Gastrointestinal conventional carcinoma with neuroendocrine differentiation: From pathological phenomenon to clinical importance (Review)

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Abstract. Conventional carcinomas that lack neuroendocrine morphological features but display detectable expression of neuroendocrine markers through immunohistochemistry are termed conventional carcinomas with neuroendocrine differentiation (NED). Distinguishing these from mixed neuroendocrine non-neuroendocrine neoplasms and amphicrine-like carcinomas is important in accurate diagnosis and clinical management. The present review systematically outlines the advancements in research on gastrointestinal conventional carcinomas with NED, focusing on their characteristics, diagnostic criteria and clinical features. The present review further summarizes the epidemiological and clinical aspects across numerous organs such as the stomach and colorectum, emphasizing common mechanisms and prognostic variability. The future prospects of gastrointestinal conventional carcinomas with NED are also discussed, concentrating on key mechanisms, molecular subtypes and ongoing clinical trials to influence future research and clinical practices.

Contents

1. Introduction

Neuroendocrine differentiation (NED) is a term used in clinical pathology to describe a number of tumor types with distinct biological implications. According to the World Health Organisation (WHO) classification and recent research, tumors exhibiting NED can be categorized into neuroendocrine neoplasms (NENs), mixed neuroendocrine non-NENs (MiNEN), amphicrine-like carcinoma (ALC) and conventional carcinoma with NED (1,2). Differentiating these tumors poses numerous challenges. First, distinguishing between conventional carcinomas with NED and ALC is difficult. Second, there is notable variability in applying diagnostic criteria among observers. Third, the evolving WHO classification criteria adds further complexity. Current TNM staging systems utilize adenocarcinoma of the respective organ for staging (2), potentially underestimating the adverse biological behavior of conventional carcinomas with NED. Therefore, a comprehensive understanding of the biological

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features of conventional carcinomas with NED is key in developing precise personalized treatment. Gastrointestinal conventional carcinomas with NED are prevalent. The present review therefore aimed to elucidate the definition, diagnosis, clinical characteristics and pathogenesis of these carcinomas and discuss future directions from the perspectives of basic mechanisms, molecular subtyping and clinical translation.

2. Definition and diagnosis of MiNEN, ALC and conventional carcinoma with NED

Classification of tumors with neuroendocrine characteristics. MiNEN neoplasms are characterized by distinct nodules or regions comprising both neuroendocrine and non-neuroendocrine components. Each component constitutes at least 30% of the tumor volume (1). ALC is defined by two possible patterns. In one pattern, individual tumor cells demonstrate both exocrine and neuroendocrine features, exhibiting exocrine glandular morphology (such as mucin secretion) alongside NED traits. In the other pattern, neuroendocrine and non-neuroendocrine cells are intimately admixed and dispersed throughout the tumor (2). Conventional carcinoma with NED is defined as a conventional carcinoma that lacks neuroendocrine morphological characteristics, such as trabecular, gyriform, solid-nesting or solid-paraganglioma-like patterns, but exhibits expression of neuroendocrine markers, including CgA, Syn or insulinoma-associated protein 1 (INSM1), detectable by immunohistochemistry (IHC). The positive cells typically appear as scattered single cells or small clusters, accounting for >30% of tumor cells. However, this threshold has not reached universal consensus (1). This threshold is borrowed from the WHO diagnostic criteria for MiNEN, but its direct application to conventional carcinoma without neuroendocrine morphology remains debated. Diagnostic thresholds vary widely across studies (3-7), from 'any positive cell' to >2, >5, >10 and 30%, with some evidence suggesting that even minor neuroendocrine components (<30%) may carry prognostic significance. These discrepancies highlight the need for further validation of the optimal cutoff.

Evolution and application of neuroendocrine markers. NED is typically determined through immunohistochemical detection of specific biomarkers, with the standard combination being CgA and Syn. INSM1 has further emerged as a promising marker for diagnosing neuroendocrine tumors, known for its high specificity. Originally identified from the insulinoma glucagon spheroid depletion library, INSM1 is a zinc finger transcription factor located on 20p11.2 without introns (8). A study by McHugh *et al* (9) demonstrated that while INSM1 has lower sensitivity (80.9%) compared with Syn (99.1%), its specificity (95.7%) surpasses that of traditional biomarkers such as Syn (86.0%), CgA (87.3%) and CD56 (86.0%). Litmeyer *et al* (3) further highlighted the utility of INSM1 in distinguishing MiNEN, neuroendocrine carcinoma (NEC) and conventional colorectal carcinomas (CRCs), particularly in cases with diffuse Syn expression. Notably, INSM1 is typically absent or expressed at low levels in conventional CRCs, aiding in the differentiation from neuroendocrine tumors. Previous investigations regarding biomarkers such as CD56, neuron-specific enolase (NSE) and pro-gastrin-releasing peptide (proGRP) for neuroendocrine tumors have been conducted, but due to their lack of specificity, they are no longer recommended as histological diagnostic markers. However, NSE and proGRP do retain importance in serological testing (10,11).

