Author's response to reviews

Title: Safety, tolerability, and impact on allergic inflammation of autologous E.coli autovaccine in the treatment of house dust mite asthma - a prospective open clinical trial

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Version: 2 Date: 13 January 2011

Author's response to reviews: see over
January 13 2010

Dear Dr. Talbot, dear Mrs. Aguera,

Thank you for giving us the chance to improve our manuscript and to share our observations with your readership.

We followed the fruitful suggestions of your reviewers and changed our manuscript in a point-by-point response to the concerns accordingly. The name of the ethical committee that gave approval for the research has been added and the conflict of interest statement has been removed from its current position on the title page to the end of the manuscript before the Reference list. As already the first version mentioned, the study was partly funded by Symbiopharm Inc.

We do appreciate all your efforts and hope that our revised version will fulfil your expectations.

Kind regards,

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ass. Professor of Paediatrics
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Dear referee No.2

Thank you so much for your friendly and constructive comments, which really helped us in improving our manuscript. We changed our manuscript accordingly: included please find two versions: one version indicating all the changes we’ve made (“with changes”), and one clear version facilitating reading.
Ref. No.2
1. Background last paragraph: the authors state that bacterial autovaccines have been used worldwide, but give only 2 references
MR: this is true – we added other references which were encompassed anyway

Ref. No.2
2. in Methods are so many parameters like body temperature or laboratory testing, described, which were measured and these parameters were not mentioned in the results, only shortly in the abstract
MR thank you for pointing out this important aspect. To provide more information, we added several tables as to clinical and laboratory safety and tolerability

Ref. No.2
3. References are missing: in discussion paragraph 4 the reference for the mouse study is missing, reference 14 is a human study, reference 15 is not osteomyelitis or furunculosis, but polypus ethmoiditis
MR we must deeply apologize. The data from the mice study are unpublished data – we’ve corrected this error. The former reference No.14 was on the immunology of allergen specific immunotherapy and doesn’t belong here. Reference 15 indeed concerns polyposis ethmoidalis; we modified the wording accordingly: “Established indications are chronic infections of the airways and associated sinuses, the skin (furunculosis), and the bones (osteomyelitis) [e.g., 15].”

Ref. No.2
4. reference 20 in references: specific should be specific
MR: sorry – we corrected also this

Ref. No.2
Discretionary Revisions:
1. It would have been nice to have more patients to have good statistics
MR there is much truth about this – and a larger placebo controlled study is on its way.

Ref. No.2
2. Results, local reactions should be better summarized in a table to have a better overview
MR yes indeed – the new manuscript shortened the text on local reactions and provides a table instead. We also modified the title, since our work does not really show an impact on clinical aspects (“efficacy”), but on markers of allergic inflammation. I think, now we are even closer to the truth.

Thank you for giving us your time and competence! We hope that the revised version fulfils your expectations.

Kindest regards, Markus A. Rose
**Dear referee No. 3,**

Thank you so much for your friendly and constructive comments, which really helped us in improving our manuscript. We changed our manuscript according your fruitful criticism; included please find two versions: one version indicating all the changes we’ve made (“with changes”), and one clear version facilitating reading.

**Ref. No.3**

The study and its report have a number of limitations that reduce the significance of its contribution to the literature. Most seriously, no control group data are available. It is therefore not clear whether the finding of a reduction in eNO response to house dust mite challenge was due to nonspecific factors, such as seasonal variation, greater medication adherence, or regression to the mean.

**MR** we share your concerns. Nonetheless, this study’s main goal was the safety and tolerability of autologous E.coli autovaccine. A bigger placebo controlled trial has been performed in the meantime. The governmental regulatory institutions wanted us to prove the safety in a detailed way before we could start with the main trial.

Taking up your criticism, we’ve even changed the title. It’s not "efficacy" that is primarily addressed, but safety and tolerability. The impact on allergic inflammation is an interesting side aspect, but – in the absence of a control group - a presentation of data on medication use and clinical effects would have been doubtful indeed.

**Ref. No.3**

**Major Compulsory Revisions**

1. The asthma patients are poorly characterized. Standards such as basal lung function, symptoms, medication, or disease history are not provided. Also, some of the GINA characterizations on p. 4 are cryptic.

**MR** you are right – we’ve added these important patient characteristics to table 1

**Ref. No.3**

2. Similarly, the documentation of asthma control and manifestations across therapy are insufficient. No information is provided on lung function, symptoms, daily activity limitations, adverse events, bronchodilator, and other medication use. Although much is made out of the Th1-Th2 switch as a rationale for this therapy, no markers of that are included in the outcome measures.

**MR** see above; now we’ve summarized these important features in the revised manuscript.

**Ref. No.3**

3. The Methods section is largely unstructured and does not distinguish between patient characterization, study design, instruments, assessments, therapy materials, and procedures. Figure 1 is also not well explained in that context and it takes a long tome to figure out that the protocol included at least 37 visits (or 41?). The rationale for a number of tests and their timing is also not provided. It is particularly strange that blood test were only taken at visit 1, 3, and 5.

**MR** we must apologize for this weak section. The revised version and figure 1 clearer point out this important background information. The protocol indeed encompassed 37 visits (41
was a former version). Blood tests on safety were performed on each visit; the wrongly mentioned blood tests on visit 1/3/5 were for special immunological features.

Ref. No.3
4. Given the very demanding protocol, it is surprising that no information on adherence is provided. Did patients come to all sessions? Also, reasons for exclusion of 6 patients after screening are not provided.

MR This is true, and we think that the impressive adherence goes with the gender distribution of our study population (mainly women ☺). We added this meaningful information on adherence and exclusion of the positively screened subjects.

Ref. No.3
5. The form in which the results are reported is suboptimal. The findings on local reactions could be presented in an overview table. It is also surprising that significance tests (which statistical tests?) are reported for eNO data, when the statistical analysis paragraph argues against inferential statistics in this small sample.

MR Yes indeed – the new manuscript shortened the text on local reactions and provides a table instead. The main statistics followed a purely descriptive approach. The secondary outcomes eNO and markers of allergy were analysed by inferential statistics - we hope that our discussion expresses the known weakness of small numbers and expresses our findings conservatively enough.

Ref. No.3
6. The information on guidelines and ethics approval is misplaced in the statistics section, should be part of the section on participants.

MR You are perfectly right. We've corrected also this.

Ref. No.3
7. With the exclusion criteria in mind the authors should clarify whether the treatment is only suitable for a very small, low severity subsample of the asthma patients.

MR The in- and exclusion criteria were meant to design a clearly defined study group for a therapy that has never been examined systematically. We also wanted to avoid an undertreatment of severely ill asthmatics – as it can be sometimes observed, when “alternative healers” are at work. We must admit that the efficacy of autovaccine treatment cannot really be judged from the data of our study.

All in all, I think with the revised version we are much closer to the truth.

Thank you for giving us your time and competence! We hope that the revised version fulfils your expectations.

Kindest regards, Markus A. Rose