Frozen in Translation: Biobanks as a Tool for Cancer Research

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Abstract: In the context of translational cancer research, biobanks are key infrastructures that provide high quality biological samples, coupled with relevant clinical and pathological information. This role can only be successfully accomplished through the implementation of standardized procedures that ensure proper collection, handling, processing, storage and recording of tissue samples, following strict legal and ethical regulations. Biobank networking is fundamental for dissemination of good practices and to help in the establishment of new infrastructures that improve the assessment of heterogeneity among tumor types and across patient cohorts. Growing demands for large number of homogenously preserved tumor tissue samples can only be met through a more intense cooperation among biobanks, facilitated by networks that foster cooperation at international level. The potential of biobanks as fundamental tools for translational cancer research can only be achieved through a concerted effort from biobankers, researchers, legislators and tissue donors that may allow for improved sample exchange.

Keywords: Biobank, tumor bank, frozen tissue, biological fluids, clinical samples, informed consent.

INTRODUCTION

The impressive and unprecedented growth of knowledge on the genesis, development and progression of cancer at molecular level would never been achieved without the support of tissue samples, representing the various tumor conditions and subtypes. Thus, the availability of standardized and homogeneously preserved (i.e., high quality) tissue samples, collected from tumors and paired normal tissue, coupled with relevant clinical and pathological information is key to translational cancer research, in its attempt to transpose to the bedside the basic findings in cell lines and animal models. This can only be achieved through the activity of large biorepositories that collect, process and store tissue remnants from diagnostic or therapeutic procedures after informed consent of cancer patients. The operation of these biobanks is largely dependent on the institutional and personal commitment of a large number of professionals, spanning from the surgical theater to the Pathology department, where most biobank storing facilities are based. Indeed, a cancer biobank results from the joint efforts of clinicians, pathologists, clinical scientists and laboratory technicians, that work in concert to provide samples for internal and/or research projects. The successful operation of a biobank depends on its structure and staffing, on the strict observation of standardized procedures, covering from collection to storing, that include both technical and ethical/legal aspects. Because sample size may determine the significance of a scientific finding, large multi-centre projects require the contribution of several biobanks that may ensure homogenous sample quality at biological and clinical level. Thus, biobank networks have emerged in response to this need and even gave rise to higher level organizations. Herein, we review some of these fundamental aspects of biobanking activity in the context of cancer research, as well as its major challenges.

The aim of this review is to provide an overview of the organization and operation of cancer biobanks and how this impacts on its contribution to translational research. Moreover, the growing need for a uniform ethical and legal framework as well as for functional biobank networks is also addressed.

CANCER BIOBANK ORGANIZATION AND STRUCTURE

An efficient biobank repository must guarantee the safe keeping of stored material, support the equipment employed, and provide a safe and effective working environment for the repository staff. In planning the design of a repository it is necessary to know not only the type of material being stored, but also the required storage and handling conditions, the projected retention periods, as well as estimated growth of specimen numbers and use of the materials. The design should also include sufficient space to accommodate the material planned for initial, future and backup storage [1].

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The main goal of establishing an organizational structure in tissue biobanks is to minimize warm ischemia time and tissue degradation. It is essential to guarantee that tissue is fit to be used for a variety of research purposes which demands that tissues should be frozen, ideally, within 20 min, which is the desired standard for human tissue preservation prior to cDNA or oligonucleotide microarray analysis [2]. Thus, it is also fundamental that the surgeon streamlines standard operating procedures to minimize warm ischemia [3]. It is of the upmost importance that the collection of remnant human tissue for research or education does not compromise the diagnostic and prognostic integrity of a specimen. Thus, Pathology is key to this process as specimens will be transferred from the operating theatre to the Pathology department [1, 3]. Consequently, tissue banking activities must be integrated into the routine surgical and pathological activities for the efficient acquisition of tissue and both teams should provide full support for this effort [3].

Infrastructure and Facilities

Biobanks should have dedicated facilities, with adequate air conditioning to maintain ambient temperature under 22°C, which is vital to achieve optimal lifespan of the mechanical refrigeration equipment. This is particularly critical for rooms containing multiple mechanical units [1, 4]. Rooms that contain liquid nitrogen (LN2) tanks should be equipped with appropriate air flow systems coupled to oxygen level alarm system to avoid the accumulation of nitrogen in case of leakage. These facilities require a constant source of electrical power, entailing the need for backup power system that should have the capacity to run for sufficient time until restoration of power supply and this should be regularly tested [4]. Security systems must be monitored and alarms must be able to be responded 24 hours a day, 7 days a week. The reaction to an alarm must occur within a time frame that prevents or minimizes loss or damage to the stored samples [4]. This may be facilitated by adequate backup capacity of low-temperature units, which must be kept, typically, within 5%– 10% of the total freezing capacity [1, 4]. Importantly, only persons assigned to the biobank operation should have access to the material, and all materials added or withdrawn should be properly documented [1, 4].

