Completed assessment forms for papers for which the Panel has found suspicion of potentially intentional scientific dishonesty of Dr. Penkowa

As indicated in the investigation report, the Panel has found suspicion of potentially intentional scientific dishonesty of Dr. Penkowa for the following 15 papers:


The Panel’s specific assessments of these papers are indicated in the forms in this annex, including the bibliographic data for the papers.
Appendix number of the paper: 3;
Altered central nervous system cytokine-growth factor expression profiles and angiogenesis in metallothionein-I+II deficient mice.

A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?
If no, please explain why scientific dishonesty of MP is not suspected for this paper.
If yes:
- Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
- Please explain why this activity may be suspected for scientific dishonesty.

According to the comments by Hidalgo the animal experiments have been performed in Barcelona. In Material and Methods some animal experiments are related to the University of Copenhagen. They may be those, published earlier and summarized in the first section of the results on background of the experiments. Overall the results from immunocytochemistry are confirmed by independent experiments done in Barcelona using ISH and PCR. Thus, the overall message of this paper seems to be correct.
The immunocytochemical data are in part problematic. This mainly relates to the data on cytokine expression. Here most results are just given as quantitative data in Figure 4. Photographic documentation is only available for Il6, bFGF, NT3 and VEGF. Considering the difficulties regarding immunocytochemistry for cytokines, one has reservations regarding the validity of the cytochemical data. Extensive controls are described in material and method, including pre-absorption.
Quantitative data were obtained from 3 mice per group. ANOVA as well as t-test. Surprisingly low standard deviations.

B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?
If yes, please write the name(s) of these co-author(s) and specify the information needed.

C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?
If yes, please specify which material should be required and perhaps how it should be examined by Panel.

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.

E. Any other comments?
One could also use this paper for analysing the procedure of handling of pathological material sent into the laboratory of Dr. Penkowa from Barcelona. Validity of immunocytochemistry and quantitative data should be checked.
Appendix number of the paper: 5
Altered inflammatory response and increased neurodegeneration in metallothionein I+II deficient mice during experimental autoimmune encephalomyelitis.

A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?
If no, please explain why scientific dishonesty of MP is not suspected for this paper.
If yes:
-Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
-Please explain why this activity may be suspected for scientific dishonesty.

The experiments are well designed, adequate numbers of mice were used. Clinical scores of EAE are lacking except one pooled data set on average and maximum scores in table 1, therefore it is hard to assess the quality of the EAE experiments in detail, but no extreme conclusions were drawn based on these EAE data. It is not clear what the score was at day 37, the day of sacrifice and sample collection. The variability in scores is quite big given the SDs. There is no ground for suspicion in these EAE data.
Animal experiments were performed elsewhere; tissues were analyzed at the lab of Dr. Penkowa. A striking accuracy is obtained in the quantification of the immunohistochemistry data (e.g. table 2: 278 ± 1 lectin positive cells per 1 mm as an average of 3 sections of 3 mice reveals a strange lack of variability). For all mice in one experiment (or for 3 mice per group?) 3 sections of brain stem, spinal cord and cerebellum were counted, cell by cell. It is not clear if 30 μm (M&M) of 3 μm thickness (legends table 2) was used.
The number and variety of funding sources is larger than would be expected for such a study, and includes unrelated funding sources like diabetes.

B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?
If yes, please write the name(s) of these co-author(s) and specify the information needed.
It would be good to have additional information on the procedure of the cell counting e.g. by interviewing Dr. Penkowa and/or the acknowledged technical assistants.

C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?
If yes, please specify which material should be required and perhaps how it should be examined by Panel.

The EAE data (disease course, difference in severity at the day of sacrifice etc.) would allow assessment of the quality of the EAE experiments.

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.
Approval of the local animal committee for the experiments would be valuable

E. Any other comments?

F. Additional assessments, based on analysis of found material (stained sections etc.)

For paper 5 (and 63), Slide boxes studied:
- MTKO-I+II m EAE, Lectin 20/11 + 27/12
- MTKO-I+II m EAE, Rab GFA, GFA
- MTKO-I+II m EAE, CD3, TNF-alpha
- MTKO-I+II m EAE, SMT IV, Hidalgo
- MTKO-I+II m EAE, TUNEL
- MTKO-I+II m EAE, ssDNA, IL6
- MTKO-I+II m EAE, SMT I+II, HE
- MTKO-I+II m EAE, NITT

General conclusion:
No HE or IHC evidence for EAE was present in the sections that I have examined, no slide similar to what was presented in figure 1 was found in the concerning slide box (relevant for figure 1)
IHC for CD3 and IL6 revealed no positive cells, IHC for TNF was atypical (relevant for figures 2 and 3)
Appendix number of the paper: 11;
Astrocyte-targeted expression of IL-6 protects the CNS against a focal brain injury.

