was also utilized, per protocol, in which 9 areas in the mouth were assessed weekly for both ulceration and erythema (see Supporting Information Table S4).[38] The instrument was scored as described with the data plotted in Figure 3A as the average peak scores summated from each of those 9 areas over time. For ulceration as a continuous scale (0-3), the use of D-met was associated with a 0.7 point reduction in the average peak ulceration score (difference: −0.70; SE 0.24; $P = .006$), which was 2.2 (0.68) for placebo and 1.5 (1.1) for D-met. Although, for erythema on a continuous scale (0-2), the use of D-met was associated with a 0.5 point reduction in peak erythema score (difference: −0.52; SE 0.18; $P = .005$), which was 1.6 (0.49) for placebo and 1.1 (0.82) for D-met.

No significant differences were found for any of the other planned secondary end points. There was no difference in hospitalization rates (3/29 [10%] placebo vs 2/29 [7%] D-

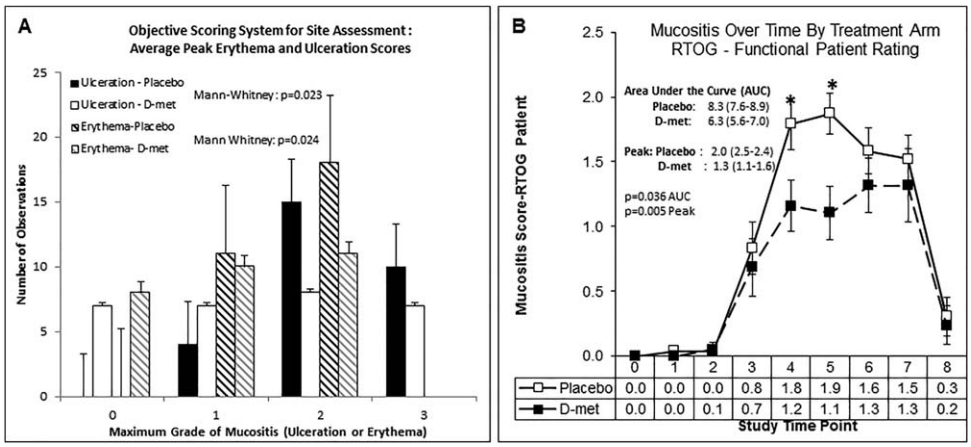

FIGURE 3 Oral mucositis by maximum grade using the Objective Scoring System for Site Assessment (mean number of observations with SE) by treatment arm for placebo or D-methionine (D-met) treatment. A, Time-dependent analysis of mucositis using the Radiation Therapy Oncology Group (RTOG) Functional Patient Rating (mean score with SE) along with calculated peak and area under the curve (AUC) B

met; $P = .64$) nor weight loss (4.4 kg; SD 3.0; placebo vs 4.2; SD 3.2; D-met; $P = .8$). Supportive therapy use was also not different for either opioid analgesics for pain control (12/29 [41%] placebo vs 9/29 [31%] D-met; $P = .62$) or the need for total parenteral nutrition (4/29 [14%] placebo vs 1/29 [3%] D-met; $P = .16$).

Per protocol, the last day of follow-up was scheduled for 30 days after the completion of RT with no difference in attendance at this time (24/29 [83%] placebo vs 21/29 [72%] D-met; $P = .35$). Treatment response was assessed by CT scan with 50% of subjects (29/58; placebo = 16; D-met = 13) having a baseline CT scan, measurable disease on this scan, and a follow-up scan at day 30 (Supporting Information Table S9). Based upon radiographic review blinded to treatment allocation, there was no difference in response rates between treatments with 62.5% (10/16) response (partial response [PR] or complete response [CR]) for placebo and 46.2% response (6/13) for D-met ($P = .48$).

