EMBO Molecular Medicine

# Haematopoietic stem cell gene therapy with IL-1Ra rescues cognitive loss in mucopolysaccharidosis IIIA

Helen Parker, Stuart M Ellison, Rebecca J Holley, Claire O'Leary, Aiyin Liao, Jalal Asadi, Emily Glover, Arunabha Ghosh, Simon Jones, Fiona L Wilkinson, David Brough, Emmanuel Pinteaux, Herve Boutin & Brian W Bigger

**Review timeline:**

| | |
|---|---|
| Submission date: | 24 July 2019 |
| Editorial Decision: | 9 September 2019 |
| Revision received: | 12 December 2019 |
| Editorial Decision: | 20 December 2019 |
| Revision received: | 15 January 2020 |
| Accepted: | 17 January 2020 |

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 9 September 2019

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

You will see from the set of comments pasted below that the two referees are overall supportive of publication. While referee 2 recommends rewriting and better structuring the paper, referee 1 is a little more critical and mentions a few technical issues, the need for clarifications and some supporting experiments that should be easily addressable. This referee also would like to see some data aimed at identifying the causal factor responsible for the observed effects. Should you have such data at hand, I would strongly encourage you to add them, however those will not be mandatory for acceptance.

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 (Comments on Novelty/Model System for Author):

the experiments are well performed, results are highly relevant for understanding neuropathology in MPS. The mouse model recapitulates the phenotype seen in patients.

Referee #1 (Remarks for Author):

In the manuscript by Parker et al., the involvement of interleukin-1beta and interleukin-1 receptor antagonist in neuroinflammation induced by MPSIIIA is described. Using lentiviral gene therapy, the authors describe an approach for reduction of neuroinflammation in a mouse model. In general, the findings are interesting and provide novel insight in the pathogenesis of MPSIIIA.
Comments:
1) In the in vitro experiments using GAGs from various sources, the authors show that MPSIII GAGs specifically induced Il1b expression, whereas wt GAGs or heparin sulfate does not. While interesting, the authors do not identify the causal factor. What is in the MPSIII GAGs preparation that causes this effect?
2) Fig 2B: what was the effect of GAG treatment at later time points?
3) In Fig. 2E, the authors state that no significant secretion of Il-1b into culture media was detected. However, the figure does show significant secretion.
4) In Fig 3C, how was expression normalized? please show not-normalized expression levels of the genes examined as well as of the gene used for normalization
5) In Fig 4J, the VCN in brain is very low. Please comment.
6) please provide a more extensive analysis of Il1b expression showing where it is expressed in the brain
7) page 15, 3rd paragraph: the authors state that MPSIIIA x IL-1R1-/- mice showed no overt differences in cognititive or neuropathological phenotype to MPSIIA x IL-1R1+/+ mice. Please specify the data. It sounds strange in the light of the results of Fig. 6
8) Fig. 6: the reduction of astrocyte or microglial activation by overexpression of hIl-1Ra or Il-1R1 knock out was about 50%. It would be good to discuss this. Which other factors could be involved in the neuroinflammation? Is it reasonable to assume that the remaining neuroinflammation does not have functional consequences?
9) Discussion page 17: 'it is secretion of Il1-b that propagates a positive feedback loop of consistent Il-1 activation ....' A feedback loop of consistent activation has not been shown here.

Referee #2 (Remarks for Author):

This is a very good and important piece of science showing that storage alone does not cause neurological problems in lysosomal storage disease, but that storage components can combine to activate neuroinflammation that does. I agree with the authors that "these data suggest that IL1 is an important mediator in the MPSIIIA inflammatory cascade, highlighting haematopoietic stem cell gene therapy using IL-1Ra as a potential anti-inflammatory therapy to treat cognitive decline in MPSIIIA and other neuronopathic lysosomal storage diseases."
However the presentation is not good and often it is hard to read. I have given some examples below. I would also commend the authors to try a schematic diagram that shows how they interpret their results and how the infammasome is activated (or not) with the different and overlapping stimuli. It would make for a very powerful paper.