A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?
If no, please explain why scientific dishonesty of MP is not suspected for this paper.
If yes:
-Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
-Please explain why this activity may be suspected for scientific dishonesty.

Animal experiments were done in Barcelona; Pathology and immunocytochemistry by Dr. Penkowa; Study shows that targeted expression of Il6 in astrocytes speeds up wound healing in cortical cryolesion.
Extensive photo-documentation, clearly showing the differences; immunofluorescence OK but less convincing;
Fig 3 is rather surprising – practically no lesion in the cortex after 20 days, despite extensive lesion at days 3 and 6 in Il6/GFAP animals; Nevertheless, the result could be explained by the decreased cell death shown in Fig 11.
Technical support by technicians from Panum is acknowledged;
Quantitative data: 3 mice per group; Anova; surprisingly low standard deviations;
Extensive immunocytochemistry controls are reported in material and method.

B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?
If yes, please write the name(s) of these co-author(s) and specify the information needed.

C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?
If yes, please specify which material should be required and perhaps how it should be examined by Panel.

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.

E. Any other comments?
Check quantitative data and immunocytochemistry controls.
Astrocyte-targeted expression of interleukin-6 protects the central nervous system during neuroglial degeneration induced by 6-aminonicotinamide.


A. Do you suspect any scientific dishonesty in MP's scientific contribution to these papers? If no, please explain why scientific dishonesty of MP is not suspected for this paper.

If yes:
- Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
- Please explain why this activity may be suspected for scientific dishonesty.

Animal experiments were done in Barcelona; Very extensive pathological analysis including immunocytochemistry for many inflammatory markers and cytokines done by Dr. Penkowa; The description of the results is consistent and the photo-documentation matches the quantitative results. Quantification is based on a very small sample size (3 animals per group!!) and shows very unusual homogeneity in the counts in individual groups. Technical help with handling the material and immunocytochemistry is acknowledged and Hanne Hadberg is co-author in this study, suggesting a major input by her technical work.

B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty? If yes, please write the name(s) of these co-author(s) and specify the information needed.

C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty? If yes, please specify which material should be required and perhaps how it should be examined by Panel.

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper? If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.

E. Any other comments?

This study is a prime candidate for further analysis of handling of the material, for immunocytochemistry and for evaluation of the sections regarding the Barcelona connection. The reason is that it contains by far the most extensive pathological and immunocytochemical analysis, and contains a lot of data dealing with immunocytochemistry with potentially dangerous markers (such as cytokines). Quantitative data should be evaluated.

F. Additional assessments, based on analysis of found material (stained sections etc.)

Astrocyte-targeted expression of interleukin 6 protects the central nervous system during neuroglial degeneration induced by 6-aminonicotinamide

We identified the relevant sections for this experiment in the boxes labelled with the key AA.
Apparently the material, sent in from Barcelona has been properly processed and cut. In this box respective sections were present stained for H&E, GFAP, Lectin, Timm’s, Perl’s, Neurotang, MBP, CD3, OHdG, Cu/Zn-SOD, F4/80, CNPase, NG2, Myeloperoxidase, NIH. It was also possible to annotate the individual sections to the respective animals in the different experimental groups. The sections stained with H&E, GFAP and Lectin reflected the patterns of changes, described in the manuscript and shown in the illustrations. This was different for other markers, such as CD3 staining, which was done only in ten of the twelve sections of this experiment. In addition the CD3 staining was not convincing and the sections did not match that what was shown in the respective figures.

