HHS Public Access

Author manuscript

Nat Rev Cancer. Author manuscript; available in PMC 2020 August 28.

Published in final edited form as:

Nat Rev Cancer. 2019 December ; 19(12): 686–697. doi:10.1038/s41568-019-0199-4.

Cancer biology as revealed by the research autopsy

Christine A. Iacobuzio-Donahue[1,2,3,*], **Chelsea Michael**[4], **Priscilla Baez**[1], **Rajya Kappagantula**[1], **Jody E. Hooper**[5], **Travis J. Hollman**[1,6]

[1]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

[2]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

[3]David M. Rubenstein Center for Pancreatic Cancer Research, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

[4]Department of Health Informatics, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

[5]Department of Pathology, The Johns Hopkins University, Baltimore, MD, USA.

[6]Parker Institute for Cancer Immunotherapy, Memorial Sloan Kettering Cancer Center, New York, NY, USA.

Abstract

A research autopsy is a post-mortem medical procedure performed on a deceased individual with the primary goal of collecting tissue to support basic and translational research. This approach has increasingly been used to investigate the pathophysiological mechanisms of cancer evolution, metastasis and treatment resistance. In this Review, we discuss the rationale for the use of research autopsies in cancer research and provide an evidence-based discussion of the quality of post-mortem tissues compared with other types of biospecimens. We also discuss the advantages of using post-mortem tissues over other types of biospecimens, including the large amounts of tissue that can be obtained and the extent of multiregion sampling that is achievable, which is not otherwise possible in living patients. We highlight how the research autopsy has supported the identification of the clonal origins and modes of spread among metastases, the extent that selective pressures imposed by treatments cause bottlenecks leading to parallel and convergent tumour evolution, and the creation of rare tissue banks and patient-derived model systems. Finally, we comment on the future of the research autopsy as an integral component of precision medicine strategies.

* iacobuzc@mskcc.org.

Author contributions

All authors researched data for the manuscript and made substantial contributions to the discussion of the content. C.A.I.-D., J.E.H. and T.J.H. wrote the manuscript. C.A.I.-D., PB., R.K., J.E.H. and T.J.H. reviewed and/or edited the manuscript before submission.

Competing interests

C.I.A.-D. and T.J.H. have received research support from Bristol-Myers Squibb. The other authors declare no competing interests.

Peer review information

Nature Reviews Cancer thanks M. Rubin, S. Turajlic and the other, anonymous, reviewers) for their contribution to the peer review of this work.

A clinical autopsy is to a post-mortem medical examination of a deceased person involving a modified surgical procedure for the purpose of establishing a cause of death. The importance of clinical autopsies for contributing to medical knowledge remains indisputable, despite their declining rates owing to their time-consuming nature, concerns related to their invasiveness, a lack of reimbursement from public or private sources, inadequate physician training, concerns about possible litigation related to post-mortem findings and the emergence of modern diagnostic methods[1]. Minimally invasive autopsies that combine CT-guided or stereotactic biopsies with radiological imaging of the deceased person are an approach to curb this trend. Indeed, initial comparisons have indicated that minimally invasive autopsies can determine the cause of death at rates similar to conventional clinical autopsy, while addressing concerns expressed by family members about the invasiveness of the conventional autopsy[2].

By contrast, some autopsies are performed primarily for the purpose of collecting one or more normal or diseased tissues to support basic or translational research. Herein we refer to these autopsies as ‘research autopsies’ to reflect the intended purpose of the procedure rather than the time or logistics used to perform it, as these can differ greatly between institutions or community programmes. Autopsies primarily performed for research purposes were first implemented for the collection of brains to study neurological and psychiatric disease at the end of the nineteenth century[3]. In the latter half of the twentieth century, a systematic approach to brain banking was initiated in the United States for collection and processing of all brains from consenting individuals[3]. Given the success of brain banks in contributing to scientific knowledge, the research autopsy approach has since been applied to another important group of diseases — cancer.

The first printed case report of cancer based on post-mortem examination was published in 1507 by the Italian physician Antonio Benivieni[4]. At the time, Benivieni did not recognize the gross findings of gastric wall thickening, loss of rugal folds and pyloric stenosis as the classic description of linitis plastica seen in the context of a diffuse-type gastric cancer[5]. In 1761 another Italian physician, Giovanni Battista Morgagni, reported 17 cases of cancer based on post-mortem examination, although his understanding of cancer biology was limited because of his personal lack of knowledge related to what metastases look like[4,6]. The first concrete example of how post-mortem examination contributed to our understanding of cancer biology was the work of Stephen Paget, who, in 1889, proposed that the processes of metastasis does not occur by chance but rather that tumour cells with metastatic propensity have a special affinity for specific organs[7]. Development of his ‘seed and soil’ hypothesis was based on review of the autopsy records of 735 women with breast cancer, in whom he documented a non-random pattern of metastasis to visceral organs and bones. In the 1970s, elegant work in mice by Fidler, Kripke and others provided experimental support for this theory, and also demonstrated that neoplasms are biologically heterogeneous and that the process of metastasis is selective rather than passive[8,9]. Subsequent analyses using similar approaches revealed the broad patterns of metastatic spread across multiple tumour types and elucidated the molecular mechanisms of organotropism[10–13]. The first autopsies performed for the purpose of contemporary translational cancer research were on decreased patients with metastatic prostate cancer in the 1990s by investigators at the University of Washington and the University of

Michigan[14,15]. Since the initial report in 2000 (REF.[14]), a variety of research autopsy programmes have been established, which have greatly expanded our insight into the lethal cancer phenotype[16–26]. In addition, a number of factors that are crucial for the successful implementation of a research autopsy programme have been identified, including scientific infrastructure, programme coordination and clinical support (BOX 1). Ultimately, a complete discussion of methods used by all programmes is difficult owing to the lack of published information regarding each programme's infrastructure. For this reason, we suggest that all programmes publish their experiences for the benefit of the scientific community.

In this Review, we discuss the utility and caveats of using post-mortem tissues for cancer research and describe the scientific insights generated from the research autopsy approach that have shaped our understanding of cancer biology, notably tumour evolution and metastasis. Finally, we discuss opportunities for using research autopsies to inform precision medicine efforts and address fundamental questions in cancer biology that have been curtailed thus far by limited access to the relevant human tissues.

Post-mortem tissues for cancer research

Collectively, the word post-mortem conjures up a number of unsubstantiated misconceptions about the quality of cancer tissues obtained from research autopsies. One common misconception regarding post-mortem tissues relates to the relationship between the length of time between death and tissue collection, known as the post-mortem interval (PMI), and tissue quality. In the clinical autopsy setting, a PMI of 24h or more is not uncommon. Owing to such concerns about tissue quality, many research autopsy programmes have aimed to perform the procedure as quickly as possible after death, leading to use of the terms rapid autopsy and warm autopsy to emphasize the shorter PMIs (anywhere from 1h to 6h) than for a clinical autopsy[14,15,24]. Although the assumption that a shorter PMI would improve tissue quality is reasonable given that it is based on the experience in handling resected tissues and biopsy samples from living patients[27], relatively limited data exist specifically within the context of material acquired after death[3,28]. For these reasons, we advocate use of the term research autopsy in place of the terms rapid autopsy and warm autopsy to increase emphasis on the goal of the procedure and remove any unconscious biases related to the time taken to perform it.

The definition of death itself is a profoundly complex issue that varies depending on ethical, legal and medical standards. However, for the purposes of this Review, we define death as the cessation of cardiopulmonary function[29–31]. Pozhitkov and Noble refer to the 48-h period after cardiac arrest as the 'twilight of death' owing to the fact that organs, tissues and cells remain functional during this period, as measured by the ongoing changes that occur in gene expression, which largely reflect a stress response to oxygen deprivation[32,33]. Although cellular death can occur within a living organism through a number of mechanisms[34], the death of the organism by cardiopulmonary arrest does not immediately lead to death of its cells[31,32,35]. This point is exemplified by rigor mortis, whereby skeletal muscle continues to metabolize ATP until all reserves are depleted[36], and forms the basis for why organ and tissue donation from deceased individuals is possible[37] and for the creation of patient-

derived cell lines, xenografts or organoid models for therapeutic testing[38–41]. Resection or biopsy specimens are no different, as the constituent cells remain viable and representative of their function in vivo for some time after removal from the body despite the loss of tissue perfusion and nutrient supply[33]. Thus, the major difference between post-mortem tissues and tissues from living patients relates to the extent that biomarkers of the stress response to oxygen and nutrient deprivation have changed before long-term preservation. Although this has not specifically been studied in a formal manner in tissues other than the brain[42], it remains conceivable that the PMI influences tumour cell-intrinsic variations in hypoxia and metabolic signalling pathways[43] and the biological features of immune infiltrates within the tumour microenvironment[44,45].

The effect of PMI on the ability to obtain meaningful data from molecular analyses has been addressed in the forensic sciences[46,47]. In this branch of investigation, the tissues obtained from deceased individuals might be exposed to prolonged periods of temperature flucuation and to excessive moisture and/or animal scavenging in addition to a prolonged PMI[48]. Despite these extreme conditions, the wealth of analytes gleaned from severely compromised tissues are impressive and include native DNA, methylated DNA, mitochondrial DNA, RNA, microRNA and microbial swabs[47,49–54]. However, such data are not a comparable reference point to that of patients with cancer who die at home, in a hospice or in a hospital, all of which have comparable ambient temperatures and humidity levels[55,56]. Cancer-related deaths are also poorly represented within forensic autopsy series[57,58]. A more comparative cohort of patients are those included in the Genotype-Tissue Expression (GTEx) project[59]. The GTEx Consortium has aimed to establish a resource database and associated tissue bank to study the relationship between genetic variation and tissue-specific gene expression in individuals who died of causes other than cancer. An important aspect of this tissue bank is that most samples were collected in the post-mortem setting, including blood for generation of lymphoblastoid cell lines and skin for fibroblast culturing. Although the presence of metastatic cancer or chemotherapy or radiotherapy within 2 years before death were exclusion criteria for the GTEx project, PMIs up to 24h were permitted[59]. Subsequent studies using the GTEx bank confirmed that RNA integrity, as reflected by RNA integrity number values, decreased with increasing PMI, and that no additional loss of RNA integrity occurred with PMIs of more than 12 h[28]. Although transcriptional signatures of the physiological stress of death are probably present, they have not interfered with the ultimate goal of the GTEx project, which is to understand how expression quantitative trait loci control gene expression[59,60]. The strongest evidence supporting the use of these post-mortem tissues is their contributions to impactful science related to tissue-specific gene expression[61–65]. Thus, it should not be unexpected that fairly high-quality DNA, RNA or protein can often be obtained from post-mortem tissues from patients with cancer with PMIs of more than 6h (reviewed in detail in REFS[66,67]). Like all well-designed research studies, an understanding of the needs of the project, the analyses to be performed and the extent that the PMI, mode of death or tissue type of interest will bias the results should be considered. As astutely summarized by Grizzle et al.[33], investigators should consider whether the tissues are fit for the intended purpose.

