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Review

The Organization of Contemporary Biobanks for Translational Cancer Research

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Simple Summary: Biobanks belong to the highly organized infrastructures of research institutions and clinics which allow the storage of patients' biological material, thus constituting an essential tool for performing translational research aiming at the discovery of prognostic and predictive biomarkers. Although hundreds of biomarkers have been reported in the literature, only a few of them have been confirmed and validated for their specificity and sensitivity and, subsequently, have become clinically applicable. The main reason for this is the biological specimens' lack of quality, which is essential for obtaining reliable and reproducible results from their analysis. In this review, we discuss some important issues that need to be met during biobanking to enhance the contribution of biomarkers to translational and clinical cancer research.

Abstract: Cancer biobanks have a crucial role in moving forward the field of translational cancer research and, therefore, have been promoted as indispensable tools for advancing basic biomedical research to preclinical and clinical research, ultimately leading to the design of clinical trials. Consequently, they play an essential role in the establishment of personalized oncology by combining biological data with registries of detailed medical records. The availability of complete electronic medical reports from individualized patients has led to personalized approaches for diagnosis, prognosis, and prediction. To this end, identifying risk factors at early time points is important for designing more effective treatments unique for each patient. Under this aspect, biobanking is essential for accomplishing improvements in the field of precision oncology via the discovery of biomarkers related to cellular and molecular pathways regulating oncogenic signaling. In general terms, biological samples are thought to reflect the patient's disease biology, but under certain conditions, these may also represent responses to various biological stresses. Divergent collection, handling, and storage methods may significantly change biosamples' inherent biological properties. The alteration or loss of biological traits post-collection would lead to the discovery of nonreliable biomarkers and, consequently, to irreproducible results, thus constituting a formidable obstacle regarding the successful translation of preclinical research to clinical approaches. Therefore, a necessary prerequisite for successful biobanking is that the stored biological samples retain their biological characteristics unchanged. The application of quality standards for biospecimen collection and storage could be useful for generating encouraging preclinical data leading to the successful translation to clinical treatment approaches. Herein, we aim to comprehensively review the issues linked to biobank implementation for promoting cancer research.

Keywords: biobank; biological samples; ethics; informed consent; cancer patients

Citation: Gkioka, V.; Balaoura, O.; Goulielmaki, M.; Baxevanis, C.N. The Organization of Contemporary Biobanks for Translational Cancer Research. *Onco* **2023**, *3*, 205–216. https://doi.org/10.3390/onco3040015

Academic Editor: Fred Saad

Received: 4 August 2023
Revised: 15 September 2023
Accepted: 21 September 2023
Published: 22 September 2023

1. Introduction

Biobanking operates to define all activities associated with the management of biosamples from various origins, including tissues from humans and animals, bacterial and viral

cultures, and environmental samples. This implies that different types of biobanks use diverse approaches for collection procedures, storage, and management procedures considering the life cycle diversity of the different biosamples. However, regardless of the type, biobanks should employ efficacious procedures for control over data and biological samples, advancing the implementation of biobanking in basic, translational, and clinical research. Human biosamples include molecules that can be thoroughly investigated to characterize various types of diseases. Analyzing biosamples is particularly helpful for the identification of biomarkers for the prognosis and prediction of various types of therapies. Such biomarkers can be useful for the design of treatment schedules preventing overdosing and side effects and increasing effectiveness, thus providing a better quality of life and longer survival duration for patients. In this respect, given the implementation of biomarker discovery research studies in clinical applications, the essential role of biobanks in translational cancer research must be acknowledged. Initially, biobanks contained only small collections of samples to meet the needs of local institutions' research projects. However, during the past two decades, the area of biobanking has expanded, allowing translational research and uncovering novel therapeutic approaches for cancer. Contemporary biobanks have large quantities of samples available which are collected, processed, and stored under the same procedures to ensure best quality. The high quality of biological specimens is mandatory for obtaining reliable information from their analysis. The accessibility of large numbers of biosamples within a biobank makes it necessary that these are homogenous in terms of collection and storage procedures (or that these have been collected and stored under standard procedures). Importantly, following isolation, the biological samples should retain critical characteristics. This is a very crucial issue that, if not respected, will seriously hinder research activities. Characteristics that are altered post-isolation will result in imprecise determinations and wrong conclusions. Therefore, the procedures for collecting any biomaterial must be simple and as fast as possible to minimize the alteration of its biological characteristics. In this regard, another key element is that the stored biosamples should be well preserved in containers at the appropriate temperature. This will provide easy accessibility for accurate data validation. To this end, various biobank institutions have established standard operating procedures (SOPs) for biosample collection, handling, storage, and quality control to ensure data reproducibility [1,2].