Problems are:
A good list of abbreviations would make for much easier reading.
There are a lot of abbreviations some of which are not expanded in a timely fashion and many similar to each other that make it very hard to follow. This starts off with IL-IRa in the title, continues NLRP3 in the abstract, Nlrp3, OC dot blot, IlRN, hCD11b.hIL1RN.WPRE (LV.IL1RN, MOI, HSC, GFAP, LAMP2, DAB, DAP1, , FITC, TRITC, GAPDH, ΔΔCT GAPDH, AAV, MyD88 knockout mice in the methods, CHC, What are lin- enriched stem cells? in figs I am guessing that standard deviations, SD in the methods is the same as STDEV in the figure legends. AAV, MyD88 knockout mice,
NLRP3's role in neuroinflammation should also be discussed in the introduction.

 

Articles missing. There are a number of these through the paper. E.g. "Lentivirus was produced and titre determined as previously described" should be, "Lentivirus was produced and the titre determined as previously described"
I do not think the phrase "according to the manufacturer's instructions" is necessary when referring to the use of kits.
Addresses required for (a gift from Dr Konstantin Dobrenis and Prof Walkley)
Results
What does "equivalent amounts based on HS quantification; Fig. EV1" mean?
"These data confirm that priming of an intracellular pro-IL-1• inflammatory response is dependent on MPSIIIA GAG acting via TLR4. This data also suggests that it is not the accumulation of MPSIIIA-specific GAG alone that is responsible for inducing the observed inflammatory response in MPSIIIA mice; instead the composition of the MPSIIIA GAG itself or a result of a co-purified GAG-binding factor must also be important." is easier to read as "These data confirm that priming of an intracellular pro-IL-1• inflammatory response is dependent on MPSIIIA GAG acting via TLR4, also suggesting that the accumulation of MPSIIIA-specific GAG alone is not responsible for inducing the observed inflammatory response in MPSIIIA mice. Instead the composition of the MPSIIIA GAG itself or a result of a co-purified GAG-binding factor must also be important."
"HS which so their" is presumably " HS which shows their"

Many ideas seem to be the wrong way around, which makes the text hard to follow. E.g. I would rewrite "An ATP assay revealed significant increases in ATP levels in MPSIIIA mice compared to WT ($p<0.05$; Fig. 3B), with ATP often released by activated immune and endothelial cells to potentiate inflammatory responses (Savage et al., 2012)" as. "ATP is often released by activated immune and endothelial cells to potentiate inflammatory responses (Savage et al., 2012). Here an ATP assay revealed significant increases in ATP levels in MPSIIIA mice compared to WT ($p<0.05$; Fig. 3B).
"Significant increases in expression of several subunits or activators of the NLRP3 inflammasome were apparent in MPSIIIA brain tissue compared to WT (Fig. 3C; $p<0.05$). This was confirmed with identification that caspase 1 activity was significantly increased in MPSIIIA brain homogenate (Fig. 3D; $p<0.001$). This suggests potential involvement of the inflammasome and activation of pyroptotic cell death, which requires caspase 1 activation." Is very clumsy and hard to follow.
Discussion.
"Amyloid beta deposits have already been evidenced in MPSIIIB cortices (Ohmi et al., 2011), and have been found co-localised to HS proteoglycans in MPSIIIA post mortem brains (Ginsberg et al., 1999). Is hard to read". Try "Amyloid beta deposits have been found in MPSIIIB cortices (Ohmi et al., 2011), and also co-localised to HS proteoglycans in MPSIIIA post mortem brains (Ginsberg et al., 1999).

