Investigating the mechanisms leading to neuronal necroptosis in Alzheimer's Disease

Project Name: Investigating the mechanisms leading to neuronal necroptosis in Alzheimer's Disease

Project Identifier: DMP_11N0722N

Grant Title: 11N0722N

Principal Investigator / Researcher: Iordana Chrysidou

Project Data Contact: iordana.chrysidou@kuleuven.vib.be

Institution: KU Leuven

Description: Alzheimer's disease (AD) is a neurodegenerative disease of the brain and the most common cause of dementia. Pathologically, AD is characterized by the extensive deposition of abnormal proteins in the brain, namely amyloid beta (Aβ) plaques and Tau tangles. While Aβ is the initiating trigger of the cascade leading to neuronal loss in AD, Tau is spatiotemporally correlated with brain atrophy, cognitive decline and necroptotic signatures. Neuronal necroptosis has recently been described in AD, and it is executed by the activation of the necrosome complex, composed of three key phosphorylated proteins: pRIPK1, pRIPK3 and pMLKL. Multiple studies indicate that pRIPK1 is the initiator of necrosome assembly. Research in our lab verifies its activation in AD patients and humanized models. However, the exact mechanism of pRIPK1-mediated activation of the necrosome complex in AD has not been investigated. Furthermore, whether this mechanism is triggered downstream or in parallel to Tau pathology is unknown. In this project, we will test a functional link between Tau and necroptosis by using stem cell engineering to generate human neurons in our chimeric AD model, and to employ proteomic and phosphoproteomic analysis in wild type human neuronal xenografts to elucidate the mechanisms that lead to activation of necroptosis in AD. This represents an unparalleled approach to elucidate the mechanisms leading to necroptotic neurodegeneration in AD.

1. General Information

Name applicant: Iordana Chrysidou

FWO Project Number & Title: 11N0722N - Investigating the mechanisms leading to neuronal necroptosis in Alzheimer's Disease

Affiliation: KU Leuven

2. Data description

Will you generate/collect new data and/or make use of existing data?

- Generate new data
- Reuse existing data

Describe in detail the origin, type and format of the data (per dataset) and its (estimated) volume. This may be easiest in a table (see example) or as a data flow and per WP or objective of the project.

Type of data	Format	How created
Microscopy images	-Digital images in raster formats: uncompressed TIFF (.tif/.tiff), JPEG (.jpg), JPEG 2000 (.jp2), Adobe Portable Document Format (.pdf), bitmap (.bmp), .gif; -Scalable vector graphics (.svg), encapsulated postscript (.eps), Scalable Vector Graphics (.svg), Adobe Illustrator (.ai);	Microscopy pictures, gel scans, graphs, illustrations, figures.
Omics data	-Next generation sequencing raw data: binary base call format (.bcl), .fastq(.gz) -Read/UMI count data: .tsv(.gz), Matrix Market format (.mtx), .loom, .rds(.gz)	Genomics, transcriptomics, proteomics
Canonical data	Nucleotide and protein sequences: raw sequence data trace (.ab1), text-based format (.fasta/.fa) and accompanying QUAL file (.qual), Genbank format (.gb/.gbk); Protein structures: Protein Data Bank format (.pdb / .pdbx);	-Nucleic acid sequences -Protein sequences -Chemical structures -Protein structures -Bacterial and viral vectors
Cell Lines	Pluripotent stem cell lines	Biobank
Biological Samples	Cryovials, samples stord at 4, -20 and -80°C	Live animals, frozen samples

The approximate volume of all the datasets included will be ~ 2TB.

