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AI Pathology and ctDNA Integration in Colon Cancer

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Executive Summary

In May 2026, the American Society of Clinical Oncology (ASCO) Annual Meeting featured a Mayo Clinic poster (Abstract 3525) reporting integration of artificial intelligence (AI) pathology and circulating tumor DNA (ctDNA) biomarkers in stage III colon cancer. This deep-learning study quantified tumor microenvironment (TME) features from digitized resection specimens and related them to postoperative ctDNA status and patient outcomes in the large phase III FOLFOX adjuvant trial (NCCTG N0147). The linkage of an algorithm-derived “digital pathology” biomarker with ctDNA – a highly prognostic liquid biopsy marker – exemplifies an emerging multimodal approach to risk stratification. We present a comprehensive analysis of this ASCO readout, situating it within the broader contexts of colorectal cancer biology, AI pathology, and biomarker regulation.

Key points include:

- **Colon Cancer and Current Practice:** Standard care for stage III colon cancer is curative resection followed by adjuvant chemotherapy (commonly FOLFOX). TNM staging remains the only formal risk stratifier (pmc.ncbi.nlm.nih.gov), even though stage III encompasses wide outcome variability (5-year DFS from ~89% in low-risk to ~31% in high-risk disease (pmc.ncbi.nlm.nih.gov)). Many patients harbor undetected microscopic residual disease (MRD), underscoring a need for more precise biomarkers beyond anatomic stage (pmc.ncbi.nlm.nih.gov).
- **Circulating Tumor DNA (ctDNA) as MRD Biomarker:** Detection of tumor-derived DNA fragments in blood after surgery is now recognized as a surrogate of MRD. Extensive recent evidence shows that postoperative ctDNA positivity strongly predicts recurrence. For example, in Mayo’s analysis of 2,260 stage III colon cancer patients from trial N0147, ctDNA was detected in 20.4% of cases and conferred dramatically worse outcomes (5-year DFS 27.7% vs. 77.1% for ctDNA-negative) (pmc.ncbi.nlm.nih.gov).

ctDNA positivity yielded hazard ratios of ~5–6 for time-to-recurrence and disease-free survival (pmc.ncbi.nlm.nih.gov). These results mirror other studies in CRC and reinforce ctDNA as a powerful biomarker (pmc.ncbi.nlm.nih.gov).

- **Tumor Microenvironment (TME) and Digital Pathology:** Tumor-infiltrating immune cells and stromal composition are known to influence colon cancer prognosis. Historically, pathologists have subjectively graded features like immune reaction and tumor budding, but AI enables objective, high-throughput quantification of dozens of morphological metrics (pmc.ncbi.nlm.nih.gov). Pioneering work has shown deep-learning models can decompose H&E images into interpretable TME components – for example quantifying TIL density, tumor-stroma ratios, tumor budding, mucin pools, and more (pmc.ncbi.nlm.nih.gov). Such features often correlate with molecular subtypes (e.g. CMS) and predict outcomes. For instance, Kather *et al.* found a CNN-derived “deep stroma” score to be an independent predictor of poor survival (pmc.ncbi.nlm.nih.gov). Mayo Clinic investigators have previously demonstrated (in an ASCO 2023 abstract) that AI-quantified tumor budding/poorly-differentiated clusters were the strongest prognostic feature in mismatch-proficient colon cancers (ascopubs.org), and that immune-related features differ sharply between dMMR vs pMMR tumors (ascopubs.org). These results affirm that AI pathology yields biologically meaningful and prognostic TME metrics.
- **ASCO 2026 Mayo Abstract – AI-TME and ctDNA:** The Mayo Clinic presentation (Sinicrope *et al.*) extended this work by linking AI-derived TME features to ctDNA status. Using the NCCTG N0147 cohort (3084 stage III patients randomized to FOLFOX ± cetuximab (pmc.ncbi.nlm.nih.gov)), the poster, per its title, examines deep-learning–quantified tumor microenvironment features in relation to postoperative ctDNA status and outcomes. The published abstract reports significant differences in histopathologic features by ctDNA status (for example, ctDNA positivity was associated with lymphovascular invasion and higher %tumor budding, %necrosis and %stroma) and risk stratification within ctDNA groups; it has no full-text component and awaits peer-reviewed publication.
- **Evidence and Data Synthesis:** We review the detailed findings underpinning this approach. Sinicrope’s JCO 2026 study of N0147 quantified ctDNA using a tissue-free methylation assay (Guardant Reveal™), which achieved >98% specificity (pmc.ncbi.nlm.nih.gov). In that study, 20.4% of patients were ctDNA positive, with high-risk clinicopathologic features (e.g. T4 status, N2, BRAF mutation) overrepresented in the ctDNA+ group (pmc.ncbi.nlm.nih.gov). Multivariable analysis confirmed ctDNA positivity as an independent predictor of adverse outcomes (adjusted HR for DFS 3.74; unadjusted HR 5.03) (pmc.ncbi.nlm.nih.gov). Meanwhile, quantification of ctDNA tumor fraction provided further granularity: higher fractional burden doubled with eventual recurrence (pmc.ncbi.nlm.nih.gov). On the tissue side, the large Gastroenterology 2022 study (“QuantCRC”) applied an algorithm to 6,468 CRC slides and found TME features varied significantly by stage (pmc.ncbi.nlm.nih.gov). For example, median tumor:stroma ratio fell from 1.3 in Stage I to 0.8 in Stage IV, and median TIL density dropped from 44.8 to 20.1 cells/mm² (all $p<0.001$) (pmc.ncbi.nlm.nih.gov). These quantitative trends align with prognostic intuition: advanced tumors tend to have denser stroma and fewer immune cells. Combining such data suggests that integration of AI-derived histology metrics with ctDNA can sharpen risk assessment beyond either modality alone.

- **Regulatory and Clinical Implications:** The combination of AI pathology and ctDNA also raises regulatory considerations. FDA classifies a “software algorithm device to assist users in digital pathology” as a Class II in vitro diagnostic device under 21 CFR 864.3750 (21 CFR 864.3750), but that classification covers diagnostic-assist image-analysis software and is not a blanket rule for every image-based prognostic score. Some ctDNA assays are FDA-approved as companion diagnostics for specific indications – for example, Guardant360 CDx is approved under PMA P200010 to identify EGFR mutations in plasma from patients with non-small cell lung cancer (FDA companion diagnostics list) – but that does not make ctDNA MRD testing in colon cancer an FDA-authorized use. Any composite “AI biomarker” would need to meet rigorous standards of analytical validity, reproducibility, and clinical utility. For comparison, NICE describes Immunoscore® (HalioDx) as an in vitro diagnostic test that uses image analysis software to measure CD3+ and CD8+ T lymphocytes in digital images of resected tumour samples (NICE MIB269). The Mayo study may be viewed as exploring a digital pathology signature linked to an independent reference (ctDNA) and to clinical outcomes, in a trial setting.
- **Future Directions:** Looking forward, this work foreshadows a new paradigm in oncology biomarkers. Multi-modal models combining genomics, radiology, and pathology (termed “surgomics” or “radiopathomics”) are being actively pursued (pmc.ncbi.nlm.nih.gov). If AI-quantified TME features consistently align with molecular markers like ctDNA, they could inform personalized therapy (e.g. selecting patients for additional treatment vs. surveillance). Large datasets like N0147 permit robust training and validation, reducing overfitting. Ultimately, the goal is to develop clinically actionable composite biomarkers – “digital twin” prognostic scores – that regulatory bodies can certify and clinicians can apply.

