TABLE 3. Surface Markers

	Pre (n = 25)	2 mo (n = 17)		7 mo (n = 7)	
	Median (Range)/μL	Median (Range)/μL	*P*	Median (Range)/μL	*P*
$CD14^+$ monocytes	234 (90–641)	376 (182–1090)	0.019*	294 (103–858)	0.16
$CD123^+$ DC $Lin1^-/CD123^+/HLA\text{-}DR^+$	6.4 (1.3–15.0)	10.5 (6.4–29.1)	< 0.001*	11.6 (9.7–35.6)	< 0.001*
$CD11c^+$ DC $Lin1^-/CD11c^+/HLA\text{-}DR^+$	13.7 (2.4–25.2)	16.1 (7.6–35.8)	0.024*	19.3 (13.2–37.7)	0.017*
NK-cell $CD3^-/CD16^+/CD56^+$	207 (91–1235)	95.6 (35.9–443)	0.004*	111 (28.4–868)	0.007*
B-cell $CD14^-/CD20^+$	178 (60.7–414)	149 (58.5–297)	0.032*	119 (49.2–201)	0.018*
$CD4^+$ T-cell $CD3^+/CD4^+/CD8^-$	619 (96.8–1652)	499 (151–959)	0.38	517 (123–869)	0.23
$CD8^+$ T-cell $CD3^+/CD8^+/CD4^-$	461 (159–811)	425 (161–856)	0.37	418 (261–549)	0.59
$CD3^+/CD4^+/CD25^+$	254 (90.7–825)	199 (53.4–510)	0.46	219 (62.0–462)	0.22
$CD4^+/CD25^+/GITR^+$	7.7 (0.29–17.6)	8.6 (1.8–27.6)	0.30	9.9 (1.0–34.0)	0.79

*Statistically significant ($P < 0.05$).
P: Statistical significance of values at 2 and 7 mo in comparison with values before (Pre) vaccination.
DC indicates dendritic cells; HLA, human leukocyte antigen; NK, natural killer.

of other patients.[27] WT1-specific lymphocytes were detectable by MLPC for the first time after 12 vaccinations in the first patient (UPN19), and no WT1-specific lymphocytes were detected throughout the course in the other patient (UPN10). Nevertheless, it is probable that the features of local immunologic status may differ from those of circulating lymphocytes in the peripheral blood.

No WT1-specific lymphocytes were detected on multimer staining in noncultured fresh whole blood. As WT1-specific lymphocytes were detected by MLPC methods, it is likely that the frequency of circulating WT1-specific lymphocytes was very low and below the detection level without expansion. WT1 vaccination is thought to have an expansion effect on precursor WT1-specific lymphocytes, as

TABLE 4. Mixed Lymphocyte Peptide Culture Analysis

No. Vaccination	Pre	2	4	6	12	30
Positive Rate	4% (1/25)	25% (5/20)	50% (8/16)	56% (5/9)	33% (2/6)	100% (1/1)
Pancreatic cancer						
1	0	0	5.26	1.86	0	
2	0	0.81				
3	0					
4	0	0	1.31	0	1.90	
5	0	0	2.75			
6	0	0				
7	0	0	0			
8	0	0	0			
9	0					
Gallbladder cancer						
10	0	0	0	0	0	
11	0	0	2.76			
12	0	0	0	1.40		
13	0	4.58				
14	0	0.56	3.02			
15	0	0				
16	0					
17	0					
Intrahepatic bile duct cancer						
18	0	29.40	9.40	0	0	3.87
19	0	0	0	0	0.35	
20	0	1.47	0.24	0.51		
21	0.16	0	0	5.29		
Extrahepatic bile duct cancer						
22	0	0	1.45	14.94	0	
23	0	0	0			
24	0	0	0			
25	0					

Percentages of Wilms tumor 1 $tetramer^+/CD8^+$ lymphocytes per $CD8^+$ lymphocytes are indicated.

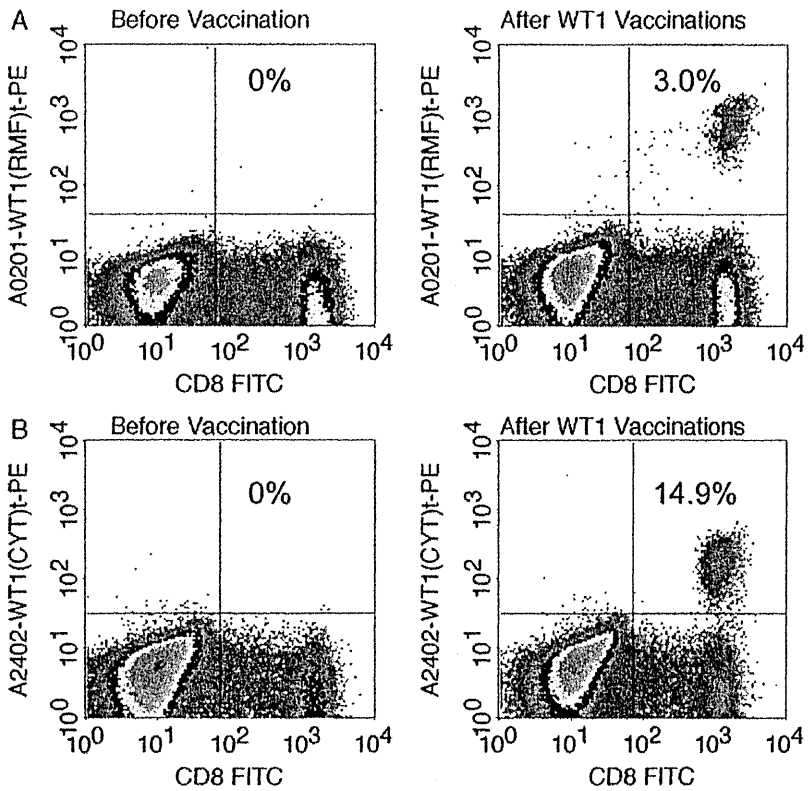

FIGURE 3. Representative results of mixed lymphocyte and peptide culture analysis. A, HLA-A 0201-positive patient with gallbladder cancer (UPN14). B, HLA-A 2402-positive patient with extrahepatic bile duct cancer (UPN22). No $CD8^+$ WT1-$tetramer^+$ cells were detected before vaccination therapy (left), whereas expansion of WT1-specific T lymphocytes was detected after vaccinations (right). FITC indicates fluorescein isothiocyanate; HLA, human leukocyte antigen; WT1, Wilms tumor 1.

only 1 patient showed positive results before vaccination, whereas 65% of patients showed positive results at least once after vaccination therapy. However, we were unable to show an apparent relationship between the therapeutic effects and the emergence of WT1-specific lymphocytes in this study. Furthermore, induction of WT1-specific lymphocytes required a long period of time in some patients, whereas WT1-specific lymphocytes disappeared during repetition of this combination therapy in some patients. Disappearance of WT1-specific T lymphocytes may be because of T-cell anergy. The optimal immunologic dose of WT1 vaccine may therefore differ among individual patients.