Further evidence supporting the quality of post-mortem tissues for use in cancer research is that xenograft, cell line or organoid models have been successfully established[14,15,24,68–70].

Although minimizing PMI facilitates the ability to create immortalized models likely by limiting the extent of cell death in the sample, biological features inherent to different tumour types also have a major role. For example, prostate cancer patient-derived xenografts (PDXs) have proven difficult to establish despite minimal PMIs and use of methodical techniques, with reported rates of engraftment of 5–10%[14,71]. Up to 18 months after implantation might be required for visible growth of metastatic prostate cancer PDXs in immuno-deficient mice[71]. By contrast, with use of similar methods, post-mortem tissues taken from pancreatic cancer metastases (all taken with a PMI of less than 6h) have demonstrated a 57% success rate for PDX formation, with visible growth typically seen within 2 months of implantation[68]. For some patients, a relatively long PMI (more than 16h) might inevitably occur, during which the remains are refrigerated at 4°C (REF.[68]). However, once in a cold-controlled environment, autolytic processes have a delayed onset and progress at a slow rate[72]. Thus, in cases in which the deceased person has spent most of the post-mortem period in refrigeration, long PMIs might conceivably facilitate retention of a level of cell viability and tissue integrity comparable to that of tissues obtained with short PMIs. In support of this notion, we note one exemplar deceased person with metastatic urothelial carcinoma in the Memorial Sloan Kettering Cancer Center Last Wish Program from whom a xenograft was established despite a PMI of more than 48h and the deceased person spending most of the post-mortem period in refrigeration (personal communication, M. Mattar, Memorial Sloan Kettering Cancer Center, USA). In some instances, only poor-quality biomolecules can be isolated from tissues collected within 1h of death[73]. In the Johns Hopkins Gastrointestinal Cancer Rapid Medical Donation Program and other experiences with brain banking programmes, this scenario is highly likely to be encountered in patients who die of a systemic infection or agonal changes in association with multiorgan dysfunction[3,73–75]. We have also noted that the integrity of biomolecules can be higher in tumour tissues than in matched normal tissues from the same patient[73], and that metastases are more likely to engraft in mice than are primary tumours[14,68], potentially reflecting the higher fitness of aggressive tumour cells in hypoxic environments after death[76,77]. Collectively, these data indicate that perimortem factors and the length of time the patient's body has been refrigerated are as influential to tissue quality as the PMI. Moreover, while we acknowledge that PMI is inversely correlated with biomolecule integrity, so many exceptions to this rule exist that it is tenuous at best.

Research autopsies and cancer

Since the first reports of autopsies on patients with cancer for translational research purposes[14,15], a range of research autopsy programmes have been developed and have yielded key insights into cancer biology — notably the dynamics of cancer evolution, early stages of carcinogenesis and therapeutic resistance — and have also facilitated the development of model systems[16–26]. This experience has also allowed refinement of research autopsy programmes, including the identification of factors that are crucial for their successful implementation (BOX 1).

Evolutionary dynamics.

Malignant neoplasms are composed of populations of cells that are subject to genetic drift, selection or competition for resources within a dynamic microenvironment[78–80]. Thus, mathematical theories developed to understand population genetics since Darwin's time are also applicable to understanding the diversity of cancer cells within a patient's body[80–84]. Metastasis is the most ominous consequence of the clonal evolution of a neoplasm and the most common cause of cancer-related death, making the research autopsy paramount to understanding evolutionary dynamics with respect to metastatic spread[85].

Research autopsies are a powerful method for studying the evolutionary biology of cancer for two reasons. First, they support multiregion (spatial) sampling to a degree that is not otherwise possible (FIG. 1). In addition to broad sampling of the primary tumour and metastases, multiple normal tissues might also be sampled and biofluids collected. The large size of each sample allows its processing for multiple downstream analyses of the same tissue, banking for future studies and distribution to the scientific community; this paradigm has been well exemplified by the GTEx Consortium[59]. Second, multiregion sampling allows 'sampling to completion' (FIG. 2), which we define as the minimum number of samples required to reasonably eliminate false negative or false positive conclusions, cognizant that the minimum number of samples required to achieve high confidence probably varies according to the question, method, tumour type and, potentially, stage of disease. At its most extreme, sampling to completion would entail deep multi-omic single-cell interrogation of all cells of the neoplasm in a single patient, including stromal and immune elements of the tumour microenvironment, to investigate genotype-phenotype correlations. Furthermore, within that patient, the neoplasm might colonize one or more sites beyond the primary tumour, including diverse metastatic sites and dormant cells in one or more tissues or organs. Accounting for potential systemic spread would, therefore, necessitate analysis of virtually all cells in the body, a feat that is technically unfeasible but theoretically possible in an autopsied patient. In reality, depending on a patient's disease burden and tumour type, the total number of cancer samples collected at autopsy might range from less than ten (for example, for a patient with a brainstem glioma) to more than 100 (for example, for a patient with metastatic pancreatic cancer). Although minimally invasive autopsies might have a role in clinical autopsies and the molecular quality appears high on the basis of RNA integrity[2,86], they would not suffice for the goals of a research autopsy for the aforementioned reasons.

Multiregion sampling is not limited to deceased patients. However, in living patients, a major factor for consideration is the tumour type and surgical procedure; for example, in clear cell renal cell carcinoma (ccRCC), high-grade serous ovarian cancer or colorectal cancer, the primary tumour and/or matched locoregional metastases are all exposed within the resection bed, allowing multiregion sampling during surgical resection[87–92]. However, for most tumour types, the research autopsy is the sole method for sampling metastases from diverse anatomical sites. In support of this concept, post-mortem multiregion sampling has provided the context with which to understand the genomic features of rapidly progressive ccRCCs in patients who were not candidates for radical nephrectomy[26]. Multiregion sampling is distinct from temporal (longitudinal) sampling, in which the same neoplasm is

biopsied over one or more intervals of time[24,93]. When these are performed together in the same patient, more robust inferences of the clonal dynamics of the neoplasm can be determined (FIG. 3).

Evolutionary features gleaned from multiregion DNA sequencing of surgically resected or autopsy samples have begun to illustrate the spatial and temporal complexity of the metastatic cancer genome[79]. In carcinomas of the breast, colon, pancreas, prostate, kidney, bladder and skin (melanoma), metastases have been found to originate from one of two patterns — a monophyletic pattern, in which all metastases in the patient derived from one common ancestor, or a polyphyletic pattern, in which multiple divergent subclones gave rise to the metastases in the patient[17,19,24,26,91,93–96]. In some instances, both patterns are present across different patients within a single tumour type[92]. Polyphyletic metastases might arise from multiple independent seeding events of a subclonal population over a period of time, or from spatially distinct subclones within the same primary tumour[92,95]. Furthermore, independent of their clonal origin, metastases might continuously seed other metastases, thereby adding to the diversity of cancer cell populations at each site[19,92]. In other tumour types, such as colorectal cancer, evolutionary patterns suggest a neutral mode of evolution, in which somatic alterations occur early, followed by expansile growth and subclonal mixing in the absence of stringent selection[97,98]. Although these aforementioned studies relied on somatic mutations to infer phylogenies, patterns of DNA methylation have also revealed features of the metastatic cancer genome. For example, a study of promoter hypermethylation of genes known to have roles in breast cancer biology revealed no appreciable differences in hypermethylation profiles between primary breast cancers and matched metastases from research autopsies[25]. In metastatic prostate cancers sampled during research autopsies, DNA hypermethylation patterns also seem to be highly concordant, whereas DNA hypomethylation occurs later during clonal evolution and is heterogeneous across metastatic sites[23,99].

These observations raise two points of interest. First, it seems that a range of evolutionary trajectories leading to lethal metastasis exist within and across tumour types[26,91,92,100]. Although the determinants of evolutionary trajectories have yet to be elucidated in a comprehensive manner using longitudinal sampling in association with multiregion sequencing or research autopsies, clues are afforded by a retrospective pan-cancer analysis of matched treatment-naive metastases derived from a variety of solid tumour types[101]. In this study, most driver gene mutations were found to be common to all metastases in the patient, and those that were not shared by all metastases were not predicted to have functional consequences. An alternative perspective is provided by data generated by TRACERx Renal, a prospective study aiming to define the spatial and temporal evolutionary trajectories of ccRCC — a neoplasm with a broad range of metastatic phenotypes and clinical outcomes[102] — through multiregion research autopsy and longitudinal tumour sampling[26,100]. In a comprehensive analysis of the evolutionary patterns associated with ccRCC metastasis from the TRACERx Renal Consortium, a small subset of patients were identified whose tumours were resistant to therapy and metastasized in an aggressive manner[26]. The phylogenies of these ccRCCs were notable for clonal driver gene mutations and low intratumoural heterogeneity, unlike most ccRCCs, which are often defined by branched evolution and intratumoural heterogeneity for one or more driver genes[26,87,100].

These aggressive forms of ccRCC parallel observations reported for metastatic pancreatic cancer[103]. For example, pancreatic cancer is known for its rapidly progressive clinical course, high metastatic propensity and therapeutic resistance, which collectively result in low overall survival[104]. Whole-genome sequencing of matched primary and metastatic samples from research autopsy participants with pancreatic cancer revealed a remarkably low degree of intratumoural heterogeneity for driver or passenger gene mutations, both within the primary tumour and across metastases, suggesting that a swift clonal sweep occurred early in the natural history of the neoplasm and before metastatic dissemination[103]. Although these examples demonstrate how the spectrum and timing of driver gene mutations might contribute to aggressive behaviour, many other cell-intrinsic and cell-extrinsic factors probably also have a role, including the tumour microenvironment, metabolic processes and the extent of immune infiltration and immunoediting[78,105].

