“CRYOSTEM Biobank: A National Prospective, Standardized Collection to Better Characterize Allogeneic Hematopoietic Stem Cell Transplantation Complications”

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CRYOSTEM was initiated in 2010 to create a multicenter biobank in the field of Hematopoietic Stem Cell Transplantation (HSCT). After initially concentrating on Graft-versus-Host Disease, CRYOSTEM has broadened its focus to all HSCT complications. Thanks to a network of 33 transplant units and 23 Biological Resources Centers, CRYOSTEM has established a standardized collection of high-quality biological samples associated with well-annotated clinical data from donors and patients pre- and post-HSCT. Plasma, dried pellets and viable cells in DMSO are isolated and cryopreserved from blood samples. Currently, the collection has reached almost 200,000 available samples coming from nearly 5,800 patients. Since 2015, CRYOSTEM has provided the national and international scientific community with these samples for large-scale research to improve the knowledge of HSCT complications.

Keywords: Hematopoietic Stem Cell Transplantation (HSCT); Graft-versus-Host Disease (GvHD); complications; cohort; biobanking network; biological resources

Funding statement: CRYOSTEM has been funded by the French government’s “National Investment Program” (call for proposals “COHORTES”, agreement ANR-10-COHO-008) and has also received financial support from INCa (call for proposals “BCB”, agreement 2013-192) and patient associations.

(1) Bioresource Overview

Project description

HSCT is the only curative treatment for numerous blood diseases (leukemia, lymphoma, aplastic anemia..) [1]. Its main complication is Graft-versus-Host Disease (GvHD), in which the donor's immune cells recognize the recipient (patient receiving the graft) as foreign. Due to several factors (growing recruitment of mismatch-related and unrelated donors and increasing recipients' age), current levels of GvHD incidence (60%) may rise over the next few years. In patients cured of their hematological diseases, GvHD is the leading cause of death post-HSCT (10 to 20%) [2]. Lack of knowledge in human GvHD physiopathology, along with limited relevant studies, explains the absence of curative treatment.

In this context, CRYOSTEM was launched in 2010, promoted by the Francophone Society for Stem Cell Transplantation and Cell Therapy (SFGM-TC), to establish a prospective and standardized multicenter cohort. In 2011, CRYOSTEM was funded with the financial support of the French government through the “National Investment Program”.

Initially focused on GvHD, CRYOSTEM has enlarged its thematics since November 2016 to all HSCT complications. CRYOSTEM’s initial main objectives were defined as follows:

- Establishing a collection of biological samples and well-annotated clinical data from patients and donors pre- and post-allogeneic HSCT;
- Supplying the national and international scientific community with these samples for large-scale research on HSCT complications and to improve biomedical knowledge in HSCT-related fields, such as oncology, hematology and immunology.

In fine, CRYOSTEM's goal is to improve transplanted patients healthcare by favoring a better understanding of HSCT complications and developing predictive tests and treatments.
To achieve these goals, CRYOSTEM has brought together 33 French transplant units and 23 Biological Resources Centers (BRCs) in a nationwide network (Figure 1), in which more than 400 health professionals work together. The national network has a positive impact on strategic logistics of patient inclusion (82% of transplanted patients – figures of the Agence de la Biomédecine 2017) and blood sample collection and processes. Indeed, nearly 65% of blood samples are processed in less than 4 hours. This operational network relies on CRYOSTEM governance (Figure 2), leading to an efficient internal communication and interactivity between all partners and to staff commitment so as to unify the different sites as one unique working whole.

Classification (1)
Human.

Species
Non Applicable.

Classification (2)
Biological samples and associated clinical data.

Context

Spatial coverage
Blood samples are collected in CRYOSTEM network transplant units and treated in the affiliated BRCs. Centers are located in Amiens, Angers, Besançon-Dijon, Bordeaux, Brest, Caen, Clermont-Ferrand, Créteil, Grenoble, Limoges, Lille, Lyon, Marseille, Montpellier, Nantes, Nice, Paris, Percy, Poitiers, Rennes, Saint-Etienne, Toulouse, Tours, Villejuif – France.

Temporal coverage
CRYOSTEM collection started on 2012, July 9th with the first patient inclusion and is currently ongoing. The last inclusion will be done on 2020, December 31st.

Temporal coverage for accessibility
Non Applicable.