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Add to the discussion references to the data conclusions are drawn from in passages like "The central importance of an inflammatory axis initiated by the TLR4-NLRP3 pathway and IL-1β was emphasised by our finding that in vitro stimulation of NLRP3 null mixed glia did not result in IL-1β secretion and that glial activation was significantly reduced and behavioural abnormalities were reversed in vivo in MPSIIIA mice deficient in IL-1R1 or over-expressing LV-mediated hIL-1Ra. We observed a pronounced reduction in astrogliosis upon inhibition of IL-1 signalling; astrocytes highly express IL-1R1, allowing them to respond to changes in the levels of central IL-1β (Srinivasan et al., 2004; Basu et al., 2004; Rothwell and Luheshi, 2000).

 

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Comments:
1) In the in vitro experiments using GAGs from various sources, the authors show that MPSIII GAGs specifically induced Il1b expression, whereas wt GAGs or heparin sulfate does not. While interesting, the authors do not identify the causal factor. What is in the MPSIII GAGs preparation that causes this effect?
We have added additional data which investigates the causal factor for MPSIIIA GAG immunogenicity. We have shown that it is predominantly HS as opposed to the DS/CS in the MPSIIIA GAG extract (Fig 2G), and that intracellular IL-1beta production is dependent on 2-O sulphation of MPSIIIA HS (Fig 2H and 2I). All GAG preparations were boiled to degrade protein and other cofactors. GAG preparations were also endotoxin tested via a LAL assay and showed no endotoxin contamination.

2) Fig 2B: what was the effect of GAG treatment at later time points?
We have repeated the in vivo experiments, whereby C57BL6 mice were intraperitoneally injected with PBS, WT GAG, MPSIIIA GAG, LPS, heparin or bovine kidney heparan sulphate. The levels of IL1b in the brain were measured 1 hour, 2 hours and 6 hours post injection via qPCR. Significant IL1b expression was observed 2 hours and 6 hours post-treatment (Fig 2B) when compared to a saline control. The effect peaked at 2 hours and declined by 6 hours.

3) In Fig. 2E, the authors state that no significant secretion of Il-1b into culture media was detected. However, the figure does show significant secretion.
Although significance was assigned post statistical analyses, the level of IL-1beta was below the limit of detection of the assay, and therefore should be classed as 0.

4) In Fig 3C, how was expression normalized? please show not-normalized expression levels of the genes examined as well as of the gene used for normalization
Gene expression was normalised by taking the Ct values (raw data) for the house keeping gene (GAPDH) and the gene being tested (e.g. IL-1b) and calculating the ΔΔCt value (change in Ct) across these, as is standardly done in Q-PCR to standardise changes in gene expression compared to a housekeeping gene and over time (Borysiewicz et al., 2009). There is no baseline value per se as we measure the difference in expression levels against a housekeeping standard. An average Ct value was calculated for the 3 replicates. The 4 values are Gene being Tested MPS3A (T3a), Gene being Tested WT (Twt), Housekeeping Gene MPS3A (H3a), and Housekeeping Gene WT (Hwt). The differences between T3a and H3a (T3a-H3a) and Twt and Hwt (Twt-Hwt) were calculated per sample. These are our ΔCt values for the MPS3A (ΔCt3a) and wildtype (ΔCtwt) conditions. The difference was then calculated between ΔCt3a and ΔCtwt (ΔCt3a-ΔCtwt) to arrive at our delta delta Ct value (ΔΔCt). Since all calculations are in logarithm base 2, every time there is twice as much DNA, your Ct values decrease by 1 and will not halve. To calculate fold change the following formula was applied 2^-ΔΔCt. **Normalisation of WT to 1, and MPSIIIA to WT.** An example of the raw Ct values is shown below

