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Efficacy of GMI, a fungal immunomodulatory protein, for head and neck cancer patients with chemotherapy-related oral mucositis

An open-labeled prospective single-arm study

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Abstract

Background: Cancer patients usually suffer from intensive chemotherapy-related oral mucositis (OM), yet limited effective treatment can rapidly alleviate OM severity.

Methods: This prospective study examined the efficacy of Reishimmune-S containing one fungal immunomodulatory protein, GMI on OM in patients with head and neck cancer. Patients with head and neck cancer and the diagnosis of chemotherapy-related OM were enrolled randomizedly to receive standard supportive care with/without Reishimmune-S 500 mg/day orally for consecutive 14 days. Due to intolerance to standard supportive care alone in the control arm, only the experimental arm with Reishimmune-S supplementation was analyzed in our trial. OM grading was evaluated as the primary outcome on day 1, 8, and 15. Secondary outcomes were absolute neutrophil counts and quality of life assessed by the EORTC-QLQ-H&N 35 questionnaire on day 1, 8, and 15.

Results: Reishimmune-S supplement significantly reduced OM grading both at day 8 and 15. Trouble with social contact and weight loss conditions were also improved by Reishimmune-S. Reishimmune-S did not significantly affect absolute neutrophil counts during the 15-day follow-up.

Conclusion: Reishimmune-S supplement potentially alleviates the severity of chemotherapy-mediated OM.

Abbreviations: 5-FU = 5-fluorouracil, FIP = fungal immunomodulatory protein, HNC = head and neck cancer, OM = oral mucositis, QoL = quality of life.

Keywords: chemotherapy, fungal immunomodulatory protein, head and neck cancer, oral mucositis, Reishimmune-S

1. Introduction

1.1. Background

Chemotherapy regimens including cisplatin, docetaxel, and 5-fluorouracil (5-FU) were usually selected as systemic therapy in any stage of head and neck cancer (HNC). Many patients suffer from common adverse effects such as oral mucositis (OM) during treatment, which may reduce patients' quality of life (QoL) and even make them self-terminate the following treatment. One article even reports that more than two-thirds

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have ulcerative mucositis among HNC patients receiving chemotherapy.[1] Therefore, how to attenuate and even prevent chemotherapy-related adverse effects including OM plays a critical role on completing a whole treatment plan.

The complicated pathogenesis of OM can be divided into multi-step biological process, which indicates that therapeutics can be developed to block involved molecular pathways.[2] The model starts from an initiation phase, to primary damage response, signal amplification, ulceration, and ultimately a healing phase. In clinic, glutamine supplement was the most common option for treating patients with OM, yet the efficacy of glutamine on reducing incidence and severity of OM in cancer patients is still controversial.[3,4] Recombinant human keratinocyte growth factor, palifermin, was also approved for patients with blood cancers receiving high dose chemoradiotherapy before bone marrow transplantation.[5] However, the effects of palifermin on patients with other cancer types who are also diagnosed with OM remain unknown, which reveals that more therapeutics should be developed to treat ulcerative mucositis. Currently, other drugs and natural compounds with antioxidant or anti-inflammatory effects also undergo clinical trials.[1]

Fungal immunomodulatory proteins (FIPs) have the potential to protect gastrointestinal mucosa from chemotherapy-induced toxicity in vitro and in vivo. One study demonstrates that 2 types of FIPs reduce pro-inflammatory cytokine productions in peripheral blood mononuclear cells and cancer cells to alleviate docetaxel-induced mice intestinal mucositis.[6] Another article presents that another FIP suppress nuclear factor kappa B activation to protect the barrier function of intestinal cells.[7] Our previous study also shows that one FIP from *Ganoderma microsporum*, GMI, protects 5-FU-mediated oral and intestinal mucosa damage in mice.[8]

1.2. Aim of study

Based on the highly preserved amino acid sequences and structures in the FIP family[9] and their multiple protective functions, we further conducted a clinical trial to examine the functions of the commercial products of GMI, Reishimmune-S, on OM. In this study, 67 patients with HNC and chemotherapy-induced OM were enrolled. Clinical OM grades, absolute neutrophil counts, and HNC questionnaires were assessed to understand whether OM conditions were improved after Reishimmune-S supplement. We aim to develop FIPs as an alternative therapy to attenuate OM in chemotherapy-treated cancer patients.

2. Methods

2.1. Study design

This was a single-institute, open-labeled, single arm, phase II trial in Chung Shan Medical University Hospital, Taiwan. Initially, the study design had double arms: patients were allocated to receive Reishimmune-S supplement plus standard supportive care (experimental arm) or standard supportive care alone (control arm). When patients were diagnosed with chemotherapy-related OM, written informed consents were received in the same day. Treatment with either Reishimmune-S plus standard supportive care or supportive care alone were started also in the same day. The first day of intervention was recorded as day 1. The total follow-up time was 15 days from the initial to the end of intervention. Clinical score, complete blood counts, and questionnaires were recorded in the follow-up. Evaluations were conducted at the day 1, 8, and 15 for the pretest (the initial of trial), during-test (during trial), and post-test (the end of trial) assessment. Since this was an open-labeled study, patients who decided to discontinue and withdraw from the study could receive other OM therapies such as glutamine supplementation. No crossover was conducted in this study. The protocols were approved by the Institutional Review Board of Chung Shan Medical University Hospital (CSMUH-IRB-CS1-16100).

