ความสัมพันธ์ของภาวะพหุสัณฐานของยีน *RRM1 ERCC1* และ *CTR1* กับการตอบสนองต่อการรักษา
และการเกิดพิษจากยาเคมีบำบัดเจมไซตาบีนและแพลทินัมในผู้ป่วยมะเร็งท่อน้ำดี
ที่ไม่สามารถผ่าตัดได้

นายสกลชาติ พงษ์มณีรัตนกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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ASSOCIATION OF *RRM1, ERCC1* AND *CTR1* POLYMORPHISMS WITH RESPONSE AND TOXICITIES OF GEMCITABINE-PLATINUM CHEMOTHERAPY IN PATIENTS WITH UNRESECTABLE CHOLANGIOCARCINOMA

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Faculty of Pharmaceutical Sciences
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Thesis Title	ASSOCIATION OF *RRM1, ERCC1* AND *CTR1* POLYMORPHISMS WITH RESPONSE AND TOXICITIES OF GEMCITABINE-PLATINUM CHEMOTHERAPY IN PATIENTS WITH UNRESECTABLE CHOLANGIOCARCINOMA
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สกลชาติ พงษ์มณีรัตนกุล : ความสัมพันธ์ของภาวะพหุสัณฐานของยีน *RRM1 ERCC1* และ *CTR1* กับการตอบสนองต่อการรักษาและการเกิดพิษจากยาเคมีบำบัดเจมไซตาบีนและแพลทินัมในผู้ป่วยมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้ (ASSOCIATION OF *RRM1, ERCC1* AND *CTR1* POLYMORPHISMS WITH RESPONSE AND TOXICITIES OF GEMCITABINE-PLATINUM CHEMOTHERAPY IN PATIENTS WITH UNRESECTABLE CHOLANGIOCARCINOMA) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ภญ. ดร.ธิติมา เพ็งสุภาพ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ภญ. ดร.ณัฏฐดา อารีเปี่ยม, อ. นพ. สืบพงศ์ ธนสารวิมล, 189 หน้า.

ยาเคมีบำบัดเจมไซตาบีนและแพลทินัมจัดเป็นสูตรยารักษามาตรฐานในการรักษาผู้ป่วยโรคมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้ แต่สูตรยามีข้อเสียคือการตอบสนองที่ต่ำและมีความหลายหลากในแต่ละบุคคล ในปัจจุบันมีหลายงานวิจัยที่ทำการศึกษาตัวชี้วัดเพื่อนำมาทำนายผลการตอบสนองจากสูตรยา แต่ผลการศึกษาเหล่านั้นยังมีความขัดแย้งกันอยู่

การศึกษาแบบ cohort นี้ ทำเพื่อศึกษาผลของภาวะพหุสัณฐานของยีน *RRM1 ERCC1* และ *CTR1* ต่อการตอบสนองของยาและการเกิดพิษจากยาเคมีบำบัดเจมไซตาบีนและแพลทินัมในผู้ป่วยโรคมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้ เลือดของผู้ป่วย 33 คนถูกวิเคราะห์หาชนิดของภาวะพหุสัณฐานด้วยวิธี Taqman allelic discrimination assay ผู้ป่วยได้รับการประเมินการตอบสนองและการเกิดพิษตามการประเมินของ RECIST version 1.1 และ CTCAE version 4.03 ตามลำดับ

ผู้ป่วยที่ได้รับการประเมิน 31 คนมีอัตราการตอบสนองร้อยละ 9.7 และ อัตราการคุมโรคร้อยละ 71 การศึกษานี้ไม่พบความสัมพันธ์ของภาวะพหุสัณฐานของยีน *RRM1 ERCC1* และ *CTR1* และการตอบสนองต่อการรักษา แต่พบว่าภาวะพหุสัณฐานของ *CTR1* มีผลต่อโอกาสเพิ่มขึ้นของ alkaline phosphatase (p=0.035)

เมื่อนำภาวะพหุสัณฐานของยีนมาศึกษาร่วมกัน ผลการศึกษาพบว่าภาวะพหุสัณฐานของยีน *RRM1* และ *CTR1* มีความสัมพันธ์กับความรุนแรงของภาวะเม็ดเลือดขาวต่ำ (p=0.048) และโอกาสเกิดภาวะเม็ดเลือดขาวชนิด neutrophil ต่ำ (p=0.039) ภาวะพหุสัณฐานของยีน *ERCC1* และ *CTR1* (CC/GG) มีผลต่อการตอบสนองต่อยาเคมีบำบัด (p=0.018) นอกจากนี้ผู้ป่วยที่มีภาวะพหุสัณฐานแบบ CT/GT ก็มีโอกาสเกิดภาวะเม็ดเลือดขาวต่ำชนิด neutropenia (p=0.024) และน้ำหนักลดลง (p=0.010) มากกว่าผู้ป่วยกลุ่มอื่นเช่นกัน

จากผลการศึกษาอาจสรุปได้ว่าภาวะพหุสัณฐานของยีนดังกล่าวมีความสัมพันธ์กับการตอบสนองและการเกิดพิษจากยาเคมีบำบัด ซึ่งอาจนำไปใช้เป็นตัวชี้วัดในการเลือกสูตรยาที่เหมาะสมให้กับผู้ป่วยโรคมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้ที่ได้รับสูตรยาเจมไซตาบีนและแพลทินัม

ภาควิชา เภสัชกรรมปฏิบัติ
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SKOLCHART PONGMANERATANAKUL: ASSOCIATION OF *RRM1, ERCC1* AND *CTR1* POLYMORPHISMS WITH RESPONSE AND TOXICITIES OF GEMCITABINE-PLATINUM CHEMOTHERAPY IN PATIENTS WITH UNRESECTABLE CHOLANGIOCARCINOMA. ADVISOR: ASSOC. PROF. THITIMA PENGSUPARP, Ph.D., CO-ADVISOR: NUTTHADA AREEPIUM, Ph.D., SUEBPONG TANASANVIMON, M.D., 189 pp.

Gemcitabine-platinum chemotherapy is the treatment of choice for unresectable cholangiocarcinoma, however, the response rate is still low and varies among individuals. Several studies have been investigated the biomarkers which can predict the response of treatment, but results are controversial.

This is a cohort study to assess the association of *RRM1*, *ERCC1*, and *CTR1* polymorphisms with response and toxicities of gemcitabine-platinum chemotherapy in unresectable cholangiocarcinoma patients. DNA from 33 patients were extracted and genetic polymorphisms were assessed by Taqman allelic discrimination assay. After third cycles of chemotherapy, treatment response and toxicities were evaluated according to RECIST version 1.1 and CTCAE version 4.03, respectively.

The response rate and tumor control rate for 31 evaluable patients was 9.7% and 71%, respectively. There were no association between genetic polymorphisms and response rate as well as tumor control rate. The results showed that *CTR1* polymorphisms were related with risk of alkaline phosphatase increasing (p=0.035). For further analysis, genetic polymorphisms were combined to observe their effects. *RRM1* and *CTR1* polymorphisms was related with risk of neutropenia (p=0.039) and severity of leukopenia (p=0.048). *ERCC1* polymorphism in combination with *CTR1* polymorphism (CC/GG) was associated with response rate (p=0.018). Carriers of CT/GT showed higher risk of neutropenia (p=0.024) and weight loss (p=0.010) than carrier of the other genotypes.

Our results suggest that genetic polymorphism combinations were related with toxicities and may be potential biomarkers for unresectable cholangiocarcinoma patients treated with gemcitabine-platinum regimen.

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Field of Study: Clinical Pharmacy
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Introduction

1.1 Background and rational

Cholangiocarcinoma (CCA) is epithelial tumors presented with cholangiocyte differentiation. They can be classified as intrahepatic (iCCA), perihilar (pCCA), or distal (dCCA) by anatomic location. CCA has poor prognosis with a median survival of 24 months after diagnosis. The 5-year survival rate is only 5%-10%. The global incidence of CCA during 1977 to 2007 varies by region, from rates of 85/100,000 in Thailand to 0.4/100,000 in some Western countries. Data from the several tumor registries in Thailand during 1998 to 2000 have shown that the incidence rates of CCA are ranged between 7.3/100,000 in Prachuap Khiri Khan and 113.4/100,000 in Udon Thani in men and between 2.1/100,000 in Songkla and 49.8/100,000 in Udon Thani in women, respectively. There are several risk factors for CCA, such as hepatobiliary fluke infection, hepatolithiasis, primary sclerosing cholangitis, and hepatitis B virus [1-4]. Besides CCA, gallbladder cancer and ampullary cancer were included in bile tract cancer. These two cancers were investigated with CCA in most of the studies because anatomic relation, similar metastatic pattern and rarity [5].

Treatment options for CCA are limited and overall survival rates are low. The only curative treatment option is surgical resection that provided not only 26 months of disease- free survival, but also 60%-65% of recurrence rates for iCCA. Recurrent factors include vascular invasion, lymph node metastasis, multiple tumors, and cirrhosis. After resection, patients with iCCA showed higher 5-year survival rate than patients with pCCA or dCCA. CCA is frequently diagnosed after metastatic or locally advanced tumor infiltrations are already presented due to non-specific symptoms and silent clinical characteristics. As a result, many patients are not candidates for surgical resection. Locoregional therapy including transarterial chemoembolization and radiofrequency ablation has only been investigated in small and mostly retrospective trials. The standard of care for advanced stage CCA and gallbladder cancer is systemic chemotherapy with gemcitabine and platinum [1, 2, 6].

Chemotherapy based on gemcitabine and platinum compound such as cisplatin or carboplatin is commonly used and counted as the standard treatment for unresectable CCA and the other bile tract cancer. A randomized phase III study showed that gemcitabine and cisplatin chemotherapy significantly improved median overall survival (OS) compared with single gemcitabine (11.7 vs 8.1 months, respectively, $p< 0.001$) without addition toxicity [7]. Another phase II trial demonstrated 10.6 months of OS in advanced CCA and another bile tract cancer patients treated with gemcitabine and carboplatin. The results are similar to the study using gemcitabine and cisplatin [8].

Although gemcitabine and platinum, especially cisplatin, chemotherapy has been considered as first line treatment in CCA patients, its efficacy is varies among individual and still unsatisfactory [9, 10]. While many patients response, some develop resistance to gemcitabine and cisplatin chemotherapy. Many researchers attempt to improve the clinical outcome of cancer patients by identification of biomarkers. Biomarkers with high predictive and prognostic value may maximize the therapeutic benefit of chemotherapy. Clinical studies focused on the efficacy of gemcitabine and cisplatin chemotherapy have revealed several candidate biomarkers, including single nucleotide polymorphisms (SNPs) of ribonucleotide reductase subunit M1 gene (*RRM1*), excision repair cross-complimentary group1 gene (*ERCC1*), and copper transporter1 gene (*CTR1*).

In previous studies, some researchers found that polymorphism of *RRM1* at position (-) 37 (rs12806698) and (-) 524 (rs11030918) are associated with response to gemcitabine. For example, Kim and colleagues in 2008 found the association of treatment response to gemcitabine and *RRM1* polymorphisms in Korean patients with non-small cell lung cancer (NSCLC) [11] and Yuan's group in 2015 also found relationship between *RRM1* polymorphisms and outcome of gemcitabine and cisplatin chemotherapy in Chinese patients with NSCLC [12]. In contrast, Feng and co-workers in 2009 found that *RRM1* polymorphisms are not associated with treatment response in NSCLC patients treated with gemcitabine and cisplatin [13].

Some studies suggest that polymorphisms of *ERCC1* (rs11615) and *CTR1* (rs12686377) are related to response to the treatment with cisplatin. Several studies

found the association between *ERCC1* polymorphisms and the treatment response of cisplatin-based chemotherapy in Chinese patients with NSCLC [14-16]. Palomba and colleagues also reported the effect of *ERCC1* polymorphisms on overall survival in breast cancer patients treated with platinum-based chemotherapy [17]. However, many studies showed no effect of *ERCC1* polymorphism on response to treatment and overall survival [18-20]. One study showed association between *CTR1* polymorphism and clinical outcome in NSCLC patients treated with cisplatin [21]

To date, no published reports or studies provide information regarding to the relationship between *RRM1*, *ERCC1*, and *CTR1* polymorphisms and both response and toxicities of gemcitabine-platinum chemotherapy in unresectable CCA patients. The objective of this study is to investigate the impacts of *RRM1*, *ERCC1*, and *CTR1* polymorphisms in unresectable CCA patients treated with gemcitabine-platinum chemotherapy.

Findings from this study may provide more knowledge about impacts of those drug metabolizing proteins' polymorphisms on the treatment response and/or toxicities. This knowledge could be used to consider the most effective and safest treatment regimen for CCA patients.

1.2 Research question

Are there any differences in treatment response rate and toxicities among CCA patients treated with gemcitabine-platinum chemotherapy in various polymorphisms of *RRM1*, *ERCC1*, and *CTR1*?

1.3 Objective

To investigate the impacts of *RRM1*, *ERCC1*, and *CTR1* polymorphisms on the treatment response and toxicities from gemcitabine-platinum chemotherapy in unresectable cholangiocarcinoma patients.

1.4 Hypothesis

1. *RRM1* polymorphism is associated with response of gemcitabine-platinum chemotherapy.
2. *RRM1* polymorphism is associated with toxicity of gemcitabine-platinum chemotherapy.
3. *ERCC1* polymorphism is associated with response of gemcitabine-platinum chemotherapy.
4. *ERCC1* polymorphism is associated with toxicity of gemcitabine-platinum chemotherapy.
5. *CTR1* polymorphism is associated with response of gemcitabine-platinum chemotherapy.
6. *CTR1* polymorphism is associated with toxicity of gemcitabine-platinum chemotherapy.

1.5 Conceptual framework

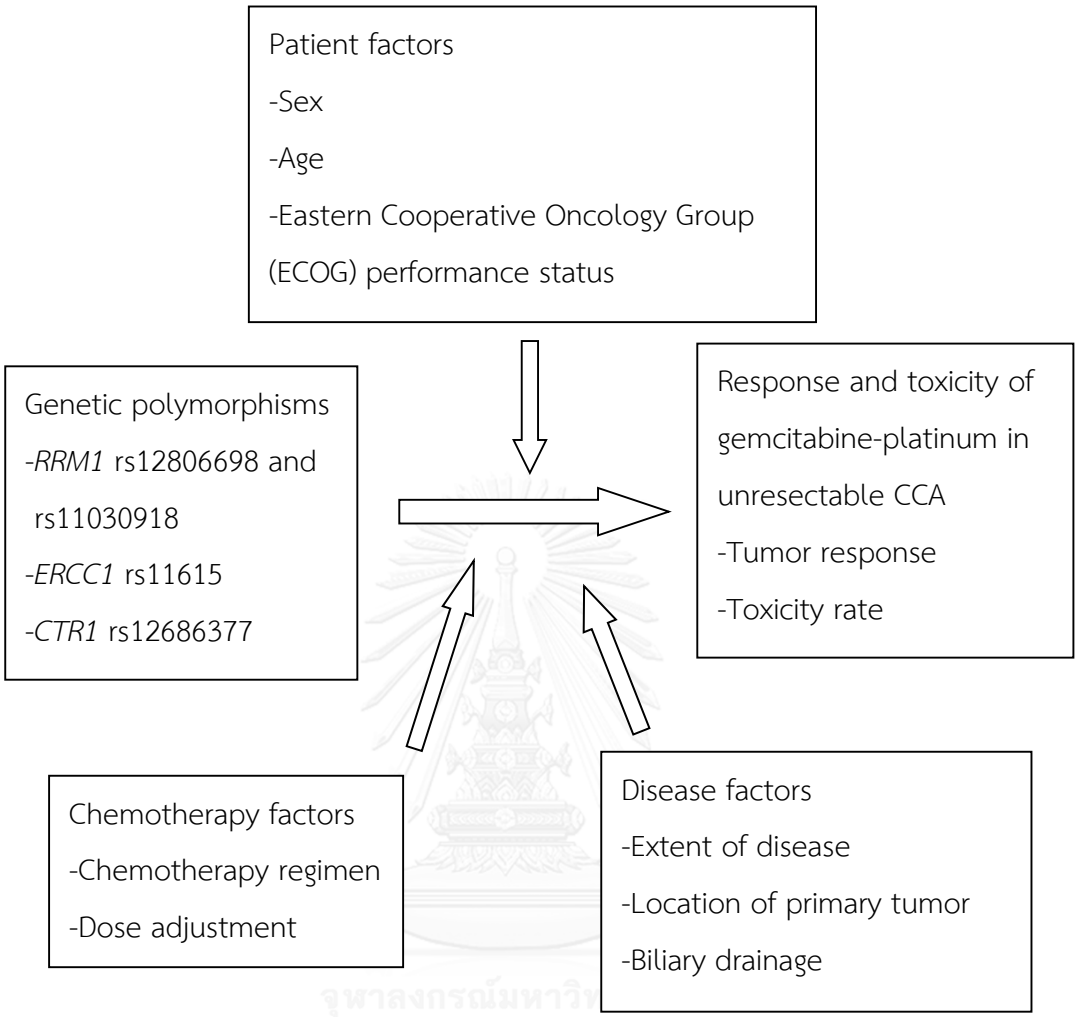

Figure 1 Conceptual framework

1.6 Operational definition

1. Patients are patients with unresectable CCA, gallbladder cancer, and ampullary cancer who have been treated at King Chulalongkorn Memorial Hospital.
2. *RRM1* polymorphism is variation in the nucleotide sequences of positions (-) 37 (dbSNP no. rs12806698) and (-) 524 (dbSNP no. rs11030918) of *RRM1* gene. Genotype (-) 37 was abbreviated as RR37CC (CC), RR37AC (AC), RR37AA (AA).

Genotype (-) 524 was abbreviated as RR524CC (CC), RR524CT (CT), RR524TT (TT).

3. *ERCC1* polymorphism is variation in the nucleotide sequences of position 19007 (dbSNP no. rs11615) of *ERCC1* gene. Three genotypes are CC, CT, TT.
4. *CTR1* polymorphism is variation in the nucleotide sequences of *CTR1* gene (dbSNP no. rs12686377). Three genotypes are GG, GT, and TT.
5. Response is the best objective tumor response. Response is classified by Response Evaluation Criteria in Solid Tumors or RECIST guideline version 1.1 [22]. There are four categories of response as mention below.
    1) Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes must have reduction in short axis to <10 mm.
    2) Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
    3) Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesion is also considered progression.
    4) Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters recorded since the treatment started.

