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PERSPECTIVE

Post-mortem tissue donation programs as platforms to accelerate cancer research

Matthew Dankner[1]*, Badia Issa-Chergui[2,4] and Nathaniel Bouganim[3,4]

[1]*Goodman Cancer Research Centre, McGill University, Quebec, Canada*
[2]*Department of Pathology, McGill University, Quebec, Canada*
[3]*Division of Oncology, McGill University, Quebec, Canada*
[4]*McGill University Health Centre, McGill University, Quebec, Canada*

**Correspondence: Matthew Dankner, Goodman Cancer Research Centre, McGill University, 1160 Pine Avenue West, Room 508, Montréal, Québec H3A 1A3, Canada. E-mail: matthew.dankner@mail.mcgill.ca*

Abstract

Given recent advances in the treatment of cancer, patients are surviving longer but frequently develop treatment-resistant and inoperable metastases. Biomedical research has advanced to the stage where in-depth study of these lesions is feasible, with the goal of further refining our understanding of metastatic dissemination, therapeutic resistance and inoperable tumors. However, there is a lack of tissue specimens derived from multiple metastatic sites within the same patient that would permit the study of these processes. Furthermore, patients with rapidly progressing or metastatic disease are rarely candidates for surgery, making those most in need of innovation and discovery extremely difficult to study. For this reason, post-mortem tissue donation programs are an approach that is quickly gaining traction in the cancer research community. Herein, we discuss what post-mortem tissue donation entails, attitudes towards these procedures, and highlight important studies already utilizing these resources. In addition, we propose future directions for use of this tissue that can directly improve clinical management of advanced cancer patients.

Keywords: rapid autopsy; rapid tissue donation; post-mortem tissue donation; cancer research

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Introduction

Cancer therapeutics are being developed at a rapid pace, with a plethora of novel targeted and immunotherapeutic agents gaining approval in the past several years [1]. Despite these improved treatments, patients' tumors often recur at distant sites many months or years after treatment. At this stage, patients are left with treatment options that are often based on molecular information derived from their primary tumor due to a lack of access to metastatic tissue samples. A body of evidence is beginning to suggest that actionable targets are frequently missed when a primary tumor is used as a proxy for treating metastases [2–4].

Surgical and biopsy specimens are the standard used by researchers to study the metastatic process and therapeutic resistance in humans. However, these specimens are often available only from a single site despite the presence of numerous metastases that are anatomically and temporally spaced. Importantly, this research paradigm selects for patients with favorable outcomes given that they are surgical candidates with limited extent of disease and adequate performance status. For example, in pancreatic adenocarcinoma, only 15–20% of patients present with resectable disease, and patients with resectable disease exhibit improved outcomes [5]. It is evident that the patients most in need of research are those who present with or develop rapidly progressing metastatic disease and are not surgical candidates, meaning no tissue is available for study. In addition, these specimens are often procured before therapy, preventing the study of recurrent disease in many instances. For this reason, a small but actively growing number of researchers have developed rapid tissue donation programs with the express purpose of obtaining tissue from

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patients with metastatic cancer after death. Such programs in combination with conventional tissue collection techniques represent the optimal paradigm for obtaining and characterizing inoperable tumors and numerous metastases from the same patient in order to understand how a patient's disease spread and evolved in response to treatment.

What is a post-mortem rapid tissue donation program?

Post-mortem rapid tissue donation programs, also called rapid autopsy or research autopsy programs (used interchangeably throughout this text), describe procedures specifically designed to retrieve live tissue from patients immediately after death [6]. To date, there are approximately 10 centers that have published studies performing these procedures on patients with metastatic cancer in North America, although there is knowledge of several other programs that have been initiated more recently. Many of these centers' programs focus on one specific cancer type and organ site [7], while some institutions have established cancer-wide programs [8]. The need to prioritize specific disease sites reflects the considerable commitment and effort required to establish such protocols, particularly in obtaining ethics approval and committed funds for these programs. However, emerging technologies and clinically relevant research questions make tissue donation programs worthwhile ventures for many centers. In this section, we outline the rapid tissue donation procedure and discuss logistical issues.