3.7 | Unplanned secondary analyses

3.7.1 | Peak and area under the time/mucositis curve

As an additional unplanned analysis, the time-dependent nature of mucositis was plotted for the patient-reported RTOG scale in Figure 3B. Mucositis on a scale of 0 to 4 is plotted from the baseline visit (0) through the weekly treatment visits[1–7] and the final follow-up appointment.[8] The integral of mucositis over time was calculated and reported as the AUC, which was higher for placebo (AUC 8.3; 95% confidence interval [CI] 7.6-8.9) as compared to D-met (AUC 6.3; 95% CI 5.6-7.0; $P = .036$). This led to a protective factor (placebo/D-met) of 1.3.

For peak mucositis there was a similar relationship with average peak value of 1.9 (95% CI 1.5-2.4) for placebo as compared to 1.3 (95% CI 1.1-1.6) for D-met, which was statistically different ($P = .005$) with a protective factor of 1.5. Peak mucositis was statistically different at weeks 4 and 5 but not at other time points. A similar relationship for time-dependent mucositis and peak mucositis with similar protective factors was seen for all 3 scales (Supporting Information Table S10).

3.8 | Missing data

One potential confounding factor is that more patients withdrew from treatment with D-met than with placebo. For those who dropped out, the mucositis score on their last assessment was compared between those with placebo or D-met for any patient who had <9 mucositis evaluations (Supporting Information Table S11). This revealed that patients who missed evaluations on the placebo arm had higher mucositis scores before missing data than those on the D-met arm (2.8-3.0 vs 1.0-1.2, all P values $< .002$). In addition, patients nonevaluable on the placebo arm had higher peak mucositis scores than placebo patients who completed treatment (2.8-3.0 vs 2.0-2.0, for all 3 scales, all P values $< .01$). In contrast, those who were not evaluable on the D-met arm did not have higher peak mucositis scores then the population that was fully evaluable and treated with D-met (1.0-1.2 vs 1.3-1.4, all P value $> .05$). However, on the D-met arm, there was a trend to those missing mucositis evaluations having higher rates of grade 1 + nausea (42% vs 18%; $P = .09$) without a difference in grade 2 or greater nausea; whereas on the placebo arm, there was no difference in grade 1 or greater than grade 1 nausea for those who completed all mucositis evaluation as compared to those who missed mucositis assessments ($P > .5$). Nevertheless, differences in timing of these mucositis evaluations in those who dropped out of therapy or did not limit the conclusions to be made were based upon an unplanned secondary analysis.

3.9 | Radiation dose and mucositis

We also evaluated the impact of RT dose on mucositis for the WHO scale (Supporting Information Table S12). By univariate regression, increasing radiation dose (<20, 20-39, 40-59, and 60-70 Gy) correlated with increasing mucositis ($P = .03$), whereas D-met was protective ($P = .0005$). On multivariate regression, the use of D-met retained significance ($P = .001$), whereas radiation dose was borderline ($P = .064$). When analyzed as the likelihood of having grade 3 to 4 mucositis by logistic regression, the use of D-met after adjusting for RT dose was associated with a substantial reduction in the rate of grade 3 to 4 mucositis (odds ratio [OR] 0.29; 95% CI 0.09-0.99; $P = .05$), whereas RT dose was not correlated with grade 3 to 4 mucositis ($P = .93$).

3.10 | Use of 5-flourouracil and mucositis

Some patients also received 5-FU (12 in the placebo group and 11 in the D-met group; Supporting Information Table S13), which was outside of the recommended protocol therapy. Logistic regression was performed to assess the rate of grade 3 to 4 mucositis as a function of treatment (placebo vs D-met) as well as the use of 5-FU (no vs yes) for the WHO scale. Overall, in this model, the use of D-met was protective of grade 3 to 4 mucositis (OR 0.29; 95% CI 0.09-0.98; $P = .047$), whereas the use of 5-FU did not influence mucositis (OR 0.72; 95% CI 0.21-2.4; $P = .60$). Similarly, when analyzing the complete WHO scale for mucositis (0–4) the use of D-met was associated with an approximately 1.0 point decrease in maximal mucositis score (difference 0.87; SD 0.23; $P = .0005$), whereas 5-FU use did not influence score (difference 0.06; SD 0.24; $P = .81$).

