Randomized, Placebo-Controlled Trial of Saforis for Prevention and Treatment of Oral Mucositis in Breast Cancer Patients Receiving Anthracycline-Based Chemotherapy

Douglas E. Peterson, DMD, PhD[1]
James B. Jones, MD, PharmD[2]
Robert G. Petit II, PhD[2]

[1] Department of Oral Health and Diagnostic Sciences, School of Dental Medicine, and Head & Neck/Oral Oncology Program, Neag Comprehensive Cancer Center, University of Connecticut Health Center, Farmington, Connecticut.

[2] MGI Pharma, Inc., Bloomington, Minnesota.

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James B. Jones, MD, PharmD, was an employee of MGI Pharma, Inc., for the duration of the study and owns MGI Pharma stock.

Address for reprints: Douglas E. Peterson, DMD, PhD, Department of Oral Health and Diagnostic Sciences, School of Dental Medicine and The Carole and Ray Neag Comprehensive Cancer Center, University of Connecticut Health Center, Farmington, CT 06030-1605; Fax: (860) 679-4760; E-mail: peterson@nso.uchc.edu

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BACKGROUND. Oral mucositis (OM) is a frequent complication of mucotoxic cancer therapy, causing significant oral pain, increased infection risk, and impaired functioning. The efficacy and safety of Saforis (glutamine) powder in UpTec for oral suspension was evaluated for the prevention and treatment of OM.

METHODS. Three hundred twenty-six patients developing World Health Organization (WHO) grade $\geq$2 OM during a chemotherapy screening cycle were randomized to Saforis (n = 163) or placebo (n = 163) 3 times/day during their next chemotherapy cycle (Treatment Cycle 1). Patients were crossed over to the alternate treatment during Treatment Cycle 2. As prespecified in the statistical plan, because of a carryover effect in Treatment Cycle 2 the primary efficacy analysis was based on Treatment Cycle 1 only.

RESULTS. Compared with placebo, Saforis significantly reduced the incidence of clinically significant WHO grade $\geq$2 OM (38.7% vs. 49.7%; $P = .026$) and severe WHO grade $\geq$3 OM (1.2% vs. 6.7%; $P = .005$) in Treatment Cycle 1. Saforis also significantly reduced the worst Oral Mucositis Assessment Scale ulceration score in Treatment Cycle 1 compared with placebo (mean, 0.23 $\pm$ 0.39 vs. 0.32 $\pm$ 0.45; $P = .013$). Patients receiving Saforis in Treatment Cycle 1 had a lower-than-expected OM incidence when crossed over to placebo in Treatment Cycle 2, indicating a significant carryover effect ($P = .027$). The incidence of treatment-emergent adverse events was similar between groups.

CONCLUSIONS. Saforis is safe and effective for preventing and treating OM in patients receiving mucotoxic cancer chemotherapy. ***Cancer*** **2007;109:322–31.**

KEYWORDS: oral mucositis, glutamine, Saforis, breast cancer, mucotoxic, chemotherapy.

Oral mucositis (OM) is a clinically significant complication of mucotoxic cancer therapy. The condition affects an estimated 5% to 40% of patients receiving standard-dose chemotherapy and >75% of patients receiving either high-dose chemotherapy with stem-cell transplantation or radiation therapy for head and neck cancer.[1–3] Clinically significant OM (World Health Organization [WHO] grade $\geq$2), which involves both erythema and ulceration of the oral mucosa, can directly affect the clinical status of the patient and results in increased pain, difficulty in swallowing, nutritional compromise, and increased risk for infection.[2–4] These clinical sequelae increase the morbidity and mortality associated with cytotoxic therapy and interfere with patient functioning and health-related quality of life. It

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is well documented that OM and its consequences increase healthcare resource utilization, eg, hospitalization, opioid analgesics for pain control, nutritional support, and antimicrobial therapy.[1–4] More recently, a symptom cluster has been described linking OM with fatigue, anorexia, and related symptoms,[5,6] perhaps from a common pathogenesis of cytokine-based neuroimmunologic derangements.[5,7] As a result, OM has emerged as an important dose-limiting toxicity in patients receiving mucotoxic cancer therapy.[2] Unfortunately, there is a limited armamentarium of pharmacologic agents that have been clearly demonstrated to either prevent the occurrence or reduce the severity and duration of clinically significant OM in well-designed controlled clinical trials. In the US, the only drug approved for clinically significant OM is palifermin (Kepivance; Amgen Inc., Thousand Oaks, CA); however, the label for this agent restricts its use to patients with hematologic malignancies undergoing high-dose chemotherapy with stem-cell transplantation.[8] Given this important unmet need, safe and effective agents that can prevent and/or treat this dose-limiting toxicity of common antineoplastic therapies are needed.