Research documentation generated by the research and technical staff or collected from online sources and from collaborators, including ethical approval documents, laboratory notes, protocols, animal husbandry data. Data will be stored in the following formats:

- Text files: Rich Text Format (.rtf), plain text data (Unicode, .txt), MS Word (.doc/.docx), eXtensible Mark-up Language (.xml), Adobe Portable Document Format (.pdf), LaTex (.tex) format;
- Quantitative tabular data: comma-separated value files (.csv), tab-delimited file (.tab), delimited text (.txt), MS Excel (.xls/.xlsx), MS Access (.mdb/.accdb);

Derived and compiled data

- Manuscripts
- Algorithms and scripts
- Software

These datasets represent an important source of information for the laboratory of the PI (including future staff), for scientists, journalists and higher education teachers working in the field of biomedical sciences, but also for non-profit organizations and industry.

3. Legal and ethical issues

Will you use personal data? If so, shortly describe the kind of personal data you will use. Add the reference to your file in KU Leuven's Register of Data Processing for Research and Public Service Purposes (PRET application). Be aware that registering the fact that you process personal data is a legal obligation.

- Yes

Short description of the kind of personal data that will be used:

We use commercially available stem cells where the genotype and gender is the only personal data known. For the use of commercial stem cell lines we follow the ethical application (S62888) with the following PRET application: G-2021-3588

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, add the reference to the formal approval by the relevant ethical review committee(s)

- Yes

Project license number: 165/2017

Project license name: Role of TAU in human-specific neurodegeneration in Alzheimer's Disease

Genetically modified organisms: All experiments are conducted according to protocols approved by the local Ethical Committee of Laboratory Animals of the KU Leuven (government license LA1210579, ECD project number 165/2017) following local and EU guidelines. Animals are housed in facilities of the Laboratory Animal Center of KU Leuven, which applies Standard Operation Procedures concerning housing, feeding, health monitoring to assure consistent care in accordance with European and national regulations and guidelines. Animal administrative, husbandry and animal welfare data are sensitive data and are stored in the LAIS database according to security procedure of KU Leuven. For the use of commercial stem cell lines we follow the ethical application (S62888) with the following PRET application: G-2021-3588

Does your work possibly result in research data with potential for tech transfer and valorisation? Will IP restrictions be claimed for the data you created? If so, for what data and which restrictions will be asserted?

- Yes

We do not exclude that the proposed work could result in research data with potential for tech transfer and valorization. VIB has a policy to actively monitor research data for such potential. If there is substantial potential, the invention will be thoroughly assessed, and in a number of cases the invention will be IP protected (mostly patent protection or copyright protection). As such the IP protection does not withhold the research data from being made public. In the case a decision is taken to file a patent application it will be planned so that publications need not be delayed.

Do existing 3rd party agreements restrict dissemination or exploitation of the data you (re)use? If so, to what data do they relate and what restrictions are in place?

- No

No third-party agreement restricts dissemination or exploitation of the data from this project. In particular, existing agreements between VIB and KU Leuven do not restrict publication of data.

4. Documentation and metadata

What documentation will be provided to enable reuse of the data collected/generated in this project?

Data will be generated following standardized protocols. Metadata will be documented by the research and technical staff at the time of data collection and analysis, by taking careful notes in the Electronic Laboratory Notebook (ELN) and/or in hard copy lab notebooks that refer to specific datasets.

Cryotubes of biological samples (brain samples, cell lines) will be labelled with a reference number that links them to our Sample Storage Manager (SSM) database.

All datasets will be accompanied by a README.txt file containing all the associated metadata (see more details below).

Clear and detailed descriptions of the protocols used will be published along with the results.

Will a metadata standard be used? If so, describe in detail which standard will be used. If no, state in detail which metadata will be created to make the data easy/easier to find and reuse.

- Yes

While specific data types might require particular metadata, as a general rule the metadata will be based on a generalized metadata schema such as Dublin Core or DataCite, including the following elements:

- Title: free text
- Creator: Last name, first name, organizationDate and time reference
- Subject: Choice of keywords and classifications
- Description: Text explaining the content of the data set and other contextual information needed for the correct interpretation of the data, the software(s) (including version number) used to produce and to read the data, the purpose ofthe experiment, etc.
- Format: Details of the file format,
- Resource Type: data set, image, audio, etc.Identifier: DOI (when applicable)
- Access rights: closed access, embargoed access, restricted access, openaccess.

