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TOPIC HIGHLIGHT

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Gastric cancer arising from the remnant stomach after distal gastrectomy: A review

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Abstract

Gastric stump carcinoma was initially reported by Balfore in 1922, and many reports of this disease have since been published. We herein review previous reports of gastric stump carcinoma with respect to epidemiology, carcinogenesis, *Helicobacter pylori* (*H. pylori*) infection, Epstein-Barr virus infection, clinicopathologic characteristics and endoscopic treatment. In particular, it is noteworthy that no prognostic differences are observed between gastric stump carcinoma and primary upper third gastric cancer. In addition, endoscopic submucosal dissection has recently been used to treat gastric stump carcinoma in the early stage. In contrast, many issues concerning gastric stump carcinoma remain to be clarified, including molecular biological characteristics and the carcinogenesis of *H. pylori* infection. We herein review the previous pertinent literature and summarize the characteristics of gastric stump carcinoma reported to date.

Key words: Remnant gastric cancer; Distal gastrectomy; Carcinogenesis; *Helicobacter pylori*; Endoscopic submucosal dissection

Core tip: Recent studies concerning gastric stump carcinoma were reviewed. Its carcinogenesis took more than 300 mo after distal gastrectomy for benign disease, in contrast to 100 mo for primary gastric cancer. Higher carcinogenetic risk was reported by molecular biological analysis in patients treated with Billroth II reconstruction than with Billroth I. Eradication of *Helicobacter pylori* in the remnant stomach improved the degree of inflammation and the pH level, and might prevent the development of carcinogenesis. Endoscopic treatment for gastric stump carcinoma has been recently reported, therefore, endoscopic surveillance should be repeated after distal gastrectomy.

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INTRODUCTION

Gastric cancer is the second leading cause of cancer-related death in Asia and the fourth most common malignan-

Table 1 Interval between primary gastric cancer and gastric stump carcinoma

Ref.	Previous disease (benign/ malignancy)	Interval (mo) (all cases)	Interval (mo) (benign/ malignancy)
Kaneko *et al*[12], 1998	21/22	180	288/118
Takeno *et al*[16], 2006	11/21		360/63
Ohashi *et al*[17], 2007		90	
Ahn *et al*[18], 2008	13/45	150	384/83
Tanigawa *et al*[19], 2010	578/309	252	
Ojima *et al*[20], 2010	17/21	180	264/108
Komatsu *et al*[21], 2012	19/14	240	360/144
Li *et al*[22], 2013	88/24		384/204

Table 2 Interval and location of gastric stump carcinoma by reconstruction

Ref.	Primary reconstruction	Interval (mo)	Location
	Billroth I / II	Billroth I / II	B- I ana/B- I non/ B- II ana/B- II non
Takeno *et al*[16], 2006	21/11	84/276	4/17/6/5
Ohashi *et al*[17], 2007	71/28		7/64/5/23
Ahn *et al*[18], 2008	26/25		11/15/16/9
Tanigawa *et al*[19], 2010	368/519	252/372	81/176/289/114
Komatsu *et al*[21], 2012	16/16	144/384	2/5/9/2
Li *et al*[22], 2013	42/70		19/23/45/25

B- I : Billroth I ; B- II : Billroth II ; ana: Anastomosis site; non: Non-anastomosis site.

cy worldwide[1,2]. The five-year survival of patients with gastric cancer is estimated to be approximately 20%, and it has been reported that only surgery, including lymphadenectomy, can provide curative effects[3-5]. However, recent advances in early detection and the development of anticancer drugs have prolonged the prognosis[6,7].

Gastric stump carcinoma was originally defined as gastric cancer arising from the remnant stomach more than five years after distal gastrectomy for benign disease[8-10]. The incidence of gastric stump carcinoma is estimated to be 1%-2%, according to the current literature[11,12]. However, most cases of gastric cancer arising from the remnant stomach after distal gastrectomy involve a second primary gastric cancer, as the rate of gastrectomy against peptic ulcers has decreased for the last three decades due to the development of gastric acid inhibitor drugs and improvements in the prognosis of patients with gastric cancer, as described above[6,7,13]. In addition, the development of endoscopic technology and periodical endoscopic surveillance has enabled clinicians to detect gastric cancer of the remnant stomach in the early stage, which may improve the unfavorable prognosis of patients with gastric stump carcinoma[12].

The characteristics of remnant gastric cancer may have changed from those previously reported in the literature. Therefore, we reviewed recent articles and attempted to clarify the modern characteristics, carcinogenesis, diagnosis and optimal treatment of remnant gastric cancer.

EPIDEMIOLOGY

In 1922, Balfore first reported that, with respect to gastric cancer arising from the remnant stomach after surgery, the most important factor affecting life expectancy after surgery for gastric ulcers is the occurrence of gastric cancer, which accounts for approximately 40% of the total number of deaths in this patient population[14]. That series included gastric cancer as well as benign ulcers as the primary lesions and reported the incidence of remnant gastric cancer to be 3% after resection of ulcerous lesions.

A population-based study of patients who underwent distal gastrectomy for benign disease was recently reported from Sweden. In that study, the incidence of remnant gastric cancer was 0.74%, which is similar to the findings of previous reports[11,12,15]. In addition, it is of interest that the incidence of gastric stump carcinoma is not higher than expected and increases only after more than 30 years after surgery for benign disease[15]. Several reports have found that it takes more than 300 mo for gastric stump carcinoma to arise from the remnant stomach after distal gastrectomy for benign disease, in contrast to the approximately 100 mo observed following gastrectomy for primary gastric cancer (Tables 1 and 2)[12,16-22].

EFFECTS OF RECONSTRUCTION DURING DISTAL GASTRECTOMY ON CARCINOGENESIS IN THE REMNANT STOMACH

It has been reported that a reduction in the level of serum gastrin and gastroduodenal reflux are factors for carcinogenesis in the remnant stomach after distal gastrectomy. This finding has also been experimentally evaluated by Miwa *et al*[23]. Billroth II reconstruction is more frequently associated with atrophic changes and an increased S phase cell count in the proliferative zone compared to that observed following treatment with Billroth I in the Wister rat model. In addition, it has been reported that intestinal metaplasia is rare. However, to the best of our knowledge, no clinical studies have compared the incidence of atrophic changes and intestinal metaplasia between patients treated with the Billroth I and II methods. The interval between primary distal gastrectomy and the diagnosis of stump carcinoma is significantly longer in patients treated with Billroth I reconstruction than in those treated with Billroth II reconstruction, according to a review of previous clinical retrospective studies[16-19,21,22].

In addition, there is a consensus that gastric stump carcinoma tends to arise from sites of anastomosis in patients treated with Billroth II reconstruction, in contrast to non-anastomotic sites in patients treated with

Table 3 *Helicobacter pylori* infection in the patients underwent distal gastrectomy

Ref.	Total infection rate	Billroth I	Billroth II	Roux-en-Y
Onoda *et al*[31], 2001	65.10%	67.10%	58.30%	
Matsukura *et al*[32], 2003	68.20%	72.20%	58.80%	
Abe *et al*[33], 2005	56.30%	55.60%	58.30%	
Chan *et al*[34], 2007	50.00%	58.60%	66.70%	26.30%

Billroth I reconstruction, and that the incidence of gastric stump carcinoma is correlated with that of gastroduodenal reflux, similar to that observed in experimental rat models[16-19,21,22].

