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EDITORIAL

Is *Helicobacter pylori* infection protective against esophageal cancer?

Rick Maity, Arkadeep Dhali, Jyotirmoy Biswas

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Abstract

Helicobacter pylori (*H. pylori*) infection affects a substantial proportion of the global population and causes various gastric disorders, including gastric cancer. Recent studies have found an inverse relationship between *H. pylori* infection and esophageal cancer (EC), suggesting a protective role against EC. This editorial focuses on the possible mechanisms underlying the role of *H. pylori* infection in EC and explores the role of gut microbiota in esophageal carcinogenesis and the practicality of *H. pylori* eradication. EC has two major subtypes: Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), which have different etiologies and risk factors. Gut microbiota can contribute to EC *via* inflammation-induced carcinogenesis, immunomodulation, lactagenesis, and genotoxin production. *H. pylori* infection is said to be inversely related to EAC, protecting against EAC by inducing atrophic gastritis, altering serum ghrelin levels, and triggering cancer cell apoptosis. Though *H. pylori* infection has no significant association with ESCC, COX-2-1195 polymorphisms and endogenous nitrosamine production can impact the risk of ESCC in *H. pylori*-infected individuals. There are concerns regarding a plausible increase in EC after *H. pylori*

eradication treatments. However, *H. pylori* eradication is not associated with an increased risk of EC, making it safe from an EC perspective.

Key Words: *Helicobacter pylori*; *Helicobacter pylori* infection; Esophageal cancer; Esophageal squamous cell carcinoma; Esophageal adenocarcinoma; Barrett's esophagus; Microbiota; Dysbiosis; Eradication

Core Tip: *Helicobacter pylori* (*H. pylori*) infection, while being a risk factor for gastric cancer, may afford protection against esophageal cancer (EC). The two major subtypes of EC, *i.e.*, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), have different etiologies and risk factors. Recent studies have unequivocally established the inverse association between *H. pylori* infection and EAC, however there was no significant association with ESCC. *H. pylori* infection may protect against EAC by inducing atrophic gastritis, altering serum ghrelin levels, and triggering cancer cell apoptosis. Contrary to prevailing concerns, *H. pylori* eradication does not increase the risk of EC.

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INTRODUCTION

Helicobacter pylori (*H. pylori*), a Gram-negative anaerobic bacterium that colonizes the stomach, affects nearly 43.9% of adults and 35.1% of children and adolescents globally[1]. Besides causing various gastric disorders such as peptic ulcer, dyspepsia, and gastritis, it is a well-established etiological and risk factor for gastric cancer[2,3]. Eradication of *H. pylori* infection reduces the risk of gastric cancer in infected individuals[2]. However, startling new evidence has come to light suggesting that *H. pylori* infection might have a protective role against esophageal cancer (EC)[2,4]. In a recent issue of the *World Journal of Gastroenterology*, we read with interest an article that investigated the prevalence of *H. pylori* infection in a retrospective cohort of EC patients from a tertiary-care hospital in Spain[5]. The study findings are consistent with recent systematic reviews suggesting an inverse relationship between *H. pylori* infection and the development of EC[4,5]. This editorial reviews the current demographics, etiologies, and risk factors associated with EC, as well as the available evidence and possible mechanisms underlying the role of *H. pylori* infection in EC. It also discusses the role of gut microbiota in esophageal carcinogenesis and feasibility of *H. pylori* eradication in light of the bacterium's inverse relationship with EC.

DEMOGRAPHICS, ETIOLOGIES, AND RISK FACTORS OF EC

EC is the seventh most common cancer and sixth-largest cause of cancer-related mortalities in the world[6]. It has various subtypes: Squamous cell carcinoma (SCC), adenocarcinoma (AC), sarcoma, small cell carcinoma, and rare varieties such as lymphomas and melanomas[7]. The two major subtypes, SCC and AC, make up the vast majority of EC cases; SCC accounts for around 85% of cases, whereas AC accounts for 14%[8]. EC is more common in men, with incidence and mortality rates two- to three-times greater than in women[7,8].

The etiologies and risk factors of EC slightly vary across the two main subtypes, although the mechanisms underlying this variation have not yet been fully determined[4]. Smoking is an established risk factor for both esophageal SCC (ESCC) and AC, whereas alcohol consumption is associated only with ESCC[4,9,10]. Obesity, particularly central obesity, can lead to gastroesophageal reflux disease, which causes esophageal AC (EAC) either directly or *via* a pre-cancerous lesion known as Barrett's esophagus[9,10]. A low intake of fruits and vegetables is associated with an increased susceptibility to EC, possibly due to the deficiency of vitamins and minerals[4,9]. Table 1 summarizes the risk factors for ESCC and EAC.

ROLE OF GUT MICROBIOTA IN ESOPHAGEAL CARCINOGENESIS

In addition to the above-mentioned risk factors, gut microbiota has been discovered to play a key role in esophageal carcinogenesis[4,11,12]. The term "gut microbiota" primarily refers to the microorganisms (mostly bacteria) that live in the human digestive system, mostly encompassing the esophageal, oral, and intestinal microbiota[12]. The esophageal microbiome can be classified into two subtypes: Type I microbiota (comprising mainly Gram-positive bacteria like *Streptococcus*) and Type II microbiota (mainly Gram-negative bacteria prevalent in dysbiotic states). The healthy

Table 1 Risk factors of esophageal squamous cell carcinoma and adenocarcinoma

Squamous cell carcinoma	Adenocarcinoma
Male gender	Male gender
Alcohol	Tobacco smoking
Tobacco smoking	Obesity (BMI > 25 kg/m^2)
HPV infection	Barrett's esophagus
Low intake of fruits and vegetables	GERD
Consumption of hot beverages, pickled vegetables, processed and red meat	Low intake of fruits and vegetables
	Consumption of processed and red meat
Low socioeconomic status	High socioeconomic status
Genetic factors: Howel-Evans syndrome, Fanconi anemia, Bloom syndrome	Genetic factors: Familial Barrett's esophagus

BMI: Body mass index; GERD: Gastroesophageal reflux disease; HPV: Human papillomavirus.

esophageal microbiome is primarily constituted by microorganisms belonging to six phyla (Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, and TM7), with *Streptococcus* as the dominant genus[13]. These microbiota achieve symbiosis with the immune system and carry out various physiological functions such as metabolism and immune maturation[12]. Any alterations in the gut microbiota can lead to the development of esophageal diseases, including EC.

Esophageal carcinogenesis can be induced by the gut microbiota *via* the following mechanisms: (1) Inflammation-induced carcinogenesis: Studies have shown that diet-induced alterations in the gut microbiota lead to increased levels of pro-inflammatory cytokines and immune cells[14]. Dysbalance between the gut microflora and immune system can disrupt the local microenvironment homeostasis and eventually lead to chronic inflammation. Persistent release of pro-inflammatory cytokines can activate toll-like receptors (TLR) and nucleotide-binding oligomeric domain-like receptors, triggering tumorigenesis[12]; (2) Immunomodulation: A taxonomic shift towards Type II microbiota has been observed in patients with gastroesophageal reflux disease (GERD) and BE. Lipopolysaccharide-producing Gram-negative bacteria can cause inducible nitric oxide synthase to be overexpressed, which impairs lower esophageal sphincter relaxation and increases intra-gastric pressure, thus predisposing to GERD, a risk factor for EC. Additionally, lipopolysaccharides can bind to TLR 4, leading to nuclear factor-kappa B activation and expression of cyclooxygenase-2 (COX-2), which blocks apoptosis and induces tumor cell proliferation and angiogenesis[14]. *Fusobacterium nucleatum* causes aberrant activation of the Wnt/β-catenin pathway, causing an increased production of chemokines that contribute to carcinogenesis and therapeutic resistance in EC[12,14]; (3) Lactagenesis: Studies have found that lactate-producing bacteria, such as *Staphylococcus* and *Lactobacillus*, are abundant in patients of GERD, BE, and EAC[14]. According to the Warburg effect, cancer cells are characterized by accelerated glycolysis and excessive lactate formation even under aerobic conditions. Lactate is thought to play a crucial role in all steps of carcinogenesis, *i.e.*, angiogenesis, immune escape, cell migration, metastasis, and self-sufficiency of cancer cells; therefore, the goal of the Warburg effect is now believed to be the augmented production of lactate (also known as lactagenesis)[15]. By converting glucose into lactate, the lactate-producing bacteria support the survival and proliferation of cancer cells[4]. Given that lactate-producing bacteria are significantly increased in EAC, the microbial contribution to lactagenesis and its effect on esophageal cells need to be explored[14]; and (4) Genotoxin production: Certain pathogens are capable of producing compounds called genotoxins, which damage the host DNA, causing cell death, oncogene activation, or downregulation of tumor suppressor genes[16]. A variety of Gram-negative bacteria (such as *Escherichia coli*, *Campylobacter*, and *H. pylori*) can produce cytolethal distending toxin, which damages structural DNA and stimulates carcinogenesis[17]. Colibactin (produced by certain members of Enterobacteriaceae) and nitrosamines are genotoxins that cause DNA damage by alkylation[17,18]. *H. pylori* produces a toxin named cytotoxin-associated gene A (cagA) that promotes production of reactive oxygen species and causes oxidative DNA damage. While cagA-positive strains of *H. pylori* have been implicated in gastric cancer, their role in EC remains unknown[17].

H. PYLORI: FRIEND OR FOE IN EC?

Till date, six meta-analyses have investigated the association between *H. pylori* infection and EC, and they have all indicated a negative correlation. While the meta-analyses have emphatically confirmed the inverse association of *H. pylori* infection with EAC, no significant association could be found with ESCC[19-24]. But significant regional variances have been observed, with certain regions (such as Asia and the Middle East) showing an inverse association and others displaying a positive association with *H. pylori* infection, especially with cagA-positive strains; these regional variances may be attributed to dietary cultures and lifestyles that differ from one region to another[19-21]. Thus, *H. pylori*'s association with ESCC is not clear and needs to be explored by further population-based studies. While analyzing the

Table 2 Summary of meta-analyses regarding the association between *Helicobacter pylori* infection and esophageal cancer

Authors	Year of publication	Number of studies	Association of *H. pylori* infection with ESCC	Association of *H. pylori* infection with EAC
Gao *et al*[19]	2019	35	No significant association in the general population: OR 0.84 (95%CI: 0.64-1.09)/OR 0.74 (95%CI: 0.54-0.97); Inverse relationship in the Middle Eastern population: OR: 0.34 (95%CI: 0.22-0.52 or 0.26-0.44); Positive association with the North American population: OR: 1.83 (95%CI: 1.17-2.87)	Inverse relationship: OR 0.55 (95%CI: 0.43-0.70)/OR 0.23 (95%CI: 0.15-0.36)
Nie *et al*[20]	2014	28	No significant association with the general population: OR 1.16 (95%CI: 0.83-1.60); Inverse association with Asian population: OR 0.74 (95%CI: 0.57–0.97); Positive association with non-Asian population: OR 1.41 (95%CI: 1.02–1.94)	Inverse relationship: OR 0.57 (95%CI: 0.44-0.73)
Xie *et al*[21]	2013	27	No significant association in the general population: OR 0.83 (95%CI: 0.63-1.03); Inverse relationship with the East Asian population: OR 0.66 (95%CI: 0.43-0.89)	Inverse relationship: OR 0.59 (95%CI: 0.51-0.68)
Islami and Kamangar [22]	2008	19	No significant association: OR 1.10 (95%CI: 0.78-1.55)	Inverse relationship: OR 0.56 (95%CI: 0.46-0.68)
Zhuo *et al*[23]	2008	195	No significant association: OR 0.80 (95%CI: 0.45-1.43), $Z = 0.75$, $P > 0.05$	Inverse relationship: OR 0.58 (95%CI: 0.48-0.70), $Z = 5.79$, $P < 0.01$
Rokkas *et al* [24]	2007	72	No significant association: OR 0.85 (95%CI: 0.55-1.33), $P = 0.48$	Inverse relationship: OR 0.52 (95%CI: 0.37-0.73), $P < 0.001$

H. pylori: Helicobacter pylori; ESCC: Esophageal squamous cell carcinoma; EAC: Esophageal adenocarcinoma; OR: Odds ratio.

association between *H. pylori* infection and the subtypes of EC, it would be prudent to consider the limitations of the meta-analyses, which include heterogeneity among study populations, confounding bias, and varying diagnostic criteria. The relevant information from all the meta-analyses has been summarized in Table 2.

The apparent protective role played by *H. pylori* in EAC can be explained by the following mechanisms: (1) Development of atrophic gastritis: *H. pylori* infection can cause atrophic gastritis; this lowers gastric acid secretion, reduces gastroesophageal reflux, and is postulated to protect against GERD, a risk factor for EAC[2,4]. Successful eradication of *H. pylori* is associated with a higher risk of GERD, especially in Asians[25]; (2) Alteration of plasma ghrelin: *H. pylori* may influence serum ghrelin levels, which is a key regulator of obesity and is known to stimulate cancer development and progression. *H. pylori* eradication is postulated to increase serum ghrelin levels, which stimulates adipogenesis and inhibits lipolysis, resulting in obesity[20,26]. By impacting the functioning of the lower esophageal sphincter, obesity predisposes to GERD, which is a risk factor for both BE and EAC[20]; and (3) Induction of cancer cell apoptosis: *In vitro*, *H. pylori* has been shown to preferentially trigger apoptosis in Barrett's-derived EAC cells over normal esophageal cells. By increasing Fas protein expression in tumor cells, *H. pylori* activates the *Fas*-caspase pathway, which leads to apoptosis by causing fragmentation of cellular DNA[27]. Table 3 summarizes the above-mentioned protective mechanisms.

In ESCC, the role of *H. pylori* infection is not entirely clear. The overexpression of COX-2 can influence the inverse association between *H. pylori* infection and ESCC. COX-2-1195G/A, a single nucleotide polymorphism, can not only modify the transcription of COX-2 but also the risk of developing ESCC. The inverse association between *H. pylori* infection and ESCC (especially in the lower third of the esophagus) is enhanced in patients carrying the COX-2-1195AA homozygous genotype[28]. On the other hand, *H. pylori*-induced atrophic gastritis and the ensuing decrease in gastric acidity may favor the proliferation of bacteria that produce nitrosamines, a known genotoxin. Gastric nitrosamines can come in contact with the esophageal mucosa and get converted into carcinogenic compounds by cytochrome P450. Thus, endogenous nitrosamines produced as a secondary effect of *H. pylori* infection may be implicated in ESCC[18]. Further studies are required to establish the roles of COX-2-1195 polymorphisms, atrophic gastritis, and endogenous nitrosamines in the pathogenesis of ESCC. The mechanisms underlying the protective role of *H. pylori* infection against EAC have been summarized in Table 3.

FEASIBILITY OF *H. PYLORI* ERADICATION

Since the dawn of humanity, *H. pylori* has coexisted with us and was previously commonly prevalent in human stomachs. With the advent of antibiotics and improved sanitation, this bacterium is fast disappearing from human populations, especially in Western nations[22].

H. pylori infection can lead to peptic ulcers, which is the main indication for eradication treatment[29]. Eradication of *H. pylori*, besides healing chronic active gastritis and peptic ulcer disease, is an effective strategy for preventing gastric cancer[2]. It comprises a regimen of antibiotics and proton pump inhibitors and has been shown to reduce the risk of developing gastric cancer by nearly 50%[2,30].

Table 3 Mechanisms underlying the protective role of *Helicobacter pylori* infection against esophageal adenocarcinoma

Mechanism	Description	Implications
Development of atrophic gastritis	The inflammatory processes in chronic *H. pylori* infection can cause gastric atrophy by loss of gastric glands and partial replacement by intestinal epithelium. This reduces the number of parietal cells which secrete hydrochloric acid, the main constituent of gastric acid	Lower gastric acidity reduces the risk of GERD and BE, risk factors for EAC
Alteration of plasma ghrelin levels	*H. pylori*-induced gastric atrophy leads to reduced gastric ghrelin production, subsequently decreasing plasma ghrelin levels. Contrastingly, eradication of *H. pylori* increases ghrelin levels, thus leading to obesity. Thus, *H. pylori* infection is inversely related to obesity	Ghrelin is a key regulator of obesity and has been implicated in the pathogenesis and differentiation of esophageal cancers. Obesity can predispose individuals to GERD, which is a risk factor for both BE and EAC
Induction of cancer cell apoptosis	*In vitro*, *H. pylori* induces apoptosis in Barrett's-derived EAC cells at a higher rate than in healthy esophageal cells. *H. pylori* activates the *Fas*-caspase cascade by increasing Fas protein expression in EAC cells, which leads to apoptosis through the fragmentation of cellular DNA	*H. pylori* infection can induce apoptosis and thus reduce the rate of esophageal cancer progression

H. pylori: *Helicobacter pylori*; GERD: Gastroesophageal reflux disease; BE: Barrett's esophagus; EAC: Esophageal adenocarcinoma.

Keeping in mind the inverse association of *H. pylori* infection with EAC, eradication treatments should have been associated with an increased incidence of EC following successful eradication. However, recent cohort studies have debunked this hypothesis, proving that *H. pylori* eradication does not increase the risk of EC[29,31]. A possible explanation is that eradication treatment cannot reverse the chronic gastric atrophy caused by *H. pylori* infection, which protects against both BE and EAC by causing diminished gastric acid secretion and gastroesophageal reflux[29,31]. The adoption of healthier lifestyles and dietary habits may be another factor contributing to the reduced incidence of EAC post-*H. pylori* eradication[29]. Thus, *H. pylori* eradication treatment is safe from an EC perspective, and there is no reason to withhold *H. pylori* eradication in cases where it is indicated[29,31]. Nevertheless, large multicentric studies with long follow-up periods are required to thoroughly evaluate this topic.

CONCLUSION

H. pylori, a Gram-negative bacterium that causes various gastric disorders, shows an inverse relationship with EC. EC has two major subtypes, *i.e.*, EAC and ESCC, which have slightly different etiologies and risk factors. Additionally, gut microbiota can contribute to carcinogenesis in four ways: Inflammation-induced carcinogenesis, immunomodulation, lactagenesis, and genotoxin production. The inverse association of *H. pylori* infection with EAC has been unequivocally confirmed, but no significant association has been observed with ESCC. *H. pylori* infection protects against EAC by inducing atrophic gastritis, influencing serum ghrelin levels, and triggering cancer cell apoptosis. While COX-2-1195 polymorphisms can modify the inverse association between *H. pylori* infection and ESCC, endogenous nitrosamines produced as a secondary impact of *H. pylori*-induced atrophic gastritis may increase the risk of ESCC. There are concerns regarding a plausible increase in EC after *H. pylori* eradication treatments. Fortunately, *H. pylori* eradication is not associated with an increased risk of EC as determined by recent cohort studies, possibly because *H. pylori*-induced atrophic gastritis cannot be reversed by eradication treatment. Thus, *H. pylori* infection affords a degree of protection against the development of EC (especially EAC), and *H. pylori* eradication treatment is safe from an EC perspective.

FOOTNOTES

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REVIEW

Modulation of host N6-methyladenosine modification by gut microbiota in colorectal cancer

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Abstract

As a research hotspot in the field of molecular biology, N6-methyladenosine (m6A) modification has made progress in the treatment of colorectal cancer (CRC), leukemia and other cancers. Numerous studies have demonstrated that the tumour microenvironment (TME) regulates the level of m6A modification in the host and activates a series of complex epigenetic signalling pathways through interactions with CRC cells, thus affecting the progression and prognosis of CRC. However, with the diversity in the composition of TME factors, this action is reciprocal and complex. Encouragingly, some studies have experimentally revealed that the intestinal flora can alter CRC cell proliferation by directly acting on m6A and thereby altering CRC cell proliferation. This review summarizes the data, supporting the idea that the intestinal flora can influence host m6A levels through pathways such as methyl donor metabolism and thus affect the progression of CRC. We also review the role of m6A modification in the diagnosis, treatment, and prognostic assessment of CRC and discuss the current status, limitations, and potential clinical value of m6A modification in this field. We propose that additional in-depth research on m6A alterations in CRC patients and their TME-related targeted therapeutic issues will lead to better therapeutic outcomes for CRC patients.

Key Words: Colorectal cancer; N6-methyladenosine; Tumour microenvironment; Gut

microbiota; Clinical application

Core Tip: This review summarizes the interactions of N6-methyladenosine (m6A) modification in colorectal cancer (CRC) with a variety of the tumour microenvironment factors such as metabolism, hypoxia, inflammation, and immunity and supports the idea that intestinal flora can influence the progression of CRC by regulating the level of m6A modification. Additionally, this review also summarizes the clinical applications of m6A modifications in CRC and suggests possible future research directions.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common malignant tumour worldwide, and its onset is associated with factors such as lifestyle and dietary habits. It is widely distributed in regions such as Asia and Latin America[1-3]. In recent years, the incidence of CRC has been steadily increasing, with a trend towards a younger age of onset[4]. The pathological mechanisms primarily involve chromosomal instability and mutations in oncogenes, tumour suppressor genes, and mismatch repair-related genes caused by CpG methylation, thereby facilitating the occurrence and progression of cancer [3,5]. Due to the continuous increase in the global incidence and mortality rates of CRC in recent decades, the prevention and treatment of this disease have become increasingly crucial for human health[6,7].

RNA modifications are widely found in various types of RNA in organisms and constitute a class of epigenetic modifications at the RNA level. These modifications include N6-methyladenosine (m6A), N1-methyladenosine (m1A), N7-methylguanosine, 5-methylcytidine, 2-o-methylation, pseudouridine, and inosine[8,9]. Currently, there are many studies on m6A modification, which refers to adenylate methylation at the 6th N position in an RNA molecule[10]. This modification is enriched mainly near the termination codon, in long internal exons, in the 5' and 3' untranslated regions, and in the shared sequence RRACH (R = G/A and H = A/C/U). Currently, m6A is the most prevalent and abundant internal chemical and epigenetic modification known in eukaryotic RNA molecules[11] and is important in the regulation of RNA splicing[12], translation[13], stability[14], and DNA damage repair[15], which in turn affects cellular differentiation[16], embryonic development[17], sex determination[18], cancer occurrence[19,20] and other processes.

The CRC tumour microenvironment (TME) is composed mainly of tumour cells, blood vessels, lymphocytes, fibroblasts, myeloid-derived suppressor cells, and signalling molecules[21]. Many proinflammatory factors and antitumour immune responses are present in localized foci in cancer patients, and both are often exacerbated and worsened by metabolic and hypoxic factors[21,22]. The onset and progression of CRC are often associated with metabolic factors in CRC, which often involve the gut microbiota[23]. The gut microbiota can regulate m6A-related enzymes (*e.g.*, methyltransferase-like 3) through their metabolites (*e.g.*, methyl donors), which can lead to the alleviation or exacerbation of CRC[24,25].

There is a strong correlation between the gut microbiota and the production and utilization of methyl donors by the body. Methyl groups are produced mainly by the single-carbon metabolic pathway and are involved in m6A RNA methylation through the folate cycle and methionine cycle pathways, and the substances that provide methyl groups are known as methyl donors. Among them, S-adenosylmethionine (SAM) is the most prominent methyl donor, and other common methyl donors are methionine, betaine, choline, folate, cobalamin, and pyridoxine[26]. All of these methyl donors can be ingested through the diet or produced from the gut microbiota, and their metabolism is also influenced by the gut microbiota. Their metabolites are involved in the synthesis of nucleotides, proteins, and lipids in the body through epigenetic mechanisms[27]. In addition, several other B vitamins are important for these pathways, and their production and metabolism are also influenced by the gut microbiota[28]. The balance of the distribution state of the gut microbiota is important for maintaining the normal functioning of human metabolism[29].

In this review, we focus on the role of the intestinal flora, which is an important factor in TME and an important upstream factor related to m6A during CRC occurrence and development, and leads to alterations in host m6A methylation levels by affecting the supply of methyl donors and other possible pathways, which ultimately affects the progression of CRC and summarize the possible underlying mechanisms. We also summarize and discuss the current status and possible future research directions of m6A RNA methylation and the intestinal flora in the clinical diagnosis, treatment and prognostic assessment of human CRC.

M6A METHYLATION IN NORMAL ORGANISMS

m6A, the most common RNA modification in organisms, mediates gene expression and is involved in many life processes. m6A modification is achieved through regulatory proteins such as writers, erasers, and readers (Table 1)[30-45], which can play roles in the methylation, demethylation, and recognition, respectively, of m6A on bound RNAs[46]. The specific processes by which m6A methylation regulates RNA modification in cells can be summarized as follows: In the nucleus, m6A methylation is accomplished by a complex consisting of METTL3, METTL14, WTAP, RBM15/15B, VIRMA, ZC3H13, or METTL16, and removal of the m6A modification is performed by the demethylation of erasers, including FTO and ALKBH5. After RNA methylation, the readers recognize m6A and perform posttranscriptional regulation, in which HNRNPs in the nucleus regulate mRNA splicing and translation, HNRNPA2B1 regulates mRNA splicing and processing, YTHDC1 regulates mRNA splicing, IGFBPs in the cytoplasm regulate mRNA stability, YTHDF1/3 and YTHDC2 regulate mRNA translation, and YTHDF2 regulates mRNA decay.

m6A RNA modification plays both a promoting and inhibitory role in CRC tumour differentiation, angiogenesis, metastasis, and immunity[47]. In addition, m6A modification, as an epigenetic regulator, can be used to detect disease progression in a variety of solid tumours, including CRC, gastric cancer, breast cancer, liver cancer, and other diseases [48]. Due to its biological function and molecular mechanism, m6A RNA is a promising target site for CRC therapy and is valuable for further research[32].

The specific process and role of m6A in the normal human body are shown in the following schematic diagram (Figure 1).

M6A MODIFICATION AND THE TME IN CRC

Numerous studies have confirmed that aberrant m6A modification of cancer-related genes is closely associated with cancer progression[49]. CRC, which is closely related to the gastrointestinal tract, is the third most common cancer in the world and the second leading cause of cancer-related death[50,51]. The focus of this review is on the associations of gut flora-mediated m6A changes in CRC patients with cancer development and progression. Hypoxia, immunity, and inflammation are also discussed as components of the TME.

Tumour progression and m6A levels in CRC patients

The onset of CRC is characterised by an invasive cancer process that originates from epithelial cells and is intricately linked to the replacement of normal tissues within the intestinal wall by cancer cells[52]. The main aetiologies of CRC include benign adenomatous malignancy and inflammatory bowel disease (IBD). This section focuses on these two aetiologies to discuss their relationship with m6A modification.

Adenoma-related CRC: The developmental trajectory of adenoma-related CRC can be succinctly described as normal mucosa–adenoma–CRC[53]. Throughout this process, numerous RNA methylation modifications, including m6A, m1A, and N2-methylguanosine, actively participate in modulating the antitumour immune function of the patient. Their regulatory role involves either fostering or inhibiting the expression of corresponding genes, thereby influencing the occurrence of CRC[54].

To date, researchers have conducted targeted experimental studies on the expression levels and potential roles of certain m6A regulators in this sequence of changes. Pan *et al*[55], after contrasting the differential expression levels of METTL3 in normal, adenoma, and CRC tumour tissues, reported significant increases in m6A and METTL3 Levels in adenoma and CRC tissues compared with normal tissues. Moreover, they reported that the m6A content was greater in adenomas than in CRC tissues. This observation suggests that METTL3 may exhibit increased expression during the adenoma-CRC process, thereby promoting the onset of CRC. By comparing the differences in ALKBH5 Levels between tumour tissues and normal intestinal mucosal tissues in the colon adenocarcinoma (COAD) patient database, Yang *et al* [56]. reported decreased expression of ALKBH5 in tumour tissues. Building upon this finding, researchers established an *in vivo* CRC metastasis model in nude mice, revealing that mice in the ALKBH5 overexpression group presented fewer lung metastatic nodules. These findings indicate that ALKBH5 potentially exerts an inhibitory effect on tumour invasion and metastasis[56]. In a study on colon cancer, researchers identified a potential correlation between low ALKBH5 expression and high YTHDF1 expression. This correlation may contribute to the transformation of cold tumours into hot tumours during the adenomatous lesion-COAD process, thereby promoting the occurrence of CRC. These effects are mediated through the modulation of the patient's immune environment, affecting the expression levels of immune cells such as CD4+ T cells, CD8+ T cells, NK cells, dendritic cells, and macrophages[57].

While some researchers have conducted experiments on certain m6A regulators, the existing reports remain quite limited. Nevertheless, data analysis allows researchers to discern differences in the expression levels of common m6A regulators between tumour and nontumor tissues in patients with COAD. Ji *et al*[58] team performed an analysis on data from The Cancer Genome Atlas (TCGA) database and revealed that regulators such as METTL3, WTAP, METTL16, VIRMA, RBM15, YTHDC1, YTHDF1/2, IGF2BP1/2/3, and FTO were expressed at relatively high levels in COAD tumour tissues, whereas ALKBH5 was expressed at relatively low levels. However, there was no significant difference in the expression of METTL14 and YTHDC2/3. Kuai *et al*[59] also utilized the TCGA database and reported significant differential expression of regulatory genes associated with YTHDF1, IGF2BP1/3, and EIFB3 in both adenomas and CRC tissues compared with normal tissues ($P < 0.0001$). Interestingly, the mRNA expression levels governed by these four related genes were found to be significantly upregulated only in CRC, with no apparent difference in adenomas. In a comprehensive analysis of data from the TCGA, Gene Expression Atlas, and Human Protein Atlas databases, Liu *et al*[60]

Table 1 Functions of N6-methyladenosine regulators

Types	Regulators	Functions	Ref.
	METTL3	Catalyses m6A modification	[30,31]
	METTL14	Provides structural support and recognizes target RNAs	[30,31]
	WTAP	Contributes to the orientation of MTC	[32,33]
	VIRMA/KIAA1429	Recruits m6A complexes to specialized RNA sites	[32,33]
m6A writers	ZC3H13	Bridges WTAP to Nito	[32,33]
	RBM15	Binds to the m6A complex and recruits to specialized RNA sites	[32,33]
	CBLL1/HAKAI	Contributes to the stabilization of MTC	[32,34]
	METTL16	Catalyses m6A modification	[35]
	FTO	Affects RNA splicing stabilization and deletes m6A modifications	[36]
m6A erasers	ALKBH5	Regulates RNA export and splicing, and deletes m6A modifications	[36]
	IGF2BPs	Enhances mRNA stability and translation	[37]
	YTHDC1	Mediates RNA splicing and export	[38]
	YTHDC2	Enhances target RNA translation and reduces RNA abundance	[39]
	YTHDF1	Enhances mRNA translation	[40]
	YTHDF2	Promotes mRNA degradation	[40]
m6A readers	YTHDF3	Synergizes with YTHDF1 and YTHDF2 to enhance translation and degradation	[40]
	ELF3	Enhances mRNA translation	[41]
	FMR1	Promotes mRNA degradation	[42]
	HNRNPs	Mediates mRNA splicing and translation	[43]
	HNRNPA2B1	Mediates mRNA splicing and miRNA processing	[44]
	ELAL1/HuR	Improve translation efficiency and stability of mRNA	[45]

m6A: N6-methyladenosine.

reported that, in contrast with normal tissues, CRC tumour tissues presented lower expression levels of METTL14. Notably, WTAP, METTL16, HNPNPC, and YTHDC1 are highly expressed in COAD but not in rectal adenocarcinoma [60]. In summary, the majority of m6A regulators play crucial roles in the normal mucosa-adenoma-CRC process.

In summary, during the process of normal mucosa evolving into adenoma and further progressing to CRC, the majority of associated m6A regulatory factors exhibit expression patterns in adenoma and CRC tissues that differ from those in normal tissues. Despite initial advancements in understanding the role of m6A modification in the pathological development of CRC, further targeted experiments are necessary to confirm the specific roles played by these m6A regulatory factors in this intricate process.

IBD-related CRC: IBD, which includes mainly ulcerative colitis (UC) and Crohn's disease (CD), is another major cause of CRC, and it also plays an important role in the development of CRC and influences the level of m6A modification in the host[19]. m6A methylation is closely associated with the development of IBD and the transition from IBD to CRC through the regulation of RNA metabolism and the immune cells in intestinal mucosal immunity[19]. Some researchers have analysed the interaction network between m6A genes and IBD risk genes and reported that there is a significant interaction between them, which also reaffirms that m6A methylation plays a broad regulatory role in the occurrence and development of IBD from the perspective of data analysis[26].

