**DMP title**

**Project Name** My plan (FWO Initial DMP) - DMP title
**Project**                         **Identifier**                         G044224N
**Principal Investigator / Researcher** Ilaria Elia
**Project Data Contact** ilaria.elia@kuleuven.be

**Description** Our immune system and, more specifically, CD8+ T cells have the ability to fight against cancer. In tumors, these cells transition from "effector" to "exhausted" T cells. Effector T cells are able to recognize and kill cancer cells, but exhausted T cells lose this anti-cancer function. Interestingly, an increase in the number of exhausted T cells is linked to cancer progression and resistance to treatment. Recent studies have shown that cellular metabolism plays a significant role in regulating the process of T cell exhaustion. Indeed, the availability of nutrients in the tumor can affect T cell differentiation and promote cancer progression. Although many metabolic targets responsible for T cell exhaustion have been identified, the metabolic differences between primary tumors and metastatic tissues make it challenging to prevent or reverse the process of exhaustion in metastases. In this project, we aim to investigate how the nutrient microenvironment of melanoma metastasis impacts T cell exhaustion. Our preliminary data suggest that a metabolic program involving the TCA cycle is involved. We will explore the relevance of this pathway in T cell exhaustion in lung and liver metastasis. Finally, we will validate our findings in mouse models and patient data sets. Our goal is to develop new strategies to prevent or reverse T cell exhaustion and improve the efficacy of immunotherapy for the treatment of metastatic cancer.

**Institution** KU Leuven

**1. Name applicant**

Ilaria Elia

**FWO project number & title**
G044224N
Exploring TCA and itaconate metabolism as drivers of CD8+ T cell exhaustion in local metastatic niches

**Affiliation**

Department of Cellular and Molecular Medicine, KU Leuven, Belgium

**2.**                         **Data**                         **description**
**2.1. Will you generate/collect new data and/or make use of existing data?**

Generate new data

**2.2. What data will you collect, generate or reuse? Describe the origin, type and format of the data (per dataset) and its (estimated) volume. This may be easiest in a numbered list or table and per objective of the project.**

We will generate new experimental data, more specifically biochemistry, flow-cytometry, cell physiology, immunochemistry data, and mouse data. We will also modulate T cell function by using genetic and pharmacological approaches. These approaches will be evaluated by western blot, RTqPCR, ELISA, proteomics data, seahorse data and flow cytometry data (.xls, .czi,.csv, .tiff, .jpeg, .fcs). Additionally, we will also measure metabolite content using mass spectrometry (.D, .CDF). All data will be generated in the host institution. When a researcher starts at KU Leuven he/she will have to sign an agreement in which he/she agrees to maintain written or digital records of all collected results (hard copy of electronic notebooks). All raw data will be stored on the university server. Furthermore, these data will generate manuscripts for

publication in peer-reviewed journals. All necessary measures will be taken to archive all biological material according to good scientific practices. The estimated volume of data is 5TB.

| Type of data | Format | Size | How created |
|---|---|---|---|
| raw data and associated statistical analysis | .xls, .pzfx | 50-200MB | Recording of count data, analysis of data by using Microsoft excel and graph pad prism |
| Raw FACs sort reports | .CSV, .fcs | 50-200MB | Reports of fluorescently stained mouse T cells for fluorescent markers and/or enrichment of CD8 T cell population |
| Analysis of FACS files | .wsp | 50-200MB | flow cytometric analysis processed in FlowJo |
| Western blot images | .xls, .pzfx | 50-200MB | Quantification of western blot images, performed using imageJ, in microsoft excel and graph pad prism. |
| qRT-PCR data | .xls, .pzfx | | Analysis of qRT-PCR data. Statistical analysis performed in graph pad prism. |
| Raw proteomics data | .RAW | 200-800MB | Samples will be acquired and processed by the Proteomics core at KU Leuven to obtain excel files |
| Analysis proteomics data | .xls | 10-20GB | Analysis of Raw proteomics data for differentially expressed proteins |
| DNA plasmids | tubes of liquid containing DNA plasmids | | DNA plasmids produced during the project, derived from existing DNA plasmids provided by commercial and non-commercial suppliers. |
| Mass spectrometry files | .D | 10-20 MB | Mass spectrometry files generated by Gas Chromatography-Mass Spectrometry |
| Mass spectrometry files after chromatogram analysis | .CDF | 50-100 KB | Files generated after identification of metabolite peaks. A Mat Lab software will be utilized to calculate the area. The resulting file will be an excel data file. |
| Seahorse files | .asyr | 50-100 KB | Files generated with the Agilent Seahorse Wave Desktop software. |
| H&E files | .qptiff | 1.5-8 GB | The data will be acquired by the "Single cell & Spatial Multiomics" core at KU Leuven and analyzed using the QuPath software. |

