A Double-blind Placebo-controlled Multicenter Phase 2 Trial to Evaluate D-methionine in Preventing/Reducing Oral Mucositis Induced by Radiation and Chemotherapy for Head and Neck Cancer.

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Abbreviations: D-met , D-methionine, L-met, L-methionine, MRX-1024, A proprietary orally available suspension of D-met, OM, oral mucositis, SCCHN, Squamous cell carcinoma of the head and neck, AUC, area under the concentration vs. time curve, Cmax, peak concentration, KPS, Karnofsky performance status, IQR, inter-quartile range, CDDP, cisplatin, SD, standard deviation, SEM, standard error of the mean, PF, protective factor.

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Abstract

Background: To test if oral D-methionine (D-met) reduced mucositis during chemoradiotherapy.

Methods: Placebo controlled double-blind randomized Phase 2 trial of D-met (100 mg/kg po BID) testing the rate of severe (grade 3-4) mucositis.

Results: Sixty patients were randomized. Grade 2+ oral pain was higher with placebo (79% *vs*. 45%, p=0.0165) while grade 2+ body odor was greater with D-met (3% *vs*. 41%, p=0.0015). Mucositis was decreased with D-met by physician (WHO, p=0.007, RTOG, p=0.009) and patient functional scales (RTOG, p=0.0023). The primary end-point of grade 3-4 mucositis on the composite scale demonstrated a decrease with D-met (48% *vs*. 24%, p=0.058) which was borderline in significance. A planned secondary analysis of a semi-quantitative scoring system noted decreased oral ulceration (2.2 *vs*. 1.5, p=0.023) and erythema (1.6 *vs*. 1.1, p=0.048) with D-met.

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

147/150 words

Statement of 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 routinely adopted. Previously it was demonstrated that D-methionine (D-met) could protect non-transformed 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 to historical controls. Here we demonstrate in a multi-institutional randomized placebo controlled phase 2 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 SCCHN.

Summary : 120 words.

Manuscript: 4854 words

Introduction

In the U.S., approximately 49,670 patients in 2017 will be newly diagnosed with cancers of the head and neck, and approximately 9,700 will die from this disease(1). The combination of chemotherapy and radiotherapy (ChemoRT) is commonly utilized in patients with squamous cell cancers of the head and neck (SCCHN). Oral mucositis (OM) is a dose-limiting side-effect of chemoRT 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 make-up of the oral cavity has also been noted to influence the development of mucostiis with the flora within the oral cavity or the cytokine response prognostic for OM(21,22).

D-methionine (D-met) is the dextro isomer of the essential amino acid, L-methionine; while MRX-1024 is a high-concentration (200 mg/ml) bio-available suspension formulation of D-met (Molecular Therapeutics Inc, Ann Arbor, MI). D-methionine is a natural micronutrient with both the D- 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 po TID-QID) while the racemic mixture has been used to treat acetaminophen overdose (10 g po over 12 hr).(27-31) The most common side-effect of oral methionine is nausea.

D-methionine was previously demonstrated in animal models to protect against oxidative stress associated ototoxicity and nephrotoxicity from cisplatin, aminoglycosides, or noise related injury.(32,33) D-methionine also protected non-transformed human cells (fibroblasts, keratinocytes, and endothelial cells) from RT associated cell death with a protective factor in clonogenic assays of 1.2-1.6. 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 to animals pre-treated 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 ($p<0.0003$).(34) More recently others demonstrated protection from radiation injury with D-met in mouse and zebra fish models.(35,36)

The long clinical use of D-met plus the pre-clinical data showing protection from mucosal injury led to a previously reported Phase I clinical trial where 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 following 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 one in 18 patients (6%) had grade 3 mucositis with no grade 4 mucositis.

We report here a randomized controlled Phase 2 trial of orally administered D-met along with concurrent weekly cisplatin and radiotherapy for SCCHN involving the oral cavity and/or oral pharynx.

Materials and Methods

Trial Design

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

Radiation Therapy

Treatment was with either a ^{60}Co teletherapy unit or linear accelerator (≥4MeV) using either 2D or 3D based CT-planning. No intensity modulated radiotherapy was used. Portal margins were shaped using cerrobend blocks or a multileaf collimator. Compensators or wedges were used to assure dose homogeneity that was ± 5% of the midplane 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 60-70 Gy over 6-7 weeks, 1.8 to 2.0 Gy once a day.

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 with 4-16 mg of ondansetron plus 5-20 mg of dexamethasone recommended; variation was allowed by institution.

Study Drug

The active pharmaceutical ingredient in MRX-1024, manufactured by stereo-specific chemical synthesis according to cGMP guidelines, is D-methionine (CAS Registry Number 348-67-4, manufactured by Natco Pharma Ltd, 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.

D-methionine - Method of Administration

D-met (200 mg/ml) or placebo were 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 BID. 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 pre-clinical data the first dose was to be taken 30-60 minutes prior to RT and the second 30-60 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 one hour prior to receiving study drug.

