Tumour budding in solid cancers

Alessandro Lugli[1] ✉, *Inti Zlobec*[1], *Martin D. Berger*[2], *Richard Kirsch*[3] *and Iris D. Nagtegaal*[4]

Abstract | Tumour budding is an emerging prognostic biomarker in colorectal cancer (CRC) and other solid cancers. Tumour buds are usually defined as isolated single cancer cells or clusters of up to four cancer cells located at the invasive tumour front. The prognostic value of tumour budding is now supported by a large body of evidence, whereas the utility of this phenotype as a predictive biomarker remains under investigation. The application of tumour budding indices in clinical practice requires a standardized scoring system that can be tailored to specific tumour types and clinical scenarios. In the context of CRC, tumour budding can be assessed according to the method agreed at the International Tumour Budding Consensus Conference (ITBCC) in 2016. Using the ITBCC scoring system, tumour budding is an independent predictor of lymph node metastasis in patients with pT1 CRC and of unfavourable survival in patients with stage II colon cancer. Regardless of the clinical scenario or tumour type, the assertion that 'the more tumour buds, the worse the clinical outcome' applies. In this Review, we provide an overview of tumour budding in solid cancers, highlighting the molecular and biological aspects of this phenomenon, including its associations with epithelial–mesenchymal transition and features of the tumour microenvironment. We also describe the available evidence demonstrating the value of tumour budding as a biomarker across various solid cancers.

Standardized and reproducible biomarkers are an important component of basic and clinical cancer research and are one of the pillars of personalized health care. The tumour, nodes, metastasis (TNM) staging system published simultaneously by the Union for International Cancer Control (UICC) and by the American Joint Committee on Cancer (AJCC) remains the gold standard for the classification of malignant tumours[1,2]. Additionally, the World Health Organization (WHO) classifications of tumours are largely focused on the pathogenetic aspects, providing histological and molecular categorizations incorporating both established and promising potential biomarkers[3].

For most tumour types, the TNM classifications include prognostic factor summary 'grids' comprising three categories, namely (1) essential, (2) additional, and (3) new and promising prognostic factors. Features in each of these categories can be related to the tumour, host or environment, with histology, age and access to treatment as respective examples. The prognostic factors assigned to these categories vary substantially depending on the anatomical location of the primary tumour, the tumour subtype and the weight of evidence supporting their prognostic value. For example, in the eighth edition of the TNM classification, perineural invasion is included as an 'essential' prognostic factor in tumours of the major salivary glands but as an 'additional' factor in tumours of the skin, colorectum or appendix[1,4–6].

Tumour budding (or 'sprouting') and its association with disease progression in patients with various solid cancers was first described by Imai in the 1950s[7]. More recently, tumour budding, typically defined as the presence of isolated single cancer cells or clusters of up to four cancer cells at the invasive tumour front (FIG. 1), has emerged as a promising prognostic biomarker across several different tumour types, predicting disease progression and unfavourable survival[8,9]. Biologically, tumour buds are part of the tumour microenvironment (TME) and are associated with epithelial–mesenchymal transition (EMT)[10]. Although most prominent at the invasive front, tumour buds can also be found within the main tumour body and, therefore, the term 'intratumoural budding' (ITB) has been introduced to distinguish this form of budding from the 'classic' peritumoural budding (PTB)[11].

The publication of the International Tumour Budding Consensus Conference (ITBCC) grading recommendations in 2017 (REF.[12]) has led to the standardization of tumour budding assessment and reporting by pathologists in the context of colorectal cancer (CRC). At present, tumour budding has the potential to influence clinical decision-making in two main scenarios. First, in patients with pT1 CRC, intermediate-grade or

[1]*Institute of Pathology, University of Bern, Bern, Switzerland.*

[2]*Department of Medical Oncology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland.*

[3]*Pathology and Laboratory Medicine, Mount Sinai Hospital, University of Toronto, Ontario, Canada.*

[4]*Department of Pathology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands.*

✉*e-mail: alessandro.lugli@pathology.unibe.ch*

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Key points

- Tumour budding is an independent prognostic factor across a variety of solid cancers.
- In general, the higher the tumour bud count, the worse the clinical outcome.
- Tumour budding is included as a prognostic factor in published cancer classification guidelines of the Union for International Cancer Control (UICC), the American Joint Committee on Cancer (AJCC) and the World Health Organization (WHO).
- Grading systems for tumour budding vary between different types of solid cancers.
- Tumour budding is strongly associated with epithelial–mesenchymal transition and various factors in the tumour microenvironment, where individual tumour buds interact with diverse components of the tumour stroma and immune system.
- The development of international, evidence-based, standardized scoring systems for tumour budding is essential for future multicentre retrospective clinical studies and prospective randomized clinical trials in order to better define the different prognostic groups.

high-grade tumour budding is an independent predictor of lymph node metastasis and is increasingly considered (along with other clinicopathological factors) when deciding on the need for radical surgery (rather than local excision of the tumour). Second, in patients with stage II colon cancer, high-grade tumour budding is a powerful adverse prognostic factor (high-risk feature) that should warrant consideration of adjuvant chemotherapy[12].

The prognostic value of tumour budding in CRC is emphasized by the inclusion of this feature as an additional prognostic factor for this disease in the most recent TNM (2017) and WHO (2019) classification schemes[1–3] and as a recommended element in the College of American Pathologists and International Collaboration on Cancer Reporting protocols for CRC histopathology[13,14]. Emerging evidence suggests that the prognostic value of tumour budding extends to other tumour types, including head and neck, breast, lung, oesophageal, stomach and urogenital tract cancers; however, tumour budding is not yet considered an additional prognostic factor in the classifications of these cancers, largely owing to the lack of disease-specific standardized scoring systems for the validation of this biomarker in retrospective multicentre studies and prospective randomized clinical trials.

In this Review, we provide an overview of the molecular and biological aspects of tumour budding, including its associations with EMT and the TME. We also highlight the emerging role of tumour budding in risk stratification across various gastrointestinal and non-gastrointestinal cancers.

Pathogenetic and molecular aspects

Activating invasion and metastasis is a hallmark of cancer[15]. During the multistep invasion–metastasis process, tumour cells undergo various biological changes that enable them to invade local tissues, intravasate into lymphatic and/or blood vessels, transit through the vascular system, extravasate into parenchymal tissues and finally seed micrometastases at distant sites. The developmental EMT programme, which is associated with increased motility, invasiveness and resistance to apoptosis, is often activated stably but transiently and to differing degrees in carcinoma cells during this process[16–18]. Tumour buds have long been hypothesized to be comprised of cells undergoing EMT. However, the investigation of tumour buds is almost always performed histologically, which limits our understanding of the dynamics of this transition. Over the past 10 years, technological advances and an increasing number of publications have provided novel insights into the relationships between features of EMT, tumour budding and the characteristics of the associated TME.

First step: tumour cell dissociation

Tumour budding is a dynamic process of tumour cell dissociation from the main tumour mass. The findings of several studies indicate a prominent role of E-cadherin, a cell–cell adhesion protein and crucial regulator of EMT, in this process. In particular, immunohistochemistry (IHC) analyses of tumour specimens from patients with CRC, pancreatic ductal adenocarcinoma (PDAC), oral squamous cell carcinoma (OSCC), endometrial cancer or oesophageal cancer have revealed diminished, and potentially completely absent, cell-surface expression of E-cadherin at the invasive tumour front and especially within tumour buds[19–25]. This loss of E-cadherin is often accompanied by a concomitant decrease in the level of β-catenin at the cell membrane and/or within the cytoplasm (but not necessarily by an increase in the level of nuclear β-catenin), suggesting that WNT pathway signalling might be activated in these cells (FIG. 2). Repressors of E-cadherin expression, including the EMT-associated transcription factors ZEB1, ZEB2, TWIST1, TWIST2, SNAI1 (SNAIL) and SNAI2 (SLUG), have also been investigated in the context of tumour budding[20,25,26]. In a histopathological evaluation of 120 PDAC specimens, budding tumour cells were found to have increased expression of ZEB1 and ZEB2 mRNA (detected using in situ hybridization) and protein, with associated decreases in the levels of membranous E-cadherin and β-catenin, as compared with bulk tumour cells[26] (FIG. 2). Tumour buds from OSCCs have been shown to overexpress SNAIL and TWIST1 (by IHC) as well as *ZEB1* (by RNA sequencing)[25,27]. Jensen et al.[25] performed an ingenuity pathway analysis to compare the gene expression profiles of laser-captured material from OSCC tumour buds with that of the corresponding central tumour regions. TGFβ was identified as a key upstream regulator of an EMT-associated gene expression signature in tumour buds, which was characterized by differential expression of 74 genes consistent with the activation of TGFβ signalling in tumour buds. Downstream of TGFβ receptor signalling, phosphorylation of SMADs induces ZEB, TWIST and SNAIL family members and thus transcriptional repression of E-cadherin[28] (FIG. 2). Overexpression of TGFβ and deregulation of SMADs have been described in tumour budding cells[29]. Furthermore, other cell adhesion molecules, including CD44 and EpCAM, are often lost from the membranes of tumour buds[30,31]. In 2019, the authors of a systematic review and meta-analysis focused on tumour budding in PDAC concluded that this morphological phenomenon is intimately associated with EMT[32].

