Author's response to reviews

Title: MIA is a potential biomarker for tumor load in neurofibromatosis type 1

Authors:

Mateusz Kolanczyk (kolanshy@molgen.mpg.de)
Victor Mautner (v.mautner@uke.uni-hamburg.de)
Nadine Kossler (kossler@molgen.mpg.de)
Rosa Nguyen (rosi.nguyen@gmail.com)
Jirko Kühnisch (jirko.kuehnisch@charite.de)
Tomasz Zemojtel (zemojtel@molgen.mpg.de)
Aleksander Jamsheer (jamsheer@wp.pl)
Eike Wegener (wegener.eike@googlemail.com)
Boris Thurisch (mail@boris-thurisch.de)
Sigrid Tinschert (Sigrid.Tinschert@tu-dresden.de)
Nikola Holtkamp (nikola.holtkamp@charite.de)
Su-Jin Park (su-jin.park@charite.de)
Patricia Birch (birch@interchange.ubc.ca)
David Kendler (kendler@ca.inter.net)
Anja Harder (anja.harder@ukmuenster.de)
Stefan Mundlos (stefan.mundlos@charite.de)
Lan Kluwe (kluwe@uke.de)

Version: 8 Date: 4 April 2011

Author's response to reviews: see over
Dear Dr. Mick Aulakh,

We are glad to hear that of manuscript: “MIA is a potential biomarker for tumor load in neurofibromatosis type 1.” MS: 1924513230504997 was now accepted in principle for the publication in journal BMC medicine.

Here we address reviewer 3 comments in the “point by point response”.

The manuscript was now amended so that the methods section follows the background section, and appears before the results section as required.

Point-by-point response to comments of the reviewers
(the original comments are in black and our response in blue)

1. This may just be a documents conversion problem, but some of the p-values reported in the section “Serum concentration of MIA in NF1 patients correlates with tumor load” (p. 9) contained commas rather than decimal points.

Comas were now replaced with decimal points.

2. Also in paragraph 3 (p. 10), the author mentioned testing the differences in MIA serum level between the groups with very low and low tumor loads excluding the outliers. It was a bit unclear if the authors simply re-ran the t-test after removing the outliers or used other procedures. It is important to note that using the t-test after the removal of outliers is technically inappropriate because the standard error estimate will be incorrect. For robust comparisons between independent groups, the authors may consider using Yuen’s test for comparing trimmed means.

We thank reviewer for this important comment. Indeed we recognized that removing outliers should be avoided. Since correlation between internal tumor load and MIA is obvious with outliers, we decided to remove that type of analysis from the manuscript as it is not crucial to the conclusion. We introduced following changes in results:

“...The patients were divided into four groups with very low (0-100 ml; n=16), low (< 350 ml; n=5), moderate (< 1000 ml; n=5) and high (> 1000 ml; n=4) internal tumor loads (Figure 3E, left). One-way ANOVA with Bonferroni’s multiple comparison test revealed significant differences between MIA serum levels in patients having very low internal tumor load and groups with high and very high internal tumor...
loads \( p<0.01 - **; p<0.001 - *** \). Also linear regression analysis revealed association between the total internal tumor load and MIA serum level \( (\text{P-value of } 1.95\times10^{-7} \text{ for the F test}) \). The line that best predicts MIA level from logarithm of total internal tumor volume defined an \( R^2 \) square of \( \sim0.64 \) (Figure 3E, right). This data indicate that elevated MIA serum level may be informative of increased internal tumor burden. Since we observed an association between total internal tumor load and number of subcutaneous tumors, a study involving a larger cohort size will be necessary to reveal relative contributions of internal, subcutaneous and possibly also cutaneous tumors to elevated MIA levels...."

Discretionary Revisions

1. In paragraph two of the same section (p. 9), the authors mentioned that MIA serum concentration was significantly higher in NF1 patients and a \( p \)-value \( (P < 0.001) \) was reported. It may be useful to specify what statistical test was being used. Was it a two-sample t-test? Moreover, as the authors pointed out, there were quite a few outliers and a bit of heterogeneity among the groups. Did the authors take that into account by using t-test with unequal variances (i.e. Welch’s test)?

Unpaired T-test with Welch’s correction was applied.

"...MIA serum concentration was independent of age and sex and (data not shown), but significantly higher in NF1 patients than in healthy controls: 15.16 ± 1.26 pg/ml vs. 4.54 ± 0.40 pg/ml \( (P<0.001 - \text{unpaired T-test with Welch’s correction, Figure 3A})..."\n
2. In paragraph 3 of the same section (p. 9), the authors reported on the ANOVA and multiple comparisons results. It may be helpful for the authors to clarify how many individual tests were being conducted as that would indicate the Bonferroni adjustment being made. Specifically, were the comparisons being done on all pairs of groups?

The comparisons were done on all pairs of groups. That was now clearly stated:

"...One-way ANOVA with Bonferroni’s multiple comparison test was done for all pairs of groups which revealed significant differences between MIA serum levels in patients having very low internal tumor load and groups with high and very high internal tumor loads \( (p<0.01 - **; p<0.001- ***)..."\n
As a minor technical note, ANOVA does not necessary have to precede multiple comparisons. Therefore, reporting on the multiple comparison results shall suffice.

We thank reviewer for this advice

3. The authors presented linear regression results on the association between the log of total internal tumor volume and MIA serum level. Have the authors considered showing the relationship in the original unit (i.e. without logarithm). That will more directly unveil whether or not the increase in MIA serum level was a linear function of total internal tumor volume.

We have considered showing the relationship in the original unit. However, as the internal tumor load volume covers a large range of values (i.e. 4-15,000), we chose to derive the log of total
internal tumor volume. The latter allowed us to more clearly illustrate (in the figure) that the increase in MIA serum level was a linear function of total internal tumor volume.