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The Good, the Bad and the Ugly - Novel Synoptic Variables in the Personalisation of Colon Cancer Treatment

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Abstract

Purpose

Standardised international reporting for colon cancer (CC) provides foundational prognostic data. However, novel histopathological variables - tumour-infiltrating lymphocytes (TILs), tumour budding (TB), and poorly differentiated clusters (PDCs) -offer superior insights into tumour biology. This review evaluates their clinical utility in risk stratification and treatment personalisation for stage I-III CC.

Methods

A comprehensive literature review was conducted to evaluate the prognostic and predictive value of TILs, TB, and PDCs in stage I-III CC.

Results

High TIL density is associated with improved oncological endpoints in stage II-III disease. Crucially, high TILs predict a survival benefit from extending adjuvant chemotherapy from three to six months in stage III disease while low TIL density in mismatch repair deficient tumours identifies a high-risk patient cohort that is otherwise overlooked. Conversely, high-grade (HG) TB and PDCs are potent independent markers of poor prognosis, outperforming traditional histopathological variables in multiple studies. In stage I disease, HG TB/PDCs significantly elevate the risk of lymph node metastasis, providing a critical metric when considering segmental resection after malignant polyp removal. In the stage II/III cohort, HG TB/PDCs correlate with diminished disease free survival and overall survival, with emerging data suggesting they may identify phenotypes resistant to standard fluoropyrimidine monotherapy.

Conclusion

TILs, TB, and PDCs provide vital, biology-focused prognostic and predictive data using cost-effective, routinely stained sections of tumour. Incorporating these variables into standard synoptic reporting and clinical guidelines will refine risk stratification and advance precision oncology in colon cancer management.

Introduction

Modern histopathological reporting of colon cancer (CC) resection specimens is standardised internationally, with International Collaboration on Cancer Reporting (ICCR) guidelines recommending 18 core and 7 non-core variables for inclusion (Table 1). Structured synoptic reporting facilitates a methodical and transparent approach to specimen examination, which has been associated with an improvement in clinical outcomes (1).

While traditional synoptic variables are fundamental to multidisciplinary decision-making across all CC patients, their application is particularly pivotal in certain clinical scenarios. These include assessing the need for segmental resection following colonoscopic removal of a malignant polyp, evaluating the indications for adjuvant chemotherapy (AC) following curative resection in stage II CC, and determining the optimal duration of AC in stage II and III CC (2, 3). There is also an emerging body of evidence highlighting the importance of variables such as mismatch repair (MMR), KRAS, and BRAF status in determining candidates who might benefit from neoadjuvant treatment (4, 5).

Advances in histopathological tumour assessment have identified variables that confer significant prognostic and predictive clinical utility in CC: tumour-infiltrating lymphocytes (TILs), tumour budding (TB), and poorly differentiated clusters (PDCs) (6-9). TILs reflect the infiltration of predominantly T cells and, less frequently, B cells into the tumour microenvironment, serving as a marker of the host's adaptive immune response and a reliable indicator of a favourable prognosis (7, 10) (Figure 1). Conversely, TB and PDCs are markers of poor prognosis theorised to represent different points along the spectrum of epithelial-mesenchymal transition. This biological process enables epithelial cells to acquire enhanced migratory capacity, resistance to apoptosis, and the ability to remodel the extracellular matrix. Morphologically, TB is defined as a group of 1-4 neoplastic cells, while PDCs represent a more advanced variant characterised by clusters of five or more neoplastic cells lacking glandular differentiation (6, 8) (Figure 2).

The aim of this review is to highlight the clinical utility of TILs, TB, and PDCs as they pertain to predicting prognosis and informing treatment strategies for non-metastatic CC.

Methods

A comprehensive narrative literature review was conducted to evaluate the clinical utility, prognostic impact and predictive value of TILs, TB and PDCs in stage I–III CC. To ensure a high standard of structural quality and methodological transparency, the formulation and reporting of this review adhere to the Scale for the Assessment of Narrative Review Articles (SANRA) guidelines. The complete SANRA checklist is available as supplementary material.

