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Proceedings of the 1st Puerto Rico Biobanking Workshop

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Abstract

The 1st Puerto Rico Biobanking Workshop took place on August 20th, 2014 in the Auditorium of the Comprehensive Cancer Center of the University of Puerto Rico, Medical Sciences Campus in San Juan Puerto Rico. The program for this 1-day, live workshop included lectures by three biobanking experts, followed by presentations from existing biobanks in Puerto Rico and audience discussion. The need for increasing biobanking expertise in Puerto Rico stems from the fact that Hispanics in general are underrepresented in the biobanks in existence in the US, which limits the research conducted specifically to understand the molecular differences in cancer cells compared to other better studied populations. In turn, this lack of information impairs the development of better diagnostic and therapeutic approaches for our population.

Dr. James Robb, M.D., F.C.A.P., consulting pathologist to the National Cancer Institute (NCI) and the Office of Biorepositories and Biospecimen Research (OBBR), opened the workshop with a discussion on the basic aspects of the science of biobanking (*e.g.*, what is a biobank; its goals and objectives; protocols and procedures) in his talk addressing the importance of banking tissues for advancing biomedical research. Next, Dr. Gustavo Stefanoff, from the Cancer Institutes Network of Latin America (RINC by its name in Spanish), explained the mission, objectives, and structure of the Network of Latin-American and Caribbean Biobanks (REBLAC by its name in Spanish), which despite limited resources and many challenges, currently accrue high quality human tissue specimens and data to support cancer research in the region. Dr. Robert Hunter-Mellado, Professor

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

PATIENT CONSENT

Declared none.

of Internal Medicine, Universidad Central del Caribe, followed with an examination of the ethical and regulatory aspects of biobanking tissues for future research, including informed consent of subjects; protection of human subjects rights; and balancing risks and benefit ratios. In the afternoon, the directors of existing biobanks in Puerto Rico (the Puerto Rico Biobank, the Comprehensive Cancer Center biobank, and an HIV-focused biobank at Universidad Central del Caribe) presented their experiences and challenges with establishing biobanks for research in Puerto Rico. In sum, this workshop presented opportunities to share knowledge in the science of biobanking, for further training, and of networking among the participants (34 from 4 different institutions), which will strengthen the collaborative links between investigators studying cancer in Latin America, the Caribbean, and the US.

Keywords

Biobank network; biobank; biobanking; biorepository; biospecimens; cancer; ethics; national cancer control institutes; standard operating procedures; translational research

1. THE FUNDAMENTAL IMPORTANCE OF HIGH QUALITY BIOSPECIMENS FOR PATIENT CARE AND RESEARCH

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INTRODUCTION

Molecular medicine and medical nanotechnology will revolutionize current medicine, that is if the culture of *H. sapiens* or infectious organisms do not prevent this exponential improvement in patient diagnosis, therapy, and safety. Advances in research in those areas are already dramatically changing the way diseases, including cancer, are diagnosed and treated; ultimately, this will lead to the development of genomically-informed personalized medical diagnostic and therapeutic approaches. However, these advances are directly dependent on high quality biospecimens for research. Inferior biospecimen quality leads to inaccurate results, which in turn may lead to incorrect treatment decisions and inferior patient care, as well as potential harm to the patient.

Cancer research programs will fail unless there are high quality biospecimens available to conduct the new generation of genomic and proteomic testing that hold the promise of personalized precision medicine. Biobanking is a complex endeavor, that requires a multidisciplinary support team working together to accrue biospecimens and their associated data following standard operating procedures (SOPs) to ensure their high quality. This team is composed of not only surgeons and pathologists, but also consenters, runners (individuals in charge of transporting the specimens from the operating room (OR) to the Pathology Department), OR nurses, pathology assistants and histotechnicians. The administration of the institutions involved must be fully supportive of the entire biobanking process, by allowing biobanking activities to take place in their premises: consenting of patients, collecting, processing and storing biospecimens. Institutional commitment is key: support

for biobanks may include provision of dedicated facilities, purchasing of equipment, development and implementation of contingency plans in case of power emergency issues, improvement of physical facilities as needed, training and recruiting key personnel, and releasing time for the biobanking directors.

All key stakeholders must be educated about the importance of their roles in the biobanking process. The primary responsibility for obtaining high quality biospecimens lies with the surgeons and pathologists and their support staff, thus it is important to engage these clinicians early on. Once in the biobank facility, laboratory personnel must handle, process and store the biospecimens according to SOPs and of documenting quality parameters to ensure their high quality. This often depends on specialized training to ensure that biospecimens are kept at ultralow temperatures or appropriate preservation media to prevent degradation of molecules. Biobank personnels are also responsible for quality control testing, studying and analyzing the biospecimens, and accurate annotation and inventory keeping. Finally, investigators using the tissues for research are responsible for appropriate management on the tissues in the laboratory, conducting well designed, well-powered experiments, and reporting accurate results. Researchers must be aware that these results may lead to treatment decisions in the future.

DEFINITIONS

The definition of a *biospecimen* is a physical biological sample derived from a human subject. Non-biological biospecimens are excluded such as prostheses, implants, pacemakers, bullets, and so forth. The products derived from a biospecimen include microscopic glass slides, paraffin blocks, DNA, RNA, proteins, metabolites, and so forth. There is ongoing discussion about whether digital images should be included¹. A *high quality biospecimen* is created and clinically annotated by using evidence-based standard operating procedures (SOPs) and maintained using an integrated quality management system within a controlled environment.