 

| | | Gapdh Ct | Il1b Ct |
|---|---|---|---|
| IIIA 9 | 99 | 16.51794243 | 33.30036926 |
| IIIA 9 | 99 | 16.30708122 | 32.96333313 |
| WT 9 | 223 | 16.17010498 | 35.6293602 |
| WT 9 | 223 | 16.44099808 | 35.70918274 |
| IIIA 9 | 104 | 16.57910728 | 32.4769516 |
| IIIA 9 | 104 | 16.48436356 | 32.32744217 |
| IIIA 9 | 202 | 15.37383556 | 32.94464874 |
| IIIA 9 | 202 | 15.29645061 | 33.01887131 |
| WT 9 | 111 | 15.97177505 | 35.08328247 |
| WT 9 | 111 | 15.87283993 | 35.1925087 |
| WT 9 | 133 | 16.53513527 | 35.45773315 |
| WT 9 | 133 | 16.74427223 | 35.8587532 |
| IIIA 9 | 112 | 16.65887451 | 33.58131409 |
| IIIA 9 | 112 | 16.75591087 | 33.31336212 |
| WT 9 | 206 | 15.7945013 | 35.12726593 |
| WT 9 | 206 | 15.68466663 | 34.94752502 |
| IIIA 9 | 133* | 16.57634163 | 32.53634644 |
| IIIA 9 | 133* | 16.53100014 | 32.64990616 |
| WT 9 | 204 | 14.71575069 | 34.39419556 |
| WT 9 | 204 | 14.72379303 | 34.3925209 |
| IIIA 9 | 178 | 16.23536873 | 33.63629532 |
| IIIA 9 | 178 | 16.25427628 | 33.92492294 |
| WT 9 | 134 | 16.67425346 | 35.67653656 |
| WT 9 | 134 | 16.6072979 | 35.47218323 |
| IIIA 9 | 205 | 17.23292351 | 33.97437286 |
| IIIA 9 | 205 | 16.99202728 | 34.21540451 |
| IIIA 9 | 221 | 16.46008873 | 33.93154144 |
| IIIA 9 | 221 | 16.32957458 | 33.7898941 |
| IIIA 9 | 222 | 15.51924515 | 33.7301178 |
| IIIA 9 | 222 | 15.8452301 | 33.87870407 |
| | NRT | Undetermined | Undetermined |
| | NRT | Undetermined | Undetermined |
| | NTC | Undetermined | Undetermined |
| | NTC | Undetermined | Undetermined |

5) In Fig 4J, the VCN in brain is very low. Please comment.
There is only a low number of cells which migrate from the periphery into the brain after a bone marrow transplant. A portion of peripheral myeloid cells are able to migrate across the blood brain barrier due to the busulfan conditioning which they receive prior to the transplant (Wilkinson et al., 2013). Others from our lab have observed a therapeutic effect with regards to neuroinflammation and behaviour following HSC gene therapy with only low VCN in the brain (Sergijenko et al., 2013; Holley et al., 2018; Gleitz et al., 2018).

6) please provide a more extensive analysis of Il1b expression showing where it is expressed in the brain
We have tried to stain for IL1b in the brain, but as the cytokine is expressed at extremely low levels and works at extremely low levels it is almost impossible to do this effectively. It is also secreted, when active, which makes cellular staining less relevant. If the reviewer is referring to expression of IL-1Ra (Fig 4L), then IL-1Ra is highly secreted, so likely has effects throughout the brain, regardless of which cells produce it. Capotondo et al., 2013 have produced a very nice IHC demonstration of microglial distribution throughout the brain following transplant, therefore we would expect distribution of IL1Ra to be also throughout the brain.

7) page 15, 3rd paragraph: the authors state that MPSIIIA x IL-1R1-/- mice showed no overt differences in cognititive or neuropathological phenotype to MPSIIA x IL-1R1+/+ mice. Please specify the data. It sounds strange in the light of the results of Fig. 6
This was an oversight in the text on our part. We have corrected the statement to 'WT × IL-1R1$^{-/-}$ and MPSIIIA × IL-1R1$^{-/-}$ mice generated were viable, however mice were housed in sterile cages due to a low level of immunodeficiency; a result of IL-1R1 knockout'.

