Journal of Pathology
J Pathol July 2022; **257:** 403–412
Published online 23 May 2022 in Wiley Online Library
(wileyonlinelibrary.com) DOI: 10.1002/path.5915

INVITED REVIEW

Beyond the snapshot: optimizing prognostication and prediction by moving from fixed to functional multidimensional cancer pathology

CJH Kramer[1], MPG Vreeswijk[2], B Thijssen[3,4], T Bosse[1]* and J Wesseling[1,5,6]

[1] *Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands*
[2] *Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands*
[3] *Division of Molecular Carcinogenesis, Oncode Institute, Netherlands Cancer Institute, Amsterdam, The Netherlands*
[4] *Department of Biomolecular Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*
[5] *Department of Pathology, Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands*
[6] *Division of Molecular Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands*

**Correspondence to: T Bosse, Department of Pathology, Leiden University Medical Center, Albinusdreef 2, 2333 ZA, Leiden, The Netherlands. E-mail: t.bosse@lumc.nl*

Abstract

The role of pathology in patient management has evolved over time from the retrospective review of cells, tissue, and disease ('what happened') to a prospective outlook ('what will happen'). Examination of a static, two-dimensional hematoxylin and eosin (H&E)-stained tissue slide has traditionally been the pathologist's primary task, but novel ancillary techniques enabled by technological breakthroughs have supported pathologists in their increasing ability to predict disease status and behaviour. Nevertheless, the informational limits of 2D, fixed tissue are now being reached and technological innovation is urgently needed to ensure that our understanding of disease entities continues to support improved individualized treatment options. Here we review pioneering work currently underway in the field of cancer pathology that has the potential to capture information beyond the current basic snapshot. A selection of exciting new technologies is discussed that promise to facilitate integration of the functional and multidimensional (space and time) information needed to optimize the prognostic and predictive value of cancer pathology. Learning how to analyse, interpret, and apply the wealth of data acquired by these new approaches will challenge the knowledge and skills of the pathology community.

Keywords: pathology; cancer; dimensions; 3D reconstruction; prognosis; prediction; biomarkers; functional pathology

Received 25 February 2022; Revised 11 April 2022; Accepted 13 April 2022

Conflict of interest statement: JW: no commercial disclosures, advisory roles: (1) Scientific Advisory Board of the Dutch Expert Centre for Screening (member), (2) Research Board KWF Dutch Cancer Society (member), (3) Scientific Advisory Board Breast Cancer Now Research Centre, The Institute of Cancer Research, London, UK (member on an ad hoc *basis), (4) Various scientific advisory roles on an* ad hoc *basis for Cancer Research UK (member on an* ad hoc *basis). No other conflicts of interest were declared.*

A brief history of pathology

The first historical description of pathology as a distinct medical specialty dates to the 15th century, when the Italian physician Antonio Benivieni bundled his patient's case and autopsy reports to produce the first 'pathology article' ('*Abditis Nonnullis ac Mirandis Morborum et Sanationum Casusis*' [About the Hidden Causes of Disease]) [1]. Pathology, both as an interest and a medical specialty, has traditionally originated from the desire of physicians to understand the relationship between the human body and disease, or, framed more exactly, between physiology and pathology. This intrinsic motivation to master the discipline led, from the 18th century on, to the appearance of an increasing number of autopsy observations in early medical journals, such as the ground-breaking work by Giovanni Morgagni [1,2].

The Leiden physician, Herman(us) Boerhaave, was the first to describe a causal relationship between medical history and pathology, basing his conclusions on extensive postmortem observations. Pathology thus emerged as a discipline that sought to understand the events that preceded autopsy. In essence, pathology sought to 'examine a *snapshot* to understand the past'.

The retrospective review of human tissue described above was, at that time, based on the gross examination of autopsy specimens. The introduction of the microscope in the late 1700s (Figure 1), innovated by Antoni van Leeuwenhoek, among others, proved a seminal moment [1,3]. The implementation and use of microscopy in daily practice was revolutionary and expanded the field from gross examination and macroscopy to the examination of the microscopic anatomy of human tissue. This eventually led to an increasing use of histology in disease classification, pioneered by the 'father

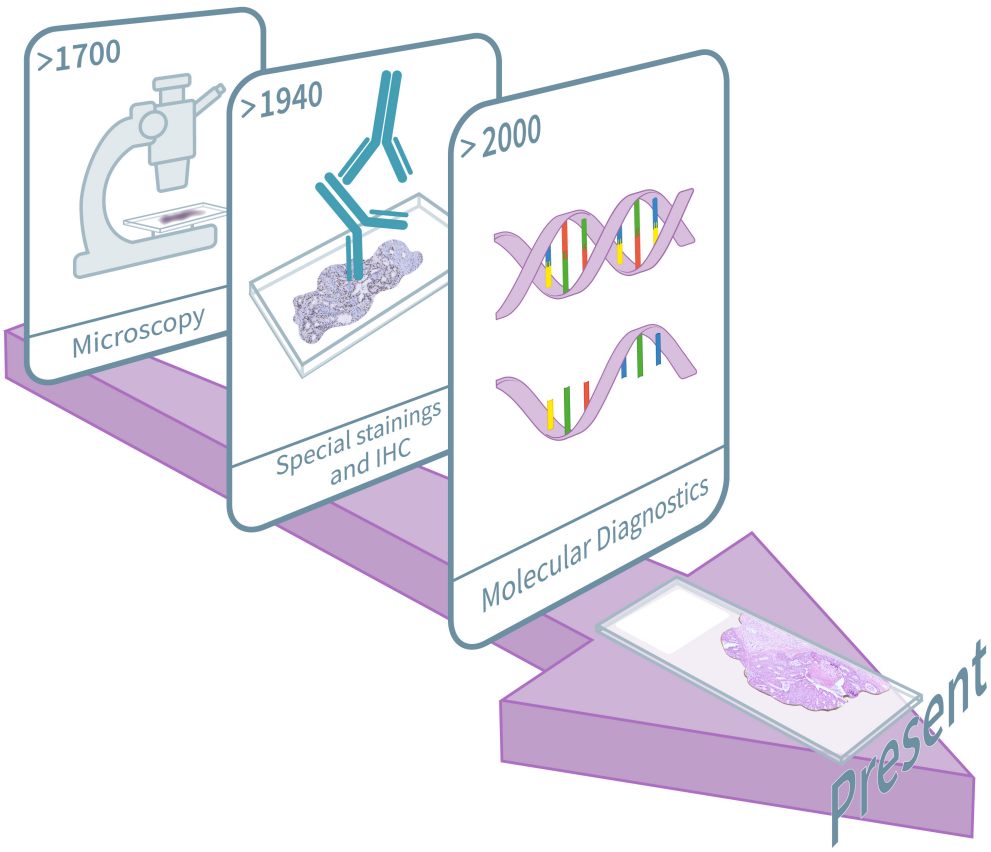

Figure 1. The three major technological breakthroughs that form the current cornerstones of pathology: (1) microscopy, (2) special and immunohistochemical (IHC) staining, and (3) molecular diagnostics.

of modern pathology' Rudolf Virchow [4], and histology has since become a cornerstone of pathology.

