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Local application of granulocyte-macrophage colony stimulating factor (GM-CSF) for the treatment of oral mucositis

G.M. Sprinzl[a,*], O. Galvan[a], A. de Vries[b], H. Ulmer[c], A.R. Gunkel, P. Lukas[b], W.F. Thumfart[a]

[a]*Department of Oto-Rhino-Laryngology-Head and Neck Surgery, University of Innsbruck, Austria*
[b]*Department of Radiotherapy, University of Innsbruck, Austria*
[c]*Departement of Biostatistics, University of Innsbruck, Austria*

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Abstract

The combination of radiation and chemotherapy administered for patients undergoing therapy for advanced head and neck neoplasms leads to a significant rise in toxic side-effects. Oral mucositis remains one of the most distressing factors leading to pain, impairment of oral nutrition, local and systemic infection and often cessation of the oncological treatment. The local and systemic administration of recombinant growth factors has revealed a potential benefit in the treatment of oral mucositis. Clinical data concerning the topical use of granulocyte-macrophage colony-stimulating-factor (GM-CSF) in the prevention and therapy of mucositis in patients undergoing radiochemotherapy for advanced cancer of the head and neck are presented in this paper. A prospective, randomised, open parallel-grouped, single centre study at a university hospital was performed. 35 patients with stage III and IV carcinomas of the head and neck were included. Statistical analysis concerning the degree of oral mucositis, the perception of pain, the incidence of secondary infections and the change in haematological parameters revealed no superiority of GM-CSF in comparison to conventional mouthwash between the two groups of patients. As a result, and faced with the tremendous costs of the regular use of a recombinant cytokine, we ended the clinical trial after 35 patients. The topical administration of GM-CSF to treat oral mucositis as a result of radiochemotherapy in patients suffering from head and neck cancer cannot be recommended. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Radiochemotherapy; Head and neck cancer; Mucositis; GM-CSF; Mouthwash

1. Introduction

The use of intensive oncological treatment protocols leads to a significant rise in the incidence of therapy-related side-effects, particularly in cases of oral mucositis [1,2]. The inflammation of the oral mucosa may be a very painful condition requiring adequate analgesic medications. As a consequence of the disruption of the mucosal barrier, local and systemic infections may develop. The intensification of oral mucositis interferes with the patient's ability to maintain oral nutrition [3]. The complications arising from severe mucositis frequently require a temporary or complete cessation of radiochemotherapy, thus preventing application or completion of the planned treatment dose [4]. In respect to data that the prolongation of overall treatment time negatively influences the radio-curability of squamous cell carcinoma of the head and neck, adequate and effective supportive treatment strategies for the management of oral mucositis are necessary [5,6]. At present, however, there is no golden standard available for the prevention and treatment of oral mucositis. In clinical practice, a diversity of different treatment strategies is used [4,7,8].

In 1999, Hejna, Brodowicz and Zielinski in the *European Journal of Cancer* [9] reviewed the preliminary data concerning the positive effects of the topical application of granulocyte-macrophage colony-stimulating-factor (GM-CSF) on the development of oral mucositis in patients treated with chemotherapy. The authors concluded that the local application of GM-CSF can successfully reduce the severity and duration of chemo-

* Corresponding author. Tel.: +43-512-504-5204; fax: +43-512-504-67-5204.
E-mail address: georg.sprinzl@uibk.ac.at (G.M. Sprinzl).

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therapy-induced oral mucositis. Concerning the potential benefit of the topical use of GM-CSF under intensified treatment strategies that combine chemotherapy and radiation for advanced carcinoma of the head and neck region, there is no statistically proven data currently available. To our knowledge, we present the first clinical trial regarding the topical application of GM-CSF on the oral mucosa in patients treated with radiochemotherapy for advanced carcinoma of the head and neck.

2. Patients and methods

Between January 1997 and October 1998, we conducted a prospective, randomised, parallel grouped phase-II clinical trial to evaluate the potential benefit of a once daily performed mouthwash containing a solution of GM-CSF (Leukomax®) in comparison to a conventional (Hydrocortisone, Pantocain) mouthwash. The study was approved by the local ethics commission; patients were included after a written informed consent. Before randomisation, patients were stratified into two treatment arms whether they received radiochemotherapy or postoperative radiation therapy alone (Table 1; Fig. 1). The study followed a non-blinded protocol.

