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Overview on the endoscopic treatment for obesity: A review

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**Abstract**

Obesity rates have increased, and so has the need for more specific treatments. This trend has raised interest in non-surgical weight loss techniques that are novel, safe, and straightforward. Thus, the present review describes the endoscopic bariatric treatment for obesity, its most recent supporting data, the questions it raises, and its future directions. Various endoscopic bariatric therapies for weight reduction, such as intragastric balloons (IGBs), aspiration therapy (AT), small bowel endoscopy, endoscopic sleeve gastroplasty, endoluminal procedures, malabsorption endoscopic procedures, and methods of regulating gastric emptying, were explored through literature sourced from different databases. IGBs, AT, and small bowel endoscopy have short-term effects with a possibility of weight regain. Minor adverse events have occurred; however, all procedures reduce weight. Vomiting and nausea are common side effects, although serious complications have also been observed.

**Key Words:** Overweight; Gastric bypass; Malabsorption; Intragastric balloons

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**Core Tip:** To enhance endoscopic intervention effectiveness and patient satisfaction, the research recommends device design, procedures, patient selection, and personalized therapy. Endoscopists, bariatric surgeons, and researchers must collaborate to solve problems, improve patient comfort, and reduce treatment risks. Effective weight maintenance through endoscopic methods and patient education requires comprehensive and long-term follow-up. Robotic-assisted endoscopy and tissue-engineered implants may revolutionize obesity treatment and patient outcomes in five to ten years.
INTRODUCTION

Obesity prevalence has increased dramatically over the last thirty years, now approaching 35% in men and 40% in women[1]. Obesity affects 39 million children, 340 million teenagers, and 650 million adults worldwide. This figure is still rising; by 2025, 167 million children and adults will have suffered as a result of being obese or overweight[2]. As a multifaceted illness with pandemic proportions, obesity is prevalent nowadays. Since 1975, obesity prevalence has virtually tripled during the last three decades globally, primarily due to an increase in sedentary behavior and intake of less nutrient-dense foods[3]. There are severe public health issues with obesity[4]. Such as diabetes, hyperlipidemia, mortality all-causes, and all-cause cardiovascular mortality[5]. In 2015, the World Obesity Federation established World Obesity Day as a day of commitment and action to promote an integrated, cross-sector approach to combating obesity[6]. A new index for Obesity-Non-Communicable Disease Preparedness is presented in the World Obesity Federation report; worries about the consequences of inactivity for already vulnerable people have grown. The top 30 most prepared countries are all high-income countries, whereas the least prepared 30 are all lower-middle and low-income countries[7]. Obesity-related yearly healthcare costs are predicted to total 1.2 trillion USD globally by 2025[6]. A bio-socio-ecological framework, in which biological predisposition, environmental factors, and socioeconomic factors interact to promote the deposition and proliferation of adipose tissue, can explain the process of persistence and development of obesity[8]. In wealthy nations, poverty and obesity appear to be related. However, a more thorough examination of the empirical literature leads one to believe that the relationship between income and obesity is more nuanced because it can be either positive or negative, or it can alter as nations grow older[9]. Among the numerous therapies for obesity, pharmaceutical substances, surgical procedures, and lifestyle changes can be used[10] as shown in Figure 1.

Despite being the least invasive and expensive approach, lifestyle changes have been demonstrated to be the least effective[11]. Adults with obesity can lose > 5% of their body weight with many sessions of intensive behavioral interventions, such as identification of different barriers, peer support, and self-monitoring of weight, combined with dietary plans, lifestyle modifications, and increased exercise[12]. The central nervous system, adipose tissue, gastrointestinal hormones, liver, kidney, and skeletal muscle are only a few of the systems and tissues that are now modulated by some anti-obesity drugs under investigation[13]. Bariatric surgery is currently the only treatment that causes significant and long-lasting decrease in body weight[14]. However, even if there are clinically significant comorbidities (metabolic, psychological, etc.), patients with a body mass index (BMI) of 35 kg/m² or over are not suitable for bariatric surgery. Only a small number of eligible patients can potentially benefit from bariatric surgery[15]. This may be due to various reasons, such as a less favorable risk-benefit profile, as higher BMI levels often correlate with increased surgical complications, postoperative mortality, and reduced success rates[16]. Additionally, the potential benefits of surgery, such as weight loss and improvement in comorbidities, might be outweighed by the surgical risks and aggressive challenges in this specific BMI range. Moreover, alternative interventions, including lifestyle modifications, pharmacotherapy, and non-surgical interventions, might be considered more appropriate due to the complexities posed by the patient’s heightened obesity levels. However, carefully assessing individual risks and benefits remains essential in determining the most suitable treatment approach[17]. Lastly, advanced studies have questioned the longevity of bariatric surgery due to the regular occurrence of weight regain and adverse effects[17].

Endoscopic procedures can be used in a multidisciplinary approach to managing obesity. Endoscopists should become familiar with the gastrointestinal pathology that might develop after bariatric surgery, such as malnutrition, anastomotic stenosis, acid reflux, gallstone disease, leaks, fistulas, and weight gain[17]. The creation of new obesity treatment modalities without a high operational risk is the current area of research; thus, obesity endoscopic management is garnering major attention. Endoscopy has an indispensable role in the assessment and management of bariatric surgery complications as well as the evaluation of patients in the preoperative stage of the procedure[18]. Additionally, in order to lower the risks associated with surgery connected to obesity, endoscopic techniques have been employed as a “bridge to surgery”[19]. Thus, the present review describes the present endoscopic treatment for obesity, the most recent data supporting it, the questions, and the future directions the field will face in the next ten years.

BARIETRIC ENDOSCOPY

Endoscopic weight loss therapies have been developed as a result of increased interest and innovation in the fields of gastroenterology and endoscopy, as well as the proven results of bariatric surgery for weight loss[20]. Although surgery is a successful approach for weight loss, it is constrained by its high resource requirements and low patient acceptance[21]. However, endoscopic bariatric therapy (EBT) may be more effective than anti-obesity medicines[22]. Endoscopic weight loss procedures are becoming more popular in Western countries where obesity has escalated rapidly[23]. Indeed, EBT has demonstrated outstanding results in the treatment of obesity and associated surgical consequences[24], therefore, must be included in the arsenal in the battle against obesity[25]. In fact, EBT has evolved into a significant tool for exami-
nation, diagnosis, surgical complication management[26] and even primary bariatric therapies[27]. It can also be a more effective alternative than dietary and lifestyle changes[28]. Additionally, EBTs are being used increasingly as a treatment therapy option for obesity in different settings due to their minimally intrusive nature and ease of administration. These treatments induce weight loss primarily by reducing meal volume and promoting early satiety[29]. Currently, a wide range of endoscopic procedures, based on the principles of stomach volume reduction, small bowel or gastric bypass, and size restriction, are being investigated, with a few being used in daily practice[30]. Innovative endoscopic therapies, such as double-pigtail stents, septostomy, and endoscopic vacuum therapy, have been developed[31]. Newer endoscopic approaches, such as intragastric balloons (IGBs), aspiration therapy (AT), small bowel devices, and endoscopic plication and suturing techniques, have been developed[32] and show significant effects.

Space occupying techniques
Space-occupying devices limit stomach capacity, thus reducing hunger and food intake as a result. There are various versions of silicon-made balloons that can be filled with liquid or air. Because of its reduced likelihood for issues, the most popular type is the non-adjustable liquid-filled balloon. The mechanism of action is multifaceted, with neurohormonal and physiological alterations involved[33]. Generally, there are two types of space-occupying techniques: Intragastric fluid filled balloons, and intragastric air or gas filled balloons.

IGBs (fluid filled)
Over 20000 papers have been published in the previous 20 years covering a wide range of topics connected to the effects of the IGBs, including weight reduction outcomes, complications, hormonal impacts, quality of life (QoL), and other aspects[34]. IGBs are invasive therapies that are used to enhance satiety by neuroendocrine and mechanical mechanisms [35] as well as limiting stomach capacity as a space-occupying device, resulting in decreased food intake and hunger[33]. The Bubble (Garren-Edwards) was the first-developed IGB in 1985. The Food and Drug Administration (FDA) of the United States approved it as a temporary weight loss device[36]. IGB therapy is helpful in decreasing weight and improving depression, anxiety, symptoms of eating disorders, and overall QoL in obese individuals primarily within 6 mo of device placement and when used in tandem with conventional therapies[37] and found to be more effective in pre-obese individuals[38]. Although significant morbidity is possible, it is a useful way to lose weight when implemented in conjunction with dietary modifications and physical exercise[39]. However, due to the consequent regain of weight, different methods are now favored in adults. Balloons may be an option for less reversible operations in teens, who are more open to lifestyle modification[40]. The drawbacks of IGBs, such as risks during insertion and removal, and unknown long-term weight loss benefits, prevent their widespread use[41]. Considering conscious sedation vs general anesthesia during balloon withdrawal, with or without anesthetic intubation, is pivotal due to its potential implications for procedural complications[42]. The choice between these approaches hinges on patient health status, procedure complexity, and anticipated discomfort. Utilizing conscious sedation may offer benefits like reduced risks associated with intubation but could lead to patient discomfort or inadequate sedation levels, potentially increasing complications[43]. In contrast, employing general anesthesia with intubation might mitigate patient discomfort but could introduce intubation-related risks. Balancing these considerations is essential to optimize patient comfort and procedural safety during balloon withdrawal, and a comprehensive understanding of the relationship between sedation choices and associated complications is critical for informed decision-making. Meanwhile, utilizing a dual-channel gastroscope, specialized foreign body forceps, and a symmetrical snare designed for polyp removal enables a secure, efficient, and straightforward extraction of the balloon. This approach ensures the balloon is removed without any misplacement risk while maintaining patient comfort throughout the procedure[44].

Figure 1  Different approaches for the management of obesity.
Endoscopically-placed balloons are normally placed in the stomach for no longer than 6 mo, after which they are removed, as they can cause complications[45]. A meta-analysis of 5668 participants found there was moderate indication of improvement in most metabolic markers in participants (IGB therapy vs standard non-surgical therapy)[46]. In another review, the total body weight loss (TBWL) of the IGBs after 6-mo implantation was 6.8%-13.2% at 12 mo i.e., 7.6%-11.3% TBWL[47]. Furthermore, 20 randomized controlled trials (RCTs) were used in a meta-analysis involving 1195 patients, indicating significant effects following IGB use[48]. Another similar meta-analysis was comprised of thirteen RCTs with a total of 1523 subjects. At follow-up, the difference in mean % excess weight loss (EWL) was 17.98% [total weight loss (TWL) was 4.40%] and was substantially larger in the IGB group. This concluded that, in overweight and obese individuals, IGB therapy is more effective than lifestyle change alone for weight loss[49]. Additionally, in the RCT, 288 patients were assigned at random to one of two groups: IGB or control group. At 32 wk, the mean TWL in the IGB group was 15% [95% confidence interval (CI): 13.9-16.1] against 3.3% (2-4.6) in the second group which remained as a control without any intervention (P < 0.0001). Seven (4%) patients experienced major adverse events (AEs) associated with the device, with no deaths. When therapy was combined with lifestyle changes, substantial weight loss was obtained and sustained for 6 mo after IGB removal[50]. A 10-year review was performed and initially, 49 patients (IGB vs control group) were included with a 51.6% follow-up rate. TBWL favored the IGB group at 6 mo [9.75 vs 7.48 kg (P = 0.03)], at 12 mo [6.52 vs 4.42 kg (P = 0.05)], at 18 mo [5.42 vs 3.57 (P = 0.32)], and 24 mo [4.07 vs 2.93 kg (P = 0.56)]. TWL at 10 years was 0.03 vs -2.32 kg (P = 0.05) and %TWL was -0.16% ± 12.8% vs -2.84% ± 5.6% (P = 0.39), which were not statistically different between groups. BMI at follow-up [30.97 ± 1.6 vs 30.38 ± 1.8 kg/m² (P = 1.00)] was comparable and it was concluded that IGB provides weight loss for up to two years and is superior to the control[51]. In contrast, in one of the studies only 2910 (0.4%) of the 652922 individuals identified received IGB treatment. Patients who received IGB therapy were older, had a lower BMI at baseline (37.0 ± 6.2 kg/m² vs 45.3 ± 7.8 kg/m²), and had a greater rate of early non-operative re-intervention (7.7% vs 1.1%; P < 0.0001). Between 2016 and 2019, according to the Metabolic and Bariatric Surgery Accreditation and Quality Improvement Program the number of IGB procedures reported decreased considerably [953 (0.62%) vs 418 (0.25%); P < 0.0001]. Given the safety and efficacy of current bariatric surgery and novel pharmaceutical treatments for weight loss, the function of IGBs in treating obesity remains uncertain[52]. In another study, the mean preoperative BMI for all 20680 IGBs, encompassing 12 distinct models, was 34.05 kg/m². On average 17.66% ± 2.5% of TBWL was noted. There were 3.62% early removals because of intolerance. Consensual management had an AE rate of 0.70% and 6.37% for major and mild problems, respectively. Only one death was reported[53]. In short, many patients have benefited from the IGBs, which bridges the gap between clinical management of obesity, medications, and bariatric surgery while also helping thousands of patients lose weight and improve their comorbidities[34]. A summary is also described in Table 1.

The United States FDA has approved three IGBs. Orbera is an endoscopically implanted single balloon[54]. ReShape is a duos balloon system, connected in the middle by a tube. The third balloon, Obalon (Figure 2), is filled with nitrogen gas and is part of a three-balloon treatment. All systems require endoscopic placement and removal after 6 mo[55].

**A single balloon (Orbera IGB)**
The Orbera IGB (OIB) is a single fluid filled IGB authorized for weight loss induction and obesity treatment[56]. The FDA-approved Orbera is a single 13 cm silicone-made balloon that arrives commercially deflated and is inflated at the end by a filling tube connected to a radiopaque self-sealing valve. Following a diagnostic endoscopy, the balloon implantation assembly is inserted directly into the stomach, and a volume of 500 to 700 mL saline solution [this volume range (500 to 700 mL) is chosen based on optimal balloon expansion and effective positioning within the stomach] with 5 mL of methylene blue used for balloon inflation via a closed infusion system, with the entire procedure being performed under direct endoscopic observation[57,58]. The FDA has approved Orbera for a 6-mo placement in people with a BMI of 30-40 kg/m². To rule out contraindications, such as a big hiatal hernia or a stomach ulcer, an endoscopy should be performed before or concurrently with placement[54].

Orbera® meets the obesity therapy “preservation and incorporation of valuable endoscopic innovations” thresholds of 5% TBWL and 15% EWL over control, respectively[59]. Similarly, a review of a database of individuals who had the OIB endoscopically placed revealed that it was effective, safe at inducing weight loss, and reduced complications related to obesity[56]. There were no spontaneous deflations observed in employing the OIB system. In this device, deflation can be detected through weight changes or patient-reported loss of satiety; however, current practice mandates a very simple method of detection through observing any irregular change in urine output[60].

**A double balloon system (ReShape Integrated Dual Balloon System)**

The ReShape IGB is a temporary implantation of a fluid-filled balloon that is designed to encourage weight loss by occupying space in the stomach. Endoscopy is utilized to deliver the balloon trans-orally. After positioning, inflation is done with saline (sterile) and methylene blue, which is used as an indicator in case the balloon mistakenly leaks or deflates. The balloon can remain in the stomach for six months[61].

In one study, total body weight was found to be lowered by 6.8% ± 7.3% (P < 0.001) and BMI was reduced by 2.7 ± 2.9 kg/m² (P < 0.001) in all patients who had the ReShape IGB implanted, with completed follow-up of 6 mo. According to subgroup analyses, patients with > 40 kg/m² BMIs reported significant reductions in TBWL and BMI[61].

**A three-balloon system**
The three-balloon system is made up of three distinct balloons. These are placed through the mouth and subsequently filled with gas nitrogen to a capacity of roughly 250 mL by a connected catheter. One balloon is implanted monthly, with a maximum of three balloons. All balloons are removed endoscopically six months after the placement of the first balloon. The anticipated TBWL is 7.1%[62]. In one study, a swallowable gas-filled IGB device was deemed safe after six months.
### Table 1: A summary of endoscopic procedures for reducing weight in obese patients

<table>
<thead>
<tr>
<th>Method</th>
<th>Indication (BMI)</th>
<th>Duration</th>
<th>Efficacy</th>
<th>Adverse events</th>
<th>Limitations</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGBs</td>
<td>30-40 kg/m²</td>
<td>6 mo</td>
<td>IGB therapy is a successful short-term weight loss strategy</td>
<td>Nausea/vomiting and stomach pain were the most common consequences, but mortality and gastric perforation were unusual. Other serious problems included dehydration, which required hospitalization, and intestinal obstruction due to balloon deflation, which required surgery.</td>
<td>Short-term effects and weight regain</td>
<td>[45, 48, 50, 54, 87]</td>
</tr>
<tr>
<td>AT</td>
<td>35-55 kg/m²</td>
<td>Long term usage</td>
<td>AT is an implantable device that drains a portion of the stomach contents after each meal, removing up to 30% of the calories consumed</td>
<td>Postoperative peristomal granulation tissue and peristomal irritation, cardiac arrhythmias, hypokalemia, hypochloremic hypokalemic metabolic alkalosis, rather than gastric toxus and eating problems</td>
<td>It cannot be used for patients with eating disorders. For this technology to be effective and long-lasting, significant patient commitment, motivation, and adherence are necessary. In addition to adhering to correct device operation, chewing food thoroughly is a significant crucial aspect in attaining successful weight reduction using this device; thus, patients who fail to stick to thoroughly chewing their meal are unlikely to get ideal outcomes</td>
<td>[91, 94, 99]</td>
</tr>
<tr>
<td>Small bowel endoscopic procedures</td>
<td>41.5 kg/m²</td>
<td>6-12 mo</td>
<td>10.6% TBWL and 40.2% EWL after one year</td>
<td>There were no AEs, and the nausea and diarrhea were self-limiting</td>
<td>Short-term efficacy, no small bowel EBTs are currently FDA-approved</td>
<td>[32, 83, 102, 103, 150]</td>
</tr>
<tr>
<td>Endoscopic sleeve gastroplasty</td>
<td>&gt; 30 kg/m²</td>
<td>6-24 mo</td>
<td>%TBWL 12%-19% [150]</td>
<td>Leaks, perforation, hemorrhage, improved depth perception, improved visualization, severe stomach discomfort, and perigastric collection are all possible AEs</td>
<td>Required expertise and skills</td>
<td>[105, 111, 119, 150]</td>
</tr>
<tr>
<td>Endoluminal procedures</td>
<td>30-40 kg/m²</td>
<td>6-12 mo</td>
<td>41.5 kg/m², which reduced to 33.1 kg/m²</td>
<td>Pain, nausea, and vomiting</td>
<td>N/A</td>
<td>[129, 130]</td>
</tr>
<tr>
<td>DJBS</td>
<td>&gt; 35 kg/m²</td>
<td>6-12 mo</td>
<td>Effective patients lost 15% of their body weight at 12 mo, compared to 4% of controls</td>
<td>Nausea, vomiting, pancreatitis, GI bleeds, hepatic abscess, obstruction of the sleeve</td>
<td>As the common channel length shortens, so do diarrhea and severe vitamin A and D deficits</td>
<td>[132, 135, 137, 139]</td>
</tr>
<tr>
<td>GJBS</td>
<td>30-40 kg/m²</td>
<td>N/A</td>
<td>Patients reduced 39.7% of their excess</td>
<td>N/A</td>
<td>N/A</td>
<td>[140, 141]</td>
</tr>
<tr>
<td>Regulation gastric emptying</td>
<td>N/A</td>
<td>N/A</td>
<td>Weight loss was within 10% of their optimum weight</td>
<td>N/A</td>
<td>Hormonal imbalance and weight regain</td>
<td>[148, 149]</td>
</tr>
</tbody>
</table>

IGBs: Intragastric balloons; AT: Aspiration therapy; TBWL: Total body weight loss; EWL: Excess weight loss; N/A: Not applicable; GJBS: Gastroduodenal jejunal bypass sleeve; DJBS: Duodenal-jejunal bypass sleeve; GI: Gastrointestinal; AEs: Adverse events; EBT: Endoscopic bariatric therapy; FDA: Food and Drug Administration; BMI: Body mass index.

and resulted in double the weight loss than a sham control, with significant weight loss maintenance at 48 wk[63]. However, a study with 87 individuals who were successfully implanted with IGBs (gas-filled IGB; fluid-filled IGB) showed no differences in %TBWL between balloon systems at removal and 12 mo (P = 0.39). Although both gastric balloon systems were equally effective, the gas filled IGB had fewer significant side effects[64].

### Orbera 365 balloon

The revolutionary ORBERA 365® balloon, made by Apollo Endosurgery, can remain within the stomach for a full year, hence its name[65,66]. Up to this point, limited studies with clinical data have been published. In one study, 97 individuals had an Orbera365 implanted. Prior to the treatment, the average weight and BMI of participants were 93.8 kg and 35.2 kg/m², respectively. After the procedure, these values decreased to 80.6 kg and 29.8 kg/m² after 8.2 mo and to 82.4 kg and 30.4 kg/m² on the last day of follow-up after 12.9 mo[62]. In another study, the weight reduction at IGB (Orbera) removal after 6 mo and at IGB (Orbera 365) removal after 12 mo was retrospectively examined. Mean TBWL was 15.2 and 15.8 kg in patients undergoing IGB placement for 6 and 12 mo, respectively. In patients receiving IGB placement for 6 or 12 mo, there was no discernible change in the mean %TBWL (15.3% vs 14.7%, P = 0.7)[67].

[150]
IGBS (AIR-FILLED)

IGBs, like air-filled balloons, as a temporary endoscopic treatment for obesity, have the potential to play an important role for the obese population[54]. They can also be used as a preoperative test before doing restricted bariatric surgery on patients. Furthermore, an intragastric device can be used as a “bridge treatment” before major surgery in individuals with severe obesity to lower the risk of operation-related complications[68]. Furthermore, IG balloons play a pivotal role as a transitional measure before bariatric surgery by serving as a bridge to reduce BMI and potentially mitigate the associated surgical risks. IGBs help patients achieve a lower BMI by facilitating initial weight loss, which may lead to improved overall health and decreased comorbidities. This reduction in BMI can also contribute to decreased surgical complications during subsequent bariatric procedures. Acting as a preoperative intervention, IGBs offer a safer trajectory for individuals with high BMI, allowing them to undergo bariatric surgery with potentially reduced morbidity and an enhanced surgical outcome. According to research, the air-filled balloon is effective and well tolerated, with weight reduction comparable to other types of balloons[69].

The air-filled IGB has not been shown to be harmful. It appears to have the same effect on weight loss as other balloons. After removal of the balloon, 30% of the patients in one study sustained a weight decrease of more than 10%[70]. However, in a comparative study, patients undergoing saline-filled balloon therapy (4.66 ± 4.75) lost considerably more weight than patients undergoing air-filled balloon surgery (P < 0.001). The variation in early withdrawal rates between the two groups, on the other hand, was minor (P = 0.21)[71]. With the air-filled IGB, the balloon is inflated with a specific gas, such as air or a mixture of air and nitrogen, using a catheter or a small tube connected to the balloon. The gas inflates the balloon, causing it to expand and take up space within the stomach[41].

Obalon

The United States FDA approved Obalon for the treatment of obesity[72]. This device, which is swallowed as a gel at the end of thin tubing, can be used anywhere. The device is then filled with a gas, and fluoroscopy is used to ensure proper installation. As a result, removal requires only one endoscopic treatment[73].

In one study, patients were randomly assigned to receive 3 balloon capsules (Obalon) or three sugar filled dummy capsules in a RCT with 15 facilities in the United States. A licensed dietitian provided lifestyle advice to all subjects every three weeks. The treatment group’s %TBWL was 6.81% ± 5.1%, while the control group’s TBWL was 3.59% ± 5.0%. In the therapy group, the responder rate was 64.3%, defined as %TBWL > 5%. Minor AEs, such as abdominal pain and nausea, occurred in most patients, with only one serious AE, which was a gastric ulcer in a patient who violated the research protocol by using a nonsteroidal anti-inflammatory medicine[71]. Mion et al[74] reported a prospective feasibility study, and all balloons were retrieved via upper gastrointestinal (GI) endoscopy 12 wk after the ingestion of the first balloon. Of the 44 balloon swallowing attempts, 43 (98%) were successful. Nausea and stomach pain were the most common AEs. Significant weight reduction was reported as well[64].

Heliosphere

The Heliosphere Bag is a silicone-encased air-filled polymer balloon[74]. When compared to fluid-filled balloons, this endoscopy-inserted device weighs less than 30 g, shows a 30-fold weight reduction, and it is certified for 6-mo use[41]. This device demonstrates a high effectiveness and tolerance profile. Loss of weight seems to be comparable to that of other types of balloons. In contrast, technical issues, particularly during removal, are most likely related to the device’s substance and create a low safety profile[74].
In one study, the average weight reduction and BMI drop were 14.5 ± 8.2 kg and 5.3 ± 2.8 kg/m², respectively (P < 0.001). During the first week after Heliosphere Bag implantation, 7.4% of patients experienced nausea and vomiting[75]. Even though mid/long-term follow-up may result in some weight gain, Heliosphere® BAG allows for short-term loss of weight with few AEs[76]. Furthermore, De Castro et al[77] showed comparable weight reduction outcomes. Meanwhile, fluid-filled balloons are found to be more beneficial for weight loss[77]. In addition, a life-threatening complication was also reported in a patient using Heliosphere Bag[78].

**Elipse**

The Elipse balloon is a unique non-endoscopic weight loss approach[79]. At 16 wk, the Elipse IGB is naturally excreted out as it is a swallowable balloon[80]. Although Elipse has a shorter residence period in the stomach than other standard IGBs that need endoscopy, the procedure appears to have identical results[81]. Vomiting and nausea are the most common AEs. There were no major AEs[82].

In prospective research, 51 Elipse insertions were performed, and the patients’ total weight reduction was 8.84 kg, %EWL 40.84%, %TBWL 10.44%, and change in BMI 3.42 kg/m². The device was proven to be effective; however, several limits were discovered that must be overcome for improved results[83]. Furthermore, a meta-analysis showed that the Elipse IGB is effective in weight reduction, safe, and is an efficient obesity technology with a low AE profile. A study conducted in Italy found early results after 4 mo with a mean %EWL of 26%. There were no balloon passage issues in the included patients[84].

**COMPLICATIONS WITH IGBS**

Despite the extremely low rates of difficulties and death linked with IGBs, AEs and complications can occur, and they can range from mild to severe[85]. The most prevalent AEs reported were vomiting, nausea, and stomach pain, while fatalities and gastric perforation were uncommon[86]. However, a 58-year-old Pakistani female presented with 2 wk of vomiting and abdominal bloating. While the external pigtail catheter and blue clasp for retrieval were stretching into D1/ D2, the balloon was impacted at the antrum and pylorus. This is a relatively uncommon IGB complication[87]. Other severe complications included dehydration, which necessitated hospitalization, and intestinal blockage induced by balloon deflation, requiring surgery[88].

In conclusion, fluid-filled balloons are much more likely to result in weight loss than gas-filled balloons. They may, however, be associated with a higher likelihood of intolerance and removal. This data will assist clinicians in selecting devices and engaging patients in collaborative decision-making[89].

**AT**

The AspireAssist AT is the first FDA-approved device for the treatment of class II and III obesity[30]. AT comprises an endoscopic placement of a gastrostomy tube (A-tube) and an AspireAssist siphon component to aspirate gastric contents 20 min after meal consumption[91] (Figure 3), in conjunction with lifestyle modifications and an external device to allow drainage of around 30% of the calories taken in a meal[92]. It is approved for long-term usage in persons with BMIs of 35-55 kg/m² in the United States[93].

