The Bio-Bank of CReSM: a tool to facilitate the research in Multiple Sclerosis.

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The poor reproducibility of published data is often due to lack of rigor in the collection of biological samples, to partial validation of the assays and to limited sharing of data, leading to a delay in transfer to real-life of the biological research and waste of huge amount of funds. In Multiple Sclerosis (MS), each biological sample needs to be related to clinical and radiological data obtained during the patient's follow up. Since 2012, Regional Referral Multiple Sclerosis Centre (CReSM), that attend to 2200 patients, has been improving collection of biological samples of healthy controls (HC), patients with MS and with other neurological diseases following standardized procedures: 20ml of cerebrospinal fluid (CSF) are collected and post-lumbar puncture headache is reduced to 1%. Collection and transport of CSF and blood samples from the bed to the bio-bank must take less than 3 hours; every step of sample handling and bio-banking is checked according to a rigorous protocol and any deviation is recorded. Collection of blood samples from HC follows the same procedure of MS patients to avoid pre-analytical differences. Samples are subdivided in several aliquots, each of them is used for a single experiment to avoid repeated thawing/re-freezing of samples. Samples of the bio-bank are combined with clinical and radiological data of the MS patients that are visited every 3-6 months: longitudinal samples and data are bio-banked for selected patients. A maximum of 6 patients or HC is banked every day. CSF cells and supernatant are stored. Each 38-ml blood sample allows the collection of serum, plasma, DNA, RNA, PBMCs in DMSO and RNA stabilizer. A barcode identification of each aliquot allows easy storage and localization of samples in the freezer. Since 2012, 2790 blood draws have been collected from more than 1300 subjects. Paired samples of CSF and blood were obtained from 869 patients. An Informed Consent approved by San Luigi Ethical Committee in 2016 regulates collection, conservation, utilization and distribution of samples and the related clinical data to scientific institutions and pharma companies for research. CRESM Bio-bank is partially supported by AISM (Associazione Italiana Sclerosi Multipla) and is active in distribution of samples to the international scientific community. Until now 6 scientific institutions used CRESM Bio-bank samples and 3 applications for samples are under evaluation.

Selection of samples for Bio-bank

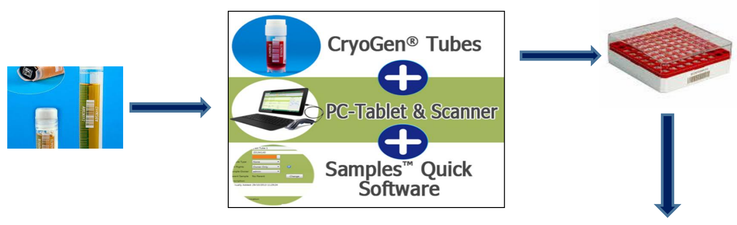
- **Patients with different subtypes of MS: RR, PP, SP, benign**
- **Post Progressive Multifocal Leukoencephalopathy (PML) and post Autologus Haematopietic Stem Cells Transplantation (AHSCT) patients**
- **MS patients treated with all available therapies at selected time points during their follow-up**
- **MS patients and Healthy Donors before, during and after pregnancy (collaboration with Obstetric Sant'Anna Hospital, Turin)**
- **Healthy Controls**

Procedures for samples collection

- **Staff dedicated to the selection, the blood draws, the handling and the data updating of the collected biological material.**
- **Refrigerate containers to store the blood draws until the start of handling**
- **Start of blood draws handling after up to 3 hours**
- **Establishment of a limited number of blood draws that can be collected for the bio-bank project every day to avoid mistakes due to the management of too many samples. Every day, a maximum of 6 samples are collected.**

Procedure for samples storage and data management

A biobanking system allows to encode each participant, to split biological fluids and cells in more aliquots and to easily track them by a serial-consecutive and unique barcode