Glutamine is a conditionally essential amino acid that has multiple well-defined functions in human biologic processes. Current evidence for the pathobiology of mucosal injury indicates that reactive oxygen species, generated from both chemotherapy and radiation therapy, play a critical role in the initiation of OM. Glutamine, a precursor for glutathione, plays a pivotal role in regulating the intracellular redox potential,[9,10] and clinical investigations indicate that glutamine inhibits other mediators of mucosal barrier injury by reducing the production of proinflammatory cytokines and cytokine-related apoptosis.[11–13] Other experimental evidence suggests that glutamine may improve mucosal barrier wound healing by increasing fibroblasts and collagen synthesis.[14] Glutamine is also critically important to meet demands for tissue repair during times of high cellular replication. In these times of increased glutamine demand caused by physiologic stress (eg, during and after cytotoxic chemotherapy), the requirements for glutamine may exceed the body's ability to produce sufficient concentrations and exogenous glutamine may be needed. Patients with advanced malignancies and those undergoing cytotoxic therapy have been reported to have a relative deficiency of glutamine.[15,16] However, previous clinical trials using traditional oral and parenteral glutamine formulations have been inconsistent and have failed to reduce the incidence and severity of OM in patients receiving a wide variety of mucotoxic therapies.[17–24] One possible explanation for these results may be insufficient delivery of glutamine to the damaged tissues of the oral mucosa, as glutamine has moderate solubility and undergoes nonenzymatic degradation under physiologic conditions.[25]

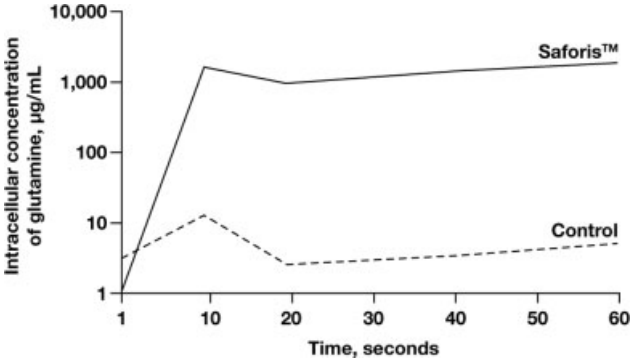

FIGURE 1. Transport of Saforis into human mucosa (CaCO) cells. Control was a saturated solution of glutamine.[26]

Saforis (MGI Pharma, Inc., Bloomington, MN) is composed of glutamine in a novel, proprietary drug-delivery system (UpTec) that is administered orally. Compared with other available forms of glutamine, Saforis has been shown to facilitate the uptake of >100 times more glutamine by epithelial oral mucosal cells.[26] The increase in intracellular glutamine levels occurs rapidly, within 10 seconds (Fig. 1).[26] The ability of Saforis to prevent OM has been documented in a well-validated animal model of mucosal injury, and significant activity was demonstrated compared with placebo.[27] It has also been studied in 4 previously reported clinical studies in various patient populations.[28–31] In cancer patients with previous chemotherapy-related OM, Saforis has been shown to significantly reduce the duration of OM compared with placebo,[29] to reduce the incidence of moderate to severe OM compared with placebo,[30] and to significantly reduce the severity of OM compared with the previous chemotherapy cycle.[31]

These studies collectively contributed to the design of the present Phase III clinical trial to determine the efficacy and safety of Saforis compared with placebo for reducing the incidence of WHO grade ≥2 OM in patients receiving mucotoxic cancer therapy for breast cancer.

MATERIALS AND METHODS

Patients

Adult patients (≥18 years of age) with an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 and histopathologically confirmed breast cancer suitable for treatment with anthracycline-based chemotherapy were eligible to participate. Eligible patients

were randomized if they experienced moderate to severe (WHO grade ≥2) OM during the screening cycle of chemotherapy and were also scheduled to receive at least 2 additional cycles of the same chemotherapy without a dose reduction. Eligible patients must have presented with normal oral mucosa (ie, WHO grade 0) at baseline, have completed any previous radiotherapy involving the oral or esophageal mucosa at least 6 weeks before study entry, and have recovered from all previous radiotherapy toxicities. All patients received acyclovir (oral 200 mg twice daily) prophylaxis during the study.

Patients were excluded if they were receiving or scheduled to receive any other topical or systemic treatments specifically targeting OM, including growth factors, cytokines, cryotherapy, sucralfate, or prostaglandins. Patients could not have uncontrolled diabetes mellitus, current evidence of alcohol or drug abuse, or active mouth or gingival sores. Patients were excluded if they were pregnant, lactating, or at risk of pregnancy or if they had participated in a clinical trial for the treatment or prevention of OM within 4 weeks of study entry.

All patients provided informed written consent and this study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice (GCP), International Committee on Harmonisation guidelines, and local ethical and legal requirements. The protocol, informed consent form, and any amendments to the protocol were reviewed and approved by the Institutional Review Board or Ethics Committee at each participating study site before enrollment of participants into the study. Centers participating in the trial were monitored by trained field associates according to GCP guidelines. Additionally, all data were entered using double entry verification and the clinical database was audited both internally and by an independent third-party auditor to ensure data quality.

