Plan Overview

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Title: IS THE CEREBROPROTECTIVE FUNCTION OF PRDM16 DURING ISCHAEMIC STROKE MEDIATED BY CONTROLLING THE TGFBETA/BMP SIGNALLING BALANCE?

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Template: KU Leuven BOF-IOF

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Project abstract:

Globally, ischaemic stroke is a leading cause of death and permanent disability and is due to cerebral artery occlusion. Since stroke is a disease of ageing, its prevalence and socioeconomic burden will continue to rise, highlighting the urgent need for effective interventions. Restoring cerebral blood flow by functionalising collateral arteri(ol)es and preserving blood brain barrier integrity is the most desirable strategy to salvage ischaemic brain tissue. We found that Prdm16 in endothelial cells plays a protective role during stroke pathogenesis, however its expression declines upon ageing. Prdm16 is known in the heart to interact with the Transforming Growth Factor (TGF)beta/Bone Morphogenetic Protein (BMP) pathways. Here, we wish to: (i) functionally validate the protective role of endothelial Prdm16; (ii) investigate whether modulation of the TGFbeta/BMP balance is part of the mechanism downstream of Prdm16; (iii) compare the effect of Prdm16 loss and ageing. Finally, we will exploit these findings to develop biomarker and therapeutic strategies to combat ischaemic stroke.

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IS THE CEREBROPROTECTIVE FUNCTION OF PRDM16 DURING ISCHAEMIC STROKE MEDIATED BY CONTROLLING THE TGFBETA/BMP SIGNALLING BALANCE?

Research Data Summary

List and describe all datasets or research materials that you plan to generate/collect or reuse during your research project. For each dataset or data type (observational, experimental etc.), provide a short name & description (sufficient for yourself to know what data it is about), indicate whether the data are newly generated/collected or reused, digital or physical, also indicate the type of the data (the kind of content), its technical format (file extension), and an estimate of the upper limit of the volume of the data.

Dataset name / ID	Description	New or reuse	Digital or Physical data	Data Type	File format	Data volume	Physical volume
		Indicate: ***N****(ew data) or* ***E****(xisting data)*	Indicate: **D**(igital) or **P**(hysical)	Indicate: **A**udiovisual **I**mages **S**ound **N**umerical **T**extual **M**odel **SO**ftware Other (specify)		Indicate: <1GB <100GB <1TB <5TB >5TB NA	
Single-nuclei RNA sequencing raw data sets	Acquired from single-cell core facility	N	D	T	.fastq files, .gz files, .bam files	<100GB	NA
Single-nuclei RNA sequencing processed datasets	Processed data allowing downstream analysis of single-cell results	N	D	I, N, T	.xls files, .jpeg files, .pdf files, .txt files, .cvs files	<1TB	NA
Microscopic images of immuno-fluorescence and histochemical stainings	Images from mouse tissues	N	D	I	.jpeg files, Zen .zvi files, .czi files, .tiff files	<1TB	NA
Macroscopic images of coronal brain sections	Images for ischaemic lesion size assessment	N	D	I	.jpeg files, .pdf files	<100GB	NA
Mouse genotyping gel pictures	Genotyping results from gel electrophoresis	N	D	I	.jpeg files	<1GB	NA
Electron microscopic images	Images from mouse tissues	N	D	I	.jpeg files, .tiff files	<100GB	NA
Ultrafast ultrasound images	Images from functional ultrasound imaging and ultrasound localisation microscopy	N	D	I	.tiff files	<100GB	NA
Laser Speckle Contrast images	Images acquired from Laser Speckle Contrast Imaging	N	D	I	.psfx files, .png files	<100GB	NA