The condition of the remnant stomach mucosa after distal gastrectomy has been biologically examined at the molecular level. Tanigawa reported that the apoptotic index, p53 labeling index and Ki-67 labeling index are significantly higher in patients treated with Billroth II reconstruction than in those treated with Billroth I reconstruction[24]. In addition, Nakachi *et al*[25] and Aya *et al*[26] demonstrated a higher frequency of microsatellite instability in patients with gastric stump carcinoma (88.9%, 43%) than in those with primary upper third gastric carcinoma (20%, 6%). Furthermore, Aya reported a significantly higher level of microsatellite instability, as well as a higher frequency of both hMLH1 and hMSH2 inactivation, in patients treated with Billroth II reconstruction than in those treated with Billroth I reconstruction[26].

Taking both clinicopathological and molecular biological changes into consideration, the Billroth I procedure is thus considered to be preferable to the Billroth II method, at least with respect to preventing the development of gastric stump carcinoma.

Roux-en-Y reconstruction has recently been adopted for reconstruction after distal gastrectomy to prevent gastroduodenal reflux. The time for which the remnant gastric mucosa is exposed to bile reflux is shorter and the degree of remnant gastritis is more mild in patients treated with Roux-en-Y reconstruction than in those treated with Billroth I reconstruction[27]. Both the latest multi-institutional randomized controlled study and a meta-analysis support this finding, and it appears that a consensus has been reached on this issue[28-30]. No reports have thus far suggested that the incidence of gastric stump carcinoma is lower in patients treated with Roux-en-Y reconstruction than in those treated with Billroth I reconstruction. However, Roux-en-Y reconstruction is preferred from the viewpoint of reducing the incidence of gastroduodenal reflux and remnant gastric mucosal injury related to gastric carcinogenesis.

HELICOBACTER PYLORI INFECTION

Helicobacter pylori (*H. pylori*) infection is a well-known major causative factor of carcinogenesis in the stomach. Nagahata reported that the rate of infection following gastrectomy gradually decreases over time. Recent studies have also examined the frequency of *H. pylori* infection in the remnant stomach after distal gastrectomy. The rate of infection ranges from 50% to 68.2% among all patients treated with distal gastrectomy, 55.6% to 72.2% among patients treated with Billroth I reconstruction and 58.3% to 66.7% among patients treated with Billroth II reconstruction (Table 3)[31-34]. Only one series has suggested the rate of infection to be lower in patients treated with the Roux-en-Y method, and further studies are thus required to clarify this issue[34]. It therefore appears that there are no significant differences between Billroth I and II reconstruction. Matsukura *et al*[32] reported that eradication with dual and triple therapy is successful in 70% and 90% of *H. pylori* patients who undergo distal gastrectomy, respectively, and that the therapeutic efficacy is the same in patients treated with and without distal gastrectomy. It has also been demonstrated that the degree of inflammation improves and the pH level normalizes following eradication of *H. pylori* in the remnant stomach[35]. Therefore, treatment with eradication of *H. pylori* in the remnant stomach is recommended to prevent the development of gastric stump carcinoma, although no significant correlations have been reported between *H. pylori* infection and carcinogenesis in the remnant stomach.

Table 4 Epstein-Barr infection in the patients with gastric stump carcinoma

Ref.	Total infection rate	Billroth I	Billroth II
Tanigawa *et al*[19], 2000	22.2%	5.9%	32.1%
Nishikawa *et al*[36], 2002	41.2%	0.0%	58.3%
Kaizaki *et al*[37], 2005	23.1%	12.5%	30.4%

EPSTEIN-BARR VIRUS INFECTION IN THE REMNANT STOMACH

Infection with the Epstein-Barr (EB) virus has been reported to be associated with various cancers, including stomach cancer. A few series have examined EB virus infection in patients with gastric stump carcinoma. According to these studies, the rate of infection ranges from 22.2% to 41.2% among all patients treated with distal gastrectomy, 0% to 12.5% among patients treated with Billroth I reconstruction and 30.4% to 58.3% among patients treated with Billroth II reconstruction (Table 4)[19,36,37]. Therefore, a higher rate of infection with the EB virus has been demonstrated in patients treated with Billroth II reconstruction.

In addition, EB-virus infection has been suggested to be correlated with the incidence of gastritis cystic polyposa and may also facilitate the development of *de novo* gastric stump carcinoma[37].

Table 5 Clinicopathologic characteristics of gastric stump carcinoma

Ref.	Patients age	pT (1/2/3/4)	pN (positive/negative)	pM (positive/negative)	pStage	5-yr survival
Takeno *et al*[16], 2006	68.7	10/22 (1,2/3,4)	12/20	4/28	21/11 (1,2/3,4)	
Ohashi *et al*[17], 2007	67	67/16/8/17	13/84		77/6/2/23	53.10%
Ahn *et al*[18], 2008		18/17/0/19	23/29	10/42		
Ahn *et al*[18], 2008	58	15/31 (1/2,3,4)	19/25	17/41	26/32 (1,2/3,4)	63.4% (3-yr)
Tanigawa *et al*[19], 2010	68	315/245/197/130	534/327	26/861		
Komatsu *et al*[21], 2012	68	10/22 (1/2,3,4)	14/13		17/15 (1/2,3,4)	
Li *et al*[22], 2013		1/3/44/64	66/46	31/81	3/16/62/31	11%

Table 6 Clinicopathological comparison between primary upper third gastric cancer and gastric stump carcinoma

Clinicopathologic chracteristics	Ikeguchi *et al*[39], 1993	*P* value	Chen *et al*[40], 1996	*P* value	Newman *et al*[41], 1997	*P* value	Komatsu *et al*[42], 2012	*P* value
pT (1/2/3/4)								
PUTGC	63/15/157/31	NS	5/30/88/20	NS	11/15/46/7	NS	69/75/54/9	0.07
GSC	4/3/7/6		0/5/13/7		7/6/11/1		10/10/7/6	
pN (negative/positive)								
PUTGC	99/167	NS	47/86	NS	24/54	NS	118/89	0.7
GSC	11/9		10/15		14/11		20/13	
M (negative/positive)								
PUTGC			127/16	NS	Nov-68	NS		
GSC			20/5		22/3			
5-yr survival								
PUTGC	62.1%	NS	25%	0.31	37%	0.1		0.67
GSC	52.5%		46%		63%			

PTUGC: Primary upper third gastric cancer; GSC: Gastric stump carcinoma; NS: Not significant.

CLINICOPATHOLOGICAL CHARACTERISTICS

The clinicopathological characteristics of gastric stump carcinoma have been analyzed in many reports, as summarized in Table 5[16-19,21,22,38]. For example, it has been reported that the prognosis of gastric stump carcinoma is unfavorable compared to that of primary gastric cancer, which may result from the more advanced stage of disease observed at diagnosis. There is currently no consensus regarding this issue based on a Japanese nationwide report of gastric cancer, although unevenness in the disease stage at diagnosis has been observed in various studies[19].

It has also been reported that there have been no remarkable changes in the number of gastric stump carcinoma patients with progressive tumor invasion. In contrast, the number of patients with progressive cancer invasion has been reported to gradually decrease in Japan since 1991, according to data for resected gastric cancer. Among patients with lymph node metastasis, there are no significant trends, as approximately half of all such patients were found to have node metastasis in a Japanese nationwide study and be negative for node metastasis in the previous literature regarding gastric stump carcinoma.

There have been several reports of prognostic analyses comparing gastric stump carcinoma and primary upper third gastric cancer[39-42] (Table 6). All such studies have suggested that there are no significant differences in either the prognosis or rate of progression between these two diseases. In contrast, it is of interest that gastric stump carcinoma exhibits a more favorable prognosis than primary upper third gastric cancer in patients with stage I or II disease and, inversely, a more unfavorable prognosis in patients with stage III or IV disease[40]. Concerning this result, Chen *et al*[40] reported that the left gastric artery is usually resected during distal gastrectomy, which may change the lymphatic flow and thereby influence the difference in prognosis observed in analyses of the cancer stage. Ikeguchi *et al*[39] also reported the incidence of jejuna mesenteric lymph node metastasis to be increased in patients with gastric stump carcinoma; these results may correlate with those of Chen. Controversially, Newman *et al*[41] reported that there are no prognostic differences between gastric stump carcinoma and upper third primary gastric cancer, even when the analysis is classified according to the cancer stage. Meta-analyses and/or multi-institutional randomized controlled studies with large series are therefore required to clarify these controversial results, although it may be difficult to conduct such studies due to the rarity of the disease.

