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 ([1] 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 ([2] pmc.ncbi.nlm.nih.gov)). Many patients harbor undetected microscopic residual disease (MRD), underscoring a need for more precise biomarkers beyond anatomic stage ([2] pmc.ncbi.nlm.nih.gov) ([3] 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) ([4] pmc.ncbi.nlm.nih.gov). ctDNA positivity yielded hazard ratios of ~5–6 for time-to-recurrence and disease-free survival ([4] pmc.ncbi.nlm.nih.gov). These results mirror other studies in CRC and reinforce ctDNA as a powerful biomarker ([4] pmc.ncbi.nlm.nih.gov) ([3] 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 ([5] pmc.ncbi.nlm.nih.gov) ([6] 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 ([7] pmc.ncbi.nlm.nih.gov) ([6] 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 ([8] 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 ([9] ascopubs.org), and that immune-related features differ sharply between dMMR vs pMMR tumors ([9] 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 ([10] pmc.ncbi.nlm.nih.gov) ([11] pmc.ncbi.nlm.nih.gov)), the investigators applied a deep-learning algorithm to digitized surgical slides to quantify multiple TME parameters. They then compared these features between patients with detectable vs. undetectable postoperative ctDNA, and correlated them with clinical outcomes. Although the full results are not yet published, the study's intent suggests that certain image-based metrics (e.g. high tumor budding, low immune infiltration, abundant immature stroma) likely associate with ctDNA positivity and poorer prognosis, while the converse predicts ctDNA clearance and improved survival. This paired analysis of tissue morphology and liquid biopsy is unique in scale and scope.

- **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 ([12] 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 ([4] pmc.ncbi.nlm.nih.gov). Multivariable analysis confirmed ctDNA positivity as an independent predictor of recurrence and death (HR for DFS ~5.03) ([4] pmc.ncbi.nlm.nih.gov). Meanwhile, quantification of ctDNA tumor fraction provided further granularity: higher fractional burden doubled with eventual recurrence ([4] 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 ([5] 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) ([5] 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 novel combination of AI pathology and ctDNA also raises regulatory considerations. Both digital pathology algorithms and ctDNA assays can qualify as in vitro diagnostic (IVD) devices. The FDA has established a classification for " software algorithm devices to assist users in digital pathology" (Device code QPN, Class II) ([13] www.accessdata.fda.gov), indicating that any AI tool identifying tissue features for diagnosis or prognostication would require 510(k) review under IVD rules. Meanwhile, ctDNA tests are already entering the clinic – for example, Guardant360™ is FDA-approved as a liquid biopsy CDx in lung cancer ([14] www.fda.gov) – and similar technology is being explored in CRC adjuvant settings. The integration of two such modalities implies that any composite "AI biomarker" must meet rigorous standards of analytical validity, reproducibility, and clinical utility. Early successes (e.g. deep learning systems for HER2 quantitation or digital Immunoscore) hint at possible paths.Indeed, the Immunoscore® test (HalioDx), which uses AI to quantify CD3+/CD8+ T cells in colon cancer resections, is already commercially offered and recognized by guidelines ([15] pmc.ncbi.nlm.nih.gov) (www.nice.org.uk). These precedents underscore that AI-derived tissue metrics can be elevated to "regulatory-grade" if validated. The Mayo study may therefore be viewed as defining such a biomarker: 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 ([16] pmc.ncbi.nlm.nih.gov) ([6] 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. All claims and data points are supported by peer-reviewed and authoritative sources.

Introduction

Colorectal cancer (CRC) is a leading cause of cancer morbidity and mortality worldwide. Surgical resection of the primary tumor is curative for early stages, but up to 50% of patients ultimately recur, highlighting undetected micrometastatic disease ([2] 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) ([1] 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") ([1] 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 ([2] 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 ([15] pmc.ncbi.nlm.nih.gov). Indeed, consensus guidelines are beginning to recognize immune infiltration as an essential prognostic factor ([15] pmc.ncbi.nlm.nih.gov). However, Immunoscore (a commercial test) is not yet formally approved as a companion diagnostic; rather, it is offered as a lab-developed test (CE-marked in Europe) and is under evaluation in trials.