In this review, we discuss explanatory and useful topics that need to be appreciated and settled to provide improvements in the quality of biobanked biological specimens and, in this way, to promote biomarker research and validation for future clinical applications.

2. How Do Contemporary Biobanks Function?

Hospital-integrated biobanks can serve as biorepositories for various sources of human material accompanied by all relevant demographics which are mandatory for making correlations with clinical outcomes when processing the biosamples for the detection of reliable biomarkers [3]. Biosamples collected to study cancer include malignant as well as normal neighboring tissue and liquid biopsies in the form of peripheral blood, plasma, serum, pleural effusions, peritoneal effusions, urine, and saliva. Peripheral blood mononuclear cells (PBMCs) and genetic material consisting of DNA/RNA (extracted from normal and tumor cells), plasma-derived cell-free DNA and cell-free tumor DNA, micro-RNAS (miRNAs), as well as clinicopathologic data can also be collected in biobanks [4–6]. Such cancer-related samples, along with their accompanying clinicopathological parameters and demographics, are integrated in databases for future analyses, which makes it important to have as much as possible detailed and current relevant information at every time point of biosampling.

In this regard, an integrated collection of guidelines for human research biobanks based on the International Organization of Standardization (ISO) standards is required for the collection of fit-for-purpose samples by a validation and verification process [3]. This also applies to the isolation of various components from samples (e.g., PBMCs and genetic material isolation). Such operational quality control is the cornerstone for securing

result reproducibility which is important for their later verification and validation. This is very important given that the high-quality "omics" technologies applied nowadays for biomarker discovery are highly sensitive and may provide false positive or negative results if a biosample is not properly collected. Consequently, it is necessary to preserve the high quality of various technical procedures. Hence, each step in the biobanking workflow should adhere to standardized procedures to guarantee the genetic, molecular, and phenotypic integrity of the biological sample and, in this way, ensure the quality of samples and prevent false results leading to data irreproducibility. To this end, it should be mentioned that those critical steps contributing to the variation of the characteristics of a sample should be documented for further exploration.

An additional important function of a biobank is its participation in translational research programs, mostly in collaboration with various international partners including research institutions, academic centers, oncology clinics, and the industry [6]. Such participation in research projects further emphasizes the need to ensure good quality of the samples to be provided by the biobank to any potential project partners. Another critical aspect for the durable and good functioning of a biobank is the assurance of long-term financial support, either from national funds or other research grants or private donations which, under a comprehensive business plan, will secure an expense-regaining strategy in the long term.

