

Building a Rapid Autopsy Program – A Step-by-Step Logistics Guide

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Protocol

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Building a Rapid Autopsy Program – A Step-by-Step Logistics Guide

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Abstract

Background: Rapid Autopsy Programs offer an opportunity to collect tissue from patients immediately after death, providing critical biological material necessary to develop more effective therapies and improve patient outcomes. Here, we present a step-by-step guide to build a cancer-focused Rapid Autopsy Program, based on our own experiences building “The Legacy Project” at the City of Hope Comprehensive Cancer Center.

Methods: The linear timeline of events is separated into four phases: 1) Building the Infrastructure, 2) Recruiting and Consenting, 3) Preparing for Death, and 4) Tissue Collection and Follow up. Important considerations and methods for adaptation are discussed throughout the protocol.

Discussion: Using these methods, we successfully collected a total of 533 specimens from 9 subjects. The average time from death to last specimen acquisition was 6.1 hours (range: 4.03 – 7.66 hours; median: 5.71 hours). A diverse team with various areas of expertise is critical for successful program implementation. Our goal herein this protocol is to provide a comprehensive framework and foundation for other institutions to use as a model.

Keywords

Rapid Autopsy Program

Warm Autopsy

Rapid Tissue Donation

Cancer Research

Metastatic

Tumor Heterogeneity

Background

Metastatic tumor heterogeneity presents a significant challenge in the clinic and is believed to drive mixed and differential responses to therapy. Better understanding of the origins of metastatic heterogeneity and tumor evolution under the pressure of therapy is needed to improve patient outcomes and develop more effective therapeutic strategies. Inaccessibility to metastatic specimens for research use has historically been the largest hurdle for addressing these themes. Collecting tissues from stage IV patients shortly after death provides a promising solution. These “rapid autopsy programs” (RAPs) are growing in popularity; however, there are only roughly 20 such programs scattered around the United States. While these programs stand to revolutionize the field of cancer research, building and maintaining RAPs presents significant logistic and financial issues that can limit successful development and implementation.

Development of our own program began with a comprehensive research phase designed to ascertain the critical components of a successful program. Initially we found ourselves overwhelmed by the lack of guidance and resources available in the field. We reviewed numerous publications from other RAPs but found that while informative on a higher level, these brief communication-styled articles did not provide the level of detail that we so desperately needed during our pre-planning processes.

Here, we present a step-by-step guide to build a cancer-focused RAP, based on our own experience developing and piloting “The Legacy Project”, at City of Hope. We present the realities and challenges of building a RAP based on our experience and discuss the nuances of addressing a spectrum of issues, ranging from basic science to end-of-life themes. We highlight common challenges as well as unexpected situations that should be considered carefully, and we explore the unpredictable circumstances of death and human nature, on both logistic and

psychological levels. Lastly, we offer detailed insights into our own solutions as well as the many subtle, yet critical, lessons learned.

Reagents & Equipment

Recruitment Material

- Softly worded introductory brochure
- Detailed step-by-step enrollment booklet for patients and families
- Consent documents

Ante-mortem Blood Collection

- Blood tubes
- De-identified labels
- Transport container

At Death

- ID bracelet
- Ice packs
- Body bag
- Bed sheet
- SOP for body preparation

Tissue Collection

- Autopsy Suite
- Specimens list / Collection plan
- De-identified labels (plus extra blank)

- Collection tubes of choice (1.5 ml, 15ml, 50, etc.)
- Media(s) of choice
- Wet ice
- Dry ice
- Large cooler for transporting specimens
- Dolly for transporting cooler
- Digital Camera
- Pen/markers
- Tissue Ink
- Blades and equipment
- PPE (Personal Protective Equipment)
- Tissue Cassettes
- 10% Formalin

Funeral Home Pick-Up

- Release documents
- Death certificate information (provided by hospice)

Key Staff and Important Stakeholders

Program Coordinator

Consenting duties and patient interactions should be restricted to a small team of individuals that is highly compassionate and able to respectfully broach sensitive topics. The Program Coordinator serves as a resource to the participant and family should any urgent issues arise, including coordination of care in consultation with the participant's clinical team. Although having a team of multiple coordinators is recommended, consistent interaction with the same

coordinator is ideal when working with each family. A close relationship helps to ensure continued engagement with the RAP and families should be given the means to contact the coordinator directly if needed after hours. In the immediate aftermath of a participant's death, the coordinator should continue to interact with the family to console them, help with paperwork, and coordinate with the funeral home. If the right person is indeed serving in this role, they too will grieve the loss of the participant, alongside the family. For this reason, dealing with multiple cases at a time, or consecutive cases back to back, can be emotionally taxing. Staff should be encouraged to "recharge" as needed between cases.

Procurement Team

Depending on the institutional setting, organizational resources, and local laws, programs may choose to utilize a diener, pathologist assistant (PA), or licensed pathologist for the tissue acquisition procedure. In our experience, finding appropriately trained and educated individuals willing to perform a "rapid" procedure was a significant challenge. We found that pathologists and PAs were unmotivated to participate, largely because of the on-call and after-hours nature of the program. Conversely, we found that while trained autopsy technicians and dieners were more eager to participate, their skills were not as sophisticated, especially when it came to lymph node systems and tissue microdissections.

Depending on the program's organizational structure and planned number of collections, procurement teams may or may not be employed as dedicated members of the RAP and may be contracted on a per-case basis. However, we recommend having at least one full-time member of the procurement team to assist with specimen processing, storage, and tracking.

Transportation

Regulations as to who may transport a decedent after death vary from state to state (see **Supplementary Table 1**). Many of the programs we surveyed stated that finding reliable and timely transportation was a significant challenge during program development. This proved to be true in our own experience and we urge new programs to consider these issues early in the development process.

Research Teams

At least one research team member must always be on-call and prepared to receive specimens during and after hours. Some programs may choose to include the research teams in the procurement process while others may tightly restrict access to the autopsy suite. Allowing research teams into the room allows them valuable input regarding specimen collection and helps ensure they receive the tissue and qualitative data they need. However, unlike medical staff, basic research personnel are likely to have little experience with human anatomy and may be ill-prepared to work with a human cadaver. Furthermore, working with a freshly deceased body presents additional psychological challenges, which may trigger new or previous trauma in untrained personnel. We advise limiting the number of untrained personnel in the autopsy suite and providing an "orientation" to all non-medical research staff, including desensitization methods such as photos or videos from autopsies, as well as an interview with an institutional psychologist to screen for previous trauma or increased risk for PTSD. Team members should be encouraged to discuss their emotional reactions with others and seek professional counseling if unsettling thoughts or memories persist. We found that holding group "debrief" sessions after each case, under the guidance of a trained psychologist, can help team members process their reactions to the procedure. Most importantly, participation in collection procedures must be voluntary and staff who decline to assist in the autopsy suite should not face negative repercussions from their supervisors.

Early-Program Champions

In the infant stages of program development, having passionate supporters to champion the program is critical. These “champions” can be physicians, scientists, or other leaders within the institution. RAPs are notoriously difficult to start and have complex logistic requirements.

Sustained enthusiasm during challenging periods is important to prevent loss of momentum and can help secure additional support from others within the institution.

Considerations – Preventing Burnout

A reliable staff is the most critical component of a RAP; without seamless coordination of countless moving parts, a program will not be successful. The 24/7 nature of RAPs and the demanding logistics require a dedicated and flexible team. Death often occurs outside working hours, in the middle of the night, and on weekends. Workplace and emotional burnout can be a real concern for these teams and competitive compensation and job flexibility should be offered to those who frequently work after hours and on-call. Depending on the size of the program and the number of cases being performed each year, multiple coordinators and procurement teams should be utilized to minimize the on-call burden placed on single individuals, prevent workplace burnout, and ensure team availability in case of emergency, illness, or scheduled time off.