Training

All repository staff must have an appropriate level of educational background, experience and specialized training, ensuring that assigned tasks are performed in accordance with the established procedures. Proper training is important for quality in specimen handling. This should be made available to all technical personnel of the biobank and performed in accordance with the standard operating procedures. The repository itself should be placed under the operational supervision of a resource manager with sufficient training and experience in tissue and molecular biology, which will have a critical role in receiving, processing and analysing requests for access to stored specimens [1, 4].

Storage Conditions

Biospecimens should be stored in a stabilized state. Therefore, samples must be stored appropriately and maintained in liquid nitrogen or in a -80ºC freezer. In selecting the storage temperature, the biospecimen types, anticipated length of storage and biomolecules of interest must be considered. Experience suggests that DNA and RNA yields will remain constant over a decade or more when tissue is long-term stored in vapor-phase liquid nitrogen freezers, which is the standard at some centers [3-5]. It is generally agreed that liquid nitrogen storage is recommended for proteomic research, but for general use, a -80ºC freezer is adequate when coupled with appropriate risk management. Temperatures at or below -80ºC are generally adequate for successful preservation of cells and tissues for extended periods of time. Also, the shelf-life increases dramatically as the storage temperature is reduced [3, 4].

It must be taken in consideration that storage of frozen tissue in upright freezers at -80ºC has some advantages in comparison to liquid nitrogen storage: greater sample accessibility, simpler installation, fewer maintenance requirements, and lower price (both at purchase and maintenance). Mechanical freezers depend on electrical power supply network, thus requiring appropriate security measures to minimize the risk of large temperature fluctuations or complete failure. It is advised that mechanical freezers are incorporated into a secure electricity supply so that in case of power failure, emergency generators will ensure continuity of supply. In addition, it is recommended that a triple-layer alarm system is installed both for mechanical freezers and liquid nitrogen repositories, which will monitor any temperature increases of more than 10% above -80ºC [3].

For optimal preservation, formalin-fixed, paraffin-embedded tissue should be stored as a block and not

sliced until analysis is imminent because degradation will occur even under the best storage conditions. Paraffin blocks should be stored at temperatures below 27 °C in light- and humidity-controlled facilities [4, 6]. In the case of biofluids, such as blood and urine, biospecimen components should be separated before storage to preserve each constituent under proper optimal conditions. Whole blood (rather than fractional) cryopreservation may be an efficient and cost-effective option for processing viable cells in large-scale studies [6].

OPERATING PROCEDURES FOR SAMPLE COLLECTION AND PROCESSING

Standard operating procedures (SOPs) constitute the backbone of the biobank activity. These must be clear, complete and easily accessible to all personal that is involved in the process of sample collection, processing and management. Thus, its influence is not restricted to the biorepository itself but it spans from surgical rooms and other areas of sample collection to sample requesters and users, as well. The main goal of SOPs is to ensure that all samples stored are of the highest quality and that its collection and availability is made in accordance with the best practices and ethical and legal regulations (Figure **1**). Some critical issues concerning biobank SOPs are detailed and discussed below.

Lag Time between Surgical Excision and Freezing of Tissue Samples

Snap freezing as soon as possible after surgery has been considered the gold standard rule to obtain high quality tissue samples. A reduced lag time between surgical excision and tissue freezing is mandatory to ensure minimal specimen degradation, so that the information that might be extracted from the sample is not biased [3]. Indeed, some studies have reported significant changes in gene and protein expression within few minutes after surgery [2, 7]. In contrast,