(2) Methods

Steps
As part of CRYOSTEM governance ISO 9001 certification, obtained in 2015 (2008 version) and renewed in 2018 (2015 version), standard operating procedures (SOPs) have been established for consents management, blood collection, samples transfer from transplant units to BRCs and samples processing so as to establish a high-quality and harmonized collection, independently at each center. SOPs are similarly applied without exception in all network centers. Furthermore, all transplant units fulfill JACIE accreditation requirements regarding HSCT quality of care and practices. CRYOSTEM affiliated BRCs are for the most ISO 9001 and/or NF 596-900 certified.

Blood samples are collected pre- and post-HSCT from patients and donors in line with a simple sampling schedule (Figure 3). A unique form, CRYOSTEM liaison document, is used to record data at blood sampling (patient ID,
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collected period, blood volume, date and hour…) and must be attached to samples. BRCs process blood samples in line with pre-analytical procedures and protocols, established in tandem with CRYOSTEM network, according to French standard NF S96-900. Moreover, CRYOSTEM governance provides key reagents to BRCs (RPMI, lysis solution, cell preparation tubes), so as to ensure a homogeneous treatment of blood samples.

Sample data, such as patient (or donor) personal information, consents and clinical data are centralized in a unique secured database: CRYOSTEM MBioLims, developed by Modul-Bio (www.modul-bio.com). CRYOSTEM MBioLims is accessible by all transplant units and BRCs personnel using unique personal user codes. Dedicated procedures have been set up to manage the database using, the data recording and centralisation.

Regarding CRYOSTEM Quality System Management (QMS), all procedures and protocols are reviewed every two years or on request. Moreover, CRYOSTEM governance proceeds each year to internal audits of transplant units and BRCs. Finally, since 2015, CRYOSTEM has set up around thirty indicators to control its processes achievement and efficacy.

Stabilization/preservation
Blood samples are collected using EDTA tubes.

Blood-derived samples (plasma, viable cells in DMSO and dried pellets) are stored in standard barcoded cryovials.

Type of long-term preservation
Frozen samples.

Storage temperature
–150°C to –190°C (vapor or liquid nitrogen).

Shipping temperature from patient/source to preservation or research use
Room temperature (18–25°C).

Shipping temperature from storage to research use
–80°C (dry ice).

Quality assurance measures
Samples quality is monitored through annual quality control campaigns, performed on a representative sampling of aliquots according to the year and the BRC. Viable cells in DMSO and dried pellets are tested blinded.

Quality criteria established as indicators for CRYOSTEM BRCs activity are the following:

- mean cell recovery and viability rates for cells in DMSO following thawing and washing, at least higher than 50% and 90% respectively (in line with literature [3, 4]).
- a ratio of optical density (OD) 260/280, reflecting DNA purity of the dried pellets, included between 1.8 and 2.0.

As far as quality controls are concerned, almost 700 aliquots of cells in DMSO and dried pellets from CRYOSTEM BRCs were tested between 2014 and 2018. Mean cell recovery and viability rates for cells in DMSO were 50% and 93%, respectively. For dried pellets, a 1.90 mean OD ratio was obtained, confirming high DNA purity. Results also highlighted the high reproducibility of the parameters tested since 2014.

Moreover, an extensive monitoring of biological and clinical data entered into CRYOSTEM MBioLims database is ensured weekly by an external clinical data technician. Liaison documents and database filling quality is reported and used as indicators in CRYOSTEM QMS and presented at each annual board meeting.

Finally, with a view of continuous improvement, CRYOSTEM MBioLims software is tailored to the biobank evolution and is regularly updated, thus resulting in homogenization of samples labelling, traceability and real-time monitoring of operations (consents import, reagents follow-up…).

Source of associated data
The liaison document attached to blood samples enables to collect several types of data:

- Socio-demographic: directly related to patients identity (anonymised). These data are collected initially at patients’ inclusion and checked at each new sampling.
– Biological: date, hour and volume of the blood collected in transplant units, date and hour of the sample processing samples in BRCs.
– Clinical: transplant date and type. Data related to acute or chronic GvHD are also recorded: targeted organs and stages according Glucksberg classification for acute GvHD; nature, classification according NIH Filipovich 2015 and evolution for chronic GvHD.
– Additional data such as transplant unit site, patient local ID and key reagents used for the blood processing.

All these data are recorded and centralized in CRYOSTEM MBioLims database.

Moreover, CRYOSTEM MBioLims is directly linked to the EBMT (European Society for Blood and Marrow Transplantation) clinical register PRoMISe. This interoperability enables CRYOSTEM and PRoMISe patients ID correlation and extraction of additional clinical data such as diagnosis and disease status pre- and post-transplant, transplant conditioning, chimerism, GvHD prophylaxis, follow-up, relapse, infections post-transplant...