nanoCT images	images acquired from computed tomography	N	D	I	.tiff files	<100GB	NA
Fluorimetric analyses	Files generated from fluorimetric measurements (ELISA, BCA protein assay, tracer leakage experiments)	N	D	N, T	.xls files	<1GB	NA
Morphometric analysis data		N	D	N	.xls files	<1GB	NA
Western blot images		N	D	I	.scn files	<1GB	NA
Statistical analysis		N	D	I, N, T	.pzfx files, .r files	<100GB	NA
Composition figures, digital images	Figures for abstracts, posters and publications	N	D	I	.eps files, Acrobat .pdf files, Adobe Indesign .indd files, Adobe Illustrator .ai files, .tiff files, .jpeg files, .png files	<100GB	NA
SOPs	Staining, qPCR, genotyping, nuclei isolation, *etc.*	N	D	T	Word .doc files	<1GB	NA
Sample inventories		N	D	T	.xlsx files	<1GB	NA
quantitative (q)RT-PCR data	from mouse and human samples	N	D	N, T	.cvs files	<1GB	NA
informed consent forms		N	D	T	.pdf files	<1GB	NA
Pseudonymised personal data humans in different formats	Demographic data of included patients	N	D	N, T	.docx, .xls files	<1GB	NA
Tissues	Paraffin embedded mouse tissues	N	P	NA	NA	NA	Approximately 300 tissue blocks
Paraffin sections	mouse origin	N	P	NA	NA	NA	30 drawers of 100 slides, stored at room temperature
Snap frozen tissues	mouse origin	N	P	NA	NA	NA	2 boxes of samples stored in -80°C freezer
Cryo embedded tissues	mouse origin	N	P	NA	NA	NA	8 boxes of samples stored in -80°C freezer

Cryo sections	mouse origin	N	P	NA	NA	NA	15 boxes of slides stored in -20°C freezer
Protein/RNA/cDNA	mouse origin	N	P	NA	NA	NA	3 boxes of samples stored in -80°C freezer
RNA	human origin	N	P	NA	NA	NA	3 boxes of samples stored in -80°C freezer

If you reuse existing data, please specify the source, preferably by using a persistent identifier (e.g. DOI, Handle, URL etc.) per dataset or data type:

We will **not reuse** existing data for this project.

Are there any ethical issues concerning the creation and/or use of the data (e.g. experiments on humans or animals, dual use)? If so, refer to specific datasets or data types when appropriate and provide the relevant ethical approval number.

- Yes, human subject data (Provide SMEC or EC approval number below)
- Yes, animal data (Provide ECD reference number below)

- **human subject data**: approval pending (due during the first project year)
- **animal data**: ECD reference N°: P140/2023

Will you process personal data? If so, please refer to specific datasets or data types when appropriate and provide the KU Leuven or UZ Leuven privacy register number (G or S number).

- Yes (Provide PRET G-number or EC S-number below)

approval pending (due during the first project year)

Does your work have potential for commercial valorization (e.g. tech transfer, for example spin-offs, commercial exploitation, …)? If so, please comment per dataset or data type where appropriate.

- No

not applicable

Do existing 3rd party agreements restrict exploitation or dissemination of the data you (re)use (e.g. Material or Data transfer agreements, Research collaboration agreements)? If so, please explain in the comment section to what data they relate and what restrictions are in place.

- No

not applicable

Are there any other legal issues, such as intellectual property rights and ownership, to be managed related to the data you (re)use? If so, please explain in the comment section to what data they relate and which restrictions will be asserted.

- No

not applicable

Documentation and Metadata

Clearly describe what approach will be followed to capture the accompanying information necessary to keep data understandable and usable, for yourself and others, now and in the future (e.g. in terms of documentation levels and types required, procedures used, Electronic Lab Notebooks, README.txt files, codebook.tsv etc. where this information is recorded).

Main results and methods will be published in peer-reviewed journals (open access as required by KU Leuven Internal Funding regulations) and all publications will be archived in Lirias, the digital KU Leuven document repository.
All **digital data** generated in the project for each WP and associated metadata will be archived digitally and a searchable database format (Excel or Access) will be implemented. Electronic lab note books will be used and templates have been designed for writing protocols/SOPs, for excel spreadsheets for raw data and (statistical) analysis. When raw data are uploaded on repositories, keywords will be affixed along with readme files containing the needed information for reuse. In the final stage of the project, a master index file with the combined metadata for each WP will be generated and archived on a non-editable drive of the host institution KU Leuven ('K drive').
All **physical data** collected during the course of the project will be stored at designated storage places (at room temperature or frozen) and location and preservation method of the biological samples (tissues, tissue sections, blood plasma, genetic material) will be documented digitally (.xlsx files).