To date, some m6A regulatory factors have been observed in experimental studies to have clear disease-promoting or -inhibiting effects on the pathogenesis of IBD. Fang *et al*[61] reported that METTL3 is highly expressed in the nuclei of intestinal epithelial cells from IBD patients, which is consistent with the oncogenic effect of METTL3 during the progression of CRC. Lu *et al*[62] utilized colitis experiments in a mouse model and reported that the absence of METTL14 in T cells led to spontaneous colitis, resulting in features such as increased inflammatory cell infiltration and increased Th1 and Th17 cytokine levels in mice, which is consistent with the oncogenic role of METTL14 during CRC progression. Similarly, Zhang *et al*[63] verified that the absence of METTL14 Led to severe colitis and suggested that m6A modification could be a potential therapeutic target for IBD. Motawi *et al*[64] compared different m6A regulators among UC patients, CD patients and healthy volunteers and reported that METTL3 was more frequently expressed in CD patients and had good diagnostic accuracy, whereas the expression of WTAP demonstrated good discrimination between UC patients and CD patients.

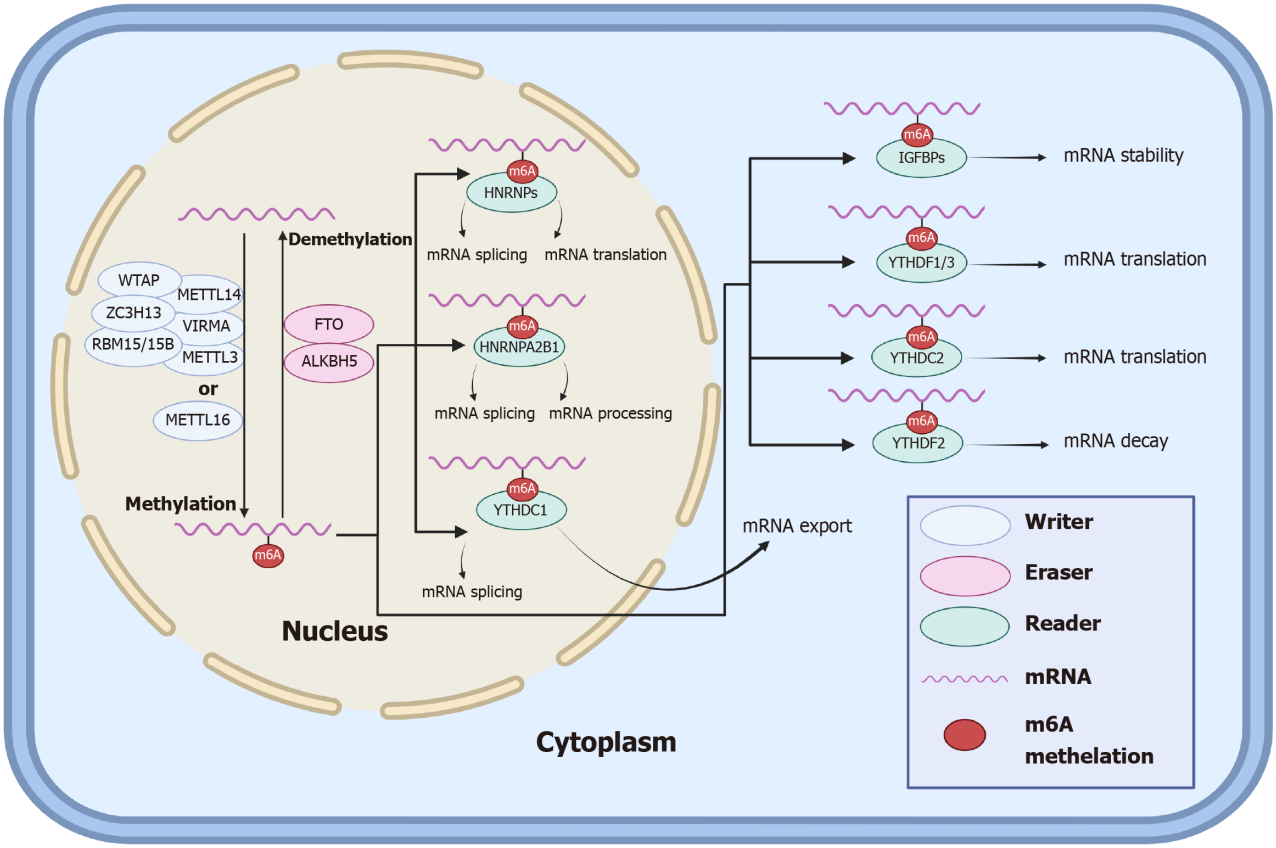

Figure 1 N6-methyladenosine modifications occurring on RNA. m6A: N6-methyladenosine. Created with BioRender.com (Supplementary material).

Some researchers have also analysed data to compare the differences in m6A modification levels between IBD patients and the healthy controls. For example, Chen *et al*[65] reported that the expression of IGF2BP1 and IGF2BP2 was lower in CD tissues than in normal tissues and that the expression of IGF2BP2 was similarly lower in UC tissues than in normal tissues.

Taken together, the results of the above experimental studies and data analyses suggest that the expression levels of multiple m6A regulators in the organisms of IBD patients differ significantly from those of healthy controls and that this difference in turn correlates with the process of the transition from IBD to CRC. In addition, dysregulation of the intestinal flora is considered one of the main reasons for the disruption of the immune response in the intestines of IBD patients and is worth exploring more deeply as a link in the pathogenesis of IBD-CRC[66].

The gut microbiota and m6A modifications in CRC patients

As a pivotal factor in the metabolic microenvironment, the intestinal flora plays a crucial role in the onset and progression of CRC; not only does the intestinal flora modulate CRC through bacterial surface receptors and the secretion of metabolites, but more significantly, it also engages in reciprocal interactions with m6A modifications through mechanisms such as gene expression programs[67]. This section primarily elaborates on the process by which the intestinal flora influences the progression of CRC by impacting m6A modification levels.

Currently, many studies have unequivocally demonstrated the intimate relationships between several intestinal flora, such as *Fusobacterium nucleatum* (*F. nucleatum*), enterotoxigenic *Bacteroides fragilis* (*B. fragilis*), and *Escherichia coli*, and the onset and progression of CRC. Additionally, other intestinal bacteria, including *Enterococcus faecalis* and *Salmonella enterica* , participate in mechanisms that promote CRC cell proliferation. Beneficial bacteria, such as *Lactobacillus acidophilus*, next-generation probiotics, such as *Akkermansia muciniphila*, and other protective bacteria, exert inhibitory effects against CRC. Several typical gut bacteria that have been described to promote or inhibit CRC development and progression are listed in Table 2[68-80].

In the process of exerting the aforementioned physiological effects, the most crucial mechanism by which the intestinal flora influences the m6A modification level is as an upstream regulator of m6A modification. Through various pathways, the intestinal flora modulates the m6A modification level, thereby either promoting or inhibiting CRC. One of the more commonly observed regulatory pathways is the methyl donor pathway. Under normal circumstances, the gut microbiota participates in the generation and metabolism of numerous methyl donors, including SAM, MET, betaine, choline, and folate. Disruption of the gut microbiota due to factors such as dietary changes alters the supply of methyl donors. SAM, the most crucial methyl donor, interacts with the writers METTL3 and METTL16, and changes in SAM levels directly impact their activity. Although METT14 Lacks methyltransferase activity, it is indirectly influenced by gut microbiota regulation, as it typically forms an MTC with METTL3 to exert methylation effects. Research has confirmed that the suppression of METTL3 in CRC cells results in the inhibition of CRC. Mechanistically, METTL3 inhibits antitumour

Table 2 The role of the gut microbiota in colorectal cancer

Gut microbiota	Roles	Functions	Mechanism	Ref.
Coriobacteriaceae	Promote	Tumorigenesis, progression↑	CPT1A-ERK axis	[68]
Clostridioides difficile	Promote	Tumorigenesis↑	Secrete toxin TcdB	[69]
F. nucleatum	Promote	Tumorigenesis, metastasis↑	modulate TME	[70, 71]
ETBF	Promote	Progression↑	Suppress the immune responses	[72]
Escherichia coli	Promote	Proliferation, progression↑	Exert toxic effects on DNA	[73]
Enterococcus faecalis	Promote	Tumorigenesis, progression↑	Secrete metabolite biliverdin	[74]
Salmonella enterica	Promote	Tumorigenesis, proliferation↑	Express secretory protein AvrA	[75]
Streptococcus bovis/gallolyticus	Promote	Tumorigenesis, progression↑	Promote inflammatory response	[76]
Blautia producta	Suppress	Tumorigenesis, progression↓	Facilitate the immune surveillance	[77]
Lactobacillus acidophilus, *Lactobacillus rhamnosus*, and *Lactobacillus casei*	Suppress	Migration, invasion↓	Reduce abnormal crypt foci	[78]
Roseburia intestinalis	Suppress	Tumorigenesis, proliferation↓	Produce butyrate and induce CD8+ T cells	[79]
Akkermansia muciniphila	Suppress	Proliferation↓	Modulate CD8+ T cells	[80]

TME: Tumour microenvironment; *F. nucleatum*: *Fusobacterium nucleatum*.

immunity and promotes CRC progression through the m6A-BHLHE41-CXCL1/CXCR2 axis[81]. One study demonstrated that METTL3 also increases the levels of m6A-modified HK2 and SLC2A1 *via* IGF2BP2/3, thereby promoting the progression of glycolysis and tumorigenesis in CRC[82]. There are limited findings regarding METTL16, and the current research suggests that METTL16 is highly expressed in CRC patients and that mechanistically, METTL16 can increase SOGA1 Levels through binding to IGF2BP1, with a consequent upregulation of PDK4, which promotes glycolytic metabolic reprogramming and the progression of CRC[83]. In addition, METTL14 affects multiple pathways to inhibit CRC progression. For example, METTL14 Levels are decreased in CRC patients, along with a significant reduction in the m6A content of total RNA, whereas METTL14 can inhibit the growth and metastasis of CRC cells *via* the miR-375/SranP1 and miR-375/YAP1 pathways[84].

In addition to the methyl donor pathway, some experiments have revealed the ability of the gut flora to alter CRC cell proliferation by acting directly on m6A and thereby altering CRC cell proliferation. For example, METTL3 promotes CRC progression by increasing the m6A level of CCNE1, whereas butyrate, a metabolite of the gut microbiota, reduces the expression of METTL3 and thus inhibits CRC[25]. However, the role of METTL3 in CRC remains controversial. Another study demonstrated that *F. nucleatum* downregulates METTL3 levels *via* the YAP/FOXD3 axis and that downregulated METTL3 promotes CRC metastasis by increasing KIF26B expression through a reduction in m6A levels[24]. However, a recent study demonstrated that although *F. nucleatum* is also enriched in CRC patients, it increases the level of METTL3 and exerts its oncogenic effects through the c-Myc pathway[85]. These reports demonstrate that the mechanism by which *F. nucleatum* regulates METTL3 and the corresponding m6A levels is contradictory and remains to be clarified by further studies. Mouse experiments have shown that m6A levels of METTL16 and its target mRNA Mat2a are downregulated under gut microbiota-deficient conditions[86], whereas METTL16 overexpression promotes the proliferation of CRC cells [87]. In addition, ETBF can directly promote CRC cell proliferation through METTL14-mediated m6A downregulation of miR-149-3p[88].

In summary, the level of METTL3 and the corresponding m6A content in CRC cells are still controversial, and the prevailing view is that the level of METTL3 and the corresponding m6A content are increased, but a few other studies have suggested that the level of METTL3 and the corresponding m6A content are decreased. This discrepancy may not account for bias in individual experimental cases, such as differences in experimental methodology or limitations in the selection of assay targets. More experiments of greater consistency may be necessary to support a clear conclusion and allow for further exploration of the possibility of additional regulatory mechanisms. Furthermore, available studies have confirmed that METTL16 Levels and corresponding m6A levels are elevated in CRC patients. However, METTL14 Levels and corresponding m6A levels were significantly decreased. Studies related to the regulation of m6A by the gut microbiota in the progression of CRC are beginning to progress, but more experimental data are still needed to support these findings.

Direct associations between the gut microbiota and erasers have rarely been reported, though it has been reported that FTO-deficient mice have increased levels of *Helicobacter*, *Lactobacillus*, and *Porphyromonas* in the intestinal tract[89]. Other upstream mechanisms have also been relatively infrequently studied, and the effects of ALKBH5 and FTO on CRC and the corresponding m6A levels are still under debate. On the one hand, some studies have indicated that high ALKBH5 expression is closely associated with poor prognosis in CRC patients and that ALKBH5 promotes CRC by binding to the downstream target AXIN2 to reduce m6A levels and subsequently suppressing the immune system through the AXIN2/Wnt/DKK1 axis[90]. On the other hand, it has also been observed that downregulation of ALKBH5 predicts a poor prognosis for CRC patients, and mechanistically ALKBH5 inhibits CRC by decreasing PHF20 m6A modification[91]. Studies on FTO have reported the two distinct findings of both promoting and inhibiting CRC progression[92-94]. After comparing the research methods and targets of the related papers, we hypothesise that ALKBH5 and FTO do not act directly on CRC tissues or cells but instead act indirectly on CRC tissues or cells by altering the m6A levels of various downstream targets, which may explain the conflicting results. In addition, studies have reported that the combination of ALKBH5 and FTO inhibits glycolysis and CRC cell proliferation and that a decrease in ALKBH5/FTO levels increases METTL3 levels while decreasing METTL14, which leads to accelerated CRC progression[95].

The mechanism by which the gut microbiota directly regulates readers has also not been reported; however, readers such as IGF2BP2 influence CRC progression *via* other upstream mechanisms. circEZH2/miR-133b first upregulates the level of IGF2BP2, which enhances the stability of CREB1 mRNA *via* IGF2BP2-mediated m6A modification, thereby promoting CRC progression[96]. YTHDF1 can inhibit antitumour immunity through the m6A-p65-CXCL1/CXCR2 axis, thereby promoting CRC progression[97]. KRT17 first degrades YTHDF2, and then YTHDF2 enhances T-cell infiltration by downregulating the level of m6A modification of CXCL10, preventing immune escape, and ultimately inhibiting CRC [98]. The lncRNA H19 binds to HNRNPA2B1 and promotes CRC metastasis by promoting epithelial-to-mesenchymal transition through the m6A/Raf-1 mRNA/Raf-ERK axis[99]. Research on the associations of the remaining readers with CRC is ongoing.

In conclusion, the gut microbiota, as an important upstream mechanism regulating m6A modification, can alter the expression levels of m6A-related enzymes and thus promote or inhibit the progression of CRC in various ways. We summarize existing upstream mechanisms regulating m6A modification levels in CRC patients and their roles in CRC progression in Table 3[100-111] (which mainly include the gut flora), and the basic process by which the intestinal flora affects m6A modification in CRC patients is shown in Figure 2 and Table 3.

Other TME factors and m6A modifications in CRC

Hypoxia: In addition to the gut microbiota, hypoxia is also closely related to the occurrence, progression, and prognosis of CRC[112]. Hypoxia is the most common component of the TME and affects a series of biological behaviours, such as genetic instability, proliferation, differentiation, metastasis, invasion, metabolism, apoptosis, and other biological behaviours of tumour cells, such as in CRC[113,114]. Moreover, hypoxia can promote neovascularization, *i.e.*, tumour angiogenesis, by activating hypoxia-inducible factor-1 (HIF-1)[115]. In addition, hypoxia can directly or indirectly affect the methylation modification of m6A[116], and m6A modification, in turn, can affect hypoxia and its inducible factors [100]. Further studies have shown that the pathway of hypoxia-regulated CRC progression is closely related to m6A regulatory factors[117], which supports the idea that hypoxia is also one of the upstream mechanisms of m6A regulation in CRC.

In a recent study that utilized controlled hypoxic conditions, activated HIF-1 induced high expression of the lncRNA STEAP3-AS1, and a large amount of STEAP3-AS1 further competed with the m6A reader YTHDF2 for binding to STEAP3 mRNA, which resulted in the protection of STEAP3 mRNA from YTHDF2-mediated degradation. Stabilized expression of STEAP3 in turn activates Wnt/β-catenin, which ultimately promotes CRC cell proliferation and invasion. In summary, hypoxia inhibits m6A-mediated mRNA degradation and plays an important role in the malignant progression and poor prognosis of CRC[118]. Another study demonstrated that hypoxia promotes ubiquitin-mediated degradation of the m6A eraser FTO, which results in lower FTO protein levels, and that low expression of FTO fails to inhibit m6A methylation of metastasis-associated protein 1 transcripts, which stabilizes mRNA expression by binding to IGF2BP2 and thereby contributes to the metastasis of CRC cells[94]. In summary, hypoxia modulates the effect of m6A on CRC and is positively associated with the malignant progression of CRC. In addition, hypoxia has an important effect on the abundance of the gut microbiota, and hypoxia can regulate the type and amount of the gut microbiota[119], which in turn may affect CRC progression through the pathways previously described.

Inflammatory response: Many clinical experiments and epidemiological studies have demonstrated that there is a complex association between inflammation and the development and progression of malignant tumours[120]. Chronic intestinal inflammation is closely associated with the development of IBD and the proliferation and metastasis of CRC [121], and IBD may increase the risk of CRC[122]. Inflammatory cells generated by long-term intestinal inflammation first activate the proinflammatory signalling pathway, which in turn releases cytokines and chemokines, ultimately forming an inflammatory microenvironment[123]. The increased methylation of m6A in the proinflammatory signalling pathway has been supported by studies[124], and it has also been shown that the expression of m6A and m6A regulatory factors may be increased in the inflammatory microenvironment[125]. In addition, some studies have summarized the relevant role of m6A in the development and progression of IBD[10], and other studies have analysed the interaction between m6A regulatory factors and IBD risk genes[26], which suggests a close relationship between m6A and IBD. In summary, intestinal inflammation also plays an important role in m6A regulation during CRC progression.

A study by Wang *et al*[126] revealed that under normal conditions, the deubiquitinase USP47 reduces the efficiency of YTHDF1-mediated c-Myc translation. Once USP47 is deficient, it leads to high c-Myc protein expression, as well as disruption of Treg cell metabolism, which in turn leads to inflammation and antitumour immunity, including anti-CRC.

Table 3 Relationships among N6-methyladenosine, colorectal cancer, and related mechanisms

m6A regulator	Upstream mechanism	Roles	Functions	Mechanism	Ref.
METTL3	Gut microbiota	Oncogene	Glycolysis, chemo-resistance↑	METTL3↑/LDHA↑	[100]
METTL3	Gut microbiota	Oncogene	Proliferation, migration, invasion↑	METTL3↑/m6A↑/YTHDF2↑/CRB3↓/Hippo↓	[55]
METTL3	Gut microbiota	Oncogene	Glycolysis, progression↑	METTL3↑/m6A/GLUT1↑/mTORC1 ↑	[101]
METTL3	Gut microbiota	Oncogene	Invasion, migration↑	METTL3↑/m6A/circ1662↑/YAP1↑/SMAD3↓	[102]
METTL3	*F. nucleatum*	Suppressor	Metastasis↑	METTL3↓/m6A↓/YTHDF2/KIF26B↑	[24]
METTL16	Gut microbiota	Oncogene	Glycolysis, progression↑	METTL14↑/IGF2BP1/SOGA1↑/PDK4↑	[83]
METTL14	ETBF	Suppressor	Proliferation↑	METTL14↓/m6A/miR-149-3p↓/PHF5A/KAT2A	[88]
METTL14	Gut microbiota	Suppressor	Proliferation, metastasis↑	METTL14↓/m6A↓/YTHDF2↓/lncRNA XIST↑	[103]
METTL14	Gut microbiota	Suppressor	Migration, invasion, metastasis↑	METTL14↓/m6A↓/YTHDF2/SOX4↑	[104]
ALKBH5	H3K27	Oncogene	Glycolysis, progression↓	ALKBH5↓/JMJD8↓/PKM2↓	[105]
ALKBH5		Oncogene	Proliferation, migration, invasion↑	ALKBH5↑/YTHDF2/RAB5A↑	[106]
ALKBH5		Suppressor	Radiosensitivity↑	ALKBH5↑/YTHDF2/circAFF2↑/Cullin-NEDD8↓	[107]
ALKBH5		Suppressor	Proliferation, migration, invasion↓	ALKBH5↑/PHF20↓	[91]
FTO		Oncogene	Chemo-resistance↑	FTO↑/YTHDF2/SIVA1↓	[92]
FTO	miR-96	Oncogene	Tumorigenesis, progression↑	AMPKα2↓/FTO↑/m6A↓/MYC↑	[93]
IGF2BP2	LINC00460	Oncogene	Proliferation, metastasis↑	IGF2BP2-DHX9↑/HMGA1↑	[108]
YTHDF1		Oncogene	Tumorigenesis, metastasis↑	YTHDF1↑/m6A/ARHGEF2↑	[109]
YTHDF2	miR-6125	Oncogene	Proliferation, growth↓	YTHDF2↓/m6A/GSK3β↑	[110]
HNRNPA2B1	MIR100HG	Oncogene	Chemo-resistance, metastasis↑	hnRNPA2B1↑/m6A/TCF7L2↑	[111]

m6A: N6-methyladenosine; *F. nucleatum*: *Fusobacterium nucleatum*.

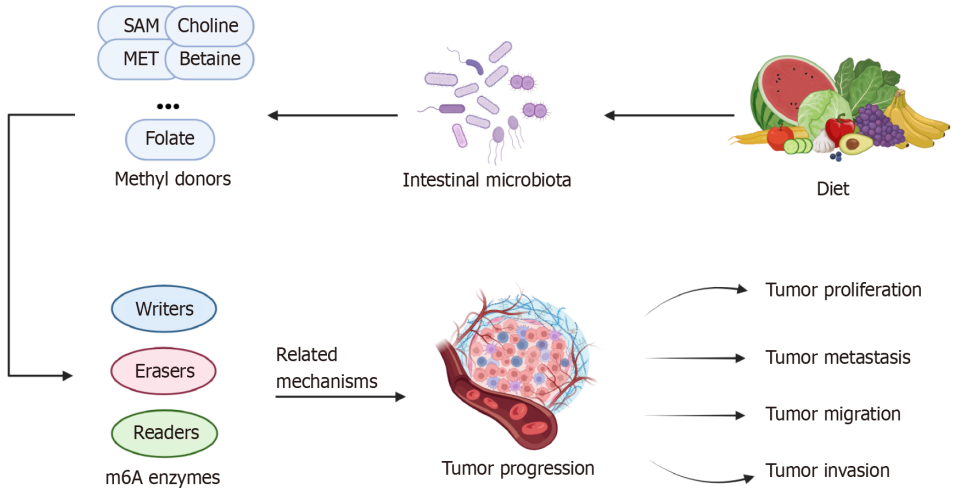

Figure 2 Diet and other environmental factors can provide nutrients for the gut microbiota to metabolize, which can affect the levels of their metabolites (various methyl donors), leading to changes in the level of N6-methyladenosine methylation in the host and ultimately triggering the occurrence of colorectal cancer, as well as the proliferation, metastasis, migration and invasion of tumours. SAM: S-adenosylmethionine; m6A: N6-methyladenosine. Created with BioRender.com (Supplementary material).

Wang *et al*[109] further showed that high expression of YTHDF1 exacerbates inflammation, which promotes the progression and metastasis of inflammatory CRC. In addition, Zhang *et al*[127] developed two isoform systems, m6AregCluster and m6AsigCluster, for assessing the association of m6A regulators with TMEs such as inflammation in CRC, which helps elucidate the mechanisms and principles involved.

Like hypoxia, intestinal inflammation also causes gut dysbiosis[128], and gut dysbiosis also regulates CRC progression through relevant pathways involving m6A. However, the difference is that gut dysbiosis also promotes intestinal inflammation[129].

Immune response: Immune factors, as important components of the TME, can cause immune tolerance and immunosuppression by regulating immune cells such as dendritic cells and T cells and their signalling pathways, which in turn contribute to the evasion of CRC cells from the host immune system and the proliferation and metastasis of CRC[19]. m6A modification decouples T-cell proliferation from cell survival by controlling the IL-7-mediated JAK-STAT signalling pathway and the TCR-mediated ERK/AKT signalling pathway[130]. Deletion of METTL3 results in decreased infiltration of CD206+ m^2-like TAMs and increased infiltration of CD103+ cDC1s and results in tumour suppressor activity, suggesting that METTL3 has a role in driving TME immunosuppression[131]. Chen *et al*[81] reported that METTL3 drives myeloid-derived suppressor cell (MDSC) accumulation, inhibits the proliferation of immune cells, such as CD4+ T cells and CD8+ T cells, and promotes CRC proliferation, which supports the role of METTL3 in driving TME immunosuppression. In METTL16-overexpressing CRC tissues, a significant decrease in the level of the proliferative biomarker Ki-67 was detected, which was accompanied by an increase in the infiltration levels of CD4+ T cells and CD8+ T cells and increased antitumour activity[132]. Similar conclusions have been reached in studies concerning changes in m6A writers in the breast cancer TME. For example, the expression of METTL14 and ZC3H13 was significantly positively correlated with CD4+ T cells, CD8+ T cells, dendritic cells, macrophages, and neutrophils in breast cancer tissues and exhibited some antitumour activity[133].

With respect to m6A erasers and altered immune responses in the TME, the lack of ALKBH5 increases the m6A modification of interferon-γ (IFN-γ) and C-X-C motif chemokine ligand 2 mRNAs, which decreases the stability of mRNAs in CD4+ T cells and increases their expression[134]. In a humanized mouse model, the knockdown of ALKBH5 was able to reduce the MDSC content, promote increased levels of NK cells, CD4+ T cells, and CD8+ T-cell infiltration, and promote tumour suppressor activity[90]. FTO evades host immune surveillance by regulating glycolytic processes in tumour tissues. Knockdown of FTO impairs tumour cell glycolytic activity, enhances the degree of CD4+ T-cell and CD8+ T-cell infiltration, and restores the immune function of CD8+ T cells (elevated levels of IFN-γ and granzyme B), thereby inhibiting tumour proliferation[135].

To date, findings regarding the associations of m6A readers with changes in immune responses in the TME have not been reported. In knockout experiments, the expression of the vast majority of m6A regulators was positively correlated with the infiltration levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in CRC tissues. High expression of FMR1, LRPPRC, METTL14, RBMX, YTHDC2, YTHDF2, and YTHDF3 in CRC patients is associated with a poor prognosis (Figure 3).

CLINICAL APPLICATIONS AND PROSPECTS OF M6A IN CRC

Currently, various types of m6A regulatory factors used as biomarkers of CRC have demonstrated great clinical value in their diagnosis and prognostic assessment, and great potential exists in their use as therapeutic targets for CRC. This section focuses on summarizing the progress of m6A in the diagnosis and prognostic assessment of CRC and discusses the possibility of m6A regulatory factors as therapeutic targets for CRC using leukaemia and other malignant cancers as examples.

Diagnosis and prognosis

On the basis of the increasing number of studies showing that aberrant m6A regulatory factors are closely associated with CRC progression, m6A regulatory factors are expected to become biomarkers of CRC and play important roles in the diagnosis and prognosis of CRC[136]. For example, many studies have reported high expression of METTL3 in CRC cells or patients and suggested a strong association with poor CRC prognosis[137,138], although a small number of studies reached the opposite conclusion[24]. Some reports have suggested that, considering the heterogeneity of METTL3, more easily detectable downstream target RNAs could be selected as new biomarkers[136], which may require more experimental data support. The results of these limited studies support that METTL16 is elevated in CRC patients[83,87]. In addition, relevant experiments have demonstrated that METTL14 Levels are downregulated in CRC patients and suggest that METTL14 Levels are negatively correlated with CRC progression[88,103]. However, studies on the erasers ALKBH5 and FTO are contradictory, and the levels of ALKBH5 and FTO fluctuate high and low in CRC tissues, making them currently unsuitable as biomarkers. Notably, the existing studies showing how readers such as IGF2BP2 and YTHDF1 affect the progression of CRC are promising. In the foreseeable future, as research clarifies how m6A regulators are expressed in CRC, the next step will be to explore how m6A regulators can be used in the clinical diagnosis of CRC and assessment of prognosis.

Targeted therapy

Among the many studies on the effects of m6A regulatory factors on CRC progression, m6A regulatory factors and related pathways are promising therapeutic targets. In addition, several studies have investigated the effects of m6A regulatory factors on chemotherapeutic resistance and sensitivity to radiotherapy, as well as synergistic effects with PD-1/PD-L1 inhibitors for the treatment of CRC. Therefore, the development of corresponding agonists or inhibitors of m6A regulatory factors seems to be a promising therapeutic strategy[139]. For example, METTL3 inhibitors are already in use, and METTL16 inhibitors and METTL14 agonists are envisioned. The roles of ALKBH5 and FTO in CRC progression are

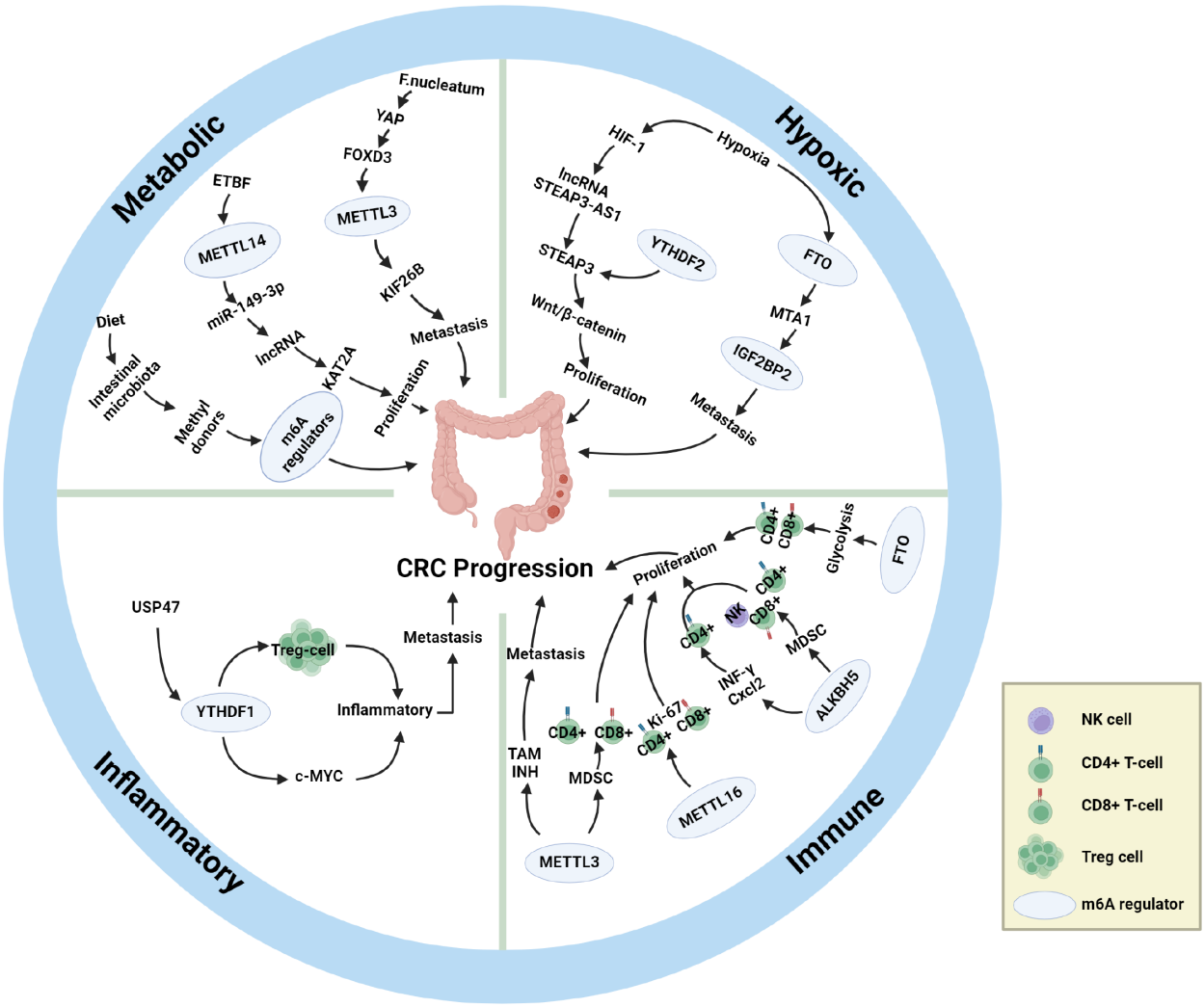

Figure 3 A summary of the role of N6-methyladenosine in the occurrence and development of colorectal cancer among the four tumour microenvironment factors discussed in the text. CRC: Colorectal cancer; HIF-1: Hypoxia-inducible factor-1; SAM: S-adenosylmethionine; m6A: N6-methyladenosine; MDSC: Myeloid-derived suppressor cell; *F. nucleatum*: *Fusobacterium nucleatum*. Created with BioRender.com (Supplementary material).

not yet clear, and whether they can be used as therapeutic targets still awaits follow-up studies. Therapeutic strategies targeting readers such as IGF2BP2 and YTHDF1, on the other hand, seem to be promising for future consideration.