A structured literature search was performed across the PubMed and MEDLINE database for English-language peer-reviewed articles published from database inception through to February 2026. Search strings utilised a combination of Medical Subject Headings (MeSH) terms and free-text keywords, including: ("colon cancer" OR "colorectal carcinoma") AND ("synoptic report" OR "histopathology report") AND ("tumour budding" OR "poorly differentiated clusters" OR "tumour-infiltrating lymphocytes"). Inclusion criteria consisted of randomised controlled trials (and their post-hoc ancillary analyses), international consensus guidelines, systematic reviews or meta-analyses and both prospective and retrospective observational cohort studies evaluating non-metastatic colon cancer. Studies focusing exclusively on rectal cancer, metastatic disease or non-human models were excluded.

The Good - Tumour Infiltrating Lymphocytes

Background

TILs comprise populations of T and B cells that infiltrate the tumour microenvironment from the systemic circulation, reflecting a critical component of the host's native anti-tumour response (Figure 1). TIL density is heavily influenced by the tumour's underlying genetic profile and overall mutational burden. MMR deficient (dMMR) tumours typically elicit a robust TIL response as a result of the high mutational burden characteristic of these tumours (7, 11).

Conventional assessment relies on haematoxylin and eosin staining to quantify TIL density as a continuous percentage (0–100%) within the peri-tumoural stroma. Although this validated approach is highly cost-effective, its reliance on visual assessment can lead to interobserver variability (7). In contrast, the digital pathology-based Immunoscore (IS) utilises automated immunostaining and proprietary digital imaging software to precisely quantify $CD3^+$ and $CD8^+$ cell densities. This method focuses on two critical regions within the tumour itself: the tumour centre (TC) and the invasive margin (IM). While combined analysis of the TC and IM in surgical specimens remains the gold standard, quantifying intra-epithelial lymphocyte density from preoperative colonoscopic biopsies offers a reliable alternative that correlates strongly with final resection specimen evaluation (12). $CD3^+$ and $CD8^+$ cells are used specifically for their high reproducibility and independent prognostic power compared to other lymphocytic subsets. Finally, the software assigns a percentile based on reference ranges established from international cohorts, stratifying patients into three prognostic tiers: low (0-25^{th} percentile), intermediate (25^{th}-70^{th} percentile), and high (>70^{th} percentile) (11, 13).

Multivariate analyses have established TILs to be a robust predictor of time to recurrence, disease-free survival (DFS), and overall survival (OS) across various international CC cohorts (7, 14, 15). These findings are further supported by large meta-analyses demonstrating that high TIL density correlates with improved OS and DFS following curative CC resection (16, 17). Although TILs are typically associated with dMMR tumours - which generally carry a superior stage-matched prognosis - their predictive value remains independent of MMR status (7, 14, 15).

Clinical Applications

Stage II Colon Cancer

Analysis of 1,434 stage II CC patients from an international cohort demonstrated that high TIL density correlates with superior five-year DFS (HR 0.33, 95% CI 0.21-0.52, P <0.0001) (14). These findings were corroborated in a smaller Austrian cohort of combined stage I/II colorectal cancer (CRC) patients which found TILs to be significantly associated with improved five-year DFS and OS (18). Despite the promising results in this cohort, TILs are yet to be formally incorporated into the European Society for Medical Oncology (ESMO), American Society of Clinical Oncology (ASCO), or National Comprehensive Cancer Network (NCCN) treatment guidelines (2, 3, 19). ESMO guidelines acknowledge the prognostic value of

TILs in stage II CC with the caveat that there is no evidence regarding treatment benefit from AC in this cohort (2) (Table 2).

Stage III Colon Cancer

Multivariable analyses from several large international stage III CC cohorts have established TILs as an independent prognostic factor for improved DFS and OS. Additionally, multiple studies have validated IS in this cohort, with a low IS conferring an increased risk of relapse and poorer DFS (20-23).