Will a metadata standard be used to make it easier to find and reuse the data?
If so, please specify which metadata standard will be used.

If not, please specify which metadata will be created to make the data easier to find and reuse.

- Yes

Metadata will be a combination of machine-generated and manually generated metadata. Metadata of raw images (file size, pixel number, acquisition date, settings, *etc.*) and qRT-PCR are captured automatically and saved on the server together with the corresponding data files. Other metadata (on quantification procedures, biochemical analysis, *etc.*) are mostly captured manually and logged in lab notebooks or in searchable Excel/Access databases. For these metadata, we will progressively design own metadata standards using http://dublincore.rog/. We will also consider archiving our data using general data repositories (https://figshare.com/ and https://zenodo.org/). RNAseq data will be uploaded to the GEO repository which uses the MIAME standard.

Data Storage & Back-up during the Research Project

Where will the data be stored?

- Shared network drive (J-drive)
- Other (specify below)
- Large Volume Storage

During the project, **digital data** will be stored at different locations, depending on the type of data and accessibility. *Non-personal data* will be stored on the researchers' computers, on the KU Leuven network editable drives where the principal investigator has reserved dedicated space for this project (the J drive for data that needs to be accessible daily and is exchangeable between KU Leuven-affiliated project participants and the L drive for longer-term storage of large data files that do not need to be frequently accessed). *Personal patient related-data* will be stored on the UZ Leuven central server. Both UZ Leuven and KU Leuven servers are compatible with GDPR regulations.

All **physical data** collected during the course of the project will be stored at designated storage places. An inventory of each storage place will be available.

How will the data be backed up?

- Standard back-up provided by KU Leuven ICTS for my storage solution

Researchers' computers are backed-up through constant **synchronisation** to the Box cloud of the KU Leuven (which provides 100 GB storage per KU Leuven researcher). The KU Leuven network drives and the UZ Leuven central server are automatically backed-up on a daily basis. Continuous KU Leuven network drive back-up is guaranteed and supervised by the KU Leuven ICT service and makes use of 'snapshot' technology. Once data sets do no longer need to be accesses and/or modified, (*e.g.*, after publication of manuscripts), archiving to a read-only KU Leuven network drive (the K drive) will be done to maintain a copy.

Is there currently sufficient storage & backup capacity during the project?

If no or insufficient storage or backup capacities are available, explain how this will be taken care of.

- Yes

not applicable

How will you ensure that the data are securely stored and not accessed or modified by unauthorized persons?

Digital data: The KU Leuven network servers allow for secure storage of *non-personal data* and are compatible with GDPR regulations. The access to the KU Leuven server is u-number and password controlled. KU Leuven ICTS services provide the option to control data access for authorised persons only (in this case, KU Leuven affiliated research lab members involved in this project). For *personal patient data* stored on the UZ Leuven central server, access will be restricted with access right management only to clinical data manager Annemie Devroye and by Prof. R. Lemmens.
All **physical data**, printed forms and notebooks are stored in the labs in locked cabinets. Access to the lab is secured and badge controlled.

What are the expected costs for data storage and backup during the research project? How will these costs be covered?

Costs for **digital data** storage and back-up during the project have been included in the research budget of the project. The current cost rates for the KU Leuven network drives are: 25€/y/100 GB block (KU Leuven Box cloud); 503.66€/y/1 TB block (J-drive) and 6.4€/y/100GB block (K-drive), 869€/y/5TB block (L-drive).

Data Preservation after the end of the Research Project

Which data will be retained for 10 years (or longer, in agreement with other retention policies that are applicable) after the end of the project?

In case some data cannot be preserved, clearly state the reasons for this (e.g. legal or contractual restrictions, storage/budget issues, institutional policies...).

- All data will be preserved for 10 years according to KU Leuven RDM policy

All **digital data** and metadata will be retained for 10 years after the project (per the requirements of Research Data Management policy of the KU Leuven). The same term will be applied to **physical data**. Long-term storage of **personal patient data** additionally requires GDPR clearance, which will be obtained upon approval from the Ethics Committee of UZ Leuven.

Where will these data be archived (stored and curated for the long-term)?

- Large Volume Storage (longterm for large volumes)

Digital data will be archived on the KU Leuven K drive for storage of read-only data.

