EMBO Molecular Medicine

Active poly-GA vaccination prevents microglia activation and motor deficits in a *C9orf72* mouse model

Qihui Zhou, Nikola Mareljic, Meike Michaelsen, Samira Parhizkar, Steffanie Heindl, Brigitte Nuscher, Daniel Farny, Mareike Czuppa, Carina Schludi, Alexander Graf, Stefan Krebs, Helmut Blum, Regina Feederle, Stefan Roth, Christian Haass, Thomas Arzberger, Arthur Liesz, Dieter Edbauer

Review timeline:

Submission date:	21 May 2019
Editorial Decision:	19 June 2019
Revision received:	11 October 2019
Editorial Decision:	11 November 2019
Revision received:	29 November 2019
Accepted:	3 December 2019

Editor: Céline Carret

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 19 June 2019

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the three referees whom we asked to evaluate your manuscript.

As you will see from the reports below, the referees find the topic of your study of potential interest. However, they raise substantial concerns on your work, which should be convincingly addressed in a major revision of the present manuscript. We would encourage you to revise the figures (ref. 1 and 3) and strengthen your findings with additional experiments as recommended by ref. 2 and 3. Further, unbiased analyses (ref. 2) and a more detailed analysis of vaccination effect in vivo (ref. 3) would be nice additions.

We would therefore welcome the submission of a revised version within three months for further consideration and would like to encourage you to address all the criticisms raised as suggested to improve conclusiveness and clarity. Please note that EMBO Molecular Medicine strongly supports a single round of revision and that, as acceptance or rejection of the manuscript will depend on another round of review, your responses should be as complete as possible.

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

Please also contact us as soon as possible if similar work is published elsewhere. If other work is published, we may not be able to extend the revision period beyond three months.

Please read below for important editorial formatting and consult our author's guidelines for proper formatting of your revised article for EMBO Molecular Medicine.

I look forward to receiving your revised manuscript.

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

This study demonstrates that immunization of a transgenic mouse model expressing the most abundant DPR protein, with ovalbumin-coupled (GA)10, induced a high titer antibody response and resulted in a reduction in poly-GA aggregation, and reduced motor symptoms and microglial activation.
The overall approach is interesting and has clinical implications. Yet, there are some key issues in the data that require attention:
Results
1. In Figure 1c, the data presented in a way that not convincing.
2. Based on Figure 2, it is not clear how the authors concluded that the treatment prevented motor deficits.
3. Figure 3 shows only Iba1 expression as a basis for the claim of microglial activation. This is not sufficient, as Iba1 is not only marker of activated microglia, but also stains monocyte-derived macrophages. Also, the phenotype of the microglia is more critical than whether they are activated.
Discussion:
The authors should tone down the conclusions with respect to the overall preventive effect. The results are impressive if the effect is only reduction of disease severity.

Referee #2 (Comments on Novelty/Model System for Author):

How relevant transgenic mouse models in FTLD is still a question? This model accumulates GA dipeptide repeats but not decreased C9orf72 expression. However, such model is not yet available.

Referee #2 (Remarks for Author):

The present work by Zhou and coll. explores the use of immunotherapy by using an active vaccination againt GA dipeptide repeats in a mouse model of FTLD.
They have tested both GA dipeptide repeats coupled to ovalbumin and polyGA aggregates as immunogens. Only GA peptides coupled to the protein carrier lead to an immune response. Such vaccination shows improvement in motor behaviour and decreased insoluble GA dipeptide repeats in the mouse model. Even if this approach is new for dipeptide repeats, such vaccination has been widely used in neurodegenerative disorders for Abeta, alpha-synuclein, tau, etc... It is not original and mostly descriptive. It does not bring any insights on DPR toxicity ou etiopathogenesis. With OMICS analyses, the authors may have uncovered new pathways, but it has not been explored.
It is an interesting study which shows nice preliminary data, but the authors have to further explore their findings (effect on cell toxicity, pathways involved, transcriptomics analysis...). Microglia is an evidence but try unbiased analyses.

Minor comments: many references are incomplete
Regarding statistical analysis, why ANOVA and not non-parametric tests?

Referee #3 (Remarks for Author):

GGGGCC repeat expansion is the most common genetic cause of both ALS and FTD. One major pathogenic mechanism is the production of dipeptide repeat (DPR) proteins through unusual RAN translation, as first reported by Dr. Edbauer and his colleagues a few years ago (Science, 2013). Indeed, numerous studies in cell cultures and animal models have demonstrated the toxicity of different DPR proteins, such as polyGA, polyGR, polyPR etc. These findings suggest that targeting DPRs may be a promising therapeutic approach.