Study Assessments, Visit Schedule

Potential study participants were screened versus the inclusion and exclusion eligibility criteria which are provided in Supplemental Table 1. All patients had to have head and neck cancer with a plan to deliver concurrent cisplatin and radiotherapy. 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 prior to 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 ChemoRT 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 (CBC) and comprehensive metabolic panel. Toxicities were evaluated by CTCAE version 3.0 at each planned visit.

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

Initially an analysis of patient reported outcomes with the FACT-H&N instrument was planned; however, due to a lack of validated instruments in several of the local dialects this aim was discontinued.

Adverse Events (AE)

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 AE as being Definitely, Probably, Possibly, Not Likely, or Unrelated to the study medication.

Serious Adverse Events (SAE) were reported to the local IRBs 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;
- Other medically significant event that may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Objectives:

- The primary objective 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.

Planned secondary objectives included:

- To determine if patients receiving D-met experience fewer complications normally associated with the development of oral mucositis compared to 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;
- To determine if patients receiving D-met obtained a similar antitumor response to radiation and chemotherapy as patients receiving placebo.

Oral Mucositis Assessments

Study personnel at each site were trained in standardized mucosal evaluations prior to opening the study. At each visit (see Figure 1), study personnel examined the oral cavity and recorded results using each of four methods for assessing oral mucositis. These included the World Health Organization (WHO) grading scale for mucositis (Supplemental Table 2), The Radiation Therapy Oncology Group (RTOG) Oral Mucositis Grading System: Gross Physician Rating (Supplemental Table 3), the RTOG Functional Patient Rating (Supplemental Table 3), and the Objective Scoring System for Site Assessment (OSSFA, Supplemental Table 4)(38).

Assessment of Tumor Response

Each patient had a CT scan of the head and neck performed within 15 days prior to beginning treatment and again 30 days after receiving their last dose of radiotherapy. 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 used to stratify patients by RECIST criteria.

Sample Size and Statistical Plan

Patients with SCCHN receiving treatment with ChemoRT were anticipated to have 70% incidence of severe (Grade 3 or Grade 4) oral mucositis. Based on the Phase 1 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 non-completing 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. The study was powered for the primary but not for the secondary objectives.

The statistical analysis plan, determined prior to 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 while 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 (AUC) 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 analysis was performed with MedCalc Statistical Software version 17.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017). All p-values of <0.05 were considered statistically significant without correction for multiple comparisons.

RESULTS

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-methionine) or a placebo. Treatment with the combination of radiation, cisplatin, and D-met/placebo continued until a total of 60-70 Gy of radiation was administered over 6 to 7 weeks, or until the patient terminated treatment for any reason.

Demographics

There were no differences between treatment arms in any clinical or demographic criteria (Table 1). All patients were of Indian ancestry with 76% male, median age of 51 years, a median KPS 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.

Treatment

The treatment delivered is outlined in Table 2. Median number of radiation fractions delivered was 31 with no difference between arms with a median total dose 62 Gy in 1.8-2.0 Gy fractions. There was no difference in the type of radiation equipment utilized (Linac *vs*. ^{60}Co, p>0.5). Patients on the placebo arm did take longer to complete all treatment (median 48 *vs*. 42 days, p=0.05). On both arms 86% of patients received at least one 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 control (60, p=0.096).

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=0.6). On the experimental arm 4/8 patients not completing treatment were for adverse events all 4 of which were from nausea and/or vomiting. There was one case of grade 1, two of grade 2, and one of grade 3. On the control arm one patient had neutropenic fever and sepsis and subsequently died on day 32 of study. This was not felt to be related to study drug (placebo).

Adverse events by maximum intensity for those reported in >10% of all patients are listed in Table 3. All patients experienced at least one adverse event of grade 1 or greater. The proportion of patient experiencing Grade 2+ AEs (27/29: 93% control *vs*. 28/29: 97% D-met, p=0.7) or Grade 3+ AEs (12/29:41% control *vs*. 10/29:34% D-met, p=0.8) were also not different between treatment arms There was greater nausea with D-met as compared to placebo (55% *vs*. 17%, p=0.005) but the majority (11/16) was Grade 1. There was no difference in Grade 2+ nausea between arms (17% *vs*. 10%, p=0.7). For grade 2 or greater AEs only pain in oral cavity (Grade 2+: 23/29 (79%) placebo *vs*. 13/29 (45%) D-met, p=0.0165) and body odor (Grade 2+: 1/29 (3%) placebo *vs*. 12/29 (41%) D-met, p=0.0015) were different between arms. There were no differences in adverse laboratory assessments (Supplemental Tables 5 and 6).

Serious adverse events per protocol are provided (Supplemental Table 7) with all SAEs deemed not related to study medication and no differences in the rate of SAEs per arm (Placebo:13, D-met:11, p>0.5). There were also no differences in significant protocol violations between arms (Supplemental Table 8). One notable violation is that 11/29 (38%) of D-met and 12/29 (41%) of placebo patients received 5-floururacil in addition to cisplatin during ChemoRT which was not part of the protocol treatment.