A *biorepository* is the infrastructure within which biospecimens are identified, collected, stored, and distributed. This includes formalin-fixed paraffin embedded (FFPE) tissue, blood, and body fluids such as urine or saliva. A *high quality biorepository* adheres to evidenced-based SOPs and published best practices for annotating, collecting, processing, storing, distributing, and retrieving distributed biospecimens if necessary. The activities that are included within a biorepository are collecting and managing clinical data, QA and QC processes, biosafety awareness, inventory management of FFPE and frozen biospecimens, and ethical-legal-societal-issues (ELSI), including informed consent of subjects.

THE BIOSPECIMEN LIFE CYCLE

It is of outmost importance to document the biospecimen life cycle and take into consideration these data when analyzing results. There are pre-analytic (*i.e.*, before time 0 = collection time), and post-analytic variables that are important to document. Pre-analytic variables include current medications, antibiotics, type and duration of anesthesia, and warm

¹I believe, if the images are not anonymized, they should be included.

ischemia time (arterial clamp time). Post-analytic variables include cold ischemia time (the time that a biospecimen is removed from the body and stays at room temperature (RT) until preservation in formalin or liquid nitrogen [LN2]), temperature of the operating room, type and time in fixative, rate of freezing, storage temperature, and size of aliquots. There are ample data showing that expression of >15% of genes and up 60% of selected proteins change >2-fold during surgery and postsurgical processing time [1]. Herein lies the importance of documenting the Cold Ischemia Time in the pathology report.

The Total Time in Formalin, when formalin is the preservative, should be less than 24 hrs if genomic testing is to be performed on the FFPE tissue. The Total Time in Formalin should be 6-72 hrs if only IHC or in situ hybridization are to be performed in the FFPE tissue. The Total Time in Formalin should also be recorded in the Pathology report.

There are alternatives to appropriate preservation of tissues in settings where, for example, access to LN2 is limited. There are various new technologies for ambient preservation of tissues and blood that have been shown to prevent nucleic acid/protein degradation [2, 3]. Always remember that high quality data begin with high quality biospecimens, which will lead to good patient care and improved patient outcomes.

THE CHALLENGES OF BIOBANKING

There are many challenges to biobanking biospecimens for research, including:

- 1). **Standardized processes:** Development of evidence-based, field-tested, standardized operating or collection protocols (SOPs). SOPs have to be developed before biospecimen collection begins. This process must start early, as it takes about a year to develop and test them. They need to be continually revised to adjust to changes. They have to be created by the people conducting the processes. There have to be SOPs for every process in the biobank, including one about writing SOPs. Discrepancies and variations from the SOPs should be documented. If a test result does not fit the clinical picture, go back to see what happened to the biospecimens when they were accrued, how they were stored, and whether deviations from SOPs occurred.
- 2). **Standardized reagents:** Use of standardized reagents will ensure standard results. For immunohistochemistry, for example, it is recommended that validated antibodies (Abs) are always used (*e.g.*, College of American Pathologists (CAP) – American Society of Clinical Oncology (ASCO) validated antibodies for Estrogen (ER), Progesterone (PgR) and HER2 receptors). Use FDA-cleared antibodies whenever possible, otherwise use appropriately validated antibodies. Follow published guidelines (*e.g.*, Principles of Analytic Validation of Immunohistochemical Assays, Guideline from the College of American Pathologists Pathology and Laboratory Quality Center [4]). Recommendation: Use weak positive controls (use 1-2+ and not 3-4+) so you do not miss positive cases.
- 3). **Accurate documentation of Cold Ischemia Time and Total Time In Formalin:** These are the two most important pre-analytic variables to record for tissue

biospecimens in the pathology report. Cold ischemia time must be recorded for each case of fresh and preserved tissues and the total time in formalin has to be recorded for tissues preserved in formalin and paraffin embedded (FFPE blocks). A good example of the role of cold ischemia time is provided by IHC intensity staining (Q score) for ER and PgR. The clinically used cutoff for a positive hormone receptor IHC results is 1% positive nuclei. It has been shown that the mean Q score declines at 1hr for PgR and 2hr for ER [5, 6] (Fig. 1). Recommendation: cold ischemia time must be less than 60 min; less than 30 min is optimal and preferred. The total time in formalin, including formalin processing time, should be 6-72 hours. The minimum formalin fixation time that is necessary for optimal IHC staining is 6 to 8 hours, but less than 24 hours if nucleic acids are to be extracted (Fig. 2). ER staining intensity/distributions at longer fixation times were similar to that observed at 8 hours [7]. The maximum time of 72 hrs is to accommodate cases that are accrued over the weekend. HER2 testing, on the other hand, is a DNA-based test. Since cancer chromatin/DNA has an abnormal structure it is more susceptible to denaturation (ischemic apoptosis). The reported incidence of genomic heterogeneity of HER2 amplification ranges from 5% to 30% cases [8-10] (Fig. 3). Recommendation: a less than 60 min cutoff of cold ischemia time to avoid loss of the HER2 signal. These standards are currently used in the United States for ER, PgR, and HER2 IHC in breast cancer biospecimens through CAP-ASCO recommendations.

