DMP title

Project Name Mechanisms of necrotic cell debris deposition and stability in acute injury - DMP title

Project Identifier 1116922N

Principal Investigator / Researcher Sara Schuermans

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Description Necrosis is a form of cell death in which cellular contents such as DNA, histones and actin are released. These components serve as danger signals and induce inflammation. Thus, necrotic debris is associated with diverse acute diseases, including drug-induced liver injury. Importantly, therapies to resolve problems caused by necrotic cell debris are currently inexistent. I propose that the burden of degrading necrotic debris, more specifically necrotic DNA, is left to soluble deoxyribonucleases (DNAses) in the bloodstream. Although DNAses are abundantly present, it is puzzling that the necrotic DNA is not cleared immediately from the injury sites and accumulates in the tissue for days. The aim of this project is to characterize biochemically the necrotic cell debris in vitro and in mouse models of acute liver injury. Moreover, I will determine why necrotic debris is resistant to degradation and as a result drives diseases. This knowledge will be used for the development of peptides to accelerate debris clearance by disassembling DNA-protein complexes. The beneficial effects of the molecules will be assessed in vitro and in mice, and our findings will be validated in samples from drug-induced liver injury patients. By accomplishing this project, I hope to unveil new mechanisms that will be valuable for developing therapies to resolve damage caused by necrotic injuries.

Institution KU Leuven

1. General Information

Name applicant

Sara Schuermans

FWO Project Number & Title

1116922N: Mechanisms of necrotic cell debris deposition and stability in acute injury

Affiliation

- KU Leuven

Laboratory of Molecular Immunology, Rega Institute for Medical Research

2. Data description

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

- Generate new 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. If you reuse existing data, specify the source of these data. Distinguish data types (the kind of content) from data formats (the technical format).

Several datatypes will be generated during this research project. The datatypes include images and movies from immunostainings, intravital microscopy, live-cell imaging, *in vitro* debris models, western blotting and mass spectrometry data. Imaging data is initially stored in the proprietary manufacturer formats (necessary). Final and publication grade images will be saved in .tif or .png formats. Videos will be stored in .mov or .mpeg formats. The imaging data, consisting of single frames, videos and 3D images, will be acquired and accumulated throughout the project objectives. We estimate 1 TB per year, up to a total of 4 TB by the end of the project. Numerical data will be generated as well, from the analysis of the images, ELISA and other spectrophotometric and biochemical assays, and saved in .xlsx format. Numerical data is estimated in the order of hundreds of megabytes per year, also cumulative up to 2 TB by the end of the project. All protocols, raw data and analyzed data produced will be cataloged in laboratory books and stored as digital files (.xlsx and .docx formats).

Objective 1: Establishment of both *in vitro* and *in vivo* models of necrosis.
Tissue and blood collection of an acute liver injury mouse model will give us numerical data in .xlsx or .csv formats (ALT and ICG assays, ELISAs). Microscopy images of both the *in vitro* debris model and liver cryosections of the mouse model will be generated in .tif

format. Image quantification will be performed and out of this, numerical data will be obtained in .xlsx or .csv formats. Biochemical LDH assays will provide us with numerical data in .xlsx or .csv formats.

Objective 2: Characterization of necrotic debris composition *in vitro* and *in vivo*.
Microscopy images and movies will be generated in .tif and .mov formats during intravital imaging. Image quantification will be performed and out of this, numerical data will be obtained in .xlsx or .csv formats. Moreover, microscopy images of both the *in vitro* debris model and liver cryosections of the mouse model will be generated in .tif format. Western blotting will generate images in .tif format. Mass spectrometry imaging data will generate folders containing the spectral files in *.d format, the X,Y coordinate position logs in *.txt format, the optical scan of the imaged section in *.tif format, and the *.mis file used to open the dataset in the proprietary Bruker FlexImaging software. Processing and data analysis of the mass spectrometry imaging data will be performed using SciLS Lab version 2016b and will yield *.sl files. MS/MS specta used to assign the relevant masses as determined by analysis of the MS images will be obtained in *.RAW format and will be exported as *.pdf files.