35 consecutive and previously untreated patients with advanced carcinoma (stage III, IV) of the oral cavity, oro- and hypopharynx participated (Table 2). The treatment plan consisted of two cycles of conventionally fractionated radiotherapy up to 30 Gray each. Daily radiation doses of 2 Gray were administered for 5 days a week. Patients received chemotherapy during the first week of each cycle (initial bolus of Mitomycin-C, dose: 10 mg/m^2; continuous infusion of 5-fluorouracil 1000 mg/m^2/day). All patients received a total dose of 60 Gray using a conventional fractionated radiation scheme of 2 Gray daily.

All patients had an oral exam before hospital admission for oncological treatment. Intra-oral, as well as panoramic, radiographs taken within the last 6 months were evaluated. Potential foci of oral infection were eliminated prior to therapy. During therapy, patients were instructed to maintain strict oral hygiene using a soft toothbrush a fluoride toothpaste and to avoid certain products such as tobacco, alcoholic beverages, very hot and cold food, as well as spicy foods. All patients participated in the oral care programme and were encouraged not to wear their dentures during radiochemotherapy. The oral care programme for all patients consisted of daily rinses for at least three times a day

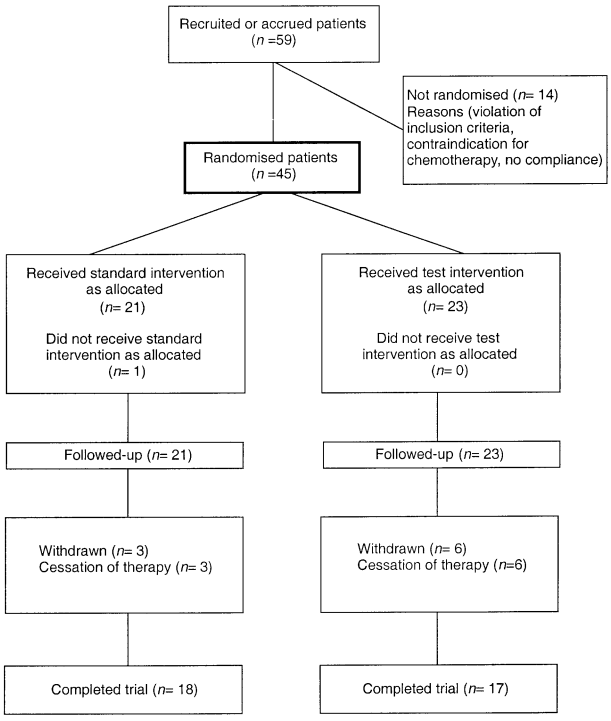

Fig. 1. Flow chart of the progress of patients through the trial (adapted from Ref. [30].

Table 1
Patient characteristics

Characteristics	GM-CSF group *n*	Control group *n*
Number of patients	17	18
Age (years) median (range)	60 (49–82)	57 (42–75)
Sex		
Male	15	12
Female	2	6
Alcohol consumption	14	14
Smoking	13	18

Table 2
Tumour location/TNM classification

	GM-CSF group	Control group
TNM		
T1	0	0
T2	4	5
T3	3	3
T4	10	10
N0	7	7
N1	3	3
N2	6	6
N3	1	2
M1	0	0
Location		
Oral cavity	4	3
Oropharynx	10	10
Hypopharynx	3	5
Larynx	0	1

GM-CSF, granulocyte-macrophage colony-stimulating factor.

using a solution containing cional kreussler and bepanthen. Patients were observed at baseline and twice a week during the first cycle of radiochemotherapy by one of two examiners using a flashlight and a tongue depressor. Mucositis was graded according to the World Health Organization (WHO)-criteria [10]. Additionally, the patients scored their perception of pain using a visual analogue scale. Blood samples were taken on a weekly basis. To determine the incidence of secondary infections, we defined infection categories (respiratory tract infection, wound infection, urinary tract infection, sepsis or sepsis syndrome, vascular catheter-related infection and intraabdominal infection) as described elsewhere [11]. Patients of both study groups received mouthwash therapy at the onset of erythema (mucositis grade 1 according to WHO-criteria) and until mucositis resolved. At this stage of therapy, additional oral antibiotics and nystatin rinses were prescribed for all patients. The use of analgesic agents, antibiotics as well as antifungal medications, was documented.