The WT1 peptide dose used in this study was larger than those used in other studies. The second dose level of 3 mg is the maximum dose that can be emulsified in a final volume of 600 μL, which we consider to be the maximum practical and realistic volume that can be injected intradermally at 6 sites (100 μL/site). The vaccine was injected intradermally to enhance immune reactivity, as the Langerhans cells that serve as antigen-presenting cells are distributed in the spinous layer of the epidermis. We were unable to determine the optimal dose for the WT1 vaccine, as the maximum tolerable dose may not be equivalent to the optimal dose, and a dose escalation study, as used in chemotherapy, is not applicable to cancer immunotherapy; thus, development of a realistic immunomonitoring system to determine the optimal vaccine dose is necessary.

Two types of 9-mer peptide, HLA-A02 and HLA-A24-restricted WT1 peptides, were used in this study. These peptides may be applied to the worldwide population, as HLA-A0201 and A2402 accounts for 57% of the Asian population, 56% of White population, and 17% of the African population.[28] The peptide earlier reported as HLA-A0201 restricted was applied to both HLA-A0201 and HLA-A0206 patients, as antigen-specific T cells against this peptide have been detected in relation to graft-versus-tumor effects in HLA-A0206-positive patients who had undergone hematopoietic stem cell transplantation in our earlier studies, thus suggesting the potency of this antigen in HLA-A0206 patients.[29] The HLA-binding motif prediction also showed that this peptide had a common anchor site with HLA-0206, which suggests that it could be applied to HLA-A0206 patients.[30]

In conclusion, although the aim of this study was to assess the safety of the combination of WT1 peptide vaccine and GEM in a small population, our observations indicated that this therapy is safe for patients with advanced pancreatic or biliary tract cancer and may provide long-term survival benefits in some patients.

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Review

Targeted Therapy for Biliary Tract Cancer

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Abstract: It is necessary to establish effective chemotherapy to improve the survival of patients with biliary tract cancer, because most of these patients are unsuitable candidates for surgery, and even patients undergoing curative surgery often have recurrence. Recently, the combination of cisplatin plus gemcitabine was reported to show survival benefits over gemcitabine alone in randomized clinical trials conducted in the United Kingdom and Japan. Thus, the combination of cisplatin plus gemcitabine is now recognized as the standard therapy for unresectable biliary tract cancer. One of the next issues that need to be addressed is whether molecular targeted agents might also be effective against biliary tract cancer. Although some targeted agents have been investigated as monotherapy for first-line chemotherapy, none were found to exert satisfactory efficacy. On the other hand, monoclonal antibodies such as bevacizumab and cetuximab have also been investigated in combination with a gemcitabine-based regimen and have been demonstrated to show promising activity. Furthermore, clinical trials using new targeted agents for biliary tract cancer are also proposed. This cancer is a relatively rare and heterogeneous tumor consisting of cholangiocarcinoma and gallbladder carcinoma. Therefore, a large randomized clinical trial is necessary to confirm the efficacy of chemotherapy, and international collaboration is important.

Keywords: biliary tract cancer; chemotherapy; molecular targeted agent

1. Introduction

Biliary tract cancer is rather common in Latin America and Asia, including Japan, while being relatively rare in European countries and the United States; approximately 16,000 patients in Japan and 5,000 patients in the United States are newly diagnosed as having this cancer each year [1-3]. The varied geographic distribution of the risk factors for biliary tract cancer, including primary sclerosing cholangitis, hepatolithiasis, congenital biliary cystic diseases, chemical agents, and hepatitis virus infections appears to contribute to the differences in the incidence rates among ethnic groups [1-4].

Bile duct cancer is subdivided according to the anatomic location of origin into intrahepatic cholangiocarcinoma, gallbladder cancer, extrahepatic cholangiocarcinoma and ampulla of Vater cancer. Although surgery currently remains the only potentially curative treatment for each of the aforementioned diseases, most patients are diagnosed at an unresectable advanced stage of the disease. While chemotherapy is applicable for all of these diseases, different carcinogenetic pathways and sensitivities to therapies have been demonstrated for each of them. The survival in patients with unresectable biliary tract cancer has been shown to differ by the tumor type, that is, gallbladder carcinoma, intrahepatic cholangiocarcinoma, or extrahepatic cholangiocarcinoma. It would, therefore, be ideal to conduct separate clinical trials in each cancer. However, it is not practical, because each of these biliary tract cancers is relatively rare. For the development of new chemotherapeutic regimens for biliary tract cancer, randomized clinical trials with an appropriate stratification strategy are required, including by the tumor types.

Despite the numerous phase II studies conducted of treatments for advanced biliary tract cancer, no accepted standard treatment for this tumor type has been established yet, because of the low incidence and small number of patients and the lack of adequately powered randomized controlled trials. A number of studies have investigated the usefulness of gemcitabine alone or tegafur/gimeracil/oteracil potassium (S-1) alone, and also gemcitabine-based combination regimens (Table 1) [5-22]. Based on the results of phase II studies, the Japanese guideline for biliary tract and ampullary carcinomas recommends gemcitabine alone or S-1 alone as the first line chemotherapy [23]. Recently, randomized controlled trials comparing the combination of cisplatin plus gemcitabine with gemcitabine alone have shown the survival benefit of the former regimen (Table 1) [24,25]. Furthermore, a randomized controlled trial among best supportive care, fluorouracil plus folinic acid and gemcitabine plus oxaliplatin (Gemox) revealed improved survival with Gemox in patients with unresectable gallbladder cancer as compared with best supportive care and fluorouracil plus folinic acid (Table 2) [26]. Thus, the combination of gemcitabine plus a platinum agent (cisplatin or oxaliplatin) has come to be recognized as standard therapy for unresectable biliary tract cancer.