With histologic examination, the focus of the specialty shifted from autopsy to the examination of formalin-fixed and paraffin-embedded (FFPE) biopsy, cytology, and carefully sampled resection specimens from living rather than deceased patients. Where the pathologist once attempted to discern and understand past disease processes, today a pathologist analyses surgical tissue with one main question in mind: what does this tissue reveal of future disease processes? The shift from the examination of deceased to living tissue has transformed the field's objectives and launched a quest for insight into the future behaviour of cells, tissue, and disease.

A second major technological breakthrough in pathology was the development and implementation of special stains (e.g. Periodic Acid-Schiff or the Alcian blue stain), and later, immunohistochemistry (IHC), with the latter now the most utilised application in surgical pathology (Figure 1). In immunostaining, the presence of specific antigens in human tissue is evaluated at the tissue and cellular level. In recent decades, the opportunity to determine the presence and localisation of proteins in tissue sections has contributed to the development of clinically-relevant biomarkers, both prognostic and predictive, allowing classifications of pathology and disease that go well beyond the morphological characteristics of tissue. This second cornerstone has improved our understanding of the disease process, as well as the past, present, and, potentially, future behaviour of cells and disease.

A combination of hematoxylin and eosin (H&E) (morphology) and IHC still provides an oversimplified view of the pathophysiological disease process, because a handful of static biological markers will inevitably fail to capture the full underlying biology. A third major breakthrough in the field of pathology, the use of 'omics' (including genomics and transcriptomics) as a diagnostic tool (Figure 1), promises to address earlier limitations. This technological revolution was facilitated by innovations in the extraction and sequencing of fragmentary DNA and RNA from FFPE specimens (Figure 1). The opportunity to routinely sequence (tumour) DNA illuminates the genotype underlying the phenotype as expressed in histology and immunostaining. The development of molecular diagnostics as the third cornerstone of modern pathology has led to an immense expansion of knowledge and facilitated the identification of potential novel treatment targets (Figure 1).

To summarise, over many decades pathology has evolved from the gross examination of autopsies to a detailed assessment of morphology, IHC, and molecular analyses (Figure 1). Additional technological advances, including electron and confocal microscopy, image analysis, polymerase-chain reaction (PCR), and *in situ* hybridisation have also significantly contributed to the field. These technological breakthroughs have yielded a wealth of novel insights, allowing improved prognostics and prediction of treatment benefit. The full potential of these novel technologies is only beginning to be explored.

The limitations of 2D and 'fixed' pathology

As discussed above, the practice of modern pathology still largely relies on a snapshot of the disease process, i.e. fixed pathology, consisting of microscopic assessment of thin (several micrometres thick) 2D tissue sections at a fixed point in time. However, a tumour exists in four dimensions (4D), i.e. three spatial dimensions (3D) (x, y, z) and a fourth dimension, time (Figure 2), so any fixed 2D representation will inevitably fail to completely describe a disease process. While specimen fixation is necessary to prevent tissue decay (Figure 2), this technique terminates the cellular processes that determine cell fate, and thus offers only a 'static' image of a tissue. FFPE tissue is a 3D derivative of a 4D object, as any tissue has a history and would have had a future if left *in situ*. Furthermore, despite the common availability of macroscopic images that seek to capture the original 3D structure before sectioning of FFPE tissue, even a sense of a third spatial dimension (z-axis) is frequently compromised or lost due to processing (Figure 2). As a consequence, disease classification and treatment decisions in surgical pathology predominantly rely on a series of static 2D H&E and IHC slides.

Unsurprisingly, information acquired from static material will quickly reach a plateau, suggesting that optimal, individualized patient management requires new techniques. Technological breakthroughs at both the dimensional (space, time) and functional levels are now on the horizon and may facilitate the pursuit of functional and multidimensional cancer pathology.

3D tumour analysis by reconstruction of H&Es and spatial genomics

The use of 2D sections in histological review limits the integration and analysis of the third, *z* spatial, dimension in current surgical pathology. Nevertheless, examples of an additional spatial dimension in daily practice are found at the patient-, organ- and tissue-levels in the form of surgical staging, macroscopic examination, and serial sectioning, respectively. Despite the fact that serial sections provide valuable additional perspective, there is still a lot to gain from additional information on the—third—spatial dimension.

Innovations at the microscopic and computational levels

An important example of spatial assessment is the 3D reconstruction of digitalized histopathological tissue slide images (Figure 3) [5–13]. The 3D reconstruction of H&E images adds a spatial dimension that helps decipher growth patterns, the spatial distribution, and the relationships of tissues, as well as the colocalisation of stromal and tumour cells. In recent years, studies have explored the 3D reconstruction of H&E images in multiple cancer types [5–10,13,14], adding a z-axis dimension to pathology review that allows pathologists to 'move through' a tissue. For example, Roberts *et al* developed a package that allowed augmentation of high-resolution 3D reconstructions [13], while Xie *et al* used a fluorescent analogue of H&E staining and light-sheet microscopy to reconstruct 3D prostate cancer biopsies [15]. Compared to conventional 2D approaches, risk stratification for clinical prostate-specific antigen (PSA)-based biochemical recurrence was more accurate when using glandular features in a computational 3D prostate biopsy [15]. That study also underlined the importance of new-generation (immunofluorescent) microscopes in the development of novel applications.

Improvements in microscope technology, in combination with computational power, have led to new spatial techniques and applications. For instance, a combination of fast chemical tissue clearing and ultramicroscopy allowed the 3D reconstruction of breast cancer resections [16]. In that study, the authors showed that procedures are reversible, allowing use of the same tissue in both spatial reconstruction and morphological review by H&E/IHC staining [16]. Similarly, second and

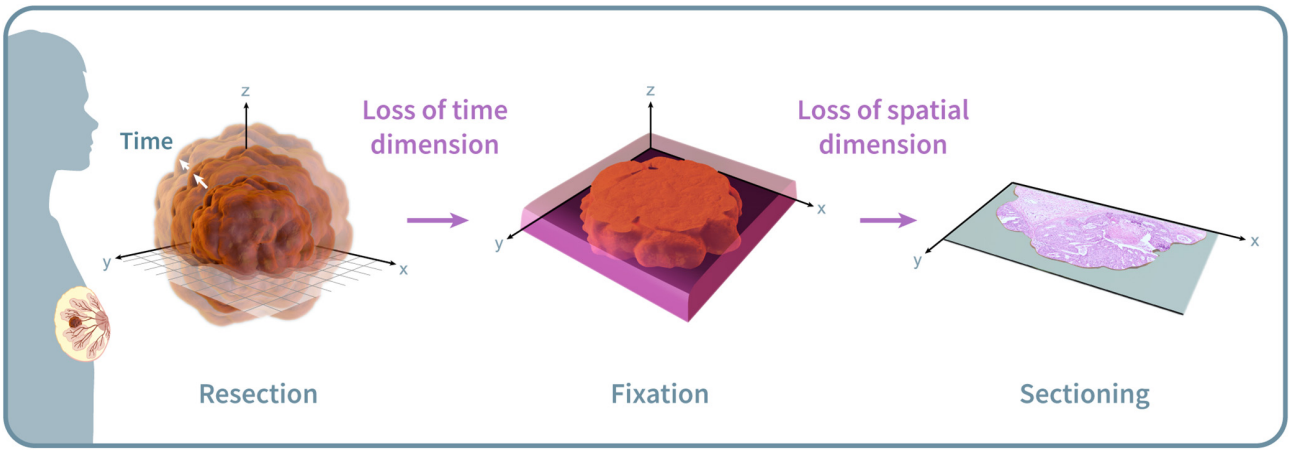

Figure 2. Loss of dimensions. Human tissue has four dimensions (4D): three spatial dimensions (3D) (x, y, z) and a temporal dimension (time). Fixation and sectioning of tissue eliminates two dimensions: the time- and z-dimensions, respectively. Current pathology practice relies heavily on the examination of a 2D snapshot of the original tissue.