8) Fig. 6: the reduction of astrocyte or microglial activation by overexpression of hIl-1Ra or Il-1R1 knock out was about 50%. It would be good to discuss this. Which other factors could be involved in the neuroinflammation? Is it reasonable to assume that the remaining neuroinflammation does not have functional consequences?
Systemic pentosan polysulfate treatment (a GAG mimetic and anti-inflammatory drug) and prednisolone (steroid) have both been shown to have effects on peripheral cytokine levels, as well as cognitive behaviour, indicating that peripheral inflammation may play a large role in the behavioural

 

abnormalities observed MPSIII murine models. We do not know whether the remaining neuroinflammation has functional consequences, the activation of microglia and astrocytes may play a protective role as opposed to destructive, we must remember that these cells will still have lysosomal burden due to GAG accumulation amongst other substrates. We have reworked the discussion to include some conjecture on this.

9) Discussion page 17: 'it is secretion of Il1-b that propagates a positive feedback loop of consistent Il-1 activation ....' A feedback loop of consistent activation has not been shown here.
We have reworded this, to reflect that this is our hypothesis for what is happening.

**Referee #2** (Remarks for Author):

This is a very good and important piece of science showing that storage alone does not cause neurological problems in lysosomal storage disease, but that storage components can combine to activate neuroinflammation that does. I agree with the authors that "these data suggest that IL1 is an important mediator in the MPSIIIA inflammatory cascade, highlighting haematopoietic stem cell gene therapy using IL-1Ra as a potential anti-inflammatory therapy to treat cognitive decline in MPSIIIA and other neuronopathic lysosomal storage diseases."

However the presentation is not good and often it is hard to read. I have given some examples below.
We have updated the introduction and discussion to clarify some of these points and make for easier reading (*).

I would also commend the authors to try a schematic diagram that shows how they interpret their results and how the infammasome is activated (or not) with the different and overlapping stimuli. It would make for a very powerful paper.
This was a great suggestion. A summary schematic diagram has been added to show how we think the inflammatory response is activated in MPSIIIA, and how our therapy attenuates such an inflammatory response.

1. A good list of abbreviations would make for much easier reading.
   There are a lot of abbreviations some of which are not expanded in a timely fashion and many similar to each other that make it very hard to follow. This starts off with IL-IRa in the title, continues NLRP3 in the abstract, Nlrp3, OC dot blot, IlRN, hCD11b.hIL1RN.WPRE (LV.IL1RN, MOI, HSC, GFAP, LAMP2, DAB, DAP1, , FITC, TRITC, GAPDH, ΔΔCT GAPDH, AAV, MyD88 knockout mice in the methods, CHC, What are lin- enriched stem cells? in figs I am guessing that standard deviations, SD in the methods is the same as STDEV in the figure legends.
   AAV, MyD88 knockout mice.

Abbreviations added as requested

2. NLRP3's role in neuroinflammation should also be discussed in the introduction.

This has been added

3. Articles missing. There are a number of these through the paper. E.g. "Lentivirus was produced and titre determined as previously described" should be, "Lentivirus was produced and the titre determined as previously described"

Updated as requested

4. I do not think the phrase "according to the manufacturer's instructions" is necessary when referring to the use of kits.

This has been removed

5. Addresses required for (a gift from Dr Konstantin Dobrenis and Prof Walkley)

4 and 5 – we will abide by journal requirements on these aspects

 

Results

6. What does "equivalent amounts based on HS quantification; Fig. EV1" mean?

We have changed the above statement to 'amounts administered based on quantity of HS detected' to clarify how much GAG was administered to mice.

7. "These data confirm that priming of an intracellular pro-IL-1• inflammatory response is dependent on MPSIIIA GAG acting via TLR4. This data also suggests that it is not the accumulation of MPSIIIA-specific GAG alone that is responsible for inducing the observed inflammatory response in MPSIIIA mice; instead the composition of the MPSIIIA GAG itself or a result of a co-purified GAG-binding factor must also be important." is easier to read as "These data confirm that priming of an intracellular pro-IL-1• inflammatory response is dependent on MPSIIIA GAG acting via TLR4, also suggesting that the accumulation of MPSIIIA-specific GAG alone is not responsible for inducing the observed inflammatory response in MPSIIIA mice. Instead the composition of the MPSIIIA GAG itself or a result of a co-purified GAG-binding factor must also be important."