The overexpression of EMT markers is not always found in tumour budding cells. In an IHC analysis of 32 intestinal-type adenocarcinomas of the sinonasal

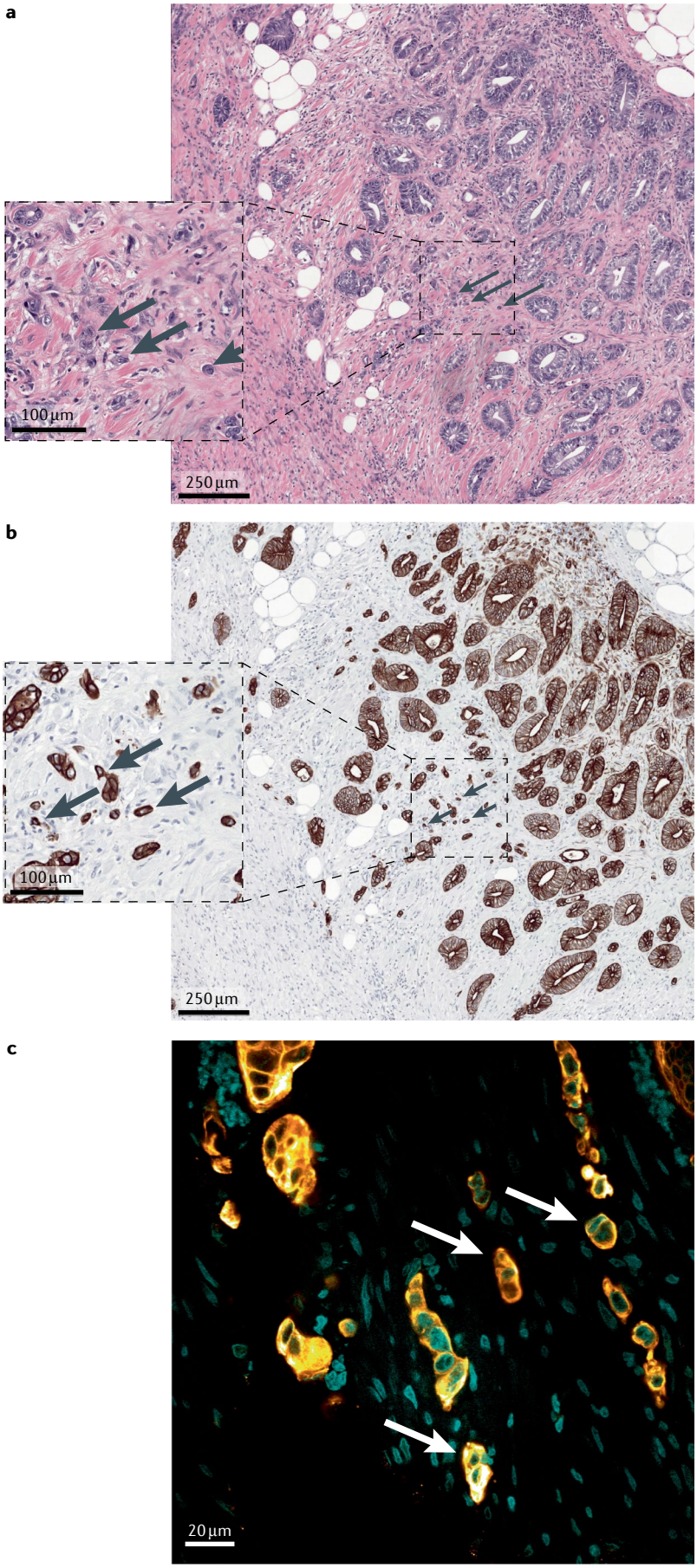

Fig. 1 | **Visualization of tumour budding by immunohistochemistry. a** | Tumour microenvironment of a primary colon adenocarcinoma specimen containing several tumour buds (arrows) visualized through haematoxylin and eosin (H&E) staining. **b** | Immunohistochemical imaging of the same colon adenocarcinoma section re-stained with a cytokeratin 8.18 stain (for the epithelial cell markers cytokeratin 8 and cytokeratin 18) reveals the same tumour budding area (arrows). The inset images in each panel show enlarged views of the tumour budding area. The budding grade of the tumour, defined according to the International Tumour Budding Consensus Conference scoring system, was BD3 (high grade). **c** | Peritumoural budding (intermediate grade; BD2) visible in a confocal immunofluorescence image of a different colon adenocarcinoma specimen with cytokeratin 8.18 (orange) and DAPI (cyan) staining (60× magnification). The invasive margin is depicted projecting outward towards the lower right-hand corner. The edge of the bulk tumour is in the upper left-hand corner. Arrows indicate tumour buds.

tract, only the expression of ZEB2 was correlated with the presence of tumour buds; no statistically significant associations were observed for E-cadherin, ZEB1, ZEB2, SLUG and SNAIL[33]. In CRCs, the expression of ZEB1, TWIST1 and TWIST2 is often not detected in tumour buds, with the upregulation of these factors instead being observed with stromal cells in regions of high-grade tumour budding[34,35], implicating cancer-associated fibroblasts (CAFs) as potential mediators of this process (FIG. 2).

Second step: buds on the move

EMT is characterized by cytoskeletal rearrangements (for example, actin reorganization), cell motility and invasion, increased cell-associated proteolytic activity and reprogramming of gene expression[36]. Evidence suggests that tumour buds share many of these traits. Gene ontology studies using RNA sequencing data from laser-capture microdissected tumour buds (and paired central tumour areas) highlight marked differences in the expression of genes involved in integrin-mediated cell adhesion, cell migration, cytoskeletal changes and extracellular matrix (ECM) degradation[37]. Of note, a monomeric form of laminin 5γ2 is often found to be increased during tumour invasion[38]. This protein is involved in the anchoring of epithelial cells to the underlying basement membrane and is overexpressed during the invasion and migration of cancer cells in vitro as well as in tumour buds, especially in patients with mismatch repair-proficient cancers[37,39]. Correspondingly, the upregulation or overexpression of laminin 5γ2 is associated with aggressive CRCs, OSCCs or squamous cell cancers (SCCs) of the lung and thus with unfavourable patient survival[40–47]. β-catenin binding to TCF and LEF family transcription factors promotes the expression of several genes, including laminin 5γ2 (REF.[48]). Accordingly, decreased membranous and increased nuclear β-catenin levels and positivity for laminin 5γ2, together with decreased E-cadherin expression, are associated with tumour budding in patients with CRC[45]. Serial sectioning and IHC analyses of CRCs has revealed that tumour budding cells extend dendritic processes