2.2. Participants, recruitment, and intervention

From June 2017 to February 2019, eligible patients were age 20 years or older, with a histologically confirmed diagnosis of any stage HNC; receiving 1 or 2 cycles of chemotherapy; and clinical diagnosis of chemotherapy-related oral mucositis. The inclusion criteria for patients' chemotherapy use histories could be neoadjuvant, postoperative, or palliative based on the HNC stage. Standard chemotherapy agents chosen for HNC included fluorouracil, platinum, and/or docetaxel. The alternative chemotherapy agent included cetuximab. The combination of chemotherapy agents could be adjusted based on each patient's condition. Exclusion criteria were impaired organ function including liver, kidney, and bone marrow; low hemoglobin levels (men <9 g/dL; women <8.5 g/dL); diarrhea for >15 days; acute infection; mental illness; and declining follow-up. Participants in the trial were randomly assigned in a 1:4 ratio to receive standard supportive care alone or plus 500 mg/day Reishimmune-S (MycoMagic Biotechnology Co., Ltd., New Taipei City, Taiwan) orally for 15 days. All patients received standard supportive care including oral hygiene with tooth paste containing fluoride, mouth rinses with 2 mg/mL chlorhexidine solution, mouth coating with water-soluble lubricants, sucralfate, and topical anesthetics. Parenteral analgesics such as opioids were used if needed for uncontrolled pain. Patients' clinical characteristics including gender, age, primary tumor site, stage, smoking, alcohol, betel chewing, chemotherapy were recorded. All patients provided written, informed consent at the time of screening.

2.3. Primary outcomes

The primary outcome was the clinical scoring of oral mucositis and ulcer severity according to the World Health Organization Oral Mucositis Grading Scale.[10]

2.4. Secondary outcomes

The secondary outcome includes absolute neutrophil counts (ANCs) and QoL. ANC was calculated based on the formula: white blood counts (/μL) × (Segment form% + Band form%). QoL was assessed at day 1, 8, and 15 using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Head&Neck35 (EORTC QLQ H&N35) version 1.0 which was designed specifically for patients with HNC.[11] Some items in this survey are grouped into 7 multi-item scales (pain, swallowing, senses, speech, social eating, social contact, and sexuality).

2.5. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences software version 20.0 (SPSS Inc.,

Chicago, IL). All quantitative data were shown as mean ± standard deviation. One-way analysis of variance (ANOVA) test was used for the change of OM clinical score and ANCs. Any 2-time point of clinical score and QoL were also analyzed using Wilcoxon signed rank test. Paired *t* test was conducted to analyze the change of ANCs in any 2-time point.

3. Results

3.1. Patients' baseline information and clinical characteristics

From June 2017 to February 2019, 97 patients were assessed as eligibility. After excluding 14 patients based on our exclusion criteria, 83 patients with HNC were randomized: 16 patients