- 4). **Heterogeneity of tissue biospecimens:** Cancer heterogeneity can lead to discordant results for biomarkers due to the diversity in cellular components, necrosis, inflammation and other parameters across the tissue. Single core biopsy samples are small and less likely to represent the heterogeneity of the tissue that may be present in the cancer as a whole (Fig. 4). Inaccurate conclusions can be obtained if looking at a single piece of tissue on a slide. Recommendation: obtain multiple core biopsies (n=2-5) and place them in one block. These multiple pieces in a single block obtained from different regions of the surgical piece will provide a better representation of the cancer. Portions of the central, middle, peripheral cancer as well as a portion of normal tissue can be put into one block. This “genomic testing” block can use a different color and should be documented in the pathology report. Do not use a core biopsy for biomarker testing if the core exhibits edge fragmentation or retraction or if crush artifacts are present. Confirmatory ER/PgR and/or HER2 testing on excision biospecimens is important, in particular when initial testing has been done on a core biopsy and the result was negative. Finally, always consider the clinical picture of the patient. If the biomarker result does not fit the clinical picture, it may be due to the quality and/or lack of representative heterogeneity of the tissue analyzed. Recommendation: Do the following in less than 30 min, if possible, after removal of the tissue from the patient:

- Ensure a Cold Ischemia Time of < 60 min.
- Ink margins per institutional protocol

- Open cancer tissue per institutional protocol
 - Take 4-5 samples from peripheral and central regions of the cancer and put into one cassette. Include one appropriate piece of normal tissue if available. Individual biospecimen size: 4-5 mm × 4-5 mm × 2-3 mm each
 - Use a unique colored cassette, possibly striped, to easily identify the block to be used for biomarker analysis. Record this block in the pathology report.
- 5). Evidence-based best practice testing and resulting: All procedures used in the biobank must have SOPs that guide every step of the process to preserve the bio-molecular integrity of specimens accrued at frozen or ambient conditions. Use evidence-based best practices for preserving and processing the biospecimen, for annotating pre-analytic and post-analytic variables, and for labeling and storing FFPE and fresh biospecimens and their derived products (DNA, RNA, etc.) (Refer to NCI's *Best Practices for Biospecimen Resources – 2011* [11]).
 - 6). **Establish a Total Quality Management Program:** The integration of a total quality management program is a very difficult process and not to be undertaken lightly. Documentation of all discrepancies and variations from the SOPs is absolutely required. Recommendation: all processes must have an SOP, including one for how to create an SOP.
 - 7). **Validate biospecimens:** It is critical to validate all “normal” and “pathologic” biospecimens, morphologically and molecularly. Recommendation: use National Cancer Institute's (NCI) Cancer Genome Atlas (TCGA) program criteria for morphologic acceptance of a tissue biospecimen. The TCGA cutoff for percentage of malignant nuclei is 60% and the percentage of ischemic necrosis is < 20%. Make sure normal is really normal. Document ANY/ALL discrepancies and abnormal findings and attach the information to the biospecimens in a format that can be reviewed by any end user.
 - 8). **Proficiency testing of all processes:** Proficiency testing of ALL the tissue collection, testing, and interpretation processes whenever possible. If no internal formal proficiency testing is available, one option is to use laboratory exchanges. Quality controls for testing and interpretation processes need to be in place. The implementation for BOTH histologic and biomolecular integrity quality assurance processes is critical. Core biopsy slides are preferred over resection slides if both are available, because the resection biomarker results are usually not as reliable as the core biopsy results due to the delayed formalin-fixation of resected biospecimens, as compared to the core biopsies. These high standards assure stakeholders and patients that accurate results are being produced, likely to lead to valid research interpretations and, ultimately, appropriate treatments.

Table 1 summarizes other challenges of biobanking that must be taken in consideration.

INTO THE FUTURE OF BIOBANKING

Nucleic Acid Extraction From FFPEs for Patient Care—A recent promising methodology, Adaptive Focused Acoustics, has been developed for optimally extracting nucleic acids (DNA and RNA) from FFPE tissue (Covaris, Woburn, MA). The determination of nucleic acid concentration and quality allows their use in a variety of downstream applications including whole genome sequencing [13]. The process uses ultrasound similar to that used in kidney stone lithotripsy, not sonication. Paraffin is actively removed from the FFPE tissue sample by the finely controlled and reproducible acoustic energy provided by ultrasonicators. A simplified workflow ensures high yield extraction of nucleic acids.

Preserving and Biobanking—The good news is that ultralow freezers (-80°C – -150°C) are becoming an acceptable alternative to LN2. There is accumulating evidence that preservation of nucleic acids is similar in quality to LN2 preservation for most clinical and research applications (personal communication); -80°C maybe even be better, but there is insufficient current data to make this determination. The new generation of ultra-cold automated freezers with LN2 purging capability for down-times, mean that there is no need to transfer biospecimens to other units in case of emergency issues such as loss of power or breakdown.

Single Cell Genomics—Recent developments in nanotechnology have led to platforms of nanofluidic channels that can isolate and analyze single fluorescent cells. The throughput allows the analysis of 100s – scalable to 1000s – cells. They are being used in studies to identify the driver pathways in cancer stem cell(s) of an individual patient to customize therapy, to identify circulating DNA and RNAs, and to track metabolic pathways using radiology tools. One example is the Fluidigm nanotechnology platform (Fluidigm SNPtrace™, Biomark HD system, City, State).

