PROTOCOL LASER MUCITE

A randomized, double-blind, multicenter phase III study evaluating the preventive and curative efficacy of low-level laser therapy (100 mW, 658 nm) on radio- and chemotherapy-induced oral mucositis in patients treated for stage III–IV head and neck cancer

A) CLINICAL TRIAL IDENTIFICATION	
STUDY TITLE	A randomized, double-blind, multicenter phase III study evaluating the preventive and curative efficacy of low-level laser therapy (100 mW, 658 nm) on radio- and chemotherapy-induced oral mucositis in patients treated for stage III–IV head and neck cancer.
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B) STUDY BACKGROUND

1. Epidemiology

Oral mucositis (OM) is one of the most common adverse effects of chemotherapy and radiotherapy, especially for head and neck cancer (HNC). Prevalence of iatrogenic mucosa lesions depends on patient characteristics (risk factors) and treatments (cytotoxic or/and radiation). This oral damage is enhanced by addition of therapy and more aggressive treatment approaches: chemotherapy in addition of radiotherapy, or targeted agents as cetuximab during radiation regimen. (1,2).

HNC represent 650 000 new cases diagnosed each year worldwide and or than 90% of them are squamous cell carcinoma (SCC). In 2012, HNC are estimated as the fifth most common cancers in France (19 000 patients/year) (3–5). Patients treated for HNC often develop severe OM: 60% of them, particularly with chemoradiotherapy (CRT), provided that OM prevalence is not under-reported (6–8). Despite an increase of oral toxicity, CRT is the treatment standard for locally-advanced HNC, demonstrating an overall survival advantage (5 year-survival increase of 12%, with chemotherapy related rate of 4,5%) compared to radiotherapy alone (7,9,10).

Several prospective studies specify that all patients reduced their quality of life (QoL) due to oral pain and mucositis; they report these side effects as the most troublesome. In addition, OM is associated with unsatisfactory treatment course (19% of interruptions) and financial burden (11–13). Moreover, unplanned radiation treatment breaks lead to lower outcome in local control rate (14).

2. Physiopathology

a. Oral mucositis (OM)

Origin and pathobiology of mucosal damage, whereas this side effect is a significant problem for patients and oncologists, is still unclear. OM represents an inflammation of oral cavity, mucous membranes are damaged, and various lesions are observed: atrophy, erythema, oedema, ulceration, bleeding. Stomatitis is a more general term for any inflammation of oral tissue (mucositis, periondotitis, dental and periapical inflammatory conditions). Mucosal damage relates to the rapid mitotic rate of epithelial cells: particularly victims of treatment 6 effects. Besides, cytotoxic and targeted drugs induce mucosal damage of the entire alimentary tract; this is called 'mucosa barrier injury'.

Several publications proposed biological models for iatrogenic OM and nowadays OM is considered as a joint damage of epithelial and connective tissues. Direct damage on dividing epithelial stem cells causes loss of epithelial regeneration. The extracellular matrix and submucosal cells are involved too, through microvascular injury (mediated by endothelial apoptosis and platelet aggregation) and connective tissue injury that precede epithelium modifications (15–17).

Besides, biological pathways may be different in chemotherapy-induced versus radiation-induced mucositis, despite of similar cellular events (18). Differences in the administration (systemic versus local treatment) and the kinetics (once three weeks versus daily) explain that OM appears within four to seven days after chemotherapy initiation, the peak is observed within two weeks. Furthermore, if chemotherapy is delivered over a short time, OM will be acute. While radiation course, OM evolves gradually due to small daily fractions, it begins after about two weeks (almost a cumulative dose of 15Gy) and becomes acute after three or four weeks (about 30Gy) and lasts for several weeks after end of treatment planning. Behind cell and tissue injuries, there is no biological isolated change but cascades of interactions across messengers, signalling and amplification among mucosal and submucosal tissue, environment: oral cavity and vascularisation.