Beyond their prognostic utility, TILs may serve as a predictive marker for AC benefit in stage III disease. Specifically, a retrospective analysis involving multiple international cohorts demonstrated that AC efficacy increased progressively with higher IS, evidenced by a significantly prolonged time to recurrence for patients treated with AC in the IS >70% cohort (HR 0.41, 95% CI 0.20-0.82, P < .009) (21). Similarly, a prospective ancillary biomarker analysis of 1,322 patients from the IDEA trial demonstrated a DFS benefit by extending FOLFOX treatment from three to six months in the intermediate/high TIL group (HR 0.53, 95% CI 0.37-0.75, P = 0.0004). This benefit remained consistent across both traditionally low (T1-3, N1) and high-risk (T4, N2) subgroups. In contrast, patients with low TIL densities derived no significant DFS benefit from the extended six-month regimen (HR 0.84, P = 0.269) (22).

Interestingly, while patients with a low IS have a higher risk of relapse, they do not appear to benefit from extending the duration of AC to 6 months. These findings challenge the traditional expectation that those with a higher risk of relapse will benefit most from additional systemic treatment and suggest that a high TIL density is required to optimally harness the therapeutic efficacy of AC, regardless of tumour prognosis. Collectively, this data supports the integration of TILs into clinical practice to personalise both the indication for and the duration of AC in stage III CC (10, 21).

MMR Deficient Tumours

While dMMR tumours are generally associated with a more favourable prognosis than their MMR proficient (pMMR) counterparts, TILs provide a more robust prediction of tumour recurrence and survival than MMR staging alone (24). Although dMMR tumours typically exhibit high TIL densities due to a high mutational burden, a distinct subset of low-TIL dMMR tumours exists. Low-TIL dMMR tumours are associated with significantly inferior outcomes compared to the high-TIL dMMR group, with a five-year DFS of 75% vs. 98% (P = 0.047) and a five-year OS of 39% vs. 75% (P = 0.008). Notably, the low-TIL dMMR group demonstrated worse outcomes than the high-TIL pMMR group (DFS 75% vs. 92%; OS 39% vs. 77%) (25). These findings challenge the assumption that dMMR status is a universal surrogate for favourable outcomes. Instead, they highlight the potential utility of integrating TIL assessment with MMR status to avoid prognostic inaccuracies and enable more informed treatment decisions.

In the era of emerging neoadjuvant immune checkpoint inhibitor (IO) use in dMMR stage III and high-risk stage II CC (4), additional prognostication within this patient cohort is important. Immunoscore-Immune Checkpoint (IS-IC) is a novel tool that measures $CD8^+$ and programmed death-ligand 1 positive ($PD\text{-}L1^+$)

cell density in addition to the physical distance and interactions between them. This tool has been validated in metastatic CRC cohorts, demonstrating a greater IO treatment benefit for the high IS-IC group. While this approach is not yet validated in non-metastatic CRC, it could potentially be utilised in the future to ensure patients selected for neoadjuvant IO are likely to achieve a favourable response (26).

Summary

TILs are associated with improved oncologic endpoints. The quantification of TIL density provides superior prognostic accuracy when integrated with traditional MMR status and TNM staging in stage II-III CC. TIL density may also act as a predictive biomarker in stage III disease, enabling clinicians to tailor the duration of AC to an individual patient's immune profile.

The Bad - Tumour budding

Background

A tumour bud is defined as a single cancer cell or a group of up to four cells at the invasive margin of a tumour (peri-tumoural budding, PTB) or within the tumour centre (intra-tumoural budding, ITB) (27). Similar to TILs, while PTB can only be accurately assessed following surgical resection where the entire invasive margin is examinable, ITB can be evaluated from pre-operative colonoscopic biopsies. TB is graded according to International Tumour Budding Consensus Conference guidelines based on the number of buds at the tumour 'hotspot' (the area exhibiting the highest density of buds). TB is classified into low grade (Bd1, 0-4 buds), intermediate grade (Bd2, 5-9 buds), and high grade (Bd3, ≥10 buds) (27, 28).

TB is an independent poor prognostic factor in CC and is typically associated with LVI, advanced TNM stage and high tumour grade (27). It is now listed as a recommended, though non-core, synoptic variable in international reporting guidelines (1, 28).

