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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

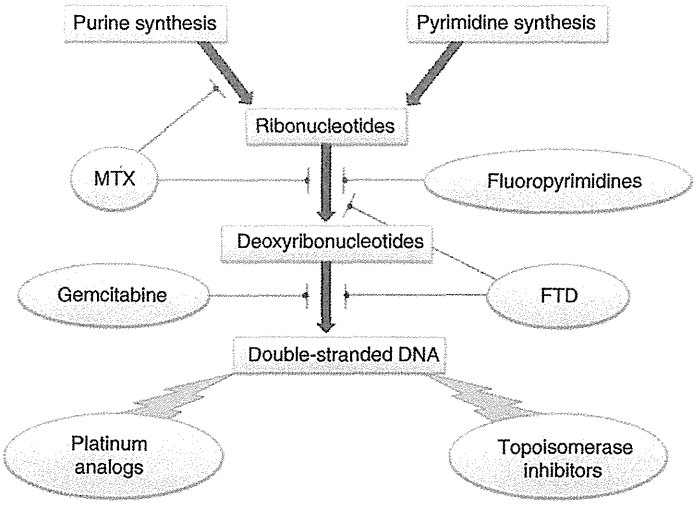

Figure 1. The mechanisms and sites of action of DNA synthesis inhibitors. The folic acid analog MTX inhibits dihydrofolate reduction, blocks thymidylate and purine synthesis, and interrupts the synthesis of DNA and RNA. Pyrimidine analogs fluoropyrimidines, such as 5-fluorouracil, inhibit thymidylate 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