First as initiation stage, reactive oxygen species are produced after radiation and drug action, and generate cells, tissues and blood vessels damages. Moreover, it creates a cascade of reactions that aggravates biological damages and simultaneous events as: DNA damage, epithelial clonogenic death, nuclear factor B NF-B and upregulation of many genes implicated in the proinflammatory cytokines production, apoptosis, activated adhesion molecules, angiogenesis, matrix metalloproteinase, ceramide pathway activation). An inflammatory infiltrate with polymorphonuclear and others inflammatory cells like T cells characterize the ulcerative phase of OM, whereas this acute inflammatory phenomenon is not observable histologically at the beginning of radiation.

Thus, five steps are needed in OM: initiation, upregulation, message generation and then ulceration and healing. Next, local proinflammatory cytokines (for instance tumour necrosis factor TNF and interleukins 1 and 6: IL1 and IL-6) increase thanks to activated transcription factors (especially nuclear factor-kappa B: NFκB).

This process is accelerated by positive feedback mechanisms, until it leads to ulceration. (2) Therefore, bacteria (gram-positive, gram-negative and anaerobic germs) can colonize oral tissues during ulcerative phase of OM; it results in macrophages activation and additional inflammatory cytokines productions. CRT-induced mucosa lesions could be explained by iatrogenic factors, but also a further development because of infectious agents (microbial colonization and systemic circulation invasion) or trauma (due to teeth or dentures). This phenomenon leads to mouth injury, oral pain, and even bacteraemia / sepsis complication in neutropenic patients.

Among risk factors of OM, oral microflora is implicated in bacteria colonisation increases severity of ulcerations but has probably a secondary role. Well-identified therapy and patient characteristics are as follows: schedule, type and posology of cytotoxic drugs, previous radiotherapy, radiation schedule (fractionation), fields and cumulative dose of radiotherapy, and concomitant use of those two antineoplastic modalities. Patient-associated factors influencing OM are: advanced age, female sex, poor oral hygiene, periodontal disease, low body mass index, weight loss before therapy, hyposalivation or xerostomia, periodontal disease, alcohol or tobacco use, immunosuppression due to comorbidities (tumour itself, diabetes mellitus, impaired renal function), periodontal disease and mucosal trauma. Genetic determinants, for instance variation in metabolizing enzymes and drug toxicity of 5-fluoro-uracile: 5FU, can also influence OM.

b. Low level laser therapy (LLLT)

LLLT is a non-invasive care for prevention and management of OM, corresponding to a simple application on mucosa of a high-density monochromatic narrow band light source with various wavelength (helium/neon or diode laser). Mechanisms of OM and healing need to be investigated to understand LLLT mode of action during and following cancer therapy. Five-phase model helps to understand the complex biology of mucositis and the rationale for therapeutic interventions as LLLT during and following cancer therapy, in combination with other recommended therapies. Efficacy may vary depending on the kind of energy and fluence, however three important effects were reported after clinical observations: analgesia, anti-inflammatory action and wound healing effect. That can explain efficiency of LLLT: OM reduction, less oral complications, better cancer treatment compliance (19,20). Actually soft laser was a widely used tool for medicine and dentistry disease or complication (pain, fibrosis, inflammation, thrombosis…) (21).

Many studies showed that LLLT during chemo- or radiotherapy was effective in preventing and treating OM (22–37). Nevertheless, further evidences are needed; in particular to find underlying photobiomodulatory mechanism on wounded tissues, for example several animal studies had already found expressive results in angiogenesis and tissue repair. Others biological effects were described: detoxification after production of oncologic treatments induced free radicals (reactive oxygen species) by acting on mitochondrial respiration, decrease of inflammation and inhibition of NFκB, activation of mitochondrial energy production, increase of collagen production, proliferation of fibroblast cells (20,38–40). Genetic polymorphisms and immune response to cell death should explain OM pathogenesis across innate and adaptive immunity activation by cell death. Lasertherapy leads also to decrease pain conduction in peripheral nerves seem and stimulate endogenous endorphins release (19). Those effects seem to be essentially dose-dependent, but not wavelength specific (41).