ENDOSCOPIC TREATMENT

Previously, radical resection was the only curable treatment for gastric stump carcinoma, as observed in the setting of primary gastric cancer. However, advancements in endoscopic diagnosis and the popularization of periodic endoscopic screening after gastrectomy have enabled clinicians to detect gastric stump carcinoma at the early

Table 7 Endoscopic submucosal dissection for gastric stump carcinoma *n* (%)

Ref.	No. of ESD cases	*En bloc* resection	Complete resection	Mortality	Delayed bleeding	Perforation
Takenaka *et al*[44], 2008	31	30 (97)	23 (74)	0	0	4 (13)
Hirasaki *et al*[45], 2008	17	17 (100)	14 (82)	0	3 (18)	0
Lee *et al*[46], 2010	13	13 (100)	12 (92.3)	0	0	0
Nonaka *et al*[47], 2013	139	131 (94)	118 (85)	0	2 (1.4)	2 (1.4)
Tanaka *et al*[48], 2013	33	33 (100)	31 (94)	0	1 (3)	3 (9)

ESD: Endoscopic submucosal dissection.

stage. Hosokawa reported that 15 patients with gastric stump carcinoma were detected among 509 patients who underwent distal gastrectomy over more than 10 years, 12 of whom were diagnosed at an early stage, and concluded that endoscopic surveillance should be repeated every two to three years after distal gastrectomy[43].

Similarly, several studies including small series of endoscopic treatment for gastric stump carcinoma have recently been reported, as summarized in Table 7[44-48]. En bloc resection and complete resection were performed in more than 90% of cases and 74%-94% of cases, respectively. Concerning complications after endoscopic treatment, there were no mortalities, and 0%-18% and 0%-13% of the patients exhibited delayed bleeding and perforation, respectively. However, morbidity, as well as the en bloc and complete resection rates, have been shown to have improved in the latest reports.

Only one study, by Nonaka *et al*[47], has reported long-term outcomes after endoscopic treatment for gastric stump carcinoma. In that study, the overall and disease-specific survival was 87.3% and 100%, respectively. Further studies using large series should thus be conducted to confirm the oncological feasibility of providing endoscopic treatment in patients with gastric stump carcinoma.

CONCLUSION

Clarifying the differences in the characteristics of gastric stump carcinoma and primary gastric cancer may enable clinicians to make an early diagnosis and improve clinical outcomes in patients with gastric stump carcinoma. In addition, multi-institutional analyses using large series may positively contribute to clarifying these issues.

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EXPERT OPINION

DNA synthesis inhibitors for the treatment of gastrointestinal cancer

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Introduction: Intensive laboratory, preclinical and clinical studies have identified and validated molecular targets in cancers, leading to a shift toward the development of novel, rationally designed and specific therapeutic agents. However, gastrointestinal cancers continue to have a poor prognosis, largely due to drug resistance.

Areas covered: Here, we discuss the current understanding of DNA synthesis inhibitors and their mechanisms of action for the treatment of gastrointestinal malignancies.

Expert opinion: Conventional agents, including DNA synthesis inhibitors such as fluoropyrimidines and platinum analogs, remain the most effective therapeutics and are the standards against which new drugs are compared. Novel DNA synthesis inhibitors for the treatment of gastrointestinal malignancies include a combination of the antimetabolite TAS-102, which consists of trifluorothymidine with a thymidine phosphorylase inhibitor, and a novel micellar formulation of cisplatin NC-6004 that uses a nanotechnology-based drug delivery system. The challenges of translational cancer research using DNA synthesis inhibitors include the identification of drugs that are specific to tumor cells to reduce toxicity and increase antitumor efficacy, biomarkers to predict pharmacological responses to chemotherapeutic drugs, identification of ways to overcome drug resistance and development of novel combination therapies with DNA synthesis inhibitors and other cancer therapies, such as targeted molecular therapeutics. Here, we discuss the current understanding of DNA synthesis inhibitors and their mechanisms of action for the treatment of gastrointestinal malignancies.

Keywords: antimetabolite, DNA synthesis inhibitor, drug delivery system, drug resistance, platinum analogs, translational cancer research

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1. Introduction

Cancer is a major public health problem in the US and other developed countries. DeSantis *et al.* reported that 1,665,540 new cancer cases are expected in the US in 2014 [1].Gastrointestinal cancer refers to malignancy of the gastrointestinal tract and accessory organs involved in digestion, including the esophagus, stomach, biliary system, pancreas, small intestine, colon, rectum and anus. An estimated 18,170 new cases of esophageal cancer, 22,220 new cases of stomach cancer, 136,830 new cases of colon and rectal cancer, 46,420 new cases of pancreatic cancer, 9,160 new cases of small intestine cancer and 33,190 new cases of liver and intrahepatic bile duct cancer will be diagnosed in 2014. Despite advances in surgery, radiation therapy, systemic chemotherapy and supportive therapies, the 5-year relative survival rates for all cancer in the US is ~ 66% for patients diagnosed between

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Article highlights.

- TAS-102 is a novel combination antimetabolite which consists of trifluorothymidine with a thymidine phosphorylase inhibitor.
- NC-6004 is a novel micellar formulation of cisplatin which uses a nanotechnology-based drug delivery system.
- The challenges of translational cancer research using DNA synthesis inhibitors include the identification of drugs that are specific to tumor cells, biomarkers to predict pharmacologic responses, identification of ways to overcome drug resistance, and development of novel combination therapies.

This box summarizes key points contained in the article.

2003 and 2009, and followed through 2010. Thus, the development of novel cancer therapeutics is urgently needed to improve cancer prognosis.

According to the American Cancer Society, cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. It is caused by the accumulation of genetic mutations and epigenetic alterations in oncogenes and tumor suppressor genes [2,3]. Cancer chemotherapy has changed since curative treatments were identified for previously fatal malignancies with rapid cell growth, such as acute leukemia [4]. As many chemotherapies affect mitosis, tumors with high growth rates are more sensitive to chemotherapy because a larger proportion of the targeted cells are undergoing cell division at any time. However, conventional chemotherapy is less effective against slow growing cancers, including gastrointestinal cancers. Additionally, intratumoral heterogeneity may contribute to the varying sensitivity of cancer cells to chemotherapy, as well as to drug resistance [5].

There are a number of strategies in the administration of chemotherapeutic drugs, including combination chemotherapy, combined modality chemotherapy, postoperative (adjuvant) chemotherapy, preoperative (neoadjuvant) chemotherapy and salvage chemotherapy. Chemotherapy is also employed as part of the multimodal treatment of cancer, such as esophageal cancer, thereby allowing for more limited surgery. Adjuvant and neoadjuvant chemotherapy can extend life and prevent disease recurrence following surgical resection of gastrointestinal cancers, including esophageal, gastric, colorectal and pancreatic cancer [6].