Clinical Applications

Stage I Colon Cancer

Substantial evidence exists to support the association of Bd3 with an increased risk of lymph node metastases (LNM) in endoscopically resected T1 CC (6, 27, 29, 30). This finding was recently corroborated by a large meta-analysis of over 25,000 patients, which reported a significantly elevated risk of LNM (RR 4.04, 95% CI 3.52-4.64, P < 0.001). Beyond LNM, this meta-analysis also identified Bd3 as a predictor of local recurrence (RR 2.35, 95% CI 1.21-4.54, P = 0.01) (31). Consequently, Bd3 is now recognised in NCCN and ESMO guidelines as a high-risk feature for endoscopically resected T1 malignant colonic polyps, providing a critical framework for clinical decision-making regarding the necessity of subsequent segmental resection (2, 19) (Table 2).

Stage II Colon Cancer

In stage II CC, Bd3 is associated with decreased DFS (32-35), recurrence free survival (RFS) (34), and OS (32, 34, 35). Updated ESMO, ASCO, and NCCN guidelines now reflect the strong prognostic impact of Bd3 by including it as a high-risk feature for recurrence in stage II CC (2, 3, 19) (Table 2).

The role of TB as a marker to inform delivery of AC in stage II CC remains unclear. The SACURA trial - a prospective study involving 991 patients - demonstrated that surgery followed by fluoropyrimidine-monotherapy AC did not provide a significant five-year RFS benefit over surgery alone, across all TB grades (36). Conversely, a retrospective single-institution analysis of 336 patients found that fluoropyrimidine-based AC was associated with a significant improvement in OS for patients with Bd1or Bd2 (HR 0.28, 95% CI 0.11–0.68, P = 0.005), whereas no OS benefit was observed in the Bd3 group (P > 0.05) (34). While these findings suggest that fluoropyrimidine monotherapy and combination AC may not be sufficient to overcome the adverse prognosis associated with Bd3 tumours, further prospective data is required to definitively clarify the utility of TB as a predictive marker in the stage II cohort.

Stage III Colon Cancer

A large post-hoc analysis of 2,048 stage III CC patients from the IDEA trial demonstrated that Bd2/3 status was an independent predictor of worse outcomes, with significant impacts on both DFS (HR 1.41, 95% CI 1.12-1.77, P = 0.003) and OS (HR 1.65, 95% CI 1.22-2.22, P = 0.001) (37). Similar results were observed in a smaller Chinese cohort of stage III CRC patients where, compared to the combined Bd1/2 group, patients with Bd3 exhibited significantly worse five-year DFS (HR 1.71, 95% CI 1.03–2.3, P = 0.038) and a trend toward poorer five-year OS (HR 1.81, 95% CI 0.97–3.34, P = 0.061). Importantly, this study demonstrated that fluoropyrimidine-based AC significantly improved outcomes compared to surgery alone within the Bd3 group (DFS, P = 0.0263; OS, P = 0.0045), whereas no survival benefit was observed between the treatment arms for Bd1 or Bd2 patients (38) (Table 2).

Pre-Operative Risk Stratification

Evaluating ITB on pre-operative colonoscopic biopsies to identify high-risk stage II/III patients suitable for neoadjuvant systemic treatment represents a promising future application (33). Multiple studies have demonstrated that ITB is strongly associated with LNM, earlier recurrence, and poorer OS (33, 39). Given the inherent inaccuracy of preoperative radiological staging in assessing suitability for neoadjuvant treatment in CC, evaluating TB at the time of initial diagnosis may better inform treatment sequencing (4, 40).

Summary

Bd3 is a validated independent prognostic marker in stage I–III CC. Potential future applications may extend to guiding AC decision making in stage II–III patients and identifying high-risk candidates for neoadjuvant therapy.