For specific datasets, additional metadata will be associated with the data file as appropriate. When depositing data in a repository, the final dataset will be accompanied by this information under the form of a README.txt document. This file will be located in the top-level directory of the dataset and will also list the contents of the other files and outline the file-naming convention used. This will allow the data to be understood by other members of the laboratory and add contextual value to the dataset for future reuse.

5. Data storage and backup during the FWO project

Where will the data be stored?

- Digital files will be stored on KU Leuven servers (“L-drive”).
- Tissue samples: Tissues will be stored locally in the laboratory and labelled with a reference

number that links them to our Sample Storage Manager (SSM) database. All human tissue samples will be registered with a Belgian biobank, in compliance with the Belgian law on human body material (dd19-12-2008).

- Cell lines: Newly created human pluripotent cell lines will be deposited in the hPSCreg database. Other cell lines will be stored locally in the laboratory, in cryotubes labelled with a reference number that links them to our Sample Storage Manager (SSM) database.

- Omics data: omics data generated during the project will either be stored on KU Leuven servers or on The Flemish Supercomputer Centre (VSC), initially in the staging area and later in the archive area.

-Genetically modified organisms: Mice will be maintained in facilities of the Laboratory Animal Center of KU Leuven, which applies Standard Operation Procedures concerning housing, feeding, health monitoring to assure consistent care in accordance with European and national regulations and guidelines. All animals will be registered in the Leuven Animal Information System (LAIS) database, along with corresponding genotyping information, ethical approval documents and animal provider receipts.

- Other biological and chemical samples: storage at 4°C, and/or as frozen samples in cryovials at -20°C and -80°C as appropriate.

- Algorithms, scripts and softwares: All the relevant algorithms, scripts and software code driving the project will be stored in a private online git repository from the GitHub account of the department (https://github.com/vibcbd).

- Nucleic acid and protein sequences: All nucleic acid and protein sequences generated during the project will be stored on KU Leuven servers. Upon publication, all sequences supporting a manuscript will be made publicly available via repositories such as the GenBank database or the European Nucleotide Archive (nucleotide sequences from primers / new genes / new genomes), NCBI Gene Expression Omnibus (microarray data / RNA-seq data / CHIPseq data), the Protein Database (for protein sequences), the EBI European Genome-phenome Archive (EGA) for personally identifiable (epi)genome and transcriptome sequences.

How is backup of the data provided?

KU Leuven drives are backed-up according to the following scheme:

- Data stored on the “L-drive” is backed up daily using snapshot technology, where all incremental changes in respect of the previous version are kept online; the last 14 backups are kept.
- Data stored on the “J-drive” is backed up hourly, daily (every day at midnight) and weekly (at midnight between Saturday and Sunday); in each case the last 6 backups are kept.
- Data stored on the digital vault is backed up using snapshot technology, where all incremental changes in respect of the previous version are kept online. As standard, 10% of the requested storage is reserved for backups using the following backup regime: an hourly backup (at 8 a.m., 12 p.m., 4 p.m. and 8 p.m.), the last 6 of which are kept; a daily backup (every day) at midnight, the last 6 of which are kept; and a weekly backup (every week) at midnight between Saturday and Sunday, the last 2 of which are kept.

- All omics data stored on the Flemish Supercomputer Centre (VSC) will be transferred on a weekly basis to the archive area which is backed up.

Is there currently sufficient storage & backup capacity during the project? If yes, specify concisely. If no or insufficient storage or backup capacities are available then explain how this will be taken care of.

- Yes

All data will be stored at servers managed by KU Leuven where storage space can be increased if necessary.