In this study, Zhou and colleagues use an active immunization approach to generate anti-GA antibodies in mice expressing (GA)149-GFP. Remarkably, they found that microglia activation in the spinal cord was largely prevented, although the reduction in polyGA inclusions was modest. This prove-of-concept study should be published (as a Correspondence, since it has limited scope), because it is the first report demonstrating beneficial effects of DPR-specific antibodies in the

ALS/FTD field, although similar approaches have been used numerous times for other neurodegenerative diseases.

In this study, they used animals that overexpress polyGA. It is expected that targeting polyGA should have some beneficial effects since it is overexpressed polyGA that causes these ALS/FTD disease-like phenotypes. It would be nice if they could demonstrate a similar beneficial effect in C9ORF72 BAC transgenic mice, where different DPR proteins as well as repeat RNAs are co-expressed and it is unclear which molecule causes neurodegeneration, such as the one reported by Dr. Ranum that shows some strong disease phenotypes. However, it is not reasonable to demand the authors to perform this time-consuming experiment at this stage, which will significantly delay the publication of this prove-of-concept study. Maybe it is feasible if they could please show whether polyGA level in the CSF is reduced after immunization, if so, the result would further strengthen their conclusion that rescue of motor function and microglia activation is largely due to extracellular polyGA.

1. The first sentence in the abstract does not seem to belong there. The point is better made in the Introduction and Discussion.
2. In the Introduction section, "neuronal cytoplasmic inclusions" should be "neuronal inclusions" since both nuclear and cytoplasmic inclusions have been reported.
3. In Figure 1B, could the authors please use different colors for mice with different treatment? Since they are all black and white, it is not obvious to tell TG-Ova-GA10 from others.
4. Figure 1C only shows a few images. This reviewer would like to suggest including some sort of quantification, which should not be difficult to do. For instance, they could count the number of neurons with inclusions per 100 cells in both control and patient brains. Also, it would be more convincing if additional brain tissues are examined, since the antisera give rise to high background signals, and/or a GA peptide blocking experiment is carried out to demonstrate the specificity of the Ova-GA10 antiserum.
5. In Figure 2B, the statistical difference between PBS and GA15 should be presented in the panel as well.
6. In Figure 3D, it is surprising to see 20% of cells in control mice show TDP-43 cytoplasmic localization. Is this number consistent with what the authors published before? In TG mice, the number is about 40%, which seems to be quite high. As Zhou and colleagues stated here: "poly-GA triggers modest TDP-43 phosphorylation and partial mislocalization of TDP-43". In contrast, the images in Figure 3C show a much lower % of cells with cytoplasmic TDP-43.
7. On Page 5, what does "ADD significance!" mean? It seems to be an error.

1st Revision - authors' response 11 October 2019

Referee #1 (Remarks for Author):
This study demonstrates that immunization of a transgenic mouse model expressing the most abundant DPR protein, with ovalbumin-coupled (GA)10, induced a high titre antibody response and resulted in a reduction in poly-GA aggregation, and reduced motor symptoms and microglial activation.

The overall approach is interesting and has clinical implications. Yet, there are some key issues in the data that require attention:

Results
1. In Figure 1c, the data presented in a way that not convincing.
Fig. 1c was done without mouse-on-mouse blocking reagents. We performed additional peptide blocking experiments using better conditions resulting in lower background (new Fig 1E) and additional show colocalization data in the new Fig EV3.

2. Based on Figure 2, it is not clear how the authors concluded that the treatment prevented motor deficits.
We reformatted the figure 2A to show cross-sectional differences at each time point with improved statistics and addition show longitudinal presentation of the data in the new Fig. EV4. Together the figures clearly show impaired performance of TG mice in the beam walk assay that is partially

prevented by Ova-(GA)10 immunization. Thus, our therapy clearly improved the motor function in TG mice dependent on development of a high anti-GA antibody titer.

3. Figure 3 shows only Iba1 expression as a basis for the claim of microglial activation. This is not sufficient, as Iba1 is not only marker of activated microglia, but also stains monocyte-derived macrophages. Also, the phenotype of the microglia is more critical than whether they are activated.
We agree that Iba1 cannot fully distinguish microglia and macrophages. Therefore, we changed the text to "microglia/macrophages" throughout the manuscript. To address the phenotype of microglia/macrophages, we now performed in depth automated morphological analysis (new Fig. EV5C/D), which confirm activation in TG-PBS mice and significant rescue upon Ova-(GA)10 immunization. The results are corroborated by transcriptome data (new Fig. 3A/B) showing partial rescue of inflammatory gene expression changes.