Dysregulation of m6A modification occurs to varying degrees in patients with various types of cancer. m6A RNA modification plays a role as tumour promoters or tumour suppressors *in vivo*. On this basis, it is possible to consider targeting different m6A writers, erasers, or readers by designing corresponding inhibitors or agonists and combining them with other therapies to enhance tumour immunity and improve clinical benefits. Although practical reports on the application of relevant drugs in CRC treatment are lacking, many drugs (*e.g.*, FTO inhibitors) have been widely investigated for the treatment of malignant diseases, such as AML, to improve the clinical benefit (Table 4).

The targeted drug STM2457 specifically inhibits METTL3, reduces the m6A levels of METTL3-dependent core leukaemia m6A substrates (including HOXA1018 and MYC19), and decreases the protein translation levels of BRD4 and SP1. Ultimately, STM2457 inhibits AML and has almost negligible toxic effects[140]. Another drug that targets METTL3, UZH1a, exerts inhibitory effects on METTL3 activity by binding to its SAM site to reduce m6A levels and inhibit mRNA transcription[141].

In addition to m6A erasers, several targeted drugs with applications in other types of cancers that could be used in attempts to treat CRC have emerged[142]. Most of these drugs are FTO inhibitors. CS1, a selective FTO inhibitor, has shown potent antileukaemic efficacy by blocking the binding of FTO to MYC, CEBPA, and RARA[143]. R-2-Hydroxyglutaric acid, a tumour suppressor drug that directly inhibits FTO, targets the FTO/m6A/MYC/CEBPA signalling pathway, increases m6A levels and has antitumour effects[144]. Meclofenamic acid is a highly selective inhibitor of FTO that is able to bind FTO and inhibit its demethylation, thus exerting anticancer effects[145]. The mechanism of action of FB23 is similar to that of CS1, which is able to negatively regulate the expression of ASB2, RARA, MYC, and CEBPA and upregulate the level of m6A in AML cells, exerting antitumour effects[146]. In Zhao *et al*'s review[147] of the therapeutic potential for liver disease, Rhein was proposed as a promising drug for the treatment of hepatocellular carcinoma, which competitively binds to the substrate-binding site of FTO and increases the m6A level, thus exerting antitumour effects. To date, few targeted therapeutic agents against ALKBH5 have been developed. 2-[(1-Hydroxy-2-oxo-2-phenylethyl)

Table 4 Summary of the molecules of action, inhibitory concentration values, and mechanisms of action of existing anticancer drugs for N6-methyladenosine-targeted therapy

Drug	Role in cancer	Cancer type	Target	IC_{50}	Mechanism	Ref.
STM2457	Tumour inhibitor	AML	METTL3	16.9 nM	m6A↓/HOXA1018↓/MYC19↓	[142]
UZH1a	Tumour inhibitor	AML	METTL3	4.6 μM	Inhibits METTL3 catalytic activity and decreases m6A level and mRNA transcription level	[143]
CS1	Tumour inhibitor	AML	FTO		m6A↑/LILRB4↓/MYC↓/CEBPA↓/RARA↑/ASB2↑	[145]
FB23	Tumour inhibitor	AML	FTO	0.8-1.5 μM	m6A↑/MYC↓/CEBPA↓/RARA↑/ASB2↑	[148]
MA	Tumour inhibitor	AML	FTO	17.4 μM	Binds to FTO and inhibits demethylation	[147]
R-2HG	Tumour inhibitor	AML	FTO		m6A↑/FTO↓/MYC↓CEBPA↓	[146]
Rhein	Tumour inhibitor	Liver cancer	FTO/ALKBH5	30 mM	Competitive binding of FTO to substrate binding sites and increased m6A levels	[149]
2-[(1-hydroxy-2-oxo-2-phenylethyl) sulfanyl] acetic acid	Tumour inhibitor	AML	ALKBH5	0.84 μM	Binds ALKBH5 and decreases m6A levels	[150]
4-{[(furan-2-yl) methyl]amino}-1,2-diazinane-3,6-dione	Tumour inhibitor	AML	ALKBH5	1.79 μM	Binds ALKBH5 and decreases m6A levels	[150]

M6A: N6-methyladenosine; IC50: Inhibitory concentration.

sulfanyl] acetic acid and 4-{[(furan-2-yl) methyl] amino}-1,2-diazinane-3,6-dione are two unnamed tentative ALKBH5 inhibitors that bind to ALKBH5 and inhibit its activity, reducing m6A levels and exhibiting antiproliferative activity in AML cell lines[148].

There is a lack of research reports on targeted therapeutic agents against m6A readers for cancer treatment, but among them, YTHDF1 and FMR1 are also expected to be new targets in CRC m6A-targeted therapies. In Transwell experiments, after the YTHDF1 gene was knocked down in CRC cells derived from CRC patients, the growth of HCT116 and HT-29 cells *in vitro* was inhibited, which significantly reduced the ability of CRC cells to migrate and invade *in vitro*[109]. The upregulation of FMR1 increased EGFR mRNA expression and activated the ERBB signalling pathway in CRC cells, promoting cancer cell proliferation and metastasis[149]. These findings suggest that m6A readers can also be used as targets for CRC therapy and have potential for targeted therapy.

On the other hand, the distribution and metabolic profile of the gut microbiota also affect the effect of tumour immunity in the body. As one of the important upstream mechanisms of m6A, the gut microbiota also has great potential as a biomarker and therapeutic target for CRC. There have been literature reviews on the research progress of gut microbiota biomarkers in the early diagnosis of CRC, especially *F. nucleatum*[128], and the application of gut microbiota therapies in the prevention and treatment of CRC, especially *via* faecal microbiota transplantation[150]. In addition, gut microbiota metabolites have a potential role in the diagnosis of CRC[151,152]. A study on the influence of the gut microbiota on m6A modification revealed that the presence of large amounts of butyrate, a metabolite of intestinal microorganisms, can reduce the level of METTL3 and the expression of cyclin E1, which in turn reduces the level of m6A in CRC cells and inhibits their proliferation and metastasis[25]. Some researchers have noted that killing *B. fragilis* and bacteria associated with mucin degradation, inflammation, and DNA methylation *via* antibiotics can reverse the dysregulated intestinal microecology in patients and inhibit CRC progression[153]. Notably, if antibiotic therapy disrupts the original intestinal ecological balance, it can instead cause an intestinal inflammatory response and exacerbate the development of intestinal tumours, causing disease progression[154]. In these cases, regulating the gut microbiota profile with drugs and combining it with m6A-targeted therapy may be a novel idea for the treatment of CRC, but antibiotics should also be used carefully to reduce the destruction of the original probiotic flora of the host to avoid disease exacerbation. In addition, the interaction between the host gut microbiota and m6A RNA modification is complex, and the mechanism is still not completely clear. The specific process of regulating the balance of the host gut microbiota to influence the level of host m6A modification and play an anticancer role as a possible therapeutic approach still needs more in-depth research (Table 4).

DISCUSSION

The morbidity and mortality of CRC in young patients have been gradually increasing in recent decades. m6A modification of RNA-mediated posttranscriptional regulation plays an important role in the development of CRC. In this

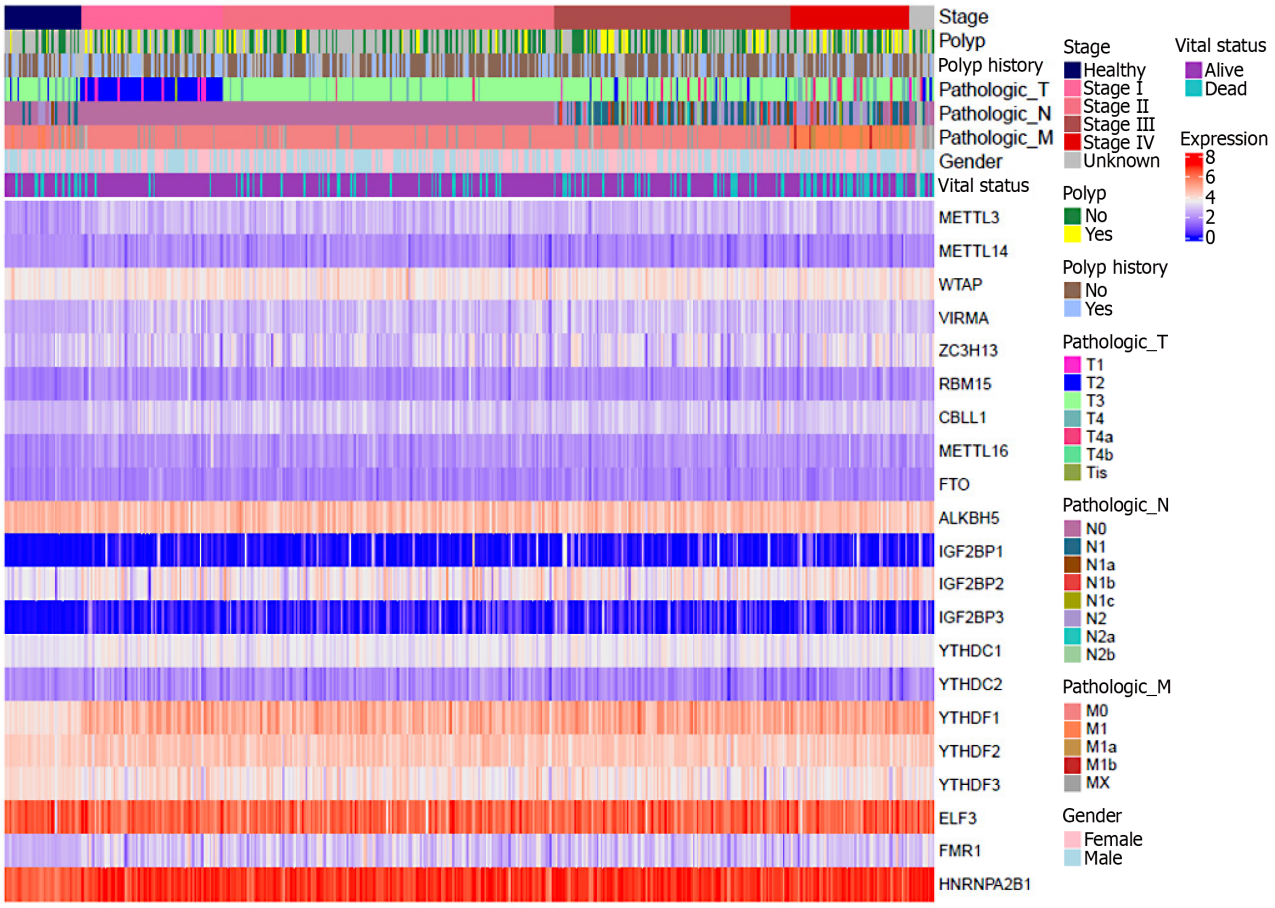

Figure 4 To analyze the correlation between different N6-methyladenosine regulators and colorectal cancer progression.

process, TME factors such as metabolism, hypoxia, inflammation, and immunity are closely related to the progression of CRC, in which the intestinal flora plays an important role.

On the basis of the above findings, we propose that the intestinal flora can participate in the regulation of m6A regulators through the methyl donor pathway and other possible pathways, which in turn affects the progression of CRC through various pathways. In other words, the intestinal flora is one of the important upstream mechanisms by which m6A modification affects CRC progression.

Notably, few studies have directly demonstrated the involvement of the intestinal flora as an upstream mechanism of m6A in the process of CRC. In addition, most existing studies have focused on the regulation of writers, such as METTL3, by the gut flora to affect CRC, and there is a relative gap in studies on the associations between the gut flora and erasers and readers. The former still needs to be supported by more high-quality experimental data, and the latter needs new studies to fill this gap. Moreover, the relationship between METTL3 and CRC progression is still debated, although most experiments support the conclusion that METTL3 is an oncogenic factor. The promotion or inhibition of CRC progression by ALKBH5 and FTO appears to be contradictory. There are relatively few studies on the direct regulation of CRC by readers such as METTL16 and IGF2BP2; however, IGF2BP2 and YTHDF2 are widely involved in the mechanism of CRC regulation by writers and erasers, and METTL14 has essentially been proven to be an oncogenic factor.

In order to verify the correctness of the above, we grouped and generated a heatmap based on the dataset of two molecular analysis-based articles on CRC[48,155], according to the gender of the patients in the samples, the origin of the tissues, the TNM classification, and the vital status. As can be seen from the heatmap below (Figure 4), compared with normal tissues: M6A regulators such as METTL3, WTAP, VIRMA, ZC3H13, CBLL1, METTL16, IGF2BP1/2/3, YTHDF1/2, FMR1, HNRNPA2B1, *etc.*, showed different degrees of increase in the expression level in the tumor tissues and exhibited oncogenic effects. The expression levels of m6A regulators such as METTL14 and YTHDC1/2 decreased to different degrees in tumor tissues and exhibited cancer-suppressing effects. The relationship between the expression levels of m6A regulators such as ALKBH5, FTO, YTHDF3, ELF3, and RBM15 and the progression of CRC is still unclear, a situation similar to the results of earlier studies by other scholars. It may be related to the sampling method of the data samples, which deserves more experiments to explore and repeat the validation.

In addition, we selected some representative CRC-associated oncogenes/tumor suppressor genes and generated a box plot based on the RNA-Seq data of COAD and rectum adenocarcinoma from the TCGA database to analyze the differences in their expression levels during CRC progression. From this box plot (Figure 5), it can be seen that compared with normal tissues: The transcript levels of KRAS and SMAD4 increased to different degrees in tumor tissues and showed oncogenic effects; the transcript levels of TP53 and PAQR3 decreased to different degrees in tumor tissues and showed cancer-suppressing effects; the transcript levels of NRAS and BRAF did not increase significantly in tumor

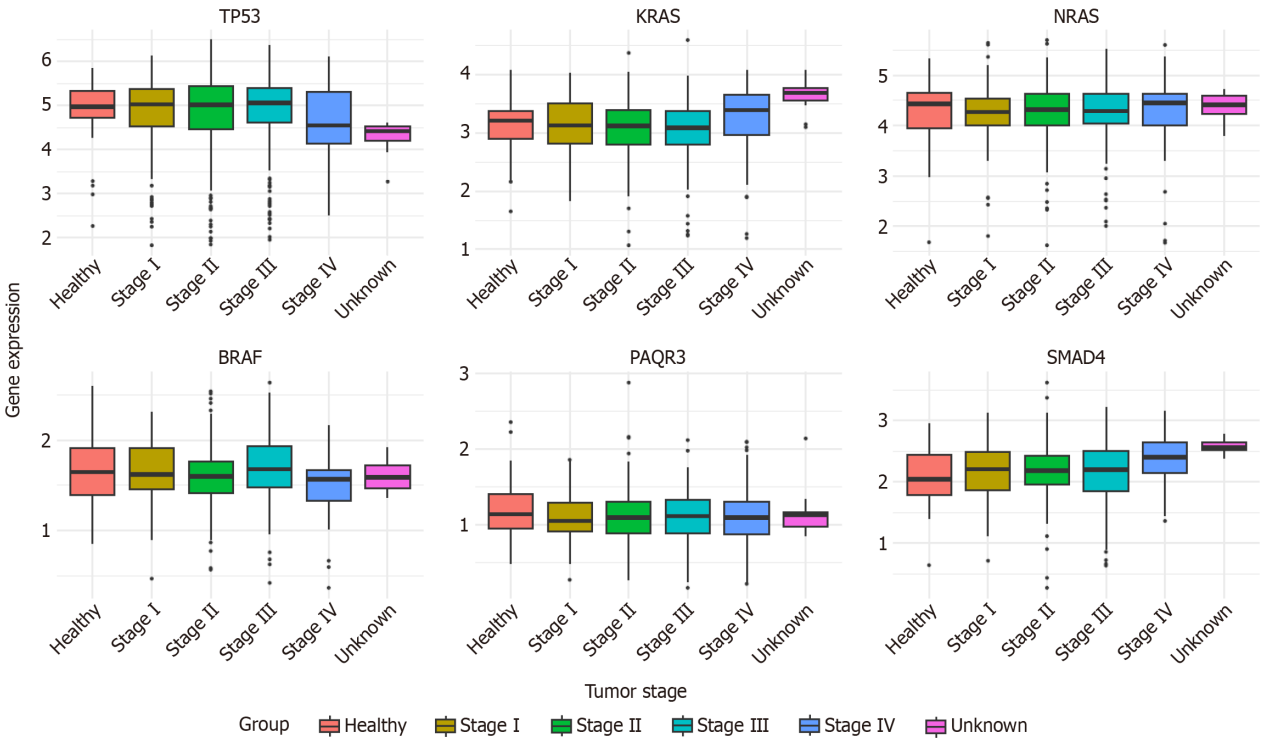

Figure 5 To analyze the correlation of different oncogenes/tumor suppressor genes with colorectal cancer progression.

tissues, which may be due to the fact that different tissues were sampled at the time of sampling. The reason for this result may be due to a certain degree of mixing of cells from different tissue sources at the time of sampling, resulting in no significant difference in expression in the final data.

CONCLUSION

On the basis of these findings above, future research can first consider more experimental studies that focus on the influence of the intestinal flora on CRC progression by altering the expression levels of different m6A regulators. Second, research can continue to elucidate the relationship between other individual m6A regulators and CRC progression, resolve controversial issues and fill in gaps in related research, which may help to explain the mechanism by which multiple regulators act together in CRC. m6A regulators and the gut microbiota have shown great potential as diagnostic biomarkers and therapeutic targets for CRC, and we highlight some of the therapeutic advances in the former. For example, although m6A-targeted therapies have not yet been applied to the treatment of CRC, METTL3 inhibitors, FTO inhibitors, and ALKBH5 inhibitors have already shown good efficacy in the treatment of other malignant tumours, especially AML. However, many challenges remain. For example, the efficacy and risk of the existing m6A regulator inhibitors used in the clinic for CRC therapy are not yet known, and other inhibitors or agonists that target m6A regulators still need to be developed. In addition, the combination of m6A-targeted therapies with gut microbiota therapies, as well as influencing the level of m6A modifications in the host by regulating the balance of the host gut microbiota and exerting anticancer effects, is promising and may help to improve the treatment of CRC.

FOOTNOTES

Author contributions: Xie C conceived and designed the study; Jiang TQ, Wang H, and Cheng WX edited the manuscript; All the authors read and approved the final manuscript.

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Abstract

Celiac disease (CD) is an autoimmune disorder exacerbated by the ingestion of gluten in genetically susceptible individuals, leading to intestinal inflammation and damage. This chronic disease affects approximately 1% of the world's population and is a growing health challenge due to its increasing prevalence. The development of CD is a complex interaction between genetic predispositions and environmental factors, especially gluten, culminating in a dysregulated immune response. The only effective treatment at present is a strict, lifelong gluten-free diet. However, adherence to this diet is challenging and often incomplete, so research into alternative therapies has intensified. Recent advances in understanding the molecular and immunological aspects of CD have spearheaded the development of novel pharmacologic strategies that should provide more effective and manageable treatment options. This review examines the latest innovations in CD therapies. The focus is on drugs in advanced clinical phases and targeting specific signaling pathways critical to the disease pathogenesis. We discuss both quantitative strategies such as enzymatic degradation of gluten, and qualitative approaches including immunomodulation and induction of gluten tolerance. Innovative treatments currently under investigation include transglutaminase inhibitors, which prevent the modification of gluten peptides, and nanoparticle-based therapies to recalibrate the immune response. These new therapies not only promise to improve patient outcomes but are also expected to improve quality of life by reducing the burden of dietary restrictions. The integration of these new therapies could revolutionize the treatment of CD and shift the paradigm from strict dietary restrictions to a more flexible and patient-friendly therapeutic approach. This review provides a comprehensive overview of the future

prospects of CD treatment and emphasizes the importance of continued research and multidisciplinary collaboration to integrate these advances into standard clinical practice.

Key Words: Celiac disease; Gluten tolerance; Enzymatic degradation; Therapeutic advances; Transglutaminase inhibitors; Tight junction modulators

Core Tip: The landscape of celiac disease treatment is evolving beyond the traditional gluten-free diet due to the challenges of strict adherence to the diet and incomplete resolution of symptoms. This review highlights emerging therapeutic strategies, including gluten sequestration and degradation, gluten tolerance induction, tight junction modulators, transglutaminase inhibitors, lymphocyte trafficking, and homing inhibitors. These novel therapies, which target specific molecular and immune signaling pathways, promise to improve patient outcomes and quality of life by reducing dietary restrictions and addressing persistent inflammation and symptoms. Further research and multidisciplinary collaboration are critical to integrate these advances into standard clinical practice.

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INTRODUCTION

Celiac disease (CD), a chronic autoimmune small bowel enteropathy triggered by gluten ingestion in genetically predisposed individuals[1], affects approximately 1% of the global population, with an increasing incidence that has been detected worldwide[2,3]. The pathogenesis of CD involves a complex interplay between genetic predisposition and environmental factors, primarily gluten, which leads to intestinal inflammation and villous atrophy. Currently, the only effective treatment for CD is a strict, lifelong gluten-free diet (GFD), which can be challenging to adhere to, and may not fully prevent the inflammatory responses and associated complications. Recent advances in understanding the molecular and immunological aspects of CD have opened new avenues for therapeutic interventions.

This review discusses the latest advances in the development of novel therapeutic approaches in CD, summarizing drugs currently in the advanced phases of clinical evaluation, targeting specific signaling pathways involved in the pathogenesis of the disease. We also discuss the potential of these drugs to change the treatment landscape for CD by offering alternatives to the dietary approach. By analyzing recent clinical trials and new research findings, we provide a comprehensive overview of the future prospects of CD treatment and how these new drugs could improve patient outcomes and quality of life.

CURRENT CHALLENGES IN CD MANAGEMENT

Gluten refers to a subgroup of wheat proteins, comprising monomeric water-soluble gliadins and multimeric water-insoluble glutenins. It also includes, in sensum latum, secalin, hordein and avenins, which are found in rye, barley, and oats, respectively[4]. Due to its viscoelastic properties, gluten is an important and ubiquitous ingredient in the food industry. Apart from the obvious foods, *i.e.* bread or pasta, it is also found in "unlikely" ones such as soups, sauces, yogurt, and frozen foods. In addition, it can be hidden in toothpaste and lipsticks, making strict adherence to a GFD very difficult[5].

Self-reported adherence to GFD ranges from 42% to 91% in adults and is about 59% in children, depending on age at diagnosis, ethnicity, cognitive, emotional and sociocultural influences, membership of an advocacy group, and regular dietetic follow-up[6,7]. It must be underlined, however, that even patients that are following a strict GFD can ingest gluten; in fact, according to some studies, up to 70%-80% of adherent patients present gluten contamination[8,9], with an average gluten exposure of about 150 mg/day, which is much higher than the considered safe amount[10-12]. Actually, many products can be contaminated with gluten during harvesting, processing, packaging, and cooking; in addition it could be difficult to control for gluten contamination while eating at restaurants or using packaged food[13,14].

GFD is also burdened by some other problems. First, even if popularity has recently improved this matter, gluten-free products can be more expensive and difficult to find than gluten-containing ones[15]. Second, gluten-free products, to be tastier, are often high in fat and sugar content and low in fibers, vitamins, and minerals increasing, on one hand, the risk of obesity and metabolic syndrome and, on the other, the risk of deficiencies[16,17]. Additionally, there is a theoretical risk for mycotoxin exposure from corn and arsenic exposure from rice in those who restrict their diet to only a few carbohydrate sources[18,19]. Moreover, adherence to GFD can have negative effects on quality of life, leading to isolation,

anxiety, depression, psychological distress, and maladaptive food attitudes and behaviors[20-22]. Finally, despite strict GFD, up to 30% of patients report persistent symptoms[23], about 30%-60% of adults will not achieve histological recovery after 1 year on a strict GFD[24], and up to 0.5% of patients with CD will progress to refractory CD[25]. Because of all of these limitations, there has been a growing interest in non-dietary treatment options in recent years, since the marketing of additional therapies could improve the response to GFD and reduce its social limitation.

NEW STRATEGIES TO TREAT CD

The therapeutic horizon for CD is expanding beyond strict adherence to a GFD, due to advances in our understanding of pathophysiology and the emergence of new pharmacologic interventions. Gluten includes two different types of proteins, namely gliadins and glutenins; the former are regarded as the main culprit in the pathogenesis of CD, and several studies have demonstrated that different portions of alpha-gliadin can trigger immunity. Indeed, amino acids 31-43 activate the innate immunity, whereas the 33-mer targets adaptive immunity[26]. Gliadins are characterized by repeated sequences of glutamines and prolamines, which are not easily processed by human digestive enzymes. This lack of digestion is particularly evident for the 33-mer peptide in CD subjects[26], allowing this peptide to cross the epithelial barrier, be deamidated by the enzyme tissue transglutaminase 2, and bind to human leukocyte antigen (HLA)-DQ2/8 molecules on antigen-presenting cells triggering gluten-specific cluster of differentiation 4 (CD4) T cells. These cells, in turn, activate CD8 T cells that cause small-intestinal mucosal injury. All of these processes are mediated by cytokines such as interferon gamma, tumor necrosis factor alpha, interleukin 2 (IL-2), IL-21, and IL-15[27].

Elucidation of the pathogenesis of CD has led to the identification of multiple possible therapeutic targets, enabling the development of innovative treatment strategies (Figure 1). These strategies can be broadly classified into quantitative approaches, which aim to reduce the gluten load that triggers the immune response, and qualitative approaches, which aim to modulate the immune system and promote gluten tolerance. Quantitative strategies are diverse (Table 1). They include the use of exogenous peptidases to enzymatically digest gluten into non-immunogenic fragments, the sequestration of gluten peptides in the intestinal lumen to prevent their interaction with the mucosal immune system, and the reduction of intestinal permeability to prevent the translocation of immunogenic peptides. Each of these strategies aims to mitigate the antigenic stimulus underlying the pathophysiologic response in CD.

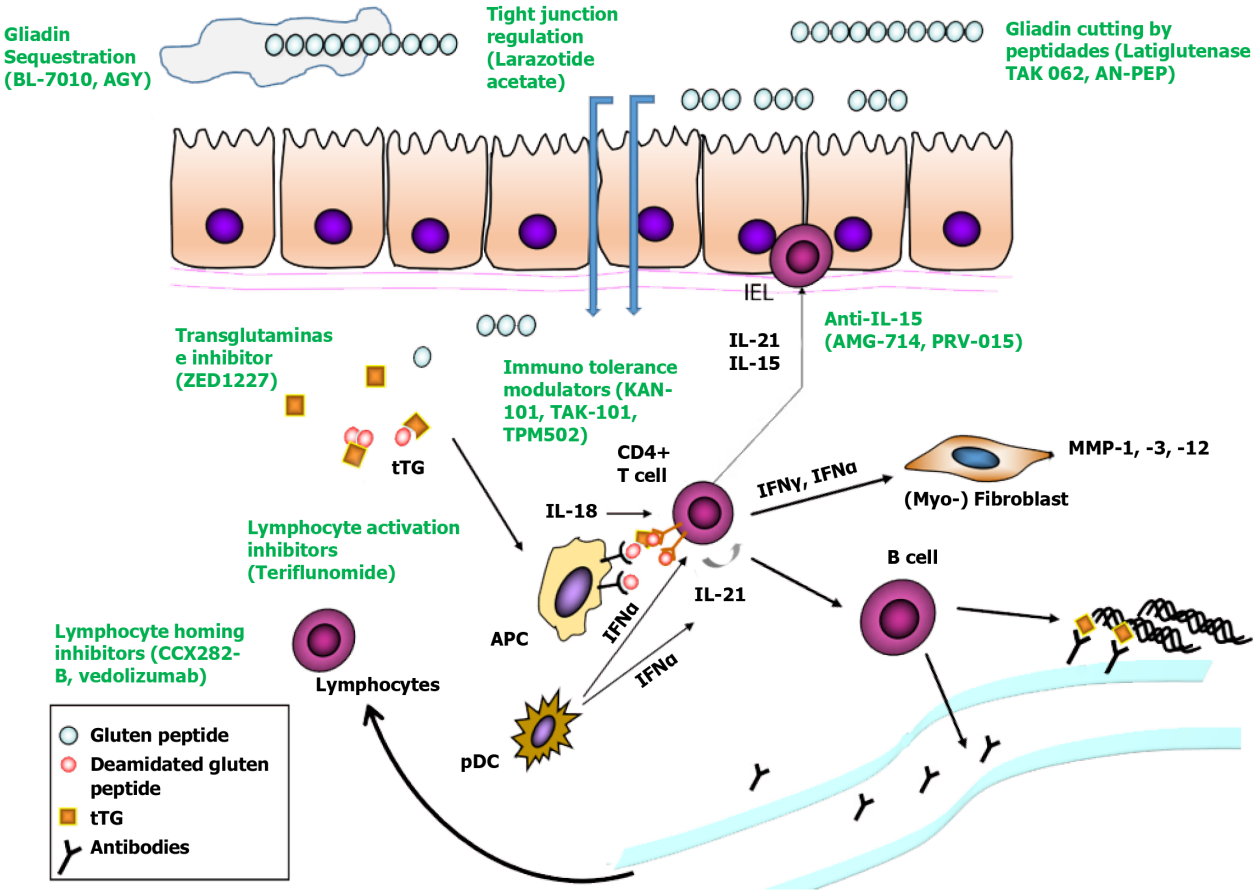

Figure 1 Mechanistic insights into innovative therapeutic approaches for celiac disease. APC: Antigen-presenting cell; IELs: Intraepithelial lymphocytes; IFN: Interferon; IL: Interleukin; MMP: Matrix metalloproteinase; pDC: Pre-dendritic cell; tTG: Tissue transglutaminase; TNF: Tumor necrosis factor.