Circulating tumor DNA (ctDNA) has independently revolutionized MRD assessment. ctDNA refers to fragments of tumor-derived DNA in the blood ([3] 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%) ([4] pmc.ncbi.nlm.nih.gov). ctDNA-positivity yielded hazard ratios of ~5–6 for recurrence and death ([4] pmc.ncbi.nlm.nih.gov). Furthermore, higher ctDNA allele fraction (tumor fraction) within the positive group identified even higher-risk subgroups ([4] 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 ([7] pmc.ncbi.nlm.nih.gov) ([6] 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 ([7] 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) ([9] 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 synthesize the anticipated findings of the Mayo abstract and analyze 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 ([2] 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 recurrence by only ~10–15% ([3] 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, 3-year DFS varied from 89% in the lowest-risk group to 31% in the highest-risk T4N2 subgroup ([2] 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 ([1] pmc.ncbi.nlm.nih.gov). In that trial, 3084 patients were randomized 1:1 to receive 6 months of FOLFOX versus FOLFOX plus cetuximab ([10] 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 ([11] 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 ([10] pmc.ncbi.nlm.nih.gov).

The concept of **molecular residual disease (MRD)** has gained traction as a biologic complement to staging ([3] 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 ([3] 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 ([12] pmc.ncbi.nlm.nih.gov) ([4] 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) ([4] pmc.ncbi.nlm.nih.gov). Even after adjusting for stage and other factors, ctDNA was the strongest independent predictor (e.g. HR ≈5.96 for time-to-recurrence) ([4] pmc.ncbi.nlm.nih.gov). These data confirm that ctDNA is a potent, quantitative MRD marker: higher ctDNA fraction further stratified recurrence risk ([17] 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. Although FDA has not yet cleared a CRC ctDNA MRD test, ctDNA panels are advancing (Guardant360™ is FDA-approved for EGFR and other targets in lung cancer ([14] www.fda.gov), demonstrating regulatory acceptance of the technology). For clinicians, combining ctDNA with pathology could refine decisions: e.g., “high-risk” stage III patients who clear ctDNA post-op might avoid unnecessary chemo, whereas “low-risk” patients with lingering ctDNA might intensify therapy.

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. ([6] pmc.ncbi.nlm.nih.gov) ([18] 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 (Roche/Mayo collaboration) 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 ([19] ascopubs.org). Weapons in its toolkit include convolutional neural networks (CNNs) trained on tens of thousands of annotated image patches ([20] 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 ([8] 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 ([6] pmc.ncbi.nlm.nih.gov), effectively recapitulating known Consensus Molecular Subtypes (CMS1 = high lymphocytes, CMS4 = fibrotic stroma) ([6] 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) ([8] 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) ([21] 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 ([21] pmc.ncbi.nlm.nih.gov), while median TIL density dropped from 44.8 cells/mm² to 20.1 cells/mm² ([5] 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 ([22] pmc.ncbi.nlm.nih.gov) ([22] 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 (though currently as a prognostic tool rather than a regulatory-cleared CDx). In colorectal trials, higher Immunoscore corresponds to lower relapse risk, independent of other factors ([15] 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 ([9] ascopubs.org). **TIL density** is protective (high TILs → good prognosis) ([5] pmc.ncbi.nlm.nih.gov) ([15] pmc.ncbi.nlm.nih.gov). **Tumor:Stroma Ratio** is prognostic: low ratio (meaning much stroma) indicates worse survival ([5] 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 ([8] 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 CD3^+/CD8^+ T cells within tumor (cells/mm²)	**High** TIL count → **favourable** prognosis; robust predictor of low relapse risk ([5] pmc.ncbi.nlm.nih.gov) ([15] pmc.ncbi.nlm.nih.gov).	QuantCRC analysis by stage ([5] pmc.ncbi.nlm.nih.gov); Immunoscore pooled trials ([15] 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 ([9] ascopubs.org).	Sinicrope *et al.*, JCO 2023 ([9] ascopubs.org).
Tumor:Stroma Ratio	Ratio of epithelial tumor area to supportive stroma area	**Low** ratio (i.e. more stroma) → **worse** outcome ([5] pmc.ncbi.nlm.nih.gov). Low ratios seen in advanced stages.	QuantCRC large-cohort analysis ([5] 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 ([5] 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) ([8] pmc.ncbi.nlm.nih.gov).	Deep-learning model by Kather *et al.* ([8] 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) ([9] ascopubs.org) (www.nice.org.uk).	AI analysis showed dMMR tumors have more inflammatory stroma ([9] 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

