INVESTIGATING THE ROLE OF ATP13A4 AS POLYAMINE TRANSPORTER IN THE CELLULAR AND IN VIVO POLYAMINE HOMEOSTASIS

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Project abstract:

Genetic ATP13A4 mutations have been implicated in a spectrum of neurodevelopmental disorders, whereas upregulation of ATP13A4 is implicated in cancer. ATP13A4 is an orphan transporter belonging to the P5B-transport ATPase family and our preliminary data show that ATP13A4 is a polyamine transporter. In this project, we will firmly establish the biochemical properties of ATP13A4 and will investigate the impact of ATP13A4 modulation on cellular polyamine uptake and homeostasis. We will focus the role of ATP13A4 in breast cancer cell models marked by high ATP13A4 levels, and we will evaluate polyamine transport inhibitors to validate ATP13A4 as a cancer drug target. Second, we will focus on astrocytes, which is the cell type that accumulates and stores polyamines in the brain, and which expresses high ATP13A4 levels. We will study astrocyte polyamine uptake and release in astrocytes, and investigate in ATP13A4 knockout mice whether ATP13A4 influences in vivo polyamine distribution and behavior. We aim to establish ATP13A4 as a regulator of brain and astrocyte polyamine homeostasis, which may explain its involvement in neurodevelopmental disorders. Understanding the physiological role of ATP13A4 will improve our understanding of the enigmatic polyamine transport system in breast cancer and astrocytes, and may validate ATP13A4 as a new therapeutic cancer target.

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DPIA

Have you performed a DPIA for the personal data processing activities for this project?

- Not applicable

GDPR

Have you registered personal data processing activities for this project?

- Not applicable

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FWO DMP (Flemish Standard DMP)

1. 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	Description	New or reused	Digital or Physical	Only for digital data Digital Data Type	Only for digital data Digital Data format	Only for digital data Digital data volume (MB/GB/TB)	Only for physical data Physical volume
EP assays (WP1)	Autoradiograms, created by Typhoon Biomolecular Imager	Generate new data	Digital	Images	.gel, .tif, .ppt	<100MB	
Radiolabelled polyamine transport assays (WP1)	Data created by scintillation counter	Generate new data	Digital	Numerical	.xlsx	<100MB	
qPCR (WP1-3)	qPCR data, created by Light Cycler machine	Generate new data	Digital	Numerical	.pcrd, .pdf, .xlsx	<100MB	
Coomassie stained gels (WP1)	Scans of gels, created by scanner	Generate new data	Digital	Images	.tif, .ppt	<100MB	
Western blots (WP1-3)	Scans of western blots, created by Bio-Rad ChemiDoc Imaging System	Generate new data	Digital	Images	.scn, .tif, .ppt	<1GB	
Flow cytometry (WP1-2)	Flow cytometry data, created by flow cytometer (Sony ID7000, BD FACSymphony, Cytek Aurora)	Generate new data	Digital	Numerical	.fcs, .csv	<1TB	
Confocal microscopy (WP1-3)	Confocal microscopy images, created by confocal microscopes like Zeiss LSM880	Generate new data	Digital	Images	.czi, .tif	<1TB	
Biochemical/cell biological read-outs (WP1-2)	Biochemical/cell biological read-outs, created by platereader (Biotek)	Generate new data	Digital	Numerical	.xpt, .xlsx	<1GB	
Metabolomics, proteomics (WP1-2)	Mass spectrometry data, secondary data origin, meaning these experiments will be performed by a core facility and we will receive an .xlsx file with the data from them.	Generate new data	Digital	Numerical	.xlsx	<1GB	

DNA/RNA sequencing (WP1-3)	Sequencing data, secondary data origin, meaning these experiments will be performed by a company/core facility and we will receive .ab1, .seq and/or .xlsx files with the data from them.	Generate new data	Digital	Numerical	.ab1, .seq, .xlsx	<100MB	
Animal behaviour read-out (WP3)	Analysis of animal behavior, secondary data origin, meaning these experiments will be performed by a core facility and we will receive an .xlsx file with the data from them.	Generate new data	Digital	Numerical	.xlsx	<1GB	
Data analysis (WP1-3)	Data analysis	Generate new data	Digital	Numerical	.xlsx, .prism	<1GB	
Statistical analysis (WP1-3)	Statistical analysis	Generate new data	Digital	Numerical	.prism	<1GB	
Microscopy slides	Microscopy slides	Generate new data	Physical				Around 500 slides stored in slide folders, will be discarded once the project is finished.
Frozen cell lines	Cryovials containing cells that were generated during the project.	Generate new data	Physical				Around 50 cryovials stored at -80°C/nitrogen barrel, will be preserved also once the project is finished.
Other biological samples	Purified protein and membrane samples, DNA and RNA samples, generated during the project.	Generate new data	Physical				Around 500 eppendorf tubes stored at -80°C, will be discarded once the project is finished.

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:

N/A.

Are there any ethical issues concerning the creation and/or use of the data (e.g. experiments on humans or animals, dual use)? Describe these issues in the comment section. Please refer to specific datasets or data types when appropriate.

- Yes, animal data

For this research project we conduct experiments on animals (mice). Ethical committee approval (167/2023) was obtained.

The human cell lines that are used in this study are well known and established cell lines. They are commercially available, the MSDS sheet can be consulted online and have been published. The laboratory of Dr. P. Vangheluwe shares an umbrella protocol for human cell lines with all other research groups within the Department of Cellular and Molecular Medicine. Ethical approval is in place (S63808).

