Author’s response to reviews

Title: Randomized clinical trial of efficacy and safety of colchicine 0.5% cream versus photodynamic therapy with methyl aminolevulinate in treatment of skin field cancerization: study protocol

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Author’s response to reviews:

Response to the Editor

Thank you for your email dated November 07, 2017 with reviewers’ comments on our paper. The comments were very helpful. We have revised the manuscript in accordance with the reviewers’ comments. All the changes in the manuscript have been marked in yellow. Below is a point-by-point response to all the issues raised by the reviewers. We hope that we have adequately addressed all the issues and that this version is now acceptable for publication.

We look forward to hear from you.

Yours Sincerely,
The authors
Responses to specific issues raised by reviewers

We thank the reviewers for their valuable comments. The comments mentioned are addressed below.

Reviewer reports:

Cristina Zane (Reviewer 1): This is an interesting trial which compare the efficacy of two skin cancertization field treatments.

Study protocol and objectives are well defined.

I have only minor concerns about this manuscript:

- please review the use of subjects: in most sentences are missing (e.g. Line 19 THEY occur... line 51 IT has a slow elimination...)

The use of subjects was revised and corrected according to English grammatical standard

- Actinic keratosis chapter, line 17: what do you mean with PLATES?

Actually, PLATES should be replaced by plaques (skin plaques)

-Check use of AK abbreviation. Isn't better to use AKs somewhere?

There were found these mistakes during the revision. They were corrected according to the regency.

- Colchicine chapter line 19-26: not meaningful sentences.

After careful reading, these sentences were changed to organize the main idea.

- Selection of participant, second point: I would add "before enrollment" at the end of the sentence

This sentence was reorganized with the objective of include this information
- please clarify if the protocol has been approved by the Local Ethic Committee

This information is written together with Clinical Trial Register at page 10, lines 19-22

- Why the authors choose to perform a single session of PDT? According to guidelines two consecutive sessions are recommended after one week to each other. Could it be a bias in comparing treatments efficacy?

Studies have been indicated that one single session of MAL-PDT shows AK counting reductions up to 80%, and 89% when two consecutive sessions are done. Basically, to thin AKs, one single session can be made with good responses, and for thick or hypertrophic AKs, two sessions is more effective (reference indicated below), which justifies the use of only a single session of MAL-PDT. Also, licensed use recommends that for AK, MAL-PDT be given as a single treatment and repeated if required after 3 months.

Ref: European guidelines for topical photodynamic therapy

part 1: treatment delivery and current indications – actinic keratoses, Bowen’s disease, basal cell carcinoma

“Actinic Keratoses (Strength of Recommendation A, Quality of Evidence I) Thin and moderate thickness AK on the face and scalp respond well to topical PDT, with typical clearance rates of 89–92% 3 months after therapy, equivalent or superior to cryotherapy, depending on protocol.44–46 One-year sustained lesion clearance rates of 78% and 63–69% have been reported following ALA-PDT (up to two treatments) and patch ALA-PDT (single treatment) respectively.32,47 Current licensed use recommends that for AK, MAL-PDT be given as a single treatment and repeated if required after 3 months, reflecting equivalent efficacy in a comparison study with double therapy 7 days apart.45 A large randomized intraindividual study of face/scalp AK in 119 patients used this protocol to compare MAL-PDT with cryotherapy.46 After the initial cycle of treatments, PDT cleared more lesions (87% vs. 76%), but with equivalent outcome after non-responders were retreated (89% vs. 86%).” (Braathen, 2012)


- I would change Figure 1, I think it is not fully clear

The figure 1 was changed to make it clearer and to include T15 evaluation either.

- Please indicate what kind of immunohistochemical probes are you going to use for your analysis

For immunohistochemistry, histological sections of 4μm thickness will be mounted on silanized slides and subjected to immunohistochemical staining for detection of Ki67 (Cell Sig. Tech., Inc., Danvers, MA, USA, mouse mAb IgG1, #9449) or p53 (Cell Sig. Tech., Inc., Danvers, MA, USA, mouse mAb Ig2b, #48818). After sections deparaffinization and rehydration will be performed the endogenous peroxidase blockade with 3% H2O2, followed by heat-induced antigen retrieval and protein blockade by treatment with milk. The sections will be incubated overnight at 4°C with the primary antibody diluted 1:300 or 1:150 for the Ki67 and p53 respectively. Signal amplification will be performed by biotin-free polymer detection system using the MACH 4® universal HRP-Polymer kit (Biocare Medical, Pike Lane Concord, CA, USA) according to the manufacturer's instructions. Lastly, 3-3' diaminobenzidine will be used as a chromogenic substrate and the sections will be counterstained with Harris hematoxylin.