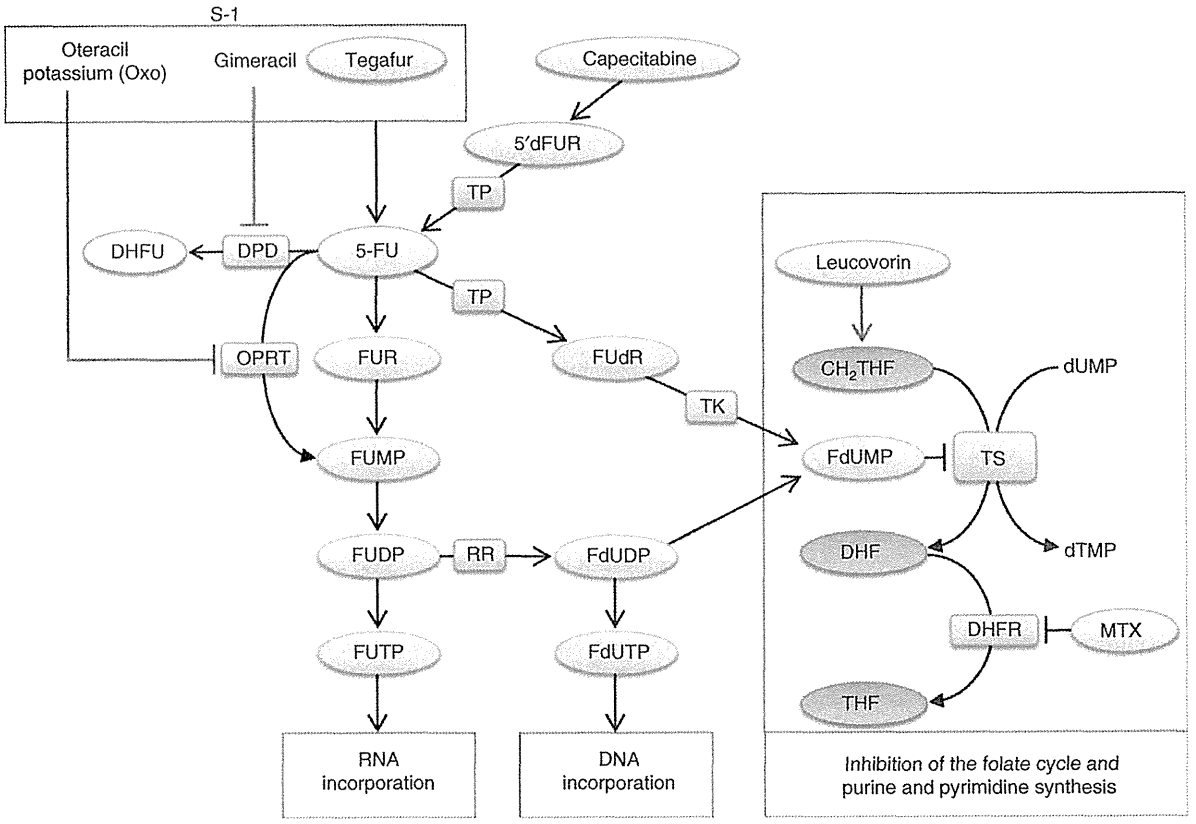

Figure 2. Summary of the metabolism of fluoropyrimidines. 5-FU is converted to three active metabolites: FdUMP, FdUTP, and FUTP. These active metabolites disrupt the synthesis of DNA and RNA and the action of TS. The main mechanism of 5-FU activation is conversion to FdUMP to inhibit TS, which plays 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

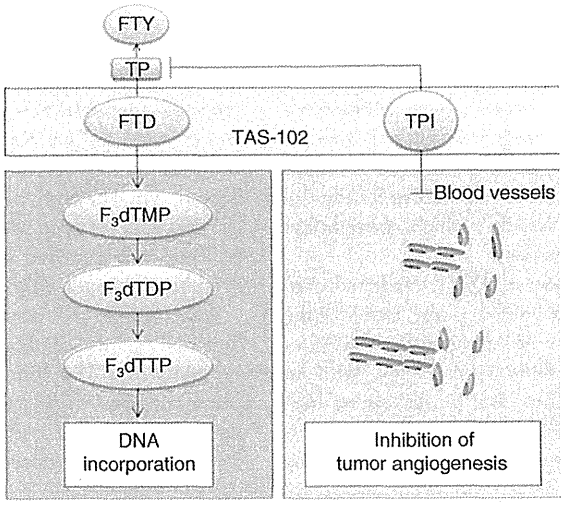

Figure 3. The mechanism of TAS-102 antitumor action. TAS-102 consists of FTD and a TPI. FTD is converted by thymidine kinase (TK) to its triphosphorylate form, F3dTTP, to incorporate into DNA and induce cytotoxicity. TPI inhibits thymidylate phosphorylase (TP) and suppresses the degradation of FTD to 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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High Level of Serum Soluble Interleukin-2 Receptor at Transplantation Predicts Poor Outcome of Allogeneic Stem Cell Transplantation for Adult T Cell Leukemia

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A B S T R A C T

The prognosis for adult T cell leukemia/lymphoma (ATL) is very poor, and only allogeneic hematopoietic stem cell transplantation (allo-SCT) has been considered to be a curative treatment for ATL. In this study, we retrospectively analyzed data for patients who had received allo-SCT for ATL in Hokkaido, the northernmost island of Japan, to determine prognostic factors. Fifty-six patients with a median age of 57 years received allo-SCT. Twenty-eight (50.0%) patients had acute type and 22 (46.4%) had lymphoma type. Twenty-three (41.1%) patients received allo-SCT in complete remission (CR), whereas the others were in non-CR. Seventeen (30.4%) patients received myeloablative conditioning and the others received reduced-intensity conditioning. With a median follow-up period of 48 months (range, 17 to 134 months), 1-year overall survival (OS) and 5-year OS rates were 55.4% and 46.1%, respectively. The survival curve reached a plateau at 22 months after stem cell transplantation (SCT). Male sex, high level of serum soluble interleukin-2 receptor (sIL-2R) at SCT, and non-CR at SCT were determined to be significant risk factors for OS. A high level of sIL-2R at SCT was a risk factor for poor OS in patients with non-CR at SCT by univariate analysis ($P = .02$), and it remained significant after adjustment by sex (hazard ratio, 2.73 [95% confidence interval, 1.07 to 7.90]). A high level of sIL-2R at SCT was also determined to be a risk factor for disease progression ($P = .02$). This region-wide study showed encouraging results for survival after allo-SCT for ATL and demonstrated for the first time that a high level of sIL-2R at SCT predicts worse SCT outcome.