Mucositis Evaluations

Patients were evaluated by the treating team at the start of treatment, weekly during RT, and then post treatment day 30 (see Figure 1). Three mucositis scales were evaluated: the WHO physicians 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 three 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=0.007 WHO, p=0.0009 RTOG) as well as a higher rate of Grade 3-4 mucositis (41% *vs*. 17% WHO, p=0.045; 48% *vs*. 21% RTOG, p=0.0285). For the RTOG Functional Patient Rating there was a lower rate of mucositis overall with D-met (p=0.0023) but the difference in grade 3-4 mucositis favoring D-met was not statistically significant (41% *vs*. 24%, p=0.16).

The primary end-point, pre-determined prior to analysis, was a reduction in the rate of grade 3-4 mucositis 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=0.058). However, the overall mucositis score (0-4) was lower with the use of D-met (p=0.0018). On the composite scale 31% (9/29) of D-met patients had grade 0-1 mucositis while this was only 3% (1/29) on the placebo arm (p=0.008). In addition, if grade 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=0.033). Finally one 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 while there were no deaths on the experimental arm.

For those who developed grade 3-4 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>0.2).

Secondary End-Points

Planned Secondary End-Points

An additional scoring system was also utilized per protocol where 9 areas in the mouth were assessed weekly for both ulceration and erythema (See Supplemental Table 4)(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 (StdError:0.24), p=0.006) which was 2.2 (0.68) for Placebo and 1.5 (1.1) for D-met. While 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 (StdError:0.18), p=0.005) 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-met, p=0.64) nor weight loss (4.4 kg (SD:3.0) Placebo *vs*. 4.2 (SD:3.2) D-met, p=0.8). 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=0.62) or the need for total parenteral nutrition (4/29 (14%) placebo *vs*. 1/29 (3%) for D-met, p=0.16).

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=0.35). 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 (Supplemental Table 9). Based upon radiographic review blinded to treatment allocation there was no difference in response rates between treatments with 62.5% (10/16) response (PR or CR) for placebo and 46.2% response (6/13) for D-met (p=0.48).

Unplanned Secondary Analyses

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 0-4 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 Area Under the Curve (AUC) which was higher for Placebo (AUC: 8.3 (95%CI:7.6-

8.9)) as compared to D-met (AUC: 6.3 (95%CI:5.6-7.0), p=0.036). 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=0.005) 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 (Supplemental Table 10).

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 less than 9 mucositis evaluations (Supplemental Table 11). This revealed that patients who missed evaluations on the Placebo arm had higher mucositis scores prior to missing data than those on the D-met arm (2.8-3.0 *vs*. 1.0-1.2, all p-values <0.002). In addition, patients non-evaluable 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 <0.01). 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 >0.05). 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=0.09) without a difference in grade 2 or greater nausea; while 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>0.5). Nevertheless, differences in timing of these mucositis evaluations in those who dropped out of therapy or did not limit the conclusions to be made based upon an uplanned secondary analysis.

Radiation Dose and Mucositis

We also evaluated the impact of RT dose on mucositis for the WHO scale (Supplemental Table 12). By univariate regression increasing radiation dose (<20, 20-39, 40-59, 60-70 Gy) correlated with increasing mucositis (p=0.03) while D-met was protective (p=0.0005). On multivariate regression the use of D-met retained significance (p=0.001) while radiation dose was borderline (p=0.064). When analyzed as the likelihood of having Grade 3-4 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 3-4 mucositis (Odds Ratio: 0.29 (95%CI:0.09-0.99), p=0.05) while RT dose was not correlated with Grade 3-4 mucositis (p=0.93).

The Use of 5-Flourouracil and Mucositis

Some patients also received 5-FU (12 in the Placebo group and 11 in the D-met group, Supplemental Table 13) which was outside of recommended protocol therapy. Logistic regression was performed to assess the rate of Grade 3-4 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 3-4 mucositis (Odds Ratio: 0.29 (95%CI:0.09-0.98), p=0.047) while the use of 5-FU did not influence mucositis (OR: 0.72 (95%CI:0.21-2.4), p=0.60). 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 (StDev: 0.23), p=0.0005) while 5-FU use did not influence score (Difference: 0.06 (StDev:0.24), p=0.81).

