Author’s response to reviews

Title: High efficacy of a dimeticone-based pediculicide following a brief application: in vitro assays and randomized controlled investigator-blinded clinical trial

Authors:
Jorg Heukelbach (heukelbach@web.de)
Doerte Wolf (doerte.wolf@cardiosec.de)
John Clark (jclark@vasci.umass.edu)
Hans Dautel (dautel@insectservices.de)
Kristina Roeschmann (k.roeschmann@Pohl-Boskamp.de)

Version: 2 Date: 04 Sep 2019

Author’s response to reviews:

Manuscript number: BDER-D-19-00034R1

Title: High efficacy of a dimeticone-based pediculicide following a brief application: in vitro assays and randomized controlled investigator-blinded clinical trial

Point-by-point reply

Dear Editor,

We thank you very much for your feedback and for considering our manuscript for publication. Please find enclosed a point-by-point response to the issues raised by the reviewers.

Best regards,

Jorg Heukelbach

Reviewer #1 (Ian Burgess):

This is a manuscript that describes the activity of a new/modified formulation of a marketed pediculicide product including clinical data that were presumably generated for both regulatory and marketing purposes. It also includes some preliminary testing data that were either generated
at an earlier date in preparation for conducting the clinical study or else the tests were conducted quite separately with some other purpose (the reason for this last statement will become apparent below). My overall impression of the manuscript is that different sections were written by different authors, as shown by style of writing, grammatical construction, etc. but none of the authors appears to have taken overall editorial control so that as a read it is somewhat disjointed, inconsistent, and in places contains duplicated or irrelevant material. Consequently, there are some logical inconsistencies throughout the text that require justification and clarification.

Answer:

We have revised the entire manuscript for language, style and logical consistency, and eliminated wordy statements and redundant information whenever possible.

Introduction

Paragraph 1, lines 64-67: I think it would make this sentence clearer if it was turned about to state that in Germany the kdr-like mutations showed a minimal impact on the permethrin treatment described by references #16 and #17.

Answer:

OK, amended as requested.

Paragraph 3: I do not see the relevance of this paragraph on hypothetical disease vector capacity of head lice to any of the aims of this study. It appears to have been "dumped" somewhat randomly in the middle of two paragraphs dealing with the need for effective products and the need to improve treatment compliance. It should be deleted.

Answer:

OK, deleted.

Lines 92-93: As above the mention of "the emerging role of head lice as potential disease vectors" is similarly irrelevant to the main point of the study. The socio-economic burden of lice or compliance with treatment regimens in Germany and other developed countries where Nyda is sold is a more pressing argument.

Answer:

Agree. The manuscript has been amended accordingly.
Lines 99-107: These sentences are more appropriately incorporated into the Methods section, where much of this wording is duplicated anyway. It would be sufficient to state here that the manuscript describes "laboratory and clinical studies to investigate the efficacy and safety of the new Nyda formulation".

Answer:

OK, amended as requested.

Line 103: It is something of a tautology to state that the permethrin-based pediculicide has a neurotoxic mode of action, given the information about resistance etc. provided earlier. Note this same statement also occurs at line 122.

Answer:

We have adjusted the statement and its wording to avoid any further misunderstandings.

Methods

Test and reference products

Lines 118-121: Why were two completely different dosage forms of permethrin used for the in vitro and clinical studies? Given that the bioavailability of an insecticide is affected to varying degrees by the formulation excipients, surely it would be logical to use the same comparator product for laboratory tests (0.5% permethrin in an alcohol vehicle) as was planned for the clinical study, especially as I suspect these laboratory data were used as corroborative evidence in the medical device submission to the German regulatory authorities.

Answer:

Within the realm of the preclinical studies, which included a permethrin-resistant louse strain, permethrin was not used as a comparator, but as internal control to confirm status of resistance of the lice. These studies have shown the in vitro efficacy of the test product within a context of permethrin resistance. As these studies were performed in the US, a permethrin product that is available within the US was used.

In the comparative clinical trial, the permethrin product was used as active comparator. Therefore, a product available on the German market, which is reimbursable and recommended by the Federal Office of Consumer Protection and Food Safety, was used.

We have rephrased the description in the Methods section to make this point clearer.

Assessment of pediculicidal activity in vitro
This section is the most puzzling of the whole manuscript.

