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Streptococcus salivarius K12 Alleviates Oral Mucositis in Patients Undergoing Radiotherapy for Malignant Head and Neck Tumors: A Randomized Controlled Trial

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ABSTRACT

PURPOSE Oral mucositis (OM) is a common debilitating toxicity associated with radiotherapy (RT) for malignant head and neck tumors. This prospective, randomized, double-blind, placebo-controlled trial aimed to evaluate the efficacy and safety of Streptococcus salivarius K12 (SsK12) in reducing the incidence, duration, and severity of severe OM (SOM).

METHODS A total of 160 patients with malignant head and neck tumors undergoing definitive or postoperative adjuvant RT were randomly assigned (1:1) to receive SsK12 probiotic (n = 80) or placebo (n = 80) at West China Hospital, Sichuan University, Chengdu, China. Patients were instructed to suck SsK12 or placebo lozenges thrice daily from the initiation to the end of RT. OM was evaluated twice a week during RT and once a week thereafter for up to 8 weeks. The primary end point was the incidence of SOM. Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0.

RESULTS Baseline patient characteristics were similar in the SsK12 and placebo groups. The incidence of SOM was significantly lower in the SsK12 group as compared with the placebo group (36.6% *v* 54.2%; $P = .0351$). The duration (median, 0.0 days *v* 7.0 days; mean, 8.9 days *v* 18.3 days; $P = .0084$) and time to develop SOM (median, not estimable *v* 42.0 days; hazard ratio, 0.55 [95% CI, 0.34 to 0.89]; log-rank test: $P = .0123$) were also improved in the case of the SsK12 group. Adverse events were similar between the groups, and mild or moderate gastrointestinal reactions (flatulence or dyspepsia) associated with the lozenges were observed in two patients in the SsK12 group. High-throughput sequencing results indicated that SsK12 inhibited opportunistic pathogens and enriched oral commensals during RT.

CONCLUSION In this prospective, randomized clinical trial, SsK12 probiotic significantly reduced the incidence, onset, and duration of SOM with a good safety profile.

ACCOMPANYING CONTENT

Article, p. 1436

Data Sharing Statement

Data Supplement

Protocol

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INTRODUCTION

Radiotherapy (RT) is an important method of treatment for malignant tumors of the head and neck and can be used alone or in combination with chemotherapy as a radical or adjuvant therapy.[1] Despite improvements in RT equipment and techniques, various acute oral complications persist, including oral mucositis (OM), dry mouth, taste dysfunction, and oral infections.[2] OM is one of the most common acute radiation-related toxicities, and approximately 50%-70% of patients experience severe OM (SOM) defined by the WHO scale as grade 3-4.[3-6] The painful inflammation and ulceration associated with OM not only profoundly affect patients' ability to eat, swallow, and speak but also decrease their tolerance to anticancer treatment, thereby impairing their quality of life (QoL) significantly and causing interruptions in their cancer treatment.[7] Although some clinical strategies for radiation-induced OM have been recommended by the Multinational Association of Supportive Care in Cancer and the International Society of Oral Oncology,[8] their efficacy and safety still need further clinical validation.

Recent evidence suggests the involvement of oral microbiota in radiation-induced OM, and modulation of oral microbiota is promising for the management of OM.[9-11] Streptococcus salivarius K12 (SsK12) is a commercially available oral

CONTEXT

Key Objective

Is probiotic Streptococcus salivarius K12 (SsK12) able to alleviate oral mucositis (OM) in patients undergoing radiotherapy (RT) for malignant head and neck tumors?

Knowledge Generated

Topical application of SsK12 significantly reduces the incidence of severe OM (SOM) in patients undergoing RT as compared with those who receive placebo. The development of SOM is delayed, and its duration is also shortened after the administration of SsK12.

Relevance *(J.P.S. Knisely)*

Head & neck cancer severe acute RT-associated OM was delayed in onset and decreased in incidence and duration by the use of a SsK12 probiotic in a prospective, randomized, double-blind clinical trial.*

*Relevance section written by *JCO* Associate Editor Jonathan P.S. Knisely, MD.

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probiotic with strong oral colonization ability, bacteriocin-like inhibitory substance (BLIS)–producing capability, and immunomodulatory properties and has been used to treat oral candidiasis, pharyngitis, tonsillitis, halitosis, and otitis media.[12-17] More importantly, data from our recent animal study demonstrated that topical use of SsK12 ameliorates radiation-induced OM in mice by modulating the oral microbiota, mainly by suppressing oral anaerobes.[18] However, the efficacy of SsK12 for treating radiation-induced OM still requires validation through well-controlled clinical trials. In this study, a prospective randomized clinical trial was designed to evaluate the effect of SsK12 on SOM in patients undergoing RT for malignant head and neck tumors. The effects of SsK12 on other radiation-related oral complications such as dry mouth, gustatory function, and microbial diversity in saliva were also investigated.

METHODS

Study Design and Patients

This prospective, randomized, double-blind, placebo-controlled trial was conducted at the West China Hospital, Sichuan University. The local institutional review board and ethics committee approved the study protocol, and the study was registered at ClinicalTrials.gov (identifier: NCT05918224). Written informed consent was obtained from each participant and family member before enrollment.