Objective 3: Determining the contribution of histones and actin to necrotic debris stability.
Numerical data will be generated by ELISA, western blotting and spectrofluorometry in .xlsx or .csv formats. Microscopy images and movies of the *in vitro* debris spot and liver cryosections treated with proteases and/or DNAse will be generated in .tif and .mov formats.

Objective 4: Creation and validation of peptides to disassemble necrotic debris.
During this objective, amino acid sequences of peptides will be generated. Debris displacement by peptides will be evaluated using western blotting, which will generate images in .tif format, and numerical data in .xlsx format. Images of labeled peptides bound to the *in vitro* debris model will be generated in .tif format. Surface plasmon resonance data related to peptide binding to debris components will be genetared in .xlsx format. Afterwards, *in vitro* and *in vivo* toxicity tests (LDH, MTT, ALT and creatinine assays, ELISAs) will generate numerical data in .xlsx or .csv formats. Finally, histology images will be generated in .tif format.

Objective 5: Validation of the debris composition in human samples and therapeutic potential of peptides in the mouse model.
Tissue and blood collection of mice with acute liver injury that are treated with peptides will provide us with numerical data in .xlsx or .csv formats (ALT and ICG assays, ELISAs). Microscopy images of liver cryosections of the mouse model will be generated in .tif format, and both microscopy images and movies will be generated in .tif and .mov formats during intravital imaging of mice. Liver samples from patients with drug-induced liver injury will be used for immunoflorescence and mass spectrometry imaging. Mass spectrometry imaging data will be generated as previously described in Objective 2, and microscopy images will be generated in .tif format. Analysis of serum samples from patients (ELISA, western blotting and single radial enzyme diffusion) will give us images in .tif format and numerical data in .xlsx and .csv formats.

Type of data	Format	Volume	How created
(Microscopy) images and movies	.tif or .png .mov or .mpeg .RAW .pdf	1 TB/year	• Intravital microscopy of APAP and control mice (WP2, WP3, WP5) • *In vitro* debris model (WP1-WP4) • Immunostainings and histology of cryosections of injured and control livers (WP1-WP5) • Western blotting data (WP2-WP5) • Mass spectrometry imaging (WP2, WP5)
Numeric data	.xlsx .csv .docx .emf .pzfx .mis .sl .txt .d	500 GB/year	• ELISA, ALT, LDH, ICG and other spectrophotometric assays (WP1-WP5) • In- and output data of solid-phase peptide synthesis (WP4) • HPLC-MS files (WP4) • Surface plasmon resonance data (WP4) • Data inserted into Graphpad Prism for analysis (WP1-WP5) • Mass spectrometry imaging (WP2, WP5)

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.

- No

We will not use personal data of the patients and healthy controls included in the study.

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

The Ethical Committee for Animal Experimentation at KU Leuven approved the use of mice during this project (P125/2019 and P128/2021). The Ethics Committee Research UZ Leuven/KU Leuven approved the use of healthy donors for the purposes of this project (S58418). Request for ethical approval for the use of patient samples will be submitted to the Ethics Committee Research UZ Leuven/KU Leuven.

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

All research data generated during this project will be secured by the need for login registration on datacenter/luna and use of u-number and password, which are also restricted. In case of potential IP establishment for one or more peptides developed in the project, the restriction will consist of omission of the peptide sequence and codenaming of the peptide. All other information will be available without restrictions. These restrictions will be lifted as soon as IP is secured.

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

4. Documentation and metadata

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

- Microscopy images: Imaging data is created by default with metadata imprinted by the image acquisition software's automatically. That includes information on user, date and time, duration of experiments, equipment parameters and imaging configurations. The metadata is saved and transferred with the original imaging file. The created data files will be organized in folders named by the date of the experiment (YYYYMMDD) followed by the researcher who performed it and the title of the experiment. The methodology and protocol of each experiment will be described in detail in a lab book.
- The numerical data obtained in quantifications and spectrophotometric analyses will be saved in excel and word formats (.xlsx and .doc), which also imprint automatically the metadata (user, date, time, equipment parameters) from those experiments. Moreover, information on quantification and experimentation parameters will be embedded by the users on the document folders in order to improve data reproducibility and maintenance. The methodology and protocol of each experiment will be described in detail in a lab book.