Based on the methodology employed, I have concluded that the test against adult body lice was possibly not conducted in Dr Clark’s laboratory but at one of the laboratories in Germany that perform louse testing. Why was testing conducted against body lice rather than against the same BR-HL isolate of head lice that is maintained by Dr Clark as the louse egg tests? It appears totally illogical to run one set of tests versus a completely susceptible laboratory colony of body lice (adults) and the other set of tests versus pyrethroid resistant head lice (eggs in this case). If resistance was an issue under scrutiny, surely testing against adult resistant lice that could adequately express any resistant trait is the logical way to evaluate the likely clinical efficacy of a product, even if it was only the comparator product?

Answer:

We do not agree with this statement, as both in vitro studies were performed according to standardized, well-established and published methods. As the presentation of the combination of different in vitro and clinical tests obviously has caused some confusion, we have reworded the respective sections to make these points clearer.

We tested different formulations on louse eggs (Dr. Clark’s lab). As neurotoxin-based products often do not show a high efficacy against louse eggs, successful treatment of lice and eggs with new formulations, such as dimeticone-based products, might still be questioned by authorities.

The tests with body lice (lab of IS Insect Services) followed a standardized protocol and an established model also used by the German Federal Office of Consumer Protection and Food Safety to evaluate efficacy of products, and results from these tests by the German authority serve as basis for the official recommendation of these products for disinfection (“Entwesungsmittelliste”). Permethrin resistance was not an issue under scrutiny.

Line 142: Why was gerbil hair used as a substrate? Surely it would be more logical to either use human hair or, since these were body lice, to place them on a clean cloth substrate similar to the one they are reared on?

Answer:

The tests with body lice were performed by a German CRO with established models and standardized protocols. There is no reason to assume that the type of hair should alter the results by any means.

Lines 145-151: The determination of mortality is also a puzzle. It is true that two of the cited references #34 and #37 utilise a criterion in which categories M, G, and D could be considered non-viable. However, the lead author of this manuscript, Dr Heukelbach, has been a strong advocate in other publications of using more stringent criteria, notably: Heukelbach J, et al. In vitro efficacy of over-the-counter botanical pediculicides against the head louse Pediculus

Therefore, I would like to question why these more stringent criteria were not applied in this study.

Answer:

As this particular determination of mortality is part of the standard protocols of the CRO that performed the experiments, these less stringent criteria were presented. However, stringent data are presented in the manuscript as well: as shown in Figure 1, the results applying more stringent criteria are very similar. We have included in the text a description of lice in the M category for the different points in time and discussed briefly the different assessments in the discussion section.

Line 151, the citation of reference #36 is incorrect because this cited article does not mention assessment of mortality. The correct reference should be: Burkhart CN, Burkhart CG. Recommendation to standardize pediculicidal and ovicidal testing for head lice (Anoplura: Pediculidae). Journal of Medical Entomology 2001; 38(2): 127-129.

Answer:

OK, corrected.

Assessment of ovicidal activity in vitro

Line 173: I believe this line should start "Besides immersion,…" not "Besides spraying,…"

Answer:

OK, corrected.

Lines 173-181: I can understand spraying of the Nyda product and of the distilled water control but I find it hard to work out how the Nix 1% permethrin product could be sprayed because it is a relatively highly viscous cream formulation that would not be easily expelled from a regular atomizer pump head in the same way as the other two fluids. Please explain.

Answer:
This is correct. The internal permethrin control was performed using the immersion protocol. We amended the respective sections in the manuscript.

Lines 178-180 (also lines 138-139): Why was the Nix 1% permethrin product shampooed off rather than just rinsed off as required by the product instructions? This could impact on the bioavailability of the permethrin.

Answer:

The wash steps followed a standardized protocol and as resistance to permethrin was not an objective of investigation, a potential impact on bioavailability of permethrin is considered negligible.

Clinical trial

Study design

Lines 195-196: This study was performed in line with reference #38 but a more recent international consensus was collected by Do-Pham, et al. Designing randomized-controlled trials to improve head-lice treatment: Systematic Review using a vignette-based method. Journal of Investigative Dermatology 2014; 134: 628-634, which could have provided a clearer endpoint by following that guideline for a 14 day follow up.

Answer:

We are aware of this discussion and would refer to this more recent consensus in future studies. This study was designed in 2015, and we have used Barker (2012) and a previous study as reference which was used for inclusion on the official recommendation of pediculicides in Germany. However, the applied standards are valid, besides standards being a matter of discussion. Some recent studies still apply 10 days as endpoint (Eertmans et al., 2019; Wolf et al. 2015).