Recently, there has been a shift toward developing novel, rationally designed and specific agents for cancer therapy [2,7,8] Among gastrointestinal cancers, there are novel molecularly targeted therapeutics, including the tyrosine kinase inhibitors imatinib and sunitinib for gastrointestinal stromal tumors [9,10], regorafenib for metastatic colorectal cancer [11] and gastrointestinal stromal tumors [12], sunitinib and everolimus for pancreatic neuroendocrine tumors and erlotinib in combination with gemcitabine for advanced pancreatic carcinoma [13]. Additionally, therapeutic monoclonal antibodies have been developed, including a humanized anti-VEGF monoclonal antibody, bevacizumab, for metastatic colorectal cancer [14], a chimeric anti-EGFR monoclonal antibody, cetuximab, for metastatic colorectal cancer [15], a human monoclonal antibody to EGFR, panitumumab, for metastatic colorectal cancer, a humanized anti-Her2 receptor monoclonal antibody, trastuzumab, for metastatic gastric or gastroesophageal junction adenocarcinoma [16,17] and a human monoclonal antibody to the Her2 receptor, ramucirumab, for metastatic gastric or gastroesophageal junction adenocarcinoma. Moreover, recombinant fusion proteins have been developed, such as ziv-aflibercept, consisting of the binding portions of VEGF from VEGF receptors 1 and 2 fused to the Fc portion of immunoglobulin G1, for metastatic colorectal cancer [18]. However, despite the remarkable successes of the molecularly targeted agents discussed above, the prognosis of gastrointestinal cancer remains poor due to drug resistance.

New therapies for gastrointestinal cancers are not likely to replace cytotoxic agents, many of which act by damaging DNA. Rather, cytotoxic agents combined with molecularly targeted drugs will continue to be used in chemotherapy for gastrointestinal cancers. Here, we discuss the current understanding of DNA synthesis inhibitors and their mechanisms of action for the treatment of gastrointestinal cancers in order to improve patient prognosis.

2. DNA synthesis inhibitors

Traditionally, cancer drugs have been discovered through large-scale testing of synthetic chemicals and natural products in proliferating animal tumor systems, including mouse allograft preclinical cancer models using murine leukemia cells, human xenograft models using immunodeficient mice and *in vitro* human cancer cell line models, such as the anticancer drug screen conducted in 60 human tumor cell lines by the United States National Cancer Institute (NCI) [4,19]. Over time, this system has evolved into one that combines both *in vitro* human cancer cell lines with human xenograft models. Most of the agents discovered in these drug screens interact with DNA or its precursors, inhibiting the synthesis of new genetic material and causing damage to DNA in both normal and malignant cells. Unfortunately, none of the screening systems have successfully predicted outcome of clinical trials [20,21].

The drugs used in cancer chemotherapy are varied in structure and mechanism of action. Most chemotherapeutic drugs work by impairing mitosis, effectively targeting fast-dividing cells. These drugs prevent mitosis through a number of mechanisms, including damaging DNA and inhibiting the cellular machinery involved in cell division. Interestingly, many of these drugs inhibit DNA synthesis.

DNA synthesis is the creation of new DNA molecules through the process of DNA replication, wherein a replication initiator protein splits the existing cellular DNA and makes a copy of each split strand. The copied strands are then joined together with their template strand to form a new DNA molecule. DNA replication proceeds in three enzymatically

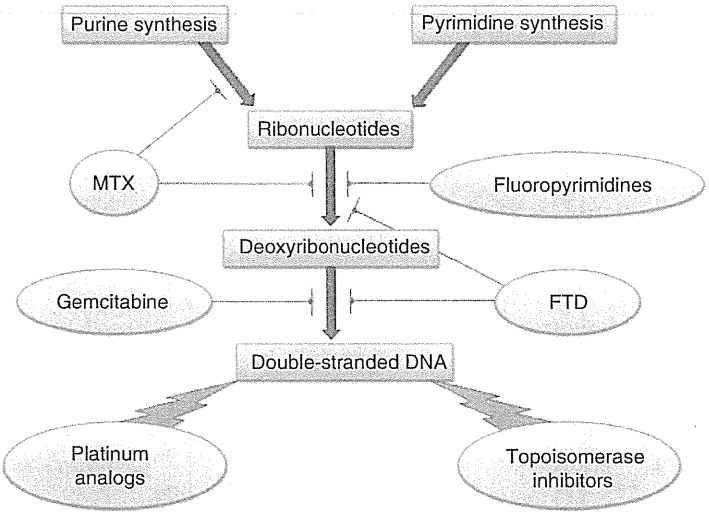

Figure 1. The mechanisms and sites of action of DNA synthesis inhibitors. The folic acid analog MTX inhibits dihydrofolate reduction, blocks thymidylate and purine synthesis, and interrupts the synthesis of DNA and RNA. Pyrimidine analogs fluoropyrimidines, such as 5-fluorouracil, inhibit thymidylate synthesis. The pyrimidine analog gemcitabine incorporates into DNA, thereby interfering with DNA synthesis. The pyrimidine analog FTD, part of the novel oral formulation TAS-102, incorporates into DNA as well as inhibits thymidine synthesis. Platinum analogs, such as cisplatin, form adducts with DNA. Topoisomerase inhibitors, such as camptothecin and epirubicin, block topoisomerase function.
FTD: α,α,α-Trifluorothymidine; MTX: Methotrexate.

catalyzed and coordinated steps: initiation, elongation and termination.

DNA synthesis inhibitors include antimetabolite analogs of folic acid, pyrimidine and purine. Figure 1 summarizes the mechanisms and sites of action of DNA synthesis inhibitors [4,22].The folic acid analog methotrexate (MTX) inhibits dihydrofolate reduction, blocks thymidylate and purine synthesis, and interrupts the synthesis of DNA and RNA. Fluoropyrimidines, a group of pyrimidine analogs that include 5-fluorouracil (5-FU), inhibit thymidylate synthesis. The pyrimidine analog gemcitabine incorporates into DNA, thereby interfering with DNA synthesis. The pyrimidine analog α,α,α-trifluorothymidine (FTD or TFT), a part of the novel oral formulation TAS-102, incorporates into DNA and inhibits thymidine synthesis [23]. Platinum analogs, such as cisplatin, form covalent adducts between platinum-DNA, which inhibit fundamental cellular processes, including DNA replication, transcription, translation and DNA repair [24].Topoisomerase inhibitors such as camptothecin and epirubicin interfere with the action of topoisomerase enzymes, which regulate the overwinding or underwinding of DNA. In this review, we discuss the antimetabolite and platinum analog DNA synthesis inhibitors in gastrointestinal cancers.

3. Antimetabolites

Antimetabolites were among the first effective chemotherapeutic agents discovered [22]. Their structures are similar to the molecules used in nucleic acid synthesis. As a result, they inhibit the enzymes needed for nucleic acid synthesis and/or are incorporated into DNA and RNA macromolecules to induce cell death during S phase, the part of the cell cycle in which DNA is replicated. Because cancer cells divide more rapidly than normal cells, antimetabolites affect the replication of cancer cells to a greater extent than normal cells. Pyrimidine analogs, purine analogs and folate antagonists are the main categories of antimetabolites.

3.1 Pyrimidine analogs

Fluoropyrimidines were developed in the 1950s following the observation that rat hepatomas used the pyrimidine uracil more rapidly than normal tissues, indicating that uracil metabolism was a potential target for antimetabolite chemotherapy [25].

5-FU is an analog of uracil with a fluorine atom at the C-5 position in place of hydrogen. 5-FU rapidly enters the cell using the same facilitated transport mechanism as uracil. Since its development by Heidelberger *et al.* in 1957, it has been used as a standard chemotherapy for solid tumors, such as gastrointestinal cancers [26]. The mechanism of 5-FU cytotoxicity has been ascribed to the misincorporation of its metabolites into RNA and DNA, and to the inhibition of the nucleotide synthesizing enzyme thymidylate synthase (TS).