Diagnostic algorithm. To aid in diagnostic workflow, a practical algorithm for distinguishing conventional carcinomas with NED from other NENs is presented in Fig. 1. The following rationale is provided for each node of diagnostic algorithm and the literature summarized in the present review: i) Node 1 (morphological assessment of neuroendocrine features), where the first step is to evaluate H&E sections for typical neuroendocrine architecture, such as nested, trabecular, insular or rosette-like growth patterns. If clear neuroendocrine morphology is present, the tumor may be a NEC, MiNEN or ALC. If such morphology is absent, the tumor is classified as a conventional carcinoma; however, NED can still be detected by IHC; ii) Node 2 (neuroendocrine marker expression), where IHC for at least two of the three validated markers (CgA, Syn and INSM1) is performed. According to the WHO classification, determination of NED requires positivity for at least two of these markers. This is because no single marker is perfectly sensitive and specific: Syn is highly sensitive but may be expressed in non-neuroendocrine tumors, CgA is specific but often lost in high-grade NENs and INSM1 combines good specificity with relatively high sensitivity; iii) Node 3 (presence of a non-neuroendocrine component), whereby if the tumor exhibits both neuroendocrine and non-neuroendocrine morphology, such as conventional adenocarcinoma or squamous cell carcinoma, it is not a pure NEN. The next distinction concerns whether the two components are spatially distinct, meaning they form separate nodules or regions or intimately admixed; iv) Node 4 (spatially distinct components, each representing at least 30% of the tumor), whereby when a tumor exhibits neuroendocrine morphology on H&E and contains both neuroendocrine and non-neuroendocrine components that form separate, recognizable areas and each accounts for at least 30% of the tumor volume, the diagnosis is MiNEN. If the neuroendocrine component comprises <30%, the tumor is classified as conventional carcinoma with a minor neuroendocrine component. Conversely, if the non-neuroendocrine component comprises <30%, the tumor is classified as NEN with a minor non-neuroendocrine component; v) Node 5 (intimate admixture with diffuse neuroendocrine marker expression involving at least 30% of cells), whereby if the tumor demonstrates neuroendocrine morphology on H&E and the neuroendocrine and non-neuroendocrine cells are intermingled throughout the tumor without separate regions and the proportion of neuroendocrine-positive cells reaches at least 30%, the tumor falls into the ALC category. In ALC, the dual differentiation is evenly distributed and the tumor is staged according to its non-neuroendocrine counterpart. The Ki-67 proliferative index should be reported for the whole neoplasm; and vi) Node 6 (neuroendocrine marker expression in scattered, isolated cells), the main focus of the present review: Conventional carcinoma with NED. Here, the tumor lacks neuroendocrine morphology on H&E. However, it exhibits widespread expression of neuroendocrine markers in scattered or isolated cells, with positive cells accounting for >30% of the tumor cell population. If the proportion of