The study also includes a large number of additional data on the expression of cytokines, molecules reflecting oxidative damage, MT 1&2 and on DNA-fragmentation. Here it was not possible to find the respective sections of this particular experiment. Detailed analysis of the immunocytochemical reactivity of the respective markers in sections from other experiments of Il6 transgenic animals treated with 6-AN or with cortical (cryo?) lesions (Boxes labelled with keys AE, SA, SB, V, X, Y) revealed a number of problems, such as the lack of specific immunoreactivity (CD4, CD3), staining, patterns which were different from those described in the papers (TNF-a, TGF-ß, NT3), or staining patterns, which were different from those, expected from the published knowledge (e.g. NITT).

Quantitative data: The manuscript contains extensive quantitative data, based on only 3 animals per experimental group. The data show a homogeneity of the values within each experimental group, which is very unusual for quantitative cell counts in in vivo experiments. From those sections with reliable immunocytochemical stains, it becomes clear that these very homogeneous data were obtained by a bias in the selection of the areas, which have been chosen for cell counting. In those sections, where immunocytochemical staining was unclear (see above), it is not clear, how the quantitative values could have been obtained at all.

Problems identified:
Control for the specificity of immunocytochemical stainings is not clear. The manuscript describes that controls with omission of primary antibodies have been made, but we found no sections in the material, which showed that. Furthermore, according to the technician, who had been instrumental in the staining (Hanne Hadberg, see acknowledgements), to the best of her memory such controls have not been performed. The pattern of staining for some of the markers show profound serum immunoreactivity, indicating non specific immunoglobulin staining by secondary antibodies, a problem which should have been identified in respective immunocytochemistry controls.

The quantitative data are highly problematic due to a bias in the selection of areas, which were evaluated. In addition, for many of the markers the staining quality appears to be insufficient for quantitative analysis.

For some of the markers used (e.g. CD3) the staining pattern in the section does not reflect, what is shown in the figures.
Appendix number of the paper: 57;
Impaired inflammatory response and increased oxidative stress and neurodegeneration after brain injury in interleukin-6-deficient mice.
Penkowa M, Giralt M, Carrasco J, Hadberg H, Hidalgo J.

A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?
If no, please explain why scientific dishonesty of MP is not suspected for this paper.
If yes:
-Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
-Please explain why this activity may be suspected for scientific dishonesty.
Extension of a previous study on cryolesions in Il6 deficient animals; Experiments done in Barcelona; Immunocytochemistry is in line with that seen in previous papers; results are described in a consistent manner; but not much new.
Technical aspects and staining of the sections is confirmed by Hanne Hadberg.
Quantitative data: 9 representative animals?

B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?
If yes, please write the name(s) of these co-author(s) and specify the information needed.

C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?
If yes, please specify which material should be required and perhaps how it should be examined by Panel.

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.
Quantitative data should be checked.

E. Any other comments?
Relatively minor extension of a previous paper
Appendix number of the paper: 65
Interferon-gamma regulates oxidative stress during experimental autoimmune encephalomyelitis.

A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?
If no, please explain why scientific dishonesty of MP is not suspected for this paper.
If yes:
- Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
- Please explain why this activity may be suspected for scientific dishonesty.

EAE was induced in 129/Sv H-2b mice with a disrupted IFNγ receptor, like in paper A23 and A138, but new EAE experiments were performed for this paper, given the group size and EAE results. In the IFNγ receptor KO, a more severe EAE was found, consistent with previous findings. In fact the mortality (table 1) in the KO animals was 13 out of 14 (and in Wt 3 out of 14). Nevertheless 3 animals per group were evaluated. Results on their clinical scores or day of “sacrifice” are not included but at least 2 of the 3 KO’s must have been moribund or dead, at the moment of tissue sampling. The animals that did not die of EAE were sacrificed at day 28. Since this model does not seem to have a recovery phase, the exact time of tissue sampling may not be critical, but the condition of the animal at the time of tissue sampling may be important.
In table 2 and 3 cell counts are given in number per 0.5 mm$^2$ with a remarkable accuracy, e.g. 258.33 ± 3.22 CD14+ cells per 0.5 mm$^2$. Dr. Penkowa did the microscopic evaluation by herself. Photographs of the stainings that were counted are not included. Detailed immunocytochemistry controls are reported in material and methods.

B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?
If yes, please write the name(s) of these co-author(s) and specify the information needed.

C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?
If yes, please specify which material should be required and perhaps how it should be examined by Panel.
Once the counting has been shown to be possible for previous papers, there is no need, for this paper, to analyse extra material.

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.

