DMP title

Project Name FWO_ DMP_1295122N_Single cell investigation of intussusceptive angiogenesis in health and disease - DMP title

Project Identifier u0134882

Grant Title 1295122N

Principal Investigator / Researcher Alessandra Pasut

Description In this project I propose to use single cell technologies to (i) investigate the cellular and molecular signature of intussusceptive angiogenesis (IA) across different species and tissues and (ii) discover novel targets to treat pathological angiogenesis.

Institution KU Leuven

1. General Information

Name applicant

Alessandra Pasut

FWO Project Number & Title

Project number:1295122N

Title: SINGLE CELL INVESTIGATION OF INTUSSUSCEPTIVE ANGIOGENESIS IN HEALTH AND DISEASE

Affiliation

- KU Leuven

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).

WP1: MODELS OF INTUSSUCEPTIEV ANGIOGENESIS (IA) & OPTIMIZATION OF PROTOCOLS

(common for both tasks 1.1. and 1.2)

1. Isolation of endothelial cells (ECs)

- Flow cytometry data analyzed by FlowJo (**.fcs** files) - processed/analyzed data: excel files **(.xlsx**)

2. Imaging of endothelial cells/vasculature

- digital images processed by the ImageJ/FiJi JAVA package (**.tiff / .jpeg**) and analyzed via excel (.**xlsx)**

WP2: SINGLE CELL RNA-SEQ ANALYSIS OF IA IN CHICKEN & MOUSE MODEL

Task 2.1.Single cell library sequencing

- Single-cell RNA sequencing analysis raw, unprocessed data files (**.fastq**) will be aligned to the mouse or chicken genome and the feature-barcode matrix will be generated using CellRanger 6.0.1 (.**csv)**

Task 2.2 and 2.3. single cell data analyses

- transcriptome-based cell clustering, differential expression analysis and function enrichment analysis, ligand-receptor interaction analysis will be performed using the Seurat package implemented in R 4.0.1 (**.csv)**

WP3: IA GENE SIGNATURE IN COVID-19 and WP4: CONSERVED IA GENE EXPRESSION SIGNATURE

(common for task 3.1. and 4.1)

- transcriptome-based cell clustering, differential expression analysis and function enrichment analysis, will be performed using the Seurat package implemented in R 4.0.1 (**.csv)**

WP5: VALIDATION- LEVEL I

Task 5.1. Validation via spatial gene analysis

- spatial analysis of the selected target(s) identifed in WP4 will be performed using the software provided by Apollo Life Sciences.
- Immunostaining will be used to confirm protein expression and images processed by the ImageJ/FiJi JAVA package (**.tiff / .jpeg**) and analyzed via excel (.**xlsx)**

Task 5.2. in silico target characterization

- in silico molecular target characterization via text mining approach will be performed using teh web application Uniapp (unicle.be) and further analyzed via excel (.**xlsx)**

Task 5.3. repurposing drugs for IA treatment

- in silico drug repurposing characterization will be performed via the web application (https://clue.io) and further analyzed via excel (**.xlsx**).

WP6: VALIDATION- LEVEL II

Task 6.1.*In vitro* functional assays (using human umbilical vein endothelial cells (HUVEC))

- EC spreading/hypertrophy (measuring the number and length of cytoplasmic protrusions and cell size diameter) excel / word (**.xlsx / .docx)**
- EC proliferation (3H-thymidine incorporation into DNA) - excel / word (**.xlsx / .docx)**
- EC barrier function/integrity (trans-endothelial electrical resistance)- excel / word (**.xlsx / .docx)**
- EC migration (wound healing assay: wound closure) - excel / word (**.xlsx / .docx).** Digital images processed by the ImageJ/FiJi JAVA package (.tiff / .jpeg)
- EC sprouting assay (spheroid assay-measurement of sprouts number and total sprout length) in excel / word (**.xlsx / .docx)**.Digital images processed by the ImageJ/FiJi JAVA package **(.tiff / .jpeg)**
- Validation of target expression via immunostaining images processed by the ImageJ/FiJi JAVA package **(.tiff / .jpeg)** and analyzed via excel **(.xlsx)**
- Validation of target expression via western blot. Digital images processed with the ImageJ/FiJi JAVA package (**.tiff / .jpeg**) and analyzed via excel **(.xlsx)**
- Validation of target expression via qRT-PCR analyses - excel files (**.csv and .xlsx file**)

Task 6.2.*In vivo* functional assays

- Analysis of neovascularization in the chick or mouse model: digital images processed by the ImageJ/FiJi JAVA package (**.tiff / .jpeg**) and analzyed via excel files (**.csv and .xlsx file**)

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

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

ECD for the pilot prazosin mouse model described in WP1 task 1.2 obtained. ECD P147/2020

We plan to apply for amendements to ECD P147/2020 and for new ethical approval required forof preclinical mouse models, scheduled for late 2023. All the necessary information will be made available in due time.

