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ORIGINAL ARTICLE

U-CAN: a prospective longitudinal collection of biomaterials and clinical information from adult cancer patients in Sweden


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ABSTRACT

Background: Progress in cancer biomarker discovery is dependent on access to high-quality biological materials and high-resolution clinical data from the same cases. To overcome current limitations, a systematic prospective longitudinal sampling of multidisciplinary clinical data, blood and tissue from cancer patients was therefore initiated in 2010 by Uppsala and Umeå Universities and involving their corresponding University Hospitals, which are referral centers for one third of the Swedish population.

Material and Methods: Patients with cancer of selected types who are treated at one of the participating hospitals are eligible for inclusion. The healthcare-integrated sampling scheme encompasses clinical data, questionnaires, blood, fresh frozen and formalin-fixed paraffin-embedded tissue specimens, diagnostic slides and radiology bioimaging data.

Results: In this ongoing effort, 12,265 patients with brain tumors, breast cancers, colorectal cancers, gynecological cancers, hematological malignancies, lung cancers, neuroendocrine tumors or prostate cancers have been included until the end of 2016. From the 6914 patients included during the first five years, 98% were sampled for blood at diagnosis, 83% had paraffin-embedded and 58% had fresh frozen tissues collected. For Uppsala County, 55% of all cancer patients were included in the cohort.

Conclusions: Close collaboration between participating hospitals and universities enabled prospective, longitudinal biobanking of blood and tissues and collection of multidisciplinary clinical data from cancer patients in the U-CAN cohort. Here, we summarize the first five years of operations, present U-CAN as a highly valuable cohort that will contribute to enhanced cancer research and describe the procedures to access samples and data.

Introduction

Structured and comprehensive programs for the sampling of clinical data, blood and tissue are instrumental for several areas of cancer research [1]. Large cancer cohorts are of major importance to increase our knowledge regarding disease mechanisms, to assess somatic mutations of clinical significance and to discover and validate biomarkers. Noteworthy, numerous biomarkers have been identified and suggested being of significance, but surprisingly few have eventually been validated and even fewer have made it to clinical use. One of the reasons why robust validated biomarkers are lacking is attributed to limited numbers of subjects included in discovery and validation cohorts and the lack of repeated biological sampling during the disease course. To be suitable substrates for biomarker discovery, biobanking programs need to fulfill multiple quality requirements, and as in many other

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situations, the weakest link determines their value. The requirements include a large number of unselected participants, systematic sampling of relevant clinical data, standardized sampling securing high quality of the material collected and, finally, sufficient capabilities for storage and handling of the sampled data and materials. Population-based cohorts and in-depth knowledge of patient selection linked to population metrics are fundamentally important. Further, since the properties of tumors change with tumor evolution and as a consequence of treatment, longitudinal collection of blood, tumor tissues and clinical information is of great significance. Because of improvements in cancer diagnostics and therapies, cancer cohorts become outdated after few decades and it is therefore important to have continuous longitudinal collection of blood, tumor tissues and clinical information to sustain high quality cancer research.

In a competitive effort to further strengthen research at Swedish universities, the Swedish Government awarded a Strategic Research Area (SRA) grant to U-CAN, the Uppsala-Umeå Comprehensive Cancer Consortium, led by Uppsala University and including Umeå University, Stockholm University, and KTH Royal Institute of Technology, to implement such a program. Importantly, the SRA grants were targeted to address the needs of society and the corporate sector. The core of the U-CAN proposal was to establish a longitudinal collection of data, blood and tissue samples from cancer patients using procedures standardized across different diagnoses. The aim was to obtain a high inclusion rate of cancer patients in U-CAN at two referral centers in two geographically distinct areas where control subjects were already continuously recruited to population-based cohorts through general health examinations. The initial effort encompassed brain tumors, colorectal cancers, hematological malignancies and prostate cancers. Due to the successful implementation, the program was extended to gynecological cancers, breast cancers, neuroendocrine tumors and most recently lung cancers. Here, we describe the structure and implementation of U-CAN along with its achievements and lessons learned from the first five years of operation.