Patients in the experimental study group received a 250 ml solution of 400 μg recombinant *Escherichia coli* GM-CSF (Molgramostim) once daily as soon as erythema was diagnosed. Patients were instructed to swish and swallow over a period of 1 h. Alternatively, the control group was treated with 250 ml solution of the conventional mouthwash containing pantocain, hydrocortisone acid, cional kreussler and bepanthen.

The Mann–Whitney test and Chi-square or Fisher's Exact test were used to compare the degree of oral mucositis, the perception of pain, the incidence of secondary infections and the change in haematological parameters between patients treated with GM-CSF and the control group. *P*-values less than 0.05 were valued as statistically significance. Due to the phase-II design of the study, corrections for multiple comparison were not performed. Data are summarised using absolute and relative frequencies, as well as medians and ranges. Perception of pain is illustrated using a box and whisker plot.

3. Results

Statistical analysis (Mann–Whitney test; Chi-square test; Statistical Package for the Social Sciences (SPSS) software version 8.0) concerning the degree of oral mucositis, the perception of pain, the incidence of secondary infections and the change in haematological parameters revealed no superiority of GM-CSF in comparison to conventional mouthwash between the two groups of patients. Therefore, or in view of the tremendous costs involved with recombinant cytokine, we ended the clinical trial after 35 patients.

Using Fisher's Exact test, the disparities regarding the distribution of male to female showed insignificant values ($P = 0.228$). All patients consumed moderate to large amounts of alcohol, comparison between the two groups did not show a statistical difference of significance ($P = 1.00$; Fisher's Exact test). There appeared to be four more smokers in the control group than in the experimental arm, statistical analysis using the Chi-square test revealed a P value of 0.045, thus indicating statistical significance.

Concerning the grading of oral mucositis, no difference of statistical significance between the two groups of patients could be found (Mann–Whitney-test, week 1: $P = 0.376$; week 2: $P = 0.451$; week 3: $P = 0.488$). The clinical course of mucositis in both of the patients groups is demonstrated in Fig. 2.

Statistical analysis did not reveal differences in the perception of oral pain between the two patients groups as shown in Fig. 3 (Mann–Whitney test, week 1: $P = 0.613$; week 2: $P = 0.386$; week 3: $P = 0.483$).

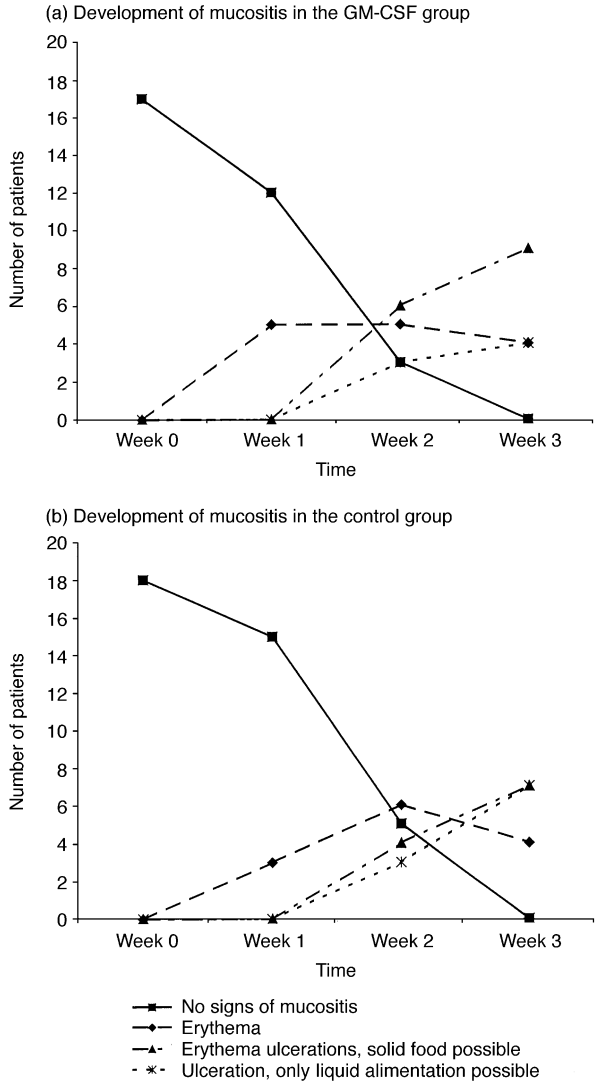

Fig. 2. Oral mucositis. GM-CSF, granulocyte-macrophage colony-stimulating factor.