The Ugly - Poorly Differentiated Clusters

Background

Traditional World Health Organisation (WHO) CC tumour grading is based on the degree of gland formation, with reduced gland formation suggesting a poorly differentiated, more biologically aggressive tumour. Colonic adenocarcinoma is considered high grade if <50% of its area contains recognisable glands. This long-standing technique has been criticised in recent years given the potential for significant interobserver variability. Furthermore, this technique suffers from an 'averaging effect' which can lead to under-grading. For example, a tumour exhibiting 90% gland formation with only a 10% non-glandular component will often demonstrate aggressive behaviour, despite being classified as low-grade (41).

PDCs have been suggested as a more reliable alternative to traditional tumour grading (8). A PDC is defined as a cluster of five or more tumour cells, typically located at the invasive front where malignant cells infiltrate the surrounding stroma. As clusters of five or more cells possess the potential for glandular organisation, a cluster must lack glandular features to be classified as poorly differentiated (Figure 2) (8). While both TB and PDCs are hypothesised to arise from underlying epithelial-mesenchymal transition, international guidelines recognise them as distinct histopathological entities that offer enhanced prognostic yield when assessed in combination (42, 43). PDC grading is standardised by counting clusters under a 20x objective lens in the field of maximum cluster density ('hotspot' technique similar to TB). Tumours are stratified into three categories: Grade 1 (<5 clusters, G1), Grade 2 (5-9 clusters, G2), and Grade 3 ($\geq$10 clusters, G3) (44).

By focusing on the least differentiated component of the tumour, PDC assessment captures a primary driver of clinical outcomes. As PDC analysis targets these high-risk focal points, it is common for tumours classified as low-grade by conventional standards to be re-stratified as high-grade based on PDC count (45, 46). Although not currently included as a core or non-core variable in ICCR guidelines, PDC analysis consistently outperforms traditional tumour grading. It delivers superior prognostic accuracy and higher interobserver reproducibility, ultimately providing a more robust assessment of patient risk (45, 46).

Clinical Applications

Stage I Colon Cancer

A recent meta-analysis of 3,909 patients with stage I CRC demonstrated that the presence of PDCs is a powerful predictor of LNM (OR 14.61, 95% CI 1.40-152.60, I^2 81%). Notably, this association was significantly stronger than conventional WHO tumour grading (OR 3.61, 95% CI 2.82-4.64, I^2 45%) (47). Beyond their utility in predicting LNM, PDCs have been further validated as an independent prognostic marker for reduced OS and DFS across multiple T1 CC cohorts (45, 48).

Stage II Colon Cancer

Shivji et al. conducted a systematic review of eight landmark papers published between 2012 and 2019, highlighting the emerging significance of PDCs in stage II disease (8). Of these, a Japanese study of 248 stage II CRC patients found that five-year DFS decreased from 96% in patients with G1 PDC to 73% in those with G3 PDC. Notably, this study observed that stage II patients with G3 PDCs had a significantly worse DFS (73%) than stage III patients with G1 PDC (95%). While possibly confounded by a higher rate of AC in the stage III cohort, this data corroborates evidence of a potential survival paradox in certain stage II subgroups (44). Statistical comparisons consistently identified PDC grade as the most powerful predictor of RFS and DFS, outperforming TNM stage, conventional histological grade, and TB (8, 44, 45).

More recently, the SACURA trial analysis validated PDCs as a powerful independent prognostic marker in stage II CC, with G3 tumours demonstrating a five-year RFS of 74.7% compared to 91.1% for the G1 cohort. Multivariate regression demonstrated that only G3 PDC remained a statistically significant predictor of RFS when compared with WHO grade and eight additional conventional histopathological variables (HR 1.54, 95% CI 1.02-2.33, P= 0.04) (Table 2) (49). Despite PDCs being a strong prognostic indicator, their predictive utility remains unproven. In the SACURA trial, fluoropyrimidine-based AC failed to provide a significant 5-year RFS benefit over surgery alone, in either the G3 (HR 0.65, 95% CI 0.34–1.24) or G1/2 cohorts (HR 0.92, 95% CI 0.63–1.24) (49).