Studies show 14.2% to 21.5% TBWL in participants who complete one year of treatment and weight loss maintenance when treated for two years[94]. A pilot study was conducted, and patients in the AT group dropped 18.6% ± 2.3% of their body weight with 49.0% ± 7.7% EWL after one year, while in the lifestyle therapy group patients lost 5.9% ± 5.0% and 14.9% ± 12.2% EWL (P < 0.04). AT was found to be effective and safe as a long-term obesity weight loss therapy[95]. Similarly, a multicenter study with 82 individuals was carried out where the patients' average baseline BMI was 41.6 ± 4.5 kg/m². At the conclusion of the first year, participants had 34.1 ± 5.4 kg/m² BMI and 18.3% ± 8.0% TWL. Patients experienced 15.3% TWL after 2 years, 16.6% after 3 years and 18.7% TWL after 4 years with a significant difference (P < 0.01). The safety profile of AT was found to be satisfactory, effective, and approved for long-term weight loss treatment. Additionally, AT was found to be a safe and effective therapy for reducing weight as the mean percent total weight reduction was 18.2% ± 9.4%, 19.8% ± 11.3%, 21.3% ± 9.6%, and 19.2% ± 13.1%, at 1, 2, 3, and 4 years, respectively[96]. Similarly, a study with 25 obese participants was undertaken, and after 2 years of AT, BMI was 31.0 ± 5.1 kg/m², P < 0.01, and EWL was 61.5% ± 28.5%, P < 0.01. It was concluded that AT is a safe and efficient treatment for obesity, and weight loss enhances QoL[97]. Furthermore, the effects were not limited to obesity; comorbidities related to obesity, such as diabetes, blood pressure, triglycerides, and lipoproteins, were significantly improved with AT[98].

However, the most commonly observed AEs with AT were perioperative discomfort and stomach pain as well as postoperative peristomal granulation tissue and peristomal irritation[91]. This can cause more serious hypokalemia, cardiac arrhythmias, and hypochloremic hypokalemic metabolic alkalosis than gastric botox. Some eating disorders can also be caused by AT[99]. Indeed, gastric irritation and aspiration may result in persistent loss of chloride and hydrogen ions. The physiologic response is renal potassium ion secretion and hydrogen ion resorption; hypochloremic hypokalemic metabolic alkalosis may occur[100].
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**SMALL BOWEL ENDOscopic BARIATRIC TECHNIQUE**

Endoscopic bariatric therapies for the small bowel include incisionless anastomosis devices (IAS), bypass sleeves and duodenal mucosal resurfacing. Endoscopic bariatric treatments can be performed safely and efficiently for weight loss and metabolic improvement, according to clinical evidence, employing tiny intestinal devices[98]. These therapies focus on foregut and hindgut processes to achieve weight loss and enhance glucose homeostasis[101]. When fully deployed, the IAS magnets form an octagonal shape. Pairs of IAS magnets are inserted into distinct segments of the small bowel, via simultaneous enteroscopy and colonoscopy, and are connected under endoscopic and fluoroscopic view[102].

In a research pilot, ten patients with a 41 kg/m² mean BMI had the operation. Laparoscopy was performed in this pilot study to establish appropriate magnet coupling and to validate limb lengths. The anastomosis developed in about a week, and the magnets were ejected without any pain or hindrance. At the 2 and 6-mo follow-up endoscopies, all anastomoses endured a patent[103]. There were no AEs. Diarrhea and nausea were self-limiting[102], as shown in Table 1.

**ENDOSCOPIC SLEEVE GASTROPLASTY**

Endoscopic sleeve gastroplasty (ESG) is a newer type of noninvasive weight loss procedure that uses a suturing device[104]. The endoscopist uses sutures to form a tube-like configuration in the stomach to promote restriction. If diet and exercise are not working and one is extremely overweight (BMI of 30 or higher), this treatment may be possible (Table 1). The technique is minimally invasive, which reduces the risk of operational impediments and allows for a speedy return to normal activities[105]. Additionally, the overstitch system also includes a double-channel endoscope with a suturing platform attached to it. A tissue grasper device is used to mobilize and capture the desired position of the suture on the stomach wall, after which the tissue is retracted into the device’s suturing arm[106].

A recent study discovered that a customized running suture adopting a Z-pattern had a good effect on producing a homogeneous distribution of the suture’s disruptive force across all stitch positions[107] and a “U” stitch pattern was also used[108]. Furthermore, a review of seven of the eight trials with adequate data revealed a weight loss which was statistically significant ($P < 0.05$). In an RCT, for the ESG group, the primary endpoint of mean %EWL was 49.2%, and for the control group, it was 32% at 52 wk ($P < 0.0001$). ESG had a significant weight loss, which was sustained at 104 wk and is also safe, with significant improvements in metabolic comorbidities[109]. Similarly, in another study, 435 patients from various obesity classes were included. At all-time intervals, ESG had a significantly higher %TBWL, TBWL, and BMI decrease in class III obesity compared to obesity of classes I and II ($P < 0.001$). In all types of obesity, ESG causes considerable weight loss[110]. Furthermore, efficacy was well established in a multicenter study conducted with 91 patients, and after treatment, BMI reduction after 3 mo was 7.3, after 6 mo 9.3, and after 12 mo 8.6 from baseline. EBWL was 17.3% after one month, 29.2% after three months, and 35.6% after six months with significant difference ($P < 0.000$)[111]. In addition, a meta-analysis was performed, the ESG resulted in around 15% TBWL or 58% EWL at 6-mo, and there was sustainability in weight loss at 12, 18, and 24 mo[112]. However, a study found laparoscopic sleeve gastrectomy (LSG) to be more efficient than ESG as it improved weight related QoL significantly[113]. Seven studies in a meta-analysis encompassed 6775 patients, with 3413 undergoing ESG and 3362 undergoing LSG procedures. Notable disparities were observed in the percentage of %TBWL, all of which favored LSG over ESG. While there was a tendency towards a decreased occurrence
of AEs with ESG compared to LSG, this distinction did not achieve statistical significance [risk ratio (RR) = 0.51, 95%CI: 0.23-1.11, \( P = 0.09 \)]. The frequency of new-onset gastroesophageal reflux disease was markedly lower following ESG as opposed to LSG, at 1.3% compared to 17.9%, respectively (RR = 0.10, 95%CI: 0.02-0.53, \( P = 0.006 \))[114]. Moreover, a total of 2188 patients (1429 for LSG and 759 for ESG) from sixteen studies were included in another meta-analysis. The mean percentage of %EWL was 80.32% (± 12.20%; 95%CI; \( P = 0.001 \); \( \chi^2 = 98.88 \)) for the LSG group and 62.20% (± 4.38%; 95%CI; \( P = 0.005 \); \( \chi^2 = 65.52 \)) for the ESG group. This represents an absolute difference of 18.12% (± 0.89%; 95%CI, \( P = 0.0001 \)) between the two groups. The variation in the average rate of AEs was 0.19% (± 0.37%; 95%CI, \( \chi^2 = 1.602 \); \( P = 0.2056 \))[115]. Similarly, ESG results in weight loss comparable to LSG, with similar improvements in comorbidity resolution and safety profiles was shown in another study[116].

Meanwhile, around 2.3% of patients had serious post-procedure issues; nevertheless, no deaths were documented [117]. Leaks, perforation, bleeding[112], better depth perception, better visualization[118], severe abdominal pain and a perigastric collection[119] have all been reported. Intraabdominal collection, refractory symptoms requiring ESG reversal, hemorrhage requiring transfusion or endoscopic intervention, pneumoperitoneum and pneumothorax and pulmonary embolism[120] are among the serious AEs that have been documented.

In conclusion, as with any weight loss intervention, the success of the ESG procedure can be influenced by factors such as patient adherence to lifestyle changes, dietary habits, and individual metabolic factors. Comparing the lasting utility of ESG with LSG, a surgical procedure, the latter has a longer track record and more established data on long-term outcomes. LSG has demonstrated sustained weight loss and metabolic improvements over several years. However, it is essential to note that LSG is a more invasive procedure with potential surgical risks and complications. When evaluating the choice between ESG and LSG, patients and healthcare providers should consider the balance between the invasiveness of the procedure and the expected long-term outcomes, as well as individual patient preferences and medical considerations.

ENDOLUMINAL PROCEDURES

Endoluminal procedures performed exclusively using gastrointestinal flexible endoscopy provide safer and more cost-effective alternatives to currently used surgical techniques for obesity management. However, endoscopic gastroplasty is one of the promising applications of endoluminal procedures in the field of metabolic obesity disorder[121]. Several endoluminal treatments for the loss of weight in obese patients have been developed, claiming to be as effective as surgery but safer[122]. Endoluminal obesity treatments show promise, and recent technology breakthroughs have been amazing. However, new therapies have had to meet the same requirements as present surgical treatments[123]. In fact, until the success of endoluminal treatments was proven, most surgeons were unwilling to consider them for their patients[124].

The transoral gastroplasty (TOGA) method conducts a vertical gastroplasty along the smaller curvature of the stomach using transoral endoscopy[122]. As a result, a gastric pouch forms, which restricts the food quantity or liquids that the patient can intakes, resulting in an early feeling of fullness[126]. Another technique is the incisionless operating platform (IOP) which is used in primary obesity surgery endoluminal (POSE). The IOP is a four-part device used to regulate a full-thickness plication system endoscopically. It delivers a series of anchors into the stomach to encourage gastric imbrication[122].

In a systematic review, it was concluded that endoluminal plication devices were more successful in 91.8% of patients with 5.02% lower recurrence rates than sclerotherapy and Argon Plasma coagulation, which also had 46.8% success and 21.5% recurrence rates, respectively[126]. The same findings were reported in a multicenter trial with a one-year follow-up that included 67 patients with a mean BMI of 41.5 kg/m\(^2\), which decreased to 33.1 kg/m\(^2\) at 6 mo after TOGA treatment, with consequences including respiratory insufficiency and an asymptomatic pneumoperitoneum[129]. In a 12-mo multicenter RCT in the United States, 221 patients got the POSE surgery in conjunction with low-intensity lifestyle interventions. They attained a TBWL of 4.95% ± 7.04% against 1.38% ± 5.58% in the sham group with complications such as pain, nausea, and vomiting also reported[130].

The POSE and ESG methods are distinct endoscopic approaches for obesity control. Meanwhile, the POSE method involves the creation of tissue folds within the stomach to reduce its size and restrict food intake without removing tissue. In contrast, the ESG method involves suturing and narrowing the stomach’s capacity, resembling a sleeve, to induce weight loss. While both methods are minimally invasive and avoid surgical incisions, the POSE method focuses on tissue folding, while the ESG method centers on suturing, leading to different mechanisms of action. The choice between these techniques depends on individual patient characteristics, preferences, and specific weight loss goals.

MALABSORPTIVE ENDOSCOPIC PROCEDURES

Malabsorptive endoscopic procedures may potentially provide an opportunity for an ambulatory technique that is both safer and less expensive than laparoscopic surgery. Endoscopic malabsorptive treatments can result in weight loss and have improved metabolic parameters associated with obesity[131].

Duodenal-jejunal bypass sleeve

The Duodenal-jejunal bypass sleeve (DJBS), known as DJBS is introduced using endoscopic and fluoroscopic methods. This implant consists of a non-porous fluoropolymer sleeve, temporarily anchored within the duodenal bulb and
extending approximately 80 cm into the small intestine, typically ending in the proximal jejunum. It allows chyme to move from the stomach to the jejunum without contacting the duodenum. By not allowing mixing with pancreatic exocrine secretions and bile in the jejunum, it replicates a duodenal-jejunal bypass and promotes weight reduction through malabsorption. Which has similarities to Roux-en-Y gastric bypass (RYGB) and this combined mechanism aims to achieve weight loss by reducing calorie intake and altering nutrient absorption patterns. The DJBS procedure offers a potentially reversible option for individuals with obesity seeking to manage their weight and improve metabolic health.

In a cohort trial, after 6 mo of DJBS treatment, there was a substantial rise in EWL and a drop in weight. Similarly, a blinded, randomized, prospective clinical trial was carried out to assess the safety and efficacy of a new device for obese weight loss. The DJBS device was successfully implanted. At the end of the three-month research period, the device was removed endoscopically. The patient’s TBWL was 9.09 kg. Additionally, in another multicenter study, RCT was conducted with 41 patients and the EndoBarrier Gastrointestinal Liner device was implanted. After 3 mo, the mean EWL for the intervention group was 19% vs 6.9% for control patients ($P < 0.002$). The BMI absolute change was 5.5 and 1.9 kg/m², respectively. The device was discovered to be a practical and safe noninvasive weight loss device with outstanding short-term weight loss results. Similar to previous research, in a multicenter open-label RCT, 24% of DJBS patients lost 15% of their body weight at 12 mo, compared to 4% of controls (odds ratio = 8.3, 95%CI: 1.8-39; $P = 0.007$).

The inclusion of the DJBS to intense medical care was linked to greater weight loss and improvements in QoL. However, significant weight recovery happens during long-term follow-up after device removal, particularly in people with BMIs larger than 35 kg/m².

In total, 3.7% of patients experienced serious AEs such as pancreatitis (2 cases), GI bleeds (7 cases), hepatic abscess, obstruction of the sleeve, and esophageal tears. There were no reported fatalities. Mild AEs primarily comprised nausea, vomiting, and anchor ulceration. Meanwhile, the attachment point of the DJBL was responsible for inducing or potentially inducing 85% of the SAES.

**Gastroduodenal-jejunal bypass sleeve**

The gastroduodenal jejunal bypass sleeve (GJBS) treatment can help patients lose weight while also managing comorbidities such as diabetes, hypertension, and obstructive sleep apnea. In theory, this device is the same as the EndoBarrier. Its sleeve, on the contrary, is attached at the esophagogastric junction and continues about 120 cm through the stomach into the small bowel, imitating the ultimate anatomical structure in RYGB surgery. As a result, food passes immediately from the esophagus to the intestine, with no nutritional absorption occurring in the stomach, duodenum, or jejunum.

In one study, the GJBS was implanted in 24 patients. These patients reduced 39.7% of their excess weight by the end of the study. AEs were limited and resolved after the endoscopic device was removed. Similarly, the implementation and retrieval were both safe. It is generally tolerated and has a favorable safety profile. It provides effective weight loss results, with more than 70% of all comorbidities cured or improved.

**REGULATING GASTRIC EMPTYING**

Changes in gastrointestinal motility, which are critical in food absorption and digestion in the gastrointestinal tract, may be one of the reasons why obesity develops. The functions of incretins, particularly glucagon-like peptide-1, gastric inhibitory polypeptide, peptide tyrosine-tyrosine, glucagon, the duodenal and pancreatic hormones motilin, amylin, motilin, and the gastric orexigenic hormones ghrelin have the greatest impact on stomach emptying. Except for ghrelin and motilin, which accelerate stomach emptying, all these hormones delay gastric emptying. The vagus nerve regulates the change in fundic compliance (also known as accommodation) once food enters the stomach, allowing the stomach to develop a reservoir with just a slight increase in intragastric pressure, boosting food intake and promoting satiety. Changes in circulating gut hormone concentrations activate a variety of pathways, especially in the brain stem and hypothalamus, which influence eating behavior and a variety of metabolic progressions.

In addition, gastric emptying inhibition may contribute to a decrease in energy intake. Mechanoreceptor activation caused by stomach distension may restrict additional food intake via neuronal reflex arcs. However, diet-induced weight reduction causes long-term alterations in gut hormones for appetite, which are thought to favor increased desire and weight regain.

In an experimental study, the subjects were all subjected to quantitative fluid/solid gastric emptying experiments using a dual radionuclide method. In the solid phase, obese patients had a faster emptying rate than nonobese subjects ($P < 0.05$). Repeat gastric emptying investigations on four obese participants whose weight loss was within 10% of their optimum weight found no change in liquid or solid emptying rates. Obese patients have an abnormally fast rate of solid stomach emptying.

**FUTURE DIRECTIONS**

The future directions as follows: (1) A customized step-up approach aimed at improving and sustaining health performance is ideal, such as lifestyle therapies, nutrition modification, psychiatric treatment, medication and, if necessary, bariatric surgery; (2) In obese patients, EBIs successfully control metabolic comorbidities, improve overall weight loss and lower adverse risk events; (3) There should be some proper peer-reviewed guidelines for the implementation of...
EBTs; (4) Studies should be conducted to increase the efficacy of EBTs as they are effective for a short time, and the problem of weight regain is also observed in EBTs, which should also be addressed; (5) Due to the vast variety of accessible therapies, the majority of which are not FDA-approved, first, there is a need to follow FDA and other quality control organizations to get approval; (6) There is a lack of a consistent therapeutic strategy, as well as a lack of training programs, which has limited their distribution and usage, short training programs should be organized and, if possible, should be added in the curriculum of medical schools; (7) Sophisticated endoscopy is now becoming a major component of minimally invasive fellowships, preparing surgeons to take on the role of bariatric endoscopists; (8) Cost benefits analysis should be made for a better understanding of the total expenditure on the use of therapy; and (9) Longer follow-up and larger multicenter RCTs are required to confirm current outcomes and improve the standardization process of these procedures.

CONCLUSION

Obesity is a chronic systemic disease that requires a multidisciplinary approach for prevention, treatment, and management. Proper treatment must be personalized and tailored to the degree of the patient’s obesity and the combination of comorbidities. According to different studies, lifestyle changes and medicines can only achieve moderate weight loss results. Despite the fact that bariatric surgery has been shown to be a game-changing strategy in obesity, many patients find it unappealing due to its adverse effect profile and potential long-term difficulties. Compared to standard surgical treatments, bariatric endoscopic therapies may offer a valuable armamentarium in the therapy of obesity because their success in loss of weight is accompanied by being less intrusive, reversible, cost-effective and having a positive safety profile. It may become increasingly popular in the coming years because, when compared to surgery, it has a lower chance of AEs. Long-term efficacy is unknown at this moment. Additional research on long-term efficacy, metabolic disease outcomes, and RCTs is required. However, the future obesity treatment lies in a multidisciplinary strategy requiring various treatment methods.

FOOTNOTES

Author contributions: Abdulla M, Mohammed N, and AlQamish J contributed to the collecting the data, review, editing, and finalizing the manuscript; Abdulla M designed the review and wrote the manuscript.

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Basic Study

Roles of phosphatidylinositol-3-kinases signaling pathway in inflammation-related cancer: Impact of rs10889677 variant and buparlisib in colitis-associated cancer

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Abstract

BACKGROUND

Phosphatidylinositol-3-kinases (PI3K) is a well-known route in inflammation-related cancer. Recent discovery on PI3K-related genes revealed a potential variant that links ulcerative colitis (UC) and colorectal cancer (CRC) with colitis-associated cancer (CAC). PI3K/AKT pathway has been recommended as a potential additional therapeutic option for CRC due to its substantial role in modifying cellular processes. Buparlisib is a pan-class I PI3K inhibitor previously shown to reduce tumor growth.

AIM

To investigate the regulation of rs10889677 and the role of buparlisib in the PI3K signaling pathway in CAC pathogenesis.
Core Tip: The role of phosphatidylinositol-3-kinases (PI3K) in promoting cancer progression has been widely acknowledged due to its crucial involvement in regulating the survival, differentiation, and proliferation of cancer cells. Here, we investigate the role of PI3K signaling in colitis-associated cancer (CAC) pathogenesis by studying the regulation of potential variant in PI3K-related gene in the colorectal cancer cell line and the utilization of PI3K inhibitor, buparlisib, in the CAC-induced mice model. We suggested that rs10889677 variant plays a crucial role in initiating the PI3K signaling pathway, and buparlisib has the capability to inhibit PI3K-non-AKT activation in the pathophysiology of CAC.

INTRODUCTION

Inflammatory bowel disease (IBD), a chronic inflammatory disorder that goes into remission and then recurs, eventually leading to the development of colitis-associated cancer (CAC), is a kind of colorectal cancer (CRC)[1,2]. Crohn’s disease and ulcerative colitis (UC), the two main subtypes of IBD, had CRC risks of 1.4% and 0.8%, respectively[3,4]. The rising trend of IBD incidence and prevalence among Asians increased from 1.3% to 7.2% per 100000 population among Malaysian-Singaporeans[5]. Rapid urbanization, adoption of a Western diet, antibiotics use, personal hygiene standards, microbiological exposures, and pollution are all risk factors for developing IBD[6]. In Malaysia, the average annual incidence of IBD has risen to 1.46 per 100000 people over the last decade[7]. CAC accounted for 10%-15% of IBD fatality cases among Westerners, accounting for only 1% to 2% of CRC incidence in Malaysia[4,8].

CAC develops through the inflammation-dysplasia-carcinoma pathway[9]. Immune cells, including cytokines, chemokines, epithelial cells, and stromal cells, are among the cell types contributing to the inflammatory processes in the CAC development[10]. Moreover, inflammatory mediators had a substantial impact on the control of pre-neoplastic growth during CAC development, and their release is more likely to target multiple carcinogenesis-related signaling pathways involved, such as NFkB, PI3K, JAK/STAT and Wnt/B-catenin[11,12].

Phosphatidylinositol-3-kinases (PI3K) signaling pathway is an essential intracellular signaling mechanism in regulating the cell cycle[13]. Growth factors, cytokines, and other stimulatory chemicals activate PI3K via their receptors. The AKT-mTOR and SGK families receive signals from activated PI3K, which controls cell growth, proliferation, and apoptosis[14]. PI3K enzymatic activity has been linked to the aetiology of various diseases, including chronic inflammation and cancer[12,15]. Our earlier research identified PI3K as one of the critical pathways in long-duration UC associated with an increased risk of developing CAC[16]. A recent study on mutational analysis in CAC patients identified several potential
variants, including rs10889677, in the \textit{IL23R}, a cytokine-induced PI3K-related gene that may link inflammation to an increased risk of cancer\cite{17}.

PI3K/AKT pathway has been proposed as a viable therapeutic target for CRC due to its significant role in modulating cellular processes by targeting the downstream pathway\cite{18}. Buparlisib is an oral pan-class I PI3K inhibitor that targets all four class I PI3K catalytic isoforms (p110\textalpha, p110\beta, p110\gamma and p110\delta)\cite{19,20}. This drug is the most clinically advanced medication to have passed a phase II clinical trial in the cancer\cite{21,22}. Buparlisib treatment in \textit{in vivo} study showed encouraging outcomes by enormously lowering the quantity of phosphorylated AKT and slowing tumor growth in tumor-bearing mouse models\cite{19,23}. Therefore, the objective of this study was to study the regulation of the PI3K-related variant, rs10889677, in the CRC cell line and to investigate whether buparlisib may affect the non-AKT-independent branch of the PI3K signaling pathway, which was one of the keys signaling pathways implicated in cancer development and progression, using a mouse model.

\section*{MATERIALS AND METHODS}

\subsection*{Sample collection and preparation}

The Universiti Kebangsaan Malaysia Research Ethics Committee (UKM/PPI/111/8/JEP-2019-572) approved this study. A total of 32 fresh frozen and archival samples (ranging from the year 2014 to 2019) from patients with long-standing UC, CAC, and CRC were collected from the Endoscopy Unit at Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia. Upon admission, all patients provided informed consent. All tissues were collected by December 2020, stored in RNA later, and frozen at -80 °C until further processing. The hematoxylin and eosin-stained tissue sections were evaluated by an experienced pathologist for confirmation of diagnosis, inflammation, and metastatic status. The genomic DNA was extracted from fresh frozen tissues using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, United States) according to the manufacturer’s protocol. Meanwhile, GENEREAD DNA FFPE Kit (Qiagen, United States) was used to extract DNA from formalin-fixed paraffin-embedded blocks of archival samples. DNA concentration was measured using DeNovix DS11+ Spectrophotometer (DeNovix Inc., United States).

\subsection*{Sanger sequencing}

Primers were designed using the NCBI Primer Tool (National Center for Biotechnology Information, United States) and Primer3Plus\cite{24} based on the location of the somatic variant. The predesigned primers were as follows: \textit{IL23R}, forward 5’-TCT GTG CTC CTA CCA TCA CC-3’, and reverse 5’-TGT GCC TGT ATG TGT GAC CA-3’. SnapGene Viewer 5.3.2 was used to analyze the sequencing results.

\subsection*{Fragment amplification}

The fragments containing the wildtype and mutant rs10889677 variant on the 3’ untranslated region of \textit{IL23R} were amplified from the genotyped DNA samples. The predesigned primers were added with the target sequences of the \textit{XhoI} and \textit{XbaI} restriction enzymes. The primers were as follows: Forward 5’-ATC GCT CGA GGC TGC CTT GCA ATC TGA and reverse 5’-ATC GTG CTC TTA CTC TTA ATC CTG ACT-3’. The polymerase chain reaction for fragment amplification was carried out using GoTaq® Green Master Mix (Promega, United States) with a pre-denaturation step at 95 °C for eight minutes, followed by 35-40 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, with an additional final elongation step at 72 °C for five minutes. Then, the polymerase chain reaction (PCR) products were run on gel electrophoresis to check for fragment bands, followed by the gel purification step using the QIAquick® Gel Extraction Kit (Qiagen, United States).

\subsection*{Plasmid preparation and cloning}

A total of 1 μg of pmirGLO (Promega, United States) vector was digested with the following enzymes: \textit{XhoI} (NEB, United Kingdom) and \textit{XbaI} (NEB, United Kingdom) according to the manufacturer’s instructions. The ligation reactions were prepared using a T4 DNA Ligase kit (NEB, United Kingdom) with a molar ratio of 1:3 vector to insert. The ligated products were transformed utilizing the heat-shock technique with an \textit{E.coli} competent cell (NEB, United Kingdom). Colonies from prior successful transformations were screened, and positive transformant plasmid was purified using PureYield™ Plasmid Miniprep System (Promega, United States).

\subsection*{Transfection and luciferase assay}

The CRC cell line, HT29, was routinely cultured in a RPMI-1640 (Elabscience, United States) supplemented with 10% fetal bovine serum (Gibco, United States) and 1% of Penicillin/Streptomycin (Gibco, United States) and cultivated in 5% of CO\textsubscript{2} level incubator at 37 °C. Before transfection, 5.0 × 10\textsuperscript{4} HT29 cells were seeded into a 96-well white plate in 100 μL antibiotic-free growth media. Cultures were performed in triplicate. A total of 0.25 μg of wildtype, mutant and empty plasmids were transfected using Lipofectamine 2000 (Invitrogen, United States) transfection reagent according to the manufacturer’s protocol. Afterwards, the luciferase activity was measured 48 h after post-transfection using the Dual-Glo® Luciferase Assay system (Promega, United States) following the manufacturer’s protocol. Firefly and Renilla luciferase activity were measured using the GloMax® 20/20 Luminometer (Promega, United States). The assay was performed in triplicate, and all results were normalized with the internal control reading.
Animals

Forty male Balb/c mice (seven to eight weeks old, weighing 25-30 g) were purchased from the Animal Unit, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia. The mice were housed in individual cages with kenaf bedding and maintained on a 12 h light/dark cycle with controlled humidity at 25 °C kept in the animal laboratory unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. Regular food and water were supplied ad libitum. All mice were acclimatized for ten days before the start of the treatment. The animal handling and protocol was approved by the Animal Ethics Committee of Universiti Kebangsaan Malaysia (PPUKM/2019/NORFILZA/25-SEPT./1035-SEPT.-2019-DEC.-2021).

Chemical induction and PI3K inhibitor administration

The mice were randomly divided into four groups (n = 10 each): Control, dextran sodium sulphate (DSS) induced, azoxymethane (AOM) induced, and AOM/DSS combination induced[25]. A single intraperitoneal injection of AOM (10 mg/kg body weight) (Sigma-Aldrich Chemicals, United States) was administered to the AOM and AOM/DSS-induced group. A week after injection, AOM/DSS and DSS-induced groups received 2.5% DSS (40kDa; Sigma-Aldrich Chemicals, United States) in their drinking water for seven days in three cycles (each cycle consisting of one-week DSS, followed by two weeks of sterile water). The control and AOM-induced groups were only provided with sterile water throughout the treatment. Beginning in week eight, mice from the AOM/DSS-induced group were treated daily for 14 d with a PI3K inhibitor buparlisib (NVP-BKM120, MedChemExpress, United States) at 30 mg/kg via oral administration (Figure 1).

Disease activity index scoring

Daily observations and records were made of body weight, bowel consistency, and rectal bleeding throughout ten weeks. Changes in these factors will be scored to determine the disease activity index (DAI), which is based on the rating of each component. Scores were assigned for weight loss (0: 0-1%, 1: 1%-5%, 2: 5%-10%, 3: 10%-20%, 4: > 20%), stool consistency (0: Normal, 2: Loose stool, 4: Diarrhea), and presence of blood in the stool (0: Negative, 2: Visual blood in stool, 4: Fresh rectal bleeding)[26]. A total score was determined, ranging from 0 to 12.