Discussion

This multi-institutional phase 2 trial was undertaken to assess if the efficacy observed in the single center Phase I trial of oral D-methionine to prevent OM could be confirmed. In planning the trial the control arm was assumed to have a 70% incidence of grade 3-4 mucositis and that following D-met it would be 10%. As such a sample size of 40 evaluable patients was needed. The observed rate of grade 3-4 mucositis was lower on the control arm than anticipated with 14/29 patients (48%) having severe mucositis while that in the experimental arm was higher than anticipated with 7/29 (24%) having severe mucositis. As a result this study did not meet its primary end-point of comparing the rate of grade 3-4 mucositis between arms based upon the composite scale ($p=0.058$). Based upon other studies it appears that the primary deficiency was that the 70% assumed rate of grade 3-4 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 $p<0.003$). In addition, no grade 4 mucositis was noted in any D-met treated patients, while 2/29 (7%) of patients had grade 4 mucositis when treated with placebo, and one 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 $p<0.009$). Another pre-planned analysis was the use of the OSSFSA to assess ulceration and erythema separately across 9 areas of the oral cavity or oropharynx where D-met resulted in lower scores for both of these planned evaluations (both $p<0.007$). It is well documented that treatment delays for SCCHN decrease local control and in a pre-planned analysis the use of D-met was associated with an approximate 6 day shorter treatment course than placebo ($p=0.05$); while 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 $p<0.0016$). Finally, unplanned analyses taking into account the time depended 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 $p<0.05$).

In the preclinical data a stronger correlation was noted between the C_{max} of Dmet 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 po, C_{max}=192 μg/mL)(37) as compared to rodents (150 mg/kg po, C_{max}= 71 μg/mL)(34) while given the longer half-life in humans (3.0 hrs *vs*. 1.0 hrs) the total exposure following oral dosing was even higher in man (AUC 793 *vs*. 211 μg * hr/mL).(34, 37) The PF observed here of 1.3-1.5 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 IV administration then after PO which could potentially have implications for further development of D-met as a radioprotector.

There were no SAE's 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 anti-emetics 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 chemoRT. 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 while more recently there has been a significant increase in

SCCHN related to human papilloma virus infection. Nevertheless, the combination of radiotherapy 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 2- or 3D conformal therapy also does not reflect current treatment standards; however, newer technologies such as parotid sparing intensity modulated RT (IMRT) have not reduced mucositis, perhaps due to spreading dose more to 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 3-4 mucositis in those getting IMRT as compared to 3D-treatments (60% IMRT *vs*. 44% 3D, $p>0.05$)(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%, $p>0.05$)(40). In this context the rate of grade 3-4 mucositis observed here using conventional RT and weekly cisplatin (48%) is consistent with these previous reports while 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 where 4 small phase 3 trials (all performed ex-US) appeared 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 two times after RT for up to 6 hours. Given the antibacterial and anti-microbial properties reported for honey it is felt that this may be its mechanism of action. A recent large phase 2 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 non-steroidal 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 tri-cyclic anti-depressant) or "Magic Mouth Wash" (lidocaine containing rinse) each compared to placebo noted decrease oral pain in the first 60 minutes with either experimental agent while those receiving doxepin had increased fatigue compared to placebo(44).

Taken together the results reported here are suggestive of a protective effect of D-methionine 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.

Figure Legends

Figure 1 : Consort Diagram

Figure 2: Maximum Mucositis score (and standard error) observed for the World Health Organization (WHO) (A), Radiation Therapy Oncology Group (RTOG) Physician (B), RTOG Functional Patient (C), or Composite Scale (D)

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

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	D-methionine (number = 29)	Placebo (number = 29)	p-value
Age: (years)			
Mean (SD)	50.2 (11.4)	47.7 (9.4)	0.4[b]
Median (Min, Max)	52 (23, 64)	50 (28, 64)	
Gender: [Number(%)]			
Male	22 (75.9)	22 (75.9)	1.0[c]
Female	7 (24.1)	7 (24.1)	
Ethnicity: [Number(%)]			
Indian	29 (100)	29 (100)	1.0[c]
Weight: (Kg)			
Mean (SD)	56.3 (10.4)	53.3 (11.0)	0.3[b]
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)	0.5[b]
Median (Min, Max)	22.0 (15.1, 30)	19.9 (11.7, 29.9)	
Karnofsky Performance Status:			
Mean (SD)	88.2 (4.7)	87.2 (4.6)	
Median (Min, Max)	90 (70, 90)	90 (80, 90)	0.8[b]
70 [Number(%)]	1 (3.4)	0	
80 [Number(%)]	3 (10.3)	8 (27.6)	
90 [Number(%)]	25 (86.2)	21 (72.4)	
Time from Diagnosis to Randomization			
Mean Days (SD)	47.8 (92.9)	34.5 (59.9)	0.5[b]
Histology/Pathology [Number(%)]			
Squamous Cell	28 (96.6)	27 (93.1)	1.0[c]
Other (Poorly differentiated carcinoma)	1 (3.4)	2 (6.9)	
Site of Primary Tumor [Number(%)][a]			
Oral Cavity	27 (93.1)	25 (86.2)	
Oropharynx	3 (10.3)	3 (10.3)	0.7[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 [Number(%)]			
I	6 (20.7)	3 (10.3)	0.16[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)	0.43[c Stage III/IV]
Not Done	1 (3.4)	0	
Sites of Metastases [Number(%)][a]			
Any	6 (20.7)	3 (10.3)	0.5[c]
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	