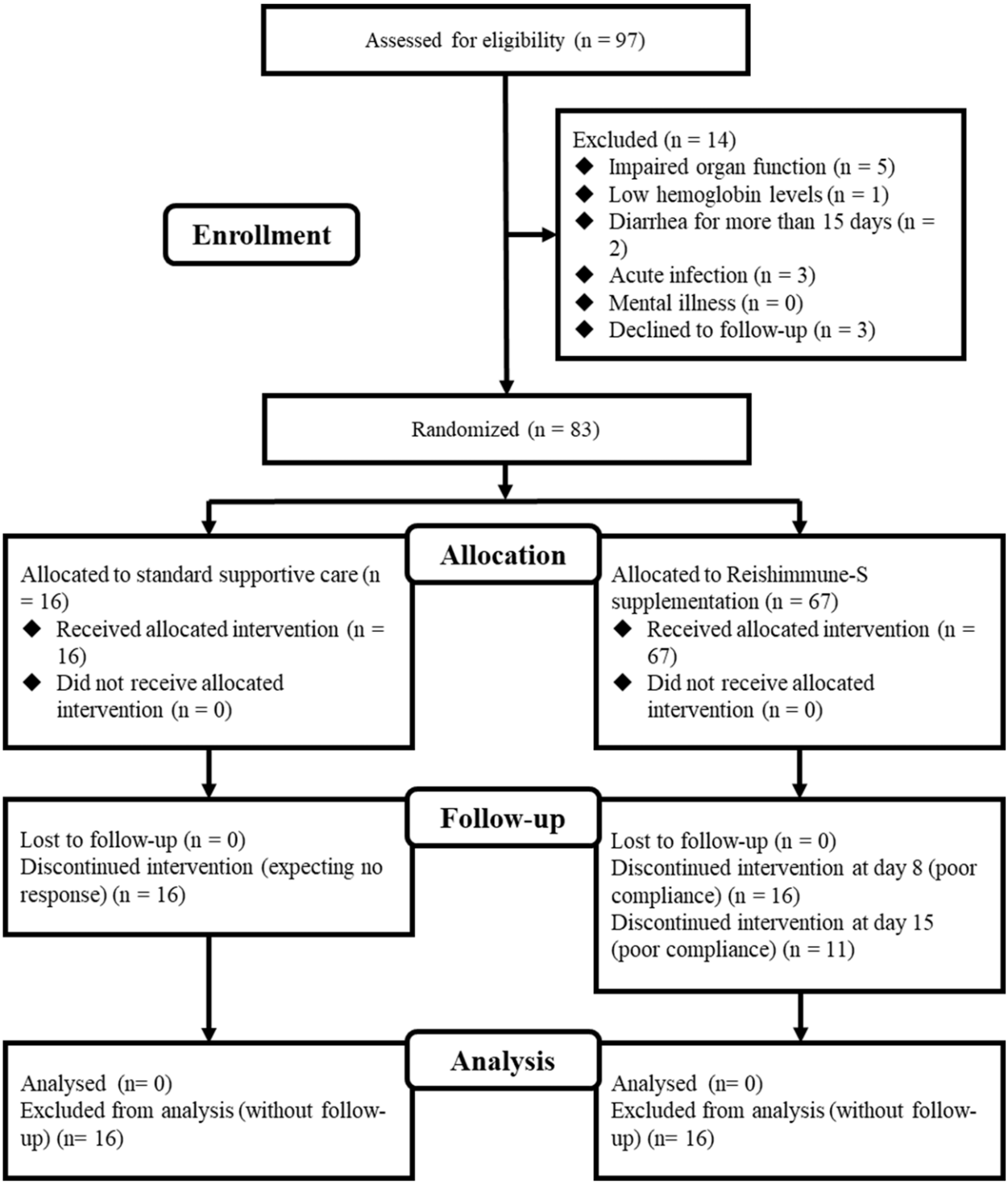

Figure 1. The schematic design of the clinical trial of Reishimmune-S in patients with head and neck cancer.

were in the control arm and 67 patients were in the Reishimmune-S arm. However, all patients in the control group did not received allocated intervention or discontinued. Since they could not tolerate the standard supportive care alone and expected that there would be no response from their treatment, they early withdrew from the trial before day 8. Only 2 patients' day 1 data (the initial) including clinical scores and questionnaire reports were received. Finally, 67 patients were enrolled into the study and all received Reishimmune-S. Sixteen and 11 patients withdrew from the trial at day 8 and 15 due to poor compliance. Only 40 patients completed the trial. All 67 patients were still included into primary and secondary outcome analyses. Due to lack of results from the control arm, only patients in the experimental arm were analyzed and the study design was shifted to single-arm. Since the purpose of this study was observing the effects of Reishimmune-S in 1 cycle of chemotherapy, the total follow-up time was 15 days. The enrollment process was shown in Fig. 1.

The clinical characteristics of the 67 patients were summarized in Table 1. The mean age was 55.3 years (range = 29–85 years). A total of 54 (81%), 49 (73%), and 52 (78%) patients were men, in stage IV, and had primary tumors in buccal and oral cavity, respectively. For common risk factors of HNC, 59 (90%), 52 (79%), and 44 (67%) patients were ex-/active smoker, alcohol user, betel nut user. For chemotherapy agents, most patients at least received platinum such as cisplatin or carboplatin (n = 65, 97%), while 37 (55%) patients also received 5-FU and/or taxane as dual or triple combination. Only one (1%) patients with tumors in soft palate and nasopharynx received cetuximab treatment rather than these 3 agents.

Table 1

Demographic data of the patients with head and neck cancer.

Factors		All patients (n = 67)
Gender	Male	64 (96%)
	Female	3 (4%)
Age	<65	54 (81%)
	≥65	13 (19%)
Primary tumor	Buccal area	21* (31%)
	Oral cavity	33* (49%)
	Nasopharynx	3† (3%)
	Oropharynx	3 (4%)
	Hypopharynx	7 (10%)
	Larynx	2 (3%)
	Unknown	1 (1%)
Stage	I–II	8 (12%)
	III	10 (15%)
	IVA–IVB	49 (73%)
Smoking	Non-smoker	7 (10%)
	Ex- or active smoker	59 (90%)
Alcohol	Never user	14 (21%)
	Ever user	52 (79%)
Betel chewing	Never user	22 (33%)
	Ever user	44 (67%)
Chemotherapy	Platinum	28 (42%)
	Platinum + 5-FU or taxane	12 (18%)
	Platinum + 5-FU + taxane	25 (37%)
	5-FU	1 (1%)
	Others‡	1 (1%)

Platinum includes cisplatin and carboplatin. Taxane includes docetaxel and paclitaxel. 5-FU = 5-fluorouracil.

* Two patients had primary tumors both in oral cavity and buccal area.