Study Design

This study was designed as a multicenter, randomized, double-blind, placebo-controlled, crossover Phase III trial conducted in Russia. Patients who developed WHO grade ≥2 OM during the screening cycle of chemotherapy were eligible for randomization to the study drug (Fig. 2) and received 1 of the following chemotherapy regimens at standard doses on a 21-day cycle: cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF); 5-fluorouracil, doxorubicin, and cyclophosphamide (FAC); or doxorubicin and cyclophosphamide (AC). The majority of patients were hospitalized during chemotherapy administration, which is customary in Russia. Eligible patients were randomized to receive either Saforis or placebo during their next cycle of chemotherapy

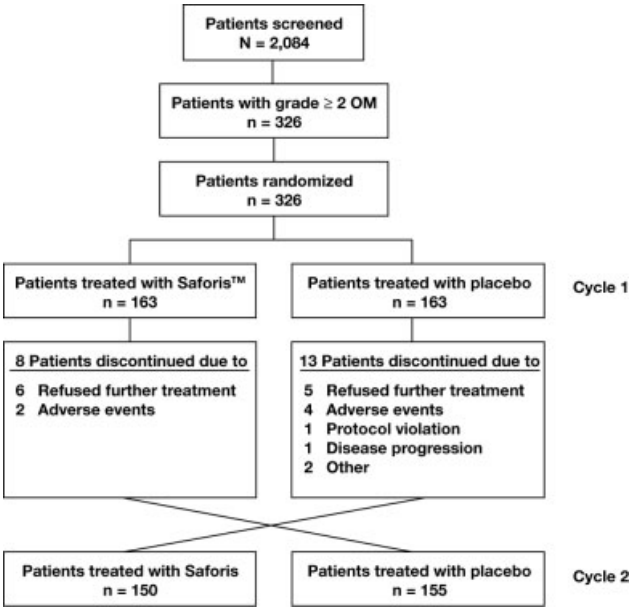

FIGURE 2. CONSORT diagram of the study design. OM, oral mucositis.

(Treatment Cycle 1). Patients were then crossed over to receive the other treatment during the subsequent cycle of chemotherapy (Treatment Cycle 2). Patients were stratified by chemotherapy regimen before randomization in a 1:1 ratio. A matched placebo was used to maintain blinding.

The primary objective of this study was to determine the efficacy and safety of Saforis compared with placebo when used to reduce the incidence of moderate to severe (WHO grade ≥2) OM associated with mucotoxic cancer therapy. Secondary objectives were to determine the treatment effect of Saforis compared with placebo on the duration of WHO grade ≥1, ≥2, and ≥3 OM and the effect of Saforis compared with placebo on another objective measure of oral mucositis, the Oral Mucositis Assessment Scale (OMAS). Mean OMAS score was calculated based on the sum of the mean ulceration score across all sites with ulceration and the mean erythema score across all sites with erythema as described by Sonis et al.[32] The worst OMAS ulceration score was defined as the average of the greatest 3 scores across the 9 evaluation sites.

Other efficacy endpoints included the intensity of oral pain and difficulty of swallowing as assessed by patient self-report using visual analog scales.

Study Drug

Saforis was administered at a dose of 2.5 g per 5 mL 3 times per day for a total daily dose of 7.5 g. The placebo formulation matched the texture and characteristics of

TABLE 1
World Health Organization Oral Mucositis Scale

Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
None	Soreness ± erythema No ulceration	Erythema, ulcers Patients can swallow solid diet	Ulcers, extensive erythema Patients cannot swallow solid diet	Mucositis to the extent that alimentation is not possible

Data from World Health Organization.[33]

the active drug and the administered dose was 5 mL 3 times per day for a total daily dose of 15 mL. The same placebo was utilized in previous trials, which demonstrated that this placebo is adequate to protect the blinding of a placebo-controlled trial and that it has no detrimental effect on the development or presence of OM in patients.

Study drug treatment began on the first day of chemotherapy and continued for 14 days after the last dose of chemotherapy in patients who did not develop OM or until 5 days after resolution of OM for patients who experienced OM or to the end of the treatment cycle. Study drug was orally swished for 30 seconds and then swallowed. Patients were instructed to refrain from eating or drinking for 30 minutes after dosing. Patients adhered to good oral hygiene practices and gently brushed their teeth twice daily, 30 minutes or more after each study drug treatment, using a soft toothbrush and fluoride toothpaste. Daily flossing and an alcohol-free fluoride rinse was recommended. No other prophylactic mouthwashes or treatments for mucositis were allowed.

Study Assessments

Signs and symptoms of OM were assessed by the investigator 3 times per week on nonconsecutive days during the screening cycle and during Treatment Cycles 1 and 2 (on Days 3, 5, 7, 9, 11, and 14), and additionally on Days 16 and 18 of each cycle if the patient developed OM. The WHO OM scale (Table 1)[33] and the OMAS were used to assess the severity of OM. Among patients who developed OM, oral assessment continued for the first 3 weeks or until mucositis returned to grade 0. Additionally, patients self-reported the ability to eat solid foods.