Table 1 : Demographic and Clinical Characteristics

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

	D-methionine (number = 29)	Placebo (number= 29)	p-value
Radiotherapy:			
Number of Fractions Received per Patient Mean (SD) Median (Min,Max)	 26.6 (9.0) 30 (1, 35)	 29.7 (5.7) 32 (10, 34)	 0.4[a]
Total Gy Administered per Patient Mean (SD) Median(Min, Max)	 52.2 (18.6) 60 (1.8, 70)	 57.8 (12.1) 64 (18, 68)	 0.2[a]
Time to Complete RT per Patient [Days] Mean (SD) Median (Min, Max)	 39.6 (14.6) 42 (1, 65)	 47.1 (12.2) 48 (13, 75)	 0.05[a]
Number (%) Treatment Device Cobalt Linear Accelerator (≥4 MV)	 10 (34) 19 (66)	 10 (34) 19 (66)	 1.0[b]
Cisplatin:			
Number (%) of Patients Receiving ≥1 Dose of Cisplatin	25 (86.2)	25 (86.2)	1.0[b]
Number of Cisplatin Doses per Patient Mean (SD) Median (Min, Max)	 3.2 (1.9) 4 (0, 6)	 3.2 (1.8) 4 (0, 7)	 0.98[a]
Study Drug:			
Number of Doses Administered per Patient Mean (SD) Median (Min, Max)	 52.7 (18.3) 60 (2, 69)	 59.5 (11.5) 64 (20, 69)	 0.096[a]
Number of Days Dosed per Patient Mean (SD) Median (Min, Max)	 26.7 (9.1) 30 (1, 35)	 29.7 (5.8) 32 (10, 34)	 0.6[a]
Dose Drug / RT Treatment Mean/Mean Median/Median	 2.0 2.0	 2.0 2.0	1.0[b]

Table 2: Summary of Study Treatment

[a] t-test

[b] Fisher's Exact Test

Table 3. Summary of Most Frequently Reported (>10%) All-Cause Adverse Events by Body System[a], Maximum Intensity[b], and Treatment Group

BODY SYSTEM [a]	D-methionine (Number = 29)					Placebo (Number = 29)				
	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 AE reported within that body system.
[b] Totals for individual AEs count each patient once. If multiple occurrences of the same AE 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 AEs of anemia, hypotension, and dyspnea resulting in his death (Grade 5)

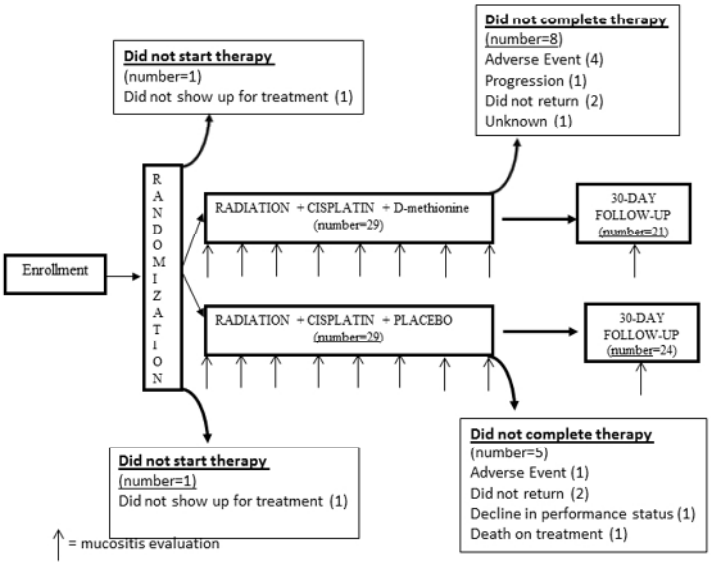

Figure 1

254x190mm (96 x 96 DPI)