† One patient had primary tumors both in soft palate and nasopharynx.

‡ Other chemotherapy agents include cetuximab.

3.2. Primary outcomes

To evaluate the clinical condition of OM, OM grades assessed by our hospital physicians were chosen as the primary outcome, and we found Reishimmune-S supplement significantly reduced OM grades. The average of OM grade in the 67 HNC patients at the initial was 1.9 ± 0.9. At day 8, the OM grade in the experimental arm significantly decreased to 1.4 ± 0.7 ($n = 51$; $P < .001$ compared with the initial) while the grade further decreased to 1.1 ± 0.6 at day 15 ($n = 40$; $P < .001$ compared with the initial) (Fig. 2A). On the other hand, both of the 2 patients in the control arm at day 1 were grade 2, which were similar to the experimental arm. For the grade distribution, after 15-day supplement, the percentage of grade 0 and 1 increased from 31.4% to 82.5% while no severe OM (grade 3 and 4) was found at the end (Fig. 2B). Wilcoxon signed rank test was also used to analyzed the before and after Reishimmune-S supplement (Fig. 2C). The OM grade at later time points was all significantly reduced when compared with the grade at former time points ($P < .001$ day 1 vs 8; $P < .001$ day 1 vs 15; $P < .01$ day 8 vs 15).

3.3. Secondary outcomes

Since our previous study discovered that FIPs alleviated docetaxel-induced leukopenia in mice,[6] we also wondered whether the Reishimmune-S reversed chemotherapy-mediated neutropenia in our cohort. Blood samples were collected and analyzed through complete blood counts. During the 15-day follow-up, patients' ANCs mildly reduced at day 15 but no significances were found (day $1 = 4961 \pm 2448$ vs day $15 = 3976 \pm 2108$, $P = .087$) through one-way ANOVA analysis (Fig. 3A). Any 2-time point of ANCs was also compared through paired t test and we found ANCs at day 15 was significantly decreased when comparing with day 1 and 8 (day 8 vs 15, $P < .01$; day 1 vs 15, $P < .01$) (Fig. 3B). Although the significant reduction of ANCs was found at the end of the trial, only 9 patients had neutropenia (ANC < 2000/μL) during the trial.

Chemotherapy-related OM usually impaired cancer patients' QoL, so HNC patients in our cohort also filled up EORTC-QLQ-H&N 35 questionnaires at day 1, 8, and 15 to understand whether Reishimmune-S intervention also alleviated OM-induced QoL impairment (Table 2). At later time points, the scores of trouble with social contact (day $1 = 6.15 \pm 2.85$, day $15 = 6.10 \pm 2.88$, $P < .05$) and weight loss (day $1 = 1.56 \pm 0.50$, day $8 = 1.34 \pm 0.48$, $P < .05$) were significantly reduced. There were no differences before and after intervention in other evaluation items in this questionnaire. For the 2 patents in the control arm, they had average of the following items at day 1: pain: 2; swallowing: 4; senses problems: 3; speech problems: 4; trouble with social eating: 4; trouble with social contact: 5; less sexuality: 5; teeth: 1.5; opening mouth: 1; dry mouth: 2.5; sticky saliva: 1.5; coughing: 1; felt ill: 1; painkillers: 1; nutritional supplements: 1.5; feeding tube: 1; weight loss: 1.5; weight gain: 1. All of these results were similar to the results from the experimental arm at day 1.

3.4. Adverse events

No obvious Reishimmune-S-related toxicity or adverse effects were found in this trial.