Stakeholders

Critical stakeholders are shown in **Table 1**.

Methods

A complete overview of the RAP protocol is shown in **Figure 1**.

Phase 1: Building the Infrastructure

1. Determine which laws apply

Each state has its own set of laws which govern many aspects of a RAP. In California for example, there are minimal laws that regulate the death industry. Laws regarding consent, transportation of a decedent, and who may perform the tissue collection procedure vary from state to state (**Figure 2; Supplementary Table 1; also see Supplementary: Determine which laws apply**).

Considerations: “Autopsy” vs. “Tissue Collection”

Autopsies and tissue collections are not subject to the same regulatory oversight. In general, a medical autopsy must be ordered by a physician and supervised by a licensed pathologist or PA. Hospitals may also require additional liability paperwork in the case of autopsies.

Additionally, consenting guidelines differ between the two procedures. Most states allow an individual to consent to donation of his/her body or tissues after death. This consent is legally binding and does not require the next-of-kin to re-consent after death. In situations where an individual dies without providing consent, the next-of-kin may donate the tissues, as a decedent’s remains become property of the next-of-kin at death. In the case of medical autopsies, most states do not allow an individual to consent to their own autopsy. Instead, it is required that the next-of-kin provides consent after death. This presents itself as a logistic challenge and could potentially result in delayed transportation of the body.

2. Assess the institutional landscape and create a steering committee

RAPs are collaborative by nature and have many stakeholders (see **Table 1**). Frequent and transparent communication and cooperation between departments is essential. The program’s early champions will be particularly important during this step, as they will drive enthusiasm and support among critical stakeholders. The steering committee should also provide input on

policies relating to sharing of data and ownership of intellectual property that is born out of the program. Depending on the organizational structure of the RAP and the institution, clearly worded collaboration contracts may be useful on projects with multiple investigators.

Consideration: Piloting the Program

During early phases of development, the ability to adapt quickly is critical. Running the program as a small scale “pilot” initially, rather than as a fully defined program, has many benefits and may offer some protection from institutional politics that may slow decision making processes. A pilot program, led by a shortlist of leadership rather than a larger committee, will also provide more flexibility and be more adaptable should unexpected issues arise. For example, the first phase of our own program included only ten metastatic breast cancer patients. Oversight was led by a small group of key stakeholders and the primary goal of the “pilot” was to assess feasibility and optimize logistics. Completion of the pilot then led to the creation of an expanded program within the institution.

3. Consult the Institutional Review Board for guidance on oversight committees, Human Subjects Research (HSR) determination, and suggested protocol structure

Because tissues are collected after death, many do not consider RAPs to be Human Subjects Research, as defined by 45 CFR 46, and argue that such programs are not subject to IRB oversight. However, if blood or clinical information is collected prior to death, an argument can be made in favor of HSR determination. Committee oversight and HSR determination may also depend on the structure of the program’s protocols: specifically, if the tissue collection and subsequent research components are tied together or housed in separate protocols. For instance, in the Legacy Program, collection of tissues and data is housed under a tissue-repository-styled umbrella program and is subject to full IRB oversight with an HSR determination. No research is conducted under this parent protocol and therefore it is not

subject to Cancer Protocol Review and Monitoring Committee (CPRMC) oversight. Conversely, research uses of collected specimens are outlined in separate “secondary-use” protocols, which are regulated by the CPRMC but may or may not be subject to IRB regulation depending on the HSR status. Other programs however choose to combine both tissue-collection and research-use into one protocol to ensure research integrity of the program itself and to simplify the oversight process. Decisions regarding protocol structure and HSR determination should be made in consultation with institution leadership and the program’s steering committee.

4. Solicit feedback from your patient population to determine the needs and unique sensitivities that should be addressed in the program

Surveying a variety of interested parties is critical to gauge public perception and level of support for the program. Focus groups should include current patients, family members, and survivors.

Considerations: Primary versus Metastatic Patients

We observed stark differences in opinions between our primary cancer survivors and those with metastatic disease, especially when it came to recruitment strategies. Primary cancer survivors were more likely to be intimidated by the program (and research in general) and insisted that the conversation should only be initiated by their primary oncologist, with whom they had a close and trusted relationship. Our metastatic survivors however, as well as their families, held a much less sensitive view of the program and felt that the conversation regarding body donation should be “normalized” in the clinic – with the topic being introduced early on in a patient’s care and that educational brochures be freely placed in waiting and exam rooms. Since metastatic patients were our target population, we leaned towards their input when making key decisions, while still attempting to balance the concerns of our more sensitive patients. Both primary and

metastatic patients agreed that removing the “taboo” around death and body donation would improve accrual and lessen the emotional impact of the initial conversation.

5. Establish eligibility criteria

Outline inclusion and exclusion criteria for the targeted patient population. Criteria should include “commute time” guidelines for patients who plan to die at home.

Considerations: Situations that warrant disqualification

In addition to clearly defined inclusion and exclusion criteria, we recommend outlining situations that may disqualify an enrolled subject from participating in the final tissue collection. These guidelines should be clearly explained to the patient and family at the time of consent to avoid confusion or disappointment should such a situation occur.

Examples include:

- Subjects who receive radioactive therapy shortly before death
- Subjects who require a forensic autopsy
- Subject those who cannot be transported to the autopsy suite within the required timeframe
- Subjects who die unexpectedly at home and are not enrolled in hospice

6. Identify vendors and order reagents

Vendors such as transportation, diener, and facility with autopsy suite should be contracted well in advanced with job expectations clearly defined. Order reagents and equipment needed for all phases.

7. Create educational material

A) *Softly worded introductory brochure that provides non-technical, high level information about the program.* This generalized brochure can be broadly distributed in waiting rooms. Readers who wish to learn more about the program should be instructed to speak to their physician or contact the program coordinator.

B) *Detailed enrollment packet should be given to participants and family who consent to the program.*

Enrolment packet should include information such as:

- Program coordinator's direct contact information
- Summary the science being done
- Summary of events before and after death
- Resource and contact information for supportive care, child life services, bereavement support
- List of local mortuaries and funeral homes
- Instructions for care taker at the time of death
- Program letter in case of emergency hospitalization at outside institution

8. Create timeline and define sequencing of events

Outline what events will occur and the appropriate timing. A general timeline of events per protocol as defined by the Legacy Project is shown in **Figure 3**. For example, consider when patients will be approached for consent – early in their disease or shortly before anticipated death? Will ante-mortem blood be collected from participants, and if so, how often? When will imaging and clinical histories be reviewed prior to death? Will participants be expected to die at

home or in the hospital? How soon after death will tissues be collected? Who is expected to inform the program about a participant's death?

Consideration: The "N of 1" Model

Having a streamlined and consistent set of processes is important, however, programs must also be flexible enough to accommodate the unpredictability of death. Rather than establishing a rigid logistics plan and applying it uniformly to all cases, we recommend that cases should be considered independently, with lessons learned from previous cases applied to the next. This "N of 1" approach allows for quick adaptation and the flexibility needed to overcome unexpected challenges, especially during periods of program development and expansion.

9. Determine budget and funding sources

Fiscal responsibility for costs associated with clinical operations, procurement reagents, tissue processing, and downstream experiments should be considered upfront. The source and use of funds should be clearly defined for each component of the program. Stakeholders should have a clear understanding of which budget components they are responsible for funding. For example, participating research labs may be responsible for all downstream processing and experimental costs.