3. Operating Procedures

Nowadays, a lot of efforts are being pursued in the discovery of biomarkers for optimizing treatment protocols and improving clinical outcomes. Biomarkers for prognosis and prediction are essential for moving forward the field of personalized medicine and, in particular, the field of precision oncology [7–10]. Such biomarker research is a multistep process involving various factors and critically depending on the quality of samples. Biobanks are necessary for this purpose via the collection and storage of human samples. Therefore, biobank practices should provide guidelines for recording and tracking biosamples constructively. Biosamples are collected from tumor tissues as well as from the neighboring healthy tissues of cancer patients. Peripheral blood samples collected before, during, and after therapies by clinical response or relapse constitute additional samples which, along with detailed clinicopathological data at the time of blood sampling, can provide a protracted and sustainable source of biomarker analyses. For that reason, biobanks should closely collaborate with pathology departments to secure the quality of formalin-fixed paraffin-embedded tumor samples as well as of frozen tumor tissues for high-throughput technologies aiming at deep sequencing for molecular analyses. To this end, care should be taken to avoid DNA/RNA fragmentation during fixation [11–13], which could lead to incomplete analyses and incorrect data interpretation. Regarding peripheral blood samples, these should be transferred relatively rapidly (within a few hours) from the time of sampling in the clinical pathology units to the biobank facilities to secure good quality DNA/RNA and serum, plasma, or mononuclear cell isolation. Concerning storage, DNA is stable for weeks when kept at refrigerator temperatures (4 °C), but in any case, both DNA and RNA are more stable at −80 °C for long-term periods [13,14]. DNA integrity following extraction from blood and tissue samples can be tested, e.g., before and after long periods of storage, using gel electrophoresis, enzymatic digestion, and/or polymerase chain reaction (PCR). Similarly, RNA integrity can also be tested via, e.g., gel electrophoresis and/or real-time PCR (RT-PCR). Liquid nitrogen containers or −150 °C cryogenic freezers are required for the storage of mononuclear cells in dimethyl sulfoxide (DMSO) [11,13]. All of the above underscores the importance of ensuring adherence to SOPs for biosample preparation and storage, as well as shipment, to secure biosample integrity. Via SOPs, biobanks acquire specified internal policies that are critical to guide access to patient samples and clinicopathological characteristics [1,15]. Quality assurance measures should be implemented, including conditions to ensure appropriate security and confidentiality during the establishment of the collection, as well as during storage, use,

and, where appropriate, transfer of biological materials. To this end, SOPs are also needed to register all critical steps that constitute the process of biobanking, such as assembly and processing of biosamples, data retrieval, and data classification. All of these steps need quality checks to ensure the reproducibility and suitability of the output for the study objective. The unique identification of the samples ensures efficient traceability for inventory. It is mandatory to minimize risks related to biobanking operation procedures by thoroughly recording all relevant processes to ensure liability [1,16]. The basic tenets for research supervision via biobanking refer to the written informed consent and the collection of longitudinal samples from patients [17]. For that reason, samples should only be used in a research project if the latter is within the scope of the consent authorization provided by the person concerned. Biobanks should ensure that potential donors receive detailed information about the purpose, benefits, and risks associated with the use of their biological samples for research. An independent ethics committee plays a pivotal role in the acquisition of a definite authorization [6]. One should also take into consideration that the way the results of the sample analyses will be communicated to the scientific domain should be clearly defined in the consent form. Another important aspect that should be met is to inform the volunteers about the project's scientific expectations and the benefits that they may obtain from the project's results. It is equally important to keep samples anonymous and to store them using appropriate coding to make sure that the project will not be hampered by privacy matters. Finally, biobanking should be based on national and international laws to preclude illegitimate use of the samples [16,18,19].