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.

- No

5. Data storage and backup during the FWO project

Where will the data be stored?

Electronical data will be stored in conformity with KU Leuven and FWO RDM policy. The research data will be stored in several locations, including on internal computer disks, at the shared local virtual drive (Rega drive), on internal computer disks, in redundant NAS (network adapted storage)-devices, on Microsoft OneDrive and on the KU Leuven central storage servers. The KU Leuven datacenters provide storage on two locations and promise high availability and disaster recovery to preserve data for a long period. Hard copy notebooks with raw data will be stored physically in our laboratory. The large raw data volumes from analysis equipment are stored redundant on hard disks in or connected to the lab computers and the workstations. The backups of the analysis data are stored on dedicated redundant NAS-devices. Also, we will use the Lirias platform as data repository for published material.

How is backup of the data provided?

We will use the central server storage of KU Leuven (Data centre ICTS Luna storage), which provides a daily automatic back up. Moreover, the data will be backed up on the Rega Institute Virtual Drives (Rega NAS (network adapted storage)) and on external hard-drives kept by the investigators.

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

Enough storage and back-up capacity is available at the systems of Rega Institute. The local shared drives on the computer offer enough storage. Additionally, KU Leuven offers storage on Microsoft OneDrive.

What are the expected costs for data storage and back up during the project? How

will these costs be covered?

Long-term data storage and costs will be managed by the principal investigator working in the project, Prof. Pedro Elias Marques. The cost for data storage is 520 Euro/terabyte/year, thus, the accumulated cost for 4 years is approximately 5200 euro. The costs will be covered by previous funding obtained by the host lab and by the bench fee offered by the FWO PhD fellowship.

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

Research data are secured by the need for login registration on datacentre/luna and use of u-number and password, which are also restricted.

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, ...).

All data generated during this project, raw or processed, will be stored for a minimum of 5 years.

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

The data will be stored redundantly during and after the research on our PCs, on external hard-drives, on the personal KU Leuven Onedrive and in the KU Leuven data centers (ICTS Luna storage and Rega NAS (network adapted storage)).

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

Long-term data storage and costs will be managed by the principal investigator of the project, Prof. Pedro Marques. The expected cost for data storage is 520 euro/terabyte/year.

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?

All the data that is not under IP protection.

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

Data will be available by access to the data storage facilities of KU Leuven and the Rega Institute as described above. Access to external users will be evaluated and authorized by Prof. Pedro Marques.

When will the data be made available?

- After an embargo period. Specify the length of the embargo and why this is necessary
- Upon publication of the research results

Data will be made available immediately after publication and clearance by Intellectual Property officers at KU Leuven.

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

Direct access to the research data will be restricted to laboratory members, project members and collaborators. External members, who are not directly related to the project, will be given access after contact and evaluation by the principal investigator, Prof. Pedro Marques.

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

Local costs are minimal. Data transfer to external partners will be a at the partners' cost.

8. Responsibilities

Who will be responsible for data documentation & metadata?

The principal investigator (Prof. Pedro Elias Marques) and the researcher (Sara Schuermans) will be responsible for the data documentation.

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

The principal investigator (Prof. Pedro Elias Marques) and the researcher (Sara Schuermans) will be responsible for the data storage and back up.

Who will be responsible for ensuring data preservation and reuse ?

The principal investigator (Prof. Pedro Elias Marques) and the researcher (Sara Schuermans) will be responsible for this.

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

The principal investigator (Prof. Pedro Elias Marques) and the researcher (Sara Schuermans) will be responsible for implementing the DMP. They will update the DMP anytime conditions change. A mid-term review will be accompanied by a detailed DMP and a final reviewed DMP will be sent along with the final report.