We have updated the text to make this clearer

8. "HS which so their" is presumably " HS which shows their"

We have changed the above statement to 'HS which shows their'.

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See above*

10. "Significant increases in expression of several subunits or activators of the NLRP3 inflammasome were apparent in MPSIIIA brain tissue compared to WT (Fig. 3C; $p<0.05$). This was confirmed with identification that caspase 1 activity was significantly increased in MPSIIIA brain homogenate (Fig. 3D; $p<0.001$). This suggests potential involvement of the inflammasome and activation of pyroptotic cell death, which requires caspase 1 activation." Is very clumsy and hard to follow.

See above*

Discussion – has been rewritten with these points taken.

11. "Amyloid beta deposits have already been evidenced in MPSIIIB cortices (Ohmi et al., 2011), and have been found co-localised to HS proteoglycans in MPSIIIA post mortem brains (Ginsberg et al., 1999). Is hard to read". Try "Amyloid beta deposits have been found in MPSIIIB cortices (Ohmi et al., 2011), and also co-localised to HS proteoglycans in MPSIIIA post mortem brains (Ginsberg et al., 1999).

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See above*

2nd Editorial Decision 20 December 2019

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed report from the referee who was asked to re-assess it. As you will see the reviewer is now supportive and I am pleased to inform you that we will be able to accept your manuscript pending minor editorial amendments including a response to the minor text change commented by referee 1.

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

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

Referee #1 (Comments on Novelty/Model System for Author):

see original review

Referee #1 (Remarks for Author):

The revised manuscript has been improved significantly. The finding that it is 2-O sulphation of HS in GAGs from MPSIIIA mice is very interesting and explains the specific activity of these GAGs in activating inflammation. The authors have addressed all comments satisfactorily. Editorial suggestions:

page 15 2nd paragraph: "...suggesting that GAGs alone prime, but do not activate inflammasome responses"
this can formally not be stated at this stage of the story, I suggest to rephrase, for example replace "suggesting" by "consistent with" and refer to the experiments below that address priming.

p 15, 3rd paragraph: suggesting that GAG should read suggesting that GAGs

p16, line 1: add ref and remove comment

discussion line 1: remove comment

Figure 2H and EV1: specify 2S, 6S, and NS in legend
Figures 2H and I are wrongly numbered in the legend

 

2nd Revision - authors' response 15 January 2020

Authors made the requested changes.

 

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

| |
|---|
| Corresponding Author Name: Brian Bigger |
| Journal Submitted to: Celine ; EMBO Molecular Medicine |
| Manuscript Number: EMM-2019-11185 |