Safety was assessed throughout the study by physical examination, including inspection of oral tissues, hematology and serum chemistry laboratory tests, and adverse event reporting. Any adverse event, whether or not related to the study drug, was reported with date and time of onset, severity (WHO Toxicity Criteria), relation with study drug (probably, possibly, or unlikely), pattern, action taken, and outcome. If the adverse event had not resolved at the time the case report forms were collected, a follow-up report was provided at a later date. If no follow-up report was provided, the investigator provided a justification. All adverse events were followed until either they resolved or the investigator determined that the event was no longer clinically significant.

Statistical Analyses

For the purpose of sample size determination, it was assumed that the proportion of cycles with occurrence of WHO grade $\geq$2 OM would be 50% in Saforis-treated cycles and 70% in placebo-treated cycles. Given these assumed proportions, the proportion of discordant pairs could have ranged from 0.2 to 0.8. Over this range, corresponding to a 0.8 proportion of discordant pairs, the maximum required sample size to achieve 90% power was 206 patients. This figure was increased to 300 to provide a greater number of patients for the purpose of evaluating safety.

The efficacy data were intended to be analyzed based on the crossover design using data from both Treatment Cycles 1 and 2, assuming no statistical evidence of an unequal carryover effect for the primary endpoint. However, because an unequal statistical carryover effect was detected, the primary efficacy analyses were conducted using results from only Treatment Cycle 1 as prespecified in the statistical plan.

The Cochran-Mantel-Haenszel (CMH) test, adjusted for center, was used to analyze the proportion of patients with WHO grade $\geq$2 OM, WHO grade $\geq$3 OM, OMAS ulceration score $>$0, and ability to eat solid foods. An analysis of variance model with terms for treatment and center was used to analyze the mean OMAS score and worst OMAS ulceration score. The Wilcoxon rank-sum test was used to assess a shift in the distribution of WHO grade of OM and to compare treatment groups with respect to patient symptom scores. Duration of OM was analyzed using a log-rank test, and patients without OM were considered to have durations of zero. Additional analyses, using descriptive statistics, were performed on the subset of patients who experienced, at a minimum, the degree of OM being analyzed.

TABLE 2
Patient Demographics and Baseline Disease Characteristics

	Sequence		
	Saforis/Placebo (n = 163)	Placebo/Saforis (n = 163)	Overall (n = 326)
Women, n (%)	163 (100)	163 (100)	326 (100)
Caucasian, n (%)	163 (100)	163 (100)	326 (100)
Median age, y (range)	50 (27–74)	50 (24–73)	50 (24–74)
Initial diagnosis		0	
Adenocarcinoma	162 (99.4)	163 (100)	325 (99.7)
Estrogen receptor-positive	1 (0.6)	0	1 (0.3)
Treatment regimen			
CAF	101 (62.0)	97 (59.5)	198 (60.7)
FAC	37 (22.7)	42 (25.8)	79 (24.2)
AC	25 (15.3)	24 (14.7)	49 (15.0)
WHO Grade OM during Screening Cycle			
Mean (SD)	2.1 (0.3)	2.1 (0.3)	2.1 (0.3)
Median (range)	2 (1–3)*	2 (1–3)*	2 (1–3)

CAF indicates cyclophosphamide, doxorubicin, and 5-fluorouracil; FAC, 5-fluorouracil, doxorubicin, and cyclophosphamide; AC, doxorubicin and cyclophosphamide; WHO: World Health Organization; OM: oral mucositis; SD: standard deviation.
* One patient in each treatment group had only grade 1 OM during the screening cycle.

RESULTS

Patients

Of 2,084 patients who were screened, 326 (15.6%) patients developed WHO grade $\geq$2 OM during the screening cycle consented and were randomized to either Saforis (n = 163) or placebo (n = 163) in Treatment Cycle 1. Baseline demographics and disease characteristics for the intent-to-treat (ITT) population are shown in Table 2. All patients were female and Caucasian; median age was 50 years (range, 24–74 years). All but 1 patient had histologically confirmed adenocarcinoma of the breast (99.7%); the remaining 1 patient was diagnosed with estrogen receptor-positive breast tumor (0.3%). The randomization groups were balanced for all baseline characteristics, including mean and median WHO grade of OM during screening chemotherapy. The majority of patients in the randomized portion of the study received CAF chemotherapy (61%) and treatment groups were balanced for chemotherapy regimen. No patients received low-level laser therapy or oral or esophageal radiation before enrollment. All subjects received prechemotherapy baseline oral examinations but no stabilizing dental treatments. No subjects were enrolled in the study if there was clinical evidence of active oral mucosal disease at baseline.