E. Any other comments?
Gradually a pattern emerges of cell counts of large numbers of immunohistochemical stainings in large numbers of sections with a remarkable accuracy. All the counting was performed by one person, Dr. Penkowa. Sometimes in a blinded fashion.
The acknowledgement of the funding indicates that a large number of resources have contributed to the immunohistochemical analysis of tissues obtained from experiments performed elsewhere.
Appendix number of the paper: 67;
Interleukin-6 deficiency reduces the brain inflammatory response and increases oxidative stress and neurodegeneration after kainic acid-induced seizures.
Penkowa M, Molinero A, Carrasco J, Hidalgo J.

A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?
If no, please explain why scientific dishonesty of MP is not suspected for this paper.
If yes:
- Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
- Please explain why this activity may be suspected for scientific dishonesty.

Animal experiments were done in Barcelona; Major input by Dr. Penkowa with respect to immunocytochemistry and quantitative expression data. The description of the results is consistent and the quantitative data match the images, shown as photomicrographs; as it is described in the manuscript the reported data appear solid and well done;
Overall the study shows, in another model of neurodegeneration (KA induced seizures), similar results as reported in other papers by Dr. Penkowa in other conditions of neurodegeneration.
Quantitative data: n=4-5 mice; ANOVA; very low standard deviations;

B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?
If yes, please write the name(s) of these co-author(s) and specify the information needed.

C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?
If yes, please specify which material should be required and perhaps how it should be examined by Panel.

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.
Quantitative data should be checked.

E. Any other comments?
Appendix number of the paper: 76;
Metallothionein-1+2 deficiency increases brain pathology in transgenic mice with astrocyte-targeted expression of interleukin 6.

A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?  
If no, please explain why scientific dishonesty of MP is not suspected for this paper.  
If yes:  
- Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.  
- Please explain why this activity may be suspected for scientific dishonesty.  

Animal experiments have been done in Barcelona. Dr. Penkowa performed (quantitative) immunocytochemical analysis; the immunocytochemical data largely reflect the ISH data done in Barcelona with exception of GFAP expression in older animals (may be explained by protein vs mRNA expression); data are well described and the quantitative immunocytochemical results match the changes in the figures.  
Technicians, involved in immunocytochemistry are acknowledged.  
Quantitative data: no animal numbers given.  
Detailed immunocytochemistry controls described in material and methods;

B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?  
If yes, please write the name(s) of these co-author(s) and specify the information needed.

C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?  
If yes, please specify which material should be required and perhaps how it should be examined by Panel.

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?  
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.

E. Any other comments?
Appendix number of the paper: 81;
Metallothionein-I overexpression alters brain inflammation and stimulates brain repair in transgenic mice with astrocyte-targeted interleukin-6 expression.

A. Do you **suspect any scientific dishonesty** in MP’s scientific contribution to these papers? If no, please explain why scientific dishonesty of MP is not suspected for this paper. If yes:
-Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
-Please explain why this activity may be suspected for scientific dishonesty.
Another study showing a protective effect of MT-I overexpression in this case in Il6/GFAP transgenic animals; It is a follow up study of paper A11; In contrast to paper A11 in this paper there were still visible cryolesions on day 20 in GFAP/Il6 mice. As in other similar papers the animal numbers for quantitative analysis are very low (3animals/group). We have some doubts, whether this is sufficient to reach the conclusions (inclusively the statistics? Overall the statistical analysis is problematic not only in this, but also in the other comparable studies (Anova; not corrected for multiple testing); Otherwise the results match the documentation in the figures. Extensive immunocytochemistry controls described in material and methods;

B. Do you find need for requesting **further information** from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty? If yes, please write the name(s) of these co-author(s) and specify the information needed.

C. Do you find need for procuring any **scientific background material** (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty? If yes, please specify which material should be required and perhaps how it should be examined by Panel.

D. Do you find need for any **other further examination** and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper? If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.

E. Any other comments?
Appendix number of the paper: 84

Metallothionein-mediated antioxidant defense system and its response to exercise training are impaired in human type 2 diabetes.

A. Do you **suspect any scientific dishonesty** in MP’s scientific contribution to these papers? If no, please explain why scientific dishonesty of MP is not suspected for this paper.
If yes:
- Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
- Please explain why this activity may be suspected for scientific dishonesty.