**Ethics Statement**

Patient documents (consent forms and supplementary information) were written with contribution of a number of patient associations. Patients are clearly informed of the biological resources and associated data later use, notably of the potential genetically researches.

Regulatory files were submitted to all official authorities and all authorizations were obtained within a year. Since then, all regulatory authorizations have been regularly updated as the network has expanded and the project has developed further (Figure 4). The authorizations duration corresponds to the time of the project accomplishment.

At the moment of recording in CRYOSTEM MBioLims database, patient identity is made anonymous and a unique ID is assigned to each patient, in the same way for each blood sample and aliquot. The database system is ongoing updating for implementation of GPDR.

Samples users have only access to anonymised data through encoded files.

**Constraints**

Non applicable.

**(3) Bioresource description**

**Object name**

Biological resources from patients and donors pre- and post-Hematopoietic Stem Cell Transplantation.

**Bioresource name**

CRYOSTEM.

**Bioresource location**

CRYOSTEM consortium consists of a central management hub (governance) located at the Institut Paoli-Calmettes, in Marseille (France) and a network of transplant units (blood collection centers) and BRCs (samples processing and storage), located across France (Table 1).

**Bioresource contact**

contact@cryostem.org.

**Bioresource URL**


**Identifier used**


**Bioresource type**

CRYOSTEM samples are derived from blood collected by any patient suffering from blood disorders, such as leukemia, lymphoma, aplastic anemia or other... and justifying a first HSCT indication.

CRYOSTEM samples are also derived from willing related donors for geno- and haplo-identical HSCT. French unrelated donors have been included in CRYOSTEM since

**Figure 4:** CRYOSTEM regulatory authorizations.
Table 1: CRYOSTEM consortium, list of the affiliated transplant units and BRCs.

<table>
<thead>
<tr>
<th>City</th>
<th>Transplant Unit (Blood collection)</th>
<th>Affiliated BRC (Blood processing and derived samples cryopreservation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angers</td>
<td>Service des Maladies du Sang, CHU Angers</td>
<td>Biothèque Patients, EFS Centre-Pays de Loire</td>
</tr>
<tr>
<td>Besançon</td>
<td>Service Hématologie, Hôpital Jean Minjoz, CHRU Besançon</td>
<td>Plateforme de Biomonitoring, EFS Bourgogne Franche-Comté, CRB Ferdinand Cabanne, Dijon</td>
</tr>
<tr>
<td>Grenoble</td>
<td>Service Hématologie, CHU Grenoble</td>
<td>CRB « Institut de Biologie et de Pathologie », CHU Grenoble</td>
</tr>
<tr>
<td>Lille</td>
<td>Service des Maladies du Sang, Hôpital Claude Huriez, CHRU Lille</td>
<td>Centre de Biologie –Pathologie Pierre-Marie Degand, CHRU Lille</td>
</tr>
<tr>
<td>Lyon</td>
<td>Service Hématologie Adulte, Hospices Civils de Lyon</td>
<td>CRB Groupe Hospitalier Lyon Sud</td>
</tr>
<tr>
<td>Bordeaux</td>
<td>Service Hématologie Clinique et Thérapie Cellulaire, Hôpital Haut-Lévêque, CHU Bordeaux</td>
<td>Biothèque Site Pellegrin, EFS Nouvelle Aquitaine</td>
</tr>
<tr>
<td>Marseille</td>
<td>Unité de Transplantation et de Thérapie Cellulaire, Institut Paoli-Calmettes</td>
<td>CRB Institut Paoli-Calmettes</td>
</tr>
<tr>
<td>Nantes</td>
<td>Service Hématologie Adulte, Hôpital Hôtel-Dieu, CHU Nantes</td>
<td>CRB CHU Nantes</td>
</tr>
<tr>
<td>Paris</td>
<td>Service Hématologie Greffe, Hôpital Saint-Louis, AP-HP</td>
<td>Cellulothèque Hôpital Saint-Louis, AP-HP</td>
</tr>
<tr>
<td>Créteil</td>
<td>Service Hématologie Clinique, Hôpital Henri Mondor, AP-HP</td>
<td>Plateforme de Ressources Biologiques, Hôpital Henri Mondor, AP-HP</td>
</tr>
<tr>
<td>Toulouse</td>
<td>Service Hématologie, Hôpital Purpan, CHU Toulouse</td>
<td>Unité mixte de Recherche Inserm U1037/Université Paul Sabatier Toulouse 3 du Centre De Recherche en Cancérologie De Toulouse, INSERM</td>
</tr>
<tr>
<td>Tours</td>
<td>Service Hématothérapie Cellulaire, Hôpital Bretonneau, CHU Tours</td>
<td>CRB de Touraine, CHU Tours</td>
</tr>
<tr>
<td>Rennes</td>
<td>Service Hématologie Clinique, Hôpital Pontchaillou, CHU Rennes</td>
<td>CRB Santé Rennes, CHU Rennes</td>
</tr>
<tr>
<td>Clermont-Ferrand</td>
<td>Service Hématologie Clinique Adulte et Thérapie Cellulaire, Hôpital Estaing, CHU Clermont-Ferrand</td>
<td>CRB Auvergne, CHU Clermont-Ferrand</td>
</tr>
<tr>
<td>Saint-Etienne</td>
<td>Département Hématologie, Institut de Cancérologie Lucien Neuwirth</td>
<td>Tumorothèque CRB 42.TTK, CHU Saint-Etienne</td>
</tr>
</tbody>
</table>

