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 over-winding 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
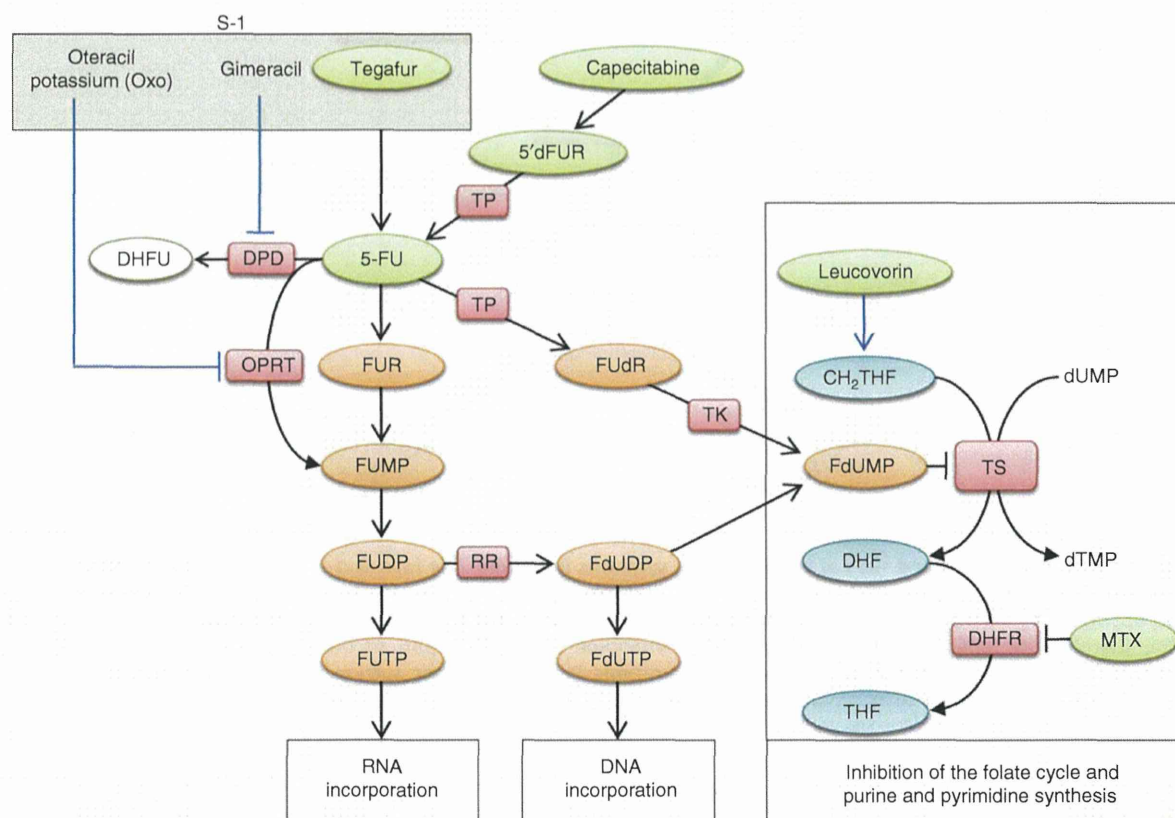
**Figure 2. Summary of the metabolism of fluoropyrimidines. 5-FU is converted to three active metabolites: FdUMP, FdUTP, and FUTP.** These active metabolites disrupt the synthesis of DNA and RNA and the action of TS. The main mechanism of 5-FU activation is conversion to FdUMP to inhibit TS, which plays 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 $\alpha$-fluoro-$\beta$-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, tegafururacil (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
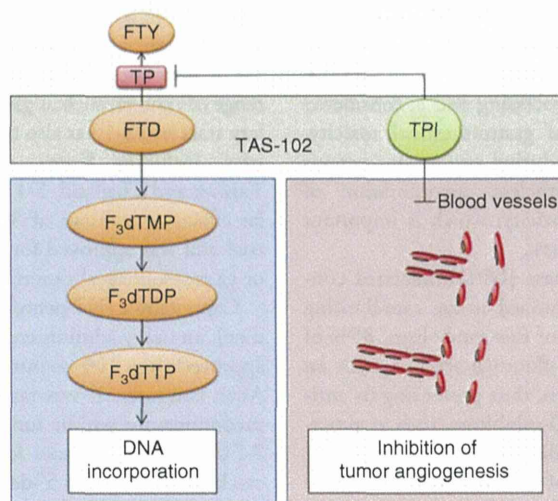
**Figure 3. The mechanism of TAS-102 antitumor action.** TAS-102 consists of FTD and a TPI. FTD is converted by thymidine kinase (TK) to its triphosphorylate form, F3dTTP, to incorporate into DNA and induce cytotoxicity. TPI inhibits thymidylate phosphorylase (TP) and suppresses the degradation of FTD 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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