Deep Freezer -80°C or Liquid Nitrogen

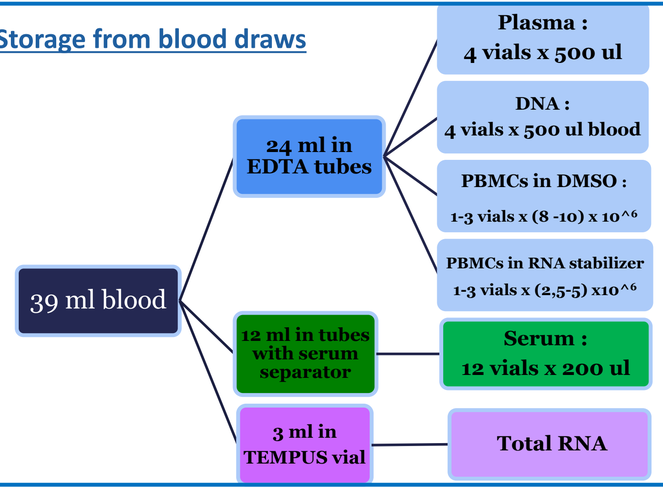
Blood draw

2 x 6 ml Vacutainer with serum separator

4 x 6 ml Vacutainer with EDTA

1 x 3 ml Tempus Blood RNA tube

Storage from blood draws



Storage of cerebrospinal fluid

Lumbar puncture → 12-18 ml → CSF : 24 vials x 500 ul

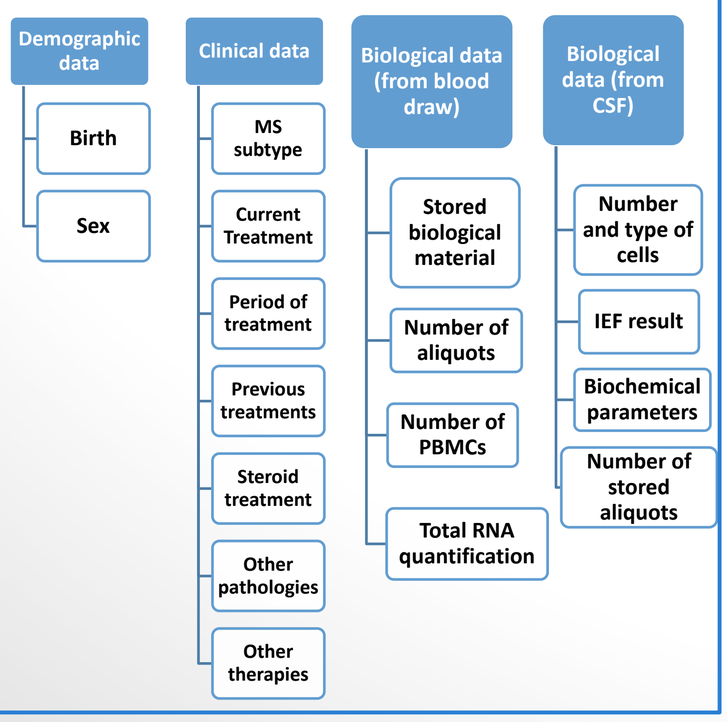
Revision and Update of the existing Informed Consent

A bioethicist has been involved in revising the existing Informed Consent (IC). The work focused on the revision of Informed Consents from different bio-banks in Italy and on the update of the existing CReSM IC. In particular, many aspects regarding the purposes of the bio-bank, the collection of data and the distribution of samples and data to other research groups have been cleared. The new IC has been approved by San Luigi Ethic Committee in July 2016.

Samples currently stored in the CReSM Bio-bank

N°	Biological material
823	CSF
2863	sera
2802	plasma
2729	DNA
3033	Total RNA
2596	PBMCs in RNA later
2639	PBMCs in DMSO

Data paired to the samples



Procedure for Bio-bank samples requested from MS Research Groups

1. **Research group should provide a description of the project for which samples are requested**
2. **Bio-bank staff evaluates the availability of stored samples**
3. **Bio-bank Scientific Committee evaluates the project**
4. **After the research project approbation and a Material Transfer Agreement (MTA) signature, Bio-bank provides the samples to the research group**
5. **The samples are free: a maintaining cost for the bio-bank will be requested, depending on the number and the type of samples**

It is strongly suggested that the data obtained using bio-banked samples are shared through a data-sharing system available to other investigators using similar samples.

Collaboration with a European bio-bank network collecting samples from MS patients treated with the new generation of monoclonal antibodies

- **Increasing importance for diagnostic, prognostic & therapeutic markers**
- **No academic consortium in neurology dedicated to cellular biobanking**
- **Field of emerging importance with little standard available**
- **Dedicated expert groups with already existing infrastructure**
- **Added value of teaming up:**
  1. **Biobanking quality/standardization issues**
  2. **"Cellular biobanking" science**
  3. **Cooperative project efforts**

Research projects realized by using the Bio-bank samples

The CReSM research group used biological material from bio-bank as follows:

1. PBMCs samples to identify by Flow Cytometry the Treg cell population in patients treated with Glatiramer Acetate compared to naïve patients and healthy controls;

2. serum samples to identify by a home-made ELISA the presence of anti-Kir4.1 antibodies as diagnostic biomarker for Multiple Sclerosis.

3. serum samples to evaluate the drug level and the immunogenicity of rituximab in MS treated patients.

External research projects that received bio-bank samples :

1. serum samples to evaluate the titer of anti-AQP4 antibodies in patients positive to anti-AQP4 antibodies as prognostic biomarker for Devic disease (Dr. Tampoia, Bari, Italy)

2. serum samples to identify the presence of anti-Kir4.1 antibodies as diagnostic biomarker for Multiple Sclerosis (Prof. Hemmer, Munich, Germany)

3. serum samples to identify the presence of anti-MOG antibodies as diagnostic biomarker for NMOSD (Dr. Jarius, Heidelberg, Germany)

4. DNA samples to evaluate the responsiveness to Fingolimod (Dr. Didonna, Prof. Oksenberg, San Francisco, USA)

5. RNA samples from Natalizumab treated patients to evaluate a change in the expression of anti-JCV antibodies (Prof. Achiron, Tel Aviv, Israel)

Bio-bank organization

Bio-bank and research

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