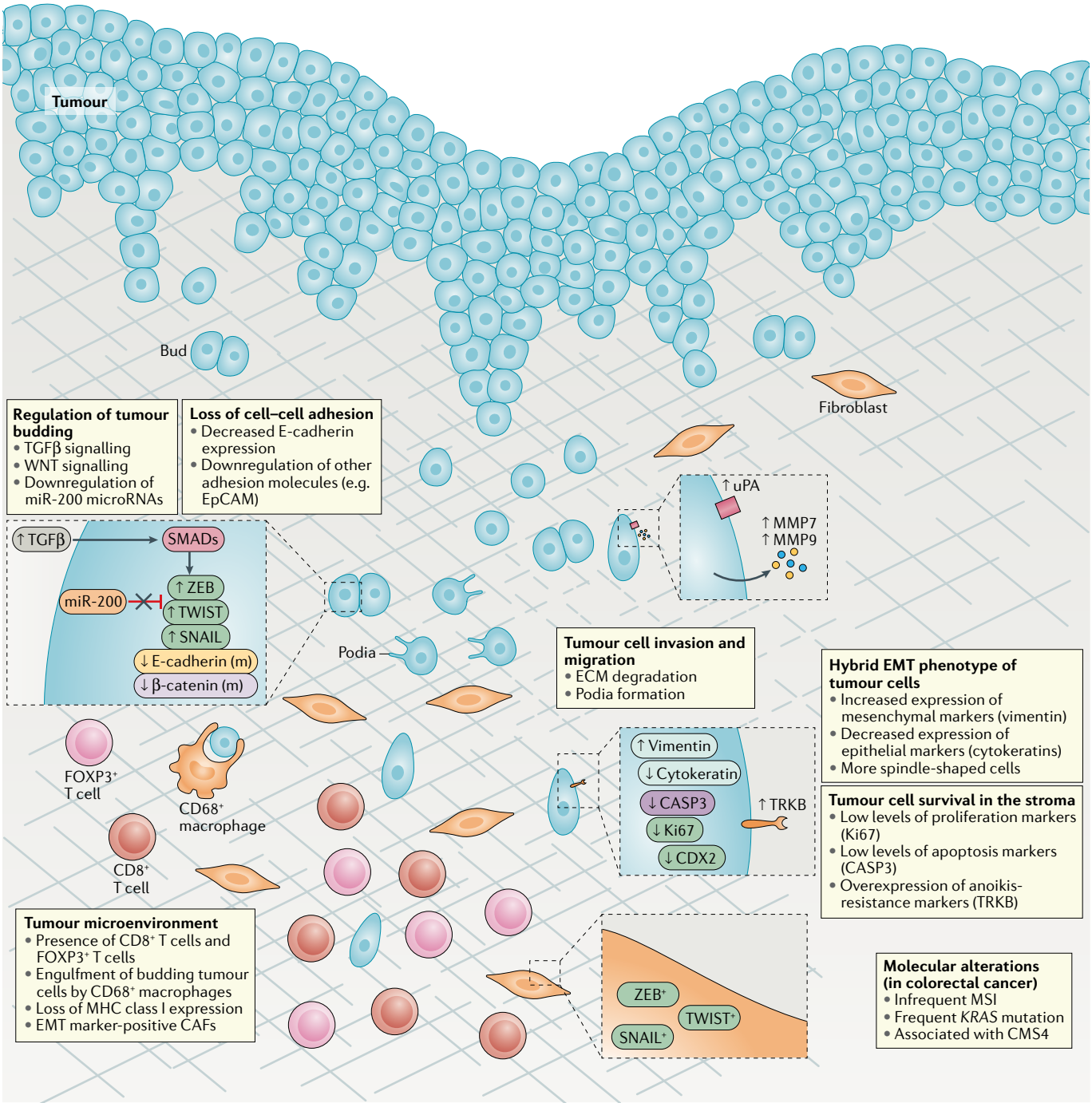

Fig. 2 | **Key processes involved in the tumour budding phenotype.** The TGFβ and WNT signalling pathways as well as microRNAs of the miR-200 family are key factors orchestrating tumour budding via the activation of repressors of E-cadherin expression such as ZEB, TWIST and SNAI1 (SNAIL), transcription factors that are associated with epithelial–mesenchymal transition (EMT). Accordingly, loss of E-cadherin and β-catenin expression and/or accumulation at the cell membrane are frequently observed in budding tumour cells. Tumour buds typically have markers of extracellular matrix (ECM) degradation and cell migration, such as urokinase plasminogen activator (uPA) and matrix metalloproteinase 7 (MMP7) and MMP9, with the tumour cells projecting invasive podia (pseudopodia). Cells of tumour buds also express low levels of both the marker of cell proliferation Ki67 and the pro-apoptotic protein caspase 3 (CASP3) but overexpress the anoikis-resistance marker TRKB. In addition, they can simultaneously express epithelial and mesenchymal proteins (such as cytokeratin and vimentin, respectively). Tumour buds are often surrounded by $CD8^+$ T cells and $FOXP3^+$ regulatory T cells and can be engulfed by $CD68^+$ macrophages. However, budding tumour cells often lose expression of MHC class I molecules on the cell surface, which constitute an immune escape mechanism. Notably, tumour buds are infrequently found in colorectal cancers with microsatellite instability (MSI), perhaps because these tumour cells harbour high numbers of mutations and neoantigens and therefore tend to be intrinsically more immunogenic. Stromal cells in regions of high-grade tumour budding, namely cancer-associated fibroblasts (CAFs), also often express markers of EMT, such as TWIST1, SNAI1 and ZEB1, and a desmoplastic reaction nearly always occurs around the tumour buds. These stromal characteristics typify the CMS4 consensus molecular subtype of colorectal cancer. EpCAM, epithelial cell adhesion molecule; m, membranous.

termed cytoplasmic podia (FIG. 2), which are hypothesized to be involved in cell adherence and movement; these podia are positive for laminin 5γ2 and are associated with vessel invasion[49,50].

The invasive front and tumour budding regions of CRCs are enriched with other markers of cell migration, including class III β-tubulin and high motility group A (HMGA) family proteins[51]. Moreover, ECM-degrading matrix metalloproteinases (MMPs), such as MMP7 and MMP9, are reportedly overexpressed in tumours with high-grade budding, in close correlation with the expression of urokinase plasminogen activator and cathepsin B (a protease that enhances the activity of MMPs and urokinase plasminogen activator)[46,52–54] (FIG. 2). In addition, known suppressors of metastasis, such as RAF kinase inhibitor protein (RKIP, also known as phosphatidylethanolamine-binding protein 1) and maspin, are often disrupted and/or downregulated in tumour buds compared with the main tumour mass[55,56].

The survival of detached cancer cells in the tumour stroma depends on mechanisms that counteract cell death, particularly anoikis (a form of apoptosis induced in anchorage-dependent cells upon loss of requisite cell–cell or cell–ECM interactions), and enable cells to thrive in a hypoxic environment. Accordingly, tumour budding cells of CRCs and gastric cancers overexpress TRKB (FIG. 2), a marker of anoikis resistance, as well as hypoxia-inducible factor 1α (HIF1α)[57–59]. Cell proliferation and migration are hypothesized to be mostly mutually exclusive processes, and thus the switch from cell proliferation to invasion might be triggered by hypoxia[60]. In line with this theory, IHC data indicate that tumour budding cells have either very low levels or an absence of proliferation markers[61–64] (FIG. 2). Furthermore, tumour budding cells often overexpress stem cell markers, such as LGR5, ALDH1 and CD44, which indicates that they might have the capacity for self-renewal, including at distant metastatic sites[31,65–67]. In fact, the prognosis of patients with CRC varies substantially depending on the profile of stem cell markers in tumour buds, suggesting heterogeneity in the invasive and metastatic potential of tumour budding cells[68]. However, the literature is much too sparse to comment further on the possible stem cell phenotype of tumour budding cells.

Taken together, evidence from IHC and RNA sequencing analyses suggests that tumour budding cells have the capacity to degrade the ECM and to invade and migrate through the surrounding stroma. Microscopically, tumour buds are often observed adjacent to or within the endothelium of either lymphatic or blood vessels — possibly caught in the process of intravasation and thus potential dissemination to distant tissues.