Eligibility criteria

Line 205: This section states "Patients >2 years of age..” Does this mean that patients had passed their second birthday but that they had not yet reached their third birthday or does it mean that as they were "over" two years they had already reached their third birthday? This question is pertinent because the SPC for Infectopedicul states that the product is for children of three years and older.

Answer:
We are sorry for this typo, which has been corrected. Children were to be included, if they were 2 years of age or older, the correct wording should be $\geq 2$ years of age.

The reference product was administered according to the most current SPC that was available in Germany before start of the study. In this version from September, 2013 it is stated that for children from the age of 2 months to 3 years a maximum dose of 25 ml must not be exceeded, which was strictly followed for children $\geq 2$ years during the conduct of the trial. A respective statement has been included in the amended manuscript.

How were patients recruited to the study?

Answer:

Patients/guardians/caretakers were informed about this study via advertisements, flyers, letters to primary schools and preschools, and internet on-screen displays. All texts used for recruitment have been approved by the responsible ethics committee. Patients/guardians/caretakers had to contact the study center to arrange an appointment, and after initial assessment of infestation and informed consent procedure patients were included and randomized. This information has been included in the amended manuscript.

Intervention

The wording of line 236 is potentially misleading. Where it states that "repeated treatments" were given it should be made clear that all participants received only two treatments. The term "repeated treatments" implies that multiple applications of product were given.

Answer:

OK, corrected.

Line 244: The specification of the "nit-comb" should be given.

Answer:

A short description of the combs used has been included.

Line 252: Was this the same specification of nit-comb for both products?

Answer:

Yes, the same comb types were used for both products. This has now been stated in the manuscript.
Informed consent, outcome measures and assessments

Lines 265-267: I do not understand why or how an assessment of reinfestation could be made at day 1 (V2) but not at day 7 (V3) and does not match up exactly to the criteria used for assessment in the cited reference #40. This assessment is understandable at day 10 (V4).

Answer:

We did not assess reinfestation at day 1, but used as one of the criteria for reinfestation on day 10 the absence of adult lice on day 1. In fact, our assessment slightly differed from the definition in the cited reference. We amended the description of the assessment likewise.

Lines 268-276: This paragraph appears unnecessarily wordy. The categories of the response ratings and observations are adequately shown in the tables of these outcomes and so only the briefest mention is required here with a statement referring the reader to the tables in the text for the characteristic criteria.

Answer:

OK, done.

Lines 293-296: If nit-combing was performed as part of the treatment and patients or their care givers were permitted to nit-comb at any time between study visits (irrespective of whether they did it or not), how could you possibly determine whether the efficacy outcome was due to the liquid treatments applied to the participants or whether the outcomes were confounded by the potentially multiple combing sessions (at least two during treatment but potentially as many as eight, if the people were really diligent)? Even if most carers failed to apply additional combing measures what is to say that the combing during treatment was not responsible for the majority of the efficacy of one or both products? This practice has made the whole study potentially meaningless from the point of view of claiming efficacy for Nyda express.

Answer:

The products were administered by the study personnel according to the instructions for use of the marketed/certified products. As both products recommend combing according to the recommendation of the Robert-Koch Institute, combs were provided and the use was documented. To evaluate, whether combing had an impact on efficacy, a post-hoc analysis was performed, which is described in the manuscript. There was no correlation observed between combing/no combing and efficacy/treatment failures.

Results

Pediculicidal activity in vitro - Ovicidal activity in vitro
Why are no data shown for lice treated using the permethrin product? Would it not be better to show numerical data for both lice and their eggs in tables rather than is these somewhat facile bar charts?

Answer:

Assessment of permethrin resistance was not an issue under scrutiny. For the in vitro experiments with eggs from permethrin-resistant lice, the permethrin-product was used as internal control for the status of resistance of the strain used. Within the experiments with body lice, no permethrin product was tested. We understand that the figure showing the “efficacy” of Nix® for eggs might be misleading. We have now omitted the Figure and amended the manuscript text likewise.