Ambient Preservation—Preserving biospecimens at ambient temperature would solve many of the challenges of biobanks, namely flash-freezing, ultra low temperature storage, and shipping. These challenges are especially significant in developing countries or emerging biobanks with limited resources. The new technologies have been shown to be as good as LN2 for preservation of both nucleic acids and proteins. Other advantages include simplification of logistics, savings in freezer space and energy, and long-term stability. Various companies have taken the lead in this area, developing products that can be used to preserve whole blood for DNA/RNA (PAXGene tubes, Qiagen, Valencia, CA; GenTegra, Pleasanton, CA; DNA/RNAstable, Biomatrix Inc., San Diego, CA), RNA in tissues (RNAlater, Life Technologies, Grand Island, NY; DNA/RNA as extracted nuclei acids (DNA/RNAstable, Biomatrix Inc., San Diego, CA), and multiple derivatives in tissues (PAXGene Tissue, BD Bioscience, San Jose, CA).

Accurate QC of Biospecimens—The quality of the biospecimen will have to be determined as soon as possible after it is collected. FFPE-derived nucleic acid (DNA, RNA) concentration and quality may be acceptable for downstream applications involving transcription and translation, but the percentage of acceptable FFPE blocks may be lower

than expected. It would be critical to determine upfront the quality of nucleic acids, the level of contamination (*e.g.*, with protein, phenol) or cross-contamination with other samples before embarking in a full project to use FFPE tissue for research. Studies have shown that 3-5% of samples are misidentified; 15% male contamination occurred in female biospecimens; and 3-15% have incorrect ancestry relatedness.

DISCUSSION

What drives biobanking science is the promise of personalized precision medicine: the ability to make diagnostic/therapeutic/prognostic decisions based on the particular molecular profile of the patient's cancer. Whether we are able to make genomically informed precision healthcare decisions or get into a molecular abyss, it will depend on the quality of the biospecimen. Using a geological analogy (Fig. 5²), we have moved from the tidelines, the “pre-molecular” testing (*e.g.*, serum albumin, measured as grams – 10⁰) and to the shelf, the early molecular tests (*e.g.*, fibrinogen/PSA, light microscopy, measured in milli / micrograms: 10^{-3/-6}). We are presently at a plateau represented by the currently available molecular tests (*e.g.*, troponins / BPN measured in nano/picograms: 10^{-9/-12}). The abyss that lies ahead is represented by future tests that can be used to measure biomarkers present at very low levels (atto/octograms: 10^{-18/-24}) thus increasing the accuracy and sensitivity of our testing capabilities. We must approach the future step by step, *via* development and implementation of evidence-based, best practice protocols for biospecimen collection, processing, annotation, storage, and privacy assurance; or, expand rapidly into the abyss—development of tests based on data obtained from biospecimens that have been inadequately processed, leading to poor patient care.

Biobanking is all about doing it right from step one: consenting the patient in a manner that ensures he/she will make an informed decision to donate tissues for research; timely accrual, processing and banking of biospecimens using rigorous procedures to ensure high quality (*i.e.*, minimize degradation of nucleic acids and proteins); releasing tissues for well-designed, appropriately powered studies; obtaining molecular data *via* translational research approaches; and using these data for genomically-informed personalized precision healthcare (diagnostic/therapeutic/prognostic) decisions.

SUMMARY

The most important thing to remember is that the Cold Ischemia Time (*i.e.*, time between removal of the biospecimen from the patient until it is dissected (if necessary) and preserved (LN2, formalin, etc.) needs to be minimized (< 30-60 min) and the Total Time In Formalin, including processing time, should be 6-72 hours for immunohistochemistry and *in situ* hybridization and < 24 hours for nucleic acid extraction. Pre-analytic and post-analytic data elements have to be carefully recorded (refer to new CAP Article on Pre-analytic Data Elements that are standardized; while 174 pre-analytic variables were identified, not all are

²Information based on experience in the National Cancer Institute's (NCI) Office of Biorepositories and Biospecimen Research, the NCI Community Cancer Center Program, and the NCI US-Latin American Research Network. The viewpoints discussed by Dr. Robb may or may not represent the views of the NCI Programs that have provided his experience that is represented in this paper.

considered critical and are thus required, some are recommended [14,15]. Biobanks must decide which data elements they can acquire at the minimum. These data elements should be implemented in their IT systems, so that they are available to investigators in case of issues with the data.

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2. LATIN AMERICA AND CARIBBEAN BIOBANK NETWORK

Gustavo Stefanoff, PhD

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Latin America offers unique opportunities to study various health issues including cancer, especially due to the particular historical, geographical and ethnic characteristics of the countries in this region. Throughout the years, several research groups have developed in most areas of health in Latin American countries. However, at present, there is a need for more cooperation of such individual groups in the development of collaborative projects to take advantage of the uniqueness of its population and the existing expertise.

In 2009, an article in TIME Magazine included biobanks as one of the “10 ideas changing the world right now” [20]. Biobanks function to support clinical and translational research and promote technological development. In Latin America, there had been an effort towards the development of a biobanking network based on UNASUR (Union of Latin American Nations by its name in Spanish)-participating countries. The Biobanks Operative Group from Latin American Network of National Cancer Institutes and Institutions (RINC by its name in Spanish) of UNASUR, also called Latin American and Caribbean Biobank Network (REBLAC by its name in Spanish), was initiated in 2007 in the agenda of the Latin American and Caribbean Alliance for Cancer Control with the initial participation of representatives from seven countries (Table 2). Since then were being incorporated to this initiative, representatives of other National Institutes of Cancer and similar institutional responsible for national control policies of cancer from 13 countries of Latin America and the Caribbean: Argentina, Brazil, Colombia, Chile, Ecuador, Peru, Uruguay, Bolivia, Venezuela, Mexico, Cuba, Panama and Puerto Rico (Table 3). This Network is already participating collaborative studies in cancer, including a study funded by the National Cancer Institute and the US-LACRN (US Latin America Cancer Research Network) Project

entitled Molecular Profiling of Stage II and III Breast Cancer in Latin American Women Receiving Standard of Care Treatment.