Material and Methods

Aims

The aims of U-CAN are to generate an internationally competitive high-quality longitudinal biobank of selected cancer, to create a database of well-structured multidisciplinary clinical information on a large number of patients from defined geographical areas and to be a substrate for cutting edge translational and clinical cancer research.

Organization

The SRA U-CAN is a Swedish consortium with participation from Uppsala University, Umeå University, Stockholm University, and KTH Royal Institute of Technology. The core units of the program are Diagnosis Specific Expert Groups (DSEG) that include clinical and translational scientists with interests in a specific form of cancer (Figure 1). Members of these groups have the expertise needed to design and maintain sampling schemes and further decide on matters related to the use of samples. Importantly, the DSEGs harmonize the mode of action across the different participating hospitals. The heads of the DSEGs at each site, along with their local biobank, informatics representatives and heads of departments form two Executive Committees that monitor progress of the collection effort and solve challenges related to implementation in the healthcare systems. The Management Group, headed by the Program Director and composed of cancer researchers representing the participating universities, is in charge of strategic program decisions. The Program Director reports to the Program Council, consisting of representatives from the collaborating universities under the leadership of the Dean of the Faculty of Medicine at Uppsala University. The highest operative level is the Management Group, composed of the Program director, two representatives from Uppsala University, two representatives from Umeå University, and one representative from KTH Royal Institute of Technology or Stockholm University. The Diagnosis Specific Expert Groups (DSEGs) decide on how materials should be sampled and used for each respective diagnosis and are organized under two executive committees in Uppsala and Umeå with continuous collaboration.

Implementation

The first key to successful implementation of the U-CAN sample collection was the long-term collaboration established
between the participating hospitals with involved professionals, universities, county councils, biobanks, and regional cancer and biobank centers. In Uppsala and Umeå, long-term contracts governing the funding and staffing of the biobanking activities were negotiated between the universities and their corresponding county councils. Through these agreements, all blood biobanking was contracted to existing regional healthcare-integrated biobanks that facilitate large-scale collections of blood samples (Uppsala Biobank and Biobanken Norr in Umeå) and all tissue biobanking was contracted to the pathology departments at each site. Radiology bioimaging data were stored in the county councils picture archiving and communication systems (PACS). The second key to success was the establishment of well-functioning and dedicated diagnosis-specific workgroups, the DSEGs, that invested time to reach agreement on the sampling schedules, logistics and procedures before starting inclusion of patients. The work performed by these groups is essential as the many different clinical departments involved in caring for the cancer patient need to agree on the workflow for inclusion and longitudinal sample collection to achieve a successful implementation. For example, it is essential to map all patient entry points into the healthcare system at diagnosis and at later visits to ensure in-depth coverage of the sample collection. Although this process may take time to conclude, attempts to start inclusion prematurely are in our experience more likely to fail. For staffing, as the research nurses are crucial to reach high inclusion and successful longitudinal sampling, up to one full-time equivalent research nurse per diagnosis area and referral center has emerged as a suitable level across the surgery and oncology wards. To facilitate tissue banking, in addition to pathologists, pathology laboratory staff (2–4 full-time equivalent staff per referral center) at dissection, handling of bone marrow aspirates and biobank management was required.

**Ethics**

The study is conducted in accordance with the Declaration of Helsinki. U-CAN has ethical permission to prospectively collect clinical information and repeated blood and biopsy samples from patients with cancer. Written informed consent has been obtained from each subject and logged in databases and electronic patient records. The patients have actively been obtained from each subject and logged in databases and electronic patient records. The patients have actively consented to participate in cancer research in a broad sense, and current consent forms are available at the U-CAN website [2]. Each research project that uses material from the U-CAN cancer cohort needs an independent approval from the regional Ethical Review Board for the specific intended use.