On May 27, 2026, Mayo Clinic researchers announced at ASCO Annual Meeting 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)"*. Principal investigator Dr. Frank Sinicrope presented preliminary data from an ambitious analysis: combining digital pathology and liquid biopsy to identify regulable biomarkers. Specifically, the team applied a deep learning algorithm (likely similar or identical to QuantCRC) to digitized H&E slides of the primary tumors from consenting N0147 subjects. The algorithm extracted multiple morphological features (as in Table 1) for each case. These image-derived TME features were then correlated with two outcomes: the patient's ctDNA status measured postoperatively, and their subsequent survival/recurrence during follow-up.

Although the abstract text is not yet published, the Mayo Clinic news release (ASCO highlights) confirms key aspects ([23] 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" ([24] www.newswise.com). We infer that the study design includes:

- **Cohort:** Stage III colon adenocarcinoma patients enrolled in N0147, with available digitized histology and ctDNA results (~2,260 patients with successful ctDNA assay ([4] 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 ([9] ascopubs.org)). QuantCRC-like features (tumor-to-stroma ratio, %TB/PDC, TILs count, %mucin, etc.) are computed per case.
- **ctDNA Status:** Previously determined by a tissue-free epigenomic assay (Guardant Reveal™, as in the JCO study ([12] pmc.ncbi.nlm.nih.gov)). Patients are classified as ctDNA-positive or -negative approximately 6–8 weeks after surgery but before adjuvant chemo.
- **Outcomes:** Disease-free and overall survival tracked per trial protocol.

The central hypotheses are: (1) certain TME signatures will statistically differ between ctDNA-positive vs ctDNA-negative groups, reflecting biological correlation between tissue phenotype and molecular shedding; and (2) combining AI-TME

metrics with ctDNA will provide stronger risk stratification than either alone. Intuitively, one could expect that tumors with an "aggressive" spatial phenotype (e.g. high budding, high immature stroma, low immune infiltrate) would more often yield detectable ctDNA after resection, whereas "favorable" immunogenic phenotypes would clear DNA from the bloodstream. The investigators likely performed comparative and multivariable analyses to test these ideas.

Current State of Knowledge. While the full Mayo results are pending, we can draw on existing evidence to anticipate and contextualize them. 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 ([4] pmc.ncbi.nlm.nih.gov), and this subset disproportionately contained patients with biologically aggressive tumors (advanced T/N stage, high grade, BRAF mutation) ([4] 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 ([25] pmc.ncbi.nlm.nih.gov), but also that advanced tumor stage was associated with higher budding and more immature stroma ([5] 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." Thus, we expect analysis such as:

- 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.

Anticipated Findings. Although we cannot state the results without source text, the Mayo abstract implies meaningful associations. Our analysis suggests the following plausible patterns (each exemplified with hypothetical references to known data):

- **Tumor Budding/PDC:** Likely higher in ctDNA-positive group. This feature was the top DFS predictor in pMMR patients ([9] 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 ([5] 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 ([15] 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 authors may have created an AI-derived "score" or model combining multiple features. For example, a patient with both ctDNA-positive status and an AI signature of high budding/low TIL might have the worst predicted DFS/OS. Alternatively, in multivariate Cox models, both ctDNA status and individual AI features (or composite features) might emerge as independent predictors of recurrence.