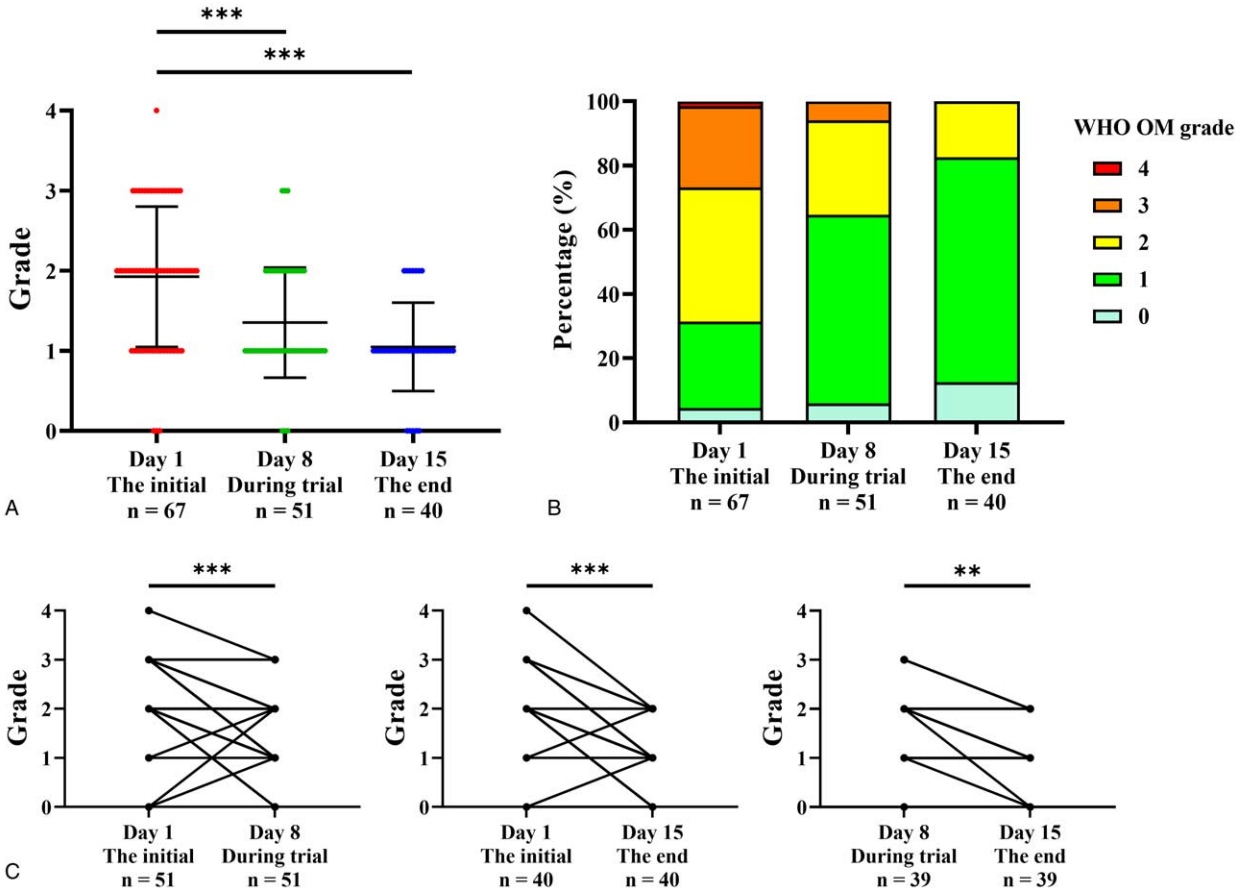

Figure 2. The change of oral mucositis clinical scores during Reishimmune-S supplement. (A) The clinical scores were evaluated and analyzed by one-way ANOVA at day 1 (the initial of trial), 8 (during trial), and 15 (the end of trial). (B) The clinical score percentage distribution at day 1 (the initial of trial), 8 (during trial), and 15 (the end of trial). (C) The clinical scores of any 2 time points were also compared respectively through Wilcoxon signed rank test. ***, $P < .001$.

4. Discussion

In the current study, 67 HNC patients who had chemotherapy-related OM were enrolled and received Reishimmune-S supplement for 14 days. For the primary outcome, Reishimmune-S significantly reduced OM clinical conditions both at day 8 and 15. For QoL evaluation, Reishimmune-S also significantly improved patients' trouble with social contact and weight loss. Based on these findings, Reishimmune-S mainly with GMI has potential to relieve chemotherapy-related OM in HNC.

Male patients accounted for >80% and were predominant in our study. The main 3 risk factors for head and neck cancer in Taiwan are smoking, alcohol use, and betal nut chewing. The majority of doing these 3 behaviors are men in Taiwan, which could explain why most patients with head and neck cancer are men. In addition, there are several epidemiological studies of head and neck cancer in East Asia including Taiwan also reports about >80% patients with head and neck cancer are men, which are consistent with our trial.[12,13]

In our results, the average initial OM grade was significantly reduced from around 2 to 1 after receiving Reishimmune-S treatment for 14 days. The consensus of maximum expression of OM is about 7 to 10 days and the total course of OM is approximately 2 to 3 weeks if patients do not receive further intervention.[14] Therefore, even though the control arm in our study was excluded for analysis due to discontinuation of all patients in this arm, we still observed a rapid relief of symptoms and severity in <2 weeks after Reishimmune-S supplementation, which might be shorter than the common course of OM. One clinical trial studying the efficacy of palifermin on chemotherapy-mediated OM in patients with hematologic cancers and found the mean OM grade was changed from about 2.5 to <1 in 14 days in the palifermin arm, which is similar to our results.[15] Palifermin also significantly reduces the incidence of severe OM (grade 3 and 4) in patients with advanced HNC who received chemoradiotherapy.[16] Even though our study had a different design and the patients enrolled into our study had already been diagnosed with OM, we still observed all cases with initially severe OM (around 25% of total patients) reduced to mild or moderate OM (grade ≤2) after 14-day Reishimmune-S supplementation. Moreover, we also observed Reishimmune-S treatment improved patients' QoL including trouble with social contact and weight loss evaluated by EORTC QLQ-H&N35 questionnaire. One research using low-level laser therapy on HNC patients with OM also demonstrates that the laser therapy improves multiple items such as pain, swallowing, trouble with social eating, dry mouth, and sticky saliva.[17] However, patients did questionnaires at the 1st, 20th, and 39th radiation therapy fraction, which indicates that the duration of follow-up time may influence the results of questionnaire. Therefore, even though there was no control arm in this study, the efficacy of GMI were