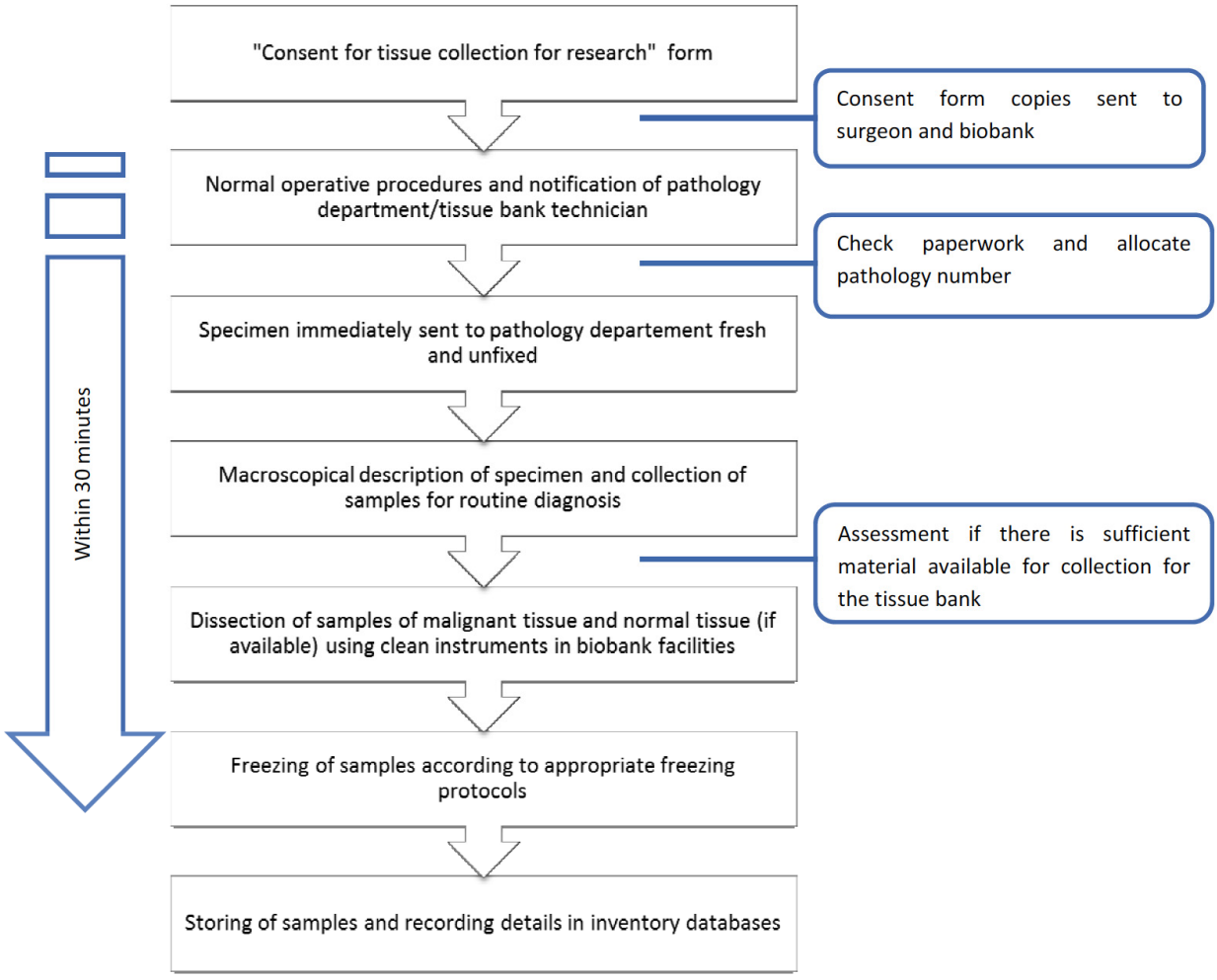

Figure 1: Simplified flow chart of specimen acquisition and storage in a cancer biobank.

other studies demonstrated no relevant or just minimal changes in gene expression according to ischemia time, although there is widespread agreement that it is prudent to snap freeze the samples as soon as possible after resection [8-10]. Based on the available data, the maximal lag time from excision to snap-freezing of tissue samples is 30 min [3]. Taking into account that this timeframe may be, in some cases, impractical, a delay time of up to 2h can still be accepted, provided that a note on the delay is annotated in the local database, so that researchers receiving the samples be informed about a factor that might affect sample quality [3].

Sample Transport

A step that significantly influences lag time from excision to snap-freezing is transport from the surgical theater to the Pathology facilities, were sample collection will take place. Transport should be as fast as possible and also made under the best preservation conditions as possible, ensuring a high sample quality. Ideally it should be made in a plastic container/bag on ice to keep them cool and, thus, delay degradation [3]. The use of refrigerated vacuum-based systems might provide an advantage for transport of specimens, extending the time from excision to sample freezing while keeping acceptable nucleic acid and protein quality [11]. Importantly, this technology also allows for an expansion of collection sites enabling a more effective networking among centers at different geographical locations.