This report synthesizes relevant data (with rigorous citations) to illuminate the Mayo Clinic’s ASCO 2026 AI-Pathology abstract. We provide historical context on colorectal cancer management, summarize state-of-the-art in ctDNA and AI pathology, analyze the evidence, illustrate with examples and tables, and discuss implications for regulatory practice and future research. Sources are cited inline; statements about the ASCO 2026 abstract rely on the published abstract (J Clin Oncol 2026;44(suppl 16):3525; ascopubs.org), a Mayo Clinic news release and a CancerNetwork report; the abstract has no full-text component.

Introduction

Colorectal cancer (CRC) is a leading cause of cancer morbidity and mortality worldwide. Surgical resection of the primary tumor is the main curative treatment, but outcomes vary widely: among stage III patients in the IDEA collaboration, 5-year DFS varied from 89% for resected T1N1a tumors to 31% for T4N2b tumors, highlighting undetected micrometastatic disease (pmc.ncbi.nlm.nih.gov). In resected stage III colon cancer, the current standard of care is surgery followed by adjuvant chemotherapy (typically 3–6 months of CAPOX or FOLFOX) (pmc.ncbi.nlm.nih.gov). Decisions on adjuvant therapy are largely guided by TNM stage and pathological features (e.g. T4 or ≥N2 designate “high-risk”) (pmc.ncbi.nlm.nih.gov). However, even within stage III there is extreme heterogeneity: the large IDEA pooled analysis showed 5-year disease-free survival (DFS) ranging from ~89% in low-risk (T1-3N1a) cases down to ~31% in high-risk (T4N2b) cases

(pmc.ncbi.nlm.nih.gov). This variability means that many patients are overtreated (receiving futile chemotherapy with toxicity) or undertreated (pursuing recurrence). Clearly, more precise biomarkers of residual disease are needed.

Conventional tissue biomarkers provide some guidance. In colon cancer, histological grade, lymphovascular invasion, and perineural invasion have limited prognostic value beyond stage. Mismatch-repair deficiency (dMMR/MSI-high) identifies a subset (~15%) with better prognosis and different therapy responses, but only a minority of stage III cases benefit from immunotherapy currently. Recently, the **Immunoscore®** has emerged as a validated TME-based assay: by digital image analysis of CD3/CD8 immunohistochemistry, it quantifies tumor-infiltrating lymphocytes and stratifies recurrence risk in stage I–III CRC (pmc.ncbi.nlm.nih.gov). Indeed, consensus guidelines are beginning to recognize immune infiltration as an essential prognostic factor (pmc.ncbi.nlm.nih.gov). However, Immunoscore (a commercial test) is not yet formally approved as a companion diagnostic; its formal regulatory status is not established by the sources reviewed here.

Circulating tumor DNA (ctDNA) has independently revolutionized MRD assessment. ctDNA refers to fragments of tumor-derived DNA in the blood (pmc.ncbi.nlm.nih.gov). When detected after curative surgery, it serves as a molecular marker of residual disease and portends poor outcome. Multiple studies have shown that ctDNA-positive status after resection strongly predicts relapse across cancer types. In colon cancer, recent evidence from large trials confirms that a tissue-agnostic (tumor-free) ctDNA assay can stratify patients: in Mayo Clinic's retrospective analysis of 2,260 stage III CRC patients (N0147 trial), 20.4% had detectable ctDNA and those patients had dramatically worse 5-year DFS (27.7%) compared to ctDNA-negative (77.1%) (pmc.ncbi.nlm.nih.gov). ctDNA-positivity yielded hazard ratios of ~4.5–6 for time to recurrence, DFS and OS (pmc.ncbi.nlm.nih.gov). Furthermore, higher ctDNA allele fraction (tumor fraction) within the positive group identified even higher-risk subgroups (pmc.ncbi.nlm.nih.gov). These findings underscore the independent prognostic power of ctDNA after surgery. Yet ctDNA is typically offered as a laboratory-developed test (not currently FDA-cleared for CRC MRD), so it is not yet standard care outside trials.

Digital pathology and AI are at the forefront of quantifying the TME from routine histology. Whole-slide imaging (WSI) allows digitization of H&E slides, and modern deep-learning algorithms can analyze features beyond human perception. For colorectal cancer, AI models can segment tumor vs. stroma, identify areas of necrosis or mucin, and count tumor buds or lymphocytes, essentially performing an "image-based cytometry" of the TME (pmc.ncbi.nlm.nih.gov). Several studies have demonstrated the prognostic value of such features. For example, a large Gastroenterology paper applied an algorithm called **QuantCRC** to thousands of CRC images and showed that features like tumor budding, stromal composition, and lymphocyte density were strongly associated with stage and survival (pmc.ncbi.nlm.nih.gov). In an ASCO 2023 abstract, Sinicrope *et al.* used AI to show that, among stage III tumors, the proportion of tumor budding/poorly differentiated clusters was the single strongest predictor of DFS in pMMR colon cancer (HR_adj ≈0.20 for lowest vs highest quartile) (ascopubs.org). These data indicate that AI-quantified pathology features capture biologically and clinically relevant signals.

Given the prognostic significance of both ctDNA and TME features, the critical question arises: *How do they relate to each other?* In particular, do AI-derived tissue biomarkers correlate with molecular evidence of MRD, and can combining them yield a more robust predictor? The Mayo Clinic researchers addressed this by linking

their AI-pathology analyses of resected colon tumors to postoperative ctDNA status. Abstract 3525 (ASCO 2026) reports exactly this: a deep-learning quantification of multiple TME parameters from N0147 trial specimens, examined in relation to ctDNA positivity and patient outcomes.

This report provides an in-depth exploration of that study and its ramifications. We begin by detailing the biological and clinical background of stage III colon cancer, TME biomarkers, and ctDNA. We then review the methodology and results of prior efforts in AI pathology (QuantCRC, Immunoscore, etc.) and the key ctDNA findings (Sinicrope JCO 2026). We summarize the reported findings of the Mayo abstract and discuss their potential impact. Throughout, we cite primary literature and authoritative sources to substantiate every claim. Tables are used to organize quantitative comparisons of TME features and of CRC biomarkers. Finally, we discuss regulatory considerations and future directions: how an AI-derived pathology biomarker can be brought to “regulatory grade,” and what this means for precision oncology.

Stage III Colon Cancer and MRD

Stage III colon cancer (tumor with regional lymph node metastases, no distant spread) carries a substantial relapse risk even after complete resection (pmc.ncbi.nlm.nih.gov). The MOSAIC trial established FOLFOX as adjuvant standard, improving cure rates compared to fluorouracil/LV. However, eradication of minimal residual disease remains imperfect: adjuvant chemo reduces the absolute recurrence rate by only ~10–15% (pmc.ncbi.nlm.nih.gov), meaning many remissions occur spontaneously and many recurrences could not be averted. In practice, chemotherapy decisions are crude. For example, the IDEA collaboration (a pooled analysis of ~12,000 patients) found that *within* stage III, 5-year DFS varied from 89% for resected T1N1a tumors to 31% for T4N2b tumors (pmc.ncbi.nlm.nih.gov). This wide spread underscores two facts: (1) TNM staging does not fully capture tumor biology, and (2) a substantial subset of stage III patients either did not have MRD (hence did not need chemo) or had aggressive micrometastases (hence chemo only partly helped).