Clinical trial

Participants and baseline demographic data

There are inconsistencies of GCP and accounting in this section, lines 403- 411. In line 406 the withdrawal was obviously a "Lost to Follow Up" and should be included in the final FAS (Intention to Treat) analysis as a treatment failure. The patient suffering the SAE should have been automatically withdrawn from any further participation and also recorded in the FAS as a treatment failure. The two non-compliant participants in the comparison group should also be included in the FAS analysis as treatment failures. So Table 3 requires correction accordingly, as does the associated statistical analysis.

Was the SAE that was initially suspected as being possibly due to use of the treatment product reported to the appropriate ethics committee and the regulatory authority, or even reported as a SUSAR, in line with the requirements of GCP? If so, this should be recorded in the manuscript and if not a good explanation of why it was not done should be provided.

Answer:

We do not agree with this point. There are no GCP inconsistencies concerning the reported clinical trial. During the blinded data review meeting, all missing visits were discussed and patients assigned to the populations to be evaluated before unblinding of the data. A withdrawal is not necessarily a treatment failure, therefore all patients with missing visits were assigned to the safety population and to the full analysis set (FAS), but were not included in the per protocol set. This is a common procedure in clinical trials.

Of the 4 patients with discontinued intervention, 2 patients from the reference group were actively excluded from the trial for non-compliance, as they used additional head lice therapy (“just to be on the safe side”), although efficacy evaluation at V2 revealed no lice. One patient in the test group withdraw consent without stating any reason (could be treatment success as well), and the patient that experienced the SAE participated in V4 on a voluntarily basis for final evaluation of infestation. The SAE was finally evaluated to be unrelated to the treatment, and all
reporting duties were fulfilled in time and in line with GCP-requirements and respective guidelines.

Lines 455-461: There is a considerable misunderstanding of louse biology in this paragraph. In lines 458-459 it states that the development of a N2-nymph from an egg between day1 (V2) and day 7 (V3) is not possible. However, if an egg were to be present on day 1 that was close to hatching the emergent nymph would have more than enough time to grow to stage 2 or even possibly stage 3 by day 7, depending upon its gender and nutritional uptake. In addition, stage 2 nymphs do not normally migrate from one host to another, this being mostly confined to stage 3 nymphs and adults because they are close to, or actually, reproductive. Young nymphs stay put and spend their time feeding and growing. Consequently, this nymph indicated lack of activity of the treatment irrespective of the status of the adult louse found at the same time.

Answer:

We have amended the respective paragraph accordingly.

Global tolerability

Please explain what "global tolerability" is. Does it mean the respondents were just happy to get rid of their lice so they would tolerate using it again? Given the number of AEs it is surprising that so many thought either of the products was "good" or "very good" so the concept requires elucidation as the terminology is vague and "woolly".

Answer:

“Global tolerability” is a common assessment within RCTs, especially with products affecting the appearance of patients. In contrast to more specific terms like “skin tolerability” or “local tolerance” the overall tolerability is documented, and a rather poor assessment of the global tolerability by the patients was further assessed by the principle investigator and documented as AE, if evaluated to be appropriate.

Discussion

Line 536: The percentage cure rate requires clarification in line with any reanalyses resulting from addressing the points about lines 403-411.

Answer:

In our opinion, no reanalyses are needed, as the assignment of patients into the populations evaluated was correct.
Questions about nit-combing and the efficacy of the Nyda product.

Answer:

As described within the paragraph “Sample size” of the manuscript, efficacy of the reference product was assessed on basis of available literature. The pre-defined minimum rate for efficacy (70%) was determined from reported cure rates for permethrin-containing products (range from 34.8% to 98.0%, mean 66.6%, approximated to a 70.0% cure rate) and was therefore selected to be the minimal acceptable cure rate 1-4. As the publications of the trials conducted in Germany involving permethrin-based products were from 2002 and 2005 and as the current status of resistance of German head lice populations to permethrin is not known, efficacy rates for permethrin-based products from other trials conducted in the EU were considered. Impact of combing was discussed in detail, as described above.

Questions about use of physically acting product like dimeticone in Germany has "diluted out" the level of resistance to pyrethroids? The fact that at least two pyrethroid-based products remain on the market under Section 18 of the German Infection Protection Act suggests that use of pyrethroids has not been eliminated and so some continued selection pressure remains on the louse population.

