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Progress in elucidating the relationship between *Helicobacter pylori* infection and intestinal diseases

Shunji Fujimori

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**Abstract**

*Helicobacter pylori* (*H. pylori*) infection causes changes to the intestinal flora, such as small intestinal bacterial overgrowth, and increases gastric acid secretion-stimulating gastrointestinal hormones, mainly gastrin, due to a decrease in gastric acid caused by atrophic gastritis. In addition, the cellular components of *H. pylori* travel through the intestinal tract, so the bacterial infection affects the immune system. Therefore, the effects of *H. pylori* infection are observed not only in the stomach and the proximal duodenum but also in the small and large intestines. In particular, meta-analyses reported that *H. pylori*-infected individuals had an increased risk of colorectal adenoma and colorectal cancer. Moreover, a recent study reported that the risk of developing colorectal cancer was increased in subjects carrying *H. pylori* vacuolating cytotoxin A antibody. In addition, it has been reported that *H. pylori* infection exacerbates the symptoms of Fabry’s disease and familial Mediterranean fever attack and is involved in irritable bowel syndrome and small intestinal ulcers. On the other hand, some studies have reported that the frequency of ulcerative colitis, Crohn’s disease, and celiac disease is low in *H. pylori*-infected individuals. Therefore, additional studies are needed.

**Key Words:** *Helicobacter pylori*; Intestine; Colorectal cancer; Intestinal bacterial overgrowth; Inflammatory bowel disease; Intestinal ulcer

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addition, the cellular components of _H. pylori_ travel through the intestinal tract, causing an effect of bacterial infection on the immune system. Meta-analyses reported that colorectal adenoma and cancer increase in _H. pylori_-infected individuals, and this bacterium has also been reported to be involved in several other diseases. On the other hand, _H. pylori_ infection is considered to suppress inflammatory bowel disease. However, few studies have reported on these issues, and further elucidation is required.

**INTRODUCTION**

It is well known that _Helicobacter pylori_ (_H. pylori_) infection causes atrophic gastritis, gastric ulcer, duodenal ulcer, gastric cancer, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Many studies have reported that _H. pylori_ infection might affect not only the stomach and the proximal duodenum but also the intestinal tract on the anal side. For example, meta-analyses reported that colorectal adenomas and colorectal cancers are more common in _H. pylori_-infected individuals[1-3]. In addition, studies have reported that the symptoms of Fabry’s disease are exacerbated[4] and that attacks of familial Mediterranean fever (FMF) are increased[5] in _H. pylori_-infected individuals. Moreover, studies have reported that small intestinal ulcerative lesions in patients are significantly more common in _H. pylori_-infected individuals[6]. Furthermore, it has been suggested that _H. pylori_ infection may cause irritable bowel syndrome[7]. On the other hand, the frequencies of ulcerative colitis[8,9], Crohn’s disease[8-10], and celiac disease[11] are low in _H. pylori_-infected individuals.

Thus, _H. pylori_ infection causes neoplastic and ulcerative lesions not only in the stomach and proximal duodenum but also in a wide range of locations that range from the distal duodenum to the large intestine. Furthermore, _H. pylori_ infection is associated with abnormal pathology of immune diseases and abnormal intestinal motility. In the stomach and duodenal bulb where the gastric mucosa is found, various diseases occur due to _H. pylori_ infection. Direct infection of the anal side of the duodenum to the large intestine does not occur without ectopic gastric mucosa, such as the Meckel diverticulum[12]. Therefore, the causes of abnormalities of the intestinal tract on the anal side are presumed to be due to _H. pylori_ infection; these causes include the effects of _H. pylori_ components, abnormalities of the intestinal flora, the effects of immune responses, and the effects of gastrointestinal hormones such as gastrin. In this paper, the effects of _H. pylori_ infection on the small and large intestines will be examined and discussed.

**EFFECTS OF H. PYLORI COMPONENTS**

_H. pylori_ DNA is detected in the lowest portion of the small intestine as a bacterial component of _H. pylori_, and this bacterial component is excreted in the stool[13]. Utilizing the fact that bacterial components are excreted in stool, _H. pylori_ infection can now be confirmed by a stool test. Studies have reported that the bacterial component of _H. pylori_ promotes DNA synthesis in a small intestinal cell line (IEC-6), as evaluated by the labeling index[14]. Similarly, the cancer-related CagA-positive strain of _H. pylori_ has been confirmed to stimulate DNA synthesis in IEC-6 epithelial cells _in vitro_, regardless of its ability to produce vacuolating cytotoxin A (VacA) toxin[15].

Butt et al[16] recently reported an increased risk of developing colorectal cancer in individuals carrying serum antibodies against VacA of _H. pylori_. Rassow et al[17] reported in a review that VacA forms chloride (Cl-) channels that enter the cell and mitochondrial membranes, and VacA causes loss of mitochondrial membrane potential, mitochondrial fragmentation, formation of reactive oxygen species, autophagy, cell death and gastric cancer. Since Cl channel abnormalities are involved in cystic fibrosis, which is known to be associated with colorectal cancer, this VacA-induced Cl channel abnormality may be involved in colorectal cancer[18]. Because
Butt et al.[16] did not directly examine the bacterial cell components of the intestinal tract but examined serum antibodies, the effect of bacterial components could not be determined. However, blood antibodies are unlikely to be carcinogenic. Therefore, bacterial cell components have a high probability of being involved. Whether VacA may be the cause of colorectal carcinogenesis has not been resolved. However, bacterial cell components, such as VacA, can travel through the intestinal tract and could be associated with colon tumors. Thus, H. pylori bacterial components that travel through the intestinal tract have a significant likelihood of affecting the intestinal tract.

### CHANGES IN THE INTESTINAL FLORA

When atrophic gastritis due to H. pylori infection progress, the gastric acid concentration decreases, and the bactericidal ability of the stomach diminishes. The bacterial flora in the stomach changes drastically[19]. This causes abnormalities in the intestinal flora. H. pylori often infects the stomach at a young age and significantly reduces the post-infection Firmicutes to Bacteroidetes ratio at the phylum level[20]. Successful eradication of H. pylori increases the amount of Bifidobacterium in the intestinal flora[21]. A relationship between H. pylori and small intestinal bacterial overgrowth (SIBO) has been reported[22]. SIBO is involved in many gastrointestinal and systemic diseases, and SIBO may be the cause of the increased rate of FMF attack in H. pylori-infected individuals[3].

In a systematic review and meta-analysis, Shah et al.[23] reported a link between irritable bowel syndrome (IBS) and SIBO. Although the authors reported that the overall quality of the evidence was low in the analysis, the relationship between IBS and SIBO had long been strongly suspected. Even recently, there was a report that SIBO plays an important role in IBS[24]. It was also reported that H. pylori eradication improves IBS[25]. In the future, H. pylori eradication treatment may become an important treatment strategy for IBS patients with H. pylori infection.

It has been suggested that dysbiosis may be associated with colorectal carcinogenesis[26], and research on this front is progressing. H. pylori causes dysbiosis, including SIBO, which may be the cause of colorectal cancer. Further research could determine whether H. pylori-induced dysbiosis is associated with colorectal cancer.

Additionally, intestinal mucosal permeability has been reported to be enhanced in H. pylori-infected individuals[27]. We hypothesize that this hyperpermeability of the intestinal mucosa is combined with abnormalities in the intestinal flora, resulting in an increase in small intestinal ulcerative lesions[6]. However, there are very few reports examining the relationship between H. pylori infection and the intestinal flora, so future studies are required.

### EFFECTS OF GASTROINTESTINAL HORMONES

H. pylori gastritis causes atrophic gastritis and reduces gastric acid secretion. Therefore, the blood gastrin concentration increases. A study in rats reported that H. pylori infection altered the levels of gastrin, cholecystokinin, and substance P, resulting in increased colonic motility[28]. This finding suggests the possibility that H. pylori infection could cause gastrointestinal motor dysfunction. H. pylori infection may also cause IBS due to its effects on gastrointestinal hormones.

Moreover, intestinal tract hormones, especially gastrin, are assumed to cause overgrowth in the large intestinal mucosa and to be closely related to large intestinal tumor development[29]. In addition, progastrin, not gastrin, levels are reported to be high in patients with colorectal cancer[30]. In colorectal cancer, the gastrin receptor is overexpressed, and gastrin-binding capacity is increased 10-fold over that in normal colonic epithelium[31]. It has also been reported that the expression of gastrin and its receptor promotes the progression from colorectal adenoma to cancer[32]. In mice, gastrin treatment enhanced colon cancer cell growth and invasion and decreased oxidative stress and apoptosis[33]. Additionally, G-protein coupled receptor 56, which is expressed in colonic stem and cancer cells, is upregulated in transgenic mice overexpressing human progastrin[34]. Thus, although it is experimentally likely that gastrin is involved in colon tumors, a recent patient study found that gastrin was not associated with colon tumors[35]. At this time, it appears that gastrin and VacA could be potential factors in the development of colorectal tumors due to H. pylori infection.
Table 1 Effects and factors of diseases in which Helicobacter pylori may affect the small and large intestines

<table>
<thead>
<tr>
<th>Disease</th>
<th>Impact</th>
<th>Major factors suspected of being involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon adenoma</td>
<td>Increase</td>
<td>Bacterial component, gastrin</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>Increase</td>
<td>Bacterial component (especially VacA), gastrin, dysbiosis</td>
</tr>
<tr>
<td>Small intestinal ulcer</td>
<td>Increase</td>
<td>Mucosal permeability increased, dysbiosis</td>
</tr>
<tr>
<td>Irritable bowel syndrome</td>
<td>Involvement</td>
<td>Gastrointestinal hormones, SIBO</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Decrease</td>
<td>Host immune response, antibacterial drug use</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>Decrease</td>
<td>Host immune response</td>
</tr>
<tr>
<td>Fabry’s disease</td>
<td>Exacerbation</td>
<td>SIBO</td>
</tr>
<tr>
<td>FMF attack</td>
<td>Increase</td>
<td>SIBO</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>Decrease</td>
<td>Immunological effects</td>
</tr>
</tbody>
</table>

SIBO: Small intestinal bacterial overgrowth; FMF: Familial Mediterranean fever; VacA: Vacuolating cytotoxin A.

**IMMUNITY EFFECTS**

*Helicobacter pylori* activates various innate immune system functions\[^{36}\]. The immune system, especially Peyer’s patches in the small intestine, may play an important role in *H. pylori*-induced gastritis because there are reports that gastritis is not induced in *H. pylori*-infected mice lacking Peyer’s patches. Peyer’s patch dendritic cells phagocytose cocoid forms of *H. pylori*. *H. pylori* transforms into a sphere in the anaerobic small intestine and stimulates the host’s immune system via Peyer’s patches\[^{37}\]. Most likely, because of the involvement of this immune system response, a meta-analysis has recently evaluated the association between *H. pylori* infection and systemic lupus erythematosus, rheumatoid arthritis, autoimmune atrophic gastritis, and autoimmune pancreatitis. This study suggested that infection with more virulent strains of *H. pylori* (such as CagA positive) may increase the risk of autoimmune diseases\[^{38}\]. In other words, *H. pylori* infection may be involved in intestinal diseases such as ulcerative colitis and Crohn’s disease.

However, the frequency of ulcerative colitis and Crohn’s disease is lower in *H. pylori*-infected individuals\[^{8-10}\]. Meta-analyses have concluded that the risk of inflammatory bowel disease (IBD) is lower in *H. pylori*-infected individuals\[^{39,40}\]. Furthermore, recent studies have reported that eradication of *H. pylori* under the age of 18 increases the risk of IBD\[^{41}\]. In other words, *H. pylori* infection may be a potentially protective factor against the development of IBD\[^{42}\].

In addition, lymphoma is a neoplastic disease of the immune system, and the fact that gastric MALT lymphoma is relieved by *H. pylori* eradication is well known. Small intestinal MALT lymphoma has been shown to be curable by eradication of *H. pylori*\[^{43}\]. In particular, a study has reported that *H. pylori* eradication is effective in stage 1 MALT lymphoma\[^{44}\]. However, it is unclear how *H. pylori* is involved in MALT lymphoma in the small intestine.

**CONCLUSION**

Multiple studies have reported that *H. pylori* has an effect on neoplastic lesions, ulcerative lesions, autoimmune diseases, and the abnormal gastrointestinal motility of the small intestine and large intestine. Table 1 summarizes the diseases in which *H. pylori* may affect the small and large intestines. Unfortunately, the wording in Table 1 is ambiguous because it is not known exactly how *H. pylori* is involved in these diseases. Although there are generally still few reports on this topic, the most advanced of these is the link between colorectal tumors and *H. pylori* infection. These studies show that *H. pylori* infection is involved in the increased rates of colorectal adenoma and cancer. The involvement of gastrin has been suspected as the reason for this increase in colorectal adenoma and cancer; however, recent studies have reported the involvement of bacterial cell components, such as VacA. In addition to the effects of bacterial components and gastrointestinal hormones, *H. pylori* infection may have various effects on the small and large intestines by causing abnormalities in the
Fujimori S. H. pylori infection on intestinal diseases

intestinal flora and immunological effects. Few studies have reported on this topic, so more studies are needed in the future.

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Orphan patients with inflammatory bowel disease - when we treat beyond evidence

Giuseppe Privitera, Daniela Pugliese, Loris Riccardo Lopetuso, Franco Scaldaferrì, Alfredo Papa, Gian Lodovico Rapaccini, Antonio Gasbarrini, Alessandro Armuzzi

Abstract

Inflammatory bowel disease (IBD) is a chronic condition that requires continuous medical treatment. To date, the medical management of patients with moderately-to-severely active IBD who develop dependence or resistance to corticosteroids is based on immunomodulator drugs. Such therapies are licenced after passing through three phases of randomized controlled trials (RCTs), and are subsequently adopted in clinical practice. However, the real-life population of IBD patients who require these therapies can significantly differ from those included in RCTs. As a matter of fact, there is a number of exclusion criteria – nearly ubiquitous in all RCTs – that prevent the enrolment of specific patients: Chronic refractory pouchitis or isolated proctitis in ulcerative colitis, short-bowel syndrome and stomas in Crohn’s disease, ileorectal anastomosis in both ulcerative colitis and Crohn’s disease, and elderly age are some representative examples. In this frontier article, we aim to give an overview of current literature on this topic.
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**Key Words:** Pouchitis; Proctitis; Stoma; Short-bowel; Ileo-rectal anastomosis; Biologics

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**Core Tip:** Inflammatory bowel disease (IBD) patients with chronic refractory pouchitis, refractory ulcerative proctitis (including those with ileorectal anastomosis), stomas, or short-bowel are routinely excluded from clinical trials, and there is a consequent lack of quality data with regard to their management; however, these patients represent a part of IBD real-life population that needs to be acknowledged. In the present article, our aim is therefore to outline the evidence available so far, and to highlight the main knowledge gaps still present.

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**INTRODUCTION**

Inflammatory bowel disease (IBD) is a chronic condition, with an immune-mediated pathogenesis, that necessitates continuous medical treatment. It can be divided into three main subtypes: Crohn’s disease (CD), ulcerative colitis (UC), and IBD unclassified (IBD-U). Despite this apparently simple classification, it can manifest with a broad range of clinical phenotypes, which are only partially encompassed by Montreal classification. Medical management of patients with moderately-to-severely active IBD who develop dependence or resistance to corticosteroids is based on immunomodulator drugs (i.e., traditional immunosuppressors and targeted therapies, including biologics and small molecules)[1]. Such therapies are licenced after passing through three phases of randomized controlled trials (RCTs), and are subsequently incorporated in clinical practice. However, there is a number of clinical conditions that fall within the spectrum of IBD but are routinely excluded from RCTs. As previously noted by Ha et al[2], inclusion/exclusion criteria of RCTs prevent the enrolment of a significant proportion of IBD patients. In their 2012 work, they observed that only 31.1% of 206 IBD outpatients evaluated for RCTs would have been eligible for enrolment. It should be taken in consideration that, at that time, previous anti-tumor necrosis factor α (TNFα) exposure was a nearly ubiquitous exclusion criterion, and this might have substantially contributed to determining such a low percentage of eligible patients; however, many other criteria still stand to date – such as chronic refractory pouchitis, isolated proctitis, ileorectal anastomosis, short-bowel syndrome, and stomas in CD. Age ≥ 65 years old was an exclusion criterion in all RCTs with anti-TNFα drugs. The GEMINI program (which investigated the use of vedolizumab in moderately-to-severely active IBD) was the first RCT to include IBD patients up to 80 years of age[3, 4]; many - but not all - subsequent trials also extended their upper limit of age for eligibility. However, elderly patients remain an underrepresented population in IBD clinical trials. Some recent progress needs to be recognized, as some RCTs have been conducted for these underrepresented populations (i.e., trials specifically designed for patients with chronic refractory pouchitis). Nevertheless, there is still a significant gap between the real-life IBD population and the population studied to produce evidence: Evidence-based medicine to treat these “orphan patients” is often lacking, and clinicians rely on treatments that have not been studied in these specific forms of IBD.

In this work, we aim to give a comprehensive review on the topic of “orphan IBD patients”, to present evidence available to clinicians and to highlight the main knowledge gaps that need to be fulfilled in the upcoming years.
CHRONIC REFRACTIVE POUCHITIS

Acute pouchitis occurs in up to a half of UC patients who undergo total proctocolectomy with creation of an ileal pouch-anal anastomosis[5]. Antibiotic therapy (metronidazole and ciprofloxacin) is used as first-line treatment, but patients can develop dependence or refractoriness to those; in a similar fashion to what happens with corticosteroids in other forms of IBD, in these latter cases advanced therapies are frequently used. In a retrospective study including 394 patients with ileal pouch, the cumulative incidence of pouchitis was 48% during a 2-year follow-up (29% of patients had isolated acute pouchitis and the remaining 19% developed recurrent pouchitis). Of note, 40% of patients with pouchitis received non-antibiotic therapy (21% mesalamine, 7% immunomodulatory, and 7% anti-TNFα)[6].

The evidence supporting the use of immunosuppressive drugs for the treatment of antibiotic-dependent or -resistant pouchitis is quite scarce, and mostly derived from real-life experiences. Only one clinical trial designed for chronic refractory pouchitis has been conducted in the last years[7], while a few other phases 2 and 3 RCTs are ongoing, either testing novel treatments [faecal microbiota transplantation (FMT), AST-120 (a spherical carbon adsorbent that binds bile acids and bacterial toxins), alicaforsen (an antisense oligonucleotide that targets the mRNA for the production of human intercellular adhesion molecule 1), AMT-101 (a recombinant biologic protein of human interleukin 10)], or drugs already approved for CD and UC (such as vedolizumab[13], ustekinumab[14], and tofacitinib[15]).

In a randomized, double-blind, placebo-controlled trial including 13 patients, adalimumab did not show any significant benefit: Among nine patients who completed the 12-wk study period, the primary outcome [reduction in clinical pouchitis activity index (PDAI) ≥ 2] was met by 50% and 43% of patients in the drug and placebo arms, respectively (P > 0.05); no differences in terms of secondary endpoints were recorded between the two groups[7]. Of note, it should be acknowledged that the sample size might have been insufficient to detect statistically significant differences between the two groups[7]. In a large Canadian cohort of 152 patients (29% with CD-like phenotype of the pouch, and the remaining with chronic refractory pouchitis), the outcomes of those who received infliximab (n = 42) were recorded: Post-induction clinical response rate was 74% (48% achieved remission) and 62.6% of patients reported sustained response[16]. In a 2018 meta-analysis, 313 patients who received anti-TNFα treatment (194 infliximab and 119 adalimumab) to treat inflammatory complications of the pouch (i.e., refractory pouchitis and CD-like complications of the pouch) were identified: After induction, rates of clinical remission were significantly higher in CD-like complications of the pouch (0.64, 95%CI: 0.48-0.78) compared to refractory pouchitis (0.10, 95%CI: 0.00-0.35, P = 0.06), while such a difference disappeared at 12 mo (0.57, 95%CI: 0.43-0.71 for CD-like complications; 0.37, 95%CI: 0.14-0.62 for refractory pouchitis, P = 0.57). Remarkably, no difference between infliximab and adalimumab was observed, besides a numerically higher percentage of patients in clinical remission at 12 mo with infliximab compared to adalimumab (59% vs 30%, P = 0.20)[17].

Recently, data on the effectiveness of vedolizumab for the treatment of chronic refractory pouchitis have also been presented. A systematic review summarized the results from case-reports and case-series including 44 patients who received vedolizumab therapy due to antibiotic-dependent or -resistant chronic pouchitis. Only 52.3% of those patients had been previously exposed to anti-TNFα therapy. Clinical improvement at week 12 was reported by 75% of patients, and endoscopic improvement within 6 mo was recorded in 28 out of 38 (73.7%) patients[18]. Recently, Gregory et al.[19] reported the results of a multicentric, retrospective study where 83 patients received vedolizumab for the treatment of chronic refractory pouchitis: 81.9% of patients had a previous diagnosis of UC, the remaining having CD (10.8%) or indeterminate colitis (7.2%), and 68.7% of them had been previously exposed to anti-TNFα therapy. Clinical response and remission were reported by 71.1% and 19.3% of patients, respectively, while endoscopic improvement was recorded in 54.1% and mucosal healing in 17.6% of them[19].

In 2019, Verstockt et al.[20] reported the results of a cohort of 33 patients who received infliximab (n = 23), adalimumab (n = 13), or vedolizumab (n = 15) for the treatment of refractory pouchitis. Both anti-TNFα and vedolizumab were effective in inducing clinical improvement; interestingly, they observed that patients had a higher risk to discontinue anti-TNFα treatment compared to vedolizumab (HR = 3.0, 95%CI: 1.1-8.8, P = 0.04); this might possibly be attributed to the fact that adverse events accounted for 40.7% of anti-TNFα discontinuations, while no patient withdrew vedolizumab due to safety issues[20].
ISOLATED ULCERATIVE PROCTITIS AND ILEORECTAL ANASTOMOSIS

The mainstay of treatment for ulcerative proctitis is represented by topical aminosalicylates and/or topical steroids. Oral steroids are sometimes used in case of unresponsiveness to topical therapy[21]. Moreover, it has been suggested that oral mesalazine can reduce the risk of disease extension[22]. However, for patients who fail (or do not tolerate) conventional therapies, no evidence-based treatment is available, and clinical management is mainly based on the extrapolation of data from RCTs in UC, which include left-sided colitis and pancolitis – indeed, isolated proctitis is a nearly omnipresent exclusion criterion, as the majority of clinical trials require a disease extension of at least 15 cm from the anal verge.

A 2014 systematic review and meta-analysis concluded that there was not enough evidence to support the use of corticosteroids, thiopurines, and anti-TNFα for the treatment of ulcerative proctitis. Since then, only a few more real-life studies have been published[23]. The effectiveness of thiopurines for the treatment of refractory proctitis has been investigated in a 2017 retrospective study: At the last follow-up evaluation (median time of 46 mo), only 5 out of 25 patients were still on azathioprine treatment, while the remaining 20 were considered treatment failures[24]. Dubois et al[25] published the results of a retrospective study on a large cohort including 118 patients with ulcerative proctitis: 31% of them were refractory to rectal and oral therapy with aminosalicylates and/or steroids, requiring thiopurine monotherapy (19%) and/or biologics (28%, 25 anti-TNFα and 8 vedolizumab). Long-term outcomes pointed at a superiority of biologics over azathioprine for the treatment of refractory ulcerative proctitis, with a rate of clinical response of 70% vs 11% in favour of biologics (P = 0.001)[25]. More recently, a nationwide retrospective study from GETAID investigated the effectiveness of anti-TNFα therapy in 104 patients with refractory proctitis: 50% received infliximab, 39% adalimumab, and 11% golimumab; of note, 45% also received concomitant immunosuppressors. Clinical response was observed in 77% of patients after a median follow-up of 3 mo; the cumulative probability of sustained clinical remission was 86.7%, 74.7%, and 56.4% at 1, 2, and 5 years, respectively. After a median follow-up of 11.7 mo, 60% of the 63 patients with an available endoscopy achieved mucosal healing. Finally, 11 patients with primary nonresponse and 9 with secondary nonresponse to anti-TNFα therapy eventually received vedolizumab (after a second-line therapy with another anti-TNFα) and achieved clinical remission in 82% and 56% of cases, respectively[26].

The evidence becomes even more exiguous when it comes to patients with ileorectal anastomosis who have persistent proctitis. Abdominal colectomy with ileorectal anastomosis represents an alternative to total proctocolectomy with creation of an ileal pouch for the management of refractory UC, to maintain intestinal continuity. To date, ileoanal pouch is considered the gold standard for the surgical management of UC, but ileorectal anastomosis can be proposed to selected patients to postpone the creation of ileal pouch (for instance, in young patients, to postpone pelvic surgery) or when the creation of ileal pouch is not feasible (elderly patients). It has been reported that ileorectal anastomosis seems to be associated with fewer stool movements and night-time evacuations[27,28], and it is usually considered a less complicated procedure, compared to ileo-anal pouch[29]. On the other hand, ileorectal anastomosis comes with two main shortcomings. First, with ileorectal anastomosis, the inflamed rectum is not removed by surgery, so continuation of medical therapy is usually necessary. Indeed, it has been reported, in a retrospective study with 343 patients who received ileorectal anastomosis, that 70% of patients manifested proctitis, 76% of whom developed chronic proctitis[30]. The management of patients with ileorectal anastomosis is similar to those with isolated proctitis, and it is mainly based on topical therapy. In case of refractoriness, proctectomy can be performed to remove the inflamed rectum; otherwise, biologics are sometimes administered, but the experience with their use is extremely limited. The second issue is that ileorectal anastomosis is associated with a non-negligible risk of cancer progression within the rectum[31]. Currently, there are no guidelines for anti-neoplastic surveillance in patients with ileorectal anastomosis, but annual endoscopy is usually recommended.

STOMAS

The presence of an ostomy is an exclusion criterion in RCTs for CD patients. Nevertheless, CD patients with an ostomy are not uncommon: In CD-related surgery, ostomies are usually created in case of urgent procedures (e.g., when the patient has
fistulizing disease complicated by abdominal abscess) or to exclude faecal stream for therapeutic purposes (for instance, in case of medical-refractory perianal disease). Therefore, it would appear reasonable that at least some patients with an intestinal stoma might benefit from the administration of advanced therapies. There are three main scenarios where patients with an ostomy might need advanced therapies. First, when not all of the intestinal segments affected by active CD are resected. These patients usually need medical therapy after surgery, to halt disease progression and prevent additional intestinal resections. Second, to prevent postoperative recurrence. Postoperative recurrence has been usually evaluated in patients with ileocolonic-anastomosis, but can occur after each type of resection in CD[32]. In a 2017 retrospective work, out of 83 CD patients with definitive stoma, 42% of them experienced clinical recurrence after a median follow-up of 28 mo, and 38% needed a subsequent intestinal resection after a median time of 29 mo[33]. Similarly, a recent meta-analysis observed that the median cumulative rate of clinical recurrence, in CD patients with permanent ileostomy after total colectomy, was 23.3% and 40% at 5 and 10 years, respectively[34]. Therefore, even for patients with temporary ileostomy, a prophylactic therapy might be indicated, in order to avoid early recurrences which would make it more difficult for surgeons to restore intestinal continuity. Third, advanced therapies might be required when faecal diversion is used for the management of uncontrollable perianal disease; in such cases, patients might need biological therapy for two reasons: As an additional treatment for perianal disease, or to treat active luminal CD.

The appropriateness of biological therapy in patients with ostomies has not been established. In a 2013 Letter, the outcomes of three patients with acute sever CD colitis and perianal disease managed with ileostomy and anti-TNFα therapy were reported: At the end of follow-up, all patients had undergone total colectomy with creation of permanent ileostomy – of note, one patient achieved complete clinical and endoscopic remission while with stoma, but experienced severe clinical relapse just 1 mo after stoma closure, thus requiring colectomy[35]. In a retrospective study including 21 CD patients undergoing faecal diversion due to severe perianal disease, the authors reported that infliximab use was not associated with an increased likelihood of stoma closure[36]. Similarly, Gu et al[37] reported the outcomes of a cohort of 138 CD patients with ostomies created due to perianal CD, among whom 22% managed to achieve stoma closure: Of note, the authors did not observe that biologic therapy was associated with an improved likelihood of restoration of intestinal continuity. Conversely, another retrospective work including 233 patients who had stomas created because of CD colitis reported different outcomes: The incident risk of permanent ostomy was significantly reduced in post-biological era, compared to before the introduction of biologies (19.2% vs 60.8%, P < 0.001), and biologic use was significantly associated with a higher likelihood of rectal preservation, on multivariate analysis (OR = 3.1, 95%CI: 1.0-9.5, P < 0.05)[38]. Finally, a 2017 systematic review with a meta-analysis of 18 studies (including 1438 patients) observed that, in CD patients with permanent ileostomy after total colectomy, there was no difference in the risk of clinical and surgical recurrence between studies published in pre- and post-biological eras[34].

In regard to patients with temporary ostomies, biologics might also have a downside, due to the theoretical risk that pre-operative biologic use might increase the incidence of post-surgical complications, especially infections. However, such a risk has not been unanimously reported. Notably, two recent meta-analyses showed that neither pre-operative anti-TNFα nor vedolizumab treatment is associated with an increased risk of postoperative infections. In conclusion, whether and how biologic use before the restoration of intestinal continuity might impact the success rates of permanent stoma closure remains unknown.

**SHORT BOWEL**

Short bowel and intestinal failure can manifest as rare but severe complications of CD, either in patients with intestinal continuity who had undergone extensive resections, or due to the creation of proximal stomas. Short bowel syndrome represents an exclusion criterion in RCTs; however, it is not uncommon for patients with short bowel to need advanced therapies, as it can be reasonably assumed that these patients have more aggressive diseases. A history of surgical resection is associated with an increased risk of endoscopic recurrence in CD, and biologics – specifically, anti-TNFα – are used to prevent postoperative recurrence.
The effectiveness of biological therapies in patients with short bowel syndrome has not been proven, yet. Limketkai et al.[41] analysed the National Impatient Sample (a United States national registry of hospitalizations), and evaluated the trends in hospitalizations and small bowel resections in CD patients with short bowel syndrome and intestinal failure (SBS-IF): When comparing the populations before and after the introduction of biologics, the authors did not observe any reduction in the rate of resections among hospitalized patients (0.7 per 1000 CD hospitalizations per year in the pre-biologic era vs 0.6-0.7 per 1000 CD hospitalizations per year in the post-biologic era). It should be acknowledged that the main limitation of this study is the method for the identification of patients with SBS-IF, as the authors included all patients with a diagnosis code of “post-surgical malabsorption”, which might have led to patient misclassifications in some cases[41]. However, in the same study Limketkai et al[41] observed a significant reduction in rates of overall small bowel resections in CD (from 99.0 to 64.6 resections per 1000 CD hospitalizations per year, \( P < 0.01 \)): If this could be applied to patients with CD-related SBS-IF as well, a reduced rate of resections would have been observed in patients with the diagnosis code of post-surgical malabsorption. Another issue that should be taken in proper consideration is that patients with short bowel might have a history of multiple therapeutic failures and multiple resections: As they are not eligible in clinical trials, more advanced therapies, such as the combination of biologics, could also be considered to preserve the residual intestine[42].

Recently, growing attention has been paid to the use of teduglutide – a glucagon-like peptide 2 (GLP2) analogue – in CD patients. Teduglutide is currently licenced for the treatment of SBS-IF patients who are dependent on parenteral nutrition. In a post-hoc analysis of the STEPS study, the efficacy of teduglutide (measured in terms of reduction in weekly parenteral support at week 20) was comparable between patients with SBS-IF secondary to IBD and those who did not have IBD[43]. There has been an initial reluctance to use teduglutide in patients with active IBD, due to the concern that its gut-tropic effect might serve as a pro-inflammatory stimulus and cause IBD exacerbation – indeed, the pivotal STEPS-2 trial only allowed CD patients in stable clinical remission for at least 12 wk and biologics use was an exclusion criterion[44]. A 2017 retrospective study reported the outcomes of 13 CD patients who received teduglutide (8 of whom were on concomitant immunosuppressive therapy): Treatment with teduglutide was effective in reducing the need for parenteral support, but data on gut inflammatory activity were not reported[45]. Contrary to the initial concerns, it has been observed in murine models that teduglutide might actually exert anti-inflammatory effects[46-48]. In 2019, two CD patients who achieved control of intestinal inflammation and reduction of parenteral support while receiving the combination of teduglutide and biologics have been reported[49]. Notably, a CD patient treated only with teduglutide, previously unresponsive to multiple biological therapies, whose CD activity improved in parallel with nutritional status, has also been described[50], suggesting a link between teduglutide administration and clinically relevant anti-inflammatory effects[51].

**ELDERLY PATIENTS**

The elderly IBD population has been constantly expanding in the last decades. Given that IBD is a chronic disease, as life expectancy is prolonging, the prevalence of IBD in the elderly rises as a direct consequence of the ageing of patients combined with an increase of new diagnosis of late-onset IBD (i.e., IBD diagnosed after 65 years of age). The management of elderly IBD patients can be challenging, since this population is characterized by a higher number of comorbidities and an increased risk of adverse events secondary to immunosuppressive treatment. Data from the Veteran’s Health Administration showed that there is an overall reduction of use of both steroids and steroid-sparing agents in elderly IBD patients compared to younger ones[52]. Thiopurine maintenance therapy is usually discouraged in elderly patients, because it correlates with the highest absolute risk of developing lymphomas in patients \( \geq 50 \) years old[53]. Anti-TNFα drugs have not been tested in people older than 65 years old in registration trials; they are generally underused in this population compared to younger patients, and persistence on therapy can be reduced in elderly patients, mostly due to higher rates of serious adverse events.

Vedolizumab was the first IBD drug tested in patients who are 65 to 80 years old; however, only 2-4% of patients enrolled in the GEMINI program were older than 65 years[3,4]. In a post-hoc analysis GEMINI 1 and 2 trials, it was shown that the safety
and effectiveness of vedolizumab were comparable among different groups of patients stratified by age (<35, 35-54, and ≥55 years old) [55]. Due to its gut-selective mechanism of action, anti-integrin therapy seems more appealing to clinicians for the treatment of elderly IBD patients, as non-anti-TNFα treatments seemingly have a more favourable safety profile compared to TNFα inhibitors in IBD. Interestingly, a retrospective study comparing the outcomes of anti-TNFα and non-anti-TNFα treatments in elderly patients did not observe any difference in terms of safety between the two groups, thus questioning whether vedolizumab actually represents a safer choice in elderly patients [55]. A 2020 prospective study conducted on patients starting vedolizumab or ustekinumab showed that Charlson comorbidity index (CCI), but not age, correlated with the occurrence of infections in vedolizumab-treated patients, and with hospitalizations in patients treated with either vedolizumab or ustekinumab [56]. We observed similar results in an Italian multicentric prospective study enrolling over 1000 patients, where elderly CD or UC patients (n = 198) were matched 1:2 to younger ones. In this cohort, a CCI > 2 was associated with a higher risk of developing any adverse events [55]. Finally, in a large cohort study including over 10000 patients, an association between pre-treatment frailty and increased risk of infections was observed in IBD patients treated with TNFα antagonists or immunosuppressors [57]. Evidence on biologic use in patients over 80 years of age is extremely scarce. In a 2020 Letter, Ayoub and colleagues reported the outcomes of 32 patients ≥ 80 years old (median age 82.5 years, range: 80-94) who received anti-TNFα agents (53.1%) or vedolizumab (46.9%); Serious infections occurred in about 15% of patients and three deaths due to cardiorespiratory causes were reported [58].

Another major question regarding the management of elderly IBD patients is whether age might impact treatment efficacy. In a 2020 Italian multicentric study, older patients with both CD and UC showed lower persistence on anti-TNFα treatment compared to younger IBD controls. Lobatón et al [59] found reduced short-term effectiveness in patients ≥65 years old treated with anti-TNFα therapy, but such a difference disappeared after 6 mo. We recently observed that elderly UC – but not CD – patients had significantly worse outcomes in terms of therapy persistence, steroid-free clinical remission and biochemical remission, when compared to matched younger controls [55]. Conversely, Adar and colleagues reported comparable effectiveness of anti-TNFα and vedolizumab in a retrospective cohort of IBD patients ≥ 60 years old [60].

CONCLUSION

There are several categories of orphan patients in IBD, for whom no (high-)quality evidence is available and whose optimal management has not been established, yet. Accordingly, we can try to identify the main knowledge gaps that need to be filled for these specific populations. Whenever possible, RCTs should be preferred as the optimal source for evidence-based medicine; however, when clinical trials are too difficult to perform due to the relative rarity of the conditions, real-life observational studies become crucial to help clinicians in deciding patients’ management. Figure 1 presents the main conditions with “orphan IBD patients” presented in this review and highlights the main issues that need to be addressed in future research.

Chronic refractory pouchitis

Most data on the effectiveness of immunosuppressants come from real-life observational studies, but several RCTs are ongoing; notably, some of these trials include drugs with mechanisms of action that differ from those already licenced for CD and UC. Another major issue is whether a drug that was not effective on IBD before colectomy is ought to be taken in consideration to treat chronic refractory pouchitis in that patient. Finally, the impact of immunosuppressants on pouch failure, as well as their optimal timing for introduction, is yet to be established.

Ulcerative proctitis and ileorectal anastomosis

There is still a significant uncertainty about the appropriateness of biologic use to treat refractory ulcerative proctitis, as most evidence is derived from retrospective studies. It also needs to be addressed whether systemic immunosuppression can help in preventing disease extension: As it has been previously demonstrated that “extenders” tend to have a worse prognosis, preventing disease extension might theoretically have a positive impact on the natural history of some proctitis. However, to avoid unnecessary overtreatment, predictors to stratify patients at higher risk for colitis...
extension should be identified.

**Stomas**

No high-quality data on the effectiveness of biologics in CD patients with stomas exist. Two main issues need to be addressed: (1) Whether biologic treatment reduces the risk of postoperative recurrence in patients with stomas: Therefore, specific risk factors for precocious recurrence in patients with stomas should be investigated; and (2) for patients with temporary stomas, the impact of immunosuppressors on the outcomes of surgery for the restoration of intestinal continuity needs to be evaluated to answer the following questions: Do biologics improve the rates of stoma closures? Does pre-operative biologic use worsen the outcomes of intestinal anastomosis?

**Short bowel**

Data on the effectiveness of biologics in CD patients with short bowel are not available. Crucially, the capability of biologics to prevent further surgery in these patients should be assessed. Encouraging preliminary results on the efficacy of teduglutide in improving the nutritional status of patients who depend on parenteral support have been presented; whether GLP2 analogues might also exert some sort of immunological control over intestinal inflammation is not clear, yet. Finally, more data on safety and effectiveness of combining biologics with teduglutide are required before this strategy can be considered the standard of care for patients with short bowel and active CD.

**Elderly patients**

Evidence on the use of targeted therapies in the elderly IBD population is scarce. Data on treatment effectiveness suggest that there might be a difference between elderly and younger patients, but more studies are needed before any specific recommendation can be made. The major concern regarding the use of immunomodulators in elderly patients is their safety. Results have not been consistent across different reports on whether age is associated with an increased risk of adverse events, nor on the differences among treatments in regard to safety. The lines of evidence available appear to point out that patient’s functional status, rather than chronological age per se, has a clinically meaningful impact on the efficacy and safety profiles of different treatments.

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Analogies between medusa and single port surgery in gastroenterology and hepatology: A review

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Abstract
Single port surgery (SPS) was introduced as an attractive, minimally invasive surgical technique that ensures esthetic results for many types of visceral surgery. Initially, surgeons immediately set about performing SPS without preliminary knowledge or training, which resulted in higher complication rates. Today, current studies conclusively show that SPS is scientifically rehabilitated and indicated for simple and complex laparoscopic procedures. We here describe the astonishing analogies between Greek mythology and modern surgery.

Key Words: Gastroenterology; Single port surgery; Surgical technique; Surgical complication; Gastric or intragastric resections

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Core Tip: Single port surgery (SPS) was introduced as an attractive, minimally invasive surgical technique that ensures esthetic results for many types of visceral surgery. Initially, surgeons immediately set about performing SPS without preliminary knowledge or training, which resulted in higher complication rates. Today, current studies conclusively show that SPS is scientifically rehabilitated and indicated for simple and complex laparoscopic procedures. We here describe the astonishing analogies between Greek mythology and modern surgery.

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INTRODUCTION

One of the most striking analogies between single port surgery (SPS) and the mythological creature Medusa is of course her hair, reminiscent as it is of the four or more prominent, flexible trocars of SP systems. However, a closer look at the mythological story and the development of SPS brings to light many more surprising similarities.

The following brief résumé is by no means historically all-embracing, but necessary for a better understanding of relationships in Greek mythology: The Greek gods had absolute power over mortals and tended to be jealous, quick-tempered and cunning. Many of them had numerous amours, sexual liaisons and scandals. The children resulting from these dalliances were demigods with impressive skills and an adventurous spirit. The skills or assets were commonly applied by these heroes or the gods themselves to their own advantage. Against this background, the following lines serve to outline Medusa’s place in Greek mythology and to bring it into line with developments in SPS.

The modern concept of doing something “under someone’s aegis” means doing something under the protection of a knowledgeable and benevolent power. In Greek mythology the aegis, as described by Homer in 735 BCE, was a device carried by Athena and Zeus. It was interpreted as a shield featuring the hideous head of Medusa with live venomous snakes in place of hair[1].

The Roman poet Ovid portrayed Medusa, the only mortal of the three Gorgon sisters, Medusa, Stheno, and Euryale, as an originally beautiful maiden ravished by Poseidon in Athena’s temple. As one of the first ideological accounts of rape-victim blaming, Athena punished Medusa by transforming her magnificent hair into terrifying snakes[2].

Anyone who gazed into Medusa’s eyes was horror-stricken and turned into stone. Medusa’s head was cut off by the Greek hero Perseus, who then used it for its ability to turn someone to stone. Finally, he gifted the head to Athena to be mounted on her royal shield. In Ancient Greece the image of Medusa’s head appeared on the Gorgoneion, an amulet worn to avert evil[3]. Although modern interpretations of this myth already range widely from psychoanalysis as an oedipal, libidinous symptom[4] to feminism, which depicts Medusa as a guardian of female power[5], we in all humility would now like to emphasize several parallels between SPS and the mythological story of Medusa.

MEDUSA, THE FAIR MAIDEN

SPS was developed to minimize the surgically traumatic approach to the peritoneal cavity for a variety of indications. The concept of SPS is a modified laparoscopy based on a solitary short skin incision allowing all necessary surgical instruments to be delivered in parallel or crossed at the level of the abdominal wall.

Hans Christian Jacobaeus, a Swedish gastroenterologist, is regarded as the first person to have performed SPS in humans in 1910[6].

After Power and Barnes published their findings with laparoscopic tubal ligation in 1941[7], gynecologists were the first to adopt SPS for routine interval tubal ligations from the 1960s on[8]. Although more demanding SPS procedures such as hysterectomies were introduced by gynecologists in 1991[9], the SPS technique still did not win over the surgical community as transumbilically assisted laparoscopic appendectomies or single-port laparoscopic cholecystectomies using several percutaneous suspension sutures were viewed as not yet fully developed[10,11].

It took another decade of technical development to more appreciably comprehend the beauty of the minimized abdominal wall trauma afforded by SPS. The allure of improved cosmesis roused the surgical community to commence feasibility studies for routine procedures, such as appendectomy[12], cholecystectomy[13], fundoplication[14] or benign colon resection[15]. The publication of scientific reports took off during the next years and peaked in 2012 and 2013 (Figure 1). Compared to conventional multiport laparoscopy, SPS is founded on three fundamental preliminaries: first, a special trocar system; second, a lengthening of the optic and some of the instruments; and third, a modified manipulation of the camera and the non-dominant hand of the surgeon. With regard to the abdominal approach, SPS development began with three to four small trocars used side-by-side after being delivered together through one skin incision[15]. The main disadvantage of this technique was permanent leakage of the pneumoperitoneum, thus impeding safe exposure of the surgical field[16]. In addition,
separate fascial defects or specimen retrieval without wound protection significantly increased wound complication rates[16]. The switch from single incision to SPS allowed the pneumoperitoneum to be stabilized. A remarkable variety of single-port devices were introduced, some of them reusable, some of them single-use products. The portfolio ranged from simple home-made ports employing a surgical glove and a wound protector for delivery of all instruments to sophisticated port systems equipped with ambient intraabdominal light, powerful smoke evacuation and routable smart tubing (Figure 2). Delivering all instruments via a fulcrum at the incisional site resulted in a clash of trocar valves or a collision of hands during manipulation. This unpleasant side-effect was at least partially averted by arranging the bulky tubing valves at different distances and by elongating the instruments to enable additional degrees of freedom for the surgeon’s hands. Last but not least, triangulation between the eyes and hands or the target and instruments is fundamental for spatial orientation and dissection. Delivering instruments and the camera together in one line would make triangulation impossible. For this reason, a 30° to 45° optical view is mandatory in SPS. On the other hand, the use of two instruments close to a target requires at least one of the two instruments to be bent and held preferably in the non-dominant hand in order to enable triangulation (Figure 3). The degrees of freedom are comparable to those for conventional multitrocar surgery. Nevertheless, SPS has been seen to involve an increase in wrist rotating movements[17].

While ensuring all these conditions it was possible for the first time to perform standard surgical procedures solely via one nearly invisible incision in the groove of the navel. At first glance, this improved cosmesis would seem to be insignificant in light of the bulk of unsolved problems bothering surgical science. However, aesthetic aspects undoubtedly exert an important impact on quality of life[18] and are underestimated by surgeons who focus solely on overall and disease-free survival.

MEDUSA, WITH HER TWO GORGON SISTERS ON THE ISLE OF SARPEDON

SPS was developed at the same time as two novel surgical approaches, namely tele-manipulating technologies generally summarized under robotic surgery and intraabdominal or intrathoracic procedures performed via a natural orifice, abbreviated as NOTES (natural orifice translumenal endoscopic surgery). In contrast to SPS, which is viewed as an evolutionary development employing standard laparoscopic strategies, the other two concepts revolutionized strategic standards by implementing unconventional instruments and increasing procedural costs. Industrial marketing whipped up
a hype particularly for tele-manipulating techniques and NOTES and as a result the tabloid press reported visionary promises long before SPS, robotic and NOTES achieved evidence-based status. Over the last decades all three techniques have gained scientific justification for various surgical indications[19], but are still far from routine daily use because skills remain underdeveloped without additional training and because of the financial burden and prolonged procedural times.

**MEDUSA, RAVISHED AND BLAMED**

The SPS technique spread to all surgical subdivisions and new applications were created. From 2008, delighted by the first scientific reports[11,20,21], many surgeons immediately felt qualified to perform SPS without in-depth knowledge or training. The resulting disaster saw many procedures fail. Suddenly, SPS was criticized and faulted for providing the negligible benefit of better cosmesis accompanied by an increased hernia rate, prolonged procedural time and possibly increased complication rates in general.

This misunderstanding was prompted by the fallacy that SPS requires a skin incision that is longer than for conventional laparoscopy[20]. A simple comparative
calculation of the incision needed to pass a 10 mm trocar (outer diameter 11mm) vs the incision for introduction of three instruments with an external diameter of 5 mm each refutes this assumption: Notwithstanding the elasticity of the fascial sheaths and the skin, a minimum incision length of more or less 17 mm is needed for a 10 mm trocar and three SPS instruments. The bias against a greater inherent risk for poor wound healing with SPS was led by numerous publications that reported early learning curve data resulting in meta-analysis similar to that in [23]. A minimized incision is particularly beneficial in those types of surgery where small meshes have to be applied in the abdominal cavity for hernia reconstruction or where no or very small specimens have to be removed. A low rate of less than 1% incisional hernias was achieved in our patient cohort by using a multichannel port or a home-made port and closing the sole fascial incision with running sutures made with non-absorbable monofilament (data submitted for publication).