    Patient who has CR or PR are classified into “responder”. Patient who has PD or SD are classified into “non-responder”. Response of patients is assessed by the physician and is recorded in patient profile.
6. Tumor control rate (TCR) refers to the total proportion of patients who demonstrate a response to treatment. The TCR is the sum of complete response, partial response, and stable disease.

7. Toxicities are adverse events caused by gemcitabine-platinum chemotherapy and occur during the chemotherapy treatment. Adverse events are assessed according to Common Terminology Criteria for Adverse Event (CTCAE) version 4.03 and are classified into grade 1-2 (mild), grade 3-4 (severe), and grade 5 (death) (appendix A) [23]. Adverse events are adverse events in blood and lymphatic system, gastrointestinal system, liver, nervous system, and kidneys.

   Adverse events in blood and lymphatic system are anemia, leukopenia, neutropenia, and thrombocytopenia.

   Adverse events in gastrointestinal system are constipation, dental caries, diarrhea, mucocitis oral, nausea, and vomiting.

   Adverse events in liver are increased blood total bilirubin (TB), increased alanine aminotransferase (ALT), increased aspartate aminotransferase (AST), increased alkakine phosphatase (ALP).

   Adverse events in nervous system are peripheral motor neuropathy, peripheral sensory neuropathy.

   Adverse event in kidneys is increased creatinine.

   Miscellaneous adverse events are fatigue, insomnia, and weight loss.

8. Gemcitabine-platinum chemotherapy is chemotherapy regimen which consists of A) intravenous cisplatin 25 mg per square meter of body surface area (mg/m^2) followed by intravenous gemcitabine 1,000 mg/m^2, each administered on days 1 and 8 every 3 weeks or B) intravenous gemcitabine 1,000 mg/m^2 on day 1 and 8, followed by intravenous carboplatin at a dose of area-under-the-curve (AUC) 5 on day 1 every 3 weeks.

   Carboplatin dose calculation

   Carboplatin dose = AUC x (GFR+25)

   GFR= [(140-age (year)] x body weight(kg) / [72 x serum creatinine (mg/dL)]

   (Multiply by 0.85 for women)

9. Single gemcitabine chemotherapy is intravenous gemcitabine 1,000 mg/m^2 on day 1, 8, and 15 every 4 weeks.
   Dosage regimen and administration date may be adjusted by patient status and/or physician opinion.

1.7 Benefits from the study

1. Confirmation of association between genetic polymorphisms and treatment response.
2. Confirmation of association between genetic polymorphisms and toxicities.
3. Prediction of treatment response and toxicities in specific patients group.
4. Extrapolation the result to other cancer types which is treated by gemcitabine-platinum chemotherapy.

Chapter 2

Literature review

2.1 Cholangiocarcinoma

Cholangiocarcinoma (CCA), biliary malignancy, is the second most common primary hepatic cancer and account for 10-20% of primary liver tumors. CCA is originated from differentiation of cholangiocyte's epithelium. CCA is classified anatomically as intrahepatic (iCCA), and extrahepatic (eCCA). Furthermore, eCCA is classified into perihilar/hilar (Klastkin tumors) and distal. In a group of CCA, hilar type is the most found followed by distal type and iCCA [24, 25]. Besides CCA, there are gallbladder cancer and ampullary cancer which were investigated with CCA in most of the studies because anatomic relation, similar metastatic pattern and rarity [5]

Despite its relative low incidence, the rate of CCA varies broadly in different geographic region. The crude incidence of eCCA and gallbladder cancer in Europe is 3.2 and 5.4/100,000 per year for male and female, respectively. The incidence of iCCA is rising and may be estimated as 0.9-1.3 and 0.4-0.7/100,000 per year for male and female, respectively. In south Italy where is high risk area in Europe, the incidence is estimate to be 4.9-7.4 and 2.9-4.3/100,000 per year for male and female, respectively. Data from the several tumor registries in Thailand during 1998 to 2000 have shown that the incidence rates of CCA are ranged between 7.3/100,000 in Prachuap Khiri Khan and 113.4/100,000 in Udon Thani in men and between 2.1/100,000 in Songkla and 49.8/100,000 in Udon Thani in women, respectively [4]. Globally, the incidence of iCCA has rising over the past 30 years, while the incidence of hilar and distal types has remained the same [1, 26].

Although the etiology of CCA is not clearly established, there are many risk factor for CCA. For example, infection with liver fluke *Opisthorchis viverrini* and *Clonorchis sinensis* has been linked with development of CCA. Hepatolithiasis, chronic biliary inflammation from calculi, Caroli's disease, primary sclerosing

cholangitis, hepatitis B virus, hepatitis C virus, and cirrhosis have been stated as potential etiology of CCA. Chronic cholecystitis and gall stones has been associated with gallbladder cancer [1, 25].

The CCA patients are presented with nonspecific symptoms such as abdominal pain, cachexia, malaise, fatigue, weight loss, and night sweat. For laboratory results, increased cholestatic parameters such as serum bilirubin, liver enzyme alkaline phosphatase, gamma-glutamyl transpeptidase, and transaminase revealed jaundice and biliary tree involvement. Serum levels of tumor marker, CA 19-9, can aid in diagnosis, but the assay has poor sensitivity. When clinical and laboratory results suggest CCA, imaging modalities including computed tomography (CT) and magnetic resonance imaging (MRI) support confirmation of diagnosis. Magnetic resonance cholagiopancreatography (MRCP) is used to assess resectability[25].

Surgical resection is the only potentially curative treatment for early stage bile tract cancer. After surgical resection, the patients with iCCA had 26 months of median time disease-free survival with 60% -65% of recurrence rate. Estimate 60% of iCCA patients survived for 5 years. For perihilar CCA, rate of 5 years survival following surgical resection with negative margin range from 11% to 41%. Only 27% of distal CCA patients survived for 5 years after surgical resection that achieve negative margin [1].

CCA is often diagnosis at advanced stage defined as unresectable (metastasis or locally advanced). Most of patients are not candidate for surgical resection. Contraindication for iCCA surgery is multifocal and for eCCA and gallbladder cancer surgery are vascular invasion of main hepatic artery, portal vein encasement or invasion of both branches of hepatic artery or portal vein, distant lymph nodes, and obviously distant metastasis [25].

For patients who are not suit for surgical resection, systemic chemotherapy is recommended. Many chemotherapy regimens have activity on advanced CCA, but evidence is inconsistent, particularly, because of small sample size and consist of vary type of bile tract cancer, pancreatic cancer or hepatocellular cancer [2]. In patients with CCA and gallbladder cancer, chemotherapy (5-fluorouracil (5-FU), folinic acid (FA), and etoposide) provided longer overall survival than best supportive care (BSC) (6 vs. 2.5 months, $p<0.01$) [27]. Another study showed the overall survival of 4.5, 4.6, and 9.5 months in gallbladder patients treated with BSC, 5-FU+FA, and gemcitabine+oxaliplatin, respectively. Transaminitis was more prevalent in patients treated with gemcitabine+oxaliplatin [28].

Gemcitabine is the main cytotoxic for some gastrointestinal cancer, especially pancreatic cancer, as a result, gemcitabine has been extrapolated to treat CCA and the other cancer in bile tract. To improve the treatment efficacy, gemcitabine is often combined with cisplatin. A pool analysis of 104 trials of patients with advanced biliary tract cancer demonstrated that the subgroup received a combination of gemcitabine and platinum had the highest response rate and tumor control rate [29]. In randomized controlled trials, which compare efficacy between gemcitabine/cisplatin and gemcitabine alone, demonstrated gemcitabine/cisplatin improved overall survival and progression free survival more than gemcitabine alone. Adverse events were similar in the two groups, except more neutropenia in the gemcitabine/cisplatin group [7]. Base on the result, gemcitabine/cisplatin is considered as the standard of care as first-line chemotherapy for CCA and gallbladder cancer.

2.2 Gemcitabine and *RRM1* polymorphism

Gemcitabine is a pyrimidine antimetabolite that has an antitumor activity. After enter into the cell, gemcitabine is metabolized to two active metabolites, gemcitabine diphosphate and gemcitabine triphosphate. These two active metabolites incorporate into DNA by replacing cytosine nucleotides; as a result, DNA replication is interrupted. Gemcitabine also inhibits ribonucleotide reductase by binding to ribonucleotide reductase subunit M1 (RRM1) which catalyzes the production of deoxynucleotide triphosphates for DNA synthesis [7, 18, 30]. The mechanism of gemcitabine was shown in figure 2 [31]. Theoretically, high activity of *RRM1* gene lead to high level of RRM1 protein expression and excess of deoxynucleotide triphosphates cause competitive displacement of gemcitabine, and result in gemcitabine resistance.

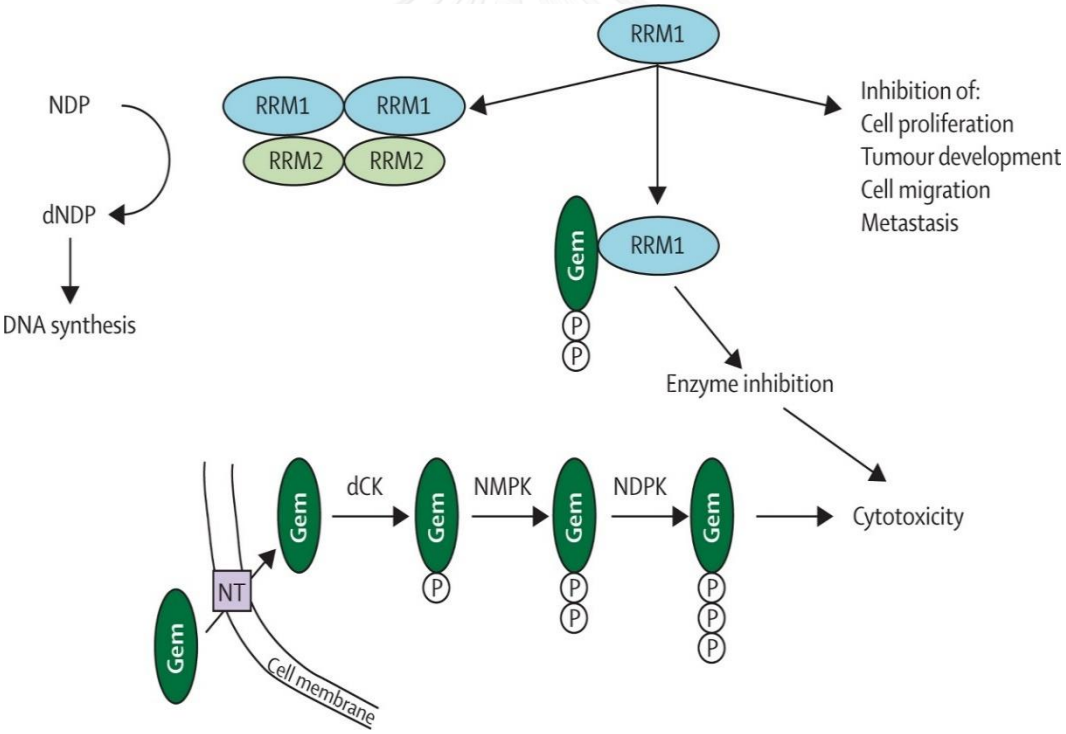

Figure 2 Mechanism of gemcitabine

Gemcitabine is known as the chemotherapy for liver and biliary tract cancer because of it is previously use in pancreatic cancer. As a single agent, gemcitabine provide overall response rate of 8%-36% with median survival of 6.3-16 months in patients with biliary tract cancer. Combination of gemcitabine and cisplatin have demonstrated response rate of 21%-35% with median survival of 8.4-11 months [32]. The treatment with a single agent gemcitabine is still reasonable option for elderly patients and patients with comorbidities or poor performance status [33].

Differences in treatment response rate of individuals are serious problem. Some researchers have demonstrated that those response is a result of genetic factor. The pharmacogenetics and genomics study proposed that the genetic diversity of cells results in the heterogeneity of cancer cells and difference in response and toxicity in individuals. Genetic predisposition as single nucleotide polymorphism (SNP) or mRNA expression may be useful tool for selection of the most effective treatment regimen. Genetic polymorphism of gene that code for drug metabolizing enzymes may partly explain for variety of response in individuals [12, 34].

Bepler and colleagues had study *RRM1* gene, especially at position (-) 37 (RR37) and (-) 524CT (RR524), and their promoter activity, polymorphism and effect on clinical outcome. They found that polymorphism of *RRM1* has been associated with level of RRM1 expression and clinical outcome in patients with lung cancer [35]. Among the already known *RRM1* polymorphisms, RR37 and RR524 seem to have the greatest important as potential predictor of treatment regimen bases on gemcitabine.

Some studies have investigated the association between genetic polymorphisms and drug response or toxicities in cancer patients receiving gemcitabine. Two single-nucleotide polymorphisms at position (-) 37 (rs12806698) and (-) 524 (rs11030918) are located in the promoter region of *RRM1* gene and are associated with prognosis [31]. In 2008, Kim and colleagues found an association between *RRM1* genotypes and clinical outcome in patients with NSCLC treated with gemcitabine-based chemotherapy. The response rate was significantly higher in the RR37AC-RR524CT group compared with the group containing other genotypes (65.5%

vs. 42.6%, respectively, p=0.039) [11]. In 2015, Yuan and colleagues' study of patients with gemcitabine-cisplatin treated NSCLC, the response rate was also significantly higher in the RR37AC-RR524CT group compared with the group contained other genotypes (p=0.009) [12]. In contrast to the previous studies, Feng and co-workers in 2009 found no association between -37AC and -524CT genotype and response rate in the group of Chinese patients receiving cisplatin and gemcitabine [13]. The same result also found in the study of Mlak and colleague in Caucasian patients with NSCLC [34]. In the term of toxicities, Yuan and colleague investigated the relationship between *RRM1* polymorphisms and toxicities, but no correlation was found [12]. Evidence for *RRM1* polymorphisms associated with response rate is lacking and controversial, therefore further studies are warranted.

2.3 Platinums and *ERCC1* and *CTR1* polymorphisms

Cisplatin, a platinum compound antitumor drug, enters the cell through both passive uptake and active transport via the copper transporter. Its activity is a result from interaction with tumor DNA and formation of crosslinks such as monoadducts, intrastrand crosslinks, interstrand crosslinks, and DNA-protein crosslinks. DNA damage results in inhibition of DNA replication and activation of apoptotic process; however, the DNA crosslink are recognized and are eliminated by nucleotide excision repair (NER) pathway. Excision repair cross-complementation group 1 (ERCC1) is the rate-limiting protein in NER pathway which is responsible for detection and removal of DNA adducts [36]. The pathway of cisplatin was shown in figure 3 [36].

Cisplatin is widely used for treatment of numerous human cancers including testicular cancer, bladder cancer, ovarian cancer, and lung cancer [37]. In patients with CCA, Valle and colleagues found that the median overall survival, median progression-free survival and tumor response were significantly higher in the cisplatin-gemcitabine group as compared with gemcitabine-only group [7].

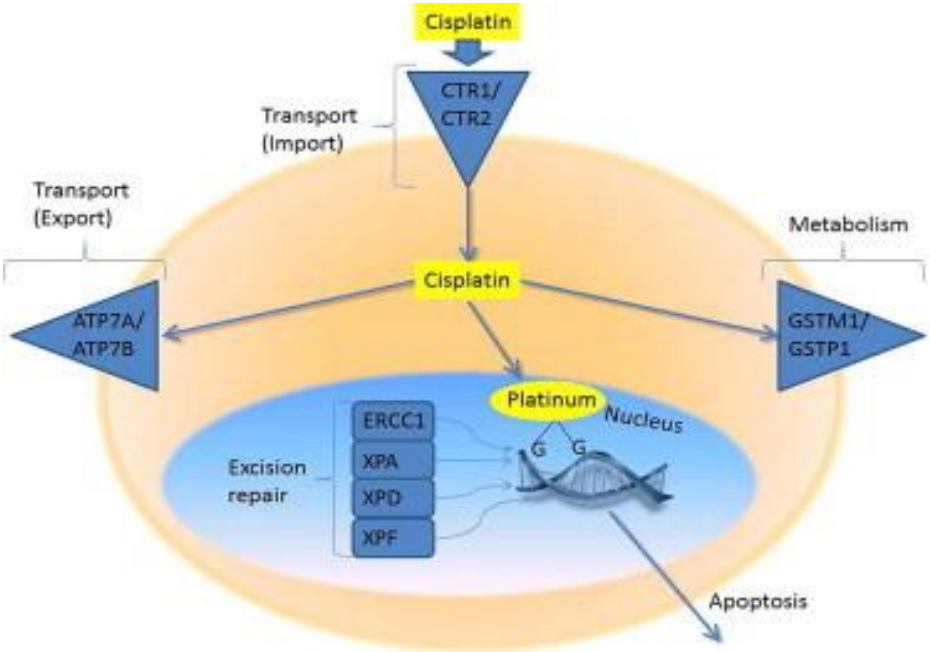

Figure 3 Pathway of cisplatin

Carboplatin, another platinum compound antitumor drug, was also demonstrated in CCA patients. A phase II single institution study conducted by Williams and team showed that activity of combination of gemcitabine and carboplatin are comparable to combination of gemcitabine and cisplatin [8].

In previous studies, some authors explained that patients' response to platinums chemotherapy was associated with SNP of *ERCC1* gene (rs11615, 19007 CT) [36]. In 2014, Lv's group found that NSCLC patients carrying CC genotype showed higher response rate to cisplatin-based chemotherapy as compared with CT+TT genotypes carriers (51.11% vs. 23.91%, respectively, p=0.007) [15]. The same result was found in two studies which NSCLC patients treated with platinum-based chemotherapy were assessed [14, 16]. For long-term outcome, Palomba and colleagues showed that the median overall survival (OS) of breast cancer patients treated with platinum-based chemotherapy was significantly higher in patients with C (CC+CT) genotype as compared with patients with TT genotype (131 vs. 66.5 months, respectively, p=0.004) [17]. However, between 2013 and 2014, Mlak's and Huang's group found no association between SNP of *ERCC1* gene and response to platinum-based chemotherapy in NSCLC patients [18, 20]. Moreover, Moxley and colleagues

also found no significant relationships between *ERCC1* genotype and OS in epithelial ovarian cancer patients [19]. In the term of toxicities, Chen and team investigated the relationship between *ERCC1* polymorphisms and toxicities, but no correlation was found [38].