The structure underlying each institution's post-mortem tissue donation program differs based on local culture, laws, and institutional practice [6]. However, general similarities apply to all programs. There are very real logistical considerations that are necessary to ensure that patients who consent to donating their tissues actually make it to the autopsy table. This involves having the infrastructure in place to organize the transport of donors from unpredictable locations such as their home, hospice or from another treatment center. In addition, coordination between the transport team and on-call pathologists and technicians is required to assure that the procedure can begin promptly after the arrival of the body [9]. In cases where the procedure cannot begin immediately after death of the donor, the body can be transferred to the morgue where it will be kept refrigerated until the procedure is ready to begin. All of these logistical aspects require oversight and come with significant associated costs, requiring dedicated funding for rapid tissue donation projects.

The maximum amount of time that is acceptable for initiation of a research autopsy remains unclear and is an open question that requires further investigation. There is a general misconception about the relationship between post-mortem interval before tissue is collected and the quality of the tissue [6]. A recent study explored nucleic acid quality in frozen samples obtained between 2 and >36 h after death, demonstrating that while RNA in normal tissues degrades in a time-dependent fashion, RNA quality is highly variable in tumor-derived tissue irrespective of post-mortem interval [10]. An examples of a case whereby a patient-derived xenograft model was successfully engrafted despite tissue being collected >48 h after death makes clear that cancer cells can remain viable long after the death of the donor [6]. Together, this demonstrates that it is worthwhile to proceed with tissue donation in all consenting patients, even if the pathology team is not immediately prepared to perform the procedure. It is likely that analysis of the quality of the DNA and RNA will be determined to be inadequate in some instances but, because of the high degree of interpatient and intrapatient variability between tissue specimens, something can likely be learnt from some of the tissues collected in all cases with the right research questions. Important future directions in this area require rigorous studies that assess the quality of tissues in patients with different post-mortem intervals that may further enlighten these decisions. Such studies may reveal important changes that occur in metabolic and immunological features of tumors and normal tissues in the post-mortem period. In addition, such studies would provide an opportunity to identify potential predictors of tissue quality for a given post-mortem interval, such as fever, sepsis, and blood oxygen saturation before death, which may be taken into consideration when designing research approaches using post-mortem tissue specimens.

Prior to death, patients and their next-of-kin are consented to be a part of a tissue donation program by their treating physician (Figure 1A,B). In many instances, this is the patient's medical oncologist or palliative care physician, but can also be a general internist, radiation oncologist or surgeon. Most groups engaged in tissue donation programs have patients consented by a medical oncologist when it is felt that the patient has accepted their prognosis and would be open to such a discussion. It is important that these discussions are held ethically [11], and in a manner that allows the patient to consent altruistically and not out of fear of receiving inferior care or disappointing

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their physician [12]. It should be noted that the specifics of consent for these procedures vary by jurisdiction due to differences in laws between sites.

After a mourning period for the family, the body is transported to the pathology suite where a pathologist and autopsy technicians perform a variation of a full autopsy, removing only organs of interest in most cases. The pathologist will use the most recent scans and records of clinical signs/symptoms of the deceased to identify organs that may contain new lesions that developed since the last scan. During the procedure, the pathology team excises lesions from a variety of sites, preserving them with a variety of methods such as flash freezing in liquid nitrogen or fixation in formalin (Figure 2). If this procedure is performed under sterile conditions using autoclaved tools and extreme care is taken to prevent contamination of the tissue, *in vitro* and *in vivo* models may be more easily established from live tissue, but utility can also be extracted from tissues isolated under normal autopsy conditions. In addition, a small amount of normal tissue and fluids such as blood or cerebrospinal fluid can be retrieved from the patient for complementary analysis. After completing the autopsy, all incisions are sutured. The body is then transported as directed by the next-of-kin, with open casket funerals remaining a possibility in all cases. Following the autopsy, members of the research team immediately begin processing the acquired clinical specimens. In a case where patient-derived xenograft models will be established, this involves engrafting immunocompromised mice with the patient's tissue. Formalin fixed tissue is processed for histological analysis, and frozen tissue is processed for DNA and/or other downstream analyses such as RNA-Seq. Specimens derived from rapid tissue donation programs may be more likely to be amenable for further analyses that require large quantities of tissue than specimens derived from conventional approaches due to full availability of tissue for research purposes. This may facilitate new approaches such as immunopeptidomics [13].