Table 1 Phase 2 studies exploring quantitative strategies in patients with celiac disease

NCT number	Study title	Study status (completion date)	Drug	Mechanism	Primary outcome measures	Sponsor	Phases
NCT01990885	Safety and systemic exposure study of BL-7010 in patients with well-controlled CD	Completed (October 2014)	BL-7010 *vs* placebo	BL-7010 interacts with α-gliadin and prevents the formation of immunogenic and cytotoxic peptides	Incidence of adverse events. For part 1, subjects were followed for up to 7 weeks from time of first administration. For part 2, subjects were followed for up to 4 weeks from time of first administration	BioLineRx, Ltd.	Phase 1, Phase 2
NCT03707730 AGY-010	A randomized, double-blind, placebo-controlled, crossover trial to evaluate safety and efficacy of AGY in CD	Unknown (December 2022)	AGY *vs* placebo	IgY antibody put into capsule form (AGY), produced from the egg yolks of superimmunized laying hens	Safety (adverse events, laboratory results, symptoms). tTGA levels measured at each visit. CD- related symptoms 14 weeks	Igy Inc.	Phase 2
NCT01917630	Evaluation of the efficacy and safety of ALV003 in symptomatic patients with CD	Completed (June 2015)	Latiglutenase (ALV003) *vs* placebo	ALV003 is a mixture of two recombinant gluten-specific proteases to contribute to the degradation of gluten into non-immunogenic fragments	Efficacy: Intestinal mucosal morphometry, change in Vh:Cd between baseline and week 12	Alvine Pharmaceuticals Inc.	Phase 2
NCT01255696	Safety and efficacy of varying methods of ALV003 administration for the treatment of CD	Completed (June 2011)	Latiglutenase (ALV003) *vs* placebo	ALV003 is a mixture of two recombinant gluten-specific proteases to contribute to the degradation of gluten into non-immunogenic fragments	Efficacy: Intestinal mucosal morphology. Safety: Tolerability of ALV003, safety evaluated at 6 weeks	Alvine Pharmaceuticals Inc.	Phase 2
NCT00959114	Safety and efficacy of ALV003 for the treatment of CD	Completed (October 2010)	Latiglutenase (ALV003)	ALV003 is a mixture of two recombinant gluten-specific proteases to contribute to the degradation of gluten into non-immunogenic fragments	Efficacy: Intestinal mucosal morphology. Safety: Tolerability of ALV003 at 6 weeks	Alvine Pharmaceuticals Inc.	Phase 2
NCT01255696	Safety and efficacy of ALV003 for the treatment of CD	Completed (June 2011)	Latiglutenase (ALV003)	ALV003 is a mixture of two recombinant gluten-specific proteases to contribute to the degradation of gluten into non-immunogenic fragments	Efficacy: Intestinal mucosal morphology. Safety: Tolerability of ALV003 at 6 weeks	Alvine Pharmaceuticals Inc.	Phase 2
NCT03585478	Latiglutenase (IMGX003) as a treatment for CD	Completed (January 22, 2021)	Latiglutenase (IMGX003)*vs* placebo	A combination of ALV001 and ALV002, a *Sphingomonas capsulata* PEP that degrade gluten proteins and reduces the immunogenic potential of gluten	The primary efficacy endpoint of this study is histologic protection as measured by EGD (Vh:Cd) at 6 weeks	Immunogenics, LLC	Phase 2
NCT04243551	Prospective, randomized, double-blind, placebo-controlled, crossover study of latiglutenase (IMGX003) in symptomatic patients with CD	Active, not recruiting (December 2023)	Latiglutenase *vs* placebo	A combination of ALV001 and ALV002, a *Sphingomonas capsulata* PEP that degrade gluten proteins and reduces the immunogenic potential of gluten	The primary efficacy endpoint of this study is the mean percent reduction in symptom severity relative to placebo at 6 months	Immunogenics, LLC	Phase 2
NCT04839575	Study of latiglu-	Terminated	DRUG: Latiglu-	A combination of	The primary efficacy	Immunogenics	Phase 2

	tenase (IMGX003) in T1D/CD patients	(December 19 2022)	tenase *vs* placebo	ALV001 and ALV002, a *Sphingomonas capsulata* PEP that degrade gluten proteins and reduces the immunogenic potential of gluten	endpoint of this study is absolute mean reduction in symptom severity relative to placebo at 6 months	LLC	
NCT05353985	A study of TAK-062 in treatment of active CD in participants attempting a gluten-free diet	Recruiting (May 6, 2025)	TAK-062 with or without simulated inadvertent gluten exposure gluten-bar	Third-generation enzyme with the ability to degrade > 99% of gluten and gluten peptides	Change in weekly CD symptom diary gastrointestinal symptom severity score from baseline (week 1) to week 12	Takeda	Phase 2
NCT00810654	Effect of *Aspergillus Niger* prolyl endoprotease (AN-PEP) enzyme on the effects of gluten ingestion in patients with CD	Completed (2009-12)	AN-PEP 160 PPU daily for 2 weeks	An enzyme that degrades both whole gluten and gluten peptides into non-immunogenic residues within minutes	Histopathological changes according to the modified marsh criteria. The presence of CD-specific antibodies (EMA, tTGA, gliadin) (1 week before start, and 2 and 6 weeks after start)	Amsterdam UMC, location VUmc	Phase 1, phase 2

AN-PEP: *Aspergillus Niger* prolyl endoprotease; CD: Celiac disease; EGD: Esophagogastroduodenoscopy; EMA: Endomysial antibodies; IgY: Avian immunoglobulin Y; PEP: Prolyl endopeptidase; T1D: Type 1 diabetes; tTGA: Tissue transglutaminase IgA; Vh:Cd: Villous height to crypt depth ratio.

On the other hand, the qualitative approaches encompass a spectrum of modalities that alter the immune system's engagement with gluten. These include the inhibition of tissue transglutaminase, which plays a crucial role in the post-translational modification of gluten peptides, thereby reducing the formation of highly immunogenic complexes. In addition, the modulation of lymphocyte migration and homing offers an opportunity to prevent the recruitment and retention of inflammatory cells in the intestinal mucosa.

New research is also addressing the potential of desensitization to gluten through advanced biotechnological methods, such as nanoparticles engineered for targeted gliadin presentation, conjugation of gluten peptides to erythrocyte membranes, and therapeutic vaccines aimed at recalibrating the immune response. In addition, the paradigm of using helminth infestation to exploit natural pathways of immune regulation represents a novel and intriguing avenue of investigation.

EMERGING THERAPIES FROM PHASE 2 TRIALS

The pursuit of novel therapeutics in the treatment of CD has led to the initiation and progression of multiple Phase 2 clinical trials.

Gluten sequestration and degradation

Gluten sequestration and degradation strategies, with a focus on enzymatic approaches, have been extensively investigated to mitigate the effects of gluten exposure in CD. These therapeutic interventions include several notable studies (Table 1). Regarding gluten sequestration, BL-7010 is a high molecular weight, non-absorbable polymer with a high affinity for gliadin[28], which is thus able to prevent the absorption of immunogenic and cytotoxic peptides. In fact, by targeting these peptides, BL-7010 could play a crucial role in reducing the inflammatory and immunogenic responses characteristic of CD. *In vitro* studies have demonstrated that BL-7010 is effective in decreasing gliadin/gluten-induced damage in cell cultures[29]. These data have also been confirmed in a mouse model expressing HLA-HCD4/DQ8 sensitized to gluten sensitization[28-30]. This drug has been assessed in NCT01990885, a randomized, double-blind study designed to evaluate the safety of single escalating doses as well as repeated administrations of BL-7010 in patients with well-controlled CD. Although the trial took place about 10 years ago, these results have not yet been published.

Another novel approach is the use of specific antibodies, such as avian immunoglobulin Y (IgY). IgY antibodies are obtained from the egg yolks of superimmunized laying hens. These antibodies are natural products with minimal toxicity, except in people with an egg allergy. They also offer a cost-effective and hygienic method of producing therapeutic agents. When the IgY antibody is formulated in capsules, it is referred to as AGY. The idea is to use these antibodies to capture gliadin peptides present in food. The NCT01765647 trial enrolled 10 patients to evaluate the potential of AGY to relieve CD symptoms and potentially reduce the burden of strict dietary control. However, the results of this study were too weak to draw definitive conclusions. The NCT03707730 trial is a randomized, double-blind, placebo-controlled, crossover study evaluating the safety and efficacy of AGY in patients with CD on GFD. The study has enrolled 169 patients, but the data are not currently available. As aforementioned, the amino acid composition of gluten in general, and of gliadins in particular, represents a difficult task for human digestive enzymes. Thus, the quantitative reduction of food gluten content relies on enzymes derived from different sources (Table 1).

Latiglutenase is a combination of two glutenases, endoprotease B, isoform-2 (EP-B2), and *Sphingomonas capsulata* prolyl endopeptidase (SC-PEP). EP-B2 is active at low pH and has specificity for the QXP sequence, abundant both in the 31-43

and 33-mer peptides[31]. SC-PEP is a proline-specific endoprotease (PEP) that attacks the carboxy end of the gliadin peptides. The two enzymes can thus act together, with EP-B2 cutting the 33-mer peptides into smaller fragments and PEP digesting their proline-glutamine links[32]. Latiglutenase (ALV003) has been investigated in various studies. The NCT01917630 Phase 2b study examined the effects of different doses of ALV003 over 12 weeks on the small intestinal mucosa and symptoms in patients with CD. The results showed no significant differences in the primary endpoint - villous height to crypt depth (Vh:Cd) ratio - nor in secondary endpoints such as intraepithelial lymphocyte counts and serologic markers between the latiglutenase and placebo groups. A post hoc analysis[33] in a subgroup of patients still positive for autoantibodies indicated a dose-dependent reduction in symptom severity, especially at the highest dose (900 mg), suggesting potential benefits for seropositive patients.

Two other studies, *i.e.* NCT01255696 and NCT00959114, investigated the efficacy, safety, and tolerability of ALV003 in patients with well-controlled CD. In NCT00959114, patients receiving ALV003 showed no significant mucosal damage after gluten challenge (2 g bread crumbs) in contrast to the placebo group, which showed signs of mucosal deterioration. Morphological changes and the number of CD3+ intraepithelial lymphocytes showed significant differences between the groups, underlining the potential of ALV003 to mitigate gluten-induced intestinal damage[34]. Similarly, NCT01255696, a Phase 2a, double-blind, placebo-controlled study, evaluated the safety, efficacy and tolerability of 6 weeks of treatment with ALV003 in patients with well-controlled CD. In summary, these studies suggest that while ALV003 does not significantly alter histologic or serologic markers of the disease in a broad cohort, it is able to mitigate gluten-related mucosal damage in patients with CD and alleviate symptoms, particularly in seropositive individuals.

Latiglutenase was employed in three other studies: NCT03585478 was a Phase 2 double-blind, placebo-controlled study assessing the efficacy and safety of a 1200 mg dose of IMGX003 in patients with CD. Participants were treated with 2 g gluten daily for 6 weeks. The primary endpoint was the change in Vh:Cd ratio, and the results indicated a lower mean change in the Vh:Cd ratio and intraepithelial lymphocyte density for IMGX003 compared to placebo, alongside reduced symptom severity[35]. NCT04243551 is a Phase 2b, multicenter, randomized, double-blind, placebo-controlled, crossover study involving symptomatic patients with CD who had been on a GFD for at least 1 year prior to the study. The study has been completed and the results are currently awaited. NCT04839575 is a prospective, double-blind, placebo-controlled, crossover study investigating the efficacy and safety of latiglutenase treatment in patients with type 1 diabetes with CD on regular gluten exposure. It was terminated due to coronavirus disease 2019 disruptions and enrollment challenges.

TAK-062 is a computer-designed enzyme based on the bacterial kumamolisin-As, obtained from *Alicyclobacillus sendaiensis*. Interestingly, the modifications enable this enzyme to target the proline-glutamine dipeptide. Its efficacy was assessed both *in vitro* and, in Phase 1 *in vivo*, showing that it is able to degrade more than 99% of gluten in complex meals [36]. These latter data are quite interesting since the assessment of gluten degradation was demonstrated by analyzing the aspirate of the stomach content, thus in a physiological condition. NCT05353985 is a Phase 2, randomized, double-blind, placebo-controlled, dose-ranging study evaluating the efficacy and safety of TAK-062 in reducing CD-related symptoms and intestinal damage in CD patients attempting a GFD. The study includes two cohorts with different treatment groups receiving TAK-062 or placebo along with simulated inadvertent gluten exposure. The multicenter study, conducted across the United States, Canada, United Kingdom, and the European Union, includes adult and adolescent participants and is ongoing.

Aspergillus niger-derived prolyl endoprotease (AN-PEP) is able to cleave immunogenic gliadin peptides (behind proline residues) into smaller, non-immunogenic peptides of about eight amino acids. This enzyme is active between a pH of 2 and 8 and is resistant to pepsin; thus, it is apt to be used to degrade gliadin ingested with food[37-39]. AN-PEP was investigated in the NCT00810654[40] trial that involved only 16 adults. AN-PEP was well tolerated and there were no serious adverse events or withdrawals. The efficacy phase showed no significant worsening of CD quality scores or antibody titers in patients who consumed gluten with either placebo or AN-PEP. Histologic and immunohistochemical evaluations also indicated stability in participants taking gluten with AN-PEP. The study concluded that AN-PEP was well tolerated, but the lack of clinical differences compared to placebo made it difficult to determine the effect of the enzyme. A double-blind, randomized, placebo-controlled trial employing commercial AN-PEP on patients on GFD has recently been published[41]. Although the patients in the treatment arm showed a reduction in symptoms, no significant difference in the level of gliadin immunogenic peptide in the stools was observed, data that could be explained by the relatively low levels detected in the run-in period. Different preparations of this enzyme are already available over the counter (OTC), but their ability to digest gluten peptides can be different, and in some cases, inferior to the pure enzyme [42]. However, patients with CD should be warned of the potential risks of relying on these OTC products, as their effectiveness may not be sufficient to prevent gluten-related damage.

Gluten tolerance

These therapies are designed to induce some form of tolerance to gluten in individuals with CD, possibly through immunomodulatory mechanisms or other pathways that reduce the pathological response to gluten. This approach does not necessarily involve the direct breakdown or sequestration of gluten but instead modifies the body's response to its presence.

A first-in-human Phase 1 study (NCT04248855)[43], performed in patients with CD on GFD, evaluated the safety and tolerability of KAN-101, a synthetic liver-targeting glycopolymer that is conjugated to a synthetic deaminated peptide domain of wheat alpha gliadin designed to induce immune tolerance to gliadin. Due to the specifics of the employed peptide, this approach is reserved to individuals carrying the HLA-DQ2.5 genotype, and the drug must be administered through the intravenous route. The mechanisms detected in preclinical studies through which KAN-101 could induce immunologic tolerance include selection of antigen-specific T cells, induction of anergy of antigen-specific T cells, and induction of regulatory T cells. The study demonstrated that KAN-101 (at increasing doses of 0.15 mg/kg, 0.3 mg/kg, 0.6

mg/kg, 1.2 mg/kg, and 1.5 mg/kg) exhibited an acceptable safety profile without any dose-limiting toxicities, and no maximum tolerated dose was identified. The rapid clearance of KAN-101 from the system and the absence of accumulation with repeated doses underscore the potential for chronic use.

Two studies focusing on KAN-101 are actively recruiting (Table 2). The first study, KAN-101-03 (NCT06001177), is a multicenter, double-blind, placebo-controlled Phase 2a trial. Its primary objective is to examine the protective effects of KAN-101 against gluten-induced histological changes in the duodenum of adult participants with CD who adhere to a GFD. Additionally, the study aims to further evaluate the safety and tolerability profile of KAN-101. In parallel, study NCT05574010 adopts a two-part, multicenter Phase 1b/2 design to evaluate the effects of KAN-101 in participants with CD on a GFD. Part A consists of an open-label, multiple ascending dose assessment to determine the safety, tolerability, and pharmacokinetics of KAN-101 in adults with histologically confirmed CD. Part B progresses to a double-blind, placebo-controlled format to characterize biomarker responses post-gluten challenge, alongside further safety, tolerability, and pharmacokinetic assessments.

The investigation of gluten tolerance in CD has led to the initiation of studies on TAK-101, which consists of gliadin encapsulated in negatively charged poly(DL-lactideco-glycolic acid) nanoparticles (Table 2). These nanoparticles, intravenously injected, are taken up by positive antigen-presenting cells localized in the liver and in the spleen. The presentation of gliadin to gliadin-specific T cells can induce tolerance through anergy and the activation of regulatory T cells. The initial clinical assessment of TAK-101 involved a Phase 1 dose-escalation study (NCT03486990). The study's outcomes indicated that TAK-101 was well-tolerated, with no serious adverse events, clinically meaningful changes in vital signs or routine clinical laboratory evaluations, indicating an acceptable safety profile[36]. The double-blind, randomized, placebo-controlled Phase 2a study (NCT03738475) was pivotal in evaluating the efficacy of TAK-101 in attenuating gluten-induced immune activation in CD. Thirty-three patients on GFD underwent a 14-day gluten challenge, with the primary endpoint being the change from baseline in circulating gliadin-specific interferon gamma (IFN-g)-producing cells, which directly correlates with the pathophysiologic immune response in CD. TAK-101 administration resulted in an 88% reduction in IFN- g-producing units compared to placebo, a statistically significant finding (P = 0.006) indicating a strong immunomodulatory effect. In addition, Vh:Cd ratio analysis revealed less deterioration in the TAK-101 group compared to placebo, although the difference did not reach statistical significance. TAK-101 also showed efficacy in modulating circulating α4β7+CD4+, αEβ7+CD8+, and gδ effector memory T cells, suggesting systemic immunomodulation[44]. Further investigation of TAK-101 is being conducted in a subsequent Phase 2 study (NCT04530123), a randomized, double-blind, placebo-controlled and dose-ranging study. This study aims to evaluate the efficacy of TAK-101 in reducing gluten-related symptoms and immune activation in adult patients with CD following a GFD and undergoing a gluten challenge; its primary completion date was expected in May 2024.

Another drug aiming at inducing gluten-tolerance is TPM502, a mixture of nanoparticles carrying three peptides each consisting of two overlapping T-cell epitopes that encompass the major gluten epitopes for HLA-DQ2.5. The ongoing NCT05660109 Phase 2a study aims to evaluate its safety, tolerability, and pharmacodynamic effects according to different doses; its primary completion date was expected in May 2024 (Table 2).

Although the studies described above have provided encouraging data, Nexvax2® is among the drugs aiming at inducing tolerance. It is a gluten peptide-based antigen-specific immunotherapy that aims to desensitize and make T cells unresponsive to gluten exposure. The first Phase 1 clinical trial (NCT00879749) confirmed its bioactivity; however a following randomized clinical trial did not show any advantage in preventing intestinal damage[27,45]. Furthermore, a Phase 2 clinical trial (NCT03644069) evaluating its efficacy on patient-reported outcomes was terminated prematurely after an interim analysis because Nexvax2® was not able to reduce acute gluten-induced symptoms.

Tight junction modulators

Research into modulators of the tight junctions in CD has focused primarily on larazotide acetate, a synthetic octapeptide that reduces the permeability of tight junctions by blocking zonulin receptors, thus preventing the opening of tight junctions in the intestinal epithelium and thereby reducing the passage of gluten peptides and the consequent immune activation[46]. Changes in zonulin levels are already detectable in the very early stages of CD and could serve as early biomarkers for the disease[47,48]. In addition to zonulin-dependent mechanisms, research has also identified zonulin-independent constitutional changes in intestinal permeability in patients with CD and their first-degree relatives[47]. These inherent permeability alterations may contribute to the development and progression of CD[47,49].

However, several Phase 2 studies have investigated the efficacy and safety of larazotide (Table 3). A Phase 2b study (NCT00492960) included 184 patients on GFD[50]. Participants received larazotide acetate (1 mg, 4 mg, or 8 mg three times daily) or placebo along with 2.7 g of gluten daily for 6 weeks. Although no significant changes in the lactulose to mannitol ratio were detected, the researchers observed that the 1 mg dose of larazotide acetate significantly reduced gluten-induced symptoms, as well as the increment in anti-tissue transglutaminase antibodies caused by gluten challenge. Thus, the study suggested that larazotide acetate may reduce gluten-induced immunoreactivity and symptoms in patients with CD undergoing gluten challenge. In the NCT00362856 study, a dose-ranging, placebo-controlled study, larazotide acetate limited gluten-induced worsening of gastrointestinal symptoms at lower doses, but not at higher doses. The study concluded that while larazotide acetate has the potential to prevent the severity of gluten-induced symptoms, its effects on intestinal permeability are unclear due to the variability of lactulose to mannitol[51]. Similarly, in a double-blind, placebo-controlled Phase 2B study (NCT01396213), three doses of larazotide acetate were evaluated as an adjunct therapy to GFD in CD patients. The 0.5 mg dose of larazotide acetate significantly reduced symptoms compared to placebo. The study concluded that larazotide acetate 0.5 mg effectively reduced signs and symptoms in CD patients adhering to a GFD, representing a successful trial of a novel therapeutic agent targeting tight junction regulation[52]. Another study (NCT00620451) evaluated the efficacy of larazotide acetate in CD. This outpatient, randomized, double-blind study aimed to evaluate the efficacy of larazotide acetate in inducing remission in active CD, but did not provide

Table 2 Phase 2 studies exploring gluten tolerance strategies in patients with celiac disease

NCT number (Acronym)	Study title	Study status (Completion date)	Drug	Mechanism	Primary outcome measures	Sponsor	Phases
NCT06001177 (SynCeD)	A study of efficacy, safety, and tolerability of KAN-101 in people with CD	Recruiting (June 2025)	KAN-101 *vs* placebo	KAN-101 acts by re-educating T cells, or tolerizing them, so they do not respond to gluten antigens	Changes from baseline in Vh:Cd as assessed by esophago-gastroduodenoscopy with biopsy after 2-week gluten challenge	Kanyos Bio, Inc., a wholly-owned subsidiary of Anokion SA	Phase 2
NCT05574010 (ACeD-it)	A study of safety, tolerability, pharmaco-dynamics, and pharmacokinetics of KAN-101 in CD	Recruiting (April 2, 2025)	Part A: Multiple ascending dose of KAN-101. Parts B and C: Participants will be randomized 1:1:1:1 to placebo and 3 treatment groups with KAN-101 doses based on information obtained from part A	KAN-101 acts by re-educating T cells, or tolerizing them, so they do not respond to gluten antigens	Severity of TEAEs assessed by common terminology criteria for adverse events (part A) at 28 days. Efficacy assessed by change in magnitude of IL-2 response pre- and post-germinal center (part B), baseline to day 15	Kanyos Bio, Inc., a wholly owned subsidiary of Anokion SA	Phase 1, Phase 2
NCT03738475	Study of the safety, pharmacodynamic, efficacy, and PK of TAK-101 in subjects with CD	Completed (July 22, 2019)	TAK-101 *vs* placebo	TAK-101, gliadin encapsulated in nanoparticles to induce gluten-specific tolerance	Change from baseline in interferon gamma spot-forming units based on results of a gliadin-specific enzyme-linked immunospot on day 20	Takeda	Phase 2
NCT04530123	Dose-ranging study of the efficacy and safety of TAK-101 for prevention of gluten-specific T-cell activation in participants with CD on a gluten-free diet	Active, not recruiting (May 29, 2024)	TAK-101 with or without gluten	TAK-101, gliadin encapsulated in nanoparticles to induce gluten-specific tolerance	Change from baseline in interferon gamma spot-forming units based on results of a gliadin-specific enzyme-linked immunospot on day 20	Takeda	Phase 2
NCT05660109	A study to assess the safety of TPM502 in adults with CD	Recruiting (May 30, 2024)	Drug: TPM502. Other: Placebo	TPM502 is a mixture of nanoparticles carrying gluten-specific antigenic peptides to the liver, to induce gluten tolerization	Incidence, severity, causality, and outcomes of TEAEs throughout the study, on average 43 days	Topas Therapeutics GmbH	Phase 2
NCT03644069	A study of the safety, efficacy and tolerability of Nexvax-2 in patients with CD	Unknown (September 2019)	Nexvax2 *vs* placebo	Nexvax2 is a therapeutic vaccine that desensitizes and induces gluten tolerance	Efficacy of Nexvax2 in reducing CD-associated GI symptoms, measured by the CD patient-reported outcome between baseline and the day of the first masked food challenge containing gluten	ImmusanT, Inc.	Phase 2

CD: Celiac disease; GI: Gastrointestinal; IL: Interleukin; TEAEs: Treatment-emergent adverse events; Vh:Cd: Villous height to crypt depth ratio.

specific results. Again, the study NCT00889473, an extension of study NCT00492960, aimed to evaluate the safety, tolerability and efficacy of larazotide acetate in a gluten challenge setting. Specific results were not presented.

Overall, these studies highlight the potential of larazotide acetate as a therapeutic agent for symptomatic relief in patients adhering to a GFD. Although results were mixed regarding its effects on intestinal permeability, its ability to alleviate gluten-related symptoms seemed to offer a promising avenue for improving the quality of life of people with CD. Thus, the findings from these Phase 2 studies laid the groundwork for further exploration in Phase 3 trials.

Table 3 Phase 2 studies on tight junction modulator (larazotide acetate) in patients with celiac disease

NCT number	Study title	Study status (Completion date)	Drug	Mechanism	Primary outcome measures	Sponsor	Phases
NCT00492960	Study to assess the efficacy of larazotide acetate for the treatment of CD	Completed (March 2009)	Larazotide acetate *vs* placebo (dietary supplement: 900 mg gluten)	Larazotide acetate intervenes by blocking the zonulin receptors and thus preventing the dissolution of the tight junction and the associated increase in intestinal permeability	Efficacy of multiple doses larazotide acetate in preventing intestinal permeability changes induced by a 6-week gluten challenge on days 7, 21, 35, 49, and 56	9 Meters Biopharma Inc.	Phase 2
NCT00362856	Safety and tolerability study of larazotide acetate in patients with CD	Completed (March 6, 2007)	Larazotide *vs* placebo	Larazotide acetate intervenes by blocking the zonulin receptors and thus preventing the dissolution of the tight junction and the associated increase in intestinal permeability	Safety endpoints assessed were adverse events. Measured at screening and on days 0, 7, 14, and 21 ('End of Study'). Efficacy of multiple dose levels of larazotide acetate in preventing intestinal permeability changes induced by gluten challenge, measured as urinary LAMA ratio the day 0-to-day 14 change	9 Meters Biopharma, Inc.	Phase 2
NCT01396213	A double-blind placebo-controlled study to evaluate larazotide acetate for the treatment of CD	Completed (August 20, 2013)	Larazotide *vs* placebo	Larazotide acetate intervenes by blocking the zonulin receptors thus preventing the dissolution of the tight junction and the associated increase in intestinal permeability	The primary efficacy endpoint was the changes in the average on-treatment (baseline to week 12) score of the CD gastrointestinal symptom rating scale	9 Meters Biopharma, Inc.	Phase 2
NCT00620451	Randomized, double-blind, placebo-controlled study of larazotide acetate in subjects with active CD	Completed (December 2009)	Larazotide acetate *vs* placebo	Larazotide acetate intervenes by blocking the zonulin receptors and thus preventing the dissolution of the tight junction and the associated increase in intestinal permeability	Assess the efficacy of larazotide acetate. Remission was defined as an improvement in the Vh:Cd ratio obtained by duodenojejunal biopsy at baseline and day 56	9 Meters Biopharma, Inc.	Phase 2
NCT00889473	Study of the efficacy of larazotide acetate in CD	Completed (April 2010)	Larazotide acetate *vs* placebo (dietary supplement: gluten 900 mg)	Larazotide acetate intervenes by blocking the zonulin receptors and thus preventing the dissolution of the tight junction and the associated increase in intestinal permeability	Response to gluten at 6 weeks	9 Meters Biopharma, Inc.	Phase 2

CD: Celiac disease; LAMA: Lactulose to mannitol; Vh:Cd: Villous height to crypt depth ratio.

Transglutaminase inhibitors

Transglutaminase, modifying gluten peptides, is essential for gluten-induced T-cell activation, and the possibility to inhibit it has been widely studied. The more promising molecule in this setting is ZED 1227, assessed by Schuppan *et al* [53] in a Phase 2, double-blind, placebo-controlled trial. In this trial, 163 patients were randomly assigned to receive 10 mg ZED 1227, 50 mg ZED 1227, 100 mg ZED 1227, or placebo during gluten challenge with a moderate amount (3 g) of daily gluten intake for 6 weeks. The primary endpoint was Vh:Cd ratio, as a marker of mucosal damage. The secondary endpoints included intraepithelial lymphocyte density, the modified Marsh-Oberhuber classification, and patient-reported outcomes measured by the Celiac Symptom Index and the Celiac Disease Questionnaire. ZED1227 significantly improved Vh:Cd ratio and attenuated intraepithelial lymphocyte density dose dependently, whereas improved Celiac Symptom Index and the Celiac Disease Questionnaire independently to the dose[53]. Due to the activity of transglutaminase in several tissues, it was important to assess that the effect was limited to the intestine. For this reason, the same group assessed the loading of ZED 1227 in the biopsies of patients treated in the Phase 2 study, showing the presence of the drug mostly in the epithelium (about 80%), with only about 20% present in the lamina propria. These data also prompted the authors to hypothesize that the drug exerts its effect mainly through an inhibition at the epithelial level [54].

Lymphocytes' trafficking and homing inhibitors

Strategies for immune modulation can include inhibition of lymphocyte proliferation, inhibition of lymphocyte trafficking and homing to the small bowel, and inhibition of the anti-inflammatory response (Table 4). CCX282-B is an

Table 4 Phase 2 studies exploring lymphocyte trafficking and homing inhibitors

NCT number	Study title	Study status (Completion date)	Drug	Mechanism	Primary outcome measures	Sponsor	Phases
NCT00540657	A Phase 2 study of CCX282-B in patients with CD	Completed (July 2008)	CCX282 *vs* placebo	CCX282-B is a chemokine receptor CCR9 antagonist that regulates migration and activation of inflammatory cells in the intestine	Evaluation of the effect of CCX282-B compared to placebo on the Vh:Cd ratio of small intestinal biopsy specimens taken from patients with CD, before and after gluten exposure	ChemoCentryx	Phase 2
NCT02929316	Vedolizumab induction may prevent celiac enteritis	Terminated (October 5, 2018)	Vedolizumab	Vedolizumab is a monoclonal antibody against integrin α4β7 that inhibit lymphocyte homing to the bowel	Histopathologic remission following induction with vedolizumab (defined as negative CD antibodies and normal duodenal biopsies) at 12 weeks	AGA Clinical Research Associates, LLC	Phase 2
NCT04806737	A Phase 2a, double-blind, randomized, placebo-controlled study on the efficacy and tolerability of a 14-day treatment with teriflunomide *vs* placebo in subjects with coeliac disease undergoing a 3-day gluten challenge	Unknown (August 15, 2022)	Oral teriflunomide *vs* placebo	Teriflunomide inhibits *de novo* synthesis of pyrimidine, performing a cytostatic effect on lymphocyte proliferation	Check the adaptive T-cell activation, evaluating the expression of CD38 on HLA-DQ: Gluten tetramer-positive T cells on peripheral blood on day 4 after a 3-day gluten challenge	Oslo University Hospital	Phase 1-phase 2
NCT02637141	A Phase 2a, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the efficacy and safety of AMG 714 in adult patients with CD	Completed (May 2, 2017)	AMG 714 *vs* placebo	AMG 714 is a monoclonal antibody an anti-IL-15, a pivotal cytokine in CD pathogenesis	Percent change from baseline in Vh:CD. To evaluate the attenuation of the effects of gluten exposure after 10 weeks of gluten challenge at week 12	Amgen	Phase 2
NCT02633020	A Phase 2a, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the efficacy and safety of AMG 714 in adult patients with type II refractory CD	Completed (May 2, 2017)	AMG 714 *vs* placebo	AMG 714 is a monoclonal antibody anti-IL-15	Percent change from baseline in the percentage of aberrant intestinal intraepithelial lymphocytes with respect to all intraepithelial lymphocytes at baseline and week 12	Amgen	Phase 2
NCT04424927 proactive	A Phase 2b, randomized, double-blind, placebo-controlled, parallel-group study to evaluate the efficacy and safety of PRV-015 in adult patients with non-responsive CD as an adjunct to a gluten-free diet	Recruiting (August 31, 2024)	PRV-015 *vs* placebo	PRV-015 is a monoclonal antibody against IL-15, a pivotal cytokine in CD pathogenesis	Efficacy of PRV-015 in attenuating the symptoms of CD in non-responsive CD as measured by the celiac disease patient-reported outcome questionnaire at 24 weeks	Provention Bio, Inc.	Phase 2
NCT05636293	Double-blind, placebo-controlled trial to establish safety and efficacy of ritlecitinib to prevent gluten-induced celiac enteropathy and symptoms in CD patients in remission	Recruiting (January 1, 2025)	Ritlecitinib *vs* placebo	Ritlecitinib is a selective Janus kinase 3 inhibitors that prevent lymphocyte activation and proliferation	Change in small intestinal histology based on Vh:Cd ratio to characterize the gluten-challenge induced changes. Patient-reported outcomes defined as CD PRO to evaluate gluten challenge-triggered symptoms. Through study completion, average of 1 year	Massachusetts General Hospital	Phase 2

CD: Celiac disease; CCR9: C-C chemokine receptor type 9; IL: Interleukin; PROs: Patient-reported outcomes; Vh:Cd: Villous height to crypt depth ratio.

orally administrated C-C chemokine receptor type 9 (CCR9) antagonist previously studied for the treatment of Crohn's disease, in which it generated contrasting results. CCR9 is expressed on circulating lymphocytes and is the key chemokine receptor determining intestinal homing[55,56]. The ligand of CCR9 is C-C chemokine ligand 25, which is expressed in the intestinal epithelium and is upregulated in the presence of inflammation[57]. A double-blind, randomized, placebo-controlled, Phase 2 study (NCT00540657) evaluated its effectiveness in mitigating the effects of gluten ingestion in patients with CD. Ninety patients were enrolled, and half of them were treated by CCX282-B 250 mg twice daily for 13 weeks. The primary outcome was the evaluation of the effect of CCX282-B compared to placebo on the Vh:Cd ratio of small intestinal biopsy specimens taken from subjects with CD, before and after gluten exposure. Secondary outcomes were the evaluation of small intestinal mucosal inflammation, celiac serology, and symptom scores. Although the study was completed in 2008, its results have never been published.