INTRODUCTION

Adult T cell leukemia/lymphoma (ATL) is a peripheral T cell lymphoma caused by human T cell lymphotropic virus type 1 (HTLV-1), and the prognoses of aggressive subtypes (acute type and lymphoma type) of ATL are very poor [1]. Although only allogeneic hematopoietic stem cell transplantation (allo-SCT) has been considered to be a curative treatment for aggressive subtypes of ATL [2], less than 40% of patients who have received allo-SCT have been cured [3-5]. We previously reported excellent outcomes for ATL patients who received allo-SCT from 2 institutions in Hokkaido, the northernmost island of Japan [6], and overall survival (OS) rate in that study was 73.3% at 3 years after allo-SCT. We, therefore, conducted a region-wide retrospective study in this area to determine prognostic factors for patients with ATL who received allo-SCT.

PATIENTS AND METHODS

Collection of Data

Clinical data for 56 patients who received allo-SCT for ATL between January 2000 and March 2012 were collected from all stem cell transplantation (SCT) centers in Hokkaido, Japan. The patients included all patients with ATL who received allo-SCT in this area. This study was conducted with the approval of the institutional review board of Hokkaido University Hospital. Conditioning regimens and other procedures of SCT were performed according to the decision of the clinicians at each center.

Definitions

Shimoyama's classification was used for the definition of ATL subtypes [7]. Neutrophil engraftment and platelet engraftment were defined as the first of 3 days with absolute neutrophil count > .5 × 10^9/L and the first of 7 days with an untransfused platelet count > 50 × 10^9/L, respectively. The hematopoietic cell transplant comorbidity index was scored by the criteria previously described [8]. Acute graft-versus-host disease (AGVHD) and chronic GVHD (CGVHD) were graded by standard criteria [9,10]. Transplantation-related mortality (TRM) was defined as any death other than death from ATL. OS was calculated from the day of SCT until death or last follow-up. Progression of ATL was defined as relapse after remission, development of new lesions, or increase in measurable disease or in the number of

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circulating leukemic cells by 25% or more [11]. Progression-free survival (PFS) was defined as survival without progression of ATL.

Endpoint and Statistical Analysis

The primary endpoint of this study was OS rate of the patients. Descriptive statistical analysis was performed using the chi-square test or Fisher's exact test as appropriate for categorical variables and using the 2-sided Wilcoxon rank-sum test for continuous variables. The probabilities of OS and PFS were estimated using the Kaplan-Meier method. Disease progression rates and TRM rates were estimated using cumulative incidence analysis and considered as competing risks, and Gray's test was used for group comparison of cumulative incidence. The effects of various patient and disease categorical variables on survival probabilities were examined using the log-rank test, and the following variables were included in subgroup analyses: age of the patients, sex of the patients, levels of serum soluble interleukin-2 receptor (sIL-2R) at diagnosis and SCT, disease status at SCT, disease subtypes, months from diagnosis to SCT, levels of lactate dehydrogenase (LDH) at diagnosis and at SCT, donor, stem cell source, intensity of the conditioning regimen, GVHD prophylaxis, and CGVHD. All *P* values were 2-sided and a *P* value of .05 was used as the cutoff for statistical significance. Multivariate analysis for OS was performed using the Cox proportional hazards regression model.