One of the next issues that needs to be addressed is whether molecular targeted agents might also be effective against biliary tract cancer. Until date, no large clinical trials using targeted agents have been conducted for biliary tract cancer, however, some of these agents appear to offer promise. In this paper, the results of preclinical experiments and clinical trials of molecular targeted therapy for the treatment of biliary tract cancer are reviewed, and the possibilities and future directions of the use of targeted agents are discussed.

Table 1. Phase II studies of gemcitabine-based regimen for unresectable biliary tract cancer.

Regimen	n	Response Rate	Median Progression-Free Survival or Time-to-Progression	Median Overall Survival	Author (Year)
Gemcitabine	25	36.0%	-	6.9 mo	Gallardo (2001) [5]
Gemcitabine	32	21.9%	5.6 mo	11.5 mo	Penz (2001) [6]
Gemcitabine	30	30.0%	7.0 mo	14.0 mo	Tsavaris (2004) [7]
Gemcitabine	40	17.5%	2.6 mo	7.6 mo	Okusaka (2006) [8]
Tegafur/gimeracil/oteracil potassium (S-1)	40	35%	3.7 mo	9.4 mo	Furuse (2008) [9]
Gemcitabine/cisplatin	30	37%	4.1 mo	4.6 mo	Doval (2004) [10]
Gemcitabine/cisplatin	40	28%	4.7 mo	8.4 mo	Thongprasert (2005) [11]
Gemcitabine/cisplatin	29	35%	3.0 mo	11.0 mo	Kim (2006) [12]
Gemcitabine/oxaliplatin	33	35.5%	5.7 mo	15.4 mo	Andre´ (2004) [13]
Gemcitabine/oxaliplatin	31	26.0%	6.4 mo	11 mo	Harder (2006) [14]
Gemcitabine/oxaliplatin	67	14.9%	3.4 mo	8.8 mo	Andre´ (2008) [15]
Gemcitabine/oxaliplatin	40	15.0%	4.2 mo	8.5 mo	Kim (2009) [16]
Gemcitabine/oxaliplatin	43	18.9%	4.8 mo	8.3 mo	Jang (2009) [17]
Gemcitabine/capecitabine	45	31%	7.0 mo	14.0 mo	Knox (2005) [18]
Gemcitabine/capecitabine	45	32%	6.0 mo	14.0 mo	Cho (2005) [19]
Gemcitabine/capecitabine	75	29%	6.2 mo	12.7 mo	Riechelmann (2007) [20]
Gemcitabine/capecitabine	44	25%	7.2 mo	13.2 mo	Koeberle (2008) [21]
Gemcitabine/S-1	35	34.3%	5.9 mo	11.6 mo	Sasaki (2009) [22]

Table 2. Randomized clinical trials of gemcitabine-based regimens for unresectable biliary tract cancer.

Regimen	n	Response Rate	Median Progression-Free Survival or Time-to-Progression	Median Overall Survival	Author (Year)
Gemcitabine	206	15.5%	5.0 mo	8.3 mo	Valle (2010) [24]
Gemcitabine/cisplatin	204	26.1%	8.0 mo	11.7 mo	
Gemcitabine	42	11.9%	3.7 mo	7.7 mo	Okusaka (2010) [25]
Gemcitabine/cisplatin	41	19.5%	5.8 mo	11.2 mo	
Best supportive care	27	0	2.8 mo	4.5 mo	Sharma (2010) [26]
Fluorouracil/folinic acid	28	14.3%	3.5 mo	4.6 mo	
Gemcitabine/oxaliplatin	26	30.7%	8.5 mo	9.5 mo	

2. Preclinical Studies of the Molecular Biology of Biliary Tract Cancer

Some growth factors, including epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR), and various signal transduction pathways that play important roles in the progression, proliferation and metastasis of various cancers have been identified. Some studies have demonstrated overexpression of EGFR and VEGFR, or mutations of their signaling pathways in

biliary tract cancer [27]. Nonomura *et al.* [28] reported overexpression rates by 32.4% of EGFR, by 59.5% of EGF, and by 89.2% of ras p21 in 37 intrahepatic cholangiocarcinomas, with all the rates being statistically significantly different as compared with those in normal tissues. Recently, Yoshikawa *et al.* [29] demonstrated EGFR, VEGF and human epidermal growth factor receptor (HER) 2 overexpression in 27.4, 53.8 and 0.9% cases of intrahepatic cholangiocarcinoma, and in 19.2, 59.2 and 8.5% of cases of extrahepatic cholangiocarcinoma, respectively. They reported the existence of a correlation between the prognosis and EGFR expression, and the survival duration of EGFR-positive patients was significantly longer than that of EGFR–negative patients, both among cases of intrahepatic cholangiocarcinoma and extrahepatic cholangiocarcinoma. Furthermore, VEGF expression has been shown to be associated with intrahepatic metastasis in cases of intrahepatic cholangiocarcinoma [29].

Biliary tract cancer includes various types of cancers, each with different molecular biological characteristics. For example, overexpression of EGFR has been reported to be observed in 10.7%, 5.1%, 12.4% and 0% of cases of intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer and ampulla of Vater cancer, respectively [30]. Furthermore, overexpression of ErbB-2 has been reported in 0%, 5.1%, 15.7% and 11.5% of cases of intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer and ampulla of Vater cancer, respectively [30]. Relationships between the presence /absence of various gene mutations and the efficacy of molecular targeted agents have been identified in various cancers; for example, the efficacy of anti-EGFR antibody was limited to colorectal cancer patients with wild-type KRAS expression in the tumor [31]. There is as yet, however, no consensus on the molecular-biologic characteristics of biliary tract cancer.

Few preclinical studies of molecular targeted agents for biliary tract cancer have been reported. In an examination conducted using human cholangiocarcinoma cell lines, ZD6474, an inhibitor of VEGFR and EGFR signaling, showed promising anticancer activity [32]. This study revealed that the absence of KRAS mutation and presence of EGFR amplification may be potentially predictive molecular markers of the sensitivity of cholangiocarcinoma to EGFR-targeted therapy [32]. Thus, therapeutically beneficial effects of molecular targeted agents against biliary tract cancer are expected.

