Reviewer’s report

Title: Kinetics of mycolactone in human skin during antibiotic therapy for Mycobacterium ulcerans disease.

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Reviewer: Tjip vd van der Werf

Reviewer’s report:

This paper adds potentially important information to existing knowledge on BU disease.

The paper is generally well written, and seems to cite most of the relevant papers in the field though there seems to be some selective referencing.

1) This study involves human subjects and – according to current Good Clinical Practice standards – should be registered as such. As there is no mention of a registration number, and this reviewer could not find the study protocol on the web, the authors should try explain where it was registered, or alternatively, why this registration requirement was not met and why perhaps registration was omitted; perhaps it would be best to explain also, when the study was designed and conducted, and when the Ethics Committee approved the study, as this current demand was not necessary before 2007, and some Ethics Committees have only recently started to insist on abiding with the GCP rule.

2) As it was impossible to see the study protocol at the registration site, it would be helpful if reviewers were provided the text of this protocol in full.

3) From the paper it is impossible to see how the sample size (number seems 80) of study participants was calculated; perhaps it was a convenience sample? Or was there a target number of adults in whom consecutive biopsies could be obtained? And if so, how many were deemed necessary to answer the primary study goal? On page 10, a median age (14yrs) is mentioned but no breakdown of numbers of adults versus children is given; please provide these details or refer to the other paper in press for further details. From the actual number of observations however, with very few observations at week 6 and notably, week 12, it seems that this paper reflects proof-of-principle, or rather, observational data alone, and not a study designed to really explore the question whether mycolactone measurement provides a biomarker to tailor treatment duration.

4) As the primary goal of the study was to explore whether mycolactone could serve as a biomarker for response to therapy, how did the study team think about the validity of their model? In a recent paper by Converse et al (PLoS Negl Trop Dis 2014), infected mouse footpads were sacrificed, homogenized and assayed in whole – thereby securing that a well defined tissue concentration would truly correlate with total amount of mycolactone present in the lesion, with a well defined inoculum of M ulcerans, and that sampling error could not be a
confounding factor of tissue concentration found; in human lesions however, this
dproblem cannot be solved – even with fairly large tissue volumes as obtained by
4mm (not 3mm; resulting in 1mL of tissue) punch biopsies. The authors should
discuss this problem in the Discussion Section. It might be that e.g., the weak
correlation between mycolactone concentration and time to healing (page 12,
top) might partly be explained by this methodological problem.

5) The authors mention that the mycolactone assay was described in detail in an
‘additional materials file but this reviewer was unable to see this material. There
have been rumours about difficulties with the extraction technique to reliably
detect mycolactone in human tissue samples. In their recently published paper
(Converse et al PNTD 2014) tissue samples were stored in 100% ethanol with no
loss of signal during shipment; could the authors explain why their transport
medium was either equally good, or should they perhaps admit that their protocol
was potentially flawed in this respect? The entire discussion about discrepant
mycolactone concentrations as measured by MSMS and cytotoxicity assays (page
13) might just as well be explained otherwise, e.g., by methodological flaws in
assay, transport medium etc. Note that in the Converse paper, fluorescent thin
layer chromatography had much lower detection level . . .

6) Initially two biopsies were taken - one to establish the diagnosis, and the other
for mycolactone detection - see page 7, top. Further on the same page 7 the
authors state that diagnostic confirmation was done by FNA – while initially no
mention was made of FNA for the diagnosis in the first place. Perhaps the
authors should explain in more detail WHICH procedures exactly were done in
their study participants, and for which purpose; as PCR already confirms the
diagnosis with a less invasive procedure than with 4 mm biopsies, the protocol
should have been crystal-clear about the necessity to perform these additional
biopsies for diagnostic reasons; again reviewing the study protocol might be
helpful for the reviewers and editors to elucidate these points. On page 11,
‘Mycolactone concentration during and after antibiotic therapy: Further biopsies
were taken at 6 and/or 12 weeks from patients whose lesions were healing
slowly (Figures 2A and 2B’ – here it seems as if indeed, up to 6 (six)
consecutive biopsies were taken in some study participants.

7) It seems – from reference 18 – that perhaps the study was primarily designed
to address yet another question – i.e., whether streptomycin as used in the
WHO standard treatment guideline could be reduced to 2 weeks – as 4 weeks
had already been shown to be non-inferior to 8 weeks of streptomycin use (ref
21).

8) The authors should provide the reference 18 for reviewers to be able to
appreciate in full the work that they refer to.

9) in their discussion, data are really over-strectched and over-interpreted in that
too many assumptions are piled. IF mycolactone kinetics are such that
antimicrobial treatment stops production, the wash-out may take several weeks -
6? 12? and stating that mycolactone PRESENCE helps tailoring treatment is
obviously incorrect. At best it might help understand antimicrobial treatment if
that treatment stops production - not presence - of mycolactone. IF their assay would show arrest of mycolactone production - e.g., expression array of mycolactone synthase activity - their statement would make sense; now it does not.

Minor comments:

Background – line 3: why do the authors mention mycolactone to be a novel polyketide molecule: are there older ones that this reviewer does not know about? Mycolactone was described almost 15 yrs ago so why should it be novel in the first place? If no reason can be given please omit the word: novel

Page 8 – diagnosis was based on . . . . PCR by FNA? Or tissues biopsy quantitative culture? Or both? The authors use the words skin and tissue as if this is entirely exchangeable – but my guess is that tissue might be preferred as with 1 mL of tissue in a biopsy, undoubtedly, subcutaneous tissue will have been sampled which is probably actually to be preferred as typically most of the micro-organisms, as well as mycolactone may be present in subcutaneous fat rather than in skin . .

Page 9 – top: . . . .which measure . . consider rephrasing; that measures . .

Page 11, top: There was a wide variation of concentration in all types of lesion with median (range) of 437ng/ml (136–2589; n=18) in nodules, 311ng/ml (148–834; n=14) in plaques, 443ng/ml (114–3020; n=45) in ulcers and 895ng/ml (457–2689; n=4) in oedematous lesions using the cytotoxicity assay (Figure 1A). If I add these numbers it is 81 (45 ulcers, 36 –non-ulcerated (plaque, nodule, edema), not 80; in table 1, not 45 but rather 44 ulcers are listed – please correct the text (or the table).

It seems that the Discussion Section overestretches the importance of the findings.

It seems more logical to simply conclude that mycolactone detection seems to correlate with healing over time, and that perhaps future studies should address the question whether the assay – provided, the threshold of detection is improved, such as the one reported by Spangenberg, Kishi and converse – could possibly help to monitor treatment and perhaps one day, tailor treatment duration?

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

no potential conflict of interest