5-FU is converted to three active metabolites (Figure 2): fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate

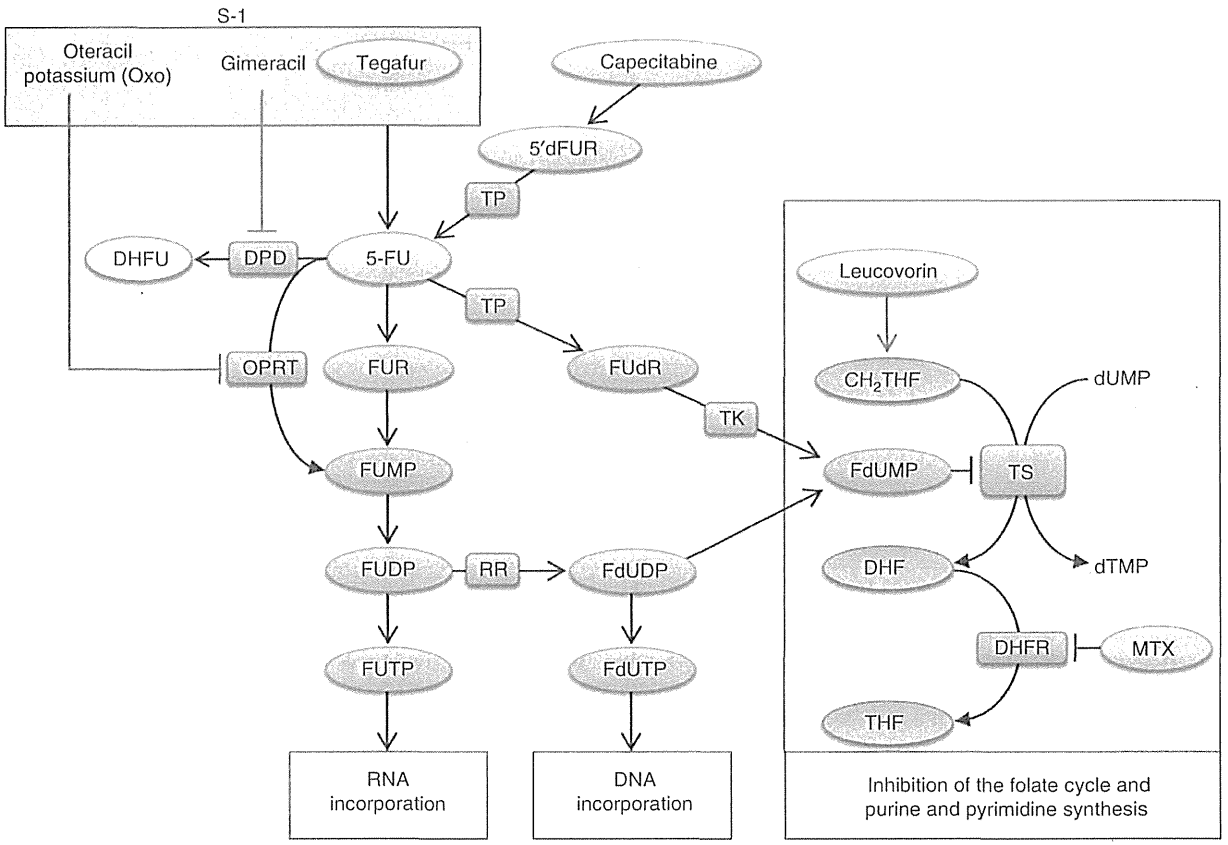

Figure 2. Summary of the metabolism of fluoropyrimidines. 5-FU is converted to three active metabolites: FdUMP, FdUTP, and FUTP. These active metabolites disrupt the synthesis of DNA and RNA and the action of TS. The main mechanism of 5-FU activation is conversion to FdUMP to inhibit TS, which plays an important role in the folate cycle and purine and pyrimidine synthesis. Addition of exogenous folate in the form of folinic acid (leucovorin) increases the intracellular pool of CH_2THF, thereby enhancing TS inhibition by FdUMP. The conversion of 5-FU to FdUMP can occur either directly via TP to FUdR and then to FdUMP by TK, or indirectly via FUR or FUMP to FUDP, and then to FdUDP and FdUMP by RR. FUDP and FdUDP can also be converted to FUTP and FdUTP to incorporate into RNA and DNA, respectively, which contributes to the cytotoxicity of 5-FU. DPD mediates the conversion of 5-FU to DHFU. Gimeracil inhibits DPD-mediated degradation of 5-FU. Capecitabine is a 5-FU pro-drug that is converted to 5'dFUR, which is then converted to 5-FU by TP. S-1 combines the 5-FU prodrug tegafur, a DPD inhibitor gimeracil, and an orotate phosphoribosyltransferase inhibitor oteracil potassium to improve the selectivity of action of 5-FU. MTX inhibits DHFR, inhibit dihydrofolate reduction to THF, and block thymidylate and purine synthesis.

5-FU: 5-Fluorouracil; CH_2THF: 5,10-Methylene tetrahydrofolate; 5'dFUR; 5'-Deoxy-5-fluorouridine; DHF: Dihydrofolate; DHFR: Dihydrofolate reductase; DHFU: Dihydrofluorouracil; DPD: Dihydropyrimidine dehydrogenase; FdUMP: Fluorodeoxyuridine monophosphate; FdUTP: Fluorodeoxyuridine triphosphate; FUDP: Fluorouridine diphosphate; FUdR; Fluorodeoxyuridine; FUMP; Fluorouridine monophosphate; FUR: Fluorouridine; FUTP: Fluorouridine triphosphate; MTX: Methotrexate; RR: Ribonucleotide reductase; THF: Tetrahydrofolates; TK: Thymidine kinase; TP: Thymidylate phosphorylase; TS: Thymidylate synthase.

(FUTP). The main mechanism of 5-FU activation is via conversion to FdUMP, leading to TS inhibition and inhibition of the folate cycle and purine and pyrimidine synthesis. Inhibition of TS by FdUMP in the presence of 5,10-methylene tetrahydrofolate (CH_2THF) results in the depletion of thymidine triphosphate and the elevation of deoxyadenosine-5'-triphosphate (dATP), which induces DNA damage, S-phase arrest and apoptosis. The addition of exogenous folate in the form of folinic acid (leucovorin) increases the intracellular pool of CH_2THF, thereby enhancing FdUMP-induced TS inhibition. Thus, 5-FU with leucovorin is a standard combination to enhance the antineoplastic activity of 5-FU [27].

The conversion of 5-FU to FdUMP can occur directly via thymidylate phosphorylase (TP)-mediated conversion to fluorodeoxyuridine, followed by thymidine kinase-mediated conversion to FdUMP. FdUMP conversion can also occur indirectly through the conversion of fluorouridine or fluorouridine monophosphate to fluorouridine diphosphate (FUDP), and then ribonucleotide reductase (RR)-mediated conversion to FdUDP and FdUMP. FUDP and FdUDP

can also be converted to FUTP and FdUTP and incorporated into RNA and DNA, respectively, which can contribute to cytotoxicity by fluoropyrimidines. Incorporation of 5-FUTP into RNA interferes with RNA processing and is considered to be the primary mechanism of gastrointestinal toxicity. It is also a dose-limiting toxicity during continuous venous administration of 5-FU. Nevertheless, incorporation of FdUTP into DNA induces cytotoxicity, which is important in the chemotherapeutic response [28].

Dihydropyrimidine dehydrogenase (DPD)-mediated conversion of 5-FU to dihydrofluorouracil is the rate-limiting step in 5-FU catabolism. Because of this catabolism, 85% of dosed 5-FU is metabolized to α-fluoro-β-alanine, with an elimination half-life of 10 – 20 min, thus preventing its antitumor effect [29].Importantly, DPD inhibitors, such as gimeracil, inhibit DPD-mediated degradation of 5-FU, enhancing its antitumor activity. Because of its enhanced antitumor activity, DPD inhibitors have been added to combination therapies such as S-1, discussed below [30]. Importantly, continuous intravenous infusion of 5-FU for 24 – 120 h achieves steady plasma concentration and has more effective antitumor activity than intravenous bolus administration [31].