Attitudes towards rapid tissue donation

Given the invasive nature of this procedure, clinicians may be disinclined to consent patients to tissue donation programs. However, several lines of evidence suggest that patients respond favorably to discussions about tissue donation. One recent study reports that, among patients approached to join a post-mortem

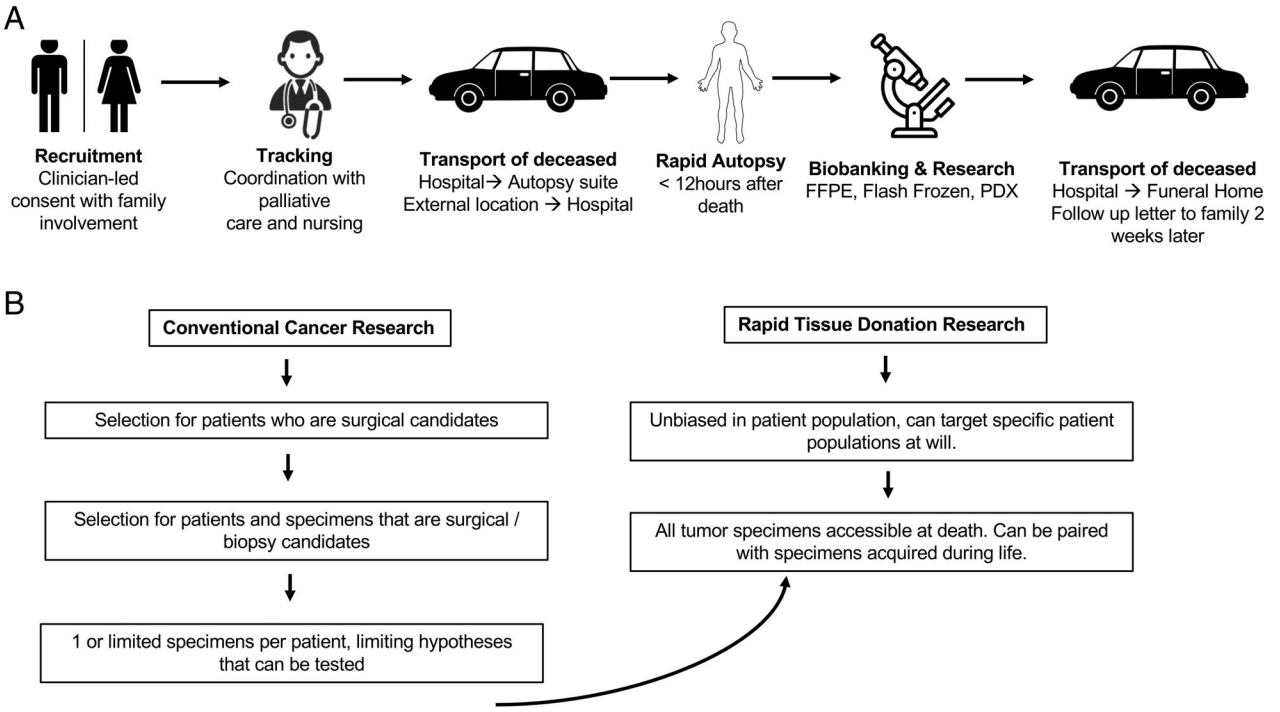

Figure 1. The logistical considerations of a rapid tissue donation program. (A) The step-by-step process of performing a rapid tissue donation procedure. (B) The interplay between conventional cancer research and rapid tissue donation programs.