Another mechanism associated with decreasing cisplatin response is the reduction of intracellular cisplatin level due to impaired import transporter, copper transporter1 (CTR1) [36]. Recent in vitro studies revealed association of CTR1 protein with resistance to platinum antitumor drugs [39]. At present time, the clinical study of association between *CTR1* polymorphisms (rs12686377) and cisplatin response is limited. In NSCLC patients treated with cisplatin-based chemotherapy, Xu and group found that two SNPs of *CTR1*, rs7851395 and rs12686377, were associated with both response rate and overall survival ($p<0.05$) [21]. In another study, Xu's group also found that rs10981694 was associated with cisplatin-induced toxicity in NSCLC patients [37]. More evidence is needed to explain the impact of *CTR1* polymorphisms in cisplatin response.

Chapter 3
Patients and Methods

3.1 Research design

This is a cohort study to assess the association between genetic polymorphisms and both response and toxicities of gemcitabine-platinum chemotherapy in unresectable cholangiocarcinoma patients. All enrolled patients in this study were recorded with basic clinical information. Written informed consents were obtained from all patients for blood sample collection. DNA was extracted from peripheral blood via the Qiagen blood kit. *RRM1*, *ERCC1*, and *CTR1* polymorphisms were assessed by Taqman allelic discrimination assay. After complete two or more cycles of chemotherapy, treatment response was evaluated according to Response Evaluation Criteria in Solid Tumors Criteria (RECIST) version 1.1. The responses were classified into four categories: complete remission (CR), partial remission (PR), stable disease (SD), progressive disease (PD). All patients were divided into two groups as responders (CR+PR) and non-responders (SD+PD). Toxicity was assessed according to Common Terminology Criteria for Adverse Event (CTCAE) version 4.03. Statistical analysis between genotypes and clinical responses or toxcities were performed by Chi square test or Fisher's exact test.

3.2 Scope of Research

This study is conducted in unresectable cholangiocarcinoma, gallbladder cancer or ampullary cancer patients treated with gemcitabine-platinum chemotherapy at King Chulalongkorn Memorial Hospital for 6 months after ethical approval.

3.3 Population and Sample

3.3.1 Population

Unresectable cholangiocarcinoma, gallbladder cancer, or ampullary cancer patients treated with gemcitabine-platinum chemotherapy at King Chulalongkorn Memorial Hospital.

3.3.2 Sample

Unresectable cholangiocarcinoma, gallbladder cancer, or ampullary cancer patients treated with gemcitabine-platinum chemotherapy at King Chulalongkorn Memorial Hospital during January 2016 to June 2016 or 6 months after ethical approval. These patients also fulfilled inclusion/exclusion criteria. The sampling technique was purposive sampling.

Inclusion criteria

1. Patients were histologically or cytologically confirmed cholangiocarcinoma, gallbladder cancer, or ampullary cancer.
2. The lesion had to be unresectable with a measurable or assessable tumor lesion.
3. Patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2.
4. Patients received or have been received gemcitabine/cisplatin or gemcitabine /carboplatin regimens. Patients received or have been received single gemcitabine, if patients were intolerant to platinum drug.
5. Patients were able to communicate about their symptoms of present illness.
6. Patients were willing to participate in the research.

Exclusion criteria

1. Patients have serious concomitant diseases, including uncontrolled hypertension, cardiac arrhythmia requiring medical treatment, myocardial infarction or active congestive heart failure in the last six months.

Sample size calculation

From the two previous studies, Kim et al. [11] and Yuan et al. [12], the prevalence (P) of responders without RR37AC-RR524CT genotypes of *RRM1* gene are 0.43 and 0.36, respectively. The relative risk (R) is between 1.54 and 2.09. Alpha-error of 0.05 (two-sided) and beta error of 0.1 were set.

$$n = \frac{Z_\alpha\sqrt{2\bar{p}\bar{q}} + Z_\beta\sqrt{p_1[1+R-p_1(1+R^2)]}^2}{[p_1(1-R)]^2} \quad \bar{p} = \frac{1}{2}p_1(1+R),\ \bar{q} = 1-\bar{p}$$

$$n = \frac{1.96\sqrt{2(0.55)(0.44)} + 1.28\sqrt{(0.4)[1+1.82-0.4(1+1.82^2)]}^2}{[(0.4)(1-1.82)]^2}$$

n= 47 patients per arm

The required sample size is estimated as 94 patients, but the target sample size is set at 105 patients (including 10% dropout).

3.4 Method

1. Patients who fulfill inclusion/exclusion criteria were asked to participate the study when they arrived to outpatient department for chemotherapy or follow-up.
2. Patients and/or legal guardians were informed about the objective and study protocol by the investigator. The investigator explained benefits/risks and answered the question until patients fully understand. Patient was freely to decide whether to participate or not before an informed consent was signed.
3. Detailed clinical information and five milliliter of venous blood were collected.
4. Genomic DNAs were extracted according to protocol using QIAamp blood kit at laboratory, Department of Medicine, Faculty of Medicine, Chulalongkorn University.
5. The treatment responses were evaluated after complete two or more cycle of chemotherapy by the physician. Chemotherapy-related toxicities were assessed in every visit of drug administration. The disease progression data

was collected form patient record and the patients were interviewed for severity of toxicities.

6. Genotypes of *RRM1*, *ERCC1*, and *CTR1* genes were determined by the Taqman allelic discrimination assay.
7. After laboratory procedure, biological samples were kept for 1 year for validate checking and will be disposed by standard method (incineration).
8. Statistical analyses were performed using SPSS version 22.0.

3.5 Buffy coat extraction

Whole blood was drawn from patients before chemotherapy administration. Five milliliter of blood was collected in Vacutainer® tube (purple-stopper) containing EDTA.

Buffy coat is a leucocyte-enriched fraction of whole blood which is a source of DNA. Preparing a buffy coat by centrifuging whole blood at 2,500 x g for 10 minutes at room temperature (15-25 °C). After centrifugation, 3 difference fractions are distinguishable: the upper clear layer is plasma, the intermediates layer is buffy coat with concentrated leucocyte, and the bottom layer contains concentrated erythrocytes. Two-hundred microliter of buffy coat was pipetted into microcentrifuge tube size 1.5 ml and stored in freezer at -20 °C until extracted DNA.

3.6 DNA extraction

Buffy coat was used for DNA extraction by ultilizing QIAamp® DNA Blood Mini kit. First, the buffy coat was equilibrated to room temperature. Then, 20 microliter QIAGEN protease was pipetted into a 1.5 ml microcentrifuge tube containing buffy coat 200 microliter. Then 200 microliter Buffer AL was added to tube and mixed by vortex mixer for 15 seconds, and incubated at 56 °C for 10 minutes. The tube was briefly centrifuged to remove drops from the inside of the lid. Next, 200 microliter of

100% ethanol was added to sample, and mixed again by vortex mixer for 15 seconds. After mixing, the tube was briefly centrifuged to remove drops from the inside of lid. The mixture was carefully applied to QIAamp Mini spin column (in a 2 mL collection tube) without wetting the rim. The tube was capped and centrifuged at 6000 x g (8000 rpm) for 1 minute. The QIAamp Mini spin column was placed in a 2 ml clean collection tube, and discarded the tube containing the filtrate. The QIAamp Mini spin column was carefully opened and 500 microliter Buffer AW 1 was added without wetting the rim. The cap was closed and was centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. The QIAamp Mini spin column was carefully opened and 500 microliter Buffer AW 2 was added without wetting the rim. The cap was closed and was centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. The QIAamp Mini spin column was placed in a 2 ml clean collection tube, and discarded the tube containing the filtrate. The tube was centrifuged at ful speed for 1 min Finally, the QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube, and discarded the collection tube containing the filtrate. The QIAamp Mini spin column was carefully opened and 200 microliter Buffer AE was added. Incubated at room temperature (15–25°C) for 1 min, and then centrifuged at 6000 x g (8000 rpm) for 1 min. For long-term storage of DNA, eluting in Buffer AE and storing at -20°C.

3.7 Genotyping

The polymorphisms were genotyped using the 5’ nuclease assay for allelic discrimination with commercially available TaqMan® genotyping assays (Applied Biosystems, USA) for amplifying and detecting specific SNP alleles in purified genomic DNA samples. The assay IDs as followed:

1. Assay ID: C__2769831_10, rs12806698 for *RRM1* (-) 37 polymorphism
2. Assay ID: C__2769829_10, rs11030918 for *RRM1* (-) 524 polymorphism
3. Assay ID: C__2532959_1_, rs11615 for *ERCC1* polymorphism
4. Assay ID: C__382585_10, rs12686377 for CTR1 polymorphism

Genotyping reactions were performed in a volume of 20 microliter containing 3 microliter of 30 nanogram genomic DNA, 0.5 microliter of 40x SNP genotyping assay, 10 microliter of 2x TaqMan Genotyping master mix, and 6.5 microliter of DNase-free water in 96-well plate. Amplification by polymerase chain reaction was performed by following program: 10 min at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 min at 60°C. After PCR amplification, perform an endpoint plate read on a StepOnePlus Real time PCR System (Applied Biosystem Inc., Foster City, CA USA. Using the fluorescence measurements made during the plate read, the SDS software plots R_n values based on the fluorescence signals from each well, then determines which alleles are in each sample.

3.8 Data collection

Data was collected during January to July 2016. When patient arrived at chemotherapy department, investigator approached for inform consent. Basic clinical data such as demographic data, diagnosis, extent of diseases, location of primary tumor, ECOG performance status score, previous treatment, chemotherapy regimen, baseline complete blood count, and baseline liver function tests were recorded in first time.

Patients were evaluated for response after 3rd or 4th cycle according to RECIST version 1.1. by the physician. We reported only the response from first assessment, if patients had more than one response results. Patients were classified into responder (CR+PR) and non-responder (SD+PD) group. We also classified them into tumor control group (CR+PR+SD) and progressive disease group (PD). In term of toxicities, both subjective and objective data were collected in every visit for chemotherapy. Information from patients record and interview was assessed and transformed into 0 to 4 grading score (0 is no symptom, 1-2 is mild, and 3-4 is severe). The highest grade of toxicities was reported. Patients with previous abnormal objective data as low complete blood count and/or high liver enzyme were defined as 0 because those abnormalities were not the effect of chemotherapy treatment. These data was

reassessed when laboratory result fluctuated to normal level. High liver enzyme with progressive disease was not counted.

3.9 Data analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS. Co., Ltd, Bangkok Thailand).

Basic clinical information of patients such as sex, extent of diseases, location of primary tumor, ECOG performance status score, and disease control rate were expressed as percentage. Continuous variable such as age, tumor marker levels, time to progression, and progression free survival were expressed as mean ± standard deviation (SD), or median and inter quartile range (IQR) or range.

Association of genetic polymorphisms with response and toxicities was evaluated using chi-square test. In 2x2 table, if one or more cells has an expected frequency of 5 or less, Fisher's exact test was used.

Table 1 Statistical analysis

Hypothesis	Variable	Statistics
RRM1 polymorphism is associated with response to gemcitabine-platinum chemotherapy.	Independent variable: genotypes Dependent variable: response rate	Chi square test or Fisher's Exact test
RRM1 polymorphism is associated with toxicity of gemcitabine-platinum chemotherapy.	Independent variable: genotypes Dependent variable: toxicity rate	Chi square test or Fisher's Exact test
ERCC1 polymorphism is associated with response gemcitabine-platinum chemotherapy.	Independent variable: genotypes Dependent variable: response rate	Chi square test or Fisher's Exact test
ERCC1 polymorphism is associated with toxicity of gemcitabine-platinum chemotherapy.	Independent variable: genotypes Dependent variable: toxicity rate	Chi square test or Fisher's Exact test
CTR1 polymorphism is associated with response to gemcitabine-platinum chemotherapy.	Independent variable: genotypes Dependent variable: response rate	Chi square test or Fisher's Exact test
CTR1 polymorphism is associated with toxicity of gemcitabine-platinum chemotherapy.	Independent variable: genotypes Dependent variable: toxicity rate	Chi square test or Fisher's Exact test

3.10 Ethical consideration

The study was approved by Institutional Review Board of Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (COA No.154/2016).

The study was conducted in accordance with The Belmont Report which consists of 3 basic ethical principles; Respect for Persons, Beneficence, and Justice.

Respect for persons- the investigator must give sufficient information on which to decide whether or not to participate, including the study protocol, the objective, benefits and risks, and a statement offering the subject the chance to ask questions. Consent to participate must be voluntarily given.

Beneficence-patients may not have direct benefit, but societal benefits might be gained from this study. Patients may have risk of pain, bleeding, bruise, and swelling at blood collecting site. Patients' data is confidential and case report form is unable to identify patients.

Justice-Patients are enrolled into the study because of inclusion/exclusion criteria. The risks and benefits of research are distributed equitably. Investigator takes precaution not to systematically select subject simply because of the subjects' easy availability or their compromised position.

Chapter 4
Results

4.1 Patients' characteristics

A total of thirty-three patients were enrolled between January and June 2016. The patients' characteristic and all basic clinical data were shown in table 2. Nineteen female patients accounted for 57.57% as the major gender groups and 14 male patients accounted for 42.42%. The median age of them was 61 years old, ranging from 38 to 89 years old. The number of cholangiocarcinoma, gallbladder cancer, and ampullary cancer were 25 (75.75%), 4 (12.12%), and 4 (12.12%), respectively. Almost half (16) of patients had metastatic stage while 10 (30.30%) and 7 (21.21 %) patients had locally advanced stage or mixed type, respectively. Eight patients (24.24%) had recurrence after previously curative treatment. ECOG performance score of 1 at baseline accounted for 84.84% of patients. Twenty-two patients (66.66%) received gemcitabine/cisplatin, and six patients (18.18%) were treated with gemcitabine/carboplatin, while the rest (5) of patients (15.15%) underwent single gemcitabine. Three patients were switched from gemcitabine/cisplatin to gemcitabine/carboplatin (two and one patients were changed after first and second cycle, respectively) because of increasing creatinine and severe vomiting. One patient was switched from gemcitabine/carboplatin to gemcitabine/cisplatin after first cycle, but the reason was unknown. Previous therapy as surgical resection, chemotherapy, or radiation were performed in 12 (36.36%), 10 (30.30%), and 7 (21.21%) patients, respectively. Biliary drainage as percutaneous transhepatic biliary drainage and biliary stent was performed in 9 (27.27%) and 8 (24.24%) patients before start chemotherapy. Twenty-one (63.63%) patients had grade 1 anemia before chemotherapy was started. All (4) of patients with gallbladder cancer were female. All (4) of patients with ampullary cancer had single gemcitabine.

Table 2 Patients' characteristics

Characteristic	N (%)
No. of patients enrolled	33
Gender	
Male	14 (42.42)
Female	19 (57.57)
Age (year)	
Median (range)	61 (38-89)
Extent of disease	
Locally advanced	10 (30.30)
Metastatic	16 (48.48)
Mixed	7 (21.21)
Primary tumor site	
Cholangiocarcinoma	25 (75.75)
Intrahepatic	9 (27.27)
Extrahepatic	
Hilar	10 (30.30)
Distal	3 (9.09)
Unspecific	3 (9.09)
Ampulla	4 (12.12)
Gallbladder	4 (12.12)
Performance status	
0	4 (12.12)
1	28 (84.84)
2	1 (3.03)

Table 2 Patients' characteristics (Continue)

Characteristic	N (%)
Chemotherapeutic regimens	
Gemcitabine/Cisplatin	22 (66.67)
Gemcitabine/Carboplatin	6 (18.18)
Gemcitabine	5 (15.15)
Previous therapy	
Surgery	12 (36.36)
Chemotherapy	10 (30.30)
Radiation	7 (21.21)

4.2 Response

A total of 151 cycles of chemotherapy were given. The median numbers of treatment cycles given to patients was 4 cycles, ranging from 1 to 8 cycles. Among the 33 patients, 31 patients were evaluable for response, 1 patient postponed his treatment because of cholangitis after chemotherapy, and 1 patient did not arrived on follow-up. One patients (3.2%) ampullary cancer patient achieved complete response (CR). Two (6.45%) patients achieved partial response (PR) were a male CCA patient treated with gemcitabine/carboplatin regimen and a female CCA patients treated with gemcitabine/cisplatin, respectively. The overall response rate (CR+PR) for 31 eligible patients was 9.7%. Stable disease (SD) and progressive disease (PD) was observed in 19 (61.3%) and 9 (29.0%) patients, respectively. The tumor control rate (TCR, CR+PR+SD) was noted to be 71.0%. The data was shown in table 3. The response rate and tumor control rate of patients did not associated with clinical factors such as age, sex, extent of disease, primary tumor site, Eastern Cooperative Oncology Group (ECOG) performance status, and chemotherapeutic regimens. The data was shown in Appendix G.

Table 3 Response rate and tumor control rate

Response N=31	n (%)	Response rate n (%)		Tumor control rate n (%)	
CR	1 (3.2)	Responder	3 (9.7)	TCR	22 (71.0)
PR	2 (6.5)	(CR+PR)		(CR+PR+SD)	
SD	19 (61.3)	Non-responder	28 (90.3)		
PD	9 (29.0)	(SD+PD)			

Long term outcomes such as median disease free survival and median time to progression were not mentioned in this study because of low number of participates, short duration of study. Some patients had stable disease with next follow-up appointment more than 6 months when data collecting finished.

4.3 Adverse events and toxicities

All patients recruited into study were evaluable for adverse events. One death was found in a 69 years-old male patient with cholangiocarcinoma who completed six cycles of chemotherapy with evidence of stable disease, but the cause of death was unknown.

Eight (24.24%) patients required hospitalization because of cholangitis, fatigue, acute renal failure, and infection. Sixty-nine cycles were hold or postponed during treatment. The most common reasons were neutropenia, thrombocytopenia, anemia creatinine rising, fatigue, and jaundice/hepatitis. Ten (30.30%) patients need two or more dose delays. In this study, dose delays were not associated with treatment response and tumor control as shown in table 4.