The RINC/UNASUR network is a geopolitical platform created with the following goals: 1) to develop a regional cancer control strategy with the support of the government; 2) to create equitable use of existing resources, and 3) to develop more appropriate public cancer policies for the populations, according to the culture and existing resources. To fulfill these goals, the RINC implemented the following specific aims: organize a regional cancer control community by sharing best practices; encourage the exchange of information and knowledge related to cancer control; identify common interests related to cancer control and seek strategies that might be shared; develop and carry out programs based on the strengths of each member country; seek coordination among the member countries in order to strengthen the management and the development of the national institutions responsible for managing cancer control; encourage commitment from all governmental levels of each member country to raise funds for regional cancer control; and, articulate alternative financial sources for the development of the RINC work plans.

The structure of this network is based on three principles: 1) identification of common challenges for cancer control in the region, 2) regional cooperation, and 3) partnerships of best practices among members. The administrative structure consists of a management council, working groups, and an executive secretariat. The management council consists of full (countries of the UNASUR) and associate members (other Latin American countries). The council is a decision-making team that discusses and makes decisions on various RINC topics. In addition, the council approves strategies and projects, which are considered appropriate to the goals of the network. All members are representatives of national institutions responsible for cancer control strategies or assigned by the government. The working groups of the RINC are responsible for its operation and implementation/coordination the working plans of the respective technical areas.

The working groups within the RINC include: cervical cancer control, cancer registries, biobanks, and breast cancer. The objective of REBLAC is the harmonization of biobanking processes through implementation of ethical requirements, technical procedures (including quality control), and management strategies. The development of this infrastructure will allow the support of high-quality collaborative projects (Fig. 6). The network had implemented these goals through annual meetings, training and capacity building seminars, technical assessments, and workshops for the implementation of common standard operating procedures.

Obviously, there are challenges to achieve the goals of harmonizing biobanking processes across countries and institutions. These challenges include: consolidation of the network's structure, implementation of a common management system, establishment of common standard operating procedures, implementation of strategies and communication tools, establishment of international collaborations, promoting of multicentric collaborative projects, identification of opportunities for funding, and the expansion of the network. Overcoming these obstacles will take substantial time and effort, recognition of differences

between countries, cooperative learning, and enhancement of the strengths while minimizing weaknesses, among others.

Participation of REBLAC/RINC in the First Puerto Rico Biobanking Workshop in San Juan, allowed Puerto Rico to demonstrate its infrastructure, activities and potential to its leadership, and become a member of this network.

3. ETHICAL AND REGULATORY ASPECTS OF INITIATING AND MAINTAINING A HUMAN SPECIMEN BIOREPOSITORY

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The scientists' work is to help people and cure disease, and we need to recognize this objective when planning and conducting our research activities. Scientists are probably well aware of what are considered violations of ethical medical procedures and research, and the rules and laws that regulate these practices. However, there are still doubts of how all these regulations apply in particular to biobanks.

The relevance of biobanks for the promotion of research is very high. Biobanking of tissues for research were recently considered by TIME Magazine as one of the "Top 10 Ideas Changing the World" [21]. Biobanks had been instrumental in the important discoveries and increased knowledge of many diseases including cancer and infectious diseases [22]. Modern uses for biorepositories include finding the cause of diseases; development and testing of new drugs or diagnostic assays; identification of those more likely to get a disease, have more serious side effects, and respond to treatment.

The general public is very concerned about potential harms of research to study subjects. There has been an exponential growth in knowledge, but at a cost to some individual, groups, and/or ethnicities. For example, knowledge of *BRCA1* mutation status not only affects the emotional well-being of the patient who has volunteered to donate their samples for research but also her parents, children, siblings, even the unborn. Knowledge of certain genetic predispositions can have serious consequences in social development, and may influence decisions about marriage and whether or not to have children. There are always risks associated to participation in research studies, not only physical but also social, financial, and psychological. Some of the risks may be unexpected. However, volunteers must be made aware of at least the foreseeable risks such that they can make an informed decision about participation in a research study.

Biobanks have to deal with a host of important and unique ethical issues including:

- 1) **Potential profit generation** through generation of commercial products (new drugs, diagnostic tests, prognostic tests). An example is Myriad Genetics, the company who designed and patented a genetic test for breast cancer based on genetic sequences obtained from patients. For many years this company monopolized this genetic test, and profited substantially from it. Recently, the

US Supreme Court ruled that genes were not eligible for patents since they were products of nature. Due to the translational nature of the research that will be conducted using these samples, it is possible that commercial products could be generated in the future using the knowledge acquired. This possibility should be informed to the subjects donating tissues for biobanks.