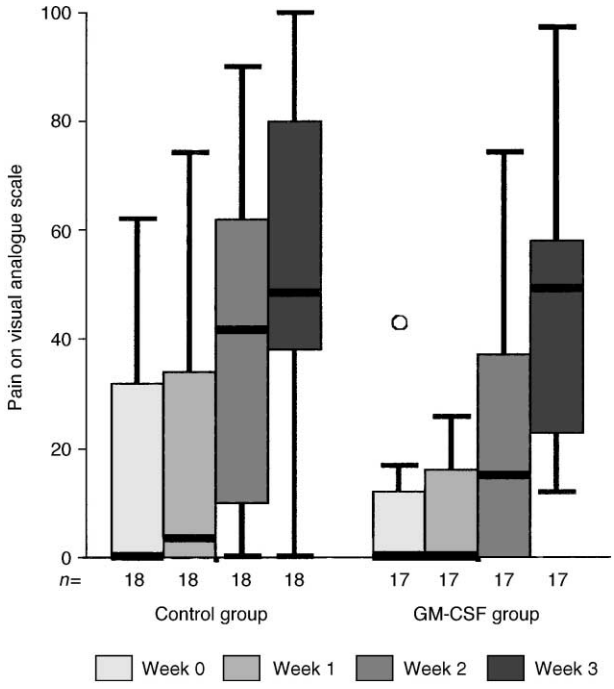

Fig. 3. Oral pain. GM-CSF, granulocyte-macrophage colony stimulating factor.

Secondary infections were noted in both of the patient groups (Table 3). Statistical analysis using Pearson Chi-square revealed an insignificant difference ($P = 0.774$).

Concerning the toxic side-effects that are associated with the application of recombinant human (rh)GM-CSF, only data concerning the systemic application are available. These include the appearance of acute self-limited neutropenia, transient dyspnoea, fever, myalgia and a capillary leak syndrome. No toxic side-effects due to the local application of rhGM-CSF were observed.

Table 3
Mucositis and infection

	Week 0	Week 1	Week 2	Week 3
Mucositis				
GM-CSF group				
No signs of mucositis	17	12	3	0
Soreness, erythema	0	5	5	4
Erythema, ulcers, can eat solids	0	0	6	9
Ulcers, requires liquid diet only	0	0	3	4
Oral alimentation not possible	0	0	0	0
Control group				
No signs of mucositis	18	15	5	0
Soreness, erythema	0	3	6	4
Erythema, ulcers, can eat solids	0	0	4	7
Ulcers, requires liquid diet only	0	0	3	7
Oral alimentation not possible	0	0	0	0
Secondary infection				
GM-CSF group	0	1	2	0
Control group	0	0	1	2

GM-CSF, granulocyte-macrophage colony-stimulating factor.

4. Discussion

4.1. Mucositis

The use of radiochemotherapy for advanced cancer of the head and neck leads to an unavoidable burden of toxicity for the patient [1,4]. In a meta-analysis from 1996, the current concepts of combined radiochemotherapy were evaluated for their toxic side-effects [2]. A significant rise in nausea, skin reaction, unplanned breaks of anticancer therapy, treatment-related death cases, bone marrow dysfunction and appearance of oral mucositis was noted. Especially in patients suffering from advanced cancer of the head and neck, the development of oral mucositis may aggravate tumour-related dysphagia and oral pain, thus exacerbating nutritional impairment [3].

Oral mucositis may be a limiting factor where intensive treatment strategies are applied. The appearance of mucositis may require the interruption of the oncological treatment, thus reducing the planned treatment-dose during radiochemotherapy [4]. Unplanned treatment breaks leading to prolonged treatment time are associated with a loss of local tumour control [4]. The reason for this may be the proliferation of clonogenic tumour cells between the given radiation fractions [5,6]. Pajak and colleagues [5] reported approximately 426 patients with inoperable head and neck cancer from two Radiation Therapy Oncology Group (RTOG) studies. The local control rate was 14% when the prolonged treatment time was more than 14 days, as opposed to 27% when the prolonged treatment time was less than 14 days.