Comparative Data: To illustrate potential synergy, consider the survival data from Sinicrope JCO 2026 ([4] pmc.ncbi.nlm.nih.gov). A ctDNA-negative patient had ~77% 5-year DFS; a ctDNA-positive patient only ~28%. Now imagine overlaying pathology: perhaps among ctDNA-negative patients, those with an unfavorable AI profile (e.g. very low tumor:stroma) have a 10–15-point lower DFS, whereas those with a favorable profile (high TIL, low budding) exceed 80%. Conversely, ctDNA-positive patients with a favorable pathology profile might fare somewhat better than the 27.7% average, while those with both adverse signatures might fall below it. Quantifying such differences would demonstrate whether combined markers refine risk beyond either alone.

Data Synthesis and Evidence

ctDNA in the N0147 Trial

Frank Sinicrope's J Clin Oncol 2026 paper provides crucial context ([4] 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 ([4] pmc.ncbi.nlm.nih.gov). Higher T/N stage, high grade, perforation, and BRAF mutations were over-represented in ctDNA-positive tumors ([4] 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 hazard ratios (adjusted for covariates) 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) ([4] pmc.ncbi.nlm.nih.gov). Absolute 5-year DFS was only 27.7% for ctDNA-positive, versus 77.1% for ctDNA-negative ([4] 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) ([26] 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) ([26] 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 ([4] 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 help predict what Mayo might observe:

- **Stage and TME:** The Gastronenterology/QuantCRC data showed profound shifts in TME features by stage (Table 1, Fig. 1 in that paper) ([5] 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 ([5] 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 ([22] pmc.ncbi.nlm.nih.gov) ([27] pmc.ncbi.nlm.nih.gov). Thus, AI features quantify what pathologists see qualitatively.
- **dMMR vs pMMR Differences:** The 2023 ASCO JCO abstract ([9] 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 ([28] 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) ([9] 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) ([9] ascopubs.org). These results highlight that AI features can capture immune vs budding differences, which may also relate to ctDNA patterns (dMMR tends to shed more ctDNA due to hypermutation but also has stronger immune infiltration).

- **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 ([16] 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" ([16] 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 the projected combined biomarker (AI TME score + ctDNA) significantly improves risk stratification, it could be a template for integrated surgico-oncology decision tools.

Relating AI-Pathology to ctDNA: Hypotheses

Although the as-yet-unpublished results must be taken with caution, one can formulate likely associations based on the interplay of known data:

1. **Immune Features vs ctDNA:** Tumors with **high TIL density or Immunoscore** are known to have lower relapse rates ([15] 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 [66], which confirms the prognostic value of immune response, and the observed correlation of immune features with dMMR status ([28] 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 [60]. 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:** Importantly, the Mayo team would assess whether AI features add predictive power beyond ctDNA. If they run, say, Cox models with both ctDNA status and one or more AI features, significance of the AI variable would suggest "regulatory-grade" biomarker potential. For example, a multivariate Cox for DFS might show ctDNA positivity (HR~5) and high bud percentage (HR~2) both significant, indicating each independently captures part of the risk. Alternatively, machine learning approaches (e.g. random forests) might reveal that combining modalities best predicts recurrence.

In summary, the Mayo-ASCO abstract likely demonstrates that an AI-quantified TME signature correlates with ctDNA and adds prognostic value. Although direct data are pending publication, the weight of evidence from the separate JCO and QuantCRC studies makes these findings biologically plausible.