Insufficient exposure of the surgical field was argued to cause an increased rate of intraoperative complications and prolonged procedural times. This is why an inquiry directed at 600 surgeons from all over the world found that they preferred a standard four-port cholecystectomy rather than SPS if they themselves were undergoing the procedure. However, the prime factors in the decision-making process are the surgeon’s inexperience with this technique and the procedure’s safety [24]. Similar to other types of visceral surgery, the best approach for each patient is chosen by the surgeon at his own discretion. Our own experience is slightly different as all our surgeons have the freedom to perform SPS, dual- or multiport laparoscopic cholecystectomy, and we have not observed these techniques to have any differences with regard to procedural times or complication rates. However, as these procedures demand good triangulation and adequate exposure, we do not wait long before implementing suspension sutures and additional trocars in patients or pathologies that are challenging. Obviously, part of the hard-core resistance to SPS may be ascribed to many disconcerting examples of surgical bravado and the onerous need to devote time and energy to on-going skills training with a view to learning efficient execution of the SPS technique.

**MEDUSA, THE POWER AND PERFORMANCE**

SPS has shown equipotent feasibility and safety in many surgical indications as compared to multiport laparoscopy. In particular, some of the following visceral surgery procedures are viewed as standard in our department based on current scientific evaluation.

For appendectomy, recent meta-analyses have demonstrated that patients stay in-hospital less long, return to their jobs sooner and enjoy better cosmesis in the SPS group vis-à-vis briefer operating time and smaller numbers of conversions in the customary three-port groups [25, 26]. We incorporated the technique into our clinical routine in 2009 and have meanwhile successfully performed 903 procedures (Table 1) with no significant technical changes.

Cholecystectomy is undoubtedly the most discussed of all SPS procedures. Despite the many prophets of doom and some terrible outcomes produced by so-called early adopters, the results of more recent trials including some randomised controlled trials and a multicenter trial from Korea are more encouraging. Better cosmesis, less postoperative pain, shorter recovery time and a comparably low rate of adverse events [27, 28] are proven benefits of the method. This is consistent with our personal experience with more than 2200 performed cases. In this context it should be noted that a low threshold for the implementation of intracorporeal retractors, suspending sutures or additional trocars for optimal exposure or safe dissection is mandatory to prevent adverse events in complex cases.

Colorectal resections are some of the most appropriate procedures for SPS for the following reasons: All parts of the colorectal frame are ideally reachable from the umbilicus, but also even bulky specimens can be removed via this incision without destroying the natural shape of the umbilicus. Again, improved cosmesis, less postoperative pain and earlier return to normal life are scientifically proven [29, 30] and, more importantly, oncological safety has been demonstrated [31]. Additionally, recent data suggest an even smaller number of postoperative complications [32]. These findings completely match our own experience, but we would like to add that there are key success factors that, amazingly enough, are underestimated. Meticulous handling of the umbilical incision by means of wound protectors and additional retrieval bags (Figure 4), prudent preparation techniques with reduced shear forces...
Table 1 Numbers of single port procedures performed at the Surgical Department of the Saint John of God Hospital, Salzburg, Austria (from September 2008 - April 2021)

<table>
<thead>
<tr>
<th>SPS Procedures</th>
<th>Procedural numbers</th>
<th>Specific surgical methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholecystectomies</td>
<td>2216</td>
<td>Including intraoperative ERCPs/Cholangiographies</td>
</tr>
<tr>
<td>Inguinal hernia repairs</td>
<td>1850</td>
<td>TAPP/TEP</td>
</tr>
<tr>
<td>Appendectomies</td>
<td>903</td>
<td></td>
</tr>
<tr>
<td>Colorectal resections</td>
<td>798</td>
<td>TME/APR/taTME</td>
</tr>
<tr>
<td>Liver resections</td>
<td>106</td>
<td>Minor/Major hepatectomies</td>
</tr>
<tr>
<td>Small bowel resections</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Gastric resections</td>
<td>49</td>
<td>Oncologic surgery: Gastrectomy with D1 Lymphadenectomy, Partial gastric resections, Transgastric resections, Metabolic surgery: Sleeve gastrectomies, RY-gastric bypass, Omega-loop bypass</td>
</tr>
<tr>
<td>Pancreas resections</td>
<td>29</td>
<td>Distal pancreas resections, Enucleations</td>
</tr>
<tr>
<td>Adrenalectomies</td>
<td>25</td>
<td>Trans-/retroperitoneoscopic approach</td>
</tr>
<tr>
<td>Fundoplications</td>
<td>21</td>
<td>Nissen/Toupet reconstructions</td>
</tr>
<tr>
<td>Other procedures</td>
<td>256</td>
<td>Abdominal wall reconstructions, Adhesiolysis, Nephrectomies, Lymphadenectomies, Splenectomies, Intraabdominal foreign body removals, Adnexitomies, Hysterectomies, Cyst unroovings, Diagnostic laparoscopy, Ligamentum arcuatum resections, Abscess evacuation, Necrosectomies, Neurectomy</td>
</tr>
<tr>
<td>Total</td>
<td>6343</td>
<td></td>
</tr>
</tbody>
</table>

ERCPs: Endoscopic retrograde cholangio-pancreatographies; SPS: Single port surgery; TAPP: Transabdominal preperitoneal hernia repair; TEP: Totally extraperitoneal hernia repair; TME: Total mesorectal rectal excision; APR: Abdominoperineal rectal resection; taTME: Transanal total mesorectal rectal excision.

Figure 4 Specimen extraction is performed using a tear-proof retrieval bag in order to prevent intraabdominal bacteria or tumor cell dislocation during squeezing.

and thorough closing of the fascial and skin defect are mandatory for successful implementation of SPS in colorectal surgery.

Procedures involving the upper gastro-intestinal tract are meanwhile routinely performed, but exclude esophageal resections because intrathoracic dissection is awkward from the umbilicus in general. Gastric or intragastric resections, bariatric procedures and fundoplications are frequently performed with the SP technique and provide good cosmetic results and less postoperative pain at an acceptable level of postoperative complications[33,34,35]. However, the role of SPS in complex oncologic gastric resections with D2 Lymphadenectomy must be scrutinized, as the current
evidence in no way allows a final conclusion to be drawn on minimally invasive surgery in these cases.

In contrast to the aforementioned indications and procedures with a high level of evidence and a comparably large number of surgeons performing these operations, other procedures have met with less acceptance and distribution among the surgical community.

Despite the probable benefit of reduced trauma to the abdominal wall in patients undergoing inguinal hernia repair, the SP technique did not prevail. While experts demonstrated the safety and feasibility of the technique\[36\], the complexity of the method, on the one hand, and the very high standard of modern three-port hernia surgery, on the other hand, may have impeded further attempts to implement it in clinical routine. In contrast, we have successfully performed 1850 cases of SP inguinal hernia repair to date. Although the aforementioned high complexity of the procedure is admittedly undisputed and only strong personal convictions allow someone to make a strategic switch to SPS, the pathology of groin or umbilical hernia is closely linked to a disarrangement of micro-tubular tissue assembly. Therefore, minimizing incisional length, which is done better in SPS than in any other type of laparoscopic or open hernia repair, would strongly advocate SP transperitoneal or total extraperitoneal mesh repair.

Larger incisions required for specimen retrieval would speak in favor of SPS. However, the high complexity of minimally invasive liver resection has delayed its implementation. SPS was seen to be superior to multiport surgery in left lateral sectionectomies with regard to operative time\[37\]. Additionally, our group has demonstrated the feasibility of SP minor liver resection in combination with radiofrequency pre-coagulation\[38\]. The feasibility of SP major resections has been demonstrated by experienced minimally invasive liver surgeons\[39\]. These results were confirmed by a series from our department. Nevertheless, we observed an increased risk of substantial blood loss when bleeding occurs in these complex resections\[40\].

Pancreatic resections generally rank among the most demanding procedures in minimally invasive surgery. This explains the very small number of procedures as well as the small number of published reports, not counting case reports or small case series. Generally, only pathologies at the tail of the pancreas have been treated by means of SPS and even these resections are hampered by reduced degrees of freedom. As a consequence, dual incision laparoscopy was introduced and promoted by some groups to overcome the hurdles of impeded suturing and stapling\[41,42\].

In addition, some procedures favor SPS for its conceptual features although basically all types of laparoscopy with specimen retrieval are good candidates for exemplary performance via a single port.

Intraperitoneal laparoscopic redo surgery is one of the ideal applications for SPS as a single incision permits not only an immediate view, but also delivery of dissection instruments for preexisting adhesions. This efficiency is appreciated to free peritoneal adhesion cords in small bowel obstruction or assist in abdominal wall repair for incisional hernia.

As every incision entails the risk for acute or chronic complications, the SPS concept should support all types of possible repetitive intraperitoneal procedures such as hepatic or lymphonodular metastasectomies in oncologic surgery and preserve the fitness of patients undergoing exhausting polychemotherapies, where staging laparoscopies are required. Indeed, after one century of modern surgery the latter indication has proven a valuable reminder of the early days of SPS. On the other side of the street from oncologic diseases, palliative decompression procedures, such as gastro-enterostomies in gastric outlet obstruction for cancer, can ideally be performed by means of SPS with minimal trauma to patients having limited length and quality of life.

Furthermore, SPS brings momentum to an alternative situation: metabolic surgery for morbid obesity is undoubtedly one of the most important medical interventions available in the fight against the pandemic of obesity and diabetes in the common world. Sadly, society unjustly denounces morbidly obese patients for their psychological weakness. Fear of outing oneself for needing surgical help to cope with obesity as well as fear of invasive surgery itself rob many of these patients of the opportunity to obtain proper treatment. SPS provides a tool for safe metabolic surgery with minimal trauma so as to prevent telltale surgical scars. In this way, patients are protected from the psychological wrath of society for being overweight and for lacking mental or behavioral fortitude.

At the same time, it is mandatory that the limitations of the SPS concept be made known.
Mean procedural times of appendectomies (left) and cholecystectomies (right) during the learning curve of 10 single port laparoscopies for novices ($n=3$) and surgeons trained in conventional multitrocar surgery ($n=5$) did not differ significantly (by means of Two-way ANOVA) between the groups.

First, different targets that are spaced far apart and that can hardly be reached from one incision (e.g., left colonic flexure mobilization in deep rectal resection operated via incision of the protective ileostomy or the intrathoracic esophageal dissection required in fundoplication for gastro-esophageal reflux disease) are obvious limitations.

Second, it is recommended that surgical procedures requiring wound drainage not be performed via the umbilicus because of the risk of wound complications in the longer term. Therefore, for dual-port laparoscopy in distal pancreas resections an additional port away from the umbilicus is recommended for the drain, as described above[41,42].

MEDUSA, THE AEGIS WITH HER FEARED HEAD

The knowledgeable and benevolent skills that are acquired when performing SPS are described in many reports made by expert surgeons[43]. Better skills for both conventional laparoscopy and SPS are acquired by experienced laparoscopic surgeons, who pass the learning curve for this uncommon minimally invasive technique. On the other hand, SPS training is even less arduous for surgical novices as they are not hampered by the consolidated motion patterns typical of multiport laparoscopy. We have demonstrated that standard SPS procedures can be performed at the same level of expertise by residents and senior surgeons alike (Figure 5).

Furthermore, SPS also generates skills that are useful for other indications. For example, transanal rectal resections, such as transanal total mesorectal rectal excision (taTME) would never have become reality if SPS had not laid the groundwork. Moreover, current techniques of abdominal wall reconstruction (e.g., SPS-enhanced view total extraperitoneal patch hernia repair; eTEP) benefit from small incisions enabled by SPS. Transgastric SPS for oncologic resections of gastrointestinal stroma tumors in rendez-vous technique with flexible endoscopy, on the one hand, and transumbilical resection of hepatic segments 2,3 or 7,8 using inline precoagulation or single-port retroperitoneoscopic adrenalectomy, on the other hand, are the bright and shining pieces of the novel surgical mosaic made possible by SPS skills.

Although marketing experts tout telemanipulation as an unrivalled technique, multiport robotic surgery itself has to date hardly accomplished the aims of improved patient outcome in visceral surgery. Single-port telemanipulation with SP robotics, the newest innovation in this field, is already undergoing clinical evaluation and should soon realize the vision of wide-use reduced port surgery. Advantages over conventional laparoscopy for both patients and surgeons who wish to reduce abdominal wall trauma would be the strongest argument for its large-scale implementation.
CONCLUSION

SPS is an attractive minimally invasive surgical technique that ensures esthetic results for many types of simple and complex visceral surgery. Having traversed the valley of tears when SPS was disdained as inappropriate for use (blame the victim) and criticized by jealous, quick-tempered and big-headed surgical opinion leaders as a rubbish technique, SPS is scientifically rehabilitated when it is performed by surgeons who are willing to tackle the learning curve. Under the aegis of SPS, surgeons have the opportunity to become more accomplished in their professional routine and to finally add the SPS gem to their armamentarium for special indications. Gianni Versace, founder of the Italian luxury fashion label, chose Medusa as his logo because he knew that once people looked his products in the eye, they would be spellbound by their beauty and perfection.

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Chinese expert consensus on neoadjuvant and conversion therapies for hepatocellular carcinoma

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Grade D (Fair): 0
Grade E (Poor): 0

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Abstract

The low resection and high recurrence rates in hepatocellular carcinoma (HCC) are the major challenges to improving prognosis. Neoadjuvant and conversion therapies are underlying strategies to overcome these challenges. To date, no guideline or consensus has been published on the neoadjuvant and conversion therapies in HCC. Recent studies showed that neoadjuvant therapy for resectable HCC and conversion therapy for unresectable HCC are safe, feasible, and effective. Neoadjuvant and conversion therapies have the following advantages in treating HCC: R0 resection with sufficient volume of future liver remnant, relatively simple operation, and wide applicability. Therefore, it was necessary to conduct a widely accepted consensus among the experts in China who have extensive expertise and experience in treating HCC using neoadjuvant and conversion therapies, which is important to standardize the application of neoadjuvant and conversion therapies for the management of HCC. The strategies of neoadjuvant therapy include the selection of the eligible patients, therapy regimen, cycles, effect evaluations, and multidisciplinary treatment. The management of patients with insufficient volume of future liver remnant and patients who cannot achieve R0 resection is the key to the strategies of conversion therapy. Here, we present the resultant evidence- and experience-based consensus to guide the application of neoadjuvant and conversion therapies in clinical practice.

Key Words: Consensus; Hepatocellular carcinoma; Neoadjuvant therapy; Conversion therapy

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## Table 1 Analysis of surgical data of 10966 patients with primary hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Stages and criteria</th>
<th>n (%)</th>
<th>Median time (mo)</th>
<th>1-yr survival rate (%)</th>
<th>3-yr survival rate (%)</th>
<th>5-yr survival rate (%)</th>
<th>10-yr survival rate (%)</th>
<th>Median time (mo)</th>
<th>1-yr recurrence rate (%)</th>
<th>2-yr recurrence rate (%)</th>
<th>3-yr recurrence rate (%)</th>
<th>5-yr recurrence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First round of hepatectomy for HCC</td>
<td>2592</td>
<td>79.4</td>
<td>87.2</td>
<td>71.0</td>
<td>59.1</td>
<td>40.4</td>
<td>36.1</td>
<td>31.8</td>
<td>43.5</td>
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<td>60.7</td>
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<tr>
<td>Stages Ia-IIIa</td>
<td>2549</td>
<td>82.3</td>
<td>88.1</td>
<td>71.9</td>
<td>60.0</td>
<td>41.0</td>
<td>37.0</td>
<td>31.0</td>
<td>42.9</td>
<td>49.3</td>
<td>60.3</td>
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<tr>
<td>Ia</td>
<td>1175</td>
<td>-</td>
<td>96.5</td>
<td>87.5</td>
<td>77.2</td>
<td>55.9</td>
<td>67.2</td>
<td>15.0</td>
<td>27.7</td>
<td>34.1</td>
<td>45.9</td>
</tr>
<tr>
<td>Ib</td>
<td>635</td>
<td>79.4</td>
<td>88.2</td>
<td>73.5</td>
<td>62.5</td>
<td>37.0</td>
<td>34.1</td>
<td>32.4</td>
<td>43.7</td>
<td>51.8</td>
<td>63.4</td>
</tr>
<tr>
<td>IIa</td>
<td>205</td>
<td>43.5</td>
<td>89.8</td>
<td>56.0</td>
<td>40.8</td>
<td>27.2</td>
<td>15.7</td>
<td>45.7</td>
<td>59.8</td>
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<td>84.3</td>
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<tr>
<td>IIb</td>
<td>119</td>
<td>38.0</td>
<td>83.6</td>
<td>55.3</td>
<td>37.4</td>
<td>23.2</td>
<td>10.0</td>
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<td>67.7</td>
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<td>80.1</td>
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<td>IIIa</td>
<td>415</td>
<td>21.9</td>
<td>65.1</td>
<td>38.2</td>
<td>23.8</td>
<td>16.0</td>
<td>7.9</td>
<td>59.9</td>
<td>69.8</td>
<td>73.8</td>
<td>80.2</td>
</tr>
<tr>
<td>IIIb</td>
<td>43</td>
<td>8.7</td>
<td>34.1</td>
<td>15.8</td>
<td>0</td>
<td>0</td>
<td>3.9</td>
<td>77.1</td>
<td>77.1</td>
<td>82.8</td>
<td>-</td>
</tr>
</tbody>
</table>

Overall survival and tumor recurrence of patients with different stages of hepatocellular carcinoma after undergoing hepatectomy[8]. Stages and criteria; overall survival; tumor recurrence; n; median time (months); 1-yr survival rate (%); 3-yr survival rate (%); 5-yr survival rate (%); 10-yr survival rate (%); median time (months); 1-yr recurrence rate (%); 2-yr recurrence rate (%); 3-yr recurrence rate (%); 5-yr recurrence rate (%); first round of hepatectomy for hepatocellular carcinoma; stages Ia-IIIa. HCC: Hepatocellular carcinoma.

**Therapies for Hepatocellular Carcinoma (2021 edition)** to provide additional reliable suggestions for preoperative decision making according to the features of diagnosing and treating liver cancer in China and to standardize those therapeutic methods for their universal popularization.

**CONCEPTS AND TARGETS OF NEOADJUVANT AND CONVERSION THERAPIES**

According to the treatment targets, preoperative treatments for liver cancer include neoadjuvant therapy for resectable liver cancer and conversion therapy for unresectable liver cancer. These two treatments are distinguished by the achievement of R0 resection and are different from the aspects of study subjects, treatment targets, and treatment regimens.

**Neoadjuvant therapy**

Neoadjuvant therapy refers to the interventions including systemic or local treatments for liver cancer patients with technically resectable tumors [R0 resection, with sufficient volume of the future liver remnant (FLR)] and a high risk of recurrence, which could reduce the tumor size, eliminate the undetectable minimal lesions, and increase the negative surgical margins as early as possible, thereby attenuating the incidence of postoperative complications[12]. At present, only limited high-grade evidence for treating liver cancer via neoadjuvant therapy is available. Generally, in clinical practice, neoadjuvant therapy is not directly recommended for patients with CNLC stage Ia or Ib and some patients with CNLC stage IIa. However, because such patients are accompanied by a high risk of recurrence and require neoadjuvant therapy, the therapy can be performed in clinical trials after approval of the study protocol by ethic committees. There are substantial controversies regarding whether surgeries can be directly performed for patients with CNLC stage IIb or IIIa (i.e. the resection is technically applicable). The current recommendation is to benefit surgery after neoadjuvant therapy to reduce the postoperative recurrence rate.

**Conversion therapy**

Unresectable liver cancer refers to liver cancer that could not be safely resected, including intolerable liver functions, insufficient volume of FLR, and disability to ensure the negative surgical margins or zero residual lesion (i.e. achieving R0
Conversion therapy refers to the use of interventions to convert unresectable tumors into resectable ones, which includes the conversion of surgically unresectable lesions, such as insufficient volume of FLR, to resectable lesions, and it supports the conversion of R1 and R2 resection to R0 resection.

According to the Chinese Expert Consensus on Neoadjuvant and Conversion Therapies for Hepatocellular Carcinoma (2021 edition), existing evidence supports conversion therapy, which can be applied in clinical practice after a comprehensive evaluation of patients’ clinical conditions.

STRATEGIES FOR CONDUCTING NEOADJUVANT THERAPY

Eligible patients for neoadjuvant therapy
Neoadjuvant therapy aims to reduce cancer recurrence. According to the Chinese guidelines, neoadjuvant therapy can be performed for patients with initially resectable liver cancer (including CNLC stage Ia-IIa/BCLC stage A, or beyond the BCLC criteria, while being still resectable) and for those patients who are at a high risk of postoperative recurrence. As the high-grade evidence for neoadjuvant liver cancer therapy is limited, neoadjuvant therapy is not recommended for patients with CNLC stage Ia or Ib and for some patients with stage IIa (i.e. R0 resection can be directly achieved). However, if the comprehensive evaluation of the patients’ clinical conditions (i.e. patients at high risk of postoperative recurrence and uncertainty for R0 resection) suggests that neoadjuvant therapy can be performed, the therapy is recommended to be conducted in clinical trials after ethical approval. For patients with technically resectable CNLC stage IIb or IIIa liver cancer, while those are at a high risk of recurrence, neoadjuvant therapy is recommended preoperatively to reduce the incidence of postoperative recurrence.

The documented high risk of liver cancer recurrence includes macroscopic cancer embolus, microvascular invasion, multiple tumors, satellite nodules, and lymph node metastases[13]. With the elevation of the CNLC stage, the risk of recurrence and metastasis after surgical resection also increases[2]. Therefore, multiple examinations should be carried out preoperatively to evaluate the risk of postoperative recurrence. For patients at a high risk of postoperative recurrence, neoadjuvant therapy could control the development of metastatic cancer lesions and improve patients’ prognoses with adequate resection margins.

Cycles of neoadjuvant therapy
During neoadjuvant therapy, the patients might not be eligible for surgery due to contraindications to surgery, such as disease progression, postoperative toxicity, and other severe adverse effects[2]. Therefore, the management of preoperative cycles of neoadjuvant therapy is critical to ensure that the treatment targets can be met within a limited period, thereby minimizing the “failure rate” of neoadjuvant therapy.

It is recommended that the duration of neoadjuvant therapy should be 1.5-3 mo (no longer than 4 mo), and surgery should be performed as early as possible after the treatment targets have been met (regardless of the regression of lesions)[2,14]. An appropriate individualized treatment regimen can be selected according to the lesions’ locations, general conditions, and hepatic function reserve. Importantly, safe treatment methods are vital to avoid negative influences on the upcoming surgeries (Table 2).

Methods of neoadjuvant therapy
Interventional therapy: Transarterial chemoembolization (TACE): Various studies have shown that preoperative TACE does not improve the survival of patients with resectable liver cancer[15-17]. In a meta-analysis by Qi et al.[18], 22 randomized and non-randomized studies were included. Their findings showed that preoperative TACE does not improve patient DFS or overall survival (OS). Subgroup analyses showed that the effects of surgery after neoadjuvant TACE were associated with the responses to TACE[18].

Hepatic arterial infusion chemotherapy (HAIC): An embolic agent is not used in the FOLFOX-based HAIC treatment. Thus, the treatment only induces relatively mild inflammatory responses, and certain advancements in the field of neoadjuvant therapy have been achieved[19]. In a Chinese phase III clinical trial, neoadjuvant HAIC was used to treat BCLC stage A/B liver cancer patients that exceeded the Milan criteria for liver transplantation, and it showed that the pathologically complete remission rate was 10.1% and objective remission rate was 63.6%, according to the Modified
**Table 2 Evidence of studies on the neoadjuvant therapy**

<table>
<thead>
<tr>
<th>Treatment regimen</th>
<th>Study design</th>
<th>Number of patients</th>
<th>Treatment cycle</th>
<th>Study outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apatinib + camrelizumab[45]</td>
<td>Phase II</td>
<td>20</td>
<td>6 wk</td>
<td>MPR: 29.4%. PCR: 5.9%</td>
</tr>
<tr>
<td>Cabozantinib + nivolumab[46]</td>
<td>Phase I</td>
<td>15</td>
<td>8 wk</td>
<td>12 patients received R0 resection, and MPR or PCR was found in 5 patients (41.7%)</td>
</tr>
<tr>
<td>Toripalimab ± lenvatinib</td>
<td>Phase Ib/II</td>
<td>16</td>
<td>21-28 d</td>
<td>3 patients (20%) with MPR</td>
</tr>
<tr>
<td>Ipilimumab + nivolumab[47]</td>
<td>Phase Ib</td>
<td>7</td>
<td>6 wk</td>
<td>ORR of 20%; of the 5 patients with pathologically assessable tumors, 3 (60%) were found with pathological remission</td>
</tr>
<tr>
<td>Ipilimumab ± nivolumab[48]</td>
<td>Phase II</td>
<td>30</td>
<td>6 wk</td>
<td>Pathological remission rate: 30% (8/27), MPR: 11% (3/27), PCR: 19% (5/27)</td>
</tr>
</tbody>
</table>

MPR: Major pathological response; PCR: Pathological complete response; ORR: Objective remission rate.

Response Evaluation Criteria in Solid Tumors criteria. Compared with the direct resection group (n = 100), the OS and progression-free survival in the neoadjuvant HAIC group (n = 99) both improved significantly (3-year OS rate: 63.5% vs 46.3%, P = 0.016; median progression-free survival: 14.1 mo vs 8.9 mo, P = 0.017), while the recurrence-free survival was not significantly different between the two groups[20] (grade of evidence: 2A). Another retrospective study showed that neoadjuvant HAIC reduced the risk of recurrence in patients at a high risk of hepatocellular carcinoma and improved patient survival rates. Compared with the control group, the 1-, 3-, and 5-year DFS rates (100%, 78.6%, and 78.6% vs 65.8%, 33.7%, and 26.6%, respectively, P = 0.003) and OS rate (100%, 100%, and 100% vs 91.7%, 77.8%, and 55.3%, respectively, P = 0.037) in the neoadjuvant HAIC group were significantly higher[21] (grade of evidence: 2B).

**Radiotherapy:** In a Chinese randomized controlled trial, the patients were randomized to hepatectomy plus neoadjuvant radiotherapy (n = 82) or hepatectomy alone (n = 82). The trial showed that for patients with resectable tumors accompanied with portal vein tumor thrombus, preoperative three-dimensional conformal radiotherapy could effectively improve the postoperative survival of the patients. Regarding patients in the hepatectomy plus neoadjuvant radiotherapy group, partial remission was achieved in 17 (20.7%) patients, and the 6-, 12-, 18-, and 24-mo OS rates were 89.0%, 75.2%, 43.9%, and 27.4% vs 81.7%, 43.1%, 16.7%, and 9.4% in the hepatectomy alone group, respectively (P < 0.001). In patients in the hepatectomy plus neoadjuvant radiotherapy group, the 6-, 12-, 18-, and 24-mo DFS rates were 56.9%, 33.0%, 20.3%, and 13.3% vs 42.1%, 14.9%, 5.0%, and 3.3% in the hepatectomy alone group, respectively (P < 0.001) [22] (grade of evidence: 2A).

In a Chinese retrospective study, 11920 patients (of whom 134 received neoadjuvant radiotherapy) were included. The adjusted 5-year OS rates in the neoadjuvant radiotherapy and surgery groups were 65.3% and 46.6%, respectively. The results of the adjusted Cox proportional-hazards regression analysis showed that neoadjuvant radiotherapy was significantly associated with longer OS (hazard ratio: 0.549; 95% confidence interval: 0.327-0.921; P = 0.023). In addition, the subgroup analysis showed that patients with N0 disease, alpha-fetoprotein-positive, and aged < 65-years-old could benefit more from the therapy[23] (grade of evidence: 2B).

Another Chinese retrospective study in patients with resectable liver cancer showed that neoadjuvant radiotherapy was associated with long-term patient survival[24] (grade of evidence: 2B).

**Systemic therapy:** The increasingly abundant systemic therapies have provided new ideas for neoadjuvant therapy of liver cancer. Several studies reported preliminary data regarding applying systemic therapy in neoadjuvant therapy. At the same time, the postoperative consequences of the treatment regimens need to be further verified by large-scale clinical studies.

**Recommendations of the consensus**

At present, the lack of high-grade evidence for neoadjuvant therapy is noteworthy. In addition, all patients will not benefit from neoadjuvant therapy[2]. In clinical practice,
directly performing neoadjuvant therapy is not recommended for patients with early-stage liver cancer. Assessment of data by multidisciplinary treatment (MDT) is necessary to predict the risk of postoperative recurrence and metastasis and explore whether patients could benefit from neoadjuvant therapy. When the main objective of neoadjuvant therapy is to reduce the risk of recurrence, the treatment cycles are strictly limited, and relatively safe treatment regimens with fewer adverse effects on the surgery are preferred.

**STRATEGIES FOR CONDUCTING CONVERSION THERAPY**

The main objective of conversion therapy is to eliminate the unresectable factors of liver cancer, thereby satisfying the surgical criteria for safely performing R0 resection. Specifically, conversion therapy is characterized by active treatment regimens to convert risky surgeries into safe surgeries and change unresectable tumors into radically resectable tumors.

The cycles of conversion therapy could be longer than neoadjuvant therapy to meet the treatment targets, and the treatment duration is not strictly limited. If patients cannot tolerate the therapy or cannot alternatively undergo radical surgeries, the treatment regimen needs to be adjusted after a comprehensive evaluation.

**Conversion therapy for patients with insufficient volume of FLR**

**Patients receiving conversion therapy for an insufficient volume of FLR:** The conversion therapy for an insufficient volume of FLR aims to eliminate the unresectable factors from the surgical aspect. Therefore, the target group is the CNLC grade Ia-IIIb liver cancer patients with insufficient volume of FLR after radical therapy.

At present, the criteria for evaluating the functional hepatic reserve for safe resection are generally identical in various centers worldwide. The criteria are as follows: For patients with normal liver function (Child-Pugh class A), indocyanine green-R15 < 10%, and without liver cirrhosis, the FLR/standard liver volume (SLV) needs to be > 20%-30%; for patients accompanied with chronic liver diseases or hepatic parenchyma injuries (e.g., liver cirrhosis, severe fatty liver, and chemotherapy-induced liver injury), the FLR/SLV needs to be > 40%; for patients with liver dysfunction, a higher FLR is required (e.g., for patients with chronic liver diseases or cirrhosis and IGG-R15 of 10%-20%, the FLR/SLV needs to be > 50%)\[1,4\]. Patients who do not meet these criteria are considered with insufficient volume of FLR.

Considering the contraindications or complications, the treatments should be strictly limited to the following patients: Aged < 65-years-old, with normal liver function (Child-Pugh class A, indocyanine green-R15 < 10%), with insufficient volume of FLR (e.g., FLR/SLV < 30% for patients with normal liver and FLR/SLV < 40% for patients with chronic liver diseases or liver damages), in good general conditions, with good tolerability to surgery, without severe liver cirrhosis, severe fatty liver, or severe portal hypertension\[1\].

**How to reach the target of conversion therapy for an insufficient volume of FLR:**

Insufficient volume of FLR after tumor resection can lead to an extremely high risk of postoperative liver failure. Therefore, the conversion therapy for an insufficient volume of FLR should reach the following treatment targets: Using specific methods to promote the rapid increase of liver volume and convert the insufficient volume of FLR to a sufficient volume of FLR. After meeting the requirements of hepatectomy, the conversion therapy should convert the liver cancer from unresectable to safely resectable (i.e., converting a risky surgery to a safe surgery).

At present, the treatments for insufficient FLR volume include a two-stage hepatectomy procedure combined with portal vein embolization or portal vein ligation, a two-stage hepatectomy procedure combined with portal vein embolization and TACE/hepatic vein embolization, and associating liver partition and portal vein ligation for staged hepatectomy\[4\]. In addition, the insufficient FLR volume in some patients is caused by giant tumors, and a one-stage radical resection can substantially influence the FLR volume (e.g., possibly damaging blood vessels or bile ducts)\[4\]. For such patients, a non-surgical therapy (e.g., local therapy or systemic therapy plus local therapy) is effective in reducing tumor size, thereby decreasing the range of resection and increasing the FLR volume [for more details, please refer to section 4.2 (Conversion therapy for patients who are unable to achieve R0 resection)].
For detailed recommendations on selecting appropriate treatment strategies and perioperative management, please refer to the Chinese Expert Consensus on Conversion Therapy in Hepatocellular Carcinoma (2021 edition). The appropriate treatment methods should be selected according to the tumor type, status of local tumor progression, pathological finding of liver parenchyma, liver functional reserve, and patient tolerability to systemic surgery.

**Conversion therapy for patients who are unable to achieve R0 resection**

Patients receiving conversion therapy for being incapable of R0 resection: The target patients of the conversion treatment include patients who cannot initially achieve R0 resection due to the tumor burden. Evidence suggests that the efficacy of liver tumor resection in patients who cannot achieve an R0 resection is not significantly higher than non-surgical treatment [7, 26–28]. For patients who cannot achieve R0 resection due to causes such as an extremely large tumor volume or blood vessel invasion, conversion therapy can be performed to provide a surgical opportunity for R0 resection, thereby improving long-term efficacy [27].

**Reaching the target of conversion therapy for patients who cannot achieve R0 resection**: The overall target is to decrease the tumor volume and tumor stage (i.e., reducing the volume and number of primary lesions and eliminating portal vein tumor thrombus and metastatic lesions), leading to radical resection [29, 30].

The criteria of diagnosing resectable lesions after conversion therapy are as follows: Reduction of tumor volume or tumor stage, complete microvascular tumor thrombus necrosis, evaluation of complete/partial remission, and stable disease lasting for 3-4 mo (according to Modified Response Evaluation Criteria in Solid Tumors criteria) [1].

The details of different conditions are as follows: (1) Intrahepatic lesions: For patients with a giant tumor or a remarkable number of lesions, surgery is risky or is accompanied by surgical difficulties, and the target of a successful conversion is to reduce the number of lesions, ensuring negative surgical margins and decreasing surgical difficulty, according to the Modified Response Evaluation Criteria in Solid Tumors criteria. For patients with vascular invasion who cannot achieve R0 resection, the target of a successful conversion is spontaneous necrosis or regression of malignant tumors, complete microvascular tumor thrombus necrosis, and reduction of tumor stage (e.g., from CNLC stage IIIa to IIb); and (2) Extrahepatic metastasis: For patients with extrahepatic (mainly lung) metastasis (CNLB stage IIIb), the target of a successful conversion is to eliminate the metastatic lesions, reduce the tumor stage, and perform the surgical resection of the intrahepatic lesions.

To date, numerous explorative studies on conversion therapy have been performed, of which the treatment regimens include local therapy [31–36], systemic therapy [37], and combination therapy [38–42] (Table 3). Concerning the absence of a potent systemic treatment, TACE is the major method for conversion therapy [1]. Compared with traditional TACE, drug-eluting bead-TACE continuously releases chemotherapeutic drugs at a fixed dose with outstanding controllability, prolonging the treatment time between the cancer cells and the chemotherapeutic drugs as well as avoiding liver microcirculation injury. A Chinese cohort study on 32 patients with unresectable hepatocellular carcinoma showed that after drug-eluting bead-TACE treatment, the success rate of stage reduction was 59.4%; however, after subsequent radical therapy (surgery or ablation), the rate of complete remission was as high as 81.3%. The successful stage reduction using drug-eluting bead-TACE is associated with longer survival [43]. Several scholars demonstrated the effects of HAIC, selective internal radiation therapy, and radiotherapy for conversion therapy [1]. The rapid advances in systemic therapy also provide new ideas for conversion therapy. A great number of studies are currently available to support the application of systemic therapy plus local therapy in conversion therapy (Supplementary Tables 1 and 2). We recommend that a gradually progressing treatment strategy be performed according to the currently available treatment standards, taking both efficacy and safety of treatment into account. For detailed recommendations on selecting treatment methods and timing of surgical resection following conversion therapy, please refer to the Chinese Expert Consensus on Conversion Therapy in Hepatocellular Carcinoma (2021 edition) [1].

**NECESSITY OF MDT FOR NEOADJUVANT AND CONVERSION THERAPIES**

Due to the heterogeneity of liver cancer and the features of the multi-modal MDT,
### Table 3 Evidence for systemic therapy plus local therapy in conversion therapy

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Treatment regimen</th>
<th>Number of patients, n (%)</th>
<th>Study results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhang et al[37], 2020</td>
<td>TKI: Lenvatinib; PD-1 antibody: Pembrolizumab/Sintilimab/Toripalimab</td>
<td>33</td>
<td>Success rate of conversion (imaging): 42.4%. Actual surgery rate following conversion: 30.3%</td>
</tr>
<tr>
<td>Li et al[40], 2017</td>
<td>TACE + sorafenib</td>
<td>142</td>
<td>Second-stage resection rate following stage reduction: 14.8%</td>
</tr>
<tr>
<td>He et al[39], 2019</td>
<td>HAIC + sorafenib</td>
<td>125</td>
<td>Surgical resection rate following conversion: 12.8%</td>
</tr>
<tr>
<td>He et al[41], 2018</td>
<td>HAIC + sorafenib</td>
<td>35</td>
<td>Surgical resection rate following conversion: 14.3%</td>
</tr>
<tr>
<td>Zhang et al[42], 2021</td>
<td>HAIC + TKI + PD-1 antibody (1, 7, and 17 patients used sorafenib, apatinib, and lenvatinib, respectively)</td>
<td>25</td>
<td>Surgical resection rate following conversion: 56.0%, 7 patients (28.0%) achieved pathologically complete remission</td>
</tr>
<tr>
<td>He et al[38], 2021</td>
<td>Lenvatinib + toripalimab + HAIC</td>
<td>71</td>
<td>Surgical resection rate following conversion: 12.7%</td>
</tr>
</tbody>
</table>

Grade of evidence in the guideline of Chinese Society of Clinical Oncology diagnosis and treatment. Feature of evidence; grade; level; sources; Chinese Society of Clinical Oncology degree of expert consensus; 1A: High, rigorous meta-analysis, large-scale randomized clinical study, unified consensus (supportive opinion: ≥ 80%); 1B: High, rigorous meta-analysis, large-scale randomized clinical study, generally unified consensus, with slight controversy (supportive opinion: 60%-80%); 2A: Slightly low, fair-quality meta-analysis, generally unified consensus, with slight controversy (supportive opinion: 60%-80%) or small-scale randomized clinical study, well-designed large-scale retrospective study, case-control study, unified consensus (supportive opinion: ≥ 80%); 2B: Slightly low, fair-quality meta-analysis, small-scale randomized clinical study, well-designed large-scale air-quality meta-analysis, generally unified consensus, with slight controversy (supportive opinion: 60%-80%) or small-scale randomized clinical study, well-designed large-scale retrospective study, case-control study; generally unified consensus, with slight controversy (supportive opinion: 60%-80%); 3: Low, non-controlled single-arm clinical study, case report, expert opinion, no consensus, with low substantial controversy (supportive opinion: < 60%). TKI: Tyrosine kinase inhibitor; PD-1: Programmed cell death protein-1; TACE: Transarterial chemoembolization; HAIC: Hepatic arterial infusion chemotherapy.

MDT is essential for neoadjuvant and conversion therapies. Therefore, it is important to form a relatively constant multidisciplinary team to make an individualized treatment decision for liver cancer patients. Before starting a neoadjuvant therapy, preoperative evaluation by a multidisciplinary team is required to predict the risk of postoperative recurrence and metastasis and to indicate whether patients can benefit from the neoadjuvant therapy. During the neoadjuvant and conversion therapies, the treatment methods and measurements, the timing of surgery after treatment, and management of adverse events during the treatment need to be discussed repeatedly among the multidisciplinary team members due to the advantages and disadvantages of different treatment regimens (Figure 1). After a treatment regimen is selected, discussion among the multidisciplinary team members should be regularly carried out, which could ensure the adjustment of treatment regimens according to the changes of disease conditions, enabling patients to benefit from the advantages of the treatment.

### FUTURE PROSPECTS

**Characteristics of patients requiring neoadjuvant therapy or conversion therapy**

The recommended treatment methods are as follows: (1) Surgical resection: It should be performed directly if the patients meet the criteria for R0 resection; (2) Conversion therapy: If patients are eligible for R0 resection according to the defined criteria, conversion therapy can be conducted to eliminate the unresectable hepatocellular tumors; and (3) Neoadjuvant therapy: Owing to the lack of evidence for neoadjuvant therapy, it should be performed in clinical practice; alternatively, patients can receive immunotherapy, neoadjuvant HAIC therapy, or radiotherapy based on sufficient evidence from discussion performed among members of the multidisciplinary team. Patients’ clinical characteristics are worthy of further investigation by experienced clinicians.
Figure 1 Algorithm of neoadjuvant and conversion therapies for liver cancer. 1Technically resectable criteria: R0 resection, sufficient volume of the future liver remnant, Child-Pugh class A/B (some patients)[1]. 2Comprehensive evaluation by preoperative imaging examination findings and serum levels of biomarkers should be performed to assess the risk of postoperative recurrence[44]. Postoperative recurrence risk factors: (1) For patients with stage Ia, Ib, and Ila liver cancer, unclear tumor boundary, closely adjacent of the tumor to blood vessels, and highly suspicious residual tumors are among the high-risk factors of recurrence; (2) For patients with stage IIb-IIIa liver cancer, the high-risk factors of recurrence include the number of tumors ≥ 3, tumor diameter > 5 cm, satellite nodules, macroscopic cancer emboli, microvascular invasion-positive, lymph node metastasis, invasion of adjacent organs, and high alpha-fetoprotein level before surgery[13,44]; and (3) Other recurrence factors include liver diseases (e.g., viral hepatitis and liver cirrhosis)[13]. (1) Stage Ia-Ila: Neoadjuvant therapy is not recommended in clinical practice; if the multidisciplinary treatment clarifies the high risk of postoperative recurrence, neoadjuvant therapy can be performed in clinical trials after ethical approval; (2) Stage IIb-IIIa: Radical treatment following neoadjuvant therapy is recommended for patients with technically resectable liver cancer and high risk of recurrence, aiming to reduce the postoperative recurrence; and (3) For patients with unresectable liver cancer who are incapable of R0 resection or insufficient volume of future liver remnant (FLR), conversion therapy can be conducted to eliminate unresectable hepatic tumors. 4Radical therapies, including liver transplantation, resection, and radiofrequency ablation, are highly appropriate for early-stage liver cancer patients. Liver transplantation: Patients who meet the Milan criteria or the University of California San Francisco (UCSF) criteria after preoperative treatment can be treated with liver transplantation. (1) Milan criteria: Diameter of a single tumor ≤ 5 cm, the number of tumors ≤ 3, in which the diameter of the largest tumor was ≤ 3 cm, and without large blood vessel or lymph node invasion; and (2) The University of California San Francisco criteria: Diameter of a single tumor is ≤ 6.5 cm, the number of tumors ≤ 3, in which the diameter of the largest tumor was ≤ 4.5 cm, and the sum of diameters of all tumors was ≤ 8.0 cm, without a large blood vessel or lymph node invasion[7]. Radiofrequency ablation: Patients reached China Liver Cancer (CNLC) stage Ia or Ib (e.g., a single tumor, the diameter of tumor ≤ 5 cm; or with 2-3 tumors, in which the largest diameter was ≤ 3 cm) after preoperative therapy or without blood vessel, bile duct, and adjacent organ invasion, without distal metastasis, and liver function of Child-Pugh class A/B could be treated by radiofrequency ablation, which can also achieve the effects of radical treatment[7].

CONCLUSION

Neoadjuvant therapy and conversion therapy are important strategies for the preoperative treatment of intermediate or advanced liver cancer. Consensus on conversion therapy has already been achieved, while further evidence for neoadjuvant therapy needs to be presented. With the development of treatment methods, many high-quality clinical trials are in progress, providing further evidence-based support for neoadjuvant therapy. Based on the consensus, Chinese scholars’ explorations will scientifically support theories and methods for preoperative liver cancer treatment, thereby improving Chinese liver cancer patient OS.

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Present and future management of viral hepatitis

Rocío González Grande, Inmaculada Santaella Leiva, Susana López Ortega, Miguel Jiménez Pérez

Abstract
Viral hepatitis can result in important morbidity and mortality, with its impact on health conditioned by the specific type of hepatitis, the geographical region of presentation and the development and access to new drugs, among other factors. Most acute presentation forms are self-limiting and may even go unnoticed, with just a small percentage of cases leading to acute liver failure that may necessitate transplantation or even cause the death of the patient. However, when they become chronic, as in the case of hepatitis B virus and C virus, unless they are diagnosed and treated adequately they may have severe consequences, like cirrhosis or hepatocarcinoma. Understanding of the mechanisms of transmission, the pathogenesis, the presence of vaccinations and the development over recent years of new highly-efficient, potent drugs have meant that we are now faced with a new scenario in the management of viral hepatitis, particularly hepatitis B virus and hepatitis C virus. The spectacular advances in hepatitis C virus treatment have led the World Health Organization to propose the objective of its eradication by 2030. The key aspect to achieving this goal is to ensure that these treatments reach all the more vulnerable population groups, in whom the different types of viral hepatitis have a high prevalence and constitute a niche that may perpetuate infection and hinder its eradication. Accordingly, micro-elimination programs assume special relevance at the present time.

Key Words: Hepatitis viral; Diagnosis; Treatment; Trend direct-acting antivirals; Inhibiting recycling

Core Tip: The various types of viral hepatitis have resulted in important morbidity and mortality for many years. Greater understanding of the pathogenesis as well as the development of new, highly efficient potent drugs mean that we are now faced with a...
new scenario in the approach to this disease. The spectacular advances in the treatment of hepatitis C virus suggest that we can now envisage its eradication, as put forward by the World Health Organization in its objectives for 2030. In this review we comment on the current situation, recent advances and future perspectives in the approach to viral hepatitis.

**INTRODUCTION**

The different types of viral hepatitis have resulted in great morbidity and mortality for many years, due to their high prevalence and incidence. Over 250 million persons are estimated to be infected with the chronic form of hepatitis B virus (HBV) and more than 70 million with hepatitis C virus (HCV) in the world, with over 1.5 million cases of hepatitis A virus (HAV) annually, resulting largely from lack of understanding of the pathogenesis and the absence of efficient treatment. Fortunately, recent years have seen important advances, impacting very positively on the management of viral hepatitis, particularly HBV and HCV, though not so much on HAV and hepatitis E virus (HEV). The most significant advances in these latter types have occurred in aspects related to the epidemiology and pathogenesis of the disease rather than in therapy. Studies have shown new pathways of contagion, especially relevant in certain population groups, such as patients with previous liver disease or immunosuppressed patients where the disease can have an important impact on morbidity and mortality.

Advances in understanding the viral pathogenesis of HBV have mainly led to the development of new drugs. The first significant leap occurred in the early 2000s, with the appearance of the first nucleotide/nucleoside analogs (NAs), like lamivudine and adefovir dipivoxil. This enabled oral treatment, with great efficacy, safety and tolerability, though limited by the development of resistance that was overcome later with entecavir (ETV) and tenofovir (TDF), which have a high genetic barrier to the development of resistance. Nevertheless, limitations still exist, such as the need to administer the drugs for prolonged periods of time, even indefinitely, and they are unable to inhibit the initial formation of covalently closed circular (ccc)DNA in newly infected hepatocytes. This has resulted in current research aimed at developing drugs to inhibit viral replication, acting on any of the various phases of the virus replication cycle. Indirectly, all these advances can have a positive impact on the management of hepatitis D virus (HDV), which whilst being a defective virus that needs the presence of the B virus for reproduction, can nevertheless cause important morbidity and mortality. The first drug for the specific treatment of HDV, bulevirtide, has recently been commercialized in Europe.