10. Refine and finalize operation protocol(s)

As institutions move through the various steps outlined in this phase (Phase 1), it may become apparent that some decisions need to be revisited and some details refined. As the program grows and new research aims are added, some details may also need to be re-optimized.

Phase 2: Recruiting and Consenting

Through the Legacy Project we defined a series of factors that are critical to successful patient recruitment and consent. These are outlined in **Figure 4**.

1. Educate physicians and other clinical staff about the program and how to best approach patients

Meet with clinical staff multiple times to present the program, offer tips, and answer questions related to the program. Repeatedly provide them with resource documents, recruiting guidelines, and frequently asked questions to help them feel comfortable approaching patients. Addressing the "taboo" of death and normalizing the concept of body donation can reduce the emotional burden of introducing the topic.

Supporting physicians in their ability to address sensitive end-of-life themes is important as patients will look to them for approval to participate in the program. Physicians with longstanding relationships with their patients may have difficulty initiating end-of-life conversations or broaching the topic of body donation. End-of-life conversations that are worded too gently can do more harm than good and may leave families unprepared for the imminent passing of the patient. Furthermore, it can be difficult to arrange consent conversations in a timely manner when families do not fully understand the gravity of the situation.

2. Define the recruitment strategy using feedback from focus group sessions and physicians

Consider the advantages and disadvantage between a broad vs. targeted recruitment. A broad strategy lends itself to normalizing the concept. This can be advantageous in situations where patients or their doctors struggle to make the transition from active therapy to comfort care at the end of life. This is also particularly true in disease modalities where the physician and

patient have long standing relationships that may span years or decades (e.g. breast cancer). The taboo surrounding conversations about death can be overcome by initiating the conversation early in a patient's care and introducing the RAP to all patients, regardless of disease status. In this way, body donation can be likened to organ donation. Normalizing these concepts early will also lessen the pressure put on the physician to introduce the RAP during end of life conversations and can limit the negative impact of the conversation on the patient's mental status. A broad strategy, however, is likely to illicit a strong response from many patients at once. Smaller or newer programs may become overwhelmed if they are unable to accommodate a large volume of study subjects. Conversely, approaching patients one by one places a greater burden on the physician and is likely to slow recruitment. However, this strategy may be advantageous for newer programs that are still optimizing processes and gaining experience. Additionally, a targeted strategy can be easily halted if recruitment needs to be temporarily paused for any reason. A blending of these two strategies can also be pursued by starting with targeted recruitment and expanding broadly as the program grows.

3. Approach and consent patients

A) The consenting process may require multiple conversations over time and key details may need to be reiterated in a sensitive, patient, and empathetic way.

Candidates and their families should be given time and space to consider relevant information and process their feelings about the RAP and end-of-life topics; and they should never be pressured to make a decision.

B) Not every patient and family will be receptive and it is important to wait for cues.

For example, past aversion to research trials are a hint that a patient is less likely to be open to body donation. However, patients who eagerly engaged in clinical trials and other research protocols are more likely to be interested in continuing in research efforts, even after death. We

found that approaching patients either very early in their care or alongside an end-of-life conversation was most effective. When death is still an abstract concept, patients can better process sensitive themes without a strong emotional response and are better equipped to appreciate the practicality and utility of body donation. At the other end of the spectrum, making the decision to donate their body to research can help patients process the reality of imminent death by giving them the “final word” in their fight against their disease. Conversely, patients who were still receiving active therapy, and whose prognoses were uncertain, were more likely to be intimidated by the concept and less likely to engage in a conversation. Furthermore, patients who are resistant or unable to engage in a preliminary end-of-life conversation are more likely to be shocked or traumatized by the rapid autopsy concept.

C) Include family in the consenting process and clarify expectations at the time of death.

Although a participant may fully commit to donation, it is ultimately the surviving family that must follow through with the decedent’s wishes and allow donation to occur. Program candidates with family who are unsupportive or dissent to participation should be considered ineligible for consent. The timing of events that follow at death should be clearly explained. Depending on how quickly the body must be transported, families should be informed if they will have limited time to spend with the decedent after death. Lastly, hospice partners can assist family members by reiterating what is expected to happen at the time of death and providing emotional and bereavement support.

D) Encourage families to make funeral home arrangements ahead of time.

It is important to have candid conversations with the participant and family about their wishes regarding funeral services. Families should be encouraged to speak with their chosen funeral home prior to death to ensure there will not be any issues in transportation. Additionally, a planned viewing may impact the tissue collection strategy. Procedures can be conducted in a

way that still allows for open casket viewings and knowing the family's intention ahead of time will ensure extra care is taken: for example, making lower chest incisions on females to allow for lower cut clothing and carefully making cranial incisions that can be easily hidden by the hair.

E) If possible, make sensitive or visible areas optional.

Participants and families should be given the opportunity to opt out of collections from visible areas, such as the face, hands, and neck, as well as other emotionally sensitive organs such as the brain. However, depending on presentation of the participant's disease, an inability to collect certain sites may impose restrictions on the specific research questions that can be asked. In these cases, it may be imperative to require participants to fully consent to the collection of any and all sites of disease.

4. Collect ante-mortem blood if applicable

A single time point or serial samples may be collected while the participant is living.

Phase 3: Preparing for Death

1. Track patient health

The frequency to which participants are monitored at the end of life is proportional to the targeted post-mortem interval (PMI), type of specimens to be collected, and the intended downstream research applications. Programs that allow collections as late as 24-48 hours after death likely do not need to monitor participants daily, or even weekly, as long as death is promptly reported. However, programs that aim to collect specimens within a few short hours after death, or with research applications requiring live cells, should closely monitor critically ill participants. Participants enrolled into hospice are best tracked by daily communication with the hospice team.

Consideration: Balancing multiple cases at once

Depending on the size of the RAP team and how closely participants are monitored, tracking multiple terminal patients at once can be challenging, requiring daily communications with hospice, RA program staff, and participating research teams. In addition to logistics, balancing multiple cases at once can be emotionally challenging; especially if a single individual is serving as the primary coordinator.

2. Confirm team member schedules and create a call tree

As death becomes imminent, it is helpful to have a calendar of which team members are available after-hours and over weekends. This calendar should be updated frequently. The RAP coordinator should verify team member contact information and create a call tree if appropriate.

3. Review imaging data and clinical history to create a “specimens of interest” list

Determine which research labs are expected to receive which tissues.

Consideration: The need to strategize compassionately

For patients and families wishing to hold viewings or open casket funerals after tissue collection, serious consideration must be made when deciding which tissues to target. For example, sampling certain areas of the skull might produce visible facial deformities and would likely cause the family to incur unnecessary reconstruction charges from the funeral home.

4. Allow research labs to review the specimen list and prioritize tissues to be collected

It is realistic that not every collaborating lab will want every specimen collected from a single participant. Additionally, depending on the specific research aims, specimens may need to be collected in a specific way, or with specific matched controls. Allow research labs the chance to review the specimen list and determine their own samples of interest.

5. Have a pre-defined collection plan

Various philosophies exist regarding how an autopsy/tissue collection should be performed and which methods are most appropriate in a given situation. Often, specific techniques are chosen based on the personal preference of the individual performing the procedure. Given the importance of both speed and accuracy in a RAP setting, we suggest creating a pre-defined collection plan using consistent methodologies. Review of participant imaging and disease history allows for streamlined sample collection. In complex cases, a sample by sample strategy is developed to keep the tissue collection team on track and ensure all relevant specimens are collected.