The conclusions of this study are largely based on immunohistochemical stainings performed in Dr. Penkowa’s laboratory. The statement to the Panel by C. Scheede-Bergdahl contains some information about unusual handling of the slides. This paper is assessed to be highly problematic. First, there is no correlation between the mRNA data from muscle and the IHC: While there was no mRNA upregulation, IHC shows strong induction of metallothionein after exercise in the control group. Second, the micrographs in figure 3 supposedly show IHC with diaminobenzidine as chromogen. However, the reaction product in the cytoplasm in B, said to represent the immunostaining after exercise in control subjects, is not brown, as would be expected, but reddish, and nuclei are very dark here, whereas almost not seen in A (which shows a very weak but more selective staining of some but not all fibers). C and D, said to represent biopsies from diabetic patients are very pale all over. This figure is problematic because the colours do not match between the different panels, and the slides should definitely be examined.

Also Figure 4, showing IHC for NITT appears problematic. Panel B (representing control subjects after exercise) shows staining of all membranes, but nothing in cytoplasm, whereas Panel D (representing diabetic subjects after exercise) shows cytoplasmic staining with not very pronounced membrane staining. Also these slides should be examined.

The semi-quantitative assessment of the MT-I+II staining reported in Table 2 is unorthodox: Instead of showing the assessment for each one of the examined subjects, which would give the reader a chance to evaluate how reproducible the observations were, the authors chose to compile the data for each group into one single assessment. The raw data should be examined.

**B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?**
If yes, please write the name(s) of these co-author(s) and specify the information needed.
We should interview first author C. Scheede-Bergdahl about how the analysis and assessments were done, and to find out who were responsible for the compilation of the assessment, i.e. who broke the code for the different slides and put the results together.

**C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?**
If yes, please specify which material should be required and perhaps how it should be examined by Panel.
Yes, see above. The slides showing MT-I+II and NITT staining should be examined. The raw data on the assessment should also be examined.

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.
No

E. Any other comments?
This appears to be a problematic paper, and if possible the histological material, as well as laboratory notes on the assessment of the slides, should be examined.

F. Additional assessments, based on analysis of found material (stained sections etc.)

Celena Scheede-Bergdahl has sent slides. They include MT-staining from two healthy controls (PK and SB) before and after exercise, and from three diabetes patients (FM, EA, BH) before and after exercise. In addition there is one slide from a diabetic individual (FJ) before exercise, and from two (FA, BJ) after exercise. PK1 (healthy before exercise) shows weak sarcolemma staining with some fiber-selective cytoplasmic staining. PK2 (after exercise shows uniform cytoplasmic staining of an intensity that is similar to that of the more darkly stained fibers in PK1, and more pronounced sarcolemma staining. SB1 and SB2 show largely identical staining although part of SB1 is more lightly stained (uneven antibody exposure?). Both slides show weak cytoplasmic staining and more prominent sarcolemma staining as well as stained nuclei. Sections on both slides suffer from freezing artifacts. The micrograph depicted in Figure 1A-B (?) in the publication is not representative for the sections from muscle biopsies taken from the control subjects PK and SB, because pre and post-exercise for the healthy individual in that figure shows very large difference between the two conditions, with a very pale section before exercise and a very dark labeling of cytoplasm and nuclei after exercise. The sections from muscle biopsies taken from the diabetic patients show throughout pale labeling. Additional slides sent by CSB contain two series on which the glass has been broken to remove the annotations, and that hence are not identifiable. One row of slides shows sections with dense sarcolemma staining, whereas the other row show sections without such labeling, but on several of the slides the sections display severe freezing artifacts. Immunoblot on the muscle biopsies performed recently by F. Dela could not confirm the IHC. The discrepancy between what is seen in the microscope from the cases that were available and the illustration shown in the paper, especially on the difference before and after exercise in healthy individuals, taken together with the fact that the data have not been reproducible, raises strong suspicions that the micrographs have been edited to strongly enhance any difference in staining intensity that may have been observed in the slides, if the photomicrographs at all are representative for this material.
**Appendix number of the paper: 104**
Metallothionein prevents neurodegeneration and central nervous system cell death after treatment with gliotoxin 6-aminonicotinamide.
Penkowa M, Quintana A, Carrasco J, Giralt M, Molinero A, Hidalgo J.