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

- The total estimated cost of data storage during the project is about 4000€ (for a final volume of 5 TB). This estimation is based on the following costs:
- The costs of digital data storage are as follows: 173,78€/TB/Year for the "L-drive" and 519€/TB/Year for the "J-drive".
- Maintaining a mouse colony alive costs about 1,200 euro per year (for 6 cages), excluding the costs of genotyping. When no experiment is planned with a particular mouse strain, and in compliance with the 3R's rule (https://www.nc3rs.org.uk), cryopreservation will thus be used to safeguard the strain, prevent genetic drift, loss of transgene and potential infections or breeding problems. Cryopreservation of sperm/embryos costs about 500 to 700 euro per genotype, plus a minimal annual storage fee (25 euro per strain for 250 to 500 embryos). Frozen specimen are kept in two separate liquid nitrogen tanks at two different sites on campus. When necessary, the costs of revitalization from cryopreserved sperm/embryos are about 1,100/600 euro.
- Electricity costs for the -80° freezers present in the labs are included in general lab costs.
- Data storage and backup costs are included in general lab cost

Data security: how will you ensure that the data are securely stored and not accessed or modified by unauthorized persons?

Both the "L-drive" and "J-drive" servers are accessible only by laboratory members and are mirrored in the second ICTS datacenter for business continuity and disaster recovery so that a copy of the data can be recovered within an hour. Access to the digital vault is possible only through using a KU Leuven user-id and password, and user rights only grant access to the data in their own vault. Sensitive data transfer will be performed according to the best practices for "Copying data to the secure environment" defined by KU Leuven. The operating system of the vault is maintained on a monthly basis, including the application of upgrades and security patches. The server in the vault is managed by ICTS, and only ICTS personnel (bound by the ICT code of conduct for staff) have administrator/root rights. A security service monitors the technical

installations continuously, even outside working hours.All private data will be rendered anonymous before processing outside the digital vault. Only the PI will be granted access to the server to deposit private data. The PI will be the only responsible for linking patient information, survey data and/or tissue samples, and will strictly respect confidentiality. All de-identified data will be exported from the database by the PI, and stored on KU Leuven servers from where it can be accessed by the research and technical staff from the laboratory.

6. Data preservation after the FWO project

Which data will be retained for the expected 5 year period after the end of the project? In case only a selection of the data can/will be preserved, clearly state the reasons for this (legal or contractual restrictions, physical preservation issues, ...).

The minimum preservation term of 5 years after the end of the project will be applied to all datasets. All datasets will be stored on the university's central servers with automatic back-up procedures for at least 5 years, conform the KU Leuven RDM policy.

Where will the data be archived (= stored for the longer term)?

As a general rule, datasets will be made openly accessible, whenever possible via existing platforms that support FAIR data sharing (www.fairsharing.org), at the latest at the time of publication. For all other datasets, long term storage will be ensured as follows:

- Digital datasets: files will be stored on the “L-drive”.
- Tissue samples: Tissues will be stored locally in the laboratory.
- Omics data: datasets will be stored on the “L-drive” or, for larger datasets, on the Vlaams Supercomputer Centrum.
- Cell lines: cell lines will be stored locally in the laboratory (-80°C).
- Vectors: As a general rule at least two independently obtained clones will be preserved for each vector, both under the form of purified DNA (in -20°C freezer) and as a bacteria glycerol stock (-80°C).
- Genetically modified organisms: All other lines that are no actively used for experiments will be cryopreserved.
- Other biological and chemical samples: storage at 4°C and/or as frozen samples in cryovials as appropriate

What are the expected costs for data preservation during the retention period of 5 years? How will the costs be covered?

The total estimated cost of data storage during the project is about 4000€ (for a final volume of

5 TB). This estimation is based on the following costs:

- The costs of digital data storage are as follows: 173,78€/TB/Year for the “L-drive” and 519€/TB/Year for the “J-drive”.
- Maintaining a mouse colony alive costs about 1,200 euro per year (for 6 cages), excluding the costs of genotyping. When no experiment is planned with a particular mouse strain, and in compliance with the 3R’s rule (https://www.nc3rs.org.uk), cryopreservation will thus be used to safeguard the strain, prevent genetic drift, loss of transgene and potential infections or breeding problems. Cryopreservation of sperm/embryos costs about 500 to 700 euro per genotype, plus a minimal annual storage fee (25 euro per strain for 250 to 500 embryos). Frozen specimen are kept in two separate liquid nitrogen tanks at two different sites on campus. When necessary, the costs of revitalization from cryopreserved sperm/embryos are about 1,100/600 euro.