Table 4 Dose delays and treatment response

	Number of dose delays		P-value
	Less than or equal 2	More than 2	Fisher's exact test
Responders	1 (50.0)	1 (50.0)	1.000
Non-responders	20 (69.0)	9 (31.0)	
Tumor control	14 (63.6)	8 (36.4)	0.677
Progressive disease	7 (77.8)	2 (22.2)	

All recruited patients were interviewed for symptoms occurred between previous and present visit, and latest laboratory results were recorded. Severity of toxicities were assessed and transform to grading system according to CTCAE version 4.03 [23]. The data from the first cycle (before received chemotherapy) was excluded as baseline, and the data from last cycle when disease was progressed was also omitted. Abnormal laboratory results in early cycles were not counted until they fluctuated to abnormal again.

The grade and distribution of hematologic toxicities are summarized in table 5. All grade anemia were recorded in 33 (100%) patients, and 8 (24.24%) of them experienced grade 3 anemia. Leukopenia and neutropenia were found in 26 (78.87%), and 20 (60.60%) patients, respectively. Grade 3-4 neutropenia was observed in 11 (33.33%) patients. Thrombocytopenia was less common hematological toxicity, which occurred in 13 (39.39%) patients, and 1 (3.03%) of them had grade 3 thrombocytopenia. Patients with severe anemia and thrombocytopenia had blood transfusion while patients with severe leukopenia and neutropenia had dose delays. There were no correlation between clinical characteristics and hematologic toxicities, except the correlation between chemotherapeutic regimens and risk of thrombocytopenia (p=0.045). Five of six patients treated with gemcitabine /carboplatin were reported events of thrombocytopenia. The data was shown in Appendix G.

Table 5 Grade and distribution of hematologic toxicities

N=33	Grade 0 [n(%)]	Grade 1-2 [n(%)]	Grade 3-4 [n(%)]
Anemia	0 (0.0)	25 (75.75)	8 (24.24)
Leukopenia	7 (21.21)	22 (66.67)	4 (12.12)
Neutropenia	13 (39.39)	9 (27.27)	11 (33.33)
Thrombocytopenia	20 (60.60)	12 (36.36)	1 (3.03)

The grade and distribution of non-hematologic toxicities are summarized in table 6. Elevated liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)) were recorded in 22 (66.67%), 18 (54.54%), 23 (69.69%) patients, respectively. However, grade 3-4 elevated liver enzymes were uncommon by found. Eleven (33.33%) patients had increased total bilirubin (TB). Grade 3 increased total bilirubin (TB) was found in 2 (6.06%) patients. Chemotherapy was delayed in patients with abnormal liver function tests and biliary drainage was done in patients with jaundice. Seven (21.21%) patients had grade 1 increased creatinine (Cr). Another one patient (3.03%) was recorded with

elevated creatinine, but level was less than 1.2 mg/dL. Gemcitabin/cisplatin regimen was switched to gemcitabine/carboplatin in 3 patients, while 4 patients had closely monitor. The results demonstrated that some clinical characteristics were related with abnormal liver function test. Chemotherapeutic regimens were associated increased AST (p=0.016) and ALT (p=0.011). Patients treated with gemcitabine/ carboplatin or gemcitabine alone had incidence of increased AST and ALT. Furthermore, we also found that male patients had higher incidence of increased Cr than female patients (p=0.026). The data was shown in Appendix G.

All grade nausea and fatigue was the most common adverse event. Almost half (16) of patients experienced nausea and 14 (42.42%) had fatigue after receiving chemotherapy. Constipation, vomiting, weight loss, and diarrhea were found in 8 (24.24%), 8 (24.24%), 7 (21.21%) and 4 (12.12%) patients, respectively. Only grade 3 maculo-papular rash was found in 3 (9.09%) patients. Uncommon toxicities such as peripheral motor neuropathy, and oral mucositis were found in 1 (3.03%) patients. The detail was summarized in table 6. The incidences of non-hematologic toxicities such as nausea, constipation, vomiting, and weight loss were not associated with the clinical characteristics. We found the association between fatigue and elder patients (p=0.029). Elder patients (more than or equal 65 years old) had higher risk of fatigue. ECOG performance status was related with diarrhea (p=0.014) and maculo-papular rash (p=0.010). Most of patients with 0-1 performance status had no experience with those symptoms. Peripheral motor neuropathy, and oral mucositis were not analyzed because of low incidence. The data was shown in Appendix G.

Table 6 Grade and distribution of non-hematologic toxicities

N=33	Grade 0 [n (%)]	Grade 1-2 [n (%)]	Grade 3-4 [n (%)]
Liver function test			
Increased AST	11 (33.33)	19 (57.57)	3 (9.09)
Increased ALT	15 (45.45)	15 (45.45)	3 (9.09)
Increased ALP	10 (30.30)	17 (51.51)	6 (18.18)
Increased Bilirubin	22 (66.67)	9 (27.27)	2 (6.06)
Increased Cr	26 (78.79)	7 (21.21)	0 (0.0)
Nausea	17 (51.51)	15 (45.45)	1 (3.03)
Fatigue	19 (57.57)	11 (33.33)	3 (9.09)
Constipation	25 (75.76)	8 (24.24)	0 (0.0)
Vomiting	25 (75.76)	7 (21.21)	1 (3.03)
Weight loss	26 (78.79)	6 (18.18)	1 (3.03)
Diarrhea	29 (87.88)	3 (9.09)	1 (3.03)
Maculo-papular rash	30 (90.90)	0 (0.0)	3 (9.09)
Peripheral motor neuropathy	32 (96.96)	1 (3.03)	0 (0.0)
Oral mucositis	32 (96.96)	1 (3.03)	0 (0.0)

Some toxicities were not able to classify their severity into 1-4 grading system because subjective data come from retrospective study. Four (12.12%) patients had insomnia. Peripheral sensory neuropathy was found in 3 (9.09%) patients. Two of them experienced after finished 8th cycle and another one occurred after first cycle of chemotherapy. Dental caries was found in two (6.06%) patients, but severity could not be assessed. The data was shown in table 7. There was relationships between ECOG performance status and insomnia (p=0.046) and peripheral sensory neuropathy (p=0.002), but the direction could not defined. Dental caries was not analyzed because of low incidence. The data was shown in Appendix G.

Table 7 Grade and distribution of non-hematologic toxicities (2)

N=33	Grade 0 [n (%)]	Grade 1-4 [n (%)]
Insomnia	29 (87.87)	4 (12.12)
Peripheral sensory neuropathy	30 (90.90)	3 (9.09)
Dental caries	31 (93.93)	2 (6.06)

Furthermore analysis on performance status and non-hematologic toxicities, the status was classified in to 0 and 1-2. There was no relationship between performance status and diarrhea (p=0.420), insomnia (p=0.062), and peripheral sensory neuropathy (p=0.330), but there was a relationship between performance status and maculo-papular rash (p=0.033). The data was shown in Appendix G.

Hematologic adverse events in each cycle

Hematologic toxicities per cycle were reported to show the effect of accumulated dose of chemotherapy on hematologic toxicities. All eligible patients were monitoring for adverse events on the first day of each cycle before chemotherapy was given. Patients with any grade hematologic toxicities were counted for rate of event per cycle.

For anemia, 27 (87.10%) patients showed anemia before cycle 1. Before cycle 2, incidence of anemia was rising approximately 10%. Most (96.15%) patients still found with anemia until before cycle 4. Four of them were recorded with grade 3-4 anemia. In late cycle, even though the number of patients in the study was decreasing, the incidence of all grade anemia was increasing. This may be due to the effect of accumulated dose on anemia. The data was summarized in table 8. The rate was higher in the late cycle. Patients with grade 3 anemia were received blood transfusion after chemotherapy.

Table 8 Rate of anemia in each cycle

Adverse event	No. of patients	No. of patients with event (%)		
Anemia		Total	Grade 1-2	Grade 3
Before cycle 1	31	27 (87.10)	26 (83.87)	1 (3.23)
Before cycle 2	31	30 (96.77)	29 (93.55)	1 (3.23)
Before cycle 3	30	29 (96.67)	25 (83.33)	4 (13.33)
Before cycle 4	26	25 (96.15)	21 (80.77)	4 (15.38)
Before cycle 5	18	17 (94.44)	14 (77.78)	3 (16.67)
Before cycle 6	17	16 (94.12)	12 (70.59)	4 (23.53)
Before cycle 7	10	10 (100.00)	10 (100.00)	0 (0.00)
Before cycle 8	7	7 (100.00)	7 (100.00)	0 (0.00)

For leukopenia, the grade of severity was collected on the first day of each cycle before chemotherapy was given. Only 2 (6.45%) patients evidenced previous leukopenia or before cycle 1. The rate increased before cycle 2 and maintained to before cycle 6. Grade 3-4 leukopenia was found before cycle 3 and before cycle 7 in the same patient. The rate of leukopenia was highest (54.55%) before cycle 7, but only 1 (9.09%) patient had severe leukopenia, as a result, chemotherapy was postponed. The number of patients was suddenly decreased during cycle 4 and cycle 5 because some of patients had progressive disease and had new treatment. The data was summarized in table 9.

Table 9 Rate of leukopenia in each cycle

Adverse event	No. of patients	No. of patients with event (%)		
Leukopenia		Total	Grade 1-2	Grade 3
Before cycle 1	31	2 (6.45)	2 (6.45)	0 (0.00)
Before cycle 2	31	7 (22.58)	7 (22.58)	0 (0.00)
Before cycle 3	30	7 (23.33)	6 (20.00)	1 (3.33)
Before cycle 4	28	8 (28.57)	8 (28.57)	0 (0.00)
Before cycle 5	18	5 (27.78)	5 (27.77)	0 (0.00)
Before cycle 6	17	4 (23.53)	4 (23.53)	0 (0.00)
Before cycle 7	11	6 (54.55)	5 (45.45)	1 (9.09)
Before cycle 8	6	2 (33.33)	2 (33.33)	0 (0.00)

Neutropenia is a subtype of leukopenia. In this study, the risk of neutropenia was correlated with the risk of leukopenia (p=0.000). The data was shown in table 10. No one had neutropenia at first visit or before cycle 1. Four (12.90%) patients appeared with neutropenia before cycle 2. At before cycle 4, the median time for disease evaluation, the highest incidence of neutropenia was found (20.83%). Five (20.83%) patients was record with grade 1-2 neutropenia. The incidence was decreased during before cycle 4 and cycle 5 because patients with neutropenia had disease progression and treatment discontinuation. Grade 3 neutropenia was found before cycle 3 and cycle 7. Chemotherapy was postponed in patients with grade 3 neutropenia. The data was shown in table 12.

Table 10 Risk of neutropenia and leukopenia

N=33	Neutropenia		P-value
Leukopenia	Grade 0	Grade 1-4	
Grade 0	7 (100.0)	0 (0.0)	0.000
Grade 1-4	6 (23.1)	20 (76.9)	

Table 11 Severity of neutropenia and leukopenia

N=20	Neutropenia		P-value
Leukopenia	Grade 1-2	Grade 3-4	
Grade 1-2	9 (52.9)	8 (47.1)	0.218
Grade 3-4	0 (0.0)	3 (100.0)	

Table 12 Rate of neutropenia in each cycle

Adverse event	No. of patients	No. of patients with event (%)		
Neutropenia		Total	Grade 1-2	Grade 3
Before cycle 1	31	0 (0.00)	0 (0.00)	0 (0.00)
Before cycle 2	31	4 (12.90)	4 (12.90)	0 (0.00)
Before cycle 3	29	4 (13.79)	3 (10.34)	1 (3.45)
Before cycle 4	24	5 (20.83)	5 (20.83)	0 (0.00)
Before cycle 5	17	2 (11.76)	2 (11.76)	0 (0.00)
Before cycle 6	17	2 (11.76)	2 (11.76)	0 (0.00)
Before cycle 7	11	1 (9.09)	0 (0.00)	1 (9.09)
Before cycle 8	7	2 (28.57)	2 (28.57)	0 (0.00)

Thrombocytopenia is the most uncommon hematologic toxicities in this study as shown in table 13. Only 2 (6.45%) patients evidenced previous thrombocytopenia. Two (6.25%) had grade1-2 thrombocytopenia before cycle 2. Five (19.23%) patients had grade 1-2 thrombocytopenia before cycle 4. Only one (5.88%) patient had severe thrombocytopenia in 6th cycle. The incident rate was higher in late cycle. This may be due to the effect of accumulated dose on thrombocytopenia.

Table 13 Rate of thrombocytopenia in each cycle

Adverse event	No. of patients	No. of patients with event (%)		
Thrombocytopenia		Total	Grade 1-2	Grade 3
Before cycle 1	31	2 (6.45)	2 (6.45)	0 (0.00)
Before cycle 2	32	2 (6.25)	2 (6.25)	0 (0.00)
Before cycle 3	29	3 (10.34)	3 (10.34)	0 (0.00)
Before cycle 4	26	5 (19.23)	5 (19.23)	0 (0.00)
Before cycle 5	19	4 (21.05)	4 (21.05)	0 (0.00)
Before cycle 6	17	5 (29.41)	4 (23.53)	1 (5.88)
Before cycle 7	11	3 (27.27)	3 (27.27)	0 (0.00)
Before cycle 8	7	3 (42.86)	3 (42.86)	0 (0.00)

4.4 *RRM1* polymorphisms

4.4.1 Prevalence

Thirty three of DNA samples were used to determine the RRM1 (-) 37, RRM1 (-) 524 polymorphisms. Among 33 cases, 13 (39.39%) had RR37CC or CC, 18 (54.54%) had RR37AC or AC, and 2 (6.06%) had RR37AA or AA. Thirteen (39.39%) evidenced RR524TT or TT, 18 (54.54%) showed RR524CT or CT, and 2 (6.06%) exhibited RR524CC or CC. There were no significant differences between the observed and expected frequencies of each genotype (p=0.1917, p=0.1917, respectively). The data was shown in table 14.

The combination frequency of genotypes are reported in table 14. RR37AC in combination with RR524CT (RR37AC-RR524CT) was the most frequent genotype and accounted for 18 cases (54.54%). The other two frequent genotypes were RR37CC-RR524TT (39.39%) and RR37AA-RR524CC (6.06%). The combination of RR37CC-RR524CT, RR37CC-RR524CC, RR37AC-RR524TT, RR37AC-RR524CC, RR37AA-RR524TT, and RR37AA-RR524CT were not found in this study.

Table 14 Observed number and expected frequencies of *RRM*1 (-) 37, *RRM1* (-) 524 polymorphisms

RRM1 (-) 37	*RRM1* (-) 524					
	TT (%)	CT (%)	CC (%)	Observed	Expected	χ^2=1.7045
CC (%)	13 (39.39)	0	0	13	14.67	P=0.1917
AC (%)	0	18 (54.54)	0	18	14.67	
AA (%)	0	0	2 (6.06)	2	3.67	
Observed	13	18	2			
Expected	14.67	14.67	3.67	χ^2=1.7045	P=0.1917	

4.4.2 Response rate and tumor control rate

The best response rates in 31 patients with different RR37 genotypes (7.7% in RR37CC, 11.8% in RR37AC, and 0.0% in RR37AA, respectively) were not significantly different (p=0.882). Patients with complete response (CR) and partial response (PR) were grouped in responder while patients with stable disease (SD) and progressive disease (PD) were grouped in non-responder. We believed that A allele in RR37 genotype was related with better response, as a result, RR37 genotype was classified into CC and AC+AA. There was no significant difference in response rate between patients with RR37CC genotype and patients with RR37AC or RR37AA genotypes (p=1.000) as shown in table 15.

Similarly, the best response rates in patients with different RR524 genotypes (7.7% in RR524TT, 11.8% in RR524CT, and 0.0% in RR524CC, respectively) were not significantly different (p=0.882). We hypothesized that existence of C allele in RR524 genotype may provide the better response. RR524 genotype was grouped as TT and CT+CC. There was no significant difference in response rate between patients with RR524TT genotype and patients with RR524CT or RR524CC genotypes (p=1.000) as shown in table 15.

Table 15 *RRM1* Polymorphisms and response rate

Genotype N=31	Responder (%) CR+PR	Non-responder (%) SD+PD	χ^2 test	P-value
RRM1 (-) 37				
CC	1 (7.7)	12 (92.3)	0.250	0.882
AC	2 (11.8)	15 (88.2)		
AA	0 (0.0)	1 (100.0)		
CC	1 (7.7)	12 (92.3)	-	1.000*
AC+AA	2 (11.1)	16 (88.9)		
RRM1 (-) 524				
TT	1 (7.7)	12 (92.3)	0.250	0.882
CT	2 (11.8)	15 (88.2)		
CC	0 (0.0)	1 (100.0)		
TT	1 (7.7)	12 (92.3)	-	1.000*
CT+CC	2 (11.1)	16 (88.9)		

*- Fisher's exact test

Furthermore, we compared response rate between RR37AC and RR37CC +RR37AA as well as RR524CT and RR524CC+RR524TT, but no significant difference was found. The data was shown in Appendix G.

We found no association between *RRM1* polymorphisms and response rate Therefore, we analyzed association between RRM1 polymorphisms and tumor control rate. Patients with complete response (CR), partial response (PR), and stable disease (SD) were grouped in tumor control. The tumor control rates in patients with difference RR37 genotypes (69.2% in RR37CC, 70.6% in RR37AC, and 100.0% in RR37AA, respectively) were not significantly different (p=0.807). There was no significantly difference in tumor control rate between patients with RR37CC genotype and patients with RR37AC or RR37AA genotypes (p=1.000). Similarly, the tumor control rates in patients with different RR524 genotypes (69.2% in RR524TT, 70.6% in RR524CT, and 100.0% in RR524CC, respectively) were not significant different

(p=0.807). There was no significant difference in tumor control rate between patients with RR524TT genotype and patients with RR524CT or RR524CC genotypes (p=1.000) as shown in table 16.

Table 16 *RRM1* Polymorphisms and tumor control rate

Genotype N=31	Tumor control (%) CR+PR+SD	Progressive disease (%)	χ^2 test	P-value
RRM1 (-) 37				
CC	9 (69.2)	4 (30.8)	0.429	0.807
AC	12 (70.6)	5 (29.4)		
AA	1 (100.0)	0 (0.0)		
CC	9 (69.2)	4 (30.8)	-	1.000*
AC+AA	13 (72.2)	5 (27.8)		
RRM1 (-) 524				
TT	9 (69.2)	4 (30.8)	0.429	0.807
CT	12 (70.6)	5 (29.4)		
CC	1 (100.0)	0 (0.0)		
TT	9 (69.2)	4 (30.8)	-	1.000*
CT+CC	13 (72.2)	5 (27.8)		

*- Fisher's exact test

Furthermore, we compared tumor control rate between RR37AC and RR37CC+RR37AA as well as RR524CT and RR524CC+RR524TT, but no significant difference was found. The data was shown in Appendix G.