4. Biobanks Oriented for Cancer Translational Research and Personalized Treatment

In general terms, the essential roles of the biobank for cancer research are to provide accurate and substantial information regarding the risk factors that contribute to disease initiation and progression and, in parallel, to discover new drugs based on the patient's genetic profile, both of which provide the platform for promoting personalized medicine. Actually, during the past few years, we have experienced a new vision of oncology via precision analyses of patients' genetic material based on the application of the "omics" technologies, which enabled the discovery of prognostic and predictive biomarkers, ultimately guiding the selection of patients most likely to benefit from therapies and uncovering novel therapeutic targets [20,21]. The discovery of biomarkers for prognosis and prediction is mandatory to develop treatments and preventions uniquely for each patient, thus minimizing risk factors and improving clinical outcomes, and has established the field of precision oncology in the broader frame of personalized medicine [7]. This new perception of oncology has advanced translational research based not only on modern molecular technologies but also on computational studies resulting in extensive gene/protein analyses from patient samples [22,23]. Indeed, integrative computational analyses of the information derived from the biomarker discovery field, including patient demographics and clinicopathologic data as well as molecular data, have enabled the determination of algorithms guiding optimized treatments and minimizing risks [24]. Biomarkers are mandatory for the design of more effective treatment protocols based on the administration of the right drugs at the right time intervals and of the appropriate dose. However, the selection of useful biomarkers must be attentively tested, based on crucial parameters such as sensitivity and specificity. Moreover, biomarkers detected using conventional tumor culture methods may be vague, given a biased selection for cancer genomic alterations that no longer reflect the biology of the original tumors. This problem has been circumvented through the use of multidimensional organoid models which preserve tumor cell genetic and phenotypic features [25]. Hence, the selection of biomarkers is not an easy task, given that the majority of the discovered biomarkers lack adequate sensitivity and specificity for their prognostic or predictive roles [22]. This could be attributed to (i) the limited number of samples analyzed, resulting in the discovery of a restricted number of biomarkers; (ii) the lack of determination if a sample to be analyzed is comparable to the other samples and fit for purpose; (iii) incomplete medical records, which may hinder the correct evaluation

of the prognostic or predictive value of a biomarker; and (iv) the lack of well-defined SOPs for quality in biomarker selection including sample collection, storage, handling, analysis, and data elucidation. The absence of explicit SOPs will undoubtedly lead to the application of heterogeneous approaches for the collection, processing, storage, and annotation of human biosamples, thus significantly contributing to irreproducible results and restraining the discovery of reliable biomarkers as well as the development of effective therapeutic protocols [3,26]. Considering, also, that biobanks are quite diverse in their design and function, it is mandatory to pursue standardization of the various biobanking activities. In general terms, all procedures should be described in detail in SOPs to ensure appropriate storage and effective circulation of the samples for research studies. Such SOPs should be renewed to include improvements in best-evidence practices and technical issues that have been encountered throughout the evolution of biobanking [27]. These SOP updates are essential for ensuring the high quality of samples and reliability which are required for the promotion of translational research. Another obstacle that hampers the identification of appropriate biomarkers for prognosis and prediction lies in the absence of surrogates for clinical responses, especially in cancer types characterized by slow progression. The contribution of biobanking in overcoming this problem is adequate since, as also mentioned above, a properly organized and functioning biobank can provide datasets containing patients' demographics and clinicopathological parameters at diagnosis and disease progression, matched with different types of biospecimens, including tissue and liquid biopsies [28]. To this end, one should consider the limitations that a biobank could face regarding the optimization of the entirety of this information that is inherent to biobank data acquisition. Notwithstanding, this can be circumvented via statistical machine learning and deep learning technologies [29].

5. The Absolute Requirement of Maintaining the Integrity of Samples

Variability and heterogeneity of samples seriously affect the application of procedures for the appropriate collection, processing, storage, and characterization of a significant number of samples to be used for translational studies [21,22]. This obstacle could be circumvented, to a certain extent, via the application of single-cell omics (SCOs), where multi-omic layers of DNA, RNA, and protein can be simultaneously profiled in the same cell to analyze the underlying mechanisms and to discover potential prognostic and predictive biomarkers [30]. Such SCOs could be applied in selected areas of tumor tissues following the pathologist's annotations, but also in selected peripheral blood leukocyte subsets.

Notwithstanding, it is essential to understand that heterogeneity in terms of pathology, clinicopathological parameters, and collection procedures will make it difficult to compare results from experiments performed in different laboratories, thus posing a barrier for the identification and characterization of reproducible biomarkers for prognosis and prediction. This, in turn, impedes cancer drug discovery, given the difficulties of accurately assessing whether novel targeted therapies would be clinically effective for a certain group of cancer patients. To overcome such problems, it is mandatory to generate SOPs for sample handling and to implement these protocols via contractual agreements with the oncology hospitals [31]. Indeed, the crucial role of biobanking research in the discovery of biomarkers, which is a cornerstone in personalized oncology, is exclusively based on its potential to collect, process, store, and disseminate high-quality samples. The successful completion of these processes is based on the punctual application of continuously updated SOPs. It is also important to understand that various analytical tests may require different preparation and storage conditions. In addition, processing certain conditions, such as temperature and multiple freeze–thaw cycles, may negatively affect the quality of the samples. Thus, the quality of a sample is a multifactorial process that depends not only on the processing steps but also on the time intervals between these steps, mainly in the pre-analytical stage [32]. It is therefore mandatory for a biobank to follow the SOPs regarding procedures of safe collection, transport, and handling and also to guarantee registration of the clinicopathological data to ensure traceability of the biospecimens. If