Understanding carcinogenesis.

Multiregion sampling in living and deceased patients has also supported study of the earliest stages of cancer development. For example, mathematical analysis of deep sequencing data generated from multiregion-sampled ccRCCs and colorectal cancers from surgery, or pancreatic cancers and wild-type *IDH* glioblastomas from research autopsies, indicates that these neoplasms arise years before clinically evident disease and diagnosis[95,106–108]. Furthermore, sequencing of discrete incidental serous tubal intraepithelial lesions of the fallopian tube in hysterectomy specimens, the precursor to high-grade serous ovarian carcinoma, revealed diverse clonal origins of tubal precursor lesions at the very early stages of tumorigenesis and that an estimated minimum of two decades is required for progression to intraepithelial carcinoma[109]. Investigation of the clonal relationships between precursor lesions and invasive cancers in the same patient has also been informative. On the basis of microscopic review of the entirely submitted pancreas in 173 consecutive autopsies with no evidence of a pancreatic neoplasm[110], the incidence and relative distribution of pancreatic intraepithelial neoplasias of different grades within the pancreas was quantified. Grade 1, grade 2 and grade 3 lesions were found in 77%, 28% and 4% of patients, respectively. However, unlike grade 1 and grade 2 lesions, which were always localized to one discrete region of a pancreatic duct, grade 3 lesions were multifocal. These data buttressed a subsequent analysis of multiregion-sampled pancreatic intraepithelial neoplasias and matched pancreatic cancers, which indicated that high-grade pancreatic intraepithelial neoplasias can migrate and colonize the ductal system, thereby providing an explanation for the high rates of local recurrence after pancreaticoduodenectomy[111,112]. In explanted livers from patients with cirrhosis, multiregional sequencing of spatially distinct regenerative nodules indicated that they contain mutations in cancer-related genes such as *ARID1A* and *TP53* (REF.[89]). However, *TERT* promoter mutations in association with mutations in cancer-related genes were seen only in synchronous hepatocellular carcinomas in the same patient, suggesting that *TERT* mutations are required for hepatic carcinogenesis.

Finally, multiregion sampling of normal tissues in the same patient has begun to elucidate the extent and dynamics of somatic mosaicism. By multiregion sampling of the normal oesophagus from nine organ donors, the number and size of mutant clones were mapped, which indicated the presence of tens to hundreds of clones within a single square centimetre

of squamous epithelium[113]. Unexpectedly, the rate of *NOTCH1*-mutant clones in the normal oesophagus was higher than reported for oesophageal cancers, indicating that the role of *NOTCH1* as a tumour suppressor gene in this tumour type is less understood than previously believed. In 2019, the extent and features of somatic clonal expansions in normal tissues from GTEx resources were reported on the basis of RNA sequencing data[114]. Mutational burden was positively associated with both age and the tissue-specific cell proliferation rate, indicating that mutations accumulate over time and in association with the number of cell divisions. Mutations in known cancer gene hotspots were also found across multiple tissues[114].

Collectively, these studies emphasize that the utility of post-mortem tissues for scientific inquiry extends beyond the biology of late-stage disease to the mechanisms of early-stage carcinogenesis.

Understanding treatment resistance.

Another major contribution of the research autopsy has been in our understanding of how a neoplasm adapts to the selective pressures imposed by therapy, specifically by parallel and convergent evolution towards the treatment-resistant genotype.

Next-generation sequencing of a primary lung tumour and multiple lymph node metastases derived after death from a patient with an exceptional response to various human epidermal growth factor receptor 2 (HER2; also known as ERBB2)-directed therapies revealed a loss-of-function *CDK12* mutation in the primary tumour that conferred increased sensitivity to adjuvant chemotherapy, potentially accounting for the favourable response observed[115]. By contrast, an *ERBB2*^{L869R} mutation was identified in one of seven lymph node metastases, and was confirmed by functional studies to have oncogenic potential and confer resistance to ERBB2-directed therapies, suggesting a subclone containing this variant was selected for by the therapeutic regimen[115]. In another study, in a patient with *EGFR*/*ERBB2*-coamplified gastroesophageal carcinoma who was resistant to the second-generation EGFR tyrosine kinase inhibitor afatinib, the progressive disease (metastasis) sampled after death showed treatment-induced selection for a subclone that had lost *EGFR* amplification compared with pretreatment biopsy samples[116].

A number of interesting findings have also been reported in breast cancer. In a patient with metastatic breast cancer containing a *PIK3CA*-activating mutation who was initially responsive to the PI3K p110α inhibitor BYL719, resistance was associated with biallelic *PTEN* loss in post-mortem tissues from all metastatic sites analysed compared with pretreatment biopsy samples[117]. One *PTEN* allele was lost in all metastases, whereas six different genetic alterations affecting the remaining *PTEN* allele were found across 14 metastases analysed, indicating that the mechanism of resistance in this patient was parallel evolution in independent metastases that converged on loss of PTEN expression. In a patient with metastatic hormone receptor-positive breast cancer who developed resistance to hormonal therapy, multiple alterations in genes associated with hormone resistance were identified compared with the pre-mortem biopsy samples, including a missense mutation in *ESR1* and three different *ERBB2* missense mutations, each of which was present in unique metastatic sites[21].

In addition to targeted therapies, research autopsies have also revealed mechanisms of resistance to chemotherapy. Indeed, a study of the mechanisms of resistance to platinum-based chemotherapy in urothelial carcinoma revealed enrichment for mutations in the gene encoding neural cell adhesion molecule L1 (L1CAM) and in integrin signalling pathway genes compared with pretreatment samples from the same patients[93]. Furthermore, ongoing clonal evolution was found to be shaped by apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like (APOBEC)-induced mutagenesis.

Investigation of mechanisms of resistance to immune checkpoint blockade is another topic of tremendous interest for which research autopsies have a role[118]. In a patient with widely metastatic melanoma who had a mixed response to the anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) antibody ipilimumab in his cutaneous metastases, no differences were found in immune infiltration, infiltrate density or infiltrate composition within responding and progressing skin metastases sampled after death[96]. However, as the effects of post-mortem stress and/or inflammatory responses on the immune contexture are unknown, the interpretation of whether this finding is representative of the host or an is artefact is unclear. Moreover, by whole-exome sequencing and phylogenetic analysis, all cutaneous metastases studied in this patient were found to be descendants of a common ancestral clone and had a high degree of genetic similarity, indicating that protein-coding genetic mechanisms alone do not fully account for the development of resistance.

Model systems.

Research autopsies have an important role in addressing fundamental needs in cancer biology. For example, in tumour types such as pancreatic cancer or prostate cancer, surgical management has a limited role in advanced-stage disease[119,120], which has led to a bias towards using surgically resected primary tumours for study. This bias is particularly strong for pancreatic cancer as only a minority of patients are surgical candidates[121]. Research autopsies have not only served as a means to obtain advanced-stage tissue from patients with these tumour types but have also facilitated the creation of cell lines and PDX models[14,68,71,122]. In other instances, investigation of rare tumour types might be limited by an overall lack of tissue to support research endeavours, which is a particular issue in paediatric oncology. As soft-tissue sarcomas such as rhabdomyosarcoma, the most common paediatric soft-tissue tumour, are uncommon[123], the collected tissues are often small biopsy samples of the primary tumour, highlighting the need for alternative methods of collection for metastatic and post-treatment disease[85,124,125]. Research autopsies have fulfilled this need by provision of ample tissues per patient from the primary and metastatic sites as well as the creation of PDX models for preclinical study[24,69]. By contrast, for diffuse intrinsic pontine glioma, a particularly rare type of paediatric tumour, pre-mortem diagnostic tissues are not available at all because a biopsy cannot be performed without putting the patient at major risk of neurological damage owing to the tumour’s location in the brainstem. Cell lines and PDXs have now been established from research autopsy materials from paediatric patients with diffuse intrinsic pontine glioma[24,126,127], thereby illustrating how research autopsies are a crucial resource for the research community.

A look to the future

A rationale that is often expressed by clinical oncologists is that the need to perform an autopsy in a patient with cancer is unnecessary because the cause of death is obvious[124,128]. In our view, this reasoning is entirely incorrect. Autopsies are unsurpassed for assessing the sensitivity and specificity of clinical diagnoses, a concept that Morgagni himself realized more than 250 years ago[6,128]. Large-scale studies spanning multiple institutions and involving thousands of autopsies have consistently indicated that more than 24% of hospital-based autopsies reveal unsuspected findings, and physicians cannot predict which of their cases will yield the unexpected results[129,130]. In light of the utility of research autopsies for understanding how cancer cells resist treatment and the expansive types of analyses that can be performed on post-mortem tissues, it stands to reason that autopsies pose an untapped opportunity for precision treatment of patients with cancer.

A practical example of how autopsies can fit into precision medicine strategies is with an adaptive clinical trial design (also known as a flexible design)[131]. Adaptive design refers to a clinical trial that uses data generated during the trial to inform prespecified modifications of the ongoing study, without undermining the validity and integrity of the trial[132]. Although ten types of adaptive trial design have been developed[133], those that rely on a biomarker-adaptive design are best suited to the incorporation of autopsy for patients who progress while receiving treatment (FIG. 4). In such a clinical trial design, consent would include the option of biopsies at the time of progression and willingness to participate in a research autopsy. The Cancer Tissue Collection After Death (CASCADE) and Posthumous Evaluation of Advanced Cancer Environment (PEACE) studies are currently operating programmes in this manner that are specific to patients with advanced or metastatic cancer[24,26]. Although the autopsy does not benefit the patient directly, it does ‘pay forward’ to subsequent patients by contributing knowledge and insight into why the treatment failed. In turn, this information would promote modification of the trial design and treatment schedule and/or incorporation of newly identified biomarkers identified using the autopsies to prolong disease-free survival in subsequently enrolled patients.