7. Data sharing and reuse

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

- No

Which data will be made available after the end of the project?

Participants to the present project are committed to publish research results to communicate them to peers and to a wide audience. All research outputs supporting publications will be made openly accessible. Depending on their nature, some data may be made available prior to publication, either on an individual basis to interested researchers and/or potential new collaborators, or publicly via repositories (e.g. negative data).

Where/how will the data be made available for reuse?

- In an Open Access repository
- Upon request by mail
- Other (specify):

Open-access publications in peer-reviewed journals, including supplemental informationAs a general rule, datasets will be made openly accessible via existing platforms that support FAIR data sharing (www.fairsharing.org). Sharing policies for specific research outputs are detailed below:

- Omics datasets will be deposited in open access repositories such as the PRIDE Archive for proteomics data, the EMBL-EBI platform for genomics and epigenomics data, or the NCBI Gene Expression Omnibus (GEO) or the EBI ArrayExpress databases for functional genomics data.
- Vectors: Upon publication, all vectors supporting a manuscript will be made publicly available via the non-profit plasmid repository Addgene, along with the corresponding DNA sequences. Addgene in turn, performs quality control on the DNA, curates the plasmids online with all relevant information (maps, sequences), and for a minimal cost (typically $65) ships the vectors upon simple request and signature of a material transfer agreement. The MTA will be prepared before depositing the vectors with the help of our organization's Tech Transfer office. For transfer between nonprofit or academic institutions, Addgene typically uses the Uniform Biological Material Transfer Agreement (https://www.addgene.org/terms/1047/). All non-published vectors and the associated documentation will be shared by the PI upon request and after signature of a material transfer agreement, at no cost except the cost of shipment.
- Cell lines: All human pluripotent cell lines supporting publications will be

registered in hPSCreg, the European human embryonic stem cell registry supported by the European Commission (https://hpscreg.eu/). Information about the deposited lines (including donor information, derivation method, availability and characterization) will also be made accessible. Registration of cell lines in hPSCreg will provide visibility, confirm ethical procurement and facilitate comparison with other hPSC lines. The PI will remain the distributor of the pluripotent cell lines. All other cell lines supporting publications will be deposited in the American Type Culture Collection (ATCC) database (https://www.atcc.org/), which is a private, non-profit biological resource center. This will provide a secure back-up for this material. Investigators can purchase cell lines from the ATCC database upon signature of a material transfer agreement (https://www.lgcstandards-atcc.org/~/media/PDFs/MTA_2.ashx) and, in some cases, of a Limited Use/Label License (e.g. for CRISPR products or iPSC materials) and/or a Customer Acceptance of Responsibility (for potentially highly pathogenic materials). Information about the cell lines (including organism, cell type, tissue, biosafety level and disease if applicable) will also be made accessible.

- Genetically modified organisms: All genetically modified organisms used in publications will be made available to researchers upon request at the time of publication.
- Other digital datasets that support publications (including image, video or audio files, electrophysiology data, cytometry data, spectroscopy data and simulation data) will be made publicly available via an open research data platform such as Mendeley Data or Zenodo.
- Synthetic and recombinant compounds: samples will be stored as appropriate in the laboratory. Within availability, they will be shared with interested researchers upon request.
- Research documentation: All protocols used to generate published data will be described in the corresponding manuscript(s), and the related documentation will be included as supplementary information. These data and all other documents (daily logs, raw data) deposited in the E-Notebook are accessible to the PI and the research staff, and will be made available upon request.
- Manuscripts: All scientific publications will be shared openly. Manuscripts submitted for publication will be deposited in a pre-print server such as bioRxiv, arXiv, Nature Precedings or ASAPbio. At the time of publication, research results will be summarized on the PI's website and post-print pdf versions of publications will be made available there if allowed by copyright agreements, possibly after an embargo as determined by the publisher. Before the end of the embargo or in cases where sharing the post-print is not allowed due to copyright agreements, a pre-print version of the manuscript will be made available. Publications will also be automatically added to our institutional repository, Lirias 2.0, based on the authors name and ORCID ID.
- Algorithms, scripts and software: All the relevant algorithms, scripts and software code driving the project will be stored in a private online git repository from the GitHub account of the department (https://github.com/vibcbd). As soon as the