- chapter of data analysis, line 36-40: repetitive sentence

The last sentence repeating the information above it was removed

Jared Jagdeo (Reviewer 2):
1. Major grammatical revisions required as the manuscript is difficult to read due to improper grammar, including spelling errors (e.g. "aminolevulinate", singular form of AKs is "actinic keratosis").

A new revision was made by American Journal Experts to improve the quality of this study protocol

2. Discussion of abstract: Consider stating the potential impact of the study.

The potential impact of the study is that colchicine is a low cost drug and this characteristics turns this drug an effective and accessible treatment for the skin field cancerization. This observation was included in discussion of the abstract (page 4, lines 46-53)

3. The flow of the Background section may be improved by changing order of introduction to first discuss AKs, then discuss field cancerization. Currently, you first discuss how skin field cancerization involves AKs next to normal skin. In the next section, you explain what an AK is and then you discuss different types of field therapy for AKs. It can be difficult for the reader to understand.

This consideration was observed, and the change was made in order to organize better the background

4. Recommend removing regular sunscreen discussion from the section regarding treatments for AKs. As stated, sunscreen use is a protective measure against formation of AKs rather than a treatment modality.

This discussion was suppressed from the text.

5. Include AK as one of the pre-malignant skin cancers that PDT targets (page 6, line 41) since AKs are the main focus of this study.

This correction was made according to this observation.

6. Recommend mentioning the adverse effects of topical colchicine (page 7, line 46). Systemic colchicine side effects are listed, but these side effects may not be applicable to topical colchicine use.
This observation was considered and the adverse events of topical use of colchicine were included.

7. Why was MAL-PDT used as a comparison instead of the other treatments mentioned (e.g. 5FU, imiquimod, etc.)? (page 8, line 24)

Recently, MAL-PDT was the treatment of skin field cancerization that had more new clinical trials in the literature for skin field cancerization and not only AKs, which made us to choose this modality of treatment.

8. Will only curetting the AK lesions treated with MAL-PDT be a confounding factor? (page 11, line 38)

Curetting the AK is part of the protocol of MAL-PDT as stated by International guidelines.

In previous studies with colchicine, AKs were not curetted. The objective of this study is to compare these two treatments in the standardized posology and procedures.


9. Distal and proximal limits on the forearm are mentioned. Will you be treating both anterior and posterior surfaces of the forearm with MAL-PDT or colchicine cream? (page 12, line 1)

Only dorsal forearms, which are the photoexposed area thus, with major risk of development of skin tumors.

10. State if the biopsy of center of the middle third of the forearm will be on the anterior or posterior side of the forearm. (page 12, line 1)

The punch biopsy will be on the dorsal side of the forearm.

11. State if biopsy will be a shave biopsy or punch biopsy. If punch biopsy, state size. (page 12, line 1)
It will be a punch biopsy 3.0 mm, and this information is written on the same place along the text.

12. How will you monitor subject's adherence to colchicine cream twice daily for 10 days? (page 12, line 10)

The adherence to the treatment will be monitored by phone calls. This information was included at the same place along the text.

13. Is assessment of adverse effects and tolerability at T15 too long of a time period for MAL-PDT? (page 12, line 10)

Some studies with MAL-PDT include adverse events like erythema, edema, and crusts, that resolve completely after 2-6 weeks, which justifies the T15 re-evaluation.

References:


14. What objective questionnaires or scales will be used to assess incidence, impact, and severity of adverse events? (page 12, line 10)

It will be considered the clinical evaluation, which will embrace: erythema, edema, crusts and vesicles, that will be graduated in absent, mild, moderate or intense.

15. In the "Interventions" section, T60 should also include assessment of adverse events and tolerability (page 12, line 11).

This recommend was accepted and this information included.

16. If the impact of the study is reducing the cost of AK treatment, consider comparing benefits and costs of MAL-PDT vs colchicine cream. What other benefits does colchicine cream have over MAL-PDT? (page 14, line 42)
colchicine has mild or moderate adverse events; short duration of treatment, specially comparing with another treatments for skin field cancerization like 5-FU and diclofenac; and is for home use, which can be positive to patients that need homecare and treatment

17. T15 should be included on consent form. (page 22)

This information was included on consent form to make it clearer to the participants.

18. T15 should also be included on Figure 1.

Figure 1 was redefined and this information was included.