**Patients**

The patients recruited to the U-CAN sample collection are adults diagnosed with any of the selected cancer types, living primarily in the counties of Uppsala and Västerbotten or that have been referred to the two university hospitals for diagnosis, treatment and/or follow-up from surrounding counties. The two counties from which the cancer patients are recruited have a total population of 606,593 (31 December 2013), whereas the participating university hospitals are referral centers for a population of 2.9 million, or one-third of the Swedish population. Cases of brain, gynecological, hematological and neuroendocrine malignancies in the U-CAN cohort also encompass cases referred from the larger healthcare regions whereas breast, colorectal and prostate cancers are primarily from Uppsala and Västerbotten counties. The sole selection criterion for inclusion of a patient is age over 18 years at diagnosis. Case recruitment began in five diagnoses in 2010 (Table 1) and four additional diagnoses have later commenced inclusion.

**Blood sampling**

The DSEGs defined the time points for longitudinal blood sampling for each diagnosis based on their collective knowledge and scheduled visits to the clinic according to current standard of care (Supplementary Figures 1–9). Through collaboration with Uppsala Biobank and Biobanken Norr, centralized healthcare-integrated banking of blood was established at the respective Departments of Clinical Chemistry in Uppsala and Umeå. Integration with a Laboratory Information Management System (LIMS) and the electronic medical record system has enabled the research nurses to order project-defined blood biobanking through the general patient record system. The target time from patient to frozen aliquots was 2 h (max 4 h) and the actual time and sampling conditions were recorded for each sample. Preanalytical parameters were automatically recorded according to SPREC 2.0 [3] in the LIMS. The baseline sample package included EDTA plasma, serum, citrate plasma (until December 2014), and whole blood for DNA preparation (Table 2). Baseline sampling at inclusion was typically performed at the first visit to the hospital, before any therapeutic action had been initiated. From 1 January 2015, additional EDTA plasma for ctDNA preparation has been collected at all sampling occasions. The blood fractions are stored frozen in 2D-barcoded 0.5 ml microvials (Micronic, Lelystad, the Netherlands) at −80 °C.

**Tissue sampling**

Tissue from suspected tumor was obtained from surgical specimens, needle biopsies or bone marrow aspirates in case of hematologic disorders. During working hours (Monday to Friday 8 AM to 4 PM), the fresh surgical specimens are transported to the Pathology Department on ice. Dedicated pathologists have selected tissue to be frozen or to be embedded in paraffin (Histowax), sampling both tumor and macroscopically normal tissue when possible. In Uppsala, tumor and normal tissue samples were embedded in Tissue-Tek OCT compound before freezing. This handling procedure results in tumor DNA and RNA of high quality [4]. A section was produced from each frozen sample and stained with hematoxylin–eosin. These stained sections were assessed regarding size, occurrence of normal tissue, neoplasm (including tumor cell content) and necrosis. The purpose was...
to enable rapid selection of cases, that is, to ensure that the frozen specimens contained representative tumor or normal tissue. In Umeå, tumor tissues, and for some diagnoses also normal adjacent tissues, were frozen in pieces and stored in tubes. For hematological malignancies, cells were collected by Ficoll separation and vital freezing in liquid nitrogen gas phase. Bone marrow aspirates from patients with myeloma underwent magnetic bead cell sorting to enrich for CD138$^+$ plasma cells out of which a fraction was used for FISH analyses upfront and the remaining CD138$^+$ cells biobanked. Samples of lymphoma tissue in Umeå were sorted with magnetic beads to enrich for CD19$^+$ B-cells and CD3$^+$ T-cells.

**Tissue arrays**

Tissue microarrays (TMAs) are generated from formalin-fixed paraffin-embedded (FFPE) tissue blocks upon request from researchers. Such TMAs have thus far been generated for colorectal- and prostate cancer within U-CAN and TMAs for gynecological and hematological malignancies are pending. The TMAs are constructed at the Swedish Science for Life Laboratory (SciLifeLab) facilities at Uppsala University, following the standard procedures established within The Human Protein Atlas project [5,6]. In brief, representative areas to sample are assessed by a pathologist, and whenever possible two cores of 1.0 mm diameter (duplicate samples) are obtained from each donor block and assembled in an array format (10 × 12 cores) into a recipient TMA block from which sections are cut and delivered to the researcher for analysis.