Bensadoun's meta-analysis in 2012, reported eleven randomized placebo-controlled trials with patients treated for HNC that the relative risk for developing OM could be significantly reduced thanks to LLLT, but dose should be between 1 to 6 Joules per point. Besides, recommendations for LLLT with intraoral applicators in curative care of OM are as follows: Red wavelength 633-685 nm; diode laser outputs of 10-150 mW; total dose per application not less than 4 J/cm2; treatment on all over the mucositis surface, identified first by a trained clinician; stationary application on a small area < 1cm2 and next moving point to point (average of 6-20 points per session) ; daily application during radiotherapy (minimum of three times a week) and until healing, calculation of application time per point using the formula : time (s) = dose (J/cm2) x surface (cm2) / power (W), for example : 40 seconds for 1 cm2 with a 100mW device. (12,42) Another major advantage of LLLT lies in the absence of reported in vivo significant toxicity.

3. Mucositis management strategies

Several clinical guidelines are available to manage mucositis and its complications: dysphagia, weight loss, oral pain, compromised scheduled antineoplastic treatment (delivery and dose)… Physician and patient can complete OM evaluation thanks to many scales and instruments. Weekly oral inspection leads to detect lesions at an early stage and facilitates successful management. Oral care and exemplar hygiene, notably stopping smoking and alcohol consumption are needed (43–46).

OM induced pain is not always palliated thanks to opioid analgesics use, and these drugs lead to other adverse effects (nausea, constipation, sleepiness…). Alternative therapies could be proposed as low level laser therapy (LLLT) (17).

Nevertheless, its role and use are still debated, despite of development of literature about laser therapy in the mucositis management. Mucositis pathogenesis could provide evidence and arguments to improve this supportive care. LLLT could reduce frequency, severity and cost of OM (better QOL, less hospital days, reduced narcotic use, less severe dysphagia and invasive nutritional support…), without adverse event and with better observance for cancer treatment (2).

Our study evaluates efficacy of low-power laser therapy during concurrent CRT for patients suffering from an advanced HNC: stage III or IV. The strength of this new multicentric phase III is that it focuses on new therapeutic standard: LLLT was used in accordance to recent recommendations. This randomized, double blind clinical trial

focus on observable OM evolution, and also secondary on subjective and functional (oral soreness, dysphagia, QOL) dimensions of OM and possible LLLT disadvantage (tolerance, risk of a local relapse).

C) STUDY INFORMATION

INDICATION	This study is intended for patients treated with concomitant chemoradiotherapy for stage III–IV head and neck squamous cell carcinoma (HNSCC).
METHODOLOGY	Randomized, prospective, national multicentre, double blind

Primary Objective and Endpoint

Primary objective	Endpoint	Evaluation time
The main objective of this phase III study was to evaluate the effectiveness of a 100mW and 658 nm diode LLLT, for the prevention and treatment of concurrent CRT-induced OM, in advanced oral or hypopharyngeal cancer patients.	Primary endpoint was LLLT outcome measured by World Health Organization (WHO) grade 3-4 OM incidence in each arm of treatment	7 weeks

SECONDARY OBJECTIVES AND ENDPOINTS

Secondary objectives	Endpoints	Evaluation time
Pain, assessed using a numeric rating scale (NRS), as well as the type and dosage of analgesic medications consumed.	Pain, assessed using a numeric rating scale (NRS), as well as the type and dosage of analgesic medications used; assessments performed weekly.	3 months
Nutritional status, evaluated through body weight, type of diet, serum albumin and prealbumin levels, and optionally by assessment of body fat mass (bioelectrical impedance analysis, skinfold measurements).	Nutritional status, evaluated by body weight and type of diet (weekly assessments), serum albumin and prealbumin levels, and an optional assessment of body composition (bioelectrical impedance analysis, skinfold thickness measurements), performed at treatment initiation and at the end of treatment.	3 months
Treatment compliance, assessed by radiotherapy prolongation, treatment duration (in days) and reasons for treatment interruptions, the number of chemotherapy cycles administered, dose modifications, and any treatment delays.	Treatment compliance, assessed by radiotherapy prolongation, treatment duration (in days) and reasons for treatment interruptions, the number of chemotherapy cycles administered, dose modifications, and any treatment delays.	3 months
Tolerance to laser therapy, evaluated using a 0–3 grading of the “tolerance” item from the treatment evaluation form	Tolerance to laser therapy	3 months
Quality of life, assessed using the EORTC QLQ-HN35 questionnaire.	Quality of life, assessed using the EORTC QLQ-HN35 questionnaire.	5 years
Locoregional control, assessed during post-treatment follow-up visits every 3 months for 1 year, then every 6 months up to 5 years.	Locoregional control, assessed during post-treatment follow-up visits every 3 months for 1 year, and then every 6 months up to 5 years.	5 years