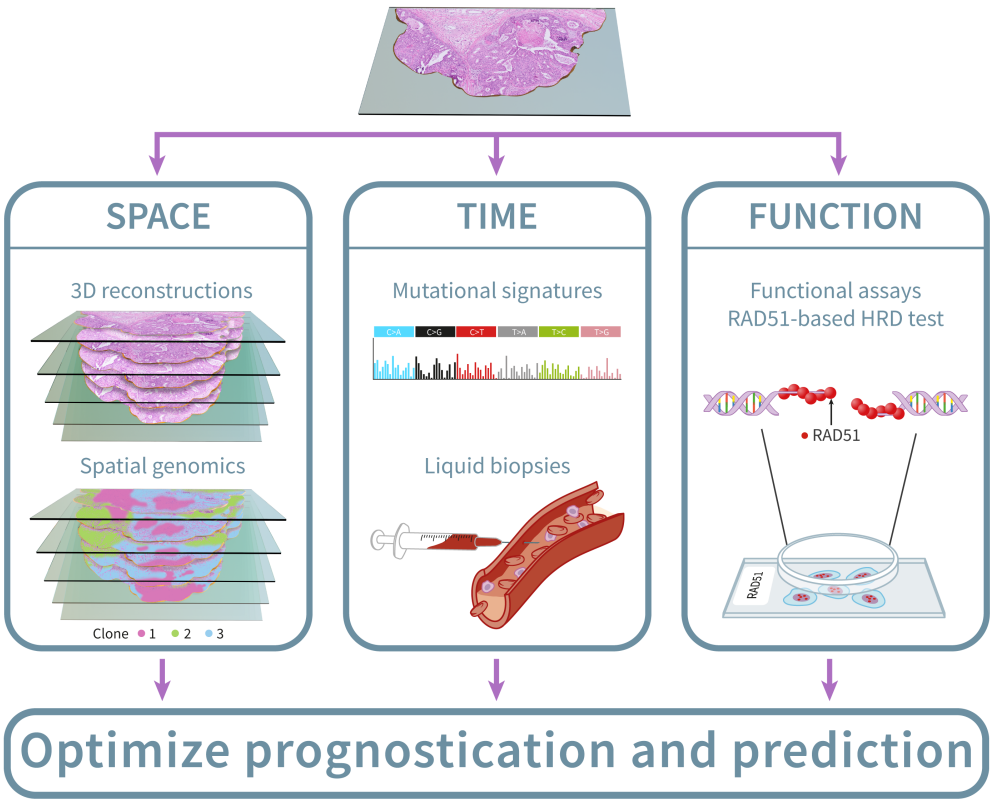

Figure 3. Towards optimal prognostication and prediction: three potential avenues of escape (space, time, and function) from the confines of the 'snapshot'.

third harmonic generation (SHG, THG) microscopy applications in breast cancer [17] capture images of fresh breast cancer tissue—without labelling—and thus help visualize relevant components such as lobules and ducts [17]. The SHG/THG images and structural information obtained were equivalent to the matching H&Es, but without the need for processing, fixation, embedding, and sectioning of tissue. These new technologies highlight the promise of an additional spatial dimension in the pathology domain. This expanded view will broaden the pathologist's perspective and help reveal novel 3D morphological features that may redefine future classification systems.

Innovations in multiomics

Progress in the field of omics has also provided new spatial insights (*spatial genomics*) (Figure 3). Zhao *et al* developed a novel spatial genomics application, 'slide DNAseq', which assesses DNA sequences from cells of intact tumour tissue specimens [18]. In addition to conventional morphological features, this technology helps illuminate tumour progression through the identification of distinct tumour subclones. Similarly, He *et al* developed a deep-learning model to link spatial transcriptomics to architectural features in breast cancers, demonstrating the ability of a deep-learning model to predict spatial gene expression from routine H&E images of breast cancers [19]. These two recent applications of *spatial genomics* allow image-based screening of tumours that show clear subclonality or intratumoural heterogeneity of biomarker expression [18,19].

This pioneering work illustrates the potential of combining state-of-the-art microscopy, digitalization, and spatial omics in the analysis of the third dimension. However, the road to clinical implementation is still long, as procedures, including storage, are relatively expensive and protocols are not yet compatible with current clinical workflows.

Integrating a time-dimension to improve prognostication and treatment response

The addition of a time dimension, either retrospective or prospective, may also help expand the current fixed view of cancer. In practice, the evaluation of *archival* FFPE tumour tissue obtained during previous resections of the same patient, or in specific cases, by sequential biopsy, already serves as a proxy for time. One example is in high-grade serous ovarian cancer patients undergoing interval debulking surgery, where both the diagnostic (pre-operative) biopsy and the (post-chemotherapy) specimen are examined. The pathological response to chemotherapy is assessed by examination of the resection specimen using a chemotherapy response score (CRS) [20]. Importantly, the CRS has clear prognostic value for progression-free survival and overall survival [21]. This pre- and post-chemotherapy specimen workflow adds a time element

that may alter the treatment regimen. In a study of chronic myeloid leukaemia, Michor *et al* used serial blood sample measurements and computational modelling to explicitly describe disease dynamics and treatment responses, revealing a biphasic treatment response that can be explained by a differential response in differentiated versus progenitor cells [22]. Thus, opportunities to use sequential tumour specimens (diagnostic biopsy and resection) already aid the examination of tumour dynamics.

The availability of archival tumour tissue underlines this ability to describe the history of a tissue prior to fixation of the latest tissue specimen. Nevertheless, a more relevant quest may be to predict a tissue's future behaviour, a concept that can be subdivided into two aspects: prognosis (natural behaviour of a tumour) and prediction (a tumour's behaviour following a specific medical intervention).

Regarding prognosis, current 2D pathology practice has multiple modalities and assays that allow for prognostication [23–25], examples of which are breast cancer, where Ki67-high cancers have a less favourable prognosis compared to Ki67-low cancers [26], and endometrial cancer, where cancers with a mutation in the exonuclease domain of *POLE* have an excellent prognosis [27–31]. In these examples, relatively simple markers have proven to be reliable prognosticators. In other instances, however, such as high-grade serous ovarian cancer, robust prognostic biomarkers have not yet been identified, and thus appear to warrant novel approaches such as the inclusion of disease progression over time. The temporal *in situ* monitoring of disease can potentially be realised through liquid biopsy.