Another component necessary for the "gut-homing phenotype" is constituted by α4β7-integrin, belonging to a heterodimeric noncovalently bound transmembrane receptor family involved in cell-cell and cell-extracellular matrix interactions. This specific heterodimer is present in more than 90% of intestinal lymphocytes, and its main ligand is the mucosal addressin cell adhesion molecule, present in the gastrointestinal tract and associated lymphoid tissue[58,59]. For this reason, a Phase 2 study (NCT02929316) aimed at evaluating if vedolizumab, a well-known monoclonal antibody against integrin α4β7, approved for the treatment of ulcerative colitis and Crohn's disease, could prevent small bowel atrophy in patients with CD after gluten challenge. However, this study was stopped in October 2018, due to lack of enrollment. Another approach could be preventing lymphocyte proliferation, possibly focusing on intestinal ones reacting to gluten peptides. In 2021 a Phase 2a, double-blind, randomized, placebo-controlled study (NCT04806737) evaluated the efficacy and tolerability of a 14-day treatment with teriflunomide in 15 subjects with CD, undergoing a 3-day gluten challenge. Teriflunomide is an orally administrated drug currently approved for the treatment of multiple sclerosis. It inhibits *de novo* synthesis of pyrimidine, performing a cytostatic effect on lymphocyte proliferation[60]. Even in this case, results are not available.

Lymphocytes involved in the pathogenesis of CD can be prompted to induce intestinal damage through the production of different mediators, including cytokines. Among them, a pivotal role has been recognized for IL-15, which can act on several cell types, including intraepithelial lymphocytes. A double-blind, Phase 2a trial (NCT02637141) investigated the effect of AMG-714, an anti-IL-15 monoclonal antibody, in patients with CD undergoing gluten challenge. In this study, 64 patients were randomly assigned to 150 mg AMG 714, 300 mg AMG 714, or placebo, administered by two subcutaneous injections every 2 weeks for 10 weeks. Patients without severe villous atrophy at baseline received also a gluten challenge. Duodenal biopsies were done at baseline and at the end of the study, in order to evaluate change in Vh:Cd ratio as primary outcome. Secondary outcomes included intraepithelial lymphocyte density, improvement in Marsh-Oberhuber score, changes in anti-transglutaminase and anti-deaminated gliadin peptide antibodies, number of bowel movements, percentage of diarrhea and changes in Gastrointestinal Symptom Rating Scale Score and in total Celiac Disease Gastrointestinal Symptom Rating Scale, questionnaires used to assess symptoms as diarrhea, indigestion, constipation, abdominal pain and reflux. The study demonstrated that Vh:Cd ratio was not significantly different between the groups of patients. However, changes in lymphocyte density and in symptoms suggest that further research of AMG 714 may be warranted in patients with non-responsive CD[61].

In fact, due to these encouraging results, the NCT02633020 trial evaluated the efficacy and safety of AMG 714 in patients with type II refractory CD. In this study 28 refractory patients with CD were randomly assigned to 8 mg/kg AMG 714 or placebo intravenous infusion on day 0, day 7, and every 2 weeks for 10 weeks. The primary outcome was to evaluate the reduction from baseline of aberrant intestinal intraepithelial lymphocytes, measured by flow cytometry after small intestinal biopsy collection. According to the study, there was no difference between the groups in terms of the primary endpoint of aberrant intraepithelial lymphocyte reduction from baseline, but there was a reduction of the number of patients with diarrhea[62].

Another ongoing Phase 2b trial (NCT04424927) is evaluating the efficacy and safety in adult patients with non responder CD on a GFD of three-dose regimens of PRV-015, which is also a monoclonal antibody against IL-15. This study is expected to be completed in August 2024. The ongoing NCT05636293 trial aims to establish the safety and efficacy of ritlecitinib, a selective Janus kinase 3 (JAK3) inhibitor, to prevent gluten-induced enteropathy and symptoms in patients with CD. JAK is a family of non-receptor tyrosine kinases, which include, in mammals, JAK1, JAK2, JAK3, and tyrosine kinase 2. Each protein has a kinase domain and binds cytokine receptors through amino terminal domains. Upon binding of the ligand to cytokine receptors, JAKs are activated and phosphorylate the receptors, allowing the binding of the signal transducer and activator of transcription family members[63]. In addition to the inflammatory response, several studies have demonstrated that JAKs are essential for intestine differentiation and damage repair[64-66]. IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 depend on both JAK1 and JAK3 to elicit their intracellular effects, and JAK3 is expressed in immune cells as well as in intestinal epithelial cells. In the trial, participants are randomized to placebo or ritlecitinib 200 mg capsule once per day and are also taking 10 g gluten once per day, for a total of 21 days, decreasing to 5 g daily after day 3 of the study if not tolerated. The primary outcome is to evaluate changes in small bowel histology based on Vh:Cd ratio, while the secondary outcomes are focused on patient-reported outcomes. The study will finish in 2025.

Helminth infestation

According to the "hygiene hypothesis", a reduction in the incidence of infectious diseases, especially of the helminth ones, can be responsible for the increasing prevalence of allergic and autoimmune diseases. From this scenario, studies arose about the possibility of suppressing the immunopathology induced by gluten and restoring tolerance in CD-inoculating patients with hookworms. NCT00671138 was a prospective, randomized, double-blinded, placebo-controlled Phase 2 trial evaluating the safety, tolerability and immunological effects of *Necator Americanus* infection in subjects with CD in remission on GFD during gluten challenge. This trial enrolled 20 patients: 10 were inoculated with hookworm and

compared with the other 10 uninfected patients. Duodenal and rectal biopsies were performed before and after gluten challenge; blood samples were also collected at the same time. Mucosal damage, systemic inflammatory response, clinical response to gluten challenge, and mucosal inflammatory response did not differ between the two groups of patients before and after gluten challenge. So even if hookworm infection is safe, it was not able to mitigate the small bowel damage induced by gluten[67]. On the other hand, a subsequent Phase 2 study (NCT01661933) aiming to establish the influence of hookworm infection in preventing intestinal damage and symptoms using escalating gluten challenges, suggested that it could promote immune regulation, provoking tolerance to gluten in CD. A more recent randomized, placebo-controlled Phase 1 trial (NCT02754609) conducted on 54 patients concluded that hookworm infection does not restore tolerance to sustained moderate consumption of gluten, but it is associated with improved symptom scores after intermittent consumption of very low gluten doses[68]. However, considering the nature of the treatment it is difficult to imagine routine clinical use of hookworm infection in the management of CD.

PROGRESS IN PHASE 3 TRIALS

Larazotide acetate, a promising therapeutic agent for CD, has been the subject of extensive clinical research, culminating in its progression to Phase 3 trials. The Phase 3 trial of larazotide acetate (NCT03569007), also known as CedLara, represented a critical step in the drug's development and aimed to evaluate its efficacy and safety in alleviating symptoms in CD patients. Whereas larazotide resulted promising in Phase 2 trials in alleviating gluten-related symptoms in patients with CD adhering to a GFD, recent developments in the ongoing Phase 3 trial have posed significant challenges. An independent statistical analysis showed that a substantial increase in the number of participants was required to achieve scientifically meaningful results. The additional need for patients was deemed too great, so the company overseeing the trial, concluded that it was not feasible to continue the trial under these conditions.

DISCUSSION

The current landscape of CD treatment is on the cusp of a paradigm shift. For decades, CD has been treated primarily dietary, with a strict GFD at its core. Although the GFD is effective for many, it comes with significant challenges, including dietary restrictions, social and psychological distress, and the risk of accidental gluten exposure. Our review highlights the need for alternative therapeutic strategies that address these unmet needs in the treatment of CD (Figure 2). The development of non-dietary therapies, such as gluten sequestrants, transglutaminase inhibitors and lymphocyte trafficking inhibitors, represents a major advance. These new therapies offer the promise of reducing the burden of strict dietary adherence and improving the quality of life for CD patients. However, there are still some challenges. While Phase 2 studies are promising, the efficacy and safety profiles of these therapies in broader patient populations need to be further validated in Phase 3 studies. For instance, therapies such as larazotide acetate and ZED 1227 have shown the potential in mitigating gluten-induced symptoms and intestinal damage, but their long-term effects and side-effect profiles need to be studied more extensively. Moreover, CD is a heterogeneous disease and individual responses to these emerging therapies may vary. Personalized medicine approaches, potentially incorporating genetic, immunological and microbiological data, could play a critical role in optimizing treatment efficacy. As these therapies are tested in clinical trials, there is a need for confirmation in Phase 3 trials for their integration into clinical practice. This integration will likely require multidisciplinary collaboration, including gastroenterologists, dietitians and patient education specialists. In addition, the role of these therapies in specific patient groups, such as patients with refractory CD or those at high risk of complications, needs to be investigated.

The advent of new therapies also brings with it ethical and social considerations. The autonomy of patients and their right to choose between dietary or pharmacological treatment must be respected. However, the development of new pharmacological treatments is costly and it is important to consider that resources should be prioritized where there is a clinical need. While respecting patient autonomy in choosing between dietary or pharmacologic treatment, it is critical to balance these options with the economic impact and practicality of making advanced treatments available to all patients who can benefit from them. Looking forward, the field of CD treatment is ready for further discoveries and innovations. Future areas of research include the development of personalized treatment strategies, long-term safety studies of new drugs, and research into adjunctive therapies to improve quality of life. In addition, ongoing research into the pathophysiology of CD may reveal novel therapeutic targets.

CONCLUSION

The horizon of CD treatment is expanding beyond dietary treatment, giving hope for better outcomes for patients. However, the path from promising clinical trial results to practical, everyday treatments is complex and requires careful consideration of efficacy, safety, accessibility, and patient preference. Continued research, patient-centered care and collaborative clinical practice will be critical to making these emerging therapies a new standard in the treatment of CD.

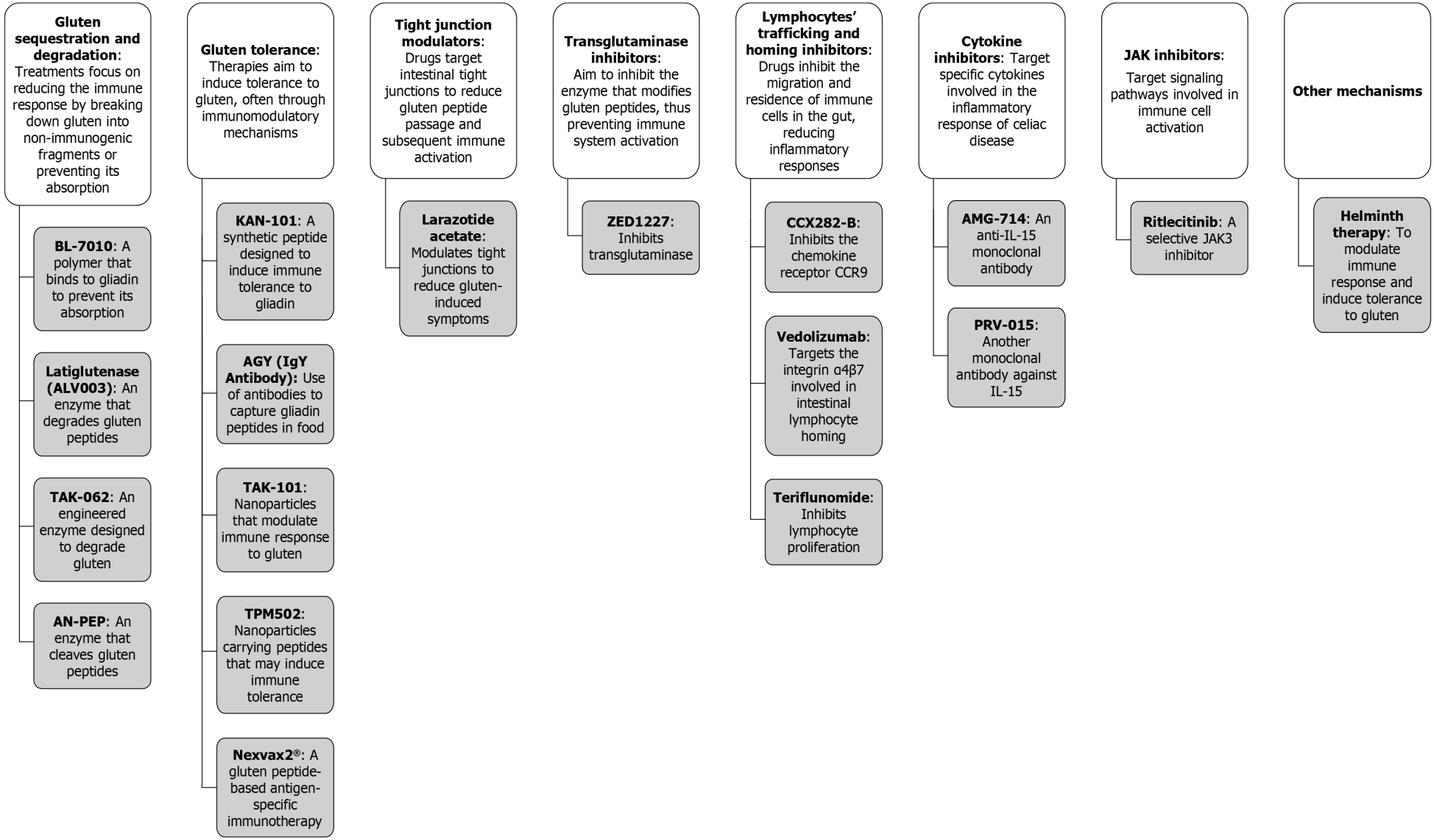

Figure 2 Emerging therapeutic strategies from Phase 2 trials in celiac disease treatment. CCR9: C-C chemokine receptor type 9; IL: Interleukin; JAK3: Janus kinase 3.

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ORIGINAL ARTICLE

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Whole-volume histogram analysis of spectral-computed tomography iodine maps characterizes HER2 expression in gastric cancer

Wei-Ling Zhang, Jing Sun, Rong-Fang Huang, Yi Zeng, Shu Chen, Xiao-Peng Wang, Jin-Hu Chen, Yun-Bin Chen, Chun-Su Zhu, Zai-Sheng Ye, You-Ping Xiao

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Abstract

BACKGROUND

Although surgery remains the primary treatment for gastric cancer (GC), the identification of effective alternative treatments for individuals for whom surgery is unsuitable holds significance. HER2 overexpression occurs in approximately 15%-20% of advanced GC cases, directly affecting treatment-related decisions. Spectral-computed tomography (sCT) enables the quantification of material compositions, and sCT iodine concentration parameters have been demonstrated to be useful for the diagnosis of GC and prediction of its invasion depth, angiogenesis, and response to systemic chemotherapy. No existing report describes the prediction of GC HER2 status through histogram analysis based on sCT iodine

maps (IMs).

AIM

To investigate whether whole-volume histogram analysis of sCT IMs enables the prediction of the GC HER2 status.

METHODS

This study was performed with data from 101 patients with pathologically confirmed GC who underwent preoperative sCT examinations. Nineteen parameters were extracted *via* sCT IM histogram analysis: The minimum, maximum, mean, standard deviation, variance, coefficient of variation, skewness, kurtosis, entropy, percentiles (1st, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 99th), and lesion volume. Spearman correlations of the parameters with the HER2 status and clinicopathological parameters were assessed. Receiver operating characteristic curves were used to evaluate the parameters' diagnostic performance.

RESULTS

Values for the histogram parameters of the maximum, mean, standard deviation, variance, entropy, and percentiles were significantly lower in the HER2+ group than in the HER2– group (all $P < 0.05$). The GC differentiation and Lauren classification correlated significantly with the HER2 status of tumor tissue ($P = 0.001$ and 0.023, respectively). The 99th percentile had the largest area under the curve for GC HER2 status identification (0.740), with 76.2%, sensitivity, 65.0% specificity, and 67.3% accuracy. All sCT IM histogram parameters correlated positively with the GC HER2 status ($r = 0.237$-0.337, $P = 0.001$-0.017).

CONCLUSION

Whole-lesion histogram parameters derived from sCT IM analysis, and especially the 99th percentile, can serve as imaging biomarkers of HER2 overexpression in GC.

Key Words: Gastric cancer; Spectral computed tomography; Iodine map; Histogram analysis

Core Tip: Anti-HER2 receptor is a critical biomarker in gastric cancer (GC). In this study, a total of 101 GC patients underwent preoperative spectral-computed tomography (sCT) and nineteen parameters were extracted from the iodine maps of sCT by histogram analysis. The relationship between parameters derived from whole volume histogram analysis of sCT and HER-2 status of GC were further investigated. Our present results indicated that parameters derived from sCT, particularly the 99th percentiles, could be utilized as an imaging biomarker in assessing the HER2 overexpression of GC. This founding would help Gastrointestinal Oncologists to develop an effective treatment strategy for patients with GC.

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INTRODUCTION

Gastric cancer (GC) is among the most prevalent malignancies of the digestive tract and is the fourth leading cause of cancer-related death worldwide[1]. Although surgery remains the primary treatment for GC, exploration to identify effective alternative treatments for individuals for whom surgery is unsuitable holds significance. Accurate biomarker characterization is critical for the development of targeted therapeutic strategies to serve as alternatives or adjuncts to chemotherapy for GC. Upon its activation through phosphorylation, the epidermal growth factor receptor (EGFR), a highly expressed cell-surface tyrosine kinase receptor, drives cancer cell proliferation and plays significant roles in the regulation of various other tumor cell functions, such as migration, differentiation, apoptosis, and adhesion[2]. HER2, a member of the EGFR family, is a 185-kDa transmembrane tyrosine kinase receptor. It has no ligand and cannot form a ligand-dependent homodimer. To initiate downstream signaling, HER2 must form heterodimers with other EGFR proteins upon their ligand binding[3]. HER2 overexpression or amplification occurs in approximately 15%-20% of advanced GC cases[4,5]. The landmark phase-3 ToGA trial demonstrated that the addition of trastuzumab use to chemotherapy improved the median overall survival of patients with HER2+ advanced GC[6]. Consequently, this combination has become the standard first-line treatment for HER2+ GC. However, a small proportion of GC cases is HER2+, and the cost of trastuzumab treatment is notably high. Currently, HER2 status is determined primarily through immunohistochemical (IHC) or fluorescence in-situ hybridization (FISH) analysis of tissues obtained from surgery or biopsy, invasive procedures that may hinder the timely provision of treatment options. Hence, a noninvasive means of

determining the HER2 status of GC cases to identify suitable candidates before treatment initiation is needed. Computed tomography (CT) is the routine imaging modality for the clinical staging of GC. Spectral-CT (sCT), which involves the acquisition of attenuation measurements from different energy spectra and the use of known attenuation changes between spectra, enables the quantification and differentiation of material compositions[7]. sCT iodine concentration (IC) parameters have been demonstrated to be useful in the diagnosis of GC and prediction of its invasion depth, lymph node metastasis, angiogenesis, and responses to systemic chemotherapy[8]. Zhao *et al*[9] explored correlations between these parameters and the HER2 status of GC, finding that the normalized venous-phase (VP) IC was a significant predictor of this status. To date, no report has described the prediction of GC HER2 status through histogram analysis based on sCT iodine maps (IMs). Given the potential of sCT for tumor evaluation, we investigated whether histogram and texture analysis of sCT IMs enabled the effective and noninvasive determination of the HER2 status of GC. We hypothesized that this combined analysis would aid the assessment of GC HER2 status.

MATERIALS AND METHODS

Patients

Consecutive patients with GC who underwent abdominal contrast-enhanced CT examinations at Fujian Cancer Hospital between April 2020 and September 2022 were included in this study. The hospital's research ethics committee approved the study protocol (No. K2022-152-01). The inclusion criteria were: (1) Histopathological confirmation of gastric adenocarcinoma; (2) Performance of abdominal sCT examination before anti-tumor treatment; and (3) Availability of complete clinicopathological data. The exclusion criteria were: (1) History of chemoradiotherapy or other anti-tumor therapy; and (2) Poor image quality or inability to measure lesions on CT images. In total, 101 patients (82 males and 19 females) with a mean age of 63.15 ± 10.00 (range, 38-88) years were included in the study. The patients' clinicopathological data are summarized in Table 1.

Table 1 Clinical characteristics between two sub-groups of patients with gastric adenocarcinoma

Clinical characteristics	HER-2 positive (*n* = 21)	HER-2 negative (*n* = 80)	*P* value
Age (years)	59.33 ± 8.534	64.15 ± 10.152	0.049
Sex			0.221
Male	19	63	
Female	2	17	
Specimen type			0.007
Gastrectomy	14	72	
Biopsy	7	8	
Tumor location			0.676
Cardia	10	30	
Body	4	16	
Antrum	7	34	
Differentiation degree			0.001
Poorly differentiated	6	55	
Non-poorly differentiated	11	18	
Lauren classification			0.023
Intestinal type	8	22	
Diffuse type	0	25	
Mixed type	5	18	

Pathological examination of HER2 status

The cases' HER2 status was assessed through IHC analysis of biopsy specimens or tumor tissues obtained during resection surgery. For tissues with HER2 scores of 2+, FISH examination was performed to confirm HER2 overexpression. The patients were allocated to HER2+ (IHC 3+ or IHC 2+ with positive FISH findings) and HER2– (IHC 0, IHC 1+, or IHC 2+ with negative FISH findings) groups.

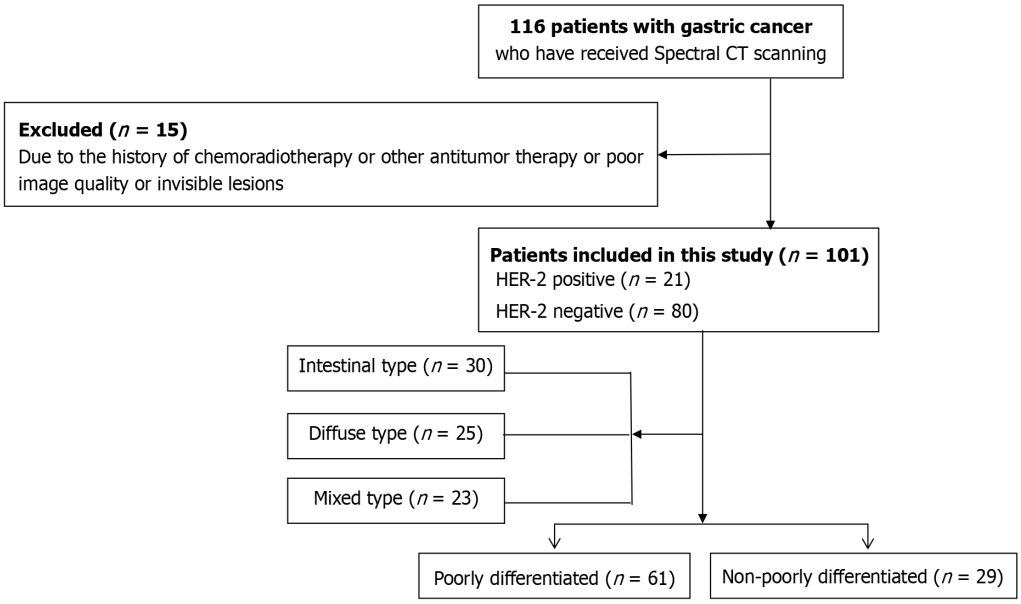

Figure 1 Diagram of study flow. CT: Computed tomography.

sCT protocol

All patients underwent bowel preparation to cleanse the gastrointestinal tract and consumed 800-1000 mL water to distend the stomach 30 minutes before sCT examination. The examinations were performed using a 256-channel sCT scanner (Revolution CT; GE Healthcare, Milwaukee, WI, United States). The acquisition parameters were: Tube voltage, 80,140 kV; tube current, 355 mA; pitch, 0.992; field of view, 500 mm × 500 mm; image matrix, 512 × 512; rotation speed, 0.8 seconds; slice thickness/gap, 1.25/1.25 mm; and reconstruction slice thickness, 1.25 mm. The nonionic contrast agent ioversol (320 mg I/mL, 1.5 mL/kg body weight; Hengrui Med, Jiangsu, China) was administered at a rate of 2.8-3.0 mL/s. Contrast-enhanced images were obtained 30 seconds and 65 seconds after contrast agent injection to capture arterial-phase and VP data.

Post-processing of sCT images and histogram analysis

The VP-enhanced IC images were converted to DICOM format for further analysis. Histogram analysis was performed using open-source image analysis software (FireVoxel; New York University, New York, NY, United States). In each case, two radiologists with 10 and 15 years of gastrointestinal CT diagnostics experience, respectively, manually delineated a region of interest (ROI) encompassing the entire GC lesion volume, avoiding areas of necrosis, bleeding, and gas. The software automatically generated a volume of interest for the calculation of whole-lesion histogram parameters, comprising the mean, minimum, and maximum; variance and coefficient of variation; SD and percentiles (1st, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 99th), lesion volume; and skewness, kurtosis, and entropy.

Statistical analyses

The statistical analyses were conducted using SPSS (version 26.0; IBM, Armonk, NY, United States). The Kolmogorov-Smirnov test was employed to assess the normality of the distributions of all histogram parameters. Continuous variables were compared between groups using the Mann-Whitney *U* test or independent-samples *t* test. Categorical variables were compared using the χ^2 test or Fisher's exact test. Receiver operating characteristic curves were generated to evaluate the parameters' predictive and diagnostic performance. Areas under the curve (AUCs), sensitivity, specificity, accuracy, and positive and negative predictive values were calculated. Inter-observer agreement on the sCT histogram measures was assessed using the intraclass correlation coefficient. *P* values < 0.05 were considered to be significant.

RESULTS

Sample characteristics and clinicopathological characteristics

The HER2+ group consisted of 21 patients [HER2+++, *n* = 16 (76.2%); HER2++ with FISH+, *n* = 5 (23.8%)] and the HER2– group comprised 80 patients [HER2–/+, *n* = 68 (85.0%); HER2++ with FISH–, *n* = 12 (15.0%)]. Histopathological examination of surgical specimens revealed 29 cases of moderately to well-differentiated GC (11 HER2+ and 18 HER2–) and 61 cases of poorly differentiated GC (6 HER2+ and 55 HER2–). Based on the Lauren classification, 30 cases of GC were of the intestinal type (8 HER2+ and 22 HER2–), 23 cases were of the mixed type (5 HER2+ and 18 HER2–), and 25 cases were of the diffuse type (all HER2–; Figure 1). The clinicopathological features of enrolled patients are presented in Table 1.

Table 2 Comparison of spectral-computed tomography histogram parameters and HER-2 status in gastric cancer

Parameters	HER-2 positive (*n* = 21)	HER-2 negative (*n* = 80)	*P* value
Min	-2.000 (-5.518, 1.000)	-0.500 (-7.750, 5.000)	0.143
Max	37.000 (29.500, 40.622)	41.000 (36.000, 50.750)	0.018
Mean	17.429 (14.923, 19.341)	21.176 (17.529, 25.899)	0.001
Std	4.383 (3.663, 4.949)	5.037 (4.285, 5.883)	0.015
Variance	19.198 (13.417, 24.489)	25.366 (18.359, 34.604)	0.015
CV	0.269 ± 0.067	0.245 ± 0.071	0.163
Skewness	-0.051 (-0.246, 0.020)	-0.064 (-0.289, 0.182)	0.569
Kurtosis	0.282 (0.020, 0.683)	0.280 (0.037, 0.617)	0.861
Entropy	2.843 ± 0.256	3.012 ± 0.259	0.009
1st percentile	6.037 (3.500, 9.000)	9.000 (6.000, 12.75)	0.007
5th percentile	10.000 (7.000, 12.000)	12.500 (10.000, 16.750)	0.003
10th percentile	11.000 (9.000, 13.500)	14.000 (12.000, 18.750)	0.003
25th percentile	14.086 (12.000, 16.500)	17.000 (14.000, 21.750)	0.002
50th percentile	18.000 (15.000, 19.500)	21.000 (18.000, 25.750)	0.001
75th percentile	20.122 (18.000, 23.000)	24.000 (21.000, 30.500)	0.001
90th percentile	24.000 (19.500, 26.000)	27.000 (24.000, 35.000)	0.001
95th percentile	24.147 (21.000, 28.000)	29.000 (25.000, 37.750)	0.001
99th percentile	28.171 (24.000, 30.500)	32.500 (28.000, 40.750)	0.001
Lesion volume	14.643 (4.214, 41.820)	9.930 (4.418, 21.191)	0.266

CV: Coefficient of variation.

The prevalence of HER2+ GC differed significantly between patients with moderately to well-differentiated malignancies and those with poorly differentiated malignancies (37.9% *vs* 9.8%, $P = 0.001$). No significant difference was found between groups according to sex or the tumor location. However, the Lauren classification and age differed significantly between groups ($P = 0.049$ and 0.023, respectively). The proportion of HER2+ GC cases detected in biopsy specimens was significantly larger than that of cases detected in resection samples (46.7% *vs* 16.3%, $P = 0.007$).

Relationships of histogram parameters to the HER2 status

Nineteen parameters were generated from the histogram analysis of the sCT IMs: The minimum, maximum, mean, SD, variance, coefficient of variation, skewness, kurtosis, entropy, percentiles (1st, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 99th), and lesion volume. The maximum, mean, SD, variance, entropy, and percentile values were significantly lower in the HER2+ group than in the HER2– groups (all $P < 0.05$; Table 2). No significant difference was observed in the minimum, coefficient of variation, skewness, kurtosis, or lesion volume.

Diagnostic performance of histogram parameters

The AUC values for the IM-derived histogram parameters ranged from 0.669 to 0.740, with sensitivities and specificities ranging from 66.7% to 85.7% and 47.5% to 71.2%, respectively. The AUC for the identification of HER2 status was largest for the 99th percentile [0.740; 95% confidence interval (CI): 0.631-0.848; 76.2% sensitivity, 65.0% specificity, and 67.3% accuracy] and smallest for the maximum (0.669; 95%CI: 0.537-0.807; 76.2% sensitivity, 53.7% specificity, and 58.4% accuracy; Table 3, Figure 2). All histogram parameters derived from sCT images correlated positively with the GC HER2 status ($r = 0.237$-0.337, $P = 0.001$-0.017; Table 4, Figure 3).

DISCUSSION

Considering the relatively low prevalence of HER2 expression in GC cases, the identification of a noninvasive imaging biomarker discriminating individuals most likely to exhibit HER2 overexpression is essential. This study was conducted to investigate the associations of sCT-derived IM histogram parameters with the GC HER2 status and their discriminatory ability. It showed that these parameters, and especially the 99th percentile, correlated significantly with the GC HER2 status. The rate of HER2 overexpression in our GC cases was 20.8%, consistent with previous findings[5]. Similar to

Table 3 Receiver operating characteristic curve results of the iodine map histogram parameters to identify HER-2 status

Parameters	AUC (95%CI)	Sensitivity (%)	Specificity (%)	Accuracy (%)	Cutoff value	*P* value	Youden index
Max	0.669	76.2	53.7	58.4	40.62	0.018	0.299
Mean	0.734	81.0	61.2	65.3	19.54	0.001	0.422
SD	0.674	85.7	50.0	57.4	5.05	0.015	0.357
Variance	0.674	85.7	50.0	57.4	25.50	0.015	0.357
Entropy	0.698	85.7	50.7	57.4	3.02	0.005	0.357
1st percentile	0.691	85.7	47.5	55.4	9.18	0.007	0.332
5th percentile	0.708	71.4	60.0	62.4	11.50	0.003	0.314
10th percentile	0.713	76.2	57.5	61.4	13.50	0.003	0.337
25th percentile	0.715	76.2	58.7	62.4	15.50	0.003	0.349
50th percentile	0.736	76.2	61.2	64.4	19.50	0.001	0.374
75th percentile	0.731	81.0	55.0	60.4	27.50	0.001	0.360
90th percentile	0.733	66.7	71.2	70.3	24.57	0.001	0.379
95th percentile	0.737	71.4	67.5	68.3	26.50	0.001	0.389
99th percentile	0.740	76.2	65.0	67.3	30.50	0.001	0.412

AUC: Area under the curve.