3. Clinical Trials of EGFR Inhibitors for Biliary Tract Cancer

Erlotinib is an orally active, potent, selective inhibitor of EGFR/HER1 tyrosine kinase, and a phase II study of erlotinib for unresectable biliary tract cancer has been reported. The results of this trial (response rate of 17% and median overall survival of 7.5 months) suggested a therapeutic benefit of EGFR blockade with erlotinib in patients with biliary tract cancer (Table 3) [33], however, no further investigation was conducted. Since lapatinib is an oral dual kinase inhibitor of EGFR and Her-2/neu, an antitumor effect of this agent against biliary tract cancer was expected. A phase II study of lapatinib for hepatocellular carcinoma and biliary tract cancer was conducted, however, no response was observed in patients with biliary tract cancer [34]. Thus, monotherapy with anti-EGFR inhibitors as first-line therapy may yield only minimum antitumor activity against biliary tract cancer. Thus, further investigation of anti-EGFR inhibitors does not appear to be warranted.

Table 3. Clinical trials of molecular targeted agents for unresectable biliary tract cancer.

Regimen	n	Response Rate	Median Progression-Free Survival or Time-to-Progression	Median Overall Survival	Author (Year)
Erlotinib	42	7%	2.6 mo	7.5 mo	Philip (2006) [33]
Lapatinib	17	0%	1.8 mo	5.2 mo	Ramananthan (2009) [34]
Gem/oxaliplatin/cetuximab	30	63%	8.8 mo	15.2 mo	Gruenberger (2010) [35]
Gem/oxaliplatin	51	16.7%(n=18)	~5.0 mo	-	Malka (2009) [36]
Gem/oxaliplatin/cetuximab	50	11.1%(n=18)	~7.0 mo	-	
Sorafenib	36	6%	2.0 mo	6.0 mo	El-Khoueiry (2007) [42]
Sorafenib	46	2%	2.3 mo	4.4 mo	Bengala (2010) [43]
Gem/oxaliplatin/bevacizumab	35	40%	7.0 mo	12.7 mo	Zhu (2010) [44]
Bevacizumab/erlotinib	53	12%	4.4 mo	9.9 mo	Lubner (2010) [45]

On the other hand, the effect of cetuximab, an anti-EGFR antibody, administered in combination with the Gemox regimen has also been investigated (Table 3) [35,36]. A phase II study of Gemox plus cetuximab showed promising efficacy, with a response rate of 63% and median overall survival rate of 15.2 months [35]. A randomized phase II study comparing Gemox plus cetuximab and Gemox alone is currently under investigation, and the results of an interim analysis were reported at the American Society of Clinical Oncology meeting in 2010 [36]. In regard to the relationship between KRAS mutation and the efficacy of cetuximab against biliary tract cancer, only 3 of the 30 patients in the phase II study of Gemox plus cetuximab had KRAS mutation in the tumor, with two of the three patients showing partial response and one showing stable disease. Thus, no definite correlation was noted between KRAS mutation and the treatment efficacy [35]. The sample size in this study was small, and further large-scale clinical trials of the combination therapy are needed to further clarify the efficacy and safety of cetuximab. Furthermore, the expression status of various key molecular targets, such as KRAS, B-raf and MEK, should be examined to identify patients with biliary tract cancer who may benefit from treatment with anti-EGFR monoclonal antibody.

4. Clinical Trials of Anti-Angiogenic Inhibitors for Biliary Tract Cancer

Vascular endothelial growth factor is related to angiogenesis and is reported as one of the important factors involved in the angiogenesis of various malignancies; VEGFR has been shown to promote the growth and metastasis of various cancers. Tumor vessels are structurally and functionally abnormal, contributing to increase of the interstitial fluid pressure within the tumor [37-39]. Anti-VEGF treatment results in pruning of the tumor vasculature, reduction in vessel tortuosity, and a drop in the interstitial fluid pressure, a process termed as vessel normalization [39]. Furthermore, it has been reported that combined use of a cytotoxic drug with anti-VEGF agent leads to a rapid decrease of the interstitial fluid pressure, which may enhance the delivery of chemotherapeutic agents to tumor

cells [39], thereby leading to tumor size reduction and improvement of the survival rates. Various inhibitors targeting VEGF or VEGFR have also been investigated for application to the treatment of biliary tract cancer.

Sorafenib, an oral multikinase inhibitor, blocks tumor cell proliferation mainly by targeting Raf/MAPK-ERK kinase/extracellular signal-regulated kinase signaling at the level of Raf kinase, and exerts an antiangiogenic effect by targeting VEGFR-2/-3. Sorafenib has been reported to exert antitumor effect on renal cell cancer and hepatocellular carcinoma, and also to show survival benefits in patients with these tumors [40,41]. Until date, the potential usefulness of sorafenib for biliary tract cancer has been investigated in two phase II studies, however, scant efficacy was noted in both trials, with a response rate of 2 and 6%, median progression-free survival of 2.0 and 2.3 months, and median overall survival of 4.4 and 6.0 months, respectively, in the two trials [42,43].

Bevacizumab, a recombinant humanized monoclonal antibody directed against VEGF, is an important therapeutic agent with promising effect against several malignancies, including colorectal, lung, breast and renal cell cancers, and has been investigated in phase II studies in combination with other agents for biliary tract cancer [44,45]. The combination of Gemox plus bevacizumab yielded promising results, with a response rate of 40% and median overall survival of 12.7 months [44]. Inhibition of VEGF and EGFR by bevacizumab and erlotinib was tested in a phase II study, and modest efficacy was noted, with a response rate of 12% and median overall survival of 9.9 months [45]. This trial was only preliminary and further investigation is expected.

The role of anti-angiogenic agents in the treatment of biliary tract cancer is still not clear. From the results of these clinical trials, anti-angiogenic agents do not appear to be promising. It is necessary to investigate the possible effect of anti-angiogenic agents against biliary tract cancer by conducting experiments *in vivo* for each agent.

5. Perspectives of Molecular Targeted Therapy for Biliary Tract Cancer

There are difficulties in conducting clinical trials for biliary tract cancer including large numbers of patients, as biliary tract cancer is a relatively rare disease, and the high frequency of complications such as obstructive jaundice or cholangitis make it difficult to recruit eligible patients. However, the curative resection rate is only 39.7%, on average, and according to retrospective studies, a half of the patients with unresectable biliary tract cancer receive chemotherapy in Japan [46,47]. Thus, chemotherapy plays an important role in the treatment of biliary tract cancer in clinical practice, and the development of an effective treatment(s) for biliary tract cancer is urgently needed. Various regimens containing molecular targeted agents are currently under investigation (Table 4).