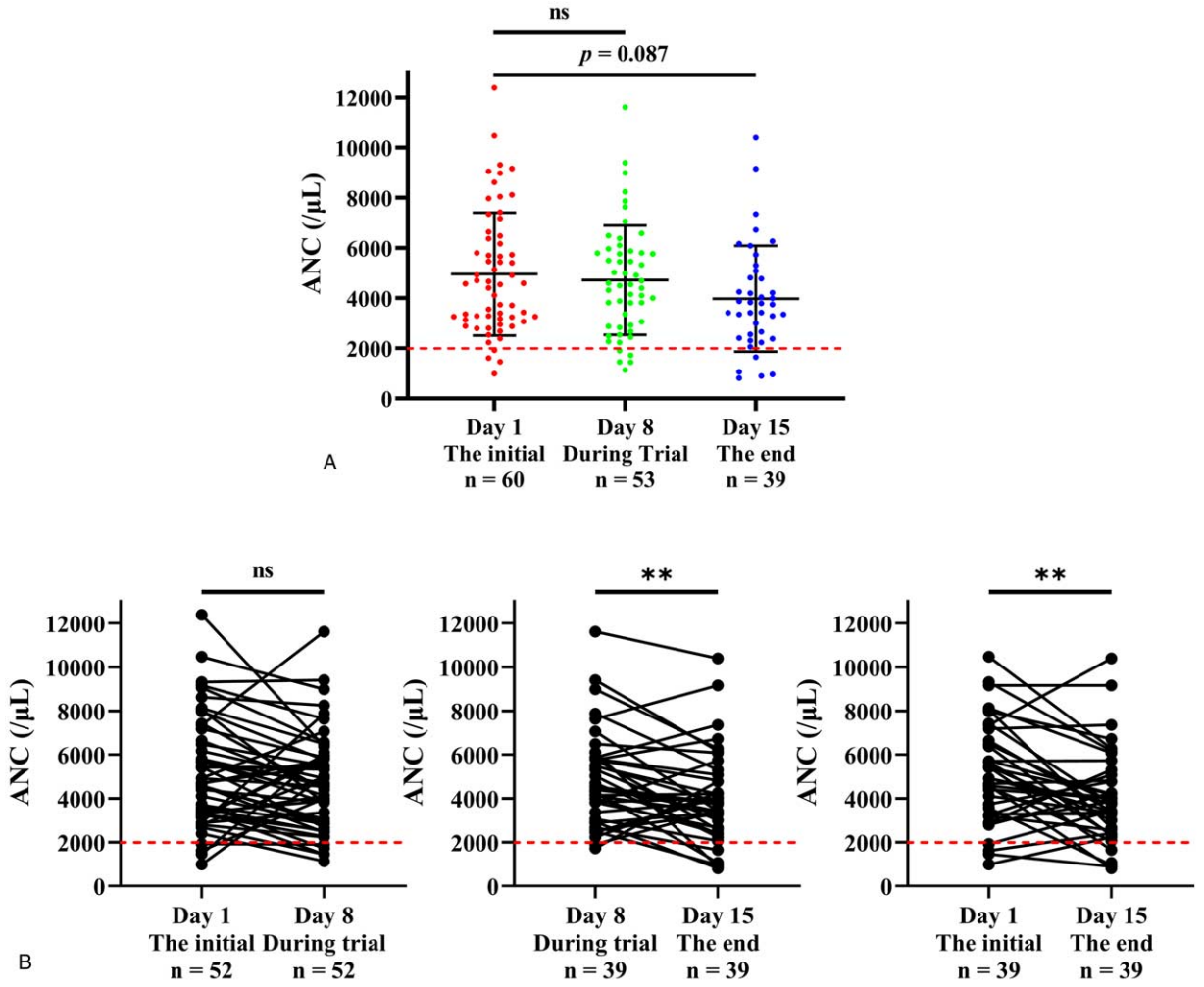

Figure 3. The change of absolute neutrophil counts (ANCs) during Reishimmune-S supplement. (A) The ANCs at day 1 (the initial of trial), 8 (during trial), and 15 (the end of trial) were compared through one-way ANOVA. (B) The ANCs of any 2 time points were also compared respectively through paired *t* test. Red dashed line means the lower limit of normal range. **, $P < .01$.

not inferior to other clinical trials studying effects of other compounds on chemotherapy-related OM. However, further closed-labeled randomized control trials should still be conducted to validate the efficacy of Reishimmune-S.

For ANC results, we did not Reishimmune-S maintain neutrophil counts and reduction of ANCs was still obviously found at day 15. We previously conducted another clinical trial to examine the effects of another FIP, Ling zhi-8 on docetaxel-mediated leukopenia in patients with advanced non-small cell lung cancer.[18] The study demonstrates that Ling zhi-8 supplementation during chemotherapy obviously reversed leukopenia and neutropenia at the 2nd and 3rd cycle of docetaxel treatment. Since this is a single-arm study design, we could not evaluate whether degree of ANC reduction in patients with Reishimmune-S supplementation is less than those without supplementation. Furthermore, due to only a small fraction of patients experiencing neutropenia in our study, we also could not assess whether Reishimmune-S containing with GMI have protective effects on chemotherapy-induced leukopenia.

