The importance of conducting well-designed clinical trials

Biobanking in clinical trials: What, When and How should I collect samples?

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Disclosure

☐ I declare that I have no conflict of interest
Biobanking in clinical trials: What, When and How should I collect samples?

- The context

- WHAT → scientific issues
  - samples
  - Quality Assurance
  - Standard Operating Procedures (SOPs)

- WHEN → clinical issues
  - translational studies
  - custodianship
  - collaboration & communication

- HOW → management issues
  - biobanking structure
  - ethical & legal consideration
  - access to biospecimens & data
Cancer: a disease of the genome

Challenge in treating tumors

- Every tumor is different
- High rate of abnormalities (driver vs passenger)
- Every cancer patient is different
Challenges in the current translational chain for biomarkers/molecular signatures development & validation

Preclinical exploratory studies
- Basic research
  - Laboratory driven
  - Bioinformatics driven

Candidate biomarker identification
- Preliminary assay
  - Clinical samples

POC validation
- Viability & feasibility assessment
- Clinical diagnostic assay
- Clinical samples
- Regulatory compliance

Clinical assay development & validation
- Assay development
  - Platform
  - Assay
  - Sample type
  - Sample processing
  - Clinical samples
  - Regulatory compliance

Limited access to samples
- Assay validation
  - Clinical samples
  - Regulatory compliance

Difficult gap for preclinical exploratory studies to cross
- Evaluation
  - IP
  - Clinical need/opportunity
  - Feasibility (availability of samples)
Annual Special Issue

TIME

10 IDEAS CHANGING THE WORLD RIGHT NOW

The global economy is being remade before our eyes. Here's what's on the horizon

- Why your job is your most valuable asset
- Repurposing the suburbs
- Survival-store shopping
- Biobanks: saving your parts
- Need land? Rent a country
- The new Calvinism
- Ecological intelligence
- Amortality: forever young
- Africa: open for business
- Reinventing the Highway
Historical context

- Biospecimen Resource = Collections or “libraries” of disease and/or normal human biospecimens
- Have existed for 100+ years
- Originated in Pathology department to confirm diagnosis and guide treatment pre/post-surgery
- No national standards exist for biospecimen resources that collect and store specimens for use in research
- No regulatory body oversees biospecimen resources
Publication activity* in the subject area of biobanking
(853 papers, 1959-2010)

*As an indication of activity in the field, the phrases ‘biobanking or biorepository or tissue bank AND cancer’ were used to search PubMed.
Number of disease-based biobanks, by therapy area (US, 1995-2008)
Relation between type of biomarker research and type of biobank

Study aims

- Genetic personal susceptibility
- Environmental exposures
- Internal dose of carcinogenic agent
- Biologically effective dose
- Early biological lesion
- Subclinical pre-invasive neoplastic disease
- Clinical disease (cancer)
- Prognostic & Predictive Markers
- Personalized Medicine

Opportunities of biomarker discovery

- Biomarkers of Susceptibility & Identity
- Biomarkers of exposure
- Biomarkers of disease

Type of biobank

- Population Banks
- Disease Oriented Banks for Epidemiology
- Disease Oriented Banks (Tumour banks)
Status of worldwide Biospecimens Resources

- 300+ million specimens, but tissue is of unknown quality
- Many biospecimen resources exist, but few “network”
- Collection methods vary, no commonly agreed standards
- Approaches to patient consent & privacy protection vary
  ➔ not all specimens are consented appropriately for today’s cancer research
- Documentation of clinical data is limited & variable
- No common IT structure links resources together
  ➔ difficult to exchange information
- Limited access to specimens exists between Institutions
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The different aspects of a Biobank

- Legal & Ethical issues
- Health Care System
- Behavioural code
- Patient
- Scientific research
- Physicians & Pathologists
- Clinical information
- Pharmra & Biotech Companies
Prepare for changes & increased requirements in biologic specimens for:

- conduct an advanced Molecular Medicine
- drive personalized treatments

Overcoming criticalities due to a limited availability of “high-quality human samples”
‘Next Generation’ sequencing instruments are providing new opportunities for comprehensive analyses of cancer genome

- Capacity greater than one Gigabase per run
- Drastic decrease in costs per genome
- Applications: DNA, RNA, chromatin (i.e. epigenome)
Lifecycle of a Biospecimen
Factors potentially having significant effect on gene expression

Uncontrollable variables

Patient
- age
- diet
- medical history
- medical treatment

Surgery
- “warm” ischemia
- anesthetic
- blood loss
- transfusion

Controllable variables

Time 0

Patient
Medical/Surgical Procedures
Tissue Acquisition
Handling/Processing
Storage

Pre-acquisition
Post-acquisition

Time 0
Collection: time to preservation, "cold ischemia"
Processing: preservation method, fixation length, processing schedule
Storage: temperature, thawing, time
Proportion of breast tumor biospecimens collected within 4 time groups at Manitoba Breast Tumor Bank (MBTB) and British Columbia Cancer Agency Tumor Tissue Repository (BCCA-TTR).