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

Yes, we foresee that our research results will have both, tech transfer and valorisation potential. The study may offer new approaches to treat pathological angiogenesis based on modulation of endothelial cells and their metabolism. Novel IP potential will be pursued, patented and managed as per the framework agreement between the KU Leuven LRD and the VIB tech transfer.

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

Currently, there are no 3rd party agreements in place that restrict dissemination or exploitation of data.

4. Documentation and metadata

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

All generated data are stored in lab notebooks (mostly digital). An online lab management system: Electronic Lab Notebook (ELN) is secured and backed up by the ICTS KU Leuven department. ELN has a logging in system that allows for easy data searching, copying data and it is very traceable with a long-term storage possibility (far beyond the required 5 years). Additionally, all standard operating procedures (experimental design, sampling, abbreviations used, lab guides, etc.) will be available as a PDF document and .docx file, organized (by the name of the researcher and exact experimentation date) into subfolders accompanied by a detailed experiment description, stored on the local network (L-drive) and BOX cloud. Therefore, all the files are, and will be, easily searchable and available for all group members and collaborators to precisely repeat experiments and allow data processing, if needed.

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

In general, no metadata standard will be used. As described above, experimental metadata will be stored in a searchable database format:

- Researcher
- Date
- Type of study (in vitro, in vivo, in silico)
- Summary followed by a detailed experimental description
- Type of generated data
- Type of analysis
- Digital tool used for analysis
- Info about data storage

Raw RNAseq data will be in FASTQ format, which is a text-based format for storing both sequencing data and its corresponding quality score. Modeling files pertaining to the different work packages will be stored in the SBML format, which is a text-based format containing various fields related to metabolic reactions, their corresponding gene and protein IDs and predicted flux information.

5. Data storage and backup during the FWO project

Where will the data be stored?

All data will be stored on the "large storage network L-drive" - KU Leuven LUNA, centrally managed by the IT department of KU Leuven: ICTS (Informatie en Communicatie: Technologie en Systemen). Additionally, a cloud-based KU Leuven Enterprise BOX is available for the secure storage, management and sharing documents between members of the research group.

How is backup of the data provided?

How is backup of the data provided?

The data will be backed up in two ways. Automatic back-up (every 24 hours) of the network L-drive is controlled by the ICTS KU Leuven department. Additionally, every researcher's computer is equipped with the Druva InSync Cloud Platform. Druva Cloud protects and manages data across all devices, and allows to perform the backup operations even every 5 minutes (managed individually - depends on the user).

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

Yes. An unlimited storage space is already available and maintained by the ICTS KU Leuven department

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

Back-up costs of 1 TB (KU Leuven ICTS): 128.39 €/year. The lab budget will cover storage & back up costs.

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

Research data are stored and managed by the KU Leuven IT department, and are accessible only by the researchers working on the project (access rights & password protection via KU Leuven login system).

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 generated data will be retained for at least 5 years after the end of the project. For publication purposes, our data will be publicly available on data repositories and published articles have an open access status.

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

Data will be stored on the archive K-drive, which is also managed by the ICTS KU Leuven department (secured with access right management).

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

Yearly storage costs of 1TB data on the K-drive: 128.39 €/year. The lab budget will cover storage & back up costs.

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

In principle, there will be no restrictions on sharing and reusing data, excluding possible specific IP rights.

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

All data will be made available after appropriate IP protection.

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

- In an Open Access repository

Data will be published using open access publications and will be available at dedicated data repositories. Unpublished research data will be accessible to the PI's group and all scientific collaborators involved in the project.

When will the data be made available?

- Upon publication of the research results

Data will be made available at the time of publication.

Who will be able to access the data and under what conditions?
When published, all the data will be uploaded in public data bases (as per journal guidelines), or will be provided to investigators on request if no public data base upload is required/possible.

What are the expected costs for data sharing? How will the costs be covered?
We do not expect any costs for data sharing to publicly available repositories.

8. Responsibilities

Who will be responsible for data documentation & metadata?
PI (Peter Carmeliet) & lab manager (Luc Schoonjans) & involved researchers

Who will be responsible for data storage & back up during the project?
The local IT-manager (Urbain Scherpereel) and the ICTS KU Leuven department.

Who will be responsible for ensuring data preservation and reuse ?
The local IT-manager (Urbain Scherpereel) and the ICTS KU Leuven department

Who bears the end responsibility for updating & implementing this DMP?
The individual researcher (Alessandra Pasut)