Answer:

As dimeticone-based products dominate the German market, we discussed that the preferred use of these products has likely decreased the selection pressure on head louse populations, thereby reducing the level of resistance in local populations. In fact, in the year of the launch of NYDA in Germany, treatments with a neurotoxin-based mode of action showed a market share of 71.1%. The current situation shows a decrease in market shares of these products to 7.1%, whereas pediculicides with a physically mode of action present a market share of 88.5% (Reference: 2006 NPI, Insight Health/ 2019 Pharmatrend national, IQVIA). Decreasing selection pressure may be a process with locally limited impact, and as a former head lice study also conducted in Germany produced an unexpected high efficacy for Goldgeist® forte (pyrethrum-based product), this local population may have a relatively low level of resistance.

Questions about repetition in this paragraph that needs editing out.

Answer:

OK, corrected.
Line 603: The Strycharz publication is incorrectly cited and should be Strycharz et al. (2012) not (2002).

Answer:
OK, corrected.

Line 645: The wording "..the only exclusion criterion that lead to non-inclusion was the absence of active infestation." is incorrect. It should read "..the only exclusion criterion that led to non-inclusion was the absence of active infestation."

Answer:
OK, corrected.

References

There are several typographical errors and transcription errors in the reference list. These should be checked thoroughly and corrected.

Answer:
We have revised the reference considering these comments.

Reviewer #2: (Michael Marks)

There is a clear need for new treatments for head lice and this paper is therefore timely.

1) I was somewhat surprised that there was no mention of either topical or systemic ivermectin in the paper. Why was this omitted?

Answer:
As ivermectin is not authorized for the treatment of head lice infestation in Germany, we felt that mentioning this therapy would not add valuable information to the manuscript.

2) I would urge caution in Lines 79-89, whilst bacteria have been isolated from head lice in some studies this does not yet prove that they do in fact act as vectors for these agents.

Answer:
We have omitted the discussion of head lice as vectors. Please also see our answers to comments of reviewer #1.

3) Why were different concentrations of permethrin used for the in-vivo and in-vitro studies?

Answer:

We are sorry that the way we presented our pre-clinical data was obviously confusing. Within the preclinical studies, which were partly performed by using a permethrin-resistant louse strain, permethrin was only used as internal control to confirm status of resistance of the lice used. In contrast, for the clinical study, the permethrin product was used as active comparator. Therefore, a product was used which is available on the German market, reimbursable and recommended by the Federal Office of Consumer Protection and Food Safety. We have revised the manuscript accordingly. Please also see our answers to comments of reviewer #1.

4) How was the sample size of the in-vivo study determined?

Answer:

Sample size estimation is explained in detail in the paragraph “Sample size” of the manuscript (lines 301-309). The design of the trial including the calculation of number of subjects was adapted from a previous trial performed with a mineral oil product 5.

According to preclinical studies with the test product and to preclinical and clinical studies with other NYDA® products, a 90% cure rate was expected for the study medication. In general, a cure rate of 70% was assumed as minimal accepted cure rate. The primary objective referred to superiority of cure rate vs. this limit.

5) Given that both treatments achieved a cure of 100% I am bit confused as to what a non-inferiority analysis is meant to achieve? Can the authors explain? Given identical outcomes a non-inferiority assessment essentaily is just dependent on the sample size (and therefore the width of the CI). I am not sure what this adds.

Answer:

The design of this study was based on a former clinical trial, conducted in Germany. For regulatory purposes we decided to evaluate non-inferiority, as has been performed within the clinical trial this study refers to, although this seems to be redundant, with both products achieving a cure rate of 100% after 2 treatments. The objective was defined during trial registration and in the statistical analysis plan, and was not changed during the course of the study.
6) Line 429 - the distribution of the number of lice per person appears nonnormally distributed (mean 1.7 ±2, range 0-13) - it might be more appropriate to give the median and IQR. The same is true with regard to nymphal instars in table 2.

Answer:

Median of adult lice and all nymphal instars is already stated within the manuscript. We added Q1 and Q3 as a measure of dispersion to the table as suggested.

7) What is meant by a serious adverse device effect? is this a typo? Or you mean this was related to the treatment? If so by what mechanism??

Answer:

No, this is not a typo. The SAE was initially evaluated by the principle investigator as product-related and reported according to the wording appropriate for medical devices (that would be a “serious adverse device effect”). More detailed evaluation also taking into account the temporal context of the event and administration of the product resulted in the assessment “not product-related”.

References mentioned:

1  Bialek, R. Studie zur Therapie der Pediculosis capitis (Kopflausbefall) mit 0, 5% Permethrin. Kinder-und Jugendarzt 36, 197-202 (2005).