Liquid biopsy-based biomarkers, including circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA) assays, have attracted a great deal of attention over the last decade following success in lung cancer (Figure 3) [32,33]. These assays show great promise in the temporal monitoring of tumours, regardless of clinical scenario (primary tumour, recurrence, metastasis, or resistance) [34]. The clinical applications of liquid biopsies include, but are not restricted to, early detection, monitoring of tumour dynamics, and identifying tumours with a high risk of recurrence [34]. Beyond lung cancer, studies have also demonstrated the role of liquid biopsies in the clinical care of female cancers, particularly breast cancer.

In recent years, multiple large pooled analyses have investigated the prognostic value of CTCs in stage I–III [35,36] and stage IV [37] breast cancer. Three meta-analyses have now reported a strong association between CTC and overall survival [35–37]. Compared to CTC-based studies, the prognostic relevance of ctDNA-based studies are less clear-cut [38–42]. Nevertheless, a meta-analysis that included patients from multiple trials with variable, mostly nonmetastatic disease (stage I–IV), found an association between ctDNA and overall survival [43]. Interestingly, the International Association for the Study of Lung Cancer (IASLC) recommends molecular analysis of a liquid biopsy in nonsmall-cell lung cancer patients with acquired resistance to a targeted inhibitor when a tissue biopsy is not possible [44]. The prognostic relevance of liquid-biopsy biomarkers in endometrial and ovarian cancer seem to be less pronounced (ctDNA: [45]; CTC: [46]), although results from large clinical studies are eagerly awaited [34]. Overall, liquid biopsy-based biomarkers have great potential to expand opportunities in the pathology domain, particularly when applied in the contexts of screening, early detection of recurrences, and/or resistance to treatment.

A more challenging task is estimating responsiveness to therapeutic compounds (prediction). Mutational signatures are one example of an innovative genomic-based modality that provides insight, albeit retrospectively, into ongoing cellular processes and their predictive relevance (Figure 3). In pioneering work, Alexandrov *et al* determined—prior to fixation—the biological mechanisms underlying the progressive accumulation of tumour-specific somatic mutations [47,48]. Based on combinations and clusters of mutation types, 21 initial single-base substitution (SBS) signatures could be defined, and particular mutational signatures linked to specific tumour processes, e.g. Signature 1 and age *or* Signature 3 and homologous recombination deficiency (HRD) [47,48]. Since the first publication in 2013, an additional 30-plus novel SBS signatures have been added to the original 21 [49], as well as doublet-base signatures (including tandem doublet, triplet, and quadruplet base substitutions) [49] and models that combine multiple genomic-based modalities (HRDetect) [50]. Interestingly, these mutational signatures have shown potential in predicting therapy response, particularly the response to platinum-based chemotherapy and PARPi (HRDetect [51,52]) or immunotherapy (tumour mutational burden [53] and microsatellite instability [54]).

Besides genomic-based platforms, '*ex vivo*' modalities such as patient-derived organoids and organotypic tumour slices have also shown promise in predicting treatment responses. Patient-derived tumour organoids artificially recapitulate tumour-specific architecture (including intratumoural heterogeneity) [55], while the lack of fixation adds a temporal dimension. In contrast to 2D cultures, organoids can often recapitulate a tumour-microenvironment [56], and once generated, their similarity to the original tumour can be confirmed by assessment of H&E and IHC staining and sequencing [57]. A notable application of patient-derived tumour organoids is the screening of potential anticancer drugs, as performed for several cancer types such as breast, liver, colorectal, ovarian, and prostate cancer [57–64]. The predictive value of patient-derived organoids in breast cancer was shown by Sachs *et al*, who used breast cancer organoids to screen multiple potential cancer drugs [60]. A relationship between genotype and drug sensitivity was confirmed in the organoids, including a clear correlation between a *BRCA1/2*-deficiency signature and sensitivity to poly(ADP-ribose) polymerase inhibitors (PARPi; olaparib and niraparib) or HER2 overexpression and a sensitivity to anti-HER2 therapies [60]. In ovarian cancer research, Jabs *et al* established primary patient-derived ovarian cancer organoids that were subsequently screened with FDA/EMA-approved, clinically-relevant compounds including carboplatin and olaparib [62]. An association

was observed between the HRD-score and sensitivity to DNA damaging agents in these organoids [62]. Despite promising results of drug screens, organoid platforms are labour-intensive and show suboptimal success rates. Although some studies have shown clear correlations between *ex vivo* (organoid) and *in vivo* (patient) drug responses [65,66], this relationship remains equivocal in most studies. Thus, despite undeniable promise, the route to clinical implementation contains numerous hurdles that must first be overcome.

Another example of a predictive *ex vivo* assay is the organotypic tumour slice [67–70], an approach that helps preserve tumour architecture, including intratumoural heterogeneity [71]. As a proof of principle, patient-derived tumour slices were cultured *ex vivo*, followed by assessment of the cytotoxic response to chemotherapy (5-FU, doxorubicin, and cyclophosphamide) [68]. The technique showed promise in identifying breast cancer patients with tumours resistant to the above-mentioned chemotherapeutic agents [68]. That *ex vivo* modalities can successfully recapitulate the tumour microenvironment was shown by Voabil *et al*, who reported that *ex vivo* reactivation of immune cells by tumour fragments was predictive of a clinical response to PD-1 blockade [72]. Despite the fact that *ex vivo* approaches, which require limited hands-on time, can potentially improve prediction of *in vivo* drug responses, high-throughput screening of anticancer drugs remains complex and challenging, and as such currently hampers clinical adaptation.

The various new tools discussed above provide a window on past cellular events prior to fixation. Delaying or avoiding fixation through the use of *ex vivo* assays or liquid biopsies is still complex, but could potentially make an important contribution to diagnostics and patient care. However, the question remains whether an *ex vivo* model can fully recapitulate the complexity of an *in vivo* tumour. Although the inclusion of a time dimension in surgical pathology still faces serious practical obstacles, it is nevertheless likely that within certain domains it will find an established position in patient management.

Functional assays to optimize prediction of outcome and treatment response

Adding a functional aspect to the static review of FFPE tissue sections could potentially aid assessment of the complex dynamics of tumoural and cellular processes, pathways, and proteins. A prototypical example of the integration of the functional in current diagnostics is by capturing ongoing cellular processes, in this case the evaluation of Ki67 immunostaining, a surrogate for cell proliferation and growth [73]. Other examples of routine immunostainings in diagnostics that reflect the functioning of a particular pathway or cellular process include the mismatch repair (MMR) proteins (MSH2, MSH6, PMS2, MLH1) [74], p53 [75,76], oestrogen receptor (ER) [77,78]/progesterone receptor (PR) [79], HER2 [80], and programmed death ligand 1 (PD-L1) [81]. The analysis of these pathways and processes has had clinical relevance to prognostication and, to some extent, prediction of treatment benefit. The success of a more dynamic assessment of cellular processes has inspired researchers to seek additional functional readouts relevant to current pathology practice.