Sample Collection and Processing

Sample Collection

During macroscopical examination, the Pathologist must decide whether tissue collection for biobanking is feasible or not. Indeed, tissue fragments for routine diagnosis are a priority, and only leftover tissue should be considered for research purposes [3]. In the context of a specimen containing a cancerous lesion, not only tumor samples but also normal tissue (at distance) and, eventually, pre-malignant lesions should be procured for freezing and storage [4].

Specimen Dissection

To avoid contamination from other tissue sources as well as from microorganisms (which might impact not only in molecular analysis results, but also on tissue degradation status), clean instruments must be used do dissect the specimen, and these should be changed, or at least cleaned, between dissecting normal and tumor tissues [3]. The use of disposable instruments may greatly facilitate this task.

Sample Size

Tissue fragments collected for the biobank are frequently very small, especially when dealing with biopsies, due to the modern tissue–sparing techniques. Ideally the tissue samples collect should have approximately $0.5cm^3$ [3]. In large surgical specimens, the main limitation is tumor size and, eventually, the amount of tumor necrosis. Nevertheless, the usual pathological rule of collecting one fragment for histopathological analysis for each centimeter of tumor largest diameter is usually compatible with tissue collection for biobanking.

Sample Containers

A variety of sample containers is currently available, from cryovials to cryomolds an cryostraws, which vary in volume and shape. An ideal and universal sample container does not exist, since it directly depends on the type and volume of the sample and on the inventory and storage system used. However, there are some criteria that all containers used for snap-freezing and storing of tissues in a biobank should meet: be specifically designed for storing biological material at ultra-low temperatures; be stable when samples are submitted to snap-freezing and stored at low temperatures for long periods of time; be as leek proof as possible [3].

Snap Freezing

There are several techniques for tissue snap-freezing, but it is highly recommended to snap-freeze the samples in pre-cooled isopentane (2- methyl butane), especially when specimen morphology needs to be preserved [1, 3]. Isopentane is a very efficient cryo-conductor, allowing for rapid freezing of the samples and causing fewer cryo-artifacts in comparison to liquid nitrogen, as isopentane remains in a liquid state during the process [3]. Although this method seems to be the most suitable, it has some disadvantages as it is time consuming, complex and poses additional biohazards [3]. To overcome some of these disadvantages, the freezing process could performed in specifically designed equipments [3].

Chemical and Biological Hazards

Manipulation of fresh human tissue always carries a risk of exposure to infectious agents. Currently, it is impossible to fully ensure the absence of high-risk agents through specific tests of all patients, thus all

tissue samples should be handled as if potential infectious. To be protected from these hazards, biobank staff must use personal protective equipment suitable and comply with general biosafety rules. Working with freezers and isopentane also carries some hazards, and, thus, biobank personnel should carry out all procedures in compliance with safety rules specific for chemicals and equipments used [3].

Sample Labelling

All samples collected need to be properly identified. During the freezing procedure, sample containers are labeled with a local inventory code or a bar code. When a bar code system is used to label the samples, this improves sample management and precise identification. As an alternative, a waterproof pen and labels able to withstand storage at low temperatures should be used. The code attributed to each sample should not be related, in any way, with pathology number or other patient identifiers. Data related with the samples must be recorded, in association with the identification code, in an inventory book and in an electronic database, with restricted access [3].

Formalin-Fixed and Paraffin Embedded Samples

In some biobanks, when material available is enough, a fragment is collected for formalin fixation and processed for paraffin embedding. This method of processing and storing tissue samples is very cheap, when compared to snap-freezing, and allows for high quality morphological studies and a range of histopathological techniques which are less likely to be successfully performed in frozen tissue samples. In practice, this procedure allows for the existence of a parallel sample that may complement studies performed in the respective frozen tissue. The collection of these samples is made along with the collection of samples for snap-freezing. Ideally the fragment that is sent to the biobank is divided in two, providing “twin” fragments, one for snap-freezing and the other for formalin fixation. Owing to this collection procedure, the formalin-fixed paraffin-embedded samples are virtually representative of the respective snap frozen samples. Thus, it might be use for morphological control of tumor cell representation and proportion, providing an unexpensive and effective quality control of biobank samples [1].