Research autopsies could and should be used with greater frequency and enthusiasm to support investigation of fundamental research questions in cancer biology. Examples of such questions include human cell and tumour atlas efforts aiming to decode all cells in the human body at the gross, macroscopic, microscopic and subcellular levels[134]. The large amount of tissues needed for these efforts to allow sufficient quality control and minimize interindividual heterogeneity is ideally suited to a research autopsy. Another important issue relates to tumour dormancy[135]. For example, as outcomes continue to improve and overall survival continues to increase in association with durable responses to new therapies in patients with systemic malignancies, the incidence of brain metastasis as a late complication is likely to increase[136]. What remains unknown is whether dormant cells universally exist in all organs or whether some tissues are more permissive of the dormant state[26,137]. By way of complete sampling of an entire organ (or all organs in a series of individuals), this admittedly tedious approach could nonetheless provide once and for all a reference of the number of dormant tumour cells in the body, thereby supporting further mechanistic studies into the biology of sanctuary sites.

Finally, the increasing complexity of datasets generated by multiregion analyses will necessitate the development of computational tools to deconvolute these data and integrate them within and across patients[138,139] (FIG. 5). A variety of tools have already been developed specifically to address these requirements for multiregion sampling datasets, predominantly with respect to phylogenetic analyses[140,141], subclonal structures[142,143] or intermetastatic seeding[144]. The GTEx project illustrates how a similar post-mortem tissue dataset has supported the development of analytical methods[145–147]. Although the GTEx project was predominantly developed to investigate genetic variation and its relationship with gene expression in normal tissues[59], it provides a robust framework that can be applied to similar studies of tissues from patients with end-stage, treatment-refractory cancer. In particular, the data that have already been generated by the GTEx project poise investigators to determine the extent that biomarkers of the tumour microenvironment or inflammatory responses reflect intrinsic features of the host or neoplasm versus an artefact of post-mortem stress. For example, as T cell functions are highly influenced by the extent of hypoxia or glucose availablility[44,45], studies of the immune microenvironment using research autopsies that are not performed with very short PMIs (1–2 h) should be interpreted with caution.

Conclusions

In conclusion, the research autopsy is an underused approach to investigate the fundamental questions in cancer biology and holds tremendous potential to inform precision medicine strategies (BOX 2). Tissues obtained in the post-mortem setting from patients with cancer represent an invaluable yet still untapped opportunity to understand the evolutionary biology of cancer and the dynamics of carcinogenesis, inform the creation of model systems and guide therapeutic development. Indeed, post-mortem tissues offer a number of key advantages over other types of biospecimens, such as diagnostic biopsy tissues or resection specimens, including the large amount of tissue that can be obtained and the extent of multiregion sampling that is achievable.

When feasible, all patients with cancer should be offered the option to participate in a research autopsy programme. From the patient perspective, participation in a research autopsy programme provides a psychological benefit at the end of life, yet clinicians remain hesitant to obtain consent for this procedure[148,149]. Methods to increase accrual to autopsy programmes include a formal logistical framework for identifying patients, providing training to caregivers on initiating the consent conversation in a sensitive manner and providing emotional support to patients, families and clinicians alike[150] (BOX 1). These efforts are expected to positively influence end-of-life care in general while supporting state-of-the-art cancer research.

Acknowledgements

The authors acknowledge grant support from the US National Institutes of Health (grants R01 CA179991 and R35 CA220508 to C.I.A.-D) and salary support from the Parker Institute for Cancer Immunotherapy to T.J.H.

References

1. Buja LM, Barth RF, Krueger GR, Brodsky SV & Hunter RL The importance of the autopsy in medicine: perspectives of pathology colleagues. Acad. Pathol 6, 2374289519834041 (2019).
2. Blokker BM et al. Conventional autopsy versus minimally invasive autopsy with postmortem MRI, CT, and CT-guided biopsy: comparison of diagnostic performance. Radiology 289, 658–667 (2018). [PubMed: 30251930]
3. Kretzschmar H Brain banking: opportunities, challenges and meaning for the future. Nat Rev. Neurosci 10, 70–78 (2009). [PubMed: 19050713]
4. Hajdu SI A note from history: the first printed case reports of cancer. Cancer 116, 2493–2498 (2010). [PubMed: 20225228]
5. Mariette C et al. Consensus on the pathological definition and classification of poorly cohesive gastric carcinoma. Gastric Cancer 22, 1–9 (2019). [PubMed: 30167905]
6. Ghosh SK Giovanni Battista Morgagni (1682–1771): father of pathologic anatomy and pioneer of modern medicine. Anat. Sci. Int 92, 305–312 (2017). [PubMed: 27629485]
7. Paget S The distribution of secondary growths in cancer of the breast. Lancet 133, 571–573 (1889).A seminal large post-mortem study of patients with breast cancer that led to the seed and soil hypothesis of metastasis.
8. Fidler IJ Metastasis: quantitative analysis of distribution and fate of tumor emboli labeled with ^{125}I-5-iodo-2′-deoxyuridine. J. Natl. Cancer Inst 45, 773–782 (1970). [PubMed: 5513503] The first study to provide experimental support for the seed and soil hypothesis.
9. Fidler IJ & Kripke ML Metastasis results from preexisting variant cells within a malignant tumor. Science 197, 893–895 (1977). [PubMed: 887927]
10. Minn AJ et al. Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. J. Clin. Invest 115, 44–55 (2005). [PubMed: 15630443]
11. Minn AJ et al. Lung metastasis genes couple breast tumor size and metastatic spread. Proc. Natl Acad. Sci. USA 104, 6740–6745 (2007). [PubMed: 17420468]
12. Minn AJ et al. Genes that mediate breast cancer metastasis to lung. Nature 436, 518–524 (2005). [PubMed: 16049480]
13. Bos PD et al. Genes that mediate breast cancer metastasis to the brain. Nature 459, 1005–1009 (2009). [PubMed: 19421193] This study and Minn et al. (*J. Clin. Invest.*, 2005), Minn et al. (*Proc. Natl Acad. Sci. USA*, 2007) and Minn et al. (*Nature*, 2005) are the first studies to illustrate the molecular mechanisms of organotropism of breast cancer metastasis.
14. Rubin MA et al. Rapid ('warm') autopsy study for procurement of metastatic prostate cancer. Clin. Cancer Res 6, 1038–1045 (2000). [PubMed: 10741732] The first published report describing the creation of a PDX from post-mortem tissue obtained from a patient with prostate cancer.
15. Morrissey C et al. Effects of androgen deprivation therapy and bisphosphonate treatment on bone in patients with metastatic castration-resistant prostate cancer: results from the University of Washington rapid autopsy series. J. Bone Miner. Res 28, 333–340 (2013). [PubMed: 22936276]
16. lacobuzio-Donahue CA et al. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. J. Clin. Oncol 27, 1806–1813 (2009). [PubMed: 19273710]
17. Liu W et al. Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. Nat Med. 15, 559–565 (2009). [PubMed: 19363497]
18. Abbosh C et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature 545, 446–451 (2017). [PubMed: 28445469]
19. Gundem G et al. The evolutionary history of lethal metastatic prostate cancer. Nature 520, 353–357 (2015). [PubMed: 25830880]
20. Savas P et al. The subclonal architecture of metastatic breast cancer: results from a prospective community-based rapid autopsy program "CASCADE". PLOS Med. 13, e1002204 (2016). [PubMed: 28027312]
21. Razavi P et al. The genomic landscape of endocrine-resistant advanced breast cancers. Cancer Cell 34, 427–438.e6 (2018). [PubMed: 30205045]

22. Yegnasubramanian S et al. DNA hypomethylation arises later in prostate cancer progression than CpG island hypermethylation and contributes to metastatic tumor heterogeneity. Cancer Res. 68, 8954–8967 (2008). [PubMed: 18974140]
23. Yegnasubramanian S et al. Hypermethylation of CpG islands in primary and metastatic human prostate cancer. Cancer Res. 64, 1975–1986 (2004). [PubMed: 15026333]
24. Pisapia DJ et al. Next-generation rapid autopsies enable tumor evolution tracking and generation of preclinical models. JCO Precis. Oncol 2017, 1–13 (2017).
25. Wu JM et al. Heterogeneity of breast cancer metastases: comparison of therapeutic target expression and promoter methylation between primary tumors and their multifocal metastases. Clin. Cancer Res 14, 1938–1946 (2008). [PubMed: 18381931]
26. Turajlic S et al. Tracking cancer evolution reveals constrained routes to metastases: TRACERx Renal. Cell 173, 581–594 (2018). [PubMed: 29656895]
27. Vaught J Biobanking comes of age: the transition to biospecimen science. Annu. Rev. Pharmacol. Toxicol 56, 211–228 (2016). [PubMed: 26514206]
28. Carithers LJ et al. A novel approach to high-quality postmortem tissue procurement: The GTEx Project. Biopreserv. Biobank 13, 311–319 (2015). [PubMed: 26484571] Description of the GTEx project, which is designed to support understanding of the relationship between genomic variation and gene expression based on a large series of post-mortem-collected tissues.
29. Farrell MM & Levin DL Brain death in the pediatric patient: historical, sociological, medical, religious, cultural, legal, and ethical considerations. Crit. Care Med 21, 1951–1965 (1993). [PubMed: 8252903]
30. De Georgia MA History of brain death as death: 1968 to the present. J. Crit. Care 29, 673–678 (2014). [PubMed: 24930367]
31. Jakušovaitė I et al. Determination of death: metaphysical and biomedical discourse. Med. 52, 205–210 (2016).
32. Pozhitkov AE & Noble PA Gene expression in the twilight of death. BioEssays 39, 1700066 (2017).
33. Grizzle WE, Otali D, Sexton KC & Atherton DS Effects of cold ischemia on gene expression: a review and commentary. Biopreserv. Biobank 14, 548–558 (2016). [PubMed: 27551929]
34. Green DR & Llambi F Cell death signaling. Cold Spring Harb. Perspect. Biol 7, a006080 (2015). [PubMed: 26626938]
35. Shemie SD & Gardiner D Circulatory arrest, brain arrest and death determination. Front. Cardiovasc. Med 13, 15 (2018).
36. Bate-Smith EC & Bendall JR Factors determining the time course of rigor mortis. J. Physiol 110, 47–65 (1949). [PubMed: 15406381]
37. Goldberg D et al. Changing metrics of organ procurement organization performance in order to increase organ donation rates in the United States. Am. J. Transplant 17, 3183–3192 (2017). [PubMed: 28726327]
38. Jimeno A et al. A direct pancreatic cancer xenograft model as a platform for cancer stem cell therapeutic development. Mol. Cancer Ther 8, 310–314 (2009). [PubMed: 19174553]
39. Tiriac H et al. Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. Cancer Discov. 8, 1112–1129 (2018). [PubMed: 29853643]
40. Pauli C et al. Personalized in vitro and in vivo cancer models to guide precision medicine. Cancer Discov. 7, 462–477 (2017). [PubMed: 28331002]
41. Aguirre AJ et al. Real-time genomic characterization of advanced pancreatic cancer to enable precision medicine. Cancer Discov. 8, 1096–1111 (2018). [PubMed: 29903880]
42. Li JZ et al. Sample matching by inferred agonal stress in gene expression analyses of the brain. BMC Genomics 8, 336 (2007). [PubMed: 17892578]
43. Lee DC et al. A lactate-induced response to hypoxia. Cell 161, 595–609 (2015). [PubMed: 25892225]
44. Xu Y et al. Glycolysis determines dichotomous regulation of T cell subsets in hypoxia. J. Clin. Invest 126, 2678–2688 (2016). [PubMed: 27294526]