One way to approach functionality at the tumour level is to divide processes into 'hallmarks of cancer', as formulated by Hanahan and Weinberg [82–84]. These hallmarks aim to capture the complexity of tumour biology that results from the dynamic and heterogeneous interaction and crosstalk of proteins and pathways in a cancerous cell, without reflecting any underlying genotype. A number of functional readouts will be reviewed here in the context of two major hallmarks of cancer ('genome instability and mutation' and 'sustaining proliferative signalling').

Functional assays related to the hallmark 'genome instability and mutation'

The hallmark that describes the initiating and subsequent genomic events in carcinogenesis is 'genome instability and mutation'. Focusing on this hallmark, the most notable example of a clinically-relevant dysfunctionality in a DNA repair pathway is HRD, which is a frequent event in breast, ovarian, and endometrial cancer [85]. Regarding aetiology, germline, or somatic PVs in *BRCA1* and *BRCA2* are the most frequent events underlying an HRD phenotype in ovarian cancer [86]. In addition to DNA-based analyses (including the HRD-related mutational signatures described in the previous section), the presence of specific proteins can serve as a proxy for the functionality of the homologous recombination (HR) DNA repair pathway. The accumulation of RAD51 protein at sites of DNA double-strand breaks is commonly used as a functional readout for HR. Both the RECAP [87–90] and RAD51-FFPE test [91–96], for example, can be considered functional HRD tests with an ability to identify HRD tumours (i.e. the absence of RAD51 accumulation in replicating tumour cells) in female cancers (Figure 3). The strength of functional HRD tests rests on their ability to identify HRD tumours, irrespective of the underlying genotype. These tests can also differentiate individual HRD and HR-proficient cells within a heterogeneous tumour and, in contrast to most DNA-based tests, are likely able to detect acquired resistance to PARPi due to restoration of HR in HRD tumours [87–89,93,94].

Functional assays related to the hallmark 'sustaining proliferative signalling'

Regarding the hallmark 'sustaining proliferative signalling', the diagnostic assessment of oestrogen receptor expression by IHC is routine, especially in breast cancer [77,78]. However, IHC-based ER expression shows limited predictive ability concerning the endocrine therapy response in breast cancer patients [97]. A novel test that better captures the functionality and activity of the ER

pathway is the quantification of mRNA expression of relevant ER-pathway-related genes [98]. Interestingly, this assay was able to improve prediction of responses to endocrine therapy [98] and may offer a superior read-out of ER-sustained proliferative signalling in a tumour. Similarly, a comparable functional mRNA-based ER pathway activity assay was assessed in endometrial cancer tissue and showed a clear association with recurrent disease [99].

The assays described above reflect the functionality of pathways and processes, including the homologous recombination DNA repair and the ER pathway, and may potentially improve prognostication and prediction in cancer. These assays enable *real-time* assessment of tumour functionality, and therefore may more accurately reflect the current state of a tumour. While some of these assays have already been implemented in current practice, we anticipate that these and other functional assays will make a profound contribution to prognostication and prediction in cancer in the coming years.

Multimodal data integration and the role of computational pathology

With the exponential increase in opportunities inherent to functional and multidimensional disease assessment, as described above, the challenge for future pathologists will be to integrate these modalities and grasp the full complexity of disease. The integration of multimodal data and the allocation of appropriate weight to each variable when constructing predictive models poses a challenge [100–102], although these can be overcome, as illustrated by recent examples for acute myeloid leukaemia [103] and myeloproliferative disorders [104]. Interestingly, the unique observational Tumour Profiler (TuPro) Study recently showed that multiomic and multimodal data, including clinicopathological, targeted NGS, CyTOF, bulk RNA, and pharmacoscopy data, can be obtained, integrated, and reviewed in multidisciplinary tumour boards in order to personalise treatment decision-making [100]. Despite the high costs of multimodal profiling, the consortium effectively challenged the current diagnostic and therapeutic infrastructure. The wealth of data obtained will potentially help identify novel diagnostic tools and/or therapeutic targets in cancer management. Beyond the limits of cancer pathology, the expansion of data inherent to innovations is also continuing in other disciplines, further contributing to complexity. Inevitably, pathologists will need to familiarise themselves with the computational possibilities available to analyse and interpret high-dimensional data to manage the wealth of the data and grasp the multidimensional continuum. The revolution in digital pathology is predominantly evoked by the opportunity to perform whole-slide imaging as well as the application of convolutional neural networks/deep-learning. The computational approaches provide pathologists with cutting-edge avenues for the entire workflow, including processing, integrating, and interpreting data. Perhaps the most promising application in this context will be the discovery of novel intrinsic features hidden in high-dimensional space, that we, humans, would have been unacquainted with. Although computational pathology allows for extracting novel—biologically relevant—clusters and subsequent information out of H&E, the input data remains of 2D origin, lacking the spatial (z-axis) and time dimensions already lost during processing. Nevertheless, reestablishing functional and/or multidimensional data, as outlined in this review, and using this as an input to deep-learning models will encourage the model's performance and help improve prognostication and prediction in cancer pathology.

Future roadblocks that may challenge the implementation of multidimensional pathology

The functional and multidimensional techniques and assays, outlined in this review, are promising, yet lack compatibility with current workflows. The current and future challenges that might hamper the implementation include infrastructural limitations, practical feasibility, regulatory issues regarding the applications, and financial- and time-investments. A crucial step in the emergence of multidimensional pathology will be the alignment of innovations with current workflows, demanding automation, standardization among laboratories, as well as integrated pathology reporting. The importance of overcoming these infrastructural needs becomes obvious when drawing a parallel with digital pathology. In spite of the endless possibilities in the world of digital pathology, the usage of high-quality pathology images in routine diagnostics is, as for now, impeded by infrastructural obstacles, including the lack of standardization and quality control of the (pre-) analytical and processing phases as well as insufficient possibilities for data storage. Interestingly, the embracement of molecular diagnostics within the field of pathology over the years illustrates that our perspective on practical feasibility is subject to change. The fact that next-generation or even whole-genome sequencing can be performed routinely in diagnostics used to be beyond our imagination. Transformations like these are driven first and foremost by a positive attitude towards an exchange of knowledge within and between domains, including the computational world. In line with that, pathology, in the end, can only deliver one piece of the puzzle required for optimal patient management. Multidisciplinary consultation to exploit the expertise of various professionals (e.g. medical oncologists and computational scientists) will be key, ensuring that each expert delivers pieces of the puzzle that are required for optimal patient management. We acknowledge that, taking the abovementioned roadblocks into consideration, the burning question remains: is clinical implementation really at the horizon for the multidimensional perspective, or are the techniques predominantly research avenues that may bring us back to the 2D setting, albeit with an advanced perspective?