Patients pathologically diagnosed with nonmetastatic head and neck malignant tumors, age 18-80 years, with an Eastern Cooperative Oncology Group performance status of ≤2, and planning to receive definitive RT or postoperative adjuvant RT at a dose of 60-72 Gy with or without concurrent chemotherapy were eligible for this study. Exclusion criteria included patients with a history of allergy to probiotics or severe allergic constitution, use of antibiotics/antifungal drugs within 1 month or antimicrobial mouthwash within 1 week before the study, poor oral hygiene and/or severe periodontal diseases, any previous RT to the head and neck region, and those deemed unsuitable for the study by the investigators (concomitant with any other severe diseases).

Random Assignment and Marking

All patients were randomly divided in a 1:1 ratio into two groups to receive either the probiotic SsK12 or a placebo via a computer-generated random assignment list using a randomized permutation block design. Methods for random assignment and marking are detailed in the Data Supplement (online only) and Protocol.

Interventions

Before the start of RT, all patients received instructions for maintaining oral hygiene, oral clinical examination, and treatment including dental filling, endodontic treatment, extraction of nonrestorable teeth, and nonsurgical periodontal treatment, if necessary. All recruited patients underwent image-guided RT at a daily dose of 1.8-2.2 Gy, five times per week (total, 6-6.5 weeks), thus receiving a cumulative tumor dose of 60-72 Gy. Clinical target volumes and organs at risk were delineated in accordance with the consensus guidelines.[19,20] The oral cavity was defined according to the recommendation in the study by Mir et al.[21] Concurrent chemotherapy consisted of cisplatin (100 mg/m^2) administered once every 3 weeks.

SsK12 lozenges (NOW Foods, USA) contained at least 1×10^9 colony-forming units of viable SsK12 cells as the active ingredient. The placebo lozenges contained sugar and starch as excipients in the active formulation. Patients were instructed to suck the SsK12 lozenges or placebo lozenges thrice daily enduring the entire RT period. The patients were

advised to avoid eating, drinking, and conducting any oral hygiene activities for at least 1 hour after using the lozenges.[22] The number of lozenges used each day was recorded in patients' diaries. During RT, supportive care such as normal oral rinses, topical analgesics, and nutritional support was allowed, whereas anti-inflammatory agents, antibiotics/antifungal agents, antimicrobial mouthwash, and other experimental systemic or topical pharmaceuticals or devices were not permitted.

Assessment

OM was evaluated by trained investigators according to the WHO Oral Toxicity Scale (Data Supplement, Table S1). OM was evaluated twice weekly during RT and once a week thereafter for up to 8 weeks. During RT, patients reported mouth and throat soreness (MTS) scores and the degree of impact of MTS on oral activities via the weekly oral mucositis questionnaire. Measures taken to ensure the consistency and accuracy of clinical data collection are detailed in the Data Supplement and Protocol.

Other clinical parameters, including hyposalivation, gustatory function, QoL, adverse events, weight loss, use of analgesics, use of intravenous hydration or total parenteral nutrition, and RT break fractions, are evaluated as detailed in the Data Supplement and Protocol.

Saliva Collection and Bioinformatics

Unstimulated whole saliva was collected at baseline (T0), during mid-term RT (T1, week 3), and at the end of RT (T2, week 6 or 6.5). Bacterial genomic DNA was extracted, and the hypervariable regions 3-4 (V3-V4) of the 16S rRNA gene were amplified for high-throughput sequencing. Bioinformatics were performed using the online Majorbio Cloud Platform (Majorbio, Shanghai, China[23]). The Benjamini-Hochberg method was used to calculate the false-discovery rate for multiple comparisons, and $P_{FDR} < .05$ was considered significant (see the Data Supplement and Protocol for details).

Outcome

The primary end point was the incidence of SOM (WHO grade 3-4). The secondary end points included the duration of SOM, time to onset of SOM, incidence and duration of OM, time to onset of OM, number of RT interruptions, and safety. The exploratory assessments included the presence of hyposalivation, changes in gustatory function, average MTS scores, incidence of analgesic use, use of intravenous hydration or total parenteral nutrition, BMI, QoL, and salivary microbiota.

Sample Size Estimation and Statistical Analysis

On the basis of the literature, the average incidence of SOM observed during head and neck RT is approximately 65%,[4] and we expected a 25% decrease in the incidence of SOM in the SsK12 group. Accordingly, 142 patients (71 patients per group) were needed (two-sided $\alpha = .05, 1 - \beta = .8$, 1:1 ratio), and considering a patient dropout rate of 10%, at least 158 patients (79 patients per group) were targeted for enrollment.