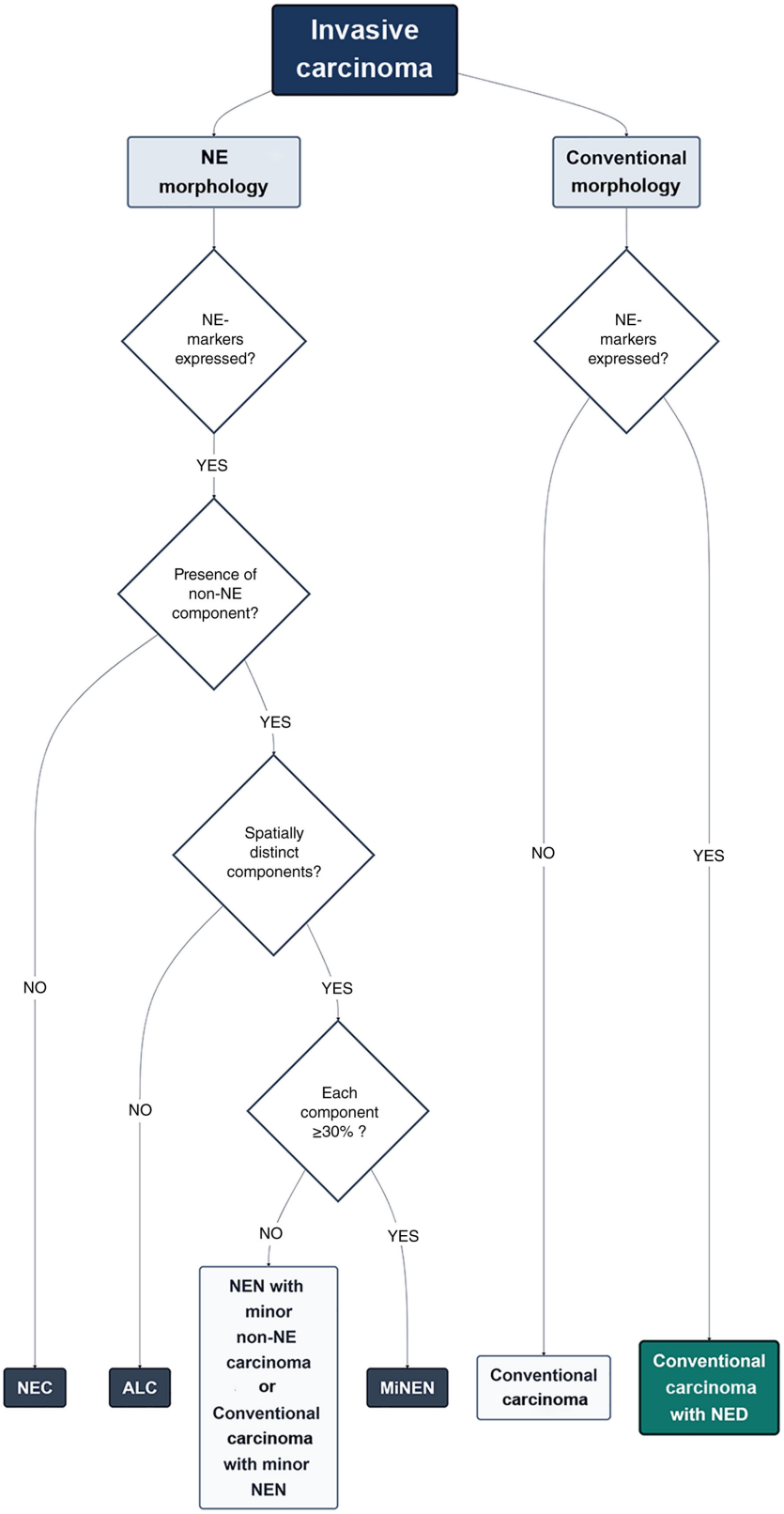

Figure 1. Diagnostic algorithm for distinguishing conventional carcinomas with NED from other NE neoplasms in the gastrointestinal tract. The diagnostic algorithm follows a stepwise approach based on World Health Organisation recommendations. First, the tumor is examined for NE morphology using H&E. If NE morphology is present, the case enters the NEN pathway. If absent, immunohistochemistry for CgA, Syn and INSM1 is performed and at least two of these three markers must be positive to determine NED. When a non-NE component coexists, the key discriminating factors are: i) Whether the components are spatially distinct and each constitutes at least 30% of the tumor, leading to a diagnosis of MiNEN; ii) whether NED is diffuse (≥30% of cells) but intimately admixed, resulting in a diagnosis of ALC; or iii) whether the tumor lacks NE morphology but shows NE markers expressed in scattered, isolated cells comprising >30% of the tumor, in which case the tumor is classified as conventional carcinoma with NED. If the proportion of positive cells is <30%, the tumor is classified as conventional carcinoma without NED. Tumors are categorized into six groups: NEC, ALC, NEN with non-NE carcinoma or conventional carcinoma with minor NEN, MiNEN, conventional carcinoma and conventional carcinoma with NED. NE markers include CgA, Syn and INSM1. NE, neuroendocrine; NED, NE differentiation; NEN, NE neoplasms; INSM1; insulinoma-associated protein 1; MiNEN, mixed NE-non-NE neoplasm; ALC, amphicrine-like carcinoma; NEC, NE carcinoma.

positive cells is <30%, the tumor is classified as conventional carcinoma without NED. Despite this, the cut-off has not yet reached universal consensus and further studies are needed to validate or refine the appropriate threshold.

Controversies and challenges surrounding diagnostic thresholds. Diagnoses of conventional carcinomas with NED presents challenges regarding inconsistent positive thresholds and differentiation from ALC. Currently, research utilizes a number of positive thresholds, ranging from 'any positive cell' to thresholds of '>2', '>5', '>10' and '30%' (7,12,13). This lack of uniformity hinders study comparisons and the establishment of standardized clinical guidelines. In addition, distinguishing between conventional carcinomas with NED and ALC is complex, as the latter necessitates determination of both exocrine and neuroendocrine characteristics, relying on the expertise and techniques of the pathologist, including electron microscopy, which are not routine in clinical practice. The clinical and pathological distinctions of ALC and its prognostic variances from conventional carcinoma with NED will be further discussed in subsequent sections regarding clinical features and cross-organ mechanisms.

3. Prognostic importance of conventional carcinomas with NED in organs of the digestive tract

Notable variations exist in the prevalence of conventional carcinomas with NED across different organs of the digestive tract, reflecting the diverse tumor biology among these organs. Discrepancies in detection rates can be attributed to variations in diagnostic criteria, positivity thresholds and study populations. Research has primarily focused on the stomach and colorectum, leaving other areas underexplored. Furthermore, divergent findings regarding the prognostic relevance of NED exist, with certain studies indicating independent prognostic value while others suggest collinearity with established high-risk factors. Table I consolidates epidemiological and prognostic data from key studies spanning numerous digestive tract organs.

Stomach. Reported prevalence rates of NED in gastric cancer vary markedly, ranging from 22.3 to 75.0%, largely depending on the diagnostic threshold applied. Early studies (14-16) employing the 'any detectable positive cell' criterion reported positivity rates as high as 75%. By contrast, studies adopting a threshold of ≥1 or 5% tumor cells reported rates of ~20-30% (5,13). NED-positive cells were found in diffuse-type and poorly differentiated adenocarcinomas. NED has been associated with deeper tumor invasion and perineural invasion. Evidence supporting the prognostic significance of NED in gastric cancer is primarily subgroup-specific. Li *et al* (13) identified an association between NED and unfavorable survival trends in well-differentiated gastric cancer. Similarly, Xu *et al* (5) reported that patients with NED and Lauren diffuse-type or stage II gastric cancer exhibited lower cumulative survival rates. However, the independent prognostic value of NED in the overall gastric cancer cohort remains uncertain and no consensus has been reached.