RESULTS

Patients and Transplantation Characteristics

Patients and SCT characteristics are summarized in Table 1. The median age of the patients was 57 years, and one half of the patients were male. Twenty-eight (50.0%) patients had acute type and 22 (46.4%) patients had lymphoma type. HTLV-1 serostatus of donors were available in 47 patients, and only 2 (4.3%) donors were positive for HTLV-1. After induction chemotherapies that were mainly CHOP or VCAP-AMP-VECP regimens [12], 23 (41.1%) patients received allo-SCT in complete remission (CR) and 33 (58.9%) patients received allo-SCT in non-CR. Nineteen patients had high level of sIL-2R at SCT. Among the patients with high level of sIL-2R at SCT, only 1 patient was in CR at SCT and the other 18 patients were not in CR at SCT. There was a correlation between disease status at SCT and sIL-2R at SCT (median, 824 U/mL [range, 351 to 2530] in CR patients versus a median of 2325 U/mL [range, 435 to 37,384 U/mL] in non-CR patients; $P = .02$). Seventeen (30.4%) patients received myeloablative conditioning, which consisted of high-dose cyclophosphamide and total body irradiation with or without VP-16, and 39 (69.6%) patients received reduced-intensity conditioning, which consisted of fludarabine with either busulfan or melphalan ±low-dose total body irradiation of 2 to 4 Gray.

Table 1
Patient and Transplantation Characteristics

Characteristics (n = 56)	Value
Age, median (range), yr	57 (37-69)
Age ≥ 60	37 (66.1%)
Sex	
Male	28 (50.0%)
Female	28 (50.0%)
Disease subtype	
Acute	28 (50.0%)
Lymphoma	26 (46.4%)
Chronic	1 (1.8%)
Smoldering	1 (1.8%)
WBC count at diagnosis, median (range), per μL	10,900 (2500-331,000)
LDH level at diagnosis, median (range), IU/L	352 (144-1736)
sIL-2R level at diagnosis, median (range), per mL	11,153 (998-116,100)
Months from diagnosis to SCT, median (range)	196 (60-3690)
≤ 3 months	7 (12.5%)
3-6 months	17 (30.4%)
> 6 months	32 (57.1%)
Disease status at SCT	
CR	23 (41.1%)
Non-CR	33 (58.9%)
PR	19 (33.9%)
REF	10 (17.9%)
REL	4 (7.1%)
LDH level at diagnosis, median (range), IU/L	218 (150-766)
sIL-2R level at SCT, median (range), U/mL	1219 (351-37,387)
HCT-CI score	
0	22 (41.5%)
1	11 (20.8%)
2	9 (17.0%)
3	6 (11.3%)
4-7	5 (9.4%)
Donor	
MRD	20 (35.7%)
MUD	22 (39.3%)
MMD	14 (25.0%)
Sex disparity	
Match	32 (57.1%)
Mismatch	24 (42.9%)
Male to female	15 (26.8%)
Female to male	9 (16.1%)
Stem cell source	
BM	39 (69.6%)
PBSC	11 (19.6%)
CB	6 (10.7%)
Conditioning regimen	
MAC	17 (30.4%)
RIC	39 (69.6%)
TBI	
Yes	40 (71.4%)
No	16 (28.6%)
GVHD prophylaxis	
CSP+MTX	22 (39.3%)
TK+MTX	25 (44.6%)
CSP alone or TK alone	7 (12.5%)

WBC indicates white blood cell; LDH, lactate dehydrogenase; SCT, stem cell transplantation; sIL-2R, soluble interleukin-2 receptor; CR, complete remission; PR, partial remission; REF, primary refractory; REL, relapse; MRD, HLA-matched related donor; MUD, HLA-matched unrelated donor; MMD, HLA-mismatched donor; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; LDH lactate dehydrogenase; HCT-CI, hematopoietic cell transplant comorbidity index; TBI, Total body irradiation; GVHD, graft-versus-host disease; CSP, cyclosporin A; MTX, methotrexate; TK, tacrolimus. Data presented are n (%) unless otherwise indicated.

* HCT-CI score was not available in 3 patients.

† PBSC were from an MRD in all cases because donation of PBSC from unrelated donors is not permitted in Japan until 2010.