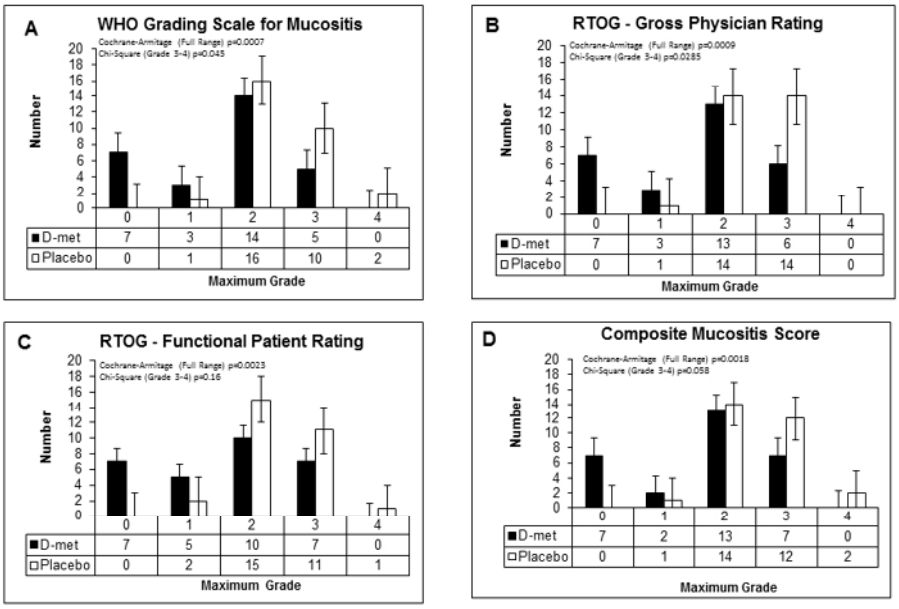

Figure 2

254x190mm (96 x 96 DPI)

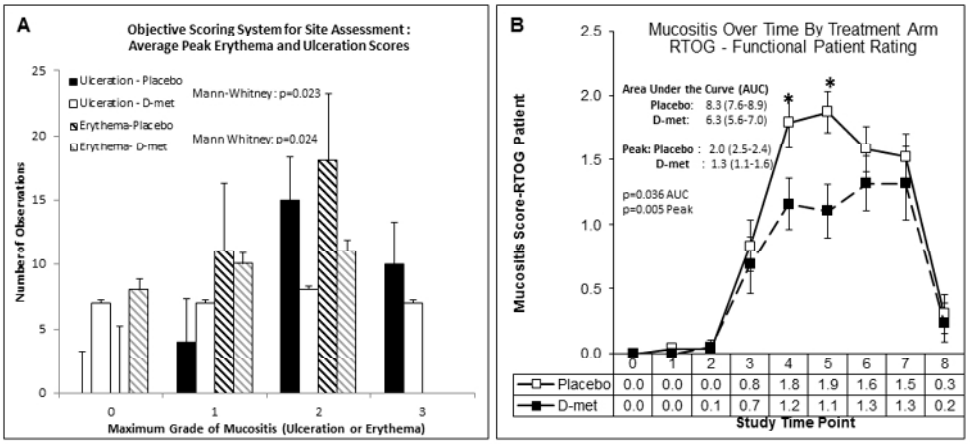

Figure 3

254x190mm (96 x 96 DPI)

Inclusion Criteria:

- Histological confirmation of head and neck cancer, first occurrence;
- Medically suited to receive primary treatment with radiation and cisplatin;
- 18-65 years;
- Radiation area should include a minimum of 50% of the oral pharynx, oral cavity, or both;
- Negative serum pregnancy test in females of child-bearing potential;
- KPS >60;
- Able to provide written informed consent.

Exclusion Criteria:

- Pregnancy;
- Breast feeding;
- T1 and T2 glottic tumors;
- Prior radiation to the head and neck region or prior chemotherapy of any type;
- History of allergic or idiosyncratic reaction to methionine, amino acid mixtures, strength formulations, egg white, other proteins, food additives;
- Simultaneous enrollment in other clinical studies;
- Other immunocompromised states,
- Current oral mucosal lesions other than from direct involvement of the underlying head and neck cancer;
- Cryotherapy to the face, head and neck region;
- Current use of any of the following oral care preparations: amifostine, chlorhexidine, sucralfate, benzydamine.

Supplemental Table 1 : Inclusion and Exclusion Criteria

Supplemental Table 2. World Health Organization Grading Scale for Mucositis (WHO)	
Grade	**Description**
0	No mucositis
1	Painless ulcer, erythema or mild soreness
2	Painful erythema, edema, ulcer, but can eat
3	Painful erythema, edema, ulcer, but cannot eat
4	Required parenteral or enteral support

Supplemental Table 3. Radiation Therapy Oncology Group Oral Mucositis Grading System (RTOG)		
Score	**Gross Physician Rating**	**Functional Patient Rating**
0	None	None
1	Erythematous sores	Mild soreness, mild dysphasia. Solid diet possible.
2	Patchy mucositis (<1/2 mucosa)	Moderate pain, moderate dysphasia. Soft diet or liquid diet possible.
3	Confluent fibrinous mucositis (>1/2 mucosa)	Severe pain, severe dysphasia. Liquids only.
4	Hemorrhage and necrosis	Requires parenteral or enteral support.