- 2) ***Custodianship***: Many important questions are raised by biobanking activities, including: Who is the owner of the donated biospecimens? Do donors get to choose how their samples and information are used? How long will the samples and information in the biorepository be kept and used for research? Will the biorepository send donors results of research done on their samples? What happens if research using samples leads to important new discoveries?
- 3) Inappropriate use of biobanks for studies on particular ethnic groups: *e.g.*, samples from Native Americans were obtained for one study, however, investigators used them for other purposes than those they were originally intended, explained and consented for. Whenever a new use of the samples is intended, the individuals must be re-consented.
- 4) ***Genetic tests in newborns***: Some nationwide pediatric programs obtain heel prick blood samples from newborns to conduct screening of genetic and metabolic diseases, and for research purposes, but questions have been raised about the informed consent process. Are parents aware that they are being consented for research on their children?

“What is a Biobank?” and Other Important Definitions

The following are important definitions generally used in biobanking:

- “biological specimens” or “biospecimens” refers to the actual biological material that is collected from a study participant, such as tissue (diseased, normal), blood (serum, plasma, buffy coat), urine, or saliva.
- “biomarker,” is a measurable biological factor that is associated with a particular medical condition and can be used as a proxy to the presence or absence of a disease. Biomarkers are generally used as the base of diagnostic assays.
- “Biodata” refers to the data associated to each biospecimens, which may include clinical, personal, behavioral or demographic information. Biorepositories generally obtain important subject data such as: i) demographics: age, sex, ethnicity, education, socioeconomic, height and weight; ii) medical history: comorbidities, treatments received, response to treatments; iii) family history of cancer and other conditions; and iv) lifestyle: smoking, diet, exercise, profession, community.

There are distinctions between a biorepository and a biobank. “Biorepository” refers to a facility used to store human specimens for research purposes, while “biobank” denotes a facility used to store tissues and the associated biodata. There are different biorepository models. The more simple ones are based on specimen and data collection that are then stored and later used for research. More complex models consist of different collection sites,

from where samples and associated data have to be transported into a central biorepository. After processing and storage, the samples are distributed to multiple researchers, also following various models of tissue sharing.

There are also important distinctions between Privacy vs. Confidentiality. “Privacy” refers to the protection of an individual from unwanted intrusions, including the acquisition of information about that individual. It is a general protection against misuse of this information by other individuals, groups, and society as a whole. “Confidentiality” refers to the protection of information about an individual that has already been provided willingly to one party; if the information is confidential, the recipient is responsible for ensuring that it is not released to others without the donor's permission. Privacy refers to the individual, while confidentiality refers to the identifiers that are attached to a biospecimen that would make it identifiable. Identifiability is a verb, an action, and refers to the ability of the investigator to know where the sample comes from, to associate data with a particular person. This is tightly linked to how close the researchers are to the process of collection of tissues. The identifiability of data can be thought of in terms of a spectrum—from data that are impossible to identify, to those that can be identified as possibly being linked to a given individual, to those for which that linkage is known with certainty. For example, if the doctor taking care of the patient is also the researcher it is possible for her/him to identify the subject even when the sample is without identifiers. A way to avoid this issue is to use separate codes that link the repository and clinical data. Also, having a data management center that is independent from the repository storage can aid in improving confidentiality. Although “coded” data are generally regarded as safe, providing the code “cannot be readily linked to a name”, it remains unclear how “readily” is defined in this context.

REGULATIONS THAT GOVERN THE ACTIVITIES WITHIN A BIOREPOSITORY

There are a number of laws and regulations governing biomedical research activities including biobanking (Table 4). Biorepositories in particular must follow several national and international regulations to ensure the ethical accrual and use of these samples in research. These include regulations by agencies such as the Office for Human Research Protections (OHRP) of the US Department of Health and Human services (HHS) (the 45 CFR Part 46 Code of conducts also known as the Common Rule), and the Food and Drug Administration (FDA). Biobanks also have to follow regulations related to Health Insurance Portability and Accountability Act (HIPAA) and Biosafety issues, regulated by Occupational Safety and Health Administration (OSHA). Whenever there is collection and subsequent use or disclosure of data for research purposes of personal health information (PHI), regulations such as the Common Rule and HIPAA apply. PHI data is usually obtained when developing correlative clinical databases and often is also associated to biospecimens that are banked for research. Whenever there is a possible development of a biomarker or therapeutic target, then FDA also oversees the study protocols.

Guidance for ethical administration of a biobank can be found in Belmont report [23], a code of ethics developed in 1979 by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Belmont Report provides an outline of three basic ethical principles in research involving human subjects: autonomy, justice and

beneficence. Autonomy refers to the right to know what is going to be done with the tissues; justice asks us to ensure that populations who were burdened with research procedures also benefit from the research results; and beneficence highlights the importance of maximizing benefits while minimizing risks. Another regulation that guides ethically sound biobanking activities sponsored by the National Institutes of Health is the Common Rule, published in 1991, consisting of a four-part Code of Federal Regulations [24]. This regulation states that if a study uses biospecimens that were already existing and collected without IDs it is not considered research and therefore there is no need to consent the subjects; it also states that once a tissue is outside the individual is no longer his/her property (*e.g.* mastectomy tissue). However, as researchers we must use common sense to question whether this may always be the case and to recognize the importance of evaluating each project independently.