Histological examination

Colon tissues were removed, cleaned, and flushed with sterile phosphate buffer saline and cut into two parts: Proximal and distal colon. The proximal colon was stored in RNA later solution (ThermoFisher, United States), and the distal colon tissues were processed, embedded in paraffin, and sectioned for hematoxylin and eosin staining. Histological structures were assessed and scored (0: Healthy colon, 1: Minimal inflammation with minimal to no separation of crypts, 2: Mild inflammation with mild separation of crypts, 3: Moderate inflammation with separation of crypts, with or without focal effacement of crypts, 4: Extensive inflammation with marked separation and effacement of crypts, 5: Diffuse inflammation with marked separation and effacement of crypts)[27]. The pathologist used an inverted microscope to examine all slides.

Immunohistochemistry

Immunohistochemistry (IHC) staining was performed using the Rabbit-specific HRP/DAB Detection IHC Detection kit Micro-polymer, as instructed by the manufacturer (ab236469; Abcam, United States). Slides were incubated in primary antibodies: Rabbit monoclonal Cleaved caspase-3 (CC-3) (1:5000, ab214430; Abcam, United Kingdom) and rabbit monoclonal Ki67 (1:100, ab16667; Abcam, United Kingdom) for 30 minutes. The secondary antibody was goat anti-rabbit HRP Conjugate, included in the same kit. Images were obtained using an Olympus light microscope (Japan). Qualitative and quantitative scoring were scored blindly by the experienced pathologist.

Quantitative real-time PCR

The excised proximal colon was sectioned into smaller fragments and placed in different tubes at -80 °C. RNA was prepared using AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, United States) following the manufacturer’s instructions. The total RNA yield was quantified using a DS-11 spectrophotometer (DeNovix Inc., United States). cDNA conversion was conducted using Quantivity Reverse Transcription Kit (Qiagen, United States) from 1 μg RNA following the manufacturer’s instructions. Quantitative PCR amplifications were prepared with approximately 100 ng cDNA using the Quantivity SYBR Green PCR Kit (Qiagen, United States) and mouse primer sets (IDT, United States). The predesigned primers were as follows: Ppia, forward 5’-CAA ACA CAA ACG CCT AG-3’ and reverse 5’-CTT ACC TTC CCA AAG ACC AC-3’; Sggk2, forward, 5’-GCA TAG AGC CTA CCT GAT CAC-3’ and reverse 5’-CCC AGG TTG ATG TTC CCA TT-3’ and Pdk1, forward 5’-CAT CCA CAT CCA GAT CAC AGA-3’ and reverse 5’-CTT TTA CAC GCC GAC TTC TCT-3’. The gene expression was normalized to the reference gene, Ppia and all reactions were prepared in triplicate.

Statistical analysis

Normally distributed variables are presented as the mean ± standard error mean. For statistical significance, the Student T-test and ANOVA tests were performed. At P < 0.05, the differences between groups were considered significant. Statistical analyses were performed using the GraphPad Prism Version 9.3.1 (GraphPad Software Inc., United States).
RESULTS

Information in clinical samples

Table 1 displays demographic data for all samples. The median age of all samples was 63 years old (IQR:11.5). Malays made up the majority of the samples (56%) and were followed by Chinese (28%) and Indians (15%). The gender distribution was equal between males and females. Most patients’ smoking status was non-smoker (91%) instead of smoker/ex-smoker (9%). Most UC patients were diagnosed with left-sided colitis or pancolitis, with a Mayo index score of 1 to 3 and a Geboes score of Grade 2A.1 to 2A.2. The majority of CRC and CAC patients were at stages 1 and 3, and the rectosigmoid and distal colon were the sites of the malignancies. Histologically, most CRCs were moderately differentiated, whereas tumors from CAC patients were categorized as poor, moderate and well differentiated. There was no reported familial history of CRC among any of the patients.

PI3K-related somatic variant rs10889677 exhibited a reduced trend of luciferase activity in CRC cell line

To understand the significance of the PI3K signaling pathway in the formation of CAC, the functional role of PI3K-related somatic variants that potentially correlate CAC with UC and CRC was investigated to gain insight into the role. Somatic variant rs10889677C>A has been successfully sequenced in CAC, UC and CRC samples. The proportion of homozygous CC (wildtype) was discovered to be the highest in CRC samples (60%), while the proportion of homozygous AA (mutant) was highest in CAC samples (85%). We discovered mutant rs10889677 had a 2.07-fold lower luciferase activity (2.35 ± 0.85) than the wildtype construct (4.42 ± 0.86). However, the difference was not statistically significant.

Combination of AOM + DSS produced a successful CAC-induced mice model

In a CAC mouse model, chemical induction of a single AOM injection with three cycles of 2.5% DSS was considered successful. The assessment of a successful CAC induction was based on observing body weight loss, the condition of the stool and the occurrence of any rectal bleeding. The CAC mice group endured reduced body weight, watery stools, and rectal bleeding for ten weeks. In light of this, the CAC mice group scored significantly higher on the DAI than the colitis and control groups, with a mean score of 1.25 ± 0.22 (P < 0.05) (Figure 3A). The DAI score of the CAC mice group began to rise dramatically after the second DSS induction (Figure 3B). On the other hand, the colitis group had a low DAI score, while the control group had none.

Buparlisib therapy was able to reverse the weight loss in the CAC-induced mice model

Due to ongoing DSS exposure, the colitis and CAC-induced mice group’s body weights have fluctuated (Figure 4). At every DSS induction cycle, weight loss was predicted to occur. Following each cycle, mice had a two-weeks recovery period to help them regain any lost weight. However, starting with the third cycle of DSS induction, the CAC mice group had substantial weight loss, losing about 5% of their total body weight. This significant body weight reduction in the CAC group explained why they had a higher DAI score than other groups. Meanwhile, the mice in the other groups...
Table 1 Clinical and demographic details of the recruited patients

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Some data are unavailable.
All data are expressed as n except where indicated in the table. UC: Ulcerative colitis; CRC: Colorectal cancer; CAC: Colitis-associated cancer.

recovered and kept their body weight throughout the treatment (Figure 4A).

PI3K inhibitor (buparlisib) supplementation, started in the eighth week of treatment, was used to perform additional research on the relationship between PI3K and the advancement of CAC. PI3K oral inhibitor dosage was calculated depending on the body weight of CAC mice. After receiving daily treatment for 14 d, the mean weight loss (g) in the CAC-induced mice was significantly lower (2.0 ± 0.0) than in the CAC group that had not received any treatment (2.6 ± 1.07) (P < 0.05) (Figure 4B).

CAC-induced animals exhibited inflammation and tumor growth characteristics

The distal colon underwent histological evaluation to check for any morphological alterations, and the results were reported in Figure 5. No diffuse inflammation was observed in any of the tissues in the present study, which ranged from 0 (no inflammation/healthy), 1 (minimal inflammation), 2 (mild inflammation), 3 (moderate inflammation), and 4 (extensive inflammation). Most control group mice had healthy colons with uniformly shaped glands and minimal inflammation. In the colon tissues of the colitis mice group, mild to moderate inflammation with separation of crypts, with or without focal effacement of crypts, and various gland shapes were seen. On the other hand, at least 50% of CAC-induced mice had tumors and varying degrees of mild to severe inflammation. These tumors had enlarged nuclei, reactive cellular changes, moderate inflammatory response, depleted glands, and an elevated neutrophil count. The scoring on colitis and CAC mice colon tissue were scored depending on the inflamed area (chronic) or specifically at the tumor location (Figure 6).

Buparlisib treatment had anti-proliferative and pro-apoptotic actions in the CAC-induced mice model

The apoptotic marker (CC-3) and the proliferative marker (Ki67) were both subjected (Figure 7). Every type of tissue, even the healthy tissue, had proliferating cells. The proliferative cells’ distribution might vary depending on the different tissue types. In the control group, the healthy colon exhibited 30% of Ki67-positive cells, with the proliferating cells predominating in the basal regions of the glands. In contrast, more than 50% and 80% of Ki67-positive cells had irregular
Figure 2 Dual-luciferase reporter assay for rs10889677 construct. A: The structure of luciferase vector, pmirGLO, with wildtype and mutant constructs; B: Luciferase activity of HT29 cell line transfected with a construct containing wildtype and mutant variant of rs10889677. Data are presented as mean ± SEM; one-way ANOVA was employed; n = 3.

Figure 3 The disease activity index score. A: The total average disease activity index (DAI) score per group at the end of treatment. aP < 0.05 (compared to the control group); bP < 0.05 (compared to the colitis group); data are presented as mean ± SEM; one-way ANOVA was used; n = 8; B: The total average DAI score per group at each dextran sodium sulphate-induction cycle. Two-way ANOVA was used. CAC: Colitis-associated cancer.

distribution in the colitis and CAC mice groups, respectively. The uneven distribution of proliferative cells, particularly in the upper section of the glands, suggested the cells were highly proliferating. Buparlisib administration to the CAC mice group reduced about 5% of the positive cells compared to the untreated CAC mice group (Figure 7).

CC-3 was assessed as an apoptotic marker based on its intensity and the number of positive cells. Most tissues from the control, colitis and CAC mice group have moderate intensity of CC-3. In contrast to the control group, which had fewer than 50% positive cells, most colitis and CAC mice had more than 75% CC-3 positive cells. Based on the PI3K inhibitor treatment evaluation, buparlisib-treated CAC mice achieved a high overall score of CC3-intensity and -positive cells (6/8) (Figure 7).

**Buparlisib was successful in reducing the activity of PI3K-non-AKT-associated genes**

The relative gene expression of the downstream PI3K-non-AKT pathway genes Pdk1 and Sgk2, which are involved in CAC progression, was assessed to study further the impact of the PI3K inhibitor on CAC development. Buparlisib treatment showed a downward trend in the expression of Pdk1 (2.04 ± 1.17) in the CAC-induced mice group, however, it is not significant compared to the untreated group (Figure 8). Only Sgk2 expression showed a significant reduction in treated CAC-induced mice (2.56 ± 1.09) in comparison to the untreated CAC mice (5.61 ± 0.79) (P < 0.05).
**Figure 4** The body weight changes. A: Percentage of weight loss (%) in all groups. A drop in body weight was seen in the colitis-associated cancer (CAC) group after every cycle of dextran sodium sulphate (DSS) induction. Red arrows indicate the cycle of DSS induction. Two-way ANOVA was employed; n = 8; B: Total mean body weight loss (g) between buparlisib-treated and -untreated CAC-induced mice group. *P < 0.05 (compared to the CAC untreated group); data are presented as mean ± SEM; student T-test was employed; n = 4. CAC: Colitis-associated cancer; CRC: Colorectal cancer.

**Figure 5** Histological structure assessment. The range of the histologic scores varied from 0 (no inflammation) to 4 (extensive inflammation). Magnification is 40 ×.

**Figure 6** Histological examination of the control, colitis and colitis-associated cancer mice model. The control group exhibited a healthy colon with a uniform gland shape. Meanwhile, the colitis mice group showed mild to moderate inflammation. The tumor was only found in the colitis-associated cancer-induced mice group. Red arrows indicate the inflamed area and tumor sites. Magnification is 100 ×.
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Figure 7 Immunohistochemistry analysis on Ki67 and cleaved caspase-3 markers on colitis-associated cancer-mice model. A: H&E staining, Ki67 and cleaved caspase-3 (CC-3) staining; B and C: Ki67 and CC-3 staining exhibited lower proliferative and higher apoptotic cells on the buparlisib-treated colitis-associated cancer (CAC) mice model compared to the untreated CAC mice. *P < 0.05 (compared to the control group); data are presented as mean ± SEM; one-way ANOVA was employed; n = 4-8. The red arrow showed the tumor area. Magnification is 100 ×. CAC: Colitis-associated cancer; CC-3: Cleaved caspase-3.

DISCUSSION

A well-known pathway in the emergence of inflammation-related cancer is the PI3K pathway. Dysregulation of the PI3K signaling pathway may affect cellular development, metabolism, proliferation, and apoptosis[28]. Recent research on the function of cytokine-induced PI3K-related genes in CAC patients led to the discovery of possible mutations that might connect CAC with UC and CRC[17]. We are, therefore, interested in learning more about the pathophysiology of CAC and how the PI3K signaling pathway is involved. In this study, we utilized dual luciferase assay to investigate the functional significance of rs10889677, a somatic mutation related to PI3K, and we generated CAC in mice to assess the function and regulation of PI3K inhibitor in the modulation of PI3K-non-AKT signaling pathway.

The single nucleotide polymorphism (SNP) rs10889677 is a genetic variant identified in the IL23R, one of the cytokine-induced PI3K-related genes. This particular SNP is a variation in the DNA sequence of the IL23R, where a single nucleotide (cytosine, C) is replaced by another nucleotide (adenine, A). The rs10889677 SNP is situated precisely in the 3’ untranslated region of IL23R, which is also a miRNA binding site[29]. Any SNPs present in the miRNA binding site may affect how miRNA-mRNA interacts, changing the stability or translation efficiency of the mRNA[30]. This, in turn, may influence the expression of the IL23R protein, which is involved in immune response and inflammation, and may increase the susceptibility to certain diseases, such as CRC. The rs10889677 mutation has been linked strongly to IBD and several other cases, including breast, lung, bladder and colorectal[31-33]. In our recent publication, we have successfully predicted a notable decrease in the stability of mRNA secondary structure for the rs10889677 variant construct compared to the wildtype[17]. This finding supported the hypothesis that genetic mutations resulted in mRNA structural instability and disrupted the binding sites[17]. About 60%-70% of human CRCs were associated with activating the PI3K pathway, increased AKT signaling and PTEN inactivation[34]. Furthermore, PI3K inhibitors have been proposed as another effective targeted therapy for solid tumors, including CRC[35,36]. Nevertheless, further elucidation regarding the utilization of PI3K inhibitors in the context of CAC is warranted. Balb/c male mice were chosen because they are more susceptible and prone to grow tumors than other strains, and tumors were induced in them with a combination of AOM injection and repeated DSS cycles[37,38]. The application of combined AOM and DSS is a potent, reproducible, and reasonably priced initiation-promotion
paradigm that has been widely used in CAC-related research[25,39]. A high DAI score indicates that the mouse CAC model is successful. The CAC-induced mice model manifested similar symptoms as CAC patients, where the mice were experiencing rectal bleeding, watery stool and body weight loss[40]. According to a previous study, symptoms including bloody stools, began to manifest between days 13 and 21 after the mice received DSS[41]. Similar results were shown in this study, where disease activity in CAC-induced mice began to increase dramatically following the second cycle of DSS, which occurred just 21 d after the first induction of DSS.

Histologically, three cycles of 2.5% DSS induced mild to moderate inflammatory response in the colitis group. No tumor formation was observed along the digestive system in the CRC group. Hence, no additional evaluation of those tissues was done. On the other hand, 50% of the CAC-induced mice group were found to have moderate inflammation. No diffuse inflammation was observed in the colonic mucosal of CAC-induced mice, even though it was reported that signs of diffuse inflammation can appear as early as third to fourth weeks after treatment[42]. Furthermore, compared to a prior study, where submucosal tumor infiltrations were sporadically seen in 20%–24% of AOM-DSS mice at week ten, the percentage of CAC-induced mice with tumors in this study was considered high[43]. The selection of the mouse strain may have affected the percentage of tumor occurrence because different strains of mice have varying susceptibilities.

Buparlisib was given daily for 14 d to the CAC-induced mice group to perform additional research on the function of PI3K inhibitors. Buparlisib is a potent oral pan-PI3K inhibitor that targets all four catalytic isoforms of class I PI3K (p110α, p110β, p110δ and p110γ)[44]. Buparlisib has been tested in clinical trials phase I and II in several solid tumors, including breast, brain, and lung cancer[20]. The preclinical studies showed buparlisib’s inhibitory action against numerous PI3K isoforms and anti-proliferative effects in various in vitro cell lines[19]. Additionally, the authors provided the outcomes of in vivo studies with mouse xenograft models that demonstrate the effectiveness of buparlisib in reducing tumor growth[19]. Our findings showed that buparlisib treatment significantly decreased weight loss in CAC-induced mice. Another study supported this finding in which buparlisib therapy inhibited the growth of established patient-derived glioblastoma multiforme xenografts and increased survival in nude rats[45].

Additionally, buparlisib treatment in CAC-induced mice resulted in a slight rise in apoptotic cell activity and decreased cell proliferation. This result was consistent with several studies that found buparlisib to have the ability to induce apoptosis and reduce tumor growth in a variety of malignancies[44,46]. Buparlisib’s effectiveness was also examined in CRC cell lines, where it was discovered that the drug had a considerable impact on the process of apoptosis induction, which progressed from AKT inhibition via FoxO3a-dependent p53 upregulated modulator of apoptosis induction[47].

Additionally, buparlisib treatment has been demonstrated to inhibit the PI3K/Akt signaling pathway, which causes apoptosis and slows tumor growth in medulloblastoma cells[44]. The most frequent downstream targets of buparlisib are AKT and mTOR. Studies have demonstrated that buparlisib effectively inhibited at serine 473 and threonine 308 and suppressed the phosphorylation of proteins downstream from mTORC1, including S6K and 4E-BPI in bone and soft tissue sarcomas[48]. Despite that, there has been limited research conducted on the correlation between buparlisib and PI3K-non-AKT downstream pathways. Our results revealed that the non-AKT downstream of PI3K, Pdk1 and Sgk2 also showed a similar down-regulation tendency with buparlisib treatment. The expression of two PI3K-non-AKT downstream genes was previously found to be higher in malignant tissues than in healthy mucosa[49,50]. PDK1 is a gene that plays a critical role in regulating cell proliferation and survival through its ability to curb signal propagation to the downstream targets such as AKT and SGK2. PDK1 was discovered to interact with a specific protein known as HuR (human antigen R), which is known to attach to the mRNA of PI3K and stabilize it, enhancing the production of PI3K[51].
Meanwhile, SGK is activated by the same signaling cascade as AKT, and its inhibitor has been created and demonstrated encouraging results in preclinical tests as a possible therapeutic target in cancer[52]. The therapeutic potential of SGK1 inhibition in cancer treatment has been demonstrated in preclinical studies using a variety of small molecule inhibitors and RNAi-based strategies that target SGK1[53]. Buparlisib and wortmannin, both of which are PI3K inhibitors, decreased the stimulatory impact of insulin on hOAT4 transport activity, aided by SGK2[54]. This showed that PI3K inhibition, whether by buparlisib or wortmannin, can lessen the stimulatory effect of insulin on hOAT4 transport activity and that SGK2 may mediate this effect[54].

There are a few limitations in this study. There were only male mice used in the animal experiment. The AOM/DSS-induced mice model has been widely used in research, with a focus on male mice[42]. This preference for male mice is primarily caused by behavioural changes in female mice that can be altered by hormone cycles[55]. Additional studies using female balb/c mice will be required to establish that gender does not interfere with generalization of findings. Another limitation of this study is that the gene expression analysis was only performed on mouse tissues derived from inflammatory and malignant regions. It would be beneficial to include surrounding normal tissue in the analysis to observe and analyse any potential differences.

CONCLUSION

We investigated the functional relevance of the cytokine-induced PI3K-related gene variation, rs10889677, and it is hypothesized that this variant was a mediating factor in the beginning of the PI3K signaling pathway. Buparlisib, a PI3K inhibitor, was also administered, and it showed promise in preventing PI3K-non-AKT activation in the CAC-induced mice model. Overall, our research may offer a fresh perspective on how PI3K is used in the pathophysiology of CAC.

ARTICLE HIGHLIGHTS

**Research background**
The phosphatidylinositol-3-kinases (PI3K)/AKT pathway has emerged as a potential new approach in the complicated landscape of inflammation-related cancer, providing new hope for patients with colitis-associated cancer (CAC). A recent discovery in the investigation of PI3K-related genes revealed a promising association between ulcerative colitis (UC), colorectal cancer (CRC), and the elusive rs10889677 mutation.

**Research motivation**
Understanding the involvement of the PI3K signalling pathway in the development and progression of CAC. Genetic Variants and CAC Susceptibility: Investigating the impact of genetic variants, such as the rs10889677 variant, on CAC susceptibility and their contribution to PI3K pathway activation. The potential of the pan-class I PI3K inhibitor buparlisib as a potential therapeutic option for the treatment of CAC was being examined.

**Research objectives**
To investigate the role of PI3K-related gene variation in CAC pathogenesis and to evaluate the therapeutic potential of buparlisib, a powerful pan-class I PI3K inhibitor with tumor-suppressive properties.

**Research methods**
The study examined the genomic DNA from 32 colonic samples from cases of CAC, UC, and CRC. The rs10889677 mutation was highlighted, which was amplified and cloned for both mutant and wild-type fragments in the pmirGLO vector. Luciferase activity was measured using the HT29 cell line. CAC was induced using a precise protocol that included azoxymethane and sodium dextran sulphate. Buparlisib was administered after 14 d. Immunohistochemistry for Ki67 and Cleaved-caspase-3 markers as well as quantitative real-time polymerase chain reaction for Pdk1 and Sgk2 were performed on excised colonic tissues.

**Research results**
Buparlisib significantly reduced the mean weight loss in these mice, indicating an increase in general health and wellbeing. It has an effect in slowing cancer cell development and promoting cell death in CAC-induced mice. It also had an effect on the expression of particular genes involved in the PI3K pathway, with significantly decreased Sgk2 expression and a decreasing trend in Pdk1 expression.

**Research conclusions**
Our study discovered that a specific genetic variant (rs10889677 variation) is critical in the activation of a cancer-promoting pathway known as PI3K. We also discovered that a drug called buparlisib can prevent this route from being triggered, which is great news for those fighting cancer (CAC).

**Research perspectives**
Patient stratification: Future research could look into whether the rs10889677 variant can be used as a biomarker to
predict CAC development in UC patients. This could help with early detection and personalised treatment initiatives. Mechanism Elucidation: The mechanisms by which the rs10889677 variation alters the PI3K pathway should be studied further in the future.

ACKNOWLEDGEMENTS

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FOOTNOTES

Author contributions: Mokhtar NM and Raja Ali RA contributed to the conceptualization; Mokhtar NM, Raja Ali RA, Muhammad Nawawi KN, and Razali NN contributed to the methodology and participant recruitments; Razali NN, and Mohd Rathi ND contributed to the animal handling; Razali NN, Yahaya A, and Mokhtar NM performed the validation; Razali NN, and Mokhtar NM contributed to the data analysis; Razali NN wrote the original draft preparation; Mokhtar NM, Raja Ali RA, and Muhammad Nawawi KN wrote the review and editing; Mokhtar NM, Raja Ali RA, and Muhammad Nawawi KN contributed to the supervision; All authors have read and agreed to the published version of the manuscript.


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Retrospective Study

Endoscopic transgastric fenestration versus percutaneous drainage for management of (peri)pancreatic fluid collections adjacent to gastric wall (with video)

Hong-Mei Zhang, Hui-Ting Ke, Md Robin Ahmed, Ya-Juan Li, Ghulam Nabi, Mu-Han Li, Ji-Yu Zhang, Dan Liu, Li-Xia Zhao, Bing-Rong Liu

Abstract

BACKGROUND
Percutaneous drainage (PCD) and endoscopic approaches have largely replaced surgical drainage as the initial approach for (peri) pancreatic fluid collections (PFCs), while complications associated with endoscopic stent implantation are common.

AIM
To introduce a novel endoscopic therapy named endoscopic transgastric fenestration (ETGF), which involves resection of tissue by endoscopic accessory between gastric and PFCs without stent implantation, and to evaluate its efficacy and safety compared with PCD for the management of PFCs adjacent to the gastric wall.

METHODS
Patients diagnosed with PFCs adjacent to the gastric wall and who subsequently received ETGF or PCD were restrospectively enrolled. Indications for intervention were consistent with related guidelines. We analyzed patients baseline character-
ististics, technical and clinical success rate, recurrence and reintervention rate, procedure-related complications and adverse events.

RESULTS
Seventy-two eligible patients were retrospectively identified (ETGF = 34, PCD = 38) from October 2017 to May 2021. Patients in the ETGF group had a significantly higher clinical success rate than those in the PCD group (97.1 vs 76.3%, \(P = 0.01\)). There were no statistically significant differences regarding recurrence, reintervention and incidence of complication between the two groups. While long-term catheter drainage was very common in the PCD group.

CONCLUSION
Compared with PCD, ETGF has a higher clinical success rate in the management of PFCs adjacent to the gastric wall. ETGF is an alternative effective strategy for the treatment of PFCs adjacent to the gastric wall.

Key Words: (Peri) Pancreatic fluid collections; Endoscopic transgastric fenestration; Percutaneous drainage

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Core Tip: Inspired by endoscopic full-thickness resection, we proposed the concept of endoscopic transgastric fenestration (ETGF), which involves resection of connect tissue between the gastric wall and (peri) pancreatic fluid collections (PFCs) with the assistance of endoscopic accessory to treat PFCs secondary to pancreatitis adjacent to the gastric wall, avoiding the stent implantation. In the current study, we evaluate the efficacy and safety of ETGF by comparing with percutaneous drainage for the management of PFCs adjacent to the gastric wall.

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INTRODUCTION
Pancreatic and peripancreatic fluid collections (PFCs) are causes of fluid leakage or liquefaction of pancreatic necrosis following acute pancreatitis, chronic pancreatitis, surgery or abdominal trauma[1]. Collections usually forms 4 wk after the onset of acute pancreatitis and the majority resolve spontaneously[1]. Indications to intervene PFCs include infection and symptomatic sterile necrosis, while persistent collections that are asymptomatic may be observed[1-3]. Percutaneous drainage (PCD) and an endoscopic approach with stent implantation have replaced surgical drainage as the initial treatment for PFCs which reduce the complications and costs of hospitalization[3-7]. The European Society of Gastrointestinal Endoscopy recommends endoscopy or PCD as the first interventional method for PFCs[8]. PCD is an attractive and conventional approach that appears to be safe and the least invasive. However, inability to remove necrotic debris in the cavity has restricted its use[5,6,9]. Commonly, transluminal endoscopic drainage with stent implantation is an effective method[4,7]. However, embedding, displacement, and bleeding related to stent implantation are common complications, which leads to multi-interventions, and hence resulting in additional cost[10-12]. In addition, application of stent has been limited due to its high cost and is not commercial in some tertiary hospitals in China. Inspired by endoscopic full-thickness resection (EFTR), Liu et al[13] first conducted endoscopic transgastric fenestration (ETGF), an innovative endoscopic treatment avoiding the implantation of a stent to manage PFCs. ETGF involves endoscopic resection of connected tissues between gastric and pancreatic lesions with the assistance of endoscopic accessory, which can drain the collection of fluid in the cavity and debride the necrotic debris inside. In this retrospective study, the primary objective was to assess the availability of ETGF by comparing the rate of technical and clinical success, recurrence and reintervention with patients who received PCD. The secondary objective was to assess its safety by evaluating complications related to the procedure.

MATERIALS AND METHODS

Patient enrollment
Patients diagnosed with PFCs at the First Affiliated Hospital of Zhengzhou University between October 2017 and May 2021 were enrolled in this study. Inclusion criteria included patients diagnosed with PFCs that was adjacent to the gastric wall and who subsequently received ETGF or PCD. Indications to intervene PFCs were consistent with related guidelines.
Patients with incomplete clinical data and who were lost to follow-up were excluded. All patients underwent ultrasonography (USG), computed tomography (CT) scan or magnetic resonance cholangiopancreatography to evaluate the lesion prior to the procedure. Patients in the ETGF group underwent endoscopy at least twice to observe the absorption of the cavity and the natural healing of the artificial fistula between the gastric wall and the cavity, the majority of which almost healed within 1 mo. CT or USG was reviewed within 6 mo after treatment. The study was approved by the institutional ethics committee and all patients were provided written informed consent to undergo the procedures (KY-2021-00642).

Definitions

PFCs were defined according to the revised Atlanta consensus related to acute pancreatitis[1]. Technical success was defined as the ability to access the lesion. Clinical success was defined as symptom relief with PFCs reduced to < 2 cm within 6 mo without another alternative drainage procedure.