Clinical guidelines currently rely on pathological stage and historic risk factors. For example, N0147 (Alliance trial) defined *high-risk* stage III as T4 and/or N2 (pmc.ncbi.nlm.nih.gov). In that trial, 3084 patients were randomized 1:1 to receive 6 months of FOLFOX versus FOLFOX plus cetuximab (pmc.ncbi.nlm.nih.gov). Patients with known KRAS mutation were given a non-randomized FOLFOX arm (cetuximab is ineffective in KRAS-mutant). Ultimately, N0147 found no DFS benefit from adding cetuximab (pmc.ncbi.nlm.nih.gov), but the trial collected rich tissue and blood samples that now allow correlative studies. (Full trial details: median follow-up ~5 years; primary endpoint DFS; it enrolled both pMMR and dMMR tumors.) Importantly, the Mayo Clinic team had access to these specimens and to centralized outcome data (pmc.ncbi.nlm.nih.gov).

The concept of **molecular residual disease (MRD)** has gained traction as a biologic complement to staging (pmc.ncbi.nlm.nih.gov). MRD refers to microscopic cancer cells or DNA left after surgery that are undetectable by imaging. In CRC, the most advanced MRD assay is ctDNA in blood (pmc.ncbi.nlm.nih.gov). Postoperative ctDNA positivity reflects that tumor DNA is still shed into the bloodstream, implying remaining viable tumor cells. Sinicrope et al. (J Clin Oncol 2026) analyzed post-surgery plasma from >2,200 N0147 patients using a tissue-free ctDNA methylation assay (pmc.ncbi.nlm.nih.gov). They found 461 (20.4%) ctDNA-positive patients, who had markedly worse outcomes: at 6-year median follow-up, 5-year DFS was only 27.7% in ctDNA-positive vs 77.1% in ctDNA-negative (HR_DFS ≈5.03) (pmc.ncbi.nlm.nih.gov). In multivariable analysis, ctDNA

positivity remained significantly associated with adverse outcomes (adjusted HR 4.33 for time-to-recurrence; the unadjusted HR was 5.96) (pmc.ncbi.nlm.nih.gov). These data confirm that ctDNA is a potent, quantitative MRD marker: higher ctDNA fraction further stratified recurrence risk (pmc.ncbi.nlm.nih.gov).

In short, ctDNA delivers “seen-into” the patient’s blood: a positive or high ctDNA means far more likely residual disease. It is now being tested prospectively (e.g. DYNAMIC, CIRCULATE, and forthcoming trials) as a way to escalate or de-escalate adjuvant therapy. ctDNA panels are advancing in other settings (Guardant360 CDx is FDA-approved as a companion diagnostic for specific plasma biomarker–drug indications, such as EGFR mutations in non-small cell lung cancer (FDA companion diagnostics list)). For clinicians, combining ctDNA with pathology may eventually help refine risk assessment, but whether ctDNA results should change adjuvant chemotherapy decisions (omitting, de-escalating or intensifying treatment) remains investigational and is being tested in prospective trials; such decisions should follow current guidelines and multidisciplinary review.

Deep-Learning TME Analysis in Colorectal Cancer

Tumors are not merely collections of malignant cells; their microenvironment – comprising stroma, immune cells, vasculature, etc. – strongly influences behavior. In CRC, a vigorous lymphocytic response portends better survival, while features like abundant stroma, extracellular mucin, and tumor budding correlate with aggression. (pmc.ncbi.nlm.nih.gov). Historically, pathologists have scored these qualitatively (e.g. “Crohn’s-like reaction”, “tumor budding present/absent”), but digital pathology paired with AI can quantify them precisely and consistently.

Modern AI models applied to H&E whole-slide images can segment regions and count cell types. For example, the QuantCRC algorithm (developed by the authors of the Pai et al. study and run on the Aiforia Create platform) detects areas of carcinoma (low-grade, high-grade, and signet-ring components) and stroma (mature, immature, inflammatory) as well as other elements like mucin, necrosis, smooth muscle, fat, and TILs (ascopubs.org). Weapons in its toolkit include convolutional neural networks (CNNs) trained on tens of thousands of annotated image patches (pmc.ncbi.nlm.nih.gov). The output is a set of features such as "Tumor:Stroma ratio", "% mucin", "% tumor budding", "% TILs", etc., each given as a fractional area or density. These features can then be correlated with clinicopathologic data.

Studies have consistently shown that such AI-extracted features have biological and prognostic meaning. In a seminal study by Kather *et al.* (PLOS Med 2019), a CNN trained on colorectal images could quantify a “deep stroma score” from H&E alone, which was an independent predictor of overall survival in TCGA colon cancer patients (pmc.ncbi.nlm.nih.gov). This work demonstrated that the CNN learned relevant morphological patterns: for instance, one output neuron responded strongly to lymphocyte-rich regions and another to desmoplastic stroma (pmc.ncbi.nlm.nih.gov), effectively recapitulating known Consensus Molecular Subtypes (CMS1 = high lymphocytes, CMS4 = fibrotic stroma) (pmc.ncbi.nlm.nih.gov). Higher activation of “stromal” outputs was associated with worse outcomes. Their combined “deep stroma” score (encompassing debris, muscle, collagen, etc.) stratified patients (multivariate HR ~1.99 for OS, $p<0.01$) (pmc.ncbi.nlm.nih.gov).

Similarly, the Mayo QuantCRC study (Gastroenterology 2022) applied its algorithm to 6,468 colon carcinomas. The analysis revealed strong associations between AI features and tumor stage/molecular subtypes. For example, quantitatively, advanced tumors had *more* tumor budding/poorly differentiated clusters (TB/PDC) and *lower* tumor:stroma ratio than early-stage tumors ($p<0.001$ for each) (pmc.ncbi.nlm.nih.gov). In concrete terms, median tumor:stroma ratio fell from ~1.3 in Stage I to ~0.8 in Stage IV CRC (pmc.ncbi.nlm.nih.gov), while median TIL density dropped from 44.8 cells/mm² to 20.1 cells/mm² (pmc.ncbi.nlm.nih.gov). These differences mirror traditional observations (higher stage tumors are often more desmoplastic and immune-poor), but AI quantifies them precisely and on a large scale. Notably, in that study a prognostic model combining QuantCRC features with stage and MMR status achieved a higher concordance for recurrence-free survival than staging alone (pmc.ncbi.nlm.nih.gov).

Even in small cohorts, AI-based TME metrics can predict therapy response. For example, deep-learning models have been used to estimate biomarkers like MSI, PD-L1, or immune cell densities that may predict immunotherapy benefit. The NICE guidance on Immunoscore explicitly notes that it uses “digital pathology alongside deep learning-based algorithms” to measure CD3+/CD8+ T cells in colon cancer resections (www.nice.org.uk). This commercial Immunoscore test (sold by HalioDx) demonstrates that AI histology is not just theoretical: it is a real-world example of a laboratory assay that uses AI image analysis to guide care. In colorectal trials, higher Immunoscore corresponds to lower relapse risk, independent of other factors (pmc.ncbi.nlm.nih.gov), underscoring how immune quantification adds value beyond classic pathology.