Transplantation Outcomes

Engraftment and GVHD

Except for 3 patients who died before engraftment, 53 (94.6%) patients achieved neutrophil engraftment at a median of 16 (range, 9 to 31) days. Platelet engraftment could be assessed in 52 patients, and 40 (76.9%) patients achieved platelet engraftment at a median of 27 (range, 14 to 415) days. All patients who achieved neutrophil engraftment were assessed for AGVHD. Overall AGVHD, grade II to IV AGVHD, and grade III to IV AGVHD occurred in 40 (75.5%), 31 (58.5%), and 8 (15.1%) of the evaluable patients, respectively. The median onset of AGVHD was 29 (range, 8 to 101) days. CGVHD was assessed in 43 patients who survived beyond day 100 after SCT. CGVHD occurred in 24 (55.8%) of the evaluable patients at a median onset day of 168 (range, 69 to 495) days, and extensive CGVHD occurred in 16 patients (37.2%).

Disease progression and TRM

Cumulative incidences of disease progression and TRM are shown in Figure 1. Fourteen (25.0%) patients showed disease progression at a median of 74 (range, 12 to 273) days after SCT. Twelve patients with disease progression after SCT died of ATL. One of the other 2 patients with disease progression died of a transplantation-related complication in

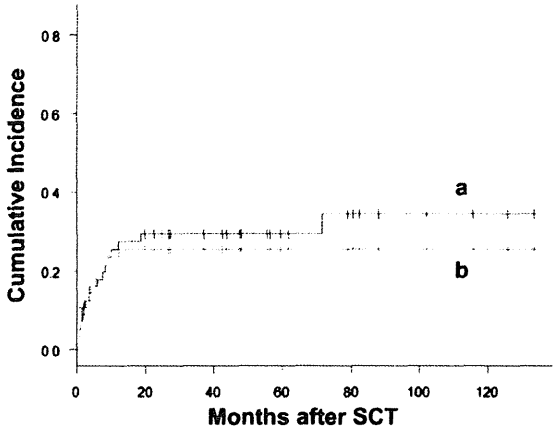

Figure 1. Cumulative incidence analyses of disease progression and TRM after SCT. Cumulative incidences of (a) TRM and (b) disease progression after allo-SCT. Disease progression and TRM were considered as competing risks.

remission and the other is alive in remission. The median time from disease progression to death was 92 (range, 32 to 399) days. Eighteen (32.1%) patients died of TRM at a median of 148 (range, 12 to 2143) days. The causes of TRM included infection (n = 6), AGVHD (n = 4), veno-occlusive disease (n = 2), CGVHD (n = 2), thrombotic microangiopathy (n = 1), cerebral infarction (n = 1), chronic renal failure (n = 1), and suicide (n = 1). Univariate analysis showed that a high level of sIL-2R at SCT (≥ 2000 U/mL) was significantly associated with disease progression ($P = .02$), whereas male sex tended to be associated with increased risk ($P = .06$). Non-CR at SCT was marginally significant for TRM ($P = .07$).

Survival

The median follow-up period for survivors was 48 (range, 17 to 134) months. One-year OS and 5-year OS rates were 55.4% and 46.1%, respectively. One-year PFS and 5-year PFS were 51.1% and 45.6%, respectively. The survival curve reached a plateau at 22 months after SCT (Figure 2). Male sex ($P = .002$), a high level of sIL-2R both at diagnosis (≥ 10,000 U/mL,