45. Lawless SJ et al. Glucose represses dendritic cell-induced T cell responses. Not. Commun 8, 15620 (2017).
46. Bär W, Kratzer A, Mächler M & Schmid W Postmortem stability of DNA. Forensic Sci. Int 39, 59–70 (1988). [PubMed: 2905319]
47. Sijen T Molecular approaches for forensic cell type identification: on mRNA, miRNA, DNA methylation and microbial markers. Forensic Sci. Int. Genet 18 September, 21–32 (2015). [PubMed: 25488609]
48. Vass A A The elusive universal post-mortem interval formula. Forensic Sci. Int 204, 34–40 (2011). [PubMed: 20554133]
49. Dumache R, Ciocan V, Muresan C, Rogobete AF & Enache A Circulating microRNAs as promising biomarkers in forensic body fluids identification. Clin. Lab 61, 1129–1135 (2015). [PubMed: 26554231]
50. Freire-Aradas A, Phillips C & Lareu MV Forensic individual age estimation with DNA: From initial approaches to methylation tests. Forensic Sci. Rev 29, 121–144 (2017). [PubMed: 28691915]
51. Woerner AE et al. Forensic human identification with targeted microbiome markers using nearest neighbor classification. Forensic Sci. Int. Genet 38 Jan, 130–139 (2019). [PubMed: 30396009]
52. Van den Berge M, Wiskerke D, Gerretsen RRR, Tabak J & Sijen T DNA and RNA profiling of excavated human remains with varying postmortem intervals. Int. J. Leg. Med 130, 1471–1480 (2016).
53. Bauer M RNA in forensic science. Forensic Sci. Int. Genet 1, 69–74 (2007). [PubMed: 19083730]
54. Budowle B, Schmedes SE & Wendt FR Increasing the reach of forensic genetics with massively parallel sequencing. Forensic Sci. Med. Pathol 13, 342–349 (2017). [PubMed: 28631109]
55. Hunt RW, D'Onise K, Nguyen AMT & Venugopal K Where patients with cancer die: a population-based study, 1990 to 2012. J. Palliat. Care 11 28, 825859718814813 (2018).
56. Gao W et al. A population-based conceptual framework for evaluating the role of healthcare services in place of death. Healthcare 6, 107 (2018).
57. Bryant VA et al. Childhood neoplasms presenting at autopsy: a 20-year experience. Pediatr. Blood Cancer 64, e26474 (2017).
58. Suzuki H, Tanifuji T, Abe N & Fukunaga T Causes of death in forensic autopsy cases of malnourished persons. Leg. Med 15, 7–11 (2013).
59. Carithers LJ & Moore HM The genotype-tissue expression (GTEx) project. Biopreserv. Biobank 13, 307–308 (2015). [PubMed: 26484569]
60. Wainberg M et al. Opportunities and challenges for transcriptome-wide association studies. Nat. Genet 51, 592–599 (2019). [PubMed: 30926968]
61. Rivas MA et al. Effect of predicted protein-truncating genetic variants on the human transcriptome. Science 348, 666–669 (2015). [PubMed: 25954003]
62. Mohammadi P, Castel SE, Brown AA & Lappalainen T Quantifying the regulatory effect size of cis-acting genetic variation using allelic fold change. Genome Res. 27, 1859–1871 (2017). [PubMed: 29021290]
63. Melé M et al. The human transcriptome across tissues and individuals. Science 348, 660–665 (2015). [PubMed: 25954002]
64. Tukiainen T et al. Landscape of X chromosome inactivation across human tissues. Nat. 550, 244–248 (2017).
65. Saha A et al. Co-expression networks reveal the tissue-specific regulation of transcription and splicing. Genome Res. 27, 1843–1858 (2017). [PubMed: 29021288]
66. Zhu Y, Wang L, Yin Y & Yang E Systematic analysis of gene expression patterns associated with postmortem interval in human tissues. Sci. Rep 7, 5435 (2017). [PubMed: 28710439]
67. Fan J & lacobuzio-Donahue CA The science of rapid research autopsy in Autopsy in the 21st Century: Best Practices and Future Diretions (eds. Hooper JE & Williamson A) 151–166 (Springer International Publishing, 2018).

68. Embuscado EEE et al. Immortalizing the complexity of cancer metastasis: genetic features of lethal metastatic pancreatic cancer obtained from rapid autopsy. Cancer Biol. Ther 4, 548–554 (2005). [PubMed: 15846069]
69. Hooper JE et al. A patient-derived xenograft model of parameningeal embryonal rhabdomyosarcoma for preclinical studies. Sarcoma 1, 826124 (2015).
70. Misuraca KL, Cordero FJ & Becher OJ Pre-clinical models of diffuse intrinsic pontine glioma. Front. Oncol 5, 172 (2015). [PubMed: 26258075]
71. Nguyen HM et al. LuCaP prostate cancer patient-derived xenografts reflect the molecular heterogeneity of advanced disease and serve as models for evaluating cancer therapeutics. Prostate 77, 654–671 (2017). [PubMed: 28156002]
72. Cocariu EA et al. Correlations between the autolytic changes and postmortem interval in refrigerated cadavers. *Rom.* J. Intern. Med 54, 105–112 (2016).
73. Fan J et al. Quantification of nucleic acid quality in postmortem tissues from a cancer research autopsy program. Oncotarget 7, 66906–66921 (2016). [PubMed: 27602498]
74. Tomita H et al. Effect of agonal and postmortem factors on gene expression profile: Quality control in microarray analyses of postmortem human brain. Biol. Psychiatry 55, 346–352 (2004). [PubMed: 14960286]
75. Harrison PJ et al. The relative importance of premortem acidosis and postmortem interval for human brain gene expression studies: selective mRNA vulnerability and comparison with their encoded proteins. Neurosci. Lett 200, 151–154 (1995). [PubMed: 9064599]
76. Gilkes DM, Semenza GL & Wirtz D Hypoxia and the extracellular matrix: drivers of tumour metastasis. Nat. Rev. Cancer 14, 430–439 (2014). [PubMed: 24827502]
77. Nobre AR, Entenberg D, Wang Y, Condeelis J & Aguirre-Ghiso JA The different routes to metastasis via hypoxia-regulated programs. Trends Cell Biol. 28, 941–956 (2018). [PubMed: 30041830]
78. Maley CC et al. Classifying the evolutionary and ecological features of neoplasms. Nat. Rev. Cancer 17, 605–619 (2017). [PubMed: 28912577] A commentary article proposing an objective set of metrics to quantify cell-intrinsic and cell-extrinsic factors that influence the clonal evolution of cancer.
79. Turajlic S, Sottoriva A, Graham T & Swanton C Resolving genetic heterogeneity in cancer. Nat. Rev. Genet 20, 404–416 (2019). [PubMed: 30918367]
80. Greaves M Darwinian medicine: a case for cancer. *Nat Rev.* Cancer 7, 213–221 (2007).
81. Wilson BA, Garud NR, Feder AF, Assaf ZJ & Pennings PS The population genetics of drug resistance evolution in natural populations of viral, bacterial and eukaryotic pathogens. Mol. Ecol 25, 42–66 (2016). [PubMed: 26578204]
82. Kreiner JM, Stinchcombe JR & Wright SI Population genomics of herbicide resistance: adaptation via evolutionary rescue. Annu. Rev. Plant Biol 4 29, 611–635 (2017).
83. Gillies RJ, Verduzco D & Gatenby RA Evolutionary dynamics of carcinogenesis and why targeted therapy does not work. Nat. Rev. Cancer 12, 487–493 (2012). [PubMed: 22695393]
84. Ibrahim-Hashim A et al. Defining cancer subpopulations by adaptive strategies rather than molecular properties provides novel insights into intratumoral evolution. Cancer Res. 77, 2242–2254 (2017). [PubMed: 28249898]
85. Spunt SL et al. The clinical, research, and social value of autopsy after any cancer death. Cancer 118, 3002–3009 (2012). [PubMed: 22006470]
86. Van Der Linden A et al. Post-mortem tissue biopsies obtained at minimally invasive autopsy: an RNA-quality analysis. PLOS ONE 9, e115675 (2014). [PubMed: 25531551]
87. Gerlinger M et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N. Engl. J. Med 366, 883–892 (2012). [PubMed: 22397650]
88. de Bruin EC, McGranahan N & Swanton C Analysis of intratumor heterogeneity unravels lung cancer evolution. Mol. Cell. Oncol 2, e985549 (2015). [PubMed: 27308463]
89. Kim SK et al. Comprehensive analysis of genetic aberrations linked to tumorigenesis in regenerative nodules of liver cirrhosis. J. Gastroenterol 54, 628–640 (2019). [PubMed: 30756187]