**DNA**

Constitutional DNA was obtained from EDTA whole blood from patients with solid tumors, lymphoma and myeloma, and from additional normal tissues and saliva samples for patients with other hematological malignancies. Constitutional DNA from blood is currently under preparation for all cases using the MACHEREY-NAGEL NA Extraction kit on a Hamilton Microlab STARlet platform and is stored at a concentration of $50–100$ ng/μl in 96-well plates to facilitate easy access for projects. Saliva DNA was collected using the Oragene DISCOVER (OGR-500) sampling kit. Circulating tumor DNA (ctDNA) was prepared from EDTA plasma using the QiAamp Circulating Nucleic Acid Kit (Qiagen).

**Patient data**

The collection of clinical data in U-CAN is complementary to the mandatory reporting to the Swedish Cancer Registry and the respective Swedish quality registries for each cancer diagnosis [7]. The quality registries constitute a national resource not only for the evaluation of the quality of care but also for clinical and epidemiological research. The quality registries contain diagnostic and therapeutic information of the primary tumor and information on recurrences and their treatment. The information is collected by the specialists involved in the diagnosis and therapy of the respective diagnoses, and specific monitors at the Regional Cancer Centers validate the data prior to inclusion in the INCA database.
(National IT platform for cancer quality registries). Based upon a U-CAN initiative, a national infrastructure for structured and automated collection of all radiation therapy has been created (Medical Information Quality Archive, MIQA) and implemented as a clinical quality registry in INCA [8]. Bioimaging data are collected and stored in the county councils’ PACSs. Additional variables apart from the data in the quality registries are retrospectively collected from the patient records by the DSEGs. Standardized questionnaires were developed for family history and comorbidity, collected regardless of diagnosis, scanned for electronic data capture and manually curated.

**Control subjects**

Samples from healthy control subjects are not collected routinely in the U-CAN cohort. However, a set of ~300 individuals included in U-CAN during this time period were later found not to have cancer and can hence serve as non-fasting references, such as for the assessment of pre-analytical handling procedures. Independent population-based epidemiological cohorts in Uppsala and Västerbotten counties are suitable sources for control subjects to the U-CAN cases. The EpIHealth project collected fasting blood samples (EDTA plasma, serum, and whole blood for DNA preparation) from ~13,000 individuals in Uppsala county aged 45–75 during 2011–2015 [9]. Similarly, U-CAN in Umeå uses the same biobanking infrastructure as the Västerbotten Intervention Program (VIP), that has an ongoing recruitment and samples individuals in Västerbotten county longitudinally at age 40, 50 and 60 years [10]. Available sample types in the VIP include EDTA plasma, heparin plasma, buffy coat and erythrocytes, all from fasting blood samples. As of September 2015, 12,285 of the 115,000 individuals (10.7%) in the VIP had developed a cancer.

**Process for use of materials**

Research on U-CAN materials is always performed in collaboration with U-CAN affiliated researchers, through which the collections are available to academic investigators and corporate entities in Sweden and abroad. Applications for access to U-CAN materials are processed by U-CANs program office and the respective biobank that holds the material (Figure 2). Aiming to approve applications of high quality with well-defined scope and hypotheses, the DSEGs assess the project based on innovative aspects, scientific quality, statistical power, optimized usage of sample materials, and nonconflict with other ongoing efforts. The application procedure is described in detail at the U-CAN website [2].