There are 2 mechanisms which may explain the protective effects of FIPs including GMI on OM found in this study. First, several studies show that FIPs alleviate chemotherapy-induced mucosa damage. One study confirms that FIP-*fve* and FIP-*gts* protect docetaxel-induced intestinal injuries.[6] Our preclinical mice models present that GMI reverses 5-FU-mediated loss of tongue epithelium and intestinal villi damage.[8] We further found GMI attenuates activation of pro-apoptotic proteins in jejunum samples. Second, FIPs also impact mucosal immune microenvironment. One article shows that fungal proteins from *Hericium erinaceus* regulate the composition and metabolism of gut microbiota to exert immunomodulatory functions.[19] Another study also demonstrates that GMI ameliorate oral submucosa fibrosis formation through inhibiting pro-inflammatory cytokines release.[20] Lin et al[21] further figures out that GMI reduces nuclear factor kappa B activation to prevent tumor necrosis factor-α-mediated inflammation.

There are some limitations in this study. First, the sample size in this study is small. However, even though some patients withdrew from the trial during Reishimmune-S treatment, we still found the significant reduction of OM clinical grades at the end of the trial. Second, this study only analyzes the single Reishimmune-S arm without the control arm. Without the

Table 2

Quality of life scores on the EORTC QLQ-HN35.

EORTC QLQ-HN35 item	Day 1 The initial n = 67	Day 8 During trial n = 50	Day 15 The end n = 39	Day 1 versus 8 n = 50 *P* value	Day 1 versus Day 15 n = 39 *P* value	Day 8 versus Day 15 n = 39 *P* value
Pain	6.95 ± 2.52	6.68 ± 2.23	6.92 ± 1.98	.445	.884	.452
Swallowing	8.02 ± 3.57	7.59 ± 3.76	7.49 ± 3.34	1.000	1.000	.937
Senses problems	4.02 ± 1.52	3.84 ± 1.50	3.85 ± 1.42	.558	.752	.809
Speech problems	4.61 ± 2.54	4.27 ± 2.35	4.46 ± 2.16	.077	.202	.570
Trouble with social eating	8.07 ± 3.73	8.07 ± 3.92	7.76 ± 3.52	.908	.721	.731
Trouble with social contact	6.15 ± 2.85	6.26 ± 2.67	6.10 ± 2.88	.154	**.044***	.599
Less sexuality	7.11 ± 1.87	6.87 ± 2.05	6.55 ± 2.26	.323	.244	.324
Teeth	1.28 ± 0.58	1.18 ± 0.39	1.33 ± 0.62	.109	.822	.083
Opening mouth	2.03 ± 0.97	2.08 ± 1.05	2.08 ± 1.06	.569	.168	.253
Dry mouth	2.56 ± 1.13	2.55 ± 1.21	2.69 ± 1.15	.855	.360	.160
Sticky saliva	2.42 ± 1.17	2.49 ± 1.17	2.74 ± 1.21	.135	**.019***	.212
Coughing	1.57 ± 0.83	1.55 ± 0.71	1.62 ± 0.75	.598	.875	.608
Felt ill	1.52 ± 0.85	1.41 ± 0.81	1.44 ± 0.72	1.000	.868	.498
Painkillers	1.64 ± 0.48	1.60 ± 0.49	1.59 ± 0.50	.659	.324	1.000
Nutritional supplements	1.83 ± 0.38	1.88 ± 0.33	1.87 ± 0.34	.322	1.000	.324
Feeding tube	1.13 ± 0.34	1.06 ± 0.24	1.10 ± 0.31	.083	.570	.160
Weight loss	1.56 ± 0.50	1.34 ± 0.48	1.59 ± 0.50	**.028***	.623	**.027***
Weight gain	1.16 ± 0.37	1.22 ± 0.42	1.08 ± 0.27	.569	.160	**.032***

* $P < .05$.

control group, we could not evaluate whether Reishimmune-S supplement was superior to placebo and whether the improvement of OM was from this intervention or patients' self-healing abilities. However, since many clinical trials studying efficacy of other natural compounds on OM observed a pattern of clinical change longer than 2 weeks, we believe it is more possible that the rapid improvement found in our study was due to the efficacy of Reishimmune-S. Third, the follow-up time in this trial was only 2 weeks, which was shorter than most of other associated clinical trials. We could not evaluate the long-term protection effects of GMI on OM condition. Further double-blind randomized control trial with placebo and Reishimmune-S groups and with larger sample size should be done to confirm the efficacy of this intervention. Nevertheless, we still discovered that Reishimmune-S supplement achieved the primary outcome to alleviate OM clinical severity and improved patients' QoL to some degree.