Colon and rectum. NED is more commonly observed in right-sided and mucinous adenocarcinomas of colon and rectum. The positive cells are usually scattered singly or in small foci. The prognostic importance of NED in colorectal cancer has been the subject of notable debate, with studies reporting conflicting findings. A number of large-scale studies have demonstrated that NED serves as an independent adverse prognostic factor, particularly in stage II colorectal cancer (4,7,17). Furthermore, two retrospective studies evaluated the prognostic value of NED in stage II colorectal cancer. Liu *et al* (18) analyzed 151 stage II patients and reported a NED incidence of 34.44% (51/151). The 5-year overall survival rate was 68% for NED-positive patients compared with 90% for NED-negative patients (P=0.001), with NED positivity identified as an independent prognostic factor (P=0.014) alongside age ≥65 years (P=0.007). Liang *et al* (4) examined 3,441 patients with stage II/III colorectal cancer and found that NED-positive stage II patients exhibited a significantly poorer prognosis (P=0.001), with NED independently predicting worse outcome (P=0.002) along with tumor differentiation (P=0.018) and nerve invasion (P<0.001). Notably, the prognosis of NED-positive stage II patients was comparable with that of patients with traditional high-risk factors (P=0.639). However, the limitations of retrospective studies should be acknowledged. It should be noted that the presence of NED does not alter the extent of surgical resection, lymph node dissection or the urgency of surgery. The decision to offer adjuvant chemotherapy in stage II NED-positive colorectal cancer might be made on a case-by-case basis within a multidisciplinary team, considering other conventional high-risk factors, including T4 disease, poor histological differentiation, lymphovascular invasion, perineural invasion, intestinal obstruction, localized perforation, indeterminate or positive resection margins, and fewer than 12 lymph nodes examined.

In addition, among BRAF V600E-mutant tumors, Fassan *et al* (6) showed that Syn positivity was associated with shorter OS (HR=2.27; 95% CI: 1.35-3.85; P=0.001) and PFS (HR=2.00; 95% CI: 1.21-3.33; P=0.006), suggesting that the prognostic impact of NED may be further modified by molecular background. Conflicting results have also been reported. Chen *et al* (19) performed multivariate Cox analysis in poorly differentiated colorectal cancer and found that only lymph node metastasis retained independent prognostic importance, while the effect of NED was closely associated with lymph node metastasis. Cho *et al* (20) observed no association between NED and disease-free survival in lymph node-negative patients. Furthermore, two large cohort studies reported no notable association between the expression of neuroendocrine markers and prognosis in conventional cancers after rigorous exclusion of MiNEN and NEC cases (3,21).

Esophagus, small intestine, appendix and anal canal. Data regarding conventional carcinomas with NED in the esophagus, small intestine, appendix and anal canal are limited. For esophageal and anal canal carcinomas, only specific case reports are available (22-24). Similarly, large-scale studies are lacking for the small intestine and appendix. Consequently, the clinical importance of NED in these sites remains to be elucidated.

Table I. Key research data on conventional carcinomas with NED in organs of the digestive tract.

A, Stomach

First author, year	Study conclusion	Sample size	NED positivity rate	Diagnostic criteria (biomarkers/thresholds)	Key findings	(Refs.)
Li *et al*, 2026	Support (subgroup)	309	22.3%	CgA, Syn and CD56 (1%)	Patients with GC and NED had a higher incidence of PNI compared with those without NED (29.0 vs. 15.8%). No significant difference in OS or RFS was observed overall, but a poorer survival trend was noted in the differentiated GC subgroup with NED (P=0.015)	(13)
Xu *et al*, 2016	Support (subgroup)	240	29.1%	CgA, Syn and SCGN (5%)	Syn positivity was associated with deeper tumor invasion, whereas SCGN positivity was associated with lymph node metastasis. Patients with NED and diffuse or stage II GC exhibited lower cumulative survival rates (P=0.006)	(5)
Blumenfeld *et al*, 1996	Descriptive research	48	75.0%	CgA, Syn, NSE, gastrin and serotonin (any positive result)	No prognostic data reported	(14)

B, Colon and rectum

First author, year	Study conclusion	Sample size	NED positivity rate	Diagnostic criteria (biomarkers/thresholds)	Key findings	(Refs.)
Syversen *et al*, 1995	Support	91	40.0%	NSE and CgA (any positive result)	CRC patients with NED exhibited a decreasing trend in median OS compared with those without NED (P=0.100)	(15)
Grabowski *et al*, 2001	Support	116	17.2%	CgA and Syn (2%)	No observed association between neuroendocrine differentiation and clinical pathological parameters. The median OS was 18.6 months in patients with NED compared with 48.9 months in those without NED (P<0.001)	(12)
Atasoy *et al*, 2003	Support	50	38.0%	CgA, Syn and NSE (any positive result)	Among the three markers, only CgA was positivity associated with tumor grade and stage and it was an independent adverse prognostic factor (P<0.05)	(25)
Liu *et al*, 2014	Support	171	41.5%	Syn and CgA (any positive result)	NED was more common in women, the 5-year OS rate was 68% in patients with NED compared with 90% in those without NED (P=0.018). The median OS was 38.6 months in patients with NED vs. 53.2 months in those without NED	(16)

Table I. Continued.