Moving from 2D to functional and multidimensional pathology

From the 15th century onwards, pioneering work and tremendous technological breakthroughs have built a strong foundation for cancer pathology. Pathologists have mastered the skill of predicting the behaviour of a tumour through evaluation of a 'static' 2D snapshot, a skill that relies heavily on the foundation of pathology: morphology-based pattern recognition, today supported by evaluation of immunostainings and—if necessary—the interpretation of targeted omics data. Following a long period of continuous innovation, we may now be reaching a plateau phase in our pursuit of optimal patient management. Nevertheless, exciting, innovative tools and techniques are on the horizon that will further support spatial or time-dependent dimensions and functional views to current cancer pathology practice; however, the concepts discussed here have wider significance and may also find applications in other—non-oncology—branches of surgical pathology.

In conclusion, *breaking free of the snapshot* will require the exploration of new avenues but offers the promise of a functional and multidimensional future pathology that supports improved prognostication and prediction.

Acknowledgements

The authors thank VTHBM Smit for careful reading of the article. The authors thank MEDACTIE.com for editing support and Maartje Kunen for the medical illustrations in the review. The work was supported by the Dutch Cancer Society, Research Project Grant (TB, 12995).

Author contributions statement

All authors contributed to the conception and design, writing, review, and/or revision of the article.

References

1. van den Tweel JG, Taylor CR. A brief history of pathology: preface to a forthcoming series that highlights milestones in the evolution of pathology as a discipline. *Virchows Arch* 2010; **457:** 3–10.
2. Ghosh SK. Giovanni Battista Morgagni (1682-1771): father of pathologic anatomy and pioneer of modern medicine. *Anat Sci Int* 2017; **92:** 305–312.
3. Gest H. The discovery of microorganisms by Robert Hooke and Antoni Van Leeuwenhoek, fellows of the Royal Society. *Notes Rec R Soc Lond* 2004; **58:** 187–201.
4. Schultz M. Rudolf Virchow. *Emerg Infect Dis* 2008; **14:** 1480–1481.
5. Jansen I, Lucas M, Savci-Heijink CD, *et al*. Three-dimensional histopathological reconstruction of bladder tumours. *Diagn Pathol* 2019; **14:** 25.
6. Liu J, Wu X, Xu C, *et al*. A novel method for observing tumor margin in hepatoblastoma based on microstructure 3D reconstruction. *Fetal Pediatr Pathol* 2020; **41**: 371–380.
7. Merrill AL, Buckley J, Tang R, *et al*. A study of the growth patterns of breast carcinoma using 3D reconstruction: a pilot study. *Breast J* 2017; **23:** 83–89.
8. Yagi Y, Aly RG, Tabata K, *et al*. Three-dimensional histologic, immunohistochemical, and multiplex immunofluorescence analyses of dynamic vessel co-option of spread through air spaces in lung adenocarcinoma. *J Thorac Oncol* 2020; **15:** 589–600.
9. Li P, Duan H, Wang J, *et al*. Neurovascular and lymphatic vessels distribution in uterine ligaments based on a 3D reconstruction of histological study: to determine the optimal plane for nerve-sparing radical hysterectomy. *Arch Gynecol Obstet* 2019; **299:** 1459–1465.
10. Booth ME, Treanor D, Roberts N, *et al*. Three-dimensional reconstruction of ductal carcinoma in situ with virtual slides. *Histopathology* 2015; **66:** 966–973.
11. Yang Y, Wu X, Leng Q, *et al*. Microstructures of the spermatic cord with three-dimensional reconstruction of sections of the cord and application to varicocele. *Syst Biol Reprod Med* 2020; **66:** 216–222.
12. McCarthy R, Orsi NM, Treanor D, *et al*. Three-dimensional digital reconstruction of human placental villus architecture in normal and complicated pregnancies. *Eur J Obstet Gynecol Reprod Biol* 2016; **197:** 130–135.
13. Roberts N, Magee D, Song Y, *et al*. Toward routine use of 3D histopathology as a research tool. *Am J Pathol* 2012; **180:** 1835–1842.
14. Korehisa S, Ikeda T, Okano S, *et al*. A novel histological examination with dynamic three-dimensional reconstruction from multiple immunohistochemically stained sections of a PD-L1-positive colon cancer. *Histopathology* 2018; **72:** 697–703.
15. Xie W, Reder NP, Koyuncu CF, *et al*. Prostate cancer risk stratification via nondestructive 3D pathology with deep learning-assisted gland analysis. *Cancer Res* 2022; **82:** 334–345.
16. Sabdyusheva Litschauer I, Becker K, Saghafi S, *et al*. 3D histopathology of human tumours by fast clearing and ultramicroscopy. *Sci Rep* 2020; **10:** 17619.
17. van Huizen LMG, Kuzmin NV, Barbé E, *et al*. Second and third harmonic generation microscopy visualizes key structural components in fresh unprocessed healthy human breast tissue. *J Biophotonics* 2019; **12:** e201800297.
18. Zhao T, Chiang ZD, Morriss JW, *et al*. Spatial genomics enables multimodal study of clonal heterogeneity in tissues. *Nature* 2022; **601:** 85–91.
19. He B, Bergenstråhle L, Stenbeck L, *et al*. Integrating spatial gene expression and breast tumour morphology via deep learning. *Nat Biomed Eng* 2020; **4:** 827–834.
20. Böhm S, Faruqi A, Said I, *et al*. Chemotherapy response score: development and validation of a system to quantify histopathologic response to neoadjuvant chemotherapy in tubo-ovarian high-grade serous carcinoma. *J Clin Oncol* 2015; **33:** 2457–2463.
21. Cohen PA, Powell A, Böhm S, *et al*. Pathological chemotherapy response score is prognostic in tubo-ovarian high-grade serous carcinoma: a systematic review and meta-analysis of individual patient data. *Gynecol Oncol* 2019; **154:** 441–448.
22. Michor F, Hughes TP, Iwasa Y, *et al*. Dynamics of chronic myeloid leukaemia. *Nature* 2005; **435:** 1267–1270.
23. Huang J, Hu W, Sood AK. Prognostic biomarkers in ovarian cancer. *Cancer Biomark* 2010–2011; **8:** 231–251.
24. Nicolini A, Ferrari P, Duffy MJ. Prognostic and predictive biomarkers in breast cancer: past, present and future. *Semin Cancer Biol* 2018; **52**(Pt 1): 56–73.
25. Coll-de la Rubia E, Martinez-Garcia E, Dittmar G, *et al*. Prognostic biomarkers in endometrial cancer: a systematic review and meta-analysis. *J Clin Med* 2020; **9:** 1900.
26. Fasching PA, Heusinger K, Haeberle L, *et al*. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. *BMC Cancer* 2011; **11:** 486.