Discussion:
The authors should tone down the conclusions with respect to the overall preventive effect. The results are impressive if the effect is only reduction of disease severity.
The reviewer is right that we did not completely prevent symptoms in GA-CFP mice. We changed the conclusion of the discussion to "active vaccination is a promising approach to reduce disease severity or even prevent ALS and FTD in people carrying the C9orf72 mutation" as requested.

Referee #2 (Comments on Novelty/Model System for Author):
How relevant transgenic mouse models in FTLD is still a question? This model accumulates GA dipeptide repeats but not decreased C9orf72 expression. However, such model is not yet available.
We agree that this model replicated only a part of *C9orf72* disease, but ample evidence suggests that poly-GA plays a critical role and is most closely linked to TDP-43 pathology (Khosravi et al, 2017; Nonaka et al, 2018, Lee et al, 2017). BAC transgenic mouse model replicating patient-length $(G_4C_2)_n$ repeats which seem to be not completely genetically stable (https://www.jax.org/strain/029099 and https://www.jax.org/strain/029102) or show no motor or cognitive phenotype (O'Rourke et al, Neuron 2015, Peters et al, Neuron 2015). Concomitant downregulation of *C9orf72* expression in BAC transgenic mice has not been reported. We clearly state the limitations of our study in the revised discussion.

Referee #2 (Remarks for Author):
The present work by Zhou and coll. explores the use of immunotherapy by using an active vaccination againt GA dipeptide repeats in a mouse model of FTLD.

They have tested both GA dipeptide repeats coupled to ovalbumin and polyGA aggregates as immunogens. Only GA peptides coupled to the protein carrier lead to an immune response. Such vaccination shows improvement in motor behaviour and decreased insoluble GA dipeptide repeats in the mouse model. Even if this approach is new for dipeptide repeats, such vaccination has been widely used in neurodegenerative disorders for Abeta, alpha-synuclein, tau, etc... It is not original and mostly descriptive. It does not bring any insights on DPR toxicity ou etiopathogenesis. With OMICS analyses, the authors may have uncovered new pathways, but it has not been explored.

It is an interesting study which shows nice preliminary data, but the authors have to further explore their findings (effect on cell toxicity, pathways involved, transcriptomics analysis...). Microglia is an evidence but try unbiased analyses.
We greatly expanded our manuscript as suggested by this and other reviewers. We now include transcriptome data from a replication cohort (new Fig. 3A/B, new Table S3-S6). Transcriptome analysis shows that poly-GA expression results in prominent neuroinflammation. These changes are partially rescued by OVA-$(GA)_{10}$ immunization. This crucial new data is now shown in the new Fig. 3A/B and Tables S3-S6. Consistent with these findings we also show that immunization reduces neuroaxonal damage using NFL immunoassays in the CSF (new Fig. 3F). Moreover, detailed analysis of the microglia morphology reveals poly-GA induced neuroaxonal damage also confirms partial rescue by immunization (new Fig. EV5B-D).

Although immunotherapy has been attempted in other neurodegenerative diseases, our manuscript provides the first proof-of-concept data for the possibility of active (and possibly passive) immunotherapy of *C9orf72* ALS and FTD. This study may rekindle the interest in active vaccination

for other neurodegenerative diseases after many failed clinical trials with passive immunotherapy. Taken together, we provide not only additional insights into poly-GA toxicity but also suggest a new therapeutic strategy.

Minor comments: many references are incomplete
We apologize for the mistakes. We checked and updated all references.

Regarding statistical analysis, why ANOVA and not non-parametric tests?
We rechecked normality for all datasets and found that ANOVA is the appropriate analysis for all but Fig. 2A/B (due to sharp drop of assay time at 3 s and wide shoulder of slow TG mice) and the new data in Fig. EV5C/D. Thus, we switched to box-plot presentation and performed pairwise Wilcox rank sum Test with Benjamini-Hochberg adjustment of p-values in the modified Fig 2A (compare Table S2 for p-values and new Fig. EV4 for longitudinal presentation). Our conclusions hold also using non-parametric testing.