(Contd.)
### Type of sampling

All patients, adult and pediatric, answering to the inclusion criteria specified below (see *“Bioresource type”*) can be included in CRYOSTEM protocol. The **Figure 5** illustrates the cohort representation in terms of age, gender and type of transplant.

Blood samples are taken pre- and post-HSCT by patients or donors in line with the sampling schedule presented above (**Figure 3**). The sampling schedule specifies systematic sampling at 100 days (s1), one (s2) and two (s3) years following transplant as well as sampling just as GvHD symptoms appear and drugs are introduced (a1/a2–c1/c2). Blood collection is integrated to patients’ health care pathway and performed in line with the European regulations into effect.

### Anatomical site

Blood is classically taken from patients and donors from arm.

### Disease status of patients/source

Patients with an HSCT indication are suffering from blood disorders (leukemia, lymphoma...). Disease type and status before transplant are available for each patient in the EBMT PRoMISE register. HSCT donors are healthy patients.

### Clinical characteristics of patients/source

Patient of any age is included in CRYOSTEM protocol if a first indication of allotransplant has been recommended. Patients’ profiles are very different from one to another. All

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**Figure 5:** CRYOSTEM cohort representation (data given as of May 31, 2019).
patients’ characteristics are available through CRYOSTEM database and PRoMISE register (age, gender, pathology nature and status before transplant).

**Size of the bioresource**
Since its beginning, a high proportion of transplanted patients have been included in CRYOSTEM protocol due to the great diligence of the medical community. In only 6 years, more than 5,700 patients and 2,300 donors have been included with an annual rhythm of almost 1,100 patients between 2013 and 2017 (as of May 31, 2019, Figure 6). In 2017, the inclusion rate was almost 82% (as a percentage of patients transplanted in CRYOSTEM network transplant units, data from the Agence de la Biomédecine, https://www.agence-biomedecine.fr/). Since January 1, 2018, CRYOSTEM plenary committee has voted a restriction of inclusions to answer budgetary constraints and to optimize cohort longevity.

**Vital state of patients/source**
Patients are alive at time of sampling. Death of transplanted patients is reported in CRYOSTEM MBioLims database.

**Clinical diagnosis of patients/source**
Included patients in CRYOSTEM protocol are suffering from various blood disorders, for which the clinical diagnosis before and after transplant is available in the EBMT PRoMISE register.

**Pathology diagnosis**
Included patients in CRYOSTEM protocol are suffering from various blood disorders, for which the pathology diagnosis is available in the EBMT PRoMISE register.

**Control samples**
Donors’ samples are considered as control samples, considering that donors have no haematological disease and are qualified beforehand by the EFS (Établissement Français du Sang) for bone marrow or peripheral blood stem cell donation.

**Biospecimen type**
Three sample types are isolated and cryopreserved from blood samples: plasma, dried pellets and viable cells in DMSO. The Table 2 summarizes the samples characteristics according CRYOSTEM internal protocols.

**Size of the bioresource**
Since 2012, nearly 17,200 blood samples have been collected (as of May 31, 2019). The Table 3 summarizes the number of available samples and aliquots per category. The Figure 7 shows the evolution in the biobank activity since 2012.