Disease-free survival, calculated from the date of randomization to the first event (recurrence or death), or up to 5 years.	Disease-free survival, calculated from the date of randomization to the first event (recurrence or death) or up to 5 years.	5 years
Overall survival, calculated from the date of randomization to death from any cause, or up to 5 years.	Overall survival, calculated from the date of randomization to death from any cause or up to 5 years.	5 years
INCLUSION CRITERIA	1) Male or female patient aged between 18 and 75 years. 2) Patient with histologically confirmed stage III–IV squamous cell carcinoma of the oral cavity, oropharynx, or hypopharynx. 3) ECOG Performance Status ≤ 2. 4) Estimated life expectancy greater than 3 months in the absence of treatment. 5) Eligible for concomitant chemotherapy with 5 fluorouracil and/or a platinum compound, or cetuximab (not contraindicated), defined as: serum creatinine < 150 µmol/L and creatinine clearance ≥ 55 mL/min (calculated using the Cockcroft–Gault formula), with creatinine clearance calculation required if serum creatinine > 120 µmol/L. 6) Adequate hematologic, renal, and hepatic function, documented within 15 days prior to randomization, with the following laboratory values: 7) Hemoglobin > 8 g/dL • Absolute neutrophil count > 1.5 × 10^9/L • Platelet count > 100 × 10^9/L • Total bilirubin < 1.5 × the upper limit of normal (ULN) • ALT/AST and alkaline phosphatase < 2.5 × ULN 8) For women of childbearing potential, use of an effective method of contraception (hormonal contraception or intrauterine device) is required.	
NON-INCLUSION CRITERIA	1) Presence of other concomitant malignancies or malignancies diagnosed within the previous five years, except for basal cell carcinoma of the skin or carcinoma in situ of the cervix. 2) Neoadjuvant chemotherapy. 3) Metastatic disease. 4) Previous radiotherapy to the head and neck region. 5) Known severe hypersensitivity to platinum compounds. 6) Any uncontrolled comorbidity (respiratory, cardiac, hepatic, or renal disease). 7) Pregnant or breastfeeding women (a serum or urine pregnancy test must be negative at study entry for women of childbearing potential).	

E) INVESTIGATIONAL TREATMENT DESCRIPTION

Radiotherapy:

All patients will be treated with conformal radiotherapy, with or without intensity-modulated radiotherapy (IMRT). The prescribed dose will be adapted to the treatment indication (definitive or postoperative) and to histopathological findings in cases of tumor resection. Radiotherapy will be prescribed in accordance with the recommendations of ICRU Report 50. The delivered dose will be 2 Gy per fraction, administered five fractions per week. All treatment beams will be delivered at each session. Radiological verification will be performed for each photon beam at the first treatment session and subsequently at least once per week.

Chemotherapy:

Several concomitant chemotherapy regimens in combination with radiotherapy are possible:

- 5-fluorouracil (5-FU) plus cisplatin
- 5-fluorouracil (5-FU) plus carboplatin

- Cisplatin alone
- Cetuximab alone

Dental care:
All patients (except edentulous patients) will undergo a pre-treatment dental and oral medicine consultation for dental assessment and rehabilitation, and for the possible fabrication of fluoride trays.
During treatment, all patients will receive standardized oral care (Appendix 4).
Dosimetric study
The dosimetric analysis will include the transverse section, as well as the frontal and sagittal planes passing through the axis of the lateral beams.

