DMP title

Project Name My plan (FWO DMP) - DMP title

Grant Title 11L7622N

Principal Investigator / Researcher Patrizia Agostinis, Francesca Rizzollo

Description Melanoma cells are endowed with an exquisite ability to reversibly dedifferentiate or phenotypic switch under exposure to cellular stress signals and drug treatments. This melanoma plasticity is a a major obstacle to successful therapy and is thought to be largely driven by non-mutational mechanisms. However, their nature remains incompletely resolved. In cancer cells, including melanoma, iron metabolism-related proteins are aberrantly regulated to support tumor-promoting processes including metabolic plasticity, DNA synthesis, and epithelial-to-mesenchymal transition. On the other side, iron is a quintessential element of ferroptosis, an emerging iron-dependent cell death modality with the ability to target mesenchymal and drug-resistant cancer cells. Therefore, processes remodeling melanoma iron homeostasis may represent promising therapeutic targets. Our preliminary data show that deregulation of a cluster of genes regulating the endo-lysosomal iron trafficking and mitochondrial iron transport is a key signature of the phenotypic switch toward the invasive drug-resistant melanoma subtype, which is coupled to the increased vulnerability of these cells to ferroptosis. Hence, in this proposal, we will use state-of-the-art microscopy and cell biology techniques to unravel the role of lysosomes, and specifically, their communication with mitochondria, as metabolic hubs in iron homeostasis, melanoma dedifferentiation, and ferroptosis vulnerability.

Institution KU Leuven

1. General Information

Name applicant

Francesca Rizzollo

FWO Project Number & Title

Iron homeostasis in melanoma plasticity and ferroptosis: a matter of contact sites? (11L7622N)

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

TYPE OF DATA	FORMAT	VOLUME	HOW CREATED
Scan of a western blot	.tiff, .jpeg, .gel	5 GB	typhoon NIR or AI600 scanner
Microscopy images	.czi, .tiff, .imaris	2 TB	acquired with different kinds of microscopes, analyzed with Imaris or ImageJ softwares
Graphs and statistics	.pzfx	10 GB	GraphPad
Presentations	.ppt and .pdf	20 GB	Power Point
Microscopy movies	.czi, .tiff, .imaris	3 TB	acquired with different kinds of microscopes, analyzed with Imaris or ImageJ softwares
Flow cytometry	.fcs	1 GB	CANTO AIG
Metabolomics	.bmp, .jpg, .csv	1 GB	mass spectrometer
Plasmids	.doc, .csv	1 MB	
Stable human cell lines, primary human cell lines	.doc, .csv	1 MB	
Antibodies	.doc, .csv	1 MB	
Chemicals	.doc, .csv	1 MB	
RNA-seq	.txt	7 MB	GSE80829

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)

- No

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. Ownership of the data generated belongs to KU Leuven and VIB in accordance with the framework agreement of both institutes. 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 or strains generated from this project. In particular, existing agreements between VIB and KU Leuven do not restrict publication of data.

There is no IP on the generated strains that would prevent us from storing the strains, performing the anticipated experiments or publishing the results.

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 and in hard copy lab notebooks that refer to specific datasets. Cryotubes of biological samples (bacterial and yeast strains) stored at -80°C will be labeled with a reference number that links to an entry in or strain database.
All datasets will be accompanied by a README.txt file containing all the associated metadata (see more details below).
The data will be generated following standardized protocols. Clear and detailed descriptions of these protocols will be stored in our lab protocol database, and 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.

- No

Metadata will include the following elements:
• Title: free text
• Creator: Last name, first name, organization
• Date 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 of the 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, open access.
Additionally, we will closely monitor MIBBI (Minimum Information for Biological and Biomedical Investigations) for metadata standards more specific to our data type.
For specific datasets, additional metadata will be associated with the data file as appropriate. 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, except for private data that will be stored on KU Leuven secure server (digital vault).
- 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.
- 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). All published vectors and the associated sequences will be sent to the non-profit plasmid

repository Addgene, which will take care of vector storage and shipping upon request.
- Cell lines: Newly created human cell lines will be stored locally in the laboratory in liquid nitrogen storage and will be deposited in the UZ Leuven-KU Leuven Biobank. Other human cell lines will be stored locally in liquid nitrogen cryostorage of the laboratory when actively used for experiments. Animal cell lines will be stored in liquid nitrogen cryostorage of the laboratory.
- Bacterial strains will be stored in a -80°C freezer in the lab of xxx. Costs are covered by general lab expenses.
- Chemical samples will be stored at 4°C and/or as frozen samples in cryovials 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).

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. Incremental backups are done daily from one 20 TB QNAP NAS to a second 20 TB QNAP NAS.

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

There is sufficient storage and back-up capacity on all KU Leuven servers:
- the “L-drive” is an easily scalable system, built from General Parallel File System (GPFS) cluster with NetApp eseries storage systems, and a CTDB samba cluster in the front-end.
- the “J-drive” is based on a cluster of NetApp FAS8040 controlers with an Ontap 9.1P9 operating system.

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

Each year €738 will be charged from our ICT service for the use of 5 TB on the L-drive (long term storage) and €51,9 will be charged each year for the use of 100 GB of the J-drive (short term storage). Back-up service is included in the price. For the K-drive (data archive) storage space of 1 TB is foreseen and will cost €128 each year, this is also expandable in blocks of 100 GB. These costs were foreseen in the application and if more the lab budget will be used to cover these expenses.

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. The costs (€156 per TB per year for “Large volume-storage”) will be covered by the lab.

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.
-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).
-Cell lines: human cell lines will be stored in the UZ Leuven Biobank (-80°C).
-Other biological and chemical samples: storage at 4°C and/or as frozen samples in cryovials as appropriate.
- Following publication, the results associated with each study will also be deposited in the Dryad repository, where they will be preserved indefinitely.

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

Each year €128 will be charged from our ICT service for the use of 1 TB on the k-drive (long term storage), back-up service is included in the price. These costs were foreseen in the budget request of the application and if more, the lab budget will be used to cover these expenses.

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).
We aim at communicating our results in top journals that require full disclosure upon publication of all included data, either in the main text, in supplementary material or in a data repository if requested by the journal and following deposit advice given by the journal. Depending on the journal, accessibility restrictions may apply.
Biological material will be distributed to other parties if requested.

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

- In an Open Access repository
- Upon request by mail

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 as the 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 that refer to specific datasets. The data will be reviewed by the principal investigator.

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.

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, and from Raf De Coster for the KU Leuven drives.

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

The PI bears the end responsibility of updating & implementing this DMP.