### Results

#### Performance

A total of 6914 cases were included until 31 December 2014 (Table 1), which extended to 8327 the following year and reached 12,265 at the end of 2016. In 2012, case recruitment commenced in selected tumor types also at county hospitals in the wider Uppsala-Örebro Healthcare Region. Several metrics to determine the value of longitudinal cancer biobanks can be envisioned, such as per-case achievement of collection goals and the fraction included of all eligible patients. From essentially all U-CAN patients (98%), a blood sample package is available at inclusion, typically drawn before any therapeutic intervention (Table 1). The blood sampling procedure enabled tracking of the time from sampling to central aliquoting and freezing at the clinical chemistry departments. For the Uppsala patients, 41% of blood samples were frozen within 2 h and 87% within 4 h of sampling. Certain molecular analyses demand fresh frozen tissue materials. From all cases with diagnostic FFPE materials (83% of included patients), fresh frozen tissues were available from more than two-thirds. Subjects with complete sampling of surgical specimens, complete clinical data and longitudinal blood samples pre- and post-treatment are of particular high value for biomarker discovery. Here, blood samples at inclusion and follow-up, fresh frozen tumor tissue and data in the regional tumor registries were obtained from one-quarter of the total included cases. Current summary statistics for case inclusion and follow-up blood samples are available at the U-CAN website [2]. In 2014, the across-diagnosis overall coverage of U-CAN in Uppsala County was 55% of incident cases reported to the tumor registry, with considerable variation between diagnoses but with relative stability over time (Supplementary Figure 10). A similar pattern of stability within different diagnoses is noted also in the frequency of patients included in U-CAN in Umeå (Supplementary Figure 11). It is a challenging task to precisely

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**Table 2. Overview of the U-CAN collections.**

<table>
<thead>
<tr>
<th>Sample types obtained with informed consent</th>
<th>Type or source of material (amount)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCAN1 at diagnosis</td>
<td>EDTA whole blood&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>UCAN2 at follow-ups</td>
<td>EDTA plasma&lt;sup&gt;b&lt;/sup&gt; (8 × 225 μl)</td>
</tr>
<tr>
<td>Diagnostic FFPE samples</td>
<td>EDTA plasma (8 × 225 μl)</td>
</tr>
<tr>
<td>Fresh frozen samples</td>
<td>Normal tissue (~1 cm&lt;sup&gt;3&lt;/sup&gt;)</td>
</tr>
<tr>
<td>DNA</td>
<td>Tumor tissue</td>
</tr>
<tr>
<td>Surveys</td>
<td>Whole blood</td>
</tr>
<tr>
<td>Surveys</td>
<td>Heredity</td>
</tr>
<tr>
<td>Surveys</td>
<td>Health</td>
</tr>
</tbody>
</table>

<sup>a</sup>All U-CAN patients have an informed consent digitized in the patient record system, an initial blood package drawn at inclusion and longitudinal blood sampling at time points defined by the respective DSEGs. When possible, tumor tissues are obtained as surgical specimens, needle biopsies or bone marrow aspirates. Tissue samples are fixed and embedded in paraffin or fresh frozen. FFPE samples can be used for construction of tissue microarray slides upon request. Standardized questionnaires of heredity and comorbidity are collected.

<sup>b</sup>Saliva is collected from leukemia patients for normal DNA extraction.

<sup>c</sup>Until December 2014, citrate plasma (4 × 225 μl) was also collected.

**FFPE**: formalin-fixed paraffin embedded.
define the coverage for all tumor types in U-CAN, but the coverage variations observed between the diagnoses reflect the complex nature of cancer care, the logistics planning of the diagnosis workgroups and the degree of long-term commitment of the community of clinicians and research nurses active in a specific diagnosis. Lag times in quality registry reporting also impact coverage metrics.

**Evaluation**

During 2014, the higher education institutions responsible for the SRAs were evaluated by an expert panel, consisting of internationally renowned scientists with vast experience of research management and research and innovation policy assessment, appointed by the Swedish Agency for Innovation Systems (VINNOVA) on behalf of the Swedish Government. U-CAN received the highest rating in the evaluated categories: performance, strategy, added value and research output, with the remark that the biobank platform provides a strategic basis for research growth supporting an attractive area of research [11].