All patients who completed the study (per-protocol set [PPS]) and all randomly assigned patients (full analysis set [FAS]) were included in the efficacy analyses. The safety analyses

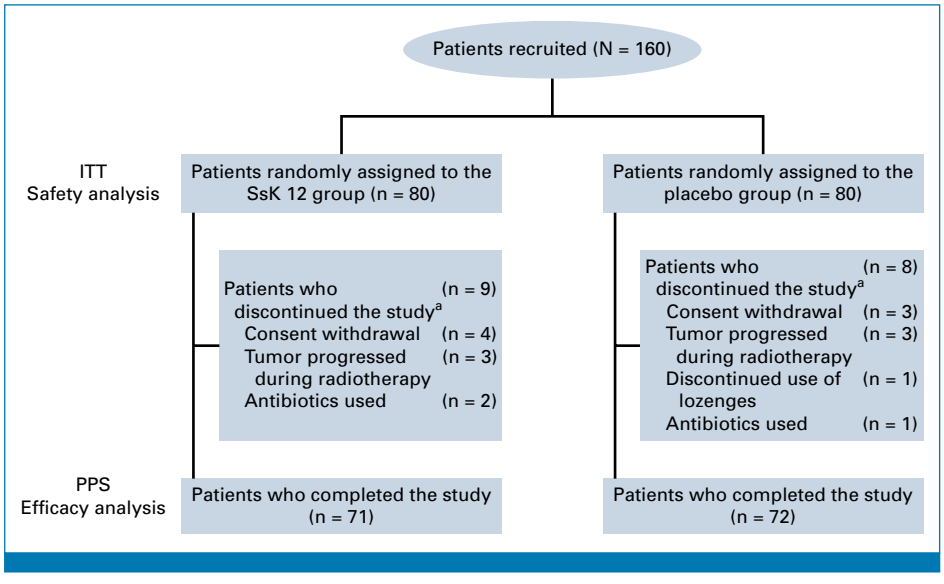

FIG 1. CONSORT diagram: patient random assignment. [a]Patients who discontinued the acute oral mucositis evaluation phase of the study. ITT, intent to treat; PPS, per-protocol set; SsK12, Streptococcus salivarius K12.

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included all randomly assigned patients who received the study medication. The duration of SOM/OM was determined as detailed in the Data Supplement and Protocol. Time to develop SOM/OM was analyzed by the Kaplan-Meier method, and log-rank tests were used to compare the curves. Hazard ratio with 95% CI was estimated using a Cox proportional hazard model. Continuous variables of the different groups were analyzed using the t-test; for nonparametric data, the comparison was performed using the Wilcoxon rank-sum test. Categorical variables were compared using the χ^2 test or Fisher's exact test. $P < .05$ was considered as significant. The SAS statistical package version 9.3 (SAS Institute, Cary, NC) was used for data analysis.

RESULTS

A total of 160 eligible patients were enrolled in the study (Fig 1). All patients were included in the safety analysis, among which 143 patients (SsK12, n = 71; placebo, n = 72) were evaluated for efficacy. Seventeen patients (11%) who discontinued the study were excluded from the PPS analysis for different reasons listed in Figure 1. The characteristics of the 17 excluded patients are presented in the Data Supplement (Table S3). The baseline characteristics of patients, who were fully evaluated, were similar between the two groups (Table 1). A majority of patients had tumors in the nasopharynx (25.87%), followed by the oral cavity (17.48%) and oropharynx (13.99%). The mean dose to oral cavity was 42.1 Gy in the SsK12 group and 42.2 Gy in the placebo group ($P > .05$; Table 1). Patients displayed good adherence in both groups, with a mean adherence rate of 97% in the SsK12 group and 96% in the placebo group.

Efficacy

The incidence of grade 3-4 SOM, which was the primary efficacy end point, was significantly reduced among patients receiving SsK12 lozenges as compared with those receiving placebo (36.6% *v* 54.2%; $P = .0351$; Table 2). The distribution of OM during each patient's observation period is shown in Figure 2. The SOM duration in the SsK12 group was significantly shorter than that in the placebo group (median, 0.0 days *v* 7.0 days; mean, 8.9 days *v* 18.3 days; $P = .0084$; Table 2). Similarly, the median duration of SOM among the patients who had SOM in the SsK12 group was also significantly shorter than that in the placebo group (19.0 days *v* 35.0 days; $P = .0485$). Similar results were obtained in the FAS (Data Supplement, Table S4). The time to develop SOM in the SsK12 group was significantly delayed as compared with that in the placebo group ($P = .0123$; Table 2; Data Supplement, Fig S2). The incidence of grade 4 OM was significantly lower in the SsK12 group vis-a-vis the placebo group (2.8% *v* 15.3%; $P = .0169$; Table 2). The administration of SsK12 effectively reduced the incidence and duration and delayed the time to onset of OM (Table 2). One patient in the SsK12 group and two patients in the placebo group missed five or more consecutive radiation fractions because of SOM (Table 2; Fig 2).

The distribution of the mean WHO OM grade and incidence of SOM during the RT course in the two groups are shown in Figure 3. As the radiation dose increased, the average OM grade of patients in the SsK12 group was consistently lower than that in the placebo group, with an increasing trend in both groups peaking at the sixth week.

Other Clinical Parameters

The mean weekly MTS and oral activity scores were slightly lower in the SsK12 group as compared with the placebo group (Data Supplement, Fig S3). The MTS index and oral activity index were also comparatively lower after SsK12 treatment versus the placebo treatment (Data Supplement, Fig S4).