COLLECTION AND PRESERVATION OF BIOLOGICAL FLUIDS

In addition to tissue, bodily fluids are the most commonly banked biospecimens, and these may include whole blood, serum, plasma, urine, saliva and bone marrow. Biobanking of the biofluid samples from different body compartments is becoming increasingly important for various purposes: risk assessment, screening, diagnosis, prognostication and prediction of response to treatment. Furthermore, blood and urine samples are widely used in several tests implemented in clinical practice, and currently act as starting point for new research on various types of neoplasms. Its accessibility and potential for acquisition of relevant information about tumor genomics, transcriptomics and metabolomics, justifies the contemporary use of “liquid biopsy” as a synonym for body fluid sample for clinical and research purposes.

There are many different protocols that may be used for these fluids biospecimen collection, and they differ primarily on the end purpose. Instead of a standard protocol, different biobanking organizations follow a set of general recommendations regarding adequate sampling, processing and storage of body fluids.

Sample Collection and Processing

Once biobanking purposes and project design are established, the type(s) of biofluid(s) to be collected should be defined so that collection and handling protocols are established. Usually, samples are separated in several aliquots appropriate to different purposes, and may be then processed differently. According to those distinct purposes, there are many factors that should be taken in consideration which may affect stability and quality of biofluids samples.

A major factor is lag time and temperature to initial processing. Although this may vary according to specimen type and its intended use, it is commonly accepted that the smaller the lag time and the lower the temperature between sampling and its processing, the lower is the risk of biomolecules degradation. Usually, if high cell viability is desired, the processing of samples should take place within 24-48 hours, and the sample should be at low-temperature environment (e.g., $4^{o}C$) [12, 13]. This is a recommendation for the majority of bodily fluids, but as previously stated, each sample type has its own particularities: blood samples collected to yield serum need to be maintained at room temperature for a minimum of 30 minutes to allow for clotting [14]. Time may be also a player that will influence the final outcome of the analysis regarding certain types of samples, such is the case of urine specimens, which may be “first morning urine samples”

– to detect substances in more concentrated solution -, "timed urine samples" – to follow a pattern of excretion of certain biomolecules -, or "fractional urine samples" – to compare the concentration of an analyte with its concentration in blood, for example [1].

Temperature is also important to the stability of biological samples during short and long-term storage. This topic is highly controversial and there are different opinions about the best temperature to store different types of samples and different components of the same sample. Generally, serum, plasma and urine are stored at -80^{o}C because they hold a large amount of soluble molecules that require low-temperatures to remain intact. The same applies to RNA, which is easily degradable at temperatures higher than -80^{o}C. Regarding isolated DNA, it is usually accepted that it remains stable at 4^{o}C for several weeks, at -20^{o}C for months, and at -80^{o}C for years [15, 16]. If the purpose is to obtain live cells, these need to be cultured up to 48h after sample collection, or cryopreserved in liquid nitrogen at -150^{o}C. It is also important to emphasize that storing samples as different aliquots helps avoid the freeze-thaw cycles that cause biomolecule degradation.

The use of anticoagulants and stabilizing agents is another important factor that should be taken into account. The choice of the specific agent to be used has to be carefully planned. Whereas certain anticoagulants are required for some purposes, others may be contraindicated, and still, in some situations, the sample needs to be in its pure state (anticoagulant-free) [17, 18]. An example is the use of citrate versus heparin-stabilized blood, in which the first is better indicated for a higher concentration of lymphocytes for culture and the other influences T-cell proliferation. EDTA is one of the most commonly used stabilizing agents, both for blood and urine biospecimens, and the general recommendation is that it should be added to the sample as soon as possible after its collection.

The choice of the collection and storage containers depends of the sample type, its volume, means of transport to the laboratory, storage facilities, and the analytical goals of the study. Moreover, overall standardization of sample labelling and its compatibility with automated platforms for processing should be considered. Nowadays, the efficiency of sample tracking has greatly increased thanks to electronic data management programs, which include sample barcoding for automatic scanning. Finally, sterility of the containers and aseptic conditions of sample collection and processing are critical, especially if RNA isolation or cell culturing is envisaged.

QUALITY CONTROL

As previously mentioned, many factors influence sample quality. Thus quality control is key for biobanking activity, ensuring that all samples are processed and stored according to the most appropriate methods and complying with local/national/international regulations. Thus, audit and testing must be performed regularly and in a standardized manner. The biorepository database may allow for random selection of cases to be analysed and for recording of results. It is recommended that 2% of new cases are assessed twice a year, during the first year, and if no significant problems are detected, then 1% of new cases each year should be reviewed thereafter [3].