manuscript is publicly available, the repository will be changed to a public repository.

- Nucleic acid and protein sequences: All nucleic acid and protein sequences generated during the project will be stored on KU Leuven servers. Upon publication, all sequences supporting a manuscript will be made publicly available via repositories such as the GenBank database or the European Nucleotide Archive (nucleotide sequences from primers / new genes / new genomes), NCBI Gene Expression
- Omnibus (microarray data / RNA-seq data / CHIPseq data), the Protein Database (for protein sequences).
- Protein structures: Upon publication, all structures supporting a manuscript will be made publicly available as supporting information and via the Protein Data Bank in Europe (PDBe) database. Coordinate sets produced by X-ray crystallography / NMR / electron microscopy / neutron diffraction / powder diffraction / fiber diffraction will be deposited to the PDBe together with the corresponding metadata (project acronym, author contact information, context and method of data collection, information about the sequence, chemistry, etc.). Datasets will be cited using the Digital Object Identifier (DOI) link generated by the PDBe, and will also be accessible via the elaborate search function available via PDBe.Data that do not support publication will be either deposited in an open access repository or made available upon request by email

When will the data be made available?

- Upon publication of the research results

As a general rule, all research outputs will be made openly accessible at the latest at the time of publication. No embargo will be foreseen unless imposed e.g. by pending publications, potential IP requirements – note that patent application filing will be planned so that publications need not be delayed - or ongoing projects requiring confidential data. In those cases, datasets will be made publicly available as soon asthe embargo date is reached.

Who will be able to access the data and under what conditions?

Whenever possible, datasets and the appropriate metadata will be made publicly available through repositories that support FAIR data sharing. As detailed above, metadata will contain sufficient information to support data interpretation and reuse, and will be conform to community norms. These repositories clearly describe their conditions of use (typically under a Creative Commons CC0 1.0 Universal (CC0 1.0) Public Domain Dedication, a Creative Commons Attribution (CC-BY) or an ODC Public Domain Dedication and Licence, with a material transfer agreement when applicable). Interested parties will thereby be allowed to access data directly, and they will give credit to the authors for the data used by citing the corresponding DOI. For data shared directly by the PI, a material transfer agreement (and a non-disclosure agreement if applicable) will be concluded with the beneficiaries in order to clearly describe the types of reuse that are permitted.

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

It is the intention to minimize data management costs by implementing standard procedures e.g. for metadata collection and file storage and organization from the start of the project, and by using free-to-use data repositories and dissemination facilities whenever possible. Data management costs will be covered by the laboratory budget. A budget for publication costs has been requested in this project.

8. Responsibilities

Who will be responsible for data documentation & metadata?

Metadata will be documented by the research and technical staff at the time of data collection and analysis, by taking careful notes in the electronic laboratory notebook (ELN) that refer to specific datasets.

Who will be responsible for data storage & back up during the project?

The research and technical staff will ensure data storage and back up, with support from Raf De Coster for the KU Leuven drives and from René Custers and Alexander Botzki for the electronic lab notebook (ELN).

Who will be responsible for ensuring data preservation and reuse ?

The PI is responsible for data preservation and sharing, with support from the research and technical staff involved in the project, from Raf De Coster for the KU Leuven drives and from René Custers and Alexander Botzki for the electronic lab notebook (ELN).

Who bears the end responsibility for updating & implementing this DMP?

The PI is ultimately responsible for all data management during and after data collection, including implementing and updating the DMP.