The incidence of analgesic use was lower in the SsK12 group; however, the difference was not statistically significant (7% *v* 11%). Approximately 45% of patients in the SsK12 group and 53% in the placebo group received intravenous hydration or total parenteral nutrition. The decrease in BMI during RT was similar between the two groups (2.18 and 2.47 in the SsK12 and placebo groups, respectively; $P = .7451$).

Furthermore, 55 (77%) and 56 (78%) patients in the SsK12 and placebo groups, respectively, experienced radiation-related grade 2 or higher hyposalivation at the end of RT, and the incidence of hyposalivation was similar between groups during the observation period (Data Supplement, Table S5). Patients' gustatory function decreased in the two groups immediately at mid-RT and was lowest at the end of treatment, with a trend toward recovery at 1 month after RT (Data Supplement, Fig S5A). The detection of all four classic tastes (sweet, bitter, sour, and salty) was affected by RT, with bitter and salty tastes being the most affected ones (Data Supplement, Fig S5B).

Oral Microbiota

A total of 420 saliva samples were collected for 16S rRNA sequencing, and 406 saliva samples were eligible for further analysis. The rarefaction curves are presented in the Data Supplement (Fig S6). No significant differences in Alpha diversity were detected within or between groups ($P > .05$; Figs 4A and 4B). The principal coordinates analysis plots showed a significant difference in the microbial community at T0 (baseline), T1 (mid-term radiation), and T2 (end of radiation) in both groups (Figs 4C and 4D); however, no significant between-group differences were observed (Data Supplement, Fig S7). The relative abundance of *Firmicutes* in the placebo group exhibited a decreasing trend, whereas it remained relatively stable in the SsK12 group during RT (Data Supplement, Fig S8). Further comparison of the relative abundance of genera at T0 and T2 showed that three of the top 30 dominant genera exhibited different trends of alteration between the two groups (Figs 4E and 4F). The relative abundance of Streptococcus significantly decreased

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TABLE 1. Baseline Characteristics of Patients Who Completed This Study

Characteristic	SsK12 (n = 71)	Placebo (n = 72)	All Patients (N = 143)	*P*
Sex, No. (%)				.0695
Male	41 (57.75)	52 (72.22)	93 (65.03)	
Female	30 (42.25)	20 (27.78)	50 (34.97)	
Age, years, mean (SD)	52 (13)	53 (12)	52 (12)	.7200
BMI, kg^2/m, mean (SD)	23 (9)	24 (11)	24 (10)	.5847
ECOG PS, No. (%)				.9976
0	42 (59.15)	43 (59.72)	85 (59.44)	
1	26 (36.62)	26 (36.11)	52 (36.36)	
2	3 (4.23)	3 (4.17)	6 (4.20)	
Tumor site, No. (%)				.9693
Nasopharynx	19 (26.76)	18 (25.00)	37 (25.87)	
Oral cavity	13 (18.31)	12 (16.67)	25 (17.48)	
Oropharynx	8 (11.27)	12 (16.67)	20 (13.99)	
Hypopharynx	7 (9.86)	7 (9.72)	14 (9.79)	
Larynx	10 (14.08)	9 (12.50)	19 (13.29)	
Others	14 (19.72)	14 (19.44)	28 (19.58)	
Tumor pathologic types, No. (%)				.8874
Squamous carcinoma	53 (74.65)	53 (73.61)	106 (74.13)	
Others	18 (25.35)	19 (26.39)	37 (25.87)	
Treatment type, No. (%)				.5389
Definitive	21 (29.58)	18 (25.00)	39 (27.27)	
Postoperative treatment	50 (70.42)	54 (75.00)	104 (72.73)	
TNM stage, No. (%)				.5792
I-II	14 (19.72)	14 (19.44)	28 (19.58)	
III	22 (30.99)	17 (23.61)	39 (27.27)	
Iva	28 (39.44)	29 (40.28)	57 (39.86)	
IVb	7 (9.86)	12 (16.67)	19 (13.29)	
Tumor primary site, No. (%)				.5640
T1	13 (18.31)	8 (11.11)	21 (14.69)	
T2	21 (29.58)	21 (29.17)	42 (29.37)	
T3	19 (26.76)	19 (26.39)	38 (26.57)	
T4	18 (25.35)	24 (33.33)	42 (29.37)	
Nodal involvement, No. (%)				.1333
N0	24 (33.80)	29 (40.28)	53 (37.06)	
N1	14 (19.72)	5 (6.94)	19 (13.29)	
N2	21 (29.58)	21 (29.17)	42 (29.37)	
N3	12 (16.90)	17 (23.61)	29 (20.28)	
Oropharyngeal cancer HPV status, No. (%)				
Positive	2 (25.00)	3 (25.00)	5 (25.00)	
Negative	6 (75.00)	9 (75.00)	15 (75.00)	
Concurrent chemotherapy, No. (%)	35 (49.30)	32 (44.44)	67 (46.85)	.5611
Tobacco use, No. (%)				.1558
Yes	33 (46.48)	42 (58.33)	75 (52.45)	
No	38 (53.52)	30 (41.67)	68 (47.55)	
Alcohol use, No. (%)				.2077
Yes	30 (42.25)	38 (52.78)	68 (47.55)	
No	41 (57.75)	34 (47.22)	75 (52.45)	
Total RT dose, cGy, mean/median	6,609/6,600	6,644/6,600	6,627/6,600	.3733
Dose to oral cavity, cGy, mean/median (range)	4,208/4,083 (1,027-6,630)	4,220/4,184 (1,032-6,585)	4,214/4,131 (1,027-6,630)	.9548

NOTE. BMI calculated as weight in kilograms divided by height in meters squared.
Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; HPV, human papillomavirus; RT, radiotherapy; SD, standard deviation; SsK12, S. salivarius K12.