A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?
If no, please explain why scientific dishonesty of MP is not suspected for this paper.
If yes:
- Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
- Please explain why this activity may be suspected for scientific dishonesty.
See suggestion under C below

B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?
If yes, please write the name(s) of these co-author(s) and specify the information needed.
No

C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?
If yes, please specify which material should be required and perhaps how it should be examined by Panel.
It is suggested that a selection of slides are checked that were immunostained for molecules that are not illustrated in the figures and for which cell counts performed by Dr. Penkowa are tabulated. This would be a selection from slides immunostained for IL-1, IL-6, IL-12, TGF-beta, NGF, NT3, MDA, 8-oxoguanine in sections from the various animals as listed in table 1

D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.
No

E. Any other comments?

F. Additional assessments, based on analysis of found material (stained sections etc.)
This is an extension study of paper A13, in which in addition the effect of transgenic overexpression of metallothionein on 6-AN lesions was analysed.
Examination of slides in boxes confirmed the existence of mouse immunostained sections. These were sections positively stained for IL-1-beta and TNF-alpha receptor in slide boxes labelled “Ø-dyr” but this appeared to be non-specific uptake of antibody by necrotic or damaged cells.
We found sections from a large number of animals, apparently reflecting the experimental groups described in the manuscript, which were dispersed in different slide boxes (see paper A13). Thus, the material sent in from Barcelona for further pathological analysis apparently have been
processed appropriately. The basic data, shown in H&E, MT1&2, GFAP and F4/80 stained sections, show what is described in the text and illustrated. In addition, the paper contains extensive quantitative data on the expression of inflammation associated markers. For those the same caveats, described before for paper A13 are valid and are here just given in the summary evaluation:
Problems identified:
Control for the specificity of immunocytochemical stainings is not clear. This manuscript describes in detail many more control experiments for immunocytochemistry, including controls with omission of primary antibodies, the use of normal rabbit, donkey and mouse serum as primary antibodies, of isotypic IgG instead of primary antibody, as well as preabsorption of primary antibodies with peptides. However, we found no sections in the material, which showed that. Furthermore, the technician, who had been instrumental in the staining (Hanne Hadberg, see acknowledgements), stated at a meeting with the Panel on 12 April 2012 that to the best of her memory such controls have not been performed. The pattern of staining for some of the markers show profound serum immunoreactivity, indicating non specific immunoglobulin staining by secondary antibodies.
The quantitative data are highly problematic, since for many of the markers the staining quality appears to be insufficient for quantitative analysis and evaluation of the sections by the panel raised doubts what was actually counted. An additional matter of concern is that the quantitative values in this paper are nearly the same (in absolute numbers) as those published in the paper A13, although they were generated in an independent experiment, performed in a different set of animals. Such a situation is highly improbable for in vivo experiments.
For some of the markers used (e.g. CD3) the staining pattern in the section does not reflect, what is shown in the figures.
Conclusion: The quantitative data are highly problematic. No control sections for the stains were found.
**Appendix number of the paper: 108**

Metallothionein treatment reduces proinflammatory cytokines IL-6 and TNF-alpha and apoptotic cell death during experimental autoimmune encephalomyelitis (EAE).

Penkowa M, Hidalgo J.


A. **Do you suspect any scientific dishonesty** in MP’s scientific contribution to these papers?  
   If no, please explain why scientific dishonesty of MP is not suspected for this paper.  
   If yes:  
   - Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.  
   - Please explain why this activity may be suspected for scientific dishonesty.

The EAE rats used in this study are the same as the ones used in paper A102 (Metallothionein I+II expression and their role in experimental autoimmune encephalomyelitis. Penkowa M, Hidalgo J. *Glia.* 2000 Dec;32(3):247-63.)  
No additional animals were used, for clinical EAE data this study refers to paper A102. Paper A102 has been reported by KU to the Danish Police in November 2010, and is thus not included in the Panel’s investigation.

B. **Do you find need for requesting further information** from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?  
   If yes, please write the name(s) of these co-author(s) and specify the information needed.

C. **Do you find need for procuring any scientific background material** (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?  
   If yes, please specify which material should be required and perhaps how it should be examined by Panel.

D. **Do you find need for any other further examination and/or documentation of possible scientific dishonesty** in any of MP’s scientific contributions to this paper?  
   If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.