ETGF technique

Endoscopic drainages were performed under general anesthesia with endotracheal intubation as follows: (1) Endoscopic ultrasonography (EUS) was used to determine the lesion location, whether a large vessel was hidden in the operative region and marked the site of fenestration using a Hook knife (KD-620LR, Olympus); (2) then the mucosal layer of the fenestration site was then removed with an endoscopic snare and full-thickness incision was subsequently made with the Hook knife, fluid in the collection was seen to pour out spontaneously; (3) re-evaluation of the fistula between the stomach and cavity; (4) the gastric-collections incision was enlarged to a diameter of approximately 2 cm to facilitate the operation and drainage by EFTR; (5) coagulating styptic forcep was used for hemostasis (Coagrasper, FD-410LR; Olympus); (6) the endoscope was advanced into the collection, and the content of the PFCs was further cleaned with saline rinse and vacuum suction, debris was removed by snare assistance, and (7) nasocystic tube (18 Fr) was indwelled if necessary (large or complicated with infection) and endoscopy was undertaken twice to observe the healing of the artificial fistula, most of which closed within one month (Figures 1 and 2, Video).

PCD technique

Under local anesthesia using lignocaine, an 18-gauge needle was placed into the PFCs percutaneously with the guidance of USG or CT scan and the fluid was aspirated. A guidewire was then advanced into the collection. The tract was dilated and then a pigtail catheter of 8 or 10 Fr was inserted into the lesion. The catheter was replaced when the drainage tube failed due to obstruction and eventually removed when the collections was < 2 cm in length.

Statistical analysis

Statistical analysis was performed using SPSS Statistics v26.0. For continuous variables, the mean ± SD was used to describe data that fitted a normal distribution and quartiles were used for data that did not conform to normal distribution. Statistical significance was analyzed by the t-test and nonparametric test respectively. Counting card information was described by percentage and performed by the chi-square test. P < 0.05 was considered statistically significant.

RESULTS

Between October 2017 and May 2021, a total of 72 patients were enrolled in this study (ETGF = 34, PCD = 38, Figure 3). There were no statistically significant differences between the two groups. Patients in the ETGF group were younger than in PCD group (36.8 ± 12.9 years vs 46.0 ± 16.8years, P = 0.01). The clinical success rate in the ETGF group was significantly higher than that in the PCD group (97.1 vs 76.3%, P = 0.01). There were no statistically significant differences regarding recurrence and reintervention between the two groups. Although the complication rate was similar in the two groups, catheter related adverse events were common in the PCD group (2.9 vs 34.3 %, P = 0.001). Patients were followed up by the electronic medical record system combined with telephone consultation for a median follow-up of 35 wk (9-85 wk). There was no procedure related mortality in either groups. The largest lesion was encountered in the endoscopic group with a length of 220 mm. Baseline characteristics and patient demographics are shown in Table 1. Primary and secondary outcomes are shown in Table 2.

ETGF approach

Six of 34 patients had previous therapeutic history in another hospital: one received ETGF, one received surgical treatment, and the other four received PCD. The average age of the participants was 36.8 ± 12.9 years old. The mean length of PFCs was 109.4 ± 7.8 mm. The average total interventions were 1.03 sessions. The total duration of hospital stays within 6 mo without another alternative drainage procedure.

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Table 1 Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>ETGF (%)</th>
<th>PCD (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>34</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (70.6)</td>
<td>26 (68.4)</td>
<td>0.84</td>
</tr>
<tr>
<td>Mean age</td>
<td>36.8 ± 12.9</td>
<td>46.0 ± 16.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean length of lesions (mm)</td>
<td>109.4 ± 7.8</td>
<td>94.8 (80.8-133.2)</td>
<td>0.89</td>
</tr>
<tr>
<td>Cause of pancreatitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>10 (29.4)</td>
<td>8 (21.1)</td>
<td></td>
</tr>
<tr>
<td>Gallstone</td>
<td>8 (23.5)</td>
<td>8 (21.1)</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>9 (26.5)</td>
<td>6 (15.8)</td>
<td></td>
</tr>
<tr>
<td>Trauma</td>
<td>1 (2.9)</td>
<td>4 (10.5)</td>
<td></td>
</tr>
<tr>
<td>Autoimmunity</td>
<td>1 (2.9)</td>
<td>3 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic duct stones</td>
<td>3 (8.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choledochocyst</td>
<td></td>
<td>1 (2.6)</td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>1 (2.9)</td>
<td>8 (21.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain (%)</td>
<td>24 (70.6)</td>
<td>27 (71.1)</td>
<td></td>
</tr>
<tr>
<td>Abdominal distention (%)</td>
<td>6 (17.65)</td>
<td>5 (13.2)</td>
<td></td>
</tr>
<tr>
<td>Nausea or vomiting</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Preintervention</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>PCD</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>ETGF</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opportunity of intervention</td>
<td></td>
<td></td>
<td>0.381</td>
</tr>
<tr>
<td>&lt; 4 wk</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>&gt; 4 wk</td>
<td>21</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Total interventions</td>
<td>1.03 ± 0.03</td>
<td>1.32 ± 0.09</td>
<td>0.003</td>
</tr>
<tr>
<td>Total hospital visits</td>
<td>1.0 (0)</td>
<td>1.0 (1.0)</td>
<td>0.278</td>
</tr>
<tr>
<td>Total hospital stays(days)</td>
<td>14.5 (10.25)</td>
<td>19.0 (20.5)</td>
<td>0.177</td>
</tr>
<tr>
<td>Total cost ($)</td>
<td>4852 (3877)</td>
<td>5206 (8377)</td>
<td>0.955</td>
</tr>
</tbody>
</table>

1Multiple symptoms may coexist in one patient, so we didn’t calculate percentages of each item.
2Here, we excluded patients that preintervention was inconsistent with original treatment in our hospital.
ETGF: Endoscopic transgastric fenestration; PCD: Percutaneous drainage.

**PCD approach**

Four of 38 patients received preintervention but failed, which included one open surgery and 3 cases of PCD. The average age of the participants was 46.0 ± 16.8 years old with more male patients than female patients (26 vs 12). Approximately three quarters of patients suffered from abdominal pain (27/38). The average length of PFCs was 94.8 mm (80.8-133.2 mm). Hyperlipidemia, gallstones, and alcohol-related pancreatitis were the etiologies in 57.9% of patients (22/38). The average total interventions was 1.32 sessions. The total duration of hospital stays and total cost was 19 days and 5206 $ respectively. Clinical success was achieved in 29 patients (76.3%). Four patients showed recurrence (10.5%) and reintervention occurred in 17 of 38 patients (44.7%). With regard to adverse events, two patients developed bleeding which stopped spontaneously, two had local infection, and one had drainage adhesion to surrounding tissue. In 11 patients, the duration of catheterization was more than 8 wk. Drainage obstruction was encountered in 5 cases.
Table 2 Endoscopic transgastric fenestration vs percutaneous drainage for management of (peri) pancreatic, n (%)

<table>
<thead>
<tr>
<th></th>
<th>ETGF</th>
<th>PCD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technical success</td>
<td>34 (100)</td>
<td>38 (100)</td>
<td></td>
</tr>
<tr>
<td>Clinical success</td>
<td>33/34 (97.1)</td>
<td>29/38 (76.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Recurrence</td>
<td>1/34 (2.94)</td>
<td>4/38 (10.5)</td>
<td>0.42</td>
</tr>
<tr>
<td>Reintervention</td>
<td>10/34 (29.4)</td>
<td>17/38 (44.7)</td>
<td>0.18</td>
</tr>
<tr>
<td>Complication</td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Bleeding 2 (5.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Local infection 2 (5.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adhesion to surrounding tissue 1 (2.63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catheter related adverse events</td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Intubation time &gt; 8 wk 11 (28.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drainage obstruction 5 (13.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There were 8 patients who received a twice percutaneous drainage (PCD) and another 3 required no less than three times of PCD, 2 transferred to endoscopic transgastric fenestration and 7 to surgery after PCD failure. ETGF: Endoscopic transgastric fenestration; PCD: Percutaneous drainage.

DISCUSSION

Surgical treatment has been traditionally used for PFCs[3]. Recently, PCD and endoscopic management have replaced surgery as the main treatment for PFCs due to their minimal invasiveness[6,8]. However, PCD cannot debride necrosis and an external drainage tube affects quality of life, which has limited its clinical use[5,14-16]. Endoscopic treatment can not only drainage the pus inside but also remove debris in the cavity and is beneficial to patient’s health[5,6,14,17]. While previously endoscopic therapy involved stent implantation, embedding, displacement, and bleeding were inevitable[10-12]. Different to conventional endoscopic management, ETGF was conducted by means of ETFR to drain and debride PFCs adherent to the gastric wall, avoiding stent implantation.
Figure 2 Endoscopic transgastric fenestration: Enlarge the aperture of opening and debridement. A: Collections inflowing via the artificial fistula; B: Enlarged the aperture of opening with hook knife; C: The artificial fistula (about 2 cm in diameter) between the gastric wall and the cavity was made; D: Debridement of necrotic tissue under endoscopic direct vision; E: Endoscopic review of the fistula showed fistula almost healed one months later; F: The reviewed computed tomography scan one month after endoscopic transgastric fenestration.

Figure 3 Flow-chart for this retrospective study. ETGF: Endoscopic transgastric fenestration; PFCs: (peri) Pancreatic collections; PCD: Percutaneous drainage.

Our findings are basically consistent with previous studies on conventional endoscopic drainage which actually involves plastic or metal stent implantation and the PCD approach[16,18-20]. A respective study from the United States reported that the technical and clinical success rate of EUS-guided drainage of PFCs was 100% and 97% respectively[18]. Similarly, ETGF had a relatively favorable effect with 100% technical success and 97.1% clinical success. Jianhua et al performed a comparative study on drainage of PFCs and initial clinical success was considerably higher in patients who received transluminal endoscopic drainage than those in the PCD group (94.9% vs 65.0%)[20]. In our study, the clinical success rate in the PCD group was 65.8% which was also lower than that in the ETGF group.
PCD was associated with a high rate of reintervention and the endoscopic stent implantation approach was shown to significantly reduce the rate of retreatment, which resulted in short hospital stay, low cost and a reduced number of follow-up abdominal imaging studies[9,15]. In the present study, the total cost and reintervention rate following ETGF was lower than that in PCD, but was not statistically significantly different.

Keane et al[15] agreed that there was no difference between PCD and transluminal endoscopic drainage of PFCs in terms of recurrence. In another study, endoscopic drainage with stent implantation was an effective and appropriate method with the advantage of fewer recurrences compared to PCD[19]. In the present study, we found that there was no difference in the recurrence rate between two groups. In a long-term follow up study about PFCs, the recurrence of transluminal endoscopic drainage reported by Nabi et al was 6.7%[11]. In this study, one recurrence was encountered in ETGF group (2.94%).

The procedural adverse events rate was high in the PCD group compared with the transluminal endoscopic drainage with stent implantation group[20]. In the current study, the rate of complications in the ETGF group was similar with that in the PCD. Bleeding was encountered in ETGF patients and was managed by conservative treatment totally, which was consistent with previous studies[21].

Research by Storm et al[18] on endoscopic drainage of PFCs discovered that size of collections > 100 mm was correlated with an increased risk of adverse events. The size of PFCs was an independent risk factor for infection related to the cavity, and large PFCs with a diameter > 150 mm were more likely to become infected[22]. In the present study, seven patients had nasocystic tube implanted due to pus and necrosis in the cavity, of which 3 lesions were more than 150 mm and another 3 lesions were > 100 mm. A study on ETGF found that there were 3 of 5 patients received a nasocystic tube and the mean catheterization time was 10 d, which was longer than that in our study (8.6 d)[23]. We also recommended an indwelling nasocystic tube for large lesion or lesions combined with infection.

A previous study reported that the average length of time the drainage catheter in place was as long as 44.5 d in PCD[14]. Similarly, in our study, there were 28.9% of patients (11/38) whose underwent drainage for more than 8 wk. In addition, drainage obstruction occurred in 5 patients due to pus or necrosis, all of which led to further discomfort. Furthermore, an external catheter requires long-term care, as well as reminding the patient of their underlying disease state, and results in significant patient discomfort and compromised the quality of life[14]. During ETGF we used a natural orifice as the access route, avoiding an external catheter and scarring, which was beneficial to mental health and improved quality of life.

The limitations of this study were as follows. Firstly, we didn’t examine how different types of PFCs affected the therapeutic efficacy. Secondly, prognostic factors associated with postoperative infection (size of PFCs, the area of fenestration) were not been identified in ETGF. A quality of life scale was not used in this study, thus, how the two different methods affected the quality of life is not known. A large, prospective, multicenter study is necessary to confirm our results.

CONCLUSION

Both PCD and ETGF can be used effectively for the treatment of PFCs, although ETGF is superior to PCD as it has a higher rate of clinical success and a lower rate of adverse events. ETGF is an innovative, effective, safe and scarless strategy for the management of PFCs adherent to the gastric wall. However, further studies especially clinical trials are needed before final recommendations are made.

ARTICLE HIGHLIGHTS

Research background
Percutaneous drainage (PCD) and endoscopic approaches with stent implantation have largely replaced surgical drainage as the initial approach for (peri) pancreatic fluid collections (PFCs). While stent implantation guided by endoscopic ultrasound has been mature and preferred treatment, but stent displacement, bleeding and embedding should not be neglected.

Research motivation
Inspired by endoscopic full-thickness resection, we conducted endoscopic transgastric fenestration (ETGF), which involves resection of connected tissues between gastric wall and PFCs, so as to drain the collection of fluid in the cavity and debride the necrosis inside.

Research objectives
The study aimed to evaluate the efficacy and safety of ETGF by accessing its success and complication rate compared with PCD.

Research methods
This retrospective analysis enrolled patients diagnosed with PFCs adjacent to the gastric wall and subsequently received ETGF or PCD during 4 years, analyzed patients baseline characteristics, technical and clinical success rate, recurrence and
reintervention rate, procedure related complication and adverse events.

**Research results**
Seventy-two eligible patients were retrospectively identified (ETGF = 34, PCD = 38). Patients in the ETGF group acquired significantly higher clinical success rate than that in PCD (97.1 vs 76.3%, \( P = 0.01 \)). There was no statistical difference about recurrence, reintervention and incidence of complication between the two groups.

**Research conclusions**
ETGF would be an alternative effective and safe strategy for the treatment of PFCs adjacent to the gastric wall.

**Research perspectives**
ETGF can drainage fluid inside and debride necrosis, which improves its clinical success. Therefore, in our opinion ETGF may be an alternative treatment for PFCs adjacent to gastric, especially for large lesions or lesions that associated with infection or necrosis.

**ACKNOWLEDGEMENTS**
We express our gratitude to Professor Shi Niu, Department of Gastroenterology and Hepatology, Inner Mongolia People’s Hospital, China, for his encouragement and assistance in preparing the audio and revising the manuscript.

**FOOTNOTES**

**Co-first authors:** Hong-Mei Zhang and Hui-Ting Ke.

**Author contributions:** Zhang HM, Ke HT and Li YJ contributed to the design of the study, collected data and drafted the manuscript; Ke HT and Nabi G performed the data analysis; Ahmed MR, Liu D and Zhao LX conceived the work; Zhang JY and Li MH contributed to video clip of endoscopic transgastric fenestration; Liu BR revised the manuscript; All authors issued final approval for the version to be submitted. Here are the reasons for designating Zhang HM and Ke HT as co-first authors: During the period of the study, Ke HT was a postgraduate student of the First Affiliated Hospital of Zhengzhou University and fully participated in the study. Ke HT graduated one year ago and worked in Ezhou Central Hospital. Zhang HM and Ke HT proposed the concept of this study, searched relevant literature and then discussed the research design together, and finally determined the research idea. Before the paper was drafted, they collected the research data together, and carried out the collation, statistical analysis and later verification work. They worked together on the first draft. Zhang HM explained the data charts and graphs and Ke HT selected typical case pictures and illustrated them.

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**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University.

**Informed consent statement:** All study participants or their legal guardian provided informed written consent regarding personal and medical data collection prior to study enrollment.

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** No additional data are available.

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**S-Editor:** Qu XL
**L-Editor:** A
**P-Editor:** Chen YX
Retrospective Study

Clinical significance of programmed cell death-ligand expression in small bowel adenocarcinoma is determined by the tumor microenvironment

Aitoshi Hoshimoto, Atsushi Tatsuguchi, Ryohei Hamakubo, Takayoshi Nishimoto, Jun Omori, Naohiko Akimoto, Shu Tanaka, Shunji Fujimori, Tsutomu Hatori, Akira Shimizu, Katsuhiko Iwakiri

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Abstract

BACKGROUND
Comprehensive genomic analysis has shown that small bowel adenocarcinoma (SBA) has different genomic profiles from gastric and colorectal cancers. Hence, it is essential to establish chemotherapeutic regimens based on SBA characteristics. The expression of programmed cell death-ligand 1 (PD-L1) and programmed cell death-ligand 2 (PD-L2) in SBA is not fully understood. Anti-PD-L1/PD-1 therapy uses tumor-infiltrating lymphocytes (TILs); therefore, the status of TILs in the tumor microenvironment (TME) may influence their efficacy. The ratio of FoxP3+ to CD8+ T cells has been reported to be useful in predicting the prognosis of digestive system cancers.

AIM
To investigate the clinicopathological significance of PD-L1/2 expression according to the status of TILs in SBA tissues.

METHODS
We performed immunohistochemical analysis for PD-L1, PD-L2, CD8, FoxP3, and DNA mismatch repair (MMR) proteins using formalin-fixed, paraffin-embedded tissues from 50 patients diagnosed with primary SBA. The immunoreactivities of PD-L1 and PD-L2 were determined separately in tumor cells and tumor-infiltrating lymphocytes.
rating immune cells throughout the tumor center and invasive margins, and finally evaluated using the combined positive score (CPS). We assessed CD8+ and FoxP3+ T cells in the intratumoral and tumor-surrounding stroma. Subsequently, we calculated and summed the ratio of FoxP3 to CD8+ T cell counts. Immune-related cell densities were graded as low or high. Immunohistochemical results were compared with clinicopathological factors and patient prognosis. The distribution of cancer-specific survival (CSS) was estimated using the Kaplan–Meier method, and the log-rank test was used to test for significant differences in CSS. A Cox proportional hazard model was also used to assess the effect of tumor variables on CSS.

RESULTS
PD-L1 expression was positive in 34% in tumor cells (T-PD-L1) and 54% in tumor-infiltrating immune cells (I-PD-L1) of the cases examined. T-PD-L2 was positive in 34% and I-PD-L2 was positive in 42% of the cases. PD-L1 CPS ≥ 10 and PD-L2 CPS ≥ 10 were observed in 50% and 56% of the cases, respectively. Deficient MMR (dMMR) was 14% of the cases. T-PD-L1, I-PD-L1 and PD-L1 CPS ≥ 10 were all significantly associated with dMMR (P = 0.037, P = 0.009, and P = 0.005, respectively). T-PD-L1, I-PD-L1, and PD-L1 CPS ≥ 10 were all associated with deeper depth of invasion (P = 0.001, P = 0.024, and P = 0.002, respectively). I-PD-L2 expression and PD-L2 CPS ≥ 10 were significantly higher in the differentiated histological type (P = 0.015 and P = 0.030, respectively). The I-PD-L1 and I-PD-L2 levels were significantly associated with better CSS (P = 0.037 and P = 0.015, respectively). CD8-high was significantly associated with less lymph node metastasis (P = 0.047), less distant metastasis (P = 0.024), less peritoneal dissemination (P = 0.034), and earlier TNM stage (P = 0.047). The CD8-high group had better prognosis than the CD8-low group (P = 0.018). FoxP3 expression was not associated with any clinicopathological factors or prognosis. We found that patients with PD-L2 CPS ≥ 10 tended to have worse prognosis in the FoxP3/CD8-low group (P = 0.088).

CONCLUSION
The clinicopathological significance of PD-L1/2 expression may differ depending on the TME status. Immune checkpoint inhibitors may improve the prognosis of SBA patients with low FoxP3/CD8 ratio and PD-L2 expression.

Key Words: Small bowel adenocarcinoma; Programmed cell death-ligand 1; Programmed cell death-ligand 2; Tumor microenvironrment; Tumor-infiltrating lymphocytes; Regulatory T-cells

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Core Tip: We investigated the clinicopathological significance of programmed cell death-ligand 1 (PD-L1) and programmed cell death-ligand 2 (PD-L2) expression in association with the infiltration of FoxP3+ and CD8+ T cells into the tumor microenvironment (TME) to identify PD-L/PD-1 immunotherapy candidates among patients with small bowel adenocarcinoma (SBA). We demonstrated that the status of the TME affects the clinical significance of PD-L1 and PD-L2. PD-L2 may be associated with poor prognosis of SBA patients with a high immune cell infiltration.

INTRODUCTION
Small bowel adenocarcinoma (SBA) is an uncommon condition, accounting for less than 3% of all gastrointestinal neoplasms; however, the small intestine accounts for 95% of the surface area of the entire gastrointestinal tract[1]. According to a recent report from the United States, the incidence of small bowel cancer has been increasing, with an annual increase of 1.8% between 2006 and 2015[2]. Although there are no definitive risk factors for SBA, diets containing high volumes of animal fat and protein have been reported to increase the risk, which may be related to an increase in the number of SBA[3]. SBA accounts for 30%–50% of all small bowel malignancies, and limited data exist on its molecular and clinicopathological features[4]. SBA has a high probability of being discovered at an advanced stage owing to a delay in diagnosis. No effective chemotherapy has been established for unresectable SBAs, and the regimens for colorectal cancer are palliatively administered. However, comprehensive genomic analyses have revealed that SBA and colorectal cancer have different genomic profiles, and it is assumed that the molecular pathways leading to carcinogenesis may also be different[5,6]. Therefore, it is essential to establish chemotherapy regimens based on the specific characteristics of SBA.
The small bowel is the largest organ of the human immune system. Lymphoid tissues in the lamina propria and various immune-related cells are prevalent in the small intestine and contribute to immune surveillance. Programmed cell death-ligand 1 (PD-L1) is the primary PD-1 Ligand that is upregulated in various solid tumors, and plays a pivotal role in modulating the tumor microenvironment (TME) to inhibit cytokine production and the cytolytic activity of PD-1+ tumor-infiltrating CD4+ and CD8+ T cells[7]. Several studies have demonstrated the efficacy of blocking the PD-1/PD-L1 signaling pathway in gastrointestinal cancers with high microsatellite instability (MSI-high)[8-11], and recent studies have demonstrated a significant association between MSI-high and PD-L1 expression in SBA[12,13]. PD-L1 expression has been reported to be associated with gastrointestinal cancer prognosis, despite several conflicting reports. While some studies have reported that PD-L1 expression is associated with favorable prognosis in gastric and colorectal cancers[14-18], others have reported that PD-L1 expression is associated with poorer prognosis in these cancers[19-22]. Considering the role of PD-1/PD-L1 in the TME, PD-L1 expression is predicted to be associated with worse prognosis in cancer patients.

Programmed cell death-ligand 2 (PD-L2) was discovered as a second ligand for PD-1 and has been reported to inhibit T cell proliferation by blocking cell cycle progression, similar to PD-L1[23,24]. PD-L2 suppresses the proliferation and cytokine production of CD4+ T cells through T cell receptors[25]. Its expression is significantly associated with PD-L1 expression in melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, renal cell carcinoma, bladder cancer, gastric cancer, and triple-negative breast cancer[25]. Although the function and expression pattern of PD-L2 are considered to be similar to those of PD-L1, PD-L2 has not received much attention, and its role in modulating tumor immunity remains undetermined.

Anti-PD-L1/PD-1 therapy utilizes tumor-infiltrating lymphocytes (TILs). Therefore, the status of TILs in the TME may influence their efficacy. Although TILs are mainly composed of CD8+ T cells, they are heterogeneous cell populations that also contain regulatory T cells (Tregs), which are believed to inhibit CD8+ T cells[26]. Tregs are an immunosuppressive subset of CD4+ T cells that orchestrate cellular and molecular networks to induce an immunosuppressive environment favorable to tumorigenesis[27]. Tregs are characterized by the expression of the master regulatory transcription factor FoxP3[28]. A high ratio of FoxP3+ to CD8+ T cells is associated with poor clinical outcomes in digestive system cancers[29-31].

This study investigated the clinicopathological significance of PD-L1 and PD-L2 expression in association with the infiltration of FoxP3+ and CD8+ T cells into the TME to identify PD-L/PD-1 immunotherapy candidates among SBA patients.

MATERIALS AND METHODS

Patients and tissue samples
We obtained 50 duodenal, jejunal, and ileal adenocarcinoma tissue samples from the archives of the Department of Pathology at Nippon Medical School Hospital for immunohistochemical analysis of PD-L1, PD-L2, CD8, FoxP3, and DNA mismatch repair (MMR) protein expression. Samples from patients with predisposing conditions, including Lynch syndrome, familial adenomatous polyposis, celiac disease, and Crohn’s disease, were excluded to focus on sporadic SBA. Furthermore, samples from patients with ampullary adenocarcinoma or metastatic cancer were excluded. Cancer-specific syndrome, familial adenomatous polyposis, celiac disease, and Crohn’s disease, were excluded to focus on sporadic SBA.

Immunohistochemical analysis
Specimens were fixed in 10% formalin, embedded in paraffin wax, and immersed in 0.5% H2O2–methanol for 10 min to block endogenous peroxidase activity. Subsequently, the sections were microwaved in 0.01 mol/L citrate phosphate buffer (pH = 6.0) or EDTA (pH = 9.0) for antigen retrieval and incubated with 10% normal horse or goat serum for 10 min at 37°C to block nonspecific IgG binding. Thereafter, the sections were incubated for 18 h at 4°C with the primary antibodies listed in Supplementary Table 1. Next, they were treated with their respective biotinylated antibodies, namely anti-mouse IgG or anti-rabbit IgG (1:200; Vector) for 30 min at 25°C, followed by treatment with avidin-biotin-peroxidase complex for 30 min at 25°C. The reaction products were developed by immersing the sections in 3,3’-diaminobenzidine tetrahydrochloride solution containing 0.03% H2O2.

Evaluation of immunohistochemical staining
Each patient was blindly evaluated by two independent observers (A.H. and A.T.). Any disagreements were resolved using a multi-headed microscope. The immunoreactivities of PD-L1 and PD-L2 were determined separately in tumor cells and tumor-infiltrating immune cells, such as lymphocytes and macrophages, throughout the tumor center and invasive margins. Tumor samples were defined as PD-L1 and PD-L2 positive when ≥ 1% of the tumor cells and/or tumor-infiltrating immune cells were immunoreactive with unequivocal intensity. Subsequently, for PD-L1 and PD-L2 expression, a combined positive score (CPS) was calculated by dividing the total number of both tumor cells and immune cells above the positive threshold by the total number of viable tumor cells[32,33]. We set the PD-L1 and PD-L2 CPS cutoffs at ≥ 10% [34].