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TABLE 2. Efficacy Results

Parameter	SsK12 (n = 71)	Placebo (n = 72)	*P*
SOM (grade 3 or 4) incidence through RT, %[a]	36.6	54.2	.0351
SOM duration, days, median/mean(SD)[b]	0.0/8.9 (15.0)	7.0/18.3 (22.5)	.0084
SOM onset, days, median[c]	NE	42.0	.0123
Grade 4 OM incidence through RT, %[a]	2.8	15.3	.0169
OM incidence through RT, %[a]	62.0	83.3	.0049
OM duration, days, median[b]	10.5	40.3	.0010
OM onset, days, median[c]	28.0	17.5	<.0001
RT treatment breaks >five consecutive fractions, No. (%)	1 (1.4)	2 (2.8)	

Abbreviations: NE, not estimable; OM, oral mucositis; RT, radiotherapy; SD, standard deviation; SOM, severe OM; SsK12, S. salivarius K12.
[a]χ^2 test or Fisher's exact test.
[b]Wilcoxon rank-sum test.
[c]Log-rank test.

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at the end of radiation in the placebo group (P = .0038; P_{FDR} = .0357), but it was unchanged in the SsK12 group (P = .1343; P_{FDR} = .3542). Conversely, the relative abundance of Selenomonas and Acinetobacter was increased at the end of radiation in the placebo group (P = .0003; P_{FDR} = .0050 for Selenomonas, and P = .0164; P_{FDR} = .0898 for Acinetobacter), whereas it was significantly decreased in the SsK12 group (P = .0055; P_{FDR} = .0386 for Selenomonas and P = .0014; P_{FDR} = .0135 for Acinetobacter; Fig 4F). The relationships between the relative abundance of the three abovementioned genera and time were further analyzed, and the relative abundance of Streptococcus was negatively correlated with time in the placebo group (coefficient = −0.0175; P = .0036; P_{FDR} = .0177), whereas it was relatively stable in the SsK12 group during RT (coefficient = 0.0095; P = .1312; P_{FDR} = .2284; Figs 4G and 4H). We also compared the top 30 dominant genera between the two groups at the end of RT. Although not statistically significant after Benjamini-