**MBTB**
- 28% (n=485)
- 32%
- 33%

**BCCA-TTR**
- 76%
- 22%
- 2%
- 1%

(Time From Excision to Cryopreservation)

Barnes R O et al. Cancer Epidemiol Biomarkers Prev 2008;17:3344-3350
BIOSPECIMENS: the possibilities are endless, however......

- Hospital staff may work differently
- Clinical pathways may be different in the different settings
- Competing priorities in clinical & academic centres
- Smaller hospitals may not have access to basic instruments
  - freezers
  - dry ice
  - liquid nitrogen

Do biospecimens for research need to be handled differently from clinical samples for diagnosis?

✓ internationally agreed protocols for handling samples?
✓ scientific evidence that differences in protocols affect biomarker expression?
RNA integrity under different storage conditions

Biobanking of fresh frozen tissue: RNA is stable in nonfixed surgical specimens

Patrick Micke¹,², Mitsuhiro Ohshima², Simin Tahmasebpoor³, Zhi-Ping Ren³, Arne Östman⁴, Fredrik Pontén⁵ and Johan Botling⁶

**RIN** (= RNA integrity number, a numerical assessment of the integrity of RNA based on its the entire electrophoretic trace) is not affected by storage conditions
Gene expression levels under different storage conditions

- ice
- room temperature
- 0.9 % NaCl
- RNA later

Expression levels (relative change compared to 0h)
Time course analysis of gene expression under different storage conditions in colon tumor samples

Consistent increased expression of histones, fosb, EGR, NFκB
Time course analysis of gene expression under different storage conditions in normal colon mucosa

Musella et al., manuscript in preparation
Although microarray-based gene expression profiling analysis has been reported as able to provide reasonably reproducible results for molecular classification of breast cancer,

3

and

4

our findings show that without thorough standardisation, these tumours cannot be classified reliably by this approach. As emphasised by Ioannidis and colleagues,

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other investigators might only be able to predict molecular subtypes accurately when a detailed description of data processing and analytical methods is provided. A roadmap similar to the one described for development and validation of therapeutically relevant genomic classifiers

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is needed for introduction of breast cancer molecular taxonomy in clinical practice. Furthermore, careful standardisation of preanalytical variables that have a direct effect on expression profiles, such as stromal component

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and tissue processing,

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are equally crucial for development of reliable and reproducible classifiers.
A basic principle

The Golden Rule

Quality of biospecimen → Quality of research

The lack of standardization of factors involved in the life-cycle of human biospecimens compromises the quality & utility of research and the advances in cancer research that depend on them.
The role of Standard Operating Procedures (SOPs)

- SOPs specify how a specimen is obtained & handled
  → judgement on what the biomaterial can be used for subsequently

- SOPs allow harmonization among studies
  → guidelines for standards:
    ✓ IARC Common minimum technical standards
    ✓ Medical Research Council, UK
    ✓ Council of Europe, Committee of Ministers
    ✓ National Cancer Institute, US
    ✓ National Cancer Center, Singapore
    ✓ Australasian Biospecimen Network
    ✓ International Society for Biological and Environmental Biorepositories
    ✓ Organization for Economic Cooperation & Development
    ✓ TUBAFROST, European Union
    ✓ BBMRI Technical Standards & Protocols for Biological Resource Centers
    ✓ RAND Corporation, Inc.
    ✓ CryoBiosystems, Inc.

JB Vaught, E Caboux, P Hainaut, Cancer Epidemiol Biomarkers Prev 2010;19:912
BIOSPECIMENS: the possibilities are endless, however ensure QUALITY!

- Samples must be collected, documented and stored according to written Standard Operating Procedures/Protocols (SOPs)

- Adherence to SOPs must be regularly checked

- If you cannot control quality at input level, must control quality at output

- SOPs enable us to collect data on how a specimen is obtained and manipulated

- If SOPs are too rigid or impractical, ....either specimens will not be collected or people will not tell the truth!

- SOPs should be developed with team involved in collection of material to ensure that they are practical
Standard principles for quality management*

- SOPs for biomaterial life cycle management
- Staff training
- Management structure
- Maintenance plans
- Infrastructures & equipment
- Safety & contingency plans
- Random biomaterial quality checks
- Document & record management
- Ethics & legal compliance

*based on OECD & IARC recommendations
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BARRIERS for Translational Studies

- Need a significant number of tumor specimens for molecular characterisations (tissue bank issue)

- Need a significant number of patients with the same predefined target(s) and (in principle) common responder genes (farmacogenetics) (highly stratified clinical trials issues)

- Need improved bioinformatics tools for meta-analysis of data bases containing results of significant number of high through-put experiments (data projection on different data-sets issue)
Why Is It So Important To Collect & Store Human Specimens?