Supplemental Table 4. Objective Scoring System for Site Assessment (OSSFSA)							
Location	**Ulceration/Pseudomembrane**				**Erythema**		
Any Site	0	1	2	3	0	1	2
Upper Lip	0	1	2	3	0	1	2
Lower Lip	0	1	2	3	0	1	2
Right Cheek	0	1	2	3	0	1	2
Left Cheek	0	1	2	3	0	1	2
Right Ventral and Lateral Tongue	0	1	2	3	0	1	2
Left Ventral and Lateral Tongue	0	1	2	3	0	1	2
Floor of Mouth	0	1	2	3	0	1	2
Soft Palate / Fauces	0	1	2	3	0	1	2
Hard Palate	0	1	2	3	0	1	2

Ulceration Scoring:
0 = No Lesion
1 = <1 cm^2
2 = 1-3 cm^2
3 = >3 cm^2

Erythema Scoring:
0 = None
1 = Mild
2= Severe

Supplemental Table 5. Summary of Patients with Abnormal Hematology Parameters, by Maximum Grade[a] Number (%) of Patients[b]

	D-methionine (Number=24[b])					Placebo (Number=28[b])				
	Grade					Grade				
Laboratory Parameter	0	1	2	3	4	0	1	2	3	4
White Blood Cell Count	19 (79.2)	4 (16.7)	1 (4.2)	0	0	21 (75.0)	6 (21.4)	1 (3.6)	0	0
Absolute Neutrophil Count Nadir	24 (100)	0	0	0	0	27 (96.4)	1 (3.6)	0	0	0
Platelet Count Nadir	24 (100)	0	0	0	0	26 (92.9)	1 (3.6)	1 (3.6)	0	0
Hemoglobin Nadir	8 (33.3)	12 (50.0)	4 (16.7)	0	0	9 (32.1)	14 (50.0)	5 (17.9)	0	0

[a] The worst post-baseline test result was graded according to the CTCAE,v3.0
[b] The total number of patients reported in a treatment group reflects the number of patients who had ≥1 post-baseline test conducted.

Supplemental Table 6. Summary of Patients with Abnormal Chemistry Parameters, by Maximum Grade[a] Number (%) of Patients

	D-methionine (Number=24[b])					Placebo (Number=28[b])				
	Grade					Grade				
Laboratory Parameter[b]	0	1	2	3	4	0	1	2	3	4
	24 (100)	0	0	0	0	23 (82.1)	4 (14.3)	1 (3.6)	0	0
Creatinine										
Total Bilirubin	22 (91.7)	1 (4.2)	1 (4.2)	0	0	27 (96.4)	1 (3.6)	0	0	0
AST	19 (79.2)	5 (20.1)	0	0	0	23 (82.1)	4 (14.3)	0	1 (3.6)	0
ALT	21 (87.5)	3 (12.5)	0	0	0	25 (89.3)	2 (7.2)	1 (3.6)	0	0

[a] The worst post-baseline test result was graded according to the CTCAE,v3.0
[b] The total number of patients reported in a treatment group reflects the number of patients who had ≥1 post-baseline test conducted.

Supplemental Table 7. Summary of All Serious Adverse Events by Body System and Treatment Group									
BODY SYSTEM	**D-methionine (Number = 29)**				**Placebo (Number = 29)**				
Term	**Total (%)**	**Grade 2**	**Grade 3**	**Grade 4**	**Total (%)**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
TOTAL PATIENTS[a] (%)	3 (10.3)	1 (3.4)	2 (6.9)	0	4 (13.8)	0	3 (10.3)	0	1 (3.4)
TOTAL SAEs	11	2	9	0	13	4	6	2	1
HEMATOLOGIC									
Neutropenia/Leukopenia	2	0	2	0	2	1	1	0	0
Hemorrhage/ Thrombocytopenia	1	0	1	0	1	1	0	0	0
Anemia	2	1	1	0	1	0	0	1	0
DIGESTIVE									
Dysphagia	1	0	1	0	2	0	2	0	0
Anorexia	1	0	1	0	1	0	1	0	0
Pain in Oral Cavity	1	0	1	0	3	1	2	0	0
OTHER BODY SYSTEMS									
Fatigue	1	0	1	0	0	0	0	0	0
Edema	1	1	0	0	0	0	0	0	0
Infection	1	0	1	0	1	1	0	0	0
Dyspnea	0	0	0	0	1	0	0	1	0
Hypotension	0	0	0	0	1	0	0	0	1

[a] Total Patients: Patients were counted once regardless of how many SAEs were reported in that patient.

Supplemental Table 8. Summary of Significant Protocol Deviations [Number (%) of Patients]			
Protocol Deviation	**D-met Number = 29**	**Placebo Number = 29**	**p-value**
Pregnancy test not done during Baseline	2 (6.9)	2 (6.9)	1.0[a]
CT scans not obtained:			
at Baseline	4 (13.8)	1 (3.4)	0.35[a]
at Follow-Up	8 (27.6)	6 (20.7)	0.76[a]
Hematology and chemistry tests not done at Baseline	4 (13.8)	4 (13.8)	1.0[a]
Patient did not receive any doses of cisplatin	4 (13.8)	4 (13.8)	1.0[a]
Patient received carboplatin instead of cisplatin	1 (3.4)	1 (3.4)	1.0[a]
Missed one or more doses of Study Medication on days radiation was given	7 (24.1)	2 (6.9)	0.079[a]
Patient received doses of 5-fluorouracil in addition to cisplatin chemotherapy	11 (37.9)	12 (41.4)	1.0[a]