27. Bosse T, Nout RA, McAlpine JN, *et al*. Molecular classification of grade 3 endometrioid endometrial cancers identifies distinct prognostic subgroups. *Am J Surg Pathol* 2018; **42:** 561–568.
28. Kommoss S, McConechy MK, Kommoss F, *et al*. Final validation of the ProMisE molecular classifier for endometrial carcinoma in a large population-based case series. *Ann Oncol* 2018; **29:** 1180–1188.
29. León-Castillo A, de Boer SM, Powell ME, *et al*. Molecular classification of the PORTEC-3 trial for high-risk endometrial cancer: impact on prognosis and benefit from adjuvant therapy. *J Clin Oncol* 2020; **38:** 3388–3397.
30. Stelloo E, Nout RA, Osse EM, *et al*. Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC cohorts. *Clin Cancer Res* 2016; **22:** 4215–4224.
31. Talhouk A, McConechy MK, Leung S, *et al*. A clinically applicable molecular-based classification for endometrial cancers. *Br J Cancer* 2015; **113:** 299–310.
32. Rolfo C, Castiglia M, Hong D, *et al*. Liquid biopsies in lung cancer: the new ambrosia of researchers. *Biochim Biophys Acta* 2014; **1846:** 539–546.
33. Saarenheimo J, Eigeliene N, Andersen H, *et al*. The value of liquid biopsies for guiding therapy decisions in non-small cell lung cancer. *Front Oncol* 2019; **9:** 129.
34. Heitzer E, Ulz P, Geigl JB. Circulating tumor DNA as a liquid biopsy for cancer. *Clin Chem* 2015; **61:** 112–123.
35. Janni WJ, Rack B, Terstappen LW, *et al*. Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer. *Clin Cancer Res* 2016; **22:** 2583–2593.
36. Bidard FC, Michiels S, Riethdorf S, *et al*. Circulating tumor cells in breast cancer patients treated by neoadjuvant chemotherapy: a meta-analysis. *J Natl Cancer Inst* 2018; **110:** 560–567.
37. Cristofanilli M, Pierga JY, Reuben J, *et al*. The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): international expert consensus paper. *Crit Rev Oncol Hematol* 2019; **134:** 39–45.
38. Fujita N, Nakayama T, Yamamoto N, *et al*. Methylated DNA and total DNA in serum detected by one-step methylation-specific PCR is predictive of poor prognosis for breast cancer patients. *Oncology* 2012; **83:** 273–282.
39. Fernandez-Garcia D, Hills A, Page K, *et al*. Plasma cell-free DNA (cfDNA) as a predictive and prognostic marker in patients with metastatic breast cancer. *Breast Cancer Res* 2019; **21:** 149.
40. Garcia JM, Garcia V, Silva J, *et al*. Extracellular tumor DNA in plasma and overall survival in breast cancer patients. *Genes Chromosomes Cancer* 2006; **45:** 692–701.
41. Fujita N, Kagara N, Yamamoto N, *et al*. Methylated DNA and high total DNA levels in the serum of patients with breast cancer following neoadjuvant chemotherapy are predictive of a poor prognosis. *Oncol Lett* 2014; **8:** 397–403.
42. Shaw JA, Guttery DS, Hills A, *et al*. Mutation analysis of cell-free DNA and single circulating tumor cells in metastatic breast cancer patients with high circulating tumor cell counts. *Clin Cancer Res* 2017; **23:** 88–96.
43. Tan G, Chu C, Gui X, *et al*. The prognostic value of circulating cell-free DNA in breast cancer: a meta-analysis. *Medicine (Baltimore)* 2018; **97:** e0197.
44. Rolfo C, Mack PC, Scagliotti GV, *et al*. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): a statement paper from the IASLC. *J Thorac Oncol* 2018; **13:** 1248–1268.
45. Pereira E, Camacho-Vanegas O, Anand S, *et al*. Personalized circulating tumor DNA biomarkers dynamically predict treatment response and survival in gynecologic cancers. *PLoS One* 2015; **10:** e0145754.
46. Liu JF, Kindelberger D, Doyle C, *et al*. Predictive value of circulating tumor cells (CTCs) in newly-diagnosed and recurrent ovarian cancer patients. *Gynecol Oncol* 2013; **131:** 352–356.
47. Alexandrov LB, Nik-Zainal S, Wedge DC, *et al*. Signatures of mutational processes in human cancer. *Nature* 2013; **500:** 415–421.
48. Alexandrov LB, Nik-Zainal S, Wedge DC, *et al*. Deciphering signatures of mutational processes operative in human cancer. *Cell Rep* 2013; **3:** 246–259.
49. Alexandrov LB, Kim J, Haradhvala NJ, *et al*. The repertoire of mutational signatures in human cancer. *Nature* 2020; **578:** 94–101.
50. Davies H, Glodzik D, Morganella S, *et al*. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat Med* 2017; **23:** 517–525.
51. Zhao EY, Shen Y, Pleasance E, *et al*. Homologous recombination deficiency and platinum-based therapy outcomes in advanced breast cancer. *Clin Cancer Res* 2017; **23:** 7521–7530.
52. Patsouris A, Diop K, Tredan O, *et al*. Rucaparib in patients presenting a metastatic breast cancer with homologous recombination deficiency, without germline BRCA1/2 mutation. *Eur J Cancer* 2021; **159:** 283–295.
53. Samstein RM, Lee CH, Shoushtari AN, *et al*. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet* 2019; **51:** 202–206.
54. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993; **260:** 816–819.
55. Clevers H. Modeling development and disease with organoids. *Cell* 2016; **165:** 1586–1597.
56. Neal JT, Li X, Zhu J, *et al*. Organoid modeling of the tumor immune microenvironment. *Cell* 2018; **175:** 1972–88.e16.
57. Liu L, Yu L, Li Z, *et al*. Patient-derived organoid (PDO) platforms to facilitate clinical decision making. *J Transl Med* 2021; **19:** 40.
58. Broutier L, Mastrogiovanni G, Verstegen MM, *et al*. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. *Nat Med* 2017; **23:** 1424–1435.
59. Gao D, Vela I, Sboner A, *et al*. Organoid cultures derived from patients with advanced prostate cancer. *Cell* 2014; **159:** 176–187.
60. Sachs N, de Ligt J, Kopper O, *et al*. A living biobank of breast cancer organoids captures disease heterogeneity. *Cell* 2018; **172:** 373–86.e10.
61. van de Wetering M, Francies HE, Francis JM, *et al*. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 2015; **161:** 933–945.
62. Jabs J, Zickgraf FM, Park J, *et al*. Screening drug effects in patient-derived cancer cells links organoid responses to genome alterations. *Mol Syst Biol* 2017; **13:** 955.
63. Roerink SF, Sasaki N, Lee-Six H, *et al*. Intra-tumour diversification in colorectal cancer at the single-cell level. *Nature* 2018; **556:** 457–462.
64. Nanki Y, Chiyoda T, Hirasawa A, *et al*. Patient-derived ovarian cancer organoids capture the genomic profiles of primary tumours applicable for drug sensitivity and resistance testing. *Sci Rep* 2020; **10:** 12581.
65. Vlachogiannis G, Hedayat S, Vatsiou A, *et al*. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* 2018; **359:** 920–926.
66. Tiriac H, Belleau P, Engle DD, *et al*. Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. *Cancer Discov* 2018; **8:** 1112–1129.
67. Carranza-Torres IE, Guzmán-Delgado NE, Coronado-Martínez C, *et al*. Organotypic culture of breast tumor explants as a multicellular system for the screening of natural compounds with antineoplastic potential. *Biomed Res Int* 2015; **2015:** 618021.
68. Naipal KA, Verkaik NS, Sánchez H, *et al*. Tumor slice culture system to assess drug response of primary breast cancer. *BMC Cancer* 2016; **16:** 78.
69. van der Kuip H, Mürdter TE, Sonnenberg M, *et al*. Short term culture of breast cancer tissues to study the activity of the anticancer drug taxol in an intact tumor environment. *BMC Cancer* 2006; **6:** 86.