The hybrid EMT phenotype

EMT is described as a reversible process, whereby the loss of epithelial characteristics and acquisition of mesenchymal traits is temporary, rather than permanent[36]. The transitional state in which cells both downregulate epithelial markers and simultaneously upregulate mesenchymal ones is often described as partial or 'hybrid' EMT. If tumour budding cells, or a subset thereof, exist in this hybrid EMT state, it follows that they should be observed to express, at some point in time, both epithelial and mesenchymal markers. Indeed, 3–22% of tumour budding cells in CRC specimens were found to co-express both cytokeratin (an epithelial marker) and vimentin (a mesenchymal marker) using two cell-sorting techniques and confocal microscopy[69]. Tumour budding cells with a spindle-like morphology and overexpression of vimentin have also been described in OSCCs and PDACs[70,71] (FIG. 2). The prognostic effect of large numbers of tumour budding cells with a hybrid EMT phenotype remains unknown but this characteristic is hypothesized to reflect an enhanced capacity for tumour dissemination and metastasis.

Tumour budding as a dynamic process

Evidence from electron microscopy studies of CRC specimens suggests that the invasive edge of tumours has a highly dynamic nature and contains a subpopulation of cancer cells with ultrastructural elements compatible with motility, including tumour buds[72]. Histomorphologically, tumour budding is associated with the so-called 'infiltrating growth pattern' of CRCs, which is characterized by finger-like tumour projections that extend into the stroma[73]. 3D reconstruction models of CRCs, PDACs, lung cancers and breast cancers show that many clusters of tumour buds are in fact still interconnected with the tumour mass via these projections and that 'true' isolated tumour buds are rare (9–22%)[74]. Thus, single-cell invasion might be an exceedingly uncommon event, and tumour budding seems to predominantly reflect collective tumour cell migration. This hypothesis is also supported by electron microscopy findings reported by Prall et al.[75]. In the 3D reconstruction study[74], the morphology of cells in tumour buds versus main tumour branches was compared, with rounded and spindle-like morphologies chosen as surrogate parameters for loss of polarization and EMT, respectively. Spindle-like cells were rare but occurred significantly more frequently in tumour buds ($P < 0.001$) than in main tumour branches. Rounded cells were more frequent than spindle-like cells and were also more frequent in buds versus tumour branches ($P < 0.001$). Tumour buds were also more frequently positive for ZEB1 and had reduced E-cadherin expression (particularly on the cell membrane) relative to cells of the main tumour branches; however, no changes in pan-cytokeratin expression were observed, possibly reflecting the hybrid EMT phenotype[74].

Molecular features of budding tumours

In 2015, Guinney et al.[76] proposed the Consensus Molecular Subtypes (CMS) classification of CRC based on a combined analysis of RNA sequencing data from various international cohorts. Specifically, they identified four different CMS of CRCs with distinct gene expression profiles, as well as a small group of unclassifiable tumours. CMS1 cancers typically have microsatellite instability (MSI), DNA hypermethylation and a high level of immune cell infiltration and activation. CMS2 tumours are considered canonical CRCs with activated WNT signalling, CMS3 encompasses a group of highly metabolic CRCs and CMS4 tumours have a

mesenchymal phenotype. Prognostically, CMS4 cancers are associated with the worst overall survival (OS) outcome. The gene expression profile of CMS4 is indeed characterized by the upregulation of mesenchymal genes and of genes involved in TGFβ signalling and EMT, as well as by ECM remodelling, angiogenesis, stromal infiltration and overexpression of β3 integrin. In an analysis of 1,320 CRCs[77], high-grade tumour budding was significantly more common in the CMS4 subgroup than in the epithelial CMS2 and CMS3 subgroups ($P<0.01$ for both comparisons). Notably, the results of laser-capture microdissection studies demonstrate heterogeneity in gene expression profiles within individual CRCs and also OSCCs, with upregulation of genes related to EMT and TGFβ signalling occurring predominantly at invasion fronts and/or tumour budding regions[25,78]. Indeed, De Smedt et al.[78] report a 'CMS-switch' from the epithelial CMS2 signature in the tumour centre to the mesenchymal CMS4 in regions of tumour budding.

Mesenchymal markers are also expressed in tumours with high chromosomal instability (CIN)[79]. In vitro, high CIN is correlated with the expression of vimentin and β-catenin, cytoskeletal reorganization, and an increase in the migratory and invasive behaviour of cancer cells[79]. This phenotype is promoted by oncogenic KRAS, which is commonly associated with CIN and has been implicated as a driver of cellular plasticity and EMT[80]. Numerous — albeit not all — studies of tumour budding in CRC have provided evidence of a close association between this process and *KRAS* mutation[49,81–83]. For example, significantly more cytoplasmic podia have been noted in *KRAS*-mutant than in *KRAS*-wild-type CRCs ($P<0.001$)[49].

MSI, which results from defects in DNA mismatch repair mechanisms, is another form of genomic instability and is mutually exclusive with CIN. Interestingly, both tumour budding[84] and laminin 5γ2 expression[40] are less common in MSI-high sporadic CRCs and hereditary nonpolyposis CRCs than in microsatellite-stable (CIN-positive) CRCs.

The presence of tumour buds seems to be, at least in part, epigenetically regulated by microRNAs (miRNAs) expressed in the tumour cells and/or surrounding stromal cells. Different miRNA signatures of tumour budding have been identified in CRCs, PDACs and OSCCs[85–88]. miRNAs of the miR-200 family are some of the most important regulators of EMT: increased expression of miR-200 miRNAs causes post-transcriptional repression of ZEB1 expression[89]. Tumour buds from both PDACs and CRCs have reduced levels of miR-200 miRNAs (particularly miR-200c) relative to the main tumour mass[86,90] in association with increased ZEB1 and ZEB2 expression (FIG. 2). By contrast, overexpression of miR-21, which is not a member of the miR-200 family, has been described in budding CRC cells and was correlated with laminin 5γ2 expression[88], potentially reflecting EMT.

The attacker versus defender model

MSI-high cancers have been hypothesized to have limited levels of tumour budding owing to the extensive inflammatory cell infiltrates that are often associated with these tumours. The MSI phenotype is associated with the generation of neoantigens that can be recognized as non-self by the immune system, thus resulting in a local immune response capable of eradicating tumour budding cells[91]. Loss of MHC class I expression, which can enable evasion of the adaptive arm of this immune response, has been reported in tumour buds and is associated with an unfavourable prognosis[92]; however, immunostaining of CRC specimens for CD68 and pan-cytokeratin has revealed engulfment of invading tumour cells (presumably tumour buds) by stromal macrophages[93], highlighting the defensive role of the innate immune system against tumour budding (FIG. 2). $CD8^+$ T cells, $FOXP3^+$ T cells and $CD68^+$ macrophages are the most frequently detected immune cells in tumour budding regions of CRCs[94]. The density of these cells in budding areas might have a major role in the stratification of patients into favourable or unfavourable prognostic subgroups[94]. All three of these cell types have previously been associated with clinical outcomes in patients with CRC and other tumour types[95–99]. In particular, the location and density of $CD8^+$ T cells within CRCs have repeatedly been shown to be favourable prognostic features (with greater tumour infiltration associated with favourable outcomes)[98] and are currently included as components in the internationally validated Immunoscore[97,100].

The interactions between tumour buds and the defences against them has been referred to as an attacker–defender model[101,102]: on the one hand, tumour buds reflect an aggressive disease phenotype and, on the other, $CD8^+$ T cells (and others factors) mediate the counterattack. Accordingly, combining tumour bud and $CD8^+$ T cell counts in a ratio, termed the budding-to-T cell score, enables the improved prediction of nodal metastasis and OS in patients with stage I–IV CRC[103,104]. Moreover, the mean number of $CD8^+$ T cells in close proximity to tumour buds (within 50 μm), determined through automated digital image analysis, has been reported to be prognostic of disease-specific survival (DSS) in patients with stage II CRC[103,104]. Beyond CRC, an integrated genomic and immunophenotypic classification of PDAC revealed an 'immune escape' subtype occurring in 54% of patients and characterized by sparse T cell and B cell infiltrates, enrichment with $FOXP3^+$ regulatory T cells, high-grade tumour budding and a poor prognosis[105].