WAIVERS OF CONSENT

Several ethical issues arise when an investigator requests a waiver of consent prior to requesting banked tissues. It is generally accepted that written consent is not required if the investigator requests a de-identified dataset for projects that do not have a specific objective (*e.g.*, preparative research to obtain preliminary data or for sample size determination). The use of “blanket”, single tiered, or general purpose consents from subjects to donate tissues for future research has been proposed. However, many experts still believe that the specific nature of the research studies to be conducted must be known at the time of sample and data collection. At the very least, subjects should be asked to opt in or opt out of future use for research. In general, no ethical issues are raised if the specimens obtained have no identifiers for which an IRB waiver of consent can be requested, but this needs to be appropriately justified, otherwise, the subjects should be re-consented.

THE PROCESS OF IRB REVIEW

IRB review of a biobanking protocol will require certain information to ensure that these activities are done in an ethical manner that protects the subjects from undue risks. This information includes:

- 1) What are the formal name, purpose, and overall goals of the repository? Who provides administrative oversight of the repository and what are the oversight mechanisms in place?
- 2) Details of the procedures for consenting subjects: How is the recruitment done and what is the process of informed consent? Details of who consents, how is the informed consent done, and where and when it occurs. Are consenters educated on human subject research ethical issues? How often, and how is this training documented?
- 3) Specimen and data collection: How are subjects identified? What information is collected with the specimen? Procedures must be in place if subjects decide to withdraw their consent and what will be done with his/her specimens/data.
- 4) Specimen and data storage/retention: How are data linked to the subject? Who will have access to identifying information? How will specimens/data be coded?

- What are the procedures for secure storage? How long will specimens/data be retained?
- 5) Specimen derivation and, processing: What types of derivatives will be obtained. Cell lines, DNA/RNA?
 - 6) Specimen and data distribution: What specimens and data will be provided to investigators? Are there policies regarding secondary distribution? Are there investigator usage agreements? Custodians of the biospecimens must not be the researcher but rather the biobank leaders.
 - 7) Ownership: Is the specimen owned by the patient, the surgeon, the hospital? Are there policies regarding commercial use of the biospecimens banked? Contingency plans must be in place in case the main investigator leaves the institution, or grant funding expires.
 - 8) Protection of subject autonomy, privacy, and confidentiality: Who has access to identifiers and key codes? What are the policies and procedures in place to protect subjects' identity? What are the policies regarding release of personal identifiers? Are there policies for assuring uses are consistent with consent? How will the repository comply with HIPAA Privacy Rule? One way is to establish an Honest Broker system. Honest brokers are keyholders who are separate from investigators and biobankers. As long as the investigator is not involved in coding the samples, confidentiality can be maintained.
 - 9) Return of research results: Are there policies regarding when, if ever, individual research results will be returned to subjects? What is the biobank's policy regarding return of aggregate, generalized research findings? What are the policies regarding sharing of results with study subjects? Whether or not results will be shared and how to reveal results? Professional groups suggest that only if clinically actionable data can be released to the subject.
 - 10) Evaluation of risk for the donor of the specimens: The risks can be classified as physical, psychosocial, invalidated research data, and group harms.
 - 11) Other special circumstances: What are the plans if the lead investigator leaves the institution or the repository loses financial support? In the case of specimens accrued from children, are there plans for obtaining consent when a child reaches age of consent for research; or is there a request for a waiver of informed consent on file?

FINAL THOUGHTS

There are still unresolved ethical issues in biobanking, such as when the professional mission of the principal investigator is in conflict – MDs who are also researchers may know the identity of the individual as a patient. IRBs must evaluate each project individually, and make determinations based on the Common Rule, HIPAA, and also the FDA if a commercial product can be derived from these tissues in the future. Researchers must strive to maintain the trust of the subjects and also of the community. They are responsible for ensuring proper and ethical use of the federal funds received for maintaining a biobank,

which are provided by the taxpayers. When the IRB committee approves the protocols of a biobank it implies its leaders are forbidden to release personal information to investigators under any circumstances. Finally, there are no laws in Puerto Rico to oversee biobanking activities, although there are laws that regulate how tissues are managed at the level of clinical pathology laboratories. This is an important area where additional regulation is needed to ensure ethical and lawful use of these specimens for research that benefits the society as a whole, while not imposing undue harm to those who altruistically donate their samples for research.

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ER, PgR Testing by IHC
Mean Q score started to decline at 1 hr mark for progesterone receptor and 2 hr mark for estrogen receptor

Khoury T, et al., Mod Pathol. 2009 Nov;22(11):1457-67

Photos: Dr. David Hicks, Univ Rochester, NY

Fig. (1).
Time to fixation (Cold ischemia time) – important for preservation of immuno reactivity.

Fig. (2).
HER2 testing IHC and FISH.

Reported incidence of genomic heterogeneity of HER2 amplification ranges from 5% to 30% cases.

Chivukula M et al. *Modern Pathology*. 2008;21:363–368.

Perez EA et al. Presented at Congress of the Future of Breast Cancer. July 22–25, 2009.

Fig. (3).

HER2 genomic heterogeneity can land to ICH/FISH discordant result: True for all biomarkers.

Picture courtesy of Dr. Ken Bloom

Fig. (4).
HER2 genomic heterogeneity can land to ICH/FISH discordant result: True for all biomarkers.