Table 4. Currently ongoing clinical trials of molecular targeted agents for biliary tract cancer.

Agent	Study	n	Primary Endpoint	Country
Gemcitabine/oxaliplatin/ sorafenib	Phase I/II	58	Progression-free survival	USA
Gemcitabine/vandetanib or placebo	R-phase II	174	Progression-free survival	Italy
Gemcitabine/oxaliplatin/ erlotinib	Phase I	22	Maximum tolerated dose, recommended dose of erlotinib	USA
AZD6244	Phase II	35	Objective response rate	USA
Gemcitabine/oxaliplatin or Gemcitabine/oxaliplatin/ cetuximab	R-phase II	100	Progression-free survival	France
Gemcitabine/cisplatin/ cediranib or Gemcitabine/cisplatin/ placebo	Phase II/III	136	Progression-free survival	UK
Gemcitabine/oxaliplatin or Gemcitabine/oxaliplatin/ cetuximab	R-phase II	120	Objective response rate	Taiwan
Folfox/cediranib	Phase II	36	Objective response rate	USA
Gemcitabine/cisplatin/ selumetinib	Phase I/II	18	Safety and tolerability, recommended dose of selumetinib	UK
Gemcitabine/cetuximab	Phase II	43	Progression-free survival	Belgium
FOLFOX6/bevacizumab	Phase II	24	Progression-free survival	USA
Gemcitabine/irinotecan/ panitumumab	Phase II	45	Progression-free survival	USA
Gemox/panitumumab	Phase II	15	Objective response rate	USA
Gemcitabine/sorafenib or Gemcitabine/placebo	R-phase II	103	Progression-free survival	Germany
Gemcitabine/capecitabine/ bevacizumab	Phase II	50	Progression-free survival	USA
Gemcitabine/cisplatin/ sorafenib	Phase II	39	Progression-free survival	USA
Trastuzumab	Phase II	32	Objective response rate	USA
Bevacizumab/erlotinib	Phase II	55	Objective response rate	USA
Sunitinib	Phase II	59	Time to progression	Korea
Gemox/erlotinib or Gemox	Phase III	180	Time to progression	Korea
Bortezomib	Phase II	35	Objective response rate	USA
Sorafenib/erlotinib	Phase II	50	Progression-free survival	USA

As mentioned above, combination therapy with gemcitabine plus cisplatin or oxaliplatin has been established as the standard first-line treatment for biliary tract cancer. The next step is focused on the usage of molecular targeted agents. There are two directions in which targeted agents can be expected to be applied in the treatment of biliary tract cancer. One is combination with standard chemotherapy

regimens as first-line therapy. Currently, combined treatment using anti-EGFR antibody with cetuximab or bevacizumab has shown promising results in phase II studies of combination with Gemox. Although a randomized phase II study of cetuximab is currently under way, a large comparison study would be needed. The other is the use of monotherapy with targeted agents as 2nd line chemotherapy. Some preclinical experiences show that VEGFR or EGFR inhibitors administered alone might be effective in the treatment of biliary tract cancer. In many patients with progressive disease receiving first-line chemotherapy with the relatively toxic regimen of cisplatin plus gemcitabine or Gemox, the general condition is poor, and serious cholangitis can easily develop. Less toxic therapy, such as monotherapy with a targeted agent, may be useful in such patients.

Biliary tract cancer, which has a heterogeneous disease background, consists of cholangiocarcinoma, gallbladder carcinoma and ampulla of Vater cancer, each having different biologic features. Molecular targeted therapy should be established based on the biologic features, and it is important to identify the characteristic biologic features of each of the aforementioned types of cancer of the biliary tract. The number of patients with each of the cancer types of the biliary tract is small, and furthermore, patients with particular biologic features may be rare. However, efficient development of targeted therapy should be advanced based on the identification of appropriate biological markers.

6. Conclusions

Effective chemotherapy is necessary to improve survival of patients with biliary tract cancer. However, biliary tract cancer is a relatively rare disease compared with other gastrointestinal cancers such as colorectal cancer or gastric cancer. It makes large clinical trials difficult to conduct in a single country. Establishment of a new standard chemotherapy using molecular targeted agents is eager, and collaboration among global clinical trials is important.

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Lessons from the comparison of two randomized clinical trials using gemcitabine and cisplatin for advanced biliary tract cancer

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Contents

Abstract

There had been no standard chemotherapy established for advanced biliary tract cancer (BTC) until 2009, when the combination of cisplatin and gemcitabine (GC) was adopted as a first line standard chemotherapy option based on the results from two randomized studies: ABC-02, a

Keywords: Biliary tract cancer; Gemcitabine; Cisplatin; Randomized; Comparison

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UK investigator-initiated trial and the largest randomized phase III study in this tumor type with 410 patients; and BT22, a Japanese, industry-sponsored, randomized phase II study with 83 patients. In this review, investigators from both studies collaborated to compare protocols, patient characteristics, and outcomes of both studies including sub-analyses of study results. Although both studies showed GC combination therapy to be more effective than monotherapy, a detailed comparison revealed disparities between efficacy and safety end-points between the studies, which did not necessarily arise from different populations but from differences in protocol design. This review provides clinicians with insights for advanced BTC clinical study design and interpretation of historical studies.

1. Introduction

The incidence of biliary tract cancer (BTC) is high in Latin America and Asia, including Japan [1,2], whereas it is relative rare in European countries and the US: 5000 new cases are diagnosed every year in the US [3] and 1200 cases in the UK [4], respectively. The varied geographic distribution of risk factors for BTC, including primary sclerosing cholangitis, hepatolithiasis, congenital biliary cystic diseases, chemical agents, and hepatitis virus infections, contributes to differences in incidence rates between ethnic groups [1,5,6]. Despite this, intra-hepatic cholangiocarcinoma, in particular, has shown a steady increase in incidence in Japan, the US, the UK and Australia since the 1970s [7].