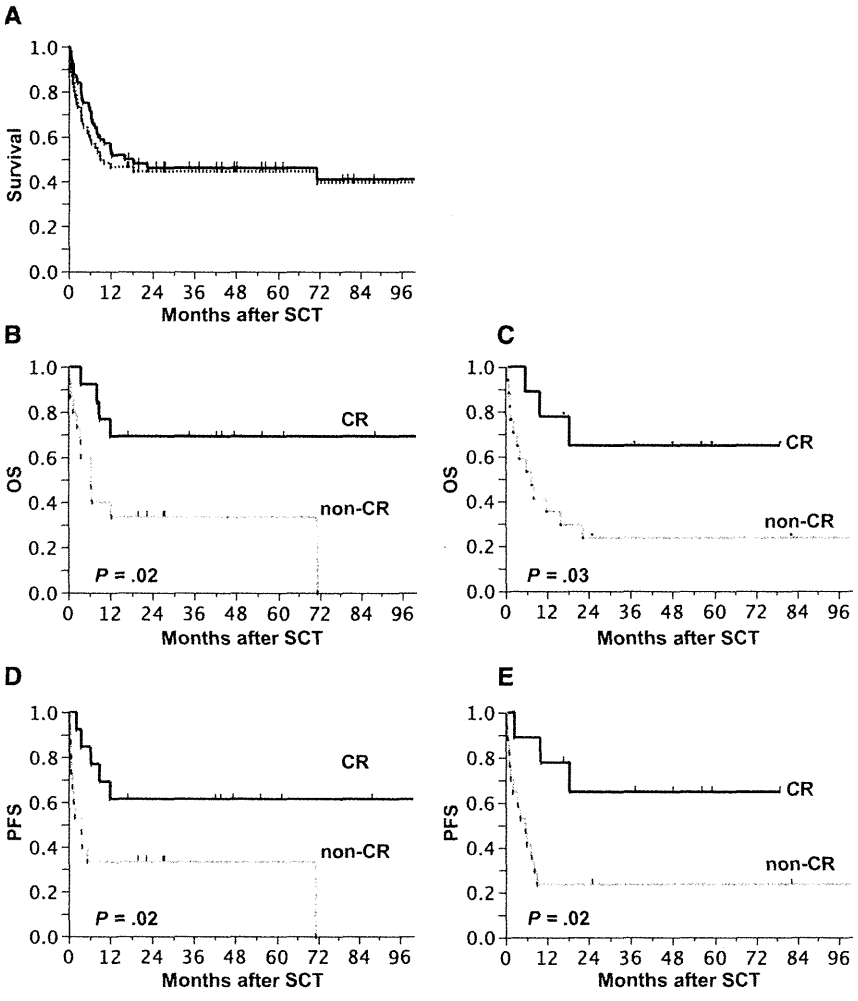

Figure 2. Survival after SCT. (A) Overall survival and progression-free survival after SCT in all patients. The solid line shows the overall survival curve and the dotted line shows the progression-free survival curve. (B) Overall survival in patients with acute type according to disease status at SCT. (C) Overall survival in patients with lymphoma type according to disease status at SCT. (D) Progression-free survival in patients with acute type according to disease status at SCT. (E) Progression-free survival in patients with lymphoma type according to disease status at SCT.

P=.02) and at SCT (≥ 2000 U/mL, P<.001) (Figure 3), and non-CR at SCT (P < .001) were identified as significant risk factors for OS by univariate analyses. Disease subtypes and other factors were not risk factors for OS. We tested several cutoff points of sIL-2R for determining the most significant cutoff points for survival and found that the cutoff levels of 10,000 at diagnosis and 2000 at SCT were most significantly associated with survival. Worse survival for male patients and patients in non-CR at SCT were confirmed by using multivariate analysis (hazard ratio, 3.40 [95% confidence interval (CI), 1.44 to 8.02] for male patients; hazard ratio, 4.45 [95% CI, 1.82 to 10.87] for non-CR patients). The levels of sIL-2R at SCT were not included in multivariate analysis, which included disease status, because the levels of sIL-2R at SCT were correlated with the disease status at SCT. In patients in non-CR at SCT, the level of sIL-2R was significantly associated with OS (P = .02) (Figure 3B), regardless of disease subtype (P = .02 for acute type and P = .01 for lymphoma type) (Figure 3C,D), and a high level of sIL-2R at SCT was determined to be a prognostic factor when it was used as an alternative variable to disease status at SCT in multivariate analysis (hazard ratio, 5.95 [95% CI, 2.14 to 17.9]). We performed multivariate analysis for non-CR patients using a level of sIL-2R at SCT and sex of the patients as variables, and a high level of sIL-2R at SCT remained significant even after adjustment by sex of the patients (hazard ratio, 2.73 [95% CI, 1.07 to 7.90]). The other variables were not confirmed to be significant by multivariate analysis.

