

## Plan Overview

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*A Data Management Plan created using DMPTool*

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**Title:** PROTEOMICS AND DEGRADOMICS APPLIED TO THE STUDY OF THE EFFECTS OF MANNOSE IN MELANOMA

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### **Project abstract:**

The high energy demand imposed by the oncogenic process has significant consequences on the metabolism of transformed cells, as well as having a significant impact on diagnostic methods and therapies associated with the treatment of neoplasms. Mannose is an epimer of glucose that is internalized through the same transporters. Recent work using tumor cell lines demonstrated that this hexose accumulates in cells in the form of the intermediate mannose-6-phosphate (M6P), due to the low expression of the enzyme phosphomannose isomerase (PMI), which converts M6P into fructose-6-phosphate (F6P), an intermediate of the glycolytic pathway. This feature significantly impacts glucose metabolism via the glycolytic pathway, the tricarboxylic acid cycle, and the pentose phosphate pathway. Recent data from our group indicate that the metabolic stress resulting from the administration of mannose to metastatic human melanoma cell lines results in a decrease in the growth rate, as well as in the activation of lysosomal degradation pathways and, possibly, of autophagy. In this context, the main objective of this project is to evaluate the effects of mannose administration on the cellular proteome of human and murine melanoma cell lines, as well as on the substrate repertoire of activated proteases (degradome). Also within the scope of this proposal is the evaluation of *in vivo* tumorigenesis in animals submitted to the injection of melanoma cells followed by the administration of mannose. Cellular models comprising distinct metabolic/mitogenic phenotypes (mutant B-Raf, A375, and non-mutant strain, WM 1366 strain, respectively) will be used, as well as a paired cell model composed of a murine melanocyte (Melan-a) and a metastatic strain (B16-F10). It is expected to correlate the results obtained with possible biological pathways relevant to the oncogenic process. From a therapeutic point of view, the results obtained will allow the

elucidation of fundamental molecular aspects that may subsidize the increase of clinical intervention strategies using this hexose (mannose) associated with therapies already used for the treatment of human melanoma.

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# PROTEOMICS AND DEGRADOMICS APPLIED TO THE STUDY OF THE EFFECTS OF MANNOSE IN MELANOMA

Experimental approaches will generate 2 main types of data:

(1) Data regarding the profile of proteins from melanoma cells (B-RAF mutant or not, cell lines A375 and WM1366, respectively) in culture conditions with (a) glucose 25 mM, (b) glucose + mannose 25 mM (final). Such data will be collected after shotgun proteomics experiment derived from these two culture conditions;

(2) Degradomics data derived from the culture conditions described above but subjected to N-terminal protein enrichment technique using Terminal Amine Isobaric Labeling of Substrates (TAILS);

The mass spectrometric data format will be made available under the original vendor (RAW) format as well as will be converted to the universal data format 'mzML'. Assembled contig data will be made available in '.fasta' format. Proteomics and data will be submitted to public repositories such as the ProteomeXchange Consortium (<http://www.proteomexchange.org/>) In addition, for bioinformatic analysis purposes, data from the above repository might also be interrogated.

Data will be locally stored, in a Microsoft Windows server, located at the Functional Proteomics Laboratory, federal University of São Paulo, São José dos Campos, Brazil. All the RAW files will be submitted to the ProteomeXchange public repository (<http://www.proteomexchange.org/>).

The main objective of this project is to evaluate the effects of mannose administration on the proteome of human malignant melanoma cells (B-Raf mutant or not), with emphasis on processes regulated by the activity of proteases.

Proteomics data will be named after the experimental conditions used in this work, as well as the corresponding biological replicate, namely: Man\_R1 (mannose, biological replicate 1), Glc\_R1 (glucose, biological replicate 1), Glc\_Man\_R1 (glucose + mannose, biological replicate 1).

Before submitting data to any public repository, each LC-MS/MS run will be evaluated based on the protein identification/quantitation which must display consistency among each biological replicate (i.e, Pearson correlation coefficient greater or equal than 0.7 for protein quantitative values).

The information on the dataset generated in this study (metadata) will be compiled in a spreadsheet, including information on RAW file size, software (and version) used for Peptide-to-Spectrum assignment, search parameters, and database size. This will be done in order to enable the reproducibility of our proteomics data analyses by any researcher worldwide.

This section is not applicable to our study.

Data will be stored locally, as described above, and will be made publicly available as soon as the publication is out (this is the only situation where data sharing might be postponed).

In addition to the public repositories mentioned above, data will be stored locally in a server located at the Functional Proteomics Laboratory, at the Institute of Science and Technology of the Federal University of São Paulo, at São José dos Campos, Brazil. A backup will be done on a weekly basis, using external hard drives.

Data in public repositories are available to the general public. Ideally, our local storage will mirror the public repositories.

All data will be retained and used throughout the development of this and other research projects at the laboratory.

At this time (December 2021), there is no budget limitation for time and effort to preserve data, as we have plenty of space on our server. However, as the project starts and data will come, it will be necessary a minimum of financial support for ordinary maintenance. However, as mentioned before, all data will be made public through public repositories of 'omics' data.

All data will be made public through public repositories of 'omics' data (i.e proteomeXchange)

No

As of today, all data handling in our server is managed by graduate students and myself. Additionally, IT personnel from the University are available for assisting in any issue.

In fact, financial support is of paramount importance for the ordinary activities of data handling and hardware updating. The IT personnel of the University is constantly assisting us in any issue that may occur with our server or with data handling.

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