Bile duct cancer is subdivided according to the anatomic location of origin into intra-hepatic cholangiocarcinoma, gallbladder cancer (GBC), extrahepatic cholangiocarcinoma and ampulla of Vater cancer and different carcinogenetic pathways and sensitivities to therapy have been suggested for each subgroup [8,9].

Despite many phase II studies for advanced BTC [10–16], there had previously been no accepted standard treatment for this tumor type because of low incidence, inclusion of patients with additional tumor types (e.g. esophago-gastric, pancreatic and hepatocellular carcinomas), small patient numbers in each study and the lack of adequately powered randomized controlled studies. A number of studies had investigated the use of gemcitabine monotherapy [17–21] as an extrapolation of activity seen in pancreatic cancer. Studies using gemcitabine and cisplatin in combination soon followed; the dose and regimen of both drugs often varied or an additional third drug was added for a triplet combination [11,14–16,22–25]. In most studies, gemcitabine was used in a 21-day cycle at 1000 mg/m^2 with the addition of cisplatin in various schedules [11,14,16,22]. However, the lack of a prospective phase III study had failed to validate any one of these regimens.

The advanced biliary tract cancer (ABC)-01 and ABC-02 studies were the result of a UK-wide collaboration under the auspices of the National Cancer Research Network, funded largely by Cancer Research UK. The BT22 study was planned in Japan following promising results from the randomized phase II (ABC-01) study (which demonstrated superior 6-month progression-free survival [57.1% vs. 45.5%] with acceptable toxicity in favor of gemcitabine 1000 mg/m^2 plus cisplatin 25 mg/m^2 vs. gemcitabine 1000 mg/m^2 alone) [13] and the announced plan to expand this to a phase III (ABC-02) study powered to detect an improvement in overall survival. Although there were some concerns in Japan about adopting the gemcitabine and cisplatin regimen (in particular the apparent low dose of cisplatin), the BT22 study design was launched to verify the same dose and regimens used in ABC-02 in Japanese BTC patients. The results of both of these studies were presented at the same international oncology meeting (American Society of Clinical Oncology, Orlando 2009).

Despite different risk factors for BTC between Western countries and East Asia, both studies showed better outcome from combination therapy vs. monotherapy (median survival time (MST) 11.7 vs. 8.3 months, $p<0.001$; and 11.2 vs. 7.7 months, $p=0.139$, in ABC-02 and BT22, respectively, although due to the patient numbers involved in each study this comparison was only statistically powered for comparison in ABC-02). The combination of cisplatin and gemcitabine became an accepted standard treatment based on these results [26,27]. This manuscript, resulting from collaboration between UK and Japanese researchers compares the ABC-02 and BT22 studies with a focus on lessons for future advanced BTC clinical study design and interpretation of historical studies.

2. Patient selection

Both studies were designed for patients with advanced (inoperable, recurrent or metastatic) disease and, as shown in Table 1, the patients' characteristics were similar in terms of age, gender and primary anatomical site with the exception of the absence of patients with ampullary cancer in the gemcitabine arm of BT22 study.

2.1. Should patients with ampullary tumors be included?

"Biliary tract cancer" is a collective term including heterogeneous sub-groups as detailed in the introduction. Neither ABC-02 nor BT22 were powered for sub-group analysis according to site of origin. Adequately powered studies with each subgroup are unlikely to be feasible due to very small patient numbers and ampullary tumors, in particular, are thought to behave differently from other BTCs. With this in mind, patients being randomized into ABC-02 were stratified at study entry for site of primary to ensure balance between treatment arms in the final analysis. If this is consistently done for future studies, it may be possible to meta-analyze data from phase III studies (depending on design) accord-

Table 1
Patients' characteristics.

	ABC-02		BT22	
	GC	G	GC	G
	$N=204$	$N=206$	$N=41$	$N=42$
Age (year, median)	64	63	65	66.5
Gender				
Male	47%	48%	44%	50%
Female	53%	52%	56%	50%
Primary site				
Gall bladder	36%	37%	37%	41%
Bile duct	60%	58%	54%	60%
Ampulla	4%	5%	10%	0%
ECOG PS				
0	32%	31%	83%	67%
1	54%	57%	17%	33%
2	13%	12%	0%	0%

GC: gemcitabine plus cisplatin; G: gemcitabine; ECOG PS: Eastern Cooperative Oncology Group performance status.

ing to subgroup and patients with ampullary tumors should, therefore, continue to be included.

2.2. *Should patients with poorer performance status be excluded from studies?*

A retrospective analysis had previously suggested little survival benefit of chemotherapy in Japanese patients with GBC and poor performance status (defined as PS $\geq$ 2) [28]. Thus, there were differences in eligibility criteria between ABC-02 and BT22 in that ABC-02 allowed patients with PS-2 to be enrolled whereas BT22 was limited to PS-0 and PS-1 patients. There were more PS-0 patients in the Japanese vs. the UK study (Table 1). Even though the number of PS-2 patients were small (12–13% in each arm) in ABC-02, exploratory subgroup analysis revealed that patients of all PS benefitted from combination therapy although, due to the smaller numbers ($n=51$) the 95%-confidence interval is wide (Fig. 1A). There does, however, appear to be a trend towards greater benefit with improved performance score [26].

The inclusion of PS-2 patients in BTC trials should be determined according to the expected toxicities of the drugs (with safety as a major concern); the regimen of gemcitabine 1000 mg/m^2 plus cisplatin 25 mg/m^2 (on days 1 and 8 of a 21-day regimen) was shown to be tolerable by PS-2 patients in ABC-02 and can therefore be applied to patients with this performance status.

However, a poor performance score may be a reflection of a patient's underlying BTC in addition to other factors. Where a reduced performance score is due solely to underlying, untreated BTC a patient may potentially benefit from gemcitabine and cisplatin therapy; and therefore PS-2 patients should not be excluded based on performance status alone.