**Table 2:** CRYOSTEM samples characteristics.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Number of aliquots</th>
<th>Volume</th>
<th>Cell quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>4 maximum</td>
<td>1 ml</td>
<td></td>
</tr>
<tr>
<td>Viable cells in DMSO</td>
<td>4 to 10</td>
<td>1 ml</td>
<td>8 to 10 million of cells/vial</td>
</tr>
<tr>
<td>Dried pellets</td>
<td>4 maximum</td>
<td>–</td>
<td>2 to 4 millions of cells /vial</td>
</tr>
</tbody>
</table>

**Table 3:** Number of available samples and aliquots in CRYOSTEM biobank as of May 31, 2019.

<table>
<thead>
<tr>
<th>As of 2019/05/31</th>
<th>Plasma</th>
<th>Viable cells in DMSO</th>
<th>Dried pellets</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Available samples</td>
<td>16,746</td>
<td>15,996</td>
<td>16,775</td>
<td>49,517</td>
</tr>
<tr>
<td>Available aliquots</td>
<td>49,665</td>
<td>65,702</td>
<td>82,924</td>
<td>198,291</td>
</tr>
</tbody>
</table>

**Figure 6:** CRYOSTEM cohort representation (data given as of May 31, 2019).

**Figure 7:** CRYOSTEM biobank dynamics since July 2012 (data given as of May 31, 2019).
real time and sample availability by transplant type and period.

**Release date**
For strategic reasons, a decision was made initially to maintain an embargo on the collection for the first three years. The main objective was to reach a critical sample mass to enable large-scale studies to begin starting with the first call for proposals. The embargo ended on April 15, 2015.

**Access criteria**
Since the end of 2014, members of the national and international scientific community, from both academia and industry, have had access to CRYOSTEM collection via annual calls for proposals. Calls for projects are ruled by general terms that specify for both CRYOSTEM and investigators their commitments and obligations, intellectual property management, terms and conditions of sample access and use, schedule and overall organization, dispute settlement, and a sample provision pricing list. The general terms are sufficiently exhaustive to lighten licensing procedures and avoid having to write licensing contracts. General terms are appended to a well-defined and detailed application file that summarizes all administrative and scientific details.

Investigators submit an application file describing the purpose of their project, all the administrative and scientific details needed for evaluation. The file specifies also the selection criteria of patients and samples required for the project and the number of aliquots. The number of aliquots needed is not limited but has to be justified and should be consistent with the project. Applications are first pre-assessed by CRYOSTEM Scientific Board, and then forwarded to international experts for review. The Scientific Board is responsible for the final selection based on the scores awarded by the reviewers. Once selected, investigators must comply with the general terms of the call for projects by signing an agreement form. Following interviews with investigators to determine sample type, number and characteristics, sample provision is coordinated by CRYOSTEM project managers.

Access costs to the collection are reviewed annually and included in the general terms for each call for projects, defining the financial conditions under which access to biological samples and clinical data can be obtained. These costs include pre-analytical processing fees, sample selection, destocking, associated clinical data export, and collection durability. They allow for BRCs to be compensated for sample retrieval and control, transplanting units for blood sample management, SFGM-TC for clinical data management, and CRYOSTEM for sample selection and centralization.

General terms and application file of the ongoing call for projects are available and downloadable on CRYOSTEM website (www.cryostem.org).

**Reuse potential**
Investigators could have access CRYOSTEM biological resources to lead fundamental or translational researches directly in the field of HSCT complications or in fields related to HSCT (hematology, cancerology, immunology, infectiology...). CRYOSTEM samples and data could be used for retrospective studies: for example to validate preliminary results obtained in smaller and/or local cohorts or in murine models, to evaluate efficacy of drugs under development...

Once selected, investigators have access to CRYOSTEM biological resources according to their selection criteria and the availability. Biological samples and associated clinical data have to be used within the scope for which CRYOSTEM has given its approval. Investigators could, within a period of 36 months following the end of their projects, ask for authorization from CRYOSTEM to use the biological resources for another research project. The new project must be submitted beforehand to CRYOSTEM Scientific Committee, who decides to reuse the biological resources for an additional scope.

Since 2015, and as of May 2019, almost 6,600 aliquots (around 4% of the collection) have been provided to researchers for the implementation of 9 national and international projects.

**Acknowledgements**
We would like to acknowledge all partners working in CRYOSTEM consortium transplant units and BRCs, including nurses, clinical research assistants, physicians, technicians, BRC managers. We would like to thank CRYOSTEM scientific and advisory boards' members. Furthermore, we thank SFGM-TC president, members and database managers. We also acknowledge the Agence de la Biomédecine.

Finally, we acknowledge CRYOSTEM funders: the Health and Biotechnologies Direction at French Government Agency, the French National Cancer Institute (INCa) and the patient associations (Association Laurette FUGAIN, IRGHET, Cent pour Sang la Vie, Vaincre la Leucémie).

**Competing Interests**
The authors have no competing interests to declare.

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Dalle Jean-Hugues: Pediatric Transplant Units Representative
Peffault de Latour Régis: Creator and Project Coordinator
Calmels Boris: Creator and BRCs Coordinator

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