**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.**

## 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 selected on the basis of previous studies to ensure adequate power (Holley et al., 2017, Sergijenko et al., 2013 & Gleitz et al., 2018). |
| 1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used. | Sample sizes were selected on the basis of previous studies to ensure adequate power (Holley et al., 2017, Sergijenko et al., 2013 & Gleitz et al., 2018). |
| 2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? | Criteria for sample exclusion were pre-established with regards to animals receiving a haematopoieitic stem cell transplant. Any animal were chimerism of below 50% was observed was excluded from the analysis, as this was deemed a failed transplant. No animals were excluded from the analysis. |
| 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. | Yes. Animals were not caged based on genotype. WT, MPSIIIA littermates were mixed. WTxIL1R1-/- and MPSIIIAxIL1R1-/- littermates were mixed. Samples were assigend ID numbers as opposed to being identified by genotype. |
| For animal studies, include a statement about randomization even if no randomization was used. | WT, MPSIIIA , WTxIL1R1-/- and MPSIIIAxIL1R1-/- littermates were randomly assigned to control or transplant groups, although transplant group allocation was also partially determined by the number of donor animals and the amount of cells avalaible for transplant.We were however sometimes limited by the number of donor animals available. |
| 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. | Biochemical and microscopic analyses were performed blinded using mouse numbers. Behavioural analyses such as hyperactivity could not be performed in an entirely blind fashion, because MPS IIIA mice exhibit obvious behavioural abnormalities. However, the Y-maze was recorded in a blind fashion and analysed at a later timepoint from video once all tests had been performed. All mouse numbers were randomised prior to the analysis and subsequently allocated to correct groups afterwards. |
| 4.b. For animal studies, include a statement about blinding even if no blinding was done | Biochemical and microscopic analyses were performed blinded using mouse numbers. Behavioural analyses such as hyperactivity could not be performed in an entirely blind fashion, because MPS IIIA mice exhibit obvious behavioural abnormalities. However, the Y-maze was recorded in a blind fashion and analysed at a later timepoint from video once all tests had been performed. All mouse numbers were randomised prior to the analysis and subsequently allocated to correct groups afterwards. |
| 5. For every figure, are statistical tests justified as appropriate? | Statistical analysis was performed using GraphPad Prism 7 software (La Jolla, CA, USA). Two-tailed parametric unpaired t-tests were applied for individual group comparisons. One-way or two-way ANOVAs were performed for multi-group analysis followed by Tukey's multi-comparisons test. Significance was set at $p<0.05$. |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. | Normality was tested via a D'Agostino-Pearson nomality test (omnibus K2) - and showed normal distribution for most of the data. Where data was skewed (ELISA data), raw values were log transformed and statistical analysis carried out on the transformed data. |
| Is there an estimate of variation within each group of data? | Yes, statistical analysis was performed with a one-way ANOVA followed by a Tukey's posthoc test. |
| Is the variance similar between the groups that are being statistically compared? | Yes, statistical analysis was performed with a one-way ANOVA followed by a Tukey's posthoc test. |

## C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

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| http://www.antibodypedia.com | Antibodypedia |
| http://1degreebio.org | 1DegreeBio |
| http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo | ARRIVE Guidelines |
| http://grants.nih.gov/grants/olaw/olaw.htm | NIH Guidelines in animal use |
| http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm | MRC Guidelines on animal use |
| http://ClinicalTrials.gov | Clinical Trial registration |
| http://www.consort-statement.org | CONSORT Flow Diagram |
| http://www.consort-statement.org/checklists/view/32-consort/66-title | CONSORT Check List |
| http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun | REMARK Reporting Guidelines (marker prognostic studies) |
| http://datadryad.org | Dryad |
| http://figshare.com | Figshare |
| http://www.ncbi.nlm.nih.gov/gap | dbGAP |
| http://www.ebi.ac.uk/ega | EGA |
| http://biomodels.net/ | Biomodels Database |
| http://biomodels.net/miriam/ | MIRIAM Guidelines |
| http://jjj.biochem.sun.ac.za | JWS Online |
| http://oba.od.nih.gov/biosecurity/biosecurity_documents.html | Biosecurity Documents from NIH |
| http://www.selectagents.gov/ | List of Select Agents |

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| 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). | Anti-glial fibrillary acidic protein (GFAP) - GA52461-2. Anti-GM2 - hybridoma clone 10-11. Anti beta amyloid 1-40 antibody clone 11A50-B10 - Biolegend Cat # 805409. Anti beta-amyloid 1-42 antibody clone 12F4 - Biolegend Cat # 805509. Goat anti human IL-1Ra - AF-280-NA. Rat anti-LAMP2 - clone GL2A7 - ab13524. Rabbit anti-NeuN - clone EPR12763 - ab177487 |
| 7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. | RAW264.7 monocyte/macrophage from ATCC (ATCC TIB-71) - cell line negative for mycoplasma. |