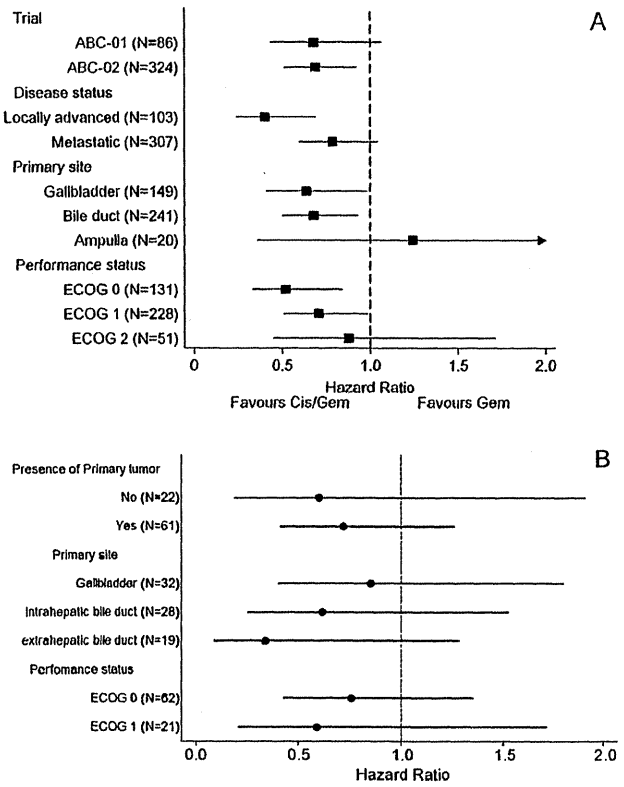

Fig. 1. Exploratory subgroup analysis in ABC-02 (A) and BT22 (B).

2.3. *What degree of liver dysfunction is acceptable for treatment of patients with gemcitabine and cisplatin?*

There is no guideline of eligibility criteria for liver dysfunction in clinical studies. For clinical studies in oncology, the language commonly used for inclusion criteria is 'patients who have adequate organ function' and adequate hepatic function is defined as total bilirubin $\leq$1.5 $\times$ upper limit of normal (ULN); ALT and/or AST and alkaline phosphatase $\leq$2.5 $\times$ ULN in general. However, because of the nature of the disease, liver function is frequently deranged in advanced BTC patients with or without the presence of liver metastases. Therefore, in the ABC-02 study, eligibility included "adequate liver function" defined as total bilirubin $\leq$1.5 times ULN; ALT and/or AST and alkaline phosphatase $\leq$5 times ULN. In BT22 this was marginally different: bilirubin $\leq$2 times ULN (and $\leq$3 times ULN if liver metastases were present), ALT/AST: $\leq$3 times ULN (and $\leq$5 times ULN if liver metastases were present).

In order to set the inclusion criteria of the study, liver toxicity due to the tested drugs should be taken into consideration. The product circular for gemcitabine reads that gemcitabine should be used with caution in patients with hepatic or renal insufficiency as there is insufficient information from clinical studies to allow for clear dose recommendations for these patient populations. With the limited information from clinical trials, treatment-related liver toxicity is not common with

gemcitabine treatment and Grade 3/4 liver dysfunction was not detected in the clinical trials with gemcitabine monotherapy for bladder cancer and non-small cell lung carcinoma [29–31]. Neither did gemcitabine at a fixed-dose-rate infusion cause additional toxicity in the patients with impaired hepatic function [32]. The predominant toxicity of cisplatin is renal rather than hepatic. In the phase I study of gemcitabine in patients with hepatic dysfunction, patients with AST elevations tolerated gemcitabine without increased toxicity, but patients with elevated bilirubin levels (1.6–7.0 mg/dL) had significant deterioration in liver function after gemcitabine therapy and authors suggested that dose reduction of gemcitabine for patients with elevated bilirubin levels [33].

Thus, in future clinical trials in BTC the same degree of AST elevation used for ABC-02 study can be applied to patients with deranged liver function, regardless of the presence or absence of liver metastases, unless there are specific safety concerns based on the pharmacokinetics (in particular hepatotoxicity) of the investigational medicinal product in question.

2.4. Which stratification factors should be employed for clinical trials for biliary tract cancer?

The use of stratification factors aims at balance of known prognostic variables between treatment arms in a randomized study. In ABC-02, randomization was stratified by performance score (PS 0–1 vs. 2), disease status at entry (locally advanced vs. metastatic disease), primary anatomical tumor site, previous treatments (surgery, biliary stenting, radiotherapy, none) and treating center; while in BT22 primary site (GBC vs. other BTC) and the presence or absence of primary tumor were used as stratification factors.

Patients with GBC receiving the combination of cisplatin and gemcitabine had a better survival in both studies. However, the impact of gallbladder as the primary tumor site on survival was different: in keeping with a number of previous reports that have suggested a poorer prognosis for patients with GBC (vs. other BTC) [10,21,34–38] the patients with GBC in BT22 also had a shorter survival time than the non-GBC patients in the study (Fig. 2B). No such survival difference was observed in the larger ABC-02 study (Fig. 2A), the results of which would suggest that it is not necessary to stratify by primary tumor site (as all sub-groups appear to benefit equally). However, continued stratification in future studies will allow this issue to be studied in more detail.

Tumor recurrence following prior resection was handled differently between the studies; being defined as a specific sub-group in BT22 but included under "metastatic" in ABC-02. Although the number of patients in BT22 is small, the prognosis appears to be different between patients with local recurrence compared to patients with non-resectable disease up-front. The impact of this variable should, therefore, be evaluated in future studies. In the gemcitabine monotherapy arm of BT22 study, MST for patients without primary tumor was 12.7 months, in comparison to 7.4 months for patients with primary tumor. Similarly, in the combination therapy arm of BT22 study, MST for patients without primary tumor was better than those with primary tumor (16.1 months vs. 9.4 months) [27]. The impact of this variable should, therefore, be evaluated in future studies. With the available evidence, stratification factor for consideration include: PS, disease status at entry (locally advanced vs. metastatic vs. recurrent), primary tumor site, previous treatment and treatment center.