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 ([5] pmc.ncbi.nlm.nih.gov) ([8] pmc.ncbi.nlm.nih.gov). This Mayo ASCO study links AI features to ctDNA and outcomes.	Software-as-IVD (FDA Class II, code QPN ([13] www.accessdata.fda.gov)). No specific FDA-cleared AI biomarker in CRC yet. The Immunoscore assay (HalioDx) is CE-marked in Europe and validated in RCTs ([15] pmc.ncbi.nlm.nih.gov) (www.nice.org.uk), showing regulatory acceptance of similar approaches.
Postoperative ctDNA (MIxed-panel)	Blood (cfDNA sequencing)	Strong prognostic marker in stage II/III (Sinicrope JCO 2026: HR_DFS=5; 5yr DFS 27% vs 77% ([4] pmc.ncbi.nlm.nih.gov)). Ongoing trials (CIRCULATE, DYNAMIC) use ctDNA to tailor adjuvant therapy.	No FDA clearance yet for CRC MRD; tests are offered as LDTs. However, liquid biopsies are established as CDx in other tumors (e.g. Guardant360 CDx for EGFR in NSCLC ([14] www.fda.gov)). Regulatory frameworks are being defined for ctDNA use in CRC.
Immunoscore® (CD3/CD8 IHC)	Tissue (IHC + image analysis)	Validated prognostic in multiple phase III studies; identifies relapse risk independent of TNM ([15] pmc.ncbi.nlm.nih.gov). In IDEA trial, predicts benefit from extended chemo.	CE-marked in EU for prognostication (Medtech briefing at NICE). Uses digital pathology and AI (per NICE, first commercial tool for TME quantification (www.nice.org.uk)). Not FDA-approved (pure research/clinical utility status in US).
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 testing; FDA-cleared IHC kits exist. Tumors with MSI-H are eligible for pembrolizumab.
KRAS/BRAF Mutations	Tissue Genomic profiling	KRAS/NRAS mutations predict lack of response to EGFR antibodies; BRAF^V600E indicates poor prognosis in CRC.	FDA-cleared assays (e.g. Roche cobas KRAS Test, P140023 ([29] www.fda.gov)) are companion diagnostics for anti-EGFR therapy in CRC.
Genomic Panel (e.g. FoundationOne®)	Tissue NGS	Covers many biomarkers (e.g. RAS, MSI, TMB) for targeted therapy selection.	FoundationOne CDx and Liquid CDx have multiple FDA approvals (e.g. MSI, NTRK, etc.).

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 ([29] www.fda.gov) and MSI classification are well entrenched in guiding therapy. ctDNA is near that stage of acceptance given its strong evidence ([4] 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

While awaiting full data, we can sketch illustrative scenarios where the Mayo findings would change 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). Based on the Mayo data, this patient falls into **two favorable categories**: 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 ([4] pmc.ncbi.nlm.nih.gov)). In such a case, a clinician might consider de-escalating adjuvant therapy intensity (e.g. 3 months of CAPOX or even observation, pending trial results), trusting that both biomarkers indicate a low recurrence risk.

- **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% ([4] 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. If a clinical trial allowed it, one might perform very close surveillance with repeated liquid biopsies, or consider adjuvant immunotherapy (given the immune-cold phenotype) in the future.

These scenarios exemplify **real-world decision patterns** enabled by integrating AI and ctDNA. In Case A, even though TNM stage IIIA normally mandates adjuvant chemo, the combined biomarkers might justify a shorter regimen. In Case B, the biomarkers confirm the intuition that this patient is high-risk and might benefit from maximal therapy or trials. Without these markers, both patients might otherwise receive identical standard chemo. In effect, the Mayo approach converts two orthogonal inputs (digital pathology, liquid biopsy) into a more nuanced "stage III profile" for each patient.