6. Prepare collection reagents and labels

Pre-labeled tubes for anticipated specimens can be prepared prior to the procedure. Extra blank labels and containers should be packed in case more tissues are collected than were initially expected. The procurement team should record the appropriate information for any additional specimens on the container label as well as the accompanying notes.

7. Coordinate with hospice

A) *Communicate the “rapid” and 24/7 nature of the RAP. Emphasize the importance of immediate communication.*

It is important that hospice partners initiate continuous care towards end of life (24 – 48 hour window) so that death can be pronounced immediately and transportation can be expedited. The role of hospice should be to minimize the responsibility of the family to care for a terminal patient, keep the coordinator updated on the participant’s health, promptly report death, and if applicable, prepare the body for transportation. A well-trained hospice team member can also provide invaluable emotional support to the family that will help prepare them to say goodbye quickly after death.

B) Create a detailed Standard Operating Procedure (SOP) that clearly states program requirements, expectations from hospice, and step by step instructions to be followed at the time of death.

Hospice companies vary in their practices. Even within the same company, offices may function differently, depending on their service location. This can pose a challenge when establishing a streamlined and uniform set of processes. Fortunately, most variances in hospice processes can be overcome through minor adjustments to accommodate RAP requirements. Family should never be asked to assist in preparing the body after death; this should be strongly emphasized in the SOP. We have provided an example SOP as a supplementary document (**SOP: Preparation of Body at Time of Death**).

C) Provide a materials kit to be used to prepare the body for transport.

This kit should include: body bag, identification tags, disposable bed sheet, and ice packs. Kits should be delivered to the hospice team and not the family.

Considerations: Make room for family

While hospice should take charge of activities relating to keeping the program updated regarding the participant's health status, informing the RAP coordinator that death has occurred, and preparing the body for transportation, occasionally, a family member may take pride in performing these actions. These family members view their involvement with the program as an act of service to their loved one and space should be made for these members to feel useful.

8. Communicate updates and timeframe to teams

As life expectancy decreases, the RAP coordinator should notify all teams, including transportation, procurement, and research, of an imminent death. An alert system can be helpful when communicating an expected timeline and gives teams adequate time to complete time

sensitive tasks such as alerting staff, preparing perishable media, un-silencing phones, booking time slots on shared laboratory equipment, and preparing ice packs if needed.

For example, participants can be assigned a code status upon hospice enrollment; “Yellow”, “Orange”, and “Red” alert:

- Yellow alert – approximately two weeks life expectancy. Updates can be delivered daily.
- Orange alert – participant is declining but not actively dying; estimated life expectancy 3-7 days. Updates can be delivered once or twice daily.
- Red alert – Participant is actively dying and death is imminent within 24-48 hours. Updates should be delivered every 4-5 hours or when there is a significant change in health status.

9. Activate teams when death occurs

As soon as a death has been reported, the RAP coordinator should refer to the *team member schedule* and *call tree* (phase 3, step 2) to notify and activate teams. After confirming transportation timeframe, the coordinator should continue to update the procurement and research teams.

Phase 4: Tissue Collection and Follow up

1. House calls and visiting after death (suggested)

The RAP coordinator should make an effort to visit the participant while on hospice. During this time, the coordinator can answer questions related to the program and make sure that the family and participant’s needs are being met. These visits also serve to strengthen the relationships with the participant, family, and hospice partner.

Depending on the consenting process, the RAP coordinator may be required to meet with the next-of-kin immediately after death. Regardless however, efforts to visit after death is reported can bring comfort to the family and can help ensure transportation happens smoothly.

2. Prepare the body for transportation

Depending on state laws, either hospice or the transporting funeral home should prepare the body for transportation. This includes removing personal articles and clothing, cleaning the body, and placing the decedent in the body bag. Ice packs may also be placed along the torso and around the head and neck to help preserve tissue integrity until collection.

To minimize variability between cases and ensure that transportation happens expeditiously, the responsible party should reference the SOP for body preparation at the time of death.

3. Transport decedent

Many states only allow funeral home directors to transport decedents and a transportation permit may be required. In these states, a close relationship with the participant's chosen funeral home or mortuary should be established prior to death to ensure transportation happens in a timely manner. In states with limited regulation, a third-party non-emergency medical transportation company is an appropriate alternative.

4. Collect tissues

A) Refer to the collection plan created in Phase 3, Step 5.

Specimen photos and notes should be recorded as needed.

B) Consider how to account for tumor heterogeneity and dividing tumor specimens between experiments.

Tumor heterogeneity can pose a problem when splitting individual specimens between labs or experiments. To account for this tumor heterogeneity when dividing samples, we suggest dividing the specimen into 3 sections, using the middle section for histological validation and analysis. By validating this middle section, rather than the tissue margin, a more accurate representation of the specimen as a whole is captured. Although not a perfect solution, this middle section can then be referenced if conflicting characteristics are observed between experiments using distinct tumor sections. To ensure that histology sections are oriented correctly, consider integrating inking into your processes. By marking the sides of each section, you can keep track of which face of the tissue block aligns to the individual sections that were distributed to each lab. This also ensures that tissue blocks are correctly oriented when tissues are mounted for slide sectioning.

C) To ensure tumor tissues can be traced back to their original organ, all samples should be grossed with a “tag” of normal tissue (or low tumor burden tissue, if normal is not available).

Specimens that have been entirely replaced by tumor can be difficult to validate, especially in terms of confirming specific tissue of origin. A normal tissue tag ensures that the specimen can be validated by pathology and that any processing errors (such as mislabeling) can be corrected if they occur.

D) Collecting and confirming lymph nodes structures can be challenging.

Lymph nodes are historically difficult to find, both in surgery and post mortem. Lymph node size and location vary wildly between subjects and fat deposits can often mimic gross lymph node structure, making accurate identification challenging. Furthermore, while tumor-draining and disease-involved nodes are often enlarged or inflamed, disease free nodes may be as small as

a single grain of rice. In a surgical setting, lymph nodes will often be marked by a pre-surgery tracer or dye injection. Additionally, most surgical lymph node specimens are discovered and isolated after surgery at the grossing bench which is not an attainable option in a RAP setting. In order to overcome this obstacle in the autopsy room, it is necessary to have someone with training, close attention to detail, and a willingness to learn and adapt. Review of the specimen histopathology can be used to assess accuracy of lymph node collection; however, this process too has its own complications. Lymph nodes with heavy tumor burden often lose their signatory structures and can be difficult to confirm microscopically. If the node has not yet been completely overtaken by tumor, presence of the node's outer capsule can aid in confirmation, in conjunction with the autopsy technician's notes, and photographs from the collection. Small, disease-free nodes should be placed in a cassette with smaller holes to minimize the risk that the tissues will be lost during processing.

5. Coordinate funeral home pick up

Once the procedure is completed, the RAP coordinator should coordinate with the funeral home to arrange retrieval of the remains. The RAP coordinator should also ensure any required administrative documents are completed.

6. Follow up with family (Recommended)

Our patient-led focus groups stressed the importance of continued follow up with donor families, weeks months, and even years after death of the participant. In the immediate aftermath of death, the RAP coordinator should continue to keep the family updated on status of the procedure and when the funeral home is expected to retrieve the body. A direct phone call is most appropriate at this time, and families should be encouraged to reach out to the coordinator if anything else is needed. Continued follow up with family provides them with the opportunity to resolve any outstanding clinical issues and to give feedback about the program and logistic

processes. After roughly two weeks, a follow-up and thank you letter can be sent to the next-of-kin. Yearly program updates, highlighting the research findings and publications made possible by the decedents, can be sent if family is interested.