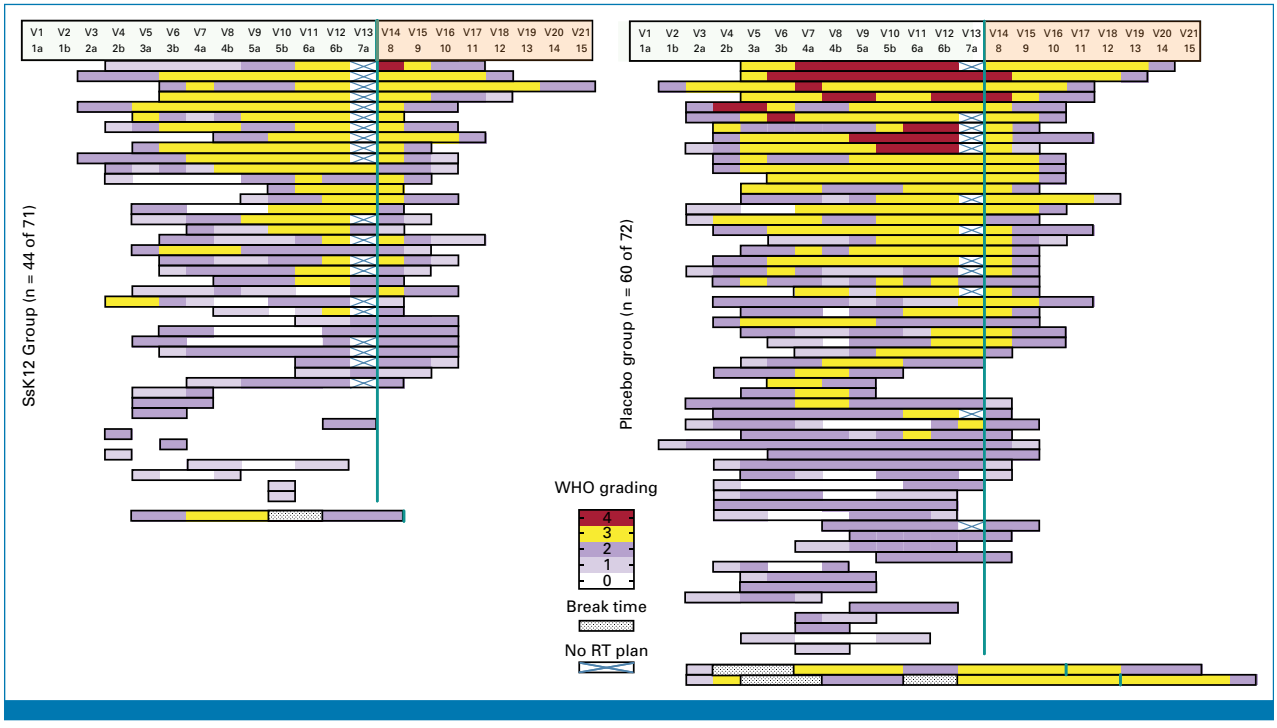

FIG 2. Swimmer plot of OM score for the subsets of patients in (left) the SsK12 group (n = 44) or (right) the placebo group (n = 60) who experienced OM. Each horizontal strip represents a patient. The top indicates the time at or after RT, whereas the vertical blue line indicates the end of RT. The bottom strip represents patients who experienced RT treatment breaks (>five consecutive fractions). OM was viewed and scored twice weekly during RT and weekly thereafter for up to 8 weeks. Dark spot, break time; mauve, WHO grade 1; OM, oral mucositis; purple, WHO grade 2; red, WHO grade 4; RT, radiotherapy; SsK12, Streptococcus salivarius K12; V, view; white, WHO grade 0; X, no RT plan; yellow, WHO grade 3.

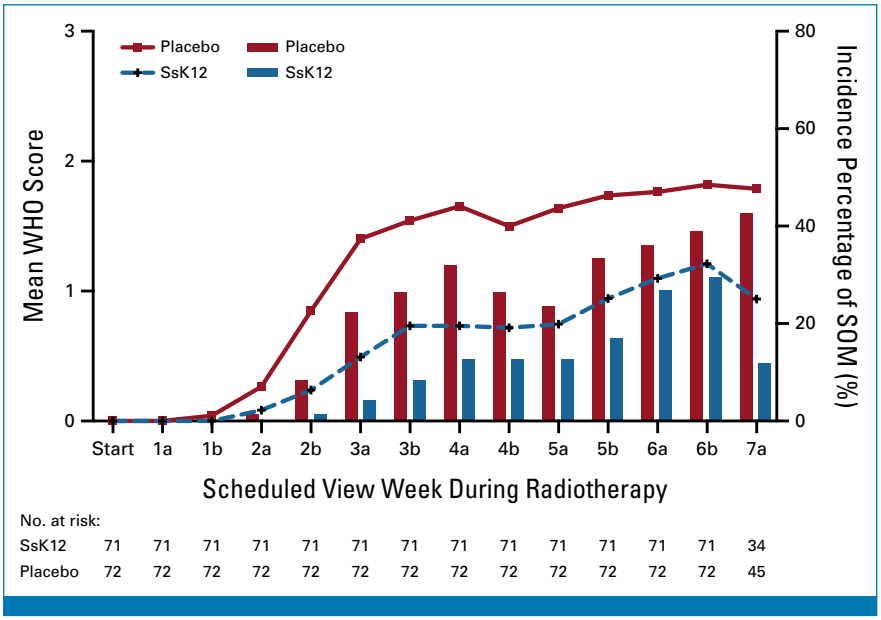

FIG 3. Mean WHO score and SOM (WHO grade 3 or 4) incidence (%) over time for SsK12 and placebo groups during radiotherapy. The bars represent the incidence of SOM, and the solid/dashed lines represent the mean WHO score. WHO scoring was performed twice per week during RT. For some patients who received 33 fractions of RT, the observation period was 6.5 weeks (7a). RT, radiotherapy; SOM, severe oral mucositis; SsK12, Streptococcus salivarius K12.

Hochberg false discovery rate adjustment, the placebo group harbored a relatively higher abundance of Peptostreptococcus and Atopobium and the SsK12 group had a higher abundance of Streptococcus and Eubacterium_brachy_ group (Data Supplement, Fig S9).

Safety

No severe adverse events (SAEs) related to SsK12 lozenges or placebo administration were observed. In the SsK12 group, 48% of the patients versus 51% in the placebo group reported at least one SAE related to RT (Data Supplement, Table S6). The AEs were evenly distributed between groups and were consistent with the known toxicities of RT (Data Supplement, Table S7). Mild or moderate GI reactions (flatulence or dyspepsia) were considered to be potentially related to the study lozenges (two patients in the SsK12 group and one patient in the placebo group). These two symptoms resolved spontaneously without any other medications.