Stage III Colon Cancer

In a cohort study of 303 stage III CRC patients, 3-year DFS ranged from 89% for G1 tumours to 59.4% for G3 tumours (HR 4.50, 95% CI 2.41-8.41, P < 0.001). Among all reported pathological prognostic factors, PDC grade was associated with the most substantial variation in DFS, positioning it as a critical metric for risk stratification (50). These findings were corroborated in a smaller 49 patient Japanese cohort where patients with seven or more PDCs had significantly shorter RFS and OS (both P <0.0001) (51).

Evidence regarding the predictive utility of PDCs for AC in stage III CC remains limited. In a single-centre study of 131 patients, Tajima et al. reported that fluoropyrimidine monotherapy AC significantly improved five-year RFS in the G1/2 PDC cohort (P = 0.0002), yet failed to provide a significant benefit in the G3 group (P = 0.068 (52). These findings suggest that PDCs may identify a biologically aggressive phenotype characterised by relative resistance to fluoropyrimidine-monotherapy AC. To date, no studies have evaluated the predictive value of PDCs in the context of fluoropyrimidine-based AC.

Pre-Operative Risk Stratification

Similar to TB, PDC assessment can be reliably performed on pre-operative biopsy specimens (53, 54). Given the prognostic accuracy of PDCs in both stage II and III disease, this represents an opportunity to enhance pre-operative risk stratification and better identify patients who may benefit from neoadjuvant systemic treatment (Table 2).

Summary

In stage I CC, PDCs are potent prognostic indicators associated with an increased risk of LNM and poorer oncological outcomes. This significance extends to stage II and III disease, where higher PDC grade correlates with diminished DFS and OS. While the prognostic value of PDCs is well-documented, their predictive utility in determining benefit from AC requires further validation in larger patient cohorts.

Conclusion

TILs are associated with improved oncological endpoints in stage II-III disease and may provide an additional tool to assist with adjuvant treatment decision making, though further research is required to define their role in stage I disease. TB and PDCs are established markers of poor prognosis across stage I–III CC. Further prospective studies are needed to determine their predictive utility in guiding AC decision making in stage II-III patients. These variables are able to be appropriately assessed by conventional H + E stained sections, providing vital prognostic and/or predictive information. It is recommended that these variables are incorporated into colon cancer histopathological reporting and treatment guidelines to facilitate a more nuanced, biology-focused approach to risk stratification and oncological precision.

Declarations

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No competing interests

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Author Contribution

S.D. - conceptualisation, literature review, manuscript writingM.W. and Z.B. - literature review and data-analysis.T.K. and A.K. - manuscript review R.V. and T.S. - conceptualisation, supervision, manuscript review All authors reviewed the manuscript and agreed with content

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Tables

Table 1: Essential and recommended items for pathology reporting of colorectal cancer (1)

Essential Items	Recommended Items
Neoadjuvant therapy	Clinical information
Operative procedure	Plane of sphincter excision
Tumour site	Plane of mesocolic excision
Tumour dimensions	Measurement of invasion beyond muscularis propria
Perforation	Tumour budding
Relation of tumour to anterior peritoneal reflection	Coexistent pathology
Plane of mesorectal excision	Ancillary studies
Histological tumour type	
Histological tumour grade	
Extent of invasion	
Lymphatic and venous invasion	
Perineural invasion	
Lymph node status	
Tumour deposits	
Response to neoadjuvant therapy	
Margin status	
Histologically confirmed distant metastases	
Pathological staging	
Ancillary studies	

Table 2: Summary of evidence for novel synoptic variables across colon cancer cohorts

	Stage I Colon Cancer	Stage II Colon Cancer	Stage III Colon Cancer
TILs	Nil	▲ DFS (14, 18) ▲OS (18)	▲DFS (20-23) ▲OS (20-23) Favourable response to AC (22)
TB	▲ LNM (6, 27, 29-31) ▲ LR (31)	▼DFS (32-35) ▼OS (32-35) Poor response to AC (34)	▼DFS (37, 38) ▼OS (37) Poor response to AC (38)
PDCs	▲ LNM (47) ▼ DFS (45, 48) ▼OS (45, 48)	▼ DFS (8, 44, 45) ▼ RFS (8, 45, 49)	▼DFS (50) ▼ RFS (51) ▼ OS (51) Poor response to AC