E. **Any other comments?**

The animal tissues included in paper 108 (and 140) are derived from the experiment published in *GLIA* in 2000 (Paper A102, which has been reported to the Police by KU, and should not be investigated by the Panel).  
The Panel has therefore not assessed paper A108 (and A140) any further, and has not investigated the found materials related to these two papers, as the result of the Police investigation into paper A102 will apply to these two papers as well.
### Appendix number of the paper: 136
Time-course expression of CNS inflammatory, neurodegenerative tissue repair markers and metallothioneins during experimental autoimmune encephalomyelitis.
Espejo C, Penkowa M, Demestre M, Montalban X, Martínez-Cáceres EM.

**A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?**
If no, please explain why scientific dishonesty of MP is not suspected for this paper.
If yes:

- *Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.*
- *Please explain why this activity may be suspected for scientific dishonesty.*

EAE experiments were performed in Spain, with mice from Janvier, France (SJL/J), immunized with PLP 139-151. 5 mice were obtained at day 7 p.i. (score 0), day 13 p.i. (score 1), day 15-19 (score 2), day 22-24 (score 3-4), day 26-29 (score 2 recovery) and day 34 – 37 (score 0, full recovery) and 2 control groups, so 40 mice altogether. Sections of 5 mice were counted per time point for 18 markers and presented as mean ± SD per 0.5 mm². Dr. Penkowa performed the counting of cells by herself according to the additional information, at least 760 sections if one section per animal was counted. In figure 1, the GFAP staining pattern is shown. It is difficult to assess from these figures if indeed astrocytes can be counted cell by cell, in particular at the stages with the largest cell counts (197.4 cells per 0.5 mm² of brain stem). Similarly there are doubts as to whether cell counts for APP are reliable and feasible in high densities as 155.4 cells per 0.5 mm² of brain stem.

Demyelination is illustrated by MBP staining. These staining patterns differ from what have been described before.

**B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?**
If yes, please write the name(s) of these co-author(s) and specify the information needed.

**C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?**
If yes, please specify which material should be required and perhaps how it should be examined by Panel.

Viewing of the sections would help to judge if the cell counts are realistic

**D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?**
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.

**E. Any other comments?**

**F. Additional assessments, based on analysis of found material (stained sections etc.)**
This paper mentions in table 1 that for 5 animals of each of the score points (6) and 2 controls 18 stainings were counted, so \(40 \times 18 = 760\) slides would have at least been analyzed.

A key (HC) was found that may be associated with this paper, but no boxes were found with stained slides labelled HC.

Our conclusion on this paper is that we were not able to investigate the original material, and the identified problems remain unsolved.
**Appendix number of the paper: 138**

Treatment with anti-interferon-gamma monoclonal antibodies modifies experimental autoimmune encephalomyelitis in interferon-gamma receptor knockout mice.

Espejo C, Penkowa M, Sáez-Torres I, Xaus J, Celada A, Montalban X, Martínez-Cáceres EM.


### A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?

*If no, please explain why scientific dishonesty of MP is not suspected for this paper.*

*If yes:*

- **Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.**
- **Please explain why this activity may be suspected for scientific dishonesty.**

Animal EAE experiments are well designed; adequate, in fact small, numbers were used; EAE data are given in a table with numbers on incidence, mean day of onset, daily score and maximum score. The day of sacrifice was day 28, but in some groups there was a 100% mortality rate before day 28. Results differ from previous EAE experiments in IFNγR -/- mice presented in app 23 (higher mortality: 100% vs 75% in app 23), but this is within the range of variability that occurs in murine EAE experiments.

The immunohistochemistry data are presented as cell counts per 1.0 mm², with a remarkable accuracy. Three sections of three animals were counted for 8 immunohistochemical stainings. Of these stainings no photographs were included, so it is hard to assess if it is possible to count individual cells with the presented accuracy. Of the staining for which photographs were presented (figure 1, 2, 3 and 4), no counts were performed.

For the treatment of anti IFNγ of IFNγR -/- mice, immunohistochemistry reveals significant differences between treated and non treated mice. This could only be established in C57BL/6 x 129Sv IFNγR -/- mice lacking the cytoplasmic domain of the receptor, since the 129 Sv IFNγR -/- mice all died after EAE induction. In figure 1, the 129Sv wildtype is shown in the upper panels, and the C57BL/6 x 129Sv IFNγR -/- mice in the lower panels. This is not mentioned in the legends.