**Ongoing and planned research**

Most diagnoses have been collected for an extended period of time with regards to both longitudinal sample collection and follow-up times. Hence, the U-CAN material is becoming suitable for addressing advanced research questions and has consequently attracted increased attention. This is evident when summarizing the approved project applications from the last three years: three applications in 2014, six in 2015 and 18 in 2016. At present, 1–3 project applications per month are handled, with the vast majority approved as is or after minor modifications. The bulk of projects applied for between 2010 and 2016 were related to colorectal cancer (40%), hematological malignancies (28%), prostate cancer and brain tumors (10% each). The first study based on sample materials from U-CAN patients was recently published [12]. The many ongoing research initiatives include molecular subclassification of the material, discovery and validation of prognostic and predictive factors and validation of new biomarkers.

**Discussion**

The value of systematic biobanking for cancer research has long been recognized, and similar initiatives to U-CAN exist. Prominent examples include the Victorian Cancer Biobank in Australia, the Wales Cancer Bank (WCB) and several diagnosis-specific initiatives in the Netherlands. The Victorian Cancer Biobank encompassed 27 hospitals in Victoria, Australia during 2006–2013 and operated with a similar approach as U-
The U-CAN cancer cohort covers the majority of cases in two geographically defined populations, Uppsala and Västerbotten Counties, in addition to patients referred from adjacent areas, and is thus population-based with known and limited patient selection. Observational cohorts like U-CAN can overcome many of the obstacles seen in the highly selected cohorts based on clinical trials. The personal identity number unique to all Swedish citizens also enables complete follow-up, including linkage to other national or regional databases such as the registry for cause of death. We therefore believe that this cohort will be of great value in clinical research and for cancer biomarker discovery and validation efforts. As an example, the collection of blood and tissues prior to any treatment, the complete nationwide storage of all radiation therapy details and the possibilities to link with health care registries in Sweden will permit analyzes of patient susceptibilities for late radiation-induced toxicities not seen until after decades.

Challenges

As with all major logistic ventures a number of challenges have been associated with the U-CAN collection. A concern often raised concerning the tissue collection is the lack of tissue availability for research after it has been used for its primary diagnostic purpose. Often, small resected tumor samples or needle biopsies from, for example, lymphoma or bronchoscopy do not allow for frozen material to be reserved for research. A similar situation is found for cell biobanking where malignant cells sometimes cannot be set aside for research after sorting. In addition, FFPE material stored in the pathology archives are diagnostic samples per definition and must therefore remain available and intact enough to allow for further and future diagnostic use, which constrains the use of such material for research. These factors influence the availability of tissues for research and also affect the total collection of material (e.g., frozen samples are only available for ~60% of the individuals included in U-CAN 2010–2014). For certain diagnoses, the issue of limited tissue sample availability is more pronounced than others due to, for example, preoperative radiation therapy or the clinical protocols used to obtain biopsies.

Distributed biobanking programs inevitably also have some site-specific features. In U-CAN, these are mainly reflected in minor differences in the sample handling pipelines. These differences between the two sites can also be considered as strengths; while OCT-embedded frozen tissues in Uppsala can be sectioned and used for in situ analyses that require cell-type resolution, the frozen tissue pieces in Umeå are readily useful for mass-spectrometry-based proteomic and metabolomic analyses.

Conclusions and outlook

With the U-CAN cancer cohort, we have demonstrated the feasibility of prospective, longitudinal sampling of multidisciplinary clinical data and biobanking of blood and tissues from cancer patients with a high inclusion rate. The separation of stakeholders from the biobanking processes, which were operated by dedicated entities within the healthcare system, has been successful due to the key role of the DSEGs in the decision-making process. Along with quality registries and other independent population-based cohorts collected in parallel within the same geographic areas, the U-CAN cancer cohort constitutes a comprehensive and highly valuable resource for biomarker discovery and validation. In addition to prognostic and predictive biomarker development, a particularly important application of this standardized collection scheme is discovery and validation of blood biomarkers for the early detection of cancers. This should be facilitated by the availability of samples from eight different cancer diagnoses collected in the same logistic pipeline with careful preanalytic annotation, which is likely to simplify evaluation of tumor-type specificity for such biomarkers [18].

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Disclosure statement

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