- Phase III clinical trials involve a large number of patients.
- Cancer treatment is moving more towards targeted therapy, but in vivo targets do not always work according to what they should do.
- Understanding biology provides evidence for why some patients respond and others do not.
- To target therapy effectively, need clinically useful markers and need biospecimens to find them.
- Access to human tissue specimens is vital in cancer research:
  - Identifying new targets
  - Understanding mechanisms of action/resistance to therapy
  - When linked with clinical data, may yield prognostic & predictive factors for treatment selection.
BIOSPECIMENS: the possibilities are endless....

- **Pre-operative**
  - core biopsies
  - blood samples
    - whole blood – *germline DNA*
    - serum/plasma
      - *circulating tumor cells/RNA/miRNA/methylated DNA*
    - other bodily fluids (urine, etc.)

- **Intra-operative**
  - bile
  - pancreatic fluid
  - interstitial fluid

- **Operative specimen**
  - snap frozen tissue
  - tissue stored in stabilizer
  - fixed tissue
Workflow pattern in a biobank

- Analysis
- Study Design
- Collection
- Collection/Annotation
- Storage
- Labeling
- Data Records
- Identification
- Processing
- Shipping

Biobanking activities
Biorepository models

- Decentralized collection and storage, with centralized bioinformatics/data management
- Decentralized collection with centralized storage and centralized bioinformatics/data management
- Centralized collection, storage, and bioinformatics/data management
Biospecimen Pathways: after the collection there are two paths a biospecimen may follow

➢ **Clinical Pathway:** This path includes diagnosis & treatment. The clinical pathway benefits the individual patient.

```
demand of human biospecimens under ideal conditions for the development of large-scale studies of clinical significance
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➢ **Research Pathway:** This path involves scientists doing research to enhance knowledge and advance cancer treatments. The research pathway benefits the broader population.