90. Zhang AW et al. Interfaces of malignant and immunologic clonal dynamics in ovarian. Cancer. Cell 173, 1755–1769 (2018).
91. Naxerova K et al. Origins of lymphatic and distant metastases in human colorectal cancer. Science 357, 55–60 (2017). [PubMed: 28684519]
92. McPherson A et al. Divergent modes of clonal spread and intraperitoneal mixing in high-grade serous ovarian cancer. Nat Genet 48, 758–767 (2016). [PubMed: 27182968]
93. Faltas BM et al. Clonal evolution of chemotherapy-resistant urothelial carcinoma. Nat Genet. 48, 1490–1499 (2016). [PubMed: 27749842]
94. Brown D et al. Phylogenetic analysis of metastatic progression in breast cancer using somatic mutations and copy number aberrations. Nat. Commun 8, 14944 (2017). [PubMed: 28429735]
95. Yachida S et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nat 467, 1114–1117 (2010).
96. Ascierto MLML et al. Transcriptional mechanisms of resistance to anti-PD-1 therapy. Clin. Cancer Res 23, 3168–3180 (2017). [PubMed: 28193624]
97. Sottoriva A et al. A Big Bang model of human colorectal tumor growth. Nat. Genet 47, 209–216 (2015). [PubMed: 25665006]
98. Sun R et al. Between-region genetic divergence reflects the mode and tempo of tumor evolution. Nat Genet 49, 1015–1024 (2017). [PubMed: 28581503]
99. Aryee MJ et al. DNA methylation alterations exhibit intraindividual stability and interindividual heterogeneity in prostate cancer metastases. Sci. Transl. Med 5, 169ra10 (2013).
100. Turajlic S et al. Deterministic evolutionary trajectories influence primary tumor growth: TRACERx Renal. Cell 173, 595–610 (2018). [PubMed: 29656894]
101. Reiter JGJG et al. Minimal functional driver gene heterogeneity among untreated metastases. Science 361, 1033–1037 (2018). [PubMed: 30190408]
102. Berquist SW et al. Systemic therapy in the management of localized and locally advanced renal cell carcinoma: current state and future perspectives. Int. J. Urol 26, 532–542 (2019). [PubMed: 30943578]
103. Makohon-Moore AP et al. Limited heterogeneity of known driver gene mutations among the metastases of individual patients with pancreatic cancer. Nat Genet. 49, 358–366 (2017). [PubMed: 28092682]
104. Borazanci E et al. Pancreatic cancer: ‘a riddle wrapped in a mystery inside an enigma’. Clin. Cancer Res 23, 1629–1637 (2017). [PubMed: 28373361]
105. Rosenthal R et al. Neoantigen-directed immune escape in lung cancer evolution. Nature 567, 479–485 (2019). [PubMed: 30894752]
106. Mitchell TJ et al. Timing the landmark events in the evolution of clear cell renal cell cancer: TRACERx Renal. Cell 173, 611–623 (2018). [PubMed: 29656891]
107. Jones S et al. Comparative lesion sequencing provides insights into tumor evolution. Proc. Natl Acad. Sci. USA 105, 4283–4288 (2008). [PubMed: 18337506] The first study to use whole-exome sequencing data to derive estimates of the evolutionary life history of a neoplasm.
108. Körber V et al. Evolutionary trajectories of IDHWT glioblastomas reveal a common path of early tumorigenesis instigated years ahead of initial diagnosis. Cancer Cell 35, 692–704.e12 (2019). [PubMed: 30905762]
109. Wu R et al. Genomic landscape and evolutionary trajectories of ovarian cancer precursor lesions. J. Pathol 248, 41–50 (2019). [PubMed: 30560554]
110. Matsuda Y et al. The prevalence and clinicopathological characteristics of high-grade pancreatic intraepithelial neoplasia autopsy study evaluating the entire pancreatic parenchyma. Pancreas 46, 658–664 (2017). [PubMed: 28196020]
111. Groot VP et al. Systematic review on the treatment of isolated local recurrence of pancreatic cancer after surgery; re-resection, chemoradiotherapy and SBRT. HPB 19, 83–92 (2017). [PubMed: 28065427]
112. Makohon-Moore AP et al. Precancerous neoplastic cells can move through the pancreatic ductal system. Nature 561, 201–205 (2018). [PubMed: 30177826]

113. Martincorena I et al. Somatic mutant clones colonize the human esophagus with age. Science 362, 911–917 (2018). [PubMed: 30337457]
114. Yizhak K et al. RNA sequence analysis reveals macroscopic somatic clonal expansion across normal tissues. Science 364, eaaw0726 (2019).
115. Biswas R et al. Genomic profiling of multiple sequentially acquired tumor metastatic sites from an “exceptional responder” lung adenocarcinoma patient reveals extensive genomic heterogeneity and novel somatic variants driving treatment response. Mol. Case Stud 2, a001263 (2016).
116. Sanchez-Vega F et al. *EGFR* and *MET* amplifications determine response to HER2 inhibition in *ERBB2*-amplified esophagogastric cancer. Cancer Discov. 9, 199–209 (2019). [PubMed: 30463996]
117. Juric D et al. Convergent loss of PTEN leads to clinical resistance to a PI(3)Kα inhibitor. Nature 518, 240–244 (2015). [PubMed: 25409150]
118. Topalian SL, Drake CG & Pardoll DM Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 27, 450–461 (2015). [PubMed: 25858804]
119. Sohal DPS et al. Metastatic pancreatic cancer: American Society of Clinical Oncology clinical practice guideline. J. Clin. Oncol 34, 2784–2796 (2016). [PubMed: 27247222]
120. Jaber Y, Reichard CA & Chapin BF Emerging role of cytoreductive prostatectomy in patients with metastatic disease. Transl. Androl. Urol 7, S505–S513 (2018). [PubMed: 30363448]
121. Winter JM et al. Survival after resection of pancreatic adenocarcinoma: results from a single institution over three decades. Ann. Surg. Oncol 19, 169–175 (2012). [PubMed: 21761104]
122. Yachida S et al. Establishment and characterization of a new cell line, A99, from a primary small cell carcinoma of the pancreas. Pancreas 40, 905–910 (2011). [PubMed: 21768923]
123. Skapek SX et al. Rhabdomyosarcoma. Nat. Rev. Dis. Prim 5, 1 (2019). [PubMed: 30617281]
124. Alabran JL et al. Overcoming autopsy barriers in pediatric cancer research. Pediatr. Blood Cancer 60, 204–209 (2013). [PubMed: 23015377]
125. Hawkins DS, Spunt SL & Skapek SX Children’s Oncology Group’s 2013 blueprint for research: soft tissue sarcomas Pediatr. Blood Cancer 60, 1001–1008 (2013). [PubMed: 23255356]
126. Monje M et al. Hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma. Proc. Natl Acad. Sci. USA 108, 4453–4458 (2011). [PubMed: 21368213]
127. Caretti V et al. Human pontine glioma cells can induce murine tumors. Acta Neuropathol. 127, 897–909 (2014). [PubMed: 24777482]
128. Sanchez H & Chamberlin G Utilizing the autopsy for quality improvement, in Autopsy in the 21st Century: Best Practices and Future Directions (eds. Hooper JE & Williamson A) 19–58 (Springer International, 2018).
129. Cabot RC Diagnostic pitfalls identified during a study of three thousand autopsies. J. Am. Med. Assoc LIX, 2295–2298 (1912).A study illustrating the rates of incidental and unappreciated clinically relevant findings found at autopsy.
130. Shojania KG, Burton EC, McDonald KM & Goldman L Changes in rates of autopsy-detected diagnostic errors over time: a systematic review. JAMA 289, 2849–2856 (2003). [PubMed: 12783916]
131. Chow S-C Adaptive clinical trial design. Annu. Rev. Med 65, 405–415(2014). [PubMed: 24422576]
132. Gallo P et al. Adaptive designs in clinical drug development—an executive summary of the PhRMA working group. J. Biopharm. Stat 16, 275–283 (2006). [PubMed: 16724485]
133. Freidlin B & Korn EL Biomarker-adaptive clinical trial designs. Pharmacogenomics 11, 1679–1682 (2010). [PubMed: 21142910]
134. Regev A et al. The human cell atlas. Elife 6, e27041 (2017). [PubMed: 29206104]
135. Manjili MH Tumor dormancy and relapse: from a natural byproduct of evolution to a disease state. Cancer Res. 77, 2564–2569 (2017). [PubMed: 28507050]
136. Chukwueke UN & Brastianos PK Sequencing brain metastases and opportunities for targeted therapies. Pharmacogenomics 18, 585–594 (2017). [PubMed: 28290769]
137. Palmieri D, Chambers AF, Felding-Habermann B, Huang S & Steeg PS The biology of metastasis to a sanctuary site. Clin. Cancer Res 13, 1656–1662 (2007). [PubMed: 17363518]

138. Beerenwinkel N, Greenman CD & Lagergren J Computational cancer biology: an evolutionary perspective. PLOS Comput. Biol 12, e1004717 (2016). [PubMed: 26845763]

139. Levitin HM, Yuan J & Sims PA Single-cell transcriptomic analysis of tumor heterogeneity. Trends Concer 4, 264–268 (2018).

140. Reiter JG et al. Reconstructing metastatic seeding patterns of human cancers. Nat. Commun 8, 14114 (2017). [PubMed: 28139641] This article presents a phylogenetic algorithm developed specifically for the analysis of multiregion-sequenced tumour samples.

141. Zaccaria S, El-Kebir M, Klau GW & Raphael BJ Phylogenetic copy-number factorization of multiple tumor samples. J. Comput. Biol 25, 689–708 (2018). [PubMed: 29658782]

142. Niknafs N, Beleva-Guthrie V, Naiman DQ & Karchin R Subclonal hierarchy inference from somatic mutations: automatic reconstruction of cancer evolutionary trees from multi-region next generation sequencing. PLOS Comput. Biol 11, e1004416 (2015). [PubMed: 26436540]

143. Malikic S, McPherson AW, Donmez N & Sahinalp CS Clonality inference in multiple tumor samples using phylogeny. Bioinformatics 31, 1349–1356 (2015). [PubMed: 25568283]

144. El-Kebir M, Satas G & Raphael BJ Inferring parsimonious migration histories for metastatic cancers. Nat. Genet 50, 718–726 (2018). [PubMed: 29700472]

145. Tang Z, Kang B, Li C, Chen T & Zhang Z GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res. 47, W556–W560 (2019). [PubMed: 31114875]

146. Ma Y & Wei P FunSPU: a versatile and adaptive multiple functional annotation-based association test of whole-genome sequencing data. PLOS Genet 15, e1008081 (2019). [PubMed: 31034468]

147. Mounir M et al. New functionalities in the TCGAbiolinks package for the study and integration of cancer data from GDC and GTEx. PLOS Comput. Biol 15, e1006701 (2019). [PubMed: 30835723]

148. Achkar T, Wilson J, Simon J, Rosenzweig M & Puhalla S Metastatic breast cancer patients: attitudes toward tissue donation for rapid autopsy. Breast Cancer Res. Treat 155, 159–164 (2016). [PubMed: 26705147]

149. Tsitsikas DA, Brothwell M, Chin Aleong J-A & Lister AT The attitudes of relatives to autopsy: a misconception. J. Clin. Pathol 64, 412–414 (2011). [PubMed: 21385895]

150. Alsop K et al. A community-based model of rapid autopsy in end-stage cancer patients. Nat. Biotechnol 34, 1010–1014 (2016). [PubMed: 27617737] A comprehensive description of the logistics of running a large-scale research autopsy programme for patients with cancer.