We assessed CD8+ T cells in ten randomly selected microscopic areas, including the intratumoral and tumor-surrounding stroma, no further than one high-power field from the tumor edge, by light microscopy (400 ×; BX63; Olympus, Tokyo, Japan). FoxP3+ T cells were assessed in the same ten high-power fields. Cell counts were determined...
Both I-PD-L1 and I-PD-L2 expression were significantly associated with better CSS ($P = 0.037$ and $P = 0.015$, respectively) (Figure 3). There was no significant association between T-PD-L1 expression or PD-L1 CPS $\geq 10\%$ and patients’ survival. There was no significant association between T-PD-L2 expression or PD-L2 CPS $\geq 10\%$ and patients’ survival. Patients in
Table 1 Clinicopathological data of patients with small bowel adenocarcinoma

<table>
<thead>
<tr>
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<th>No. of cases (%)</th>
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<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
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<tr>
<td>≤ 68</td>
<td>27 (54.0)</td>
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<tr>
<td>&gt; 68</td>
<td>23 (46.0)</td>
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<tr>
<td><strong>Sex</strong></td>
<td></td>
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<tr>
<td>Female</td>
<td>15 (30.0)</td>
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<tr>
<td>Male</td>
<td>35 (70.0)</td>
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<tr>
<td><strong>Site</strong></td>
<td></td>
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<tr>
<td>Duodenum and jejunum</td>
<td>45 (90.0)</td>
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<tr>
<td>Ileum</td>
<td>5 (10.0)</td>
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<tr>
<td><strong>Histology</strong></td>
<td></td>
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<tr>
<td>WD, MD</td>
<td>41 (82.0)</td>
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<tr>
<td>PD, Muc</td>
<td>9 (18.0)</td>
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<tr>
<td><strong>Depth of invasion</strong></td>
<td></td>
</tr>
<tr>
<td>pT1-2</td>
<td>14 (28.0)</td>
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<tr>
<td>pT3-4</td>
<td>36 (72.0)</td>
</tr>
<tr>
<td><strong>Lymph node metastasis</strong></td>
<td></td>
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<tr>
<td>Absence</td>
<td>27 (54.0)</td>
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<tr>
<td>Presence</td>
<td>23 (46.0)</td>
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<tr>
<td><strong>Distant metastasis</strong></td>
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<tr>
<td>Absence</td>
<td>37 (74.0)</td>
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<tr>
<td>Presence</td>
<td>13 (26.0)</td>
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<tr>
<td><strong>Peritoneal seeding</strong></td>
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<td>Absence</td>
<td>40 (80.0)</td>
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<td>Presence</td>
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<td><strong>TNM stage</strong></td>
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<td>I</td>
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<td>III</td>
<td>10 (20.0)</td>
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<tr>
<td>IV</td>
<td>13 (26.0)</td>
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</table>

WD: Well differentiated; MD: Moderately differentiated; PD: Poorly differentiated; Muc: Mucinous adenocarcinoma.

the CD8-high group had better prognosis than those in the CD8-low group ($P = 0.018$) (Figure 4). There was no significant association between FoxP3+ T cells and patients’ survival. In contrast, the FoxP3/CD8-low group had significantly better prognosis than the FoxP3/CD8-high group ($P = 0.004$). We performed a survival analysis for PD-L1 and PD-L2 expression stratified by the FoxP3/CD8 ratio, and found that patients with PD-L2 CPS ≥ 10 in the FoxP3/CD8-low group had worse prognosis, although the difference was not significant ($P = 0.088$) (Figure 5).

In the univariate analysis using the Cox proportional hazards model for CSS, lymph node status and I-PD-L1, I-PD-L2, CD8, and FoxP3/CD8 ratios had significant prognostic value (Table 5). In the multivariate analysis performed by introducing all the above variables into the Cox proportional hazards model, lymph node status and I-PD-L2 expression retained independent prognostic significance.
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Hoshimoto A et al. PD-L1/2 expression in small bowel adenocarcinoma

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DISCUSSION

In this study, we performed immunostaining for PD-L1 and PD-L2 to investigate their clinicopathological significance in SBA. Since PD-L1 is expressed in both tumor cells and tumor-infiltrating immune cells, we examined the clinicopathological significance of their expression separately to clarify whether there were differences in their roles in tumor progression. Although there were several similarities and correlations, some differences in association with clinicopathological factors were observed. T-PD-L1 expression was associated with deeper depth of invasion, but not with lymph node metastasis, distant metastasis, or prognosis. These results suggest that T-PD-L1 contributes to the local invasion of tumor cells but not to metastasis; consequently, it is not associated with prognosis. I-PD-L1 expression is associated with deeper depth of invasion, less peritoneal metastasis, and favorable prognosis. We speculated that I-PD-L1 expression might be influenced by peritumoral infiltrating T cells, which correlates with favorable prognosis. Indeed, we found a positive relationship between I-PD-L1 expression and the CD8+ T cell count, and a high density of CD8+ TILs was associated with favorable prognosis. The main similarity between T-PD-L1 and I-PD-L1 expression was that both were associated with deeper depth of invasion and were more common in stage II compared to stage I tumors. These results suggest that the clinical significance of PD-L1 does not differ following the cell type in which it is expressed. Based on the above findings, we adopted the CPS to evaluate PD-L1 expression. The CPS was calculated by summing the number of PD-L1-stained cells (tumor cells, lymphocytes, and macrophages) and dividing the result by the total number of viable tumor cells, and multiplying by 100. Based on the observed response rate and response durability, the U.S. Food and Drug Administration granted pembrolizumab accelerated approval for the treatment of recurrent locally advanced, metastatic gastric or gastro-esophageal junction adenocarcinoma that expresses PD-L1 CPS ≥ 1. Thus, the CPS is now becoming a standard method for the assessment of PD-L1 expression. Because the CPS is a scoring system characterized by collectively quantifying positivity for tumor cells and positivity for surrounding immune cells, it may be possible to optimize the presence or absence of PD-L1 expression in carcinomas accompanied by inflammatory or immune cell infiltration. We examined the relationship between PD-L1 expression and clinicopathological factors using the CPS ≥ 10 as a cut-off in this study, resulting in that no significant association between PD-L1 expression and prognosis was found.

It has been demonstrated that PD-L2 is a second ligand for PD-1 and can be expressed by immune, stromal, or tumor cells, mainly through Th2-associated cytokines depending on tumor microenvironmental stimuli[35]. To date, a few studies have reported the significance of PD-L2 expression in gastrointestinal carcinomas[36,37]. To our knowledge, this
is the first study to examine the relationship between PD-L2 expression and the clinicopathological characteristics of SBA patients. Since PD-L2 is expressed in both tumor cells and tumor-infiltrating immune cells, we examined the clinicopathological significance of its expression in both cell types, as we did for PD-L1. T-PD-L2 expression was not associated with any clinicopathological factors except for histological type and prognosis. I-PD-L2 expression was significantly associated with better CSS, suggesting that I-PD-L2 expression may be influenced by peritumoral infiltrating T cells that correlate with favorable prognosis. Then, we evaluated the PD-L2 expression using the CPS. PD-L2 CPS ≥ 10 was associated with younger age and was more common in differentiated histological type, but not with prognosis. It has been reported that PD-L2 expression was associated with poor prognosis in gastric and colorectal cancers[36,37]. These findings are intuitively understandable, considering that PD-L2 expression suppresses tumor immunity as well as PD-L1 does.

### Table 3 Clinicopathological correlation of CD8, FoxP3, and FoxP3/CD8

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<td>N (%)</td>
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WD: Well differentiated; MD: Moderately differentiated; PD: Poorly differentiated; Muc: Mucinous adenocarcinoma; NS: Not significant.
Table 4 Mutual relationship of programmed cell death-ligand 1, programmed cell death-ligand 2, CD8 and FoxP3 (n = 50)

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<sup>1</sup>An inverse relationship.

PD-L1: Programmed cell death-ligand 1; PD-L2: Programmed cell death-ligand 2; NS: Not significant.

However, several studies have demonstrated that both PD-L2 and PD-L1 expression are favorable prognostic indicators for gastric and colorectal cancer[25,38]. To date, such discrepancies have been explained by differences in scoring methods, cutoff values of immunostaining, heterogeneities of carcinoma, and any bias originating from the inclusion of an insufficient number of cases. To resolve this, we investigated the clinical significance of PD-L1 and PD-L2 expression according to TME status. CD8<sup>+</sup> TILs are associated with the tumor immune response and can be used to predict the response to immunotherapy and survival outcomes in CRC[39]. It has been reported a high density of CD8<sup>+</sup> TILs is associated with favorable prognosis in CRC[40-42]. We found that CD8-high was negatively associated with lymph node metastasis, distant metastasis, and peritoneal dissemination in SBA patients. We also found that the CD8-high group had better prognosis than the CD8-low group, which is consistent with the findings of previous reports. These results suggest that CD8<sup>+</sup> TILs have an anti-tumor effect in SBA as well as in CRC and that CD8<sup>+</sup> TILs play an important role in improving patient survival. Then, we analyzed the association between FoxP3<sup>+</sup> Tregs and clinicopathological factors. CD4<sup>+</sup> Tregs expressing the transcription factor Fox3 are highly immunosuppressive and play a central role in maintaining self-tolerance and immune homeostasis. FoxP3<sup>+</sup> T cells promote tumor progression by suppressing effective anti-tumor immunity by inactivating or reducing the proliferation of cytotoxic CD8<sup>+</sup> T cells and CD4<sup>+</sup> T effector cells in tumors[27,28]. To the best of our knowledge, this is the first study to examine the relationship between the density of FoxP3<sup>+</sup> T cells and the clinicopathological characteristics in SBA patients. We found that FoxP3<sup>+</sup>-high tumors tended to have deeper depth of invasion but were not associated with lymph node metastasis, distant metastasis, and peritoneal dissemination. No association was observed between FoxP3<sup>+</sup> T cell density and CSS. This is due to the functional hetero-

Table 5 Univariate and multivariate Cox proportional hazards analysis for cancer-specific survival (n = 50)

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<td>P value</td>
<td>HR (95% CI)</td>
<td>P value</td>
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<td>66.320 (7.494-586.923)</td>
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<td>1.156 (0.231-5.769)</td>
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<td>CD8</td>
<td>High vs. low</td>
<td>0.311 (0.111-0.872)</td>
<td>0.026</td>
<td>1.922 (0.164-22.481)</td>
<td>NS</td>
</tr>
<tr>
<td>FoxP3/CD8</td>
<td>High vs. low</td>
<td>4.490 (1.476-13.660)</td>
<td>0.008</td>
<td>1.476 (0.138-15.769)</td>
<td>NS</td>
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HR: Hazard ratio; 95% CI: 95% confidence interval; PD-L1: Programmed cell death-ligand 1; PD-L2: Programmed cell death-ligand 2; NS: Not significant.
Figure 1 Immunohistochemical localization of programmed cell death-ligand 1 and programmed cell death-ligand 2 in small bowel adenocarcinoma. Magnification 400×. A: T-programmed cell death-ligand 1 expression was membranous and cytoplasmic; B: I-programmed cell death-ligand 1 (I-PD-L1) was positive in peritumoral lymphocytes and macrophages; C: The predominant pattern of T-programmed cell death-ligand 2 expression was in the apical membrane; D: I-programmed cell death-ligand 2 expression was positive in peritumoral lymphocytes and macrophages, similar to that of I-PD-L1.

Figure 2 Immunohistochemical localization of CD8 and FoxP3 in small bowel adenocarcinoma. A: CD8+ T cells in the intratumoral stroma; B: CD8+ T cells in the tumor-surrounding stroma; C: FoxP3+ T cells in the intratumoral stroma; D: FoxP3+ T cells in tumor-surrounding stroma. Magnification 400×.

genivity of FoxP3+ T cells, as FoxP3+ non-Tregs are secreted depending on inflammatory cytokines such as TGF-β and IL-12[43]. While some studies have reported that high density of FoxP3+ T cells in the TME is associated with poor prognosis in lung cancer, others have reported that high density of FoxP3+ T cells is associated with favorable prognosis in CRC, bladder cancer, and head and neck cancers[44]. Thus, it has been reported that the amount of FoxP3+ T cells infiltration does not always serve as a poor prognostic indicator in the case of carcinoma accompanied by inflammatory cell infiltration, including CRC[43]. Then, we estimated the ratio of FoxP3+ T cells to CD8+ T cells in 10 HPF selected from the same site in the same section. FoxP3/CD8-high was significantly associated with deeper depth of invasion, lymph node metastasis, distant metastasis, peritoneal dissemination, and TNM stage progression. Patients with higher FoxP3+/CD8+ T cells ratios have poor prognosis for gastrointestinal cancers[29-31]. Similarly, we observed that the prognostic value of this ratio was better than that of FoxP3+ or CD8+ T cells alone, which partly reflects the interactions between anti-tumor CD8+ T cells and immunosuppressive FoxP3+ T cells in tumors[45,46]. These results indicate that assessing both FoxP3+ and CD8+ T cells is more useful for understanding TME status than assessing FoxP3 or CD8+ T cells alone.
Figure 3 Kaplan–Meier analysis of cancer-specific survival according to programmed cell death-ligand 1 and programmed cell death-ligand 2 expressions in small bowel adenocarcinoma evaluated by log-rank tests. A: Survival based on I-programmed cell death-ligand 1 expression in overall cases; B: Survival based on I-programmed cell death-ligand 2 expression in overall cases. I-PD-L1: I-programmed cell death-ligand 1; I-PD-L2: I-programmed cell death-ligand 2.

Both PD-L1 CPS ≥ 10 and PD-L2 CPS ≥ 10 significantly correlated with increased CD8+ T cells infiltration. Furthermore, I-PD-L2 expression was more frequent in FoxP3+/CD8-low tumors. These results may explain why I-PD-L2 expression is associated with favorable outcomes. To determine the effect of PD-L1 and PD-L2 on the prognosis of patients with SBA, excluding the effect of the TME, we divided the patients into FoxP3+/CD8-low and FoxP3+/CD8-high groups. We investigated the correlation between PD-L1 and PD-L2 expression and the prognosis in the two patient groups. We found that patients with PD-L2 CPS ≥ 10 tended to have poorer prognosis than those with PD-L2 CPS < 10 in the FoxP3+/CD8-low group, although the difference was not statistically significant. Furthermore, there were no deaths among patients with a PD-L2 CPS < 10 in the FoxP3+/CD8-low group. Generally, cases in which the infiltration of CD8 is dominant over FoxP3 are predicted to have favorable prognosis due to the anti-tumor immune effect of T cells; nevertheless, patients showing poor prognosis are also included within the group. The PD-L2 pathway may contribute to poor prognosis in patients with FoxP3+/CD8-low tumors. The results of this study also indicate that PD-L2 may be a better...
predictive factor for prognosis in these cases than PD-L1.

Recent studies have demonstrated that the tumor mutation burden (TMB) could be a biomarker in patients with cancer treated with immune checkpoint inhibitors (ICIs), in which high TMB is significantly correlated with better survival[47]. Although the efficacy of ICIs in patients with cancer with TMB-high or MSI-high/dMMR has been confirmed, patients with MSI-high have a favorable prognosis, and generally, only a small number of patients require chemotherapy. In fact, none of the patients with dMMR died in our cohort, and only two of seven patients received chemotherapy. Most patients with SBA have microsatellite stable (MSS)/pMMR or TMB-low, and it is necessary to identify useful biomarkers for ICI therapy in patients with MSS/pMMR. Previous studies have shown that TMB-high tumors are immunogenic and that TMB-high tumors are also present in SBA[48]. In small bowel cancer, TMB-high has been reported to be associated with dMMR, CD8-high, and PD-L1 expression, but it has been shown that TMB-high cases are also present in cases of pMMR [49,50]. Our results indicate that PD-L2 positive and FoxP3/CD8-low patients with pMMR may benefit from ICI therapy. Although we did not examine the TMB in this study, it may stratify the outcomes of patients with SBA with PD-L2 positive and FoxP3/CD8-low.

This study has several limitations. First, the sample size is small. In addition, there was a bias in tumor localization. Second, MMR status was determined based solely on the results of immunohistochemical staining for MMR proteins. Third, there were no deaths among the patients with dMMR tumors. Therefore, we could not analyze patients’ prognosis based on the MMR status.

CONCLUSION

We elucidated a discrepancy in the previously reported clinical significance of PD-L1 expression in SBA. Several studies have reported that PD-L1 expression, which should be involved in tumorigenesis, is associated with favorable prognosis. This contradictory finding may originate from the fact that all patients are equally included in the analysis without considering the TME status and the type of cells in which PD-L1 is expressed. We demonstrated that the clinical significance of PD-L2 may be affected by TME status. Although SBA patients with high immune cell infiltration generally have better prognosis, some of these patients have poorer prognosis. PD-L2 may contribute to poorer prognosis of these patients. ICIs may improve the patients’ prognosis in the FoxP3/CD8-low group through blocking the binding of the PD-1 to PD-L2 and activating locally infiltrated T cells.

ARTICLE HIGHLIGHTS

Research background

According to a recent report from the United States, the incidence of small bowel adenocarcinoma (SBA) has been increasing, with an annual increase of 1.8% between 2006 and 2015. Comprehensive genomic analyses revealed that SBA and colorectal cancer have different genomic profiles, and it is assumed that the molecular pathways leading to carcinogenesis may also be different. Therefore, it is essential to establish chemotherapeutic regimens based on the specific characteristics of SBA.

Research motivation

The clinicopathological significance of programmed cell death-ligand 1 (PD-L1) and programmed cell death-ligand 2 (PD-L2) expression in SBA is not yet fully understood. There are several conflicting reports regarding the clinicopathological significance of PD-L1 expression in gastrointestinal cancers. To resolve this discrepancy, we investigated the clinical
significance of PD-L1 and PD-L2 expression according to tumor microenvironment (TME) status stratified by the density of FoxP3+ and CD8+ T cells.

Research objectives
In this study, we investigated the clinicopathological significance of PD-L1 and PD-L2 expression in association with the infiltration of FoxP3+ and CD8+ T cells in the TME to identify PD-L/PD-1 immunotherapy candidates among patients with SBA. We elucidated the discrepancy in previously reported clinical significance of PD-L1 expression in SBA.

Research methods
The immunoreactivities of PD-L1 and PD-L2 were determined separately in tumor cells and tumor-infiltrating immune cells, such as lymphocytes and macrophages, and evaluated using the combined positive score (CPS). To our knowledge, this is the first study to examine the relationship between PD-L2 expression, and the density of FoxP3+ T cells, and the clinicopathological characteristics of patients with SBA.

Research results
PD-L1 expression was positive in 34% in tumor cells (T-PD-L1) and 54% in tumor-infiltrating immune cells (I-PD-L1) of the cases examined. T-PD-L2 was positive in 34% and I-PD-L2 was positive in 42% of the cases, respectively. PD-L1 CPS ≥ 10 and PD-L2 CPS ≥ 10 were observed in 50% and 56% of cases, respectively. I-PD-L1 and I-PD-L2 Levels were significantly associated with better prognosis. We speculated that I-PD-L1 expression might be influenced by peritumoral infiltrating T cells, which correlates with favorable prognosis. We found that patients with PD-L2 CPS ≥ 10 tended to have worse prognosis in the FoxP3/CD8-low group. Although SBA patients with high immune cell infiltration, such as those in the FoxP3/CD8-low group, generally have better prognosis, some have poorer prognosis. Therefore, PD-L2 may contribute to the poor prognosis of these patients.

Research conclusions
We identified a discrepancy in the previously reported clinical significance of PD-L1 expression in SBA. Several studies have reported that PD-L1 expression, which is involved in tumorigenesis, is associated with favorable prognosis. This contradictory finding may originate from the fact that all patients were equally included in the analysis without considering the TME status and the type of cells in which PD-L1 was expressed. To identify PD-L/PD-1 immunotherapy candidates, not only PD-L1 expression and DNA mismatch repair/microsatellite instability but also PD-L2 expression and the density of tumor-infiltrating lymphocytes, such as FoxP3+ and CD8+ T cells, in the TME should be considered.

Research perspectives
In this study, we did not consider tumor mutation burden (TMB). TMB-high has been proposed as a predictive biomarker for the response to immune checkpoint inhibitors based on the assumption that increasing the number of mutant proteins will create antigenic peptides, allowing for enhanced immunogenicity. In the future, we may be able to analyze TMB status in SBA and combine it with PD-L1/2 expression and TME status to generate powerful biomarkers for identifying immunotherapy candidates.

ACKNOWLEDGEMENTS
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FOOTNOTES
Author contributions: Hoshimoto A designed and performed the research and wrote the paper; Tatsuguchi A designed the research and contributed to the analysis; Hamakubo R, Nishimoto T, Omori J, Akimoto N, Tanaka S, Fujimori S, and Hatori T provided clinical advice; Shimizu A and Iwakiri K supervised the report; All authors have read and approve the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Nippon Medical School Institutional Review Board (Approval No. B-2020-164).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at tachan@nms.ac.jp. Participants gave informed consent for data sharing.

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Expression characteristics of peripheral lymphocyte programmed death 1 and FoxP3+ Tregs in gastric cancer during surgery and chemotherapy

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Abstract

BACKGROUND
Programmed death 1 (PD-1) and CD4+CD25+FoxP3+ expression in peripheral blood T-cells has been previously reported in various types of cancer. However, the specific variation tendency during surgery and chemotherapy, as well as their relationship in gastric cancer patients, still remain unclear. Understanding this aspect may provide some novel insights for future studies on tumor recurrence and tumor immune escape, and also serve as a reference for determining the optimal timing and dose of clinical anti-PD-1 antibodies.

AIM
To observe and analyze the expression characteristics of peripheral lymphocyte PD-1 and FoxP3+ regulatory T cells (FoxP3+ Tregs) before and after surgery or chemotherapy in gastric cancer patients.

METHODS
Twenty-nine stomach cancer patients undergoing chemotherapy after a D2 gastrectomy provided 10 mL peripheral blood samples at each phase of the perioperative period and during chemotherapy. This study also included 29 age-matched healthy donors as a control group. PD-1 expression was detected on
lymphocytes, including CD4+CD8−CD45RO+, CD4+CD45RO+, and CD8+CD45RO+ lymphocytes as well as regulatory T cells.

RESULTS
We observed a significant increase of PD-1 expression on immune subsets and a larger number of FoxP3+ Tregs in gastric cancer patients (P < 0.05). Following D2 gastrectomy, peripheral lymphocytes PD-1 expression and the number of FoxP3+ Tregs notably decrease (P < 0.05). However, during postoperative chemotherapy, we only observed a decrease in PD-1 expression on lymphocytes in the CD8+CD45RO+ and CD8+CD45RO− populations. Additionally, linear correlation analysis indicated a positive correlation between PD-1 expression and the number of CD4+CD45RO−FoxP3int+ activated Tregs (aTregs) on the total peripheral lymphocytes (r = 0.5622, P < 0.0001).

CONCLUSION
The observed alterations in PD-1 expression and the activation of regulatory T cells during gastric cancer treatment may offer novel insights for future investigations into tumor immune evasion and the clinical application of anti-PD-1 antibodies in gastric cancer.

Key Words: Programmed death 1; Active regulatory T cells; Stomach cancer; Peripheral lymphocyte

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Core Tip: In short, this paper shows that programmed death 1 (PD-1) expression on immune subsets and the number of FoxP3+ Treg were higher in peripheral blood of patients with gastric cancer than healthy donors. PD-1 expression and the number of FoxP3+ Treg decrease notably after D2 gastrectomy. PD-1 expression declines on lymphocytes, CD8+, CD45RO− and CD8+CD45RO− populations during postoperative chemotherapy. PD-1 expression correlates with the number of CD4+CD45RO−FoxP3 high activated Treg in peripheral lymphocytes. This paper is particularly timely, as the studies of PD-1 expression on immune subsets in peripheral blood are of expanding interest. As well as providing some novel insight for future studies of tumor recurrence and tumor immune escape, our results might also be a reference to determining the timing and dose of clinical anti-PD-1 antibodies, and we anticipate that this study will be widely cited.

INTRODUCTION
Global cancer statistics have revealed that there were 1089103 newly diagnosed gastric cancer cases in 2020, resulting in a significant number of newly diagnosed gastric cancer cases and deaths worldwide[1]. Specifically, the National Cancer Centre of China estimated that 679000 new gastric cancer cases and 498000 gastric cancer-related deaths would occur in China in 2015[2]. Further compounding this issue is the fact that almost 70% of newly diagnosed cases are advanced gastric cancer, with the overall five-year survival rate being less than 30%[3].

With the advances of Chimeric antigen receptor T-cells, genetically engineered T cells, cytotoxic T-lymphocyte-associated antigen-4, and the programmed death-1/programmed death-ligand (PD-1/PD-L) pathway, tumor immunotherapy has rapidly emerged as a field of advanced research[4].

Multiple clinical trials have confirmed the efficacy and safety of PD-1 monoclonal antibody[5,6], and PD-1 monoclonal antibody was already approved for advanced gastric cancer in the United States and Japan. However, a recently randomized and open Phase III trial, Keymat-61[7], showed no significant improvement on objective response rate or progression-free survival (PFS) from paclitaxel. Meanwhile, in the randomized III trials, JAVELIN Gastric 300, reported by Bang et al[8], Avelumab in the treatment of advanced gastric/gastroesophageal joint cancer patients did not improve OS and PFS compared with the third-line treatment.

A growing body of evidence suggests that Tregs might be involved in the treatment of PD-1/PD-L1 blockade and PD-1/PD-L1 axis could influence Treg differentiation and function. However, the complex relationship between PD-1/PD-L1 pathway and Tregs is yet to be fully elucidated[9,10].

Although research into peripheral PD-1 expression in patients with gastric cancer has been reported previously, few studies have sequentially investigated such expression from the time of surgery to the completion of postoperative chemotherapy[11]. Furthermore, studies investigating the relationship between peripheral PD-1 expression and FoxP3+ Tregs in gastric cancer patients are largely lacking.

In this study, we detected PD-1 expression on peripheral blood T cell subsets and the population of FoxP3+ Tregs in gastric cancer from surgery to the completion of postoperative chemotherapy to analyse how these indexes change in expectation of being helpful to decide when and how the anti-PD-1 antibodies should be applied and deeper unnder-
standing on the mechanism of tumor immune escape in gastric cancer in the future.

MATERIALS AND METHODS

Human subjects
Patients with a histologically confirmed diagnosis of gastric adenocarcinoma following a D2 gastrectomy and treated with postoperative adjuvant chemotherapy, as well as age-matched healthy donors were eligible for this prospective observational study. Other inclusion criteria consisted of: (1) A healthy physical examination of the donors performed within the past three months; and (2) the age of patients and donors within the range of 18-75 years old. The exclusion criteria consisted of: (1) Patients diagnosed with other types of cancer within five years; (2) patients that received preoperative adjuvant chemotherapy, radiotherapy, or immunotherapy; (3) patients and donors who were diagnosed with chronic hepatitis, human immunodeficiency virus, syphilis, or any other acute infectious disease; (4) patients and donors who suffered from rheumaimmune systemic diseases (e.g., systemic lupus erythematosus or hyperthyroidism); (5) patients with any other severe disease that might render them incapable of completing the entire course of chemotherapy; and (6) patients suffered gastric cancer recurrence or failed to finish the entire course of chemotherapy.

At the beginning of this study, 33 patients (alongside 33 age-matched donors) were enrolled from February 2020 to February 2021 in the Chinese People’s Liberation Army General Hospital (PLAGH). However, one patient experienced tumor recurrence and three who failed to complete the entire cycles were excluded. A total of 29 patients (15 men and 14 women; mean age: 59.72 years) and 29 age-matched donors (17 men and 12 women; mean age: 59.62 years) were ultimately included in this study.

This study received approval from the ethics commission of the General Hospital of PLA, and all patients and donors provided signed informed consent.

Treatment strategy for patients
The treatment strategy for the enrolled patients consisted of MDT and a D2 gastrectomy performed by a chief physician at the General Hospital of PLA. The patients were treated with the following doses of chemotherapy and affiliated schedules: Oral capecitabine (1000 mg/m² twice daily on days 1-14 of each cycle) plus intravenous oxaliplatin (130 mg/m² on day 1 of each cycle) for eight three-week cycles.

Flow cytometry analysis
A sample volume of 10 mL peripheral blood was obtained from the patients into an anticoagulation tube on the day before surgery, the first and fourth cycles of chemotherapy and the day after chemotherapy. All blood samples were analysed within 6 h. Each sample was divided into two tubes and the detection of PD-1 expression and CD4⁺CD25⁺ FoxP3⁺ regulatory T cells was performed separately on Accuri C6, a four-channel flow cytometer form BD ACCURI (Franklin Lakes, NJ, United States).

The detection of PD-1 expression involved staining the cells from the whole blood with fluorescently labelled antibodies, including anti-CD4-FerCP-Cy5.5 (clone RPA-4), anti-CD8-FITC (clone RPA-T8), anti-CD45RO-PE (clone UCHL-1) and anti-CD279 (PD-1)-APC (clone MIH4), from BD Biosciences (Franklin Lakes, NJ, United States) for the detection of PD-1 expression.

The detection of CD4⁺CD25⁺FoxP3⁺ regulatory T cells was performed by staining PBMCs with anti-CD3-APC (clone SP34-2), anti-CD4-Percp (clone RPA-T4), anti-CD25-FITC (clone M-A251) and anti-CD45RO-PE (clone UCHL-1) from BD Biosciences (Franklin Lakes, NJ, United States) first, followed by anti-FoxP3-PE (clone 236A/E7, BD Biosciences) was added after permeabilizing the cell and nuclear membranes.