Low-level laser therapy:
The device used will be a diode laser with an output power of 100 mW and a wavelength of 658 nm. Application will be performed after each radiotherapy session, in an appropriate room (low ambient lighting, facilities allowing ENT examination), on all mucositis lesions of grade ≥ 2.
The application is painless, athermal, odorless, and completely silent. The patient will wear protective goggles to ensure retinal safety.
The operator will also wear protective goggles that allow visualization of the beam and its boundaries. Application times will be controlled using a timer. Prior to application, the laser probe will be covered with a single-use protective film (condom).
Macroscopically involved tumor areas will be excluded from the treatment fields.
The laser will be applied at a distance of ≤ 15 mm from the mucosal surface, sweeping all areas affected by grade ≥ 2 mucositis.
The delivered energy dose will be 4 J/cm². The optical fiber will be held perpendicular to the mucosal surface throughout the application. The treatment duration for a given area will be determined using the corresponding calculation chart:
t (s) = energy (J/cm²) × surface area (cm²) / power (W)
The total treatment time will be a few minutes, depending on the extent of the surface area to be treated.

Sham device:
The procedure will be identical to that used in Arm A, except that the laser device will not be operational. The application duration will be approximately one minute.
All applications will be performed by a single operator per center. Outcome assessments will be carried out on a weekly basis by another physician (or a specifically trained nurse) at each center, who will be unaware of whether the treatment was administered, in order to maintain blinding.
Assessments will be recorded on a dedicated case report form specifying: the degree of mucositis (WHO classification), pain level (numeric rating scale, NRS), body weight (kg), type of diet (RTOG scale), ongoing analgesic treatment(s), quality of life (patient-completed questionnaire), and any treatment interruptions (days) or therapeutic modifications (number of chemotherapy cycles, dose adjustments).l care will be prescribed to patients.

F) STATISTICAL CONSIDERATIONS	
SAMPLE SIZE DETERMINATION	The primary objective of this study is to demonstrate the preventive and therapeutic efficacy of low-level laser therapy on mucositis induced by concomitant chemoradiotherapy in patients treated for stage III–IV head and neck cancer. According to the literature, the proportion of grade 3–4 mucositis (WHO classification) in patients with head and neck cancer treated with chemoradiotherapy is at least 67% ([2], [15]). The use of low-level laser therapy is expected to reduce this

	rate by 30%, which would correspond to the incidence of grade 3–4 mucositis observed in patients treated with radiotherapy alone. With a two-sided alpha level set at 5% and a statistical power of 90%, 43 patients per treatment arm are required to demonstrate a statistically significant difference. Assuming that 15% of patients will be non-evaluable (loss to follow-up, death, or other reasons**), a total of 100 patients** will be included, i.e. 50 patients per treatment arm. Randomization will be balanced in a 1:1 ratio and stratified according to the investigational center and the treatment administered.
DESCRIPTION OF ANALYSIS SETS	The statistical analysis will be performed at the Clinical Oncology Evaluation Center (Centre d'Évaluation Clinique en Oncologie) of the Centre Paul Papin in Angers. All analyses will be conducted according to the intention-to-treat principle (patients will be analyzed in the treatment arm to which they were randomized, regardless of the treatment actually received). For the primary endpoint, the rate of grade 3–4 mucositis will be estimated in each treatment group and presented with its confidence interval. A chi-square test, or Fisher's exact test when appropriate, will be used to assess differences between the low-level laser therapy arm and the control arm. For secondary endpoints, including nutritional impact (weight, type of diet, etc.), type of chemotherapy, pain intensity (NRS), treatment compliance, quality of life, and locoregional response, the non-parametric Mann–Whitney test will be used for quantitative variables, and chi-square or Fisher's exact tests for qualitative variables, to compare the two study groups. The Kaplan–Meier method will be used to analyze overall survival and progression-free survival, as well as to assess the time to onset and duration of grade 3–4 mucositis in each treatment arm. Comparisons between groups will be performed using the log-rank test. All statistical analyses will be conducted using SPSS software, and statistical significance will be set at a two-sided p-value of 0.05.

G) STUDY CALENDAR	
DURATION OF INCLUSION PERIOD	90 months
TREATMENT DURATION	2 months
POST TREATMENT FOLLOW UP	60 months