Will you process personal data? If so, briefly describe the kind of personal data you will use in the comment section. Please refer to specific datasets or data types when appropriate.

- No

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

Do existing 3rd party agreements restrict exploitation or dissemination of the data you (re)use (e.g. Material/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.

- Yes

Related to WP1-O2: The used polyamine transport inhibitors are under MTA from Dr. O. Phanstiel, UCF while the AMXT-1501 analogs are under MTA from Dr. M. Burns, Aminex Therapeutics. These compounds are strictly for the use in our lab and when publishing data concerning the polyamine transport inhibitors, Dr. O. Phanstiel and/or Dr. M. Burns should be mentioned on the paper.

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

2. 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).

Each researcher keeps a digital lab notebook with a description of all performed experiments. The lab notebook contains information on how data were generated: the composition, method, temperature, incubation conditions, and a reference to the loading conditions of the considered material. In addition, modifications to protocols are written down in the lab notebook and the place where the material is being stored is also mentioned in the lab notebook. The digital lab notebook is stored on the J-drive and, once the research project has ended, will be stored on the K-drive for long-term storage.

Raw data files of read-outs, analyzed data and statistical analyses are being stored on the J-drive and will be transferred to the K-drive at the end of the research project. The name of the folder of the saved data refers to the date, project, specifications and version of the data. All folders are organized on the j-drive according to the project, results, proposal, papers, presentation, administration, … Each researcher has access to his/her folder on the j-drive and a common folder of the lab containing protocols, list of plasmids, list of antibodies, list of cell lines. Only the PI and the lab manager have access to all folders.

Furthermore, data will be made available to the broad audience as publications in peer reviewed journals. All data, constructs, cell and animal models will be made available upon reasonable request.

Will a metadata standard be used to make it easier to find and reuse the data? If so, please specify (where appropriate per dataset or data type) which metadata standard will be used. If not, please specify (where appropriate per dataset or data type) which metadata will be created to make the data easier to find and reuse.

- No

For now, no metadata standard will be used.

3. Data storage & back-up during the research project

Where will the data be stored?

Each researcher will store his/her data in their personal folder on the j-drive. This is our data repository for short term storage which is expandable, fast and the data can be modified by the researcher itself. Only the PI and the lab manager have access to all the folders of the j-drive. When a paper is published, the data will be moved to our k-drive. This is our repository for data archiving, for long term storage. Only the PI and the lab manager can move the data to this drive, but data on this drive cannot be modified or deleted. Only the ICT service can do this.

How will the data be backed up?

An automatic back-up service is provided by our ICT service.

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

Regarding the j-drive (short term storage), 1 TB of storage space is foreseen for our research group and this is expandable in blocks of 100 GB. Regarding the k-drive (data archive) a storage space of 100 TB is foreseen and this is also expandable in blocks of 100 GB.

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

Data are not saved locally on laptop/desktop but are stored in the KU Leuven secure data center.
Only two people have access to all folders: the PI and the lab manager. Each researcher has access to his own folder on the j-drive and has read only access to the data on the long-term storage (k-drive). Non-authorized persons can't access or modify the data.

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

Each year €173.40 will be charged from our ICT service for the use of 1 TB on the k-drive (long term storage) and €519.00 will be charged each year for the use of 1 TB of the j-drive (short term storage). Back-up service is included in the price. These costs were foreseen in the application and if more the lab budget will be used to cover these expenses.

4. Data preservation after the end of the research project

Which data will be retained for at least five 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 stored for at least five years after the end of the research project.

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

Once the research project is finished, the data will be archived on the K-drive. This is our repository for data archiving and long term storage.
Only the PI and the lab
manager can move the data to this drive, but data on this drive cannot be modified or deleted. Only the ICT service can do this.

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

Each year €173.40 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.

5. Data sharing and reuse

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

- Yes, in an Open Access repository

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

N/A

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 in the comment section per dataset or data type where appropriate.

- No

Where will the data be made available? If already known, please provide a repository per dataset or data type.

All the data on which publications were based on will be made publicly available. All datasets generated or analyzed in this study will be deposited at Zenodo. All data will
be moved to our long-term storage (=data archive) where the data is available under a read only mode for everybody within the lab and only the PI (Peter Vangheluwe) and the lab manager (Marleen Schuermans) have access to that drive. Data that are important for future applications and publications will not be made available.

When will the data be made available?

Upon publication of the research results.

Which data usage licenses are you going to provide? If none, please explain why.

Creative Commons Attribution 4.0 International

Do you intend to add a PID/DOI/accession number to your dataset(s)? If already available, you have the option to provide it in the comment section.

- Yes

All datasets generated or analyzed in this study will be deposited at Zenodo where every upload is assigned a DOI.

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

Each year €173.40 will be charged from our ICT service for the use of 1 TB on the K-drive (long term storage) and €519.00 will be charged each year for the use of 1 TB of
the J-drive (short term storage). These costs and the publication costs of the data will be covered by the lab.

6. Responsibilities

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

During the research, individual co-workers are responsible to collect and store data on a dedicated personnel drive. The data will be reviewed by Peter Vangheluwe (PI).

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

Peter Vangheluwe (PI) and ICT service of KU Leuven

Who will manage data preservation and sharing?

Marleen Schuermans (lab manager) and Peter Vangheluwe (PI)

Who will update and implement this DMP?

Peter Vangheluwe (PI) bears the end responsibility of updating and implementing this DMP.