Greater than 90% of patients completed the study, with little difference in the rate of discontinuation during treatment with Saforis or placebo. During Treatment Cycle 1, 8 (5%) patients discontinued treatment with Saforis, and 13 (8%) patients discontinued treatment with placebo. The majority of patients were compliant with the dosing regimen. Among patients who missed $\geq$1 dose of Saforis (n = 71) or placebo (n = 69) during Treatment Cycle 1, the mean number of missed doses were 2.9 and 4.0, respectively. Among patients who missed $\geq$1 dose of Saforis (n = 58) or placebo (n = 59) during Treatment Cycle 2, the mean number of missed doses were 3.2 and 2.8, respectively. The majority of patients in the Saforis/placebo sequence (95%) and in the placebo/Saforis sequence (90%) received $\geq$1 concomitant medication during Treatment Cycle 1. There were no statistically significant differences between sequence groups with regard to number of patients using >1 concomitant medication or number of patients using a specific medication. Nearly all patients were receiving at least 1 concomitant medication during Treatment Cycle 1, most commonly ondansetron, metoclopramide, ascorbic acid, and prednisolone.

Incidence and Severity of Oral Mucositis (ITT Analysis: Treatment Cycle 1)

During Treatment Cycle 1 the incidence of WHO grade $\geq$2 OM was significantly reduced for patients treated with Saforis compared with patients treated with placebo (38.7% vs 49.7%; $P = .026$; Fig. 3). Analysis of the severity of OM in Treatment Cycle 1 showed a statistically significant ($P = .042$) shift in the distribution of maximum grade toward lower grade in the Saforis arm (Table 3). Although overall incidence of WHO grade $\geq$3 OM was low, the incidence was significantly lower in the Saforis arm compared with the placebo arm. Only 2 (1.2%) patients treated with Saforis compared with

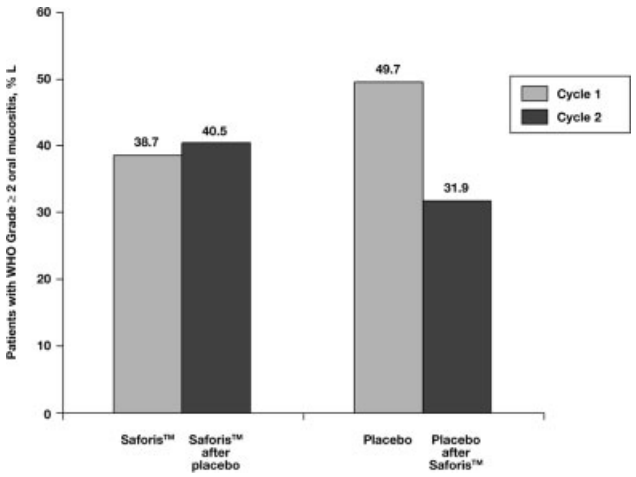

FIGURE 3. Percentage of patients responding to treatment with Saforis versus placebo in Treatment Cycles 1 and 2.

TABLE 3
Maximum Severity of Oral Mucositis by Treatment Group in Treatment Cycle 1

Maximum WHO grade	Patients, n (%) Saforis (n = 163)	Placebo (n = 163)	*P*
0	52 (31.9)	50 (30.7)	.042*
1	48 (29.4)	32 (19.6)	
2	61 (37.4)	70 (42.9)	
3	2 (1.2)	11 (6.7)	.005†

WHO indicates World Health Organization.
* Overall shift in the distribution of maximum oral mucositis grade using the Wilcoxon rank-sum test, adjusted for center.
† Cochran-Mantel-Haenszel test, adjusted for center.

11 (6.7%) patients in the placebo arm developed WHO grade ≥3 OM during Treatment Cycle 1 ($P = .005$; CMH test adjusted for center). Treatment with Saforis was also associated with a statistically significant decrease in worst ulceration score in Treatment Cycle 1 compared with patients receiving placebo ($P = .013$; Table 4). The proportion of patients with OMAS ulceration score >0 was significantly lower in the Saforis group than in the placebo group ($P = .025$). Analysis of the incidence of WHO grade ≥2 OM at each assessment time point throughout Treatment Cycle 1 is shown in Figure 4. Saforis consistently reduced the proportion of patients with WHO grade ≥2 OM.

Ability to Eat Solid Foods and Subject Assessment of Pain

Patient self-assessment of the ability to eat solid foods during Treatment Cycle 1 showed a statistically significant difference in the proportion of patients who could eat solid foods in the Saforis group compared with the placebo group (97.5% vs 91.9%; $P = .039$). No treatment differences were observed with respect to intensity of oral pain or swallowing difficulty.

TABLE 4
Oral Mucositis Assessment Scale

	Treatment Cycle 1 Intervention Saforis (n = 163)	Placebo (n = 163)	*P*
Oral mucositis score, mean (SD)	0.22 (0.29)	0.26 (0.34)	.200
Worst ulceration score, mean (SD)	0.23 (0.39)	0.32 (0.45)	.013*
Ulceration score >0, n (%)	63 (38.7)	81 (49.7)	.025†

SD indicates standard deviation.
* From analysis of variance with terms for center and treatment.
† From Cochran-Mantel-Haenszel test adjusted for center.