70. Holliday DL, Moss MA, Pollock S, *et al*. The practicalities of using tissue slices as preclinical organotypic breast cancer models. *J Clin Pathol* 2013; **66:** 253–255.
71. Meijer TG, Naipal KA, Jager A, *et al*. *Ex vivo* tumor culture systems for functional drug testing and therapy response prediction. *Future Sci OA* 2017; **3:** FSO190.
72. Voabil P, de Bruijn M, Roelofsen LM, *et al*. An ex vivo tumor fragment platform to dissect response to PD-1 blockade in cancer. *Nat Med* 2021; **27:** 1250–1261.
73. Tan PH, Bay BH, Yip G, *et al*. Immunohistochemical detection of Ki67 in breast cancer correlates with transcriptional regulation of genes related to apoptosis and cell death. *Mod Pathol* 2005; **18:** 374–381.
74. Stelloo E, Jansen AML, Osse EM, *et al*. Practical guidance for mismatch repair-deficiency testing in endometrial cancer. *Ann Oncol* 2017; **28:** 96–102.
75. Köbel M, Ronnett BM, Singh N, *et al*. Interpretation of P53 immunohistochemistry in endometrial carcinomas: toward increased reproducibility. *Int J Gynecol Pathol* 2019; **38**(Suppl 1): S123–S131.
76. Marks JR, Davidoff AM, Kerns BJ, *et al*. Overexpression and mutation of p53 in epithelial ovarian cancer. *Cancer Res* 1991; **51:** 2979–2984.
77. Harvey JM, Clark GM, Osborne CK, *et al*. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 1999; **17:** 1474–1481.
78. Sieh W, Köbel M, Longacre TA, *et al*. Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study. *Lancet Oncol* 2013; **14:** 853–862.
79. Mohsin SK, Weiss H, Havighurst T, *et al*. Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. *Mod Pathol* 2004; **17:** 1545–1554.
80. Wolff AC, Hammond ME, Schwartz JN, *et al*. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007; **131:** 18–43.
81. Herbst RS, Soria JC, Kowanetz M, *et al*. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 2014; **515:** 563–567.
82. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100:** 57–70.
83. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144:** 646–674.
84. Hanahan D. Hallmarks of cancer: new dimensions. *Cancer Discov* 2022; **12:** 31–46.
85. Knijnenburg TA, Wang L, Zimmermann MT, *et al*. Genomic and molecular landscape of DNA damage repair deficiency across the cancer genome atlas. *Cell Rep* 2018; **23:** 239–54.e6.
86. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, *et al*. Homologous recombination deficiency: exploiting the fundamental vulnerability of ovarian cancer. *Cancer Discov* 2015; **5:** 1137–1154.
87. de Jonge MM, Auguste A, van Wijk LM, *et al*. Frequent homologous recombination deficiency in high-grade endometrial carcinomas. *Clin Cancer Res* 2019; **25:** 1087–1097.
88. Meijer TG, Verkaik NS, Sieuwerts AM, *et al*. Functional *ex vivo* assay reveals homologous recombination deficiency in breast cancer beyond BRCA gene defects. *Clin Cancer Res* 2018; **24:** 6277–6287.
89. van Wijk LM, Vermeulen S, Meijers M, *et al*. The RECAP test rapidly and reliably identifies homologous recombination-deficient ovarian carcinomas. *Cancers (Basel)* 2020; **12:** 2805.
90. Naipal KA, Verkaik NS, Ameziane N, *et al*. Functional ex vivo assay to select homologous recombination-deficient breast tumors for PARP inhibitor treatment. *Clin Cancer Res* 2014; **20:** 4816–4826.
91. Castroviejo-Bermejo M, Cruz C, Llop-Guevara A, *et al*. A RAD51 assay feasible in routine tumor samples calls PARP inhibitor response beyond BRCA mutation. *EMBO Mol Med* 2018; **10:** e9172.
92. Cruz C, Castroviejo-Bermejo M, Gutiérrez-Enríquez S, *et al*. RAD51 foci as a functional biomarker of homologous recombination repair and PARP inhibitor resistance in germline BRCA-mutated breast cancer. *Ann Oncol* 2018; **29:** 1203–1210.
93. van Wijk LM, Kramer CJH, Vermeulen S, *et al*. The RAD51-FFPE test; calibration of a functional homologous recombination deficiency test on diagnostic endometrial and ovarian tumor blocks. *Cancers (Basel)* 2021; **13:** 2994.
94. Waks AG, Cohen O, Kochupurakkal B, *et al*. Reversion and non-reversion mechanisms of resistance to PARP inhibitor or platinum chemotherapy in BRCA1/2-mutant metastatic breast cancer. *Ann Oncol* 2020; **31:** 590–598.
95. Llop-Guevara A, Loibl S, Villacampa G, *et al*. Association of RAD51 with homologous recombination deficiency (HRD) and clinical outcomes in untreated triple-negative breast cancer (TNBC): analysis of the GeparSixto randomized clinical trial. *Ann Oncol* 2021; **32:** 1590–1596.
96. Eikesdal HP, Yndestad S, Elzawahry A, *et al*. Olaparib monotherapy as primary treatment in unselected triple negative breast cancer. *Ann Oncol* 2021; **32:** 240–249.
97. Selli C, Dixon JM, Sims AH. Accurate prediction of response to endocrine therapy in breast cancer patients: current and future biomarkers. *Breast Cancer Res* 2016; **18:** 118.
98. Inda MA, Blok EJ, Kuppen PJK, *et al*. Estrogen receptor pathway activity score to predict clinical response or resistance to neoadjuvant endocrine therapy in primary breast cancer. *Mol Cancer Ther* 2020; **19:** 680–689.
99. van Weelden WJ, van der Putten LJM, Inda MA, *et al*. Oestrogen receptor pathway activity is associated with outcome in endometrial cancer. *Br J Cancer* 2020; **123:** 785–792.
100. Irmisch A, Bonilla X, Chevrier S, *et al*. The Tumor Profiler Study: integrated, multi-omic, functional tumor profiling for clinical decision support. *Cancer Cell* 2021; **39:** 288–293.
101. Boehm KM, Khosravi P, Vanguri R, *et al*. Harnessing multimodal data integration to advance precision oncology. *Nat Rev Cancer* 2022; **22:** 114–126.
102. Sammut SJ, Crispin-Ortuzar M, Chin SF, *et al*. Multi-omic machine learning predictor of breast cancer therapy response. *Nature* 2022; **601:** 623–629.
103. Gerstung M, Papaemmanuil E, Martincorena I, *et al*. Precision oncology for acute myeloid leukemia using a knowledge bank approach. *Nat Genet* 2017; **49:** 332–340.
104. Grinfeld J, Nangalia J, Baxter EJ, *et al*. Classification and personalized prognosis in myeloproliferative neoplasms. *N Engl J Med* 2018; **379:** 1416–1430.