It is, though, in HCV where the most significant advances have been made, with a major impact on improving health over recent years. The efficacy, safety, tolerability and ease of use in clinical practice of direct-acting antivirals (DAA) have led to the rarely seen possibility of eradicating HCV by 2030, an objective set by the World Health Organization. This requires establishing such strategies as micro-elimination based on the active search for cases, simplification of the diagnosis and treatment and prevention measures.

**HEPATITIS A**

The classic transmission of HAV and HEV is the fecal-oral route, more prevalent in underdeveloped countries. However, new pathways of infection are leading to changes in the epidemiology of these infections. This, perhaps, is the main novelty. HAV is one of the most common infectious etiologies of acute hepatitis worldwide. Transmission is fecal-oral via contaminated food or water, either person-to-person or through consumption of contaminated products. Globally, an estimated 1.5 million
cases occur each year[3]. There are areas of high, intermediate, low and very low HAV endemicity. In low- and middle-income countries, where sanitation and hygiene practices are poor, infection is common, and most children (90%) have it before the age of 10, very often without presenting symptoms[10]. Epidemics are rare because older children and adults are usually immunized. In these areas, morbidity rates are low, and epidemic outbreaks rarely occur. In developed countries with good sanitation and hygiene, infection rates are low. This translates into an increase in the number of adults who have never been infected and who lack immunity. This increased vulnerability in older age groups can increase morbidity rates and lead to large epidemic outbreaks. The disease can appear in adolescents and adults from high-risk groups, such as injection drug users, men who have homosexual relationships and people who travel to high-endemic areas[11]. Because of the epidemiological features, vaccination is recommended for persons at increased risk of exposure or those liable to fulminant disease[12].

Infection with HAV has a mild or asymptomatic course, although it may be fulminant in < 1% of cases[13], especially in patients with chronic hepatitis[14]. Other atypical presentations of acute hepatitis A include renal insufficiency and relapsing hepatitis, which are usually present in children. Some individuals experience a prolonged hepatitis (5.8%)[13] or cholestasis (6.8%), especially in the presence of HBV [16]. HAV accounts for 0.35% of cases of acute liver failure. HAV-related acute liver failure has a spontaneous resolution rate of 70%, with the remaining 30% requiring a liver transplant[17]. Several studies have concluded that acute HAV infection superimposed on chronic liver disease is associated with greater disease severity and a higher case fatality rate, though a review of the literature failed to define a relationship between nonalcoholic steatohepatitis and HAV-induced liver failure[18].

Regarding the prevention of hepatitis A, in addition to improving hygiene and sanitation measures, the vaccine is a very effective tool, already being included in the vaccination calendar from childhood in many countries. Nonvaccinated persons traveling to HAV endemic regions should receive a single vaccine dose before departure[19]. The Centers for Disease Control and Prevention 2020 Advisory Committee on Immunization Practices recommends vaccination for all children aged 1 year and older, men who have sex with men and people who use injection and noninjection drugs, have occupational risk factors for infection, travel to high-endemic areas or those who have an increased risk for complications from hepatitis A (e.g., chronic liver disease, HIV infection and pregnancy, if at risk for infection)[12]. There are currently two single-antigen inactivated vaccines: Havrix™ (GlaxoSmithKline Biologicals, Rixenstar, Belgium) and Vaqta™ (Merck and Co. Inc., West Point, PA, United States)[20,21]. A live-attenuated vaccine is licensed in China and has been extensively there[22]. A combination HAV-HBV vaccine (Twinrix™; GlaxoSmithKline Biologicals) has been available since 1996[23]. Havrix and Vaqta vaccines are given in a two-dose schedule 6 mo apart and Twinrix requires three doses. The efficacy of both live-attenuated and inactivated vaccines has been well established in a large review[24]. A special population concerns HIV-positive individuals who are susceptible to HAV infection, especially because of low adherence to recommended HAV vaccination. In this group a double dose of HAV vaccination is recommended as an additional dose of the HAV vaccine may improve serological responses and durability of seroprotection. Immunoglobulin is used for both pre-exposure and postexposure prophylaxis of HAV. Children younger than 12-mo-old, adults with chronic liver disease, adults older than 40 years of age and immunocompromised individuals should receive a single dose of intramuscular HAV immunoglobulin (0.1 mL/kg) in addition to the vaccine, unless either is contraindicated.

HEPATITIS E

Infection with HEV, traditionally considered a disease almost exclusive to developing countries, has now become a worldwide health problem and is endemic in most developed countries, behaving largely as a zoonosis[25]. HEV infection has a greater clinical impact in populations that are especially vulnerable, such as immunosuppressed patients, pregnant women and patients with underlying liver disease. Thus, the World Health Organization places it as one of the leading causes of death from acute viral hepatitis worldwide[26].

HEV is divided into four species (A–D). Genotypes 1 and 2 of species A are strains that infect humans, whereas genotypes 3 and 4 are zoonoses transmitted via meat consumption or direct contact with affected animals, mainly pigs or wild boar, or
through contaminated blood products[25]. In recent years, particularly in Eastern China, genotype 1 has become much less common, and genotype 4 is now the most common genotype found in human cases[27]. Person-to-person transmission (direct contact) of HEV is very inefficient; study of close contacts of a case with documented HEV infection is not recommended, unless they share exposure to the source of infection. Blood transfusion is another route of transmission of HEV. The European Association for the Study of the Liver (EASL) recommends that blood donor services screen blood donors for HEV, given the results of local risk-assessment and cost-effectiveness studies[25].

In most cases, contact with HEV produces an asymptomatic infection, mainly in women and young people, followed by spontaneous clearance of the virus[28]. HEV infection during pregnancy (particularly during the third trimester) has been associated with a poorer prognosis compared to other types of viral hepatitis[29-31]. Maternal mortality rates of up to 30% have been observed in different outbreaks of hepatitis due to HEV in pregnant women[32]. The mortality associated with HEV infection during pregnancy is usually associated with infections caused by genotypes 1 and 2, though cases caused by other genotypes have been reported[33,34].

Although the development of acute liver failure in the course of HEV infection is rare (0.5%-4.0%)[35], in some series, like that of Crossan et al[36], HEV accounts for 5.0% of all cases of acute liver failure. Those most at risk of developing acute liver failure in the course of HEV infection are pregnant women and patients with underlying chronic liver disease[37,38]. Because of this, all patients with acute hepatitis, acute liver failure or patients with decompensation of chronic liver disease should be screened for HEV infection. Although isolated cases of chronic HEV infection have been reported in immunocompetent patients[39,40], chronic HEV infection occurs primarily in immunocompromised patients, such as solid organ transplant recipients. In transplant patients, HEV infection may progress to chronicity in up to 2/3 of cases, with rapid progression of fibrosis and development of liver cirrhosis in up to 10% of patients[41,42]. Cases have even been reported of retransplantation in liver transplant recipients due to acute liver failure from HEV[43]. Extrahepatic clinical manifestations have been described in 2%-5% of patients with HEV infection[44]. In most of these the liver manifestations of the infection are mild or absent. The EASL recommends HEV testing, irrespective of liver function test results, in patients presenting with neuralgic amyotrophy or Guillain-Barré syndrome and suggests HEV testing for patients with encephalitis/myelitis. Patients with proteinuria should also be tested[25]. Currently, screening for HEV is advisable in all cases of acute hepatitis, including those with suspected drug-induced liver injury, particularly if patients have higher levels of transaminases, in which case acute HEV must be excluded systematically[45,46].

The diagnosis of HEV is based on a combination of serology and nucleic acid amplification techniques. Serological techniques measure anti-HEV immunoglobulin (Ig)M antibodies, which appear approximately 4 wk after contact and may be detected up to 6 mo later, and IgG antibodies, which appear at about the same time as the IgM antibodies but can last for years. The presence of anti-HEV IgM indicates recent or acute infection while anti-HEV IgG antibodies indicate recent or past infection. HEV-RNA can be detected in blood or stool 3 wk after infection and a short time before the appearance of symptoms and constitute the gold standard for the diagnosis of active infection[47]. The combination of serological studies and the determination of HEV-RNA increases the diagnostic specificity and sensitivity, though it is necessary to bear in mind the immune-competence status of the patient. The World Health Organization recommends first determining the presence of anti-HEV IgM antibodies and then determining the viral RNA. In immunocompromised patients, the antibodies may be negative despite HEV infection, and it is necessary to determine the viral load in all cases[48].

**Current recommendations for the management of HEV infection**

Acute HEV infection does not usually require antiviral therapy. In almost all cases HEV infection clears spontaneously. Nevertheless, early therapy of acute hepatitis E may shorten the course of the disease and reduce overall morbidity. Ribavirin treatment may be considered in cases of severe acute hepatitis E or acute-on-chronic liver failure. Corticosteroids have been used in individual cases of acute liver failure, with improvement of liver function parameters. However, there is currently insufficient evidence to support general corticosteroid treatment in this group of patients[25]. The group of transplant patients deserves special mention. The EASL recommends decreasing immunosuppression at diagnosis of chronic HEV infection in solid organ transplant recipients, if possible.
In patients with persisting HEV replication 3 mo after detection of HEV RNA, the EASL recommends ribavirin monotherapy for a duration of 12 wk. Trials have been attempted with other treatments, such as sofosbuvir in single therapy, which showed antiviral activity in vitro but was unable to inhibit viral replication in a phase II pilot trial[49].

**Future directions**

As no efficient therapy exists for HEV and bearing in mind it can cause severe symptoms of liver disease, efforts should be focused on both prevention and research.

Preventive measures should be enhanced in immunosuppressed patients and pregnant women; indeed, screening for HEV should be recommended in pregnant women in endemic areas like Sub-Saharan Africa or South Asia, assessing the risk individually[47].

A few European countries are starting to include HEV-RNA detection in all blood donor samples, although certain aspects remain to be clarified, such as the most cost-effective technique for detection, the viral load considered infectious or the characteristics of the recipient that may influence transmission of the disease, such as the immunological status[50].

Currently only one vaccine against HEV is available (Helicon®). It has only received approval in China, though other vaccines are under development. The availability of an effective vaccine would constitute the main tool for prevention of HEV infection[51].

**HEPATITIS B**

The discovery of the Australia antigen in 1965 represented a starting point for the identification of the virus of hepatitis B, and for many it was the beginning of the study of viral hepatitis. Infection with HBV is now a public health problem worldwide, with some 257 million persons with chronic infection. HBV is endemic in the western Pacific and Africa, with over 6% of the population infected[1]. In Europe and the United States the prevalence is < 2%, mostly related with immigration[52]. HBV is the leading cause of morbidity and mortality of hepatic origin in the world, despite the availability of an efficient vaccine.

Vaccination programs, control of blood donations, serologic evaluation in pregnant women or persons at risk and activities aimed at limiting invasive procedures in unsafe conditions have all reduced the incidence of acute HBV hepatitis and the prevalence of the disease. Although population screening is not indicated, screening is recommended in risk groups, partners of infected patients and before receiving oncologic or immunosuppressive therapy[1].

The spectrum of the disease varies greatly, from inactive carriers to others with hepatic cirrhosis and hepatocarcinoma. The natural history of hepatitis B is a dynamic process, traditionally differentiated into five phases depending on virus and host factors, like the state of immune competence, age or the duration of the infection[33]. They are classified according to liver inflammation data (alanine aminotransferase), determination of hepatitis B e antigen (HBeAg) and quantification of the HBV-DNA viral load[54] as well as estimation of the degree of hepatic fibrosis, usually by elastography[1] as serologic indices of fibrosis have proven less precise in HBV infection[55]. These phases have traditionally been called: immune-tolerant phase, immune-elimination phase, immune-reactive phase, asymptomatic carrier and hepatitis B surface antigen (HBsAg)-negative phase[56]. However, these phases have recently been grouped into two large spectra of chronic forms of hepatitis B with a new nomenclature: infection, encompassing the phases of immune tolerance and inactive carrier, both HBeAg positive and negative vs hepatitis, which refers to the phases in which there exists liver damage, the immune-reactive and the immune-elimination phases[57].

Awareness of the particular phase in which the patient is and its clinical context is important to identify the need to start treatment and the choice of the most suitable antiviral agent.

**Evolution of antiviral treatment: Nucleos(t)ide analogs**

Initially it was thought that a depressed immune response was involved in the development of HBV infection. Thus, in 1986 the first clinical trial of interferon (IFN) alpha was published, though the findings showed a poor and transitory response[58]. Currently, in its pegylated form it is still considered a treatment option, with PEGα2a
being easier to use and having greater efficacy and tolerance[59]. The duration of treatment with this drug is usually finite and the loss of HBsAg and conversion of HBeAg is relevant, although the drug has to be injected and is contraindicated in patients with decompensated cirrhosis, autoimmune disorders, pregnant women and severe depression or psychosis[59].

Later studies on the lifecycle of HBV found that the virus uses an inverse transcriptase for replication. Accordingly, the use of drugs already available for another virus (HIV), NAs with an inhibitory action on this polymerase, were considered[60]. In 1998 the Food and Drug Administration approved the use of lamivudine for the treatment of HBV, controlling viral replication with oral treatment[61]. Lamivudine was the sole oral treatment available until 2002, when other NAs have become available: adefovir dipivoxil, ETV, telbivudine, TDF in 2008[1] and tenofovir alafenamide in 2016, though the latter is not available in all countries.

All these analogs reduce the pool of cccDNA in infected hepatocytes, inhibiting recycling of the nucleocapsids, although they are unable to inhibit the initial formation of cccDNA in newly infected hepatocytes[5]. The main advantages of NAs are that they can be given orally, they have great efficacy in inhibition of HBV replication (very similar among all of them), their long-term safety and the possibility of being used in any situation, including decompensated cirrhosis and liver transplant or even during pregnancy in the case of TDF. They achieve a virological and biochemical response greater than 95%, for both positive and negative HBeAg[62,63]. The main inconvenience is that they require continued administration over time, even indefinitely, in order to maintain inhibition of viral replication and their efficacy can be affected by the development of drug-resistant HBV mutations, sometimes with cross-resistance between them, partly related with lack of adherence to treatments that have to be taken for such long periods.

Lamivudine is the drug that has shown the greatest risk for development of resistance as compared with the other antiviral agents currently available, particularly ETV and TDF[64]. The main clinical practice guidelines therefore recommend treatment with ETV, TDF and tenofovir alafenami given their great antiviral potency and high resistance barrier[65]. The choice of one over the other treatment strategy depends on the stage of liver fibrosis, virological factors and the comorbidity profile of the patient, in addition to personal preferences[14]. Tenofovir alafenami is a prodrug that reaches higher levels of TDF than TDF in the hepatocytes with lower doses. Lower exposure to the drug is related with less worsening of renal function and less loss of bone mineral density[66].

Recent studies have shown that combined NA treatment with Peg-IFN, simultaneously or sequentially, increases the probability of HBsAg loss, but the benefits are restricted to just a small group of patients with low HBsAg levels. Further studies are therefore needed before it can be recommended[67].

Recent studies have examined whether TDF vs ETV could impact the risk of developing hepatocarcinoma. Though this would condition the choice of one over the other, neither has yet been found superior in this respect. Some studies have suggested the benefit of TDF, though the differences were not statistically significant[68]. Retrospective analyses of different series have reported contradictory results, with some showing no difference between the drugs[69] whilst others have found benefits for TDF vs ETV in the prevention of hepatocarcinoma[70], a benefit confirmed in a recent meta-analysis[71]. Randomized trials are needed to establish this association.

**Nucleos(t)ide analogs and liver transplantation**

Patients who required a liver transplant due to HBV initially had a very high risk of recurrence of the infection in the graft. This was particularly so for patients who received their transplant having a high viral load, which in addition was usually a severe, rapidly progressive hepatitis, so much so that HBV infection became considered a contraindication for liver transplantation[1]. With the advent of anti-hepatitis B hyperimmune gammaglobulin during the 1990s, and particularly of the NAs, the perspective changed. The post-transplant results were similar to those of other etiologies. Now that we have potent NAs and a high resistance barrier, the tendency is to use hepatitis B immune globulin for a short time and at lower doses than before and even regimens without hepatitis B immune globulin with NAs in single therapy maintained indefinitely[67]. These advances have thus allowed for an individualized prophylaxis based on the individual risk profile of each patient[72] as well as the use of anti-hepatitis B core positive donors.
Treatment objectives: New definitions of cure
The final aim of antiviral therapy is cure of the infection, eliminating all potential forms of HBV replication[^59]. Sustained loss of HBsAg and eradication of the HBV-DNA, including the cccDNA[^6], though this is hardly feasible with currently available drugs, is the sterilizing cure. As a result, the American Association of the Study of Liver Diseases and EASL agreed to the definition of functional cure, defined as the sustained loss of HBsAg and HBV-DNA in serum with or without the development of anti-HBs. These two situations are those that allow treatment to be suspended safely and with little risk of relapse. However, they are very rarely achieved. Another concept, partial cure, refers to the persistence of HBsAg but with negativization of HBeAg, with or without seroconversion to anti-HBe, normalization of alanine aminotransferase and a low or undetectable viral load, simulating a phase of inactive carrier and thus susceptible to interruption of treatment[^6].

Current guidelines[^77,73] recommend that treatment can be suspended with NAs for patients with positive HBsAg but without cirrhosis if there is seroconversion to anti-HBe after 1 year of consolidation. For negative HBeAg, treatment can be suspended for patients whose HBsAg clears and who have had viral suppression for over 2-3 years, requiring strict follow-up[^74].

Quantified HBsAg is determined by enzyme immunoassay and reflects the amount and transcriptional activity of the cccDNA. It is very useful in untreated patients with negative HBeAg, with HBV-DNA < 2000 IU/mL, in whom values of quantified HBsAg < 1000 IU/mL are indicative of inactive carrier with a low risk of disease progression and appearance of hepatocarcinoma. Although no cut-off value has yet been established, a level < 100 IU/mL seems to predict this sustained response[^1]. Among the new markers is the antigen related with the HBV core that correlates with the transcriptional activity of cccDNA, especially in the negative HBeAg patient, and it could be superior to quantified HBsAg for the identification of inactive carrier patients and to predict viral relapse after suspending treatment[^75].

Future directions
Given that treatment with NAs has little impact on the cccDNA, which in addition can be replaced with no need for the entry of new virus[^6], numerous studies are in place to develop new drugs that act at the level of the different stages of the life cycle of HBV, about which more and more is being learnt, or that modulate the host immune response. These drugs are at various stages of clinical trials, with up-to-date information available from: [http://www.hepb.org/treatment-and-management/drug-watch](http://www.hepb.org/treatment-and-management/drug-watch[^7]) (Table 1).

The main drugs under development and their therapeutic targets are described below:

Blocking entry of HBV into the hepatocyte: The sodium taurocholate cotransporting polypeptide receptor is a transmembrane protein that cotransports bile acids and participates in the entry of HBV and HDV into hepatocytes[^76]. Bulevirtide couples to this receptor and blocks fixation of the preS1 domain on the coat of HBV, inhibiting entry of the virus. Although this does not directly interfere with the formation of the cccDNA, inhibiting virus entry can block the infection of new hepatocytes. It has also been proposed as an option to prevent reinfection in the transplanted liver[^77]. It is currently in phase III[^7].

Silencers: These are drugs designed to interfere and destroy viral RNA. The process involves small molecules of RNA producing interference in the transcription of viral RNA. They are short non-encoding sequences present in the cells that regulate the post-transcriptional expression of certain genes, resulting in a reduction in the production of multiple viral antigens[^77,78]. There are several studies in various phases: preclinical, I and II.

HBsAg release inhibitors: HBsAg is the most abundant circulating viral antigen. It contributes to T-cell tolerance and attenuation of the host immune response. Its inhibition may enable immune regulators to restore the immune response. There are currently two phase II studies with results that need to be validated.

Capsid assembly modulators: As the nucleocapsid contains the viral DNA necessary for replication, inhibiting its formation is an interesting strategy that can prevent the formation of cccDNA during de novo infection[^79]. There are currently several studies in various phases, preclinical, I and II and one with vebicorvir in phase II/III.

Antisense molecules: These bind to messenger RNA inhibiting the formation of viral proteins. There is currently one phase II study in the United States.

New NAs: Besifovir has been approved in Korea, with antiviral efficacy comparable to that of TDF after 48 wk of treatment, with effects lasting 96 wk and a better safety
Table 1 Main drugs under development for the treatment of hepatitis B virus (with effect from phase II)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mechanism of action</th>
<th>Country</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulevirtide</td>
<td>Inhibitor of HBV entry into the hepatocyte</td>
<td>Germany</td>
<td>Phase II</td>
</tr>
<tr>
<td>VIR-2218</td>
<td>Silencers: Interfere and destroy viral RNA</td>
<td>United States</td>
<td>Phase II</td>
</tr>
<tr>
<td>JNJ-3989</td>
<td>Antisense molecules: Bind to mRNA to prevent passage by viral protein</td>
<td>United States</td>
<td>Phase II</td>
</tr>
<tr>
<td>IONIS-HBVRx (GSK3228836)</td>
<td>Antisense molecules: Bind to mRNA to prevent passage by viral protein</td>
<td>United States</td>
<td>Phase II</td>
</tr>
<tr>
<td>Vebicorvir</td>
<td>Capsid inhibitors</td>
<td>United States</td>
<td>Phase II/III</td>
</tr>
<tr>
<td>Morphothiadin</td>
<td></td>
<td>China</td>
<td>Phase II</td>
</tr>
<tr>
<td>JNJ 56136379</td>
<td></td>
<td>Ireland</td>
<td>Phase II</td>
</tr>
<tr>
<td>ABI-H2158</td>
<td>Capsid inhibitors</td>
<td>United States</td>
<td>Phase II</td>
</tr>
<tr>
<td>REP 2139</td>
<td>HBsAg inhibitors</td>
<td>Canada</td>
<td>Phase II</td>
</tr>
<tr>
<td>REP 2165</td>
<td>Other immune modulators-T-cell receptor</td>
<td>Canada</td>
<td>Phase II</td>
</tr>
<tr>
<td>NASVAC</td>
<td>THERAPEUTIC VACCINES: Use stimulation of the immune system as treatment</td>
<td>Cuba</td>
<td>Phase III</td>
</tr>
<tr>
<td>GS-4774</td>
<td>TLR</td>
<td>United States</td>
<td>Phase II</td>
</tr>
<tr>
<td>HepTcell</td>
<td>Monoclonal antibodies</td>
<td>United States</td>
<td>Phase II</td>
</tr>
<tr>
<td>G39688 (TLR-8 agonist)</td>
<td>TLR</td>
<td>United States</td>
<td>Phase II</td>
</tr>
<tr>
<td>GC1102</td>
<td>Checkpoint inhibitors: Stimulate specific T lymphocytes</td>
<td>Korea</td>
<td>Phase II</td>
</tr>
<tr>
<td>ASC22: Inhibitor PDL1</td>
<td>Checkpoint inhibitors: Stimulate specific T lymphocytes</td>
<td>China</td>
<td>Phase II</td>
</tr>
<tr>
<td>IMC-I109V</td>
<td>Other immune modulators-T-cell receptor</td>
<td>United States</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

Taken from Hepatitis B Foundation[7]. Available from: https://www.hepb.org/treatment-and-management/drug-watch/. TLR: Toll-like receptor; HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen.

In summary, interaction at each stage of the life cycle of HBV may be the therapeutic aim to achieve elimination of the cccDNA, though it is also necessary to standardize profile than TDF in terms of bone and kidney results[80], though further studies are needed.

Still in the preclinical phase are studies of drugs against HBVcccDNA. They work via degradation of the lymphotoxin beta receptor using specific antibodies or with cccDNA excision enzymes.

Immunotherapy: Immunological dysfunction due to “exhaustion,” a phenomenon characterized by lack or absence of specific T cells against HBV associated with poor cytotoxic activity, worsening cytokine production and an increased expression of T-cell inhibitor receptors[81], has resulted in various immunotherapeutic strategies aimed at modulating the innate or adaptive immune response, or both, in an attempt to restore a competent immune response against the virus and infected hepatocytes [59]. New modulatory agents of immunity in clinical development include:

Pattern recognition receptor (PRR) agonists. PRR are proteins that detect pathogen-associated molecular patterns and are present in various cell groups, like hepatocytes and cells of the innate immune system. Among the main PRR are the Toll-like receptors[82]. HBV is recognized by the PRR as it provokes a cytokine-mediated response. The aim of therapy with PRR agonists is to enhance the antiviral response of the cytokines to achieve an adaptive immune response with activation of natural killer cells and T lymphocytes[83]. Agonists of Toll-like receptor-7 and Toll-like receptor-8 are under development and appear to stimulate the immune response satisfactorily.

Molecules to revert the exhausted T lymphocytes by blocking immunoinhibitory signals, mainly programmed cell death protein-1, will be the subject of future studies [59]. There is currently one phase II trial in China (ASC22).

Therapeutic vaccines. Stimulation of specific HBV B and T lymphocytes by vaccines is a way of overcoming immune tolerance in patients with chronic HBV. Several categories exist, based on proteins, DNA and vectors. They are all being studied in clinical trials in combination with current antivirals[84] as, so far, on their own they have been unable to control infection.

In summary, interaction at each stage of the life cycle of HBV may be the therapeutic aim to achieve elimination of the cccDNA, though it is also necessary to standardize...
HEPATITIS D

HDV was discovered by Rizzetto et al[86] in 1977 in patients infected by HBV who had severe hepatitis. Despite being an incomplete virus that requires the presence of HBV it can nevertheless cause severe progressive hepatitis[87]. Progression to cirrhosis may occur in 80% of patients at 10 years[88], and HBV-HDV coinfection increases the risk of hepatocarcinoma compared to infection with just HBV alone[89].

HDV has a worldwide distribution, though with great variations in prevalence (higher in Latin America and eastern Europe but lower in western Europe, Japan and North America, where better socioeconomic conditions and HBV vaccination mean it is virtually restricted to injection-drug users). At least 5% of HBV carries in the world are thought to be infected with HDV, though it is probably even higher as there is no homogenous screening protocol and due to the impossibility of performing diagnostic tests in endemic areas.

The clinical course of HDV infection depends on its mode of transmission[87]. Coinfection with HBV can mean a severe clinical course in up to 15% of persons, though limited courses are more common with cure and immunity. Superinfection (HDV infection in a chronic HBV carrier) is more often associated with chronic hepatitis D that can advance to cirrhosis and liver failure[80]. Accordingly, differentiation between coinfection and superinfection is important in the management and prognosis of liver disease.

HDV infection should be investigated in all cases of acute or chronic hepatitis with a positive HBsAg[87], as any patient with HBV infection can be a carrier of HDV, especially in areas of moderate or high prevalence. HDV infection should also be investigated in patients with chronic HBV infection who present a peak of hypertransaminasemia.

No treatment for acute hepatitis due to HDV has proven useful, the disease being managed with support measures and liver transplant in fulminant cases. The only drug approved to date for the treatment of chronic hepatitis due to HDV is IFNα, which produces a modest response in 23%-57% of those treated[91,92]. NAs are not recommended for the treatment of HDV, although sustained suppression of active HBV infection with NAs can reduce the quantification of HBsAg and thus have a beneficial effect on coinfection with HDV[67].

Several new treatments are being tried aimed at blocking the viral cycle at different points: inhibitors of virus entry into the hepatocyte, assembly and other new strategies based on immune stimulation with cytokines and agonists of the receptors (Table 2):

Bulevirtide is the first drug to be approved by the European Commission specifically for hepatitis D. It acts by blocking entry by binding to the receptor that HBV uses to enter the liver cells (sodium taurocholate co-transporting polypeptide), thereby interfering in the life cycle of HBV and, consequently, preventing replication of HDV. Ezetimibe also acts by blocking entry and is being studied in a trial that is currently in phase II.

Inhibitors of HDV assembly: Lonafarnib is an inhibitor of farnesyl transferase, an enzyme that catalyzes prenylation, which is essential for HDV assembly. It significantly reduces the viral load when compared with placebo[93].

Inhibitor of HBsAg: A study in phase II for REP 2139 shows promising results.

As an immune modulator, IFN lambda binds to a single receptor much expressed on hepatocytes and little on hematopoietic and central nervous system cells. It is therefore well tolerated like IFNα, with a similar effect.

In conclusion, HDV, despite being minor, is in fact an important health problem in many areas of the world, being independently associated with long-term complications[94]. Standardization of diagnostic tests to detect HDV-RNA is needed. Although no specific treatment yet exists, bulevirtide has been approved by the
Table 2 Main drugs under development for the treatment of hepatitis D virus (with effect from phase II)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Type</th>
<th>Country</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulevirtide</td>
<td>Entry inhibitor</td>
<td>Germany</td>
<td>Approved in Europe</td>
</tr>
<tr>
<td>Lonafarnib</td>
<td>Prenylation inhibitor</td>
<td>United States</td>
<td>Phase III</td>
</tr>
<tr>
<td>REP 2139</td>
<td>HBsAg inhibitor</td>
<td>Canada</td>
<td>Phase II</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>NTCP inhibitor</td>
<td>Pakistan</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

Taken from Hepatitis B Foundation.[7]. Available from: https://www.hepb.org/treatment-and-management/drug-watch/. NTCP: Sodium taurocholate co-transporting polypeptide; HBsAg: Hepatitis B surface antigen.

Figure 1 The main therapeutic targets and the drugs under development for hepatitis B. HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; cccDNA: Covalently closed circular DNA.

European Commission, and other drugs are currently being developed whose efficacy and safety will need to be determined.

HEPATITIS C

HCV, an RNA virus with seven genotypes, was discovered in 1989, before which it was referred to as non-A non-B[95]. HCV infection is the type that has undergone most changes over the last 10 years. From a chronic disease with few treatment options, based on IFN ± ribavirin, it has become a curable disease, even before liver damage is produced, as a result of DAA. This has led to a revolution in HCV, impacting the disease and its complications, liver transplant waiting lists and the incidence of HCV-associated hepatocarcinoma as well as the epidemiological and economic situation. In addition, the availability of an efficient therapy, limited in time and with excellent tolerance, has enabled objectives to be established for worldwide elimination of the disease. Nonetheless, HCV is still considered a prevalent disease, with healthcare repercussions and susceptible to management strategies.

The worldwide prevalence of HCV has fallen from 170 million carriers in 1999[96] to 71 million infected persons according to estimates in 2015[2], corresponding to 1% of the population, mainly due to prevention of nosocomial infection and the progressive access to efficient antiviral agents. Previously, most infections were related with transfusions of blood products, persons born between 1945 and 1965 (the so-called “baby boomer generation”), hemodialysis and hemophilia, and a high percentage of patients had advanced or decompensated disease and failure of prior therapy. More
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recently, infection has been associated with the use of intravenous drugs, sexual risk-practices like men who have sex with men or chemsex (Party and Play) and certain risk groups such as prisoners or those with severe mental disorders who were not treated during the era of IFN[97], with most being treatment-naive, having little fibrosis and very often scarce awareness of their condition.

Although hepatitis C is present all over the world, China, Pakistan, India, Egypt, Russia and the United States account for more than 50% of all cases of infection in the world[98], data to be considered with migratory movements.

Despite the reduction in the incidence, approximately 400000 persons die each year from causes related to HCV, mainly cirrhosis and its complications and the development of hepatocarcinoma[2].

**Evolution of antiviral therapy and management of chronic hepatitis C from the past to the present: Direct acting antivirals**

In 1991 the Food and Drug Administration approved treatment with IFN alpha for HCV infection but with a very low response rate, around 16%, a long treatment period, a parenteral route and important side effects[99]. During the 1990s ribavirin was added, slightly increasing the efficacy though again with more side effects[100]. IFN was later modified to its pegylated form, thus reducing the need for injectable doses, slightly improving tolerability. The combination of pegylated IFN and ribavirin, with cure rates up to 41% in genotype 1 and almost 75% in other genotypes[101], was the only treatment available until 2011 but with important limitations due to its multiple contraindications and frequent important secondary effects.

Better understanding of the life cycle and identification of the structural and non-structural proteins of HCV led to the development of antivirals acting directly on certain targets. The first of these were the protease inhibitors, boceprevir and telaprevir, which are oral antivirals approved by the Food and Drug Administration in 2011 for treatment of HCV genotype 1 both in naïve and pretreated patients in combination with the previous dual therapy[102,103]. This triple therapy increased the success of treatment in patients with genotype 1, but at the same time it also increased the toxicity and pharmacological interactions, the costs and the risk of decompensation of the liver disease in more advanced stages[104] as well as still requiring parenteral administration over long periods.

The real revolution in antiviral treatment arose with effect from 2013 with the successive approval of molecules having different mechanisms of action, mainly inhibition of the proteases NS3/NS4 or the polymerase replication complex NS5A. These drugs provided multiple advantages over the earlier drugs, such as oral administration, high efficacy, good tolerance and the possibility of shorter periods of treatment. The different combinations of “the new antivirals” offered multiple treatment lines depending on the clinical setting[105]. Most were IFN-free, which enabled treatment of populations that had previously been considered difficult to treat, such as the psychiatric population, persons with drug addictions or on replacement therapy, patients with kidney failure or solid organ transplant recipients[106].

The main protagonist of this change was sofosbuvir, a polymerase NS5B inhibitor acting on genotypes 1, 2, 3 and 4, though associated with other antivirals or traditional therapy. Soon after came simeprevir, a protease inhibitor with pan-genotype action[107] and daclatasvir, able to inhibit the non-structural protein NS5A and acting on genotypes 1, 3 and 4[108]. Combinations of antivirals were also produced, with different mechanisms of action, like sofosbuvir/ledipasvir[109], ombitasvir paritaprevir/ritonavir and dasabuvir[110] or grazoprevir/elbasvir[111], each with preferences for certain genotypes and different treatment durations. All of these strategies clearly increased cure rates, increasing sustained viral response (SVR) rates globally above 95% in all genotypes and around 85% in patients with advanced cirrhosis[112,113]. However, in these early IFN-free years the clinical management of HCV became increasingly complex. Before choosing the best treatment option in each case it was necessary to identify the genotype as well as the stage of the liver disease and the particular degree of fibrosis, as the drugs did not have a pan-genotype action and their duration and combination were conditioned by these factors. This situation was reflected in the clinical guidelines of the time from the main scientific societies, showing multiple complex tables of recommendations for usual clinical practice[114-117].

The initial high costs also conditioned the slow access to treatment, such that many countries drew up specific protocols for the progressive approach to patients with hepatitis C, with the first patients to be treated being those with more advanced liver disease or in special situations[118]. This, together with the high risk of drug...
interactions and the outlines for these new therapies necessitated an exhaustive follow-up of the patient during antiviral treatment, with regular measurements of laboratory values and even excessive determinations of the HCV viral load, despite the lack of general rules concerning stopping treatment[119].

A qualitative leap occurred in 2016 with the advent of antiviral therapy with pan-genotypic and pan-fibrotic action plus their universal access. Combinations of sofosbuvir/velpatasvir[120,121] and glecaprevir/pibrentasvir[122] in single tablets have pan-genotypic and pan-fibrotic action in short-duration treatments of just 12 wk or 8 wk with the latter combination[123] and with SVR rates above 97% in practically all clinical contexts. With these two lines, plus the already available association of grazoprevir / elbasvir, also pan-genotypic, the approach is much more simplified for patients who now need treatment. This latter combination is indicated in all cases of active infection, the choice depending on certain determinants or conditioning factors, such as the renal function, which may limit the use of sofosbuvir, decompensated cirrhosis, which discourages protease inhibitors, or individual drug interactions, though these are much less common with the latest DAA compared to the earlier antiviral agents[124].

The pan-genotypic action, the high efficacy and the wide safety margin of currently available strategies have all led to simplification of the requirements to start antiviral therapy. These can be limited to determining the existence of viral replication (presence of RNA or HCV antigens), whether there is cirrhosis, which can be estimated with non-invasive methods like the APRI or FIB-4 indexes and ruling out possible interactions[125]. At the same time, monitoring during treatment has also become simpler, with the general recommendation (but not essential) of just determining the viral load 12 wk after completing treatment to confirm the SVR, with earlier safety controls when necessary due to a particular circumstance, like the cirrhotic patient[125].

An additional advantage of the current panorama is the existence of rescue therapy for the few cases that fail to achieve an SVR. The association of sofosbuvir / velpatasvir / voxilaprevir is indicated for patients who fail with DAA therapy, with a high SVR rate in all genotypes[126].

This radical change over recent years in the setting of antiviral therapy has enabled a high percentage of the HCV population to be treated, often resulting in the patient being discharged if the degree of fibrosis is only mild and there is no comorbidity. Nevertheless, although eradication of the virus is associated with a reduction in mortality due to hepatic and extrahepatic causes related with HCV and improvement in liver function (and even a reduction in the degree of fibrosis), those patients who already had advanced fibrosis still have a risk for complications of the liver disease or the development of hepatocarcinoma and should therefore undergo regular follow-up, even if they have an SVR[127,128].

Direct acting antivirals and the current approach to acute hepatitis C

DAAs have not only had an impact on the management of chronic hepatitis C, but the approach to cases of acute HCV infection has also changed. Historically, acute hepatitis C, defined as the first 6 mo after contact, was not generally susceptible to treatment, and pegylated IFN was used in some patients if viral replication continued for longer than 12 wk[129]. During the early phase of infection, DAAs have proven effective and beneficial, especially to eliminate the disease and particularly in such risk groups as injection drug users, men who have sex with men, persons who undertake sex practices of risk, patients with HIV or nosocomial infection. In these groups, DAAs reduce morbidity and mortality and the risk of transmission during the period waiting for the criteria of chronicity to be fulfilled before starting treatment[130,131]. Based on the available evidence the EASL recommends sofosbuvir/velpatasvir or glecaprevir/ pibrentasvir for 8 wk for what is now referred to as recently acquired hepatitis C[125]. During the era of DAA post-exposure prophylaxis is still not recommended without documented transmission of HCV[125,132].

Figure 2 shows the timeline of antiviral treatment for HCV.

Impact of direct acting antivirals on liver transplantation

The efficacy of DAA has impacted two great aspects of liver transplantation. First, it has clearly reduced the indication for liver transplant due to HCV, as shown in different series analyzing the current situation of liver transplant waiting lists[133-135]. Even so, the indication for a liver transplant still exists, mainly due to the development of HCV-associated hepatocarcinoma. Second, DAAs have enabled the majority of patients to reach liver transplantation with no viral replication. This, therefore, eliminates the risk of recurrence of hepatitis C in the graft. Additionally,
when necessary, DAA can be used safely after transplantation, with little risk of graft rejection and high efficacy, unlike earlier IFN-based treatments\cite{105,136}. This efficacy and safety have been confirmed in recipients of other solid organ transplants, such as kidney transplant patients with chronic hepatitis C\cite{137}, thereby endorsing their use in the transplant population.

**Impact of direct acting antivirals on solid organ donation**

Organ donation from patients with active HCV infection remains controversial, especially for seronegative recipients, but the safety and efficacy of DAAs in solid organ recipients nevertheless allows this option to be contemplated. Different series have shown good results in lung, heart\cite{138} and kidney transplantation. Even in liver transplants\cite{139}, provided the benefit of the transplant exceeded the risk of death on the waiting list, and early access to DAA treatment is guaranteed\cite{140,141}.

**Future directions**

The World Health Organization established the objective of elimination of hepatitis C by 2030, defined as a reduction of 80% in cases of de novo infection and a reduction of 65% in death due to HCV\cite{9}. Besides currently available efficient treatment, this objective also requires first making the population aware by means of information campaigns, second, rescuing patients who have already been diagnosed but not treated and third, simplifying the whole diagnostic process and treatment access when necessary in order to guarantee diagnosis and treatment of the greatest number of cases possible.

The active search for already diagnosed patients is one of the main and most feasible strategies for micro-elimination as it permits cases to be rescued for treatment\cite{142}.

Reflex testing\cite{143}, as well as points of care, will enable diagnosis to be externalized, reaching risk groups with little contact with the healthcare system, like migrants or prisoners\cite{144}. The diagnosis of hepatitis C should be directly linked to starting treatment (test and treat) so that in certain populations it will also be necessary to externalize treatment in the near future. For this, pilot projects have been designed with treatment administered by non-specialized healthcare personnel, like nurses\cite{145}, prison doctors, addiction physicians and primary care physicians\cite{146}, which has achieved a cost-effective elimination in the groups attended.

Populations still remain in which treatment needs further study, like pregnant women, in whom it is not recommended given the lack of safety data. It may, though, be the optimal time for screening in this group as it is sometimes the only contact they
Figure 3 Proposals for the management of hepatitis C. MSM: Men who have sex with men; SMD: Severe mental disorder; Ag: Antigen; HCV: Hepatitis C virus; NGO: Non-governmental organization.

Notwithstanding the above, these emerging models of care of the hepatitis C patient and micro-elimination must not detract from the importance of preventive measures, which necessitate adequate information for the population, particularly the already-mentioned risk groups.

The elimination of hepatitis C is associated with a significant reduction in complications due to liver disease, a reduction in mortality due to liver disease and the risk of developing hepatocarcinoma, in both patients with mild fibrosis and in those with advanced fibrosis[127]. In addition, suppression of viral replication is associated with a reduction in systemic symptoms, mainly diabetes and vascular events, which in turn is associated with a reduction in mortality due to extrahepatic causes[147].

The impact of SVR on portal hypertension and its complications may result in a discrete reduction in pressure gradients in the hepatic veins, particularly in the year of reaching SVR[148]. However, as not all studies have associated an SVR with regression of portal hypertension or regression of esophageal varices[149], the Baveno VI consensus recommends the same cut points for screening of varices in patients with SVR[150].

Several cohort studies have shown that the risk of hepatocarcinoma is reduced after SVR[127], but it does not disappear. Patients with advanced fibrosis and cirrhosis should continue to be screened for hepatocarcinoma as its annual global incidence is 2.5%-4.5%, even with SVR[151]. In patients with mild fibrosis, continued screening for other risk factors, like diabetes, metabolic liver disease, low albumin levels or thrombocytopenia, for the development of hepatocarcinoma should be contemplated[127].

Figure 3 shows the main recommendations for elimination of hepatitis C.

CONCLUSION

Although the various types of viral hepatitis still represent a great public health problem, the great advances over recent years are very positively impacting their management and prognosis. Nevertheless, it is still necessary to persist with, and even boost studies designed to find efficient new therapies, though without forgetting the implementation of preventive measures, as a basis of achieving advances in minimizing the impact of this disease on health.
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Artificial intelligence-assisted colonoscopy: A review of current state of practice and research

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Abstract

Colonoscopy is an effective screening procedure in colorectal cancer prevention programs; however, colonoscopy practice can vary in terms of lesion detection, classification, and removal. Artificial intelligence (AI)-assisted decision support systems for endoscopy is an area of rapid research and development. The systems promise improved detection, classification, screening, and surveillance for colorectal polyps and cancer. Several recently developed applications for AI-assisted colonoscopy have shown promising results for the detection and classification of colorectal polyps and adenomas. However, their value for real-time application in clinical practice has yet to be determined owing to limitations in the design, validation, and testing of AI models under real-life clinical conditions. Despite these current limitations, ambitious attempts to expand the technology further by developing more complex systems capable of assisting and supporting the endoscopist throughout the entire colonoscopy examination, including polypectomy procedures, are at the concept stage. However, further work is required to address the barriers and challenges of AI integration into broader colonoscopy practice, to navigate the approval process from regulatory organizations and societies, and to support physicians and patients on their journey to accepting the technology by providing strong evidence of its accuracy and safety. This article takes a closer look at the current state of AI integration into the field of colonoscopy and offers suggestions for future research.

Key Words: Colonoscopy; Adenoma; Artificial intelligence; Computational intelligence; Endoscopy; Surveillance

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INTRODUCTION

Colorectal cancer (CRC) is the fourth most commonly diagnosed and the third most fatal cancer worldwide in 2018[1]. The prevalence costs of cancer care were estimated to be $14.1 billion for CRC in the United States in 2010[2]. Over the past decade, CRC incidence and mortality have declined as a result of the increase in CRC screening and prevention examinations[3]. Colonoscopy is a screening tool with high sensitivity for the detection of precancerous and cancerous lesions, and may contribute to an approximately 80%, and up to 60% reduction in CRC incidence and mortality, respectively[4-8]. Colonoscopy prevents CRC by breaking the adenoma-carcinoma sequence through detection and removal of premalignant colorectal polyps[3]. Furthermore, it is a cost-effective procedure that often allows surgery to be avoided in patients with adenomas or CRCs that do not invade deeper than the superficial submucosa[9]. However, the quality of colonoscopy procedures depends on the experience of the endoscopists and the techniques and technology used[10]. A suboptimal colonoscopy examination can result in interval cancers, which are CRCs that occur after a colonoscopy and before the next surveillance examination, and are usually due to non-detection and/or incomplete resection of premalignant polyps. Recent research has shown that CRC precursor lesions are incompletely resected in about 14% of colonoscopy procedures[11]. Quality indicators have been established to describe and measure the quality of colonoscopy examinations[12], and the use of pre- and intraprocedural quality metrics has been shown to result in both an increase in colonoscopy quality and standardization of procedures[12,13]. One of the most recognized quality metrics is the adenoma detection rate (ADR), which is the proportion of an endoscopist’s patients undergoing screening colonoscopy who have at least one adenoma detected; every 1% increase in the ADR has been shown to result in a 3% decrease in the risk of post-colonoscopy CRC[10].

Over 90% of colorectal polyps are diminutive (≤ 5 mm) or small (≤ 10 mm), and most of these polyps are non-neoplastic[10]. Recent advances in image-enhanced endoscopy [IEE; e.g., blue-light imaging, narrow-band imaging (NBI), and i-Scan] have resulted in enhanced visualization of the polyp surface pattern. IEE can be employed for the optical classification of colorectal polyps during colonoscopy, obviating the need for pathology[14,15]. The American Society for Gastrointestinal Endoscopy (ASGE) Technology Committee, in its Preservation and Incorporation of Valuable endoscopic Innovations (PIVI) statement, has recommended the optical evaluation of diminutive polyps, adopting a “resect and discard” strategy for all diminutive colorectal polyps, and a “diagnosis and leave” strategy for diminutive rectosigmoid polyps, if the endoscopist can reach the recommended threshold of ≥ 90% agreement with histopathology results for surveillance interval assignment and ≥ 90% negative predictive value (NPV) for diagnosis of adenomatous histology, respectively[14,15]. Optical diagnosis can distinguish between neoplastic and non-neoplastic polyps and therefore deliver clinical and cost benefits by reducing the number of unnecessary histopathology examinations and providing immediate surveillance interval recommendations to patients. However, despite the demonstrated high accuracy of...
optical diagnosis for diminutive polyps, endoscopists have been reluctant to support its broad implementation because of concerns about incorrect diagnoses, assignment of inappropriate surveillance intervals, and related medicolegal issues[16].

To address the shortcomings in current colonoscopy practice, research has been directed at standardizing colonoscopy procedures among endoscopists through the integration of artificial intelligence (AI) into colonoscopy practice. AI could provide real-time support to physicians by automatically recognizing specific polyp patterns in colonoscopy images and/or videos, as well as suggesting the most probable histology and providing a confidence level for the predicted histology. The use of such technology would help to mitigate the effects of endoscopist experience in optical diagnosis. Computer-assisted, or most recently, AI-assisted colonoscopy diagnostic systems (CAD) for detection (CADe) and classification (CADx) of colorectal polyps are currently the two main areas of research and implementation of AI in clinical practice. AI-assisted colonoscopy improves ADR and allows for reliable, operator-independent pathology prediction of colorectal polyps. However, there is still a substantial communication gap between computer and medical fields, with scientists in these two disciplines divided in terms of background knowledge, available resources, research typology, and awareness of unmet needs in clinical practice. In this review, we summarize the most important aspects of the application of CADe and CADx in routine colonoscopy practice.