B, Colon and rectum

First author, year	Study conclusion	Sample size	NED positivity rate	Diagnostic criteria (biomarkers/thresholds)	Key findings	(Refs.)
Guo *et al*, 2020	Support	94,291	Not reported	Not reported	The presence of NED was significantly associated with lymph node metastasis. In multivariable and propensity score analyses, NED remained an independent adverse prognostic factor, with a more pronounced effect observed in lymph node-positive patients	(17)
Fassan *et al*, 2021	Support	159	22.0%	Syn (20%)	Metastatic lesions showed a higher rate of Syn positivity compared with primary lesions. Syn positivity was independently associated with shorter OS and PFS. The Syn-positive group had a poorer treatment response compared with the Syn-negative group (66.7 vs. 36.8%)	(6)
Liang *et al*, 2025	Support	3,441	8.3% (total population) and 5.5% (phase II)	CgA and Syn (2%)	Patients with NED had a worse prognosis and were identified as an independent risk factor (P=0.002). In stage II patients, NED positivity was equally important as traditional high-risk factors	(4)
Sabella *et al*, 2025	Support	663	4.1%	Syn (30%)	Syn positivity was associated with right colon, G2 grade, mild TILs and BRAF mutations. The presence of Syn expression in ≥30% of tumor cells forming glands independently predicted poor OS and DFS	(7)
Chen *et al*, 2017	Opposition	70	51.4%	CgA and Syn (any positive result)	In multivariate analysis, only lymph node metastasis was identified as an independent prognostic factor (P<0.001). NED positivity was associated with lymph node metastasis (P=0.006)	(19)
Cho *et al*, 2010	Opposition	89	30.3% (CgA) and 77.5% (Syn)	CgA and Syn (2%)	No significant association was found between NED and any clinicopathological parameters except preoperative CEA. NED positivity was not associated with DFS	(20)
Konukiewitz *et al*, 2021	Opposition	1,002	23.8%	Syn (1%)	Syn positivity was associated with lymph node metastasis, UICC stage and lymphatic infiltration, but not with prognosis. Only MiNEN and NEC were associated with poor survival	(21)

Table I. Continued.

B, Colon and rectum						
First author, year	Study conclusion	Sample size	NED positivity rate	Diagnostic criteria (biomarkers/thresholds)	Key findings	(Refs.)
Litmeyer *et al*, 2023	Opposition	1,033	14% (INSM1), 24% (Syn) and 11% (CgA)	Syn, INSM1 and CgA (any positive result)	The presence of NED showed no association with prognosis	(3)

NED, neuroendocrine differentiation; GC, gastric cancer; CRC, colorectal cancer; OS, overall survival; RFS, recurrence free survival; PFS, progression free survival; DFS, disease free survival; SCGN, secretagogin; PNI, perineural invasion; TILs, tumor infiltrating lymphocytes; MiNEN, mixed neuroendocrine non-neuroendocrine neoplasms; NEC, neuroendocrine carcinoma; INSM1, insulinoma-associated protein 1.

Sources of prognostic divergence. A number of factors contribute to the divergent prognostic findings reported in the literature. These can be broadly categorized into differences in diagnostic criteria, heterogeneity in study populations and variability in study design.

First, diagnostic criteria vary markedly across studies. Both the choice of neuroendocrine markers and the positivity thresholds differ. Regarding marker selection, early studies often employed numerous markers, including CgA, Syn, NSE and GRP (14,15,25), whereas more recent studies have favored Syn or INSM1 based on their higher specificity (3,7,21). The sensitivity-specificity trade-off between markers means that studies using different marker combinations may capture distinct patient populations. Regarding positivity thresholds, the reported thresholds range from 'any detectable positive cell' to ≥30% (7,13,16,19,26). Studies using lower thresholds may inadvertently include 'background noise', potentially diluting the true prognostic effect of NED. Conversely, studies using higher thresholds may select cases that more closely resemble MiNEN, thereby overestimating the adverse impact of NED.

Second, heterogeneity in study populations contributes to conflicting findings. Key differences include disease stage, histological subtype, molecular background and treatment exposure. In colorectal cancer, NED consistently predicts poor outcome in stage II disease (4,16), but its independent prognostic value in stage III-IV disease remains uncertain, with some studies showing an effect (12,17) and others suggesting collinearity with lymph node metastasis (19,21). In lymph node-negative patients, NED has shown no association with disease-free survival (20). Regarding histological subtype, studies focusing on poorly differentiated cancers report higher NED prevalence (13,19,25), but the poor prognosis of these cancers may confound the effect of NED. Regarding molecular background, Fassan *et al* (6) demonstrated that Syn positivity predicts worse survival specifically in the BRAF V600E-mutant subgroup. suggesting that the prognostic impact of NED may be restricted to certain molecular contexts. Finally, treatment exposure is also important: Studies incorporating patients who received neoadjuvant therapy report higher NED rates and stronger associations with poor survival (27,28), whereas those limited to treatment-naïve surgical cohorts may underestimate the prognostic impact.