5. Conclusion

In conclusion, supplying with Reishimmune-S mainly containing GMI reduces chemotherapy-associated OM grading in 14 days and improves QoL including trouble with social contact and weight loss based on the EORTC QLQ H&N35 questionnaire. Therefore, Reishimmune-S has potential to be used in attenuating chemotherapy-related OM.

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All authors have agreed to the published version of the manuscript.

References

[1] Blakaj A, Bonomi M, Gamez ME, Blakaj DM. Oral mucositis in head and neck cancer: evidence-based management and review of clinical trial data. Oral Oncol 2019;95:29–34.
[2] Sonis ST. The pathobiology of mucositis. Nat Rev Cancer 2004;4:277–84.
[3] Huang CJ, Huang MY, Fang PT, et al. Randomized double-blind, placebo-controlled trial evaluating oral glutamine on radiation-induced oral mucositis and dermatitis in head and neck cancer patients. Am J Clin Nutr 2019;109:606–14.
[4] Peng TR, Lin HH, Yang LJ, Wu TW. Effectiveness of glutamine in the management of oral mucositis in cancer patients: a meta-analysis of randomized controlled trials. Support Care Cancer 2021;29:4885–92.
[5] Riley P, Glenny AM, Worthington HV, et al. Interventions for preventing oral mucositis in patients with cancer receiving treatment: cytokines and growth factors. Cochrane Database Syst Rev 2017;11:1–156. CD011990.

[6] Ou CC, Hsiao YM, Hou TY, Wu MF, Ko JL. Fungal immunomodulatory proteins alleviate docetaxel-induced adverse effects. J Funct Foods 2015;19(part A):451–63.
[7] Chen YH, Shin JY, Wei HM, et al. Prevention of dextran sulfate sodium-induced mouse colitis by the fungal protein Ling Zhi-8 via promoting the barrier function of intestinal epithelial cells. Food Funct 2021;12: 1639–50.
[8] Li CH, Ko JL, Ou CC, et al. The protective role of GMI, an immunomodulatory protein from Ganoderma microsporum, on 5-fluorouracil-induced oral and intestinal mucositis. Integr Cancer Ther 2019;18:1–10. 1534735419833795.
[9] Li QZ, Wang XF, Zhou XW. Recent status and prospects of the fungal immunomodulatory protein family. Crit Rev Biotechnol 2011;31:365–75.
[10] Sonis ST, Elting LS, Keefe D, et al. Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. Cancer 2004;100(9 suppl):1995–2025.
[11] Bjordal K, Hammerlid E, Ahlner-Elmqvist M, et al. Quality of life in head and neck cancer patients: validation of the European Organization for research and treatment of cancer quality of life questionnaire-H&N35. J Clin Oncol 1999;17:1008–19.
[12] Huang CC, Hsiao JR, Lee WT, et al. Investigating the association between alcohol and risk of head and neck cancer in Taiwan. Sci Rep 2017;7:1–13. 9701.
[13] Lee YA, Li S, Chen Y, et al. Tobacco smoking, alcohol drinking, betel quid chewing, and the risk of head and neck cancer in an East Asian population. Head Neck 2019;41:92–102.
[14] Chaveli-Lopez B, Bagan-Sebastian JV. Treatment of oral mucositis due to chemotherapy. J Clin Exp Dent 2016;8:e201–9.
[15] Spielberger R, Stiff P, Bensinger W, et al. Palifermin for oral mucositis after intensive therapy for hematologic cancers. N Engl J Med 2004;351:2590–8.
[16] Le QT, Kim HE, Schneider CJ, et al. Palifermin reduces severe mucositis in definitive chemoradiotherapy of locally advanced head and neck cancer: a randomized, placebo-controlled study. J Clin Oncol 2011;29:2808–14.
[17] Antunes HS, Herchenhorn D, Small IA, et al. Phase III trial of low-level laser therapy to prevent oral mucositis in head and neck cancer patients treated with concurrent chemoradiation. Radiother Oncol 2013;109: 297–302.
[18] Wu MF, Wu CH, Chen CW, Lin YS, Chen DC, Ko JL. Effect of Ling Zhi-8 supplementation on docetaxel-induced leukopenia and thrombocytopenia in patients with advanced non-small cell lung cancer. Chung Shan Med J 2018;29:123–32.
[19] Diling C, Chaoqun Z, Jian Y, et al. Immunomodulatory activities of a fungal protein extracted from Hericium erinaceus through regulating the gut microbiota. Front Immunol 2017;8:1–22. 666.
[20] Lee PH, Hsieh PL, Liao YW, Yu CC. Inhibitory effect of GMI, an immunomodulatory protein from Ganoderma microsporum, on myofibroblast activity and proinflammatory cytokines in human fibrotic buccal mucosal fibroblasts. Environ Toxicol 2018;33: 32–40.
[21] Lin CH, Hsiao YM, Ou CC, et al. GMI, a Ganoderma immunomodulatory protein, down-regulates tumor necrosis factor alpha-induced expression of matrix metalloproteinase 9 via NF-kappaB pathway in human alveolar epithelial A549 cells. J Agric Food Chem 2010; 58:12014–21.