What are the expected costs for data preservation during the expected retention period? How will these costs be covered?

The cost rate for storage on the K drive is 6.4€/year/100 GB. To store a total of 5 TB for 10 years, the estimated cost hence is 3,200 €. Costs will be allocated to the project budget.

Data Sharing and Reuse

Will the data (or part of the data) be made available for reuse after/during the project?
Please explain per dataset or data type which data will be made available.

- Yes, as restricted data (upon approval, or institutional access only)
- Yes, as open data
- Other (specify below)

Main findings of the research with all supporting processed data will be made available through publication in peer-reviewed journals with open access policies (as required by KU Leuven internal funds regulations). All manuscripts will also be deposited in the KU Leuven Lirias digital repository. Raw RNAseq data will be made available publicly on the GEO website upon acceptance of the manuscript. Other raw data related to published manuscripts may be available upon specific request as will be stated in a data availability statement included in the published manuscripts.

If access is restricted, please specify who will be able to access the data and under what conditions.

All KU Leuven-affiliated researchers involved in the project will have access to **non-personal data** on the KU Leuven servers through their u-number and accompanying personal password. **Personal patient data** will only be shared only with certain third parties (as will be specified in the GDPR addendum to the informed consent form) if needed, thereby always ensuring the privacy of the donors.

Are there any factors that restrict or prevent the sharing of (some of) the data (e.g. as defined in an agreement with a 3rd party, legal restrictions)?

Please explain per dataset or data type where appropriate.

- Yes, privacy aspects

Personal patient data will only be shared with certain third parties (as will be specified in the GDPR addendum to the informed consent form) if needed, thereby always ensuring the privacy of the donors.

Where will the data be made available?

If already known, please provide a repository per dataset or data type.

- Other data repository (specify below)
- Other (specify below)

Main findings of the research with all supporting processed data will be made available through publication in peer-reviewed journals with open access policies (as required by KU Leuven internal funds regulations). All manuscripts will also be deposited

in the KU Leuven Lirias digital repository. Raw RNAseq data will be made available publicly on the GEO website upon acceptance of the manuscript.

When will the data be made available?

- Upon publication of research results
- Other (specify below)

Data will be made available as publications at logical points during the course of the project when the research questions have been sufficiently addressed. Other data (including physical data) will be made available upon request, where considered appropriate, following publication.

Which data usage licenses are you going to provide?

If none, please explain why.

- CC-BY 4.0 (data)

All papers will be published in open access journals (according to KU Leuven internal funds regulations) under a Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) licence.

Do you intend to add a persistent identifier (PID) to your dataset(s), e.g. a DOI or accession number? If already available, please provide it here.

- Yes, a PID will be added upon deposit in a data repository

For raw snRNAseq data, a **GEO accession number** will be provided.

What are the expected costs for data sharing? How will these costs be covered?

For sharing digital data, no sharing costs are foreseen. For sharing physical data, Material Transfer Agreements will have to be put in place which will be mutually signed. Shipping costs would be covered by either party (through the C1 budget in case of the provider) as long as the costs are low, however, significant sharing costs will be expected to be borne by the requestor.

Responsibilities

Who will manage data documentation and metadata during the research project?

PhD students (R. Schellingen), post-docs (H. Kemps) and technicians (P. Vandervoort, M. Lox) will have the daily responsibility of record keeping of all data (digital, paper and biological samples). They will also be responsible for a correct and accurate data entry and recording of metadata. The PI's (Aernout Luttun and An Zwijsen) and the clinical data manager (Annemie Devroye) will be responsible for management of data documentation and metadata during the project.

Who will manage data storage and backup during the research project?

PhD students (R. Schellingen), post-docs (H. Kemps) and technicians (P. Vandervoort, M. Lox) will have the daily responsibility of record keeping of all data (digital, paper and biological samples). They will also be responsible for a correct and accurate data entry and recording of metadata. The PI's (Aernout Luttun and An Zwijsen) and the clinical data manager (Annemie Devroye) will be responsible for data storage and back-up during the project.

Who will manage data preservation and sharing?

The PI's (Aernout Luttun and An Zwijsen).

Who will update and implement this DMP?

The PI's (Aernout Luttun and An Zwijsen).