Discussion

Benchmarks and Expected Results

Using this protocol, we collected a total of 533 specimens from 9 metastatic breast cancer subjects. The average time from death to acquisition of the last specimen was 6.1 hours (range: 4.03 – 7.66 hours; median: 5.71 hours). Total number of specimens collected from each participant ranged from 38-75, with an average of 60 across all patients; the number of tumor-positive specimens collected ranged from 12-46, with an average of 29; the number of non-cancer specimens collected ranged from 25-45, with an average of 31.

Despite initial concerns that accrual would be challenging, we found that nearly all patients who were approached expressed interest in learning more about the program and the vast majority wanted to participate. Complicated family dynamics was the primary reason that patients declined or were not consented, highlighting the importance of the family's perception of the program and their relationship with the program staff. During our pilot study, a total of 19 patients were approached for consent over a period of 13 months (**Table 2**). Of these, ten (53%) were consented and nine successfully donated tissues at the time of death. Only one fully consented patient did not undergo the collection procedure due to the family's failure to notify our team at the time of death.

Of the nine candidates who were not consented, two patients immediately declined when approached by their oncologists, three declined after consideration with their families, one had family approach us at the time of consent stating their desire to not participate despite the

patient's interest, one patient declined after learning her chosen funeral would not retrieve her body without a hefty surcharge, and two interested patients passed away suddenly before consent. The two cases in which patients passed away suddenly occurred early in program development. At the time, we did not have a mechanism by which next-of kin can consent after death. In response to this, we have modified our processes to ensure that similar situations can be accommodated should they occur again.

At the time of tissue procurement, one third of subjects exhibited clinically unidentified diseased sites in organs not commonly associated with breast cancer metastases including ovary, kidney, and pancreas. In two other instances, "resolved" bone specimens (as measured by absence of FTG uptake in PET/CT imaging) were later determined to be >30% tumor positive when assessed by H&E.

As a preliminary assessment of tumor heterogeneity, a subset of samples was profiled for estrogen, progesterone, and Her2 receptors, as well as the Ki67 antigen by immunohistochemistry. Consistent with clinical hypotheses regarding disease heterogeneity, we observed variable expression of the estrogen, progesterone, and Her2/neu receptors across metastatic locations within patients.

While these preliminary findings generate more questions than answers regarding mechanisms of metastatic progression and resistance to therapy, they highlight the utility of rapid autopsy in a research setting. We suggest that many unanswered clinical questions can be addressed through interrogation of post-mortem tissues and we urge research institutions to thoughtfully consider adoption of the RAP model.

Balancing the post mortem interval (PMI)

Concerning the PMI, the time from death to completion of the collection procedure, goals should be driven by the specific research intents. Although DNA may remain relatively stable in specimens collected 24-48 hours after death, RNA quality declines with increasing PMI (Fan et al. 2016) and altered transcriptional patterns have been observed in the hours following death (Ferreira et al. 2018). Results from specimens taken further out should be interpreted with caution. Live cell experiments, cytokine signaling, and single cell sequencing experiments are much more sensitive to PMI and specimens intended to be used for these applications should be collected as quickly as possible. Decisions regarding PMI should be made based on feedback from collaborating research labs.

Big Data Challenges

While RAPs may open the door to seemingly unlimited experimental possibilities, a sudden over-abundance of tissue and data can be difficult to manage. This, compounded with the various omic-datasets that can be generated from a single tissue, sets the stage for a number of “big data” challenges that must be overcome by the research teams. Comprehensive teams with experts in wet-lab experiments, bioinformatics, and statistics are critical to answering the complex research questions asked of this kind of data.

What insights are left to see at death if the “war” has already been lost?

An important question that should be asked of RAP specimens is whether the disease state at the time of death is reflective of clinically relevant disease or is largely composed of artifacts of death. Further research in the RAP setting is needed to answer this complicated question. Co-interrogation of ante-mortem tumor and blood samples paired with RAP specimens can provide insight. Efforts can also be made to collect post-mortem tumor tissues from patients who die from other causes than their disease.

Conclusion

We conclude that each RAP is unique and is driven by fundamental differences in: institutional governances and resources, state laws, IRBs, and patient populations. These differences highlight the need to tailor RAPs and to consider the specific needs of patients, researchers, and the overall strategic goals of the institution. Importantly, these findings highlight the need to use a tested framework and protocol when planning to establish a RAP. We hope that the framework provided herein this text assists others in building their own successful programs.

List of Abbreviations

ER – Estrogen Receptor

Her2 - Human Epidermal growth factor Receptor 2

HSR – Human Subjects Research

IRB – Institutional Review Board

PA – Pathologist’s Assistant

PMI – Post Mortem Interval

PPE – Personal Protective Equipment

PR – Progesterone Receptor

RAP – Rapid Autopsy Program

SOP – Standard Operating Procedures

Declarations

Ethics approval and consent to participate

This study was approved by City of Hope’s Institutional Review Board under study number 17503. Participants were enrolled either by self-consent prior to death, or had their body donated after death by next-of-kin.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no competing interests

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Author Contributions

JRW and PPL conceived and initiated program development. JRW funded this work through the Waisman Innovation Fund, privately funded by grateful patients wishing to advance research in the field of metastatic breast cancer . ERB directed and implemented the various aspects of the program and coordinated operations, under the supervision of JRW and PPL. KI performed all post-mortem tissue collection activities and provided expert advice in post-mortem logistics. All authors provided significant input regarding the manuscript's scope and purpose. ERB wrote the manuscript's primary text and constructed the figures and tables, with contributions from KI.

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Figure Legends

Figure 1: RAP protocol overview

A summary of the four protocol phases and their respective steps. Phase 1 involves program development and organizational structure. Phase 2 focuses on patient education and recruiting. Phase 3 involves tracking participants, communicating with family and hospice, and preparing for the tissue collection. Finally, Phase 4 describes what should happen at death, during the collection procedure, and the follow-up after.

Figure 2: Determine Which Laws Apply

State and local laws dictate which regulations apply to various aspects of the program. Requirements for consenting, transporting the decedent, and performing the procedure should be verified during Phase 1. We have compiled a state-by-state breakdown summary, which we provide in the supplementary materials.

Figure 3: Example Timeline of Events

General timeline of events per protocol for a RAP as defined by the Legacy Project
Example timeline showing the sequence of events in relation to life expectancy and time of death. Depending on the circumstances of each patient's disease, some events may occur out of order.

Figure 4: Critical factors to successful patient recruitment and consent within a RAP

Summary of key decisions and actions that are viewed positively or negatively by patients and their families. These guidelines were developed based on conversations with target patient populations.

Table 1: Summary of Program Stakeholders and Their Respective Roles

Stakeholder	Interest/Responsibility
Medical Oncology	Responsible for identifying patients and serving as a bridge of trust as patients are introduced to RAP staff. Many patients prefer to be approached by their primary oncologist first. Continues to provide standard of care regardless of enrollment into the RAP, serves as the patient's primary medical advocate, and acts in best interest of the patient. Honest and realistic communication with patient and family regarding prognosis and life expectancy is critical.
Radiology	Reviews patients' imaging histories throughout course of disease. Provides summary highlights of disease history in the context of remission, progression, and relapse - by organ. Gives recommendations regarding areas of interest.
Supportive Care	Aid in the identification of candidate patients. Are available to meet with patients struggling to cope with end of life issues. Meeting with research staff involved in collections to ensure coping appropriately.
Patient and Family Advocates	Serve as stakeholders in project planning meetings. Provides input on recruitment strategies and educational handouts.
Case Management	Aid in identifying candidate patients. Ensure enrolled patients are placed in hospice programs compatible with the RAP. Facilitate introduction and initial communication between RAP and chosen Hospice company.
Social Work	Aid in identifying candidate patients. Serve as a bridge of trust as patients are introduced to RAP staff. Help patient and family process key information related to the program requirements and obligations.