Quantity and quality of nucleic acids (DNA and RNA) extracted from banked biospecimens can easily be assessed by the determination of the optical density (OD) at the 260nm and 280nm wavelengths. Generally, an OD 260/280 ratio equal to or greater than 1.8 is commonly accepted as a good indicator of the purity and integrity of DNA or RNA, namely the absence of contaminating proteins. Other wavelength readings may provide additional information, such as the OD 260/240 and OD 260/320 ratios, which inform on contaminants other than protein (e.g., EDTA, phenol, alcohol) [19-21]. Other common methods for assessing DNA integrity (despite its quantity) are agarose gel electrophoresis or amplification of a specific well-known sequence by polymerase chain reaction (PCR). Regarding RNA integrity, the most widely used test is the ratio of 28S to 18S ribosomal RNA (rRNA), or, more recently, the measurement of the RNA Integrity Number (RIN) [22]. Checking for nucleic acids integrity should take place periodically and always before the start of any project involving the respective samples.

The assessment of the biospecimens protein content and quality is also of major importance to provide accurate pathological and molecular information. The protein yield may be easily evaluated though the bicinchoninic acid (BCA) assay [23]. Besides histomorphological tissue evaluation, specific immunohistochemistry testing may provide an insight on the protein integrity of the sample. Ki-67, WT1, p53 and E-chaderin are examples of specific markers that are commonly and periodically used to immunohisto-chemically assess protein expression integrity in stored frozen samples [16, 24].

Eventually, biofluid specimens (e.g., urine, serum, plasma) may require an assessment of its integrity after storage, by means of detection and quantification of specific analytes, such is the case of the determination of hemoglobin content to evaluate hemolysis [25].

ETHICAL AND LEGAL ISSUES

The same way scientific progress is inevitable so it is ethical evaluation [26]. In recent years, biobanks have undergone rapid proliferation and have become increasingly complex structures. Consequently, they pose a wide spectrum of ethical and legal issues to researchers, donors and managers. In fact, the increasing number of studies on biobanking is paralleled by an increment in publications dealing with the numerous ethical aspects related to them [27]. With the expected further rapid development of the field, these issues will likely continue to arise and accumulate, thus requiring constant re-appraisal and continuing discussion [28].

Informed Consent

An informed, voluntary and valid consent is required for all biomedical research and biobanking is not an exception. It aims to protect patients' rights and autonomy and to help maintaining public trust in patient care and scientific progress [29]. However, consents in biobanking have certain specificities, in particular due to long-term storage of biomaterials and data that can be used for future projects which may not be specified at the time of sample collection. Hence, modifications to the consent process were introduced to respect a person's autonomous decision-making capacity, while also addressing these future research issues. The actual discordance on the most adequate type of consent is problematic, because the lack of proper consent may prevent samples from ever being used at all [30]. Due to the unpredictability of research projects, many biobanks ask donors to provide a "broad consent", instead of the standard narrow consent to one specific investigation. These should include information concerning aspects related to future research, like international sharing, property rights, commercial use and data protection, focusing on how the research differs from routine medical care, thus having the advantage of avoiding re-contacting donors throughout the study. Other biobanks opt for a more recent approach known as "dynamic consent", favoring an interactive process between both parties, giving participants the opportunity to state their preferences concerning their data [31]. A blanket consent is also an option for certain biobanks, but studies suggest that despite most donors wanting to donate to biobank research they may have moral, religious, and cultural concerns about the use to which their specimens are put, which may affect their willingness to give this type of consent [32].

A few empirical studies indicate that some research participants also have misconceptions about participating in biobank research, showing that consent procedures might not be explicit enough [33]. This initial lack of understanding of critical information included in the consent form will likely worsen over time as participants' memories fade and as the scientific complexity involving biobanking and international sharing increases [34]. Finally, most authors also support the idea of participants being able to withdraw their consent, but the extent of what exactly can be withdrawn and at which point of the study remains controversial [35].

Privacy

Research with remnant tissue can only be pursued when data about the patient is available. However, the link between these two types of information presents a major threat to individual's privacy, and this is considered by some as the major harm associated with biobanks [36]. In principle, only fully anonymized or partially anonymized but coded residual tissue can be used in an exchange program, to avoid access to personal information, especially from insurance companies [37]. Because fully anonymized samples limit the research utility by avoiding the potential to transform biobanks into longitudinal epidemiologic studies [38], most authors agree that coded information is safe enough to ensure a satisfactory level of privacy [39].