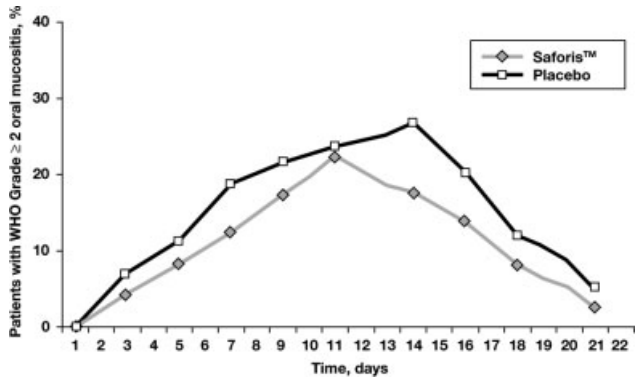

FIGURE 4. Percentage of patients with World Health Organization (WHO) grade ≥2 oral mucositis at each time point during Treatment Cycle 1 by treatment group.

Carryover Effect: Treatment Cycle 2

Clinical trials using a crossover design assume that the effects of the drug used in the first cycle will not affect the outcome of the second cycle or that the 2 carryover effects will be equal.[34] Because the test of the hypothesis of equal carryover effects for the 2 treatment sequence groups (Saforis followed by placebo and placebo followed by Saforis) detected a statistically significant difference in carryover effects ($P = .0269$ for the ITT population), the assumption of equal carryover effects was rejected and supported the prespecified plan to analyze the trial as a single-treatment-cycle, parallel-group-design study.

The incidence of WHO grade ≥2 OM in patients receiving Saforis for the first time was consistent between Treatment Cycles 1 and 2 (38.7% Cycle 1 and 40.5% for Cycle 2); however, the incidence of WHO grade ≥2 OM among placebo-treated patients was

TABLE 5
Most Commonly Reported Treatment-Emergent Adverse Events Occurring in Treatment Cycle 1 and Treatment Cycle 2

	Patients, n (%)			
	Saforis (n = 306)		Placebo (n = 314)	
Preferred term	Related*	Unrelated	Related*	Unrelated
At least 1 event	32 (10.5)	173 (56.5)	35 (11.1)	181 (57.6)
Nausea	27 (8.8)	100 (32.7)	26 (8.3)	108 (34.4)
Vomiting NOS	5 (1.6)	32 (10.5)	6 (1.9)	33 (10.5)
Dry mouth	16 (5.2)	1 (0.3)	13 (4.1)	1 (0.3)
Anorexia	2 (0.7)	11 (3.6)	1 (0.3)	15 (4.8)
Leukopenia NOS	0	41 (13.4)	0	45 (14.3)
Weakness	0	29 (9.5)	0	30 (9.6)
Asthenia	0	25 (8.2)	0	27 (8.6)
Alopecia	0	27 (8.8)	0	25 (8.0)
Fatigue	0	23 (7.5)	1 (0.3)	24 (7.6)
Neutropenia	0	18 (5.9)	0	24 (7.6)

NOS indicates not otherwise specified.
* Related includes probable, possible, and missing relationship.

significantly lower in Cycle 2 (31.9%) compared with Cycle 1 (49.7%; $P = .0269$, Fig. 3). Patients who were treated with placebo in Treatment Cycle 2 were exposed to Saforis for approximately 19 days in their prior Treatment Cycle 1 and then received chemotherapy on average approximately 2 days later. Therefore, the apparent effectiveness of placebo in the second cycle is more suggestive of a carryover effect.

Safety

Adverse events were analyzed for Treatment Cycles 1 and 2. The most commonly reported treatment-emergent adverse events included nausea, vomiting, leukopenia, weakness, asthenia, alopecia, fatigue, neutropenia, dry mouth, and anorexia (Table 5), all of which typically occur in cancer patients receiving cytotoxic chemotherapy. The majority of these events were mild to moderate in severity and considered unrelated to study drug. Treatment-emergent adverse events that were considered possibly or probably related to study drug (primarily nausea) were reported by 10.5% of patients during a Saforis cycle and 11.1% of patients during a placebo cycle. Five patients (1.6%) experienced serious adverse events while receiving placebo; no patient experienced a serious adverse event while receiving Saforis. Overall, the safety profile of Saforis was comparable to that of placebo.

DISCUSSION

This trial demonstrated that Saforis reduced the incidence of clinically significant OM and reduced the severity of OM compared with placebo in patients receiving mucotoxic chemotherapy for the treatment of breast cancer. Moreover, the efficacy of Saforis was consistently observed across multiple endpoints.