DISCUSSION

A previous retrospective study on allo-SCT for ATL in Japan [4] demonstrated 3-year OS of 36.0%, and a prospective study on allo-SCT using a reduced-intensity conditioning regimen showed 5-year OS of 34.0% [5]. The 5-year OS rate in the present study was 46.1% and the survival curve reached a plateau at 22 months after SCT. Although the results of the present study are worse than the results we previously reported [6], the difference in results is probably due to the selection bias of the patients or might simply reflect a multi-institutional study versus a selected institutional study.

In previous nationwide studies on ATL in Japan, advanced age, male sex, non-CR at SCT, poor performance status, SCT from unrelated donors, or SCT using cord blood were associated with poor survival after allo-SCT [3,4]. Multivariate analysis in this study confirmed that male patients and patients in non-CR at SCT were at risk for poor OS. There were no differences in characteristics of the patients and SCT between male and female patients (data not shown), and the incidence of disease progression after allo-SCT was increased in male patients with marginal significance (P = .06). There has been no report showing worse survival in male patients after chemotherapy for ATL. It is thus tempting to speculate that this difference is due to the difference in allogeneic immune responses between male and female recipients after allo-SCT.

Although a high level of sIL-2R has been reported to reflect disease progression of ATL [13,14], the clinical significance of sIL-2R for patients who received allo-SCT remains to be determined. In this study, a high level of sIL-2R at SCT was identified as a significant risk factor for OS by univariate analysis. We did not include sIL-2R at SCT in the multivariate analysis because the level of sIL-2R at SCT was stringently correlated with disease status at SCT. However, a high level of sIL-2R at SCT was determined to be a prognostic factor when it was used as an alternative variable to disease status at SCT in multivariate analysis, and a high level of sIL-2R at SCT was a risk factor for OS in patients with non-CR at SCT, regardless of the sex of the patient. Only sIL-2R at SCT was identified as a risk factor for disease progression. Thus, sIL-2R at SCT could be a useful surrogate marker for disease status. Although transplantation outcomes in non-CR patients were inferior to those in CR patients, as has been previously reported [3,4], the level of sIL-2R at SCT was significantly associated with OS in non-CR patients, indicating that sIL-2R level at SCT could be used as a decision-making parameter for selection of allo-SCT for patients in non-CR. Additional chemotherapies or a

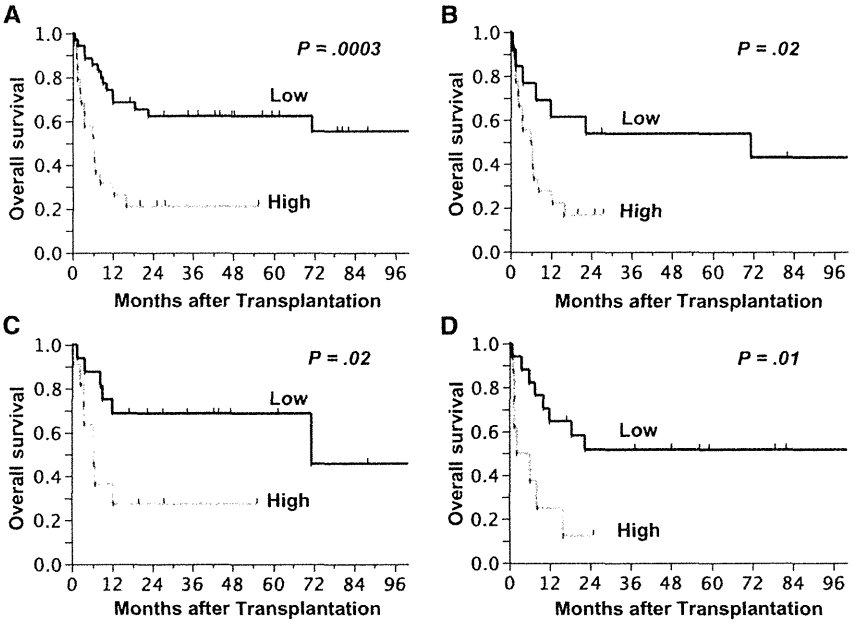

Figure 3. Overall survival according to level of serum sIL-2R at SCT. A high level of serum sIL-2R at SCT was defined as 2000 U/mL or higher. (A) All patients. (B) Patients who were in non-CR at SCT. (C) Patients with acute type of ATL. (D) Patients with lymphoma type of ATL.

novel anti-CCR4 antibody therapy [15] before SCT for patients who have high level of sIL-2R may improve the outcome of allo-SCT, although this hypothesis needs to be tested in a prospective study.