Oral administration of chemotherapeutic drugs can achieve steady plasma concentration and is beneficial in that it enables patients to receive treatment as outpatients and to maintain their quality of life. An oral formulation of fluorouracil was developed in the 1970s [32].Tegafur or ftorafur (1-(2-tetrahydrofuryl)-5-FU), an oral prodrug metabolized in the liver to 5-FU by cytochrome P450 2A6, was developed by Giller *et al.* [33,34]. In order to optimize the therapeutic activity of tegafur, the first DPD inhibitory fluoropyrimidine, tegafur-uracil (UFT), was developed, and tegafur and the DPD inhibitor uracil were combined at a molecular ratio of 1:4, respectively [35]. The addition of uracil to tegafur has been shown to enhance the fluorouracil concentration in tumor tissues versus normal tissues. Ota *et al.* reported in the results of a Phase II study that UFT is well tolerated, with antitumor activity in a wide variety of solid tumors [36]. Daily oral administration of UFT and leucovorin achieved similar antitumor efficacy in colon cancer compared with intermittent intravenous administration of 5-FU and leucovorin [27,37]. UFT is now approved in over 50 countries as a cancer therapy, most commonly for advanced colorectal cancer, to replace 5-FU.

S-1 is oral fluoropyrimidine that combines the 5-FU prodrug, tegafur, a DPD inhibitor, gimeracil and an orotate phosphoribosyltransferase (OPRT) inhibitor, oteracil potassium (Oxo), at a molecular ratio of 1:0.4:1, respectively. It was developed in Japan by Shirasaka *et al.* [30]. Animal experiments suggest that Oxo is distributed at high levels in the digestive tract after oral administration, thereby relieving the gastrointestinal toxicity induced by 5-FU [38,39]. Thus, S-1 improves the selectivity of 5-FU action, prolongs the efficacious 5-FU concentration in the blood through its DPD inhibitor, gimeracil, and reduces toxicity through the OPRT inhibitor, Oxo [40]. A pharmacokinetic study of orally administered S-1 by Hirata *et al.* revealed that S-1 has a similar effect to continuous intravenous infusion of 5-FU [39]. S-1 is now approved in Japan as a monotherapy for a wide range of cancers, such as gastric, colorectal, pancreatic and biliary tract [41]. S-1 has also been approved in other Asian countries, including Korea, China, Singapore, Hong Kong, Taiwan and Thailand. S-1 combined with cisplatin had similar effects as infusion of 5-FU with cisplatin in the FLAGS trial and was approved for the treatment of advanced gastric or gastroesophageal cancer in the EU in 2011 [42].

Capecitabine (N4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine), an orally administered 5-FU pro-drug, has been already approved in > 100 countries including many European and Asian countries. It was rationally designed to generate 5-FU predominantly within tumor cells [43,44]. It is converted to 5-FU by three enzymes located in the liver and tumors. It can be metabolized to 5′-deoxy-5-fluorocytidine by carboxylesterases in the liver, converted to 5′-deoxy-5-fluorouridine (5′dFUR) by the cytidine deaminases in the liver and tumor tissue or converted into 5-FU by thymidine phosphorylase (TP), which is present in high concentration in tumors and their microenvironment. When combined, capecitabine can have antitumor effects once metabolized. As capecitabine is at least equivalent to 5-FU in terms of safety and efficacy, it can be used as a substitute for intravenous 5-FU [45]. Combination of capecitabine and oxaliplatin has been shown to be consistent with FOLFOX (oxaliplatin plus infusion of 5-FU and leucovorin) treatment for patients with metastatic colorectal cancer. Recently, Hong *et al.* showed that a combination of S-1 plus oxaliplatin is also consistent with a combination of capecitabine and oxaliplatin as first-line chemotherapy in patients with metastatic colorectal cancer [46]. These results indicate that the oral 5-FU prodrugs capecitabine and S-1 can be a substitute for infused 5-FU.

3.2 Thymidine analogs

TAS-102 is a novel oral nucleoside antineoplastic agent consisting of the thymidine analog, FTD, and a thymidine phosphorylase inhibitor (TPI) (5-chloro-6-(2-iminopyrrolidin-1-yl) methyl-2, 4 (1H, 3H)-pyrimidinedione hydrochloride), which inhibits degradation of FTD by TP in the liver [47,48]. FTD was first synthesized by Heidelberger *et al.* in 1964 [49]. This group demonstrated that FTD can be phosphorylated by thymidine kinase to its active monophosphate form [50]. Importantly, in preclinical studies and clinical trials, TAS-102 was active in 5-FU resistant tumors [47,51]. TAS-102 has several mechanisms of action (**Figure 3**) [52]. FTD incorporates into DNA and can inhibit TS to induce cytotoxicity [23,47,53]. Further, TPI enhances the bioavailability of FTD and can also inhibit angiogenesis [54,55]. TP, which is inhibited by TPI, was originally identified as a platelet-derived endothelial cell growth factor, which is present in high concentrations in tumors and their microenvironment [56]. As TPI inhibits the proliferation of endothelial cells, the secretion of antiangiogenic factors by cells with high TP expression, and TP-induced

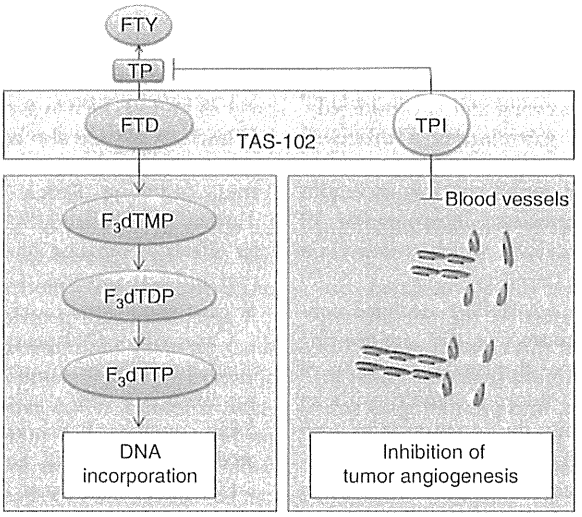

Figure 3. The mechanism of TAS-102 antitumor action. TAS-102 consists of FTD and a TPI. FTD is converted by thymidine kinase (TK) to its triphosphorylate form, F3dTTP, to incorporate into DNA and induce cytotoxicity. TPI inhibits thymidylate phosphorylase (TP) and suppresses the degradation of FTD to enhance the bioavailability of FTD. TPI also inhibits tumor angiogenesis, which is the proliferation of a network of blood cells that penetrates into cancerous growths, supplying nutrients and oxygen and removing waste products.

FTD: α,α,α-Trifluorothymidine; FTY: Trifluorothymine; F3dTDP: Trifluoromethyl deoxyuridine 5′-diphosphate; F3dTMP: Trifluoromethyl deoxyuridine 5′-monophosphate; F3dTTP: Trifluoromethyl deoxyuridine 5′-triphosphate; TP: Thymidylate phosphorylase; TPI: Thymidine phosphorylase inhibitor.

angiogenesis, TPI can be considered a potential antiangiogenic therapy [54,55,57]. The antitumor activity of TAS-102 in 5-FU-resistant tumors might be explained by the differences between FTD and 5-FU, as well as by the antiangiogenic effects of TPI.