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

## D- Animal Models

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| 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. | Species: Mouse. B6.Cg-Sgshmps3a/6J mice backcrossed for over 20 generations to a C57BL/6J background. Mice were maintained by heterozygote breeding and offspring genotyped. Wild-type (WT) and mutant (MPSIIIA) littermates were from the breeding of this strain. 1 day old WT pups were used to produce mixed glial cultures. Tissue from female WT and MPSIIIA mice were used for gene expression analysis at 9 months of age. Female WT and MPSIIIA mice were also used as controls for the main in vivo study. 6-8 week old female MPSIIIA mice were used as recipients during HSCT. Due to conditioning with busulfan, recipient mice are kept in autoclaved cages throughout the tranplantation protocol, and fed irradiated chow and acdified water. B6.Cg-Sgshmps3a/6J mice were also backcrossed into the Pep3 CD45.1 congenic background (B6.SJL-Ptprca Pepcb/BoyJ; Jackson Laboratories) and 6-12 week old female MPSIIIAxPep3 offspring used as donors during HSCT. 6 month old female MPSIIIAxIL1R1-/- and WTxIL1R1-/- mice were generated by crossing the B6.Cg-Sgshmps3a/6J heterozygote mice with the IL-1R1-/-1 FAIE C57BL/6 background (generated by Immunex, Seattle, USA and backcrossed to C57 by Dr Martin Nicklin, Sheffield). Offspring were then intercrossed to obtain third generation WT andMPSIIIA offspring deficient in IL-1R1. IL-1R1 defcient mice are kept in autoclaved cages throughout the tranplantation protocol, and fed irradiated chow and acdified water due to an inherent level of immunodeficiency. We are grateful to Dr Emmanuel Pinteaux for providing the IL-1R1-/- mouse sperm. Nlrp3-/- (Cias1-/-) mice were generated by Dr Vishva Dixit (Genentech) as per Mariathasan et al., 2006. We are grateful to Dr Dave Brough for providing Nlrp3-/- 1 day old pups to prepare mixed glial cultures. 16 week old female C57BL/6 mice were bought from Envigo, UK. All mice apart from those mentioned are fed normal chow and water. All mice are kept in individually ventilated cages. All mice were housed in a temperature and light controlled environment, with regular 12/12 hour light/dark cycle. |
| 9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments. | All animal work was performed with local ethical approval and in accordance with Home Office regulations. All Schedule 1 procedures and experimental procedures were performed in accordance with the Animals (Scientific Procedures) Act, 1986 (UK), under project licences PPl 40/3658 and POC3AEEB0, and approved by the Univeristy of Manchester Ethical Review Process Committee. |
| 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. | All animal experiments reported in this study are adequately reported based on ARRIVE guidelines. |

## E- Human Subjects

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| 11. Identify the committee(s) approving the study protocol. | Patient samples were gathered under the clinical trial GENISIS2013, EUDRACT number 2013-001479-18. Patient samples were gathered with informed consent from affected and unaffected subjects under NHS Research ethical approval REC 08/H1010/63 granted to Prof. B Bigger. All experiments conform with the principles set out in the WMA declaration of Helsinki. |
| 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. | Patient samples were gathered with informed consent from affected and unaffected subjects under NHS Research ethical approval REC 08/H1010/63 granted to Prof. B Bigger. All experiments conform with the principles set out in the WMA declaration of Helsinki. |
| 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. | No patient photos included |
| 14. Report any restrictions on the availability (and/or on the use) of human data or samples. | None |
| 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

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| 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'.<br><br>Data deposition in a public repository is mandatory for:<br>a. Protein, DNA and RNA sequences<br>b. Macromolecular structures<br>c. Crystallographic data for small molecules<br>d. Functional genomics data<br>e. Proteomics and molecular interactions | Human IL1RN cDNA (secreted isoform): https://www.uniprot.org/uniprot/P18510#P18510-1<br>Transcript: IL1RN-005 ENST00000409930<br>From Ensembl website (note isoform 5 ensembl = isoform 1 uniprot)<br>177aa secreted form (according to www.uniprot.org IL1RN) (531)<br>Isoform 1 (identifier: P18510-1) |
| 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). | N/A |
| 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. | N/A |

## G- Dual use research of concern

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| 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. | No |