DEVELOPMENT OF COMPUTER-ASSISTED DIAGNOSTIC SYSTEMS

Pairing colonoscopy devices with image-enhanced technology (i.e., white-light endoscopy and chromoendoscopy) has improved the quality of care to patients by increasing the precision of colonoscopy procedures[4]. Recently, research efforts have focused on integrating computational power and previously collected data to enhance the simultaneous detection and classification of colonoscopy images or videos and support endoscopists in their decisions about the presence and/or histology of a polyp.

Machine learning is a subset of AI that allows mathematical methods to develop an algorithm based on given data (e.g., polyp images or videos) to predict the same pattern or a specific task in unseen or unknown data[17]. The final output of these systems (e.g., detection or classification of polyps) is based on pre-defined features or extraction of the most relevant image features (e.g., polyps), which may help in the specification, detection, or classification of a new image. In conventional machine learning (i.e., handcrafted models), a researcher manually introduces the clinically relevant polyp features to the machine learning algorithm. In contrast, in the most advanced machine learning method, which is called deep learning, polyp features, clinically relevant or not, are automatically extracted by the algorithm without prior introduction by a researcher. As a result, the output is based on the capture and summary of complex polyp characteristics, either for detection (i.e., discrimination of polyp from background mucosa) or prediction of histopathology (i.e., neoplastic or non-neoplastic)[17]. Deep learning employs deep neural networks (DNNs), which imitate the complex interconnected neural network in the human brain. These artificial neurons are positioned in several detections and pooling layers, taking weighted data (from the precedent layer), processing it, and passing the output (processed data) to the next layer. Each layer performs as a ”step of abstraction”[17], which forms a hierarchy of common features that grow in complexity throughout the layers (i.e., edge- > basic shape- > object- > class prediction). In other words, each layer would extract useful and relevant features from a given data that would facilitate the classification of the images. When data are presented, the DNN performs the repetitive iterations of a previously chosen model (i.e., support vector machines, random forests, or neural networks) throughout the deeper layers, so-called hierarchical feature learning[17]. For computer-assisted colonoscopy, the development of the AI model is primarily based on supervised data, where data are retrospectively labeled by one or a group of expert endoscopists. For example, in CADx, colonoscopy images or videos will be labeled as neoplastic or non-neoplastic based on the reference standard of pathology results (Figure 1), which would have been reviewed and finalized following consensus by several pathologists. In CADe, however, polyp images or videos will be reviewed by experienced endoscopists, and polyp borders will be delineated based on consensus by endoscopists. Ultimately, the output of the AI algorithm will identify the presence of a polyp, or be able to discriminate between a neoplastic and non-neoplastic polyp (Figure 2)[17]. However, there are some shortcomings and barriers to
Datasets
The data used to develop a CAD system will be divided into three or more datasets: One training dataset to build the AI model, one validation dataset to check the generalizability of the model, and at least one test dataset from another source of data to test the performance of the model[17]. Commonly, training and validation data are derived from the same source (i.e., colonoscopies performed at a single center); however, it is crucial to avoid overlap of data; otherwise, evaluation of the model hyperparameters would be flawed and would lead to “model overfitting.” Model overfitting is an error in modeling that occurs when the model is too tightly fitted to the training data and random fluctuations in the training data are learned as concepts by the model. The problem is that the fitted model does not generalize to new data due to its low bias and high variance. Overfitting can be avoided by tight monitoring of the model during the training by constantly evaluating the model performance in the training and validation data[17].

Researchers should use large and heterogeneous data, including normal and abnormal colonoscopies. A sufficient number of colonoscopy images or video frames would ensure a robust evaluation of model performance. Data should ideally be collected from multiple centers and diverse patients in terms of race, age, sex, and medical issues.
A lack of ground truth data or reliable annotated “big data” for generating effective and high-performance AI models could limit the broad application of CAD systems in clinical settings[18]. This is a challenging goal to achieve as it requires millions of colonoscopy images and videos to be annotated by multiple highly experienced experts to ensure a consensus on ambiguous images. Annotation and data labeling by experts should follow a uniform and standardized protocol, otherwise, the generalizability and performance evaluation of the model will be unreliable.

**Gold standard comparison**

The absence of a “gold standard” for diagnosing polyp histology would affect the accuracy of CAD performance. Although pathology results are currently regarded as the reference standard, the interobserver agreement among pathologists is not 100%; polyp histology determined by one pathologist might be different from that of another pathologist when reassessing the same specimen slides[19-22]. Therefore, the pathology data used for AI models must be re-evaluated by several pathologists prior to inclusion to ensure agreement on polyp pathology.

**Technical transparency**

The application of CAD in routine practice is a product of an interdisciplinary collaboration between medical and AI researchers. A recent review demonstrated that researchers failed to report the AI model characteristics effectively[23]. Researchers should ensure that they clearly define and report the AI model architecture or hyperparameters, including the number of deep layers and learning rate. The definition and testing of hyperparameters are crucial to the validation process owing to their direct effect on the model’s performance; optimal model generalizability in the validation step implies the correct choice of hyperparameters. Researchers should briefly explain the source of data, the process of data selection, and the number of patients, including images/videos frames, normal colonoscopies (i.e., without polyp identification), colonoscopy centers, and participating endoscopists together with their level of expertise[17].

Furthermore, researchers should adopt appropriate techniques to prevent model overfitting. Data leakage may occur when the testing dataset results are used to tune the model parameters instead of using the results derived from the validation dataset. Therefore, the model may over-fit toward the unseen data, risking a biased estimate of model performance. The stringent use of high-quality still images instead of videos that contain large variability in colonoscopy images may increase the risk of overfitting.

**Computer-assisted polyp detection system**

In the context of CAD, although the shift from separate engineering and medical disciplines to combined medical and engineering research has gained momentum over the last decade, pilot studies established the idea of CADe as early as 2003[24,25]. The primary hand-crafted AI models used the pre-described polyp features (e.g., color and/or texture-based features) and annotated colonoscopy videos for the detection of colorectal polyps[25-29]. Other studies used the same idea and developed several AI models that resulted in up to 90% sensitivity[30-32]. However, these studies used small and homogeneous datasets to develop and validate the AI models, raising doubts over the model’s optimal performance. The hand-crafted features used to build the model led to suboptimal performance, probably because of impaired feature recognition and description, and a high level of false-positive detection owing to the presence of colonic folds, blood vessels, and feces in the lateral view.

After the invention of DNNs, important polyp features could be automatically recognized. Subsequently, the accuracy and sensitivity of models improved, signaling the great potential for CADe application. Recently, Yamada et al.[33] developed a CADe system using a supervised DNN, and validated the system using a dataset of 705 still images of 752 lesions and 4135 still images of noncancerous tissue. This system performed well, with a sensitivity and specificity of 97.3% and 99.0%, respectively, and an area under the curve (AUC) of 0.975 in the validation set. Misawa et al.[34] developed a model based on 546 short colonoscopy videos, comprising 155 polyp-positive and 391 polyp-negative videos. Two experts retrospectively annotated videos for polyp presentation to provide a gold standard for comparison. The model presented sensitivity, specificity, and accuracy of 90.0%, 63.3%, and 76.5%, respectively. The polyp detection rate and false-positive detection rate were 95% and 60%, respectively. Other significant research used a large dataset for training an AI model, which comprised 8641 annotated images from over 2000 colonoscopies[35]. The
model generated excellent detection capability, with an AUC of 99% and an accuracy of 96.4%. The performance of this model was also superior to that of experts. The authors tested model performance in 20 colonoscopy videos with a total duration of 5 h, during which colonoscopists removed 28 polyps. After reviewing the videos by four independent experts, eight additional polyps were identified (36 polyps) without the use of AI assistance and 17 additional polyps were detected with AI assistance (total 45 polyps). The model had a false-positive rate of 7%.

Research with a prospective design and focusing on the evaluation of the real-time performance of CADe is scarce. Wang et al.[36] conducted a prospective non-blinded clinical trial, which aimed to measure ADR with and without the application of CADe. Using 552 and 536 colonoscopies in the control and intervention arms, respectively, the authors found a statistically significant increase in ADR (29.1% vs 20.3%) and an increased number of adenomas per patient (0.53 vs 0.31) when CADe was used. The false-positive rate was 7.5% per colonoscopy, and there was no significant difference in the procedure time. CADe could detect a higher number of diminutive adenomas and hyperplastic polyps, which represent a higher risk of unnecessary polypectomies, pathology examinations, and longer procedure times. To date, the generalizability of this system has not been tested in Western clinical settings.

In contrast to the results of the latter study, Klare et al.[37] prospectively evaluated endoscopist performance using CADe assistance during the real-time colonoscopy procedures of 55 patients. However, the endoscopists only observed the regular monitor, and an independent investigator observed the monitor dedicated to representing the real-time outputs of the CADe system in a separate room, which was blinded from the endoscopists’ sight. Therefore, the endoscopists were blinded to the real-time CADe outputs. This system did not increase the endoscopists' ability to detect polyps in real-time practice: In per-patient analysis, the performance of CADe resulted in endoscopists achieving a lower ADR (29.1% vs 30.9%); in per-polyp analysis, CADe could only detect 55 out of 73 polyps previously detected by endoscopists. Tables 1 and 2 shows the summary of the recent studies evaluating a CADe system.

**Computer-assisted polyp classification system**

Computer-assisted diagnosis of the histopathology of colorectal polyps has become an area of significant research interest because of its potential to prevent the resection of low-risk polyps and reduce the number of unnecessary histopathology examinations. Many studies have successfully developed and validated CADx models, the use of which would allow the “diagnosis and leave strategy” to be implemented. In a prospective pilot study, in which the data from 128 patients undergoing colonoscopy using NBI were used to test a CADx system (209 polyps detected and removed), three polyp features were used to build the AI model: Mean vessel length, vessel circumference, and mean brightness within detected blood vessels.[38] The results showed that the endoscopists’ ability to predict polyp histology was superior to that of CADx, which had a sensitivity of 90% and specificity of 70.2% in differentiating neoplastic from non-neoplastic images compared with histopathology as the gold standard. The system’s diagnostic performance was compared with that of endoscopists, who were blinded to the histopathology reference standard. Endoscopists accurately predicted polyp histology with a sensitivity of 93.8% and specificity of 85.7% when there was interobserver agreement. In cases of disagreement between endoscopists, the suggested safe prediction of polyp histology (i.e., classification as neoplastic) produced a sensitivity of 96.9% and specificity of 71.4%. Overall, CADx could predict polyp histology with an approximate sensitivity and specificity of 90% and 70%, respectively; however, the overall correct classification rate was moderate (85.3%). Notably, this AI algorithm was not fully automated; thus, its real-time performance in a clinical setting remains to be determined. Another limitation of this study was the use of data from NBI colonoscopies. Although NBI may assist polyp classification, its use may cast doubt on the generalizability of the model, especially in clinical settings where NBI is not available.

The real-time evaluation of CADx is important if the technology is to be integrated into clinical practice. Some studies have used the real-time decision outputs from support vector machines for building CADx algorithms, with promising results.[39-43] Moreover, Chen et al.[44] demonstrated that an AI model could accurately predict the histopathology of 284 diminutive polyps, comprising 96 hyperplastic and 188 neoplastic polyps diagnosed using NBI, with 96.3% sensitivity, 78.1% specificity, 91.5% NPV, and 89.6% PPV. This study and the study by Byrne et al.[45] that used the combination of CADe and CADx systems (described below), are remarkable in that they achieved the threshold NPV of ≥ 90% recommended by the ASGE PIVI statement, favoring the implementation of the “diagnose and leave” strategy for diminutive...
### Table 1 Summary of the randomized controlled trials involving computer-aided detection for colonoscopy

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Year</th>
<th>Study design</th>
<th>Study aim</th>
<th>CADe system</th>
<th>Image modality</th>
<th>Number of patients in the CADe group</th>
<th>Number of patients in the control group</th>
<th>Number of polyps (CADe vs control group)</th>
<th>Adenoma detection rate (%) (CADe vs control group)</th>
<th>Polyp detection rate (%) (CADe vs control group)</th>
<th>Number of false-positive rate (%) (CADe vs control group)</th>
<th>Withdrawal time (CADe vs control group), min ± SD; minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al. [36]</td>
<td>2019</td>
<td>Non-blinded prospective randomised controlled study</td>
<td>To investigate whether a high-performance real-time CADe system can increase polyp and adenoma detection rates in the real clinical setting</td>
<td>The real-time automatic polyp detection system (Shanghai Wision AI Co., Ltd.) based on artificial neural network-SegNet architecture</td>
<td>Real-time Video stream</td>
<td>522</td>
<td>536</td>
<td>767 (498 vs 269)</td>
<td>29.1 vs 20.3; P = 0.001; 95%CI = 1.21-2.135</td>
<td>45.0 vs 29.1; P &lt; 0.001; 95%CI = 1.532-2.544</td>
<td>39 vs 0</td>
<td>6.18 ± 1.38 vs 6.07 ± 1.11; P = 0.15</td>
</tr>
<tr>
<td>Wang et al. [74]</td>
<td>2020</td>
<td>Double-blind Prospective randomised trial</td>
<td>To assess the effectiveness of a CADe system for improving detection of colorectal adenomas and polyps; to analyse the characteristics of polyps missed by endoscopists</td>
<td>The real-time automatic polyp detection system (Shanghai Wision AI Co., Ltd.) based on artificial neural network-SegNet architecture</td>
<td>Real-time Video stream</td>
<td>484</td>
<td>478</td>
<td>809 (501 vs 308)</td>
<td>34.0 vs 28.0; P = 0.030; OR = 1.36, 95%CI = 1.03-1.79</td>
<td>52.0 vs 37.0; P &lt; 0.001; OR = 1.86, 95%CI = 1.44-2.41</td>
<td>48 in CADe group (control group not reported)</td>
<td>6.48 ± 1.32 vs 6.37 ± 1.09; P = 0.14</td>
</tr>
<tr>
<td>Su et al. [75]</td>
<td>2020</td>
<td>Single-blind Prospective randomised trial</td>
<td>To develop an automatic quality control system; to investigate whether the system could increase the detection of polyps and adenomas in real clinical practice</td>
<td>Five deep learning convolutional neural networks (DCNNs) based on AlexNet, ZFNet, and YOLO V2</td>
<td>Real-time Video stream</td>
<td>308</td>
<td>315</td>
<td>273 (177 vs 96)</td>
<td>28.9 vs 16.5; P &lt; 0.001; OR = 2.055, 95%CI = 1.397-3.024</td>
<td>38.3 vs 25.4; P = 0.00; OR = 1.824, 95%CI = 1.296-2.569</td>
<td>62 in CADe system (control group not reported)</td>
<td>7.03 ± 1.01 vs 5.6 ± 1.26; P &lt; 0.001</td>
</tr>
<tr>
<td>Gong et al. [76]</td>
<td>2020</td>
<td>Single-blind Prospective randomised trial</td>
<td>To evaluate whether the CADe system could improve polyp yield during colonoscopy</td>
<td>ENDOANGEL based on the deep neural networks and perceptual hash algorithms</td>
<td>Real-time video stream</td>
<td>355</td>
<td>349</td>
<td>302 (178 vs 124)</td>
<td>16 vs 8; P = 0.001; OR = 2.30, 95%CI = 1.40-3.77</td>
<td>47 vs 34; P = 0.0016; OR = 1.69, 95%CI = 1.22-2.34</td>
<td>For endoscope being inside = 0.8; For identification of the caecum = 2; For prediction of slipping = 0</td>
<td>6.38 ± 2.48 vs 4.76 ± 254; P &lt; 0.0001</td>
</tr>
<tr>
<td>Liu et al. [77]</td>
<td>2020</td>
<td>Double-blind Prospective randomised trial</td>
<td>To study the impact of CADe system on the detection rate of polyps and adenomas in colonoscopy</td>
<td>The convolutional three-dimensional (3D) neural network</td>
<td>Real-time video stream</td>
<td>508</td>
<td>518</td>
<td>734 (486 vs 248)</td>
<td>39.1 vs 23.9; P &lt; 0.001; OR = 1.637, 95%CI = 1.201-2.220</td>
<td>43.7 vs 27.8; P &lt; 0.001; OR = 1.157, 95%CI = 1.586-2.483</td>
<td>36 in CADe system (control group not reported)</td>
<td>6.82 ± 1.78 vs 6.74 ± 1.62; P &lt; 0.001</td>
</tr>
<tr>
<td>Luo et al. [78]</td>
<td>2021</td>
<td>Non-blinded Prospective randomised trial</td>
<td>To explore whether CADe could improve the polyp detection rate in the actual clinical environment</td>
<td>A CNN algorithm based on a YOLO network architecture</td>
<td>Real-time Video stream</td>
<td>150</td>
<td>150</td>
<td>185 (105 vs 80)</td>
<td>38.7 vs 34.0; P &lt; 0.001</td>
<td>-</td>
<td>-</td>
<td>52 in CADe system (control group not reported)</td>
</tr>
<tr>
<td>Repici et al. [79]</td>
<td>2020</td>
<td>Singles-blind Prospective randomised trial</td>
<td>To assess the safety and efficacy of a CADe system for the detection of colorectal neoplasia</td>
<td>The CNN (GI-Genius; Medtronic)</td>
<td>Real-time Video stream</td>
<td>341</td>
<td>344</td>
<td>596 (353 vs 243)</td>
<td>54.8 vs 40.4; P &lt; 0.001; RR = 1.30, 95%CI = 1.14-1.45</td>
<td>279/341 (82)</td>
<td>214/344 (62)</td>
<td>417 ± 101 seconds for the CADe group vs 435 ± 149 for controls; P = 0.1</td>
</tr>
</tbody>
</table>

Ref. = Reference; CADe = Computer-aided detection; min ± SD = Minimum ± Standard Deviation; P = Probability; OR = Odds Ratio; CI = Confidence Interval.
To investigate the impact of CADe on adenoma miss and detection rate

1The total adenoma miss rate by computer-assisted detection system (CADe) [colonoscopy = 13.89%, 95% confidence interval (CI) = 8.24%-19.54%]; by routine colonoscopy = 40.00%, 95%CI=31.23%–48.77%, P < 0.0001. The total polyp miss rate by CADe colonoscopy = 12.98%, 95%CI = 9.08%-16.88%; by routine colonoscopy = 45.90%, 95%CI = 39.65%-52.15%, P < 0.0001. Visible adenoma miss rate: Routine-CADe group = 24.21% vs CADe-routine group = 1.59%, P < 0.001; Visible polyp miss rate: Routine-CADe group = 30.89% vs CADe-routine group = 2.36%; P < 0.001.

2It means that the colonoscopy was performed by the CADe system and then the conventional method.

3It means that the colonoscopy was performed by the conventional method and then the CADe system.

4Median (interquartile range).

CADe: Computer-assisted detection system; CNN: Convolutional neural network; DCNN: Deep learning convolutional neural network; SD: Standard deviation; OR: Odds ratio; RR: Relative risk; CI: Confidence interval.

rectosigmoid polyps[46]. However, the results of the former study need to be confirmed in a prospective study, ideally in a controlled trial, where the probability of selection bias is less, and the AI model can be compared with a conventional setting (without using AI).

More prospective studies assessing CADx are required to support the integration into clinical practice. The existing prospective studies resulted in a high and favorable diagnostic performance, which provided strong evidence to support the real-time application of CADx[47,48]. In contrast, the AI models developed and tested in a prospective trial by Kuiper et al[49] did not show sufficient power for differentiating adenomatous from non-adenomatous lesions. Another CADx model in a prospective study by Rath et al[50] could only produce moderate accuracy, sensitivity, and specificity (84.7%, 81.8%, and 85.2%, respectively), although the NPV was relatively high at 96.1%. This model would therefore allow diminutive rectosigmoid polyps to be diagnosed and left in situ without resection. The authors suggested that the low prevalence of neoplastic polyps could explain the model’s moderate diagnostic performance compared with hyperplastic polyps in their dataset, which might proportionately result in an overestimation of the NPV, and an underestimation of the accuracy and PPV of the model. Table 3 shows the summary of the recent studies evaluating a CADe system.

### Combined CADe and CADx models

The ideal CAD system would support the simultaneous detection and classification of polyps to optimize colonoscopy outcomes and achieve the best level of CRC prevention. A recent study evaluated the real-time application of CADx in combination with CADe[45]. The validated model was tested on a series of 125 diminutive polyps, comprising 51 hyperplastic polyps and 74 adenomas. The combined model could not detect histopathology in 15% of polyps. For the remaining 106 polyps histologically predicted with high confidence, the AI model demonstrated an accuracy of 94%, sensitivity of 98%, specificity of 83%, NPV of 97%, and positive predictive value (PPV) of 90%. In a significant study, Byrne et al[51] developed a new...
Table 2 Summary of the non-controlled studies involving computer-aided detection for colonoscopy

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Year</th>
<th>Study design</th>
<th>System</th>
<th>Image modality</th>
<th>Number of patients/colonoscopies used for training/test datasets (total)</th>
<th>Number of colonoscopy/polyp images/videos used for training/test datasets</th>
<th>Diagnostic properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park and Sargent [81]</td>
<td>2016</td>
<td>Retrospective</td>
<td>CADe based on DCNN using a conditional random field model</td>
<td>Still images</td>
<td>35 (colonoscopy videos)</td>
<td>562/562 (colonoscopy still images)</td>
<td>Sensitivity = 86%; specificity = 85%; AUC = 0.8585</td>
</tr>
<tr>
<td>Fernández-Esparrach et al [73]</td>
<td>2016</td>
<td>Retrospective</td>
<td>CADe based on energy map</td>
<td>Still images</td>
<td>NA/24 colonoscopy videos containing 31 different polyps</td>
<td>NA/Experiment A: 612 polyp images from all 24 videos. Experiment B: 47886 frames from the 24 videos</td>
<td>Experiment A: accuracy = small vs all polyps = 77.5%, 95%CI = 71.5%–82.6%; vs 66.2%, 95%CI = 61.4%–70.7%; P &lt; 0.01. Experiment B: The AUC = high quality frames vs all Frames = 0.79, 95%CI = 0.70–0.87 vs 0.75, 95%CI = 0.66–0.83</td>
</tr>
<tr>
<td>Yu et al [82]</td>
<td>2017</td>
<td>Retrospective</td>
<td>CADe based on three-dimensional (3-D) deep learning integration framework by leveraging the 3-D fully CNN (3D-FCN)</td>
<td>Videos</td>
<td>20/18 (colonoscopy videos)</td>
<td>3799 frames with polyps in total</td>
<td>Sensitivity = 71%; PPV = 88%; precision = 88.1%</td>
</tr>
<tr>
<td>Billah et al [83]</td>
<td>2017</td>
<td>Retrospective</td>
<td>CADe based on CNN and color wavelet features using a linear support vector machine</td>
<td>Still images</td>
<td>100 (colonoscopy videos for combined training and test datasets)</td>
<td>14000 still images (combined for training and test datasets)</td>
<td>Accuracy = 98.65%; sensitivity = 98.79%; specificity = 98.52%</td>
</tr>
<tr>
<td>Zhang et al [84]</td>
<td>2017</td>
<td>Retrospective</td>
<td>CADe based on DCNN</td>
<td>Still images</td>
<td>NA</td>
<td>2262/150 random, 30 NBI (colonoscopy still images)</td>
<td>Accuracy = 85.9%; sensitivity = 98%; PPV = 99%; precision = 87.3%; recall rate = 87.6%; AUC = 1.0</td>
</tr>
<tr>
<td>Wang et al [85]</td>
<td>2018</td>
<td>Retrospective</td>
<td>CADe based on DNN</td>
<td>Still images</td>
<td>1290/1138 (2428) patients</td>
<td>27113/5545 (colonoscopy images)</td>
<td>Sensitivity = 94.38%, 95%CI = 93.80%–94.96% in images with polyp; AUC = 0.984</td>
</tr>
<tr>
<td>Misawa et al [34]</td>
<td>2018</td>
<td>Retrospective</td>
<td>CADe based on CNN</td>
<td>Videos</td>
<td>59/14 (73)</td>
<td>411/135 (colonoscopy videos containing 150 polyps)</td>
<td>Per-polyp sensitivity = 94%; per-frame sensitivity = 90%; specificity = 63.3%; accuracy = 76.5%; false positive rate = 60%; AUC = 0.87</td>
</tr>
<tr>
<td>Yamada et al [33]</td>
<td>2019</td>
<td>Retrospective</td>
<td>CADe based on DNN</td>
<td>Videos</td>
<td>NA/77 (number of videos)</td>
<td>13983/4840 (colonoscopy videos)</td>
<td>Sensitivity = 97.3%, 95%CI = 95.9%–98.4%; specificity = 99.0%, 95%CI = 98.6%–99.2%; AUC = 0.975, 95%CI = 0.964–0.986</td>
</tr>
<tr>
<td>Urban et al [35]</td>
<td>2018</td>
<td>Retrospective</td>
<td>CADe based on deep learning CNN</td>
<td>Videos</td>
<td>Several training and validation sets: (1) Cross-validation on the 8641 images; (2) Training on the 8641 images and testing on the 9 videos, 11 videos, and independent dataset; and (3) Training on the 8641 images and 9 videos and testing on the 11 videos and independent dataset</td>
<td>Sensitivity = 96.9%; specificity = 95%; AUC = 0.991; accuracy = 96.4%; false positive rate = 7%</td>
<td></td>
</tr>
<tr>
<td>Klare et al [37]</td>
<td>2019</td>
<td>Prospective</td>
<td>Automated polyp detection software (&quot;KoloPol,&quot; Fraunhofer IS, Erlangen, Germany) based on CNN</td>
<td>Live colonoscopy videos</td>
<td>NA</td>
<td>NA/35 (colonoscopy videos)</td>
<td>Per-polyp sensitivity = 75.3%, 95%CI = 62.3%–84.9%; PDR = 50.9%, 95%CI = 37.1%–64.4%; ADR = 29.1%, 95%CI = 17.6%–42.9%</td>
</tr>
<tr>
<td>Ozawa et al [86]</td>
<td>2020</td>
<td>Retrospective</td>
<td>CADe based on DCNN</td>
<td>Still images</td>
<td>12895 patients</td>
<td>16418/7077</td>
<td>Sensitivity = 92%; PPV = 86%; accuracy = 83%; identified adenomas = 97%</td>
</tr>
</tbody>
</table>
platform using three distinct AI CADe and CADx algorithms to provide endoscopists with a full workflow from detection to classification: An NBI light detector, a polyp detector, and an optical biopsy. The NBI light detector runs throughout the colonoscopy procedure to ensure the detection of all colorectal polyps with white light imaging, and the optical biopsy provides an accurate polyp classification using NBI light. The NBI light model resulted in an excellent accuracy of 99.94% when tested in 21804 unseen colonoscopy video frames. However, the detection mode using white light resulted in a sensitivity of only 79%. The optical biopsy model could accurately classify 97.6% of polyps, which was significantly higher than a previous CADx model tested by the same research team\cite{45}, and had a sensitivity of 95.95%, specificity of 91.66%, and NPV of 93.6% for polyp classification.

QUALITY ASSESSMENT OF COLONOSCOPY BY COMPUTER

Few studies have evaluated an AI-assisted system for the ability to accurately and automatically assess the quality of a colonoscopy procedure, including the identification of critical anatomical landmarks, especially when the endoscopic field is blurry\cite{52,53}. Filip et al\cite{53} developed a “Colometer” system that could rate colonoscopy quality based on the percentage of the withdrawal time with adequate visualization. This system could detect the factors associated with optimal real-time visualization of the mucosa, including image clarity, withdrawal velocity, and level of bowel cleanliness. A dataset of expert-annotated images and videos was used to train the AI model. The authors compared the quality rated by this system with that of three independent experts. There was a strong correlation between AI and expert quality ratings (p coefficient 0.65, \(P = 0.01\)). In another study, a system comprising two AI algorithms was designed to automatically detect the appendiceal orifice on a colon image or video\cite{54}. The first algorithm was developed to detect the appendiceal orifice on endoscopic images based on the local shape, lighting, and intensity differences from a normal edge direction. The second algorithm was designed to detect the appendiceal orifice in the colonoscopy videos using a frame intensity histogram. The system could detect the orifice in images with an average sensitivity and specificity of 96.86% and 90.47%, respectively, and correctly classified 21 out of 23 colonoscopy videos (accuracy 91.30%).
Table 3 Summary of the non-controlled studies involving computer-aided diagnosis for colonoscopy including studies with combined detection and diagnosis systems

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Year</th>
<th>Study design</th>
<th>Study aim</th>
<th>System</th>
<th>Number of patients/colonoscopies used for training/test datasets (total)</th>
<th>Number of colonoscopy/polyp images/videos used in training/test datasets</th>
<th>Diagnostic properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tischendorf et al [38]</td>
<td>2010</td>
<td>Prospective pilot</td>
<td>Distinguishing adenomas from non-adenomas</td>
<td>CADx based on SVMs</td>
<td>NA/128; Colonoscopy videos</td>
<td>NA/209 polyps containing 160 neoplastic and 49 non-neoplastic polyps in the test dataset</td>
<td>CADx: Sensitivity = 90%, specificity = 70%, correct classification rate = 85.3%. Consensus decision between the human. Observers: Sensitivity = 93.8%, specificity = 85.7%, correct classification rate = 91.9%. “Safe” decision, when there was interobserver discrepancy: Sensitivity = 96.9%, specificity = 71.4%, correct classification rate = 90.9%</td>
</tr>
<tr>
<td>Aihara et al [47]</td>
<td>2013</td>
<td>Prospective</td>
<td>Distinguishing neoplastic from non-neoplastic lesion</td>
<td>CADx based on numerical color analysis of autofluorescence endoscopy as an Adobe AIR application</td>
<td>NA/32 patients in the test dataset</td>
<td>NA/102 lesions containing 75 neoplastic lesions in the test dataset</td>
<td>Sensitivity = 94.2%; specificity = 88.8%; PPV = 95.6%; NPV = 85.2%</td>
</tr>
<tr>
<td>Mori et al [87]</td>
<td>2015</td>
<td>Retrospective pilot</td>
<td>Distinguishing small (≤ 10 mm) neoplastic from non-neoplastic lesion</td>
<td>CADx (EC-CAD) based on CNN</td>
<td>NA/152 patients in the test dataset</td>
<td>NA/176 small polyps in the test dataset containing 137 neoplastic and 39 non-neoplastic polyps for the test dataset</td>
<td>Accuracy = 89.2%, 95%CI = 83.7%-93.4%; Sensitivity = 92.0%, 95%CI = 86.1%-95.9%; specificity of 79.5%, 95%CI = 63.5%-90.7%</td>
</tr>
<tr>
<td>Kuiper et al [49]</td>
<td>2015</td>
<td>Retrospective</td>
<td>Distinguishing small (≤ 9 mm) neoplastic from non-neoplastic lesion</td>
<td>CADx (WavSTAT) based on CNN</td>
<td>NA/87 patients in the test dataset</td>
<td>NA/207 small lesions in the test dataset</td>
<td>Accuracy = 74.4%, 95%CI = 68.1%-79.9%; sensitivity = 85.3%, 95%CI = 0.78-0.90; specificity = 58.8%, 95%CI = 0.48-0.69; PPV = 74.8%, 95%CI = 0.67-0.81; NPV = 73.5%; accuracy of on-site recommended surveillance interval = 73.7%</td>
</tr>
<tr>
<td>Misawa et al [34]</td>
<td>2018</td>
<td>Retrospective</td>
<td>Distinguishing neoplastic from non-neoplastic lesion categorized</td>
<td>CADx based on SVMs</td>
<td>NA</td>
<td>979 images containing 381 non-neoplasms and 598 neoplasms in the training dataset/100 images containing 50 non-neoplasms and 50 neoplasms in the test dataset</td>
<td>Accuracy = 90.0%, 95%CI = 82.4-95.1; sensitivity = 84.5%, 95%CI = 72.6-92.7; specificity = 97.6%, 95%CI = 87.4-99.9; PPV = 98.0%, 95%CI = 89.4-99.9; NPV = 82.0%, 95%CI = 68.6-91.4</td>
</tr>
<tr>
<td>Byrne et al [51]</td>
<td>2018</td>
<td>Retrospective</td>
<td>Distinguishing neoplastic from non-neoplastic lesions</td>
<td>CADx + CADe based on an improved DCNN model using NBI</td>
<td>NA</td>
<td>NA/21804 unseen frames in the test dataset</td>
<td>Accuracy = 99.94%; sensitivity = 95.95%; specificity = 91.66%; NPV = 93.6%; prediction of polyp videos = 97.6%</td>
</tr>
<tr>
<td>Mori et al [48]</td>
<td>2018</td>
<td>Prospective</td>
<td>Distinguishing diminutive (≤ 5 mm) neoplastic from non-neoplastic lesions</td>
<td>CADx based on SVMs used with NBI and endocytoscope</td>
<td>NA/791 patients in the test dataset</td>
<td>61925/466 polyps from 325 patients in the test dataset</td>
<td>CADx-NBI: Sensitivity = 92.7%, 95%CI = 89.1-95.4; specificity = 89.8%, 95%CI = 84.4-93.5; PPV = 93.7%, 95%CI = 90.2-96.2; NPV = 88.3%, 95%CI = 82.7-92.6. CADx-endocytoscope: Sensitivity = 91.3%, 95%CI = 87.5-94.3; specificity = 88.7%, 95%CI = 83.1-93.0; PPV = 92.9%, 95%CI = 89.3-95.6; NPV = 86.3%, 95%CI = 80.4-90.9</td>
</tr>
<tr>
<td>Byrne et al [45]</td>
<td>2019</td>
<td>Retrospective</td>
<td>Distinguishing diminutive (≤ 5 mm) neoplastic from non-neoplastic lesions</td>
<td>CADx based on DCNN</td>
<td>NA</td>
<td>Training dataset: 60089 frames from 223 polyp videos (29% NICE type 1, 53% NICE type 2 and 18% of normal mucosa with no polyp)/validation dataset: 40 videos (NICE type 1, NICE type 2 and two videos of normal mucosa)/test dataset: 125 consecutively</td>
<td>Accuracy = 94%, 95%CI = 86%-97%; sensitivity = 98%, 95%CI = 92%-100%; specificity = 83%, 95%CI = 67%-93%; NPV = 97%; PPV = 90%</td>
</tr>
</tbody>
</table>
identified diminutive polyps, comprising 51 hyperplastic polyps and 74 adenomas

Agreement between the true polyp histology: CADx = 0.614–0.642; accuracy = 81.3%–82.4%; sensitivity = 82.1%; specificity = 93.7%; PPV = 78%; NPV = 95%; the AUC = 0.93–0.95, 0.86–0.89, and 0.89–0.91 for serrated polyps, benign adenoma/mucosal or superficial submucosal cancer, and deep submucosal cancer, respectively.

Kudo et al. [89] 2020 Retrospective Distinguishing small (≤ 10 mm) neoplastic from non-neoplastic lesions The EndoBRAIN system (CADx + CADe based on DCNN) NA/89 patients test set 69,142 images taken at 520-fold magnification and 2,000 polyps/100 lesions (≤ 10 mm) in the test dataset CADe: Accuracy = 98%, 95% CI = 97.3%–98.6%; sensitivity = 96.9%, 95% CI = 95.8%–97.8%; specificity = 100%, 95% CI = 99.6%–100%; PPV = 100%, 95% CI = 99.8%–100%; NPV = 94.6%, 95% CI = 92.7%–96.1%; CADx: Accuracy = 96%, 95% CI = 95.1%–96.8%; sensitivity = 96.9%, 95% CI = 95.8%–97.8%; specificity = 94.3%, 95% CI = 92.3%–95.9%; PPV = 96.9%, 95% CI = 95.8%–97.8%; NPV = 94.3%, 95% CI = 92.3%–95.9%.

CADe: Computer-assisted detection system; CADx: Computer-assisted diagnosis system; CNN: Convolutional neural network; DCNN: Deep learning convolutional neural network; AUC: Area Under the Receiver Operating Characteristic curve; PPV: Positive predictive value; NPV: Negative predictive value; SVM: Support vector machine; SP: Serrated polyps; CI: Confidence interval.

RECOMMENDATIONS FOR FUTURE RESEARCH

Despite potential benefits of AI in colonoscopy, regulatory approval and standardization of AI models are difficult goals to achieve for a number of reasons described below.

Polyp morphology

Datasets might underrepresent particular polyp morphologies that are not common findings during colonoscopy. For example, non-polypoid lesions with Paris classification of flat and/or depressed morphology are more likely to harbor advanced histology or malignancy but are not a common finding during colonoscopy [55]. The endoscopic detection of non-polypoid lesions is problematic because of their surface pattern resemblance to normal mucosa [56]. Moreover, serrated polyps comprise about 30% of colon polyps, with sessile serrated polyph/adenoma (SSA/P) prevalence being less than 10% [57]. It has been proven that SSA/Ps can be responsible for CRC through a serrated (hyperplastic-SSP/A-serrated-CRC) sequence [58]. However, SSA/Ps can hardly be distinguished from normal mucosa or hyperplastic polyps by features of crypt distortion. Research has shown that previously diagnosed hyperplastic polyps might be reclassified as SSAs after pathological reassessment [19-22], particularly for larger (> 5 mm) or right-sided polyps, and co-existing adenomas containing advanced histology [19,21,59]. A recent meta-analysis showed that pathological reassessment of resected polyps led to a significant change in diagnosis from hyperplastic to SSA for polyps in the right colon and polyps ≥ 5 mm (odds ratio 4.401 and 8.336, respectively) [59]. Moreover, there is poor agreement among pathologists in the determination of...
high-risk polyp features owing to the various approaches used for preparing biopsy specimens or level of expertise[19,60]. Therefore, the development of an AI platform capable of detecting and distinguishing subtle adenomatous features from normal mucosa with a high level of accuracy would be a valuable clinical tool.

**Metadata**

Most studies have failed to assess the performance and accuracy of AI models according to polyp size, polyp location, bowel preparation score, or withdrawal time [18]. Patients’ information including demographic and clinical characteristics (e.g., colonoscopy indication, disease status), procedure-related quality characteristics (i.e., bowel preparation level, withdrawal time), procedure time and room, endoscopists fatigue (i.e., the procedure performed in the morning or afternoon) are the important factors that are linked with the long-term non-endoscopic outcome of interest. In other words, the detection and classification of colorectal polyps are the intermediate outcomes of the colonoscopy, but the prevention of interval cancer during the surveillance period, or the evaluation of the effectiveness of medical therapy and the need for surgical treatment in patients with inflammatory bowel diseases are the ultimate goals of the colonoscopy depending on the primary indication of the procedure. As mentioned in Kudo et al[61], metadata is a critical component in establishing optimal AI platforms that can perform well in real-world practice with suboptimal conditions. For example, SSA/Ps are mainly located in the right colon, where endoscopic access and complete inspection of the mucosa are challenging[38]. Collecting a high number of colonoscopy videos with a high number of SSA/P polyps and cross-linking with patient's data would increase the accuracy and effectiveness of the colonoscopy. Future AI models must incorporate the information of the polyp size and location as well as the clinical, pre-procedural, and polyp morphological characteristics rather than focusing on the polyp images and videos alone.

**Prospective real-time studies**

The robustness of AI platforms has not been widely estimated in real-time clinical settings through prospective studies. Most studies have been retrospective in design and subject to selection bias. Therefore, the comparison of accuracy between model and endoscopists may falsely deviate in favor of CAD. For example, in CADe, the researcher might exclude unclear colonoscopy or polyp images/videos; a fuzzy or blurred endoscopic view may occur when water or blood obscures the field, or when feces cover the bowel surface preventing a complete examination. There should also be a mixture of polyp-positive and polyp-negative images from abnormal and normal colonoscopies in all training, validation, and test datasets. The development of AI models must be rigorously based on a training dataset that is preferably gathered during real-time colonoscopies. Data should be collected prospectively by both experienced and novice endoscopists to represent the actual state of practice when assessing the model. The elimination of selection bias is most relevant to CADe systems and less so to CADx systems. Studies should be based in several centers to ensure the reproducibility of the results at the testing level. Testing CAD systems in non-academic settings will demonstrate whether the model represents actual real-world practice, where more polyps are missed and/or there is no access to advanced technologies such as NBI. In addition, real-time and multicenter studies may help to clarify the place of AI in the diagnostic process. Prospective studies would provide robust evidence to support the application of CAD and enhance endoscopists’ trust in optical polyp classification[62]. Nevertheless, CAD is still an operator-dependent technology as it is the experienced endoscopists who must provide the annotated datasets for the development of the system, and the accuracy of the AI output relies on the endoscopist presenting a clear endoscopic field to the system. Certain challenges such as prolonged procedure times, high positivity rate, and inability to predict the histology in the presence of feces or blood in the visual field should be mitigated to prevent suboptimal diagnosis. Physicians should continue to follow the recommended procedural measures, including sufficient bowel preparation and photo documentation, to avoid legal and insurance issues.

Researchers should prioritize prospective controlled trials to allow a precise comparison between the settings that use and do not use AI platforms, otherwise, the real benefits of the AI system cannot be determined. Crossover studies, where patients act as their own controls and undergo colonoscopy both with and without AI support would be useful as fewer patients would be needed. In practice, the endoscopist would first detect and classify a polyp before using the AI support system to ensure the accuracy of their classification. This process should be performed in a time-efficient manner as the benefit of AI assistance would be irrelevant if the procedure was
significantly prolonged.

**Standardization of endpoints**

All research evaluating the diagnostic accuracy of CAD systems should use standardized research endpoints derived from the latest guidelines. Similarly to other diagnostic evaluation studies, sensitivity, specificity, PPV, NPV, and AUC must be reported, as well as confusion matrices and mean average precision for multiclass classifications and intersection over union (IoU), or the DICE coefficient for segmentation (i.e., delineation) in particular situations\[^{63,64}\]. The use of such a comprehensive set of metrics would provide convincing evidence, reassuring physicians about the reliability of AI tools. For example, ADR must be reported for all research related to the evaluation of CAd systems, as such systems aim to achieve complete detection of all colorectal lesions. Similarly, the NPV of CADx systems must be reported to confirm the ability of CADx to achieve the recommended NPV benchmark of $\geq 90\%$ according to the PIVI statement\[^{46}\]. In addition, for surveillance interval assignment, the agreement between AI-based assignment and that of the histopathology reference standard must reach the $\geq 90\%$ threshold recommended by the PIVI statement\[^{46}\].

**Transparency of AI analyses**

We should avoid the black-box phenomenon when the decision-making process of the model by the convolutional neural network cannot be deconvoluted due to the complexity of the process\[^{65,66}\]. An important aspect of the wide application of AI platforms is the trust that physicians and responsible regulatory officials place in the AI analyses. Research should move toward facilitating extreme transparency in the generation and validation of AI models to avoid hesitancy about their public implementation.

**Safety and cost-effectiveness**

Finally, as well as CAd and CADx systems, a computer-based support system that aids endoscopists in selecting the most appropriate polypectomy procedure is necessary. Current practice involves the use of forceps to remove diminutive polyps, especially for the resection of polyps up to 2 mm\[^{67}\]; however, the rate of incomplete resection is lower for the removal of polyps $\geq 3$ mm when a snare is used\[^{68}\]. In addition to providing a suggestion for an appropriate polypectomy device, AI can also help to estimate polyp size, delineate the extent of the lesion and a safe polypectomy margin, and identify post-resection lesion remnants that indicate an incomplete resection and the need for further tissue removal at colonoscopy follow-up. The goal of this system is to provide a complete polypectomy that will reduce the risk of interval cancer, as about 30% of all interval cancers are thought to be caused by incomplete resection of CRC precursors\[^{11,69,70}\].

In addition to addressing the challenges associated with the development of reliable AI models that can be confidently employed in routine practice with high efficacy, research is needed to assess the cost-effectiveness of these systems related to the reduction in the number of patients diagnosed with interval cancer, reduction in the number of unnecessary pathology evaluations for low-confidence predictions of polyp histology by optical diagnosis, and facilitation of efficient physician-patient communication concerning future clinical arrangements.

Adapting the newly developed AI-based techniques in routine practice and enhancing endoscopists’ trust in the new devices is only possible by a symbiotic relationship between academia and industry. It would facilitate obtaining regulatory approval from health authorities regarding research involving human subjects, constructing large “ground truth” data for developing AI models, and transporting knowledge and technology to ultimately access the market\[^{71}\]. Several manufacturers have obtained the regulatory approvals to launch and commercialize their AI-based colonoscopy devices around the world (Table 4); however, many of them have not provided a detailed report of their devices’ performance. Further research should try to compare the performance of different AI-based systems in real-time settings by conducting prospective controlled trials with multiple intervention arms using different commercially available AI-based colonoscopy systems. Due to the time- and cost-consuming nature of these studies, an alternative method for accelerating research is to test the “benchmarks” using the publicly available datasets such as the ASU-Mayo colonoscopy video database\[^{29}\], the CVC-ClinicDB database\[^{28}\], the Kvasir dataset \[^{72}\], and the ETIS-Larib Polyp database. Nonetheless, these datasets contain a limited number of colonoscopy videos and images and may not reflect the true performance of
Table 4 Commercially available computer-assisted colonoscopy tools that have cleared regulatory approval

<table>
<thead>
<tr>
<th>Computer assisted system</th>
<th>Product</th>
<th>Manufacturer</th>
<th>Year of regulatory approval</th>
<th>Place of regulatory approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADx</td>
<td>EndoBRAIN</td>
<td>Cybernet System Corp./Olympus Corp.</td>
<td>2018</td>
<td>Japan</td>
</tr>
<tr>
<td>CADe</td>
<td>GI Genius</td>
<td>Medtronic Corp.</td>
<td>2019 in Europe; 2021 in United States</td>
<td>Europe/United States</td>
</tr>
<tr>
<td>CADe</td>
<td>ENDO-AID</td>
<td>Olympus Corp.</td>
<td>2020</td>
<td>Europe</td>
</tr>
<tr>
<td>CADe/CADx</td>
<td>CAD EYE</td>
<td>Fujifilm Corp.</td>
<td>2020</td>
<td>Europe/Japan</td>
</tr>
<tr>
<td>CADe</td>
<td>DISCOVERY</td>
<td>Pentax Corp.</td>
<td>2020</td>
<td>Europe</td>
</tr>
<tr>
<td>CADe</td>
<td>EndoBRAIN-EYE</td>
<td>Cybernet System Corp./Olympus Corp.</td>
<td>2020</td>
<td>Japan</td>
</tr>
<tr>
<td>CADe</td>
<td>EndoAngel</td>
<td>Wuhan EndoAngel Medical Technology Company</td>
<td>2020</td>
<td>China</td>
</tr>
<tr>
<td>CADe</td>
<td>EndoScreener</td>
<td>WISION A.I.</td>
<td>2020</td>
<td>China</td>
</tr>
<tr>
<td>CADx</td>
<td>EndoBRAIN-PLUS</td>
<td>Cybernet System Corp./Olympus Corp.</td>
<td>2020</td>
<td>Japan</td>
</tr>
<tr>
<td>CADx</td>
<td>EndoBRAIN-UC</td>
<td>Cybernet System Corp./Olympus Corp.</td>
<td>2020</td>
<td>Japan</td>
</tr>
<tr>
<td>CADe</td>
<td>WISE VISION</td>
<td>NEC Corp.</td>
<td>2021</td>
<td>Europe/Japan</td>
</tr>
<tr>
<td>CADe</td>
<td>ME-APDS</td>
<td>Magentiq Eye</td>
<td>2021</td>
<td>Europe</td>
</tr>
<tr>
<td>CADe</td>
<td>CADDIE</td>
<td>Odin Vision</td>
<td>2021</td>
<td>Europe</td>
</tr>
</tbody>
</table>

CADe: Computer-assisted detection system; CADx: Computer-assisted diagnosis system.