Other stromal factors are implicated in the tumour budding phenotype. Not only are EMT markers, such as SNAIL, TWIST1, TWIST2, ZEB1 and ZEB2, expressed by CAFs in regions of tumour budding (FIG. 2), but also the density and type of desmoplasia are linked to cancer invasion[34,106]. Buds from CRCs are predominately found within desmoplastic regions composed of 'immature' stroma that is associated with a high incidence of recurrence in the liver and lungs (as well as other sites) and with unfavourable OS, suggesting that this microenvironment is more permissive to tumour cell budding and invasion[106].

Understanding the immune escape mechanisms of tumour buds as well as the signals from the tumour stroma that influence budding has the potential to inform novel treatment strategies. Thus, the interactions

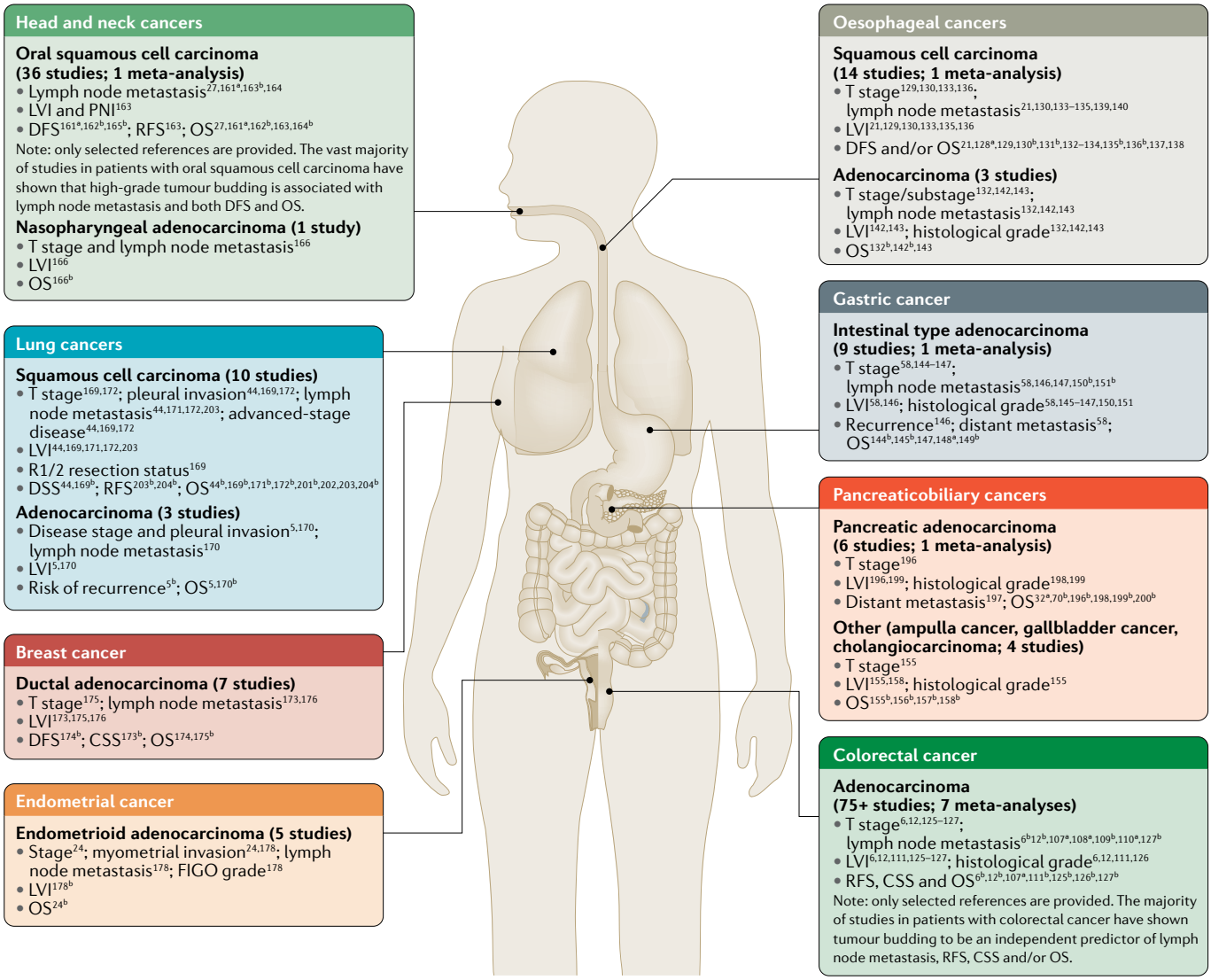

Fig. 3 | **An overview of the prognostic associations of tumour budding in cancers arising at various anatomical sites.** Associations between high-grade tumour budding and a higher T stage, lymph node metastasis, adverse histological features unrelated to disease stage, and unfavourable clinical outcomes are summarized. The number of published studies and meta-analyses evaluating the prognostic implications of tumour budding in each type of cancer are noted, and references are provided for the studies that revealed statistically significant associations with the particular pathological features and clinical outcomes reported in the figure. Definitions of high-grade budding varied across different studies and across different anatomical sites. CSS, cancer-specific survival; DFS, disease-free survival; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; LVI, lymphovascular invasion; OS, overall survival; PNI, perineural invasion; RFS, relapse-free survival. [a]Meta-analysis. [b]Independent association on multivariate analysis.

between CAFs and tumour buds as well as between other cell types need to be further explored.

Prognostic relevance of tumour budding

Gastrointestinal cancers

Colorectal cancer. Tumour budding is a long-established prognostic biomarker in CRC as emphasized by inclusion of this feature as an additional prognostic factor for this disease in the most recent WHO classification of tumours[3]. Various meta-analyses have demonstrated the prognostic value of this biomarker in different clinical settings, including oesophageal, gastric and pancreaticobiliary cancers as well as CRC[107,108] (FIG. 3). Until recently, however, the application of this biomarker in clinical practice was limited by the lack of a standardized assessment and reporting methodology. This deficiency was addressed at the ITBCC in 2016, during which consensus was reached on a standardized definition of tumour buds (single tumour cells or cell clusters of up to four tumour cells), histopathology method (buds counted in the 'hotspot' on a haematoxylin and eosin (H&E)-stained specimen using a 20× objective lens, followed by normalization to a field area of 0.785 mm^2) and clinically relevant cut-off values (low (0–4 buds), intermediate (5–9 buds) and high (≥10 buds), termed tumour budding grades BD1–BD3, respectively), based on a review of the existing evidence from retrospective studies and meta-analyses[12]. This grading system has been validated in >26 studies, including at least 12,754 patients with CRC since it was published in 2017.

In the context of CRC, tumour budding could potentially be considered relevant in three different clinical

scenarios: (1) determining the risk of lymph node metastasis in patients with early stage CRC and thereby informing on the need for radical surgery (that is, with lymph node dissection)[108–110]; (2) identifying patients with high-risk stage II colon cancer, which is a potential indication for adjuvant therapy[107,111]; and (3) as an indicator of metastasis and a lack of response to neoadjuvant therapy if detected in pre-treatment biopsies[112–114]. The latter scenario requires a different methodological approach: in surgical specimens, PTB at the invasive front of the tumour is traditionally evaluated; however, in biopsy specimens, ITB should be determined[11]. Currently, no standardized approach exists for assessing ITB in biopsy specimens, and thus tumour budding is not routinely assessed in this setting. Nonetheless, accumulating evidence indicates that the assessment of tumour budding in preoperative biopsy samples can be of prognostic utility across several different tumour types[112–119]. Several issues need to be addressed before the evaluation of tumour budding in preoperative biopsy specimens can be routinely implemented. First, scoring systems based on robust data need to be developed and might differ by cancer type. Second, the associated clinicopathological end points, such as the presence of metastases, extent of tumour regression after neoadjuvant therapy, and/or disease-free survival (DFS) and OS, need to be clearly defined. Third, the required number, size, quality and depth of the biopsy specimens must be established, all of which might vary between different tumour types.