Community Education	Facilitate discussions with Patient Advocates. Guide development of study handouts and recruitment materials.
Pathology	Responsible for histological assessment of collected specimens.
Basic Research	Provide scientific proposals for new projects. Perform experiments. Assist in sample collection if needed.
Hospitalists	Aid in identifying candidate patients. Serve as a bridge of trust as patients are introduced to RAP staff. Provide Legacy team with updated and real time information regarding patient's health status.
Hospice	Responsible for notifying RAP team IMMEDIATELY after death and preparing body for transportation. Provide support to both patients and families dealing with end-of-life issues. Provide RAP team with updated and real time information regarding patient's health status. Strongly encouraged to provide continuous care towards last 48 hours of life expectancy. Declares death, provides details of death to funeral home, and signs final death certificate.
Transportation	Responsible for transporting body immediately after death, within one hour's notice. Expected to be professional and compassionate when dealing with a decedent's family.
Funeral Homes	Retrieve body from Hospital. Coordinates with RAP staff and Nursing Administration to schedule pick up.
Participants, Friends, & Family	Must be fully supportive and in agreement of patient's wishes to donate. Responsible for informing RAP or Hospice staff of sudden change in health status. Have final authority to decline donation.
Nursing Administration	Assists in discharge paperwork and release of body.

Research	Administrative and operational interests.
Operations	

Table 2: Summary of patients approached for consent and reasons for screen failure.

Candidate	Consent Status	Tissue Collected	Reason Unconsented or Declined
01	Not Consented	No	Patient and family interested but patient died suddenly, prior to consent. Program logistics have been modified in response.
02	Consented	Yes	
03	Declined	No	Patient lived further than 1 hour away and wished to die at home. Chosen funeral home would not agree to retrieve body from hospital without significant surcharge to family.
04	Consented	Yes	
05	Not Consented	No	Patient and family interested but patient died suddenly, prior to consent. Program logistics have been modified in response.
06	Consented	Yes	
07	Consented	Yes	
08	Declined	No	Did not want to discuss any end of life topics.
09	Not Consented	No	Patient was interested but family declined at the time of consent.
10	Declined	No	Patient interested but was already enrolled in another body donation program.
11	Consented by Next of Kin	Yes	
12	Consented by Next of Kin	Yes	
13	Declined	No	Patient was interested but family declined at the time of consent.
14	Consented by Next of Kin	Yes	
15	Consented	No	Death was not reported by family.
16	Consented	Yes	
17	Declined	No	Patient did not want to discuss end of life topics.
18	Not Consented	No	Patient was interested but family declined at the time of consent.
19	Consented by Next of Kin	Yes	