In early clinical studies of FTD performed in the 1960s, different schedules of intravenous FTD administration were evaluated in patients with metastatic breast cancer and colorectal cancer. These studies showed that, although FTD had antitumor efficacy, it also causes severe myelosuppression and has a short elimination half-life. However, further clinical development of FTD has not been undertaken as the oral administration of FTD combined with TPI showed an improvement in the pharmacokinetic profile of FTD and the antitumor activity of FTD [48].

Several independent Phase I studies of patients with solid tumors were used to optimize TAS-102 dosage [58-62]. In 2012, Yoshino *et al.* presented evidence for the activity of TAS-102, as compared with placebo, for the treatment of patients with metastatic colorectal cancer who are refractory or intolerant to standard chemotherapy in a randomized comparative Phase II trial [51]. TAS-102 also appeared to be generally well tolerated. Importantly, these trials showed that the KRAS status may not directly affect the antineoplastic activity of TAS-102 because the mechanism of TAS-102 action involves direct incorporation of FTD into DNA. Based on the results of these clinical studies [51,62], TAS-102 was approved in Japan for the treatment of advanced metastatic colorectal cancer in March 2014. More recently, a global Phase III trial of TAS-102 in patients with refractory metastatic colorectal cancer met the primary efficacy end point of statistically significant improvement in overall survival versus placebo. The median overall survival time was 7.1 months (95% CI: 6.5 – 7.8) and 5.3 months (95% CI: 4.6 – 6.0) for TAS-102 and placebo-treated patients, respectively [63]. Future studies will help to delineate the mechanism of action of TAS-102 in tumors and the tumor microenvironment, and will identify biomarkers to predict those patients who would benefit most from treatment with TAS-102.

3.3 Cytidine analogs

Gemcitabine (2′,2′-difluoro 2′-deoxycytidine; dFdC) is an important cytidine analog for the treatment of gastrointestinal cancers, whereas other cytidine analogs, such as cytosine arabinoside (Ara-C), 5-azacytidine and 5-aza-2′-deoxycytidine (decitabine), are active in hematological malignancies. Gemcitabine was studied in a Phase I clinical and pharmacology trial in 1991, and has become an important drug for patients with several cancers, including pancreatic and non-small cell lung cancer [64,65]. The effect of gemcitabine is cell phase specific as it kills cells undergoing DNA synthesis and blocks the progression of cells through the boundary between the initial growth G1 phase and the S phase, in which DNA is synthesized [66].

Gemcitabine enters cells through the cell membrane via nucleoside transporters and is effectively accumulated in high concentrations in cells due to its relatively slow elimination half-life. Gemcitabine is converted intracellularly to the active metabolite, difluorodeoxycytidine, through a series of

sequential phosphorylations. In the first rate-limiting step, deoxycytidine kinase converts gemcitabine to gemcitabine monophosphate (dFdCMP). Subsequent phosphorylations lead to the accumulation of gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP), which are both active metabolites. dFdCTP can interfere with DNA synthesis by competing with endogenous dCTP for incorporation into replicating DNA. In contrast, dFdCDP is a potent inhibitor of RR, which results in inhibition of deoxyribonucleotide triphosphate synthesis, specifically dATP. Importantly, gemcitabine could be a potent radiation sensitizer [67,68]. *In vivo* and *in vitro* studies have demonstrated that gemcitabine can enhance the antineoplastic activity of ionizing radiation in various cancer cells [68-71]. In addition, results from clinical trials suggest that gemcitabine functions as a radiosensitizer in patients [67]. Recently, Loehrer *et al.* demonstrated improved overall survival with the addition of radiation therapy to gemcitabine treatment in patients with localized unresectable pancreatic cancer, with acceptable toxicity [72].

3.4 Folic acid analogs

MTX (2,4-diamino-N10-methyl propylglutamic acid) is the most studied folate antagonist and is an effective therapeutic agent for many malignancies, as well as autoimmune diseases [73]. MTX acts as an inhibitor of dihydrofolate reductase, which is the enzyme required for the maintenance of the intracellular pool of THF. As THF and its metabolite, CH_2THF, are required for the synthesis of purines and thymidylate, MTX interrupts the synthesis of DNA and RNA, as well as other metabolic reactions.

4. Platinum analogs

Platinum-containing antineoplastic drugs are coordination complexes of platinum and have been widely used in the treatment of a variety of human cancers. The cytotoxic potential of platinum compounds was discovered in 1965 by Rosenberg *et al.* [74]. They cause the crosslinking of DNA through the formation of various adducts, including monoadducts, interstrand crosslinks, intrastrand crosslinks and DNA–protein crosslinks in cancer cells. Additionally, they interfere with the replication of DNA to stop the division of the cells and induce cytotoxicity [75]. Cisplatin is activated intracellularly through aquation of one of the two chloride groups, and subsequently covalently binds to DNA, forming DNA adducts. Clinical development of platinum analogs had been started, including cisplatin in the 1970s, carboplatin in the 1980s and oxaliplatin in the early 2000s.

4.1 Cisplatin

Cisplatin (*cis*-dichlorodiammineplatinum (II)) is the prototype of the platinum family of agents used to treat cancer. Cisplatin chemotherapy is curative in testicular cancer and is effective in lung, gynecological, gastrointestinal and genitourinary cancers, as well as cancers of the head and neck. For instance, in advanced biliary cancer, combination therapy of cisplatin and gemcitabine was associated with a significant survival advantage without the addition of substantial toxicity compared with gemcitabine alone, in Phase III trial [76]. Nevertheless, cisplatin has significant limitations. It is often necessary to discontinue cisplatin treatment because of adverse toxicities, such as nephrotoxicity, gastrointestinal toxicity such as nausea and vomiting, neurotoxicity, hematological toxicity and irreversible ototoxicity. Furthermore, cisplatin is effective only for a specific range of cancers.

Resistance to cisplatin can result from decreased accumulation, increased inactivation by cellular glutathione or an increased ability of the cells to tolerate cisplatin–DNA adducts [75,77-79]. Decreased accumulation of cisplatin is induced by a decrease in the active transport of the drug into cells through the copper transporter CTR1. Alternatively, accumulation can be inhibited through increased drug export from the cells through the copper exporters, ATP7A and ATP7B, and the glutathione S-conjugate export GS-X pump (MRP2 or ABCC2). The increased ability of cells to tolerate cisplatin-damaged DNA is induced by an increase in nucleotide excision repair and decrease in DNA mismatch-repair activity.

In addition to cisplatin, multiple platinum derivatives were tested in clinical trials. To date, only a few platinum analogs, such as carboplatin (*cis*-diammine-[1,1-cyclobutanedicarboxylato] platinum (II)) and oxaliplatin (1*R*,2*R*-diaminocyclohexane oxalatoplatinum (II)), have received worldwide approval for cancer therapy.

4.2 Carboplatin

Carboplatin has nearly the same range of clinical efficacy as cisplatin and is less toxic to the kidneys and more toxic to the bone marrow [75]. As the chloride groups in carboplatin have been changed, resulting in better delivery to cells and fewer side effects, it overcomes cisplatin-related toxicities, such as nephrotoxicity and neurotoxicity [80,81]. Early clinical studies with carboplatin reported that carboplatin is not nephrotoxic and reduces emesis compared with cisplatin [82]. In 1989, carboplatin was approved by the United States Food and Drug Administration (FDA) for ovarian cancer, and it has replaced cisplatin in the treatment of several malignancies. Recently, van Hagen *et al.* showed that preoperative chemotherapy with carboplatin, paclitaxel and radiation improved survival among patients with potentially curable esophageal or esophagogastric-junction cancer compared with surgery alone [6].