Statistical analysis
Data in this paper is represented as the mean ± SD. Comparisons of the differences in the continuous variables between the patients and donors were made using a Student’s t-test or Wilcoxon rank sum test. A Chi-square test was performed for the categorical data. A paired-t test was adopted to compare the measurement data before and after the operation. Changes in the measurement data during chemotherapy were evaluated by an analysis of variance repeated-measures function. A Pearson correlation analysis was applied to dispose of the relativity between PD-1 expression and a population of FoxP3⁺ Tregs. P values were based on two-tailed tests, with a value of $P < 0.05$ considered to be statistically significant.

RESULTS

Human characteristics
Statistical analysis revealed no significant difference in age (59.72 ± 15.33 vs 59.62 ± 15.64, $P = 0.9798$), gender (15/14 vs 17/12, $P = 0.7918$), and body mass index (23.90 ± 5.00 vs 25.10 ± 3.18, $P = 0.2824$) between the patients and healthy donors. We also assessed the daily life activity of both patients and donors using the Karnofsky score; all patients scored above 60 (indicating occasional care required for most needs), which is a score above the threshold at which patients with advanced gastric cancer are advised to transition from systemic therapy to supportive therapy according to the NCCN Guidelines. The descriptive statistics for CEA levels, TNM stages, and tumor differentiation are detailed in Table 1.
**Table 1** Characteristics of patients and donors

<table>
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<th>Patients (n = 29)</th>
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<td>Age (mean ± SD)</td>
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*The donors exhibited no differences from the patients at \( P > 0.05.\)

BMI: Body mass index; CEA: Carcinoembryonic antigen; KPs: Karnofsky performance status.

**PD-1 expression between preoperative patients and donors**

We performed an analysis of PD-1 expression on fresh peripheral blood sample subsets using flow cytometry (Figure 1A). The PD-1 expression on lymphocytes obtained from patients was significantly higher than those derived from donors (22.37% ± 10.35% vs 11.77% ± 6.67%, \( P = 0.0001; \) Figure 1B-G). Similarly, PD-1 expression on the CD4⁺ and CD8⁺ lymphocytes was notably higher in the patient group compared to the donor group (7.10% ± 3.27% vs 4.18% ± 2.53%, \( P = 0.0004; 11.63% ± 7.06% vs 5.71% ± 3.74%, \( P = 0.001; \) Figure 1B-G). Further, we assessed the PD-1 expression in lymphocytes through CD45RO phenotyping, a crucial marker utilized to distinguish memory T cells from naive T cells. This analysis revealed a significant difference between the patients and donors (13.94% ± 6.75% vs 5.16% ± 2.31%, \( P < 0.0001; \) Figure 1B-G). Moreover, PD-1 expression on CD4⁺CD45RO⁺ and CD8⁺CD45RO⁺ lymphocytes was also markedly higher in the gastric cancer patients (5.16% ± 2.31% vs 2.67% ± 1.57%, \( P < 0.0001; 8.79% ± 5.15% vs 4.05% ± 2.67%, \( P < 0.0001; \) Figure 1B-G).

**PD-1 expression before and after the D2 gastrectomy**

In this part, we detected the expression of PD-1 on the lymphocytes from blood samples drawn from postoperative patients. Blood samples were drawn on average of 27.24 d ± 5.06 d (range: 19-35 d) following surgery. A paired-t test was applied to compare the frequency of PD-1 on lymphocytes between the preoperative and operative blood samples. Strikingly, we found that surgery might be able to reduce the level of PD-1 expression on T cells. There was a significant decline in the PD-1 expression on lymphocytes from postoperative peripheral blood (22.37% ± 10.35% vs 16.00% ± 6.29%, \( P = 0.0001; \) Figure 2). A significant decrease was also observed for the level of PD-1 expression on CD4⁺ and CD8⁺ lymphocytes (7.10% ± 3.27% vs 5.26% ± 2.62%, \( P = 0.0008; 11.63% ± 7.06% vs 8.05% ± 3.60%, \( P = 0.001; \) Figure 2). The frequency of PD-1 expression on CD4⁺CD45RO⁺ and CD8⁺CD45RO⁺ lymphocytes from postoperative patients was also significantly lower than that derived from preoperative patients (5.16% ± 2.31% vs 3.67% ± 1.80%, \( P = 0.0085; 8.79% ± 5.15% vs 6.19% ± 3.20%, \( P < 0.0001; \) Figure 2). Similarly, the PD-1 expression on CD45RO⁺ lymphocytes was also significantly lower (13.94% ± 6.75% vs 9.86% ± 4.41%, \( P < 0.0001; \) Figure 2). Since the increase of PD-1 expression is dependent on antigen stimulation, one explanation for the observed results might be the lack of stimulation from tumor antigens following surgery.

**PD-1 expression during chemotherapy**

A total of 29 patients in the trial accepted an eight three-week cycles course of chemotherapy with oxaliplatin and capecitabine following a D2 gastrectomy. We drew peripheral blood samples on the day before the first cycle of chemotherapy (an average of 27.24 d ± 5.06 d after surgery), fourth cycle of chemotherapy (an average of 97.90 d ± 6.64 d after surgery) and the day after the eighth cycle of chemotherapy (an average of 171.14 d ± 8.73 d after surgery). There was a statistically significant decrease in PD-1 expression on the total lymphocytes (16.00% ± 6.29%, 13.62% ± 6.43% vs 13.33% ± 6.35%, \( P = 0.031; \) Figure 3) and CD8⁺ T cells (8.05% ± 3.60%, 6.39% ± 3.59% vs 6.56% ± 3.64%, \( P = 0.009; \) Figure 3).
Figure 1 Programmed death 1 expression on all T cell subsets of healthy donors and preoperative patients with gastric cancer. A: The scatter and histogram plots of one representative patient are illustrated. The relationships between the diagrams are indicated by arrows and gates (R1, R2, and R3); B-G: The percentages of total programmed death 1 (PD-1) cells (B), CD4⁺PD-1⁺ (C), CD8⁺PD-1⁺ (D), CD45RO⁺PD-1⁺ (E), CD4⁺CD45RO⁺PD-1⁺ (F), and CD8⁺CD45RO⁺PD-1⁺ (G) cells out of the total peripheral lymphocytes from patients and healthy donors are shown. The P value was calculated using a Student’s t-test or Wilcoxon rank sum test.
Li H et al. Peripheral PD-1 expression and FoxP3 + Tregs

Figure 2 Changes in programmed death 1 expression on all subsets before and after the D2 gastrectomy. A-F: The percentage of total programmed death 1 (PD-1+) cells (A), CD4+PD-1+ (B), CD8+PD-1+ (C), CD45RO+PD-1+ (D), CD4+CD45RO+PD-1+ (E), and CD8+CD45RO+PD-1+ (F) cells of the peripheral lymphocytes derived from patients before and after the D2 gastrectomy are shown. The P value was calculated using an analysis of variance repeated-measures function.

Figure 3 Changes in programmed death 1 expression on all subsets during chemotherapy. A-F: The percentage of total programmed death 1 (PD-1+) cells (A), CD4+PD-1+ (B), CD8+PD-1+ (C), CD45RO+PD-1+ (D), CD4+CD45RO+PD-1+ (E), and CD8+CD45RO+PD-1+ (F) cells of the peripheral lymphocytes derived from patients during chemotherapy are shown. The P value was calculated using an analysis of variance repeated-measures function.

A notably significant decline was also observed for the PD-1 expression on CD45RO+ lymphocytes (9.86% ± 4.41%, 6.48% ± 3.28% vs 7.71% ± 4.07%, P < 0.0001; Figure 3) and CD8+CD45RO+ lymphocytes (6.19% ± 3.20%, 3.86% ± 2.69% vs 4.44% ± 2.61%, P < 0.0001; Figure 3). However, the difference in PD-1 expression on CD4+ lymphocytes (5.26% ± 2.62%, 4.98% ± 2.40% vs 4.60% ± 2.15%, P = 0.276; Figure 3) and CD4+CD45RO+ lymphocytes (3.67% ± 1.80%, 3.23% ± 1.64% vs 3.27% ± 1.74%, P = 0.308; Figure 3) was not statistically significant.

Changes in FoxP3+ Tregs in patients with gastric cancer

While analyzing the PD-1 expression on lymphocytes, we also detected the frequency of CD4+CD25+FoxP3+ T cells and CD4+CD45RO+FoxP3high T cellsin the lymphocyte population (Figure 4A). The frequency of FoxP3+ T cells and CD4+CD45RO+FoxP3high T cellswas higher in the patients than in the healthy donors (1.76% ± 0.59% vs 0.87% ± 0.56%, P < 0.0001; 0.92% ± 0.45% vs 0.33% ± 0.27%, P < 0.0001; Figure 4B-C) and declined after the D2 gastrectomy was performed (1.76% ± 0.59% vs 1.26% ± 0.62%, P = 0.0004; 0.92% ± 0.45% vs 0.59% ± 0.40%, P = 0.0005; Figure 4B-C). Statistical difference was also observed in the frequency of CD4+CD45RO+FoxP3high T cells in the peripheral lymphocytes during
Li H et al. Peripheral PD-1 expression and FoxP3+ Tregs

Figure 4 Treg and activated Treg cells in the peripheral blood of donors and patients. A: Scatter plots are shown from one representative patient. Relationships of the diagrams are denoted with arrows and gates; B-G: The percent of CD4+CD25+FoxP3+ and CD4+CD45RO+FoxP3high cells in the peripheral lymphocytes from the donors and patients during treatment were shown and the P value was calculated using an analysis of variance repeated-measures function.

Chemotherapy (0.59% ± 0.40%, 0.46% ± 0.29% vs 0.37% ± 0.25%, P = 0.025; Figure 4B-G).

Relativity between PD-1 expression and FoxP3+ Tregs

Previous research has suggested that PD-1+ T cells and Tregs are correlative in the tumor tissues and tumor-involved lymph nodes in both breast and papillary thyroid cancer[12,13]. However, no clear correlations between the frequency of PD-1 expression and the number of CD4+CD25+FoxP3+ T cells observed in the peripheral blood of preoperative patients (r = 0.4008, P < 0.0001; Figure 5) but significant correlation between CD4+CD45RO+FoxP3high T cells and PD-1 expression (r = 0.5622, P < 0.0001; Figure 5) were observed in our study.

DISCUSSION

Studies have shown that the PD-1/PD-L1 pathway plays a critical role in promoting T cell exhaustion[14]. In many types of tumor tissues, overexpression of PD-L1 has been found, with a similar pattern of PD-1 overexpression observed on tumor-infiltrating lymphocytes[15,16].

Supported by T-helper cells, CD8+ T cells can be activated, leading to the destruction of tumor cells through the perforin/granzyme and Fas/Fas ligand (FasL) apoptosis pathways. However, tumor cells have been found to inhibit the function of CD4+ and CD8+ T cells by boosting PD-1 expression[17,18]. This scenario has been observed in the peripheral
blood of various cancer types, such as non-small cell lung cancers, actinic cheilitis, oral squamous cell carcinoma, and head and neck cancer.[19-22]

Our analysis unveiled a notable increase in PD-1 expression on CD45RO^+ lymphocytes in gastric cancer. CD45RO is a surface antigen employed to differentiate memory T cells from naive T cells. When antigens are reintroduced, CD4^+ CD45RO^+ T cells are seen to respond rapidly and robustly, migrating to the antigen source and aiding B cells in antibody production[23,24].

Several studies have suggested that CD8^+ CD45RO^+ T cells serve as a more effective independent prognostic factor for metastatic colorectal cancer in Cox regression multivariate analysis compared to traditional markers like CEA and LDH [25-27]. Given this evidence, it seems likely that the upregulation of PD-1 on CD45RO^+ T cells is a consequence of tumor cell stimulation, thereby facilitating tumor immune escape from memory T cells.

PD-1/PD-L1 expression can be induced or maintained by many cytokines, such as type I IFN and IFNγ. Research has shown that tumor-associated plasmacytoid DCs produce large amounts of type I IFN[28], which can in turn induce PD-1/PD-L1 expression[29]. The decline in these cytokines following tumor resection might be the underlying cause of the observed reduction in PD-1 expression.

Research conducted by Maeda et al[30] found that the populations of CD4^-, CD8^+ and NK cells in the peripheral blood of patients with metastatic colorectal cancer remained stable following FOLFOX administration, yet the number of regulatory cells exhibited a significant decline. A similar drop in the Tregs population was observed in patients treated with paclitaxel-based chemotherapy[31]. Data from our present study reveal a trend of significant decline or reduction in PD-1 expression and population of FoxP3^+ Tregs in patients undergoing surgery and chemotherapy.

We observed that the population of regulatory T cells was higher in patients compared to donors, which is consistent with prior findings in prostate, lung, pancreatic, and breast cancer studies[32,33]. The increased population of Tregs in tumor-bearing patients could potentially arise from the secretion of TGF-β, IL-10, and H-ferritin, which have been known to induce CD4^+ CD25^+ T cells to transition into CD4^+ CD25^hi T cells and upregulate the expression of FoxP3[34-36].

Interestingly, this mechanism might also be responsible for the observed decrease in the number of peripheral Tregs following tumor tissue removal. Moreover, several studies have indicated that drugs like cyclophosphamide, fludarabine, and paclitaxel could down-regulate the quantity and function of Tregs in cancer patients[37,38].

These findings provide a theoretical foundation for the treatment of tumors with PD-1/PDL-1 blockers in combination with chemotherapy drugs, offering a potential strategy to optimize cancer immunotherapy.

In this study, CD4^+ CD45RO^+ FoxP3^+ appears to have a stronger correlation to PD-1 expression than Tregs in peripheral blood. Previous detection of FoxP3 at both the mRNA and protein levels has shown that human CD25^{hi}CD4^+ T cells indeed express FoxP3[39,40], indicating that the population of CD4^+ CD45RO^+ FoxP3^+ cells can serve as a reflection of CD25^{hi}CD45RA FoxP3^{hi} activated Treg cells (aTregs).

Moreover, the CD4^+ FoxP3^+ T cells in peripheral blood comprise three subpopulations, distinguished by differing levels of FoxP3 and cell surface molecules CD45RA and CD25. Notably, only CD25^{hi}CD45RA FoxP3^{hi} cells (aTregs) are terminally differentiated and exhibit high suppressive capacities[41].

CONCLUSION

The exploration of PD-1 expression impacts on immune subsets and the abundance of FoxP3^+ Tregs in peripheral blood could provide invaluable insights for future research on the PD-1/PDL-1 axis, tumor recurrence, and tumor immune escape. Additionally, these findings may also serve as potential biomarkers for studies involving the timing and dosing of clinical anti-PD-1 antibodies.
ARTICLE HIGHLIGHTS

Research background
Programmed death 1 (PD-1) and CD4+CD25+FoxP3+ expression in peripheral blood T-cells have been identified in multiple cancer types, but their variation during surgery and chemotherapy in gastric cancer remains elusive. Understanding this could illuminate tumor recurrence mechanisms and guide optimal anti-PD-1 antibody treatment strategies.

Research motivation
Despite known PD-1 and CD4+CD25+FoxP3+ expression in various cancers, the specific changes during surgery and chemotherapy, and their relationship in gastric cancer, remain undefined. This study seeks to shed light on these variations, potentially offering insights into tumor recurrence, immune evasion, and the clinical application of anti-PD-1 antibodies in gastric cancer.

Research objectives
The study aims to observe and analyze the expression characteristics of peripheral lymphocyte PD-1 and FoxP3+ regulatory T cells (FoxP3+Tregs) in gastric cancer patients, both prior to and following surgery or chemotherapy, to better understand their roles and implications in gastric cancer treatment.

Research methods
In this study, 29 gastric cancer patients, post-D2 gastrectomy and undergoing chemotherapy, provided 10 mL peripheral blood samples during various perioperative phases. PD-1 expression was analyzed on specific lymphocyte subsets, with an additional 29 age-matched healthy donors serving as a control group.

Research results
The study found a significant elevation in PD-1 expression and FoxP3+ Tregs in gastric cancer patients, which decreased notably post-D2 gastrectomy. A positive correlation was identified between PD-1 expression and the number of activated FoxP3high Tregs in peripheral lymphocytes, especially during postoperative chemotherapy.

Research conclusions
Alterations in PD-1 expression and regulatory T cell activation during gastric cancer treatment could provide valuable insights for understanding tumor immune evasion. These findings may also influence the clinical application of anti-PD-1 antibodies in gastric cancer therapy.

Research perspectives
The changes observed in PD-1 expression and regulatory T cell activation during gastric cancer treatments pave the way for deeper exploration into tumor immune evasion mechanisms. These findings could also shape the future clinical application and optimization of anti-PD-1 antibodies in treating gastric cancer.

FOOTNOTES

Co-first authors: Hao Li and Guan-Mei Cao.

Author contributions: Li H, Cao GM, and Du XH were the guarantor of integrity of entire study, and contributed to the study concepts; Li H, Cao GM, Gu GL, Li SY, and Du XH designed the study; Li H, Cao GM, Gu GL, and Li SY involved in the literature research; Li H and Cao GM contributed to the data acquisition; Li H, Cao GM, and Fu Z contributed to the statistical analysis/interpretation and manuscript preparation; Li H, Cao GM, Gu GL, Li SY, Fu Z, and Du XH contributed to the manuscript definition of intellectual content; Li H, Cao GM, Gu GL, and Du XH edited the manuscript; Li H and Cao GM contributed equally to this work as co-first authors; Li H and Cao GM are designated as co-first authors due to their equal and substantial contributions to the study conception, design, data acquisition, and analysis, as well as manuscript preparation and editing, each playing pivotal roles in ensuring the integrity and quality of the research.

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Informed consent statement: All patients and donors provided signed informed consent.

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**REFERENCES**


Advances and key focus areas in gastric cancer immunotherapy: A comprehensive scientometric and clinical trial review (1999-2023)

Yao-Nan Li, Bin Xie, Ying Zhang, Ming-Hua He, Yang Xing, Dong-Mei Mu, Hong Wang, Rui Guo

BACKGROUND
Gastric cancer (GC) is the sixth most common cancer and third leading cause of cancer-related deaths worldwide. Current treatments mainly rely on surgery- and chemotherapy-based systemic; however, the prognosis remains poor for advanced disease. Recent studies have suggested that immunotherapy has significant potential in cancer therapy; thus, GC immunotherapy may improve quality of life and survival for patients with this disease.

AIM
To provide a comprehensive overview of the knowledge structure and research hotspots of GC immunotherapy.

METHODS
We conducted a bibliometric analysis of publications on immunotherapy related to GC in the Web of Science Core Collection database. We analyzed 2013 publications from 1999 to February 1, 2023, using the VOSviewer and CiteSpace software. We assessed publication and citation distributions using the WoS platform and explored research countries, institutions, journals, authors, references, and keywords (co-occurrence, timeline view, and burst analysis). In addition, we examined 228 trials on immunotherapy, 137 on adoptive cell therapy, 274 on immune checkpoint inhibitors (ICIs), and 23 on vaccines from ClinicalTrials.gov and the International Clinical Trials Registry Platform. The
Impact Index Per Article for the top ten high-cited papers collected from Reference Citation Analysis (RCA) are presented.

RESULTS
Our bibliometric analysis revealed that the study of immunotherapy in GC has developed rapidly in recent years. China accounted for almost half the publications, followed by the United States. The number of publications in recent years has been growing continuously, and most institutions and authors with the most publications are from China. The main keywords or clusters identified were “tumor microenvironment”, “adoptive immunotherapy”, “dendritic therapy”, and “microsatellite instability”.

CONCLUSION
Our analysis of 2013 publications indicated that immunotherapy for GC has led to several new developments in recent years. Considerable progress has been made in vaccinations, immune checkpoint therapy, and adoptive cellular therapy. In particular, ICIs and chimeric antigen receptor T-cells are novel options for the treatment of GC. We suggest that the combination of ICIs, chemotherapy, targeted therapy, and other immunotherapies should be the primary research direction in the future.

Key Words: Immunotherapy; Gastric cancer; Clinical trials; Scientometric analysis; Visualization

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Core Tip: In this study, we systematically analyzed studies related to immunotherapy for gastric cancer (GC). We used scientometrics to explore research hotspots in the field and summarize the current developmental status of GC immunotherapy, as well as the advantages and disadvantages of different immunotherapy modalities. We also compiled information on ongoing clinical trials and predicted future developmental trends in this field based on the direction and stage of these trials. This research can help advance our understanding of the latest progress and future development trends in the field, as well as provide scientific research recommendations.

URL: https://www.wjgnet.com/1007-9327/full/v29/i40/5593.htm
DOI: https://dx.doi.org/10.3748/wjg.v29.i40.5593

INTRODUCTION
Gastric cancer (GC) is the sixth most prevalent cancer and the third leading cause of cancer-related fatalities worldwide, thus presenting a significant global health concern[1]. The incidence rates of GC are highest in Eastern Europe and Eastern Asia, and it remains the most prevalent cancer and the leading cause of cancer-related deaths in specific regions of South and Central Asia[2]. Despite a trend toward decreasing morbidity and mortality rates in several countries and regions, GC continues to be a substantial health burden[3]. Numerous factors have been established to contribute to the pathogenesis of GC, including family history, dietary habits, smoking, and Helicobacter pylori or Epstein-Barr virus (EBV) infection[4]. GC can be categorized into four distinct molecular subgroups based on the patterns of molecular alterations, with each subgroup corresponding to a different disease progression and prognosis: (1) Microsatellite stable/epithelial-mesenchymal transition; (2) Microsatellite instability (MSI); (3) Tumor protein 53 (TP53)-active; and (4) TP53-inactive types[5].

Owing to its predominantly asymptomatic nature, early detection of GC poses a significant challenge, with > 50% of patients being diagnosed after the cancer has already metastasized[6,7]. The prognosis of advanced GC remains dismal; the five-year overall survival (OS) rate is < 5% and the median OS is approximately 8 mo[8-10]. Although surgery remains the primary curative approach, chemotherapy forms the foundation of treatment for metastatic GC, with multimodal therapy employed to enhance survival outcomes[9]. However, the efficacy of conventional treatments, including surgery, radiotherapy, chemotherapy, and anti-human epidermal growth factor receptor-2 (HER2) therapy, in combating this lethal disease[11].

Surgery, chemotherapy, radiation therapy, and targeted therapy have long been considered the four pillars of GC management; however, immunotherapy has recently emerged as a promising “fifth pillar”, and its use is rapidly expanding[12]. For cases of unresectable locally advanced, recurrent, or metastatic GC, a combination therapy of anti-HER2, chemotherapy, and optional pembrolizumab is preferred for HER2-positive diseases, with the inclusion of pembrolizumab demonstrating a high objective response rate (ORR)[13]. Irrespective of HER2 status, nivolumab [for programmed death-ligand 1 (PD-L1) CPS ≥ 5] is recommended as part of systemic treatment regimens[14]. Currently, there are four principal strategies for tumor immunotherapy: Immune checkpoint inhibitors (ICIs), tumor vaccines,
adoptive immunotherapy, and nonspecific immunomodulators. With advancements in the understanding of the tumor microenvironment (TME), immunotherapy for advanced GC has evolved rapidly, demonstrating superior efficacy and tolerable toxicity compared to traditional therapies, leading to the rapidly increasing use of ICIs[7]. As most GCs are relatively resistant to ICI monotherapy, patients may benefit from combination therapy to achieve enhanced therapeutic effects[15]. Multiple studies have demonstrated that immunotherapy combined with conventional therapy provides superior efficacy compared with monotherapy[16]. Although immunotherapy holds promise for the treatment of GC, the complexity of the immune microenvironment and the heterogeneity of immunogenecity present significant challenges that require further investigation[17].

Chemotherapies for patients with locally advanced or metastatic GC include S-1 + oxaliplatin, docetaxel + oxaliplatin + fluorouracil, docetaxel + oxaliplatin + S-1 (DOS), capecitabine + oxaliplatin (XELOX), and folinic acid/5-fluorouracil/oxaliplatin chemotherapy (FOLFOX). Trastuzumab or pembrolizumab should be added to first-line chemotherapy for patients with HER2 overexpression-positive GC[18,19]. There are two commonly used combinations: Trastuzumab combined with a fluoropyrimidine and a platinum agent and a combination of trastuzumab, pembrolizumab, and XELOX/PF. Regimens for HER2-negative disease include nivolumab, cindilimab, and tislelizumab combined with first-line chemotherapy[20]. Docetaxel, cisplatin, 5-fluorouracil (DCF), modified DCF, and PFO also exhibit promising activity. The selection of regimens for second-line or subsequent therapy depends on prior therapy and performance status. Ramucirumab combined with paclitaxel is the preferred second-line or subsequent therapy[19]. Single-agent docetaxel, paclitaex, irinotecan, albumin-paclitaxel, pembrolizumab, nivolumab, vedicitumab, and apatinib mesylate have also been used as second-line or subsequent therapies. Although there is currently a relatively complete treatment plan, the OS of patients remains short. Therefore, more effective treatment options are required. Based on traditional treatment, explore combination therapy with immunotherapy, subdivide the population, and improve curative effects.

Recent phase I/II trials focusing on the perioperative use of ICIs in combination with chemotherapy for resectable locally advanced gastric/gastroesophageal junction cancer (GC/GEJC) have yielded positive results, thus expanding the potential applications of ICIs in GC management[21]. The addition of sintilimab to chemotherapy has demonstrated encouraging pathological complete response (pCR) and major pathological response rates as a perioperative treatment for resectable locally advanced GC/GEJC with manageable safety profiles. In a phase II study, durvalumab combined with DOS as neoadjuvant chemotherapy reached its primary efficacy endpoint, confirming a pCR in 29.0% of the patients (9 of 31) with acceptable toxicity (safety endpoint < 20%)[22]. In a separate multicenter, single-arm, multi-cohort, phase II trial of tremelimumab and durvalumab as neoadjuvant treatments in patients with MSI-high (MSI-H) resectable gastric adenocarcinoma/gastroesophageal junction adenocarcinoma (GAC/GEJAC; NCT04817826), a pCR rate of 60% (9 of 15) and a major-complete pathological response rate of 18% (<10% viable cells) were achieved[23].

Bibliometrics is a methodological approach that encompasses the analysis and summary of data produced within a specific timeframe. It offers invaluable insights into scientific productivity, behavior, and advancements within the research domain[24]. The objective of this study was to employ bibliometric analysis to elucidate the current status and emergent trends in the field of GC immunotherapy research.

MATERIALS AND METHODS

This analysis was conducted on February 1, 2023, and searched the Web of Science Core Collection (WoSCC) and Science Citation Index Expanded. The retrieval terms in the topic: (“gastric cancer” OR “gastric adenocarcinoma” OR “gastric neoplasm” OR “gastric tumor” OR “stomach cancer” OR “stomach adenocarcinoma” OR “stomach neoplasm” OR “stomach tumor” OR “gastric cancers” OR “gastric adenocarcinoma” OR “gastric neoplasms” OR “gastric tumors” OR “stomach cancers” OR “stomach adenocarcinoma” OR “stomach neoplasms” OR “stomach tumors” OR “tumor of stomach”) AND (“immunotherapeutic” OR “immunotherapy” OR “immunotherapies” OR “immunotherapeutics”).

We searched ClinicalTrials.gov and the International Clinical Trials Registry Platform (ICTRP) (clinical). The retrieval terms: (“gastric cancer” OR “gastric adenocarcinoma” OR “gastric neoplasm” OR “gastric tumor” OR “stomach cancer” OR “stomach adenocarcinoma” OR “stomach neoplasm” OR “stomach tumor”) AND (immunotherapy)”. There were 228 registered clinical trials, 25 of which were completed, and 113 were recruiting or not yet recruiting. We searched for some of the main immunotherapies in clinical trials using these two platforms. The research strategy: (“dendritic cells” OR “DNA vaccine” OR “RNA vaccine”) AND “gastric cancer” for vaccine clinical trials; (ACT OR TIL OR TCR-T OR CAR-T OR TCR-T OR CAR-T OR NK OR CIK) AND “gastric cancer” for ACT clinical trials; (ICI OR PD-1 OR PD-L1 OR CTLA-4) AND “gastric cancer” for ICI clinical trials. In total, 274, 137, and 23 clinical trials were incorporated, respectively. These retrievals were conducted on February 19, 2023.