Third, study design and statistical power further influence results. Small single-center studies (25) may lack statistical power to detect modest effects, whereas large database analyses (17) provide greater precision but may lack detailed pathological data, including the specific diagnostic thresholds used. A previous meta-analysis attempted to synthesize available evidence (29), but heterogeneity in inclusion criteria limits the generalizability of their conclusions.

Fourth, diagnostic confusion with other neuroendocrine entities further complicates interpretation. On the one hand, when conventional carcinomas with NED are not rigorously distinguished from MiNEN, the poor prognosis associated with MiNEN may be erroneously attributed to NED. A number of studies (3,21,30) have emphasized that only MiNEN and NEC are associated with poor survival, whereas the expression of neuroendocrine markers in conventional carcinomas does not independently impact survival (3). On the other hand, the rare entity of ALC adds another layer of complexity. Although large-scale survival data for this entity are lacking, available case series suggest a grade-dependent prognosis: High-grade tumors exhibit shorter survival times and higher metastatic rates, whereas low-grade tumors demonstrate improved outcomes (31-33). The exclusion of ALC from the majority of analyses further contributes to the uncertainty surrounding the prognostic role of NED in conventional carcinomas.

Collectively, the divergent prognostic findings reflect not a single issue but a confluence of factors, including differences in diagnostic criteria, heterogeneity in study populations, variability in study design and diagnostic confusion with related entities. While numerous large-sample studies support the independent prognostic value of NED in stage II colorectal cancer (4,7,17), the effect in other settings remains contested and its importance may be confounded by the aforementioned factors.

4. Treatment response characteristics of conventional carcinomas with NED

Chemosensitivity. Bozkaya *et al* (34) conducted a comparative analysis of the response to the modified docetaxel, cisplatin and fluorouracil regimen in 35 cases of gastric adenocarcinoma with NED and 356 cases without NED. The objective response rates were 50.0 and 41.0% for the two

groups, respectively. The median progression-free survival durations were 7.6 and 7.5 months, respectively, indicating no significant difference. These findings suggest that conventional chemotherapy remains effective for gastric adenocarcinoma with NED.

Changes in therapeutic efficacy of anti-angiogenic therapy. Dost Gunay *et al* (35) analyzed 123 cases of advanced colorectal cancer and found that the efficacy of bevacizumab combined with chemotherapy may be diminished in patients with NED, an effect potentially attributable to increased infiltration of tumor-associated macrophages (TAMs). This finding suggests that the presence of NED may be associated with altered responsiveness to anti-angiogenic therapy. Notably, a previous study demonstrated that gastric cancer with NED remains responsive to conventional chemotherapy (26), though whether this holds true for colorectal cancer with NED requires further investigation.

Treatment-induced NED. Enhanced expression of neuroendocrine markers following neoadjuvant therapy, particularly in colorectal cancer and gastroesophageal adenocarcinoma, has been observed (27,28). A study by Dodington *et al* (28) involving 218 samples of gastric and esophageal adenocarcinoma resection post-neoadjuvant therapy revealed NED in 59% of residual tumors. Patients with NED exhibited a significantly shorter median OS compared with those without (22.5 vs. 48.8 months). NED has been identified as an independent prognostic risk factor. This observation prompts key clinical inquiries: Whether this phenotype arises from clonal selection due to treatment pressure, undetected pre-existing elements or therapy-induced lineage transformation. It is important to note that currently there are no reliable preoperative imaging features or serum biomarkers (including CgA or proGRP) that can predict the presence of NED before surgery. Thus, NED status can only be determined postoperatively on resected specimens or biopsies. Surgeons should not alter their preoperative planning based on suspicion of NED, as it cannot be reliably diagnosed prior to pathological examination. Further investigation is therefore warranted.

5. Integration of commonalities and mechanisms across organs

Common origin: Conventional carcinomas with NED arise from differentiation plasticity rather than independent clonal evolution. Zhang *et al* (36) and Wang *et al* (37) conducted clonal analyses in colorectal and gastric cancers, respectively. Their findings indicated that scattered NED cells shared consistent genetic alterations with adjacent adenocarcinoma cells, suggesting a common clonal origin. By contrast to MiNEN, where neuroendocrine components exhibit additional genetic changes (38,39), the genetic profiles of NED cells and adjacent adenocarcinoma cells in conventional carcinomas are more homogeneous.

Lineage tracing studies have provided insights into the cellular origin of neuroendocrine cells in colorectal neoplasia (40,41). Humphries *et al* (41) used mitochondrial DNA mutation analysis and immunofluorescence staining to study human colorectal adenomas. This study established that pluripotent stem cells within a single adenoma crypt can give rise to both mucinous secretory cells and neuroendocrine cells. This finding suggests that, at the premalignant stage, neuroendocrine cells and epithelial cells may share a common stem cell origin (41). Whether this lineage association is maintained during malignant transformation remains to be elucidated, but it offers a plausible explanation for the shared clonal origin observed in conventional carcinomas with NED.

The discrepancy indicates that conventional carcinomas with NED primarily demonstrate the plasticity of tumor cell differentiation rather than distinct clonal evolution events. Factors within the tumor microenvironment, such as inflammation, hypoxia and therapeutic stress, may trigger the transient expression of neuroendocrine markers in certain adenocarcinoma cells without enduring genetic alterations. This mechanism of differentiation plasticity also offers a theoretical rationale for 'treatment-induced NED', where therapeutic interventions including chemotherapy and radiotherapy may prompt some adenocarcinoma cells to acquire neuroendocrine characteristics in response to microenvironmental cues.