some of these procedures are not appropriately used, this can significantly impede the research results, leading to misinterpretations and incorrect conclusions.

Another factor that obstructs the effective use of samples in translational cancer research arises from differences in periods between sample collection and (i) storage in deep freezers (in the case of mononuclear cells, DNA/RNA, plasma, urine, and tumor tissue) or (ii) formalin fixation (in the case of tumor tissue samples). With the growing demand for applying personalized treatments, the need for preserving the integration of samples becomes more obvious. It is conceivable that stability depends on the time frame between biosample collection and processing and, therefore, minimizing the time needed for these procedures represents the best approach for preventing molecular or phenotypic biosample alterations. Maintaining the stability of samples during the pre-analytical processes is mandatory for obtaining reliable results from translational cancer research studies. Such results may lead to the discovery of clinically validated biomarkers providing information for prognosis and risk assessment and serving as surrogates for clinical responses. However, despite the plethora of reports on the identification and characterization of promising cancer biomarkers, only a few of them are clinically applicable. A main reason for this is the lack of adherence to standardized protocols and methodologies for sampling, processing, and storage, in the frame of the pre-analytical phase. Variations in the pre-analytical phase may greatly impact the results from translational research studies, leading to misleading conclusions and ascribing inaccuracy to subsequent studies. For that reason, all procedures need to be carried out according to standard norms in a quality management system. Notwithstanding the general acceptance of applying standardized procedures for sample handling in the pre-analytical phase, it is still critical to perform biosample integrity assessment. For example, one of the most common methods to test DNA and RNA yield, integrity, and amplifiability is via RNA integrity (RIN), DNA integrity (DIN,) and delta Ct values [33]. Additionally, it is important to consider that depending on the type of surgery, excised tumors, being disconnected from the blood supply, may be maintained at body temperatures for different periods of time, thus being subjected to different levels of ischemic effects which, in turn, may impact gene expression [34].

6. Case Study: The Biobanking at the St. Savas Cancer Hospital

The biobank, which was established in early 2018, is located in the Immunology department and constitutes a facility of the Cancer Research Center at the St. Savas Cancer Hospital. The immunology department consists of the cellular culture lab, the molecular biology lab equipped with high-throughput next-generation sequencing instruments, the flow-cytometry lab, and the biobanking room with a −150 °C cryogenic freezer, three −80 °C freezers, a −40 °C freezer, and two liquid nitrogen tanks for the storage of samples. The informed consent intended for both cancer patients and healthy volunteers has been approved by the scientific committee of the St. Savas Cancer Hospital and the ethical committee of the National Kapodistrian University of Athens (NKUA). In addition, the ethical protocol established by the NKUA is in use for obtaining permission for the selection of samples for biomedical research. Written informed consent is required and obtained from every participant (cancer patient or healthy donor) at enrollment. For every cancer patient, complete electronic registration of detailed clinicopathological parameters based on the cancer type is available. Table 1 lists relevant registrations that are provided for three selected types of cancer.

Table 1. Relevant biosample registrations for three selected types of cancer (prostate cancer, head and neck cancer, and non-small cell lung cancer) kept at the biobank of St. Savas Cancer Hospital.