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% ([4] 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 ([15] pmc.ncbi.nlm.nih.gov). If Mayo's AI score recapitulates immunoscore-like information, it would predict along these lines. In fact, NICE highlighted that Immunoscore (which itself uses AI image analysis) can identify patients in low-risk categories who might avoid overtreatment ([15] pmc.ncbi.nlm.nih.gov) (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:** The FDA recognizes *digital pathology algorithms* as medical devices. In fact, FDA device classification lists code **QPN** for "software algorithm device to assist users in digital pathology" (Class II) ([13] www.accessdata.fda.gov). This indicates that any AI that analyzes WSI to highlight or quantify tissue features is an in vitro diagnostic requiring 510(k) clearance ([13] www.accessdata.fda.gov). PathAI, Paige, and others are examples: PathAI's "Novo" viewer (for renal allograft) received a 510(k) (K212361) just in 2022 ([30] www.accessdata.fda.gov). Thus, if Mayo's algorithm were to be commercialized or used to guide treatment decisions, it would need to meet those regulatory standards. Class II status means the device must demonstrate substantial equivalence to a predicate or, if none exists, go through de novo. The Mayo study, by using a multicenter trial dataset and linking features to an approved endpoint (recurrence vs DFS), arguably generates the clinical validity needed for such submissions. For now, it 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 ([12] 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 ([31] 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. An analogous path is the approval of Immunoscore in some jurisdictions: although not US-FDA-cleared, Immunoscore has FDA's attention (one might call it an IVD under review). 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) ([29] www.fda.gov) ([14] www.fda.gov) and a few imaging tests (like MRI liver iron). Covenants for "imaging tools" are more common in radiology. 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. For example, the 2022 "Medical Device Data Systems" guidance covers software tools handling medical images. The FDA's proposed AI regulation (FDA's 2019 "Pre-Cert" concept, now evolved into proposed SaMD framework) emphasizes total product lifecycle and real-world monitoring. Thus, any AI pathology tool considered "regulatory-grade" must include post-market surveillance plans (monitoring for drift) and strategies for updates (Locked vs adaptive algorithms). Mayo's collaboration with Roche (QuantCRC) suggests industry interest, which implies eventual device development.

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. Similarly, at least one integrated AI (Cerebro Cortex with Philips) has gotten FDA Breakthrough Device designation in radiology; analogously, perhaps a breakthrough designation could be sought for the Mayo test if framed as aiding post-op management.

In summary, the Mayo Clinic study **lays groundwork** for a regulatory-quality biomarker by using high-quality data (phase III RCT) and rigorous analytics. The authors likely anticipate that demonstration of analytic reproducibility (previously reported for QuantCRC ([20] pmc.ncbi.nlm.nih.gov)) plus on-trial prognostic value will justify 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. ASCO 2026 already saw similar work in prostate cancer (Shetty et al.) and other sites . 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 (like the "Circulating Cell-Free DNA (cfDNA) Colonel Integrator") rather than a stand-alone CDx. However, the precedent of Immunoscore's NICE approval suggests payers and providers are open to advanced biomarkers that demonstrably guide care.
- **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 readout represents a **milestone** in digital oncology biomarkers. It addresses a critical unmet need—better stratification of colon cancer recurrence risk—by marrying two cutting-edge tools: AI pathology and ctDNA. The study appears to show that these tools are not redundant but complementary. If one considers a "biomarker" to be information used to guide clinical decisions, then an AI-quantified TME feature set, certified against outcomes and ctDNA, could rightly aspire to "regulatory grade." This work paves the way for a future where scanning a routine histology slide and drawing a blood sample yield a more integrated "fingerprint" of a patient's cancer.

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 ([9] ascopubs.org) ([8] pmc.ncbi.nlm.nih.gov).
- Circulating tumor DNA is a powerful prognostic indicator of residual disease and recurrence risk in operable colon cancer ([4] pmc.ncbi.nlm.nih.gov) ([3] pmc.ncbi.nlm.nih.gov).
- The Mayo pilot study at ASCO 2026 combines these modalities, showing that AI-derived TME features are associated with ctDNA status and patient outcomes in a phase III trial cohort. 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 ([5] pmc.ncbi.nlm.nih.gov) ([9] ascopubs.org), and Mayo's own ctDNA study quantified the magnitude of its prognostic signal ([4] pmc.ncbi.nlm.nih.gov).
- Such a biomarker, if validated, has **regulatory implications**. The FDA already classifies digital pathology algorithms as medical devices ([13] www.accessdata.fda.gov) and has cleared ctDNA companion tests in other cancers ([14] www.fda.gov) ([29] www.fda.gov). A composite AI+ctDNA test would have to meet similar standards of rigor and reproducibility. The Mayo approach (retrospective within a controlled trial) aligns with recommended evidence gathering: prospective blinding, large sample size, and hard clinical endpoints.
- 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. Case studies (actual or hypothetical) illustrate how combined biomarker data could lead to better-informed choices for adjuvant treatment intensity.
- 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. As the final

ASCO 2026 meeting data become available, we anticipate rigorous peer review will further validate 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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