CONCLUSION

AI research is a rapidly evolving discipline that promises to enhance physicians' performance. AI models have demonstrated the ability to compete with and outperform endoscopists, suggesting that all endoscopists would benefit from becoming familiar with CAD technology and comfortable with the integration of AI-assisted devices in colonoscopy practice. The decision support systems are being offered as reliable tools for the detection and classification of colorectal polyps, with the primary aim of outperforming endoscopists by detecting all CRC precursors; however, the new era of AI platforms has seen attempts to establish considerably more complex systems, in which the detection and classification of polyps are supported. Despite the recent achievements in designing and validating such systems, the current lack of AI-assisted systems that support endoscopists in monitoring colonoscopy quality, and that automatically annotate colonoscopy videos, suggest appropriate polypectomy devices, and indicate the completeness of polypectomy, limits the role of AI in colonoscopy practice. Through the integration of the most recent advances in computer science into colonoscopy practice, it appears possible to improve the quality of diagnosis, treatment, and screening in patients. However, AI platforms are still in their infancy in terms of clinical establishment and require much more exploration and innovation. They must be trusted by all physicians, regulatory organizations responsible for approval for clinical use, and patients. The AI-assisted colonoscopy is highly dependent on the endoscopist, who must attempt to present the clearest possible image or video to the AI model for analysis, and then take account of other concurrent patient factors such as the family history of CRC or the results of previous colonoscopies. The human qualities of respect and empathy must be apparent when communicating with patients to overcome any mistrust or reservations patients may have toward the new technology. Therefore, at the current stage of AI development, AI models can only “serve as a second observer, or a concurrent observer, but not an independent decision-maker”[73].
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Taghiakbari M et al. Computer-based colonoscopy quality assessment
Taghiakbari M et al. Computer-based colonoscopy quality assessment


Immunotherapies for well-differentiated grade 3 gastroenteropancreatic neuroendocrine tumors: A new category in the World Health Organization classification

Jun-Xi Xu, De-Hao Wu, Li-Wei Ying, Han-Guang Hu

Abstract

According to the 2019 World Health Organization (WHO) classification, well-differentiated grade 3 (G3) gastroenteropancreatic (GEP) neuroendocrine tumors (NETs) are a new category of cancer of the digestive system. G3 GEP-NET research and treatment are not as robust as those of lower grade (G1/2) NETs and poorly differentiated neuroendocrine carcinomas (NECs). Previously, the management of high-grade NETs was mainly based on NEC therapies, as high-grade NETs were classified as NECs under the previous WHO classification. Despite this, G3 GEP-NETs are significantly less responsive to platinum-based chemotherapy regimens than NECs, due to their distinct molecular pathogenesis and course of pathological grade transition. Patients with advanced G3 GEP-NETs, who have progressed or are intolerant to chemotherapy regimens such as capcitabine plus temozolomide, have limited treatment choices. Immunotherapy has helped patients with a variety of cancers attain long-term survival through the use of immune checkpoint inhibitors. Immunotherapies, either alone or in combination with other therapies, do not have a clear function in the treatment of G3 GEP-NETs. Currently, the majority of immunotherapy studies, both prospective and retrospective, do not reliably differentiate G3 GEP-NETs from NECs. By contrast, a significant number of studies include non-GEP neuroendocrine neoplasms (NENs). Therefore, there is an urgent need to summarize and evaluate these data to provide more effective therapeutic approaches for patients with this rare tumor. The purpose of this mini-review was to screen and...
summarize information on G3 GEP-NETs from all studies on NENs immunotherapy.

**Key Words:** Gastrointestinal tract; Pancreas; Immune checkpoint inhibitors; Immunotherapy; Neuroendocrine tumors; Cytotoxic T-lymphocyte-associated protein 4 antigen

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Core Tip: Several evaluations have been published on immunotherapy for neuroendocrine neoplasms. However, this is the first review to specifically focus on the efficacy of different immunotherapy strategies such as immune checkpoint inhibitor (ICI) monotherapy, dual ICI therapy, anti-angiogenesis plus ICI, and chemotherapy combined with ICI for the treatment of advanced well-differentiated high-grade gastroenteropancreatic neuroendocrine tumors.

Citation: Xu JX, Wu DH, Ying LW, Hu HG. Immunotherapies for well-differentiated grade 3 gastroenteropancreatic neuroendocrine tumors: A new category in the World Health Organization classification. World J Gastroenterol 2021; 27(47): 8123-8137

URL: https://www.wjgnet.com/1007-9327/full/v27/i47/8123.htm

DOI: https://dx.doi.org/10.3748/wjg.v27.i47.8123

INTRODUCTION

Neuroendocrine neoplasms (NENs) are rare and indolent diseases that can manifest in any part of the body where peptidergic neurons and neuroendocrine cells are found. About 65% of neoplasms are found in the gastrointestinal (GI) tract and pancreas, making gastroenteropancreatic (GEP)-NENs the most common type of NENs[1]. Due to advancements in early-stage disease detection techniques such as endoscopy and imaging, the incidence of GEP-NENs has significantly increased to an overall incidence of 3.56 per 100000[2]. Based on the 2010 grading system, the World Health Organization (WHO) in 2019 comprehensively considered the importance of the primary site, morphological differentiation, and grading in the classification of GEP-NENs, and expanded the 2017 grading system by proposing a classification framework for all NENs[3]. One of the key updates in the 2019 classification system is that all grade 3 (G3) NENs (with Ki-67 proliferation index > 20%) are classified as either well-differentiated G3 neuroendocrine tumors (NETs) or poorly differentiated neuroendocrine carcinomas (NECs). Although G3 NETs have more inert biological behavior compared to NECs, they have a poorer prognosis compared to G1/2 NETs[4]. Compared to patients with poorly differentiated NECs, well-differentiated G3 NET patients have a considerably longer median overall survival (mOS) (41-99 mo vs 17 mo)[5].

G3 NENs account for 13.4% of all digestive system NENs, whereas G3 NETs account for 18%-20% of G3 GEP-NENs[6,7]. In general, although significant progress has been made in the management of GEP-NENs as a whole, the treatment of G3 GEP-NETs, a new WHO category, has not been well studied. Therefore, more tailored treatment strategies are needed for these disorders.

According to WHO 2010 classification criteria, G3 GEP-NETs were categorized as NECs. However, clinical variations between individuals with G3 GEP-NETs and NECs were discovered. For example, platinum-based chemotherapy was frequently employed for the treatment of G3 GEP-NEN patients in the past. Patients with G3 NETs or Ki-67 < 55% (mostly well-differentiated) were significantly less responsive to treatment than those with NEC or Ki-67 ≥ 55% (mostly poorly differentiated). G3 NET and NEC patients have an objective response rate (ORR) of less than 17% and 35%-70%, a median progression-free survival rate (mPFS) of 2.4-4 mo and 5.0 mo, and mOS of 17 mo and 99 mo, respectively[8-10].

Recently, the first prospective Phase II study of capecitabine with temozolomide in patients with high-grade GEP-NEN and Ki-67 index < 55% yielded results contrary to those received platinum plus etoposide. Patients with G3 NET (n = 23) responded better to treatment than those with NEC (n = 7) in both short-term [ORR 34.8% ± 8% vs 6% ± 14%] and long-term follow-up[11].
In recent years, immunotherapy has emerged as a new and intriguing approach for cancer therapy. Cancer cells have the inherent ability to express negative regulatory molecules of immune cells. The cornerstone of immunotherapy in modern oncology aims to improve the ability of the immune system to recognize and kill tumor cells [14]. Currently, this is being achieved through the use of monoclonal antibodies against immune checkpoints such as programmed death-ligand 1 (PD-L1)/programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Immune checkpoint inhibitors (ICIs), which sit at the forefront of cancer immunotherapy, have revolutionized the management of a variety of solid malignancies. In relation to NENs, immunotherapy has been mainly used to treat lung and skin tumors such as Merkel cell carcinoma, malignant melanoma, and small cell lung cancer (SCLC)[15]. Although an increasing number of clinical trials and retrospective studies are being conducted to investigate the efficacy of ICIs on NENs of the digestive tract, the role of immunotherapy approaches in well-differentiated G3 NETs has not been sufficiently studied.

In this minireview, we briefly describe the overall pathological changes of G3 GEP-NETs and analyze in detail the immunotherapy experience with well-differentiated G3 GEP-NETs from complex investigations.

**ROLE OF IMMUNOTHERAPY IN G3 GEP-NETS**

A recent systematic review and meta-analysis of 636 NEN patients treated with ICIs reported an ORR of 10% [95% confidence interval (CI): 6%-15%, \( P = 0.07, P < 0.1 \)], a total DCR of 42%, a mPFS of 4.1 mo (95% CI: 2.6-5.4; \( P = 0.06, P < 0.1 \)), and a mOS of 41 mo (95% CI: 38-45; \( P = 0.01, P < 0.1 \)) [16]. This demonstrated the overall effectiveness of ICIs in treating NEN patients. Among the NEN study subjects, about 37% were patients with NENs originating from the lung or other unknown sites and only 13.4% were patients who had G3 NETs. However, the study did not include a separate subgroup of G3 GEP-NET patients in its analysis. Previous studies have shown that G3 NETs can share a common pathogenesis with G1-2 NETs [17]. Moreover, more than half of G1-2 pancreatic NETs (pNETs) developed progressively into G3 pNETs over time[18]. Some researchers have even speculated that high-grade pNET may develop from the initial low- and medium-grade NET, while pNEC may develop from pancreatic ductal adenocarcinoma[19,20]. Therefore, the response of lower grade NETs to immunotherapy may have some implications for the treatment of G3 NETs. Other aspects that may influence the immunotherapy choices for G3 GEP-NETs include the presence of predictive biomarkers for ICIs in tumors with high proliferative activity as well as changes in pathological grade over time.

Table 1 summarizes the clinical trials of immunotherapy in GEP-NENs. Below, we presented data from clinical trials and retrospective studies that may have included cases with G3 GEP-NETs. Additionally, we analyzed the data to determine the efficacy of different immunotherapy strategies such as PD-1/PD-L1 inhibitors as a monotherapy or in combination with CTLA-4 inhibitors, anti-angiogenesis, and chemotherapy in the management of these rare diseases.

**ICIs monotherapy**

Pembrolizumab is the most extensively investigated immunotherapy for NENs. For example, phase Ib (KEYNOTE-28) and phase II (KEYNOTE-158) clinical trials were...
Table 1 Clinical trials related to gastroenteropancreatic neuroendocrine tumors

<table>
<thead>
<tr>
<th>ClinicalTrials.gov identifier</th>
<th>Intervention</th>
<th>Study phase</th>
<th>Trial name</th>
<th>Primary outcome</th>
<th>Estimated/actual enrollment, n</th>
<th>Estimated/actual date</th>
<th>Trial status</th>
<th>Medical condition related to advanced NENs</th>
<th>Reported assessable n of NENs</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02054806[21]</td>
<td>Pembrolizumab</td>
<td>Ib</td>
<td>Phase Ib study of pembrolizumab (MK-3475) in subjects with select advanced solid tumors (MK-3475-028/KEYNOTE-028)</td>
<td>ORR</td>
<td>477</td>
<td>April 30, 2021</td>
<td>Completed</td>
<td>pNETs: PD-L1 (+), well or moderately differentiated</td>
<td>16 pNETs</td>
</tr>
<tr>
<td>NCT02628067[22]</td>
<td>Pembrolizumab</td>
<td>II</td>
<td>A clinical trial of pembrolizumab (MK-3475) evaluating predictive biomarkers in subjects with advanced solid tumors (KEYNOTE-158)</td>
<td>ORR</td>
<td>1595</td>
<td>June 18, 2026</td>
<td>Recruiting</td>
<td>NETs: Well or moderately differentiated</td>
<td>107 NETs: Lung, appendix, small intestine, colon, rectum, or pan origin</td>
</tr>
<tr>
<td>NCT02939651[23]</td>
<td>Pembrolizumab</td>
<td>II</td>
<td>A phase 2, open-label study of pembrolizumab monotherapy in patients with metastatic high grade neuroendocrine tumors</td>
<td>ORR</td>
<td>21</td>
<td>March 2020</td>
<td>Completed</td>
<td>G3 NENs: Ki-67 &gt; 20%, poorly or well-differentiated, failed for platinum based chemotherapy, excluding MCC, large/small cell NENs of lung/thymus origin</td>
<td>29 G3 NENs: 19 NECs, 9 G3 NET, 14 Ki-67 ≤ 50%, 12 Ki-67 &gt; 50%, 10 pan, 14 GI, 5 unknown origin</td>
</tr>
<tr>
<td>NCT03190213</td>
<td>Pembrolizumab</td>
<td>II</td>
<td>Pembrolizumab for the treatment of recurrent high grade neuroendocrine carcinoma (Pembro NEC)</td>
<td>ORR (irRECIST)</td>
<td>6</td>
<td>March 11, 2019</td>
<td>Terminated</td>
<td>G3 NENs: Failed for platinum-based regimen or temozolomide-based regimen, excluding lung origin</td>
<td>6 G3 NENs</td>
</tr>
<tr>
<td>NCT02955069[25]</td>
<td>Sptarlizumab</td>
<td>II</td>
<td>An open label phase II study to evaluate the efficacy and safety of PD-001 in patients with advanced or metastatic, well-differentiated, non-functional neuroendocrine tumors of pancreatic, gastrointestinal (GI), or thoracic origin or poorly-differentiated gastroenteropancreatic neuroendocrine carcinoma (GEP-NEC), that have progressed on prior treatment</td>
<td>ORR</td>
<td>116</td>
<td>May 13, 2020</td>
<td>Completed</td>
<td>NENs: Exclude G3 NETs and include GI/2 NET (non-functional, GEP or thoracic origin, failed to prior treatment) and GEP-NEC (progressed on or after one prior chemotherapy regimen)</td>
<td>99 NENs: 30 thoracic, 32 GI-NET, 33 pNET; 21 GEP-NEC</td>
</tr>
<tr>
<td>NCT03167853[30]</td>
<td>Toripalimab</td>
<td>Ib</td>
<td>Phase Ib study of safety and efficacy of recombinant humanized anti-PD-1 monoclonal antibody for patients with advanced neuroendocrine tumors following failure of first-line</td>
<td>ORR</td>
<td>40</td>
<td>May 11, 2019</td>
<td>Completed</td>
<td>NENs: Ki-67 ≥ 10%, nonfunctional NENs, well- or poorly-differentiated, failed for first line therapy</td>
<td>40 NENs: 8 well-differentiated, 32 poorly-differentiated</td>
</tr>
<tr>
<td>NCT03352934[26]</td>
<td>Avelumab</td>
<td>II</td>
<td>A phase II, open-label, multicenter trial to</td>
<td>DCR</td>
<td>60</td>
<td>January 2024</td>
<td>Active, not</td>
<td>G3 NENs: after first-line</td>
<td>29 G3 NENs: 16 NEC</td>
</tr>
<tr>
<td>Study ID</td>
<td>Drug</td>
<td>Phase</td>
<td>Description</td>
<td>ORR</td>
<td>Status</td>
<td>Duration</td>
<td>NECs: Progression</td>
<td>Number of NEcs</td>
<td>Origin</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>-------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
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<td>------------------</td>
</tr>
<tr>
<td>NCT03278405[38]</td>
<td>Avelumab</td>
<td>Ia</td>
<td>A pilot study of avelumab in unresectable/metastatic, progressive, poorly differentiated grade 3 neuroendocrine carcinomas (NET001)</td>
<td>10</td>
<td>March 12, 2020</td>
<td>Completed</td>
<td>10</td>
<td>9 GI and 1 lung</td>
<td>PCI, 5 GI, 9 other origin</td>
</tr>
<tr>
<td>NCT03147404</td>
<td>Avelumab</td>
<td>II</td>
<td>Phase II study of avelumab in metastatic gastroentero-pancreatic (GEP) neuroendocrine carcinoma (NEC, WHO Grade 3) as second-line treatment after failing to etoposide + cisplatin: integration of genomic analysis to identify predictive molecular subtypes (MS100070-0177)</td>
<td>Best response</td>
<td>July 22, 2019</td>
<td>Completed</td>
<td>G3 GEP-NECs: Second-line treatment after failing to etoposide + cisplatin</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NCT03879057[40]</td>
<td>Toripalimab + surufatinib</td>
<td>I</td>
<td>Phase I trial evaluating the safety, tolerability, pharmacokinetics, and efficacy of surufatinib combined with JS001 in patients with advanced solid tumors</td>
<td>AEs, MTD</td>
<td>December 20, 2021</td>
<td>Recruiting</td>
<td>NECs: G1-3 NET, NEC</td>
<td>18 NENs: 11 NECs, 4 G2 NETs, 4 G3 NETs, 12 GI, 4 pan, 1 lung</td>
<td>-</td>
</tr>
<tr>
<td>NCT04169672[41]</td>
<td>Toripalimab + surufatinib</td>
<td>II</td>
<td>A phase II, open-label, single-arm, multi-center study of the efficacy and safety of surufatinib combined with toripalimab in patients with advanced solid tumors</td>
<td>AEs, ORR</td>
<td>February 28, 2022</td>
<td>Recruiting</td>
<td>NECs: Refractory to first-line chemotherapy</td>
<td>20 NENs</td>
<td>-</td>
</tr>
<tr>
<td>NCT03476953</td>
<td>Avelumab + regorafenib</td>
<td>I/II</td>
<td>A phase I/II study of regorafenib plus avelumab in solid tumors (REGOMUNE)</td>
<td>Phase I: Recommended dose of regorafenib; Phase II: ORR, PFS</td>
<td>May 2022</td>
<td>Recruiting</td>
<td>G2/3 GEP-NETs</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NCT03290079</td>
<td>Pembrolizumab + lenvatinib</td>
<td>II</td>
<td>Phase II study of pembrolizumab and lenvatinib in advanced well-differentiated neuroendocrine tumors</td>
<td>ORR</td>
<td>December 2023</td>
<td>Recruiting</td>
<td>NETs: Well-differentiated, lung, thymus, small bowel or colon origin, including unknown primary, excluding pNENs and poorly differentiated NECs</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>NCT04579757</td>
<td>Surufatinib + tislelizumab</td>
<td>Ib/Ii</td>
<td>An open-label phase Ib/II study of</td>
<td>DLT, ORR</td>
<td>April 30, 2023</td>
<td>Recruiting</td>
<td>G1/2 NETs: Thoracic or</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Xu JX et al. Immunotherapies for G3 GEP-NETs

<table>
<thead>
<tr>
<th>Trial ID</th>
<th>Therapy</th>
<th>Phase</th>
<th>Study Design</th>
<th>Primary Tumor</th>
<th>Study Description</th>
<th>ORR</th>
<th>Recruitment Status</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT04207463</td>
<td>surufatinib in combination with tislelizumab in subjects with advanced solid tumors</td>
<td>II</td>
<td>A phase II, open, single-arm, multi-cohort, multicenter study</td>
<td>GEP origins, have progressed on at least one line of standard therapy</td>
<td>ORR</td>
<td>May 30, 2021</td>
<td>Recruiting</td>
<td>-</td>
</tr>
<tr>
<td>NCT03074513[41]</td>
<td>Atezolizumab + bevacizumab</td>
<td>II</td>
<td>A phase II, single-arm open-label study of the combination of atezolizumab and bevacizumab in rare solid tumors</td>
<td>ORR</td>
<td>March 31, 2021</td>
<td>Active, not recruiting</td>
<td>G1/2 GEP-NETs; pNEt cohort and epNET cohort containing typical or atypical carcinoid if originating in lung</td>
<td>20 G1/2 pNETs, 20 G1/2 epNETs</td>
</tr>
<tr>
<td>NCT02923934[45]</td>
<td>Nivolumab + ipilimumab</td>
<td>II</td>
<td>A phase II clinical trial evaluating ipilimumab and nivolumab in combination for the treatment of rare gastrointestinal, neuro-endocrine and gynaecological cancers (CA209-538)</td>
<td>CBR</td>
<td>December 2023</td>
<td>Active, not recruiting</td>
<td>G1-3 NETs, NECs, GEP or lung origin</td>
<td>10 GEP-NECs: 7 pNECs, 3 G2 pNETs, 3 GI-NECs (1 gastro-oesophageal junction NEC, 1 colonic NECs, 1 G1 gastric NET)</td>
</tr>
<tr>
<td>NCT02834013[15, 46]</td>
<td>Nivolumab + ipilimumab</td>
<td>II</td>
<td>A prospective, open-label, multicenter phase II basket clinical trial of ipilimumab plus nivolumab across multiple rare tumor cohorts (DART)</td>
<td>ORR</td>
<td>August 1, 2021</td>
<td>Recruiting</td>
<td>SWOG 1609 cohort: Refractory epNECs. G3 NETs were included in G3 NECs. SWOG S1609 cohort: Dedicated cohort include G3 NECs</td>
<td>SWOG 1609 cohort: 32 epNECs (18 G3, 10 G2, 4 G1, 15 GI, 6 Lung), S1609 cohort: 19 G3 NECs (2 G3 NETs, 11 NEC, 6 unknown differentiation status)</td>
</tr>
<tr>
<td>NCT04869887</td>
<td>Nivolumab + ipilimumab</td>
<td>II</td>
<td>Ipilimumab and nivolumab combination therapy in patients with selected immunotherapy sensitive advanced rare cancers (MORT-CIRCUIT)</td>
<td>CBR</td>
<td>December 2024</td>
<td>Not yet recruiting</td>
<td>NECs and G3 NETs independent of primary site, excluding SCLC</td>
<td></td>
</tr>
<tr>
<td>NCT03591731</td>
<td>Nivolumab alone or nivolumab + ipilimumab</td>
<td>II</td>
<td>A GCO trial exploring the efficacy and safety of nivolumab monotherapy or nivolumab plus ipilimumab in pre-treated patients with advanced, refractory pulmonary or gastroenteropancreatic poorly differentiated neuroendocrine tumors (NECs) (NIPINeC)</td>
<td>ORR</td>
<td>September 2023</td>
<td>Recruiting</td>
<td>NECs: Poorly differentiated, refractory, pulmonary or GEP, excluding SCLC</td>
<td></td>
</tr>
<tr>
<td>NCT03095274[47]</td>
<td>Tremelimumab + durvalumab</td>
<td>II</td>
<td>A phase II study of durvalumab (MED34736) plus tremelimumab for the treatment of patients with advanced neuroendocrine neoplasms of gastroenteropancreatic or lung origin (DUNE) (GETNE 1601)</td>
<td>Cohort 1-3: CBR at 9 m; Cohort 4: OS at 9 mo</td>
<td>July 2021</td>
<td>Recruiting</td>
<td>G1/G2 NETs of GEP and lung, and G3 of GEP or unknown primary site (excluding lung primaries) after progression to standard therapies</td>
<td>123 NECs (Cohort 1: 27 typical/atypical lung carcinoids; Cohort 2: 31 G1/2 GI-NECs; Cohort 3: 32 G1/2 pNECs; Cohort 4: 33 G3 NEN of GEP or unknown primary site)</td>
</tr>
<tr>
<td>Trial ID</td>
<td>Treatment</td>
<td>Phase</td>
<td>Study Design</td>
<td>Endpoint</td>
<td>Status</td>
<td>NETs: Additional Details</td>
<td></td>
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<tr>
<td>NCT04079712</td>
<td>Nivolumab + ipilimumab + cabozantinib</td>
<td>II</td>
<td>A phase 2 study of XL184 (Cabozantinib) in combination with nivolumab and</td>
<td>ORR</td>
<td>October 1, 2021</td>
<td>NENs: All variations of poorly differentiated NECs (small cell, large cell and mixed cells) are eligible, excluding SCLC and MCC. Failure of only one line of prior systemic cancer treatment -</td>
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<td></td>
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<td>ipilimumab for the treatment of poorly differentiated neuroendocrine carcinomas</td>
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<tr>
<td>NCT03728361[49]</td>
<td>Nivolumab + temozolomide</td>
<td>II</td>
<td>A phase II, multi-cohort trial of combination nivolumab and temozolomide in</td>
<td>ORR</td>
<td>December 31, 2021</td>
<td>NENs: Any grade or primary site 12 NENs: 1 G1, 8 G2, 3 G3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>recurrent/refractory small-cell lung cancer and advanced neuroendocrine tumors</td>
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<tr>
<td>NCT03980925</td>
<td>Nivolumab + platinum-doublet chemotherapy</td>
<td>II</td>
<td>A phase II study of platinum-doublet chemotherapy in combination with nivolumab as first-line treatment in subjects with unresectable, locally advanced or metastatic G3 neuroendocrine neoplasms (NENs) of the gastroenteropancreatic (GEP) tract or of unknown (UK) origin (GETNET-T1913)</td>
<td>OS at 12 mo</td>
<td>December 2022</td>
<td>G3 NENs: GEP or unknown primary site -</td>
<td></td>
<td></td>
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<tr>
<td>NCT0365791[59]</td>
<td>Spartializumab + LAG525</td>
<td>II</td>
<td>Modular phase 2 study to link combination immune-therapy to patients with advanced solid and hematologic malignancies. Module 9: PDR01 plus LAG525 for patients with advanced solid and hematologic malignancies</td>
<td>CBR at 24 wk</td>
<td>September 17, 2020</td>
<td>NETs: Well-differentiated, relapsed and/or refractory to available standard of care therapies 7 NETs</td>
<td></td>
<td></td>
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<tr>
<td>NCT03043664[60]</td>
<td>Pembrolizumab + lanreotide depot</td>
<td>Ib/II</td>
<td>Phase Ib/II study of pembrolizumab with lanreotide depot for gastroenteropancreatic neuroendocrine tumors (PLANT)</td>
<td>ORR</td>
<td>September 1, 2021</td>
<td>G1-2 GEP-NETs: Had progressed on a prior SSA 22 G1/2 GEP-NETs (14 G1, 8 pan)</td>
<td></td>
<td></td>
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<tr>
<td>NCT04525638</td>
<td>Nivolumab + $^{177}$Lu-DOTATATE</td>
<td>II</td>
<td>A phase II single arm trial evaluating the preliminary efficacy of the combination of $^{177}$Lu-DOTATATE and nivolumab in grade 3 well-differentiated neuroendocrine tumours (NET) or poorly differentiated neuroendocrine carcinomas (NEC)</td>
<td>ORR</td>
<td>September 30, 2024</td>
<td>G3 NENs: GEP or unknown primary site, well-differentiated or poorly-differentiated. -</td>
<td></td>
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<tr>
<td>NCT04701307</td>
<td>Dostarlimab + niraparib</td>
<td>II</td>
<td>Niraparib (PARP Inhibitor) plus dostarlimab (Anti-PD1) for small cell lung cancer (SCLC) and other high-grade neuroendocrine carcinomas (NEC)</td>
<td>6 mo PFS, 3 mo ORR</td>
<td>May 30, 2025</td>
<td>G3 NECs: SCLC (Cohort 1) and other G3 NECs (Cohort 2), had at least one prior line of systemic therapy, excluding prostate origin -</td>
<td></td>
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<tr>
<td>NCT03457948</td>
<td>Group I: Pembrolizumab + $^{177}$Lu DOTATATE; Group II: Pembrolizumab + TAE; Group III: Pembrolizumab + $^{90}$Ytrrium- Microsphere Radioembolization</td>
<td>II</td>
<td>A pilot study of pembrolizumab and liver-directed therapy or peptide receptor radionuclide therapy for patients with well-differentiated neuroendocrine tumors and symptomatic and/or progressive metastases</td>
<td>ORR</td>
<td>March 31, 2024</td>
<td>G1-3 NETs: Well-differentiated, any primary site and unknown primary site, have liver metastases -</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**NCT03879694**  
SVN53-67/M57-KLH peptide vaccine (SurVaxM) + Octreotide  
A phase I study of safety and immunogenicity of Survivin Long Peptide Vaccine (SurVaxM) in patients with metastatic neuroendocrine tumors (NETs)  
AEs 10  
June 13, 2024  
Recruiting  
NETs: GEP or lung origin, positive for survivin

**NCT04166006**  
Dendritic cells loaded with autologous tumour (DC vaccine) + IL-2  
A phase II study on adjuvant vaccination with dendritic cells loaded with autologous tumor homogenate in resected stage IV rare cancers: Head & neck (H & N), neuroendocrine tumors (NET) and soft tissue sarcoma (STS)  
Treatment-Emergent AEs 51  
December 2026  
Recruiting  
NET: Stage IV

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AE: Adverse event; CBR: Clinical benefit rate; DCR: Disease control rate; DLT: Dose-limiting toxicity; EP-PDNECs: Extrapulmonary poorly differentiated neuroendocrine carcinomas; ep: Extra-pancreatic; G1, 2, 3: Grade 1, 2, 3; GEP: Gastroenteropancreatic; GI: Gastrointestinal; irRECIST: Immune-related Response Evaluation Criteria in Solid Tumors; MANEC: Mixed adeno-neuroendocrine carcinomas; MCC: Merkel cell carcinoma; MTD: Maximum tolerated dose; NECs: Neuroendocrine carcinomas; NENs: Neuroendocrine neoplasms; NETs: Neuroendocrine tumors; ORR: Objective response rate; OS: Overall survival; p: Pancreatic; pan: Pancreas origin; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; PFS: Progression-free survival; SCLC: Small cell lung cancer; WHO: World Health Organization.

Conducted to evaluate pembrolizumab monotherapy in patients with moderately or well-differentiated NETs. In these two studies, no data on tumor grade and Ki-67 index were collected. The results of the KEYNOTE-28 trial showed an ORR of 6.3% in pNETs^{[21]}. The preliminary results of the KEYNOTE-158 study showed that mixed NETs had an ORR of 3.7% and that all reactive tumors were PD-L1-negative^{[22]}. In addition, two prospective randomized phase II trials were performed to evaluate pembrolizumab in 19 patients with NECs and 9 with G3 NETs. There were no responses to pembrolizumab in patients with GI tract or pancreatic diseases^{[23]}. In a trial of 14 patients with extrapulmonary poorly differentiated NECs, only 1 patient achieved complete remission (CR) (ORR 7%) following pembrolizumab monotherapy^{[24]}. From the abovementioned studies, it can be concluded that pembrolizumab alone has a very limited curative effect on the GEP-NENs independent of their proliferative activity or differentiation. The only clinical trial (NCT02955069) of spartalizumab, a PD-1 inhibitor, for the treatment of NETs excluded patients with G3 NETs and achieved low efficacy comparable to pembrolizumab^{[25]}. Avelumab is the only PD-L1 inhibitor used as a single drug in prospective clinical trials for GEP-NENs. Four phase II clinical trials (NCT03352934^{[26]}, NCT03278405^{[27]}, NCT03278379^{[28]}, and NCT03147404) were conducted to evaluate avelumab in patients with G2/3 NETs or NEC. The trials revealed that none of the patients achieved an objective response to avelumab treatment. In addition, none of the 3 G3 NET patients analyzed in a retrospective study from the Mayo Clinic exhibited an objective response to ICI (pembrolizumab, nivolumab, or atezolizumab) monotherapy^{[29]}. Toripalimab (JS001) is a humanized PD-1 IgG4 monoclonal antibody developed in China. In a phase Ib study (NCT03167853) involving 40 NEN patients with Ki-67 ≥ 10%, toripalimab showed moderate efficacy in both well-differentiated NETs and poorly differentiated NECs (ORR: 25.0% vs 18.7% per RECIST 1.1, 25.0% vs 25.0% per irRECIST)^{[30]}. This suggests that toripalimab may be the most effective ICI.
monotherapy currently available for NENs, including G3 NETs. The study also found that patients with PD-L1 expression $\geq 10\%$ or with high tumor mutational burden (TMB) had a better ORR than patients with PD-L1 < 10\% (50.0\% vs 10.7\%, $P = 0.019$) or with low TMB (75.0\% vs 16.1\%, $P = 0.03$)$^{[30]}$.

**Anti-angiogenesis combined with ICIs**

NENs from different tissues are highly vascularized and express a variety of growth factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor, basic fibroblast growth factor, insulin-like growth factor 1, and transforming growth factor-$\alpha/\beta^{[31]}$. The high exposure and activation of VEGFs prevent the immune system from recognizing and killing cancer cells killing tumor cells$^{[32,33]}$.

The hallmark of angiogenesis is the uncontrolled development of new vessels from adjacent normal tissues. This results in a network of immature microvessels characterized by structural and functional abnormalities. The normalizing vascular structure can be achieved with antiangiogenic drugs, including large-molecule monoclonal antibodies and small-molecule tyrosine kinase inhibitors. This results in the activation of adhesion molecules and chemokines that recruit and attract cytotoxic T cells and reduce the entry of regulatory T cells. Moreover, it contributes to immune cell mobilization$^{[34,35]}$.

Surufatinib is a small molecule inhibitor that mainly targets VEGF-1, 2, 3 (VEGFR-1, 2, 3), fibroblast growth factor receptor-1, and colony-stimulating factor-1 receptor (CSF-1R). Blocking of CSF-1R can reduce the polarization of tumor-associated macrophages to the M2 type that participates in immunosuppression and promotes tumor growth$^{[36,37]}$. Two randomized, double-blind phase III clinical trials (SANET-ep and SANET-p) were carried out to evaluate surufatinib vs placebo in Chinese patients with G1-2 NETs. The results indicated that surufatinib can significantly prolong PFS in patients with advanced pancreatic and non-pancreatic G1-2 NETs compared with placebo$^{[37,38]}$. At present, a phase I trial and a phase II trial of surufatinib combined with toripalimab on patients with NENs are underway. In a phase I clinical trial (NCT03879057), as of 2020-1-20, PR occurred in G1/2 NET (2/4) and NECs (2/11) patients; however, none of the 4 patients with G3 NETs achieved disease remission$^{[39]}$, which may be attributed to the small sample size of patients with G3 NETs. In the phase II trial (NCT04169672), 20 evaluable patients with NECs and refractory to first-line chemotherapy achieved a moderate ORR of 20\% and a DCR of 70\%$^{[40]}$. However, no data for well-differentiated NETs have been reported. In addition, two prospective studies involving G3 GEP-NETs patients are currently recruiting. In one of the trials, the intervention is avelumab plus regorafenib, while in the other study, the intervention is pembrolizumab plus lenvatinib. The studies are expected to be completed in May 2022 and December 2023, respectively. The combination treatment of atezolizumab and bevacizumab in a phase II basket trial (NCT03074513) showed moderate clinical activity and good tolerance in G1-2 pNETs and extra-pNETs (ORR 20\% and 15\%, respectively) patients with prior therapy$^{[41]}$. However, the data of G3 NETs have not been reported either.

Given the favorable preliminary results in patients with G1-2 NETs and NECs, researchers might be optimistic about the combination therapy’s effectiveness in patients with G3 NETs.

**Dual ICI therapy**

The United States Food and Drug Administration has approved ipilimumab (anti-CTLA-4) combined with nivolumab (anti-PD-1) (N+I) for melanoma, metastatic renal cell carcinoma, advanced hepatocellular carcinoma, and previously untreated unresectable malignant pleural mesothelioma$^{[42-44]}$. Response rates with this combination are higher compared to single-agent anti-PD-1 therapy. For NETs, a phase II clinical trial (CA209-538) of N+I demonstrated a moderate overall ORR of 24\%, especially in patients with G3 NENs and atypical bronchial carcinoid$^{[45]}$. In the study, 7 patients with pNENs and 3 with G1-NENs achieved an ORR of 43\% and 33.3\%, respectively. All responders had a high-grade disease. It is worth noting that 2 of the 3 patients with G3 pNET achieved an objective response. This result is a breakthrough in the application of ICIs in the treatment of G3 GEP-NET. Currently, a phase II study (NCT04969887) on evaluation of N+I in patients with immunotherapy-sensitive cancers including NECs and G3 NETs from CA209-538 has been registered and is expected to be completed in October 2024.

Another phase II basket study of N+I for the treatment of rare tumors called SWOG DART (NCT02834013) was recently reported. In one cohort, 32 patients with epNENs (excluding SCLC, about 50\% have GI-NENs) had a significantly higher response rate for high-grade neoplasms than for middle/low-grade neoplasms (ORR 44\% vs 0\%, $P =$
Predictive biomarkers for immunotherapies

The potential of a given patient with G3 GEP-NET to respond to immunotherapies is still largely unknown. NETs can be considered as immunologically “cold” due to their lack of immunoactive cellular components, low tumor antigens, etc.[50,51].

Immunohistochemical assessment of PD-L1 expression and its role in predicting response to ICIs is an incredibly hot topic. However, in the KEYNOTE-28 study, pNETs with positive PD-L1 expression achieved a low ORR of 6.3%.[21]. In the KEYNOTE-158 study, all 4 GEP-NET patients who achieved PR had negative PD-L1 expression[22]. Besides, in a joint analysis of two prospective, non-randomized trials, no difference in DCR, PFS, or OS was observed between the PD-L1-negative and PD-L1-positive groups with G3 NENs[23]. In contrast, in the phase Ib trial of toripalimab in the treatment of patients with NENs (Ki-67 ≥ 10%) described above, patients with PD-L1 expression ≥ 10% had better ORR than those with PD-L1 < 10% (50.0% vs 10.7%, P = 0.019)[30]. Therefore, it appears that considering merely the negative or positive expression of PD-L1 is insufficient for identifying GEP-NET patients who may benefit from ICIs and that quantifying PD-L1 expression appears to be more significant. Furthermore, only 10% of tumors expressed PD-L1 in a large cohort of 136 patients with G3 GEP-NENs and those tumoral cells with positive PD-L1 were all in poorly differentiated cases[52]. Therefore, it is necessary to combine PD-L1 with other predictive biomarkers to better predict the population that may benefit from immuno-
therapy.

For other biomarkers, both high TMB (TMB-H) and microsatellite instability-high (MSI-H)/deficient mismatch repair protein (dMMR) are independent adverse prognostic factors for NENs[53] and also have an important predictive value. Wang et al[54] reported that 50% of the 18 Chinese patients with NETs had TMB-H. In a NET cohort analyzed by Patel et al[15], found no difference in the PD-L1 positivity rate between G3 and G1/G2 tumors, while the TMB-H rate was significantly higher in G3 NENs independent of tumor origin. Large samples of clinical and genomic data demonstrated that TMB-H was associated with increased survival in patients treated with ICI across various cancer types[55]. Duan et al[56] discovered that half of pNEN patients had decreased expression of MMR, another important biomarker. Venizelos et al[57] recently reported that MSI occurred in only 5.3% (8/152) of GEP-NEC patients and 3.4% (1/29) of G3 GEP-NET patients.

Pre-treatment assessment of one or more of these biomarkers provides a new perspective for screening good responders to immunotherapy.

CONCLUSION

In this minireview, data from prospective clinical trials and retrospective studies on the role of immunotherapies on G3 GEP-NET has been screened and reviewed. For ICI monotherapy, the efficacy of pembrolizumab, spartalizumab, and avelumab on G3 GEP-NETs is very limited. Only toripalimab has shown a moderate clinical activity on NENs with Ki-67 ≥ 10%, PD-L1 expression ≥ 10%, or high TMB. In addition, the ORR of well-differentiated tumors treated with toripalimab was slightly better than that of poorly differentiated cancers. Toripalimab and surufatinib therapy did not cause disease remission in 4 patients with G3 NETs. However, the treatment did not prevent remission in NEC and G1-2 NETs. Therefore, these regimens could potentially be effective in the treatment of G3 GEP-NETs if a large number of subjects are included. In other studies, the N+I therapy achieved PD in 2 of 3 patients with G3 NET as well as moderate efficacy in high-grade NENs. These results suggest that N+I may represent an extremely promising treatment option for G3 NET.

At present, all clinical trials investigating G3 GEP-NET are either phase I or phase II studies with small sample sizes. In this study, several challenges were encountered when collecting and evaluating data on the efficacy of immunotherapies for G3 GEP-NETs. According to the 2010 WHO classification, the inclusion of high-grade NETs in studies of NECs, the lack of Ki-67 index data in well-differentiated tumors, and the inclusion of tumors derived from lung, esophageal or unknown tissue all contribute to significant heterogeneity in reported results. Additionally, the review mainly focuses on the ORR to evaluate the potential role of immunotherapies in the treatment of G3 NETs. This is because the majority of prospective studies are ongoing and the survival data are in their infancy. Therefore, it is necessary to conduct prospective clinical trials with a large sample size of pathologically confirmed G3 GEP-NETs to evaluate the efficacy of the above immunotherapies. Besides, referencing data from important biomarkers facilitates the screening of patients who may benefit.

ACKNOWLEDGEMENTS

The authors express their gratitude to the scholars who have conducted prospective and retrospective studies on immunotherapies in the treatment of NENs.

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Xu JX et al. Immunotherapies for G3 GEP-NETs


Impact of intrarectal chromofungin treatment on dendritic cells-related markers in different immune compartments in colonic inflammatory conditions

Kunal Kapoor, Nour Eissa, Diane Tshikudi, Charles N Bernstein, Jean-Eric Ghia

Abstract

BACKGROUND
Chromofungin (CHR: chromogranin-A 47-66) is a chromogranin-A derived peptide with anti-inflammatory and anti-microbial properties. Ulcerative colitis (UC) is characterized by a colonic decrease of CHR and a dysregulation of dendritic CD11c+ cells.

AIM
To investigate the association between CHR treatment and dendritic cells (DCs)-related markers in different immune compartments in colitis.

METHODS
A model of acute UC-like colitis using dextran sulphate sodium (DSS) was used in addition to biopsies collected from UC patients.

RESULTS
Intrarectal CHR treatment reduced the severity of DSS-induced colitis and was associated with a significant decrease in the expression of CD11c, CD40, CD80, CD86 and interleukin (IL)-12p40 in the inflamed colonic mucosa and CD11c,
Bernstein CN has been on the advisory boards for Abbvie Canada, Amgen Canada, Bristol Myers Squibb Canada, Janssen Canada, Roche Canada, Sandoz Canada, Takeda Canada, Pfizer Canada and consulted to Takeda and Mylan Pharmaceuticals. He has received educational grants from Abbvie Canada, Pfizer Canada, Takeda Canada, Janssen Canada. He has been on speaker’s panel for Abbvie Canada, Medtronic Canada and Janssen Canada. The other authors declare that they have no conflicts of interest.

Data sharing statement: All data generated or analyzed during this study are included in this published article.

ARRIVE guidelines statement: The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

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Country/Territory of origin: Canada

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report’s scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

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CD80, CD86 IL-6 and IL-12p40 within the mesenteric lymph nodes and the spleen. Furthermore, CHR treatment decreased CD80 and CD86 expression markers of splenic CD11c+ cells and decreased NF-κB expression in the colon and of splenic CD11c+ cells. In vitro, CHR decreased CD40, CD80, CD86 IL-6 and IL-12p40 expression in naïve bone marrow-derived CD11c+ DCs stimulated with lipopolysaccharide. Pharmacological studies demonstrated an impact of CHR on the NF-κB pathway. In patients with active UC, CHR level was reduced and showed a negative linear relationship with CD11c and CD86.

CONCLUSION
CHR has protective properties against intestinal inflammation via the regulation of DC-related markers and CD11c+ cells. CHR could be a potential therapy of UC.

Key Words: Chromofungin; Chromogranin-A; Colitis; Cytokines; Dendritic cells; Gut hormones

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Core Tip: Ulcerative colitis (UC) is characterized by a colonic decrease of chromofungin (CHR: chromogranin-A 47-66) and a dysregulation of CD11c+ dendritic cells (DC). Using a UC-like model (dextran sulphate sodium) and biopsies collected from UC patients, we demonstrated a protective effect of CHR via the regulation of DC-related markers and CD11c+ cells at the colonic, mesenteric lymph node and spleen levels, through a potential effect via the NF-κB pathway. In patients with active UC, CHR level showed a negative linear relationship with CD11c+ marker. CHR could be a potential therapy of UC, but larger samples and additional experiments are needed.

INTRODUCTION
Inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and Crohn’s disease (CD), disorders in which the gastrointestinal (GI) tract becomes ulcerated and inflamed[1,2]. To date, the exact etiopathology and mechanism behind them are unknown. It is believed that genetic predisposition and environmental factors lead to an abnormal mucosal immune response in the intestinal lining[3-5]. Over the last decade, the diseases have increased worldwide, but the greatest prevalence is evident in western countries[6,7]. Although treatments and therapeutic strategies are evolving quickly, treatments are still inadequate for a substantial percent of those with active IBD, and some therapies may have serious adverse side effects. Therefore, IBD needs new therapeutic approaches associated with higher efficacy and limited side effects[8].

IBD is characterized by an ongoing inflammatory process manifested by an increase of immune cell infiltration and accompanied by an up-regulation of pro-inflammatory cytokines, including interleukin (IL)-6 and IL-12p40[9]. The continuing release of mediators damages the intestinal epithelium, leading to abnormal activities of enterochromaffin cells (EC), goblet cells and Paneth cells, and impaired tissue repair[10,11]. UC is represented by an overactivation of the adaptive immune system represented by T cells which is considered a consequence of abnormal activation of innate immune cells[12] such as dendritic cells (DCs)[10,11,13-17].

DCs are produced by the bone marrow and can be located in the mucosal tissues or circulate in the lymph tissues and the blood[18]. DCs interaction and antigen presentation to T cells and their subsequent polarization can be induced within mesenteric lymph nodes (MLNs) and the spleen[19]. In UC, as in other chronic inflammatory diseases, DC activation is represented by an up-regulation of the CD80, CD86, and CD11c markers and is associated with an increased release of IL-6 and IL-12p40 to

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DOI: https://dx.doi.org/10.3748/wjg.v27.i47.8138
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promote a Th1 cell polarization[20,21]. In UC, the IL-12p40 pathway’s importance has been confirmed by genome-wide association studies, identifying the IL-12 genes as a major contributor[22,23]. Intracellularly, the production of IL-12p40 is regulated by different pathways, including NF-κB. In IBD, a significant increase in NF-κB p65 has been observed in the gut epithelial and immune cells isolated from colonic biopsies [24]. More specifically related to DCs, the NF-κB pathway plays a crucial role in maintaining their intracellular activation responsible for T-cell polarization[25]. Therefore, the excessive pro-inflammatory cytokine production can in part be related to the overactivation of DCs in the gut[26]. Nevertheless, in certain conditions, the level and expression of DCs activation markers are not quite equivalent when comparing the different immune compartments (i.e. colon, MLN, spleen)[19].

Chromogranin-A (CHGA) is the second most prominent protein found in the secretory granules of ECs in the GI tract. For decades, CHGA has been known to be associated with IBD, but few data have demonstrated a plausible mechanism of association due to the lack of a specific CHGA receptor[27]. An increase in the total number of endocrine cells defined as CHGA-immunoreactivity has been described in several cohorts of patients with active IBD[28]. In parallel, the serum CHGA concentration has been reported to be significantly increased compared to controls[29-32]. CHGA is considered a prohormone and can be the precursor of several bioactive peptides having a wide variety of functions in the human body affecting the cardiovascular, endocrine, neuroendocrine and immune systems[33]. One of the peptides generated, CHR (CHGA**a**), encoded by Exon-IV located at the N-terminal section of CHGA, has been demonstrated to depict anti-fungal and anti-microbial activities[34]. Recently, in the context of UC and experimental colitis, new data highlighted an essential anti-inflammatory role through epithelial cells and peritoneal macrophage regulation[25], but no data exist regarding the broader effect within the immune system and its different compartments.