```
demand of human biospecimens even for small high-quality series for basic researches
```
### Different types of biomaterial collection

<table>
<thead>
<tr>
<th>Biomaterial collected for research purposes only</th>
<th>Biomaterial collected as a part of routine clinical practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very high quality criteria</td>
<td>Variable quality</td>
</tr>
<tr>
<td>High scientific value</td>
<td>Required for biomarker validation</td>
</tr>
<tr>
<td>Few samples fulfill criteria</td>
<td>Available in sufficient quantity</td>
</tr>
<tr>
<td>Strong selection bias</td>
<td>Available for larger patient cohorts</td>
</tr>
<tr>
<td>Very expensive</td>
<td>Affordable cost</td>
</tr>
<tr>
<td>Not relevant for medical routine</td>
<td>Necessary for clinical application</td>
</tr>
</tbody>
</table>

The type of biomaterial collection depends on the assay/question

Some difficulties to second future developments in Science…but technologies are ever improving
BIOSPECIMENS: endless possibilities within a clinical trial....

Pre- and post-treatment specimens

Tumor biopsy
- mRNA
  - Gene profiling
  - RT-PCR
- DNA
  - FISH
  - Mutation analysis
  - Methylation

FFPE tissue
- mRNA
- Protein
  - IHC
  - .......
- RT-PCR

Blood
- mRNA
  - ELISA
  - Proteins
  - SNPs
  - Circulating tumor cells
BIOSPECIMENS: the possibilities are endless, however ensure QUALITY!

- Clinical trials mostly planned for post-operative patients (= access to material restricted to that from the diagnostic record: you have to work with what you can get!)

- Pathology departments understaffed and poorly resourced (= provision of material for clinical trials not a priority)

- Preservation of morphology and ability to assess resection margins important for diagnosis

- Specimens often placed in fixative in Surgery Departments
  - access to frozen tissue
  - alternative methods of preservation
  - access only to formalin-fixed paraffin-embedded tissue

- FFPE block a treasure!
  - morphologic studies and IHC
  - RNA-ISH/qRT-PCR
  - miRNA & mRNA arrays
Why consider biologic material collection prospectively?

To best support collection and translational research project success:
- secure quantity & quality
- biomaterial collection may be done in an optimal collection setting based on patient’s consent and local site resources, feasibility & willingness
- allow planning for appropriate logistic & financial support
- logistics + collection guidelines prior to first patient registration

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Diagram:
- 100% samples
- study acceptance
- Patient consents
- shipped samples
- passed QC (‘useful samples’)
- Statistical sample size considerations?
How will the best practices benefit cancer research?

- Improving the quality of biospecimens
  
  - more reliable research results

- Standardized practices
  
  - more comparable results across studies and researchers allowed to use multiple biospecimen resources within a single studies

- Standardized access policies and encouraging sharing of resources
  
  - greater research access to specimens
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Research Ethics

Confidence in biomedical research

Interest in/of Research

Confidentiality

Autonomy of researcher opinion

Autonomy of patient opinion

Safety
Ethical & legal issues

Today the main problems have to do with:

- meaning & implications of the concept of donation
- the extent of informed consent
- withdrawal of consent and its consequences
- protection of personal data (privacy)
- guarantees for individuals and the Community
- availability of information useful for the patient and/or family members
- possibilities and modalities of data identification
- research acknowledgement

Main Actors involved

- PATIENTS
- Governs/Regions (rules/legal aspects for investigations on human materials)
- Charities supporting research
- Ethics Committees
- Comprehensive Cancer Centers/Hospitals
- Investigators
- Biotech & Pharma Companies
Broad consent

Partially restricted consent

Multi-layered consent

Specific informed consent
General principles

- Biospecimens can be obtained and/or used for Research only following a full consent by the Patients. Such a consent should be:
  - informed
  - voluntary & spontaneous

- Researches can and must be performed only if potential benefits overcome possible damages to the Donor of the biologic material

- Researches should start only after the approval by IRB & Ethics Committee that oversee:
  - scientific aspects
  - privacy/confidentiality procedures
  - biorepository practices

- Limit access to codes linking patient identifying information to their tissue specimens through physical and/or cyber tools
General principles

- Personal information should be treated with the maximal protection

- The Donor, if required, must be informed about research results

- In the informed consent form, possible financial and economic benefit from the Research for the Institution should be mentioned
A one-time general informed consent by a 2-step decision procedure
What do research participants want?

<table>
<thead>
<tr>
<th>Author</th>
<th>Survey participants</th>
<th>Consent forms (#)</th>
<th>Valuable results</th>
<th>Consent for research: future</th>
<th>Consent for research: unlimited</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT Chen (2005)</td>
<td>Healthy &amp; affected individuals</td>
<td>1670</td>
<td>78%</td>
<td>87%</td>
<td>83%</td>
</tr>
<tr>
<td>Kettis-Lindbland (2007)</td>
<td>Population</td>
<td>6000</td>
<td>49%</td>
<td>72%</td>
<td>72%</td>
</tr>
<tr>
<td>T Malone (2002)</td>
<td>Cancer patients</td>
<td>5411</td>
<td>40%</td>
<td>94%</td>
<td></td>
</tr>
<tr>
<td>Our data (unpublished)</td>
<td>Cancer patients</td>
<td>1950</td>
<td>81%</td>
<td>98%</td>
<td>97%</td>
</tr>
</tbody>
</table>

- Patients/healthy individuals support the future research use of their biological samples
- A simply binary choice might allow individuals to control use of their samples, simplify consent forms, foster important researches
Prioritization to researches based on merit review by IRB, Ethics Committee & standardized criteria

- Policies to prevent the last sample distribution and researcher monopolization of samples
- Researcher feedback on particular sample/shipment
- Evaluation of repository performance through Committees/ review groups

Approval & prioritization of researches by IRB & Ethics Committees
Access to biospecimens & data

- Develop clear policies for specimen and data access
- Develop clear guidelines for sample distribution and clinical data sharing (*protocol-specific requirements to be met before other access is considered*)
- Ensure that investigators have timely, equitable and appropriate access, without undue administrative burden
- Charge for specimens only to recover costs
- If a resource needs to close, announce the availability of specimens for transfer
- Restrict access to subjects identities and medical, genetic, social and personal histories via data access system with defined privilege levels
Take Home Message

- Collecting biospecimens in clinical trials is essential for translational studies & targeted therapies
- Patients broadly supportive – including generic consent for future studies
- Detailed planning and an understanding of the clinical pathway are essential
- Develop robust methods that work on routine clinical samples: FFPE holds may treasures!
- Build quality assurance into every step
The pan-European Scale of BBMRI

Preparatory phase 27 months
Funding 5 mio €

- 51 Participating institutions
- 210 Associated organisations
- 30 Countries

Rete Italiana Biobanche Oncologiche

Responsible: Angelo Paradiso - Giovanni Migliaccio

Chi siano
Background
Obiettivi del Progetto
Contatti

Documenti
Pubblicazioni

Biobanche e Privacy
Consenso informato
Leggi e Normative

Area riservata

Link
- Allianza contro il cancro
- Eurobiobank
- European Bio-banking and Biomolecular Resources Federation (EBBRI)
- Ministero della salute
- WHO - World Health Organization