Table 4 Correlations between spectral-computed tomography histogram parameters and HER-2 status of gastric cancers

Parameters	Correlation coefficient	*P* value
Max	-0.237	0.017
Mean	-0.329	0.001
SD	-0.244	0.014
Variance	-0.244	0.014
Entropy	-0.278	0.005
1st percentile	-0.270	0.006
5th percentile	-0.293	0.003
10th percentile	-0.300	0.002
25th percentile	-0.303	0.002
50th percentile	-0.332	0.001
75th percentile	-0.325	0.001
90th percentile	-0.328	0.001
95th percentile	-0.333	0.001
99th percentile	-0.337	0.001

previous findings, a larger proportion of HER2+ cases was detected in biopsy specimens than in resection samples. This difference may be attributed to small sample sizes or the superior fixation of biopsy specimens, as proposed by Wang *et al* [10]. Previous studies of the correlation between HER2 overexpression and tumor location have yielded inconsistent findings[11], and these variables did not correlate in the present study. Additionally, as in the present study, previous studies have revealed consistent associations of a greater frequency of HER2 overexpression with the intestinal histological subtype of GC and moderate to high degrees of differentiation[12-14]. sCT has emerged as a valuable tool in various clinical studies of cancer[15,16], as it provides information on blood flow and quantitative lesion parameters based on IMs. sCT enables the assessment of actual iodine deposition in tissues and the indirect quantification of lesion blood-vessel density and blood volume. Several reports emphasize the diagnostic and predictive potential of sCT for GC, including the modality's use for detailed evaluation, histological differentiation, Lauren classification, the prediction of lymph node metastasis, the assessment of angiogenesis, the determination of the Ki-67 antigen expression level, and the

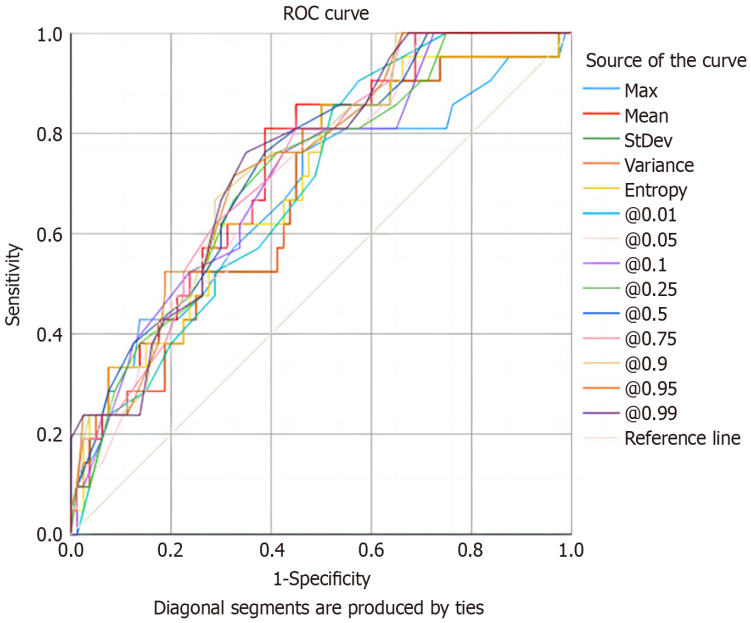

Figure 2 Receiver operating characteristic curves of the diagnostic performance of spectral computed tomography histogram parameters in the identification of HER2 status. The 99th percentile had the best overall area under the curve among parameters (0.740), with 76.2% sensitivity, 65.0% specificity, and 67.3% accuracy. ROC: Receiver operating characteristic.

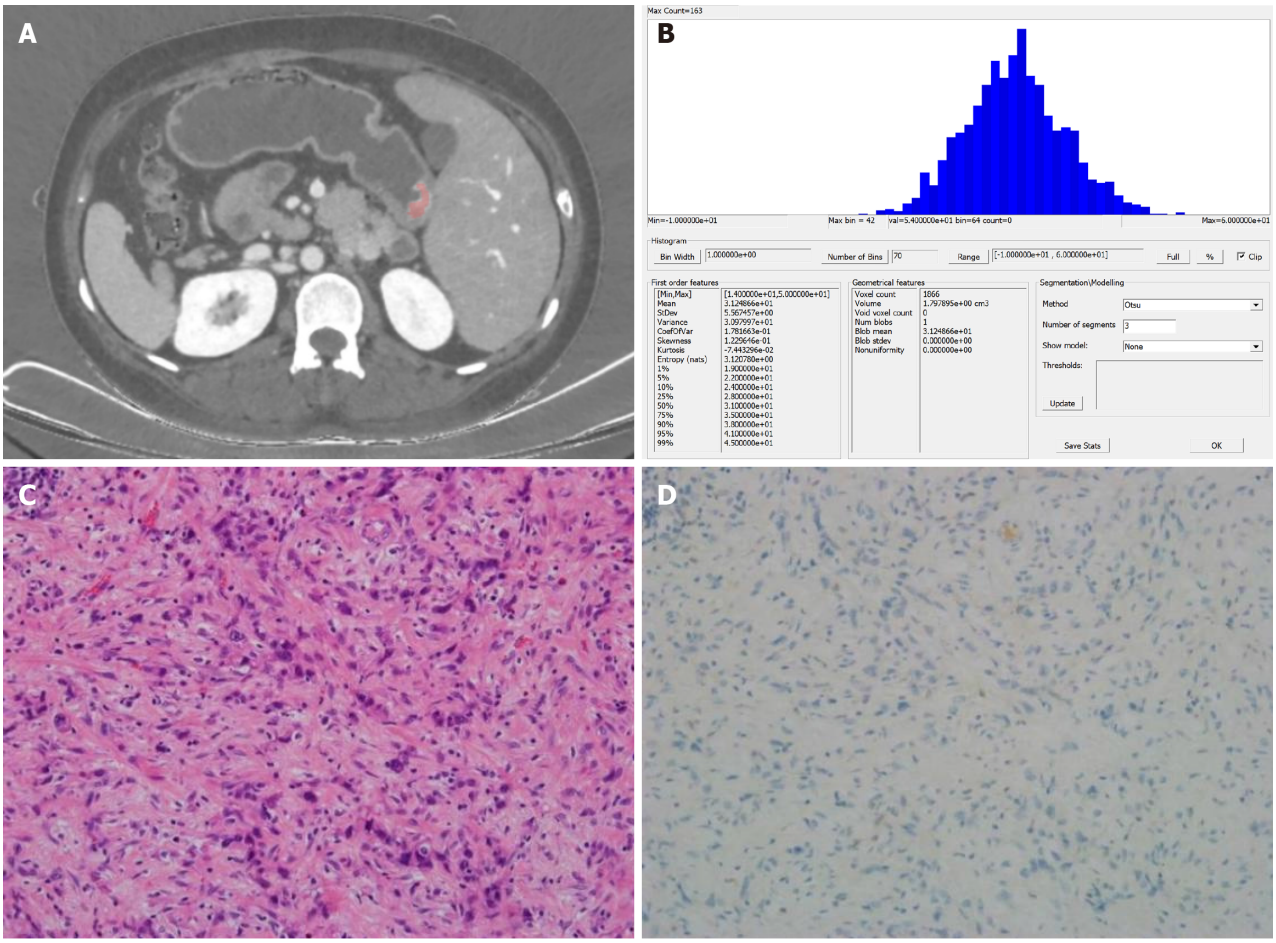

Figure 3 Images from a 43-year-old woman with poorly differentiated gastric adenocarcinoma. A: Portal venous-phase spectral computed tomography image showing clear (pink) enhancement of the lesion, located at the gastric antrum; B: Histogram of parameter distributions for the whole tumor (minimum = 14.000, maximum = 50.000, mean = 31.249, standard deviation = 5.567, skewness = 0.123, kurtosis = 0.074, 1st-99th percentiles = 19.000, 22.000, 24.000, 28.000, 31.000, 35.000, 38.000, 41.000, and 45.000, respectively); C and D: Microscopic pathological (HE staining, 200 ×) and immunohistochemical images,

respectively, showing a poorly differentiated adenocarcinoma with a Lauren classification of diffuse type, vascular and neural invasion, and negative HER2 staining.

evaluation of response to neoadjuvant chemotherapy[16-19]. However, assessments of the use of sCT to determine the HER2 status of GC cases are limited. Zhao *et al*[9] reported a strong correlation of the tumor and normalized VP ICs with HER2 overexpression. Histogram analysis has become a standard tool in the diagnosis and evaluation of differentiation and treatment response of various cancer types, including GC[19-22]. The analysis of IM histogram parameters enables the estimation of the iodine distribution, reflecting the spatial distribution of gray values and providing a comprehensive view of tumor heterogeneity[23,24]. This study is the first in which HER2 overexpression in GC was identified using sCT-derived histogram features. These histogram characteristics can serve as imaging-based biomarkers that aid the selection of patients most likely to benefit from anti-HER2 targeted therapy. In this study, we focused primarily on first-order histogram parameters, which are considered to be more repeatable and stable than higher-order features. To mitigate sampling errors stemming from ROI delineation within tumors, we characterized histogram features for entire target lesion volumes. Values for the variance, skewness, kurtosis, entropy, and percentiles (1st, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 99th) were significantly lower in the HER2+ group than in the HER2– group. These findings suggest that these parameters could help clinicians devise more personalized therapeutic strategies for GC cases. This study has several limitations. First, the sample was small and the study lacked an external validation cohort. Additionally, as the study was preliminary, a limited number of sCT parameters was examined. The consideration of a more comprehensive set of sCT parameters and high-throughput radiomics features extracted from sCT images may significantly enhance the predictive power of this approach.

CONCLUSION

The results of this study suggest that quantitative parameters derived from whole-lesion histogram analysis of sCT IMs can serve as biomarkers of HER2 overexpression in GC. The use of these biomarkers could help oncologists noninvasively distinguish cases likely to be sensitive to anti-HER2 therapy and aid in clinical decision making.

FOOTNOTES

Author contributions: Xiao YP, Ye ZS and Chen YB designed the study; Zhang WL, Sun J, Zeng Y, Chen S, Wang XP and Chen JH performed the research; Huang RF and Zhu CS contributed new reagents and analytical tools; Zhang WL, Sun J, Ye ZS and Xiao YP analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript. Zhang WL and Sun J contributed equally to this work as co-first authors. Xiao YP and Ye ZS worked together to design the study and supervise the entire research process. Xiao YP is a radiologist who was responsible for conducting the spectral computed tomography examinations and managing the imaging database. Ye ZS is a surgical oncologist who was responsible for the clinical treatment of patients with gastric cancer and the management of the clinical database. Moreover, Xiao YP participated in the data analysis and manuscript writing and revision. Thus, Xiao YP and Ye ZS are co-corresponding authors for this paper.

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SYSTEMATIC REVIEWS

Survival outcomes in early-onset oesophageal adenocarcinoma patients: A systematic review and meta-analyses

Ashleigh Russell, Shauna Mitchell, Richard C Turkington, Helen G Coleman

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Abstract

BACKGROUND

The incidence of oesophageal adenocarcinoma (OAC) has been reported to be increasing in many countries. Alongside this trend, an increase in incidence of early-onset OAC, defined as OAC in adults aged under 50 years, has been observed. It is unclear whether survival outcomes for early-onset OAC patients differ from older age groups.

AIM

To investigate survival outcomes in early-onset OAC patients.

METHODS

Ovid Medline and Embase were searched from inception to January 2022 for relevant studies relating to early-onset OAC and survival outcomes. Results regarding the overall five-year survival and risk of death of younger and older patients with OAC were extracted and pooled using meta-analyses to produce pooled estimates and 95%CIs where possible.

RESULTS

Eleven studies which compared survival of early-onset OAC, defined as age at diagnosis of < 50 years, with older patients were included. A narrative review of median and mean survival demonstrated conflicting results, with studies showing early-onset OAC patients having both better and worse outcomes compared to older age groups. A meta-analysis of five-year survival demonstrated similar outcomes across age groups, with 22%-25% of patients in the young, middle and older age groups alive after five years. A meta-analysis of four studies demonstrated that early-onset OAC patients did not have a significantly increased risk of death compared to middle-aged patients (hazard ratio 1.12, 95%CI: 0.85-1.47).

CONCLUSION
Results suggest that early-onset OAC patients do not have a significantly different survival compared to older patients, but further population-based research, taking into account stage and treatment, is required.

Key Words: Early-onset cancer; Early-onset oesophageal adenocarcinoma; Survival; Cancer epidemiology; Systematic review; Meta-analysis

Core Tip: In this systematic review, we investigated survival outcomes in early-onset oesophageal adenocarcinoma (OAC) (< 50 years) compared to older age groups. Eleven studies were included. A narrative review of median and mean survival demonstrated conflicting results. Meta-analyses of 5-year survival and risk of death demonstrated no significant difference in survival between younger and older OAC patients. Current evidence in this area has limitations, and up-to-date population-based research is required.

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INTRODUCTION

There is concern that the incidence of oesophageal adenocarcinoma (OAC) in patients under 50, described as early-onset OAC, is increasing. However, data regarding survival of younger patients with OAC is sparse.

Globally, while increasing age remains a major non-modifiable risk factor for cancer, the incidence of early-onset cancers, largely accepted to be in adults aged under 50 years, is increasing[1]. This includes an observed increase in the incidence of gastrointestinal malignancies such as colorectal, oesophageal, gastric and hepatobiliary cancers[2-4].

Despite oesophageal squamous cell carcinoma (OSCC) being more common globally (88% of cases)[5], a striking increase in oesophageal OAC incidence has been reported in developed countries, such as the United States and Europe [6,7]. Worryingly, the United Kingdom has the highest incidence of OAC cases in the world[8]. In addition to the increase in OAC, an increase in incidence of early-onset OAC, defined as OAC in adults aged under 50 years, has been observed[9, 10]. A population-based cohort in the Netherlands, consisting of 59584 patients, demonstrated the incidence of early-onset OAC to have tripled from 1989 to 2018, while OSCC cases declined in this age group[7].

OAC usually develops in the lower third of the oesophagus and the gastro-oesophageal junction, with risk factors including obesity and gastro-oesophageal reflux disease[11]. A poor prognosis is observed, with the overall five-year survival rate for oesophageal cancer between 15%-20%, even with treatment[12,13]. These low survival rates are likely due to a combination of late diagnosis, intrinsic resistance to systemic therapy and the limited efficacy of surgical resection.

Younger patients tend to present at a more advanced stage at diagnosis compared to those diagnosed later in life. A single centre, retrospective study found that 33.3% of patients in the younger age category (< 50 years old) presented with stage IV OAC, compared to the 20.6% of the oldest age category (> 70 years old)[14]. Another population-based study in the Netherlands observed that OAC patients under 50 years old also presented with distant metastasis more often in comparison to older patients (50.5% *vs* 44.7%), and that tumour differentiation also varied between age groups[15].

Reports of survival estimates in patients with early-onset OAC compared with older patients have resulted in contrasting findings to date. Some studies report that due to the advanced stage and aggressiveness of the tumours seen that the prognosis of these patients is almost always worse than their older counterparts[16]. In contrast, another study found that the overall survival, as well as stage-specific survival was higher in those who were younger[17]. A Dutch study which included only resectable cases found no difference in 5-year disease specific survival[18].

Given the conflicting evidence to date, the aim of this systematic review was to investigate survival in OAC patients according to age at diagnosis.

MATERIALS AND METHODS

A protocol was composed, and the reporting of this systematic review designed, using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[19]. The protocol included: The review question, search strategy, inclusion criteria, type of quality assessment, the strategy for data analysis, and the 'population, intervention, comparator, and outcome' criteria. These are expanded below.

Study population

The population of interest was patients diagnosed with OAC aged less than 50 years. Survival outcomes were compared between older patients and those < 50 years with a diagnosis of OAC where possible, with older patients being considered the control group. Studies only reporting survival outcomes of those < 50 years were also included. In addition, studies were included if the cut off for early-onset OAC was less than 50 years.

Outcome

Overall survival estimates (*e.g.* those recorded as net, relative and observed) within patients aged < 50 years at the time of diagnosis of OAC.

Search strategy

The electronic databases Embase (Reed Elsevier PLC, Amsterdam, Netherlands) and Medline (United States National Library of Medicine, Bethesda, MD, United States) were searched from week of inception to 12 January 2022 for relevant studies relating to the age of diagnosis of OAC and the survival outcomes documented. The search strategy identified studies that contained at least one keyword or Medical Subject Heading term relating to young age, survival outcomes and oesophageal cancer. The full search terms are available in Supplementary Table 1. A rapid review of studies published up to mid-2024 did not identify any further relevant articles that met our inclusion criteria.

Inclusion and exclusion criteria

Population-based studies were included, along with population representative studies, for example, large database studies or using cancer registry data. Real-world studies considering consecutive patients were also included.

Clinical trial studies were excluded, in order to reduce the impact of selection biases or volunteer biases being present. Conference abstracts, case reports and review papers were also excluded. The exclusion criteria also removed any studies which did not specifically focus on the patients under the age of 50 years old. The search strategy was restricted to include English language articles and human studies only.

The reference lists of several studies, which included the correct age categories < 50 years were also searched for any relevant articles, but this did not identify any further studies.

Data extraction

Articles from the search were imported into Covidence, and duplicates were removed. Titles and abstracts were reviewed independently by at least two authors (Turkington RC, Coleman HC, and Mitchell S), to remove irrelevant studies. The full text of all selected articles was read independently by at least two authors, to determine whether they met the inclusion criteria. Any discrepancies were resolved by discussion among the reviewers of the text, with the third reviewer involved if required.

Methodological quality was evaluated using the Newcastle-Ottawa Scale (Supplementary Table 2). Studies were assessed on eight items, categorised into three groups: The selection of the study groups; the comparability of the groups; and the ascertainment of either the exposure or outcome of interest.

Statistical analysis

A narrative synthesis was assembled after a thorough and critical review of each study. Where it was deemed suitable, the association between the age of diagnosis of OAC and survival was summarised in meta-analyses by comparing the survival outcomes of those < 50 years old and those > 50 years old reported in the studies. Outcome measures were evaluated in three ways:

Firstly, the median and mean survival of patients < 50 years old and those > 50 years old diagnosed with OAC was extracted and summarised narratively since it was not possible to combine these in meta-analyses.

Secondly, the overall 5-year survival of early-onset OAC and older patients, *i.e.*, the proportion of patients still alive after 5 years, was also extracted from relevant studies. A meta-analysis was performed on these results, using the Freeman-Tukey method. Meta-analyses were also performed using the Score (Wilson) method and exact CI method, with consistent results observed across all three methods, demonstrating the robustness of the results. I^2 values were applied used to assess heterogeneity between study results.

Finally, the hazard ratios (HR) for risk of death, adjusted and unadjusted, and their 95%CI were extracted from relevant cohort studies. Random-effects meta-analyses were applied to combine adjusted HR and 95%CI from studies reporting on the risk of death in early-onset OAC patients, compared with older adults. The random effects model included study specific weights, which were calculated and then scaled to percentages. I^2 values were used to assess heterogeneity between study findings.

All meta-analyses were conducted using Stata version 16 (StataCorp, College station, TC, United States).

RESULTS

The search strategy identified 2261 papers, and 911 duplicates were removed, leaving 1350 articles for screening. Following title and abstract screening, 128 studies were eligible for full text review. Following full text review, 117 studies were excluded. Reasons for exclusion included the wrong patient population, wrong condition, inappropriate study design or outcomes, and are shown in Figure 1. Any conflicts which arose were resolved through discussion and received

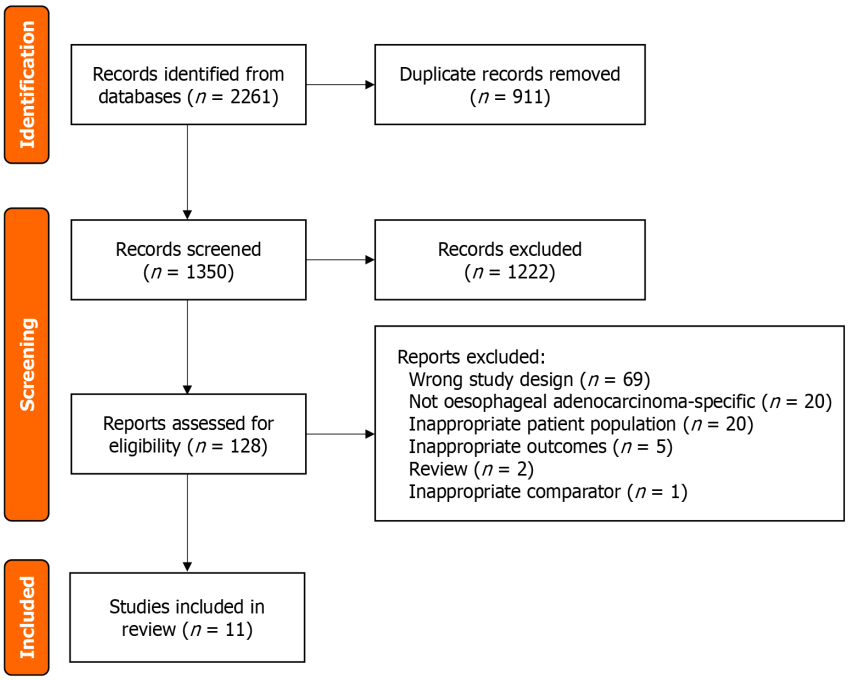

Figure 1 Flow chart of the selected articles included in the review.

a final vote on whether or not to be included. After all screening processes had taken place, eleven studies were included in the review[14-17,20-26].

The characteristics of the included studies are summarised in Table 1. Six studies included population-based register cohorts[15,17,20-22,26], two were single centre retrospective cohorts[14,25], two were consecutive case studies[23,24], and one was a retrospective cohort[16]. Nine studies were conducted in the United States, four being from the Surveillance, Epidemiology, and End Results (SEER) database[17,20,22,26], while one was conducted in the Netherlands[15] and Sweden[21].

Median and mean survival

To compare the difference in median survival between those diagnosed with OAC < 50 years old and those > 50 years old we analysed three of the eleven studies[15-17,23]. One study compared the mean survival of these patients, and a summary of these results can be seen in Table 2[14]. Together these results show inconsistent differences in survival between age groups.

There were three studies[14,16,17] which demonstrated a significant difference in survival between early onset OAC and patients diagnosed at an older age. However, these studies concluded in different directions on whether younger age at the time of diagnosis impacts survival. Boys *et al*[16] reported a significantly shorter median survival in younger patients (< 40 years old) at 17 months compared to older patients (> 40 years old) at 30 months. In contrast, Kolb *et al*[17] observed a lower median survival in older patients at 10.4 months with median survival in younger patients being 15.2 months. Sawas *et al*[14] reported that patients in the middle-aged (51-70 years old) category had the longest mean survival in comparison to shorter survival in the older (> 70 years old) and younger (< 50 years old) age categories. It is important to note that the age cut-offs and comparator older age groups differed in each study, limiting the interpretation of the results.

Overall five-year survival

To compare the difference in overall 5-year survival between early-onset OAC patients and older age groups, we analysed five studies[14,15,20,22,24], with four being included in meta-analyses. The results demonstrated a similar proportion of patients still alive after 5 years in the young, middle and old age groups (Table 3 and Figure 2A). The study carried out by Haiyu *et al*[22] could not be included in the meta-analysis due to the use of the same database (SEER) and a similar time frame as Codipilly *et al*[20] which used the years 2000-2011 for the reported results.

Meta-analyses results are shown in Figure 2A. The proportion of younger patients still alive five years after diagnosis was 22% (95%CI: 0.18-0.27) compared with 25% (95%CI: 0.19-0.31) in older patients, with high evidence of heterogeneity (83.49% and 98.56% respectively). Sawas *et al*[14] and Codipilly *et al*[20] considered older patients to be over 70 years old and this could not be separated in the statistical analysis. These studies also reported on the middle age category, those aged 50-70, separately and the overall effect size in this group was 22% (95%CI: 0.21-0.23); similar to the pooled analysis of the other two age groups.

Haiyu *et al*[22] compared the relative survival rates of younger patients and older patients from 1984-2013. For the purpose of this review, we extracted information from Haiyu *et al*[22] on survival rates in patients aged 20-44 and 55-64 years in the most recent time period analysed (2000-2013). The survival rates for both age categories in the first six months

Table 1 Characteristics of studies investigating survival outcomes in early-onset oesophageal adenocarcinoma patients

Ref.	Publication year	Country	Study design	No. of cases	No. of cases < 50 (%)	Recruitment period	Age categories (years)	Stage	Follow-up (Y/N)	Specific survival outcome	Confounders considered
Boys *et al* [16]	2015	United States	Retrospective cohort	772	42 (5)	1990-2013	< 40, > 40	I-IV	Y	Median overall survival	Sex, race, staging, year of diagnosis
Kolb *et al* [17]	2020	United States	Population-based Register	114123	10271 (9)	2000-2017	< 50, 50-69, > 70	I-IV	Y	Median survival, HR of death	Treatment, staging, race
Codipilly *et al*[20]	2021	United States	Population based register	25813	2183 (8)	1975-2011	< 50, 50-69, > 70	I-IV	Y	Overall 5 year survival	Sex, race, staging, year of diagnosis
Xie *et al* [21]	2017	Sweden	Population-based cohort	5140	212 (4.1)	1961-2014	< 50, 50-59, 60-69, 70-79, > 80	Not recorded	Y	HR of death	Sex
Haiyu *et al*[22]	2019	United States	Population-based register	16474	Crude number not reported	1984-2013	20-44, 45-54, 55-64, 65-74, > 75	Not recorded	Y	5-year relative survival	Sex, Socio-economic status, race
Hashemi *et al*[23]	2009	United States	Consecutive case series	242	31 (12.8)	1994-2004	< 50, > 50	0-IV	N	Median survival	Sex, family history
Portale *et al*[24]	2004	United States	Consecutive case series	263	32 (12.1)	1992-2002	< 50, > 50	I-III	N	5-year survival rates	
Sawas *et al*[14]	2019	United States	Single centre retrospective study	682	105 (15.4)	2009-2012	< 50, 51-70, > 70	I-IV	N	Mean survival, 5-year survival rates, HR of death	Sex, staging, intestinal metaplasia, Charlson comorbidity index
Strauss *et al*[25]	2020	United States	Single centre retrospective study	630	65 (10.3)	1991-2018	< 50, > 50	I-IV	Y	Overall survival, HR of death	Sex, race, pathology grade
van Nistelrooij *et al*[15]	2014	Netherlands	Population-based register	13331	1466 (10.9)	2000-2011	< 50, > 50	I-IV	Y	Median overall survival, 5-year overall survival	Sex, staging
Yang *et al* [26]	2016	United States	Population-based register	2601	94 (3.6)	1988-2011	< 45, 45-59, 60-74, > 75	I-III	Y	HR of death	Sex, race, lymph nodes examined

HR: Hazard ratio; Y: Yes; N: No.

were similar, averaging at approximately 75%. However, the five-year survival differs between the age categories, with a lower proportion of younger patients still alive five years after diagnosis (19.4%) compared with older patients (22.6%).

Hazard ratios for risk of death over time

To compare the HRs between those diagnosed with OAC < 50 years old and those > 50 years old we analysed five studies (Table 4)[14,17,21,25,26], with four being included in a meta-analysis (Figure 2B). Together the results show an inconsistent risk of death between age groups. The study by Xie *et al*[21] was excluded from the meta-analysis due to the use of a different reference group (all other studies used middle aged patients as the reference group). Kolb *et al*[17] and Yang *et al*[26] both included data from SEER database, however each study covered different time periods with little overlap and so could be included in the meta-analysis.

Three studies had significant results, with Kolb *et al*[17] finding that patients with early-onset OAC had a reduced risk of death compared to 50-69 year olds (adjusted HR 0.94, 95%CI: 0.92-0.96). In contrast, Sawas *et al*[14] and Yang *et al*[26] found that early-onset OAC patients had an increased risk of death compared to middle age groups (adjusted HR and 95%CI: 1.34 (1.02-1.75) and 1.35 (1.02-1.80) respectively). Strauss *et al*[25] reported no association between the age of diagnosis of OAC and overall survival. The one study that used early-onset OAC patients as the reference group, published by Xie *et al*[21] found that patients who were middle-aged (50-69 years old) or older-aged (> 70-80 years old)

Table 2 The median and mean survival of younger, middle-aged and older patients with oesophageal adenocarcinoma

Ref.	Young age	Middle age	Older age	Significance
Median survival				
Boys *et al*[16][1]	17 months	N/A	30 months	$P = 0.04$
Kolb *et al*[17][2]	15.2 months	15.1 months	10.4 months	$P < 0.1$
Hashemi *et al*[23][3]	21.1 months	N/A	22 months	Not significant
Mean survival				
Sawas *et al*[14][4]	4 ± 4.2 years	5 ± 3.9 years	3.6 ± 3.2 years	$P = 0.03$

[1]Young age < 40 years and old age ≥ 40 years.
[2]Young age < 50 years, middle aged 50-69 and old age ≥ 70 years.
[3]Young age ≤ 50 years and old age > 50 years.
[4]Young age ≤ 50 years, middle age 51-70 years, old age > 70 years.
N/A: Not applicable.

Table 3 The proportion of patients alive at five years in young, middle-aged and older patients diagnosed with oesophageal adenocarcinoma

Ref.	5-year survival (%) (proportion of patients still alive)			
	Young age	Middle age	Older age	Significance
Codipilly *et al*[20][1]	19.7	21.4	12.3	$P < 0.01$
Portale *et al*[24][2]	32.6	N/A	45.5	Survival similar
Sawas *et al*[14][3]	34.3	49.9	33.3	$P < 0.01$
Van Nistelrooij *et al*[15][2]	18.2	N/A	16.4	$P = 0.021$
Haiyu *et al*[22][4]	19.4	N/A	22.6	$P < 0.05$

[1]Young age < 50 years, middle-age 50-69 years, old age ≥ 70 years.
[2]Young age ≤ 50 years, old age > 50 years.
[3]Young age ≤ 50 years, middle age 51-70 years, old age > 70 years.
[4]Young age 20-44 years, old age 55-64 years.
N/A: Not applicable.

Table 4 Hazard ratios of risk of death and 95%CI for young, middle-aged, and older patients diagnosed with oesophageal adenocarcinoma

Ref.	Hazard ratios for risk of death and 95%CI					
	Age categories (years)	Young age	Age categories (years)	Middle age	Age categories (years)	Older age
Kolb *et al*[17]	< 50	0.94 (0.92-0.96)	50-69	Ref. (1.0)	≥ 70	1.24 (1.21-1.26)
Xie *et al*[21]	< 50	Ref. (1.0)	50-59	1.19 (0.99-1.43)	70-79	1.26 (1.06-1.50)
			60-69	1.09 (0.91-1.30)	≥ 80	1.66 (1.38-1.99)
Sawas *et al*[14]	≤ 50	1.34 (1.02-1.75)	51-70	Ref. (1.0)	> 70	1.70 (1.36-2.10)
Strauss *et al*[25]	< 50	0.53 (0.17-1.69)	> 50	Ref. (1.0)	N/A	N/A
Yang *et al*[26]	< 45	1.35 (1.02-1.80)	45-59	Ref. (1.0)	≥ 75	1.52 (1.28-1.81)
			60-74	1.11 (0.97–1.26)		

N/A: Not applicable.