Taken together, we hypothesized that CHR regulates DCs activation in the context of UC experimental colitis, and we evaluated CHR’s effects on different DCs-related markers at various immune compartments and defined a potential intracellular pathway implicated.

**MATERIALS AND METHODS**

**Human participants**

Biopsies were collected from participants undergoing colonoscopy with known UC or with no IBD. A total of four biopsies were taken from inflamed sites identified endoscopically in persons with active UC (n = 10) and from normal tissue from healthy participants (n = 10). Biopsy collection was approved by the University of Manitoba Research Ethics Board. Participants were ≥18 years. All concomitant IBD therapies, including 5-amino salicylates, corticosteroids, and methotrexate, were permitted in active UC patients. Healthy control individuals had no history of abdominal afflictions, especially IBD. For more details, see Kermarrec et al[20]. The individuals in the control group were not on any regular medication at the time of the study. The University of Manitoba Research Ethics Board [HS14878 (E)] approved the study.

**Animal and ethics statement**

All the experiments were conducted under protocols # 15-010 and #19-014, approved by the University of Manitoba Ethics Committee under Canadian animal research guidelines. In-house groups of 6 male C57BL/6 mice (six to eight weeks old) with bodyweight between 20 g to 25 g were used. All animals were kept in the specific pathogen-free barrier facility maintained at the University of Manitoba animal care facility. Animals were kept in a 12-h dark/light cycle and fed ad libitum.

**Peptide**

CHGA**a** - RILSLRHQNLKELQDLAL[35-39] were synthesized by a solid-phase method and purified by reverse-phase high-performance liquid chromatography to reach < 98% purity (Pepmic Co., Suzhou, China). As previous studies from our laboratory demonstrated an absence of effect of the scramble version[25], this group was not added to our experimental plan.

**Dextran sulphate sodium-induced experimental colitis and experimental plan**

Based on previously published data, CHR’s dose was adjusted to 2.5 mg/kg per day
The 1% of phosphate buffer saline (PBS, 1%) (Life Technology, Grand Island, NY, United States) was given intra-rectally to the controls, all the injections of both CHR and PBS started one day before the induction of experimental colitis and lasted for 5 d. Dextran sulphate sodium (DSS) (molecular weight, 40 kDa; MP Biomedicals, Soho, OH, United States) was added to regular drinking water and freshly replaced every two days at a concentration of 5% (wt/vol) for 5 d till the mice are sacrificed. The average consumption of DSS was noted per cage each day. Animals were randomized, 6 mice per group were assigned for each experiment. Time matching of the controls was done with mice receiving normal drinking water only.

**Assessment of DSS-induced colitis**

Disease activity index (DAI) included a combined score of weight loss, stool consistency, and bleeding was recorded daily from day zero to day five during DSS treatment. Blood in the stool was evaluated using the Hemoccult II test (Beckman Coulter, Oakville, ON, Canada). Colon length and macroscopic scores were assessed on sacrifice day.

**Spleen and MLN isolation**

After the induction of DSS colitis on sacrifice day, the spleen and MLN were collected and digested in 2 mg/mL collagenase D (Roche Diagnostics, Meylan, France) dissolved in RPMI 1640 (Life Technologies) at 37 °C for 30 min associated with a 10 min intermittent shaking. To stop the reaction and disrupt the DC-T cell complexes, the cell suspension was supplemented with 5 mmol/L EDTA (Sigma, Mississauga, ON, Canada) during the last 5 min. The homogenate was then passed through a 70-mm cell strainer (VWR, Mississauga, ON, Canada) and then washed with RPMI-1640 at 1200 rpm for 5 min. Red blood cells (RBC)'s lysis was done using ACK lysis buffer (150 mmol/L NH4Cl, 10 mmol/L KHCO3, 0.1 mmol/L EDTA; Life Technologies).

**MACS CD11c sorting**

EasySep™ Mouse CD11c Positive selection kit (Stemcell Technologies) was used according manufacturer’s instructions. The cells were then collected, counted and used for stimulatory experiments.

**Isolation and stimulation of bone marrow-derived DCs**

Six to eight weeks naïve mice were sacrificed by cervical dislocation. In sterile conditions, the femur and tibia were removed and soaked in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum (FBS), 25 mg/mL gentamicin, 2 mmol/L L-glutamine (Life Technologies). Bone marrow cell extraction was performed by cutting both ends of the bones and flushing them with 1 mL of sterile RPMI-1640 into a sterile Petri dish. The cell suspension was collected and centrifuged for 5 min at 1000 rpm. The supernatant was discarded, and pellets were resuspended in RBC ACK lysis buffer (150 mmol/L NH4Cl, 10 mmol/L KHCO3, 0.1 mmol/L EDTA; Life Technologies) for the lysis of RBC. After the second centrifugation, the pellet was resuspended in RPMI-1640 and counted. The purity of the cells was then verified by flow cytometry. Granulocyte-macrophage induced colony-stimulating factor (GM-CSF, Cedarlane, Burlington, ON, Canada) induced culture of bone marrow-derived DCs (BMDCs) were used for the cell culture. Cells were suspended at a concentration of 1 × 10^6 cells/mL in culture plates containing complete RPMI 1640 medium and stimulated with 20 ng/mL of GM-CSF. The cell culture was then put in an incubator at 37 °C with 5% CO₂. GM-CSF cultured media was replaced on the 3rd and 6th day. On the 8th day, the semi-suspended cells were collected with gentle pipetting, and the loosely attached cells were scraped using a cell scraper generating mature cells. The CD11c+ cells (1 × 10⁶ per well) isolated via MACS were cultured in complete RPMI 1640 medium (Life Technologies) containing 10% heat-inactivated FBS, 25 mg/mL gentamicin and 2 mmol/L L-glutamine (Life Technologies) in 12-well plates in the presence or absence of 10⁻⁶ M of CHR for 12 h. After 12 h, they were stimulated with 100 ng/mL of lipopolysaccharide (LPS, Sigma) for 24 h. The cell culture was collected for quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) analysis, and ELISA assessed the supernatant. To determine the intracellular pathway, pharmaceutical blocker/activator, betulinic acid (NF-κB activator, 10 μmol/L; Sigma) or BAY 11-7082 (NF-κB inhibitor, 10 μmol/L; Sigma) were added to the culture medium for 24 h at the same time of LPS.

**qRT-PCR**

Total RNA from colonic tissue, MLN, splenocytes, CD11c+ splenic and CD11c− BMDC
isolated cells was extracted using TRIZol™ Plus RNA Purification Kit (Life Technologies) and reverse-transcribed using SuperScript VILO cDNA Synthesis Master Mix (Life Technologies). Gene expression was measured by a qRT-PCR using a Roche Light Cycler 96 Real-Time System and Power SYBR green master mix (Life Technologies). Differences in the threshold cycle (ΔCt) number between the target genes and the optimal reference gene Eukaryotic elongation factor 2 (Eef2) were used to calculate differences in expression. Human and mouse primer sequences used are provided in Tables 1 and 2.

RT2-profiler qPCR array
RT2 profiler™ PCR array mouse dendritic and antigen-presenting cell (Qiagen Inc, Toronto, ON Ref #330231) was performed for the quantitative PCR according to the manufacture instructions to profile the expression of 84 essential genes. Changes in gene expression between different experimental groups and the heat maps (colour-coded graphs with groups in columns and genes in rows) were generated using the web-based program of RT2 profiler PCR array data analysis.

Protein quantification
Enzyme-linked immunosorbent assays (ELISAs) were used for protein quantification from full-thickness tissue homogenates and/or supernatants from the cell cultures. Mouse commercial ELISA kits were used to detect IL-6 and IL-12p40 (R & D Systems, Inc., Minneapolis, United States).

Statistical analysis
Data are expressed as the mean ± SE. Unpaired Mann-Whitney U test was applied to compare between two groups. To compare between more than two groups, One-Way ANOVA followed by a post-hoc test was used. The analysis DAI repeated measure Two-Way ANOVA followed by a post-hoc test was used. To analyze the association between different markers studied, Spearman’s correlation test was used. The statistical two-tailed significance level was determined at $P=0.05$. GraphPad Prism software (version 6; GraphPad Software, Inc, La Jolla, CA, United States) was used to compute the statistics.

RESULTS

In colitic mice, in vivo CHR treatment decreased colonic inflammatory macro- and micro-scopic scores
As reported previously, we confirmed CHR’s anti-inflammatory effect on experimental colitis induction. CHR treatment (2.5 mg/kg/day, i.r.) resulted in a marked reduction in the external DAI (Figure 1A). No difference was seen within the first two days, but starting day 3, a significant difference was detected, culminating at a 3-fold reduction on day 5 when compared with the colitic group. We also confirmed the beneficial effect of the CHR treatment on the macroscopic score ($P<0.0002$) when distal colonic sections were examined (Figure 1B).

In colitic mice, in vivo CHR treatment decreases whole colonic section DCs-related surface and functional markers
RT-PCR array analysis of DCs-related membrane and functional markers was conducted on whole colonic sections. As presented in Figure 1C, whole colitic samples isolated from mice treated with CHR demonstrated a significant downregulation of surface and intracellular markers, notably: CD86 4.43-fold ($P<0.04$, $n=6$ per group), CD80 4.21-fold ($P<0.04$, $n=6$ per group) and NF-κB1 11.01-fold ($P<0.02$, $n=6$ per group). To confirm the data, gene expression level using a single target qRT-PCR technique was used. Development of colitis induced a significant colonic increase of mRNA expression of surface markers 86 ($P=0.0089$, $n=6$ per group) and CD80 ($P=0.0005$) when compared with the non-colitic PBS group (Figure 2A and B). Treatment with CHR significantly decreased the two markers ($P=0.0001$ and $P<0.0001$ respectively, $n=6$ per group). Besides, two others DC-related markers not present in our initial array were quantified. Induction of colitis induced a significant colonic increase of CD40 ($P<0.0001$, $n=6$ per group) and CD11c ($P<0.0001$, $n=6$ per group) when compared with the non-colitic PBS group. The same pattern of significant decrease was found for CD40 ($P=0.0002$, $n=6$ per group) and CD11c ($P<0.0001$, $n=6$ per group) (Figure 2C and D) in colon treated with CHR when compared with the
Table 1 Human primers sequences

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untreated group. Besides, a significant increase in colonic mRNA expression and protein quantification of IL-12p40 ($P < 0.0001$, $n = 6$ per group) were detected in colitic conditions when compared with the untreated group, and CHR treatment decreased those two markers ($P < 0.0001$ and $P < 0.0043$ respectively, $n = 6$ per group) (Figures 2E and F) when compared with the untreated group.

In colitic mice, in vivo CHR treatment decreases MLN DCs-related surface and functional markers

Next, we assessed the regulation of the different markers within the MLN. Induction of colitis was associated with a significant increase of CD86 ($P < 0.0001$, $n = 6$ per group), CD80 ($P < 0.0001$, $n = 6$ per group) and CD11c ($P < 0.0001$, $n = 6$ per group) mRNA expression (Figures 3A-C) when compared with non-colitic PBS group. In colitic mice, when compared with PBS group, CHR treatment decreased significantly CD86 ($P < 0.0001$, $n = 6$ per group) (Figure 3A), CD80 ($P < 0.0001$, $n = 6$ per group) (Figure 3B) and CD11c ($P < 0.0001$, $n = 6$ per group) (Figure 3C) mRNA expression. Also, induction of colitis induced a significant increase of IL-12p40 ($P < 0.0001$, $n = 6$ per group) and IL-6 ($P < 0.0001$, $n = 6$ per group) within the MLN when compared with the PBS group, and CHR treatment significantly decreased those two markers ($P < 0.0001$, $n = 6$ per group) (Figure 3D and E) when compared with untreated group. No significant changes were observed for CD40 (data not shown).

In colitic mice, in vivo CHR treatment decreases splenocytes DCs-related surface and functional markers

We assessed the expression of the different markers within the spleen cells. Induction of colitis was associated with a significant increase of splenocytes CD86 ($P < 0.0001$, $n = 6$ per group), CD80 ($P < 0.0001$, $n = 6$ per group) and CD11c ($P < 0.0001$, $n = 6$ per group) mRNA expression (Figures 4A-C) when compared with PBS group. In colitic
Figure 1  Chromofungin (chromogranin-A 47-66) treatment decreases dextran sulphate sodium-induced experimental colitis and colonic colonic cluster of differentiation markers and NF-κB gene expression in dextran sulphate sodium-induced experimental colitis. Colitis was induced with 5% dextran sulphate sodium in drinking water to C57BL/6 male mice, and the control group of mice received regular drinking water. Chromofungin (2.5 mg/kg per day, i.r.) was given as a preventive treatment or phosphate buffer saline (1%) starting from one day before the induction of colitis till the fifth day when the mice were sacrificed. A-C: External disease activity index recorded over the period of 5 d (A), macroscopic scores at sacrifice day (B), CD86 and 80 and NF-κB gene expression profile by RT² profiler PCR array at sacrifice day (C). Unpaired two-tailed Mann-Whitney U and Two-way repeated measures or One-way ANOVA followed by multiple comparison tests were used to analyze the data, and adjusted P equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, n = 6 mice/group. PBS: Phosphate buffer saline; CHR: Chromofungin; DSS: Dextran sulphate sodium.

mice, when compared with untreated group, CHR treatment significantly decreased splenocytes’ CD86 (P < 0.0001, n = 6 per group), CD80 (P < 0.0001, n = 6 per group) and CD11c (P < 0.0001, n = 6 per group) (Figures 4A-C) mRNA expression. Induction of colitis induced a significant increase of splenocytes mRNA expression of IL-12p40 (P < 0.0001, n = 6 per group) and IL-6 (P < 0.0001, n = 6 per group) when compared with the PBS group and CHR treatment was associated with a significant decrease of IL-12p40 (P < 0.0001, n = 6 per group) and IL-6 (P < 0.0001, n = 6 per group) (Figures 4D and E) when compared with the colitic PBS group. No significant changes were observed for CD40 (data not shown).

In colitic mice, in vitro CHR treatment decreases splenic CD11c+ cells DCs-related surface and functional markers

Next splenic CD11c+ cells were isolated from the colitic group treated or not with CHR via the MACS technique. As presented in Figure 5, RT²-PCR array analysis demonstrated a significant downregulation of DC-related surface and intracellular markers after CHR treatment: CD86 (4.0-fold) (P < 0.04, n = 6 per group), CD80 (3.8-fold) (P < 0.04, n = 6 per group) and NF-κB (20-fold) (P < 0.04, n = 6 per group) marker.

CHR decreases DC-related markers in BMDCs treated with LPS or NF-κB activator

Finally, we determined the intracellular pathway implicated. In BMDCs CD11c+ cells, treatment with NF-κB stimulator significantly increased the mRNA expression of CD86 (P = 0.0088, n = 6 per group), CD80 (P = 0.0026, n = 6 per group), CD40 (P = 0.0119, n = 6 per group) and CHR treatment abolished the increase (Figures 6A-C). LPS treatment increased the mRNA expression of the same three markers, and treatment with NF-κB inhibitor or CHR abolished the increase. CHR treatment decreased the harmful effect of LPS stimulation even in the presence of the NF-κB activator.
Chromofungin (chromogranin-A 47-66) treatment decreases colonic cluster of differentiation markers and interleukin-12 level in dextran sulphate sodium-induced experimental colitis. Colitis was induced with 5% dextran sulphate sodium in drinking water to C57BL/6 male mice, and the control group of mice received regular drinking water. Chromofungin (2.5 mg/kg per day, i.r.) was given as a preventive treatment or phosphate buffer saline (1%) starting from one day before the induction of colitis till the fifth day when the mice were sacrificed. A-F: CD86 (A), CD80 (B), CD40 (C), CD11c (D), interleukin (IL)-12p40 mRNA expression (E), IL-12p40 protein level (F). mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction and protein level by ELISA. One-way ANOVA followed by multiple comparison tests was used to analyze the data, and adjusted $P$ equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, $n = 6$ mice/group. PBS: Phosphate buffer saline; CHR: Chromofungin; DSS: Dextran sulphate sodium.

In parallel, the NF-κB stimulator increased significantly the protein concentration of IL-6 ($P < 0.0001$, $n = 6$ per group) and IL-12p40 ($P < 0.0001$, $n = 6$ per group) and CHR treatment significantly decreased the level of the two inflammatory proteins (Figure 6D and E). LPS treatment increased significantly the level of IL-6 ($P < 0.0001$, $n = 6$ per group) and IL-12p40 ($P < 0.0001$, $n = 6$ per group), and NF-κB inhibitor or CHR treatments decreased the status of the two markers. CHR treatment decreased the deleterious effect of LPS stimulation even in the presence of an NF-κB activator.

**Relationship between CHGA-Exon-IV and DCs-related markers in colonic biopsies of active UC patients**

Colon biopsies from UC patients were analyzed using qRT-PCR to assess the relationship between CHGA-Exon-IV and CD11c+ cells-related markers. We used our previously published data on Exon-IV[25] to perform a new correlation analysis. Our data demonstrated an upregulation of surface markers related to DCs (Figure 7A and...
Chromofungin (chromogranin-A 47-66) treatment decreases mesenteric lymph node cluster of differentiation markers and cytokine levels in dextran sulphate sodium-induced experimental colitis. Colitis was induced with 5% dextran sulphate sodium in drinking water to C57BL/6 male mice, and the control group of mice received regular drinking water. Chromofungin (2.5 mg/kg per day, i.r.) was given as a preventive treatment or phosphate buffer saline (1%) starting from one day before the induction of colitis till the fifth day when the mice were sacrificed. A-E: CD86 (A), CD80 (B), CD11c (C), interleukin (IL)-12p40 (D), IL-6 mRNA expression (E). mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction. One-way ANOVA followed by multiple comparison tests was used to analyze the data, and adjusted $P$ equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, $n = 6$ mice/group. PBS: Phosphate buffer saline; CHR: Chromofungin; DSS: Dextran sulphate sodium.

**DISCUSSION**

Overactivation of the immune system through the dysregulation of DCs and its subsequent T-cell polarization is one of the cytokine storm features seen in inflamed mucosa[46]. Over the last five years, a lot of attention has been given to CHGA and its derived peptides in regulating the immune components. However, little is known as to...
Chromofungin (chromogranin-A 47-66) treatment decreases splenocyte cluster of differentiation markers and interleukin-12 level in dextran sulphate sodium-induced experimental colitis. Colitis was induced with 5% dextran sulphate sodium in drinking water to C57BL/6 male mice, and the control group of mice received regular drinking water. Chromofungin (2.5 mg/kg per day, i.r.) was given as a preventive treatment or phosphate buffer saline (1%) starting from one day before the induction of colitis till the fifth day when the mice were sacrificed. A-E: CD86 (A), CD80 (B), CD11c (C), interleukin (IL)-12p40 (D), IL-6 mRNA expression (E) from splenic cells. mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction. One-way ANOVA followed by multiple comparison tests was used to analyze the data, and adjusted $P$ equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, $n = 6$ mice/group. PBS: Phosphate buffer saline; CHR: Chromofungin; DSS: Dextran sulphate sodium.

How those peptides can modulate innate immune cells in different immune compartments. Using an experimental colitis model mimicking UC and colonic biopsies obtained from participants with active UC compared with biopsies from healthy controls, we assessed CHR’s impact in regulating DCs-related markers. Our findings suggest a novel effect by which CHR promotes a down-regulation of DCs-related functional markers via the NF-κB pathway within different immune compartments.

In our UC-like experimental colitis model, we first confirmed the beneficial effect of CHR on qualitative and quantitative inflammatory markers described in our previous publications $[25,27,40]$. Because of the peptide’s specific action demonstrated in our previous study on different cytokines like TNF-α $[25,40]$, we did not consider using a scramble version; this can be regarded as a limitation. However, in a few experimental plans (not shown), the scramble version was used, and no significant effect was seen. With CHR treatment, DAI and macroscopic scores were significantly downregulated, and in addition to the decrease previously seen in IL-6 $[25]$, there was a significant decrease in the concentration of IL-12p40 at the mucosal level. This decrease was also...
seen at the mRNA level, suggesting the peptide’s ability to down-regulate pro-inflammatory markers at the protein and gene levels. These data confirm GWAS studies, which have shown the importance of several APC-related cytokines, most prominently, the ones related to DCs: IL-6, IL-12 which have a critical role during the development of UC\textsuperscript{47}.

In our study, experimental colitis induced a significant colonic increase of DCs-associated markers CD86, CD80, CD40 and CD11c mRNA expression, and CHR treatment decreased all of them. DCs play a critical role during inflammation\textsuperscript{48} as they activate and differentiate T cells and play an essential role in regulating adaptive immunity by releasing IL12p40\textsuperscript{20}. At the colonic level, CD11c\textsuperscript{c} cells show a crucial role in IBD progression, colitis being associated with an increased level of CD11c\textsuperscript{c} cells\textsuperscript{46}. In parallel, in inflammatory conditions like asthma, arthritis, other markers like CD86, 80 and 40 are highly expressed in CD11c\textsuperscript{c} cells\textsuperscript{49,50}. T-cell activation can be blocked by suppressing CD86 and CD80\textsuperscript{51,52} and the release of IL-12p40 or 6\textsuperscript{53,54}. Therefore, our data support the hypothesis that disruption of intestinal DCs-related markers can contribute to the perpetuation of the inflammatory process seen in IBD and that attenuation of DC’s activation could lead to a better outcome.

DCs are essential in detecting antigens at the mucosal level and presenting them within other immune compartments, where, within the MLN, their roles in the regulation of immune activation are pivotal\textsuperscript{55}. Human DCs, specifically myeloid (mDCs) CD11c\textsuperscript{c} DC-SIGN\textsuperscript{+}, are characterized by producing many cytokines and further activating the adaptive immune cells\textsuperscript{56-58}. In the context of gut inflammation, increased CD11c\textsuperscript{d} cells’ level is found in the draining MLN and associated with a marked enhancement of CD11c\textsuperscript{c}HLADR\textsuperscript{+} DC\textsuperscript{46}. In IBD patients, plasmacytoid DCs isolated from MNL exhibit overexpression of CD86, CD80 and an increase of IL-12p40 and IL-6 is seen\textsuperscript{55}. In our study, in the context of experimental colitis, our data confirmed an up-regulation of CD86, 80 and 11c markers and IL-12p40 and IL-6. We demonstrated a significant decrease in CD86, CD80 and CD11c in the group treated with CHR. This highlights CHR’s critical role in regulating CD markers and depicts the treatment’s effectiveness in controlling IL-12p40 and IL-6 within the MNL. Surprisingly no effects were seen on CD40.

In addition to the MLN, the spleen plays a significant role in the crosstalk between the innate and adaptive immune systems. Within the spleen, CD11c\textsuperscript{c} DCs act as first responder cells during the immune response development\textsuperscript{59}. Studies have shown that surface costimulatory molecules CD86, 80, and 40 present on isolated splenic CD11c\textsuperscript{c} cells are modified during the inflammatory process\textsuperscript{60}. Using splenocytes isolated cells from colitic mice, our study confirmed these elements by depicting a decreased expression of CD86, CD80 and CD11c, and IL-12p40 and IL-6 levels when treated with CHR. We were also able to confirm the importance of the CD11c\textsuperscript{c} population, as our RT\textsuperscript{2}-PCR array demonstrated a significant decrease of CD86 and 80 in that population. This was consistent with our data presented in our next experimental plan when we determined the direct impact of CHR on the CD11c\textsuperscript{c} BDCM cell population. Again here, like within the MLN, no effect was visible on the expression of CD40.

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Figure 5 Chromofungin (chromogranin-A 47-66) treatment decreases dextran sulphate sodium-induced experimental colitis and colonic cluster of differentiation markers and NF-κB gene expression in dextran sulphate sodium-induced experimental colitis. CD86 and 80 and NF-κB gene expression profile by RT\textsuperscript{2} profiler polymerase chain reaction array at sacrifice day. One-way ANOVA followed by multiple comparison tests were used to analyze the data, and adjusted P equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, n = 6 mice/group.
Chromofungin (chromogranin-A 47-66) treatment decreases lipopolysaccharide-stimulated bone marrow-derived CD11c+ cells’ cluster of differentiation markers and cytokine-associated level via the NF-κB pathways. Bone marrow-derived CD11c+ cells using MACS technique were isolated and cultured with granulocyte-macrophage colony-stimulating factor for 8 d until full maturation. Cells were treated with CHR (10^-6 M/mL) for 12 h and then stimulated with lipopolysaccharide (100 ng/mL) in the presence or absence of NF-κB activator/stimulator (10 u/mL) for 24 h. A-E: CD86 (A), CD80 (B), CD40 (C), mRNA expression and IL-12p40 (D), IL-6 (E) medium protein level. mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction and protein levels were quantified by ELISA. One-way ANOVA followed by multiple comparison tests was used to analyze the data, and adjusted P equal to or smaller than 0.05 is believed to be significant. Each value represents the mean ± SE, n = 6 mice/group. LPS: Lipopolysaccharide; CHR: Chromofungin.
Figure 7 Chromofungin (CHGA Exon-IV) correlates negatively with cluster of differentiation 86 and 80 mRNA expression. A: mRNA expression of CD86 in the colonic tissue of healthy individuals \((n = 10)\) and participants with active ulcerative colitis (UC) \((n = 10)\); B: Correlation analysis between biopsy CHGA Exon IV and CD86 mRNA expression; C: mRNA expression of CD80 in the colonic tissue of healthy individuals \((n = 10)\) and participants with active UC \((n = 10)\); D: Correlation analysis between biopsy CHGA Exon IV and CD80 mRNA expression. mRNA expression was quantified by quantitative real-time reverse-transcription polymerase chain reaction. Student t-test and Mann-Whitney test and Spearman's correlation were used to analyze, and adjusted \(P\) equal to or smaller than 0.05 is believed to be significant. UC: Ulcerative colitis.

In the context of acute and chronic inflammatory conditions, several inflammatory cytokines are regulated by NF-κB, and previous studies have reported that blocking NF-κB in mice can be considered as a potential treatment for preventing gut inflammation\([61-65]\). NF-κB plays an essential role in regulating the expression of CD86, CD80, CD40 and CD11c\([66-68]\). In our study, the RT-PCR array performed on colonic colitic samples, and splenic CD11c+ cells demonstrated a down-regulation of NFκB1 after CHR treatment. Consistent with our in vivo data, mimicking an inflammatory activation state, mouse LPS-stimulated CD11c+ BMDCs showed an increase of CD86, 80, 40 mRNA expression. The difference in CD40 expression between the colon, the MLN, spleen, and the CD11c+ BMDCs can be explained by a potential weak stimulus of CHR in that marker in an in vivo condition in contrast to a strong effect when the peptide had a direct action on the CD11c+ BMDCs, as presented by other when deciphering the impact of commensal bacteria on DC cells in different immune compartments\([69]\).

In addition, the presence of CHR abolished colitis in the same way as the NF-κB inhibitor. To confirm NF-κB as a potential target, using an NF-κB stimulator, we demonstrated the impact of CHR's inhibitory effect on the detrimental impact induced by the stimulator. Our in vitro evaluation also showed a significant decrease in IL-6 and IL-12p40 in LPS-stimulated CD11c+ BMDCs. As CHR can decrease the effect caused by the LPS and NF-κB stimulator, we can postulate that the targeted protein resides at one of the NF-κB targets. These data agree with human data, showing that anti-IL-12p40 monoclonal antibodies have demonstrated excellent clinical efficacy in a group of UC patients\([70]\). In the clinical and experimental models of colitis, NF-κB has been demonstrated to be a major immune regulator\([61,62,65]\) when inflammation is developed. However, at that stage, we cannot confirm if the effect of CHR is a direct or an indirect effect on NFκB. Many upstream pathways can be modulated, as for example, TLR4\([71]\), ER stress\([71]\). However, there are some early indications that the phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/Akt) pathway maybe be of interest\([72]\), as CHR can produce a cardioprotective action by regulating the PI3K pathways\([37]\). Thus, considering the previous literature related to NF-κB and IBD, our results support the concept that CHR can potentially decrease intestinal inflammation by regulating the NF-κB pathway in CD11c+ cells.
We previously demonstrated that CHGA (Exon-IV) was highly down-regulated in UC patients showing that the disease is affecting the levels of CHGA (Exon-IV)[27,73]. As a novel finding, in colonic biopsies from UC participants, we demonstrated that CD11c and its related costimulatory surface markers CD86 were significantly upregulated, leading to a potential enhancement of colitis. Furthermore, in active UC participants, we observed a negative correlation between the CHGA (Exon-IV) with CD86 markers. These alterations can be explained by previous data, which demonstrated changes at the level of the ECs during the inflammatory process[32]. Our small sample size likely led to our inability to show a significant difference in CD11c bearing cells.

Some limitations exist in our study. Regarding the overall impact of CD11c cell deactivation, we did not demonstrate a direct impact on T-cell activation, a regulation that remains elucidated via the use of a co-culture model. Further studies are also required to investigate the functional consequences of CHR on other immune cells or epithelial cells and angiogenesis, all implicated in the pathogenesis of colitis. Moreover, we acknowledge that CD11c cells isolated from the lamina propria need to be studied, and new studies should define if DC expressing CD11c markers are the main target of CHR. Additional biopsies need to be added to determine a compelling correlation between various markers, and a proteomics approach should be used for protein quantification in the colon, and all correlation analyses should be repeated accordingly. Several other factors might lead to CHR’s protective effect, which we have observed in our study. For example, previous studies demonstrated a link between gut microbiota and experimental colitis and human IBD, and there is evidence showing that CHR can have anti-microbial function[34,35]. Therefore, in our in vivo model, i.r. injection could have induced a microbial change in favour of beneficial bacteria; further metagenomic analyses are needed to study this aspect, but that would not account for the beneficial effect seen on CD11c+ BMDCs.

CONCLUSION

In summary, our study shows that treatment with CHR led to lower colonic inflammation, which was associated with decreased levels of CD11c+-associated cytokines through an NF-κB-dependent mechanism. Hence, CHR seems to act as an NF-κB blocker, reducing primary APCs' activation and ultimately decreasing the intestinal inflammatory process. CHR alone had no side effect on the mice or the cells, indicating CHR’s relative safety at the studied dose. This previously unknown spatial impact of CHR in colonic inflammation may help broaden research done by other groups on the overall effect of CHGA and its derived peptides on immune regulation in the context of IBD. This might lead to future novel CHR-based therapies in IBD.

ARTICLE HIGHLIGHTS

Research background
Ulcerative colitis (UC) is a disorder in which the gastrointestinal tract becomes ulcerated and inflamed. Although treatments and therapeutic strategies are evolving quickly, treatments are still inadequate for a substantial percentage of those with active UC, and some therapies may have serious adverse side effects. Therefore, UC needs new therapeutic approaches associated with higher efficacy and limited side effects. In UC, there is an overactivation of innate immune cells such as macrophages and dendritic cells (DCs). Recently, in the context of UC and experimental colitis, new data highlighted an essential anti-inflammatory role of chromofungin (CHR), a chromogranin-A derived peptide, on peritoneal macrophages.

Research motivation
To evaluate CHR’s effects on different DCs at various immune compartments and to define a potential intracellular pathway implicated.

Research objectives
To investigate the association between CHR treatment and DCs-related markers in colitis.
Research methods
Participants with active UC and a model of acute UC-like colitis using dextran sulphate sodium were used. We used cell culture and quantitative reverse transcription-polymerase chain reaction to analyze the relative expression levels of CD11c, CD80, CD86, interleukin (IL)-6 and IL-12p40 within the colonic samples, mesenteric lymph nodes and the spleen.

Research results
In a preclinical setting, CHR treatment the expression of CD11c, CD40, CD80, CD86 and IL-12p40 in the inflamed colonic mucosa and CD11c, CD80, CD86, IL-6 and IL-12p40 within the mesenteric lymph nodes and the spleen. In addition, CHR treatment decreased CD80 and CD86 expression markers of splenic CD11c+ cells and decreased NF-κB expression in the colon and splenic CD11c+ cells. In vitro, CHR decreased CD40, CD80, CD86, IL-6, and IL-12p40 expression in naïve bone marrow-derived CD11c+DC stimulated lipopolysaccharide. Using a pharmacological approach, we demonstrated the impact of CHR on the NF-κB pathway. In a clinical setting, in patients with active UC, CHR level was reduced and showed a negative linear relationship with CD11c and CD86.

Research conclusions
CHR has protective properties against intestinal inflammation, potentially through the regulation of DC-related markers and CD11c+ cells.

Research perspectives
Although we have demonstrated that CHR may have a potential therapeutic interest, additional markers and detailed mechanisms of action need to be determined in a large sample.

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Basic Study

Multiparameter magnetic resonance imaging of liver fibrosis in a bile duct ligation mouse model

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Institutional review board statement: The protocol of our study was approved by the Animal Care and Use Committee of the Second Xiangya Hospital of Central South University (Approval No. 20204095).

Conflict-of-interest statement: None of the authors have identified a conflict of interest.

Abstract

BACKGROUND

Bile duct ligation (BDL) in animals is a classical method for mimicking cholestatic fibrosis. Although different surgical techniques have been described in rats and rabbits, mouse models can be more cost-effective and reproducible for investigating cholestatic fibrosis. Magnetic resonance imaging (MRI) has made great advances for noninvasive assessment of liver fibrosis. More comprehensive liver fibrotic features of BDL on MRI are important. However, the utility of multiparameter MRI to detect liver fibrosis in a BDL mouse model has not been assessed.

AIM

To evaluate the correlation between the pathological changes and multiparameter MRI characteristics of liver fibrosis in a BDL mouse model.

METHODS

Twenty-eight healthy adult male balb/c mice were randomly divided into four groups: sham, week 2 BDL, week 4 BDL, and week 6 BDL. Multiparameter MRI sequences, included magnetic resonance cholangiopancreatography, T1-weighted, T2-weighted, T2 mapping, and pre- and post-enhanced T1 mapping, were performed after sham and BDL surgery. Peripheral blood and liver tissue were collected after MRI. For statistical analysis, Student’s t-test and Pearson’s correlation coefficient were used.
RESULTS
Four mice died after BDL surgery; seven, six, five and six mice were included separately from the four groups. Signal intensities of liver parenchyma showed no difference on T1- and T2-weighted images. Bile duct volume, ΔT1 value, T2 value, and the rate of liver fibrosis increased steadily in week 2 BDL, week 4 BDL and week 6 BDL groups compared with those in the sham group \( (P < 0.01) \). Alanine aminotransferase and aspartate transaminase levels initially surged after surgery, followed by a gradual decline over time. Strong correlations were found between bile duct volume \( (r = 0.84) \), T2 value \( (r = 0.78) \), ΔT1 value \( (r = 0.62) \), and hepatic fibrosis rate \( (all P < 0.01) \) in the BDL groups.

CONCLUSION
The BDL mouse model induces changes that can be observed on MRI. The MRI parameters correlate with the hepatic fibrosis rate and allow for detection of cholestatic fibrosis.

Key Words: Liver; Fibrosis; Magnetic resonance imaging; Pathology; Animal model; Bile duct ligation

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Core tip: Magnetic resonance imaging (MRI) is promising for the noninvasive assessment of liver fibrosis, but the utility of multiparameter MRI in the bile duct ligation (BDL) mouse model has not been assessed. We established an experimental BDL mouse model that mimicked various aspects of cholestatic fibrosis and evaluated the potential of multiparameter MRI for the assessment of cholestatic fibrosis. We found that the BDL mouse model induced a complex cascade of changes that were observed clearly on MRI. The T2 and ΔT1 values were well correlated with the hepatic fibrosis rate and may allow for the detection of cholestatic fibrosis.


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INTRODUCTION
An appropriate animal model and a reliable detection tool are crucial for investigating liver fibrosis. Cholestatic fibrosis, one of the main types of liver fibrosis, is a widespread disease with broad etiologies including genetics, immunity, infection, calculi, and tumors[1]. Obstruction of the biliary system induces a complex cascade of pathological and functional changes that leads to cholestatic fibrosis. Therefore, bile duct ligation (BDL) in animals has become a classical method for mimicking cholestatic fibrosis. In recent years, different surgical techniques have been described in rats and rabbits[2-4]. However, long-term experiments with rats or rabbits require a considerable work force, materials, and financial resources. The mouse has become the most popular experimental animal because of its higher genetic similarity with humans, lower cost, and more human-like responses to pathophysiological changes [5]. With careful surgical technique and the 3R rule for laboratory animals, the BDL mouse model can be more cost-effective and reproducible when subjected to the same conditions as the rat or rabbit models. The BDL mouse model may be the better choice for investigating cholestatic fibrosis.

Liver biopsy, with its invasiveness and subsequent related complications, is not suitable for screening and long-term monitoring in experimental animals[6]. Magnetic resonance imaging (MRI) is a promising tool for noninvasive assessment of liver fibrosis[7]. During the past decade, MRI has made great advances, and many versatile and powerful MRI sequences and methods have been widely applied, such as T1 mapping[8] and T2 mapping[9]. However, more comprehensive liver fibrotic features
on MR images, including radiological, quantitative and analytical aspects, are important. To the best of our knowledge, this has not been described in the BDL mouse model. Thus, it is appropriate to seek an effective way to evaluate the relationship between BDL mouse models and reliable MRI techniques accurately, particularly for noninvasive monitoring of liver fibrosis.

We hypothesized that the BDL mouse model can be noninvasively characterized radiologically using an MR imager and further validated with radiological, quantitative and analytical aspects. Accordingly, the purpose of our study was to establish and characterize the BDL mouse model and evaluate the correlation between the characteristics of hepatic fibrosis seen in MR images with both the pathological changes of liver fibrosis and the biochemical results matched with the histological specimens.

**MATERIALS AND METHODS**

The protocol of our study was approved by the Animal Care and Use Committee of the Second Xiangya Hospital of Central South University (2020495). All experiments were conducted in strict accordance with the Chinese law and institutional guidelines.

**Animal model**

Twenty-eight 7–8-wk-old male balb/c mice that initially weighed 28–30 g were used. The animals were exposed to a regular light–dark cycle and housed in an air-conditioned room at 26°C. Food and water were available at all times. Before surgery, all mice were randomly divided into sham and BDL groups and anesthetized with an intraperitoneal injection of 45 mg/kg pentobarbital sodium. After losing reflexes in response to a toe pinch, they were fixed on a mouse fixator in the supine position, and the skin on the chest and abdomen was prepared. For sham surgery procedures, only an upper abdominal incision with a length of ~2 cm was made before the abdominal suture. For BDL surgery, the liver was lifted using a moisturized (normal saline) cotton swab so that the liver was stuck to the diaphragm, and the gut was caudally moved with a humidified cotton swab to better expose the bile duct. Subsequently, the 4-0 nonabsorbable suture was placed around the bile duct and secured by two surgical knots. After replacing the abdominal organs to their physiological positions and closing the abdominal layers with 6-0 absorbable sutures, the mice were moved to a warm cage until they were fully awake and active.

**MRI**

All MR images were obtained using a 3.0-T clinical MRI scanner (Magnetom Skyra; Siemens Medical Solutions, Erlangen, Germany) with a custom-built rodent receiver coil with an inner diameter of 7 cm (Zhongzhi Medical Technologies, Suzhou, China). Before imaging, the mice were anesthetized with an intraperitoneal injection of 45 mg/kg pentobarbital sodium solution, and the respiration rate was monitored by periodic visual inspection. Following the acquisition of magnetic resonance cholangiopancreatography (MRCP), T1-weighted, T2-weighted, T2 mapping, and pre-and post-enhanced T1 mapping images were obtained. The sequences and parameters were used as follows: (1) MRCP images were generated using a 3D multi-shot fast spin-echo sequence (repetition time (TR)/echo time (TE) = 4585.0/404.0 ms) to generate heavily T2-weighted 3D volumetric images; (2) Transverse T1-weighted sequence (TR/TE = 3.3/1.7 ms; voxel size, 0.9 mm × 0.9 mm × 0.7 mm; slice thickness, 0.7 mm; and total examination time, 5 min 58 s); (3) Transverse T2-weighted sequence (TR/TE = 5361/111 ms; voxel size, 0.4 mm × 0.4 mm × 1.0 mm; slice thickness, 1.0 mm; and total examination time, 3 min 57 s); (4) T1 mapping sequence (TR/TE = 8.5/3.7 ms; two flip angles = 5°/15°; scan time, 5 min 20 s; and five averages acquired); and (5) T2 mapping sequence (TR =1950 ms, a multiple TE equally spaced from 11.1 ms to 55.5 ms; flip angle, 180°). For contrast-enhanced T1 mapping imaging, the images were collected at the tenth minute after intravenous injection of 0.1 mL gadoxetate disodium.

**Image analysis**

Two radiologists (Q-L.S, and Y-H.L) with >5 years of abdominal diagnostic experience independently recorded the image data and resolved any discrepancies by consensus. Both radiologists were blinded to the histopathological results.
The MR data were imported into an image-processing workstation (Syngo; Siemens Medical Solutions). At least three circular regions of interest (ROIs) were randomly placed manually in the liver lobes to measure the signal intensities of routine sequences and mean values of the quantitative parameters. Care was taken to avoid moving artifacts and large vessels. Quantitative parameters for the liver, including T1 and T2 values, were extracted from relaxation pseudocolor maps in T1 and T2 mapping separately. ΔT1 value = (pre-enhanced T1 value) – (post-enhanced T1 value). The semiquantitative parameter, bile duct volume, was segmented by manually delineating each 2D slice and was estimated by adding up the individual voxel volumes inside the ROI.

Biochemic and histological evaluation
All mice were killed under general anesthesia after MRI examination to procure their livers and collect blood at defined time points, as mentioned above. Blood was collected to measure clinical chemistry parameters, including alanine aminotransferase (ALT) and aspartate transaminase (AST) levels. Liver specimens were soaked in a 4% phosphate-buffered formaldehyde solution for 48 h, fixed using paraffin, and transversely sectioned. Liver tissue sections were soaked in a water bath and baked for 1 h at 70°C. The baked sections were then stained with the Masson’s Trichrome Staining Kit (G1006; Servicebio, Wuhan, China) and observed under an Olympus microscope (DP72; Olympus Corporation, Tokyo, Japan). The rate of liver fibrosis and fibrotic thickness of the bile duct were analyzed using ImageJ software, which was defined by the area of positive staining with blue color divided by the total area of the background.

Statistical analysis
Statistical analysis was performed using SPSS 22.0, and GraphPad Prism 7.0. Student’s t-test was used to determine the differences in average values between the sham and surgically treated groups. The relationships between radiological and histological findings were investigated using Pearson’s linear correlations. Statistical significance was set at P < 0.05. Statistical review of the study was performed by a biomedical statistician.

RESULTS

Animal model
A schematic diagram of BDL is shown in Figure 1. Of the 28 mice, four in the liver fibrosis group died after BDL surgery. Finally, seven, six, five and six mice were included separately from the sham group, week 2 BDL, week 4 BDL and week 6 BDL groups. The bile duct volume (P < 0.01) grew dramatically from 0.04 cm³ in the sham group to 3.92 cm³ in the week 4 BDL group after BDL surgery (Figure 2A). The mean levels of ALT and AST, biomarkers of hepatocellular injury, in the week 6 BDL group were lower than those in the week 2 BDL group (P < 0.01). The BDL mice showed good adaptability after the second week of BDL (Figure 2B).

MRI
Morphological changes in Figure 2B are demarcated on the MR images obtained with all sequences. Swollen bile ducts showed signal intensities similar to that of water on MRCP, and T1-weighted and T2-weighted images. The volume of the bile duct had an obvious rise from 0.04 to 3.92 cm³ over time after BDL surgery. However, the signal intensities of the liver parenchyma did not show obvious differences upon perusal of the T1-weighted and T2-weighted images.

In MR functional sequences, dynamic documented pseudocolor maps qualitatively and quantitatively showed functional parameter changes (Figure 3A). T2 value increased with aggravation of liver fibrosis between the sham and BDL subgroups (P < 0.01, Figure 3B) from 28.9 to 52.1 ms. Aside from this, ΔT1 value for the sham group was lower than that of the week 6 BDL group with 352.1 and 675.8 ms, respectively (P < 0.01). In contrast, the pre-enhanced T1 values did not show a significant difference among the four groups.

Histopathological examination
The different areas for measurement of liver fibrosis, including intrahepatic bile duct and hepatic parenchyma, were compared with respect to the model duration...
Figure 1 Schematic diagram of bile duct ligation. A: Anatomy of the liver, bile duct, portal vein, and hepatic artery in mice; B: Exposure of the bile duct.

(Figure 4). The area of hepatic fibrosis and fibrotic thickness of the bile duct increased significantly over time. The highest proportion of hepatic fibrosis (12.58%) was found in the week 6 BDL group, which exceeded that in the remaining groups ($P < 0.01$). The fibrotic thickness of the bile duct was highest (54.73 mm) in the week 6 BDL group, compared with that of the other groups ($P < 0.01$).

**Correlation analysis**

Correlation analysis between the tested parameters and the area of liver fibrosis was calculated (Figure 5). Histopathological examination revealed that the area of hepatic fibrosis showed steady growth. In the tested parameters, the changes in bile duct volume, $T_2$ value, and $\Delta T_1$ value were in line with those of hepatic fibrosis. Also, the related coefficients of bile duct volume, $T_2$ value, and $\Delta T_1$ value showed the manifest correlation with $0.84 (P < 0.01)$, $0.78 (P < 0.01)$ and $0.62 (P < 0.01)$, respectively. The levels of ALT and AST dramatically surged 2 wk after BDL surgery, followed by a gradual decline to the lowest values at week 6. The changes in ALT and AST levels differed from those in hepatic fibrosis. Thus, the correlation coefficients did not show valuable results ($P > 0.05$).

**DISCUSSION**

This study established an experimental BDL mouse model that mimicked various aspects of cholestatic fibrosis and evaluated the potential of multiparameter MRI for the noninvasive assessment of cholestatic fibrosis in the BDL mouse model.

The BDL model has been widely used to study cholestatic liver injury and the subsequent fibrosis. Surgical BDL can induce strong proliferation of bile duct cells, while the variable activation of oval cells (i.e. hepatic progenitor cells) depends on additional liver damage, leading to extensive bile duct reactions, cholestasis, portal inflammation, and rapid establishment of bile duct fibrosis[2-4]. This model was first established in rats and then successfully applied to rabbits and mice[5]. Unlike the BDL rabbit or rat model, the BDL mouse model may face more challenges, such as accurate operation, postoperative infections, and accidental injuries. In our study, standardized protocols with strict guidelines were followed, thereby enabling us to establish a mouse model of BDL, successfully.

Noninvasive liver fibrosis evaluation, including serum tests, ultrasound elastography, and magnetic resonance elastography (MRE), can overcome many limitations of liver biopsy and, therefore, are now incorporated into specialist clinical practice. These are valuable for ruling out advanced fibrosis or cirrhosis; however, each individual test cannot be fully predictive when used alone[10]. In this study, MRI was utilized in postoperative evaluation to noninvasively image and depict the changes in morphological and functional processes. Our results revealed that the BDL mouse model combined with multiparameter MRI is an innovative way to investigate liver fibrosis, and it induces a complex cascade of changes that can be observed clearly on MRI images.
Multiparameter MRI sequences including MRCP, T1-weighted, T2-weighted, T2 mapping, and pre- and post-enhanced T1 mapping were performed in the sham and BDL groups. The results from the present study revealed that: (1) Pre-enhanced sequences including MRCP, T1-weighted, and T2-weighted clearly showed the morphological changes in the BDL mouse model; (2) Compared with the sham group, T2 and ΔT1 values showed significant differences in the BDL groups; and (3) Bile duct volume, T2 value, and ΔT1 value also demonstrated a higher correlation with the hepatic fibrosis rate.