Historically, management of OM in cancer patients has been limited to supportive care—including pain control, nutritional support, hydration, and wound care—and to simple, nonspecific interventions such as cryotherapy and compounded mouthwashes that include a topical local anesthetic agent. Although these interventions may be beneficial, they are not directed to the fundamental mechanistic changes associated with the pathophysiology of OM, nor do they prevent OM. The current paradigm for mucosal injury in cancer patients is based on a complex cascade of mucosal tissue changes that appears to be initiated within hours of exposure to cytotoxic agents (Fig. 5).[2,23,35] This model provides an important framework for conceptualizing and testing possible interventions directed at various targets or points along the mucosal injury cascade. Glutamine has for many years been viewed as a potentially valuable agent for reducing mucosal injury in cancer patients.[15,22] Given the role of reactive oxygen species and cytokines in the mucosal injury cascade, it is logical to hypothesize that glutamine may provide therapeutic benefit in cancer patients at risk for OM. However, several studies using simple formulations of oral glutamine that do not facilitate uptake by mucosal cells did not demonstrate a definitive benefit.[17–23] By contrast, Saforis facilitates rapid uptake of glutamine and delivers glutamine directly to mucosal cells at risk for injury from mucotoxic cancer therapy.

There are several potential mechanisms by which Saforis may protect epithelial cells from mucotoxic cancer therapy, including providing them with the fuel to recover from previous damage and stimulating collagen production. As noted previously, glutamine is a conditionally essential amino acid that has multiple functions in the human body. Most important and in the context of mucosal injury, glutamine is a precursor for glutathione, which increases intracellular redox potential[9,10]; it inhibits proinflammatory cytokines and cytokine-related apoptosis[11–13]; and it increases synthesis of collagen by fibroblasts, which is important for healing (Fig. 5).[14,23]

In addition, a statistically significant carryover effect in this study was observed that may reflect a possible biologic effect of Saforis in patients receiving placebo in Treatment Cycle 2. This finding is intriguing and suggests that pretreatment with Saforis in Treatment Cycle 1 may have protected patients from OM during the subsequent chemotherapy cycle, perhaps through multiple wound-healing pathways. Although epithelial lesions may resolve clinically between courses of anticancer