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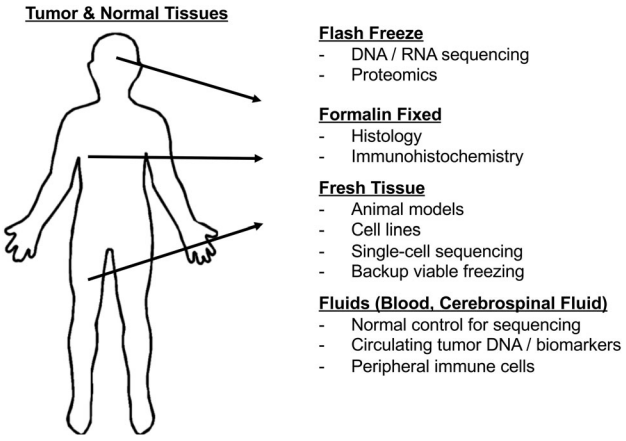

Figure 2. Biobanking tissues from post-mortem tissue donation procedures. Tissue taken from tumor and normal tissues can be stored in a number of different ways (flash freezing, formalin fixation, fresh tissue) to allow different experimental pipelines to be performed. Fluids, such as blood, urine or cerebrospinal fluid can also be collected and biobanked.

tissue donation program, 93% expressed interest, with the remaining patients citing impact on family members or religious concerns as reasons for declining [9]. Of the 37 patients expressing interest, 32 ultimately gave consent, with the remaining 5 having rapid clinical deterioration precluding participation. Another study surveyed patients with metastatic breast cancer and affirmed these results, revealing that 87% were willing to donate tissue at death [14]. Surprisingly, the majority of these patients believed the preferred time for doctors to begin discussion about tissue donation should be during the early stages of their disease [14]. Together, it is clear that patients are open minded about the idea of rapid tissue donation and require education programs to inform them on all aspects related to the procedure [15].

In contrast with the positive attitudes expressed by patients towards tissue donation after death, health care professionals are less inclined towards participation. In one study that explored healthcare providers' knowledge and attitudes about rapid tissue donation, the majority were either uncomfortable (17.8%) or unsure if they felt comfortable (42.2%) discussing rapid tissue donation with cancer patients [16]. This point was further emphasized by another study demonstrating that, while 93% of parents of deceased pediatric cancer patients would have consented to a research autopsy, the most significant barriers to conducting such autopsies were physicians' reluctance to ask and their lack of awareness about such programs [17]. Recent work from our group further revealed this disconnect between healthcare professionals and patients [18]. While surveyed patients were very enthusiastic towards rapid tissue donation, healthcare professionals, while also enthusiastic, expressed greater concern than the patient population as a result of a lack of education about rapid tissue donation. This makes clear the strong need to include the health care team in educational programs to provide logistic details and promote the importance of such initiatives.

Together, existing evidence suggests that patients are open to discussing rapid tissue donation with their physicians. It is likely that specific training programs, educational rounds and information sharing campaigns are necessary to educate clinicians on the benefits of such programs as well as methods of consenting patients in an ethical manner. Clinicians can then in turn educate their patients about the details underlying the procedure and the significant research potential that rapid tissue donation presents for improving the care of future patients [19].

Research opportunities

Given the tremendous challenges in establishing functional rapid tissue donation programs, the benefit to future patients must be worth the effort involved. Fortunately, we are at a time in the evolution of cancer research whereby such tissue is more valuable than ever. With existing capability to perform bulk and single-cell DNA and RNA sequencing, as well as the improvement of mouse xenografting techniques, in-depth genetic and functional analysis of patient material is now possible [8,20,21]. This is particularly relevant in the current treatment setting, where new therapeutic agents are continually gaining approval, and the growing problem of therapeutic resistance and adverse effects on healthy tissue has yet to be fully elucidated. In this section, we highlight the questions answered by studies that have been performed using rapid autopsy tissue and discuss novel avenues that can be explored prospectively.