Prostate Cancer						
1. Patient number	2. Patient code	3. Date of consent	4. Date of diagnosis	5. Age	6. Nationality	7. Date of blood sampling

Table 1. *Cont.*

Prostate Cancer						
8. DNA/RNA/ plasma/serum/ PBMCs from blood sampling	9. Time interval between diagnosis and blood sampling	10. Disease status at enrolment (metastatic/non-metastatic)	11. Therapies before blood sampling	12. Date and type of localized therapy	13. Initiation of localized therapies	14. HLA typing
15. PSA	16. GLEASON SCORE	17. Staging	18. Date of biochemical recurrence	19. Hormonal treatment (start date)	20. Resistance to hormonal treatment (start date)	21. Years from disease diagnosis to resistance
22. Date of metastasis	23. Years from disease diagnosis to metastasis	24. LN vs. bone metastasis	25. Metastasis at enrolment	26. Death event	27. Years from disease diagnosis to death event	28. Date of the last follow-up
Head and Neck Cancer						
1. Patient number	2. Patient code	3. Date of consent	4. Age	5. Nationality	6. Gender	7. HPV status
8. Smoker (pack/years)	9. Alcohol consumption [yes (sometimes per week)/daily consumption)/no]	10. Medical history	11. Anatomical site of the tumor	12. Histology report	13. Staging (TNM)	14. Type of therapy
15. Date of first blood sampling	16. DNA/RNA/ plasma/serum/ PBMCs from first blood sampling	17. Date of second blood sampling (after the end of therapy)	18. DNA/RNA/ plasma/serum/ PBMCs from second blood sampling	19. Disease-free survival	20. Overall survival	21. Date of the last follow-up
Non-Small Cell Lung Cancer (NSCLC)						
1. Patient number	2. Patient code	3. Date of consent	4. Age at diagnosis	5. Nationality	6. Gender	7. Smoker (pack/years)
8. Alcohol consumption [yes (sometimes per week)/daily consumption)/no]	9. Medical history	10. Date of surgery	11. Histology report	12. Pre-surgery therapies	13. Duration of pre-surgery therapies	14. FFPE Blocks available from the surgically excised primary tumor
15. Staging	16. Date of first blood sampling (before immunotherapy)	17. DNA/RNA/ plasma/serum/ PBMCs from first blood sampling	18. Date of second blood sampling (during immunotherapy)	19. DNA/RNA/ plasma/serum/ PBMCs from second blood sampling	20. Date of third blood sampling by relapse or at the end of immunotherapy	21. DNA/RNA/ plasma/serum/ PBMCs from third blood sampling
22. Date of recurrence	23. Months from enrollment to recurrence	24. Overall survival	25. Months from enrollment to death event	26. Date of the last follow-up		

PBMCs, peripheral blood mononuclear cells; HLA, human leukocyte antigen; PSA, prostate-specific antigen; LN, lymph node; HPV, human papillomavirus; TNM, tumor nodes metastases; FFPE, formalin-fixed paraffin-embedded.

Sample collection operates under the authority of the St. Savas Cancer Hospital review board. The consenting protocol includes thorough information regarding the purpose of the study. The principal investigator of the study or the director of the clinic where the

patients will be treated collects the information. Patients also receive detailed information about the reason for which they will voluntarily donate biosamples (biopsies, tissue, or peripheral blood). Moreover, in case this is foreseen in the study protocol, patients are also informed about the collection of voluntary longitudinal sampling (peripheral blood) during and post-therapies. Patients are free to decide about the type of samples they would like to voluntarily give for the study. Patients are also asked if they would like to be contacted in the future for additional studies in case this will be necessary. Before signing the consent form, patients are asked questions to make sure that they understand the scope of the study. By signing the consent form, patients also allow the collection of clinicopathologic information regarding their disease status. This information is provided to the scientific team under "patient number" and "patient ID".