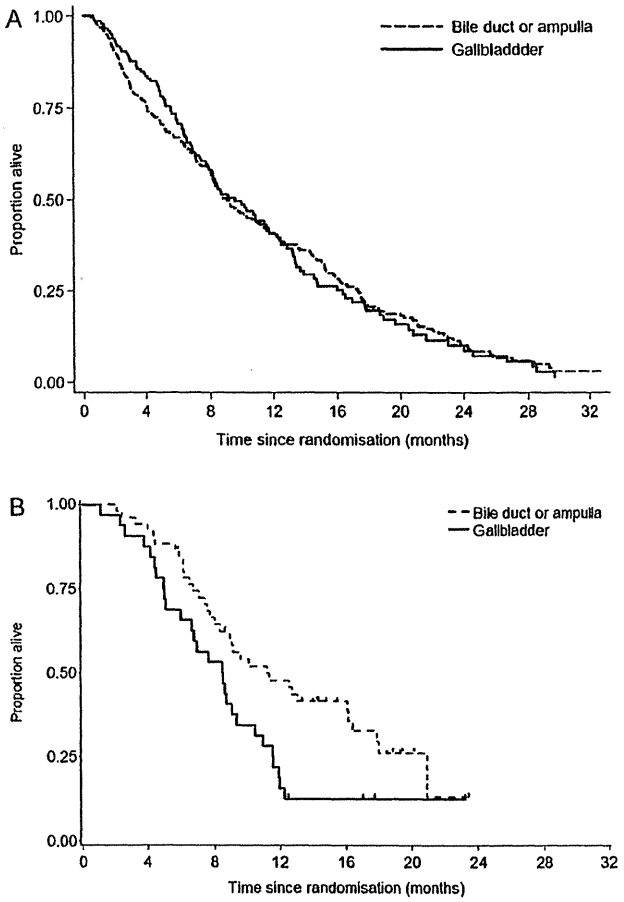

Fig. 2. Kaplan-Meier curves for overall survival comparing gallbladder and non-gallbladder patients in ABC-02 (A) and BT22 (B).

3. Protocol design

3.1. What is the optimal duration of treatment for future clinical trials?

The intended treatment periods were different between the two studies: 24 weeks in ABC-02 and 48 weeks in BT22. Neither study individually aimed to assess the duration of treatment (≤24 weeks vs. >24 weeks); and comparison between the studies needs to be undertaken with caution. A number of themes do emerge, however. The median duration on treatment was similar 13.0 weeks and 16 weeks for gemcitabine monotherapy and 19.7 weeks and 18 weeks

Table 2
Summary of treatment efficacy.

	ABC-02		BT22	
	GC	G	GC	G
	$N=204$	$N=206$	$N=41$	$N=42$
Median duration of treatment	19.7 weeks	13.0 weeks	18 weeks	16 weeks
Median PFS	8.4 months	6.5 months	5.8 months	3.7 months
Median OS	11.7 months	8.3 months	11.2 months	7.7 months
Post-study treatment	17.7%	17.5%	73.2%	78.6%
Tumor response	$N=132$	$N=148$	$N=41$	$N=42$
CR	0.7%	0.8%	0%	0%
PR	25.0%	15.2%	19.5%	11.9%
SD	53.4%	53.4%	48.8%	38.1%
PD	18.9%	25.0%	22.0%	40.5%
Response rate (CR + PR)	25.7%	15.9%	19.5%	11.9%
Disease control rate (CR + PR + SD)	79.1%	71.2%	68.3%	50.0%

GC: gemcitabine plus cisplatin; G: gemcitabine; PFS: progression free survival; OS: overall survival; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

for cisplatin/gemcitabine in ABC-02 and BT22, respectively, regardless of doubling the intended duration of treatment in BT22. Continuous therapy is known to increase the risk of cumulative toxicity and, of note, all patients in BT22 with a partial response at 48 weeks had already achieved this response by 24 weeks. In clinical practice, the risk to benefit ratio of treatment beyond 24 weeks needs to be based on individual patient's circumstances including ongoing tumor responsiveness to chemotherapy, cumulative toxicities, performance status, quality of life and patient choice. Within clinical trials flexibility needs to allow for occasional patients to continue therapy if they are still responding to treatment or where, in the treating clinician's opinion, ceasing treatment is considered undesirable.

3.2. Deciding on optimal frequency of radiographic assessment

In clinical trials for BTC (often sponsored by pharmaceutical companies), 6-weekly efficacy assessments are most commonly used [16,34,35,39,40] although the frequency can vary from 4 to 12 weeks [10,14]. Tumor imaging by CT or MRI was performed at 12 weeks and 24 weeks in ABC-02 (where overall survival was the primary end-point) and every 6 weeks in BT22 (where one-year survival rate was the primary end-point). This difference in scanning frequency contributed towards a shorter observed PFS in BT22 than that observed in ABC-02 (Table 2).

The adoption of a new therapy or regimen as a standard treatment option usually depends on demonstration of a survival advantage in a prospective phase III study. However, within phase II studies a number of end-points are used as "surrogates" including response rate and PFS, amongst others. PFS (a composite endpoint that includes death and evidence of radiological progression) has been shown to correlate better with overall survival than response rate in BTC [36]. More frequent scanning (e.g. every 6–8 weeks), resulting in a more accurate PFS is therefore appropriate within phase II studies. However, in routine daily practice, a 2–3 month-interval of CT is reasonable and feasible and ABC-02 has demonstrated that 12-weekly scanning is sufficient within phase III studies.

In future, overall survival may also be influenced by the emergence of second-line therapies with increased interest in the treatment of advanced BTC. Should this become the case, study design (balancing scientific accuracy and patients' best interests) will need to reflect this and PFS (with more frequent imaging) may become a critical end-point in phase III studies for each line of therapy.

3.3. Ensuring appropriateness of toxicity comparisons

At first glance, Japanese patients experienced greater toxicity (particularly hematological toxicity) in BT22 than the UK patients in ABC-02 (Table 3). However, the frequency of laboratory tests was different between the studies: patients in BT22 were recalled weekly for toxicity assessment, including the treatment-free week of every cycle; this was not the case for ABC-02. In BT22 study, 21 patients in GC arm (12 decreased platelets, 4 decreased hemoglobin, 5 neutropenia) and 9 patients in G arm (3 decreased hemoglobin, 5 neutropenia, and 1 increased platelets) experienced Grade 3/4 hematotoxic events only on the treatment free weeks, which might contribute to the increased frequency of adverse events shown in Table 3. This is an important issue to bear in mind when interpreting data from historical studies.

Hematological toxicities, including neutropenia, are common in gemcitabine-based chemotherapy. Previous studies of cisplatin and gemcitabine combination chemotherapy in other tumor types demonstrated that day 15 is the nadir for this combination [41–43]. Despite this, the detection of the nadir count does not necessarily alter clinical management; for example, prophylactic antibiotics are not routinely used in the UK and the ABC-02 study protocol did not, therefore, mandate day 15 testing for the gemcitabine plus cisplatin arm.