Table 1 (below) summarizes salient AI-derived TME features in CRC and their known associations with outcome. Many of these features appear in Sinicrope’s ASCO 2026 work and prior JCO 2023 abstract. For instance, **Tumor Budding/PDC** (clusters of undifferentiated tumor cells at the invasive front) is a well-known adverse factor (ascopubs.org). **TIL density** is protective (high TILs → good prognosis) (pmc.ncbi.nlm.nih.gov). **Tumor:Stroma Ratio** is prognostic: low ratio (meaning much stroma) indicates worse survival (pmc.ncbi.nlm.nih.gov). AI methods also capture intangible patterns: the “Deep Stroma Score” combining multiple stromal elements was an independent negative prognostic factor (pmc.ncbi.nlm.nih.gov). The convergence of these studies paints a consistent picture: a TME that is immune-rich, epithelial-predominant and glandular tends to be indolent, whereas a stroma-rich, bud-rich, immune-poor TME signals aggression.

Feature	Description (AI-quantified)	Association with Prognosis	Evidence/Source
Tumor-Infiltrating Lymphocytes (TILs)	Density of tumor-infiltrating lymphocytes per mm² of tumor on H&E-stained slides (QuantCRC); Immunoscore instead scores CD3+/CD8+ T-cell density by immunohistochemistry	**High** TIL count → **favourable** prognosis; robust predictor of low relapse risk (pmc.ncbi.nlm.nih.gov).	QuantCRC analysis by stage (pmc.ncbi.nlm.nih.gov); Immunoscore pooled trials (pmc.ncbi.nlm.nih.gov).
Tumor Budding / PDC	Percent area of “buds”/poorly-differentiated clusters in tumor	**High** TB/PDC → **worse** DFS; strongest predictor in pMMR CRC (ascopubs.org).	Sinicrope *et al.*, JCO 2023 (ascopubs.org).
Tumor:Stroma Ratio	Ratio of epithelial tumor area to supportive stroma area	**Low** ratio (i.e. more stroma) → **worse** outcome (pmc.ncbi.nlm.nih.gov). Low ratios seen in advanced stages.	QuantCRC large-cohort analysis (pmc.ncbi.nlm.nih.gov).

Feature	Description (AI-quantified)	Association with Prognosis	Evidence/Source
Mucin Content	% area of extracellular mucin pools in tumor	Mixed; high mucin can indicate mucinous subtype (worse prognosis) or be neutral in isolated form. * (Not primary driver)	QuantCRC showed higher mucin in proximal/dMMR tumors (pmc.ncbi.nlm.nih.gov).
Deep Stroma Score	CNN-derived composite of stromal components (e.g. collagen, muscle, debris)	**High** deep stroma score → **worse** OS (HR≈2.0) (pmc.ncbi.nlm.nih.gov).	Deep-learning model by Kather *et al.* (pmc.ncbi.nlm.nih.gov).
Inflammatory Stroma	Proportion of stroma that is lymphocyte-rich "inflammatory" type	**High** inflammatory stroma (i.e. high immune response) → **better** outcome (dMMR often shows this) (ascopubs.org) (www.nice.org.uk).	AI analysis showed dMMR tumors have more inflammatory stroma (ascopubs.org); Immunoscore (NICE) (www.nice.org.uk).

Table 1. Selected AI-quantified tumor microenvironment features in colorectal cancer and their prognostic implications (high vs low).

Table 1 encapsulates key digital pathology biomarkers. Notice that high immune content (TILs, inflammatory stroma) is beneficial, whereas features of dispersion and stroma relate to poor outcome. These patterns are grounded in biological intuition: for example, abundant tumor budding (EMT-like transition) portends metastasis, and a "cold" immune microenvironment fails to restrain tumor cells. Importantly, these features can now be systematically extracted by AI, enabling unbiased stratification.

The Mayo Clinic ASCO 2026 Study: AI-TME Meets ctDNA

A Mayo Clinic news release previewed the researchers' presentations at the 2026 ASCO Annual Meeting, including a poster (Abstract 3525) titled *"A deep learning approach to quantify tumor microenvironment features associated with postoperative ctDNA status and outcomes in a phase 3 FOLFOX-based adjuvant colon cancer trial (N0147; Alliance)"*, with Dr. Frank Sinicrope listed as presenter. The release gives only the title, session, time and presenter; the methods and results described below come from the published abstract.

The abstract is published in the Journal of Clinical Oncology meeting supplement (ascopubs.org), and the Mayo Clinic news release (ASCO highlights) confirms the session details (www.newswise.com). In the poster session on May 30, 2026 (Gi Cancer – Colorectal and Anal track), Sinicrope's team reported "A deep learning approach to quantify tumor microenvironment features associated with postoperative ctDNA status and outcomes" (www.newswise.com). The abstract describes the study design as follows:

- **Cohort:** Stage III colon adenocarcinoma patients enrolled in N0147, with digitized H&E whole-slide images and postoperative ctDNA results (1,817 cases met quality control in this analysis, a subset of the 2,260 evaluable patients in the earlier JCO ctDNA paper (pmc.ncbi.nlm.nih.gov)).

- **TME Quantification:** Use of an AI segmentation model to parse each slide into components (tumor epithelium of various grades, stromal categories, necrosis, mucin, and TILs as per prior Mayo descriptions (ascopubs.org)). QuantCRC (Aiforia Technologies) was applied to extract quantitative histopathologic features, and 15 pathologist-defined features were analysed against outcomes.
- **ctDNA Status:** Previously determined by a tissue-free epigenomic assay (Guardant Reveal™, as in the JCO study (pmc.ncbi.nlm.nih.gov)). Outcomes are stratified by postoperative ctDNA status assessed by a tissue-free assay (Guardant Reveal).
- **Outcomes:** Disease-free and overall survival tracked per trial protocol.

The abstract reports statistically significant differences in histopathologic features by ctDNA status (ascopubs.org). Features associated with ctDNA positivity included lymphovascular invasion and increased %high grade, %tumor budding, %necrosis and %stroma, while ctDNA-positive cases had lower %inflammatory stroma and fewer %signet ring cells (all $p<0.006$). A CancerNetwork report on the presentation likewise describes ctDNA-negative tumors as having greater tumor-infiltrating lymphocyte density and more inflammatory stromal features, and states that the combination of ctDNA and QuantCRC features improved prognostic discrimination (cancernetwork.com). Because this is a conference abstract without a full-text component, peer-reviewed publication is needed to confirm these findings.

Current State of Knowledge. Because only a conference abstract is available, existing evidence helps contextualize the reported results. The prior JCO work by the same group established how the tissue-free ctDNA assay behaves in N0147: only ~20% of patients have detectable ctDNA (pmc.ncbi.nlm.nih.gov), and this subset disproportionately contained patients with biologically aggressive tumors (advanced T/N stage, high grade, BRAF mutation) (pmc.ncbi.nlm.nih.gov). Conversely, the AI pathology analysis has independently shown that certain features map to these aggressive subtypes. For example, QuantCRC revealed that proximal (right-sided) tumors – which are more often dMMR and BRAF-mutant – had higher mucin and inflammatory stroma than distal tumors (pmc.ncbi.nlm.nih.gov), but also that advanced tumor stage was associated with higher budding and more immature stroma (pmc.ncbi.nlm.nih.gov). It is plausible that within N0147, ctDNA-positive cases skew toward those advanced features.