Concerning the pathogenesis of oral mucositis, multiple factors seem to influence the mucosal breakdown and the development of the clinical condition termed mucositis [4,12]. The oral epithelial cells undergo a rapid renewal lasting seven to 14 days under physiological conditions. Cytostatic agents and radiation interfere with the renewal capacities of the oral mucosa. Direct stomatotoxicity of radiochemotherapy leads to cellular damage specifically of the rapidly dividing basal layer cells of the oral epithelium, thus leading to exposure and injury of the underlying stroma. Atrophy of the epithelium, destruction of collagen, glandular degeneration and finally ulceration develop. The release of cytokines such as Interleukin-1 (Il-1) and tumour-necrosis-factor-α (TNF-α), leads to an extension of the destructive process [12]. The direct effects on the oral mucosa usually develop within 5 to 7 days after the beginning of radiation or chemotherapy [4]. In the patient who is not suffering from myelosuppression, the lesions heal within 2 to 3 weeks. The indirect stomatotoxicity induced by the systemic effect of chemotherapy leads to an aggravation of oral mucositis. The disruption of the continuity of the oral epithelium results in an increased risk of secondary local or even systemic infection [4,13].

Morphologically, mucositis begins with the development of erythema. As therapy continues, desquamative lesions appear and present as solitary and finally confluent, white, elevated patches. These very painful pseudo-membranous ulcerations are associated with dysphagia and odynophagia and therefore interfere with the patient's ability to maintain oral nutrition. Deficiencies in the nutritional status may potentially interfere with overall cellular regeneration and renewal, thus decreasing the capacity of regeneration of the oral mucosa. The exposure of the underlying connective tissue aggravates oral pain. Oral infections due to bacterial, viral and fungal agents lead to an exacerbation of mucositis [4].

4.2. GM-CSF

GM-CSF is a glycoprotein that is produced by a variety of human cells, some of which include cells of the haematopoietic environment such as fibroblasts and endothelial cells and cells of the immune system (macrophages, stimulated T-cells) [14]. The recombinant factor induces stimulation of the growth of granulocytes, macrophages and eosinophilic cell formations [14]. In 1989, Bussolino and colleagues [15] established the influence of GM-CSF on the migration and proliferation of endothelial cells. The authors concluded that this molecule appears to act as mediator outside the haematopoietic system [14,15]. Among non-haematopoietic cells, the receptor for GM-CSF was found on endothelial cells and tumour cell lines such as melanoma and lung cancer cells. The function of non-haematopoietic receptors remains unclear [16].

Clinical observation led to a discussion about a possible beneficial effect of locally applied GM-CSF on the mucosa of the oropharynx [14]. However, the expected mechanism of action of topically applied GM-CSF so far remains unknown.

4.3. Systemic administration

Chi and colleagues [17] performed a randomised, cross-over study to prospectively evaluate the effects of subcutaneously (s.c.) applied GM-CSF in the reduction of chemotherapy-induced oral mucositis. The results exposed a significant decrease regarding the incidence, mean duration and severity of oral mucositis following the application of chemotherapy. In another study, 29 patients were treated with s.c. injections of GM-CSF during chemotherapy for head and neck cancer [18]. The authors found that application of GM-CSF was effective in preventing oral mucositis. Similar results regarding the s.c. injection of GM-CSF were also reported from Kannan and colleagues [19] who administered GM-CSF to 10 patients undergoing radiotherapy for carcinoma of the head and neck. Makkonen and colleagues [20] conducted a randomised clinical trial to evaluate the potential effectiveness of the s.c. administration of GM-CSF. Two groups of 20 head and neck cancer patients were treated with sucralphate alone or in combination with GM-CSF to alleviate oral mucositis. The authors found no evidence indicating that s.c. GM-CSF reduces the severity of radiation-induced mucositis.

Regarding the intravenous (i.v.) administration of GM-CSF, positive effects have been reported. Gordon and colleagues [21] found that GM-CSF significantly shortened the duration of oral mucositis in children undergoing chemotherapy for haematopoietic stem-cell transplantation. However, the authors concluded that GM-CSF does not appear to influence the severity of mucositis.

Only few data are available in the current literature concerning the use of GM-CSF against oral mucositis for patients treated with radiochemotherapy for head and neck cancer. In a small pilot study conducted by Ausili-Cefaro and colleagues [22] the s.c. application of GM-CSF led to an alleviation of oral mucositis.