<u>Tidelands</u>	“premolecular” testing serum albumin (grams: 10^0)
<u>Shelf</u>	early molecular tests fibrinogen/PSA & light microscopy (milli/micrograms: $10^{-3/-6}$)
<u>Plateau</u>	current molecular tests Troponins/BNP (nano/picograms: $10^{-9/-12}$)
<u>Abyssal plane</u>	future tests (atto/yoctograms: $10^{-18/-24}$)

1. Approach the future via the Blake Ridge (step by step) with development and implementation of evidence-based, best practice biospecimen annotating, collecting, processing, storing, and privacy protocols
- OR
2. Expand rapidly into the abyss: excellent tests with inadequately annotated, collected, processed, stored, & private biospecimens = **poor patient care**

<http://oceanexplorer.noaa.gov/explorations/04etta/background/profile/profile.html>

Fig. (5).
Genomically-informed precision healthcare or molecular abyss? It is all in the biospecimen.
Figure from <http://oceanexplorer.noaa.gov/explorations/04etta/background/profile/profile.html> (last accessed 1.12.2015), modified by Dr. James A. Robb

Fig. (6).
The objectives of REBLAC.

Table 1

Other Challenges of Biobanking Specimens for Research.

Challenge	Recommendation
Clinical Annotation	Use Information technology (IT) to automate data entry and annotation of biospecimens as much as possible, to minimize errors. The more you can validate the clinical information, the more valuable is the biospecimen.
Data elements	Must be captured using standardized, coded, and discrete data elements; biospecimen distribution; retrieval, packaging, shipping and receiving must be carefully annotated. It is also important to document freeze- thaw events of each biospecimen.
Biospecimen Distribution	Validated SOPs must be used for retrieval, packaging, shipping, and receiving biospecimens. The freeze-thaw cycle is often very destructive if not properly performed and documented! The transport temperature must be documented. Beware of the “glass transition zone” or vitrification of samples. Vitrified tissue samples that undergo sudden cooling can turn into a solidified, “glassy” state characterized by ice formation in due to transient infringing of the Tg (glass transition temperature, -137°C), which could lead to loss of the sample [12].
Integrated quality management system	Every biorepository process used from consenting to distribution needs to be documented with an SOP in an integrated quality management system for QC and QA.

Table 2

Timeline of Efforts to Create a Cancer Control Community and Promote Regional Cooperation in Latin America.

Year	Outcomes
June 2006	Common challenges and goals were identified during the 1st International Forum for Leaders for Cancer Control, (Mexico City)
November 2007	The Latin America and Caribbean Alliance for Cancer Control was created during the 2nd International Forum for Leaders for Cancer Control (Brazil) The Latin American and Caribbean Tumor Bank Network was created. The initial goal was to establish a cooperative milestone to allow design strategies to consolidate Tumor banks in member countries.
May 2008	The Treaty of the Union of the South American Nations (UNASUR) was signed (Brazil)
December 2008	American Council of Health (UNASUR Health) was created, (Brazil)
April 2010	UNASUR Health recognized the importance of a network of cancer control institutions (Ecuador)
September 2010	The Declaration of Buenos Aires ratified the creation of the National Cancer Control Institutes and Institutions in Latin America (Argentina)
July 2011	The Network of National Cancer Institutes was created (RINC/UNASUR) with the implementation of the Executive Secretariat in Rio de Janeiro and the Development of Working Groups. (Brazil)
September 2011	The Latin American and Caribbean Tumor Bank Network is incorporated into the RINC as the Operative Group of Biobanks

Table 3

Institutions Participating in the Biobanks Operative Group from RINC/UNASUR

Country	Institutions
Argentina	Instituto De Oncología “Angel H. Roffo”, Buenos Aires
	Hospital de Pediatría “Prof. Juan P. Garrahan”, Buenos Aires
	Hospital General de Niños “Ricardo Gutierrez”, Buenos Aires
Brazil	Instituto Nacional de Câncer (INCA), Rio de Janeiro
Bolivia	Instituto Oncologico del Oriente Boliviano (IOOB), Santa Cruz de la Sierra
Chile	Hospital del Salvador, Santiago de Chile
	Hospital Luis Tisne, Santiago de Chile
	Hospital Clínico San Borja Arriarán, Santiago de Chile
	Hospital Luis Calvo Mackenna, Santiago de Chile
Colombia	Instituto Nacional de Cancerología (INC), Bogotá
Cuba	Instituto Nacional de Oncología y Radiobiología (INOR), La Habana
Ecuador	Instituto del Cáncer SOLCA, Cuenca
México	Instituto Nacional de Cancerología (INCan), Ciudad de México
Panama	Instituto Oncológico Nacional (ION), Ciudad de Panamá
Peru	Instituto Nacional de Enfermedades Neoplásicas (INEN), Lima
Puerto Rico	Centro Comprensivo de Cáncer de la Universidad de Puerto Rico (CCCUPR), San Juan
	Universidad de Ciencias de la Salud de Ponce
Uruguay	Hospital Central de las Fuerzas Armadas (HCFFAA), Montevideo
Venezuela	Instituto anatomopatológico “José A. O’ Daly” - Universidad Central de Venezuela, Caracas

Table 4

Overview of Applicable Regulations and Policies.

Regulation ^a	Summary
45CFR46	Protects subjects from research – informed consent process
HIPAA	Not for research use, guidelines to regulate research use, fights of the subjects participate in research. HIPAA applies if clinical, identifiable data are obtained to create the repository and if these data are used in projects.
FDA	It is important to recognize when does it apply. FDA regulations deal with all biomedical inventions that may lead to a product that would need to be patented, for example data that will lead to a new drug or a diagnostic/prognostic biomarkers.
OSHA	Guidelines on biosafety issues of handling human samples

^a Sometimes these regulations clash and herein lies many of the difficulties in this area.