4 | DISCUSSION

This multi-institutional phase II trial was undertaken to assess if the efficacy observed in the single center phase I trial of oral D-met to prevent OM could be confirmed. In planning the trial, the control arm was assumed to have a 70% incidence of grade 3 to 4 mucositis and that after D-met it would be 10%. As such, a sample size of 40 evaluable patients was needed. The observed rate of grade 3 to 4 mucositis was lower on the control arm than anticipated with 14 of 29 patients (48%) having severe mucositis, whereas that in the experimental arm was higher than anticipated with 7 of 29 patients (24%) having severe mucositis. As a result, this study did not meet its primary end point of comparing the rate of grade 3 to 4 mucositis between arms based upon the composite scale ($P = .058$). Based upon other studies, it seems that the primary deficiency was that the 70% assumed rate of grade 3 to 4 mucositis on the control arm (as reported for the phase I trial[37] was higher than observed on the control arm of the current study; although, the rate we did observe is more in line with other published clinical trials. As a result, statistical significance was not obtained for the primary end point.

Of note, a number of planned and unplanned complementary analyses of mucositis were also undertaken with strong support for reduced mucositis in patients treated with D-met. This included decreased mucositis when looking at all 4 scales utilized over their full range (WHO, RTOG physician, RTOG functional patient, and the composite scale, all $P < .003$). In addition, no grade 4 mucositis was noted in any patient treated with D-met, whereas 2 of 29 of patients (7%) had grade 4 mucositis when treated with placebo, and 1 patient died secondary to sepsis on the placebo arm (potentially related to grade 4 mucositis). If grade 4 mucositis is addressed separately from grade 3, then all 4 scales would also support a protective effect of D-met (all $P < .009$). Another preplanned analysis was the use of the Objective Scoring System for Site Assessment to assess ulceration and erythema separately across 9 areas of the oral cavity or oropharynx, in which D-met resulted in lower scores for both of these planned evaluations (both $P < .007$). It is well documented that treatment delays for SCCHN decrease local control and in a preplanned analysis the use of D-met was associated with an approximate 6 day shorter treatment course than placebo ($P = .05$); whereas patients missing treatment on the placebo arm had higher mucositis scores then patients remaining on treatment consistent with treatment breaks for mucositis in the placebo arm (all $P < .0016$). Finally, unplanned analyses taking into account the time-dependent exposure of mucositis as the AUC as well as the impact of both RT dose and the use of 5-FU concurrent with cisplatin and RT all supported a protective effect of D-met (all $P < .05$).

In the preclinical data, a stronger correlation was noted between the peak plasma concentration (C_{max}) of D-met and radiation protection factor (R^2 0.94) as compared to D-met AUC (R^2 0.31; D.A.H. unpublished data). Peak serum concentrations were higher in humans (100 mg/kg p.o.; C_{max} 192 μg/mL) [37] as compared with rodents (150 mg/kg p.o.; C_{max} 71 μg/mL),[34] whereas given the longer half-life in humans (3.0 hours vs 1.0 hours), the total exposure after oral dosing was even higher in humans (AUC 793 vs 211 μg*hr/mL).[34,37] The protective factor observed here of 1.3 to 1.5 is lower than that predicted based upon extrapolating from a comparable C_{max} in rodents, which would have been 2.1.[34] Nevertheless, given the much longer half-life in man (and correspondingly much higher AUC), this is still most consistent with radiation protection correlating best with peak serum concentration. Notably, in rodents, peak serum concentrations were markedly higher after i.v. administration then after p.o., which could potentially have implications for further development of D-met as a radioprotector.

There were no SAEs noted with the use of D-met, although 4 patients did withdraw from the study due to nausea/vomiting (most grade 1-2). This is consistent with previous reports of pharmacologic doses of methionine. As a result, it is recommended that antiemetics that are active in the setting of mildly emesis-inducing drugs be utilized prophylactically if D-met is going to be administered, as outlined herein.