4.4. Topical administration

For topical use of GM-CSF in oral mucositis, only case reports and pilot studies are currently available. It has been suggested that a mouthwash of GM-CSF might be useful to either improve or prevent oral mucositis induced by oncological therapy. Ovilla and colleagues [23] compared two groups of 10 bone-marrow transplant patients undergoing pretransplant conditioning regimens. One group was treated with a daily mouthwash of GM-CSF. The authors speculated that GM-CSF re-established the cell cycle of the oral epithelium, thus promoting healing of oral mucositis. De la Torre and colleagues [24] found strong treatment tolerance in 2 patients suffering from AIDS and receiving radiotherapy for Kaposi's sarcoma in the head and neck region when using a GM-CSF mouthwash simultaneously. In yet another study, positive effects were reported on the topical administration of GM-CSF in 17 patients irradiated for head and neck cancer [25]. Ibrahim and colleagues [26] published similar data regarding the usefulness of a GM-CSF mouthwash for cancer patients undergoing chemotherapy. Rovirosa and colleagues [27] conducted a trial to evaluate the effectiveness of a mouthwash of GM-CSF in the healing of oral ulcers, control of pain and weight loss. 12 patients received conventionally fractionated, curative radiotherapy for head and neck cancer up to 72 Gray. Patients gargled with a solution of 300 μg of GM-CSF in 250 cc of water once a day, as soon as oral ulceration was diagnosed. The results were compared with a historical control group. In the GM-CSF group, oral ulcerations healed in 75% of the patients during therapy. A notable improvement in pain control and a

Table 4
Use of GM-CSF for prevention and treatment of chemotherapy (C)- and/or radiotherapy (R)-induced oral mucositis

Disease	Treatment	No. patients	Application	Results	Study protocol	Author	Year
Head and Neck	R/C	11	Systemic	+ Severity	Retrospective, open consecutive grouped	Ausili-Cefaro [22]	1995
Head and Neck	C	20	Systemic	+ Severity and duration	Prospective, randomised, open parallel grouped	Chi [17]	1995
Head and Neck	R/C	29	Systemic	+ Prevention	Retrospective, control group, non-randomised	Rosso [18]	1997
Head and Neck	R	10	Systemic	+ Mucosal protection	Prospective, no control group	Kannan [19]	1997
Head and Neck	R	24	Topical	+ Healing ulcers and weight loss	Retrospective, open consecutive grouped	Rovirosa [27]	1998
Head and Neck	R	17	Topical	+ Severity, + healing	Prospective, no control group	Nicolatou [25]	1998
Head and Neck	R/C	35	Topical	No effect	Prospective, randomised, open parallel grouped	Sprinzl [29]	1999
Head and Neck	R	40	Systemic	No effect	Prospective, randomised, open parallel grouped	Makkonen [20]	2000
Various Neoplasms	R/C	26	Systemic	+ Duration; no effect severity	Retrospective, open consecutive grouped	Gordon [21]	1994
Various Neoplasms	BMT	10	Topical	+ Healing	Prospective, non randomised, parallel grouped	Ovilla [23]	1994
Breast Cancer	C	45	Topical	No effect	Prospective, randomised, double-blind	Cartee [28]	1995
AIDS	R	2	Topical	+ Mucosal toxicity	Case report	De La Torre [24]	1997
Various Neoplasms	C	30	Topical	+ Severity and duration	Prospective, no control group	Ibrahim [26]	1997

BMT, bone marrow transplant; GM-CSF, granulocyte-macrophage colony-stimulating factor.

weight loss reduction could be shown. Cartee and colleagues [28] performed a randomised, double-blind, dose-ranging study and found no effect of locally applied GM-CSF for breast cancer patients receiving high dose chemotherapy. In Table 4, the protocols and the outcome of the mentioned clinical trials are summarised.

5. Conclusion

Behavioural factors such as cigarette smoking, drinking alcohol, bad dentition and poor oral hygiene should be taken into account to optimise the treatment outcome of mucositis therapy. Currently, it is uncertain which treatment should be regarded as the therapy of choice. As a result of our investigation, the *topical* use of GM-CSF for the treatment of oral mucositis induced by radiochemotherapy for patients suffering from head and neck cancer cannot be recommended. Hence, studies have shown that *systemic* application of GM-CSF may potentially reduce the severity and duration of oral mucositis. However, further studies regarding the investigation of systemic use of GM-CSF need to be prospective, randomised and double-blinded to draw final conclusions about the usefulness of treatment regimens containing recombinant cytokines.

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