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GGGGCC repeat expansion is the most common genetic cause of both ALS and FTD. One major pathogenic mechanism is the production of dipeptide repeat (DPR) proteins through unusual RAN translation, as first reported by Dr. Edbauer and his colleagues a few years ago (Science, 2013). Indeed, numerous studies in cell cultures and animal models have demonstrated the toxicity of different DPR proteins, such as polyGA, polyGR, polyPR etc. These findings suggest that targeting DPRs may be a promising therapeutic approach.

In this study, Zhou and colleagues use an active immunization approach to generate anti-GA antibodies in mice expressing (GA)149-GFP. Remarkably, they found that microglia activation in the spinal cord was largely prevented, although the reduction in polyGA inclusions was modest. This prove-of-concept study should be published (as a Correspondence, since it has limited scope), because it is the first report demonstrating beneficial effects of DPR-specific antibodies in the ALS/FTD field, although similar approaches have been used numerous times for other neurodegenerative diseases.

In this study, they used animals that overexpress polyGA. It is expected that targeting polyGA should have some beneficial effects since it is overexpressed polyGA that causes these ALS/FTD disease-like phenotypes. It would be nice if they could demonstrate a similar beneficial effect in C9ORF72 BAC transgenic mice, where different DPR proteins as well as repeat RNAs are co-expressed and it is unclear which molecule causes neurodegeneration, such as the one reported by Dr. Ranum that shows some strong disease phenotypes. However, it is not reasonable to demand the authors to perform this time-consuming experiment at this stage, which will significantly delay the publication of this prove-of-concept study. Maybe it is feasible if they could please show whether polyGA level in the CSF is reduced after immunization, if so, the result would further strengthen their conclusion that rescue of motor function and microglia activation is largely due to extracellular polyGA.
We thank this reviewer for the encouragement and enthusiasm. We now mention in the discussion that repeating these experiments in *C9orf72* BAC models will be critical, but this experiment is unfortunately too time-consuming for a revision.

We also agree that measuring poly-GA reduction in the CSF would improve the translational impact of our study, but we so far failed to detect any poly-GA in the CSF of our mice (and patients), although our immunoassay clearly detects reduced poly-GA levels in brain homogenates. Thus, we cannot make direct conclusions on extracellular poly-GA levels. The dominant effects of Ova-$(GA)_{10}$ immunization on inflammatory changes as shown by transcriptomics and immunohistochemistry suggest that the induced antibodies reduce microglia activation presumably by acting on extracellular poly-GA or reducing neuronal damage by acting on intracellular poly-GA (see revised discussion).

Importantly, we provide new exciting data on neurofilament light chain (NFL) as clinically established biomarker for neuroaxonal damage. Importantly, GA-CFP mice show elevated NFL levels in CSF, which are reduced upon OVA-$(GA)_{10}$ immunization (new Fig. 3F).

1. The first sentence in the abstract does not seem to belong there. The point is better made in the Introduction and Discussion.
We removed the first sentence of the abstract as suggested.

2. In the Introduction section, "neuronal cytoplasmic inclusions" should be "neuronal inclusions" since both nuclear and cytoplasmic inclusions have been reported.
We changed the text as requested.

3. In Figure 1B, could the authors please use different colors for mice with different treatment? Since they are all black and white, it is not obvious to tell TG-Ova-GA10 from others.
We modified Fig. 1B and all other relevant figures (revised Fig 2A, EV1 and EV4) to include a consistent color code for the six different experimental groups.

4. Figure 1C only shows a few images. This reviewer would like to suggest including some sort of quantification, which should not be difficult to do. For instance, they could count the number of neurons with inclusions per 100 cells in both control and patient brains. Also, it would be more convincing if additional brain tissues are examined, since the antisera give rise to high background signals, and/or a GA peptide blocking experiment is carried out to demonstrate the specificity of the Ova-GA10 antiserum.
This is an excellent suggestion. We quantified the number of poly-GA positive neurons per 100 cells (new Fig. 1D). Additionally, we show colocalization of poly-GA aggregates labeled with a commercial rabbit anti-GA antibody and our mouse sera (new Fig. EV3). Finally, we performed blocking experiments using GST-$(GA)_{15}$ antigen to confirm specificity (new Fig. 1E).

5. In Figure 2B, the statistical difference between PBS and GA15 should be presented in the panel as well.
We included this important information in the revised Fig. 2A and Table S2.

