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## Plan Overview

*A Data Management Plan created using DMPonline*

**Title:** Investigation into macrophage reprogramming following clearance of apoptotic cells.

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**Template:** DCC Template

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### Project abstract:

This project is focused on an important aspect of immune homeostasis in the human body known as efferocytosis. In brief, 0.4% of the approximate 40 trillion cells in the adult human body are estimated to die every day, undergoing a form of programmed cell death known as apoptosis. These apoptotic cells are cleared via a process known as efferocytosis, performed by immune cells, namely macrophages. Once these cells are phagocytosed and subsequently digested, reprogramming events are triggered within the macrophage leading to a shift towards an anti-inflammatory, or 'wound healing', phenotype. This process is crucial to the resolution of inflammation, and thus, key in maintaining immune homeostasis. While defects in efferocytosis have been linked to numerous diseases such as systemic lupus erythematosus and inflammatory respiratory disorders, it remains unclear how macrophage reprogramming following efferocytosis contributes or even exacerbates these inflammatory conditions. This project aims to address this knowledge gap by applying this novel workflow to examine changes in *ex vivo* macrophage proteome following the phagocytosis of apoptotic cells using stable isotope labelling by amino acids in cell culture (SILAC) to separate the macrophage and apoptotic cell proteomes. This will then be combined with ultra-sensitive mass spectrometry to allow for deep proteome coverage. Here, alveolar macrophages will be collected directly from bronchoalveolar lavages following an inflammatory respiratory challenge in recruited volunteers; after which this workflow will be applied. This will be the first study to investigate proteome changes in *ex vivo* macrophages from humans following efferocytosis, and hence will provide an unparalleled view into how deficiencies in macrophage reprogramming may contribute to disease.

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# Investigation into macrophage reprogramming following clearance of apoptotic cells.

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## Data Collection

### What data will you collect or create?

The project will primarily produce mass spectrometry-based datasets obtained from cell-based assays using ex vivo cells. Mass spectrometry data will be acquired and stored as .RAW files or proprietary Bruker Tims data format (TDF). This is the preferred format to store the data prior to analysis as all the metadata is inclusive in these files and they are easily read by multiple software. Additionally, we cannot change the file output of the mass spectrometer. The data will then be transformed for publication into mzXML or mzML files and processed for analysis in .csv or R format.

Due to the large size of raw mass spectrometry file (1-2 gigabytes per file), Matthias Trost provides up to 100 terabytes of storage on the Newcastle University research data warehouse which is backed up regularly.

### How will the data be collected or created?

The data will be acquired at Newcastle University, Faculty of Medical Sciences, in the laboratory of Professor Matthias Trost in accordance with best practice. This includes necessary controls (positive, negative, quality) as well as optimal conditions to generate the data and sufficient replicates for appropriate statistical analysis.

Regarding organisation, consistent naming conventions will be used where folder, subfolders and files will all begin with yyyy/mm/dd. All file and folder names will be kept short but informative so that they describe the file/folder accurately. Versioning of files will be standardised by v1.0, v2.0 etc. Folders will be indexed according to experiment type to limit clutter and confusion.

## Documentation and Metadata

### What documentation and metadata will accompany the data?

All raw data will be stored along with metadata that will include information about the experimental and instrument conditions. As mentioned above, the files or folders will be labelled with the date, name of the experimenter and more detailed information about the experiment so that it can be easily found and understood. In addition, an electronic lab notebook (Benchling) will be used to keep a record of the experiments that have been undertaken and it will also contain information about the storage location of the raw data (file/folder names) and the corresponding analysis.

## Ethics and Legal Compliance

### **How will you manage any ethical issues?**

There are no ethical considerations for this project as no identifiable information will be collected. This project will be solely focused on generating data on cellular material from which we have received no identifiable information.

### **How will you manage copyright and Intellectual Property Rights (IPR) issues?**

The data will be owned by Newcastle University and there are currently no copyright and Intellectual Property Rights (IPR) issues to consider.

If these issues arise in the future I, Benjamin Raymond, will be responsible for revising this DMP.

## **Storage and Backup**

### **How will the data be stored and backed up during the research?**

All data will be entered on computers within the Newcastle University internal network. Additionally, the data will be deposited in the Newcastle University research data warehouse which is based at two physically different locations and provides storage as well as backup solutions. The data will be archived for at least 10 years on a server with a regular backup routine.

### **How will you manage access and security?**

Data will be stored on computers with secure password access only. These computers will never be left logged in while unattended. The data is also stored on a secure Newcastle University research data warehouse where only members of Matthias Trost's lab have access. Collaborators will not be given direct, local, access to this data but when required secure data sharing of analysed data (not raw data) with collaborators and partners will be facilitated via Newcastle University's Microsoft Office 365/Teams account.

## **Selection and Preservation**

### **Which data are of long-term value and should be retained, shared, and/or preserved?**

All data will be kept for at least 10 years as described above. Raw data however, will be stored indefinitely unless advised differently by Matthias Trost as they have the ability to be reanalysed by others in the future.

### **What is the long-term preservation plan for the dataset?**

For storage on Newcastle University research data warehouse, Matthias Trost pays for up to 100 terabyte storage that ensures there is enough space.

Additionally, there are several online mass spectrometry data repositories available that allow the upload of large mass spectrometry datasets. Proteomics datasets will be uploaded in ProteomeXchange associated public repositories like PRIDE for which there are no associated costs.

## **Data Sharing**

### **How will you share the data?**

The data will be shared by publications in peer-reviewed journals and also during national and international presentations in scientific meetings. Approved data will be shared as supporting material with journals for publication. As mentioned above, data will also be uploaded to ProteomeXchange associated public repositories like PRIDE.

### **Are any restrictions on data sharing required?**

No restrictions on the aforementioned data sharing strategies is required as of now.

## **Responsibilities and Resources**

### **Who will be responsible for data management?**

I, Benjamin Raymond will be solely responsible for implementing the DMP and ensuring it is reviewed and revised. Matthias Trost, who pays for Newcastle University research data warehouse storage will be responsible for maintaining access to these.

### **What resources will you require to deliver your plan?**

No additional specialist expertise is required and no hardware/software not already available is required. No charges will be applied by data repositories.