Re-Contacting Participants

Bioethicists have raised concerns about the idea of researchers having limited interactions with donors and have criticized biobanking practices that seek one-time permission for all future sample uses [40]. There is, indeed, controversy regarding re-contacting participants, as it may be quite expensive or even lead to unnecessary distress for them. Many authors consider unethical to deny donors the opportunity to receive significant clinical information unfolded by research, especially if it can improve its life [41]. Although an outstanding level of public support and will to participate in biobanks has been observed, some

studies have shown that most individuals want to receive regular updates and that they are comfortable with relatively inexpensive and convenient methods of communicating with researchers [42].

Benefit Sharing

Another issue that is rarely discussed but that is also very sensitive is benefit sharing, especially financial benefit from research results, as it can be distributed by many participants in biobank projects. Authors mainly agree that donors should not be paid, as they will eventually benefit from the results of the research in terms of better diagnosis or treatment of diseases [43].

Problems for Biobank Managers

Most literature on these ethical and legal issues has relied on perspectives of people outside the biobank management. Little attention was directed towards those who operate biobanks from inside. However, biobanking also raises problems to its managers, such as the fate of specimens if the biobank closes or if funding is limited. Biobank creation should be carefully designed to meet scientific needs but also planned according to sustainable business models and rules to handle specimens and data if the biobank needs to be terminated [44].

Legislation and International Sharing

Investigation on tissue samples requires collection of data from individuals on a large scale to achieve statistical power, making international collaboration both a scientific and an ethical imperative [45]. However, sharing of data and samples remains challenging, as significant variation persists among legal and ethical regulations governing biobanks in different jurisdictions. Recent laws on biobanking pose, indeed, many problems: they are often inflexible and conservative, extend to types of research far from the reality of biobanking, exhibit tremendous variability between nations, are reactive in nature, and very few provide an adequate encouragement for international access. The diversity observed is related to aspects like the distinct moral traditions, health research administrative structure and ethics review processes among countries and also biobank establishment at different speeds in different nations, with legislation being adopted at different times, in a reactive way.

Over the past years, there has been an avalanche of recommendations and guidelines on research with tissue and accompanying data [46]. This proliferation, however, has not led to consensus: despite being self-harmonizing to some extent and more adaptable than legislation, many statements avoid giving much regulatory guidance and also diverge on many important issues throughout countries, not clearly providing for international access and lacking the normative force to influence biobanking practice. Also, guidelines might even conflict with applicable laws, leaving researchers with the option of breaching one or the other [47].

Suggestions of harmonization of operating practices and networking from the European Science Foundation to the European Biobank Community have been issued, leading towards harmonization of methods, approaches, and tools used in biobanks, but not of the regulations; such a consensus would be hard, if not impossible, to be achieved due to the extent of variation of legislation between countries [48, 49]. The need to respect regulations and at the same time meet the need of sharing tissue between nations led to a coordinating rule based on the so-called “home-country principle”: if tissue may legitimately be used for a certain kind of research in the country where it was taken out and under whose jurisdiction the patient falls, it may also be used for such research in the country where it is sent to, even if in that other country other regulations would apply for research with residual tissue taken from patient under their jurisdiction [37].

All in all, as in any other ethical discussion, the general principles of autonomy, beneficence, non-maleficence, and justice are mandatory topics in the biobanking field. However, one should not envisage these principles as barriers to potentially life-changing progress as biobanks might represent a shift in thinking from individual to population-based understanding of health and disease [50].

BIOBANK NETWORKING

Unity makes strength. Thus, biobank networks are a means to amplify the effort of sample collection and provide a wider range and number of tumor types to allow for more specific and detailed analysis, with superior statistic power to detect meaningful differences across tumor types. Indeed, multicenter cohorts are need for the discovery and validation of diagnostic, prognostic and predictive biomarkers, as well as for the study of rare tumors. In these cases, even when a biobank is associated with several hospitals/institutions, the number of samples collected

may not be large enough to accomplish specific project aims. Moreover, international multicenter studies can reveal different geographic landscapes for a tumor subtype, for instance. Thus, biobank networks and consortia have emerged over the last decade, stimulating high-quality sample and expertise exchange among researchers (http://www.p3g.org/; http://biospecimens.cancer.gov/programs/cahub/default.asp).