151. Siminoff LA et al. Impact of cognitive load on family decision makers' recall and understanding of donation requests for the genotype-tissue expression (GTEx) project. J. Clin. Ethics 29, 20–30 (2018). [PubMed: 29565794]

152. Hoadley KA et al. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. Cell 158, 929–944 (2014). [PubMed: 25109877]

Box 1 |

Implementation of a research autopsy programme

The successful implementation of a research autopsy programme is contingent on three major factors—scientific infrastructure, programme coordination and clinical support.

Scientific infrastructure

The first consideration is the need for a scientific infrastructure. Most programmes exist at cancer centres where the necessary resources and infrastructure are already in place, although implementation through a coordinated community-based approach is also feasible[14,15,68,150]. Minimal staffing requirements for the procedure include a dedicated professional to perform the procedure, staff to collect the samples in a systematic manner, pathology department support for review of the materials collected and a long-term storage solution. Some programmes provide 24-h coverage, whereas others operate successfully with coverage during business hours only[15,68,150].

Programme coordination

The second need is for a dedicated programme coordinator. This individual should be responsible for interacting with clinical teams, providing administrative support, tracking consented patients, arranging transport of the deceased individual to and from the morgue should death occur outside the hospital and follow-up with the families of each participant to reinforce the importance and appreciation of the deceased person's participation. Depending on the structure of the programme, this programme coordinator might also pursue consent from participants. Ideally, consent should occur before death, rather than after death, as the emotional toll is lower and the chance for receiving consent is higher[151]. As this crucial role is both physically and emotionally demanding, this responsibility should ideally be shared between at least two individuals[150].

Clinical support

Third, support is needed from the clinical caretakers of patients to introduce the possibility of participation in a research autopsy programme. From the experience of more than one programme, this aspect is crucial and might be the major hurdle to be overcome for accrual of participants[85,124,150]. The reasons for lack of clinician support are multifactorial, including lack of awareness that such programmes exist, reluctance to ask the patient or family for consent, misconceptions related to how a research autopsy differs from a clinical autopsy, lack of awareness among families that the autopsy can be limited to a particular region of the body and fear of litigation[85,124,128].

Box 2 |

Key take-home messages

- The post-mortem interval, use of post-mortem refrigeration, mode of death and biological features of a tumour might independently influence biospecimen integrity.
- Post-mortem tissue can be used to establish immortalized preclinical models of rare tumour types or tumour types for which pre-mortem samples are unavailable.
- Research autopsies allow multiregion sampling to an extent that is not otherwise possible by biopsy or resection sampling in a living patient.
- Multiregion sampling via a research autopsy allows ‘sampling to completion’, defined as the minimum number of samples required to reasonably eliminate false negative or false positive conclusions.
- Multiregion sampling supports investigation of the natural history of a neoplasm and can, therefore, reveal the earliest events of carcinogenesis.
- Research autopsies are unparalleled for understanding intratumoural heterogeneity and the mechanisms of treatment resistance.
- Research autopsies are an underused method for optimization of personalized medicine approaches.
- The amount of tissue and the extent of sampling afforded by research autopsies have and will continue to support fundamental insights into cancer biology.
- An ongoing need exists for the development of computational methods to unravel the complexity of data generated by analysis of multiregion-sampled neoplasms.

Rapid autopsy

An autopsy that is performed within 2h of cardiopulmonary arrest.

Warm autopsy

An autopsy that is performed so rapidly that the deceased person’s body has not yet cooled to room temperature.

Somatic mosaicism

The presence of two or more genetically distinct populations of cells within an individual.

Tumour dormancy

A state in which viable cancer cells remain quiescent for a prolonged period.

Sanctuary sites

Tissues within the body in which cancer cells are protected from pharmacological agents or other therapies.

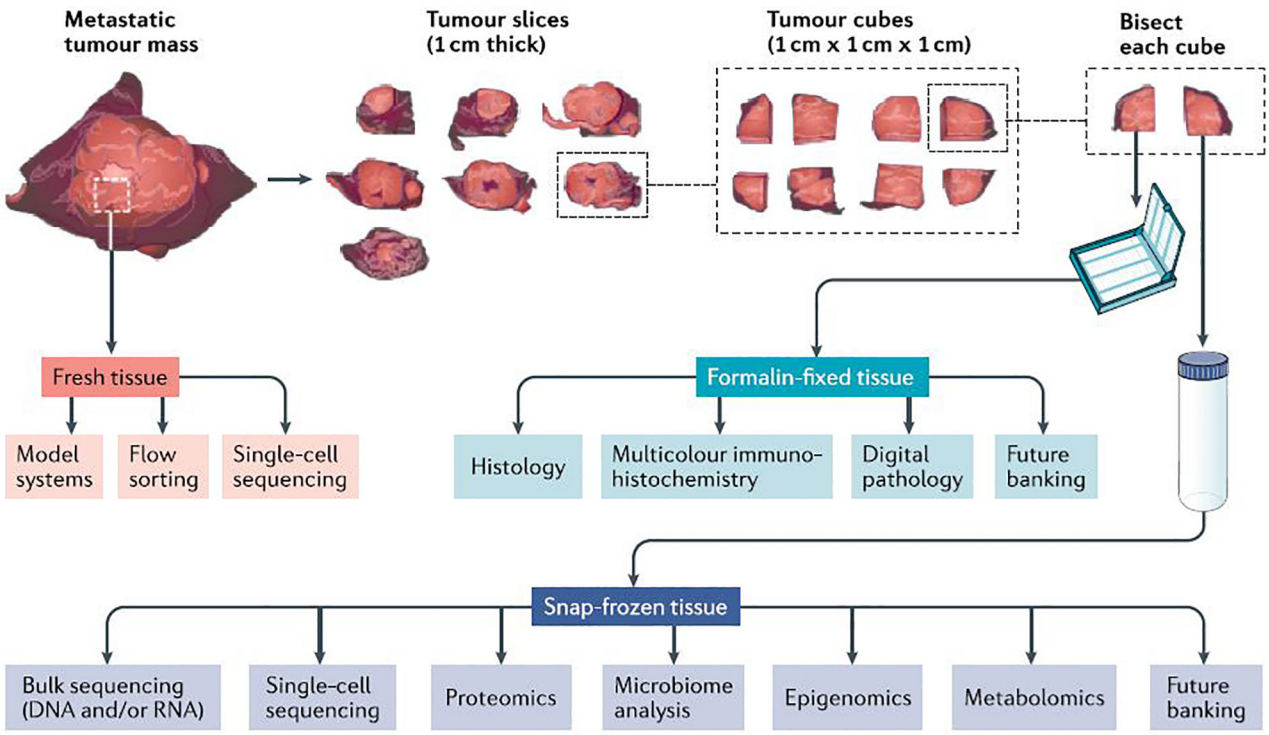

Fig. 1 |. Methods and rationale for multiregion sampling.

Depiction of an example of multiregion sampling of an autopsy-derived solitary metastasis based on previously described methods[95]. The large circumscribed mass is sectioned into 1-cm-thick slices, each of which is further sectioned into 1cm × 1cm × 1cm cubes. The cubic piece of tissue is then bisected along the long axis, with one half fixed in formalin and embedded in paraffin (green cassette) and the other half snap frozen in liquid nitrogen in a cryovial. Fresh samples might also be taken before, during or after multiregion sampling, and can be used for the creation of model systems (such as patient-derived xenografts or organoids), for flow sorting and isolation of cell types of interest and/or for disaggregation to facilitate single-cell sequencing. Formalin-fixed samples can be used for histological assessment, immunohistochemistry (including multicolour labelling) and digital pathology and banked for future use and distribution. Snap-frozen tissues can be used for a range of additional downstream analyses in which snap freezing is the preferred mode of preservation, and can also be banked.