In previous studies [11-13, 40], polymorphism of RRM1 (-) 37 and (-) 524 were combined and were categorized. The researchers had study the effect of heterogeneous genotypes at both position on treatment response. Patients with heterogeneous genotypes at both position (RR37AC-RR524CT) were selected and compared with patients with the others genotypes. Only three combinations (RR37AC-RR524CT, RR37CC-RR524TT, and RR37AA-RR524CC) were found in this study.

Seventeen patients were grouped as RR37AC-RR524CT and fourteen patients were grouped as RR37CC-RR524TT and RR37AA-RR527CC (the others). There was no significant difference in response rate (p=0.488) and tumor control rate (p=1.000) between 2 groups. The data was shown in table 17. The data was shown in Appendix G. *RRM1* polymorphisms, both single and combination, may not be a good predictor for treatment response.

Table 17 *RRM1* polymorphism combination and treatment response and tumor control rate

Genotype N=31	Responder (%) CR+PR	Non-responder (%) SD+PD	P-value
RR37AC-RR524CT	2 (11.8)	15 (88.2)	1.000*
The others	1 (7.1)	13 (92.9)	
	Tumor control (%) CR+PR+SD	Progressive disease (%)	
RR37AC-RR524CT	12 (70.6)	5 (29.4)	1.000*
The others	10 (71.4)	4 (28.6)	

*- Fisher's exact test

4.4.3 Adverse events and toxicities

4.4.3.1 Hematologic toxicities

After hematologic toxicities were recorded and translated to 1 to 4 grading scale, the most severe grade of hematologic toxicities of each participants was selected. *RRM1* polymorphisms and risk of toxicities, as well as severity of toxicities, were analyzed. There was no association between *RRM1* polymorphisms and both risk and severity of hematologic toxicities. The data was shown in Appendix G. However, we found a trend of significant difference in severity of leukopenia between carriers of RR37CC genotype and carriers of RR37AA or RR37AC genotypes. Carriers of RR37CC genotype are likely to have higher incidence of mild leukopenia (p=0.063). The same trend was appeared in carriers of RR524TT genotype and carriers of RR524CT or RR524TT. The data was shown in table 18.

Table 18 *RRM1* Polymorphisms and severity of leukopenia

Genotype N=26	Grade 1-2 (%)	Grade 3-4 (%)	χ^2 test	P-value
Leukopenia				
RRM1 (-) 37				
CC	8 (72.7)	3 (27.3)	4.625	0.099
AC	14 (100.0)	0 (0.0)		
AA	1 (100.0)	0 (0.0)		
CC	8 (72.7)	3 (27.3)	-	0.063*
AC+AA	15 (100.0)	0 (0.0)		
RRM1 (-) 524				
TT	8 (72.7)	3 (27.3)	4.625	0.099
CT	14 (100.0)	0 (0.0)		
CC	1 (100.0)	0 (0.0)		
TT	8 (72.7)	3 (27.3)	-	0.063*
CT+CC	15 (100.0)	0 (0.0)		

*- Fisher's exact test

4.4.3.2 Non-hematologic toxicities

After non-hematologic toxicities were recorded and translated to 1 to 4 grading scale, the most severe grade of non-hematologic toxicities of each participants was selected. In our study, severe (3-4) grade non-hematologic toxicities were uncommon found. Therefore, we assessed only *RRM1* polymorphisms and risk of toxicities. There was no association between *RRM1* polymorphisms and the risk of abnormal liver function tests. This may suggest that there was no effect of *RRM1* polymorphisms on liver function tests. In addition, we also found no relationship between *RRM1* polymorphisms and other toxicities such as nausea, fatigue, fever, constipation, vomiting, and weight loss. The data was summarized in Appendix G.

4.5 *ERCC1* polymorphism

4.5.1 Prevalence

Thirty-three of DNA samples were used to determine the *ERCC1* polymorphism. CC homozygous variant of *ERCC1* polymorphism was found in 18 patients (54.54%), CT heterozygous variant was found in 13 patients (39.39%), and TT homozygous variant was found in 2 patients (6.06%). No statistically significant difference was observed between the observed and expected frequencies of each genotype (Table 19).

Table 19 Observed number and expected frequencies of *ERCC1* polymorphism

ERCC1	Observed (%)	Expected	
CC	18 (54.54)	18.19	χ^2=0.0297
CT	13 (39.39)	12.62	P=0.8631
TT	2 (6.06)	2.19	

4.5.2 Response rate and tumor control rate

ERCC1 protein is responsible for detection and removal DNA and platinum crosslinks. We hypothesized that activity of platinum antitumor drugs was related with function of ERCC1 protein. In our study, only 26 patients received gemcitabine-platinum treatment and evaluated for treatment response were selected. The best response of patients was summarized in table 20. No patients had complete response (CR). The response rate is 7.7 %. Low response rate may related to chemotherapy resistance of cancer cells. Most (65.4%) patients had stable disease. Therefore, tumor control rate was 73.1%.

Table 20 Response rate and tumor control rate in patients received gemcitabine/ platinum

Response N=26	n (%)	Response rate n (%)		Tumor control rate n (%)	
CR	0 (0.0)	Responder	2 (7.7)	TCR	19 (73.1)
PR	2 (7.7)	(CR+PR)		(CR+PR+SD)	
SD	17 (65.4)	Non-responder	24 (92.3)		
PD	7 (26.9)	(SD+PD)			

After single gemcitabine regimen was excluded, CC, CT, and TT genotypes of *ERCC1* polymorphism were found in 15 (57.70%), 11 (42.30%), and 0 (0.0%), respectively. We found no difference in response rate between 2 genotypes (p=0.492). According to previous studies [14-16], CC genotype provided better response, therefore, genotypes of *ERCC1* gene were grouped as CC and CT+TT; however, there was no significant difference in response rate between two groups (p=0.492) as shown in table 21.

Table 21 *ERCC1* polymorphism and response rate

Genotype N=26	Responder (%) CR+PR	Non-responder (%) SD+PD	P-value Fisher's exact test
ERCC1			
CC	2 (13.3)	13 (86.7)	0.492
CT	0 (0.0)	11 (100.0)	
TT	0 (0.0)	0 (0.0)	
CC	2 (13.3)	13 (86.7)	0.492
CT+TT	0 (0.0)	11 (100.0)	

We found no difference in response rate among genotypes because of low response rate. Therefore, tumor control rate (CR+PR+SD) and *ERCC1* polymorphisms were further analyzed. Genotypes of *ERCC1* gene were grouped as CC and CT+TT as previous section; however, there was no significant difference in tumor control rate between two groups (p=0.407) as shown in table 22. Stable disease, the major group in tumor control, was high in both groups.

Table 22 *ERCC1* polymorphism and tumor control rate

Genotype N=26	Tumor control (%) CR+PR+SD	Progressive disease (%)	P-value Fisher's exact test
ERCC1			
CC	12 (80.0)	3 (20.0)	0.407
CT	7 (63.6)	4 (36.4)	
TT	0 (0.0)	0 (0.0)	
CC	12 (80.0)	3 (20.0)	0.407
CT+TT	7 (63.6)	4 (36.4)	

4.5.3 Adverse events and toxicities

4.5.3.1 Hematologic toxicities

Among 33 patients, only 28 patients were treated with gemcitabine/cisplatin or gemcitabine/carboplatin and had toxicities assessment. Patients were group as CC and CT+TT according to potential of treatment response. Genotypes and risk as well as severity of hematologic toxicities were analyzed. All patient had at least grade 1 anemia after received chemotherapy. Therefore, the risk of anemia and polymorphism were not analyzed. There was no correlation between *ERCC1* polymorphism and both risk and severity of hematologic toxicities. The data was shown in table 23 and table 24. The result suggested that *ERCC1* polymorphism were not related with hematologic toxicities.

Table 23 *ERCC1* polymorphism and risk of hematologic toxicities

N=28	Grade	CC (%) n=15	CT+TT (%) n=13	P-value
Anemia	1-4	15 (53.6)	13 (46.4)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	11 (52.4)	10 (47.6)	1.000*
	0	4 (57.1)	3 (42.9)	
Neutropenia	1-4	8 (50.0)	8 (50.0)	0.718
	0	7 (58.3)	5 (41.7)	
Thrombocytopenia	1-4	8 (72.7)	3 (27.3)	0.137*
	0	7 (41.2)	10 (58.8)	

*- Fisher's exact test

Table 24 *ERCC1* polymorphism and severity of hematologic toxicities

	Grade	CC (%)	CT+TT (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	11 (52.4)	10 (47.6)	1.000
	3-4	4 (57.1)	3 (42.9)	
Leukopenia (n=21)	1-2	10 (52.6)	9 (47.4)	1.000
	3-4	1 (50.0)	1 (50.0)	
Neutropenia (n=16)	1-2	3 (37.5)	5 (62.5)	0.619
	3-4	5 (62.5)	3 (37.5)	
Thrombocytopenia (n=11)	1-2	7 (70.0)	3 (30.0)	1.000
	3-4	1 (100.0)	0 (0.0)	

4.5.3.2 Non-hematologic toxicities

After non-hematologic toxicities were recorded and translated to 1 to 4 grading scale, the most severe grade of non-hematologic toxicities of each participants was selected. In our study, severe (3-4) grade non-hematologic toxicities were uncommon found. Therefore, we assessed only *ERCC1* polymorphisms and risk of toxicities. There was no association between *ERCC1* polymorphisms and the risk of liver enzyme increasing. This may suggested that there was no effect of *ERCC1* polymorphisms on liver function tests. In addition, we also found no relationship between *ERCC1* polymorphisms and other toxicities such as nausea, fatigue, fever, constipation, and weight loss. The data was shown in Appendix G. In particular, the patients with CC genotype had higher rate of vomiting than the patients with other genotypes, but there was no statistical significance (p=0.084). The data was shown in table 25.

Table 25 *ERCC1* Polymorphism and risk of vomiting

N=28	Grade	CC (%)	CT+TT (%)	P-value (Fisher's exact test)
Vomiting	1-4	6 (85.7)	1 (14.3)	0.084
	0	9 (42.9)	12 (57.1)	

4.6 *CTR1* Polymorphisms

4.6.1 Prevalence

Thirty-three of DNA samples were used to determination of *CTR1* polymorphism. There was 14 (42.42%) GG homozygous variant, 17 (51.51%) GT heterozygous variant, and 2 (6.06%) homozygous variant. There was no significant difference between the observed and expected frequencies of each genotype (Table 26).

Table 26 Observed number and expected frequencies of *CTR1* polymorphism

CTR1	Observed (%)	Expected	
GG	14 (42.42)	15.34	χ^2=1.1577
GT	17 (51.51)	14.32	P=0.2819
TT	2 (6.06)	3.34	

4.6.2 Response rate and tumor control rate

CTR1 protein is responsible for platinum uptake into the cell. We assumed that intracellular level and activity of platinum antitumor drugs was related with function of CTR1 protein. In our study, 26 patients who were received gemcitabine-platinum therapy and were evaluated for treatment response were chosen. No patients had complete response (CR). The response rate is 7.7%. Low response rate may related to chemotherapy resistance of cancer cells. Most (65.4%) patients were non-responder with stable disease.

After single gemcitabine regimen was excluded, GG, GT, and TT genotypes of *CTR1* polymorphism were found in 9 (34.62%), 16 (61.54%), and 1 (3.84%) patients, respectively. In previous study [21], TT genotype was more likely to be resistant to chemotherapy, as a result, genotypes of *CTR1* gene were grouped as GG and GT+TT to balance the number of patients. The result showed that there was no significant difference in response rate between two groups (p=0.129) as shown in table 27.

Table 27 *CTR1* polymorphism and chemotherapy response

Genotype N=26	Responder (%)	Non-responder (%)	χ^2 test	P-value
CTR1				
GG	2 (22.2)	7 (77.8)	4.093	0.129
GT	0 (0.0)	16 (100.0)		
TT	0 (0.0)	1 (100.0)		
GG	2 (22.2)	7 (77.8)	-	0.111*
GT+TT	0 (0.0)	17 (100.0)		

*- Fisher's exact test

In the term of tumor control rate (CR+PR+SD), genotypes of *CTR1* gene were grouped as GG and GT+TT; however, there was no significant difference in tumor control rate between two groups (p=0.188) because tumor control rate was high in both groups as shown in table 28.

Table 28 *CTR1* polymorphism and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	χ^2 test	P-value
CTR1				
GG	5 (55.6)	4 (44.4)	2.316	0.314
GT	13 (81.3)	3 (18.8)		
TT	1 (100.0)	0 (0.0)		
GG	5 (55.6)	4 (44.4)	-	0.188*
GT+TT	14 (82.4)	3 (17.6)		

*- Fisher's exact test

4.6.3 Adverse events and toxicities

4.6.3.1 Hematologic toxicities

Among 33 patients, only 28 patients were treated with gemcitabine and platinum and had toxicities assessment. Patients was group in GG and GT+TT according to potential of treatment response. Genotypes and risk as well as severity of hematologic toxicities were analyzed. All patient had at least grade 1 anemia after received chemotherapy. Therefore, the risk of anemia and polymorphism were not analyzed. There was no relationship between *CTR1* polymorphism and both risk and severity of hematologic toxicities. However, we found that *CTR1* polymorphism was almost significantly related with the risk of neutropenia (p=0.05). Patients with GG genotype had lower incidence of neutropenia than patients with GT/TT genotypes. The data was shown in table 29 and table 30.

Table 29 *CTR1* polymorphism and risk of hematologic toxicities

N=28	Grade	GG (%) n=10	GT+TT (%) n=18	P-value (Fisher's exact test)
Anemia	1-4	10 (35.7)	18 (64.3)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	6 (28.6)	15 (71.4)	0.207
	0	4 (57.1)	3 (42.9)	
Neutropenia	1-4	3 (18.8)	13 (81.3)	0.050
	0	7 (58.3)	5 (41.7)	
Thrombocytopenia	1-4	3 (27.3)	8 (72.7)	0.689
	0	7 (41.2)	10 (58.8)	

Table 30 *CTR1* polymorphism and severity of hematologic toxicities

	Grade	GG (%)	GT+TT (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	9 (42.9)	12 (57.1)	0.364
	3-4	1 (14.3)	6 (85.7)	
Leukopenia (n=21)	1-2	6 (31.6)	13 (68.4)	1.000
	3-4	0 (0.0)	2 (100.0)	
Neutropenia (n=16)	1-2	2 (25.0)	6 (75.0)	1.000
	3-4	1 (12.5)	7 (87.5)	
Thrombocytopenia (n=11)	1-2	2 (33.3)	4 (66.7)	1.000
	3-4	1 (20.0)	4 (80.0)	

4.6.3.2 Non-hematologic toxicities

There was the association between *CTR1* polymorphisms and the risk of liver enzyme increasing. The data was shown in Appendix G. In particular, there was the association between *CTR1* polymorphism and the risk of ALP increasing (p=0.035) as shown in table 31. The result showed that patients with GG genotype had lower risk of ALP increasing compared with patients with GT or TT genotype (p=0.035) after the confounder like progressive disease was eliminated.

Table 31 *CTR1* polymorphism and risk of ALP increasing

N=28	Grade	GG (%)	GT+TT (%)	P-value (Fisher's exact test)
Increased ALP	1-3	4 (21.1)	15 (78.9)	0.035*
	0	6 (66.7)	3 (33.3)	

* P-value of <0.05 was considered statistically significant.

There was no association between *CTR 1* polymorphisms and the risk of non-hematologic toxicities such as nausea, fatigue, fever, constipation, and vomiting. The data was shown in Appendix G. In particular, the patients with GT+TT genotype evidenced higher rate of vomiting than the patients with GG genotypes, but there was no statistical significance (p=0.062). The data was shown in table 32.

Table 32 *CTR1* polymorphism and risk of weight loss

N=28	Grade	GG (%)	GT+TT (%)	P-value (Fisher's exact test)
Weight loss	1-4	0 (0.0)	6 (100.0)	0.062
	0	10 (45.5)	12 (54.5)	

4.7 Combination of *RRM1* and *ERCC1* polymorphisms

4.7.1 Prevalence, response rate, and tumor control rate

To observe the combined effect of genotypes of *RRM1* and *ERCC1* genes on response rate and tumor control rate, 26 patients treated with gemcitabine/platinum and had treatment outcome were chosen. RR37AC-RR524CT of *RRM1* polymorphisms and CC genotypes of *ERCC1* polymorphism provided higher response rate in previous studies [11, 12, 14-16, 40]. Eight patients were classified into RR37AC-RR524CT/CC and eighteen patients were classified into the other genotypes based on previous results. Patients carried RR37AC-RR524CT/CC and patients carried the other genotypes showed no significant difference in response rate as well as tumor control rate (p=0.529 and p=0.375, respectively). In further analysis, presenting of A allele in RR37 genotype or C allele in RR524 genotype with CC genotype of *ERCC1* gene was assessed. However, no difference in response rate or tumor control rate was found (p=0.529 and p=0.375, respectively). Most of Patients were non-responder without RR37AC-RR524CT/CC. The data was shown in table 33 and 34.

Table 33 Combination of *RRM1* and *ERCC1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/CC	1 (12.5)	7 (87.5)	0.529
The others	1 (5.6)	17 (94.4)	
RR37AC-RR524CT+ RR37AA-RR524CC/CC	1 (12.5)	7 (87.5)	0.529
The others	1 (5.6)	17 (94.4)	

Table 34 Combination of *RRM1* and *ERCC1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/CC	7 (87.5)	1 (12.5)	0.375
The others	12 (61.1)	6 (38.9)	
RR37AC-RR524CT+ RR37AA-RR524CC/CC	7 (87.5)	1 (12.5)	0.375
The others	12 (61.1)	6 (38.9)	

4.7.2 Adverse events and toxicities

4.7.2.1 Hematologic toxicities

RR37AC-RR524CT/CC was chosen according to potential of treatment response. Presenting of RR37AC-RR524CT/CC was not associated with hematologic toxicities except the risk of thrombocytopenia. The results showed that RR37AC-RR524CT/CC was related with risk of thrombocytopenia (p=0.030). Carriers of RR37AC-RR524CT/CC had higher proportion of all grade thrombocytopenia than carriers of the other genotypes as shown in table 35. However, this may not reveal the real relationship between genotype and toxicity because chemotherapy regimen was also related with thrombocytopenia as shown in previous analysis of this study. RR37AC-RR524CT/CC showed no correlation with the other hematologic toxicities. The data was shown in table 35. The analysis between genetic combination and hematologic toxicities were shown in Appendix G.