In conclusion, although the current study has several limitations that should be considered when reviewing the findings, including the use of a retrospective design and a small number of patients, it showed encouraging results of allo-SCT for patients with ATL in both CR and non-CR with low levels of sIL-2R at SCT.

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Epstein-Barr Virus–infected Cells in IgG4-related Lymphadenopathy With Comparison With Extranodal IgG4-related Disease

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Abstract: IgG4-related lymphadenopathy with increased numbers of Epstein-Barr virus (EBV)-infected cells has been reported but not fully described. We analyzed 31 cases of IgG4-related lymphadenopathy and 24 cases of extranodal IgG4-related diseases for their possible relationship with EBV. Other types of reactive lymph nodes (22) and angioimmunoblastic T-cell lymphoma (AITL) (10) were also studied for comparison. EBV-encoded RNA (EBER) in situ hybridization revealed EBER$^+$ cells in 18 of 31 cases (58%) of IgG4-related lymphadenopathy. Increased EBER$^+$ cells were found in only 4 of 22 (18.1%) non–IgG4-related reactive lymphoid hyperplasia in patients of a similar age ($P = 0.002$) and in only 5 of 24 (21%) extranodal IgG4-related biopsies ($P = 0.006$). Interestingly, all patients with EBER$^+$ progressively transformed germinal center–type IgG4-related lymphadenopathy had systemic lymphadenopathy and/or extranodal involvement. AITL also is associated with EBV, and IgG4-related lymphadenopathy sometimes mimics the morphology of AITL; however, the number of IgG4$^+$ cells in AITL was significantly less than that in IgG4-related lymphadenopathy ($P < 0.001$). Increased numbers of regulatory T cells are seen in IgG4-related disease; however, there was not a significant difference between the EBER$^+$ and EBER$^-$ cases. In conclusion, the presence of increased numbers of EBV-infected cells in IgG4-related lymphadenopathy, compared with other reactive lymphadenopathy or extranodal IgG4-related disease, suggests that there may be a relationship at least between nodal IgG4-related disease and EBV. It is important to avoid overdiagnosing these cases as malignant lymphomas or EBV-related lymphoproliferative disorders.

Key Words: IgG4-related lymphadenopathy, IgG4-related disease, Epstein-Barr virus

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IgG4-related disease is a recently recognized systemic syndrome with unique clinicopathologic features, which frequently include lymphadenopathy.[1] Five histologic patterns are recognized in lymph nodes. The type with progressively transformed germinal centers (PTGCs) has distinct clinical and pathologic features.[2] Although PTGC-type IgG4-related lymphadenopathy is often localized in submandibular lymph nodes and usually asymptomatic, as we previously reported, it usually persists and relapses, and some cases can progress to involve extranodal sites and/or have systemic lymphadenopathy. In other cases, prominent interfollicular expansion with increased immunoblasts and vascular proliferation are observed, which can mimic malignant lymphoma, especially angioimmunoblastic T-cell lymphoma (AITL).[1] Increased forkhead box P3-positive (FOXP3$^+$) regulatory T cells (Tregs) are usually observed in IgG4-related disease. Although the pathogenesis of IgG4-related disease remains to be solved, cytokines produced by Treg and type 2 helper T cells (Th2) are considered to play an important role.[3]

Epstein-Barr virus (EBV) is a common human herpes virus that infects >90% of the adult human population. After primary infection at an early age, the virus persists in a small population of B cells for life. Immunocompetent hosts are asymptomatic, and their lymph nodes show few EBV-encoded RNA–positive (EBER$^+$) cells, whereas those

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