Unraveling the mechanisms of therapeutic resistance, heterogeneity and tumor evolution

With an influx of new therapeutic agents, particularly immunotherapeutics, medical oncologists have a broader arsenal of drugs at their disposal than ever before. While this allows patients to experience longer progression free- and overall survival, resistance to treatment occurs in many cases [22]. Since tumors are

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under constant evolutionary pressure guided by the host immune system, the tumor microenvironment, and host exposures such as therapies, tumor evolution and heterogeneity allow for resistance to develop [23]. Rapid tissue donation programs represent valuable platforms for studying therapeutic resistance, heterogeneity and tumor evolution because they offer the opportunity to sample multiple regions of the same tumor, different tumors, and biological fluids in the same patient, and can be compared with previously collected specimens.

While a multitude of resistance mechanisms have been uncovered using biopsies and surgically resected material, several studies have begun employing post-mortem tissue to study these mechanisms and identify potential therapeutic avenues to overcome therapeutic resistance. These studies have led to improved understanding of therapeutic resistance to FGFR inhibitors in cholangiocarcinoma [24], PI3K inhibitors in breast cancer [25], afatinib in trastuzumab-resistant *HER2* amplified esophagogastric cancer [26], endocrine-resistant breast cancer [27], PD1-inhibitor resistant melanoma [28] and chemotherapy resistant urothelial carcinoma [29]. These studies together used sequencing approaches to compare pre-treatment specimens with post-mortem treatment resistant tissues and identified novel molecular mediators of resistance.

Tumor evolution has been studied using autopsy specimens in the context of metastases from prostate and breast cancer, revealing that DNA hypermethylation is more similar between metastatic sites in the same patient than between different patients [30–32]. Furthermore, in untreated patients, metastases originating from different primary sites express nearly identical driver mutations and targetable features to their matched primary tumor [33,34].

Such studies would not have been possible without rapid tissue donation and emphasizes the importance of these programs. As costs for sequencing and analysis drop, such approaches can be applied more broadly and in a large enough cohort of patients to reveal population level trends in tumor evolution in the context of a particular therapeutic. In addition, immunological analyses such as cytokine profiling and immunopeptidomics can be performed to refine our understanding of resistance to immune checkpoint blockade and other emerging treatment modalities after controlling for tissue quality in the post-mortem interval. Studies that investigate immune function in autopsy specimens, while valuable for preliminary understanding of an entity [35], must be corroborated with freshly acquired tissues. Single-cell RNA sequencing is an emerging technology that can also be powerfully applied to post-mortem tissues and allow us to better understand tumor heterogeneity and evolution [36].

To this effect, we encourage collaboration between industry and academia through rapid tissue donation programs by linking them to clinical trials *via* a unique informed consent process. Even if only a subset of patients in a trial agree to rapid tissue donation and ultimately donate tissue *post mortem*, this allows for the study of carefully monitored patients with matched pre-treatment and post-treatment biobanked samples. This would provide controlled and well annotated datasets which can act as valuable platforms for transformative research.

Understanding metastatic organotropism, the contribution of the metastatic microenvironment, and dormancy

Metastasis, the process whereby tumor cells escape the primary site to colonize distant organs, accounts for over 90% of deaths from solid cancers [37]. Therefore, understanding functional mediators of this process that promote the various steps of the metastatic cascade is of crucial importance [38]. Having samples from metastases from multiple distinct sites from the same patient, repeated across many patients with both similar and different tumor types and treatments, is a tool that has the potential to change the paradigm of metastasis research (Figure 3A). Recent examples can be found in the context of pancreatic cancer, with two studies exploring metastases from multiple sites and multiple patients using next generation sequencing approaches [34,39]. While several other studies using rapid tissue donation programs have been published in breast, prostate and pancreatic cancer patients [40–47], it is evident that this is a field in its infancy that has not yet been explored using the breadth of tissue that may be possible to include in datasets, nor the new techniques established by emerging technologies.

Prior to the advent of using rapid autopsy tissue to study metastasis, exploration of candidate genes thought to be involved in metastatic organotropism began with mouse models and was validated using human material [48,49]. Obtaining post-mortem tissue donation samples allows the identification of reliable gene or pathway candidates that can then be validated and functionally investigated using mouse and cell models. This inversion of the paradigm researchers apply to study metastasis would rapidly hasten our understanding of this process in the context of human disease in an unbiased, high throughput and expedited fashion.