A key benefit of the Mayo approach is **biological interpretability**: linking two orthogonal biomarkers could reveal how histology and molecular findings reflect common biology. If ctDNA-positive patients tend to have AI-histology signatures of immunosuppression (e.g. low TIL, high fibrotic stroma), that would support the notion that an immune-cold microenvironment fails to clear residual cells, allowing ctDNA release. Alternatively, if a certain TME pattern strongly predicts ctDNA status independent of other factors, it might serve as a surrogate MRD indicator even when blood samples are not obtained. Conversely, if the AI features do **not** differ between ctDNA groups, that would suggest that tissue morphology and molecular shedding capture different aspects of tumor risk. Both outcomes would be informative.

Reporting on both associations and outcomes is crucial. The abstract title implies that the team studied not only the correlation of AI features with binary ctDNA status, but also “and outcomes.” The abstract reports risk stratification within ctDNA groups; the questions below frame the kinds of analysis involved:

- Among ctDNA-negative patients, can AI features further sub-stratify risk? Example: *Even if ctDNA is undetectable, does a patient with very high tumor budding by AI still have a higher recurrence rate than a ctDNA-negative patient with low budding?*
- Among ctDNA-positive patients, are there AI features that differ between those who do recur versus those who remain disease-free?

These questions aim to validate whether AI pathology adds prognostic information above ctDNA alone, and whether it can refine risk within each molecular group.

Hypotheses from Prior Studies. The bullets below are hypotheses drawn by analogy from prior studies, not reported findings; the abstract's reported results are summarized above and take precedence where they differ:

- **Tumor Budding/PDC:** Likely higher in ctDNA-positive group. This feature was the top DFS predictor in pMMR patients (ascopubs.org), and ctDNA positivity often marks aggressive biology. Thus, we expect ctDNA-positive tumors to have larger %TB/PDC on average, linking two adverse markers.
- **Stromal Composition:** Possibly ctDNA-positive cases show **lower** tumor:stroma ratio (i.e. more stroma, possibly more “immature” stroma). In QuantCRC data, advanced tumors (with poor outcomes) had increasingly low tumor:stroma ratios (pmc.ncbi.nlm.nih.gov). If ctDNA tracks recurrence risk robustly, it may align with those features.
- **Immune Infiltrate (TIL/Immunoscore):** We hypothesize that immune-rich (high-TIL) tumors are more often ctDNA-negative. Historically, high TIL is prognostic of long DFS (pmc.ncbi.nlm.nih.gov). If a potent immune response already eliminated most tumor clones, ctDNA would not be detected. Conversely, an immune-evaded tumor could both lack TILs and release ctDNA. Thus, there may be an **inverse correlation** between algorithmic TIL density and ctDNA positivity.
- **Combined Risk Score:** The abstract does not describe a composite AI score; it reports that QuantCRC features enable risk stratification within ctDNA groups (ascopubs.org). A CancerNetwork report states that adding QuantCRC features to clinical and ctDNA data added 0.018 to the C-index for DFS and 0.022 for OS (cancernetwork.com).

Comparative Data: To illustrate potential synergy, consider the survival data from Sinicrope JCO 2026 (pmc.ncbi.nlm.nih.gov). A ctDNA-negative patient had ~77% 5-year DFS; a ctDNA-positive patient only ~28%. The abstract reports that, among ctDNA-negative patients, reduced intratumoral TIL density and reduced tumor necrosis identified subgroups with inferior outcomes (TTR, DFS, OS), and that within ctDNA-positive cases a higher %immature tumor bed was associated with adverse outcome (ascopubs.org).

Data Synthesis and Evidence

ctDNA in the N0147 Trial

Frank Sinicrope's J Clin Oncol 2026 paper provides crucial context (pmc.ncbi.nlm.nih.gov). This tissue-free epigenomic ctDNA assay (Guardant Reveal™) was applied to 2,260 stage III CRC patients from N0147, sampled after surgery. Key findings were:

- **Detection Rate:** ctDNA was positive in 20.4% of patients (pmc.ncbi.nlm.nih.gov). Higher T/N stage, high grade, perforation, and BRAF mutations were over-represented in ctDNA-positive tumors (pmc.ncbi.nlm.nih.gov), aligning with factors for residual disease.
- **Prognostic Power:** ctDNA-positive status conferred dramatically worse outcomes. At 6.1 years median follow-up, the unadjusted hazard ratios for ctDNA-positive vs -negative were 5.96 for time-to-recurrence, 5.03 for DFS, and 4.45 for overall survival (all $p<0.0001$); in multivariable analysis the adjusted hazard ratios were 3.74 for DFS, 4.33 for time-to-recurrence, and 3.17 for overall survival (pmc.ncbi.nlm.nih.gov). Absolute 5-year DFS was only 27.7% for ctDNA-positive, versus 77.1% for ctDNA-negative (pmc.ncbi.nlm.nih.gov). These statistics emphasize ctDNA as one of the strongest prognostic factors.
- **Tumor Fraction:** Among the 461 ctDNA-positive cases, their estimated tumor fraction (TF) roughly doubled in those who recurred or died versus those who did not ($p=0.0002$) (pmc.ncbi.nlm.nih.gov). This means not only the binary status but the quantitative burden of ctDNA adds nuance: higher ctDNA levels imply even higher risk.
- **Genomic Correlates:** Guardant360™ targeted profiling on ctDNA revealed that mutations in genes like FLT1 and PREX2 were enriched in patients who recurred (odds ratios ~8–9) (pmc.ncbi.nlm.nih.gov), suggesting certain mutational profiles are linked to relapse propensity.

In sum, Sinicrope **et al.** provide a data-driven profile of MRD in stage III CRC. Importantly for our topic, this delineates the background on which to interpret the Mayo ASCO abstract: we know which clinical/pathologic features accompany ctDNA positivity, and we know the quantitative impact on survival (pmc.ncbi.nlm.nih.gov). Any AI-derived TME signature will be assessed against these benchmarks.

Digital Pathology Findings

Quantitative examples from prior AI pathology studies provide background for the features examined in the Mayo analysis:

- **Stage and TME:** The Gastroenterology/QuantCRC data showed profound shifts in TME features by stage (Table 1, Fig. 1 in that paper) (pmc.ncbi.nlm.nih.gov). Advanced stage III/IV tumors had more tumor budding (median %TB/PDC rising from 0.7% in stage I to 2.1% in stage IV) and more immature stroma (median 34% to 46%) than stage I (pmc.ncbi.nlm.nih.gov). Importantly, TILs declined (median

44.8 → 29.4 cells/mm² from Stage I to III). When these features were incorporated into prognostic models, adding QuantCRC data improved prediction accuracy (Harrell's C-index) over TNM alone (pmc.ncbi.nlm.nih.gov). Thus, AI features quantify what pathologists see qualitatively.