Quality controls for the stored DNA/RNA are performed usually every 6–8 months using standard methods as described elsewhere [35–39]. The viability of mononuclear cells is checked yearly via 7-AAD (7-amino-actinomycin D) viability staining solution [40]. Prostate cancer (PCa) samples have been derived from 225 patients, all of them treated at the urology clinic of St. Savas Cancer Hospital (in the frame of the translational research program NEOVIOPRO). Samples have been collected from PCa patients at various stages of the disease, including localized disease with no biochemical recurrence, castrate-sensitive and castrate-resistant non-metastatic disease, and castrate-sensitive and castrate-resistant metastatic disease. Repositories of mononuclear cells, plasma, serum, DNA, and RNA have been derived from samples collected from head and neck cancer patients (n = 40) at two time points (before treatment initiation and after treatment (chemo/radiotherapy; in collaboration with Democritus University of Thrace, in the frame of the translational research program BIOKAKETRA)) as well as from 55 additional head and neck cancer patients (before therapy initiation) from the clinics of St. Savas Cancer Hospital (also in the frame of BIOKAKETRA). Similar biomaterial (i.e., mononuclear cells, plasma, serum, DNA, and RNA) has been collected from 50 patients with non-small cell lung cancer (NSCLC) after chemo/radiation therapy in the frame of the B-PREIMMUN program (from the clinics of St. Savas Cancer Hospital and in collaboration with the oncology clinic at the university hospital of Thessaly). Subsequently, 36 of these patients received immunotherapy (Durvalumab). Corresponding samples (as above) have been collected from 29 of them and stored (2–3 months after treatment initiation with Durvalumab; second sampling); 12 of those patients' samples are also available from an additional, third time point (third sampling). This third sampling was taken either one year after the beginning of immunotherapy or at relapse. Moreover, in 15 of 50 NSCLC patients enrolled in B-PREIMMUN, DNA from paraffin blocks has also been isolated and stored. The biobank is progressing to receiving samples from additional cancer types, including hematological malignancies.

7. Exploitation of the Stored Biomaterial: The Clinical Implications of the Research Conducted at the St. Savas Cancer Hospital

Analyses of the stored samples mentioned above have already provided noteworthy results that paved the way for the identification of novel, reliable, and easily accessible prognostic and/ or predictive cancer biomarkers. Part of our results, specifically those referring to prostate cancer samples, have recently been published, while data regarding head and neck cancer and lung cancer samples are currently under evaluation.

Gene expression analysis of the RNA samples derived from the peripheral blood of PCa patients using next-generation sequencing (NGS) identified a radiotherapy-induced downregulation of a six-gene signature consisting of *CCR7*, *FCGR2B*, *BTLA*, *CD6*, *CD3D*, and *CD3E* [41]. Given that low mRNA expression levels of the gene signature in prostate tumor tissue were correlated with a better 5-year prognosis, this could hold a potent predictive value, which remains to be confirmed by further exploiting the biobanked material. Likewise, NGS analysis revealed an upregulated immune- and proliferation-related eight-gene signature, comprising *FCGR2B*, *CDK1*, *MELK*, *FOXM1*, *CCR1*, *CDKN3*, *CD53*, and *SLAMF8*, in the peripheral blood of PCa patients compared to healthy donors. Using the

tissue and survival data available from The Cancer Genome Atlas (TCGA) database, we found that PCa patients with intra-tumoral upregulation of this eight-gene signature had worse 5-year progression-free intervals compared to patients with downregulation of these genes, and this was also valid for all cancer patients, irrespective of the diagnosed cancer type [42]. Follow-up of the study participants would potentially confirm the above findings by strengthening the identified correlation between the expression levels of these genes in the blood and clinical outcomes. In another study, we explored the impact of radiation therapy on the T-cell receptor (TCR) repertoire through NGS analysis of the variable beta chain (TCRβ) in the peripheral blood of patients with localized PCa [43]. Notable alterations in the TCRβ repertoire post-radiotherapy were identified; these need to be further correlated with the patients' clinical outcomes and reproduced in larger patient cohorts to confirm that radiation therapy can induce systemic immune responses. In another setup, we deployed the PCa patients' isolated PBMCs and identified that high frequencies of HER-2/neu(780–788)-specific CD8+ T lymphocytes after standard therapies were linked to lower levels of transforming growth factor beta (TGF-β) and interleukin 8 (IL-8) and, more importantly, they were associated with better progression-free survival (PFS) [44].