Importantly, risk stratification according to the ITBCC grading system strongly depends on the clinical scenario[12]. Specifically, both BD2 and BD3 are risk factors for lymph node metastasis in patients with pT1 (stage I) CRC, whereas only BD3 is associated with an increased risk of recurrence and mortality in those with stage II CRC[12]. These observations were the main reason for adopting a three-tier rather than a potentially simpler two-tier grading scheme. Moreover, a three-tier system better reflects the nature of tumour budding as a continuous variable.

Results from two studies using the ITBCC grading system[120,121] have validated the association between intermediate and high-grade tumour budding (BD2–3) and an increased risk of lymph node metastasis in patients with pT1 CRC (TABLE 1), in line with the results of meta-analyses involving data on tumour budding obtained using a variety of different definitions and methodologies[108]. The ITBCC grading system has also been used to analyse the prognostic value of tumour budding in a total of 1,978 patients with stage II CRC included in five independent studies[122–126] (TABLE 1). The results consistently demonstrate favourable outcomes in patients with low-grade tumour budding (BD1), accounting for between 36%[123] and 83%[122] of patients, with a 5-year DSS of 89–98%. Patients with intermediate-grade (BD2) or high-grade tumour budding (BD3) have a significantly worse 5-year DSS of 52–80%. Most studies confirmed the importance of tumour budding through multivariate analyses. These findings suggest that patients with BD3 tumours, in particular, might be candidates for adjuvant therapy. Indeed, in the SACURA trial, adjuvant therapy with tegafur-uracil was associated with a trend towards reduced 5-year recurrence rates in patients with stage II colon cancer: 10.3% versus 14.8% with surgery alone in those with BD2 tumour budding ($P = 0.189$) and 21.0% versus 26.4% with surgery alone in those with BD3 tumour budding ($P = 0.295$)[126]. Additionally, the ITBCC grading system has been successfully validated in a retrospective analysis of 952 primary-operable stage I–IV CRCs in which high-grade primary tumour budding was associated with TNM stage, venous invasion and reduced cancer-specific survival (CSS)[127].

Table 1 | **Prognostic validation of the ITBCC tumour budding grades in pT1 and stage II CRC**

Study (year of publication)	*n*	Disease setting	Clinical end point	End point data according to ITBCC tumour budding grade (%)			*P* value	Ref.
				BD1	BD2	BD3		
Backes et al. (2018)	148	Pedunculated pT1 CRC	Metastasis[a]	20	35		0.04	120
Barel et al. (2019)	312	pT1 CRC	Lymph node metastasis	6	21		0.016	121
Lee et al. (2018)	135	Stage II colon cancer	DSS	89	73	52	0.001[b]	125
Nearchou et al. (2019)	446	Stage II CRC	DSS[c]	87	69	64	0.028[d]	123
Romiti et al. (2019)	174	Stage II colon cancer	DSS	98		80[e]	0.008[f]	124
Slik et al. (2019)	232	Stage II CRC	DSS	90	55	70	0.001[f]	122
Ueno et al. (2019)[g]	991	Stage II colon cancer	RFS	91	85	74	<0.001[f]	126
van Wyk et al. (2019)	445	Stage II CRC	CSS	NA	NA	NA	<0.001[f,h]	127

ITBCC tumour budding grades are assigned through analysis of a single 'hotspot' on a haematoxylin and eosin-stained specimen using a 20× objective lens, followed by normalization to a field area of 0.785 mm^2, with grades defined as follows: BD1: 0–4 tumour buds per hotspot; BD2: 5–9 tumour buds per hotspot; BD3: ≥10 tumour buds per hotspot. All studies were retrospective, and 5-year disease-specific survival (DSS) or relapse-free survival (RFS) is reported, unless otherwise specified. *P* values are for comparisons across all tumour budding grades, unless otherwise noted. CRC, colorectal cancer; CSS, cancer-specific survival; ITBCC, International Tumour Budding Consensus Conference; *n*, number of patients; NA, not available. [a]Case–control study that included both patients with lymph node metastases and patients with distant metastases. [b]Multivariate analysis not performed. [c]DSS at 9.3 years. [d]Only a statistically significant prognostic marker on univariate analysis. [e]Without chemotherapy. [f]Independent prognostic marker on multivariate analysis. [g]Prospective study. [h]*P* value refers to BD3 versus BD1/2.

Oesophageal and gastric cancers. The results of several studies have linked high-grade tumour budding in oesophageal SCCs (ESCCs) with adverse clinical outcomes[21,128–138], lymph node metastasis[21,130,133–135,139,140], T stage[129,130,133,136] and other adverse histological features[21,129,130,133,135,136] (FIG. 3). High-grade tumour budding was an independent predictor of adverse outcomes on multivariate analysis in some of these studies[130,131,135,136] but not in others[133,134,139]. In most studies, tumour buds were counted in the hotspot on H&E-stained specimens using a 20× objective lens[21,129–132,135,136,141], with a cut-off of ≥5 buds most frequently used to define high-grade tumour budding[21,129–132]. However, other researchers have leveraged cytokeratin IHC[133] or applied different cut-offs (≥3 buds)[21,135,136] or different approaches to classification, for example, based on the presence versus the absence of tumour budding[134,139,140] or averaging of tumour budding counts over multiple fields[137,138]. Tumour budding has the potential to aid clinical decision-making in the context of superficially invasive ESCC, whereby the ability to predict lymph node metastasis might help to select patients for radical surgery (that is, extended oesophagectomy with lymphadenectomy) following endoscopic tumour resection. Indeed, evidence from several studies demonstrates that tumour budding is associated with lymph node metastasis in patients with early stage ESCC[21,134,135,139–141], although independent predictive value remains to be established. Thus, large multicentre studies are required to determine the optimum methods and thresholds for defining high-grade tumour budding in ESCC and to establish its independent predictive value before this biomarker is ready for use in clinical decision-making.

Evidence indicates that tumour budding might also be predictive of adverse outcomes in patients with adenocarcinomas of the oesophagus and gastroesophageal junction (FIG. 3). In a study of 287 oesophageal and gastroesophageal junction adenocarcinomas, tumour budding was associated with T stage, lymph node metastasis and poor tumour differentiation and was found to be an independent predictor of OS[132]. Similarly, results from an analysis of 210 surgically resected pT1 oesophageal adenocarcinomas demonstrated that tumour budding is an independent predictor of lymph node metastasis and OS[142]. In a third study[143], ITB (but not PTB) was associated with decreased OS, although this association was lost on multivariate analysis. Further studies are needed to better define the prognostic role of tumour budding in oesophageal adenocarcinomas.

Findings of numerous studies have linked tumour budding with adverse clinical outcomes[58,144–149], T stage[58,144–147], lymph node metastasis[58,146,147,150,151], lymphovascular invasion[58,147] and histological grade[58,145–147,150,151] of gastric cancers (FIG. 3). These associations predominantly apply to intestinal-type gastric cancers because most diffuse-type gastric cancers would be classified as positive for tumour budding owing to their discohesive growth pattern[8,147]. The potential of tumour budding to influence clinical decision-making might be greatest in the early stage disease setting, in which predicting lymph node metastasis could facilitate the selection of patients for radical gastrectomy following endoscopic tumour resection. In a series of 276 patients with well or moderately differentiated, tubular or papillary-type submucosal early stage gastric cancers undergoing radical gastrectomy, tumour budding was found to be an independent predictor of lymph node metastasis[150]. The prevalence of lymph node metastasis was 26% (39/150) in patients with tumour budding compared with 6.3% (8/126) in those without, and was as low as 3.7% (4/109) in those without both tumour budding or lymphovascular invasion[150]. Tumour budding was also independently associated with lymph node metastasis in a smaller series of patients with pT1a or pT1b gastric cancer ($n = 126$)[151]. Methodologies and cut-offs for defining high-grade tumour budding in gastric cancer have varied widely, thus limiting the role of tumour budding as a prognostic biomarker in current clinical practice. As with oesophageal cancers, the optimization and validation of this biomarker in large, multicentre cohorts is required for its implementation in the management of gastric cancer.