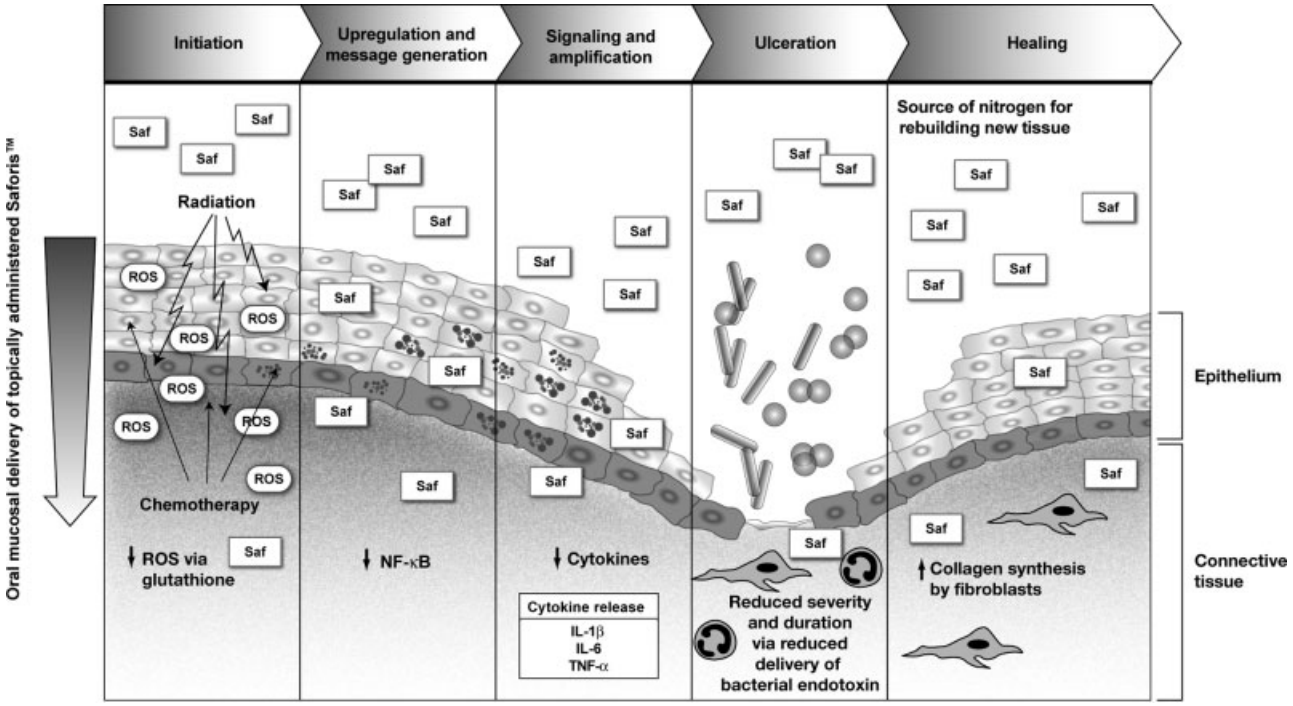

FIGURE 5. Proposed effects of Saforis in relation to the current model of the mucosal injury process as described by Sonis.[23] During initiation, chemotherapy and radiation damage DNA directly and through production of reactive oxygen species (ROS); intracellular glutamine neutralizes ROS via glutathione, thus preventing further damage. During up-regulation and message generation, intracellular signaling molecules such as NF-κB induce expression of genes involved in inflammation and fibronectin breakdown; during this phase, intracellular glutamine decreases NF-κB expression. During signaling and amplification, a variety of proinflammatory cytokines are produced that cause extended tissue injury; during this phase, intracellular glutamine down-regulates these cytokines, particularly TNF-α, thus closing the inflammatory feedback loop. In addition, intracellular glutamine reduces apoptosis of epithelial cells throughout these initial phases. During ulceration, breakdown of the mucosal epithelium results in significant pain; colonization by microorganisms, thus increasing the risk of sepsis; and secretion of inflammation-mediated proinflammatory cytokines from macrophages in response to bacterial endotoxin. During this phase, intracellular glutamine reduces inflammation, stimulates infiltrating lymphocytes, and provides metabolic energy for new protein synthesis. Finally, during healing, the oral mucosa is reestablished, but the patient remains at higher risk for oral mucositis. During healing, intracellular glutamine promotes collagen biosynthesis by fibroblasts and provides a source of nitrogen for rebuilding normal healthy mucosal tissue, resulting in more robust healing and reduced risk of subsequent mucositis. Saf, Saforis; NF-κB, nuclear factor kappaB; IL, interleukin; TNF-α, tumor necrosis factor-alpha.

therapy, the nascent epithelial cells may still be rapidly dividing and differentiating as part of the normal healing process.[2] These rapidly dividing cells are likely more susceptible to cytotoxic agents, thus increasing the potential of recurring mucosal damage during the next course of chemotherapy. Thus, patients on multiple-cycle chemotherapy may be at risk for more severe OM in subsequent cycles, particularly if they developed severe OM during their previous cycle of chemotherapy. However, not every patient who previously developed WHO grade $\geq$2 OM develops a comparable degree or severity of OM during their next chemotherapy cycle, as observed in this study. For example, despite the fact that 100% of all patients who were entered into the treatment phase of this clinical trial had documented WHO grade $\geq$2 OM during the screening cycle, only 50% of patients in the placebo arm developed WHO grade $\geq$2 OM during Treatment Cycle 1. This outcome illustrates the variable clinical expression of OM within patients despite repeated mucotoxic chemotherapy cycles, as well as across patients receiving the same chemotherapy regimen. Further studies of Saforis in patients receiving multiple cycles of mucotoxic chemotherapy, without a crossover design, will be important.

Although a statistically significant carryover was observed in this study, the clinical significance of this observation is unclear. One hypothesis is that Saforis may interrupt the cycle of mucosal damage and reduce the incidence of OM not only in the treatment cycle when it was administered but also in the next treatment cycle when patients were no longer receiving active drug. This would be consistent with the hypothesis that Saforis facilitates more rapid and complete repair of mucosal damage, thus rendering the epithelium more resistant to further oxidative damage. Alternatively,

there may have been insufficient time between the last dose of Saforis and crossover to placebo to allow a return to baseline tissue levels of glutamine; indeed, based on the dosing guidelines, a subset of patients may have continued treatment with Saforis through the end of Treatment Cycle 1.

Saforis offers several potential advantages over certain other therapies, including its ease of use and a favorable safety profile. The oral swish-and-swallow regimen integrates well with current established foundations of oral care for cancer patients (including oral hygiene practices) delivers the drug directly to the target tissue, and may be more convenient than intravenous therapy. Several studies,[29–31] including the present study, have demonstrated the excellent safety profile of Saforis, which is comparable to that of placebo. The total daily dose of Saforis used in the present study was within the range of dietary glutamine consumed by an adult on a high-protein diet (approximately 8 g per day). At this dose, the amount of glutamine ingested has been shown to present no safety risk; 30 g or more per day is often administered as a supplement to total parenteral nutrition without negative effect.

In conclusion, there continues to be a considerable need for safe, convenient, and cost-effective agents that can prevent or reduce the severity of OM in cancer patients at risk. Molecularly targeted therapies are being developed as expanded knowledge of the pathophysiology of mucosal injury emerges. Saforis is a promising new agent that has demonstrated significant clinical benefit in patients at risk for OM, and it is a safe, easily administered oral therapy. Moreover, Saforis could be complementary if not synergistic with other agents that are directed at specific molecular targets. Such strategies may result in the substantial reduction of not only the incidence and severity of OM, but also, possibly, in other symptom complex toxicities that share common pathophysiologic pathways in these cancer patients.

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