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|---|---|---|---|
| Immunostaining files | .qptiff | 5-15 GB | The data will be acquired by the "Single cell & Spatial Multiomics" core at KU Leuven and analyzed using the QuPath software. |

# 3. Ethical and legal issues

**3.1. Will you use personal data? If so, shortly describe the kind of personal data you will use. Add the reference to the file in KU Leuven's Record of Processing Activities. Be aware that registering the fact that you process personal data is a legal obligation.**

No

**3.2. 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.

All experiments on mice are conducted according to institutional, national and European animal regulations. The lab already has ethical clearance files approved by the animal ethics committee at the KU Leuven (P054/2022).

**3.3. Does your research 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?**

No

No third-party agreement restricts dissemination or exploitation of the data from this project.

# 4. Documentation and metadata

**4.1. What documentation will be provided to enable understanding and reuse of the data collected/generated in this project?**

Documentation and metadata associated with the different experiments will be meticulously described in electronic notebooks. The researcher will outline all different steps including experimental setup, protocol, method of data collection, employed controls, and analysis.

**4.2. Will a metadata standard be used? If so, describe in detail which standard will be used. If not, state in detail which metadata will be created to make the data easy/easier to find and reuse.**

At the moment, the lab does not make use of a standard metadata. To make the data easy to find, we will specify: (1) title of the project, (2) name of the researcher, (3) data type, (4) raw data and analyzed data, (5) data created and modified, (6) file size, (7) equipment and product reference. Depending on the nature of the data additional metadata are collected. Mass spectrometry: (8) columns used, (9) solvents. FACS: (8) channel used, (9) fluorescence.

**5. Data storage and backup during the project**

**5.1. Where will the data be stored?**

All data will be stored in the host laboratory at KU Leuven. The data will be reported on the electronic lab notebooks. The researcher will indicate all necessary information (experimental setup, protocol, method of data collection, employed controls, and analysis) as well as a cross-reference to electronic storage of data to ensure other people can easily find and reproduce all data sets. The data will be stored in the lab archives for at least 10 years. All raw data will be stored on the KU Leuven L drive (large storage server).

**5.2. How will the data be backed up?**

Backup is secured daily on the central servers of the university.

**5.3. 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
The laboratory has already purchased 5TB of storage capacity which can be extended on demand.

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

The current cost of the L-drive (5TB) is €569.2 per year. These costs are financed through grant applications.

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

To ensure secure data storage and to avoid modification by unauthorized persons, we have: i) controlled physical access to the building, ii) controlled digital access to the L-drive, iii) encrypted communications, iv) password access to all computer.

**6.      Data      preservation      after      the      end      of      the      project**
**6.1. Which data will be retained for the expected 10 year period after the end of the project? If only a selection of the data can/will be preserved, clearly state why this is the case (legal or contractual restrictions, physical preservation issues, ...).**

All data will be retained for at least 10 years after the project.

**6.2. Where will these data be archived (= stored for the long term)?**

Digital data will be archived on the electronic storage (L-Drive).

**6.3. What are the expected costs for data preservation during these 10 years? How will the costs be covered?**

Digital data storage costs are included in general lab costs/paid at the department level (in case of retirement). The cost for storage on the L-drive is €569.2 per year. These costs are financed through grant applications.

**7.                    Data                    sharing                    and                    re-use**
**7.1. 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 or because of IP potential)?**

No

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

All data are available upon publication of the results. Bioinformatic data set will be deposited publicly.

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

In an Open access repository or upon request.

**7.4. When will the data be made available?**

Upon publication of the results

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

Published data are accessible to all.

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

Most of the data are put without cost on public repositories.

**8. Responsibilities**

**8.1. Who will be responsible for the data documentation & metadata?**

The individual researcher will have the responsibility for data documentation and metadata.

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

The individual researcher and the PI will be responsible for data storage and backup. However, the maintenance of servers and the integrity of data stored on these servers will be controlled by the ITC services of the university.

**8.3. Who will be responsible for ensuring data preservation and sharing?**

The host institute's IT team is responsible for digital preservation.

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

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