Real-life example of biobanking: Results of the PATH Biobank

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Disclosures

PATH Foundation has received donations from and/or has had sponsoring relations with the following companies: Amgen, AstraZeneca, GlaxoSmithKline, MSD, Novartis, Pfizer, Pierre Fabre, Roche and vfa.

Introduction

Since 2002, PATH Foundation has kept a biobank collecting high-quality fresh frozen breast cancer (BC) specimens adhering to uniform SOPs at seven certified breast cancer centres in Germany. Research groups from academia and industry can obtain samples after application and review. PATH Biobank is a not-for-profit organisation and asks for a cost recovery fee in exchange for sample allocation to sustainably finance the expenses it incurs.

Methods

Decentralized liquid nitrogen biobank

- PATH's samples are stored at 7 certified breast cancer centres in Germany (Figure 1)
  - tumor samples with ≥ 3 mm edge length
  - Corresponding, normal adjacent tissue samples with ≥ 3 mm edge length
  - Blood serum samples with ≥ 1 ml volume
- All samples are stored "fresh frozen" in the vapour phase of liquid nitrogen

Figure 1: PATH's 7 sample source sites in Germany

Central database

- Data storage using Oracle® software and LIMS developed in-house
- Standardized forms for patients' informed consent
- Approval by ethical review commission of the Uniklinikum Bonn
- Central survey of patient reported follow-up data

Using PATH Biobank as a resource

Researchers from academic or industrial circles may apply for sample allocation by submitting a proposal. Sample requests are reviewed by independent experts. PATH's managing board decides on sample allocation advised by PATH's board of trustees and scientific board. A material transfer agreement is signed, which also includes a cost recovery fee and reimbursement for logistics. For more information, please send an e-mail or visit: **www.path-biobank.org**
info@stiftungpath.org

Results of the PATH Biobank

Since 2004, more than 8,000 breast cancer patients have given their informed consent to the storage and analysis of their tissue and blood serum for research purposes [1]. Breast cancer tissue samples from 58% of donors could be stored due to the size of the surgically removed tissue specimen. In addition, normal adjacent tissue is available from 62% of donors and blood serum aliquots can be derived from 91% of patients. In total, a number of 7,132 tumor tissue samples (donated by 4,817 individuals), 7,983 normal, adjacent tissue samples and 15,257 blood serum samples could be stored in the biobank for research purposes (some of which is redundant storage). Figure 2 shows the total number of patients who have given their informed consent together with the distinct number of individual donors who respectively contributed to the different sample types.

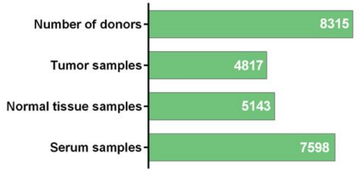

Figure 2: Numbers of donors and samples

Since 2008, all sample donors have been contacted individually to directly survey patient reported follow-up data. By now, information on the course of disease and therapy from 4.195 women has been collected. Median follow-up time is 38.5 months. If donors are lost to follow-up, missing survival data can be inquired at registry offices.
By using the annotating data sets, it is possible to classify 96% of donors into the intrinsic molecular subtypes of breast cancer in accordance with the St. Gallen Criteria [2]. For example, 66% of the cases belong to the Luminal A subgroup; the distribution of all PATH cases is shown in Figure 3. The UICC-stages are used as an alternative way to subgroup the patients (Figure 4).

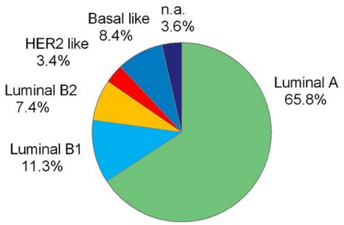

Figure 3: Distribution by intrinsic molecular subtypes*
* total number of cases 7,184; excluded: cases with neoadjuvant therapy, loco-regional recurrence or DCIS

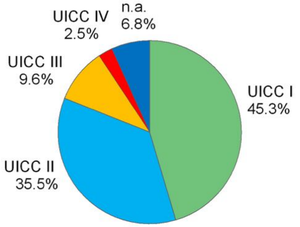

Figure 4: Distribution by UICC stages*

Example from real life

In 2008, PATH Biobank started to support research groups by providing breast cancer samples and/or data. Since then, 16 different research projects have used PATH Biobank as a resource. The frequency of requests and resulting co-operations have been increasing as well as the number of manuscripts and articles depicting results obtained while working with PATH samples. In 2015, three co-operations with PATH Biobank led to scientific publications [3, 4, 5].
Each time, samples are allocated to scientists, a material transfer agreement is concluded which comprises feedback on quality control parameter measurements and results where applicable and feasible.
PATH Biobank received quality control data from 7 different projects.

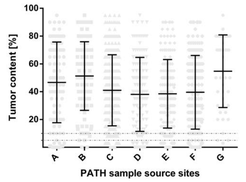

Figure 5: Sample tumor content according to source site

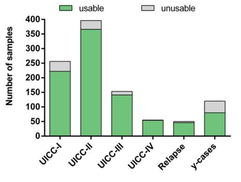

Figure 6: Sample usability according to BC stage

One major quality characteristic of fresh frozen tumor tissue samples is the actual tumor content. The pathologists at PATH's sample source sites macroscopically assess tumor content prior to snap freezing and biobanking. In addition, researchers are encouraged to reassess the tumor content of the allocated samples as a first step of the analysis by reviewing sections taken from the samples by microscopy. Results concerning tumor content are available for 1,039 samples studied in 4 different projects and are depicted in Figure 5, showing values related to sample source sites (error bars indicate statistical mean and standard deviation).

Only 11% of tumor tissue samples were classified as insufficient (due to project specific inclusion criteria which ranged from 5% - 10% tumor content, cf. [6, 7]). Tumor content depended on clinical conditions and staging (ranging from 34% failure in samples derived from neo-adjuvantly treated patients to 2% in cases with staging UICC IV (cf. Figure 6).

As a further parameter for quality control measurements, the RNA Integrity Number (RIN) was determined in 4 projects working with 240 samples. Values ranged from 2.9 – 10.0 with an average RIN score of 8.4 and a standard deviation of 1.2. The values shown in Figure 7 relate to sample source sites.

Only one project using blood serum samples shared data on the cfDNA yield. The 120 samples had an average volume of 826 µl. cfDNA yield ranged from 173 – 1776 ng (average 819 ng, standard deviation 278 ng).

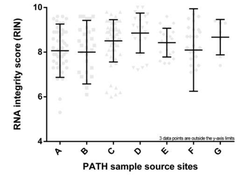

Figure 7: Sample RIN score according to source site

As an outlook for 2016, five ongoing research projects are currently using PATH Biobank as a resource and in addition scientists asked for support of 3 different studies; details are currently being discussed.

Literature

[1] Waldmann, A., et al.; Patients and Methods of the PATH Biobank – a Resource for Breast Cancer Research. Geburtsh Frauenheilk 74, 361-369, 2014
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Conclusion

With its detailed clinical and follow-up data, PATH Biobank is a valuable, scientific resource for breast cancer research based on tissue and blood serum, e.g. biomarker studies. Since 2008, various research projects have successfully been conducted using PATH Biobank's samples. Both, the quality and diversity of samples as well as the integrity of annotating data sets, made it possible to achieve the scientific purposes of these projects