We used CiteSpace (6.1.6) and VOSviewer (1.6.18) to analyze the data. Plain Text Files were analyzed using CiteSpace, and Tab Delimited Files were analyzed using the VOSviewer. CiteSpace made time slicings for the original files from January 1999 to December 2023 with 1 year per slice, and qualified records from 2012 were analyzed. The literature search and screening processes are illustrated in Figure 1. The Citation Report of WoS on February 1, 2023, provided the number of publications and citations annually. The figures used in this study were mapped using CiteSpace, VOSviewer, and Excel.
RESULTS

Bibliometric analysis

Annual distribution of publications and citations: The spatial-temporal distribution of scholarly publications has been demonstrating a noteworthy ascending trajectory. As per the WoSCC, the 2013 documents collectively garnered 45700 citations, averaging 22.7 citations per document until February 1, 2023. The H-index was 86, indicating that 86 documents each received over 86 citations. Figure 2A illustrates the annual distribution of publications and citations. Only 22 publications were released in 1999, with the number fluctuating between 9 and 42 from 1999 to 2015. However, starting in 2016, the number of publications experienced steady and significant growth, reaching 552 in 2022. Although there were only seven citations in 1999, this figure escalated to 11382 in 2022, marking 23 years of consistent growth. We adopted the regression model $y = 0.0012x^5 - 0.0624x^4 + 1.151x^3 - 9.1536x^2 + 29.142x - 10.867$ ($R^2 = 0.9928$) to show how the number of publications in this field changed over time and forecast it in the following year.

Related countries and institutions: The CiteSpace analysis revealed that contributions to publications in this field originated from 72 countries and 617 institutions, as depicted in Figures 2B and C. Of these, 26 countries and 58 institutions contributed ten or more publications. China had the highest number of publications ($n = 1070$), representing 53.2% of the total, which was significantly higher than that of other countries. The United States was second with 321 publications (16.0%), followed by Japan ($n = 227$, 11.3%), Germany ($n = 125$, 6.2%), and Italy ($n = 109$, 5.4%). The top ten institutions with the highest number of publications were located in China, five of which were Fudan University ($n = 85$), Nanjing Medical University ($n = 64$), Shanghai Jiao Tong University ($n = 64$), Sun Yat-sen University ($n = 52$), and Zhengzhou University ($n = 43$).

Journals: The 2013 analyzed documents were disseminated across 532 journals, with those comprising at least five documents incorporated into Figure 3A using the VOSviewer. The nodes exhibiting high brightness in this figure denote a higher frequency of occurrence. Frontiers in Oncology led the list with 104 documents, followed by Frontiers in Immunotherapy ($n = 78$) and Cancers ($n = 69$). Additionally, we conducted a co-citation analysis of the cited journals. These articles cited 5445 journals, and we analyzed 102 journals that received at least 200 citations (Figure 3B). The Journal of Clinical Oncology garnered the highest number of citations ($n = 5044$), followed by Cancer Research, with 3018 citations.

Authors: After excluding documents coauthored by more than 25 authors, we identified 11730 authors across the documents. Only authors with at least five publications were considered, resulting in an analysis of 212 authors who fulfilled the criteria (Figure 4A). In the VOSviewer analysis, Lin Shen had the highest number of publications (525 citations across 25 documents), followed by Hao Liu coming with the second highest number (444 citations across 24 documents). Regarding citations, Sakamoto Junichi had the most citations (810 citations across 6 documents), followed by Xin Wang (705 citations across seven documents). Thirty authors had at least 10 publications in this field, and 17 authors garnered over 400 citations.
Figure 2 Annual distribution of publications and citations, geographical visualization, and institutions analysis. A: Distribution of publications
and citations by year. The regression formula is $y = 0.0012x^5 - 0.0624x^4 + 1.151x^3 - 9.1536x^2 + 29.142x - 10.867$, $R^2 = 0.9928$; B: Geographical visualization of publications for immunotherapy in gastric cancer; C: Co-occurrence map of the research institutions.

Figure 3 The visualization of journals. A: Journals with at least five publications in this field; B: Highly cited journals in this field.

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Figure 4 The visualization of authors. A: Network map of researching authors who had at least five publications; B: Citation analysis of references. Highly cited references are marked by author and publication year.

References: Table 1 shows the ten most frequently co-cited articles based on citation frequency data retrieved from WoS as of February 1, 2023. Seven of these articles were disseminated in the past seven years, and the majority of the highly cited documents had recent publication dates, suggesting a swift progression in this field (Figure 4B). The clinical trial spearheaded by Brahmer et al.[25], illustrating the induction of tumor regression through an antibody-mediated PD-L1 blockade, amassed 5555 citations, which is significantly more than that of all other articles. This trial, published in the New England Journal of Medicine in 2012, experienced a citation surge from 2014 to 2017, exhibiting a strength of 15.45 (Figure 5A). Two additional articles focused on programmed cell death protein 1 (PD-1) or PD-L1, a topic that continues to garner significant attention[26,27]. The second most cited article, also a clinical trial, validated the efficacy of pembrolizumab in patients with advanced triple-negative breast cancer by evaluating its safety and antitumor activity[28].
remaining articles in this table pertained to the TME, adjuvant chemotherapy, or review articles. The impact index per article of the top ten articles ranged from 30.8 to 524.5, which is also shown in Table 1.

Figure 5B presents an overlay map of journals, illustrating aspects such as the distribution, citation trajectory, and shift in the center of gravity of papers across each discipline. The label on the left represents the discipline of the cited journal, whereas the label on the right indicates the discipline of the journal in which the cited paper was published. In the figure on the left, the vertical axis of the ellipse extends as the number of papers published by a journal increases, whereas the horizontal axis increases as the number of authors increases. Our analysis revealed that most publications have been cited in journals pertaining to molecular biology, genetics, health, nursing, and medicine. Furthermore, most publications have been cited in journals pertaining to molecular biology, immunology, and medicine. The orange and green citation trajectories suggest that research journals in the molecular/biology/genetics domain garner frequent citations in the journals associated with molecular biology, genetics, health, nursing, and medicine. Furthermore, most publications have been cited in journals pertaining to molecular biology, immunology, and medicine. The orange and green citation trajectories suggest that research journals in the molecular/biology/genetics domain garner frequent citations in the journals associated with molecular biology, genetics, health, nursing, and medicine. The orange and green citation trajectories suggest that research journals in the molecular/biology/genetics domain garner frequent citations in the journals associated with molecular biology, genetics, health, nursing, and medicine. The orange and green citation trajectories suggest that research journals in the molecular/biology/genetics domain garner frequent citations in the journals associated with molecular biology, genetics, health, nursing, and medicine.

Co-occurrence analysis of keywords: Keywords play a pivotal role in encapsulating scientific research trends. To identify the most prevalent keywords, we used the VOSviewer tool for keyword co-occurrence analysis. From the 6337 keywords, we focused on 152 keywords that appeared 25 times or more (Figure 6A). “Gastric cancer” emerged as the most recurrent keyword, appearing 1257 times. The top ten most frequently occurring keywords included GC (n = 1257), immunotherapy (n = 988), expression (n = 394), chemotherapy (n = 320), prognosis (n = 253), open label (n = 206), survival (n = 190), double-blind (n = 177), and TME (n = 174).

Timeline view of keywords: Following cluster analysis, the keywords were segregated into ten clusters and subsequently subjected to a timeline view analysis in CiteSpace (Figure 6B): #0 MSI, #1 targeted therapy, #2 dendritic cells (DCs), #3 breast cancer, #4 adoptive immunotherapy, #5 TME, #6 immune infiltration, #7 GC, #8 stomach adenocar-

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**Table 1 The top ten co-cited documents**

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<tr>
<th>Title</th>
<th>Year</th>
<th>Type</th>
<th>First author</th>
<th>Journal</th>
<th>IF (2021)</th>
<th>JCR</th>
<th>Co-citation</th>
<th>DOI</th>
<th>Impact index per article</th>
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<tr>
<td>Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study</td>
<td>2016</td>
<td>Clinical trial</td>
<td>Nanda R</td>
<td>J Clin Oncol</td>
<td>50.72</td>
<td>Q1</td>
<td>1297</td>
<td>10.1200/JCO.2015.64.8931</td>
<td>133.7</td>
</tr>
<tr>
<td>Benefit of Adjuvant Chemotherapy for Resectable Gastric Cancer A Meta-analysis</td>
<td>2010</td>
<td>Review</td>
<td>Paolletti X</td>
<td>JAMA</td>
<td>157.34</td>
<td>Q1</td>
<td>604</td>
<td>10.1001/jama.2010.534</td>
<td>47.9</td>
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<td>m(6)A regulator-mediated methylation modification patterns and tumor microenvironment infiltration characterization in gastric cancer</td>
<td>2020</td>
<td>Article</td>
<td>Zhang B</td>
<td>Mol Cancer</td>
<td>41.44</td>
<td>Q1</td>
<td>442</td>
<td>10.1186/s12943-020-01170-0</td>
<td>134.0</td>
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<td>PD-1(+) regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer</td>
<td>2019</td>
<td>Article</td>
<td>Kamada T</td>
<td>Proc Natl Acad Sci U S A</td>
<td>12.78</td>
<td>Q1</td>
<td>428</td>
<td>10.1073/pnas.1822001116</td>
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<td>Tumor Microenvironment Characterization in Gastric Cancer Identifies Prognostic and Immunotherapeutically Relevant Gene Signatures</td>
<td>2019</td>
<td>Article</td>
<td>Zeng DQ</td>
<td>Cancer Immunol Res</td>
<td>12.02</td>
<td>Q1</td>
<td>418</td>
<td>10.1158/2326-6066.c18-0436</td>
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<td>PD-L1 expression in human cancers and its association with clinical outcomes</td>
<td>2016</td>
<td>Review</td>
<td>Wang X</td>
<td>Onco Targets Ther</td>
<td>4.35</td>
<td>Q2</td>
<td>412</td>
<td>10.2147/OTT.8105862</td>
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<td>Deep learning can predict microsatellite instability directly from histology in gastrointestinal cancer</td>
<td>2019</td>
<td>Article</td>
<td>Kather JN</td>
<td>Nat Med</td>
<td>87.24</td>
<td>Q1</td>
<td>400</td>
<td>10.1038/s41591-019-0462-y</td>
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<td>The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: results of a prospective randomized phase II/III trial</td>
<td>2010</td>
<td>Clinical trial</td>
<td>Heiss MM</td>
<td>Int J Cancer</td>
<td>7.32</td>
<td>Q1</td>
<td>349</td>
<td>10.1002/ijc.25423</td>
<td>30.8</td>
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</tbody>
</table>

JCR: Journal Citation Reports; IF: Impact Factor.
A: Top 25 references with the strongest citation bursts

<table>
<thead>
<tr>
<th>References</th>
<th>Year</th>
<th>Strength</th>
<th>Begin</th>
<th>End</th>
<th>1999 - 2023</th>
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</table>

Figure 5 References burst analysis and dual-map overlay of journals. A: Top 25 references with the strongest citation bursts are sorted by strength; B: Dual-map overlay of journals. The colored curve represents the reference path; the nature of each region is defined by the journal of the corresponding region.

cinoma, and #9 colorectal cancer. Each cluster comprised multiple closely associated words with smaller ranks or cluster numbers that contained more keywords. The premier cluster (#0) had the largest number of keywords, showing minimal past activity with two minor bursts but became more active in the recent decade. Cluster #1 followed a trend akin to that of cluster #0, signifying that MSI and targeted therapy have drawn significant interest. Clusters #2, #3, and #4 were notably active around 2000 and have undergone minor bursts in recent years. The term “tumor microenvironment” first appeared in 2010 in cluster #5 and has maintained activity in recent years.

Burst analysis of keywords: Following the burst analysis, we selected 25 keywords from the burst keywords based on burst strength and initiation time (Figures 7A and 1B). The keywords exhibiting the highest burst strengths included “Adoptive immunotherapy” (strength = 17.37), “dendritic cells” (strength = 16.79), and “carcinoma” (strength = 12.14). Three keywords that initiated bursting in the past 5 years and were identified as hotspots encompassed “mismatch repair deficiency” (initiated in 2018), “tumors” (initiated in 2019), and “plus chemotherapy” (initiated in 2021). The emergence of new burst keywords and prevalence of strong bursts in recent years have led to rapid developments in this field.
Clinical trials

The clinical trial with the earliest enrolment commenced in 1987, and was a randomized controlled trial predicated on preoperative serum glycoproteins assessing the efficacy of immunotherapy in GC (JPRN-UMIN000037472). The annual distribution of clinical trials (Figure 8A) indicated a sharp recent progress in the study of GC immunotherapy. The peak in the number of clinical trials occurred in 2019 ($n = 37$), with high numbers observed in the subsequent three years (2020 $n = 32$, 2021 $n = 35$, 2022 $n = 30$). The trials were predominantly in phase I ($n = 46$), phase I/II ($n = 44$), or phase II ($n = 75$).
Figure 7 Analysis of keywords burst. A: Top 25 keywords with the strongest citation bursts are sorted by strength; B: Top 25 keywords with the strongest citation bursts sorted by starting year.

We have consolidated the clinical trials of phase II/III ($n = 4$), III ($n = 4$), and IV ($n = 2$) in Table 2, offering scholars an update on the latest advancements in this field. The status of the clinical trials is shown in Figure 9B, with a significant number of trials either recruiting ($n = 92$) or not yet recruiting ($n = 21$). The three primary immunotherapies for GC, namely adoptive cell therapy (ACT), ICI, and vaccination, were subjected to separate clinical trial searches (Supplementary material, Figures 8B and 9C).
### Table 2 Clinical trials in phase 3 and phase 4 (or phase 2/3)

<table>
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<tr>
<th>No.</th>
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<th>Status</th>
<th>Phases</th>
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<th>Title</th>
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<td>1</td>
<td>NCT00503321</td>
<td>Terminated</td>
<td>Phase 2/3</td>
<td>October 1, 2006</td>
<td>Phase II Study of TS-1 Therapy and TS-1+PSK Therapy Against Advanced Gastric Carcinoma</td>
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<td>2</td>
<td>EUCTR2017-004896-30-IE</td>
<td>Not recruiting</td>
<td>Phase 3</td>
<td>July 6, 2018</td>
<td>A Randomized, Active-Controlled, Blinded, Phase III Clinical Trial of BM-986213 (Fixed Dose Combination of Relatlimun [anti-LAG-3] and Nivolumab) in Combination with Chemotherapy versus Placebo in Combination with Chemotherapy as First-Line Treatment in Participants with Unresectable, Locally Advanced or Metastatic LAG-3 Positive Gastric or Gastroesophageal Junction Adenocarcinoma</td>
</tr>
<tr>
<td>3</td>
<td>NCT04078152</td>
<td>Active, not recruiting</td>
<td>Phase 4</td>
<td>September 5, 2019</td>
<td>Durvalumab Long-Term Safety and Efficacy Study</td>
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<tr>
<td>4</td>
<td>ChiCTR2000039110</td>
<td>Recruiting</td>
<td>Phase 4</td>
<td>October 14, 2020</td>
<td>Effect of immunotherapy combined with chemotherapy on gastric cancer</td>
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<tr>
<td>5</td>
<td>NCT05002686</td>
<td>Recruiting</td>
<td>Phase 2/3</td>
<td>August 7, 2021</td>
<td>Safety and Efficacy of Sintilimab in Combination With Chemoradiation Followed by D2 Surgical Resection in Patients With Advanced Gastric Cancer With Retropertioneal Lymph Node Metastasis</td>
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<td>6</td>
<td>NCT05152147</td>
<td>Recruiting</td>
<td>Phase 3</td>
<td>December 2, 2021</td>
<td>A Study of Zanidatamab in Combination With Chemotherapy Plus or Minus Tislelimizum in Patients With HER2-positive Advanced or Metastatic Gastric and Esophageal Cancers</td>
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<td>7</td>
<td>NCT05270824</td>
<td>Not yet recruiting</td>
<td>Phase 3</td>
<td>March 1, 2022</td>
<td>Study Evaluating Neoadjuvant Immunotherapy Increasing CD8+ Cell Infiltration in Advance Gastric Adenocarcinoma</td>
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<td>8</td>
<td>NCT05325228</td>
<td>Recruiting</td>
<td>Phase 2/3</td>
<td>April 4, 2022</td>
<td>Study of Tislelimizum in Combination With SOX for the Treatment of Gastric Cancer With Liver Metastases</td>
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<td>9</td>
<td>NCT05677490</td>
<td>Not yet recruiting</td>
<td>Phase 3</td>
<td>January 6, 2023</td>
<td>mFOLFRINOX Versus mFOLFOX With or Without Nivolumab for the Treatment of Advanced, Unresectable, or Metastatic HER2 Negative Esophageal, Gastroesophageal Junction, and Gastric Adenocarcinoma</td>
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<tr>
<td>10</td>
<td>NCT05699655</td>
<td>Not yet recruiting</td>
<td>Phase 2/3</td>
<td>March 1, 2023</td>
<td>Tislelimizum Combined With Apatinib and Oxaliplatin Plus S1 Vs Oxaliplatin Plus S1 as Neoadjuvant Therapy for Borrmann IV, Large Borrmann III Type and Bulky N Positive Advanced Gastric Cancer</td>
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### DISCUSSION

In recent years, there has been a surge in the bibliometric analysis of articles across various fields. Tools such as CiteSpace and the VOSviewer enable raw data visualization, offering comprehensive and intuitive data representation. Cancer is a persistent medical challenge that has caused researchers to work globally toward enhancing treatments to improve progression-free survival (PFS) and OS. GC is one of the most prevalent cancers and requires more effective and targeted treatments. Bibliometric analysis of documents pertaining to GC immunotherapy elucidated the current research hotspots, thereby providing researchers with insights and directions. This analysis incorporated every related article from the WoSCC database until December 31, 2022, offering a visual and systematic overview. It summarizes and analyzes the countries, institutions, authors, keywords, and references. Clinical trials included every related trial from ClinicalTrials.gov and ICTRP until February 18, 2023.

### Bibliometric analysis

With regard to the volume of documents produced by each country, China held a dominant position, contributing 1070 documents, accounting for 53.2% of the total. The ten institutions with the highest publication counts were all located in China. The substantial population base of China facilitates a larger number of researchers and institutions in related fields, thereby leading to a higher volume of literature production. Furthermore, China’s substantial investment in GC immunotherapy research underscores the promising future of this field. In addition to the patient count correlating with the large population base, the incidence of GC in China significantly surpasses that in the United States and United Kingdom[29]. Elevated rates of smoking and *Helicobacter pylori* infection in China could contribute to a higher incidence of GC. The extent of early cancer screening in China remains unclear.

The United States holds the second position with regard to the volume of documents produced, contributing 16.0% of the publications, and is potentially linked to factors such as obesity, alcohol consumption, and high-fat diets. Japan ranked third (11.3%). The high incidence of GC in Japan mirrors that in China, likely because of Asian dietary habits, including high salt intake and excessive nitrite consumption, which are associated with a high incidence of digestive system tumors.

Co-citation analysis revealed that Sakamoto Junichi had the highest citation count (810 citations across six documents). His research has primarily focused on immunochemoay and adjuvant chemotherapy, with a meta-analysis of the benefits of adjuvant chemotherapy for resectable GC with over 600 citations. Oba et al.[30] also highlighted the therapeutic effects of polysaccharide K, lentilin, and OK-432 in GC[31,32]. This highlights the pivotal role of chemotherapy in GC treatment and ongoing advancements in research on the integration of immunotherapy and chemotherapy. Xin Wang, with 705 citations across seven documents, held the second position. His contributions include the development of
clinical guidelines for GC diagnosis and treatment and a review of PD-L1 expression in human cancers and its correlation with clinical outcomes, which has received over 400 citations. PD-L1-related research has emerged as a significant focus in recent years, offering new prospects for GC immunotherapy, which will be discussed in subsequent sections.

Keyword analysis
Keyword analysis, excluding less-specific terms, revealed several significant keywords and clusters. Notably, clusters #0 (MSI), #2 (DCs), #4 (adoptive immunotherapy), and #5 (TME) stood out; these were also keywords with notable bursts. Moreover, keyword “mismatch repair deficiencies” have garnered considerable attention. We delved into these categories in detail. Because of the substantial progress and potential of ICIs reported in recent studies, we discuss them separately.

TME (#5 TME): Among the top ten most frequently occurring keywords in this study, three only surfaced in recent years: “open label” (first appearance in 2015, 206 occurrences), “double-blind” (first appearance in 2015, 177 occurrences), and “tumor microenvironment” (first appearance in 2017, 174 occurrences). Open-label and double-blind denote two contrasting types of experiments. In open-label trials, both investigators and participants are aware of the treatment status, whereas in double-blind trials, neither party knows the treatment status. The frequent appearance of these two keywords likely reflects the growing number of immunotherapeutic drugs entering clinical trials in recent years. Pembrolizumab and nivolumab have shown efficacy against certain types of GC[33,34]. Toripalimab, currently under evaluation for its safety and efficacy in treating advanced GC resistant to chemotherapy, is also part of an ongoing phase III randomized trial assessing the combined therapy of toripalimab and XELOX[33,35].

The sixth keyword cluster, represented by “tumor microenvironment”, gained prominence in 1999 and has received consistent attention since. The TME refers to non-tumor cells and their metabolites and secretions within the tumor, including immune cells such as myeloid suppressor cells, tumor-infiltrating lymphocytes (TILs), macrophages, stromal fibroblasts, endothelial cells, extracellular matrix components, growth factors, and cytokines[36]. Five highly cited articles
in this study featured “tumor microenvironment” as a keyword, focusing on tumor-associated macrophages (TAMs) or pyroptosis. Macrophages are primarily categorized into two phenotypes, classically activated (M1) and alternatively activated (M2). M1 macrophages exhibit antitumor effects; however, M2 macrophages promote angiogenesis and tumor progression. TAMs, comprising M2 and a fraction of M1 cells, foster pro-angiogenic and immunosuppressive signals in gastric tumors, thereby presenting potential therapeutic targets[37,38].

Another study highlighted the significant role of RNA N6-methyladenosine modifications in shaping the diversity and complexity of the TME[39]. Pyroptosis aids cytotoxic lymphocytes in eliminating tumor cells and reprograms the TME toward an immunostimulatory state[40]. A recent study has suggested that the GC microenvironment of patients may provide a more accurate prediction of chemotherapy sensitivity, thereby enabling improved staging and prognostic assessment of patients[41]. The role of TME in GC continues to garner interest.

MSI (#0 MSI) and defective mismatch repair (mismatch repair deficiency): MSI arises from the inability to repair replication errors in microsatellite sequences owing to defective mismatch repair (dMMR). When these errors accumulate significantly, they result in MSI-H, a subtype of GAC constituting up to 22% of the cases[42]. The keyword “mismatch repair deficiency” experienced a burst from 2018 to 2020, with a burst strength of 10.82, ranking eighth (Figure 7A). As
depicted in Figure 6B, MSI ranks first in the cluster, signifying its high association and occurrence. The MSI-driven cancer pathway prompts tumor cells to produce abnormal and potentially immunogenic neoantigens, suggesting high antigenic potential in MSI GC[43,44]. Studies have established the independent prognostic significance of dMMR (P = 0.0001), with patients with metastatic GC exhibiting defective MMR systems demonstrating improved prognoses[45]. MSI cancer, characterized by extensive expression of immune checkpoint ligands and robust immunogenicity, exhibits heightened sensitivity to immunotherapy, albeit with potential resistance to chemotherapy[45,46]. The significance of the MSI-H subtype is well established, with numerous recent studies focusing on this subtype. Both nivolumab as a standalone treatment and in combination with ipilimumab have demonstrated antitumor activity with a tolerable toxicity profile in chemotherapy-resistant gastroesophageal adenocarcinomas. Moreover, pembrolizumab has received regulatory approval as a therapeutic alternative for MSI-H tumors[47,48]. Emerging evidence indicates a potential correlation between MSI-H and PD-L1 expression in various cancers, suggesting that MMR defects may serve as predictors of ICI therapy[45].

DC (#2 DCs): “Dendritic cells” constituted the third cluster in Figure 6B, exhibiting a significant burst from 2001 to 2013, as indicated by the second highest burst strength (Figure 7A, strength = 16.79). DCs are antigen-presenting cells essential for the induction and regulation of adaptive immune responses. DCs can phagocytose and process antigens and present these antigens to naïve T-cells, resulting in the activation, differentiation, and polarization of T-cells toward specific effector functions. In GC immunotherapy, DCs play a key role in the activation and regulation of antitumor immune responses[49]. DCs can be used as adjuvants or vaccine adjuvants to enhance antitumor immunity by presenting tumor-specific antigens to naïve T-cells[49,50].

DC-based vaccination and ACT are the two main types of cellular immunotherapies used in GC[50]. Our analysis revealed that 23 clinical trials have focused on GC vaccines, with new trials initiated annually since 2012, as depicted in Figures 8B and 9C. Vaccines have surfaced as a pivotal modality in cancer immunotherapy, with DC-based vaccination being the predominant approach, constituting 20 out of 23 clinical trials. In 1990, Inaba et al.[51] demonstrated that injection of in vitro antigens from DCs could sensitize normal mice to protein antigens. A decade later, researchers discovered that DCs can elicit antitumor responses[52]. A study in 2015 illustrated that DCs, when loaded with tumor RNA, can stimulate lymphocytes to differentiate into effector cells that respond to tumors and are capable of eliminating tumor cells[53]. The fusion of GC cells and DCs significantly enhances their ability to stimulate anti-tumor immune responses and prevents their proliferation into newly implanted tumors in vivo, highlighting the potential of DCs as a safe and effective anti-tumor vaccine[54]. Recombinant adenovirus-bearing secondary lymphoid tissue chemokine-modified DCs could serve as adjuvants to induce a robust immune response against GC[55]. DCs derived from cord blood in combination with cytokine-induced killer (CIK) cells have also been clinically utilized for the treatment of GC, showing significant disease-free survival (DFS, P = 0.0448) and OS (P = 0.0646) rates[56]. As of February 6, 2023, we identified 18 trials on ClinicalTrials.gov using the keywords “gastric cancer” and “dendritic cells”, with seven completed, nine with unknown status (indicating that the study had passed its completion date and its status had not been verified for over two years), one active, and one recruiting.

ACT (#4 adoptive immunotherapy): Adoptive immunotherapy was identified as the fifth cluster (Figure 6B, #4 adoptive immunotherapy) and experienced a surge in research from 1999 to 2016, as evidenced by its high strength (Figures 7A and B, strength = 17.37). A total of 137 clinical trials pertaining to ACT were initiated during this period, with relevant trials conducted almost annually for the past two decades (Figures 8B and 9C). ACTs involve the transfer of immune cells, such as lymphocytes or DCs, to a patient to enhance the antitumor immune response. In GC immunotherapy, ACTs generate effector T-cells that specifically target tumor antigens and induce long-term antitumor immunity[57]. ACTs can also enhance the function of regulatory T-cells and improve patient outcomes[50,57].

ACT has a rich history demonstrating its importance and progression in the field of immunotherapy. Currently, four types of ACTs have made significant contributions to international research: (1) TIL therapy; (2) Engineered T-cell receptor (TCR) therapy; (3) Chimeric antigen receptor T-cell (CAR-T) therapy; and (4) Natural killer (NK) cell therapy. CIK cell therapy, another form of ACT, is considered a promising approach for next-generation tumor-adaptive cell immunotherapies, with CIK cells often referred to as precision-guided missiles that target tumor cells[58].

First, we investigated TIL therapy. Unlike several types of cell immunotherapies that use blood-derived cells, TIL therapy leverages immune cells extracted directly from tumor tissues, thereby enhancing the capacity of these cells for tumor recognition. TILs play a critical role in curbing tumor growth and adjusting therapeutic response in cancer patients and could potentially act as predictive markers, providing insights into patient response to treatment and prognosticizing survival outcomes[59,60]. GC can be stratified into four distinct TME subtypes based on the assessment of PD-L1 expression and TILs: (1) PD-L1+/TIL+, associated with the best PFS [Hazard ratio (HR = 2.044)] and OS (HR = 1.993); (2) PD-L1-/TIL+, linked with the poorest survival outcomes; (3) PD-L1+/TIL-; and (4) PD-L1+/TIL+[61]. Increased counts of T-bet+ TILs were found to be correlated with non-invasion of the muscle layer (P = 0.0138), smaller tumor size (P = 0.0202), and early Union for International Cancer Control stage (P = 0.0196), and studies have demonstrated that higher numbers of T-bet+ TILs are associated with longer median PFS (41 vs 26 mo, P = 0.0481) and median survival time (MST) (55 vs 32 mo, P = 0.0455)[62].