Pancreatic and biliary tract cancers. The prognostic role of tumour budding in PDAC was systematically analysed in a meta-analysis reported in 2019, which included data from a total of 613 patients involved in six eligible studies[32]. High-grade tumour budding, defined as at least 10 tumour buds per high power field, was detected in 40.9% of patients and was associated with an increased risk of disease recurrence and of all-cause mortality: RR 1.61 (95% CI 1.05–2.47; $P = 0.03$) and 1.46 (95% CI 1.13–1.88; $P = 0.004$), respectively[32].

Efforts have been made to better understand the pathogenetic mechanisms of PDAC through molecular characterization. Bailey et al.[152] reported the molecular classification of PDAC on the basis of transcriptional signatures, which enabled the identification of four disease subtypes: squamous, pancreatic progenitor, immunogenic and aberrantly differentiated endocrine exocrine. Puleo et al.[153] have also proposed a classification system based on gene expression analysis of formalin-fixed PDAC specimens that includes five subtypes, namely pure basal like, stroma activated, desmoplastic, pure classical and immune classical. The disease subtypes identified in these two studies reflect both tumour-related and host-related factors and were associated with differences in clinical outcome, thus underlining the importance of the TME — including the immune landscape — in the prognosis of patients with PDAC. The interaction of tumour buds and immune cells in solid cancers underscores the importance of the immune landscape to classifications of disease subtypes. In PDAC, three immune phenotypes have been described[102]: (1) an immune-escape phenotype characterized by high numbers of $FOXP3^+$ regulatory T cells and M2-like macrophages and lower numbers of $CD3^+$ and $CD4^+$ or $CD8^+$ T cells, $CD20^+$ B cells, and M1-like macrophages, together with miRNA dysregulation and high-grade EMT-like tumour budding[102]. This phenotype has molecular and clinical similarities with the squamous PDAC subtype described by Bailey et al.[152] and is associated with unfavourable clinicopathological features and a poor prognosis[102]. (2) An immune-rich phenotype that has the opposite profile of immune cells

and tumour budding and is therefore associated with a better prognosis[102] (sharing characteristics with the pancreatic progenitor subtype described by Bailey et al.[152]). (3) An immune-exhausted phenotype defined by the upregulation of PD-L1 expression in association with an immune-rich TME, loss of DNA mismatch repair proteins and often high-grade EMT-like tumour budding, in association with a poor prognosis relative to other PD-L1-upregulated carcinomas[102] (compatible with the immunogenic subtype reported by Bailey et al.[152]). The characteristics of the immune-exhausted phenotype suggest that the immunosuppressive mechanisms convert a TME that is typically not permissive to tumour budding into a tumour budding-permissive TME, potentially providing a therapeutic opportunity for PD-1 or PD-L1 inhibitors. These findings are in keeping with the attacker–defender model of tumour budding and suggest that composite tumour budding and immune cell scores might have added prognostic value as demonstrated in the context of CRC[97,101–103,154].

Tumour budding has also been detected in ampullary and gallbladder cancers and in intrahepatic and extrahepatic cholangiocarcinomas[8,155–158]. In these tumour types, high-grade tumour budding, according to the definitions proposed by Ueno et al.[159], Kai et al.[160] and the ITBCC[12], is associated with tumour progression and unfavourable OS on multivariate analysis[8,155–158] (FIG. 3).

In summary, tumour budding seems to be a promising prognostic biomarker in patients with pancreatic or biliary tract cancers. Currently, however, the lack of an international standardized scoring system and the small number of published studies precludes the implementation of tumour budding in routine clinical practice.

Non-gastrointestinal cancers

Head and neck cancers. In a meta-analysis that included 16 studies involving a total of 2,341 patients with OSCC[161], high-grade tumour budding according to variable definitions was significantly associated with lymph node metastasis (OR 7.08, 95% CI 1.75–28.73) and with both reduced DFS (HR 1.83, 95% CI 1.34–2.50) and OS (HR 1.88, 95% CI 1.25–2.82 across all disease stages), compared with low-grade tumour budding; several other studies have produced similar findings (FIG. 3). In a series of 246 patients undergoing primary resection of tongue SCC, high-grade tumour budding was an independent variable predicting unfavourable DFS and OS[162]. In a study involving 200 patients with OSCC[163], tumour budding was correlated with lymphovascular and perineural invasion and was significantly associated with the presence of lymph node metastases in those with early stage disease ($P = 0.042$) and shorter recurrence-free survival in the entire cohort ($P \leq 0.0001$); budding was also an independent predictor of lymph node recurrence in patients with resected stage I and II disease (adjusted HR 3.89). Xie et al.[164] applied the ITBCC scoring system to tongue SCC specimens and found that a greater degree of tumour budding was correlated with adverse clinicopathological features, such as lymph node metastases, depth of invasion and unfavourable OS. Additionally, incorporation of the degree of tumour budding into the 2017 WHO histopathological grading system resulted in increased prognostic value (with regard to DFS and DSS) in patients with early stage tongue SCC[165].

A few studies have evaluated the biological aspects of tumour budding in head and neck cancers. In a small retrospective cohort comprising 56 patients with surgically treated OSCC (encompassing all stages), the presence of tumour budding correlated with the expression of SNAIL as well as lymph node metastasis and unfavourable OS[27]. Through RNA sequencing, Jensen et al.[25] identified a distinct gene expression signature in budding cells characterized by increased expression of well-established EMT transcription factors, such as ZEB1 and PRRX1, relative to that observed in central tumour cells (although no difference was found in levels of TWIST or SLUG expression). Furthermore, the tumour budding cells had upregulation of genes involved in TGFβ signalling[25]. These findings suggest that disrupting TGFβ-driven EMT might be a promising strategy to improve the treatment of cancer.

High levels of expression of the stem cell marker aldehyde dehydrogenase 1 (ALDH1) have been reported in tumour budding cells of nasopharyngeal carcinomas[166] and in areas of OSCCs with high-grade budding[167]. In addition, high-grade tumour budding in OSCC correlates with the expression of the stem cell marker CD44, which regulates cell proliferation and migration[65]. These observations suggest that tumour budding cells have stem cell-like properties[168].

Lung cancer. In 354 patients with resected primary lung SCC, high-grade tumour budding (according to the ITBCC criteria) was associated with larger tumours, higher UICC/AJCC pT, pN and disease stages, mediastinal lymph node metastasis, pleural invasion, an R1/2 resection status, and unfavourable progression-free survival, DSS and OS[169]. Using a different grading system, Yamaguchi et al.[170] identified statistically significant correlations between tumour budding and lymph node metastasis, tumour stage, lymphovascular or pleural invasion, and OS in 181 patients with small (≤3 cm in diameter) stage I–III lung adenocarcinomas. Tumour budding cells had reduced levels of E-cadherin, β-catenin and laminin 5γ2 expression as well as reduced levels of the differentiation marker surfactant protein A compared with tumour cells located in 'nests'. The predominant papillary subtype was associated with tumour budding, whereas an absence of tumour budding was correlated with the bronchioloalveolar subtype[170]. Similar associations of tumour budding with adverse clinicopathological features and markers of an aggressive disease biology were observed in a series of 217 patients with resected primary lung SCC (stage IA–IIIA)[44]. Among patients in this cohort, 5-year OS was higher in those without detectable tumour budding than in those with tumour budding (64.0% versus 45.6%; $P < 0.001$)[44]. These findings were mirrored by those of Masuda et al.[171] in 103 patients with resected primary lung SCC: again, the presence of tumour budding was associated with both lymphatic invasion and lymph node metastasis, which translated into unfavourable OS[171]. More recently, Kadota et al.[172] confirmed tumour budding as

an independent prognostic factor for OS in 485 patients with resected stage I–III SCC non-small-cell lung carcinomas. Additionally, high-grade tumour budding was independently associated with an increased risk of recurrence (HR 1.61, 95% CI 1.13–2.29; $P = 0.008$) in a study including 524 patients with resected stage I lung adenocarcinoma[5].