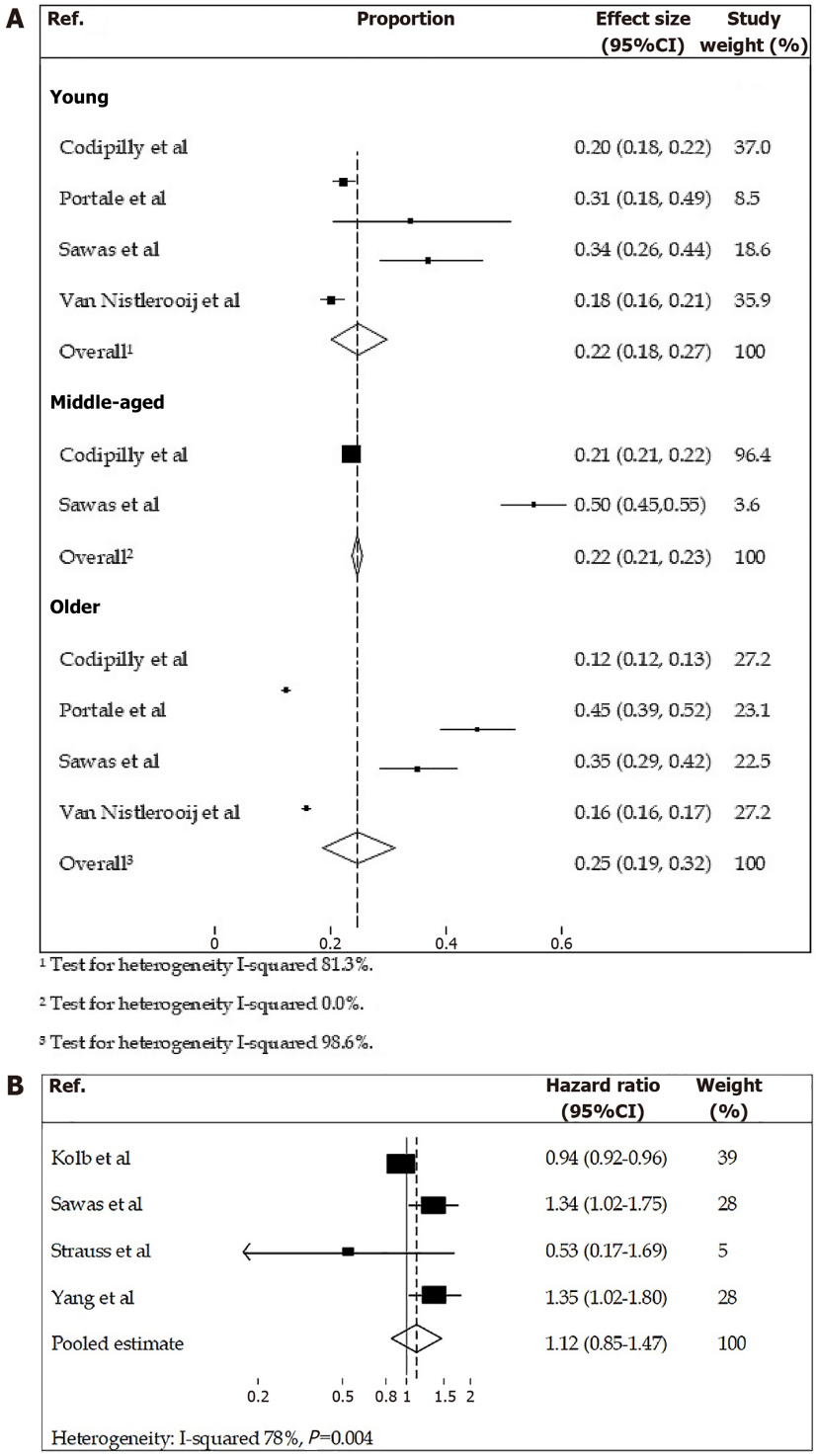

Figure 2 Forest plots. A: Forest plots showing overall five-year survival of young, middle-aged, and older patients diagnosed with oesophageal adenocarcinoma (with effect size being the proportion of patients alive); B: Risk of death in adults diagnosed with early-onset oesophageal adenocarcinoma compared with older adults.

had an increased risk of death compared to younger (< 50 years old) OAC patients.

As shown in Figure 2B, the comparison of the pooled estimate for risk of death for younger patients in comparison to middle-aged patients was not statistically significant, HR 1.12 (95%CI: 0.85-1.47), with high evidence of heterogeneity (78%).

Quality assessment was carried out using the Newcastle-Ottawa Scale (Supplementary Table 2) with no studies excluded based on quality assessment.

DISCUSSION

We performed a systematic review of eleven studies which investigated the survival outcomes of younger patients (< 50 years old) and older patients diagnosed with OAC. A narrative review of median and mean survival demonstrated conflicting results, with studies showing early-onset OAC patients having both better and worse outcomes compared to older age groups. A meta-analysis of five-year survival demonstrated similar outcomes across age groups, with 22%-25% of patients in the young, middle and older age groups alive after five years. Finally, a meta-analysis of risk of death demonstrated that early-onset OAC patients did not have a significantly increased risk of death compared to middle-aged patients (HR 1.12, 95%CI: 0.85-1.47). Overall, our findings showed no clear difference in survival in OAC between age groups.

Despite Kolb *et al*[17] and Yang *et al*[26] using data from the same database, SEER in the United States, their HR for risk of death was different. It can be assumed these contradictions in findings were due to the differing time periods which data was taken from, with little overlap seen. The reduced risk of death in younger patients reported by Kolb *et al*[17] was from the more recent data set, which is likely due to improvements in cancer treatment over time. Encouragingly, it should be noted from Haiyu *et al*[22] that five-year survival rates improved over time from 1984 to 2013, almost doubling in the most recent decade studied, depending on age. This improvement in overall survival of patents reflects the developments made in diagnosis and treatment in the past decades, such as access to endoscopy, improvement in surgery and the introduction of neoadjuvant treatment[27].

Early-onset OAC has been observed to present at a more advanced stage than older patients, which was demonstrated in a number of studies included in this systematic review[17,20]. For example, Codipilly *et al*[20] found that 84.9% of early-onset cancer patients had regional/distant disease compared with 77.6% and 67.8% in middle age and older age groups respectively[20]. Despite this, our results demonstrate overall five-year survival was similar in younger and older patients. This may be because younger patients are more likely to receive aggressive cancer treatment in the presence of advanced stage of disease or have less comorbidities. This has been demonstrated in early-onset colorectal cancer, where young patients are more likely to receive chemotherapy and radiotherapy, as well as multimodality treatment, than older patients[28-30]. However, whether higher rates of treatment are given to early-onset OAC patients compared to older patients is yet to be determined.

The reason why younger patients present with a more advanced stage of OAC is still unknown and debated, but it may be that younger patients experience a delayed diagnosis[24]. This could be because young patients do not seek medical attention for their symptoms, or they do seek medical attention but physicians initially attribute their symptoms to benign conditions such as gastro-oesophageal reflux disease. There may also be a reluctancy to refer these younger patients for endoscopy and screening, which is needed to diagnose OAC, due to a lack of concern or a perception that oesophageal cancer is a disease of the elderly[31]. There is an urgent need to raise awareness of the observed increasing incidence of early-onset OAC, both in the general public and healthcare professionals.

Another reason why younger patients present at more advanced stage is the possibility of a more aggressive subtype, which should also be taken into consideration. The increase in incidence of early-onset OAC, along with differing clinical features compared to older patients, raises questions about the pathogenesis and molecular pathways that lead to the development of the disease. It has been shown that early-onset OAC has stronger associations with recurrent gastro-oesophageal reflux and obesity compared to later-onset OAC[32], suggesting age specific risk factor profiles. Furthermore, it may be that early-onset OAC carries distinct biological differences compared to late-onset OAC. Recently, a large, single-centre retrospective analysis of oesophageal, gastric and gastro-oesophageal junction tumours demonstrated that early-onset cases had a preponderance for gastric tumours but had a reduced frequency of genomic and microsatellite instability compared to average onset disease[33]. A study investigating the molecular profile of OAC found no overall difference in mutational load between early-onset OAC and late-onset OAC, but did demonstrate several additional mutations seen in early-onset OAC (including *APC* and *CTNNB1*) which were not seen in conventional OAC[34]. Further research is needed into the molecular profile of the disease in different age groups.

The majority of cases of early-onset OAC appear to be sporadic rather than hereditary, with germline mutations being demonstrated in 21% of cases of early-onset oesophagogastric cancer (< 50 years) compared to 13.8% in older patients [35]. This study also included gastric cancer cases and further research is needed into the role of germline mutations in OAC, in order to identify those at risk of a cancer diagnosis at a young age and to inform prevention strategies.

Since our literature search, two further papers have been published which include survival outcomes in early-onset OAC, although neither would have met our inclusion criteria. Firstly, a large United States-based single centre study published in 2023 investigated oesophagogastric cancers (n = 218 early-onset cases), and did not find a significant difference in stage distribution or survival between early-onset and older patients[33]. Of note, this study also included gastric cancer, which comprised 64% of the early-onset group, and included non-adenocarcinoma pathology (although these numbers were small). As such, further studies are needed on presenting symptoms, time to diagnosis and pathology of early-onset OAC specifically. Secondly, a Swedish study using a population-based cancer register (n = 470 early-onset OAC, defined as < 55 years) found that 5-year relative survival was 20% in early-onset OAC compared to 16% in later-onset OAC, a similar proportion to our meta-analyses results[36].

In summary, there are a number of potential reasons for the observed variation in early-onset OAC survival. This includes biological differences in OAC across age groups, such as stage at diagnosis and molecular characteristics. Predictors of poor survival observed include ethnicity, treatment modality, tumour differentiation and comorbidity[17]. Different time periods covered by included studies will reflect developments in treatment over the decades, with more favourable outcomes being observed in later studies. Disparities in healthcare access may also be contributing–most of the studies included used data from the United States, where adults aged over 65 years are eligible for Medicare, leaving younger patients at a disadvantage. However, many factors such as ethnicity and socioeconomic status also affect access

to healthcare and so firm conclusions cannot be drawn about this in our study.

This systematic review had several strengths. To our knowledge, this is the first systematic review undertaken to investigate survival in early-onset OAC. Excluding studies set within a trial setting which investigated age and outcomes in cancer patients, reduced the impact of selection biases and any possible volunteer biases. An evaluation of the quality of the studies was also carried out using the Newcastle-Ottawa Scale quality assessment, a robust and recognised tool.

This systematic review has some limitations. The search strategy which was conducted was limited to English language only, and findings may not have been representative of all the evidence available. Several of the studies defined 'young age' and 'older age' at different thresholds. Studies which consider a middle-aged category overlap with older age categories in other studies. These differences could not be separated in the statistical analysis and so could have affected the results seen. Several of the studies could not be included in the meta-analyses for various reasons; including the use of the same database or not reporting outcomes in a consistent manner.

CONCLUSION

In summary, our results suggest that early-onset OAC does not have a significantly different survival compared to older patients, but interpretation is limited due to a small number of studies over a long time period. Other factors, such as pathological characteristics and treatment receipt, also influence OAC outcomes and need to be taken into account. The inconsistency of the findings from the retrieved studies suggests the need for a more thorough population-based study with patients of all ages receiving similar treatment types. There is an urgent need for research to better understand the complex factors affecting survival such as inherited *vs* sporadic cases, stage of disease and pathology, as well as decision making by clinicians and patients and their influence on treatment uptake and survival for a cancer that, until now, has been mostly studied in older age groups.

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FOOTNOTES

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CASE REPORT

Early gastric composite tumor comprising signet-ring cell carcinoma and mucosa-associated lymphoid tissue lymphoma: A case report

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Abstract

BACKGROUND

Composite tumors are neoplasms comprising two distinct, yet intermingling, cell populations. This paper reports a rare phenomenon where early gastric signet-ring cell carcinoma (SRCC) and gastric mucosa-associated lymphoid tissue (MALT) lymphoma coexist within the same lesion.

CASE SUMMARY

A 40-year-old woman presented to the West China Hospital for examination, which revealed a whitish, shallow, and uneven mucosal lesion in the stomach. The lesion was diagnosed as a poorly differentiated adenocarcinoma, including SRCC with atypical lymphoid hyperplasia associated with *Helicobacter pylori* infection, based on histopathological examination of the biopsy specimen. The lesion was excised using segmental gastrectomy. However, histological examination of the surgical specimen confirmed that it was a poorly differentiated gastric adenocarcinoma with features of SRCC and MALT lymphoma. These two entities were stage I and coexisted in the same lesion.

CONCLUSION
It is uncommon for gastric SRCC and MALT lymphoma to coexist without distinct borders. Surgical resection is effective for these lesions.

Key Words: Gastric cancer; Composite tumors; Early stage; Signet-ring cell carcinoma; Mucosa-associated lymphoid tissue lymphoma; Case report

Core Tip: This paper reports a rare case of an early composite tumor involving the simultaneous occurrence of gastric signet-ring cell carcinoma (SRCC) and mucosa-associated lymphoid tissue lymphoma. A "composite tumor" is a unique phenomenon that combines two distinct tumor types into a single lesion without clear borders. When signet-ring cells are present in gastric B-cell lymphoma, it is crucial to carefully distinguish between lymphoma-associated signet-ring cell changes and gastric SRCCs. The former exhibits inconspicuous epithelial cell atypia and is mostly negative for p53 on immunohistochemistry, with positive E-cadherin expression, whereas the latter exhibits the opposite pattern.

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INTRODUCTION

Among the most common cancers worldwide, gastric cancer is the third largest cause of cancer-related death[1]. Notably, specific gastric cancer lesions may contain components of signet-ring cell carcinoma (SRCC). However, the incidence of SRCC, a distinctive subtype of gastric cancer, has consistently increased, accounting for 35% to 45% of the incidence of gastric adenocarcinoma[2]. Pathological classification of SRCC of the stomach corresponds to the low adhesion type classified by the World Health Organization, diffuse type by the Lauren classification, undifferentiated type classified by the Nakamura classification, and poorly differentiated adenocarcinoma classified by the Japan Gastric Cancer Association [3]. Mucosa-associated lymphoid tissue (MALT) lymphomas occur in organs outside the lymph nodes, such as the lungs, salivary glands, and gastrointestinal tract. Gastric MALT lymphomas account for 40% to 50% of primary gastric malignant lymphomas[4]. Although there have been reports of SRCC combined with MALT lymphoma, most of these have described two lesions that existed independently[5-9]. Herein, we report a composite tumor involving early gastric SRCC and MALT lymphoma, where the two histological patterns lack a distinct boundary.

CASE PRESENTATION

Chief complaints

A 40-year-old woman came to West China Hospital for a routine physical examination.

History of present illness

The patient's medical history was unremarkable, with no recent weight loss or symptoms like abdominal pain, vomiting, nausea, or diarrhea.

History of past illness

The patient had a medical history of appendectomy 30 years previously and a cesarean section 5 years ago.

Personal and family history

The patient denied using alcohol, smoking cigarettes, or abusing any illicit drugs and had no personal history of peptic ulcer, family history of gastric cancer, or MALT lymphoma.

Physical examination

The patient appeared well on physical examination, with a body mass index of 20 kg/m^2. An examination of the abdomen revealed a midline cesarean section scar and an oblique surgical scar from the prior appendectomy on the right lower abdomen. No abdominal tenderness or masses were observed. There was no palpable spleen or liver, and no nodes were palpable.

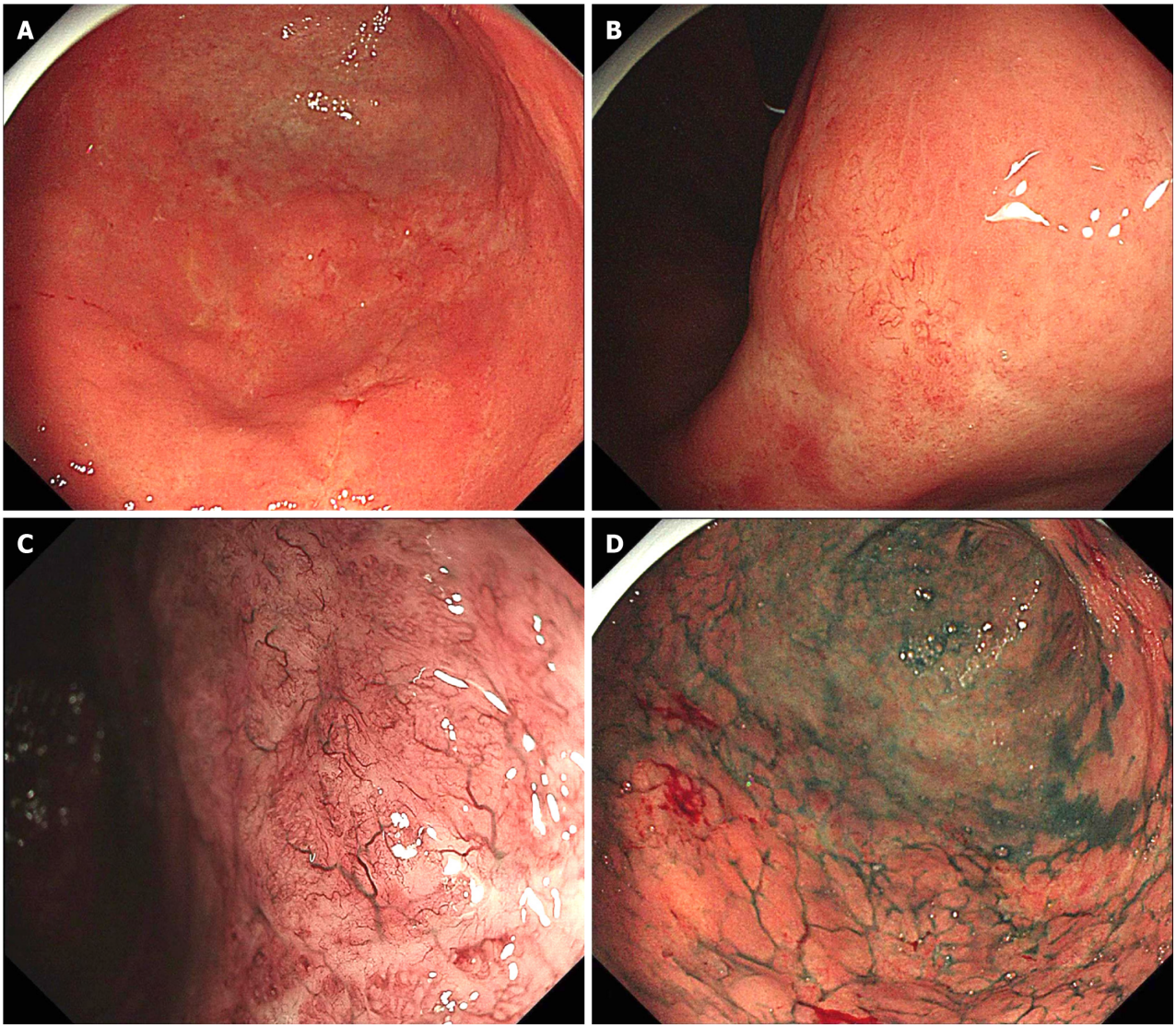

Figure 1 Endoscopic features of lesions. A: Whitish, shallow, and uneven mucosa lesions with minimal spontaneous bleeding at gastric antrum; B: The tumor invades gastric antrum and angular notch; C: Magnifying endoscopy with narrow-band imaging reveals typical tree-like appearance microvessels; D: Indigo carmine chromoendoscopy delineates a poor-demarcated lesion with an irregular margin.

Laboratory examinations

Hematological tests suggested normocytic anemia [112 g/L (normal, > 115 g/L)]. Laboratory investigations, including liver and kidney function tests, and tumor markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9, showed normal results. However, serum levels of pepsinogen (PG) 2 reached 31.2 ug/L (normal, 3–15 ug/L), and the PG1/2 ratio was low [4.04 (normal, 7-20)]. The 13C-urea breath test was positive.

Imaging examinations

Abdominal contrast-enhanced computed tomography revealed no significant thickening or mass shadow in the gastric wall, but enlargement of the hepatoduodenal ligament and para-abdominal aortic lymph nodes was evident.

Endoscopy of the upper gastrointestinal tract revealed a whitish, shallow, and uneven mucosal lesion (type IIc according to the Paris endoscopic classification) with minimal spontaneous bleeding. The lesion was located in the lower part of the stomach, including the angular notch, the greater curve of the gastric antrum, and the junction of the gastric antrum and body (Figure 1A and B). Magnifying endoscopy with narrow-band imaging (ME-NBI) revealed an irregular and partially absent microsurface along with tree-like appearance (TLA) microvessels (Figure 1C). The demarcation line, which may have distinguished the boundary between the gastric cancer and normal mucosa, was poorly defined. Indigo carmine chromoendoscopy revealed a poorly demarcated lesion with irregular margins (Figure 1D). Biopsy of the lesion subsequently confirmed it to be a poorly differentiated adenocarcinoma, including SRCC with atypical lymphoid hyperplasia (ALH), and was positive for *Helicobacter pylori* (*H. pylori*) infection in the gastric antral junction. In addition, endoscopic biopsy specimens of the posterior wall of the gastric antrum revealed ALH and follicular colonization, and immunohistochemical staining confirmed B-cell predominance. Distal gastrectomy with D2 Lymphadenectomy was performed after informed consent was obtained from the patient.

Postoperative pathological examination revealed no residual cancer tissue at the surgical margin. The resected specimen was a single lesion situated at the junction between the gastric antrum and body on the anterior wall, involving the lesser curvature of the stomach. Macroscopically, a superficial elevated (0-IIa) type and superficial depressed (0-IIc) type early gastric cancer, measuring 4.0 cm × 5.0 cm, was observed. The lesion was characterized by slight mucosal protrusions and scattered small erosions with clear boundaries. Histologically, numerous lymphoid cells diffusely infiltrated within the laminae propria mucosae. The lymphoid cells had small to medium-sized, slightly irregular nuclei with moderately dispersed chromatin and inconspicuous nucleoli. Few reactive lymph follicles were scattered. These tumorous lymphocytes infiltrated around lymph follicles in a marginal zone distribution and spread out to form confluent areas (Figure 2A). Simultaneously, multifocal atypical epithelial cells were observed. These atypical epithelial cells were isolated or arranged in small aggregates without well-formed glands. Some atypical cells presented a signet-ring cell appearance, characterized by a central globoid droplet of cytoplasmic mucin with an eccentrically placed nucleus (Figure 2B). Partial signet-ring cells formed delicate microtrabecular pattern. Immunohistochemical analyses revealed gastric lymphoid hyperplasia and heterotopic epithelial cells. The atypical lymphoid cells were positive for CD20, CD79a, p53 (partially), and negative for CD3, CD5, CD43, CD10, cyclin D1 and CD23. The Ki-67 index was 10%-20% (Figure 2C). Atypical epithelial cells were positive for CEA, pan-cytokeratin (pan-CK), p53 (partially), and Ki-67 (partially; Figure 2D). Monoclonal immunoglobulin heavy chain (*IGH*) and immunoglobulin Kappa light chain (*IGK*) gene rearrangements were detected. Moreover, pathology results for the lymph node tissue removed by surgery exhibited no tumor metastasis. Overall, the pathology examination revealed that the single lesion was an early-stage SRCC with MALT extranodal marginal zone B-cell lymphoma. Both tumors invaded within mucosal lamina propria.

FINAL DIAGNOSIS

The final diagnosis was T1N0M0 SRCC of the stomach coexisting with MALT lymphoma.

TREATMENT

In addition to the standard quadruple anti-*H. pylori* therapy, as described in the diagnostic workup, the patient underwent distal gastrectomy with D2 lymphadenectomy and was administered intraperitoneal chemotherapy with 0.5 g of 5-fluorouracil and 1 mL of carboplatin during surgery. Intraoperative gastroscopy revealed smooth and normal residual gastric mucosa.

OUTCOME AND FOLLOW-UP

The patient made a good recovery and was discharged on the 10th postoperative day. At the six-month follow-up, there was no evidence of recurrence, and no additional adjuvant therapy was required.

DISCUSSION

Herein, we report a rare case of gastric SRCC complicated by MALT lymphoma. In a review of previous cases, it was found that in 2014, George *et al*[6] documented a mixed lesion of poorly differentiated gastric SRCC and MALT lymphoma due to gastrointestinal bleeding. However, the patient had lymph node metastases when the lesion was discovered. In a separate study, Suwa *et al*[7] described a case involving gastric MALT lymphoma coexisting with early poorly differentiated adenocarcinoma, which included SRCC as part of the adenocarcinoma component. However, these two lesions were independent of one another and existed in different locations in the stomach. Additionally, Yacoub *et al* [8] reported collision tumors that comprise a well-differentiated gastric cancer adjacent to MALT lymphoma, with 10% SRCC. In this case, such a "collision tumor" represents the parallel development of two different pathological types of tumors, and it is believed that *H. pylori* is the cause of simultaneous occurrence. Distinguishing our case from the above-mentioned cases is that we report a composite tumor with SRCC and MALT lymphoma at an early stage. Composite tumors are characterized by the coexistence of two tumors with ill-defined boundaries. The corresponding collision tumor was composed of two tumors with different histological forms and clear margins[9].

Furthermore, the occurrence and development of MALT lymphoma are related to various signaling pathways, the most representative of which is the nuclear factor-κB (NF-κB) signaling pathway[10]. In particular, in the case of *H. pylori* infection, the bacteria bind to gastric epithelial cells *via* adhesion molecules, and nearby epithelial and immune cells recognize *H. pylori* through both membrane and intracellular receptors, triggering apoptosis and NF-κB-dependent inflammatory responses. Chronic *H. pylori* infection not only induces persistent inflammation in the gastric mucosa and stimulates lymphocyte proliferation, but also leads to genetic alterations. These genetic changes are primarily characterized by chromosomal translocations, resulting in the formation of fusion genes such as *AP12-MALT1*, *BCL10-IGH*, and *BIRC3-MALT1* in B cells, which subsequently activate the NF-κB signaling pathway[10,11]. More interestingly, a single-cell analysis of SRCC demonstrated significant enrichment of the NF-κB signaling pathway in SRCC cells[12]. These

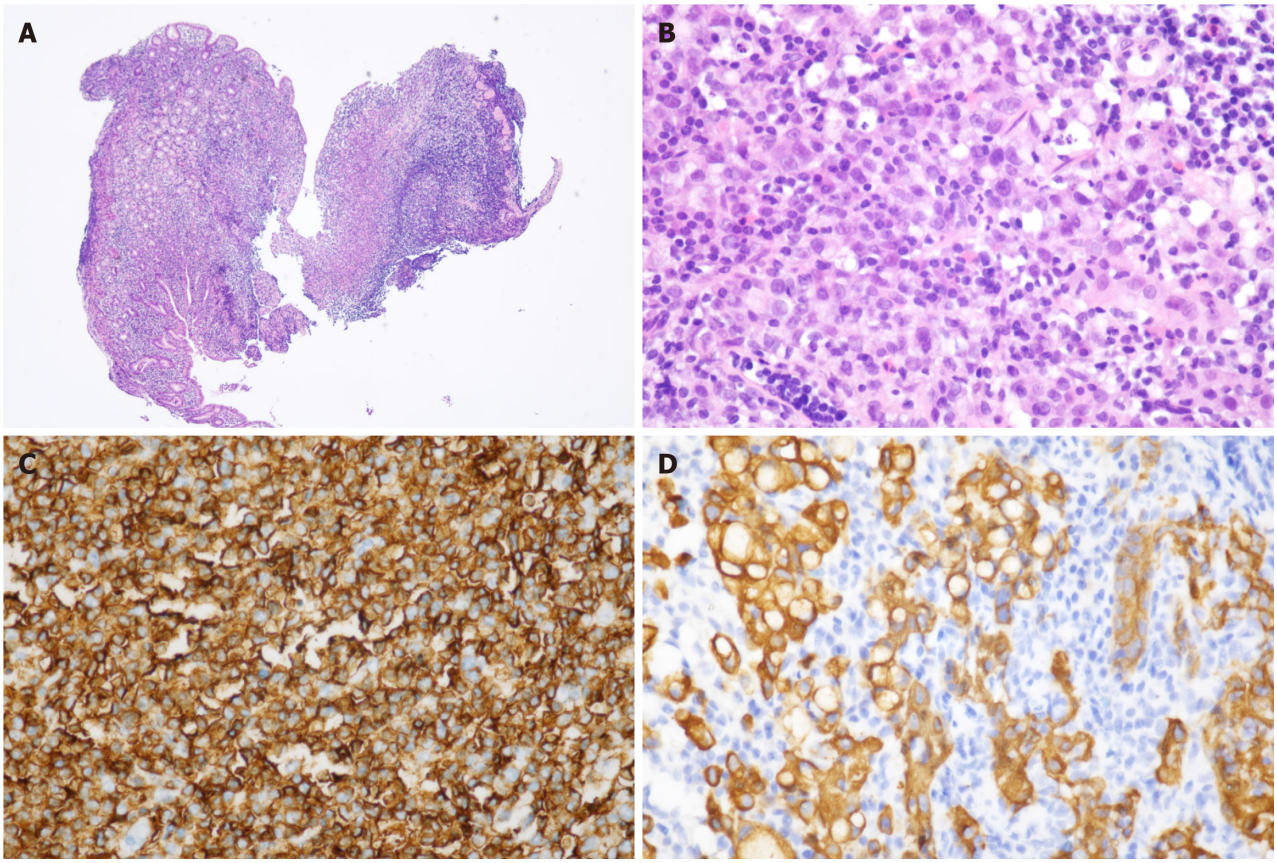

Figure 2 Histopathology of the stomach specimens removed through surgery. A: Low-power micrograph of gastric tumor (hematoxylin and eosin stain, × 40); B: High-power micrograph of signet ring cells and lymphoid follicular hyperplasia (hematoxylin and eosin stain, × 400); C: CD20 stain shows strong and diffuse cytomembrane immunoreactivity confirming gastric mucosa-associated lymphoid tissue lymphoma (× 400); D: Pan-cytokeratin stain shows strong and diffuse cytoplasmic immunoreactivity confirming signet-ring cell carcinoma (× 100).

studies indicate that the involvement of the immune response, particularly through the NF-κB signaling pathway, may play a crucial role in the coexistence of MALT lymphoma and SRCC under *H. pylori* infection. However, further research with larger sample sizes and more comprehensive studies is needed to elucidate the specific mechanisms underlying these conditions.

Our case report provides additional information for the early identification and study of concomitant SRCC and MALT lymphoma, especially regarding endoscopic manifestations and pathological features. Previous studies have shown that ME-NBI has a significantly higher sensitivity and specificity for gastric tumors than white light endoscopy[13]. We encourage using ME-NBI for comprehensive observation in cases where the coexistence of two distinct tumor types is suspected. The endoscopic findings of superficial flat-type early-stage gastric SRCC are mainly based on the destruction of the glands and the presence of typical microvessels found under ME-NBI. Previous studies have summarized the typical microvessels into three types: Wavy microvessels, corkscrew pattern, and raimon vessels[14,15]. On the other hand, "intersection traffic" and "pebble" signs are characteristics of the microsurface pattern of MALT lymphoma. Additionally, MALT lymphoma microvascular morphology frequently exhibits TLA[16]. In this case, we report that ME-NBI revealed typical TLA-like microvascular structures; however, the pathological result of the biopsy was SRCC. This discrepancy may be attributed to MALT usually accumulating in the submucosal layer rather than in the mucosal layer, making MALT rarely diagnosed on biopsy[17].

Signet-ring cells represent a morphological description of cellular proliferation under various conditions. They can be observed in mucous-producing epithelial tumors and various cell types, including endocrine cells, histiocytes, lymphoid cells, melanocytes, and squamous cells. This wide distribution presences span both reactive processes and benign or malignant tumors. Signet-ring cells have been identified in some cases of primary gastric B-cell lymphoma[18]. Notably, Zamboni *et al*[19] reviewed the surgical and biopsy specimens of 70 cases of MALT lymphoma and found signet-ring cell change (SCC) in 20 of the 70 cases of MALT lymphoma. They characterized SCC by an abundant pale cytoplasm and a small peripheral nucleus with insignificant nucleoli, without heterotypic or mitotic activity. The cells in SCC had high positive for E-cadherin expression but negative p53 and Ki-67 results. In contrast, cells in SRCC exhibited strong positivity for p53 and a lack of or weak positivity for E-cadherin. Routine immunohistochemical staining for p53, Ki-67, and E-cadherin can help distinguish between SCC and SRCC[18]. In the present case, the expression of pan-CK, CEA, p53, and Ki-67 in heterotopic epithelial cells, combined with general morphology, supported the diagnosis of SRCC rather than SCC in MALT lymphoma. When two types of tumors are present simultaneously, the diagnosis can only be confirmed after surgery. Although the occurrence of early gastric SRCC and MALT lymphoma is uncommon, early detection and timely intervention can yield a favorable prognosis. This underscores the importance of gastroenterologists

being vigilant during routine gastroscopic procedures, devoting close attention to suspicious lesions, conducting multiple biopsies, and capturing lesions as much as possible.

CONCLUSION

In summary, we report a rare case of an early composite tumor, including gastric SRCC and MALT lymphoma. For suspected lesions, detailed observation with ME-NBI should be incorporated into endoscopic examination, with multiple biopsies to evaluate. It is crucial to distinguish SCC observed in gastric B-cell lymphoma from gastric SRCC, as SCC typically exhibit minimal epithelial atypia, are p53-negative, and show positive E-cadherin expression, which contrasts with the pattern in gastric SRCC. Surgical resection is effective for early-stage SRCC combined with MALT lymphoma.