Second, we examined engineered TCR therapies. TCR-engineered T-cells require the presentation of antigens by specific major histocompatibility complex (MHC) molecules for activation, which presents certain constraints on their clinical applications[63]. Initial trials with TCR therapy showcased cancer regression; however, the associated toxicities were severe, underscoring the need for caution when implementing high-avidity TCRs[10,64]. Later clinical trials achieved efficacy without substantial toxicity by using T-cells redirected against cancer testis antigens[65,66,66]. A 2022 study profiled the hypervariable complementarity determining region 3 of the TCR beta chain in the peripheral blood of GC patients to identify new biomarkers[67].
Thirdly, we turn our attention to CAR-T therapy. Emerging over the past 30 years, CAR-T-cell therapy is a relatively novel approach, evolving from prior clinical applications of ACT, including TILs[11]. CAR-T-cells, a subset of genetically engineered T-cells, can identify specific antigens, and their cytotoxic effects operate independently of MHC interactions [50]. The first-generation CAR, constructed in 1993, comprises an extracellular antigen recognition domain-commonly a single-chain Fragment variant derived from an antibody-a transmembrane domain, and the intracellular T-cell activation domain of CD3[68,69]. The second-generation CAR introduces a costimulatory domain, enhancing antitumor effects and promoting the establishment of immunological memory in patients with hematological tumors[69,70]. Third-generation CAR integrates two tandem costimulatory molecules[11]. The fourth-generation CAR adopts a more intricate design that aims to mitigate off-target toxicity and immunosuppression and boost the anti-tumor transport activity targeted by solid tumors[11]. Various potential targets for GC therapy, including claudin 18.2, mesothelin, ANTXR1 (TEM8), and MUC3A, have been identified, and claudine18.2-specific CAR-T-cell therapy is currently being explored in clinical trials[71,72]. As of February 6, 2023, a search of ClinicalTrials.gov using the keywords “gastric cancer” and “CAR T” yielded 37 trials, 23 of which were recruiting or not yet recruiting. Numerous related experiments will be conducted in the coming years, likely catalyzing rapid advancements in CAR-T therapy.

NK cell therapy was also considered. Patients with digestive cancers frequently demonstrate elevated PD-1 expression in both peripheral and tumor-infiltrating NK cells, and this heightened expression could potentially signify an unfavorable prognosis[73]. A decade ago, a study revealed that among patients with GAC, those with high concentrations of NK cells exhibited higher survival rates than those with low concentrations (P = 0.0027, HR = 0.343)[74]. As NK cell therapy continues to evolve, researchers have begun to integrate NK cells with other forms of immunotherapy to investigate the potential improvements in patient outcomes. A recent animal study employed a combination of interleukin (IL)-2-activated NK cells and anti-PD-1 therapy, demonstrating that this synergistic approach could hinder gastric tumor progression and promote tumor immune cell infiltration[75]. Moreover, various strategies involving CAR NK cell therapy have demonstrated efficacy in the treatment of certain tumor types[76-78].

Finally, CIK cell therapy is discussed. As illustrated in Figure 7A, the term “cytokine-induced killer cells” experienced a research publication surge from 2013 to 2017, with a burst strength of 11.39, whereas “ciik cells” followed a similar trend from 2012 to 2017, with a burst strength of 10.75. CIK cells have several attributes that make them a promising therapeutic avenue for cancer treatment, including the potent antitumor capabilities of T lymphocytes and the non-MHC-restricted tumoricidal activity characteristic of NK cells. The earliest research document we obtained featuring the terms “cytokine-induced killer cells” or “CIK” dates back to a 2006 study, which indicated that the total remission rate (CR + PR + MR) in the group treated with a combination of chemotherapy and CIK cells was higher than in the group treated with chemotherapy alone (56.3% vs 48.0%) [79]. A clinical trial in 2010 showed that GC patients treated with CIK cell adoptive immunotherapy combined with chemotherapy had a significantly longer survival time than those treated with chemotherapy alone (MST: 49 m vs 27 m, P < 0.05; 2- and 5-year survival rates: 73.5% vs 52.6%, 40.4% vs 23.9%, P < 0.05). Another report evaluated 11 studies proving that adjuvant immunotherapy with CIK cells may prevent recurrence and improve the quality of life and PFS rate in patients with cancer [80,81]. However, a recent meta-analysis revealed that the incorporation of cellular immunotherapy into chemotherapy resulted in a statistically significant improvement in OS and DFS only in patients with stage III GC[82].

Despite the significant anti-tumor effects demonstrated by CAR-T therapy across various iterations, numerous challenges remain in its widespread application. Future research in this area will likely concentrate on the development of more potent CAR-T-cells with enhanced anti-tumor activity and mitigated toxicity[83]. Despite the significant anti-tumor effects demonstrated by CAR-T therapy across various iterations, there are still numerous challenges to its widespread application. Future research in this area will likely concentrate on the development of more potent CAR-T-cells with enhanced anti-tumor activity and mitigated toxicity[84]. NK cells display high anti-tumor activity and antibody-dependent cytotoxicity. However, their therapeutic application is limited by the challenge of generating large quantities of highly pure and functional NK cells[7,85]. In contrast, immunotherapies involving CIK cells and TILs have a significant potential for cancer treatment. Our analysis shows that CIK cells exhibit potent anti-tumor activity, and that combination therapies incorporating CIK cells demonstrate improved efficacy in patients with GC. TILs immunotherapy has also been extensively used in advanced GC treatment[7]. TILs offer several key advantages, including lower off-target toxicity and increased specificity, making them superior in addressing tumor heterogeneity in comparison to other ACT therapies; they can also regulate the immune response by releasing cytokines, which subsequently bolster anti-tumor immunity[86]. Moreover, TIL levels are prognostic indicators[87].

ICIs: Clinical research on ICIs is the most advanced and widespread cancer immunotherapy. These inhibitors have shown notable efficacy in the treatment of various solid and hematological cancers, often leading to durable, long-lasting responses that are typically well tolerated by patients[88]. The interaction of PD-1 (also referred to as CD279) with PD-L1 (also denoted as CD274 or B7-H1) can induce T-cell dysfunction, exhaustion, and tolerance, thereby inhibiting the PD-1/PD-L1 and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4, also known as CD152) signaling pathways, T-cell function can be rejuvenated, leading to an increase in the destruction of tumor cells[17,89]. Studies over the past 20 years have indicated that inhibiting PD-L1 can boost antitumor immunity[90-92]. In fact, it has been demonstrated that most patients with GAC express CTLA-4 on their tumor cells (CTLA-4 86.6%, PD-L1 44.9%) and those who tested negative for CTLA-4 or PD-L1 had a significantly better mean OS than those who tested positive (CTLA-4: 62.0 vs 44.4 m, P = 0.018; PD-L1: 54.2 vs 39.1 m, P = 0.011)[93]. Contrary to a previous study, another study demonstrated that the expression of PD-L1 was associated with improved DFS and OS in GC (PD-L1+ vs PD-L1- tumors, 5-year DFS rate, 82.6% vs 66.9%; 5-year OS rate, 83.0% vs 69.1%, P < 0.05)[94]. These contrasting findings could be due to differences in the ethnic backgrounds of the patients (Caucasians in the first study and Asians in the second) or variations in the antibodies used in each study, indicating the need for larger studies to...
clarify the roles of PD-L1 and CTLA-4 in GC.

As of February 18, 2023, 274 clinical trials were registered at ClinicalTrials.gov and ICTR, focusing on ICIs, which is a considerably larger number than those related to ACT and vaccine therapies (Figure 9C). The first clinical trials centered on ICIs began to emerge in 2013, and their counts saw a steady increase from 2015 to 2019, maintaining a high level over the last four years (Figure 8B). ICIs have received growing academic attention and have shown robust potential in GC immunotherapy. Some PD-1 inhibitors with relatively advanced research include avelumab, pembrolizumab, nivolumab, tisllizumab, camrelizumab, atezolizumab, and ipilimumab, function in combination with CTLA-4.

Published studies indicate that therapy using ICIs may present less toxicity, better tolerance than chemotherapy, and potentially superior outcomes. In trials with GC patients, avelumab did not outperform chemotherapy in terms of OS or RFS, but it did showcase a more manageable safety profile [grade ≥ 3 treatment-related adverse events (AEs): 9.2% vs 31.6% and 12.8% vs 32.8%, NCT02625623 and NCT02625610][95,96]. Pembrolizumab, which has undergone phase III clinical trials (NCT02370498 and NCT02494583), showed promising results; compared to chemotherapy, it demonstrated a trend toward improved OS and ORR while reducing the occurrence of treatment-related AEs[97,98].

In recent years, notable advances have been made in the combination of ICIs and chemotherapy. In patients with advanced GAC, the combination of tisllizumab and chemotherapy results in sustained responses and manageable side effects[99]. Similarly, a combination of Camrelizumab and CAPOX has demonstrated encouraging anti-tumor effects and manageable toxicity in patients with metastatic or advanced GAC, having successfully completed phase II clinical trials (NCT03469557 and NCT03472365)[100]. The effectiveness of ipilimumab in combination with chemotherapy has been assessed in both phase II and III clinical trials (NCT03241173 and NCT03126110, respectively).

The HER2, also known as Neu or ErbB2, is part of the epidermal growth factor receptors family, which encodes the transmembrane glycoprotein p185. It was initially discovered to have high expression in GC in 1986 and has since become a significant focus in translational cancer research due to its role in the HER2 signaling pathway[101,102]. For HER2-positive GCs, the preferred treatment regimen often includes a combination of pembrolizumab, trastuzumab, and chemotherapy[13]. A recent clinical trial (NCT03409848) demonstrated that combination therapy with trastuzumab, nivolumab, and FOLFOX was more effective than previous treatment protocols in treating HER2-positive esophagogastric adenocarcinoma[103].

**Clinical trial**

We screened 228 clinical trials on GC immunotherapy identified in a preliminary search and further analyzed 174 eligible clinical trials. There were 38 phase I clinical trials, 40 phase I/phase II clinical trials, 71 phase II clinical trials, three phase II/phase III clinical trials, four phase III clinical trials, and two phase IV clinical trials. For status, 25 clinical trials were completed: 20 were active but not recruited, 72 were recruited, 17 were not yet recruited, and the others were unknown or difficult to classify.

**Completed trials:** There are 25 completed clinical trials were completed, all of which were in phase I or II. After some immunotherapeutic drugs have proven effective, researchers have begun to explore the potential of multiple immunotherapy combinations.

Monoclonal antibodies (mAbs) are the predominant form of immunotherapy used to manage GC. The anticipated synergies arise primarily from the combination of anti-PD-1 and anti-CTLA-4 antibodies, a therapeutic pairing that has been extensively explored by numerous researchers, as indicated by associated clinical trials (NCT02340975, NCT02983045). Moreover, the inclusion of anti-HER2 antibodies in this treatment paradigm has also been investigated (NCT03409848). Beyond ICIs, other emergent forms of immunotherapy, such as ACT, have also been initiated in clinical trials. The completed trials included an array of treatment strategies. These include the integration of autologous DC-CIK cell immunotherapy with chemotherapy (NCT01783951), implementation of CAR-T immunotherapy (NCT02850536), and combination of NK cell therapy with ICIs (NCT03319459, NCT03841110). A phase II clinical trial (ChiCTR-OCH-12002610) investigating postoperative immunomodulatory therapies involving tumor lysate-loaded DCs, in vitro DC-activated T-cells, and activated T-cells in conjunction with chemotherapy demonstrated significantly improved mean survival rates in patients with operable colorectal cancer (59.74 ± 3.21 years vs 49.99 ± 2.54 years, P = 0.034), though its applicability to GC has not been substantiated with published results[104].

**Trials active, not recruiting:** Eighteen active clinical trials that completed recruitment primarily employed a therapeutic approach involving the synergistic use of multiple ICIs alongside chemotherapy, with notable targets, including PD-1/ PD-L1, CTLA-4, HER2, and LAG3 (NCT03662659, NCT03647969, NCT03443856, NCT04062656). A phase I/II clinical trial (NCT03936688) preliminarily corroborated the tolerability of an immunotherapeutic approach based on invariant NK T cells and PD-1+CD8+ T-cells for lung adenocarcinoma, suggesting their potential applicability to other solid tumors[105].

**Trials in recruiting:** With 66 clinical trials currently recruiting, the discovery of novel cellular immunotherapeutic approaches continues to facilitate the development of increasingly effective immune cells, and consequently, the integration of more cellular immunotherapies into clinical trials. Four clinical trials focused on peptide vaccines (NCT03784040, KCT0005481, NCT05269381, and NCT05311176), all of which implemented treatment regimens that incorporated a combination of vaccines and ICIs or the addition of chemotherapy. Notably, a phase II clinical trial was initiated to explore the potential of IMU-131, a B-cell peptide vaccine, in combination with anti-vascular endothelial growth factor receptor (VEGFR) and anti-PD-1 antibodies (NCT05311176).

Current research indicates that, although the combination of DC-CIK with S-1 does not yield a statistically significant advantage over S-1 in combination with cisplatin (P = 0.892), DC-CIK exhibit good tolerability, suggesting their potential as an alternative in cases where multi-agent chemotherapy is not tolerated[106]. Therefore, researchers continue to explore more efficacious cellular immunotherapies. T-cells stimulated by Claudin18.2 peptide demonstrate potent anti-
tumor activity and are emerging as a hope of a beacon and a focal point of interest in the landscape of GC cellular immuno-
therapy, with corresponding clinical trials already underway (NCT04683939)[107].

Zanidatamab (ZW25) is an asymmetrically structured antibody that binds to two non-overlapping HER2 epitopes. A phase III clinical trial aims to examine the efficacy of ZW25 in combination with chemotherapy, both with and without tislelizumab, as a first-line treatment for patients with advanced or metastatic HER2-positive gastroesophageal adenocarcinomas (NCT01552147). Investigations involving ZW25 as a stand-alone treatment or in conjunction with chemotherapy or ICIs are ongoing. On May 29, 2019, the United States Food and Drug Administration (FDA) approved ZW25 as a first-line treatment in standard-of-care chemotherapy for patients with HER2-overexpressing gastroesophageal adenocarcinoma[102]. ZW49, an antibody-drug conjugate (ADC) derivative of ZW25, utilizes the proprietary cytotoxic and cleavage adapters of the Zymework. A Phase I clinical trial (NCT03821233) is currently underway to explore the potential of ZW49 in patients with HER2-positive malignancies.

IL-2 is known for its ability to foster the growth of diverse immune cell populations, modulate cellular differentiation, and depending on the cellular milieu, either promote survival or induce apoptosis. Being the first cytokine to gain FDA approval for cancer treatment, IL-2’s role in strategies that synergistically enhance immune responses is garnering increasing interest. Several clinical trials have investigated the synergistic use of interleukins with other treatments, including IL-2 combined with an anti-PD-1 antibody (NCT05086692), and AU-007 (interleukin antibodies) in conjunction with aldesleukin (NCT05267626). IL-15, which is similar to IL-2, is currently under investigation in a phase II clinical trial (NCT04847466) that examines the combination of N803 (an IL-15 agonist), pembrolizumab, and PD-L1 CAR NK cells. Additionally, an ongoing investigation involves a combination of KK-1C1 TCR-T cells and aldesleukin (NCT05483491). However, no associated literature has been published on these trials. The future utility and progress of interleukins in cancer therapy warrants further investigation.

**Trails not yet recruiting:** Sixteen trials have not yet started recruitment, and most of them were phase II trials, with some phase I and III trials being planned. One or more ICIs combined with chemotherapy continue to be the most frequently studied, but some new treatment strategies are also being explored.

Research has demonstrated that the addition of anti-PD-1 antibodies to anti-HER2 antibodies and chemotherapy substantially diminishes tumor size and significantly enhances the ORR to 74.4%, as opposed to 51.9% without anti-PD-1 antibodies in HER2-positive GC[13]. In recent years, simultaneous blockade of PD-1 and HER2 has emerged as a significant area of research. Studies on VEGFR continue to progress. The anti-VEGFR2 antibody, ramucirumab, has demonstrated clinical efficacy in GC, both as a standalone therapy and in combination with chemotherapy[15]. Furthermore, clinical trials have been initiated to investigate the combination of anti-VEGFR and anti-PD-1 antibodies with and without chemotherapy (NCT05585580 and NCT05721651).

Olaparib, a PARP inhibitor approved for the treatment of ovarian cancer, is currently being explored for its potential clinical utility in GC. A phase I/II clinical trial (NCT0492211) has investigated the combination of olaparib, pembrolizumab, and paclitaxel in patients with GC. Over the past decades, 5-fluorouracil has remained a mainstay in the realm of chemotherapy. Recently, researchers have introduced NUC-3373, which has demonstrated a more favorable safety profile in patients[108]. A phase I/II clinical trial is currently centered on the use of NUC-3373 in conjunction with other agents in patients with advanced solid tumors, including GC (NCT05714553). IRAK4 can induce T cell dysfunction, making it a promising and novel target for immunotherapy[109]. A phase I clinical trial (NCT05187182) is currently investigating the use of CA-4948 (an IRAK4 inhibitor) combined with chemotherapy and ICIs for untreated unresectable gastric and esophageal cancer. A phase II clinical trial (NCT06718222) focusing on SHR-A1811 (an ADC drug targeting HER2 and TOPI) combined with SHR-1701 (a PD-L1 and TGFβR2 inhibitor) and chemotherapy in advanced HER2-positive GC is being conducted, which we believe will be significant.

**The trends of clinical trials:** (1) Discovering novel biomarkers to subclassify patients and exploring more specific treatment options. NCT0593419, ChiCTR2100052367, NCT02757391, NCT03158571; (2) Integration of immunotherapy with surgery, radiotherapy, and chemotherapy. NCT04688801, chemotherapy + immunotherapy ± radiotherapy after surgery; (3) Transition from single-agent to multi-agent therapy. NCT01552147, trastuzumab (anti-HER2)/ZW25 (anti-
HER2) + tislelizumab (anti-PD-1) + chemotherapy; (4) Combination therapy involving various immunotherapies such as ICI, ADC, and ACT. NCT05269381, vaccine + pembrolizumab (anti-PD-1, ICI) + chemotherapy; NCT05671822, SHR-A1811 (HER2, ADC) + SHR-1701 (PD-L1 and transforming growth factor-β double antibody) + capcitabine + oxaliplatin; NCT05313906, RC48 (HER2, ADC) + AK105 (anti-PD-1) + cisplatin; (5) Discovery of new ICIs. NCT05187182, CA-4948 (IRAK4/FLT3 inhibitor) + FOLFOX + PD-1 inhibitor ± trastuzumab (anti-HER2); NCT05714553, NUC-3373 (thymine synthase inhibitors) + leucovorin+ pembrolizumab/docetaxel.

**Current status and future perspectives**

Through this study, we have understood that: (1) Immunotherapy has become an important treatment modality for GC, representing the greatest advancement since chemotherapy and anti-HER2 therapy; (2) Immunotherapy has evolved from a sole focus on replacing chemotherapy to a concept of combined therapy; (3) Immunotherapy has developed from a simple pursuit of efficacy to one that balances efficacy and toxicity; and (4) Immunotherapy has progressed from the use of ICIs to the exploration of CAR-T therapy. For future research, we suggest: (1) Continued exploration of new targets; (2) Investigation into new prognostic and predictive biomarkers for immunotherapy, enabling individualized precision treatment; (3) Exploration of the optimal treatment modalities for immunotherapy in combination with ADC therapy; (4) Investigation into the application scenarios for immune therapy bispesific antibodies; and (5) Further development of CAR-T therapy targets and reduction of CAR-T therapy-related toxicities.
Currently, there are many limitations and challenges in GC immunotherapy research that need to be overcome in future studies. Firstly, GC is highly heterogeneous, both inter- and intratumorally. This variability affects the response to immunotherapeutic agents and poses challenges for identifying universal targets. Comprehensive genomic and transcriptomic analyses could identify reliable biomarkers and offer a more individualized treatment approach. Secondly, current biomarkers like PD-L1 expression, and MSI are not wholly predictive of the treatment response. So the development and validation of new biomarkers or a set of biomarkers are essential for better patient stratification and response prediction. Thirdly, immunotherapies have shown limited efficacy in the late stages of GC thus far. Combining immunotherapy with other treatment modalities such as chemotherapy or targeted therapy could potentially synergize to improve outcomes. Fourthly, the use of ICIs can lead to autoimmunity and other side effects. Therefore, developing methods for early identification and management of AEs is crucial, or discovering new lower toxic agents. Fifthly, the lack of clinically relevant animal models (e.g., TIL, ACT, TME/TIME) for GC hampers the pre-clinical evaluation of immunotherapeutic strategies. The development of patient-derived xenograft models and organoids could enhance the translational potential of pre-clinical findings.

**Trends in publications and significant breakthroughs:** As presented in Figure 2A, discernible surges in both publications and citations characterize the years 2016-2017 and 2020, with a pronounced resurgence beginning in 2020. These periods align with seminal studies and landmark events in the field. For instance, a 2015 meta-analysis reported that PD-L1 overexpression was correlated with adverse prognoses in solid tumors[110]. Furthermore, in 2016, a distinguished trial (NCT02447003) examining the antitumor efficacy and safety of pembrolizumab amassed 1298 citations, culminating in the FDA’s subsequent approval for HER2-positive GC treatment on May 5, 2021[28,48]. Post-2020, propelled by technological advancements, breakthroughs such as the use of single-cell RNA sequencing in GC cells and transcriptome analysis of peritoneal cancer samples from patients with GAC have emerged, enriching our understanding of tumor biology and uncovering novel immunotherapy targets[111,112]. Notably, EBV-positive status has been identified as a potential biomarker for EBV-associated GC, paving the way for future studies[113].

**Evolving clinical trial landscape:** With only ten phase III or IV clinical trials and 113 in either the recruiting stage or yet to commence (Figures 9A and B), immunotherapy application in GC is rapidly evolving and requires for further investigation. Analysis of ongoing trials portends emerging directions in the field, emphasizing the continuance of surgery, chemotherapy, and radiotherapy as foundational modalities, supplemented by significant advancements in targeted therapy and immunotherapy[12,114].

**Monotherapy and combination therapies:** The current therapeutic armamentarium includes monotherapies with ICIs and combination regimens that demonstrate efficacy against solid tumors, including GC. Specifically, ICIs have become prevalent in the treatment of advanced GC, with all analyzed phase III and IV trials incorporating anti-PD-1 antibodies, highlighting this avenue as a focal research point[114].

**Anticipated future developments:** Concurrently, advancements in anti-HER2 and anti-VEGFR therapies are steadily progressing, with research anticipated to encompass a wide spectrum, including ICI monotherapies, various ICI combinations, and integration with ADCs. ADCs, which bridge the specificity of mAbs with the potency of cytotoxic agents, hold immense potential as therapeutic modalities for GC, with ongoing trials investigating their synergistic application with chemotherapy. These collective developments indicate a well-defined direction for future research, affirming a promising era for immunotherapy in the field of GC.

**CONCLUSION**

In conclusion, this study delineates a clear trajectory in GC therapy, from the present use of monotherapies and chemo/target therapy to future integrative and combinatorial approaches, with CAR-T technologies at the forefront. It encapsulates the potential for enhanced therapeutic paradigms and heralds a new era of precision medicine for GC treatment. The findings presented here highlight substantial progress as well as pinpoint the direction for continued research and clinical innovation, aligning with the rigorous academic standards of premier oncological journals.

**Strengths and limitations**

**Strengths:** This study offers systematic and specialized insights into the treatment and current status of GC by merging published literature with clinical research. By comprehensively articulating GC immunotherapy, readers can decode this complex subject into multiple dimensions. This inclusive approach synthesizes the current understanding and offers a valuable reference for future investigations, thereby enriching the collective knowledge of this field.

**Limitations:** Literature selection: Although extensive literature related to immunotherapy for GC was included, enabling comprehensive and objective conclusions, our search was restricted to articles and reviews within the WoSCC, using limited terms. Although this strategy emphasizes high-impact data, potentially enhancing the accuracy of our analysis, it may also lead to incomplete literature retrieval. Time constraints: Conducted in early 2023, our literature review spans from 1999 to the present, imposing time limitations that may create delays in reflecting the most recent advancements. Citation bias: Newly published critical research findings may lack substantial citation numbers, thereby obscuring their value in our data analysis.

In summary, the strengths of this study lie in its systematic and specialized approach, providing a multidimensional understanding of GC immunotherapy, whereas the limitations primarily pertain to literature selection, temporal
boundaries, and potential citation bias. These factors must be considered when interpreting the findings and their implications for future research and clinical practice.

ARTICLE HIGHLIGHTS

Research background
Gastric cancer (GC) ranks sixth in incidence among all cancers, and is the third leading cause of cancer-related deaths worldwide. However, there are significant limitations to the treatment of GC, and more effective and less toxic treatment options are required to prolong the survival of patients with GC. Immunotherapy has recently shown rapid development for the treatment of GC and has great potential.

Research motivation
In recent years, scientometrics has been applied to analyze literature related to certain fields to identify hotspots and predict future trends. However, to the best of our knowledge, there have been no previous studies on scientometric analyses in the field of GC immunotherapy.

Research objectives
To present a comprehensive review of the knowledge framework and research hotspots in the field of GC immunotherapy, we aimed to assist scholars in promptly understanding the current research status and latest developments in this field and provide new ideas and directions for future studies.

Research methods
Publications related to GC immunotherapy between 1999 and 2023 were retrieved from the Web of Science Core Collection database on February 1, 2023. An analysis of the 2013 relevant articles retrieved using CiteSpace and VOSviewer was conducted. We get Impact Index Per Article from Reference Citation Analysis (RCA). Additionally, we searched for clinical trials on ClinicalTrials.gov and the International Clinical Trials Registry Platform, and analyzed ongoing clinical trials in this field to predict future developmental trends.

Research results
Through a literature search, we included 2013 relevant articles in this study and used the scientometric software CiteSpace and VOSviewer to analyze 11730 authors, 617 institutions, 71 countries, 726 keywords, citations, and the emergence and timeline of certain information. We have provided specific explanations for the main keywords and analyzed the significance of these research hotspots, including “tumor microenvironment”, “microsatellite instability”, “mismatch repair deficiency”, “dendritic cells”, and “adoptive immunotherapy”. Because immune checkpoint inhibitors (ICIs) play an important role in GC immunotherapy, we have also provided a separate discussion on them. Additionally, we classified the retrieved clinical trials based on the type of immunotherapy and stage of disease and further analyzed the research hotspots and trends in this field in the coming years. The integration of literature and clinical trials has enabled the production of comprehensive and objective research findings.

Research conclusions
Through the use of novel research methods, ideas, and software, we gained innovative and comprehensive insights into the current status and future trends in GC immunotherapy. Our research findings summarize the current state of development in this field and facilitate understanding and learning among scholars. Additionally, this article identifies future developmental directions and trends in this field and provides scientific research ideas for researchers to promote the development of GC immunotherapy.

Research perspectives
Burst keywords and clusters, including tumor microenvironment, microsatellite instability, mismatch repair deficiency, dendritic cells, and adoptive immunotherapy represent the current frontiers of research on immunological factors in cirrhosis. ICIs have also been studied. The combination of multiple drugs and immunotherapeutic methods has received increasing attention.

FOOTNOTES

Author contributions: Wang H and Guo R conceived the study and critically revised the manuscript; Li YN designed the study, performed statistical analyses, and drafted the manuscript; Xie B, Zhang Y, He MH, Mu DM, and Wang H designed the study and wrote the manuscript; Li YN, Guo R, and Wang H performed article retrieval and data interpretation; Mu DM conducted statistical assessment; and all the authors have read and agreed to the final version of the manuscript.

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