Oral mucositis continues to be a significant burden for patients treated for SCCHN with combined CRT. The current study was undertaken in India where consumption of betel nut leads to a high rate of squamous cell cancers involving the oral cavity and oropharynx. However, in western countries, alcohol and tobacco-related SCCHN were traditionally more prevalent, whereas more recently there has been a significant increase in SCCHN-related to human papillomavirus infection. Nevertheless, the combination of RT and chemotherapy is still associated with oral or pharyngeal mucositis in a high proportion of patients regardless of patient heritage or the causative agent for their SCCHN.[2] In addition, the treatment utilized here with CT planned 2D or 3D conformal therapy also does not reflect current treatment standards; however, newer technologies, such as parotid-sparing IMRT have not reduced mucositis, perhaps due to spreading the dose more to the mucosal surfaces with IMRT. The only phase III trial comparing 3D-conformal RT to parotid-sparing IMRT reported a numerically higher but not significantly different rate of grade 3 to 4 mucositis in those getting IMRT as compared to 3D-treatments (60% IMRT vs 44% 3D; $P > .05$).[39] Similarly, in a randomized trial, the use of every 3-week cisplatin (100 mg/m^2) also correlated with a higher (albeit not statistically different) rate of oral mucositis when compared to weekly cisplatin (30 mg/m^2; 53% vs 40%; $P > .05$).[40] In

this context, the rate of grade 3 to 4 mucositis observed here using conventional RT and weekly cisplatin (48%) is consistent with these previous reports, whereas that with the addition of D-met (24%) is lower. As a result, the protective effect of D-met potentially identified herein likely is still applicable even with different demographic and treatment-related characteristics.

A number of other agents have been reported recently as to their ability to mitigate oral mucositis. Most prominently is topical honey, in which 4 small phase III trials (all performed outside the United States) seemed to show significantly reduced mucositis as compared to placebo or best standard of care with the most common regimen being topical honey administered before and 2 times after RT for up to 6 hours. Given the antibacterial and antimicrobial properties reported for honey, it is felt that this may be its mechanism of action. A recent large phase II trial performed by the RTOG in patients receiving thoracic RT, however, did not note a benefit of Manuka Honey using either liquid or lozenge formulation as compared to best standard of care in reducing esophagitis.[41] Benzydamine (a locally acting topical nonsteroidal anti-inflammatory) was also demonstrated to decrease OM when compared to saline mouth wash daily during RT with the greatest effect in reducing oral pain.[42] Caphasol, which is marketed to lubricate the mouth for xerostomia, did not result in any decrease in mucositis when provided during RT.[43] Finally, in a single dose study, the use of doxepin (a tricyclic antidepressant) or "Magic Mouth Wash" (lidocaine-containing rinse) each compared to placebo noted decreased oral pain in the first 60 minutes with either experimental agent, whereas those receiving doxepin had increased fatigue compared to placebo.[44]

Taken together, the results reported here are suggestive of a protective effect of D-met in preventing OM. Although the study did not achieve its primary end point, the remainder of the data are robust and supportive of an effect. Further studies of D-met powered to assess tumor response as well as mucosal protection are warranted.

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CONFLICT OF INTEREST

B.D.R. and A. R. had a financial interest in Molecular Therapeutics Inc, which has now been disincorporated. P. S. was employed by Molecular Therapeutics Inc. S.B. was a paid consultant for Molecular Therapeutics Inc and was employed by Clinical Evaluation and Testing Services, Bangalore, India. K.C.M.C. and Southern Illinois University hold a patent on the use of D-methionine to decrease normal tissue toxicity. A.E. acted as a paid consulted to Molecular Therapeutics Inc. K.C.L. has no financial disclosures to declare. B.P. worked and was funded through a grant to St John's Medical College.

AUTHOR CONTRIBUTIONS

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Data analysis: Ross, Rehemtulla, Sunkara, Borgonha, Eisbruch, Hamstra

Manuscript preparation: Ross, Rehemtulla, Sunkara, Borgonha, Campbell, Eisbruch, Lee, Phillip, Hamstra

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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