4.3 Oxaliplatin

Oxaliplatin has broader spectrum of antineoplastic activity than cisplatin and has, at least partially, overcome cisplatin resistance [83]. Oxaliplatin was first reported by Kidani *et al.*, who showed that adding different amino groups than found in cisplatin resulted in the formation of a bulkier DNA crosslink [84]. Oxaliplatin also showed a different sensitivity profile

than cisplatin in the NCI 60-cell human tumor panel [85]. Whereas cisplatin is effective in upper gastrointestinal malignancies, such as esophageal cancer and stomach cancer, oxaliplatin is much more effective in colorectal cancer. One reason for this difference is that the accumulation of oxaliplatin seems to be less dependent on CTR1. Further, mismatch repair recognition proteins do not recognize oxaliplatin-DNA adducts. Finally, differences between oxaliplatin-DNA adduct structures and cisplatin-DNA adduct structures may affect the cancers in which they are effective [75,86-88].

A 1992 clinical study of oxaliplatin with an infusion of 5-FU and leucovorin showed promising effects in patients with metastatic colorectal cancer [89]. In 2002, oxaliplatin was approved in the US for the treatment of colorectal cancer. Oxaliplatin, in combination with other anticancer agents, is currently the standard of care for advanced stage colorectal cancer. Although oxaliplatin has not been extensively studied in other malignancies and its range of effectiveness is fully unknown, it has recently been used for the treatment of pancreatic cancer. Conroy *et al.* showed that the combination chemotherapy regimen consisting of oxaliplatin with irinotecan and an infusion of 5-FU with leucovorin (FOLFIRINOX) was associated with survival advantage in patients with metastatic pancreatic cancer compared with the first-line therapy, gemcitabine [90].

4.4 NC-6004

Regimens including cisplatin are widely used for cancers, including gastric, lung, testicular, gynecological and genitourinary [75,91]. Currently, the use of targeted drug delivery systems (DDS) is being investigated for the specific accumulation of drugs in tumors [92]. This drug-targeting method is based on the principles of enhanced permeability and retention, and it is hoped it will lead to the development of antineoplastic drugs with greater therapeutic effects and fewer adverse effects [93]. In this approach, the drug accumulates in the tumor tissue by taking advantage of the pathophysiological characteristics of the tumor, including hyperplasia and hyperpermeability of tumor blood vessels. These characteristics can facilitate the extravasation of nanoparticles containing chemotherapeutic drugs. Importantly, because the nanoparticles are too large to pass though the smaller holes found in healthy tissue, they are less prone to leak from intact blood vessels.

NC-6004 (nanoplatin) is an innovative new drug containing cisplatin-incorporated micellar nanoparticles, which are composed of PEG-poly (glutamic acid) block co-polymers through a polymer–metal complex. NC-6004 is expected to reduce the drug toxicity of cisplatin and to increase antitumor efficacy. The basic nanotechnology of this formulation was invented by Kataoka and Nishiyama *et al.* [94,95]. Preclinical development of NC-6004 has been in progress in Japan [96]. Matsumura and Maeda demonstrated in 1986 that polymeric micelles containing cisplatin are preferentially distributed to tumors through the enhanced permeability and retention effect [97]. Further, Uchino *et al.* showed that NC-6004 had significantly lower toxicity than cisplatin and greater antitumor activity [98]. On the basis of these results, the first administration of NC-6004 in patients with advanced solid tumors in Phase I clinical study has been carried out in the UK [99]. A Phase I/II clinical study of NC-6004 in patients with advanced pancreatic cancer has been completed in Taiwan and Singapore. A Phase III study combining NC-6004 and gemcitabine for the treatment of advanced pancreatic cancer is also ongoing in Taiwan, Singapore, Hong Kong, China and Korea.

5. Conclusion

The recent decade has shown marked progress in how cancer is studied and how new therapies are developed [2,7,8]. However, despite advances in the treatment of cancer, including gastrointestinal malignancies, many patients still succumb to their disease due to drug resistance. In addition, many agents that were promising in preclinical studies fail to demonstrate similarly promising clinical activity as single agents in clinical trials. One of the major challenges on the road toward improved prognosis lies in the identification of combinations of novel molecularly targeted agents with conventional chemotherapy, including DNA synthesis inhibitors that overcome drug resistance. There is an urgent need for future clinical trials designed around novel combination therapies to achieve a higher response rate and longer remissions. To date, there are a vast number of laboratory, preclinical and clinical studies of DNA synthesis inhibitors, as well as novel molecularly targeted agents that hint for a synergistic approach. Efforts to examine patient samples from both tumors and healthy tissues are important to identify biomarkers to improve patient classification and, if possible, introduce personalized therapy for gastrointestinal cancers [3,100]. Translational cancer research to develop novel cancer therapeutics in gastrointestinal cancers will depend on close collaboration between basic researchers and clinicians, which will help to identify biomarkers, overcome drug resistance and improve the prognosis of patients and their quality of life.

6. Expert opinion

More than 50 years after the appearance of DNA synthesis inhibitors, such as antimetabolites, these drugs remain the most active category of anticancer drugs available and the standard therapeutics that new drugs are compared with. Although there has been a shift toward developing novel, rationally designed and specific therapeutics, the prognosis of gastrointestinal cancer still remains poor due to drug resistance. There are novel molecularly targeted agents in gastrointestinal cancers, including tyrosine kinase inhibitors such as imatinib, sunitinib, egorafenib, erlotinib, as well as therapeutic monoclonal antibodies such as bevacizumab, cetuximab and trastuzumab. Therefore, efforts to discover novel agents, as well as

novel chemotherapy combinations using molecularly targeted agents with conventional antineoplastic agents, have become increasingly important.

There are many challenges that must be overcome to identify novel DNA synthesis inhibitors. These include identifying ways to specifically and efficaciously target tumor cells, reducing chemotherapeutic toxicity, the development of biomarkers to predict pharmacological responses, rationally designing and testing combination therapies, and overcoming drug resistance.

The development of DDS is one method that could improve the low specificity of DNA synthesis inhibitors in cancer cells. There are two main concepts in DDS, either active targeting or passive targeting. Active targeting involves monoclonal antibodies or ligands to tumor-related receptors. Passive targeting systems can be achieved through enhanced permeability and retention effects. NC-6004, a cisplatin-incorporated polymeric micelle, is a promising drug using DDS technology in gastrointestinal cancer. A Phase III study evaluating the combination of NC-6004 and gemcitabine in advanced pancreatic cancer is ongoing.

The identification of biomarkers that define drug sensitivity, as well as drug toxicity, is a promising therapeutic strategy. Importantly, appropriate clinical trial designs are necessary in order to identify biomarkers to predict the clinical responses to new drugs. Phase I studies are needed to establish that the new drug inhibits the target molecule in the tumor. Phase II or III studies are required to obtain data for determining predictive biomarkers that will identify patients with tumors that are affected by the drug, thus allowing for the development of therapy-specific diagnostic tests. Efforts to examine patient samples from not only tumors, but also normal tissues, by various methods based on biochemistry, genetics, cytogenetics and epigenetics are important to identify biomarkers to improve patient classification and, if possible, introduce personalized therapy for gastrointestinal malignancies. Caution is needed against over reliance on the biomarker strategy to predict drug sensitivity as intratumor heterogeneity has been identified in various cancers, including gastrointestinal malignancies, and has important implications for acquired drug resistance.

The challenges to improved prognosis can be found in the identification of both promising therapeutic agents and combination therapies to overcome drug resistance. Translational cancer research will design novel combination therapies rationally in order to achieve a higher response rate and longer remissions.

Translational research to develop novel cancer therapeutics in gastrointestinal tumors will depend on close collaboration between basic researchers and clinicians, which will help to identify biomarkers, overcome drug resistance and improve the prognosis of the patients and their quality of life.

Declaration of interest

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