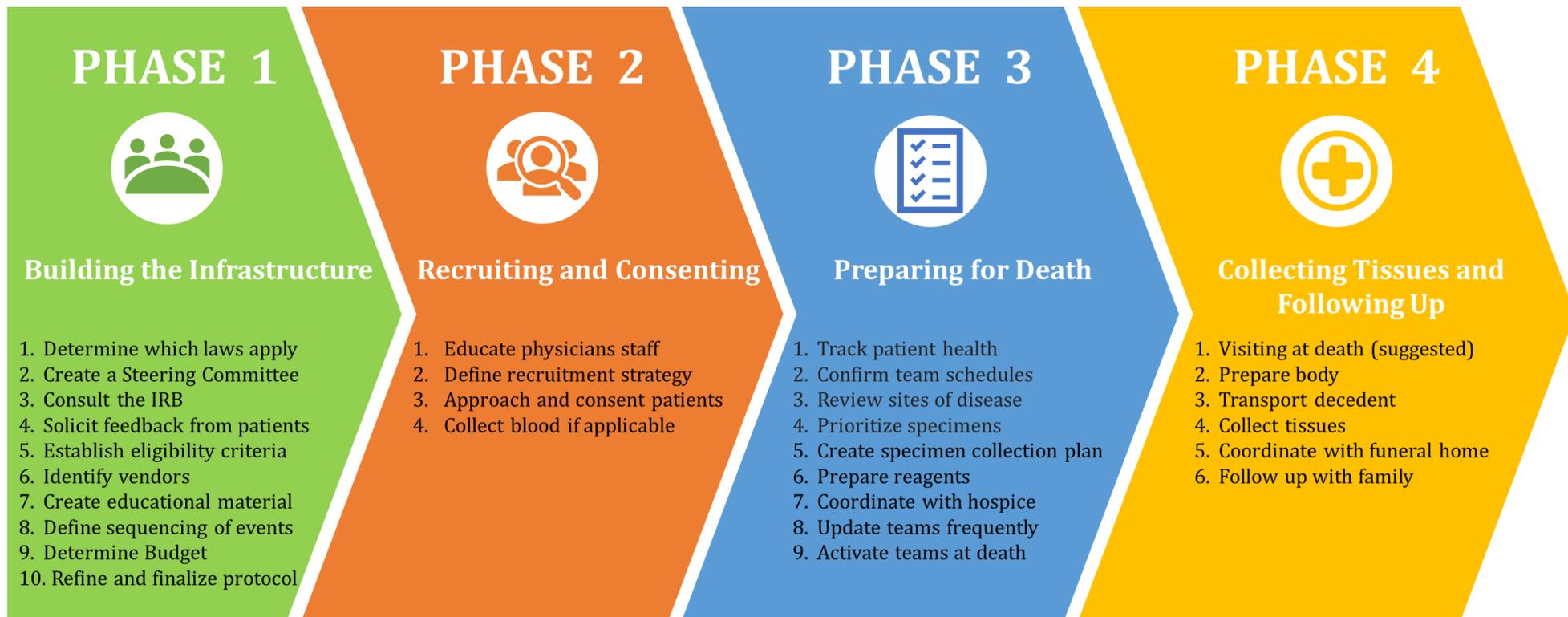


Figure 1

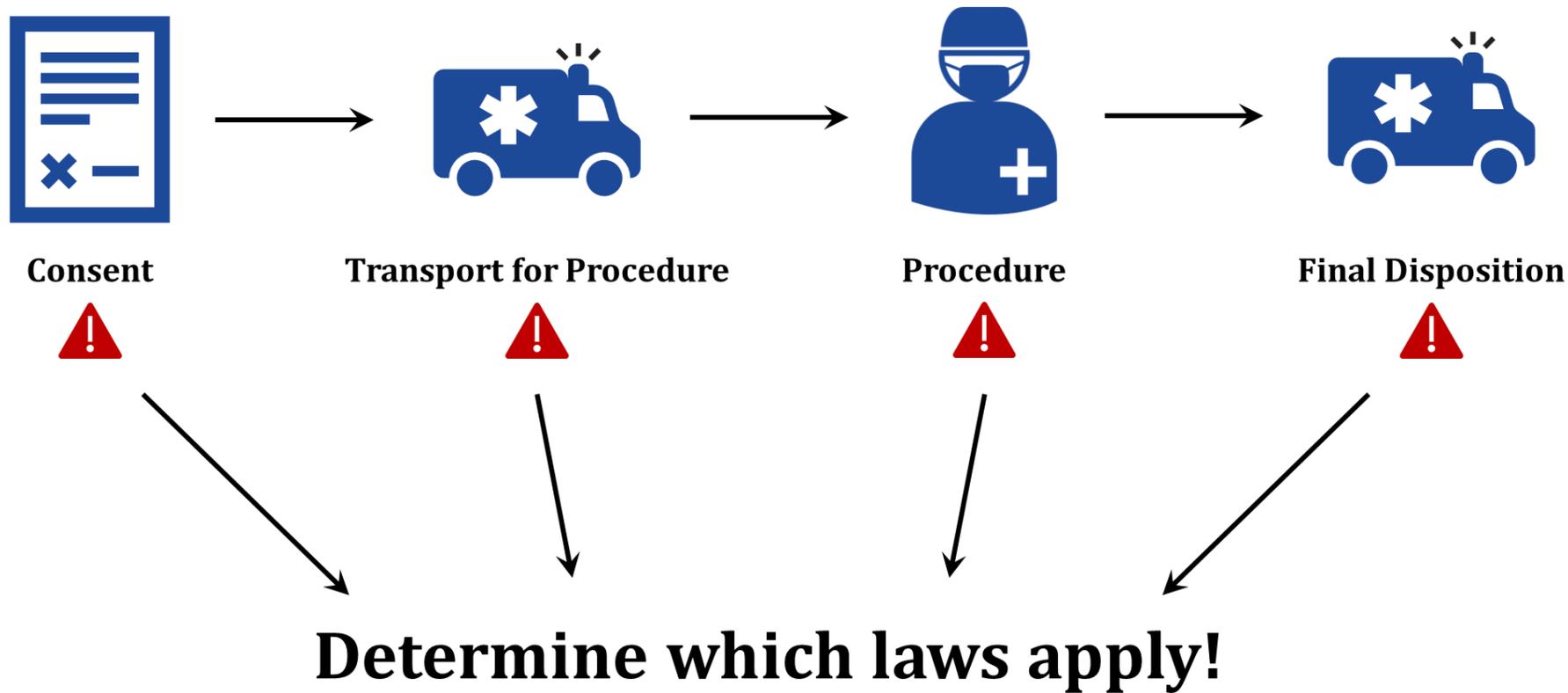


Figure 2

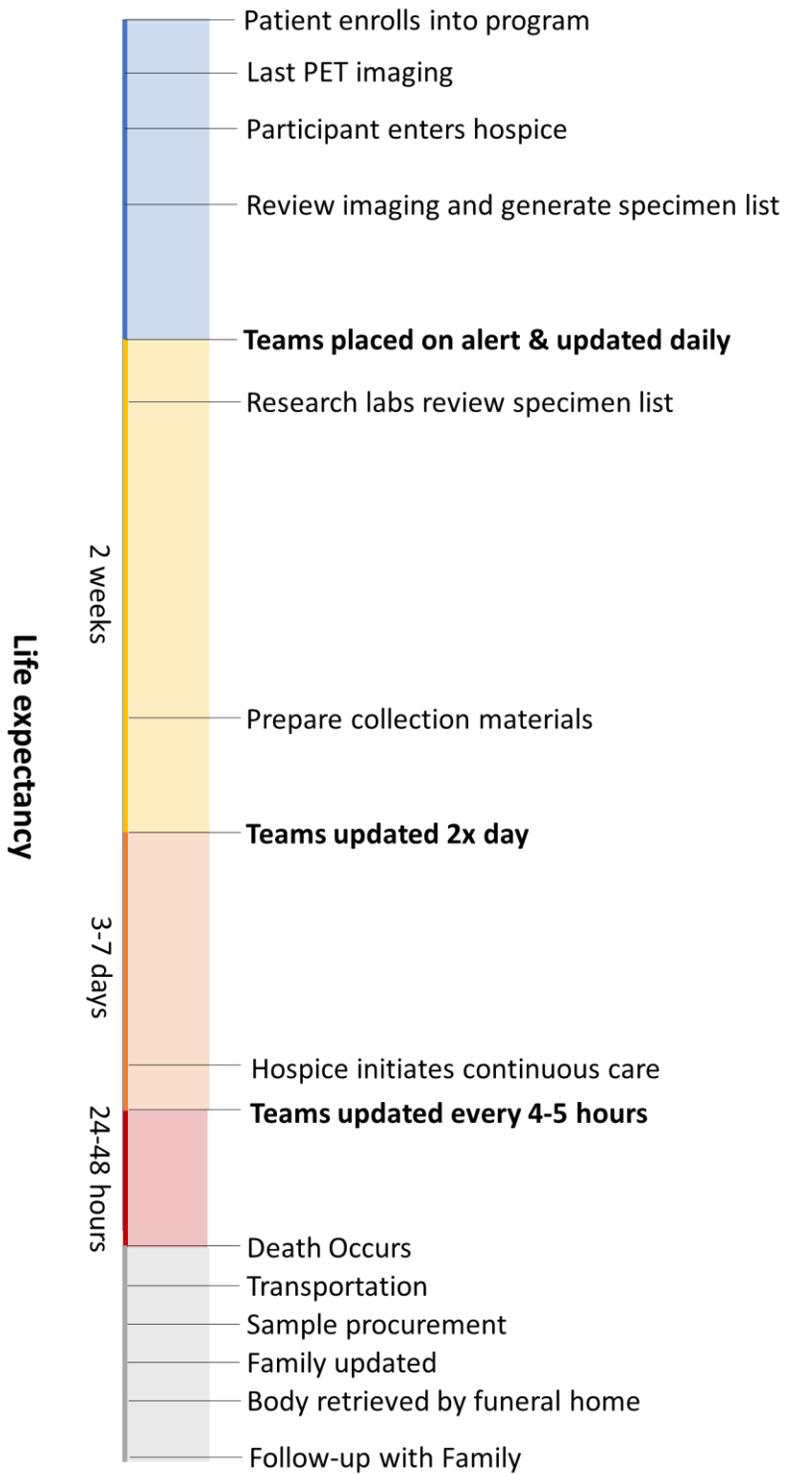


Figure 3

✓ DO

- DO use the words “donation”, “tissue collection”, and “pass away”.
- DO approach patients who have expressed interest in participating in research studies and are coping well with their diagnosis.
- DO have the clinical care team initiate discussions early on.
- DO engage family members in the consenting process.
- DO develop education materials (brochures, flyers)
- DO make ante-mortem blood and sensitive organ collection optional.
- DO give family members the final decision and authority to dissent after death.
- DO follow up with family after death, send program updates and emerging research.

✗ DO NOT

- DO NOT use the words “autopsy”, “tissue harvesting”, “expired”.
- DO NOT approach every patient; instead, wait for cues.
- DO NOT have discussions about RAP during initial appointments.

Figure 4

Figures



Figure 1

RAP protocol overview. A summary of the four protocol phases and their respective steps. Phase 1 involves program development and organizational structure. Phase 2 focuses on patient education and recruiting. Phase 3 involves tracking participants, communicating with family and hospice, and preparing for the tissue collection. Finally, Phase 4 describes what should happen at death, during the collection procedure, and the follow-up after.