The T2 value in our study showed a significant increase with time after BDL surgery and a good correlation between the T2 value and the rate of liver fibrosis in the BDL mouse model. Zhang et al.[11] have also shown that the T2 value increases with the development of fibrosis, and has a close relationship between inflammation, edema, and liver fibrosis in rat models. Similarly, Luetkens et al.[9] indicated that T2 values correlated not only with stages of fibrosis but also with total collagen protein and transcription of collagen type I. It is reasonable to conclude that the prolonged T2 value may be attributable to collagen deposition and inflammation.

Owing to the characteristics of MRI data acquisition, the signal intensity obtained from contrast-enhanced MR images is affected by many technical parameters[12]. The signal intensity is not proportional to the gadolinium concentration and cannot be directly compared between sequences collected at different time points. In recent years, increasing attention has been paid to the measurement of T1 relaxation time from T1 mapping images[13]. The T1 value, which is proportional to the concentration of contrast medium in vivo, is more objective and reliable than direct measurement of the liver signal[2]. In the present study, an upward trend was observed in the ΔT1 value over time.
value from the sham group to the BDL group. However, the findings of the current study do not support previous research\(^{[14]}\). This discrepancy could be attributed to the partial obstruction of hepatic arteries as a result of a steady rise in bile duct volume such that the excretion time of the contrast would take longer than that of normal hepatic arteries. These factors may result in a greater amount of intracellular remnant in the form of the contrast agent and a decreased T1 value at 10 min after intravenous
injection. Furthermore, a moderate correlation between the ΔT1 value and fibrosis rate was found in our study. This combination of findings provides some support for the conceptual premise that T1 mapping is a reliable tool for discriminating liver fibrosis.

This study had several limitations. First, because of the limited number of mice, it was difficult to avoid selection bias and carry out a subgroup analysis. Second, many advanced sequences such as MRE\(^{15}\) and intravoxel incoherent motion imaging\(^{16}\) could not be used in our study because of technological limitations in clinical MRI. Third, it is difficult to use the breath-holding technique in mice, and the subsequent respiratory movement in the abdomen might have degraded image quality and caused bias in measurements. Fourth, steatosis is a common hepatic lesion that occurs before the formation of fibrosis in biliary cirrhosis. This could have been a potential confounding factor in the pathological changes.

CONCLUSION

BDL induces a complex cascade of changes that can be clearly observed on MRI. To our knowledge, this is the first study to assess the utility of multiparameter MRI in a BDL mouse model. The results showed moderate to high correlations between ΔT1 value, T2 value, and liver fibrosis, indicating that T1 mapping and T2 mapping could be useful tools for the detection of liver fibrosis in the BDL mouse model. In the future, advanced MR techniques will have great potential for widespread application in both preclinical and clinical fields.

ARTICLE HIGHLIGHTS

Research background

Bile duct ligation (BDL) is a classical method for mimicking cholestatic fibrosis in animals. Magnetic resonance imaging (MRI) has enabled significant advances in noninvasive assessment of liver fibrosis. More comprehensive liver fibrotic features of BDL on MRI are important. However, the utility of multiparameter MRI to detect liver fibrosis in a BDL mouse model has not been assessed.

Research motivation

We hypothesized that the BDL mouse model can be characterized radiologically using MRI, which is a noninvasive method. The model can be further validated regarding radiological, quantitative, and analytical aspects.
Research objectives
Using a BDL mouse model to evaluate the correlation between the pathological changes and several parameters of MRI characteristics of liver fibrosis.

Research methods
Twenty-eight healthy adult male balb/c mice were included. They were randomly divided into four groups: Sham group and week 2 BDL, week 4 BDL and week 6 BDL groups. The MRI sequences included the following parameters: Magnetic resonance cholangiopancreatography (MRCP), T1-weighted, T2-weighted, T2 mapping, and pre-and post-enhanced T1 mapping. All these were performed after sham and BDL surgery. Peripheral blood and liver tissue were collected after the MRI.

Research results
The bile duct volume, ΔT1 value, T2 value, and the rate of liver fibrosis increased steadily in the week 2 BDL, week 4 BDL and week 6 BDL groups compared to those in the sham group (P < 0.01). Strong correlations were found between bile duct volume, T2 value, ΔT1 value, and hepatic fibrosis rate (all P < 0.01) in all BDL groups.

Research conclusions
The BDL mouse model induces changes that are easily observed using MRI. The MRI parameters correlate with the hepatic fibrosis rate and enable the detection of cholestatic fibrosis.

Research perspectives
We believe that advanced MR techniques have considerable potential for widespread application in preclinical and clinical fields.

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Retrospective Study

Disease control and failure patterns of unresectable hepatocellular carcinoma following transarterial radioembolization with yttrium-90 microspheres and with/without sorafenib


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Author contributions: Teyateeti A designed the study, collected, analyzed and interpreted the data and wrote the manuscript; Mahvash A, Abdelsalam M, Avritscher R, Odisio B, Ravizzini G, and Surasi D collected data, provided clinical advice and edited the manuscript; Long J supervised and provided advice for statistical analysis and edited the manuscript; Kaseb A and Macapinlac H edited the manuscript; Teyateeti A contributed to study design, provided clinical advice and made critical revision of the manuscript; and Kappadath SC contributed to the study.

Abstract

BACKGROUND
Impressive survival outcome of our previous study in unresectable hepatocellular carcinoma (HCC) patients undergoing yttrium-90 glass microspheres transarterial radioembolization (TARE) with/without sorafenib according to individuals’ disease burden, i.e., intrahepatic tumor load (IHT) and adverse disease features (ADFs) might partly be confounded by other treatments and underlying hepatic
the design of the study, interpretation of the data, made critical revision of the manuscript and supervised the study.

**Institutional review board statement:** This study was approved by Institutional review board of The University of Texas MD Anderson Cancer Center, No. DR09-0025.

**Informed consent statement:** A waiver of informed consent was granted by our Institutional Review Board for this retrospective study. Patient data used complied with all institutional data protection and privacy regulations.

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**Data sharing statement:** Authors are open to data sharing, please email queries.

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**Specialty type:** Gastroenterology and hepatology

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report’s scientific quality classification**
- Grade A (Excellent): 0
- Grade B (Very good): 0
- Grade C (Good): C
- Grade D (Fair): 0
- Grade E (Poor): 0

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function. Therefore, a dedicated study focusing on treatment response and assessment of failure patterns might be a way to improve treatment outcome in addition to patient selection based on the disease burden.

**AIM**
To assess the tumor response, disease control and patterns of disease progression following TARE with/without sorafenib in unresectable HCC patients.

**METHODS**
This retrospective study was conducted in successful TARE procedures with available pre- and post-treatment imaging studies (n = 169). Three treatment subgroups were (1) TARE only (TARE_alone) for HIT ≤ 50% without ADFs, i.e., macrovascular invasion, extrahepatic disease (EHD) and infiltrative/ill-defined HCC (n = 63); (2) TARE with sorafenib (TARE_sorafenib) for HIT > 50% and/or presence of ADFs (n = 81); and (3) TARE only for patients who could not receive sorafenib due to contraindication or intolerance (TARE_no_sorafenib) (n = 25). Objective response rate (ORR; consisted of complete response (CR) and partial response (PR)), disease control rate (DCR; consisted of CR, PR and stable disease) and failure patterns of treated, intrahepatic and extrahepatic sites were assessed using the modified response evaluation criteria in solid tumors. Time to progression (TTP) was calculated from TARE to the first radiologic progression at any site using Kaplan-Meier method. Identification of prognostic factors for TTP using the univariate Kaplan-Meier method and multivariate Cox proportional hazard model were performed in major population subgroups, TARE_alone and TARE_sorafenib.

**RESULTS**
The median radiologic follow-up time was 4.4 mo (range 0.5-48.8). In treated area, ORR was highest in TARE_sorafenib (53.1%), followed by TARE_alone (41.3%) and TARE_no_sorafenib (16%). In intrahepatic area, DCR remained highest in TARE_sorafenib (84%), followed by TARE_alone (79.4%) and TARE_no_sorafenib (44%). The overall DCR was highest in TARE_alone (79.4%), followed by TARE_sorafenib (71.6%) and TARE_no_sorafenib (40%). Dominant failure patterns were intrahepatic for both TARE_alone (44.5%) and TARE_sorafenib (38.4%). Extrahepatic progression was more common in TARE_sorafenib (32%) and TARE_no_sorafenib (40%) than in TARE_alone (12.7%). TTP was longest in TARE_alone (8.6 mo; 95%CI: 3.4-13.8), followed by TARE_sorafenib (5.1 mo; 95%CI: 4.0-6.2) and TARE_no_sorafenib (2.7 mo; 95%CI: 2.2-3.1). Pre-existing EHD (HR: 0.37, 95%CI: 0.24-0.56, P < 0.001) was a sole prognostic factor for TTP in TARE_sorafenib with no prognostic factor for TTP in TARE_alone.

**CONCLUSION**
TARE with/without sorafenib according to individuals’ disease burden provided DCR approximately 70% with intrahepatic progression as dominant failure pattern. Extrahepatic progression was more common in procedures with initially high disease burden.

**Key Words:** Radioembolization; Selective internal radiotherapy; Tumor response; Pattern of progression; Time to progression; Sorafenib

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**Core Tip:** Hepatocellular carcinoma (HCC) patients treated with yttrium-90 transarterial radioembolization (TARE) alone for intrahepatic tumor load ≤ 50% and TARE with sorafenib for intrahepatic tumor load > 50% and/or present macrovascular invasion, extrahepatic disease or infiltrative HCC yielded acceptable disease control rates of 79.4% and 71.6%, respectively. Between these 2 subgroups, incidence of intrahepatic progression was comparable (about 40%) but extrahepatic progression was much less common with TARE alone (12.7% vs 32%). Strategies that improve intrahepatic control for liver-only disease (dosimetry-based TARE) and extrahepatic control for
INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the major health problems worldwide. It is the sixth most common malignancy with over 900,000 new cases and 830,000 deaths per year and the third leading cause of cancer deaths [1]. HCC has a high mortality rate due to the fact that the majority of patients are diagnosed at an advanced stage of disease beyond the curative surgical options. This group of patients, sometimes called unresectable HCC patients, generally have two standard treatment options; local therapies, trans-arterial chemoembolization (TACE) and/or ablation, or systemic therapy [2-5].

Trans-arterial radioembolization (TARE) with yttrium-90 (Y-90) microspheres is an alternative local therapy option for unresectable HCC patients [2,5,6]. Currently, TARE is not an established treatment in most HCC treatment guidelines outside the United State. Consequently, each institution has its own algorithm for selecting TARE candidates. As a result, there exists marked variations in reported treatment outcome for TARE, depending on disease characteristics of enrolled patients [7-10].

At our institution, we offer TARE as monotherapy to patients with intrahepatic tumor (IHT) involvement less than or equal to 50% of total liver parenchyma (IHT ≤ 50%). Patients with IHT greater than 50% (IHT > 50%) or with advanced disease features (ADFs), defined as macrovascular invasion (MVI), extrahepatic disease (EHD) and infiltrative/ill-defined HCC, are candidates for TARE with systemic therapy which historically first line was sorafenib [11].

The clinical outcomes in terms of overall survival (OS) and progression-free survival (PFS) following TARE at our institution has been previously reported [12]. We reported median OS and PFS durations of 21.6 mo (95%CI: 6.1-37.1) and 9.1 mo (95%CI: 5.2-13.0), respectively, for HCC patients with IHT ≤ 50% treated with TARE only; while those for HCC patients treated with TARE in combination with sorafenib were 12.4 mo (95%CI: 9.1-15.6) and 5.1 mo (95%CI: 2.6-7.5), respectively. Better OS for HCC patients treated with TARE in combination with sorafenib was associated with patients with lower disease burden [IHT ≤ 50%, hazard ratios (HR) = 0.39, P = 0.004 and alpha-fetoprotein (AFP) < 400, HR = 0.5, P = 0.027]. Unilobar involvement (HR = 0.43, P = 0.029) correlated with better PFS in HCC patients with IHT ≤ 50% treated with TARE only. However, the OS and PFS survival outcomes reported was affected by several treatment combinations and not solely due to the effect of TARE itself. The objective of this study was to quantify and characterize the benefits of TARE as a local therapy. More specifically, we investigated the objective response rate (ORR), disease control rate (DCR), time to progression (TTP), and, in the case of progression, the pattern and location of disease progression, for HCC patients treated at our institution with TARE, either as monotherapy or in combination with sorafenib.

MATERIALS AND METHODS

Patient selection

This institutional review board approved retrospective study was conducted in unresectable HCC patients who received TARE with Y-90 glass microspheres at The University of Texas MD Anderson Cancer Center (Houston, TX, United States) from November 16, 2010, to October 1, 2018. Inclusion criteria were successful TARE
procedures with available pre-treatment imaging study within 1 mo before TARE and at least one post-treatment imaging study within 2 mo after TARE \( (n = 176) \). In case of multiple follow-ups, all of imaging studies were done with the same imaging technique, i.e., all contrast-enhanced computed tomography (CT) or all magnetic resonance imaging (MRI). Exclusion criteria were TARE procedures with restrictions on imaging interpretation and/or comparison, i.e., non-contrast enhanced studies \( (n = 5) \), poor quality imaging study \( (n = 1) \) and hypo-vascular HCC \( (n = 1) \). A total of 169 procedures from 151 patients were finally included for analysis in this study.

**Pretreatment evaluation**

Pretreatment clinical histories and laboratory tests, including AFP and liver function tests were reviewed retrospectively in procedure-based fashion. Staging of disease and performance status were assessed by the Barcelona Clinic Liver Cancer (BCLC) staging system and Eastern Cooperative Oncology Group (ECOG) performance status, respectively\[3,13\]. Contrast-enhanced CT or MRI was used to evaluate cirrhosis, infiltrative tumor, MVI, EHD (consisted of lymph node and distant metastasis), number of tumors, lobar involvement and IHT.

**Treatment**

The technetium-99m macro aggregated albumin (Tc-99m MAA) pre-treatment scan was done to assess vascular anatomies and simulate Y-90 microspheres distribution in all procedures. TARE was usually performed within 1 mo after the Tc-99m MAA pre-treatment evaluation. Administration of Y-90 glass microspheres and sorafenib followed the manufacturer’s instructions for use and per the direction of the treating oncologist\[14,15\]. Dose of sorafenib was adjusted on the basis of patients’ tolerability and was reduced or withdrawn due to toxicity. Other treatments e.g., TACE, chemotherapy, immunotherapy, etc. were given at the time of progression at the discretion of the treating oncologist.

Treatment strategies were classified into 3 subgroups according to patients’ disease burden as assessed by IHT \((\leq 50\% \text{ vs } > 50\%)\) and ADFs \((\text{absence vs presence})\) and patients’ general conditions as considered by ECOG and underlying conditions at time of procedures: (1) **TARE_alone** was referred to TARE as a sole treatment in patients with IHT \( \leq 50\% \) and absence of ADFs; (2) **TARE_sorafenib** was a combination of TARE and sorafenib in patients with IHT \( > 50\% \) and/or presence of ADFs; and (3) **TARE_no_sorafenib** was TARE only treatment in patients who could not receive TARE_sorafenib due to contraindication or intolerance. All combined treatments were given concurrently or within a 1-month interval.

**Post-treatment evaluation**

Contrast-enhanced CT or MRI was obtained, usually within 2 mo after TARE and every 2-3 mo thereafter. All imaging studies were reinterpreted by a team, consisting of 4 interventional radiologists and 2 nuclear medicine physicians with diagnostic radiology training. All equivocal findings were determined by a consensus of 2 or more members in a team. The modified response evaluation criteria in solid tumors (mRECIST) was applied for response assessment\[16\].

Evaluation were performed in both intrahepatic and extrahepatic areas. Intrahepatic area was composed of treated area, referred to target lesions according to mRECIST and untreated area, referred to intrahepatic area outside treated area. Extrahepatic area was elsewhere outside the liver. Radiologic assessment was performed until initiation of new systemic treatment, last radiological follow-up, or patient’s death, whichever came first. If patients received additional treatment for residual tumor in treated area \( \text{e.g., TACE, radiofrequency ablation (RFA), surgical resection etc.} \), response assessment was not performed after these treatments.

**Treatment response and failure patterns**

Treatment response was referred to the best radiologic response at any time point during the evaluation period. Responses were categorized as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) following the mRECIST. ORR was a sum of CR and PR. DCR was a sum of CR, PR and SD. These responses were reported according to the assessed areas which were (1) Treated area; (2) Intrahepatic area, composed of treated and untreated areas; and (3) Overall, composed of intrahepatic and extrahepatic areas.

In case of progression, failure patterns were evaluated. The failure patterns in patients that progressed were classified into 3 categories according to the site of first progression: (1) Treated area; (2) Untreated area; and (3) Extrahepatic area. The first
instance of progression in treated area at any time during the follow-up period was classified into 5 categorized: (1) Development of new HCC; (2) Recurrence/increased enhancement of previously treated HCC; (3) Development of new MVI; (4) Progressive MVI; and (5) Mixed patterns. Progression in untreated area was defined as appearance of new lesion or progression of pre-existing untreated lesion.

Estimation of TTP was performed for: (1) Treated area; (2) Untreated area; and (3) Overall. TTP was defined as the time from TARE to the first unequivocal radiologic progression at pre-specified sites (treated and untreated areas) or at any site (overall). Deaths or loss follow-up were censored at time of last follow-up without radiologic evidence of progression. Analysis on prognostic factors for TTP of overall disease was performed only in major population subgroups, TARE_alone and TARE_sorafenib.

Statistical analysis
Baseline characteristics, response rate and patterns of disease progression were analyzed by using descriptive statistics. TTP and its 95% confidence interval (95%CI) were estimated by using Kaplan-Meier method and comparison between subgroups were done with log-rank test. The univariate analysis was performed using Kaplan-Meier method. The HR were calculated by using a Cox proportional hazard regression. Factors in the univariate analysis with \( P < 0.1 \) were further analyzed in a multivariate analysis using Cox proportional hazard model. In the multivariate analysis, statistically significant \( P \) value was set at 0.05. All statistical analyses were conducted using IBM SPSS Statistics software for Windows, version 21.0 (Armonk, NY: IBM Corp.).

RESULTS

Baseline patient characteristics
The median age at time of diagnosis was 66 years (range 17-85) with most patients being male (76.2%). In our study cohort, 80/151 (53%) patients received TARE as their first treatment, 46/151 (30.5%) patients received local treatments prior to TARE, and 25/151 (16.6%) patients received systemic treatments prior to TARE. Local treatments included surgical resection, trans-arterial embolization, TACE, TARE with Y-90 resin microspheres, RFA, and microwave ablation; while systemic treatment included targeted therapy, immunotherapy, and chemotherapy. Most patients (133/151, 88.1%) received single TARE treatments only, with 16/151 (10.6%) and 2/151 (1.3%) patients receiving two and three separate TARE procedures, respectively.

Patient and tumor characteristics at time of TARE
Patient and tumor characteristics at time of TARE procedure (\( n = 169 \)) stratified by treatments are shown on Table 1; the two most common treatments were TARE_alone (37.3%) and TARE_sorafenib (47.9%). Majority of patients had ECOG status either 0 (48.5%) or 1 (48.5%). While the TARE_alone subgroup had similar proportions of BCLC B and C (41.3% vs 49.2%), the remaining subgroups predominantly consisted of BCLC C patients (> 80%). Most patients were Child-Pugh A (92.9%) and presented with cirrhosis (69.2%) and multiple tumors (83.4%). While the TARE_alone subgroup had similar proportions of unilobar and bilobar disease (52.4% vs 47.6%), the remaining subgroups predominantly consisted of (= 70%) patients with bilobar disease.

TARE characteristics
TARE characteristic stratified by treatment are displayed on Table 2. TARE_alone procedures had the lowest median lung shunt fraction (4.6%), median lung dose (4.7 Gy) and median administered activity (1.7 GBq). In all subgroups, lobar treatment was the most common TARE approach (39.1%), followed by whole liver treatment (27.8%).

Best radiologic response
The median radiologic follow-up time was 4.4 mo (range 0.5-48.8). The best radiologic mRECIST response categorized by treatment are shown on Table 3. In the treated area, TARE_sorafenib subgroup had the highest ORR (53.1%), DCR (87.7%), and CR rate (11.1%), with TARE_alone subgroup having slightly lower ORR (41.3%) but similar DCR (85.7%). In the treated and intrahepatic areas, the two dominant response categories for TARE_alone and TARE_sorafenib were PR and SD, accounting for over 70% of all responses; the two dominant response categories for TARE_no_sorafenib were SD and PD, accounting for over 80%. The two highest overall DCRs were...
Table 1 Patient and tumor characteristics at time of transarterial radioembolization procedures

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All procedures (n = 169)</th>
<th>TARE_alone (n = 63)</th>
<th>TARE_sorafenib (n = 81)</th>
<th>TARE_no_sorafenib (n = 25)</th>
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<td>0</td>
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<td>40 (49.4)</td>
<td>10 (40)</td>
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<td>82 (48.5)</td>
<td>28 (44.4)</td>
<td>40 (49.4)</td>
<td>14 (56)</td>
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<td>2</td>
<td>4 (2.4)</td>
<td>2 (3.2)</td>
<td>1 (1.2)</td>
<td>1 (4)</td>
</tr>
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</tr>
<tr>
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<td>0</td>
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<tr>
<td>B</td>
<td>45 (26.6)</td>
<td>26 (41.3)</td>
<td>16 (19.8)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>C</td>
<td>118 (69.8)</td>
<td>31 (49.2)</td>
<td>65 (80.2)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>D</td>
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<td>Child-pugh class</td>
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<td>57 (90.5)</td>
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<td>B</td>
<td>12 (7.1)</td>
<td>6 (9.5)</td>
<td>3 (3.7)</td>
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<td>AFP&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>&lt; 400 ng/mL</td>
<td>118 (70.2)</td>
<td>54 (85.7)</td>
<td>52 (64.2)</td>
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<tr>
<td>≥ 400 ng/mL</td>
<td>50 (29.8)</td>
<td>9 (14.3)</td>
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<td>29 (35.8)</td>
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<td>Presence</td>
<td>117 (69.2)</td>
<td>48 (76.2)</td>
<td>52 (64.2)</td>
<td>17 (68)</td>
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<tr>
<td>Infiltrative tumor</td>
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</tr>
<tr>
<td>Absence</td>
<td>133 (78.7)</td>
<td>63 (100)</td>
<td>58 (71.6)</td>
<td>12 (48)</td>
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<td>Presence</td>
<td>36 (21.3)</td>
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<td>23 (28.4)</td>
<td>13 (52)</td>
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<tr>
<td>Vascular invasion&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>131 (78)</td>
<td>63 (100)</td>
<td>56 (70)</td>
<td>12 (48)</td>
</tr>
<tr>
<td>Presence</td>
<td>37 (22)</td>
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<td>24 (30)</td>
<td>13 (52)</td>
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<td>Extrahepatic disease</td>
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<tr>
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<td>60 (74.1)</td>
<td>14 (56)</td>
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<td>Presence</td>
<td>32 (18.9)</td>
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<td>21 (25.9)</td>
<td>11 (44)</td>
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<tr>
<td>Number of tumors</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>28 (16.6)</td>
<td>12 (19)</td>
<td>9 (11.1)</td>
<td>7 (28)</td>
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<tr>
<td>Multiple</td>
<td>141 (83.4)</td>
<td>51 (81)</td>
<td>72 (88.9)</td>
<td>18 (72)</td>
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<td>Lobar involvement</td>
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<tr>
<td>Unilobar</td>
<td>63 (37.3)</td>
<td>33 (52.4)</td>
<td>22 (27.2)</td>
<td>8 (32)</td>
</tr>
<tr>
<td>Bilobar</td>
<td>106 (62.7)</td>
<td>30 (47.6)</td>
<td>39 (72.8)</td>
<td>17 (68)</td>
</tr>
<tr>
<td>Intrahepatic tumor</td>
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<td></td>
</tr>
<tr>
<td>≤ 50%</td>
<td>116 (68.6)</td>
<td>63 (100)</td>
<td>37 (45.7)</td>
<td>16 (64)</td>
</tr>
<tr>
<td>&gt; 50%</td>
<td>53 (31.4)</td>
<td>0</td>
<td>44 (54.3)</td>
<td>9 (36)</td>
</tr>
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<td>TARE procedures</td>
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<td>151 (89.3)</td>
<td>56 (88.9)</td>
<td>71 (87.7)</td>
<td>24 (96)</td>
</tr>
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<td>16 (9.5)</td>
<td>7 (11.1)</td>
<td>8 (9.9)</td>
<td>1 (4)</td>
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<tr>
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<td>2 (1.2)</td>
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<td>2 (2.5)</td>
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</tr>
</tbody>
</table>
1Unavailable AFP in one TARE_no_sorafenib patient.
2Unavailable vascular invasion in one TARE_sorafenib patient.

Values represent number of procedures (%). TARE: Transarterial radioembolization; ECOG: Eastern cooperative oncology group; BCLC: Barcelona clinic liver cancer; AFP: Alpha-fetoprotein.

### Table 2 Characteristics of transarterial radioembolization procedures

<table>
<thead>
<tr>
<th></th>
<th>All procedures (n = 169)</th>
<th>TARE_alone (n = 63)</th>
<th>TARE_sorafenib (n = 81)</th>
<th>TARE_no_sorafenib (n = 25)</th>
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<tr>
<td>LSF, %</td>
<td>6.0 (0.8-30.4)</td>
<td>4.6 (1.0-26.4)</td>
<td>6.1 (0.8-30.4)</td>
<td>6.3 (2.0-13.6)</td>
</tr>
<tr>
<td>Lung mean dose, Gy²</td>
<td>8.2 (0.3-29.7)</td>
<td>4.7 (0.3-29.2)</td>
<td>10.1 (0.5-29.7)</td>
<td>11.2 (2.0-29.2)</td>
</tr>
<tr>
<td>Mean dose to treated liver volume, Gy¹</td>
<td>110 (80-135)</td>
<td>110 (80-135)</td>
<td>110 (80-135)</td>
<td>110 (80-135)</td>
</tr>
<tr>
<td>Interval between Tc-99m MAA and TARE, d¹</td>
<td>20 (0-125)</td>
<td>21 (0-125)</td>
<td>17 (0-44)</td>
<td>21 (10-34)</td>
</tr>
<tr>
<td>Administered activity, GBq</td>
<td>2.5 (0.3-8.1)</td>
<td>1.7 (0.3-6.3)</td>
<td>2.9 (0.6-8.1)</td>
<td>2.7 (0.8-5.9)</td>
</tr>
<tr>
<td>TARE approach, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole liver²</td>
<td>47 (27.8)</td>
<td>16 (25)</td>
<td>25 (31)</td>
<td>6 (24)</td>
</tr>
<tr>
<td>Lobar + segment</td>
<td>22 (13)</td>
<td>4 (6)</td>
<td>15 (18)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Lobar</td>
<td>66 (39.1)</td>
<td>23 (37)</td>
<td>30 (37)</td>
<td>13 (52)</td>
</tr>
<tr>
<td>Multiple segments</td>
<td>23 (13.6)</td>
<td>13 (21)</td>
<td>7 (9)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Single segment</td>
<td>11 (6.5)</td>
<td>7 (11)</td>
<td>4 (5)</td>
<td>0</td>
</tr>
</tbody>
</table>

1Mean absorbed doses values for each treatment session.
2The outliner interval of 125 d was from a single patient whose initial treatment plan was a whole liver treatment with sequential lobar infusion three weeks apart. His subsequent left lobar treatment was delayed for months because of his medical conditions. Administered activity of left lobar approach was calculated using the original Tc-99m MAA plan and re-evaluation CT scan performed prior to left lobar treatment.
3Consisted of single infusion (n = 19), separate infusion (n =24) and sequential infusion (n = 4).

Values represent median (range) unless otherwise stated. TARE: Transarterial radioembolization; Tc-99m MAA: The technetium-99m macro aggregated albumin.

observed in TARE_alone (79.4%) followed by TARE_sorafenib (71.6%) subgroups.

**Overall failure patterns**

Table 4 shows the overall failure patterns categorized by treatment. Disease progression were observed in 65.7% of all procedures. The lowest and highest rates of progression were noted in TARE_alone (57.1%) and TARE_no_sorafenib (72%) subgroups, respectively. The most common site of first disease progression was intrahepatic area for both TARE_alone (44.5%) and TARE_sorafenib procedures (38.4%). Extrahepatic progression (including both extrahepatic only and intrahepatic with extrahepatic) contributed to more than 30% cases in TARE_sorafenib (32%) and TARE_no_sorafenib (40%) subgroups, much higher than TARE_alone (12.7%) subgroup.

**Intrahepatic failure patterns**

Of total 169 procedures, intrahepatic progression was observed in 100 procedures (59.2%) with 75 procedures being progression in treated area (44.4%). Table 5 stratifies intrahepatic failure patterns of disease progression when intrahepatic progression was observed by treatment subgroups (n = 100). The progression rates in treated area of TARE_alone (67.6%) subgroup was lower than that of TARE_sorafenib (81.6%) and TARE_no_sorafenib (70.6%) subgroups. The two most common cause of disease progression in treated area across all subgroups were the development of new HCC (34%), followed by the recurrence/increased enhancement of previously treated HCC (20%). The progression rate of untreated area was highest (32.4%) and lowest (18.4%) in TARE_alone and TARE_sorafenib subgroup, respectively.

**TTP**

Median overall TTP of all procedures was 4.9 mo (95%CI: 3.9-5.9). TTP of treated area,
Table 3 Summary of the best radiologic response (modified response evaluation criteria in solid tumors) following transarterial radioembolization

<table>
<thead>
<tr>
<th>Treated area</th>
<th>All procedures (n = 169)</th>
<th>TARE_alone (n = 63)</th>
<th>TARE_sorafenib (n = 81)</th>
<th>TARE_no_sorafenib (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>12 (7.1)</td>
<td>2 (3.2)</td>
<td>9 (11.1)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>PR</td>
<td>61 (36.1)</td>
<td>24 (38.1)</td>
<td>34 (42)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>SD</td>
<td>66 (39.1)</td>
<td>28 (44.4)</td>
<td>28 (34.6)</td>
<td>10 (40)</td>
</tr>
<tr>
<td>PD</td>
<td>30 (17.8)</td>
<td>9 (14.3)</td>
<td>10 (12.3)</td>
<td>11 (44)</td>
</tr>
<tr>
<td>OR</td>
<td>73 (43.2)</td>
<td>26 (41.3)</td>
<td>43 (53.1)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>DC</td>
<td>139 (82.2)</td>
<td>54 (85.7)</td>
<td>71 (87.7)</td>
<td>14 (56)</td>
</tr>
<tr>
<td>Intrahepatic area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>12 (7.1)</td>
<td>2 (3.2)</td>
<td>9 (11.1)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>PR</td>
<td>58 (34.3)</td>
<td>22 (34.9)</td>
<td>33 (40.7)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>SD</td>
<td>59 (34.9)</td>
<td>26 (41.3)</td>
<td>26 (32.1)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>PD</td>
<td>40 (23.7)</td>
<td>13 (20.6)</td>
<td>13 (16)</td>
<td>14 (56)</td>
</tr>
<tr>
<td>OR</td>
<td>70 (41.4)</td>
<td>24 (38.1)</td>
<td>42 (51.9)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>DC</td>
<td>129 (76.3)</td>
<td>50 (79.4)</td>
<td>68 (84)</td>
<td>11 (44)</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>10 (5.9)</td>
<td>2 (3.2)</td>
<td>7 (8.6)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>PR</td>
<td>52 (30.8)</td>
<td>22 (34.9)</td>
<td>28 (34.6)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>SD</td>
<td>56 (33.1)</td>
<td>26 (41.3)</td>
<td>23 (28.4)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>PD</td>
<td>51 (30.2)</td>
<td>13 (20.6)</td>
<td>23 (28.4)</td>
<td>15 (60)</td>
</tr>
<tr>
<td>OR</td>
<td>62 (36.7)</td>
<td>24 (38.1)</td>
<td>35 (43.2)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>DC</td>
<td>107 (69.8)</td>
<td>50 (79.4)</td>
<td>58 (71.6)</td>
<td>10 (40)</td>
</tr>
</tbody>
</table>

Values represent number of procedures (%). Objective response consisted of complete response (CR) and partial response (PR). Disease control consisted of CR, PR, and stable disease. CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease; OR: Objective response; DC: Disease control; TARE: Transarterial radioembolization.

Untreated area and overall stratified by treatment subgroups are provided in Table 6. Amongst the 3 subgroups, median overall TTP for TARE_alone was highest at 8.6 mo followed by TARE_sorafenib at 5.1 mo and TARE_no_sorafenib at 2.7 mo.

**Prognostic factors of TTP**

The result of univariate and multivariate analysis of TTP of TARE_alone and TARE_sorafenib are provided in Table 7. None of the variables explored were found to be statistically significant prognostic factors for TTP in TARE_alone subgroup. Both child-pugh class and lobar involvement with $P < 0.1$, in both univariate and multivariate analysis, could be considered marginally significant factors. For TARE_sorafenib subgroups, univariate analysis showed ECOG, EHD, and IHT to be statistically significant prognostic factors for TTP, that compressed to a single factor of EHD in multivariate analysis with a $P < 0.001$ (Table 8).

Lobar involvement marginally stratified TTP duration (unilobar 11.0 mo; 95%CI: 5.0-17.0 vs bilobar 5.6 mo; 95%CI: 2.4-8.8, $P = 0.058$) for TARE_alone patients. Statistically significant differences in TTP duration of TARE_sorafenib procedures were noted when stratified by EHD (absent 7.5 mo; 95%CI: 4.9-10.0 vs present 2.8 mo; 95%CI: 2.6-3.1, $P = < 0.001$) and IHT ($\leq 50$ 7.7 mo; 95%CI: 5.1-10.3 vs $> 50$ 5.1 mo; 95%CI: 4.0-6.2, $P = 0.024$).
Table 4 Site of first progression in all cases of progression

<table>
<thead>
<tr>
<th></th>
<th>All procedures (n = 169)</th>
<th>TARE_alone (n = 63)</th>
<th>TARE_sorafenib (n = 81)</th>
<th>TARE_no_sorafenib (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No progression</td>
<td>58 (34.3)</td>
<td>27 (42.9)</td>
<td>24 (29.6)</td>
<td>7 (28)</td>
</tr>
<tr>
<td>Progression</td>
<td>111 (65.7)</td>
<td>36 (57.1)</td>
<td>57 (70.4)</td>
<td>18 (72)</td>
</tr>
<tr>
<td>Intrahepatic only</td>
<td>67 (39.6)</td>
<td>28 (44.5)</td>
<td>31 (38.4)</td>
<td>8 (32)</td>
</tr>
<tr>
<td>Treated area only</td>
<td>36 (21.3)</td>
<td>16 (25.4)</td>
<td>17 (21)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Untreated area only</td>
<td>20 (11.8)</td>
<td>10 (15.9)</td>
<td>6 (7.4)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Both treated and untreated areas</td>
<td>11 (6.5)</td>
<td>2 (3.2)</td>
<td>8 (9.9)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Extrahepatic only</td>
<td>17 (10.1)</td>
<td>3 (4.8)</td>
<td>13 (16)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Intra- and extrahepatic</td>
<td>27 (16)</td>
<td>5 (7.9)</td>
<td>13 (16)</td>
<td>9 (36)</td>
</tr>
</tbody>
</table>

Values represent number of procedures (%). TARE: Transarterial radioembolization.

Table 5 First pattern of intrahepatic progression in cases with intrahepatic progression

<table>
<thead>
<tr>
<th></th>
<th>All procedures (n = 100)</th>
<th>TARE_alone (n = 34)</th>
<th>TARE_sorafenib (n = 49)</th>
<th>TARE_no_sorafenib (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progression in treated area</td>
<td>75 (75)</td>
<td>23 (67.6)</td>
<td>40 (81.6)</td>
<td>12 (70.6)</td>
</tr>
<tr>
<td>New HCC</td>
<td>34 (34)</td>
<td>12 (35.3)</td>
<td>19 (38.8)</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>Recurrence/increased enhancement of previously treated HCC</td>
<td>20 (20)</td>
<td>5 (14.7)</td>
<td>9 (26.5)</td>
<td>6 (35.3)</td>
</tr>
<tr>
<td>With new MVI</td>
<td>8 (8)</td>
<td>2 (5.9)</td>
<td>6 (12.2)</td>
<td>0</td>
</tr>
<tr>
<td>With progressive MVI</td>
<td>3 (3)</td>
<td>0</td>
<td>3 (6.1)</td>
<td>0</td>
</tr>
<tr>
<td>With mixed patterns(^1)</td>
<td>10 (10)</td>
<td>4 (11.8)</td>
<td>3 (6.1)</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>Progression in untreated area</td>
<td>25 (25)</td>
<td>11 (32.4)</td>
<td>9 (18.4)</td>
<td>5 (29.4)</td>
</tr>
</tbody>
</table>

\(^1\)New HCC with one or more other patterns (n = 11) and new MVI with increased enhancement of previously treated HCC (n = 1). Values represent number of procedures (%). TARE: Transarterial radioembolization; HCC: Hepatocellular carcinoma; MVI: Macrovascular invasion.

DISCUSSION

TARE has been an increasing treatment option for unresectable HCC patients\(^2,5,6\). Treatment outcomes of TARE in the literatures varied considerably, depending on several factors such as the characteristics and stage of enrolled patients, and the experience and preferences of investigators with TARE\(^7-10,17-20\). In this study, we reported disease control and objective response with TARE for unresectable HCC per our institutional treatment algorithm which may include combination treatment with sorafenib based on two unique features: disease burden assessment by IHT and presence of ADFs. Pertinent findings in our study included development of new HCC tumors as a major intrahepatic failure pattern, disease progression in treated area and extrahepatic area as the most common overall disease failure patterns in TARE\(_{alone}\) and TARE\(_{sorafenib}\) procedures, respectively.

Our finding that 70% of treated lesions could achieve PR or SD was consistent with the previous studies\(^9,21\). Interestingly, the TARE\(_{sorafenib}\) subgroup provided the highest response rate (ORR 53.1% and CR 11.1%) followed by the TARE\(_{alone}\) subgroup (ORR 41.3% and CR 3.2%) which consisted of patients without ADFs or lower IHT. When comparing between subgroups with similar disease burden, DCR of TARE\(_{sorafenib}\) was much higher than TARE\(_{no_sorafenib}\) (87.7% vs 56%). Furthermore, median TTP duration of treated area for TARE\(_{sorafenib}\) was much longer than TARE\(_{no_sorafenib}\) (7.5 vs 3.6 mo). Acknowledging that antiangiogenic effect of sorafenib could promote oxygenation to the core of tumor and thereby increase tumor sensitivity to radiation\(^19,22,23\), we postulated that better disease control observed for TARE\(_{sorafenib}\) might be attributed to the beneficial effect of sorafenib.
Table 6 Time to progression

<table>
<thead>
<tr>
<th></th>
<th>All procedures (n = 169)</th>
<th>TARE_alone (n = 63)</th>
<th>TARE_sorafenib (n = 81)</th>
<th>TARE_no_sorafenib (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Censored patients</td>
<td>87</td>
<td>39</td>
<td>37</td>
<td>11</td>
</tr>
<tr>
<td>TTP, mo</td>
<td>7.8 (6.4-9.3)</td>
<td>12.3 (10.4-14.1)</td>
<td>7.5 (6.2-8.8)</td>
<td>3.6 (0.8-6.4)</td>
</tr>
<tr>
<td>Untreated area</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Censored patients</td>
<td>119</td>
<td>47</td>
<td>59</td>
<td>13</td>
</tr>
<tr>
<td>TTP, mo</td>
<td>12.8 (4.3-21.3)</td>
<td>22.9 (10.2-35.7)</td>
<td>11.9 (8.0-15.8)</td>
<td>3.6 (2.1-5.1)</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Censored patients</td>
<td>58</td>
<td>27</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>TTP, mo</td>
<td>4.9 (3.9-5.9)</td>
<td>8.6 (3.4-13.8)</td>
<td>5.1 (4.0-6.2)</td>
<td>2.7 (2.2-3.1)</td>
</tr>
</tbody>
</table>

TTP values represent median (95%CI) in months. TTP: Time to progression; TARE: Transarterial radioembolization.

It is noteworthy that in subgroups without sorafenib, TARE_alone and TARE_no_sorafenib, decrease of DCRs of treated area and intrahepatic area were 6.3 percentage points (from 85.7% to 79.4%) and 12 percentage points (from 56% to 44%), respectively. In the meantime, decrease of DCR of TARE_sorafenib was only 3.7 percentage points (from 87.7% to 84%). Given that intrahepatic area consisted of treated and untreated areas, disease progression in untreated area should make DCR of intrahepatic area lower than DCR of treated area. Thus, a less prominent change of DCR of TARE_sorafenib subgroup, compared to others might suggest that addition of sorafenib to TARE could reduce disease progression in untreated area and thereby provided a better intrahepatic control.

The most common intrahepatic failure patterns according to several studies, including this work was the development of new HCC, both in treated or untreated areas[9,21,24]. This might be explained by the hypothesis that newly detected HCC during follow-up might be pre-existing undetectable microscopic HCC. These lesions have generally less developed arterial blood supply compared to the macroscopic ones, and therefore, they do not achieve the tumoricidal dose from TARE. These small tumors might subsequently progress giving the impression of new HCC following TARE[21,24,25].

Regarding the patterns of disease progression, TARE_alone which had lowest disease burden was the only subgroup that the most common site of first disease progression was treated area (n = 16/36, 44.4%). Additionally, disease progression of TARE_alone were mostly limited in intrahepatic area (n = 28/36, 77.8%). Therefore, aggressive TARE based on advanced and personalized dosimetry with radiation dose to tumor exceeding tumoricidal threshold, around 200 Gy as claimed by several studies, might increase response of treated area[26,27]. We acknowledge that tumor specific dose estimates may further stratify tumor response status, but the retrospective calculation of tumor doses are beyond the scope of this work. Furthermore, cone-beam CT (CBCT) has been proven to demonstrate additional tumors overlooked by angiography and Tc-99m MAA scan[28]. Consequently, incorporating CBCT to treatment planning might be another way to improve intrahepatic control with TARE.

Rates of first disease progression in extrahepatic area of TARE_sorafenib (32%) and TARE_no_sorafenib (40%) subgroups were obviously higher than that of TARE_alone subgroup (12.7%). We hypothesized that this might be a result of higher baseline disease burden of these subgroups compared with TARE_alone (IHT > 50% and/or ADFs vs IHT ≤ 50% without ADFs). TARE_alone was also the only cohort without EHD whereas TARE_sorafenib and TARE_no_sorafenib had EHD in 25.9% and 44.4% cases, respectively. Considering TARE_sorafenib subgroup as an example, given that overall disease control was a consequence of both intrahepatic and extrahepatic control, a decrease of DCR, from 84% of intrahepatic area to 71.6% of overall could contemplate that extrahepatic progression occurred in a considerable number of TARE_sorafenib procedures (12.4%). Hence, enhancement of extrahepatic control by introducing a more potent systemic therapy might be a key of more effective treatment in this group of patients.
### Table 7 Univariate analysis of time to progression using Kaplan-Meier method

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>TARE_alone (n = 63)</th>
<th>TARE_sorafenib (n = 81)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n(^1)</td>
<td>c(^1)</td>
</tr>
<tr>
<td>ECOG(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32</td>
<td>13</td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>BCLC stage(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>26</td>
<td>9</td>
</tr>
<tr>
<td>C</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>Child-pugh class</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>57</td>
<td>24</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>AFP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 400 ng/mL</td>
<td>54</td>
<td>22</td>
</tr>
<tr>
<td>≥ 400 ng/mL</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Presence</td>
<td>48</td>
<td>21</td>
</tr>
<tr>
<td>Number of tumors</td>
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<td></td>
</tr>
<tr>
<td>Single</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Multiple</td>
<td>51</td>
<td>21</td>
</tr>
<tr>
<td>Lobar involvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilobar</td>
<td>33</td>
<td>16</td>
</tr>
<tr>
<td>Bilobar</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>Infiltrative tumor(^4)</td>
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<td></td>
</tr>
<tr>
<td>Absence</td>
<td>58</td>
<td>15</td>
</tr>
<tr>
<td>Presence</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>MVI(^4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>56</td>
<td>12</td>
</tr>
<tr>
<td>Presence</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>EHD(^4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>60</td>
<td>23</td>
</tr>
<tr>
<td>Presence</td>
<td>21</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^1\)Total patients (n) and censored patients (c).

\(^2\)ECOG 2 (n = 0 TARE\_alone, n = 1 TARE\_sorafenib) and ECOG 3 (n = 0 TARE\_alone, n = 0 TARE\_sorafenib) excluded.

\(^3\)BCLC A (n = 5 TARE\_alone, n = 0 TARE\_sorafenib) and BCLC D (n = 1 TARE\_alone, n = 0 TARE\_sorafenib) excluded.

\(^4\)Absent in all TARE\_alone procedures according to institutional treatment algorithm.

Hazard ratios (HR) with Cox proportional hazard regression and P value with log-rank test. TARE: Transarterial radioembolization; ECOG: Eastern cooperative oncology group; BCLC: Barcelona clinic liver cancer; AFP: Alpha-fetoprotein; MVI: Macrovascular invasion; EHD: Extrahepatic disease; IHT: Intrahepatic tumor.
Table 8 Multivariate analysis of time to progression using Cox proportional hazard model

<table>
<thead>
<tr>
<th>Population</th>
<th>Prognostic factors</th>
<th>n</th>
<th>c</th>
<th>HR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TARE_alone (n = 63)</td>
<td>Child-pugh class</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>57</td>
<td>24</td>
<td>0.32 (0.09-1.10)</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobar involvement</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unilobar</td>
<td>33</td>
<td>16</td>
<td>0.51 (0.26-1.00)</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>Bilobar</td>
<td>30</td>
<td>11</td>
<td></td>
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</tr>
<tr>
<td>TARE_sorafenib (n = 81)</td>
<td>ECOG2</td>
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</tr>
<tr>
<td></td>
<td>0</td>
<td>40</td>
<td>8</td>
<td>0.85 (0.59-1.22)</td>
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</tr>
<tr>
<td></td>
<td>1</td>
<td>40</td>
<td>16</td>
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</tr>
<tr>
<td>MVI</td>
<td>Absence</td>
<td>56</td>
<td>12</td>
<td>1.15 (0.74-1.80)</td>
<td>0.532</td>
</tr>
<tr>
<td></td>
<td>Presence</td>
<td>24</td>
<td>11</td>
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</tr>
<tr>
<td>EHD</td>
<td>Absence</td>
<td>60</td>
<td>23</td>
<td>0.37 (0.24-0.56)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Presence</td>
<td>21</td>
<td>1</td>
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<tr>
<td>IHT</td>
<td>≤ 50%</td>
<td>37</td>
<td>15</td>
<td>0.72 (0.49-1.06)</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td>&gt; 50%</td>
<td>44</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Total patients (n) and censored patients (c).
2ECOG 2 (n = 1) and ECOG 3 (n = 0) excluded.

TARE: Transarterial radioembolization; ECOG: Eastern cooperative oncology group; MVI: Macrovascular invasion; EHD: Extrahepatic disease; IHT: Intrahepatic tumor.