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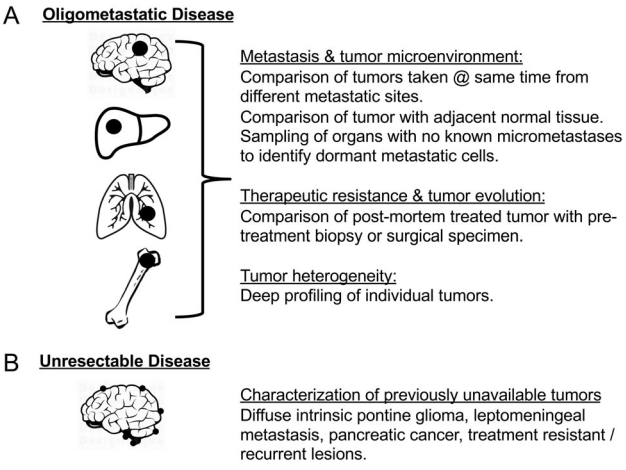

Figure 3. Applications of post-mortem tissue donation specimens. (A) In the context of patients with oligometastatic disease, specimens can be collected from the tumor and normal tissues to interrogate the metastatic cascade, microenvironment, tumor evolution, therapeutic resistance, and heterogeneity. (B) Unresectable tumor specimens, such as diffuse intrinsic pontine glioma or disseminated leptomeningeal metastases can be isolated and studied in the post-mortem setting, whereas this would not be possible using live tissue donors.

Molecular characterization of previously inaccessible tissue

Many tumors are inaccessible to biopsy or surgical resection. In tumor types that can be accessed in only a fraction of cases due to rapid clinical deterioration or anatomic location, there is a clear selection bias in the types of cancer that researchers can study. This is particularly important given that tumors not readily surgically resectable are, in fact, the ones that require the most in-depth study in order to develop alternative treatments (Figure 3B). It has already been demonstrated that unresectable primary renal cell carcinomas (RCC) can be sampled in the context of a post-mortem tissue donation program to yield valuable information relevant to understanding the aggressiveness of the patient's disease [50]. It is a distinct possibility that tissue obtained through rapid autopsies for primary brain tumors and central nervous system metastases can reveal insights not possible with surgically resected specimens. This is evident through a series of recent papers studying diffuse intrinsic pontine glioma [51–56], a tumor type that cannot be surgically excised due to its anatomic localization in the brain stem and diffuse infiltrative growth pattern [57]. Researching understudied entities such as these has the potential to lead to important discoveries leading to improved patient outcomes in a short time.

In addition to inoperable end-stage tumors, precancerous disease can also be studied using post-mortem tissue. An exemplar of this is a study that compared precursor pancreatic lesions with cancerous lesions, demonstrating that preneoplastic cells migrate through the pancreatic duct to form neoplasia [58]. Prospectively, other pre-cancerous lesions that are not routinely surgically resected can be studied with post-mortem specimens, including ductal carcinoma *in situ* of the breast. This can also extend to improving our understanding of cancer cell dormancy in the metastatic niche, a phenomenon whereby small deposits of metastatic cells can exist within an organ but do not actively proliferate to establish micrometastases for a variable length of time [59].

Conclusions

Rapid post-mortem tissue donation has become more prominent in recent years, which is evident by an increase in the number of medical/research centers performing this procedure and the papers now being published using these tissues. While patients are open to the idea of rapid tissue donation, the healthcare team is often uncomfortable discussing the possibility with patients. These barriers can be overcome through the establishment of educational programs for health care professionals that outline the potential gains such programs present, as well as the appropriate methods that should be followed to consent patients. As the research community continues to develop tools to study a high volume of specimens with precision and low cost, the immense potential of rapid tissue donation can be fully realized (Figure 1B). It is the strong conviction of stakeholders in rapid tissue donation programs that such initiatives guided by close collaboration between patients, the healthcare team and researchers will ultimately lead to major discoveries leading to improved outcomes for patients with cancer.

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Author contributions statement

MD drafted the text. BIC and NB contributed intellectual discussion and feedback on the text.

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