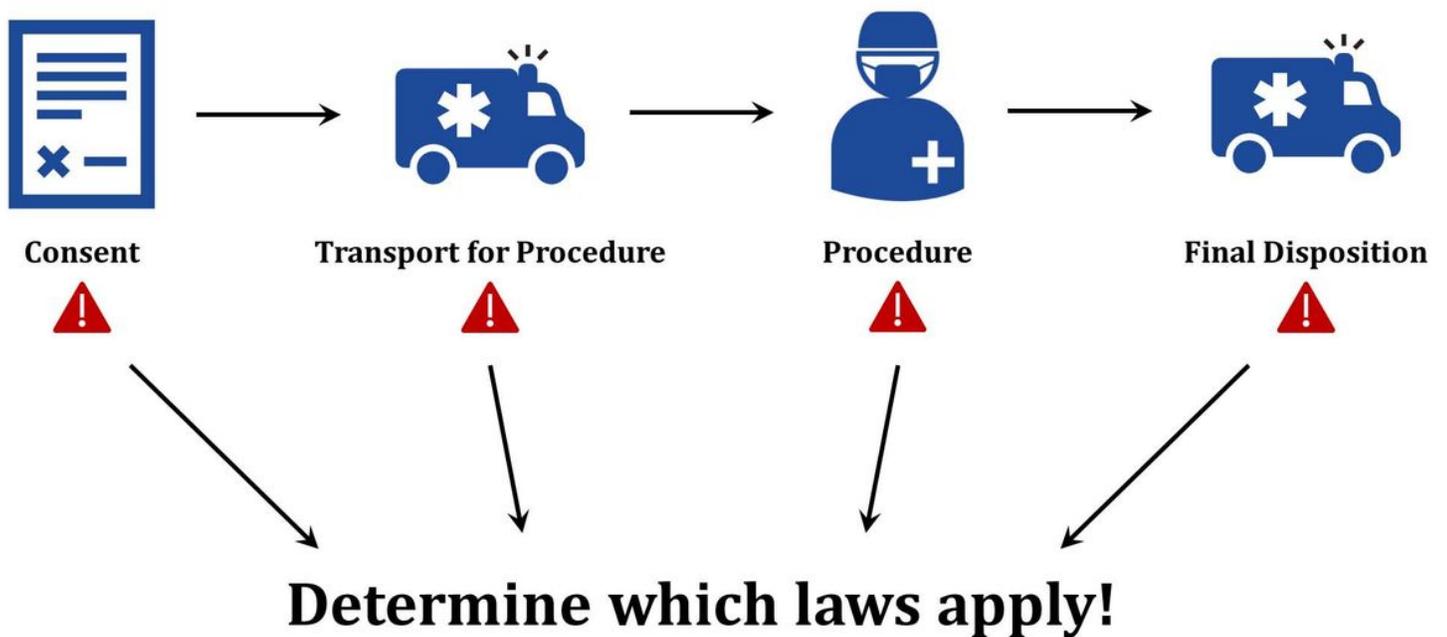


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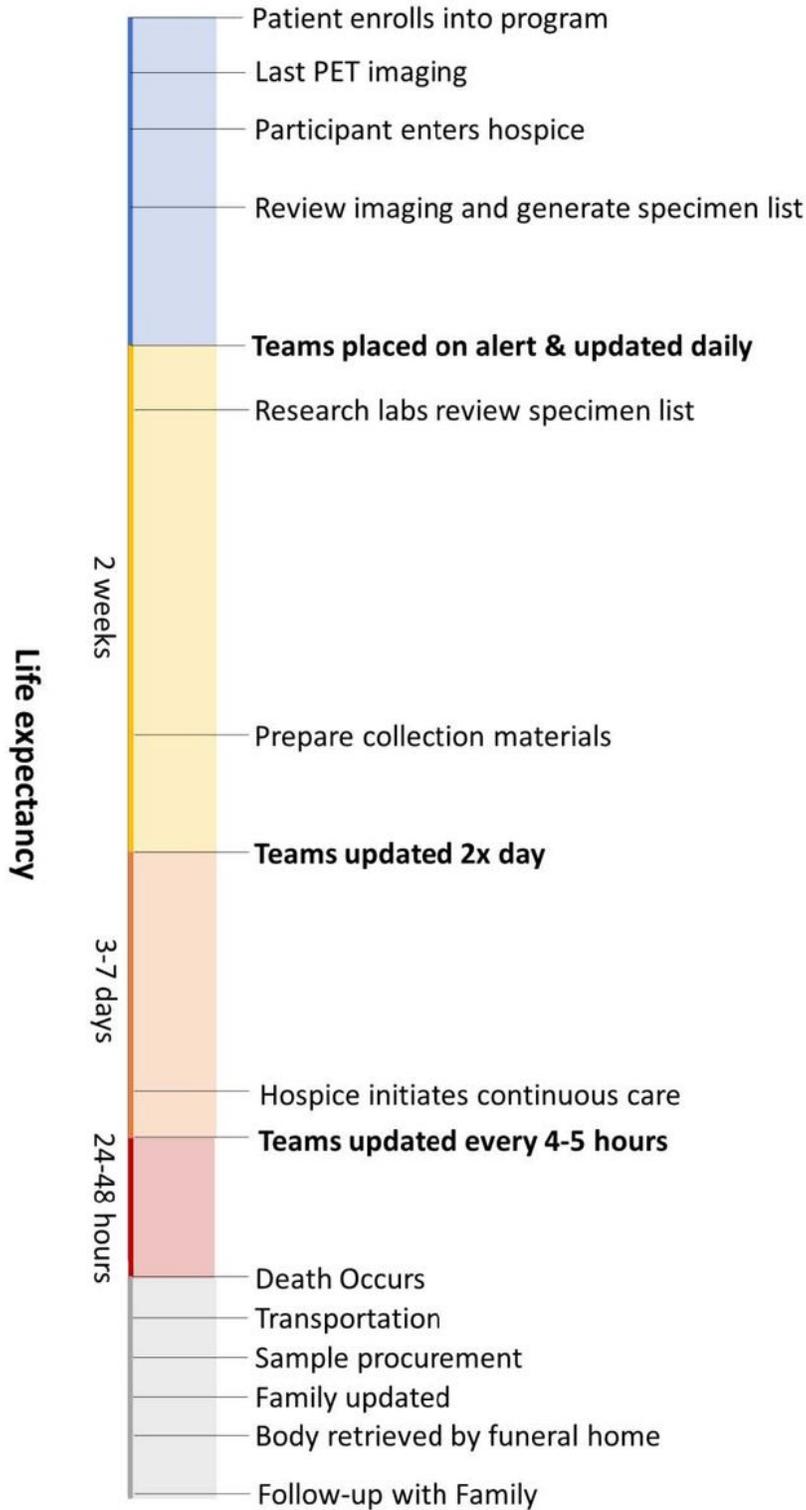


Figure 3

Example Timeline of Events. General timeline of events per protocol for a RAP as defined by the Legacy Project Example timeline showing the sequence of events in relation to life expectancy and time of death. Depending on the circumstances of each patient’s disease, some events may occur out of order.

 DO	 DO NOT
<ul style="list-style-type: none"> • DO use the words “donation”, “tissue collection”, and “pass away”. • DO approach patients who have expressed interest in participating in research studies and are coping well with their diagnosis. • DO have the clinical care team initiate discussions early on. • DO engage family members in the consenting process. • DO develop education materials (brochures, flyers) • DO make ante-mortem blood and sensitive organ collection optional. • DO give family members the final decision and authority to dissent after death. • DO follow up with family after death, send program updates and emerging research. 	<ul style="list-style-type: none"> • DO NOT use the words “autopsy”, “tissue harvesting”, “expired”. • DO NOT approach every patient; instead, wait for cues. • DO NOT have discussions about RAP during initial appointments.

Figure 4

Critical factors to successful patient recruitment and consent within a RAP. Summary of key decisions and actions that are viewed positively or negatively by patients and their families. These guidelines were developed based on conversations with target patient populations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [RAPProtocolSupText.pdf](#)
- [SupplementaryHospiceSOP.pdf](#)
- [supplementarytable1.pdf](#)