- **dMMR vs pMMR Differences:** The 2023 ASCO JCO abstract (ascopubs.org) (Sinicrope et al.) identified differences in AI features by MMR status. For example, dMMR tumors (n=191) had *higher* inflammatory stroma and TIL counts than pMMR tumors, consistent with immune-reaction phenomenon (ascopubs.org). Conversely, pMMR tumors had higher tumor budding as a relative feature. In that study, stratified analysis showed: for pMMR cases, lower tumor budding quartile (Q1 vs Q4) was associated with much better DFS (HR_adj=0.20, p=0.01) (ascopubs.org). For dMMR tumors, the AI "tumor area" feature (including mucin and TB/PDC) was independently prognostic (HR_adj=0.45, p=0.01) (ascopubs.org). These results highlight that AI features can capture immune vs budding differences, which may also relate to ctDNA patterns.
- **Integration and Multimodality:** The broader oncology literature is increasingly focused on multimodal biomarkers. A recent review notes that combining radiomics (imaging), pathomics (pathology), and genomics can yield better prognostic models (pmc.ncbi.nlm.nih.gov). In particular, integrated modeling of the tumor immune microenvironment is a key direction: "modelling of microenvironmental and immune correlates is an active direction that radiomics and pathomics may help approximate at scale" (pmc.ncbi.nlm.nih.gov). Mayo's work fits into this paradigm: tissue histology (pathomics) and ctDNA (circulating genomics) together probe the same biology from two angles. If a combined biomarker (AI TME features + ctDNA) is confirmed in peer-reviewed analyses to improve risk stratification, as the abstract's within-ctDNA-group stratification suggests, it could be a template for integrated surgico-oncology decision tools.

Relating AI-Pathology to ctDNA: Hypotheses

The published abstract of the Mayo poster reports only limited, specified associations between AI-derived TME features and ctDNA status, and it does not report hazard ratios or a combined AI-plus-ctDNA model; the following items are hypotheses drawn by analogy from other studies, not findings, and peer-reviewed results are pending:

1. **Immune Features vs ctDNA:** Tumors with **high TIL density or Immunoscore** are known to have lower relapse rates (pmc.ncbi.nlm.nih.gov). If the AI algorithm correctly quantifies TILs, we would expect **ctDNA-negative cases to show higher AI-TIL scores** on average than ctDNA-positive cases. In other words, an active immune microenvironment may clear residual disease, preventing ctDNA release. Conversely, ctDNA-positive tumors might exhibit immune "cold" signatures (low TIL, low inflammatory stroma). This hypothesis follows from the established prognostic value of immune response and the observed correlation of immune features with dMMR status (ascopubs.org).
2. **Tumor Budding vs ctDNA:** High **tumor budding/poorly differentiated clusters (TB/PDC)** reflects an invasive phenotype. In Sinicrope's 2023 study, TB/PDC was the largest driver of poor DFS. It is plausible that **ctDNA-positive patients have higher TB/PDC**. This would mean the AI-derived %TB/PDC is significantly larger in the ctDNA(+) group. If confirmed, it links an AI morphological marker

to molecular dissemination. Statistically, one could test for difference in mean %TB/PDC between ctDNA+ and ctDNA– cohorts; a multivariable model could then assess TB/PDC as independent of other covariates.

3. **Stroma Composition vs ctDNA:** Tumor:stroma ratio and stroma maturity may also correlate with MRD. For instance, a low tumor:stroma ratio (i.e. predominance of desmoplastic stroma) was characteristic of higher-stage tumors in the QuantCRC data. If ctDNA-positive patients tend to have more occult metastases, they might also tend to have higher stromal fraction (lower ratio). Similarly, details like “immature stroma” or fibrotic content could be higher in ctDNA(+) cases. However, it is not clear-cut biologically whether stroma directly promotes or inhibits shedding; it may simply correlate with aggressiveness.
4. **Composite Scores:** The researchers may have constructed an overall “AI-risk score” from multiple features (e.g. a weighted sum of budding, TIL, stroma). If so, this composite might be strongly predictive of ctDNA positivity and outcomes. For instance, among ctDNA-negative patients, those in a high AI-risk tertile might still have moderate relapse rates (~40–50% DFS), whereas those in a low AI-risk tertile might enjoy DFS similar to low-stage predictions (~85%). Among ctDNA-positive patients, an AI-risk stratification could further split survival curves. Ultimately, one might envision a 2×3 stratification (ctDNA ± crossed with AI risk levels) yielding 6 groups with distinct prognoses.
5. **Prognostic Independence:** The abstract reports multivariable Cox models adjusted for KRAS, BRAF V600E and MMR status in which %tumor budding, %mucin, %signet ring cells, %inflammatory tumor bed and %inflammatory stroma were significantly associated with adverse outcomes irrespective of ctDNA status, with significant interactions with ctDNA status for LVI and %signet ring cells (ascopubs.org). The abstract does not give hazard ratios and does not by itself establish a regulatory-grade biomarker.

In summary, the published Mayo abstract reports only limited associations between QuantCRC-derived TME features and ctDNA status, without hazard ratios or a combined model. The separate JCO and QuantCRC studies make a broader association between AI-quantified TME features and ctDNA biologically plausible, but the extensions described in this section remain hypotheses until peer-reviewed results are available.

Data Integration and Tables

To illustrate the above points, we present *Table 2* contrasting key biomarkers and their clinical/regulatory contexts in stage III CRC. This highlights how AI-pathology and ctDNA compare with established markers:

Biomarker / Test	Modality	Clinical Evidence (Stage III CRC)	Regulatory Status / Notes
Digital Pathology AI (Quantitative TME)	Tissue H&E (WSI + AI)	Emerging research; shown to predict DFS in trials (e.g. QuantCRC, Immunoscore). AI features correlate with recurrence (pmc.ncbi.nlm.nih.gov). This Mayo ASCO study links AI features to ctDNA and outcomes.	Software-as-IVD (FDA Class II, 21 CFR 864.3750, for diagnostic-assist image-analysis software (eCFR)). No FDA-authorized AI prognostic biomarker in CRC is identified here. NICE describes the Immunoscore assay (HalioDx) as an in vitro diagnostic test that applies image analysis software to digital images of CD3+/CD8+ stained tumour samples (NICE).
Postoperative ctDNA (blood-based MRD testing)	Blood (cfDNA sequencing)	Strong prognostic marker in stage II/III (Sinicrope JCO 2026: HR_DFS≈5; 5yr DFS 27% vs 77% (pmc.ncbi.nlm.nih.gov)). Ongoing trials (CIRCULATE, DYNAMIC) use ctDNA to tailor adjuvant therapy.	Use of ctDNA to guide adjuvant therapy in CRC remains investigational in the trials cited. Liquid-biopsy companion diagnostics exist for other tumors (e.g. Guardant360 CDx for EGFR in NSCLC (www.fda.gov)).
Immunoscore® (CD3/CD8 IHC)	Tissue (IHC + image analysis)	Validated prognostic in multiple phase III studies; identifies relapse risk independent of TNM (pmc.ncbi.nlm.nih.gov). In IDEA trial, predicts benefit from extended chemo.	Described in NICE medtech innovation briefing MIB269 (2021), which states that it uses digital pathology and AI-based algorithms to assess CD3+ and CD8+ T-cell density (NICE summary); the briefing is an evidence summary.
MMR Status / MSI	Tissue (IHC or PCR)	dMMR (MSI-high) stage III CRC has better prognosis; predictive for immunotherapy benefit. dMMR ~15% of stage III.	Standard-of-care biomarker testing to identify dMMR/MSI-high tumors.
KRAS/BRAF Mutations	Tissue Genomic profiling	KRAS/NRAS mutations predict lack of response to EGFR antibodies; BRAF^V600E indicates poor prognosis in CRC.	FDA-approved companion diagnostic (e.g. Roche cobas KRAS Mutation Test, PMA P140023, for KRAS codon 12/13 mutations in colorectal cancer tissue with cetuximab or panitumumab (FDA CDx list)).
Genomic Panel (e.g. FoundationOne®)	Tissue NGS	Covers many biomarkers (e.g. RAS, MSI, TMB) for targeted therapy selection.	FoundationOne CDx (PMA P170019) is FDA-approved as a companion diagnostic in colorectal cancer tissue to identify KRAS wild-type (and, for panitumumab, NRAS wild-type) tumors for cetuximab or panitumumab; its other approved indications are listed on FDA's companion diagnostics list (FDA CDx list).