The most well-known networks of biobanks that store cancer patients' biological samples are summarized in Table **1**. These networks mostly follow the virtual biobank model, providing the member biobanks a similar database organization, sometimes with specific software, as well as uniform SOPs and quality control guidelines (http://www.eurobiobank.org/; http://biospecimens.cancer.gov/programs/cahub/default.asp; http://www.tubafrost.org/). Samples are stored in each member biobank and the network interface provides a sample search method and facilitates the contact between researchers and biobanks (http://biospecimens.cancer.gov/programs/cahub/default.asp; http://www.tubafrost.org/). Biological material and associated clinical information may be shared only among member biobank research groups (OECI-Tubafrost) (http://www.tubafrost.org/), or may be available upon request and project evaluation for external researchers (Australasian Biospecimen Network – Oncology, Canadian Tumour Repository Network, EuroBioBank network) (http://abrn.net/; https://www.ctrnet.ca/; http://www.eurobiobank.org/).

Finally, international biobank networks may be further connected at higher level and scale. Examples include BBMRI, which is an active collaborator of EuroBioBank (http://bbmri-eric.eu/; http://www.euro-

Table 1: Biobank Networks / Consortia, Collecting Biological Samples from Cancer Patients (Exclusively or in Association with other Diseases)

Designation	Number of Members	Starting year	Website
Australasian Biospecimen Network – Oncology (ABN-Oncology)	26 (biobanks)	2005	http://abrn.net/
BBMRI-ERIC	16 European Union Member States and IARC-WHO	2013 *	http://bbmri-eric.eu/
Canadian Tumour Repository Network (CTRNet)	6 (tumour banks)	2004 §	https://www.ctrnet.ca/
Cancer Human Biobank (caHUB) Project of the Biorepositories and Biospecimen Research Branch (BBRB), National Cancer Institute (NCI)	Not stated	2005	http://biospecimens.cancer.gov/programs/cahub/default.asp
EuroBioBank network (Coordinated by the Telethon Foundation)	25	2001	http://www.eurobiobank.org/
European, Middle Eastern & African Society for Biopreservation and Biobanking (ESBB)	64	2010	http://www.esbb.org/index.html
International Society for Biological and Environmental Repositories (ISBER)	8 partner associations	1999	http://www.isber.org/
Nordic Biobank Network	7**	2011	http://www.ntnu.no/biobanknorge/nordic-biobank-network
OECI-TuBaFrost	11 founding members; Under OECI auspices presently	2003	http://www.tubafrost.org/
Public Population Project in Genomics and Society (P^3G)	membership from over 40 countries	Not stated	http://www.p3g.org/
UK Biobank	Not stated	2006	http://www.ukbiobank.ac.uk/

BBMRI-ERIC: Biobanking and BioMolecular resources Research Infrastructure – European Research Infrastructure Consortium; OECI: Organisation of European Cancer Institutes; * 2008 as Biobanking and BioMolecular resources Research Infrastructure – Preparatory Phase (BRMI-PP); § Sample collection since 1993; ** BBMRI.se in Sweden, BBMRI.fi in Finland, BBMRI.no in Norway, BBMRI.ee in Estonia, Biobank Denmark, researchers from Iceland and Faroe Islands.

biobank.org/), BBRMI-ERIC and Nordic Biobank Network, that share some member biobanks (http://www.ntnu.no/biobanknorge/nordic-biobank-network), CTRNet, Australasian Biospecimen Network Association and ESBB, which are members of ISBER (http://www.isber.org/), and BBMRI-ERIC, ISBER and P^3G among others integrate the Forum for International Biobanking Organizations (FIBO) (www.isber.org/?IBO; p3g.org/fibo). Although the boundaries and aims of those organizations are sometimes diffuse and overlapping, their existence clearly demonstrates the need for joint international efforts in biobanking in demand for the increasing need of high-quality tumor samples that fuels basic and translational cancer research.

CONCLUDING REMARKS

Biobanking in the context of cancer research has emerged in recent years as a fundamental activity to provide high-quality, clinically and pathologically annotated tissue samples to researchers that aim to fight the global threat of cancer through a deeper knowledge of its biology at molecular level. This effort has been fundamental not only for an improved understanding of the mechanisms underlying the genesis of cancer, but also for the development of novel screening, diagnostic, prognostic, predictive and therapeutic tools. Through networking, standardized procedures that may ensure high sample quality have been defined and constitute an excellent guide to the establishment and development of institution-based biobanks, as well as its networks. However, it seems that the potential of this amazing tool for translational cancer research has not been fully exploited, probably due to legal and ethical constraints that may limit international cooperation. This might be overcome through a concerted effort from biobankers, researchers, legislators and, most important, from tissue donors, to reach a consensus on the facilitation of sample exchange while preserving the fundamental rights of those that constitute the utmost focus of cancer research: the patients.

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