Table 35 Combination of *RRM1* and *ERCC1* polymorphisms and risk of hematologic toxicities

N=28	Grade	RR37AC-RR524CT/CC (%) n=8	The others (%) n=20	P-value (Fisher's exact test)
Anemia	1-4	8 (28.6)	20 (71.4)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	6 (28.6)	15 (71.4)	1.000
	0	2 (28.6)	5 (71.4)	
Neutropenia	1-4	6 (37.5)	10 (62.5)	0.401
	0	2 (16.7)	10 (83.3)	
Thrombocytopenia	1-4	6 (54.5)	5 (45.5)	0.030*
	0	2 (11.8)	15 (88.2)	

* P-value of <0.05 was considered statistically significant.

Table 36 Combination of *RRM1* and *ERCC1* polymorphisms and severity of hematologic toxicities

	Grade	RR37AC-RR524CT/CC (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	6 (28.6)	15 (28.6)	1.000
	3-4	2 (28.6)	5 (28.6)	
Leukopenia (n=21)	1-2	6 (31.6)	13 (68.4)	1.000
	3-4	0 (0.0)	2 (100.0)	
Neutropenia (n=16)	1-2	2 (25.0)	6 (75.0)	0.608
	3-4	4 (50.0)	4 (50.0)	
Thrombocytopenia (n=11)	1-2	5 (50.0)	5 (50.0)	1.000
	3-4	1 (0.0)	0 (0.0)	

4.7.2.2 Non-hematologic toxicities

There was no association between polymorphism combinations and risk of abnormal liver function tests. In addition, we found no relationship between combination of *RRM1* and *ERCC1* polymorphism and the toxicities such as nausea, fatigue, constipation, and vomiting. The data was shown in Appendix G. This findings suggested that *RRM1* polymorphisms in combination with *ERCC1* polymorphism were not a predictive biomarker for toxicities in patients treated with gemcitabine/ platinum.

4.8 Combination of *RRM1* and *CTR1* polymorphisms

4.8.1 Prevalence, response rate, and tumor control rate

To observe the combined effect of genotypes of *RRM1* and *CTR1* genes on response rate and tumor control rate, 26 patients treated with gemcitabine/platinum and had treatment outcome were chosen. Four patients and twenty-two patients had RR37AC-RR524CT/GG and the other genotypes, respectively. The classified was based on previous studies which demonstrated that RR37AC-RR524CT and GG provided the higher response rate than another genotypes [14-16, 21]. However, patients carried RR37AC-RR524CT/GG and patients carried the other genotypes showed no significant difference in response rate as well as tumor control rate (p=0.289 and p=0.287, respectively). In further analysis, presenting of A allele in RR37 genotype or C allele in RR524 genotype with CC genotype of *ERCC1* gene was assessed, however, no difference in response rate or tumor control rate was found (p=0.354 and p=0.101, respectively). The data was shown in table 37 and table 38.

Table 37 Combination of *RRM1* and *CTR1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/GG	1 (25.0)	3 (75.0)	0.289
The others	1 (4.5)	21 (95.5)	
RR37AC-RR524CT+ RR37AA-RR524CC/GG	1 (20.0)	4 (80.0)	0.354
The others	1 (4.8)	20 (95.3)	

Table 38 Combination of *RRM1* and *CTR1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/GG	2 (50.0)	2 (50.0)	0.287
The others	17 (77.3)	5 (22.7)	
RR37AC-RR524CT+ RR37AA-RR524CC/GG	2 (40.0)	3 (60.0)	0.101
The others	17 (81.0)	4 (19.0)	

4.8.2 Adverse events and toxicities

4.8.2.1 Hematologic toxicities

RR37AC-RR524CT/GG was chosen according to potential of treatment response. Presenting of RR37AC-RR524CT/GG was not associated with hematologic toxicities. We further analyzed another combinations. We found that RR37AC-RR524CT/GT was related with risk of neutropenia (p=0.039), as shown in table 39. Patients with RR37AC-RR524CT/GT showed higher rate of all grade neutropenia than patients with the other genotypes.

Table 39 Combination of *RRM1* and *CTR1* polymorphisms and risk of neutropenia

N=28	Grade	RR37AC-RR524CT/GT (%) n=9	The others (%) n=19	P-value (Fisher's exact test)
Neutropenia	1-4	8 (50.0)	8 (50.0)	0.039*
	0	1 (8.3)	11 (91.7)	

* P-value of <0.05 was considered statistically significant.

In addition, RR37CC-RR524TT/GT was associated severity of leukopenia (p=0.048). Carriers of RR37CC-RR524TT/GT had higher rate of grade 3-4 leukopenia than carriers of the other genotypes (Table 40). The data of genetic combinations and hematologic toxicities was shown in Appendix G.

Table 40 Combination of *RRM1* and *CTR1* polymorphisms and severity of leukopenia

N=21	Grade	RR37CC-RR524TT/GT (%) n=5	The others (%) n=16	P-value (Fisher's exact test)
Leukopenia	1-2	3 (15.8)	16 (84.2)	0.048*
	3-4	2 (100.0)	0 (0.0)	

* P-value of <0.05 was considered statistically significant.

4.8.2.1 Non-hematologic toxicities

Although we found no association between RR37AC-RR524CT/GG and non-hematologic toxicities, we continuously analyzed another combinations. There was an association between RR37AC-RR524CT/GT and peripheral sensory neuropathy (p=0.026). All of patient with RR37AC-RR524CT/GT evidenced peripheral sensory neuropathy both during and after treatment. The data was shown in table 41.

Table 41 Combination of *RRM1* and *CTR1* polymorphisms and risk of peripheral sensory neuropathy

N=28	Grade	RR37AC-RR524CT/GT (%) n=9	The others (%) n=19	P-value (Fisher's exact test)
Peripheral sensory neuropathy	1-4	3 (100.0)	0 (0.0)	0.026*
	0	6 (24.0)	19 (76.0)	

* P-value of <0.05 was considered statistically significant.

4.9 Combination of *ERCC1* and *CTR1* polymorphisms

4.9.1 Prevalence, response rate, and tumor control rate

To observe the combined effect of genotypes of *ERCC1* and *CTR1* genes on response rate and tumor control rate, 26 patients treated with gemcitabine/platinum and had treatment outcome were chosen. Four patients and twenty-two patients were respectively classified into CC/GG and the other genotypes based on previous studies as the high response rate genotypes [14-16, 21]. CC/GG were associated with response rate (p=0.018). Patients with CC/GG showed higher rate of responsive disease compare to patients without CC/GG. No association between CC/GG and tumor control rate was found (p=1.000). The data was shown in table 42 and table 43.

Table 42 Combination of *ERCC1* and *CTR1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
CC/GG	2 (50.0)	2 (50.0)	0.018*
The others	0 (0.0)	22 (100.0)	

* P-value of <0.05 was considered statistically significant.

Table 43 Combination of *ERCC1* and *CTR1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
CC/GG	3 (75.0)	1 (25.0)	1.000
The others	16 (72.7)	6 (27.3)	

4.9.1 Adverse events and toxicities

4.9.1.1 Hematologic toxicities

There was no association between genetic polymorphism combination and hematologic toxicities, except neutropenia. We found the association between CT/GT and risk of neutropenia (p=0.024). Patients with CT/GT showed higher rate of all grade neutropenia than patients with the other genotypes as shown in table 44. The data was shown in Appendix G.

Table 44 Combination of *ERCC1* and *CTR1* polymorphisms and risk of neutropenia

N=28	Grade	CT/GT (%) n=6	The others (%) n=22	P-value (Fisher's exact test)
Neutropenia	1-4	6 (37.5)	10 (62.5)	0.024*
	0	0 (0.0)	12 (100.0)	

* P-value of <0.05 was considered statistically significant.

4.9.1.2 Non-hematologic toxicities

We found no correlation between polymorphism of *ERCC1* and *CTR1* and non-hematologic toxicities such as fatigue, nausea, constipation and vomiting. However, patients with CT/GT showed higher risk of weight loss than patients with the other genotype (p=0.010). The data was shown in table 45.

Table 45 Combination of *ERCC1* and *CTR1* polymorphisms and risk of weight loss

N=28	Grade	CT/GT (%) n=6	The others (%) n=22	P-value (Fisher's exact test)
Weight loss	1-4	4 (66.7)	2 (33.3)	0.010*
	0	2 (9.1)	20 (90.9)	

* P-value of <0.05 was considered statistically significant.

4.10 Triple combination of three polymorphisms

4.10.1 Prevalence, response rate, and tumor control rate

We selected the genotypes from three genes which provide the higher response rate according to previous studies [11, 12, 14-16, 21, 40]. RR37AC-RR524CT was chosen from RRM1 (-) 37 and RRM1 (-) 524 polymorphisms, while CC and GG were taken from *ERCC1* polymorphism and *CTR1* polymorphism, respectively. Only 2 (7.70%) patients from 26 patients had specific set of genotypes, RR37AC-RR524CT/CC/GG. However, No association between triple combination and response rate as well as tumor control rate (p=0.151 and p=0.529, respectively). The data was shown in table 46 and table 47

Table 46 Combination of *RRM1*, *ERCC1*, and *CTR1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/CC/GG	1 (50.0)	1 (50.0)	0.151
The other	1 (4.2)	23 (95.8)	

Table 47 Combination of *RRM1*, *ERCC1*, and *CTR1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/CC/GG	1 (50.0)	1 (50.0)	0.529
The other	17 (70.8)	7 (29.2)	

4.10.2. Adverse events and toxicities

4.10.2.1 Hematologic toxicities

Twenty-eight patients treated with gemcitabine and platinum and evaluated for toxicities were selected. There was no association between triple combination and both risk and severity of hematologic toxicities. The data was shown in table 48 and 49.

Table 48 Combination of *RRM1*, *ERCC1*, and *CTR1* polymorphisms and risk of hematologic toxicities.

N=28	Grade	RR37AC-RR524CT /CC/GG (%) n=2	The other (%) n=26	P-value (Fisher's exact test)
Anemia	1-4	2 (7.1)	26 (92.9)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	1 (14.3)	6 (85.7)	0.444
	0	1 (4.8)	20 (95.2)	
Neutropenia	1-4	1 (6.3)	15 (93.8)	1.000
	0	1 (8.3)	11 (91.7)	
Thrombocytopenia	1-4	2 (18.2)	9 (81.8)	0.146
	0	0 (0.0)	17 (100.0)	

Table 49 Combination of *RRM1*, *ERCC1*, and *CTR1* polymorphisms and severity of hematologic toxicities.

	Grade	RR37AC-RR524CT /CC/GG (%)	The other (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	2 (9.5)	19 (90.5)	1.000
	3-4	0 (0.0)	7 (100.0)	
Leukopenia (n=21)	1-2	1 (5.3)	18 (94.7)	1.000
	3-4	0 (0.0)	2 (100.0)	
Neutropenia (n=16)	1-2	0 (0.0)	8 (100.0)	1.000
	3-4	1 (12.5)	7 (87.5)	
Thrombocytopenia (n=11)	1-2	1 (16.7)	5 (83.3)	1.000
	3-4	1 (20.0)	4 (80.0)	

4.10.2.2 Non-hematologic toxicities

There was no association between triple polymorphism combinations and risk of abnormal liver function tests. In addition, we found no relationship between triple combination and the toxicities such as nausea, fatigue, constipation, and vomiting. The data was shown in Appendix G. This findings suggested that triple polymorphism combinations were not a predictive biomarker for toxicities in patients treated with gemcitabine/platinum.

Chapter 5
Discussion

Cholangiocarcinoma (CCA) is a malignancy from epithelial cell of bile tract. CCA is rare in Western countries but more common in Asia, especially in the northeast of Thailand, where the incidence rate have been highest in the world. Surgical resection is the curative treatment, but most of patients was diagnosed at the advanced stage. Systemic chemotherapy is usually recommended for patients with unresectable CCA [1].

Systemic chemotherapy, gemcitabine and cisplatin, has become the new standard first line regimen on the basic of result from the ABC-02 study [7]. The ABC-02 study by Valle and team demonstrated that gemcitabine plus cisplatin provided the longer median overall survival compared with single gemcitabine regimen. In addition, the study of William and colleague showed that gemcitabine/carboplatin also provide similar efficacy in unresectable CCA and other biliary tract cancer patients [8].

To provide the better response rate to the patients, selection of patients to appropriate chemotherapy regimens is important. Previous researchers had suggested the association between the polymorphisms of genes involved in drug pathway and treatment response. Even the impact of genetic alteration is still unknown, but the genetic variation may change the structure or function of protein in drug metabolism pathway and may result in drug sensitivity or drug tolerance.

This study has been conducted to investigate the association of single nucleotide polymorphisms with response and toxicity of gemcitabine-platinum in thirty three patients with unresectable CCA. Our results reported that *RRM1*, *ERCC1*, and *CTR1* polymorphisms were not associated with response rate as well as tumor control rate. Some genetic polymorphisms were almost correlated with toxicities.

The response rate and toxicities events

Among 33 patients, partial response (PR) were found in 2 patients. Number of responder in analysis which involved *ERCC1* or *CTR1* polymorphism was lower than total analysis. In contrast, most of patients had stable disease (SD) or progressive disease (PD), as result, more patients were non-responders than responders. High number of non-responders was a result from chemotherapy resistant property which is the characteristic of CCA and other bile tract cancers. Furthermore, we assessed tumor control rate to see the difference between tumor control (CR+PR+SD) group and progressive disease group. The overall response rate and tumor control rate were 9.7% and 71.0% which were lower than in the study of Valle [7] and William [8]. Valle reported only tumor control rate as 81.4%, but William demonstrated response rate and tumor control rate as 31.1% and 75.6%, respectively. William's study also showed higher response rate in gallbladder group but our study and Valle's study found no difference in response rate among different primary tumor sites. The reason could be explain by the high number of gallbladder patients which had more rapidly progressive disease in William's study than our study.

For hematologic toxicities, the incidence of grade 3-4 neutropenia (33.33%) was as almost high as in William's study (37%) using gemcitabine/carboplatin. Severe anemia (24.24%) in the study was higher than previous studies. Those 2 hematologic toxicities (neutropenia and anemia) were more frequently found than we expected. Most (80%) of our patients were treated with gemcitabine/ cisplatin or single gemcitabine, we expected the incidence of toxicities should be more likely to the study of Valle. The discrepancies were explained by different supportive care provided by different sites.

For liver function tests, severe increased liver enzyme level was respectively reported as 9.09%, 9.09%, and 18.18% for increased AST, increased ALT, and increased ALP. Valle's group only reported severe increased ALT as 9.6% which consistent with our study while William and co-worker reported severe transaminitis (increased AST/ALT) and increases ALP as 4%. The inconsistencies may be explained by the difference of study design. In the 2 clinical studies, their patients were routinely monitor for abnormal laboratory results and adverse events were probably

solved at early phase. The other severe non-hematologic toxicities such as fatigue, maculo-papular rash was reported in 9.09% while uncommon toxicities like nausea, vomiting, weight loss and diarrhea was noted between 0% - 3% of patients. Our study showed lower incidence of grade 3-4 non-hematologic toxicities than in previous studies. The reason could be explained by our patients' performance status which better than the patients in two previous studies [7, 8].

Prevalence of polymorphisms

DNA from peripheral blood was extracted and determined for genotypes of *RRM1* polymorphisms at position of (-) 37 and (-) 524. The genotypes from both genes were analyzed individually and in combination. According to the result, those patients who had homozygous variants of RRM1 (-) 37 also possessed homozygous variants of RRM (-) 524. The same result was observed in heterozygous variants. Combination between homozygous variant and heterozygous variant of each position may be found in study with larger sample size.

In this study, RR37AC-RR524CT was the most frequent combination and accounted for 54.54%. The prevalence was different from the previous studies in Asian non-small cell lung cancer (NSCLC) patients which RR37AC-RR524CT was in range between 26.76%-36.20% [11-13, 40]. Our result was consistent with the study in Poland which RR37AC and RR524CT was 54.5% and 52.7% of NSCLC patients, respectively [34]. We found only three major combinations, while previous researches with a large number of patients showed more types of combinations. We expected that our frequent of combinations are similar to the frequent in Asian study if the number of participants are higher.

For prevalence of *ERCC1* polymorphism, more than half of patients (54.54%) had CC genotype which was slightly higher than in previous studies in Asian NSCLC patients (46.63%-49.45%) [14-16], while prevalence of CT and TT genotypes were consistent with previous studies and were in range between 37.42%-43.96% and 6.59%-16.30% of patients, respectively. In Caucasian NSCLC patients, proportion of CC genotype was smaller than in Asian patients as report in Mlak and team's study (11.3%) [18] and Sullivan and team's study (16.22%) [41]. Prevalence of *ERCC1*

polymorphisms in this study may similar to the prevalence in the other Asian study, if more participants were recruited.

For prevalence of *CTR1* polymorphism, 51.51% of patients carried GT genotype similar to the finding in the study by Xu and team among Chinese NSCLC patients [21]. However, another two genotypes from our study was different from Xu and colleague's study. Xu showed prevalence of GG and TT genotypes as 27.66% and 20.92%, respectively, while we reported GG and TT genotypes as 42.42% and 6.06%, respectively. The study of *CTR1* polymorphism and clinical outcome is still limited.

Association of genetic polymorphisms and response rate

For *RRM1* polymorphisms, we believed that difference in single nucleotide on gene may provide different structure or function of RRM1 proteins, as a result, cancer cell or normal cell was more sensitized to gemcitabine. Our results showed no association between *RRM1* polymorphisms and response to chemotherapy as well tumor control. In contrast, previous studies which *RRM1* polymorphisms of (-) 37 and (-) 524 were studied revealed that RR37AC-RR524CT was associated with better response rate in NSCLC patients treated by gemcitabine-base regimens [11, 12, 40]. The reason could be explain by the difference site of tumor and low number of responders. Patients with NSCLC were more sensitized to gemcitabine-based regimens. Consequently, more patients responded to chemotherapy. These finding lead us to believe that *RRM1* polymorphisms may not be used to predict response rate in unresectable CCA and other bile tract cancers patients treated with gemcitabine-based regimen.