The iNOS staining is in ramified cells, while normally it is in round/oval cells. In figure 3D the TUNEL staining seems in the cytoplasm with a nucleus non-stained, rather than nuclear. But this is hard to assess in the available photograph.

No scale bars are given for the figures.

The number and variety of funding sources is remarkable. It is not clear if this study was in fact funded by all these sources. It is not clear if the funds are given for specific projects, for which a proposal was written.

### B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?

*If yes, please write the name(s) of these co-author(s) and specify the information needed.*

Dr. Penkowa, technical assistants and/or photographers (as mentioned in acknowledgements) may be able to provide insight as to how the immunohistochemistry was quantified.
C. Do you find need for procuring any **scientific background material** (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?
If yes, please specify which material should be required and perhaps how it should be examined by Panel.

D. Do you find need for any **other further examination** and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?
If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.
Sections may reveal the possibility to count cell by cell, the immunopositive cells.

E. Any **other comments**?
Dr. Penkowa had a major contribution to this paper; 4 figures and all quantification of IHC data

F. **Additional assessments, based on analysis of found material** *(stained sections etc.)*
Found slide boxes that may hold sections on which paper 138 may be based:
1. EAE/MS iILFN-rec-KO +/- Med.Beh. T-Dyr-Lektin, HE
2. EAE/MS iILFN-rec-KO +/- Med.Beh. T-Dyr-Lektin
3. EAE/MS iILFN-rec-KO +/- Med.Beh. T-Dyr. IL-6, CD3
4. EAE/MS iILFN-rec-KO +/- Med.Beh. T-Dyr-GFA
5. EAE/MS iILFN-rec-KO +/- Med.Beh. T-Dyr. Mn-SOD, Nitt
6. EAE/MS iILFN-rec-KO +/- Med beh. T-dyr, Hidalgo
(These boxes may also hold sections relevant for paper 23 and 65)

Overall conclusion after examination of the available material:
There were indeed tissue sections of all groups
Most of the IHC is of poor quality;
3 out of 4 figures could not be matched/checked with the available material.
### Appendix number of the paper: 140

Treatment with metallothionein prevents demyelination and axonal damage and increases oligodendrocyte precursors and tissue repair during experimental autoimmune encephalomyelitis. Penkowa M, Hidalgo J.


**A. Do you suspect any scientific dishonesty in MP’s scientific contribution to these papers?**

If no, please explain why scientific dishonesty of MP is not suspected for this paper.

If yes:
- Please specify the scientific work (e.g. experiments, sample collection, analyses, results) and/or parts of the paper which may be suspected for scientific dishonesty.
- Please explain why this activity may be suspected for scientific dishonesty.

The EAE rats used in this study are the same as the ones used in paper A102 (Metallothionein I+II expression and their role in experimental autoimmune encephalomyelitis. Penkowa M, Hidalgo J. *Glia.* 2000 Dec;32(3):247-63.)

No additional animals were used, for clinical EAE data this study refers to paper A102. Paper A102 has been reported by KU to the Danish Police in November 2010, and is thus not included in the Panel’s investigation.

**B. Do you find need for requesting further information from any of the co-authors – for the Panel’s further examination and/or documentation of suspicion or rejection of scientific dishonesty?**

If yes, please write the name(s) of these co-author(s) and specify the information needed.

**C. Do you find need for procuring any scientific background material (e.g. histological samples, images, data and lab records) – for the Panel’s further examination and/or documentation of suspicion of scientific dishonesty?**

If yes, please specify which material should be required and perhaps how it should be examined by Panel.

**D. Do you find need for any other further examination and/or documentation of possible scientific dishonesty in any of MP’s scientific contributions to this paper?**

If yes, please specify the scientific work and/or parts of the paper that should be further examined and perhaps how it should be examined.

**E. Any other comments?**

The animal tissues included in paper 140 (and 108) are derived from the experiment published in *GLIA* in 2000 (Paper A102, which has been reported to the Police by KU, and should not be investigated by the Panel).

The Panel has therefore not assessed paper A140 (and A108) any further, and has not investigated the found materials related to these two papers, as the result of the Police investigation into paper A102 will apply to these two papers as well.