DISCUSSION

Microbiota plays a pivotal role in the development and progression of radiation-induced OM.[24,25] Recent studies have shown that alterations in genus abundance, particularly the decrease in oral commensals and enrichment of Gram-negative bacteria throughout RT, are associated with the onset and severity of OM,[9-11,26] suggesting that rescuing or countering the alterations of specific taxa would be promising for the management of this disease. Probiotics are live microorganisms that modulate microecology and confer health benefits to the host.[27] Previous clinical trials have shown the effectiveness of probiotics, including Lactobacillus brevis CD2 and other probiotic combinations (Bifidobacterium longum, Lactobacillus lactis, and Enterococcus faecium) in the management of radiation-induced OM.[28,29] However, the beneficial effects of Lactobacillus brevis CD2 in reducing the incidence of SOM were not confirmed in a recent clinical trial.[30] Therefore, clinical trials using alternative probiotics in larger cohorts are needed to extensively evaluate the efficacy of probiotics in the management of radiation-induced OM, with a particular focus on the incidence of SOM. SsK12 can modulate oral microbiota by producing BLISs and has exhibited good immunomodulatory properties.[31,32] Our recent data showed that SsK12 effectively ameliorates radiation-induced OM in mice, suggesting its potential application in this disease.[18]

Here, we conducted a prospective randomized clinical trial that demonstrated the effectiveness of using SsK12, wherein it significantly reduced the incidence and severity of OM in patients with malignant head and neck tumors undergoing RT. Furthermore, trends in the secondary end points, that is, the duration and time to the onset of SOM, supported this observation. Moreover, SsK12 did not increase the RT-associated toxicity.

In this study, we monitored dynamic changes of oral microbiota during the course of RT. During treatment, we found that the relative abundance of Streptococcus continuously decreased, whereas that of Selenomonas and Acinetobacter

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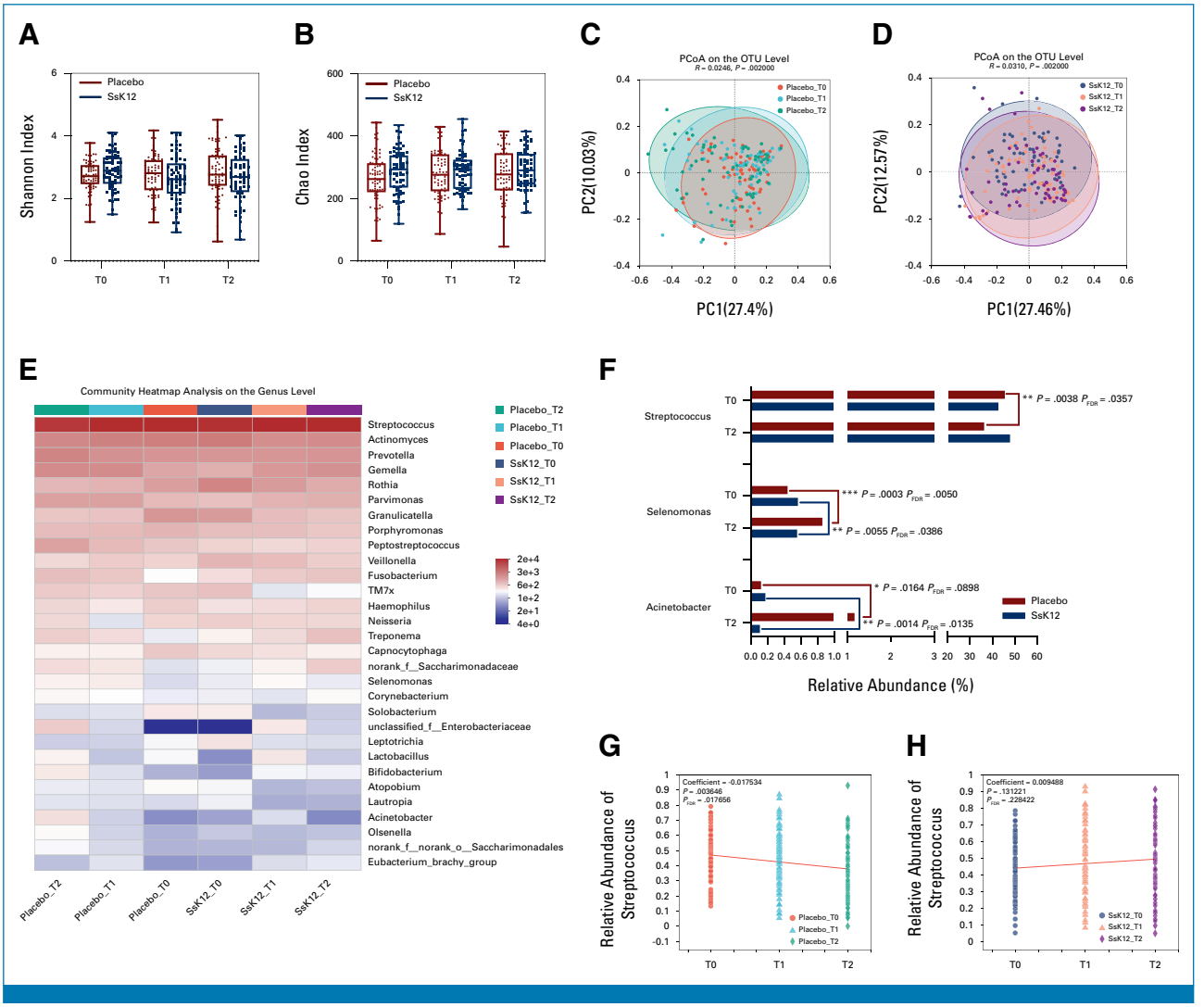