Despite these prognostic associations, methodological inconsistencies between the studies, especially regarding cut-offs for defining high-grade versus low-grade budding, again limit the application of tumour budding as an adverse histological factor in clinical practice. Thus, a validated and standardized scoring method for tumour budding in both adenocarcinomas and SCCs of the lung is essential to clarify the role of this emerging prognostic biomarker and its potential to guide therapeutic decision-making.

Breast cancer. In a series of 474 patients with invasive ductal carcinoma, tumour budding correlated with adverse clinicopathological features, including lymphatic invasion, lymph node involvement, a high tumour stromal content, a low level of inflammatory infiltration and, ultimately, unfavourable CSS[173]. Tumour budding grade (defined as G1 (<5 buds), G2 (5–9 buds) or G3 (≥10 buds) per 0.95 mm^2 field) was significantly associated with DFS on multivariate analysis ($P = 0.009$) and OS on univariate analysis ($P < 0.001$) in 146 patients with operable invasive ductal breast cancers[174]. In 160 patients with surgically treated invasive ductal carcinoma, high-grade budding (≥8 buds) was associated with lymphovascular invasion, larger tumours and reduced OS in both univariate and multivariate analyses as compared with low-grade budding (<8 buds)[175]. Tumour budding cells had decreased membranous E-cadherin and nuclear Ki67 levels but increased vimentin levels compared with those of cancer cells located in the tumour centre[175]. Salhia et al.[176] investigated the utility of ITB and PTB as predictors of lymph node involvement using a series of 148 invasive ductal breast cancer resection specimens and 99 matched preoperative biopsy samples. A high number of PTB (average of >4 buds comprising 1–5 tumour cells across 10 high-power fields) in resection specimens was associated with both lymphatic invasion and lymph node metastasis. In the biopsy samples, high-grade tumour budding (≥10 buds per high-power field) was correlated with venous invasion[176]. Interestingly, Laedrach et al.[177] demonstrated differences in progesterone (but not oestrogen receptor or HER2) expression between tumour budding cells and non-budding tumour cells. Notwithstanding, further research is required to determine the utility of tumour budding as a prognostic factor in routine clinical practice.

Endometrial cancer. Only a small number of studies have evaluated tumour budding in endometrial cancer (FIG. 3). In a series of 95 patients with this disease, high-grade tumour budding (defined as ≥5 budding foci per field) was associated with advanced tumour stage, myometrial invasion and reduced OS in univariate and multivariate analyses relative to low-grade budding[24]. Tumour budding has also been associated with depth of invasion, an advanced FIGO grade, lymphovascular invasion and lymph node-positive disease in a retrospective analysis of 96 endometrioid carcinomas; however, no statistically significant association with OS was observed[178]. The tumour budding cells had a loss of oestrogen receptor and progesterone receptor expression as well as reduced expression of E-cadherin[178]. Further studies are required to determine the prognostic value of tumour budding in endometrial cancers.

Cervical cancer. The prognostic value of tumour budding in early stage SSCs and adenocarcinomas of the uterine cervix has also been investigated. In a cohort predominantly comprising women with SSCs, high-grade tumour budding (≥5 buds) was found to be an independent prognostic factor for DFS and OS[179]. However, in women with adenocarcinomas, tumour budding was associated with unfavourable DFS and CSS on univariate but not on multivariate analysis[180]. Additionally, the degree of tumour budding has been combined with cell nest size in a scoring system for the histopathological grading of cervical cancers[181]. This novel grading system was independently predictive of DFS, DSS and OS, with superior prognostic performance compared with the conventional WHO grading system and was proposed as an additional histopathological parameter for daily routine diagnostics[181].

Urothelial cancer. The prognostic value of tumour budding in urothelial carcinomas has only been investigated in a series of 60 patients with muscle-invasive bladder cancer[182]. No correlation was found between tumour budding and tumour necrosis, lymphovascular invasion, perineural invasion, metastasis, progression-free survival or OS[182].

Methodological considerations

Tumour budding is typically assessed using H&E-stained specimens, although the use of pan-cytokeratin IHC has also been frequently used. Both techniques have advantages and disadvantages. Morphological and cytological atypia as well as cellular context can be better appreciated with H&E staining; however, tumour buds can be difficult to identify on a background of peritumoural inflammation and activated fibroblasts can resemble and therefore be misinterpreted as tumour buds. Pan-cytokeratin can highlight tumour buds (FIG. 1b) and improves interobserver agreement at the patient level, although tumour areas containing fragmented glands are distracting and must be avoided in the budding count[183].

Historically, definitions, scoring methods and grading schemes for tumour budding have varied widely. For example, tumour buds have been defined as single cells or clusters of up to either 4 or 5 cells, depending on the publication. Numerous different methods have been used to categorize tumour budding, including those proposed by Hase et al.[184] (qualitatively defined as none/minimal, moderate or severe), Ueno et al.[185] (negative or positive status defined by <5 buds and ≥5 buds, respectively, within a 20× objective area of 0.785 mm^2), and Nakamura et al.[186] (none, half, two-thirds or greater than two-thirds of the invasive tumour margin

with budding) and have involved the analysis of single hotspots or a 10-hotspot approach, on H&E-stained or pan-cytokeratin specimens, at 20× or 40× magnification. These scoring systems have been summarized elsewhere[187,188]. The ITBCC guidelines[12], based on evidence primarily from large-cohort studies conducted in Japan, recommend reporting absolute budding counts in an area of 0.785 mm^2 on H&E-stained specimens within a single hotspot and grading the severity as BD1 (0–4 buds), BD2 (5–9 buds) or BD3 (≥10).

Interestingly, work by Bokhorst et al.[189] highlights some of the challenges that expert pathologists face in identifying individual tumour buds, chiefly whether or not a 'candidate' tumour bud is indeed a true bud. Computational pathology approaches could potentially help to improve the standardization of budding counts as well as automating this process. Several groups have published algorithms for the detection and quantification of tumour buds, which have been applied to the analysis of budding in CRC[88,104,122,190–193], muscle-invasive bladder cancer[194] or OSCC[195]. All of these algorithms are based on pan-cytokeratin IHC or immunofluorescence; automated or semi-automated machine learning approaches using H&E-stained specimens are yet to be reported.

Conclusions

The prognostic value of tumour budding in patients with CRC is now well established. In addition, emerging evidence suggests that tumour budding has prognostic value in an expanding list of gastrointestinal and non-gastrointestinal malignancies. The availability of the standardized ITBCC grading system for CRCs has supported the status of tumour budding as an additional prognostic factor in the 2017 UICC/AJCC TNM classification and has facilitated the inclusion of tumour budding in the 2019 WHO classification of tumours[1,3] and as a recommended element in the CRC pathology protocols of the College of American Pathologists and the International Collaboration on Cancer Reporting[13,14]. Since the publication of the ITBCC recommendations in 2017 (REF.[12]), the proposed scoring method has been validated in 25 retrospective studies as well as in a prospective study, with two main clinical implications: (1) the tumour budding grade, along with other clinicopathological factors, can aid the selection of patients with pT1 CRC for radical surgery; and (2) high-grade tumour budding is a high-risk feature in patients with stage II CRC and can warrant the consideration of adjuvant chemotherapy.

Less published data are available regarding tumour budding in non-CRCs, although the body of biological, molecular, pathogenetic and clinical evidence suggests that the mantra 'the more tumour buds, the worse the clinical outcome' applies to all solid cancers. Internationally standardized and validated grading systems for the assessment of tumour budding in individual cancer types will be essential to further advance the field by providing a solid basis for multicentre retrospective or prospective clinical studies.

Biologically, tumour budding is intimately associated with EMT, and tumour buds are a key component of the TME. The aggressive biology of tumour buds is closely related to an increased capacity for tumour cell dissociation, migration and infiltration. The interaction between tumour buds as a tumour-related factor and immune cells as a host-related factor reflects the attacker–defender model, with important prognostic and, potentially, therapeutic implications. Promising avenues for current and future research include the application of digital pathology to improve the accuracy and reproducibility of tumour budding assessment in clinical practice and the delineation of the molecular and pathogenetic mechanisms underpinning tumour budding, a deeper understanding of which might be a starting point for the development of 'anti-budding therapies'.

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