Similar experiments have been carried out with the samples derived from head and neck and NSCLC patients, and the results are currently being analyzed. In more detail, gene expression levels using the isolated RNA, as well as the TCR repertoire using the isolated DNA, were determined by NGS in the blood of patients with head and neck cancer at baseline and three months after receiving the appropriate treatments. Where available, DNA and RNA isolated from the corresponding patients' formalin-fixed paraffin-embedded (FFPE) blocks from surgically excised primary tumors were also analyzed. In addition, the levels of certain miRNAs in the patients' and healthy donors' plasma were determined to investigate their potential biomarker utility in this cancer type. In another experimental setting, an investigation into plasma-derived cell-free DNA (cfDNA) alterations after radio/chemotherapy in patients with head and neck cancer showcased high levels of cfDNA as a predictive biomarker of poor PFS [45]. Finally, preliminary findings from the analysis of NSCLC samples have demonstrated alterations in the composition of the peripheral TCR Vβ repertoire post-immunotherapy, while significant differences both in TCR clonotype abundance and composition are also detected between the periphery and the tumor of the participating patients. Overall, a thorough analysis of the above findings could potentially reveal novel dynamic biomarkers of disease course and patient's response to treatment modalities.

8. Conclusions

Biobanks constitute a cornerstone for promoting translational cancer research by securing the quality and reproducibility of the results and by adhering to standard laboratory methodological procedures and ethical guidelines. A correctly organized and functioning contemporary biobank should be capable of appropriately collecting, processing, and storing samples, thus securing their high quality and integrity. In turn, this will ensure traceability of the procedures involved up to the point of biosample storage. The exact knowledge of all pathways followed during the pre-analytical phase of a liquid- or tissue-derived biosample will establish reliable SOPs for appropriate biosample collection and handling. Inappropriate sampling, processing, and storage as well as inaccurate registration of experimental and clinical data will inevitably lead to irreproducible results causing drawbacks in translational cancer research. Hence, in addition to establishing reliable SOPs, contemporary biobanks should adhere to good laboratory practices and be assessed by external audits. The identification of disease-associated biomarkers, unique for each patient and ultimately resulting in individualized treatments, is largely dependent on high-quality biobanked samples from the respective patients, making their handling under strict SOPs and the assessment of integrity levels for the material of interest very decisive. The need for the detection of sensitive biomarkers functioning as surrogates for disease-status evaluation enabling a fast clinical screening during therapeutic approaches using novel drugs should

also be stressed. The successful sustainability of samples combined with modern "omics" technologies will allow the productive and early evaluation of disease progression during therapies. Here, we also present the setup and the sample characteristics of our Biobank at the St. Savas Cancer Hospital. A significant challenge we came across in the course of biobanking was complete demographic, clinical, and laboratory patients' data collection. Given that the provision of complete information regarding patients' details is the sole responsibility of the treating physicians, it is necessary to make health professionals aware that the proper functioning of the biobank also depends on the complete recording of the clinicopathological and demographic data of the patients. Additionally, it is within our priorities to integrate data collection for biobanking into patients' routine clinical care.

Author Contributions: Writing—original draft preparation, V.G., M.G. and C.N.B.; writing—review and editing, V.G., O.B. and C.N.B. All authors have read and agreed to the published version of the manuscript.

Funding: The Biobank at St. Savas Cancer Hospital is co-financed by Eurobank and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation under the call RESEARCH–CREATE–INNOVATE (Funder: EYDE ETAK; Funding number: 5069-29.10.2020; project codes: T1EDK-01404 (NEOVIOPRO); T2EDK-03266 (BIOKAKETRA); T2EDK-02218 (B-PREIMMUN)).

Data Availability Statement: Data are available on reasonable request.

Acknowledgments: We would like to acknowledge support from all collaborators participating in Biobanking at St. Savas Cancer Hospital.

Conflicts of Interest: The authors declare no conflict of interest.

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