ERCC1 protein was a key enzyme in repairing process of damaged DNA after being attacked by platinum drugs. Polymorphisms of *ERCC1* probably lead to increased activity of ERCC1 protein and promoted recovery of cancer DNA, as a result, cancer cells were tolerated with chemotherapy. We found no relationship between *ERCC1* polymorphism and response rate as well as tumor control rate. High tumor control rate was found in both groups. Inconsistency to previous studies conducted in NSCLC patients treated by platinum-based regimens, patients with CC

genotype had higher response rate than patients with CT or TT genotypes [14-16]. The reason could be explain by the difference tumor organ and unbalance distribution between responders and non-responders. These finding imply that response of patients is not associated with *ERCC1* polymorphism.

CTR1 is a membrane transporter which responsible for platinum uptake into cell. It is assumed that modified CTR1 protein by genetic variation had influenced on intracellular drug concentration and platinum resistance. In our study, no genotype provided better response rate and tumor control rate. However, In Xu and team's study which conducted in NSCLC patients treated by platinum-base regimens, patients with TT genotype showed higher incidence of resistance as higher non-responder than patients with GG or GT genotype. The reason could be explain by the higher platinum drug dosage for NSCLC patients and unbalance distribution between responders and non-responders. These finding lead us to believe that *CTR1* polymorphism may not predict response rate in unresectable CCA and bile tract cancer patients treated with gemcitabine and platinum regimen.

Association of genetic polymorphisms and toxicities

Our study provide new finding that was not mention in other studies, we found a trend of significance relationship between *RRM1* polymorphism and severity of leukopenia. CC genotype showed higher incidence of mild leukopenia than the other genotypes, but there was no statistical significant difference. Two studies have established the relationship between *RRM1* polymorphisms at position of (-) 37 and (-) 524 and toxicities. Zhang and co-worker reported *RRM1* (-) 37 polymorphism in NSCLC was related with all grade 3-4 toxicities including hematologic and non-hematologic toxicities, but type of hematologic toxicities was not demonstrated. They also stated the relationship between *RRM1* polymorphisms and grade 3-4 vomiting as well [42]. Zhang and co-worker may not found the association, if toxicities were individually analyzed. Yuan and colleagues demonstrated no correlation between incidences of toxicities and *RRM1* polymorphism when combined with *GSTP1* polymorphism in NSCLC [12]. This is the first study to examine

the association between polymorphisms of *RRM1* and toxicities in unresectable CCA and bile tract cancer patients.

No correlation between *ERCC1* polymorphisms and toxicities were found in this study. Our current findings are consistent with previous studies. Several studies have been investigated the association between *ERCC1* polymorphism and toxicities, but none of study have reported the association [38, 41, 43]. It is seem that *ERCC1* polymorphism may not affect the risk and severity of toxicities in CCA and bile tract cancer patients treated with gemcitabine and platinum.

For *CTR1* polymorphism, no study has been assessed the association between this genetic polymorphism and toxicities. Our study showed that *CTR1* polymorphism was correlated with risk of increased ALP. These finding suggest that *CTR1* polymorphism may be a useful biomarker to predict some abnormal liver function test.

For unusual toxicities, we found one patient with tubular injury. An 89 year-old woman with gallbladder cancer had potassium level as 3.2 mEq/L which lower than normal range at second cycle. She was prescribed a KCl elixir as potassium supplement. After 1 week chemotherapy postpone, her potassium level was low as result from previous visit (3.3 mEq/L) and KCl elixir was prescribed. After third cycle, she was admitted to hospital and was diagnosed with cisplatin induced tubular injury and low level of electrolytes, especially magnesium and potassium. After disorder was corrected, she started forth cycle with gemcitabine/cisplatin. Magnesium and calcium supplement was given and electrolytes level were closely monitored.

In the term of genetic polymorphism, the patient mentioned above had RR37CC-RR524TT genotype of *RRM1* polymorphisms, CC genotype of *ERCC1* polymorphism, and GG genotype of *CTR1* polymorphism. We assumed that those *ERCC1* polymorphism may related with translation of malfunction ERCC1 protein and DNA adduct was not removed, while *CTR1* polymorphism probably associated with over intake of cisplatin and intracellular level of cisplatin was increased. Two mechanism possibly occurred in the proximal tubules of kidney, as a result, tubules were injured and potassium level was low.

Combined genetic polymorphisms and outcome

We believed that genetic polymorphism combination is one method of patient selection to appropriate chemotherapy regimen. We found some associations between treatment outcomes and genetic polymorphisms when genotypes of 2 genes were combined. In previous study, Mlak and team investigated *ERCC1* and *RRM1* polymorphisms and efficacy of treatment in NSCLC patients treated by gemcitabine and platinum. The genetic combination of both gene was related with tumor control rate while individual genetic polymorphism showed no correlation with treatment efficacy [18]. Patient with RR37CC and TT genotype showed higher tumor control rate than patients with the other genotypes. In contrast, Oh and co-worker found that carriers of RR37AC-RR524CT and CC genotypes showed higher response rate than carriers of the other combinations. In our study, the combinations of *RRM1* and *ERCC1* polymorphisms were not related with response rate and tumor control rate because of low number of responders and different dosage of chemotherapy. On the other hand, we found the association between genetic combination and risk of thrombocytopenia. RR37AC-RR524CT /CC, which provided high response rate in previous study, showed high risk of thrombocytopenia in our study.

The combination of *RRM1* and *CTR1* polymorphisms was not correlated with response rate and tumor control rate. On the other hand, there were correlation between combinations and toxicities. RR37AC-RR524CT/GT was related with risk of neutropenia and peripheral sensory neuropathy, while RR37CC-RR524TT/GT was related with severity of leukopenia. This findings presented GT genotype of *CTR1* polymorphism in combination with any genotype of *RRM1* polymorphisms lead to toxicities. In previous analysis, GT+TT was not related with hematologic toxicities and peripheral sensory neuropathy. These may be conclude that GT genotype represents the higher risk of toxicities and *RRM1* polymorphisms determine the type of toxicities.

The last combination which both polymorphisms are involved in platinum pathway. We expected to see more obviously effect of the genotypes on the outcomes. Theoretically, genotype of *ERCC1* which provide low function ERCC1

protein and genotype of *CTR1* which translate high function CTR1 protein are the best combination for treatment response. However, we found the relationship between response rate and combination of *ERCC1* and *CTR1* polymorphisms. CC/GG genotype showed higher response rate than the other genotypes. In term of toxicities, the combination of *ERCC1* and *CTR1* polymorphisms was associated with risk of neutropenia. CT/GT genotype was correlated with higher risk of toxicities than the other genotypes combinations. Our findings were consistent with previous study in term of response rate, but contrary to our assumption in the term of toxicities. We assume that effect of genetic polymorphisms, which influence on cancer cells, also influence on normal cells, consequently, patients with good response to chemotherapy have severe toxicities from chemotherapy as well. Presenting of C allele of *ERCC1* polymorphism or G allele of *CTR1* polymorphism was related with response rate and may related with some toxicities. In other words, patients with CC/GG combination probably had benefits from chemotherapy like high response rate and low incidence of toxicities.

We selected the most effective genotypes from each gene to assess the relationship between triple combination and treatment outcome. Nevertheless, no association between combination and treatment response or toxicities was found. The results can be explained by strict criteria and low number of carrier.

Conclusion

These findings provide evidence that genetic polymorphisms may be a useful predictor for treatment response and toxicities in unresectable CCA and the other bile tract cancer patients treated with gemcitabine and platinum. Patients with GG genotypes of *CTR1* polymorphisms had lower risk of increased ALP than patients with GT or TT genotype. Therefore, patients with GT or TT should be routinely monitored for liver function. Carriers of RR37AC-RR524CT/GT genotype had higher risk of neutropenia and peripheral sensory neuropathy than carrier of the others genotypes, we suggested to educate those patents about self-hygiene and sign of hospital visit. Patients with RR37CC-RR524TT/GT genotypes were had higher rate of grade 3-4 leukopenia than patients with the other genotypes. Care giver should educate those

patients about self-hygiene and sign of infection and closely monitor complete blood count. Carriers of CC/GG genotypes had better response than carriers of the other genotypes, as a result, those carries were supposed to be treated by gemcitabine/platinum regimen. Lastly, patients with CT/GT genotype had more chance to neutropenia and weight loss than patients with the other genotypes. We suggested to give those patients education about self-hygiene and nutrition support to maintain their status. The findings of this study are restricted to a small sample size and distribution in genetic polymorphism and treatment response. Larger sample size and long term outcome are advisable for further work to confirm to the association.

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APPENDICES

Appendix A

Common Terminology Criteria for Adverse Events					
Adverse Event	1	2	3	4	5
Blood and lymphatic system					
Anemia	Hemoglobin (Hgb) <LLN-10.0 g/dL; < LLN-6.2 mmol/L; <LLN-100 g/L	Hgb <10.0 - 8.0 g/dL; <6.2 - 4.9 mmol/L; <100 – 80 g/L	Hgb <8.0 g/dL; <4.9 mmol/L; <80 g/L; transfusion indicated	Life-threatening consequences; urgent intervention indicated	Death
Neutropenia, Neutrophil count decreased	<LLN - 1500/mm^3; <LLN - 1.5 x10^9 /L	<1500 - 1000/mm^3; <1.5 - 1.0 x10^9 /L	<1000 - 500/mm^3; <1.0 - 0.5 x10^9 /L	<500/mm^3; <0.5 x 10^9 /L	-
White blood cell decreased	<LLN - 3000/mm^3; <LLN -3.0 x10^9 /L	<3000 - 2000/mm^3; <3.0 – 2.0 x10^9 /L	<2000 - 1000/mm^3; <2.0 - 1.0 x10^9 /L	<1000/mm^3; <1.0 x 10^9 /L	-
Thrombocytopenia, Platelet count decreased	<LLN - 75,000/mm^3; <LLN -75.0 x 10^9 /L	<75,000 - 50,000/mm^3; <75.0 - 50.0 x 10^9 /L	<50,000 - 25,000/mm^3; <50.0 -25.0 x 10e9 /L	<25,000/mm^3; <25.0 x 10^9 /L	-

Common Terminology Criteria for Adverse Events					
Adverse Event	1	2	3	4	5
Gastrointestinal system					
Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental ADL	Obstipation with manual evacuation indicated; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Dental caries	One or more dental caries, not involving the root	Dental caries involving the root	Dental caries resulting in pulpitis or periapical abscess or resulting in tooth loss	-	-

Common Terminology Criteria for Adverse Events					
Adverse Event	1	2	3	4	5
Gastrointestinal system (continue)					
Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Mucocitis Oral	Asymptomatic or mild symptoms; intervention not indicated	Moderate pain; not interfering with oral intake; modified diet indicated	Severe pain; interfering with oral intake	Life-threatening consequences; urgent intervention indicated	Death

Common Terminology Criteria for Adverse Events					
Adverse Event	1	2	3	4	5
Gastrointestinal system					
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated	-	-
Vomiting	1 - 2 episodes (separated by 5 minutes) in 24 hrs	3 - 5 episodes (separated by 5 minutes) in 24 hrs	>=6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death

Common Terminology Criteria for Adverse Events					
Adverse Event	1	2	3	4	5
Liver					
Increased blood bilirubin	>ULN - 1.5 x ULN	>1.5 - 3.0 x ULN	>3.0 - 10.0 x ULN	>10.0 x ULN	-
Increased alanine aminotransferase (ALT)	>ULN - 3.0 x ULN	>3.0 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Increased aspartate aminotransferase (AST)	>ULN - 3.0 x ULN	>3.0 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Increased alkaline phosphatase (ALP)	>ULN - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Kidney					
Increased creatinine	>1 - 1.5 x baseline; >ULN - 1.5x ULN	>1.5 - 3.0 x baseline; >1.5 - 3.0x ULN	>3.0 baseline; >3.0 - 6.0 x ULN	>6.0 x ULN	-

Common Terminology Criteria for Adverse Events					
Adverse Event	1	2	3	4	5
Nervous system					
Peripheral motor neuropathy	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL; assistive device indicated	Life-threatening consequences; urgent intervention indicated	Death
Peripheral sensory neuropathy	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL; assistive device indicated	Life-threatening consequences; urgent intervention indicated	Death

Common Terminology Criteria for Adverse Events					
Adverse Event	1	2	3	4	5
Skin					
Rash maculo-papular	Macules/papules covering<10% BSA with or without symptoms (e.g., pruritus, burning, tightness)	Macules/papules covering 10 - 30% BSA with or without symptoms (e.g., pruritus, burning, tightness); limiting instrumental ADL	Macules/papules covering >30% BSA with or without associated symptoms; limiting self care ADL	-	-

Common Terminology Criteria for Adverse Events					
Adverse Event	1	2	3	4	5
Miscellaneous					
Fatigue	Fatigue relieved by rest	Fatigue not relieved by rest; limiting instrumental ADL	Fatigue not relieved by rest, limiting self care ADL	-	-
Insomnia	Mild difficulty falling asleep, staying asleep or waking up early	Moderate difficulty falling asleep, staying asleep or waking up early	Severe difficulty in falling asleep, staying asleep or waking up early	-	-
Weight loss	5 to <10% from baseline; intervention not indicated	10 to <20% from baseline; nutritional support indicated	>=20 from baseline; tube feeding or TPN indicated	-	-

Appendix B

แบบเก็บข้อมูล

ลำดับผู้ป่วย.....วันที่.....

เพศ.....วันเกิด(วัน/เดือน/ปี).....

โรคประจำตัว.....

ระยะของโรค 1) Locally advanced 2) Metastatic 3) Mixed

ตำแหน่งของโรค 1) Intrahepatic CC 2) Hilar CCA 3) Distal CCA

4) Unspecific CCA 5) Gallbladder CA 6) Ampullary CA

วันที่ได้รับยาเคมีบำบัดครั้งแรก.....รอบ-วันที่การรักษาปัจจุบัน (Cxdy).....

ECOG PS 1) 0 2) 1 3) 2

ประวัติการทำ Biliary drainage 1) เคย 2) ไม่เคย

ระดับCEA (ng/ml) (ก่อนการรักษา)..... (หลังการรักษา....รอบ).....

ระดับCA 19-9 (U/ml) (ก่อนการรักษา).....(หลังการรักษา....รอบ).....

ผลการตอบสนอง 1) CR 2) PR 3) SD 4) PD 5) NA

ผู้ป่วยจัดอยู่ในกลุ่ม 1) Responder 2) Non-responder

ผลการตรวจ Genotype

RRM1 (-) 37 Polymorphisms

1) RR37CC 2) RR37AC 3) RR37AA 4) ระบุไม่ได้

RRM1 (-) 524 Polymorphisms

1) R5247CC 2) RR524CT 3) RR524TT 4) ระบุไม่ได้

ERCC1 Polymorphisms

1) CC 2) CT 3) TT 4) ระบุไม่ได้

CTR1 Polymorphisms

1) GG 2) GT 3) TT 4) ระบุไม่ได้

ผลทางห้องปฏิบัติการและความรุนแรงของการเกิดพิษจากยา

รอบที่ วันที่	ก่อนให้ยา	1 (.../.../...)		2 (.../.../...)		3 (.../.../...)		4 (.../.../...)		5 (.../.../...)		6 (.../.../...)	
ผลทางห้องปฏิบัติการ		1	8	1	8	1	8	1	8	1	8	1	8
Gemcitabine													
Platinum (.....)													
Weight													
Hgb													
Hct													
WBC													
Neutrophil count													
ANC													
Platelet count													
Total Bilirubin													
Direct bilirubin													
ALT													
AST													
ALP													
Creatinine													
BUN													
CEA													
CA 19-9													

รอบที่ วันที่	ก่อนให้ยา	1 (.../.../...)		2 (.../.../...)		3 (.../.../...)		4 (.../.../...)		5 (.../.../...)		6 (.../.../...)	
ความรุนแรง		1	8	1	8	1	8	1	8	1	8	1	8
Anemia													
Neutropenia													
Thrombocytopenia													
↑Bilirubin													
↑ALT													
↑AST													
↑Creatinine													
Oral mucocitis													
Diarrhea													
Nausea													
Vomiting													
Peripheral motor neuropathy													
Peripheral sensory neuropathy													
อาการอื่นๆ													

ผลทางห้องปฏิบัติการและความรุนแรงของการเกิดพิษจากยา (single gemcitabine)

รอบที่ วันที่	ก่อนให้ยา	1 (.../.../...)			2 (.../.../...)			3 (.../.../...)			4 (.../.../...)			5 (.../.../...)		
ผลทางห้องปฏิบัติการ		1	8	15	1	8	15	1	8	15	1	8	15	1	8	15
Gemcitabine																
Weight																
Hgb																
Hct																
WBC																
Neutrophil count																
ANC																
Platelet count																
Total Bilirubin																
Direct bilirubin																
ALT																
AST																
ALP																
Creatinine																
BUN																
CEA																
CA 19-9																

รอบที่ วันที่	ก่อนให้ยา	1 (.../.../...)			2 (.../.../...)			3 (.../.../...)			4 (.../.../...)			5 (.../.../...)		
ความรุนแรง		1	8	15	1	8	15	1	8	15	1	8	15	1	8	15
Anemia																
Neutropenia																
Thrombocytopenia																
↑Bilirubin																
↑ALT																
↑AST																
↑Creatinine																
Oral mucocitis																
Diarrhea																
Nausea																
Vomiting																
Peripheral motor neuropathy																
Peripheral sensory neuropathy																
อาการอื่นๆ																

Appendix C

เอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในโครงการวิจัย
(Information sheet)

ชื่อโครงการวิจัย ความสัมพันธ์ของภาวะพหุสัณฐานของยีน *RRM1 ERCC1* และ *CTR1* กับการตอบสนองต่อการรักษาและการเกิดพิษจากยาเคมีบำบัดเจมไซตาบีนและแพลทินัมในผู้ป่วยมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้

ผู้ทำวิจัย เภสัชกรสกลชาติ พงษ์มณีรัตนกุล นิสิตระดับปริญญาโท ภาควิชาเภสัชกรรมปฏิบัติ สาขาวิชาเภสัชกรรมคลินิก จุฬาลงกรณ์มหาวิทยาลัย