FOOTNOTES

Author contributions: Jia YF and Chen FF contributed equally to manuscript writing and editing, and data collection; they make the equal contribution; Yang L, Ye YX, and Gao YZ contributed to conceptualization and supervision; Yang JL and Zhang WY contributed equally to revise the article and supervision; all authors have read and approved the final manuscript.

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LETTER TO THE EDITOR

Review on article of preoperative prediction in chronic hepatitis B virus patients using spectral computed tomography and machine learning

Yao-Qian Yuan, Qian-Qian Chen

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Abstract

This letter comments on the article that developed and tested a machine learning model that predicts lymphovascular invasion/perineural invasion status by combining clinical indications and spectral computed tomography characteristics accurately. We review the research content, methodology, conclusions, strengths and weaknesses of the study, and introduce follow-up research to this work.

Key Words: Gastric cancer; Spectral computed tomography; Perineural invasion; Lymphovascular invasion; Machine learning

Core Tip: Accurate preoperative assessment of gastric cancer staging and tumor aggressiveness is critical for the development of individualized treatment. Previous studies have shown that lymphovascular invasion (LVI) and perineural invasion (PNI) can predict tumor invasion and patient prognosis; therefore, preoperative LVI and PNI assessment can help oncologists identify high-risk categories of gastric cancer patients preoperatively and predict outcomes. This letter comments on a published study that showed that the accurate preoperative identification of LVI/PNI in gastric cancer can be achieved by merging clinical markers with portal venous and equilibrium phase spectral computed tomography characteristics.

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TO THE EDITOR

For gastric cancer, perineural invasion (PNI) and lymphovascular invasion (LVI) are significant prognostic variables, suggesting a higher risk of metastasis and poor prognosis. Clinical professionals can determine high-risk patients and make treatment decisions with the use of precise preoperative LVI/PNI status. Nevertheless, the accuracy of previous models that solely used computed tomography (CT) scans to predict LVI or PNI was restricted.

Spectral CT imaging, which offers a wide range of quantitative characteristics, can transition from studying macroscopic morphology to microscopic quantitative issues[1]. Prior research has shown that the prognosis, staging, lymph node metastases, and histological classification of patients with gastric cancer may all be evaluated quantitatively using spectral CT imaging[2-4]. According to Ren *et al*[5], LVI and PNI could be evaluated using energy-based spectral CT parameters, histological grading, Borrmann grading, and cancer antigen 125. Few studies have examined the efficacy of predicting preoperative LVI and PNI in gastric cancer patients using machine learning and spectral CT imaging. To better address this clinical problem, this study used a machine learning approach in which the optimal fusion of clinical markers and spectral CT parameters could more precisely anticipate preoperative LVI/PNI status in gastric cancer patients.

MACHINE LEARNING MODEL

The purpose of this letter is to comment on the machine learning model that incorporates spectral CT parameters and clinical indicators to accurately anticipate LVI/PNI status.

We read with interest the article published in *World Journal of Gastroenterology* by Ge *et al*[6]. The retrospective dataset utilized for this investigation included 257 gastric cancer patients [validation cohort (n = 85); training cohort (n = 172)]. First, quantitative spectral CT characteristics were retrieved from the delimited tumor sites, together with various clinical indicators such as cytotactin/tenascin (TN) stages, serum tumor markers, and CT-detected extramural vein invasion (CT-EMVI). Subsequently, informative clinical and spectral CT parameters were chosen by a two-step feature selection procedure within a 10-fold cross-validation loop that combined information gain ranking and correlation-based techniques. The area under the receiver operating characteristic area under the curve (AUC) was used to assess the efficacy of a nomogram model based on logistic regression (LR) that was created to predict LVI/PNI status.

A statistically significant difference was observed in the prevalence of CT-EMVI positive status (P < 0.05), CT-N positive status, and CT T3-4 stage between the LVI/PNI-positive group in both the validation and training cohorts. Following LR analysis, the training group's preoperative CT-EMVI, CT-T stage, the ratio of standardized iodine concentration of equilibrium phase (EP-NIC), and single-energy CT values of 70 keV of venous phase (VP-70 keV) were found to be independent affecting factors. CT-T and CT-EMVI had AUC of 0.793 and 0.762, respectively, the AUC of EP-NIC and VP-70 keV were 0.824 and 0.888, respectively, and were marginally higher.

This study used a machine learning system to assess CT-determined TN stage, quantitative spectral CT parameters, CT-EMVI, and blood tumor markers. Feature reduction and LR analysis showed that the histological LVI/PNI status could be independently predicted by the VP-70 keV CT value, CT-EMVI, CT-T stage, and EP-NIC.

There were some limitations to this study. First, there were differences in the number of patients in the LVI/PNI-positive and negative groups, and the sample size was small. Second, other histological tumor types were not examined; only gastric adenocarcinoma. Third, conventional clinicopathological parameters were not taken into account by this prognostic model. Fourth, the results may not be generalizable because the study was conducted at a single center. To confirm that using these predictive models more widely is clinically feasible, a multicenter study is required.

Subsequent research will rely on verifying the therapeutic utility of a noninvasive spectral-CT-based machine learning model in preoperative risk assessment through a prospective multicenter investigation. Further research may examine how this model might be incorporated into standard clinical practice to evaluate its effects on patient management, especially in terms of identifying patients who might profit from more intensive preoperative treatment plans. In the future, research on spectral CT imaging may enhance and expand its prognostic powers, which could lead to better results and more personalized treatment plans for patients with gastric cancer.

The combination of artificial intelligence (AI) and medical imaging is helpful for preoperative prediction of gastric cancer, and many novel techniques are emerging. Huang *et al*'s team used CT deep learning features and clinical data to predict malnutrition in patients with gastric cancer[7]. Fan *et al*'s team used alexander networks, extreme learning machines to optimize a new hybrid method for detecting early gastric cancer[8]. Fan *et al*'s team used positron emission CT/CT and augmented CT radiomics and clinical variables for machine learning analysis of noninvasive prediction of LVI in gastric cancer[9].

CONCLUSION

These studies demonstrate the potential of AI techniques for preoperative prediction of gastric cancer, especially in analyzing complex medical imaging data. As these technologies continue to evolve and their effectiveness and clinical applications are being validated through clinical trials and studies, more innovative methods may be developed in the future to improve the accuracy of preoperative gastric cancer prediction.

FOOTNOTES

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LETTER TO THE EDITOR

Physician-dependent diagnosis delay in Crohn's disease: A pseudo-proposition or not?

Yan Zeng, Jun-Wen Zhang, Jian Yang

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Abstract

The challenge of diagnosis delay in inflammatory bowel disease (IBD) has emerged as a significant concern for both patients and healthcare professionals. The widely accepted notion that there is an extended time frame from the onset of symptoms to the definitive diagnosis is often attributed to the heterogeneity of IBD and the non-specificity of clinical manifestations. Specific to patients with Crohn's disease, the issue of delayed diagnosis appears to be more pronounced across different regions globally. The intricate interplay of real-world factors has led to debates regarding the primary contributors to these diagnostic delays. Drawing a comparison solely between patients and physicians and implicating the latter as the predominant influence factor may fall into a simplistic either-or logical trap that may obscure the truth. This letter, grounded in published evidence, explores areas for improvement in a forthcoming paper within the field, hoping to pinpoint the culprit behind the diagnosis delay issue for IBD patients rather than simply attributing it to so-called "physician-dependent factors". Our objective is to motivate healthcare providers and policymakers in relevant fields to reflect on strategies for addressing this problem to reduce diagnostic delays and enhance patient outcomes.

Key Words: Inflammatory bowel disease; Crohn's disease; Diagnosis delay; Influencing factors; Culprit

Core Tip: This letter centers on the pressing matter of diagnostic delays in inflammatory bowel disease, particularly Crohn's disease. Drawing on a comprehensive evaluation of a forthcoming paper in the field, our editorial posits that addressing the current diagnostic delays in Crohn's disease hinges on recognizing the myriad complex real-world factors contributing to the issue, particularly emphasizing those behind the so-called "physician-dependent factors".

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TO THE EDITOR

The diagnostic journey for inflammatory bowel disease (IBD), particularly Crohn's disease (CD), is often fraught with delays, a reality that has significant implications for patient outcomes and healthcare delivery[1,2]. The prolonged time from symptom onset to a confirmed diagnosis is a well-documented issue in the field, with factors ranging from the disease's heterogeneity and non-specific clinical presentations to variations in healthcare systems and physician practices [3]. A forthcoming paper reporting a 10-year prospective study of delayed diagnosis in German IBD patients found that CD exhibited a more pronounced delay in diagnosis compared to ulcerative colitis (UC), highlighting the complexity of the diagnostic challenge[4]. However, the study's conclusion asserts: "Compared to patient-dependent factors, the longer diagnostic delay in CD patients compared to UC patients is physician-dependent"[4].

We analyzed previous studies on diagnosis delay in CD up to June 2024, utilizing reference citation analysis (https://www.referencecitationanalysis.com/), a unique artificial intelligence system for evaluating citations in biomedical literature. First, the current wording of this mentioned conclusion is prone to misinterpretation, potentially leading to the erroneous belief that the longer diagnostic delay in CD patients is mainly due to physician-related issues, which is not the whole truth or the primary truth. This oversimplification does not accurately reflect the complexity of the situation. Instead, it may inadvertently cast physicians as the primary culprits and the scapegoats for the causes of the diagnostic delay. It is widely known that IBD patients, especially CD patients, present with non-specific symptoms and significant heterogeneity, requiring the collaborative involvement of experienced gastroenterologists, endoscopists, radiologists, and pathologists[5,6]. Therefore, the diagnostic process is cautious and fraught with challenges, and cannot be achieved overnight. Although the authors have acknowledged certain limitations of the study, such as the omission of smoking habits, education level, and disease complications, the survey questionnaire used in the study to explore factors that may directly or indirectly affect the delay in the diagnosis of IBD patients still overlooks a multitude of real-world factors that are prevalent and cannot be ignored, such as the inherent complexity of CD, the heterogeneous patient-related factors, comorbidities, patient's socioeconomic status, the accessibility of medical facilities (including waiting times for colonoscopy and pathological diagnosis), the quality of medical services, and their local insurance policies and referral system regarding IBD[3,7-10]. These factors undoubtedly shape the diagnostic journey and cannot be disregarded in a comprehensive understanding of the issue.

We argue that placing undue emphasis on physicians as the primary cause of delayed diagnoses in IBD may distract from the other significant factors that necessitate intervention. Moreover, it could precipitate a rushed, low-quality, or uncompleted diagnostic process, potentially shortening the necessary differential diagnosis and trial treatments and ultimately compromising patient health and well-being[11]. Furthermore, this perspective may erode trust in healthcare providers, exacerbating the existing challenges in diagnostic delays. Therefore, this issue warrants a comprehensive and thoughtful examination.

We acknowledge the possibility that the abovementioned issues were not the authors' intention but may reflect a limitation in the study's conceptualization. The design may have inadvertently pitted patient-related factors against those attributed to physicians, an oversimplification that does not reflect the multifaceted nature of IBD diagnosis delay. It may seem intuitive to consider patient and physician factors as opposing forces (the factors contributing to the delayed diagnosis of IBD are other than patient-dependent factors, the remainder being physician-dependent factors); however, this dichotomy overlooks the intricate interplay of variables that influence diagnostic timelines. The most easily overlooked aspects are the characteristics of the disease itself and the individual variation between patients.

Second, we highlight the potential oversight in this forthcoming paper's inclusion criteria. The authors mentioned the enrollment of 513 IBD patients in a 10-year duration, with 18 subsequent exclusions for indeterminate colitis and irritable bowel syndrome (IBS), raising questions about the initial cohort's diagnostic process[4]. Were the diagnostic procedures, such as colonoscopies, pathological examinations, and necessary differential diagnosis, uniformly conducted following the diagnostic criteria at that time[12]? This crucial aspect requires clarification; otherwise, any subsequent discussion of diagnostic delays would be akin to building on quicksand. Moreover, suppose the "513 patients with IBD" mentioned by the authors in their study design were only "suspected" cases of IBD. In that case, it is noteworthy that, in addition to the indeterminate colitis and IBS mentioned by the authors, there were no other cases typically misdiagnosed as IBD in the 10-year study cohort, such as intestinal tuberculosis, Behçet's disease, and intestinal lymphoma, which seems to contradict our clinical experience and published evidence[8,13].

Furthermore, the study's methodology, characterized as a post-enrollment survey of the 513 patients with IBD, aligns more closely with a retrospective study design than the "prospective study" described in the abstract. While noted by reviewers, this distinction has not been adequately addressed or rectified, which is crucial for accurately representing the study's approach. Additionally, towards the end of the discussion section in paragraph four, the analysis of diagnostic gastroscopy referenced as Table 3 should correspond to Table 4 in the manuscript. It is puzzling that the authors included gastroscopy as a variable in their multivariate analysis; however, they failed to directly analyze the more crucial factors of colonoscopy quality and waiting times among IBD patients[14]. Furthermore, the authors' discussion regarding using gastroscopy findings to assess the accessibility of diagnostic endoscopic procedures for different patient groups appears to diverge from the core issue. A more direct approach would be to examine indicators with greater clinical significance and potential for future interventions, such as the waiting time for a colonoscopy following a doctor's medical order and the interval between acquiring pathological specimens from a colonoscopy and receiving the pathological diagnosis.

Overall, this forthcoming paper remains highly commendable, its strengths shining despite the areas where refinement is possible. As highlighted by the authors, it still holds valuable guidance and reference value for gastroenterologists worldwide. This 10-year retrospective analysis, focused on the delayed diagnosis of IBD patients in Germany, addresses a critical data gap for European and mainly German patients. The study identifies risk factors for delayed diagnosis related to patient waiting times and physician diagnosis intervals. Additionally, it underscores disparities in relative risk factors between patients with CD and UC[4].

Returning to addressing the hot issue of delayed diagnosis in IBD, a multifaceted approach is required, mainly encompassing medical regimes, human resources, and financial support. These aspects are often conflated or mistakenly attributed to "physician-dependent factors". To begin with, continuously improving medical regimes is essential. It includes revising the medical insurance system to ensure timely and comprehensive IBD diagnostics coverage. Additionally, establishing an efficient referral system for patients with suspected IBD is crucial. This system should facilitate quick and seamless transitions between primary care physicians and specialists, ensuring that patients receive prompt and appropriate care. Second, investing in human resource development is vital, which entails enhancing the education and training of general practitioners and common people in recognizing the early signs of IBD. Furthermore, forming a well-staffed, multidisciplinary IBD management team, including skilled gastroenterologists, endoscopists, and pathologists, is necessary. This team can ensure timely diagnosis and long-term management, improving overall patient outcomes. Third, adequate financial support is crucial, which includes securing funds to cover necessary patient examinations, referrals, and the maintenance of the IBD management team. Ensuring financial sustainability will help in providing consistent and high-quality care to patients. Should the necessary financial resources fail to materialize to safeguard the rights that physicians are striving to uphold, the possibility of rectifying diagnostic delays across various diseases, including IBD, stands as an unachievable goal once a strike is called[15].

Although fundamentally addressing the root causes of IBD diagnosis delays is not a task that can be achieved globally in a single step, at the very least, we can identify these deeper underlying causes and avoid attributing all issues solely to "physician-dependent factors". By considering the socioeconomic conditions and health service needs of different regions, we can take steps within our capabilities to gradually advance in the right direction towards resolving the current diagnostic delays.

CONCLUSION

In conclusion, the contention that physician-dependent factors are the relatively primary cause of the prolonged diagnostic delay in CD than UC is an oversimplification that fails to capture the intricate nature of the problem. While physicians undoubtedly play a pivotal role in the diagnostic process, the diagnostic delay in CD is shaped by a constellation of factors, encompassing the disease's intrinsic complexity, patient-specific variables, systemic limitations within healthcare, and the dynamic evolution of diagnostic criteria and technologies. A nuanced understanding of these multifaceted influences is imperative for effectively tackling the diagnostic challenges in IBD, particularly for CD, expediting the diagnostic timeline and ultimately improving patient outcomes.

FOOTNOTES

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LETTER TO THE EDITOR

Endoscopic polidocanol foam sclerobanding for the treatment of Grade II-III internal hemorrhoids: The focus of clinical practice

Yu-Yan Zhang, Bing Hu

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Abstract

We have read the article by Qu *et al* with great interest, as it presents an integration of endoscopic polidocanol foam sclerotherapy with rubber band ligation in patients with Grade II-III internal hemorrhoids. The authors conducted a prospective, multicenter, randomized study to evaluate the long-term symptomatic and endoscopic efficacy of this combined intervention. In this discussion, we focus on the procedural steps of this combined strategy and suggest potential avenues for future research.

Key Words: Internal hemorrhoids; Endoscopic therapy; Polidocanol foam; Sclerotherapy; Rubber band ligation; Sclerobanding

Core Tip: Hemorrhoid disease is common, and multiple intervention methods exist. Qu *et al* conducted a prospective, multicenter, randomized study to demonstrate the effectiveness and safety of combining endoscopic sclerotherapy and rubber band ligation in managing Grade II-III internal hemorrhoids. We aim to share our concerns about the specific procedures of this combined strategy and to highlight some potential directions for subsequent research.

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TO THE EDITOR

Internal hemorrhoids are very common anorectal diseases. Injection sclerotherapy (IS) and rubber band ligation (RBL) are commonly utilized techniques for managing hemorrhoids[1]. Some researchers have observed enhanced outcomes by combining these two techniques, which has been verified by retrospective studies[2,3]. The introduction of flexible endoscopy has improved the maneuverability and convenience of the IS and RBL procedures, enabling direct visualization and documentation. We are interested in the study conducted by Qu *et al*[4], which explored the application of the combined IS and RBL approach within an endoscopic framework.

COMMENT

This study compared the outcomes of endoscopic IS combined with RBL and simple endoscopic RBL in a cohort of 195 patients diagnosed with Grade II-III internal hemorrhoids. The findings indicated that the combined approach offered prolonged satisfaction and effective alleviation of prolapse recurrence and postprocedural pain within 24 hours. The research methodology employed was multicenter, prospective, randomized, and single-blind, thereby reducing the risks of performance and detection biases. This high-quality evidence is expected to serve as a reliable reference for clinical treatment decision-making. Nevertheless, unresolved issues warrant further discussion and investigation. Despite both methods being combined, variations in the sequence of procedural steps may influence the safety, efficacy, and convenience of operational implementation.

In the retrospective study conducted by Pata *et al*[2], which included 97 cases of Grade II-III internal hemorrhoids with a median follow-up duration of 12 months, the treatment involved RBL followed by IS with 3% polidocanol foam. The results revealed the absence of intraoperative adverse events or severe complications, as well as no instances of mortality or readmission. The authors explained the effectiveness of this procedure; specifically, the 3% polidocanol injection may prevent early slippage of the rubber band, reduce the risk of delayed bleeding, and increase fibrosis at the ligated site, and the preplaced rubber band may prevent the spread of polidocanol foam into the surrounding tissues, thereby reducing significant complications, especially in the anterior area (such as abscess, acute prostatitis, and sepsis).

In this study[2] and another retrospective study[3], IS was implemented first, followed by RBL. Chew *et al*[3] investigated the results of 6739 patients who underwent an injection of 5% phenol in almond oil followed by band ligation. The study revealed a low recurrence rate of 16% and an overall complication rate of 3.1%, with minor bleeding being the major complaint. Qu *et al*[4] implemented a combined strategy endoscopically, utilizing 1% polidocanol foam as the sclerosant, in a prospective, multicenter, randomized study. Compared with simple endoscopic RBL, the combination of endoscopic IS followed by RBL resulted in a lower postprocedural hemorrhoid severity score [2.0 (range: 1.0-3.0) *vs* 3.0 (range: 2.0-3.0), $P < 0.001$], a lower recurrence rate (11.2% *vs* 21.6%, $P = 0.038$) at the 12-month follow-up, and decreased postprocedural pain within the first 24 hours after the procedure. Both studies share the same validity assumption, as submucosal injection aids in elevating the mucosa, facilitating ligation, and preventing aspiration of the muscularis propria. This, in turn, reduces postoperative pain resulting from visceral innervation and promotes increased fibrosis to improve symptom relief.

Nevertheless, endoscopists may harbor concerns during the procedure. Specifically, will the tension escalate postinjection, thereby complicating the application of RBL? Could the compression of the rubber band facilitate the spread of surplus sclerosant to adjacent regions? As delineated in this article, to what extent may the occurrence of postoperative bleeding increase due to mucosal injury induced by the injection?

Moreover, the IS and RBL techniques play a synergistic role in the combination strategy. The different implementation orders may imply different functional principles. Do these differences extend to safety and efficacy? To what degree does this combined strategy contribute to increased procedural time and costs? Additionally, what disparities exist between the trans anal and endoscopic approaches in this procedure? Is the endoscopic approach the optimal method for implementing the combination strategy? Further evidence is needed to address these inquiries, which will assist endoscopists in enhancing their clinical application and practice.

Various sclerosant solutions have been utilized in the management of hemorrhoids. Polidocanol, a local anesthetic with analgesic properties, also facilitates fibrosis. The ideal concentration for hemorrhoid treatment, whether it is 1% (as employed in this study) or 3%[2,5], remains uncertain. This sclerosant shows promise, and determining the optimal concentration and method of application to maximize effectiveness is crucial[6,7].

CONCLUSION

This study offers high-quality evidence supporting the endoscopic treatment of internal hemorrhoids. We anticipate that the authors will continue to contribute to further research and insights in this area.

FOOTNOTES

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LETTER TO THE EDITOR

Anti-tumor efficacy of *Calculus bovis*: Suppressing liver cancer by targeting tumor-associated macrophages

Ishita Kathuria, Bhupesh Singla

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Abstract

Despite significant advances in our understanding of the molecular pathogenesis of liver cancer and the availability of novel pharmacotherapies, liver cancer remains the fourth leading cause of cancer-related mortality worldwide. Tumor relapse, resistance to current anti-cancer drugs, metastasis, and organ toxicity are the major challenges that prevent considerable improvements in patient survival and quality of life. *Calculus bovis* (CB), an ancient Chinese medicinal drug, has been used to treat various pathologies, including stroke, convulsion, epilepsy, pain, and cancer. In this editorial, we discuss the research findings recently published by Huang *et al* on the therapeutic effects of CB in inhibiting the development of liver cancer. Utilizing the comprehensive transcriptomic analyses, *in vitro* experiments, and *in vivo* studies, the authors demonstrated that CB treatment inhibits the tumor-promoting M2 phenotype of tumor-associated macrophages *via* downregulating Wnt pathway. While multiple studies have been performed to explore the molecular mechanisms regulated by CB, this study uniquely shows its role in modulating the M2 phenotype of macrophages present within the tumor microenvironment. This study opens new avenues of future investigations aimed at investigating this drug's efficacy in various mouse models including the effects of combination therapy, and against drug-resistant tumors.

Key Words: *Calculus bovis*; Liver cancer; M2-like tumor associated macrophages; Wnt/β-catenin pathway; Tumor environment

Core Tip: *Calculus bovis*, a traditional animal drug used in China, has been recognized for its therapeutic effects across various organ systems, including the central nervous, cardiovascular, respiratory, and digestive systems. Recent studies have also suggested its anti-tumor potential. While previous studies have explored the mechanisms of action of its active compounds, this study provides novel insights into its anti-tumor potential using a liver cancer xenograft model. M2 macrophages are associated with tumor progression because they promote tumor growth, angiogenesis, and metastasis while inhibiting effective anti-tumor immune responses. This study, for the first time, demonstrates that *Calculus bovis* modulates the tumor environment by governing M2-tumor-associated macrophages in a Wnt pathway-dependent manner, thereby suppressing tumor growth.

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TO THE EDITOR

Liver cancer and treatment strategies

Liver cancer is one of the most prevalent cancers worldwide and accounts for over 800000 deaths annually[1]. Treatment for liver cancer is tailored to individual needs, considering the tumor stage, the extent of the underlying disease, and the expected patient's response to available therapies[2]. For early-stage liver cancer, therapeutic interventions include surgical resection of tumor-bearing liver parts, transplantation with a healthy donor liver, ablation therapies to destroy tumors, and other modalities to kill cancer cells[3-5]. However, treatment options for intermediate- and advanced-stage cancer include transarterial embolization to block the blood supply to tumors, multiple kinase inhibitors such as sorafenib and lenvatinib, and immune checkpoint blockers like atezolizumab/bevacizumab[5-10]. These treatment strategies despite decreasing yearly mortality rate and improving patient survival, often possess significant adverse effects. Additionally, patients may experience tumor reoccurrence, metastasis, resistance to treatments, and liver toxicity[11-14]. These challenges indicate the limitations of current therapies and suggest the urgent need for more effective and safer therapeutic alternatives.

Role of tumor-associated macrophages and β-catenin pathway in liver cancer

The heterogeneity of the tumor immune microenvironment (TME) significantly contributes to tumor metastasis, relapse, and drug resistance[15]. To develop effective treatment regimens and successfully treat primary liver cancer, it is crucial to understand the TME composition both at baseline and during treatment. The TME consists of various types of immune cells including T and B lymphocytes, regulatory T cells, macrophages, neutrophils, dendritic cells, natural killer cells, platelets, and mast cells, as well as non-immune components such as cancer-associated fibroblasts, adipocytes, endothelial cells, pericytes and lymphatic endothelial cells and the extracellular matrix[16]. Additionally, the liver cancer TME comprises hepatic stellate cells, myeloid-derived suppressor cells[17], and liver sinusoidal endothelial cells[18]. In order to support their growth, cancer cells induce a tumor-supportive environment by reprogramming non-cancerous cells, remodeling the extracellular matrix, and altering the vasculature. Tumor-associated macrophages (TAMs), the most abundant immune cell population within a tumorigenic liver, are key players in sustaining cancer cell growth and invasiveness, often correlating with poor prognosis[19]. TAMs exhibit a high level of plasticity, differentiating into either tumor-promoting (M2) or tumor-regressing (M1) phenotypes. Inflammatory stimuli, such as interferon-gamma or microbial products like lipopolysaccharide molecules induce a 'classical activated' or M1-like phenotype, characterized by increased antigen-presenting capability, high cytotoxic activity, expression of pro-inflammatory cytokines, and activation of T helper 1 immune response. Conversely, growth factors [interleukin (IL)-4, IL-13, IL-10] and T helper 2-related cytokines in the TME promote alternative activation of macrophages into an M2-like phenotype. These M2 macrophages are marked by high expression of cytokines such as IL-10, transforming growth factor-β (TGF-β), and CCL17 and have poor antigen-presenting ability. These anti-inflammatory M2-TAMs possess immunosuppressive properties and promote cancer cell growth and invasiveness, making them a viable therapeutic target[20].

The Wnt/β-catenin pathway plays a crucial role in adult tissue homeostasis and embryonic development. Dysregulated activation of this pathway is responsible for the development of multiple diseases, including cancer[21]. Modulated Wnt/β-catenin signaling is one of the main genetic alterations in the pathogenesis of liver cancer. Gain-of-function mutations in the *CTNNB1* gene encoding β-catenin and loss-of-function *AXIN1* mutation occur in a significant number of human liver cancer patients[22]. The Wnt/β-catenin pathway comprises four components: Extracellular (Wnt proteins: Wnt3a, Wnt1, and Wnt5a), membranous (Wnt receptors: Frizzled and lipoprotein receptor-related protein 5/6), cytoplasmic (β-catenin, AXIN1, casein kinase 1, *etc.*), and nuclear (β-catenin translocates to the nucleus and induces the transcription of downstream target genes)[23]. Activation of Wnt receptors by Wnt proteins stabilizes β-catenin, promoting its nuclear translocation and leading to transcription of downstream target genes. In the absence of Wnt signaling, β-catenin undergoes cytoplasmic degradation, thereby preventing this signaling cascade[24].

Major findings, future directions, and conclusions

Huang *et al*[25] investigated the anti-tumor potential of *Calculus bovis* (CB), a well-known animal drug made from the dried gallstones of *Bos taurus domesticus* Gmelin cows[26]. Previous studies have shown that CB inhibits liver tumor growth by modulating the viability of primary liver cancer cells and inducing their apoptosis[27,28]. Further, a derivative of CB has been reported to reduce hepatic and gut injury in an estrogen-induced cholestasis rat model by regulating inflammation, oxidative stress, apoptosis, and bile acid profiles[29]. Huang *et al*[25] utilizing both *in vitro* studies and *in vivo* liver cancer xenograft mouse models, elucidated the mechanisms by which CB suppresses M2-TAM polarization and inhibits tumor growth, making this study highly informative. The authors identified lithocholic acid as a key pharmacological component in CB extract, and glycohyodeoxycholic acid in CB-enriched serum. Using bioinformatics and docking studies to determine underlying molecular mechanisms, they prioritized the Wnt pathway due to its important role in cell proliferation, apoptosis, invasion, and tissue homeostasis, all of which are linked to liver cancer progression and immune modulation[30-33]. The study demonstrated that CB inhibits the tumor-promoting M2 polarization of TAMs by suppressing Wnt/β-catenin signaling, thus shifting the TME towards regression. This effect is supported by the elevated expression of genes associated with M2-TAMs, including, CCL2, IL-10, TGF-β, and Arg-1. *In vitro* experiments further revealed reduced migratory and invasion capabilities of HepG2 cells when treated with CB serum (M2-TAM conditioned medium). The involvement of the Wnt/β-catenin signaling pathway was investigated using a Wnt agonist SKL2001, which reversed CB's effects on TAM polarization.

While the study provides strong evidence supporting CB's anti-tumor effects through M2-TAM polarization and Wnt pathway inhibition, further research is needed. It would be interesting to explore the involvement of other molecular signaling pathways identified in the transcriptomic sequencing analysis, such as Phosphoinositide 3-kinase-Akt, Ras-associated protein1, and Ras in liver cancer development and CB's potential effects on these pathways[34-36]. Besides, Wnt signaling involves nuclear translocation of the β-catenin for regulating the expression of target genes, and several Wnt proteins (nineteen) have been identified till date, the in-depth effects of CB on the levels of these Wnt proteins and β-catenin nuclear translocation would provide further insights[23]. Although Huang *et al*[25] substantiated their findings with complementary *in vitro* and *in vivo* experiments and transcriptomic data, some areas require future investigations. The most abundant active compound identified in the study, lithocholic acid (CB extract) has been demonstrated to have anti-cancer properties[37,38]. Similarly, glycohyodeoxycholic acid (CB-serum), a bile acid derivative, has inhibitory effects in the carcinogenesis of various organs[39]. These findings suggest that these active constituents of CB may be responsible for the inhibition of liver cancer in CB-treated animals. For future research, ursodeoxycholic acid, a secondary bile acid with established anti-tumor activities and already used in clinics for the treatment of gallstones, biliary cirrhosis, and hepatic dysfunction should be used as a control to compare CB's anti-tumor effects[40-43]. Moreover, determining the levels of M1-[Inducible nitric oxide synthase, cluster of differentiation (CD) 80, CD86, and human leukocyte antigen-DR)[44] and M2-(CD206, CD204, and CD163)[45] TAMs using multiple markers would be informative. Angiogenesis, the formation of new blood vessels, is crucial for cancer progression as it supplies nutrients, oxygen, and growth factors to the tumors[46]. Additionally, efferocytosis, the process by which macrophages clear apoptotic and cancer cells, promotes a shift to an M2-like phenotype. This shift can inhibit anti-tumor activity and support angiogenesis through vascular endothelial growth factor production[47,48]. Future studies are needed to understand the effects of CB on angiogenesis, comparing it to known angiogenesis inhibitors like Sorafenib, and on efferocytosis to gain deeper insights into the mechanisms. Additionally, safety studies assessing hematological, liver, and renal function are also important[49]. Besides, this study opens new avenues of research focusing on the effects of CB in combination with commercially available anti-tumor therapies, in other models of hepatocellular carcinoma (syngeneic orthotopic with/without underlying liver cirrhosis), on immune cells other than macrophages, and bioavailability and pharmacokinetics of CB.

In conclusion, Huang *et al*[25] effectively demonstrate that CB exerts its anti-tumor effects by inhibiting M2-TAM polarization, eventually reducing the migratory, invasion, and proliferative capacities of hepatocytes. Further, CB modulates the M2 phenotype by inhibiting Wnt pathway. However, future studies are warranted to better understand the mechanisms and its safety profile with longer-term therapy.

FOOTNOTES

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