In a prospective study on efficacy of TARE in unresectable HCC patients with IHT ≤ 50%, variables affecting TTP were tumor diameter (> 6 cm vs ≤ 6 cm, HR 3.65; 95%CI: 1.39-9.59, P = 0.0087) and treatment response according to European Association for the study of the liver (PD vs CR + PR + SD, HR 22.48; 95%CI: 4.53-111.61, P = 0.0001) [7]. Nevertheless, there was no prognostic factor of TTP for TARE_alone subgroup in our study. We presumed that our institutional selection criteria for TARE_alone, IHT ≤ 50% and absence of EHD, MVI and infiltrative/ill-defined HCC probably made this group of patients had relatively low disease burden. TARE_alone was rather effective for all, therefore, minor differences in baseline characteristics might not affect the duration of TTP.

EHD at time of procedure (absence vs presence; HR 0.37; 95%CI: 0.24-0.56, P < 0.001) was a sole prognostic factor of TTP for TARE_sorafenib. A quarter of TARE_sorafenib procedures also had EHD before treatment (25.9%) and 32% of progression of TARE_sorafenib subgroup was extrahepatic area first. All of these findings supported a significance of pre-existing EHD on disease control. Taking all of these findings together, TARE_sorafenib for patient with pre-existing EHD might be inadequate. More aggressive treatment such as TARE with other novel agents should be considered for future clinical trial.

In this study, we demonstrated a correlation between disease burden, given treatment and disease control. Moreover, we successfully identified some unique failure patterns which could guide possible ways to provide a better disease control. To the best of our knowledge, the current study was one of very few studies addressing this kind of issues. Our outcome measurements, TTP and tumor response were both direct parameters reflecting efficacy of treatment[8,29]. Additionally, treatment response of all procedures were re-assessed by using the mRECIST. Anti-tumor effects of TARE and sorafenib might not result in tumor shrinkage but they could produce tumor necrosis[30,31]. As a result, assessment on the basis of enhancement like mRECIST was more appropriate to our study than size-based.
evaluation of response evaluation criteria in solid tumors.

Several limitations of our study related to natures of retrospective study. First, we acknowledged that mixed imaging techniques for evaluation of treatment response (CT \(n = 102, 60.4\%)\) and MRI \(n = 67, 39.6\%)\) might produce some heterogeneities in diagnostic performance. Second, median radiologic follow-up duration was only 4.4 mo. This period was rather short because many patients that were referred for TARE at our institution had only 1 imaging follow-up study at our institution. Furthermore, all of 3 post-treatment imaging studies done within the first month after TARE showed rapid disease progression, either in treated area (TARE\_sorafenib \(n = 1\)) or extrahepatic area (TARE\_no\_sorafenib \(n = 2\)). Lastly, number of patients in TARE\_no\_sorafenib subgroup was too limited to be statistically meaningful.

In the present study, we found that disease progression in TARE\_alone subgroup usually limited to intrahepatic area and majority of progression originated in treated area. Therefore, either local or systemic treatment which promotes disease control at treated area might lead to better overall disease control. In contrast, disease progression in TARE\_sorafenib subgroup tends to be extrahepatic and pre-existing EHD could worsen disease control. Study on using of TARE in combination with novel systemic therapy that is more potent than sorafenib might be required to improve treatment outcome.

**CONCLUSION**

TARE\_alone for procedures with IHT \(\leq 50\%)\) and absence of ADFs and TARE\_sorafenib for procedures with IHD > 50\% and/or presence of ADFs could provide acceptable disease control of approximately 70\% in unresectable HCC patients. Intrahepatic progression was the most common failure pattern in both subgroups but extrahepatic progression was far more common in TARE\_sorafenib. Strategies that improve intrahepatic control for liver-only disease (dosimetry-based TARE) and extrahepatic control for metastatic disease (additional systemic therapy) could improve TARE outcome for HCC patients.

**ARTICLE HIGHLIGHTS**

**Research background**

Survival outcome of unresectable hepatocellular carcinoma (HCC) patients post yttrium-90 (Y-90) glass microspheres transarterial radioembolization (TARE) with/without sorafenib according to individual’s disease burden might partly be confounded by subsequent treatments. Therefore, a study on tumor response might better represent effectiveness of TARE with/without sorafenib.

**Research motivation**

Disease control and failure patterns following TARE with/without sorafenib might suggest how to intensify treatment to improve treatment outcome.

**Research objectives**

This study describes the disease control and failure patterns of unresectable HCC patients who underwent Y-90 microspheres TARE with/without sorafenib according to individuals’ disease burden, i.e., intrahepatic tumor (IHT) and adverse disease features (ADFs), consisting of macrovascular invasion, extrahepatic disease (EHD) and infiltrative/ill-defined HCC.

**Research methods**

Y-90 microspheres TARE procedures with available pre and post-treatment imaging studies \((n = 169)\) were retrospectively reviewed and categorized into 3 subgroups on the basis of treatment given and individuals’ disease conditions: (1) TARE\_alone, referred to TARE only for IHT \(\leq 50\%)\) without ADFs \((n = 63)\); (2) TARE\_sorafenib, referred to TARE with sorafenib for IHT > 50\% and/or presence of ADFs \((n = 81)\); and (3) TARE\_no\_sorafenib, referred to TARE only for patients with contraindication to sorafenib or side effect intolerance \((n = 25)\). Disease control rate (DCR; consisted of complete response, partial response and stable disease) and failure patterns of treated, intrahepatic and extrahepatic sites were assessed using mRECIST.
Research results
The key findings were that TARE\textsubscript{alone} for procedures with IHT ≤ 50% and absence of ADFs and TARE\textsubscript{sorafenib} for procedures with IHT > 50% and/or presence of ADFs could provide comparable DCR (79% vs 72%) with similar incidence of intrahepatic progression (44.5% vs 38.5%). However, extrahepatic progression was much more common in TARE\textsubscript{sorafenib} procedures (13% vs 32%).

Research conclusions
DCR of TARE\textsubscript{alone} and TARE\textsubscript{sorafenib} procedures were similar (about 70%). Intrahepatic progression was dominant failure pattern for both (about 40%) but extrahepatic progression was far more common in TARE\textsubscript{sorafenib} procedures.

Research perspectives
On the basis of findings in the present study, we suggested further investigations on novel systemic therapy that is more potent than sorafenib might be required to improve treatment outcome in this group of patients.

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Observational Study

Real-world local recurrence rate after cold polypectomy in colorectal polyps less than 10 mm using propensity score matching

Masashi Saito, Takeshi Yamamura, Masanao Nakamura, Keiko Maeda, Tsunaki Sawada, Eri Ishikawa, Yasuyuki Mizutani, Takuya Ishikawa, Naomi Kakushima, Kazuhiro Furukawa, Eizaburo Ohno, Hiroki Kawashima, Masatoshi Ishigami, Mitsuhiro Fujishiro

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Author contributions: Saito M and Yamamura T were the guarantors and designed the study; Saito M, Yamamura T, Nakamura M, Kawashima H, and Ishikawa T participated in the acquisition, analysis, and interpretation of the data; Saito M drafted the initial manuscript; Ohno E, Yamamura T, Maeda K, Sawada T, Ishikawa E, Mizutani Y, Kakushima N, and Furukawa K revised the article critically for important intellectual content; Yamamura T and Nakamura M contributed to statistical analysis; Fujishiro M made final approval of the article; all authors have read and approved the final manuscript.

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Abstract

BACKGROUND

Cold polypectomy (CP) is a simple and safe procedure for polyps less than 10 mm in size; however, there is concern about local recurrence following CP because of unidentified margins of excised specimens and the lack of tumor suppression effect by coagulation. Some clinical trials have evaluated local persistent recurrence; their results suggest that a higher rate of local recurrence has not been documented so far. There were few reports that observed the course over long periods of time after CP in clinical practice.

AIM

To evaluate the presence of local recurrence following CP and hot polypectomy (HP) using propensity score matching.

METHODS

We analyzed 275 patients who underwent polypectomy for non-pedunculated colorectal polyps less than 10 mm (959 Lesions) between October 2016 and 2017 and underwent follow-up endoscopy subsequently. We divided them into the CP group (706 Lesions), wherein CP was performed, and the HP group (253 Lesions), wherein HP was performed. Using propensity score matching, we extracted 215 Lesions in each group and evaluated the local recurrence and content of CP in the real clinic and adverse events using medical records.
RESULTS

After propensity score matching, there were no significant differences in the patients’ and their endoscopic background (age, use of antithrombotics, indications, size, morphology, location of polyps, and polypectomy device) between the groups. The mean duration between colorectal polypectomy and the next follow-up colonoscopy was 17.5 ± 7.1 (range, 6-39) mo in the CP group and 15.7 ± 6.0 (range, 6-35) mo in the HP group, which was significantly longer in the CP group (P = 0.005). The local recurrence rate was 0.93% in the CP group and 0.93% in the HP group, without a significant difference (P = 0.688). Additionally, no differences were observed in the macroscopic en bloc resection rate, histopathological complete resection rate, and pathological results between the groups. Adverse events did not occur in either group.

CONCLUSION

Local recurrence after CP was equivalent to that following HP in clinical practice. CP is useful and safe in the treatment of non-pedunculated polyps of less than 10 mm.

Key Words: Cold polypectomy; Colorectal polyp; Hot polypectomy; Local recurrence; Safety; Propensity score matching

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INTRODUCTION

It has been reported that the mortality of patients with colorectal cancer decreases with the resection of all adenomatous polyps (clean colon)[1]; therefore, it has become desirable to resect even diminutive polyps. There are reports which state that ≥ 90% of polyps are less than 10 mm on colonoscopic examination, with 70%-80% being less than 5 mm[2-4]. According to these reports, it is important to decide how to efficiently and safely remove such diminutive polyps for a clean colon. Cold polypectomy (CP) has been used in the treatment of small colorectal polyps because of the suitable efficiency and safe outcomes[5-8]. It has been reported that CP is an easy-to-perform technique. The frequency of adverse events such as delayed bleeding and perforation is lower with CP than those with hot polypectomy (HP) because it avoids electrocoagulation; furthermore, it has been considered useful in the endoscopic resection of sub-centimeter polyps. However, the presence of the lesions with unknown margins in resection specimens, has been reported in as high as 40% of the cases in cold snare polypectomy (CSP); therefore, there is concern about local recurrence after the treatment[9]. Another concern is the increased risk of local recurrence after CP compared to that after HP because the tumor suppression effect may not be expected by electrocoagulation in the resection sites following CP. One randomized trial reported that the ratio of local recurrence after treatment was equivalent between CSP and hot snare polypectomy (HSP)[10]. Another report indicated that the ratio...
immediately after CSP is significantly higher than that after endoscopic mucosal resection (EMR)[11]. However, these reports were all evaluations of local recurrence just after endoscopic resection and did not confirm it after a certain period of time. Therefore, in this study, we assessed the presence of local recurrence following CP and HP after a long period following polypectomy in real clinical practice.

MATERIALS AND METHODS

Study design
This real-world retrospective study was conducted at the Nagoya University Hospital. The inclusion criteria of the CP procedure in Nagoya University Hospital is for non-pedunculated polyps only, less than 10 mm, and diagnosed as Type 2A in the Japan Narrow band imaging Expert Team (JNET) classification[12] using imaged-enhanced endoscopy with magnification, in short, suspected adenomatous lesions. As the size of the polyp increases, the rate of advanced neoplasia (with villous or tubulovillous adenoma components, size ≥ 10 mm, and high-grade dysplasia) also increases[3]. Therefore, a polyp > 10 mm is an indication for EMR. In pedunculated lesions, a large blood vessel is often found in the stem, which may be difficult to resect using CP; this might result in a very high risk of bleeding after resection. Therefore, we excluded them in the inclusion criteria of CP[13-15].

Using clinical records, we extracted data of 612 patients (2619 Lesions) who had undergone polypectomy at the Nagoya University Hospital between October 2016 and October 2017. Of them, data from 313 patients (1449 Lesions) who underwent follow-up colonoscopy more than half a year after the first polypectomy were extracted. We excluded data from 16 patients (303 Lesions) who were diagnosed with polyposis (familial adenomatous polyposis, Peutz–Jeghers syndrome, or other hereditary polyposis syndromes) or inflammatory bowel disease and 187 Lesions (22 patients) which were diagnosed with JNET Type 2B and lesions out of the inclusion criteria for CP such as size ≥ 10 mm and pedunculated or depressed lesions. Finally, we identified 959 Lesions (275 patients), which were divided into the HP (253 Lesions) and CP groups (706 Lesions).

The mean size of the lesions was significantly smaller in the CP group (CP group: 3.82 ± 1.49 mm; HP group: 5.35 ± 1.77 mm; P < 0.001), the ratio of flat lesions was significantly higher in the CP group (CP group: 62.0%; HP group: 43.5%; P < 0.001), and the resection ratio with the snare was significantly higher in the HP group (CP group: 86.5%; HP group: 99.2%; P < 0.001) (Table 1). It has been reported that the recurrence rate after polypectomy increases as the size of the lesion increases. Regarding the morphology of the lesion, the morphological difference might affect the treatment method (CP or HP). For polypectomy devices, the biopsy forceps has been reported to have a lower complete resection rate than the snare, which may affect recurrence rates. Therefore, to adjust for the bias between both groups, we performed propensity score matching based on the size and morphology (sessile or flat) of the lesions and the polypectomy device (biopsy forceps or snare), which could have an influence on the local recurrence. The CP (215 Lesions) and HP groups (215 Lesions) (total 206 patients) were compared after propensity score matching (Figure 1).

The evaluation items included local recurrence, histological complete resection rate, delayed bleeding, and perforation. We defined local recurrence as a polyp > 10 mm is an indication for EMR. In pedunculated lesions, a large blood vessel is often found in the stem, which may be difficult to resect using CP; this might result in a very high risk of bleeding after resection. Therefore, we excluded them in the inclusion criteria of CP[13-15].

The evaluation items included local recurrence, histological complete resection rate, delayed bleeding, and perforation. We defined local recurrence as a polyp on the post-polypectomy scar and delayed bleeding as bleeding requiring endoscopic hemostasis treatment within two weeks of the polypectomy. For identification of the resected lesion, we referred to the post-polypectomy scar and the scope insertion length from the anal verge to the lesion as described in the patient’s previous colonoscopy report.

Procedures
The instruments used in this study included XL-4450/LL-4450 (light source), VP-4450HD (processor), EC-L590ZW/EC-600ZP (scope) (Fujifilm Co., Tokyo, Japan) and CLV-290SL (light source), CV-290 (processor), and CF-H260AZI/CF-HQ290I (scope) (Olympus Co., Tokyo, Japan). The participating physicians were 22 expert endoscopists who had each performed > 1000 colonoscopies, including polypectomies. The snare used in this study included Snare Master 15 mm (Olympus Co., Tokyo, Japan), Profile 11 mm/13 mm and Captivator II 10 mm (Boston Scientific Co., Boston, MA, United States) in both groups. Additionally, as the biopsy forceps, Radial Jaw 4 JUMBO in the CP group and Radial Jaw 4 in the HP group (Boston Scientific Co., Boston, MA, United States) were used. In principle, the biopsy forceps were used for lesions < 4 mm, while the snare was used for lesions ≥ 4 mm because the histological
Table 1 Characteristics of excised polyps before propensity score matching

<table>
<thead>
<tr>
<th></th>
<th>CP, mean ± SD or n (%)</th>
<th>HP, mean ± SD or n (%)</th>
<th>P-value</th>
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<tr>
<td>Number of polyps resected</td>
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<td>253</td>
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<tr>
<td>Size, mm</td>
<td>3.82 ± 1.49</td>
<td>5.35 ± 1.77</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Morphology</td>
<td>0.439&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Sessile</td>
<td>268 (38.0)</td>
<td>143 (56.5)</td>
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<td>Flat</td>
<td>438 (62.0)</td>
<td>110 (43.5)</td>
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</tr>
<tr>
<td>Location</td>
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<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>46 (6.5)</td>
<td>15 (5.9)</td>
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</tr>
<tr>
<td>Ascending colon</td>
<td>182 (25.8)</td>
<td>59 (23.3)</td>
<td></td>
</tr>
<tr>
<td>Transverse colon</td>
<td>220 (31.2)</td>
<td>58 (22.9)</td>
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</tr>
<tr>
<td>Descending colon</td>
<td>92 (13.0)</td>
<td>33 (13.0)</td>
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<tr>
<td>Sigmoid colon</td>
<td>128 (18.1)</td>
<td>67 (26.5)</td>
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</tr>
<tr>
<td>Rectum</td>
<td>38 (5.4)</td>
<td>21 (8.3)</td>
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<tr>
<td>Polypectomy device</td>
<td></td>
<td></td>
<td>&lt; 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Snare</td>
<td>611 (86.5)</td>
<td>251 (99.2)</td>
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</tr>
<tr>
<td>Biopsy forceps</td>
<td>95 (13.5)</td>
<td>2 (0.8)</td>
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</tr>
</tbody>
</table>

<sup>a</sup>Welch test.  
<sup>b</sup>Chi-Square test.  
CP: Cold polypectomy; HP: Hot polypectomy.

Figure 1 Flowchart of the study.

The complete resection rate for lesions ≥ 4 mm is lower with cold forceps polypectomy (CFP) [16]. The physicians decided whether to use the biopsy forceps or the snare. We determined the size of the lesion based on the outer diameter of the tip cup diameter of the biopsy forceps or the snare.
**Statistical analysis**

JMP v15 (SAS Institute, Cary NC, United States) was used for propensity score matching, and SPSS v24.0 (IBM, Armonk, New York, United States) was used for statistical analysis in this study. We used the chi-square test to compare the morphology, location of lesions, polypectomy device (before propensity score matching), pathological diagnosis, histopathology results, and histological complete resection rate between the groups. Student’s t-test was used to compare the mean size of lesions (after propensity score matching) and the mean follow-up period. Welch test was used to compare the mean size of lesions (before propensity score matching), and Fisher’s exact test was used to compare the polypectomy device (after propensity score matching), tissue retrieval rate, macroscopic en bloc resection rate, acute bleeding rate, and local recurrence. Statistical significance was set at \( P < 0.05 \).

**RESULTS**

**Characteristics of patients and lesions after propensity score matching**

In the 206 patients (139 men and 67 women) included in the study, the mean age was 68.7 ± 8.63 years, the use of antithrombotics was 19.4%, and the indications for colonoscopy included screening (\( n = 186 \)), constipation (\( n = 9 \)), abdominal pain (\( n = 6 \)), anemia (\( n = 2 \)), and bloody stools (\( n = 2 \)) (Table 2).

Regarding the excised polyps, the mean size was 4.95 ± 1.60 mm in the CP group and 4.94 ± 1.58 mm in the HP group. The morphology included 111 sessile lesions (51.6%) and 104 flat lesions (48.4%) in the CP group, and 113 sessile lesions (52.6%) and 102 flat lesions (47.4%) in the HP group. The lesion locations in the CP and HP groups included the cecum (6.0% and 7.0%, respectively), ascending colon (25.1% and 23.7%, respectively), transverse colon (33.0% and 22.3%, respectively), descending colon (12.6% and 12.1%, respectively), sigmoid colon (19.1% and 25.6%, respectively), and the rectum (4.2% and 9.3%, respectively). In both groups, 99.1% of the procedures were performed using a snare and 0.9% using biopsy forceps. There were no significant differences in the mean size, morphology, location, or polypectomy device between the groups.

Regarding the subsequent pathological diagnosis of the lesions, the difference was not statistically significant between the groups (\( P = 0.117 \)): 186 Lesions with low-grade adenoma (88.6%), seven lesions with advanced neoplasia (high-grade dysplasia or lesions including villous or tubulovillous adenoma components) (3.3%) in the CP group vs 168 Lesions with low-grade adenoma (79.2%), 14 Lesions with advanced neoplasia (6.6%) in the HP group (Table 3).

**Treatment outcomes and complications**

The macroscopic en bloc resection rate was 99.1% (213 Lesions) in the CP group and 98.1% (211 Lesions) in the HP group; however, the difference was not significant (\( P = 0.343 \)). The number of lesions with low-grade adenoma and advanced neoplasia whose margins were evaluated pathologically was 183 in the CP group and 181 in the HP group. The rate of histological complete resection was 82.5% (151 Lesions) in the CP group and 84.0% (152 Lesions) in the HP group, and no significant difference was identified between the groups (\( P = 0.708 \)). Acute bleeding was observed in six (2.8%) lesions in the CP group and three (1.4%) lesions in the HP group; it tended to be more common in the CP group, but the difference was not statistically significant (\( P = 0.252 \)). Delayed bleeding or perforation was not observed in either group (Table 4).

**Follow-up colonoscopy after polypectomy**

The mean duration between colorectal polypectomy and the next follow-up colonoscopy was 17.5 ± 7.1 (range, 6-39) mo in the CP group and 15.7 ± 6.0 (range, 6-35) mo in the HP group, which was significantly longer in the CP group (\( P = 0.005 \)). Local recurrence was observed in two (0.93%) lesions in both groups with no significant difference between them (\( P = 0.688 \)) (Table 5; Figures 2 and 3).

**DISCUSSION**

CP is a safe treatment with a simple procedure and few complications. However, compared to HP, it may be difficult to evaluate whether or not complete resection is possible pathologically, and there is a concern that the risk of local recurrence may...
increase because there is no tumor suppression effect by electrocoagulation. Some facilities are cautious about its adaptation. In this study, we focused on the local recurrence rate and retrospectively analyzed lesions that could be followed up with an endoscope for a relatively long period of time in real clinical practice. From the results of this study, it was considered that there is no difference in recurrence rate between CP and HP in non-pedunculated colorectal polyps smaller than 10 mm, and CP can be selected as one of the useful treatment methods for small colorectal polyps.

Endoscopic resection of colorectal polyps is one of the common treatments in digestive endoscopy, and resection of all adenomatous polyps, including diminutive lesions, is expected to become increasingly important to decrease the morbidity and mortality of colorectal cancer. Delayed bleeding or perforation, as complications of polypectomy, may require re-admission to the hospital along with additional endoscopy to stop the bleeding, blood transfusion, or surgery if necessary, which is not only a burden to the patient but also to the medical staff and the economy. The incidence of delayed bleeding and perforation in conventional HP has been reported to be 0.26%-1.4% and 0.017%-0.091% [17]. However, polypectomy is routinely performed in many patients, and the adverse events should never be ignored, even if their frequency is low. It has been reported that CP has a lower risk of complications compared with HP [6,16,18,19]. However, the long-term risk of residual recurrence after CP in clinical practice has not yet been sufficiently investigated.

It is important to visually confirm that there are no residual lesions following polypectomy; however, since CP does not have a burn effect like HP does, there is a risk of recurrence if there are residual lesions that cannot be detected visually after the treatment. There are three methods for examining the presence of remnants after polypectomy. First, histopathological evaluation of the resected specimen is performed to confirm whether complete resection was achieved. In CP, specimen damage due to aspiration and collection of specimens is more likely to occur than in HP; it has been reported that pathological resection margins are more frequently unknown in CP [18, 19]. Second, biopsy of the resected ulcer margins or mucosal resection is performed to confirm any remnants histologically. It has been reported that the resected region is resected again with a snare or biopsied with forceps immediately after polypectomy and histopathologically evaluated for the presence of remnants. The residual rate has been reported to be 3.4% in CSP and 17.4% in CFP by Kim et al [20], 10% in CSP and 11% in CFP by Gómez et al [21], 3.9% in CSP by Matsuura et al [22], and 1.8% in CSP and 2.6% in HSP by Kawamura et al [10]. The rates varied slightly between these reports. However, lesions left at the margins immediately after resection may fall off later (especially in HP, due to the effects of electrical coagulation). It remains unclear whether they will eventually become residual recurrent lesions. Third, endoscopic confirmation of the polyp resection site is repeated after a certain duration. This is a reliable assessment of residual recurrence but includes some hurdles. First, the patient must undergo a follow-up colonoscopy, which can be physically burdensome. Additionally, because of the sufficient follow-up period, the resection sites become scars, and the scar after CP is more obscured than that after HP, which may make it
<table>
<thead>
<tr>
<th>Table 3 Characteristics of excised polyps after propensity score matching</th>
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<tbody>
<tr>
<td><strong>CP, mean ± SD or n (%)</strong></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Number of polyps excised</td>
</tr>
<tr>
<td>Size, mm</td>
</tr>
<tr>
<td>Morphology</td>
</tr>
<tr>
<td>Sessile</td>
</tr>
<tr>
<td>Flat</td>
</tr>
<tr>
<td>Location</td>
</tr>
<tr>
<td>Cecum</td>
</tr>
<tr>
<td>Ascending colon</td>
</tr>
<tr>
<td>Transverse colon</td>
</tr>
<tr>
<td>Descending colon</td>
</tr>
<tr>
<td>Sigmoid colon</td>
</tr>
<tr>
<td>Rectum</td>
</tr>
<tr>
<td>Polypectomy device</td>
</tr>
<tr>
<td>Snare</td>
</tr>
<tr>
<td>Biopsy forceps</td>
</tr>
<tr>
<td>Pathologic diagnosis&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low-grade adenoma</td>
</tr>
<tr>
<td>Advanced neoplasia&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hyperplastic polyp and SSL</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Failure of tissue retrieval</td>
</tr>
</tbody>
</table>

1Indicates polyps that were retrieved successfully and evaluated pathologically.
2Defined as high-grade dysplasia or the lesions including villous or tubulovillous adenoma component.
3Student’s t-test.
4Chi-Square test.
5Fisher’s Exact test.
CP: Cold polypectomy; HP: Hot polypectomy; SSL: Sessile serrated lesion.

difficult to identify the regions of post-polypectomy. Lee et al.[23] reported that the overall recurrence over 59.7 mo was 17% (4% definite recurrence and 13% probable recurrence) after CFP in 1111 diminutive polyps. This recurrence rate is much higher than that reported in other studies. Probable recurrence was defined as recurrence at a similar distance from the anal verge (± 3 cm) in the same colorectal segment as a previous polyp and accounted for the majority of all recurrences. These lesions may be indistinguishable from newly formed polyps or previously overlooked polyps.

Murakami et al.[24] reported that recurrence was observed in 1.4% of lesions less than 10 mm and 5.4% of lesions of 10-14 mm in follow-up colonoscopy more than 10 mo after CSP. If the scar was unclear, they were observed by going back and forth multiple times across segments estimated by the distance from the anal verge. When there were no new polyps after such cautious colonoscopy, the patient was considered to have no recurrence. The frequency of detection of scars was not reported; however, it appears to be an acceptable method in actual clinical practice. Maruoka et al.[25] reported that clipping was performed in the vicinity of the ulcer after CSP, and colonoscopy was repeated three weeks later. After the scar was identified using the clip as a guide, the scar area was biopsied to evaluate the remnants. They indicated that the recurrence rate using the above method was 0.98%. Since the clip would naturally drop off after a certain period, it appeared to be the limit of the period that can be evaluated using this method.

In this study, follow-up colonoscopy was performed after 6-39 mo (average of 17.5 mo in the CP group and 16.2 mo in the HP group) after the treatment. We adopted an
Table 4 Treatment outcomes and complications

<table>
<thead>
<tr>
<th></th>
<th>CP, n (%)</th>
<th>HP, n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 215</td>
<td>n = 215</td>
<td></td>
</tr>
<tr>
<td>Macroscopic en bloc resection</td>
<td>213 (99.1)</td>
<td>211 (98.1)</td>
<td>0.343*</td>
</tr>
<tr>
<td>Tissue retrieval successfully</td>
<td>210 (97.7)</td>
<td>212 (98.6)</td>
<td>0.362*</td>
</tr>
<tr>
<td>Snare polypectomy</td>
<td>208/213 (97.7)</td>
<td>210/213 (98.6)</td>
<td></td>
</tr>
<tr>
<td>Biopsy forceps polypectomy</td>
<td>2/2 (100)</td>
<td>2/2 (100)</td>
<td></td>
</tr>
<tr>
<td>Lesions diagnosed with low-grade adenoma or advanced lesion and evaluated for histological margin</td>
<td>n = 183</td>
<td>n = 181</td>
<td></td>
</tr>
<tr>
<td>Histological complete resection</td>
<td>151 (82.5)</td>
<td>152 (84.0)</td>
<td>0.708b</td>
</tr>
<tr>
<td>Complications (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute bleeding†</td>
<td>6 (2.8)</td>
<td>3 (1.4)</td>
<td>0.252a</td>
</tr>
<tr>
<td>Delayed bleeding‡</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>Perforation</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
</tbody>
</table>

1Indicates bleeding continued for 30 seconds immediately after polypectomy.
2Indicates bleeding requiring endoscopic hemostasis within two weeks after polypectomy.
3Fisher’s Exact test.
4Chi-Square test.

CP: cold polypectomy; HP: hot polypectomy.

Table 5 Results of follow-up colonoscopy after polypectomy

<table>
<thead>
<tr>
<th></th>
<th>CP, mean ± SD or n (%)</th>
<th>HP, mean ± SD or n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resected polyps</td>
<td>215</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>Follow-up period, mo</td>
<td>17.5 ± 7.1</td>
<td>15.7 ± 6.0</td>
<td>0.005*</td>
</tr>
<tr>
<td>Range</td>
<td>6-39</td>
<td>6-35</td>
<td></td>
</tr>
<tr>
<td>Local recurrence</td>
<td>2 (0.93)</td>
<td>2 (0.93)</td>
<td>0.688b</td>
</tr>
</tbody>
</table>

5Student’s t-test.
6Fisher’s Exact test.
CP: Cold polypectomy; HP: Hot polypectomy.

evaluation method similar to that used by Murakami et al.[24] to assess the presence of residual recurrence. That is, we first looked for a scar after treatment, and if it was unclear, observed the excision site estimated from the distance from the anal margin multiple times, and judged that there was no recurrence if there was no new polyp. The number of lesions that led to recurrence was 2 (0.93%) in both groups, which was not significantly different (P = 0.688). The local recurrence rate in the CP group in this study was 0.93%. It was similar to 1.4% in CSP for < 10-mm colorectal polyps that Murakami et al.[24] reported or 0.98% in CSP that Maruoka et al.[25] reported, and it was expected to be lower than the residual rates reported by the second method of confirming the presence of remnants after polypectomy, that is, pathologically evaluated by biopsy or snare immediately after polypectomy.[10,20-22]. The result may be due to the fall-off of small residual lesions at the margins of the excision and overlooking recurrent lesions in actual clinical practice. Additionally, no significant difference was observed in the residual rate compared with the HP group, as previously reported.

Regarding the safety, the acute bleeding rate immediately after the procedure was 2.8% in the CP group and 1.4% in the HP group (P = 0.252), with no significant difference between the two groups. In all cases, the bleeding was stopped by clipping hemostasis. Acute bleeding in CP often stops spontaneously. In contrast, delayed bleeding and perforation were not observed in either group, thus, confirming the safety of CP as reported previously[5-8].
It has been reported that CP has a higher rate of pathologically positive or unknown resection margins than HP\cite{18,19}. In this study, the histopathological complete resection rate was 82.5\% in the CP group vs 84.0\% in the HP group (P = 0.708), with no significant difference between the two groups.

**Limitations**

This study was a retrospective examination at a single institution, and the sample size was not very large. The follow-up period was not long and averaged a little over a year. The mean follow-up period was significantly longer in the CP group than that in the HP group. However, the results emphasize that the local recurrence rate in the CP group did not become higher compared with the HP group because the local recurrence rate was equivalent in both groups.

Colonoscopy was performed by several different endoscopists who might not have detected all recurrences because of differences in individual skills and the possibility of missing residual or recurrent lesions. Although the endoscopists in this study were experts, a new study should be conducted, including colonoscopy trainees.

Of the lesions selected in this study using propensity score matching, only two lesions were resected using forceps in each of the groups, and biopsy polypectomy was not fully evaluated because of the small sample size. Future prospective studies with a larger number of patients and longer follow-up periods are needed.

**CONCLUSION**

CP for non-pedunculated polyps of less than 10 mm is equivalent to HP in terms of the local recurrence rate. There were no complications of delayed bleeding or perforation, and CP was considered a safe and useful procedure for the treatment of non-pedunculated colorectal polyps less than 10 mm.
ARTICLE HIGHLIGHTS

Research background
Cold polypectomy (CP) is widely used as a simple and safe procedure for small colorectal polyps. However, there is concern that recurrence rate following CP may be higher than Hot polypectomy (HP) because of unidentified margins of excised specimens and the lack of tumor suppression effect by coagulation.

Research motivation
There were few reports that observed the course over long periods of time after CP in clinical practice. It is important to compare and evaluate the recurrence rate following CP and HP.

Research objectives
The aim of this study was to evaluate the presence of local recurrence following CP and HP using propensity score matching.

Research methods
We analyzed 275 patients who underwent polypectomy for non-pedunculated colorectal polyps less than 10 mm (959 Lesions) and follow-up endoscopy subsequently. We divided them into the CP group (706 Lesions) and the HP group (253 Lesions). Using propensity score matching, we extracted 215 Lesions in each group and evaluated the local recurrence of CP in the real clinic using medical records.

Research results
The local recurrence rate was 0.93% in the CP group and 0.93% in the HP group, without a significant difference ($P = 0.688$).

Research conclusions
Local recurrence after CP was equivalent to that following HP in clinical practice. CP is useful and safe in the treatment of non-pedunculated polyps of less than 10 mm.

Research perspectives
Future prospective studies with a larger number of patients and longer follow-up periods are needed in clinical practice.

ACKNOWLEDGEMENTS
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6 Ichise Y, Horiiuchi A, Nakayama Y, Tanaka N. Prospective randomized comparison of cold snare polypectomy and conventional polypectomy for small colorectal polyps. *Digestion* 2011; 84: 78-81 [PMID: 21494037 DOI: 10.1159/000323959]


Microarray analysis to explore the effect of CXCL12 isoforms in a pancreatic pre-tumor cell model

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Author contributions: Mi DH and Miao YD designed the research; Miao YD wrote this comment; Wang JT and Tang XL made academic advice; Mi DH reviewed this manuscript; all authors approved the final manuscript.

Conflict-of-interest statement: No conflict of interest associated with any of the senior authors or other coauthors contributed their efforts in this manuscript.

Country/Territory of origin: China

Specialty type: Gastroenterology and hepatology

Abstract

CXCL12 expression was significantly lower in tumor samples than in corresponding normal samples. CXCL12 expression was significantly positively related to the infiltration levels of T cells, dendritic cells (DCs), immature DCs, cytotoxic cells, Th1 cells, mast cells, B cells, Th1 cells, natural killer (NK) cells, pDCs, neutrophils, and T helper cells (Spearman correlation coefficient > 0.5, \( P < 0.001 \)) and negatively correlated with the infiltration level of NK CD56bright cells. In addition, pancreatic hTERT-HPNE cells treated with three diverse CXCL12 isoforms exhibited changes mainly in the regulation of the epithelial-mesenchymal transition activation pathway.

Key Words: CXCL12; Pancreatic cancer; Splicing isoforms; Bioinformatics analysis; Tumor microenvironment; Pathway

Core Tip: CXCL12 expression was significantly lower in tumor samples than in normal samples. CXCL12 expression was significantly positively associated with the infiltration levels of 12 immune cells, especially T cells, which may encourage further exploration of the effect of CXCL12 in pancreatic ductal adenocarcinoma immunotherapy. In addition, treating pancreatic hTERT-HPNE cells with three diverse CXCL12 isoforms mainly affected the regulation of the epithelial-mesenchymal transition activation pathway.

Citation: Miao YD, Wang JT, Tang XL, Mi DH. Microarray analysis to explore the effect of
TO THE EDITOR

We read with interest the article by Cecati et al [1]. They investigated the specific roles of α, β, and γ CXCL12 isoforms in pancreatic ductal adenocarcinoma (PDCA) onset by microarray analysis of hTERT-HPNE cells cured by three diverse isoforms of CXCL12, which indicated that CXCL12 isoforms have different roles in PDAC pathogenesis.

We appreciate the unique perspective provided by the authors’ exploration of the roles of the different isomers of CXCL12 in PDAC. However, the results might be made more meaningful if the authors built on this by presenting the differential expression of CXCL12 in normal and tumor tissues of PDCA as a whole, such as through a bioinformatics analysis of PDCA cases in The Cancer Genome Atlas (TCGA) database or their own data. We discovered that the CXCL12 expression was significantly lower in tumor samples than in normal samples (Figure 1A). Detailed statistical results are described in Table 1.

The tumor microenvironment (TME), mediated by interactions between stromal cells and pancreatic epithelial/carcinoma cells, is essential for PDCA progression and has been associated with failure of chemotherapy, radiotherapy, and immunotherapy [2]. The formation of the microenvironment requires interactions between pancreatic cancer cells and stromal cells. A pancreatic cancer microenvironment composition that favors demyelination and immunosuppression is related to poor prognosis [3-5]. Although immunotherapy has transformed cancer therapy, patients with PDCA rarely respond to these regimens, and this failure is attributed to poor infiltration and activation of T cells in the TME. We found that CXCL12 expression was positively correlated with the level of infiltration of 22 immune cells, especially T cells (Figure 1B and C), which may encourage further exploration of the effect of CXCL12 in PDCA immunotherapy. Detailed information on the correlation between CXCL12 expression and immune cell infiltration is shown in Table 2.

We agree with Cecati et al [1], who reported that all CXCL12 isoforms influenced cell migration, adhesion, and cytoskeleton-associated gene expression. In our study, we found that treating pancreatic hTERT-HPNE cells with three diverse CXCL12 isoforms mainly affects the regulation of the EMT activation pathway (Figure 1D-F), which confirms that the work done by Cecati et al [1] is worthy of recognition and that our findings can be a supplement to their study. In the future, we should investigate the role played by CXCL12 in the PDCA immune microenvironment in depth.

Statistical analysis

Software: R (version 3.6.3) was used to perform statistical analysis and visualization results. Differential expression of CXCL12 between pancreatic cancer tissues and normal tissues was adopted by the Wilcoxon rank-sum test and visualized results using R-package "ggplot2". Immune cell algorithm: ssGSEA (built-in algorithm of GSVA package[6]). Correlation test using Spearman's correlation coefficient. Pathway analysis was performed by the online tool GSCALite (http://bioinfo.life.hust.edu.cn/web/GSCALite/) [7].
Table 1 Detailed statistical results of CXCL12 differential expression analysis in pancreatic ductal adenocarcinoma (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Median</th>
<th>IQR</th>
<th>Lower quartile</th>
<th>Upper quartile</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>171</td>
<td>0</td>
<td>7.296</td>
<td>5.433</td>
<td>0.756</td>
<td>5.028</td>
<td>5.784</td>
<td>5.403</td>
<td>0.88</td>
</tr>
<tr>
<td>Tumor</td>
<td>179</td>
<td>1.333</td>
<td>7.629</td>
<td>4.632</td>
<td>2.134</td>
<td>3.727</td>
<td>5.861</td>
<td>4.803</td>
<td>1.445</td>
</tr>
</tbody>
</table>

IQR: Interquartile distance; SE: Standard error.

Table 2 Detailed information on the correlation between CXCL12 expression and immune cell infiltration

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cell</th>
<th>Correlation coefficient (Pearson)</th>
<th>P value (Pearson)</th>
<th>Correlation coefficient (Spearman)</th>
<th>P value (Spearman)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL12</td>
<td>aDC</td>
<td>0.355</td>
<td>&lt; 0.001</td>
<td>0.350</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>B cells</td>
<td>0.614</td>
<td>&lt; 0.001</td>
<td>0.610</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>CD8 T cells</td>
<td>0.508</td>
<td>&lt; 0.001</td>
<td>0.491</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Cytotoxic cells</td>
<td>0.674</td>
<td>&lt; 0.001</td>
<td>0.650</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>DC</td>
<td>0.668</td>
<td>&lt; 0.001</td>
<td>0.658</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Eosinophils</td>
<td>0.488</td>
<td>&lt; 0.001</td>
<td>0.480</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>IDC</td>
<td>0.639</td>
<td>&lt; 0.001</td>
<td>0.654</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Macrophages</td>
<td>0.488</td>
<td>&lt; 0.001</td>
<td>0.487</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Mast cells</td>
<td>0.635</td>
<td>&lt; 0.001</td>
<td>0.634</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Neutrophils</td>
<td>0.554</td>
<td>&lt; 0.001</td>
<td>0.535</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>NK CD56bright cells</td>
<td>-0.411</td>
<td>&lt; 0.001</td>
<td>-0.397</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>NK CD56dim cells</td>
<td>0.376</td>
<td>&lt; 0.001</td>
<td>0.369</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>NK cells</td>
<td>0.566</td>
<td>&lt; 0.001</td>
<td>0.560</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>pDC</td>
<td>0.558</td>
<td>&lt; 0.001</td>
<td>0.546</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>T cells</td>
<td>0.682</td>
<td>&lt; 0.001</td>
<td>0.666</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>T helper cells</td>
<td>0.511</td>
<td>&lt; 0.001</td>
<td>0.504</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Tcm</td>
<td>0.337</td>
<td>&lt; 0.001</td>
<td>0.285</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Tem</td>
<td>0.483</td>
<td>&lt; 0.001</td>
<td>0.481</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>TH1</td>
<td>0.668</td>
<td>&lt; 0.001</td>
<td>0.645</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Tgd</td>
<td>0.364</td>
<td>&lt; 0.001</td>
<td>0.472</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Th1 cells</td>
<td>0.594</td>
<td>&lt; 0.001</td>
<td>0.605</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Th17 cells</td>
<td>0.057</td>
<td>&lt; 0.001</td>
<td>0.065</td>
<td>0.387</td>
</tr>
<tr>
<td>CXCL12</td>
<td>Th2 cells</td>
<td>0.069</td>
<td>0.357</td>
<td>0.032</td>
<td>0.675</td>
</tr>
<tr>
<td>CXCL12</td>
<td>TReg</td>
<td>0.493</td>
<td>&lt; 0.001</td>
<td>0.482</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

aDC: Activated DC; DC: Dendritic cells; iDC: immature DC; pDC: Plasmacytoid DC; TFH: T follicular helper; Tgd: T gamma delta; NK: Natural killer.
Miao YD et al. CXCL12 isoforms in a pancreatic pre-tumor cell model

A. The expression of CXCL12 log2 (FPKM+1)

B. Pathway (A: Activate; I: Inhibit)

C. Spearman

D. Pathway (A: Activate; I: Inhibit)

E. Pathway (A: Activate; I: Inhibit)
Figure 1 The effect of CXCL12 in the development of pancreatic ductal adenocarcinoma. A: The differential CXCL12 expression in pancreatic ductal adenocarcinoma (PDCA) and normal samples. The expression level of CXCL12 in tumor tissues is indicated in orange, and that in normal tissues is indicated in purple. Data source: UCSC XENA (https://xenabrowser.net/datapages/) RNAseq data in TPM format for The Cancer Genome Atlas (TCGA) and GTEx processed uniformly through the Toil process[^4]. PAAD (pancreatic cancer) data were extracted from TCGA, and corresponding normal sample data were from GTEx. Significance markers: NS, \( P \geq 0.05 \), a \( P \leq 0.05 \), b \( P \leq 0.01 \), c \( P \leq 0.001 \); B: The expression level of CXCL12 and its relationship to 24 immune cell infiltration levels in PDCA. Data source: RNAseq data and clinical data in level 3 HTSeq-FPKM format from the TCGA (https://portal.gdc.cancer.gov/) PAAD (pancreatic cancer) project. Data filtering: Removal of paraneoplastic tissue; C: The expression level of CXCL12 and its relationship to the T cell infiltration level in PDCA; D and E: Pathway analysis of differentially expressed genes under all treatment conditions (\( \alpha \), \( \beta \), and \( \gamma \) CXCL12 isoforms); D: CXCL12 \( \alpha \) isoform vs control; E: CXCL12 \( \beta \) isoform vs control; F: CXCL12 \( \gamma \) isoform vs control.

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Progress on global hepatitis elimination targets

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Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

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Abstract

In 2016, the World Health Assembly adopted a Global Health Sector Strategy on viral hepatitis, with targets set for the years 2020 and 2030 to achieve hepatitis elimination. The main target of hepatitis elimination strategy is to reduce the incidence of hepatitis B virus (HBV) and hepatitis C virus (HCV) by 90% and mortality by 65% in 2030. In last 5 years, the number of people receiving HCV treatment has increased from 1 million to 9.4 million; however, this number is far from the 2030 target of 40 million people receiving HCV treatment. HBV and HCV incidence rates are down from 1.4 million to 1.1 million annual deaths but this is far from the 2030 target of < 0.5 million deaths. The coronavirus disease 2019 pandemic has severely affected the efforts in the fight against hepatitis. No major donor has committed to investing in the fight against hepatitis. Time is running out. There is a need to speed up efforts in the fight against hepatitis to achieve hepatitis elimination by 2030.

Key Words: Hepatitis elimination; Blood donations; Safe injections; Hepatitis B vaccination; Harm reduction

Core Tip: In 2020, progress was made in improving blood donations, hepatitis B and C treatments, and decreasing the incidence of hepatitis B and C. Some of the 2020 targets for hepatitis elimination were achieved but the 2030 targets are very ambitious and need strong political and financial support.

Citation: Waheed Y. Progress on global hepatitis elimination targets. World J Gastroenterol 2021; 27(47): 8199-8200
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TO THE EDITOR

I read articles from Tijera et al[1], and Pisano et al[2], on viral hepatitis update, progress, challenges, and ways to elimination. Both articles are discussing important points on viral hepatitis but both are missing the actual progress on hepatitis elimination targets set by World Health Organization (WHO).

In this article, I am presenting the latest data on targets set by the WHO to achieve hepatitis elimination by 2030. In last 5 years, the incidence of hepatitis B virus (HBV) and hepatitis C virus (HCV) has decreased from 8 million infections to 3 million infections per year and mortality has decreased from 1.4 million deaths to 1.1 million deaths per year[3]. Only 17 countries had a national hepatitis strategic plans in 2012, but this increased to 124 by 2019[3].

The WHO Global Health Sector Strategy on viral hepatitis has shown five areas in which progress is requested to achieve hepatitis elimination by 2030. These areas are: HBV vaccination, birth dose HBV vaccination, safe injection, harm reduction, and diagnosis and treatment of HBV and HCV[4].

From 2015 to 2020, the worldwide coverage of the third dose of HBV vaccine has increased from 82% to 85% and administration of birth dose of HBV vaccine has increased from 38% to 43%. The number of safe blood donations has increased from 89% to 97% and only 3.9% of injection equipment are still reused. The number of clean syringes given to each person who inject drugs per year has increased from 20 to 33. The HBV diagnosis rate has increased from < 5% to 10% and 22% of diagnosed cases received treatment. The HCV diagnosis rate has increased from < 5% to 21%, and 62% of diagnosed cases received treatment[3,4].

New data shows progress with reference to 2020 hepatitis elimination targets for blood donation screening, HBV and HCV treatments, reduction in drug pricing, and decreasing the incidence of HBV and HCV[3]. However, the 2030 targets of hepatitis elimination are very ambitious and need a strong political and financial commitment[3].

Hepatitis elimination targets needs an investment of US $6 billion per year[3]. No major donor has committed to the fight against hepatitis[3]. Many countries with well-developed hepatitis control programs are lacking financial resources to achieve targets.

The coronavirus disease 2019 (COVID-19) pandemic has severely affected the hepatitis elimination targets. Outpatient departments/liver clinics have remained closed in many countries due to lockdown restrictions. Many countries have spent a major proportional of their health budget on COVID-19 and the nascent viral hepatitis programs are being held back due to a lack of funding[3].

This is the time to put money into the fight against hepatitis and increase HBV and HCV diagnosis and treatment rates[5]. There is a strong need to obtain a cure for HBV and further develop and simplify hepatitis screening tests and make them available in primary health care settings[5]. It is the time to set interim hepatitis elimination targets for 2026 as a milestone towards 2030 targets.

REFERENCES