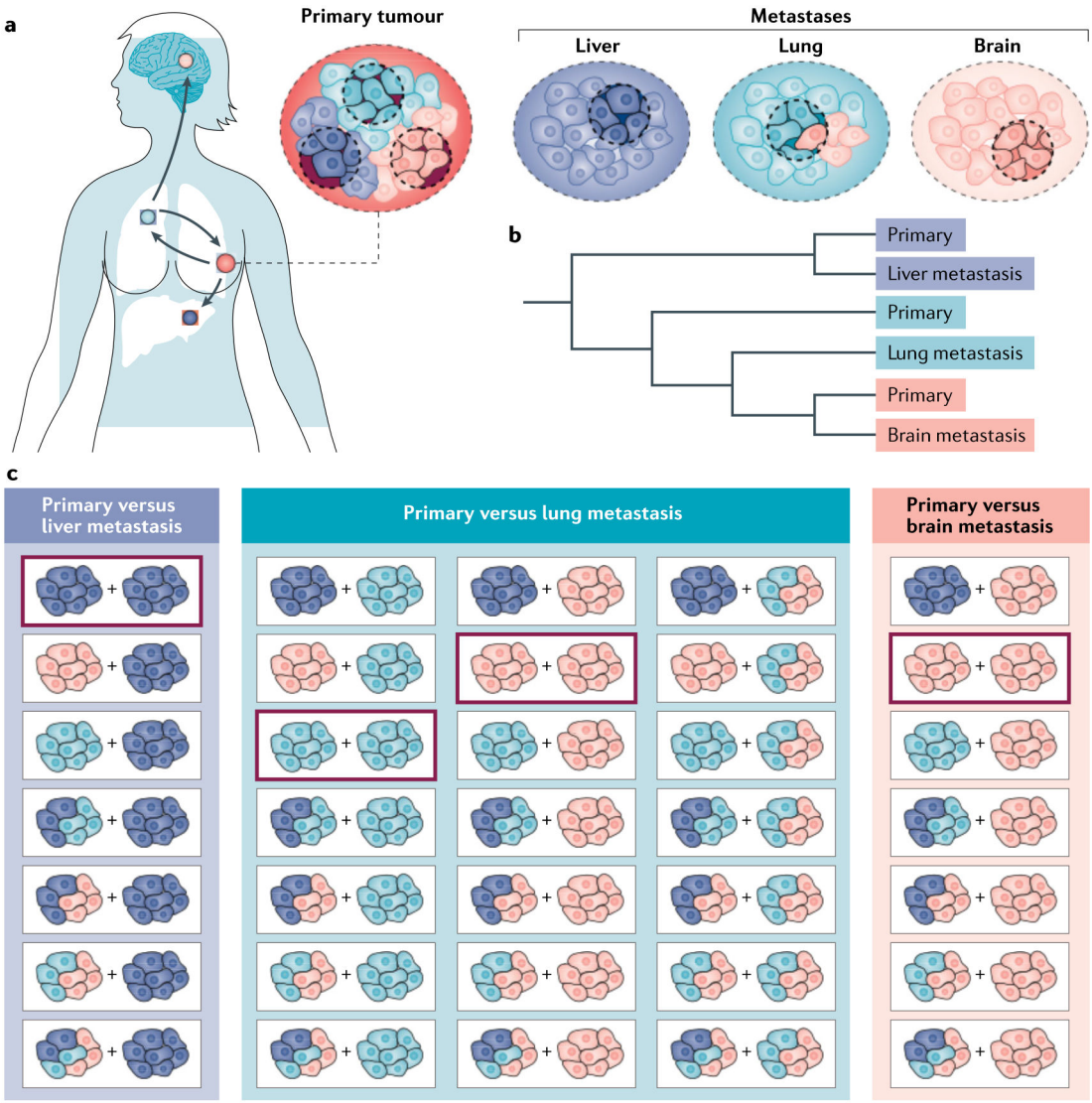

Fig. 2 |. Multiregion sampling to understand the evolutionary biology of cancer.

a | In this hypothetical example, a patient presents with stage IV breast cancer with metastases to the liver, lung and brain. The natural history of this carcinoma is that the liver and lung metastases originated from divergent subclones (blue and green cells) in the primary tumour. A subclonal expansion in the lung metastasis (pale orange cells) seeded a brain metastasis and also seeded back to the primary tumour, resulting in a genetically heterogeneous primary tumour mass containing three distinct subclonal populations, **b** | Multiregion sampling of the same hypothetical patient. At autopsy, three samples are taken from the primary tumour in an unbiased manner that happen to include cells from each subclone, and one sample is taken from each of the three metastases (a conservative example). Hashed black circles indicate from where these samples were taken. In this hypothetical example, phylogenetic analysis of deep sequencing data reveals the phylogenetic relationship of each of the six samples to each other. This tree does not indicate the seeding events that occurred, which would require additional computational analyses. The phylogenetic tree and branch lengths are not drawn to scale, **c** | Possible interpretations

of a single biopsy of the primary tumour and a single biopsy of each of the metastases (matched pair analysis) are shown, illustrating the sampling error caused by a single-region biopsy. Given that three subclonal populations are present that are located in spatially distinct regions of the primary tumour and metastatic sites, 35 interpretations are possible. Rectangles outlined in red indicate those comparisons for which a low amount of diversity might be inferred.

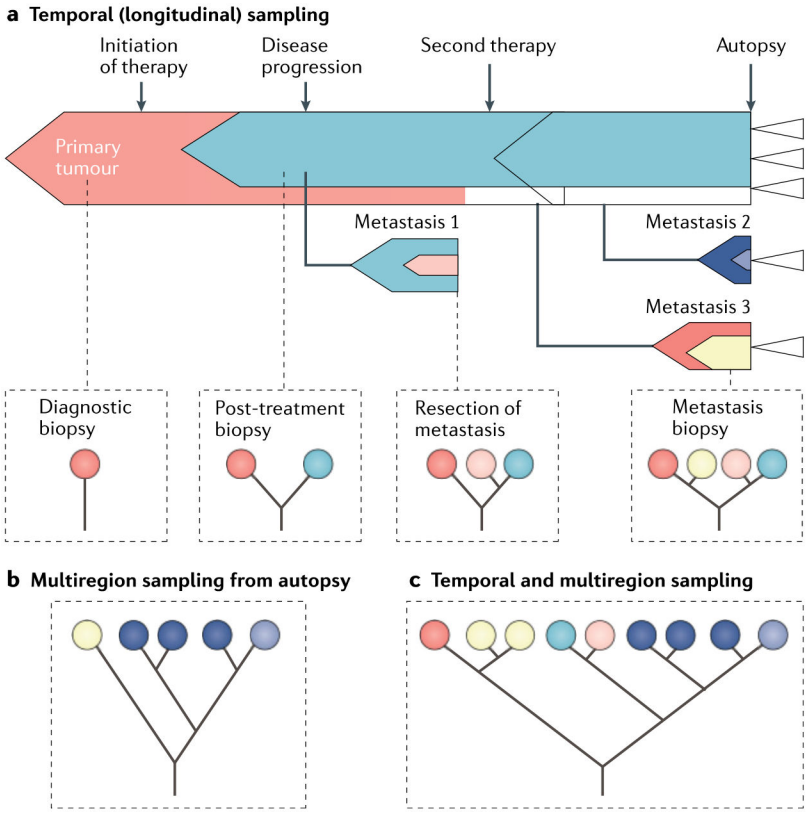

Fig. 3 |. Interpretation of evolutionary dynamics relative to the sampling method.

a | The clonal evolution of a neoplasm, during which four longitudinal samples (dotted lines) are taken, including samples from diagnostic biopsy, post-treatment biopsy, metastasectomy and resection of a late-emerging metastasis. The approximate timing of initiation of therapy, disease progression and death (autopsy) are also shown, and samples taken during autopsy are indicated by open arrowheads. Following the diagnostic biopsy, each subsequent sample increases the resolution of phylogenetic analyses. The caveat of this approach is that the interpretation is biased by the samples used. For example, the lack of a second sample of the primary tumour or from metastasis 2 limits inferences of a late subclonal event (dark blue clone), **b** | On the basis of multiregion sampling at autopsy, the phylogenetic analyses are more reflective of the dominant lethal subclone (dark blue) that emerged after two failed therapies but fail to capture the timing of emergence of subclones that were detected by longitudinal sampling (for example, in metastasis 1). **c** | Combination of the samples obtained by temporal sampling and multiregion sampling at autopsy reveal the complete evolutionary history of the neoplasm, illustrating that the combination of these approaches can yield more robust inferences of the clonal dynamics of the neoplasm.

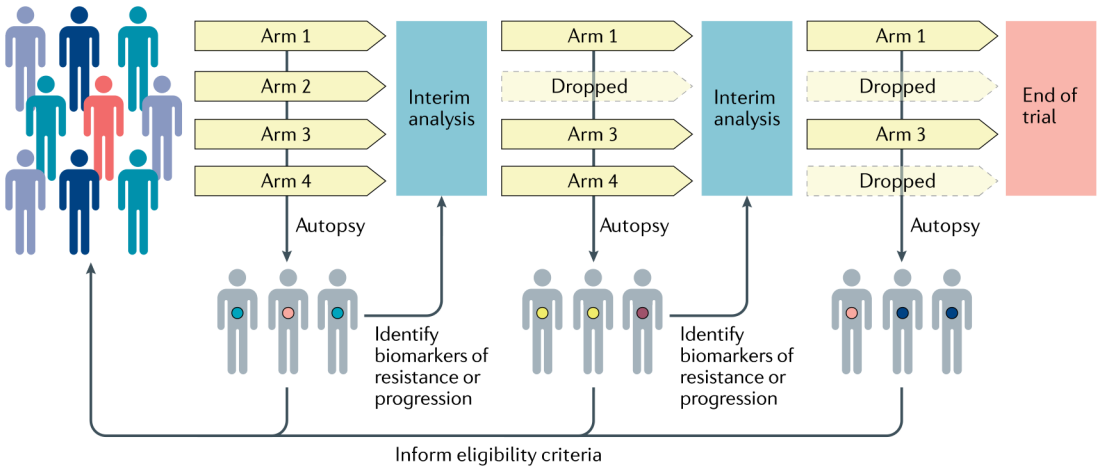

Fig. 4 |. Incorporation of research autopsies into biomarker-driven adaptive clinical trials.
This hypothetical biomarker-driven adaptive clinical trial begins with randomization to four drug regimens (arms 1–4). Patients who progress while receiving treatment are offered a research autopsy, in which a subset of patients will elect to participate. Interim analyses are performed with the goal of identifying the regimens that might be the most successful on the basis of treatment responses, and could be improved by using research autopsies to identify mechanisms and biomarkers of treatment resistance and progression. Such data from autopsies would also be expected to inform eligibility criteria for prospectively accrued patients.

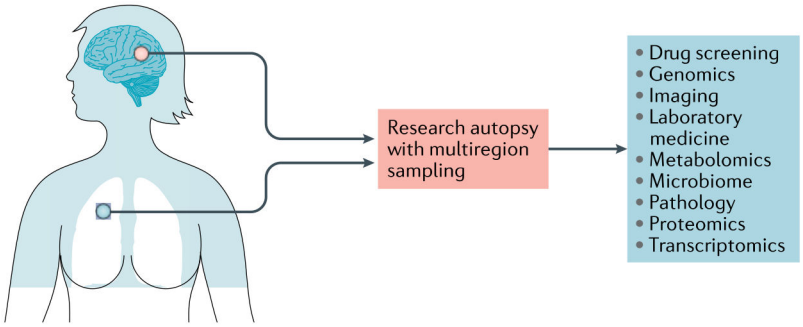

Fig. 5 |. Integration of multimodal data to maximize understanding of lethal cancer.
Many important initiatives have focused on one type of analysis in a large cohort of patients[59,152]. By contrast, research autopsies generate ample amounts of tissue to enable all types of analyses to be performed within the same patient, and even in the same piece of tissue. Thus, the scale of data possible from research autopsies requires computational efforts and innovation to maximize the use of this information and reveal biological aspects of lethal cancer that were not previously appreciated.