Table 2. Selected biomarkers and diagnostics in stage III colorectal cancer: source, validation, and regulatory status.

Table 2 underscores the novel nature of AI-pathology as a biomarker. Existing tests like KRAS genotyping (www.fda.gov) and MSI classification are well entrenched in guiding therapy. ctDNA is increasingly studied for this role given its strong evidence (pmc.ncbi.nlm.nih.gov), and immunoscore represents a forerunner of pathology-based AI in commercial use (www.nice.org.uk). By contrast, Mayo's "regulatory-grade AI biomarker" is still at the investigation stage. The comparison shows that for a new biomarker to ascend to regulatory grade, it must achieve analytical validity (consistent image analysis), clinical validity (predicting outcomes as in this study), and ideally demonstrate utility in decision-making. The meticulous design (phase III trial setting) suggests the Mayo team is aiming for exactly such rigor.

Case Studies and Clinical Implications

The following hypothetical scenarios are illustrative only and are not drawn from the abstract; they show how findings of this kind could inform discussion of patient management. Consider two hypothetical patients with stage III colon cancer:

- **Case A (Low-risk AI, ctDNA-negative):** A 60-year-old patient underwent curative resection of a T3N1a (stage IIIA) colon cancer. AI analysis of the pathology slide shows abundant tumor epithelium, sparse stroma (high tumor:stroma ratio), low percentage of tumor buds, and very dense CD8+ lymphocyte infiltration. Postoperative blood tests show no detectable ctDNA (tissue-free assay). In this hypothetical, the patient would have two favorable features: a strong immune microenvironment and negative MRD. According to Sinicrope JCO findings, their 5-year DFS would be expected near the high end of the range (approaching ~80% or more (pmc.ncbi.nlm.nih.gov)). Even so, such a patient would be managed according to current guidelines for stage III disease; shortening or omitting adjuvant chemotherapy on the basis of AI pathology plus ctDNA is investigational and is not supported by the data reviewed here.
- **Case B (High-risk AI, ctDNA-positive):** Another patient, age 55, has T3N2 (stage IIIC) disease. Digital histology reveals extensive tumor budding and a desmoplastic, immune-poor stroma (low AI-derived TIL score). The same ctDNA assay comes back **positive**. Individually, each factor signals high risk: historically, even stage IIIC patients have much lower DFS, and Sinicrope's data show ctDNA-positive 5-year DFS around 28% (pmc.ncbi.nlm.nih.gov). Combined, these markers solidify the prognosis as very poor. Clinically, one would advocate full 6-month FOLFOX (the trial standard) or even new adjuvant approaches. Perhaps this profile might qualify the patient for experimental plasma-driven escalation trials. Any intensification beyond standard adjuvant therapy would be investigational and would be considered only within a clinical trial.

These hypothetical scenarios illustrate questions that integrating AI pathology and ctDNA might one day address; they do not reflect validated decision rules, and no Mayo results on the combination are available.

Such cases are consistent with outcomes in the literature. For instance, in Sinicrope's cohort, ctDNA-negative patients had a 5-year DFS of ~77% (pmc.ncbi.nlm.nih.gov) – akin to the good-prognosis group – while ctDNA-positive were around 28%. Similarly, Immunoscore data (analogous to high AI-TIL) show long-term DFS approaching 90% in the top TIL quartile (pmc.ncbi.nlm.nih.gov). If Mayo's AI score recapitulates immunoscore-like information, it would predict along these lines. In fact, NICE's evidence summary says that Immunoscore (which itself uses image-analysis software) could potentially lead to fewer people having more, or less, chemotherapy than is needed (www.nice.org.uk).

These "case studies" also emphasize perspective: in a clinical trial, patients like Case A might be eligible for shortened therapy arms, while Case B might need novel interventions. From an imaging standpoint, the integration also has operational meaning. Pathology labs would need WSI capability and AI tools, and clinicians would get a composite report. Meanwhile, blood-based assays like Guardant Reveal are already run clinically. The synergy of these two diagnostic veins is what the Mayo ASCO abstract points toward.

Regulatory-Grade Biomarker Considerations

Describing the Mayo AI signature as a “regulatory-grade biomarker” raises important regulatory science questions. The FDA and other agencies are increasingly accommodating software and AI tools as medical devices, but stringent evidence is required. We outline key regulatory considerations:

1. **Device Classification and Approval Pathway:** FDA regulates software algorithms that analyze whole slide images as in vitro diagnostic devices. Under 21 CFR 864.3750, a “software algorithm device to assist users in digital pathology” is a Class II (special controls) device intended to evaluate scanned pathology whole slide images and to provide information about the presence, location and characteristics of areas of the image with clinical implications, to assist the user in determining a pathology diagnosis (21 CFR 864.3750). That classification describes diagnostic-assist image-analysis software; it should not be read as a blanket rule that every image-based prognostic score requires 510(k) clearance, and the appropriate pathway for a composite prognostic biomarker would depend on its intended use and would need to be confirmed with FDA. For now, Mayo’s AI-derived feature set is an investigational research tool.
2. **Analytical Validation:** A “regulatory-grade” assay must have tight performance specs. For ctDNA, the Guardant Reveal assay cites >98% specificity and high sensitivity (pmc.ncbi.nlm.nih.gov). For AI pathology, reproducibility across scanners, staining variations, etc., must be shown. QuantCRC underwent internal validation against pathologists on 30 images (pmc.ncbi.nlm.nih.gov). For approval, the algorithm would need evidence that its segmentation and quantification are robust, ideally as reported in its instructions-for-use (not yet published). FDA expects protocols for training set diversity, overfitting control, and real-world testing. The clinical study helps indirectly by showing it works on trial data, but a technical validation report would be required.
3. **Clinical Validation as a Biomarker:** Biomarkers must link to meaningful outcomes. Here, Mayo presents exactly that: AI feature → ctDNA status and survival. If the results show strong independent correlation with recurrence, one could in principle pursue FDA “biomarker qualification” (CDER) or PMA/De Novo for a specific claim (e.g. prognostic risk stratifier). However, regulators have yet to handle purely digital-image biomarkers extensively. If fully validated, an AI TME score could be offered to clinicians after clearing regulatory milestones. Crucially, it must demonstrate not just retrospective correlation but also prospective utility (e.g. does treatment change based on it improve outcomes?). That is a tall bar.
4. **Companion Diagnostics vs Risk Stratification:** FDA’s official CDx list (approved companion diagnostics) currently includes no pathology image algorithms for CRC. It does list many genetic tests (KRAS, NGS panels) (www.fda.gov) and a few imaging tests (like MRI liver iron). The absence of digital pathology CDx reflects the novelty: to date, no drugs require an image-based biomarker. However, one could envision future scenarios: e.g. if an adjuvant drug is effective only in patients with certain TME profiles, the AI test could become a “companion” for selecting therapy. Even without a drug-CDx label, FDA encourages “complementary diagnostics” (non-treatment obligatory tests) if beneficial. The word **“regulatory-grade”** suggests that the Mayo group is thinking ahead: their AI signature might need to meet the same validation rigor as established IVDs if it were to be used in trials or practice.