FIG 4. Effects of SsK12 treatment on salivary microbiota during RT. (A and B) Alpha diversity of oral microbial estimated by Shannon index and Chao index. (C and D) Beta diversity calculated using weighted UniFrac by the PCoA plot and further confirmed by ANOSIM analysis. (E) The heatmap of the relative abundances of the top 30 bacterial genera (on average). (F) Genera showing a divergent change trend at T0 and T2 within each group (Wilcoxon rank-sum test). (G and H) The correlations between the relative abundance of *Streptococcus* and time throughout the treatment regimen. The correlations were calculated using MaAsLin. All *P* values were adjusted for multiple comparisons using BH-FDR, and P_{FDR} < .05 was considered significant. *P < .05, **P < .01, ***P < .001. ANOSIM, analysis of similarities; BH-FDR, Benjamini-Hochberg false discovery rate; FDR, false discovery rate; OTU, operational taxonomic unit; PC, principal component; PCoA, principal coordinates analysis; Placebo-T0, before the treatment of RT plus a placebo; Placebo-T1, the middle of the treatment of RT plus a placebo; Placebo-T2, the end of the treatment of RT plus a placebo; SsK12, Streptococcus salivarius K12; SsK12-T0, before the treatment of RT plus the probiotic S. salivarius K12; SsK12-T1, the middle of the treatment of RT plus the probiotic S. salivarius K12; SsK12-T2, the end of the treatment of RT plus the probiotic S. salivarius K12.

increased in the placebo group, with the administration of SsK12 appearing to rescue or reverse this trend. Most Streptococcal species are oral commensals. A lower abundance of Streptococcus was associated with a shorter average time to the onset of SOM.[26] Consistently, patients who subsequently developed ≥grade 2 OM reported a lower abundance (26%) of Streptococcus.[10] Our study found that the application of SsK12 countered the decrease in Streptococcus during RT, which partially explained the protective effect of this probiotic. Selenomonas is a cluster of Gram-negative bacteria implicated in periodontitis[33] and OM.[9] Acinetobacter is a group of Gram-negative bacteria that are usually associated with opportunistic infections.[34,35] In the current study, the use of SsK12 reversed the increasing trend of Selenomonas and Acinetobacter during RT, which may also benefit mucosal homeostasis. Hence, we speculated that the use of SsK12 could benefit the management of OM by maintaining the abundance of commensal Streptococcus while inhibiting opportunistic pathogens during RT.

In addition to OM, we found that patients who received SsK12 had slightly lower MTS scores, oral activity impairment, additional nutritional intake, and reduced BMI than those in

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the placebo group; however, the differences were not statistically significant. This is partly because other complications of RT, such as sore throat, hyposalivation, taste dysfunction, nausea, and vomiting, also affect the QoL of patients with malignant tumors. Most patients in both groups developed hyposalivation and taste dysfunction, which is consistent with the results of previous studies.[36,37] This suggests holistic management of patients undergoing head and neck RT.

To our knowledge, this is the first study to evaluate the effectiveness of the oral probiotic SsK12 in preventing and treating radiation-induced OM. The major strength of this randomized trial is the strict inclusion and exclusion criteria, standardized RT delivery and supportive care, and the use of a placebo and double-blind design with minimized bias. In addition, previous studies have evaluated the efficacy of probiotics in the treatment of radiation-induced OM but without a longitudinal track of oral microbiota. Furthermore, compared with palifermin (keratinocyte growth factor-1), the only agent approved by the US Food and Drug Administration for minimizing OM in patients with hematologic malignancies undergoing hematopoietic cell transplantation and receiving chemotherapy or RT, SsK12 is cost-effective, is easy to use, and has a good safety profile with only mild or moderate GI reactions, thereby favoring its daily use in patients with malignant tumors undergoing RT.

This study has a few limitations. Some patients undergoing RT/chemotherapy may develop systemic infections because of immune suppression; in such cases, antibiotic intervention is needed, which may compromise the clinical outcome of probiotic treatment for OM. In addition, the placebo group had more T4 and N3 cases (although not statistically significant), which could potentially lead to dietary changes in patients because of dysphagia and sore throat, thereby decreasing the patient's resistance to mucositis. In addition, some patients reported difficulty in sucking lozenges because of dry mouth. An improved design for the form or delivery of probiotics is warranted in future studies. Furthermore, this study was conducted at a single cancer center and therefore requires multi-institutional validation.

In conclusion, the current study has demonstrated that the use of SsK12 can significantly reduce the incidence and severity of OM in patients with malignant head and neck tumors receiving RT with a good safety profile. The use of SsK12 has the potential to become a novel strategy for the oral mucosal care of patients receiving RT.

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EQUAL CONTRIBUTION

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CLINICAL TRIAL INFORMATION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Streptococcus salivarius K12 Alleviates Oral Mucositis in Patients Undergoing Radiotherapy for Malignant Head and Neck Tumors: A Randomized Controlled Trial

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