[a]=Fisher's exact test

Supplemental Table 9. Objective Response Rate via RECIST Criteria Based on a Subset of 29 Patients with a Baseline CT Scan, a Follow-Up CT Scan, and Measurable Disease at Baseline [Number (%) of Patients]		
Response Category	**D-methionine Number = 13**	**Placebo Number = 16**
Complete Response	2 (15.4)	1 (6.2)
Partial Response	4 (30.8)	9 (56.2)
CR + PR	6 (46.2)	10 (62.5)

		AUC^{all} data	AUC^{full} data	p-value	AUC Protective Factor (all/full)	Mean Peak^{all} data	Mean Peak^{full} data	p-value	Peak Protective Factor (all/full)
RTOG Functional Patient Rating	Placebo	8.3	7.6	0.035 all	1.3 / 1.3	2.0	1.9	0.0056 all	1.5 / 1.4
	D-met	6.3	5.6	0.036 full		1.3	1.3	0.0048 full	
RTOG Gross Physician Rating	Placebo	8.9	8.3	0.012 all	1.4 / 1.4	2.0	2.0	0.0014 all	1.4 / 1.4
	D-met	6.7	5.9	0.034 full		1.4	1.4	0.0014 full	
WHO Grading Scale for Mucositis	Placebo	8.5	7.9	0.017 all	1.4 / 1.4	2.0	1.9	0.002 all	1.5 / 1.4
	D-met	6.3	5.6	0.038 full		1.3	1.3	0.002 full	

Supplemental Table 10: Peak and Time Dependent Analysis of Mucositis By Treatment Arm as Well as Protective Factors

The total time dependent response of mucositis to chemoradiotherapy with or without D-met was evaluated (as seen for Figure 3A which was for the Functional Patient Scale from the RTOG scale) using all 3 scales. This score was calculated as the integral of mucositis over time and expressed as the Area Under The Curve (AUC). Given the secondary nature of the analysis it was performed in two ways.

First, there were nine time points at which mucosal evaluation was performed (baseline, weekly during RT for up to 7 weeks, and at 30 days post RT). For the time dependent analysis reported here a patient was included in the analysis only if mucosal evaluation was performed at a minimum 6 of 9 data points (Placebo: 24/29 patients and D-met: 20/29 patients which was not different between groups, Chi-square: p=0.6) this is reported as AUC^{all} or Peak^{all}.

Second, analysis was also limited to those in whom all 9 data points were available which is reported as AUC^{full} and Peak^{full} (Placebo: 23/29 patients and D-met: 18/29 patients, Chi-square: p=0.5).

The protective factor was calculated as the value for AUC or Peak mucositis score with Placebo divided by the score with D-met where a value >1.0 would indicate lower mucositis with D-met.

Overall there was a higher AUC reflecting higher exposure to mucositis in the placebo arms on all three scales as compared to the D-met arm with protective factors of 1.3-1.4. Similarly, there were higher peak mucositis scores across all analyses for those treated with Placebo than those treated with D-met with protective factors of 1.4-1.5. No significant differences were noted between any of the scales. Comparisons for average AUC and average Peak were evaluated with student's t-test.

	RTOG Functional Patient Rating	RTOG Gross Physician Rating	WHO Grading Scale for Mucositis
Placebo	3.0 (0.63)	2.8 (0.41)	2.8 (0.75)
D-methionine	1.1 (1.22)	1.2 (1.07)	1.0 (1.12)
t-test	0.0014	0.0013	0.0015

Supplemental Table 11: Mucositis score on last evaluable day for those with <9 scores

Supplemental Table 12. Summary of Maximum WHO Oral Mucositis Scores by Cumulative Radiation Exposure [Number of Patients]

Cumulative Radiation Exposure (Gy)	D-met (N = 29) Grade					Grade 3-4 D-met	Placebo (N = 29) Grade					Grade 3-4 Placebo	Overall Grade 3-4
	0	1	2	3	4		0	1	2	3	4		
<20	3	0	0	0	0	0% (0/3)	0	0	0	1	0	100% (1/1)	25% (1/4)
20-39	1	0	2	0	0	0% (0/3)	0	0	1	0	1	50% (1/2)	20% (1/5)
40-59	0	2	3	1	0	17% (1/6)	0	0	3	4	0	57% (4/7)	38% (5/13)
60-70	3	1	9	4	0	24% (4/17)	0	1	12	5	1	32% (6/19)	28% (10/36)
Overall	7	3	14	5	0	17% (5/29)	0	1	16	10	2	41% (12/29)	29% (17/58)

Supplemental Table 13: Grade 3-4 Mucositis as a Function of 5FU Use and D-met		
	Percentage with Grade 3-4 Mucositis Based on WHO Scale	
	No 5FU	5FU
Placebo	47.1% (8/17)	33.3% (4/12)
D-methionine	16.7% (3/18)	18.2% (2/11)