6. In Figure 3D, it is surprising to see 20% of cells in control mice show TDP-43 cytoplasmic localization. Is this number consistent with what the authors published before? In TG mice, the number is about 40%, which seems to be quite high. As Zhou and colleagues stated here: "poly-GA triggers modest TDP-43 phosphorylation and partial mislocalization of TDP-43". In contrast, the images in Figure 3C show a much lower % of cells with cytoplasmic TDP-43.
We apologize for the confusion. We performed a blinded analysis of cytoplasmic TDP-43 mislocalization rather than of mature aggregates (which are not found in these mice). We now clearly marked the cells we counted for the analysis in the revised figure (now EV5E). In our initial characterization of the GA-CFP mice we had focused on phosphorylated TDP-43 using an immunoassay performed by collaborators (Schludi et al, 2017).

7. On Page 5, what does "ADD significance!" mean? It seems to be an error.
We removed this editing comment from the revised manuscript.

2nd Editorial Decision 11 November 2019

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. It's my pleasure to let you know that we have now received the enclosed reports from the referees that were asked to re-assess it. As you will see the reviewers are supportive of publication and we will be able to accept your manuscript pending final editorial amendments.

I look forward to reading a new revised version of your manuscript as soon as possible.

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

The authors adequately addressed the concerns raised by this referee

Referee #2 (Comments on Novelty/Model System for Author):

The etiopathogenesis is not fully established but the choice of this model does not affect the quality of the work

Referee #2 (Remarks for Author):

The authors have answered most of the comments

2nd Revision - authors' response 29 November 2019

Authors made the requested editorial changes.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Dieter Edbauer
Journal Submitted to: Embo Mol Medicine
Manuscript Number: EMM-2019-10919-V2

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	sample sizes were predetermined according to the original charactization of the mouse line (Schludi et al, 2017). Sample sizes were selected as allowed by the animal license and availability of tissue.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	sample sizes were predetermined according to the original publication where the line was fully charaterized (Schludi et al, 2017). Sample sizes were selected as allowed by the animal license and availability of tissue.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	no samples were excluded
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	blood sampling and immunization was performed in a random order. Sample distribution for immunoassay was randomized. Application of antibodies for immunohistochemitry staining or immunoflurescent staining was performed in a randomized order.
For animal studies, include a statement about randomization even if no randomization was used.	blood sampling and immunization was performed in a random order. Behavior test were pregrouped by gender and tested in a centain order to minimize the behaviral bias, so cannot be randomized.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Investigators were blinded to genotype during data collection by the use of a number code for each animal. Investigators were blinded to patient genotpye during data collection by the use of a number code for each donor.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Investigators were blinded to genotype during data collection by the use of a number code for each animal.
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. Data distributions were checked for normality by the Shapiro-Wilk test and homogeneity of variances was checked by the F test or the Brown-Forsythe test. One-way ANOVA with Turkey correction to compare more than two groups. Data for Figure 2A, 2B and S5C/D were not normally distributed and non-parametric tests were performed: Pairwise Wilcoxon rank sum with Benjamini-Hochberg correction was applied for group comparisons (Fig 2A, S5C/D) using R, Kruskal-Wallis Test with Dunn's correction (Fig 2B) using Prism.

Is there an estimate of variation within each group of data?	yes
Is the variance similar between the groups that are being statistically compared?	Yes.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	All the antibodies except monoclonal anti-GA clone 1A12 were purchased from commercial sources, clone number and manufactory information is given in the method section.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HEK293 cells were purchased by Life Technology and were regularly tested for mycoplasma contamination.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	(GA)149-CFP expressing transgenic (TG) mice have been characterized in detail (Schludi et al, 2017). Animal handing and animal experiments were performed in accordance to institutional guidelines approved by local animal laws and housed in standard cages in pathogen free facility on a 12 h light/dark cycle with ad libitum access to food and water.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Animal handing and animal experiments were performed in accordance to institutional guidelines approved by local animal laws
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Yes

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	All patient materials were provided by the Neurobiobank Munich, Ludwig-Maximilians-University (LMU) Munich.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Yes
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	n.a.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	n.a.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	n.a.
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	n.a.
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	n.a.

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Yes. RNAseq data was uploaded to Gene Expression Omunibus as indicated in the manuscript.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	source data provided for Fig 2A, 2B, 2D, 2E, 3D, 3F.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	n.a.
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	Original MATLAB script for automated microglia morphology analysis can be downloaded at https://github.com/isdneuroimaging/mmqt as reported previously (Heindl et al, 2018).

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	n.a.