5. **Existing Guidance:** The FDA has issued several guidances relevant to AI and digital pathology. Regulatory expectations for AI-enabled software continue to evolve. Any AI pathology tool considered "regulatory-grade" must include post-market surveillance plans (monitoring for drift) and strategies for updates (Locked vs adaptive algorithms).
6. **Real-World Evidence and Ongoing Trials:** The FDA is open to Real-World Evidence (RWE) for devices. The Mayo ASCO results, from a controlled trial, have strong validity, but utility in community settings remains to be shown. Prospective trials could test whether using the AI+ctDNA info to guide therapy improves outcomes (e.g., omitting chemo in ultra-low risk, or adding novel agents in high risk). Such prospective data would cement it as a practice-altering biomarker.

In summary, the Mayo Clinic study **lays groundwork** for a regulatory-quality biomarker by using high-quality data (phase III RCT) and rigorous analytics. Demonstration of analytic reproducibility (previously reported for QuantCRC (pmc.ncbi.nlm.nih.gov)) plus on-trial prognostic value would support further development. Whether this AI pathology signature ultimately becomes part of a cleared diagnostic remains to be seen; but it illustrates the path: design a mature algorithm, validate in trials, link to outcomes, then engage with regulatory framework for software IVDs.

Discussion and Future Directions

The integration of deep-learning pathology with liquid biopsy represents a frontier in precision oncology. The Mayo abstract points to several future implications:

- **Enhanced Risk Stratification:** If validated, combining AI-TME scoring with ctDNA status yields a multi-dimensional risk index. This could replace or augment TNM staging. In practice, each resected patient could receive a report stating: "AI Pathology Score = X (high/low), ctDNA = +/−, 5-year recurrence risk = Y%." Such information could guide tailored adjuvant regimens beyond our current binary approaches.
- **Design of Clinical Trials:** Using these biomarkers as stratification factors could improve trial efficiency. For example, an adjuvant trial might enroll only ctDNA-positive patients, or stratify randomization by AI-risk group. Alternatively, de-escalation trials (testing shorter chemo) could restrict to those with favorable biomarker profiles. Such designs are already underway using ctDNA alone; incorporating AI-pathology could refine them further.
- **Extension to Other Cancers:** While this study focuses on colon cancer, the concept is general. In any solid tumor, digital pathology features and ctDNA could synergize. For instance, in resected lung cancer, AI quantification of immune infiltration combined with ctDNA MRD might predict recurrence. Cross-tumor digital signatures may have common patterns, and regulatory frameworks could eventually consider tissue-agnostic digital biomarkers as well as molecular ones.
- **Technical Advances:** The success of this approach will drive improvements in both domains. For AI pathology, better models (e.g. multi-spectral immunofluorescence, 3D analysis) might capture more TME detail. For ctDNA, more sensitive multi-omic assays (methylation, fragmentation, ultra-deep sequencing) are in development to detect MRD even earlier. Combining such enhanced assays could further improve predictive power.

- **Clinical Adoption:** Ultimately, adoption of an AI+ctDNA diagnostic will hinge on demonstrating improved patient outcomes (clinical utility). Realistically, this will require prospective validation. It may begin as an ingredient of risk models rather than a stand-alone CDx. NICE's 2021 medtech innovation briefing on Immunoscore (MIB269) is an evidence summary, and it lists key uncertainties, including that none of the published studies included people having treatment in the NHS; uptake of advanced biomarkers will depend on demonstrated clinical utility.
- **Ethical and Practical Aspects:** A biomarker more refined than stage raises tough questions: will payers cover longer or shorter chemo based on it? How to counsel patients and handle false positives/negatives? There is also concern that AI models trained on one center's images might not generalize across labs. Mayo's use of Alliance trial samples does help (multi-site accrual), but broader validation in diverse pathology labs will be needed.

In conclusion, the Mayo Clinic's ASCO 2026 poster (Abstract 3525) addresses a recognized need—better stratification of colon cancer recurrence risk—by pairing AI pathology with ctDNA. The published abstract (J Clin Oncol 2026;44(suppl 16):3525) reports that QuantCRC-derived TME features differ by postoperative ctDNA status and enable risk stratification within ctDNA groups, and a CancerNetwork report says the combination improved prognostic discrimination; as conference-abstract findings, they still require peer-reviewed publication and independent validation.

Conclusion

The Mayo Clinic's ASCO 2026 abstract on AI-assisted pathology and ctDNA in stage III colon cancer synthesizes multiple cutting-edge domains into a cohesive biomarker strategy. We have examined the background biology, the methodology, and the evidence surrounding this approach. Key takeaways include:

- Deep-learning analysis of tumor histology can objectively quantify features of the tumor microenvironment (including immune infiltration, stroma, and tumor architecture) that have known prognostic value (ascopubs.org) (pmc.ncbi.nlm.nih.gov).
- Circulating tumor DNA is a powerful prognostic indicator of residual disease and recurrence risk in operable colon cancer (pmc.ncbi.nlm.nih.gov).
- The Mayo poster (Abstract 3525) is titled as examining AI-derived tumor microenvironment features in relation to postoperative ctDNA status and outcomes in N0147, and the published abstract reports significant differences in histopathologic features by ctDNA status and risk stratification within ctDNA groups; as a conference abstract without a full-text component, it still awaits peer-reviewed confirmation. This integrated biomarker could refine risk assessment beyond either modality alone.
- Extensive pre-existing data support the plausibility and importance of this integration: prior work shows AI pathology features map onto outcomes (pmc.ncbi.nlm.nih.gov) (ascopubs.org), and Mayo's own ctDNA study quantified the magnitude of its prognostic signal (pmc.ncbi.nlm.nih.gov).

- Such a biomarker, if validated, has **regulatory implications**. FDA classifies diagnostic-assist digital pathology algorithms as Class II in vitro diagnostic devices (www.ecfr.gov) and has approved ctDNA-based companion diagnostics for specific indications in other cancers (www.fda.gov). A composite AI+ctDNA test would have to meet similar standards of rigor and reproducibility. The Mayo approach is a retrospective analysis of specimens and data from a prospective phase III trial, with a large sample (N=1817) and hard clinical endpoints (time to recurrence, DFS, OS); prospective validation is still required.
- The broader impact could be profound: **multi-modal biomarker integration** embodies the precision oncology ideal. Future clinical practice might routinely incorporate AI pathology scores and ctDNA assays to tailor therapy. Whether combined biomarker data could inform adjuvant treatment intensity would need to be tested in prospective trials.
- Several perspectives converge on this advancement: pathologists see a tool to capture microscopic features systematically; oncologists gain a more granular risk stratification; regulators are prompted to define how to qualify AI biomarkers; and patients ultimately stand to benefit from truly personalized care.

In summary, the Mayo Abstract 3525 linking deep-learning TME quantification to ctDNA status is more than an academic curiosity – it signifies a step toward *regulatory-grade* AI biomarker deployment. By anchoring claims in peer-reviewed evidence and analyzing all dimensions, this report underscores both the promise and the challenges ahead. Because these findings come from a conference abstract, peer-reviewed publication and independent validation will be needed to confirm and refine these insights. The fusion of AI imaging and ctDNA heralds a new era in oncology diagnostics, and Mayo Clinic's contribution is at the forefront of this transformation.

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