Molecular regulatory mechanisms and tumor microenvironment remodeling of NED. Chen *et al* (42) proposed that colorectal cancer with NED releases neuroendocrine granules, which influence neighboring colorectal cancer cells through paracrine signaling, activating the PI3K/Akt pathway and thereby promoting tumor invasion and metastasis. Ladaika *et al* (43) elucidated an epigenetic regulatory mechanism underlying NED development in mucinous colorectal cancer, whereby lysine-specific demethylase 1 (LSD1) and REST corepressor 2 (CoREST2) facilitate STAT3 demethylation, enhancing its chromatin binding capacity and promoting intestinal endocrine cell differentiation (43,44). *In vivo* experiments demonstrated that CoREST2 knockdown reduces the growth and lung metastasis of transplanted tumors. This mechanism was identified specifically in mucinous adenocarcinoma. Whether similar or distinct pathways operate in other histological subtypes warrants further investigation.

In parallel with these intrinsic signaling pathways, accumulating evidence has implicated the tumor microenvironment in NED-associated progression. Zeng *et al* (45) and Zou *et al* (46) independently determined an association between NED and increased TAMs infiltration, fostering an immunosuppressive tumor microenvironment in colorectal cancer and gastric cancer, respectively. Zeng *et al* (45) showed that neuroendocrine-like cells recruit TAMs through C-X-C motif chemokine ligand (CXCL)-10 and CXCL11 chemokines, a finding supported by experiments overexpressing CgA and Syn in colon cancer cell lines. Li *et al* (47) demonstrated that colon cancer cells with NED overexpressing CgA transmit lnc-HOXB8-1:2 through exosomes, acting as a competing endogenous RNA to sequester hsa-miR-6825-5p, upregulate C-X-C motif chemokine receptor 3 (CXCR3) expression, induce M2 polarization and TAMs infiltration, thus forming a feedback loop that promotes tumor progression.

Zou *et al* (46) conducted a comprehensive analysis of the immune microenvironment in gastric cancer with NED. This study observed that gastric cancer with NED exhibits more pronounced immunosuppressive characteristics compared