TILs = tumour infiltrating lymphocytes, TB = tumour budding, PDCs = poorly differentiated clusters, LNM = lymph node metastases, DFS = disease free survival, OS = overall survival, AC = adjuvant chemotherapy, LR = local recurrence, RFS = recurrence free survival

Figures

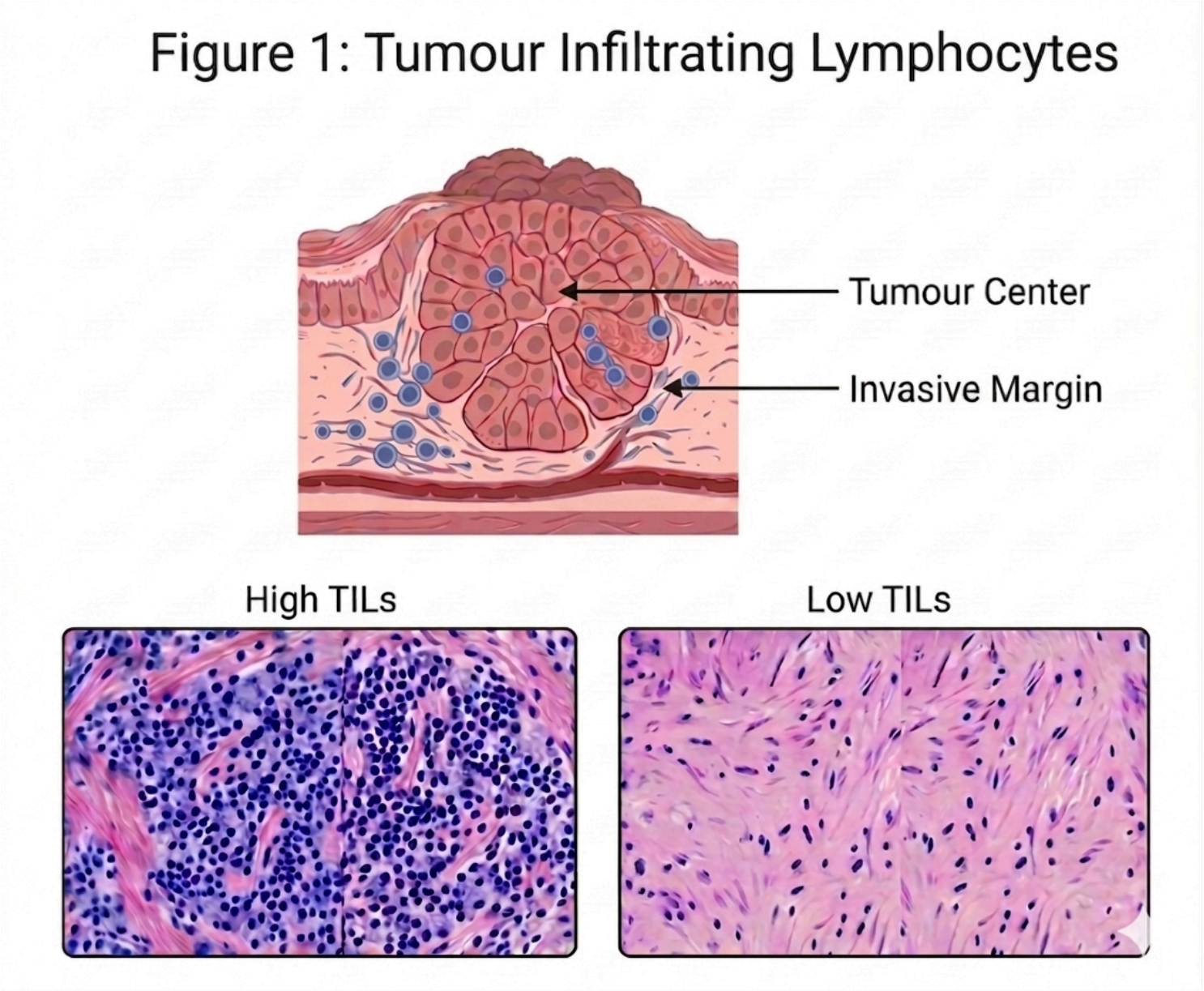


Figure 1

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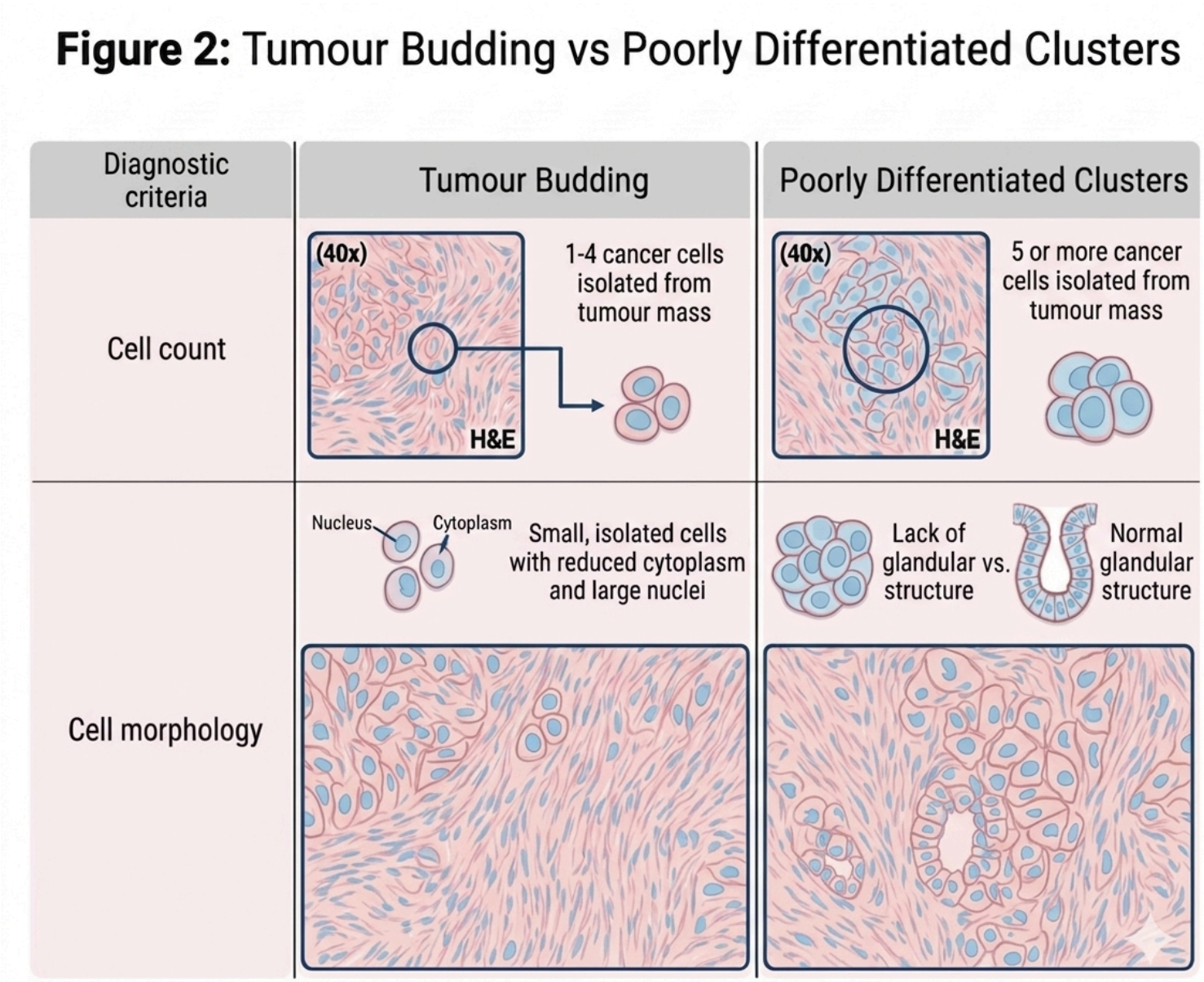


Figure 2

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