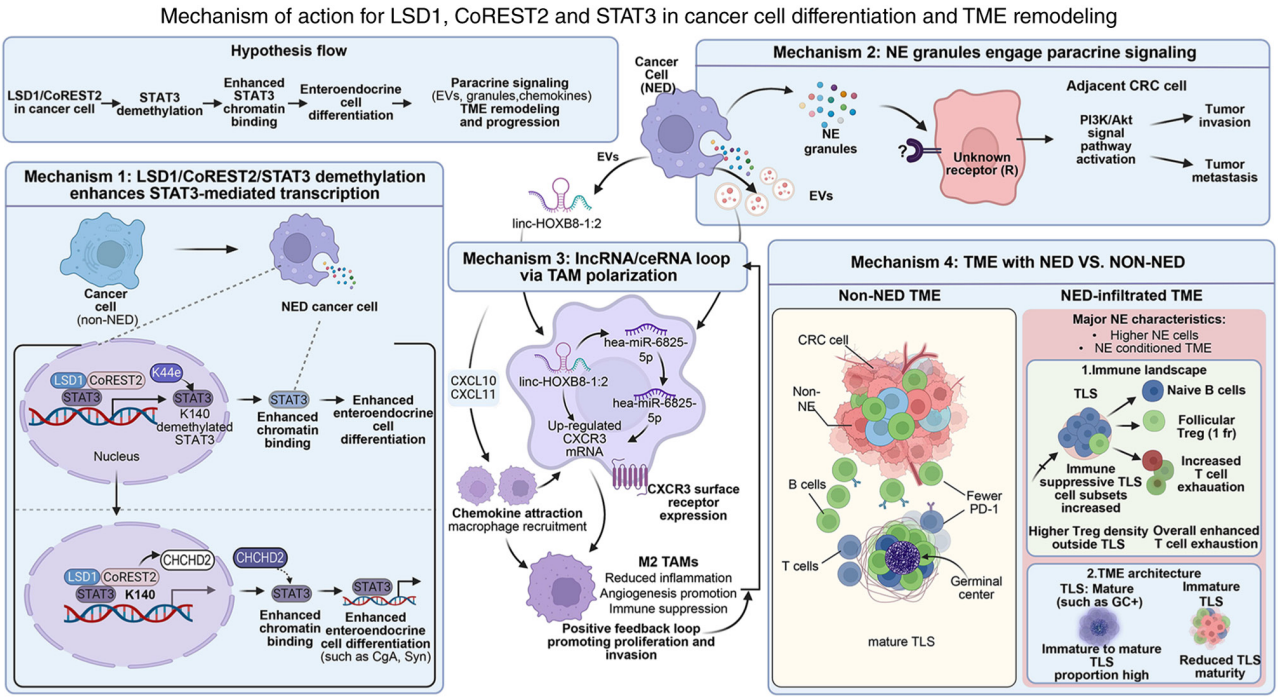

Figure 2. Molecular mechanisms and TME regulation in conventional carcinoma with NED. The LSD1/CoREST2 complex mediates STAT3 demethylation, which induces NED in cancer cells. These differentiated cells promote invasion and metastasis by releasing NE granules that activate the PI3K/Akt pathway in adjacent cancer cells through paracrine signaling. Concurrently, cells recruit macrophages through CXCL10 and CXCL11 and deliver exosomal lnc-HOXB8-1:2, which acts as a ceRNA for miR-6825-5p, upregulates CXCR3 and induces M2 polarization of macrophages, thereby establishing a pro-tumorigenic positive feedback loop. Furthermore, this differentiation markedly remodels the immune repertoire, characterized by increased but immature TLS, enrichment of naïve B cells and regulatory T cells and exacerbated T cell exhaustion, ultimately resulting in a highly immunosuppressive microenvironment. TME, tumor microenvironment; NE, neuroendocrine; NED, NE differentiation; EVs, extracellular vesicles; TLS, tertiary lymphoid structures; CRC, colorectal cancer; lnc/lncRNA, long non-coding RNA; miR, micro-RNA; LSD1, lysine-specific demethylase 1; CoREST2, REST corepressor 2; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C chemokine receptor; ceRNA, competing endogenous RNA; TAMS, tumor associated macrophages; CHCHD2, coiled-coil domain helix 2; Treg, regulatory T cell.

with gastric cancer without NED. Specifically, there is an increase in tertiary lymphoid structures (TLS) but with reduced maturity, higher densities of immature B cells and follicular regulatory T cells within TLS, elevated numbers of regulatory T cells outside TLS and a higher proportion of T cell exhaustion. These findings suggest that similar immune microenvironment alterations may occur in adenocarcinomas of the colon, pancreas, lungs and prostate. This indicates that NED may have a universal impact on tumor microenvironment interactions across different organs. This shared mechanism may contribute to the prognostic implications of conventional carcinomas with NED, as schematically summarized in Fig. 2.

6. Future directions and clinical translation

Unresolved core scientific questions. A number of key questions regarding conventional carcinomas with NED remain unanswered despite decades of research: i) What are the fundamental transcription factors and epigenetic mechanisms responsible for inducing neuroendocrine phenotypes in adenocarcinoma cells?; ii) how do microenvironmental cues such as hypoxia, inflammation and therapeutic stress trigger NED through intracellular signaling pathways?; iii) what is the molecular basis and clinical implications of the discordance in NED status between primary tumors and metastatic sites?; iv) are there distinct metabolic features associated with NED and can these be therapeutically targeted?; and v) how can one effectively differentiate between conventional carcinoma with NED and ALC and do their molecular distinctions support varied treatment approaches?.

Key directions for future research. Basic research should aim to focus on elucidating the molecular switches governing lineage plasticity. Systematic investigation of neuroendocrine lineage determinants such as achaete-scute Family BHLH transcription factor 1, neurogenic differentiation factor 1 and POU class 2 homeobox 3 in NED, using single-cell transcriptome sequencing and organoid models, will help clarify their roles. The LSD1/CoREST2/STAT3 axis represents a promising target and further exploration of DNA methylation, histone modification and chromatin remodeling in NED development is warranted.

Clinical translation should prioritize three areas. First, standardization of diagnostic criteria, including unified positivity thresholds and observer consistency, is important in enabling meaningful comparisons across studies. Second, prospective clinical trials are needed to validate the independent prognostic value of NED in stage II colorectal cancer and to determine whether the presence of NED should guide adjuvant therapy decisions. Third, given the association between NED and increased TAM infiltration, combination strategies targeting both angiogenesis and TAMs warrant investigation in patients with advanced colorectal cancer and NED. In addition, although prospective data are lacking, a cautious

approach may be to consider closer postoperative surveillance for NED-positive patients with stage II colorectal cancer or for those who develop NED after neoadjuvant therapy. Such surveillance could include more frequent imaging (for example, every 3-4 months for the first 2 years) and serial monitoring of serum tumor markers such as CgA or proGRP, recognizing that the clinical utility of these markers in this setting remains unproven.

7. Conclusions

Gastrointestinal conventional carcinomas with NED represent a distinct entity characterized by the absence of neuroendocrine morphology but detectable expression of neuroendocrine markers. Accumulating evidence has indicated that NED arises from differentiation plasticity rather than independent clonal evolution, with the PI3K/Akt and LSD1/CoREST2/STAT3 pathways serving as key regulatory axes. While NED holds independent prognostic importance in stage II colorectal cancer, its role in other settings remains controversial, largely due to heterogeneity in diagnostic criteria, study populations and diagnostic confusion with associated entities.

Clinical practice faces a number of challenges, including distinguishing NED from ALC, observer variability in diagnostic thresholds and evolving classification concepts. Addressing these issues requires standardized diagnostic guidelines, wider adoption of high-specificity markers such as INSM1 and prospective trials to validate the prognostic and therapeutic implications of NED. Ultimately, unifying diagnostic criteria will be key in transforming NED from a pathological observation into a clinically actionable biomarker, enabling a shift from mere identification to active intervention.

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Authors' contributions

TF conceived the idea of the present review. CZ, ST, DZ and WW prepared the figures and tables. CZ, ST, DZ, YP, JX, SL, WW and TF performed the literature search, wrote the manuscript and approved the submission of the article. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

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Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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