สถานที่วิจัย โรงพยาบาลจุฬาลงกรณ์

บุคคลที่สามารถติดต่อเมื่อเกิดเหตุฉุกเฉินระหว่างการวิจัย

1. เภสัชกรสกลชาติ พงษ์มณีรัตนกุล
   ที่อยู่ ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
   254 ถนนพญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพมหานคร 10330
   โทร 086-8906988
2. อาจารย์ นายแพทย์สืบพงศ์ ธนสารวิมล
   ที่อยู่ ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
   254 ถนนพญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพมหานคร 10330
   โทร 02-2564533
3. รองศาสตราจารย์ เภสัชกรหญิง ดร.ธิติมา เพ็งสุภาพ
   ที่อยู่ ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
   254 ถนนพญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพมหานคร 10330
   โทร 081-9377350
4. อาจารย์ เภสัชกรหญิง ดร.ณัฏฐดา อารีเปี่ยม
   ที่อยู่ ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
   254 ถนนพญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพมหานคร 10330
   โทร 081-6222858

เรียน ผู้เข้าร่วมโครงการวิจัยทุกท่าน

ท่านได้รับเชิญให้เข้าร่วมในโครงการวิจัยนี้เนื่องจากท่านเป็นผู้ป่วยมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้ ก่อนที่ท่านจะตัดสินใจเข้าร่วมในการศึกษาวิจัยดังกล่าว ขอให้ท่านอ่านเอกสารฉบับนี้อย่างถี่

ถ้วน เพื่อให้ท่านได้ทราบถึงเหตุผลและรายละเอียดของการศึกษาวิจัยในครั้งนี้ หากท่านมีข้อสงสัยใดๆ เพิ่มเติม กรุณาซักถามจากผู้ทำวิจัย หรือแพทย์ผู้ร่วมทำวิจัยซึ่งจะเป็นผู้สามารถตอบคำถามและให้ความกระจ่างแก่ท่านได้

ท่านสามารถขอคำแนะนำในการเข้าร่วมโครงการวิจัยนี้จากครอบครัว เพื่อน หรือแพทย์ประจำตัวของท่านได้ ท่านมีเวลาอย่างเพียงพอในการตัดสินใจโดยอิสระ ถ้าท่านตัดสินใจแล้วว่าจะเข้าร่วมในโครงการวิจัยนี้ ขอให้ท่านลงนามในเอกสารแสดงความยินยอมของโครงการวิจัยนี้ ท่านจะได้รับสำเนาใบยินยอมที่เก็บไว้ 1 ฉบับ

เหตุผลความเป็นมา

โรคมะเร็งท่อน้ำดี คือโรคที่เกิดจากเซลล์เยื่อบุผนังของท่อน้ำดีมีการเจริญเติบโตที่ผิดปกติ การศึกษาอุบัติการณ์ของโรคมะเร็งท่อน้ำดีทั่วโลกทำให้ทราบว่า ประเทศไทยมีอุบัติการณ์ของโรคสูงที่สุด โรคมะเร็งท่อน้ำจัดเป็นโรคที่มีการพยากรณ์ของโรคที่ไม่ดี ผู้ป่วยส่วนมากมักมาพบแพทย์เมื่อโรคมีการลุกลามจึงทำให้ผู้ป่วยไม่สามารถเข้ารับการผ่าตัดได้ ผู้ป่วยในกลุ่มนี้จึงควรได้รับการรักษาด้วยยาเคมีบำบัด คือ เจมไซตาบีนและแพลทินัม แต่ผลการตอบสนองก็มีความแตกต่างกันในแต่ละบุคคลและยังให้ผลที่ยังไม่น่าพอใจ ผลการศึกษาในก่อนหน้านี้พบว่า ภาวะพหุสัณฐานของยีนหรือความหลากหลายทางพันธุกรรมมีความสัมพันธ์กับการตอบสนองต่อยาเคมีบำบัด จึงนำมาสู่การศึกษาผลของความหลากหลายทางพันธุกรรมของยีนอาร์อาร์เอ็มวัน (*RRM1*) อีอาร์ซีซีวัน (*ERCC1*) และซีทีอาร์วัน (*CTR1*) ต่อผลการตอบสนองและการเกิดพิษจากสูตรยาเจมไซตาบีนและแพลทินัมในผู้ป่วยโรคมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้

วัตถุประสงค์ของการวิจัย

วัตถุประสงค์จากการวิจัยในครั้งนี้คือเพื่อศึกษาผลของความหลากหลายทางพันธุกรรมของยีนอาร์อาร์เอ็มวัน (*RRM1*) อีอาร์ซีซีวัน (*ERCC1*) และซีทีอาร์วัน (*CTR1*) ต่อผลการตอบสนองและการเกิดพิษจากสูตรยาเจมไซตาบีนและแพลทินัมในผู้ป่วยโรคมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้

จำนวนผู้เข้าร่วมในโครงการวิจัย คือ 105 คน

การวิจัยยังไม่มีแหล่งทุน

วิธีการที่เกี่ยวข้องกับการวิจัย

หลังจากท่านให้ความยินยอมที่จะเข้าร่วมในโครงการวิจัยนี้ ท่านจะได้รับตรวจและรักษาตามปกติ โดยแพทย์จะเป็นผู้คัดกรองว่าท่านมีคุณสมบัติที่เหมาะสมที่จะเข้าร่วมในการวิจัย

สำหรับงานวิจัยครั้งนี้ท่านจะได้รับการเจาะเลือดทางหลอดเลือดดำปริมาณ 5-10 ซีซี (หนึ่งถึงสองช้อนชา) จำนวน 1 ครั้ง หลังจากนั้นเลือดของท่านจะถูกนำไปสกัดแยกดีเอ็นเอ (DNA) เพื่อนำไปวิเคราะห์หาลักษณะความหลากหลายทางพันธุกรรมของยีนอาร์อาร์เอ็มวัน (*RRM1*) อีอาร์ซีซีวัน (*ERCC1*) และซีทีอาร์วัน (*CTR1*) หลังจากนั้นเมื่อท่านมาพบแพทย์ตามรอบการรับยา ท่านจะได้รับ

การติดตามเหตุการณ์ไม่พึงประสงค์จากยาเคมีบำบัดและจะมีการเก็บข้อมูลจากเวชระเบียน จากนั้นข้อมูลที่ได้จะนำไปวิเคราะห์ทางสถิติ ท่านควรจะต้องอยู่ในโครงการวิจัย 4 เดือนหรือจนกว่าจะมีผลการประเมินการรักษา

ผู้วิจัยจะไม่แจ้งผลการตรวจความหลากหลายทางพันธุกรรม จนกว่าจะมีหลักฐานชัดเจนว่ามีความสัมพันธ์กับการตอบสนองและการเกิดพิษจากยา

ความรับผิดชอบของอาสาสมัครผู้เข้าร่วมในโครงการวิจัย

ขอให้ท่านแจ้งผู้ทำวิจัยในกรณีที่อาการผิดปกติต่าง ๆ ที่เกิดขึ้น หรืออาการอื่น ๆ ที่พบร่วมด้วย ระหว่างที่อยู่ในโครงการวิจัย ถ้ามีการเปลี่ยนแปลงเกี่ยวกับสุขภาพของท่าน ขอให้ท่านรายงานให้ผู้ทำวิจัยทราบโดยเร็ว

ความเสี่ยงที่ได้รับจากการเจาะเลือด

ท่านมีโอกาสที่จะเกิดอาการเจ็บ เลือดออก ช้ำจากการเจาะเลือด อาการบวมบริเวณที่เจาะเลือดหรือหน้ามืด และโอกาสที่จะเกิดการติดเชื้อบริเวณที่เจาะเลือดพบได้น้อยมาก

ความเสี่ยงที่ไม่ทราบแน่นอน

ท่านอาจเกิดอาการข้างเคียง หรือความไม่สบาย ซึ่งอาการข้างเคียงเหล่านี้เป็นอาการที่ไม่เคยพบมาก่อน เพื่อความปลอดภัยของท่าน ควรแจ้งผู้ทำวิจัยให้ทราบทันทีเมื่อเกิดความผิดปกติใดๆ เกิดขึ้น

หากท่านมีข้อสงสัยใดๆ เกี่ยวกับความเสี่ยงที่อาจได้รับจากการเข้าร่วมในโครงการวิจัย ท่านสามารถสอบถามจากผู้ทำวิจัยได้ตลอดเวลา

ประโยชน์ที่อาจได้รับ

ท่านจะไม่ได้รับประโยชน์ใดๆจากการเข้ารับการวิจัยครั้งนี้ แต่ข้อมูลที่ได้จากการวิจัยสามารถเป็นแนวทางในการพิจารณาการรักษาผู้ป่วยมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้ด้วยยาเคมีบำบัดเจมไซตาบีนและแพลทินัมตามความหลากหลายทางพันธุกรรมของยีนอาร์อาร์เอ็มวัน (*RRM1)* อีอาร์ซีซีวัน (*ERCC1*) และซีทีอาร์วัน (*CTR1*) ให้เหมาะสมในผู้ป่วยแต่ละรายได้ในอนาคต

อันตรายที่อาจเกิดขึ้นจากการเข้าร่วมในโครงการวิจัยและความรับผิดชอบของผู้ทำวิจัย

หากพบอันตรายที่เกิดขึ้นจากการวิจัย ท่านจะได้รับการรักษาอย่างเหมาะสมทันที ผู้วิจัยยินดีจะรับผิดชอบค่าใช้จ่ายในการรักษาพยาบาลของท่าน และการลงนามในเอกสารให้ความยินยอมไม่ได้หมายความว่าท่านได้สละสิทธิ์ทางกฎหมายตามปกติที่ท่านพึงมี

ค่าตอบแทนสำหรับผู้เข้าร่วมวิจัย

ท่านจะไม่ได้รับเงินค่าตอบแทนจากการเข้าร่วมในการวิจัย แต่ท่านจะได้รับค่าเดินทางรวมทั้งหมด 300 บาทต่อคนในวันที่เจาะเลือด

การเข้าร่วมและการสิ้นสุดการเข้าร่วมโครงการวิจัย

การเข้าร่วมในโครงการวิจัยครั้งนี้เป็นไปโดยความสมัครใจ หากท่านไม่สมัครใจจะเข้าร่วมการศึกษาแล้ว ท่านสามารถถอนตัวได้ตลอดเวลา การขอถอนตัวออกจากโครงการวิจัยจะไม่มีผลต่อการดูแลรักษาโรคของท่านแต่อย่างใด

การปกป้องรักษาข้อมูลความลับของอาสาสมัคร

ข้อมูลที่อาจนำไปสู่การเปิดเผยตัวท่าน จะได้รับการปกปิดและจะไม่เปิดเผยแก่สาธารณชน ในกรณีที่ผลการวิจัยได้รับการตีพิมพ์ ชื่อและที่อยู่ของท่านจะต้องได้รับการปกปิดอยู่เสมอ โดยจะใช้เฉพาะรหัสประจำโครงการวิจัยของท่าน

จากการลงนามยินยอมของท่านผู้ทำวิจัย หากท่านต้องการยกเลิกการให้สิทธิ์ดังกล่าว ท่านสามารถแจ้ง หรือเขียนบันทึกขอยกเลิกการให้คำยินยอม โดยส่งไปที่ เภสัชกรสกลชาติ พงษ์มณีรัตนกุล ที่อยู่ ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 254 ถนนพญาไท แขวงวังใหม่ เขตปทุมวัน กรุงเทพมหานคร 10330 หากท่านขอยกเลิกการให้คำยินยอมหลังจากที่ท่านได้เข้าร่วมโครงการวิจัยแล้ว ข้อมูลส่วนตัวของท่านจะไม่ถูกบันทึกเพิ่มเติม อย่างไรก็ตามข้อมูลอื่น ๆ ของท่านอาจถูกนำมาใช้เพื่อประเมินผลการวิจัย และท่านจะไม่สามารถกลับมาเข้าร่วมในโครงการนี้ได้อีก ทั้งนี้เนื่องจากข้อมูลของท่านที่จำเป็นสำหรับใช้เพื่อการวิจัยไม่ได้ถูกบันทึก

จากการลงนามยินยอมของท่านผู้ทำวิจัยสามารถบอกรายละเอียดของท่านที่เกี่ยวกับการเข้าร่วมโครงการวิจัยนี้ให้แก่แพทย์ผู้รักษาท่านได้

การจัดการกับตัวอย่างเลือดที่เหลือ

ขอเก็บตัวอย่างเลือดสำหรับตรวจซ้ำ เพื่อยืนยันความถูกต้องของผลการทดลองเป็นระยะเวลา 1 ปี หลังจากนั้น ตัวอย่างเลือดจะถูกทำลายทำลายตามวิธีมาตรฐาน (เผาด้วยระบบไร้ควัน) ทันที

หากท่านไม่ได้รับการชดเชยอันควรต่อการบาดเจ็บหรือเจ็บป่วยที่เกิดขึ้นโดยตรงจากการวิจัย หรือท่านไม่ได้รับการปฏิบัติตามที่ปรากฏในเอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในการวิจัย ท่านสามารถร้องเรียนได้ที่ คณะกรรมการจริยธรรมการวิจัย คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ตึกอานันทมหิดลชั้น 3 โรงพยาบาลจุฬาลงกรณ์ ถนนพระราม 4 ปทุมวัน กรุงเทพฯ 10330 โทร 0-2256-4493 ต่อ 14, 15 ในเวลาราชการ

ขอขอบคุณในการร่วมมือของท่านมา ณ ที่นี้

Appendix D

เอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัย
(Consent form)

การวิจัยเรื่อง ความสัมพันธ์ของภาวะพหุสัณฐานของยีน *RRM1 ERCC1* และ *CTR1* กับการตอบสนองต่อการรักษาและการเกิดพิษจากยาเคมีบำบัดเจมไซตาบีนและแพลทินัมในผู้ป่วยมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้

วันให้คำยินยอม วันที่.....เดือน.....พ.ศ.....

ข้าพเจ้า นาย/นาง/นางสาว.....

ที่อยู่.....

ได้อ่านรายละเอียดจากเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัยวิจัยที่แนบมาฉบับวันที่..... และข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยโดยสมัครใจ

ข้าพเจ้าได้รับสำเนาเอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนามและ วันที่ พร้อมด้วยเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัย ทั้งนี้ก่อนที่จะลงนามในใบยินยอมเข้ารับการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย ระยะเวลาของการทำวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัย และแนวทางรักษาโดยวิธีอื่นอย่างละเอียด ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัยจนมีความเข้าใจอย่างดีแล้ว โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้ารับทราบจากผู้วิจัยว่าหากเกิดอันตรายใด ๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่เสียค่าใช้จ่าย

ข้าพเจ้ามีสิทธิที่จะบอกเลิกเข้าร่วมในโครงการวิจัยเมื่อใดก็ได้ โดยไม่จำเป็นต้องแจ้งเหตุผลและการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคหรือสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะเมื่อได้รับการยินยอมจากข้าพเจ้าเท่านั้น บุคคลอื่นในนามของบริษัทผู้สนับสนุนการวิจัย คณะกรรมการพิจารณาจริยธรรมการวิจัยในคนอาจได้รับอนุญาตให้เข้ามาตรวจและประมวลข้อมูลของข้าพเจ้า ทั้งนี้จะต้องกระทำไปเพื่อวัตถุประสงค์เพื่อตรวจสอบความถูกต้องของข้อมูลเท่านั้น โดยการตกลงที่จะเข้า

ร่วมการศึกษานี้ข้าพเจ้าได้ให้คำยินยอมที่จะให้มีการตรวจสอบข้อมูลประวัติทางการแพทย์ของข้าพเจ้าได้

ผู้วิจัยรับรองว่าจะไม่มีการเก็บข้อมูลใด ๆ เพิ่มเติม หลังจากที่ข้าพเจ้าขอยกเลิกการเข้าร่วมโครงการ วิจัยและต้องการให้ทำลายเอกสารและตัวอย่างที่ใช้ตรวจสอบทั้งหมดที่สามารถสืบค้นถึงตัวข้าพเจ้าได้

ข้าพเจ้าเข้าใจว่า ข้าพเจ้ามีสิทธิ์ที่จะตรวจสอบหรือแก้ไขข้อมูลส่วนตัวของข้าพเจ้าและสามารถยกเลิกการให้สิทธิในการใช้ข้อมูลส่วนตัวของข้าพเจ้าได้ โดยต้องแจ้งให้ผู้วิจัยรับทราบ

ข้าพเจ้าได้ตระหนักว่าข้อมูลในการวิจัยรวมถึงข้อมูลทางการแพทย์ของข้าพเจ้าที่ไม่มีการเปิดเผยชื่อ จะผ่านกระบวนการต่าง ๆ เช่น การเก็บข้อมูล การบันทึกข้อมูลในแบบบันทึกและในคอมพิวเตอร์ การตรวจสอบ การวิเคราะห์ และการรายงานข้อมูลเพื่อวัตถุประสงค์ทางวิชาการ รวมทั้งการใช้ข้อมูลทางการแพทย์ในอนาคตหรือการวิจัยทางด้านเภสัชภัณฑ์ เท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความเต็มใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

.....ลงนามผู้ให้ความยินยอม

(.....) ชื่อผู้ยินยอมตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

การจัดการกับตัวอย่างทางชีวภาพ

☐ ไม่มีตัวอย่างชีวภาพ

☐ มีแต่ไม่มีการขอเก็บ

☐ มีและขอเก็บตัวอย่างชีวภาพที่เหลือไว้เพื่อการวิจัยในอนาคต

ข้าพเจ้า ☐ ยินยอม

☐ ไม่ยินยอม

ให้เก็บตัวอย่างชีวภาพที่เหลือไว้เพื่อการวิจัยในอนาคต

.....ลงนามผู้ให้ความยินยอม

(.....) ชื่อผู้ยินยอมตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้าได้อธิบายถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการไม่พึงประสงค์ หรือความเสี่ยงที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัย อย่างละเอียด ให้ผู้เข้าร่วมในโครงการวิจัยตามนามข้างต้นได้ทราบและมีความเข้าใจดีแล้ว พร้อมลงนามลงในเอกสารแสดงความยินยอมด้วยความเต็มใจ

.....ลงนามผู้ทำวิจัย

(เภสัชกรสกลชาติ พงษ์มณีรัตนกุล) ชื่อผู้ทำวิจัย ตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

.....ลงนามพยาน

(.....) ชื่อพยาน ตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

Appendix E

Protocol Number 599/58

INSTITUTIONAL REVIEW BOARD
Faculty of Medicine, Chulalongkorn University
1873 Rama IV Road, Patumwan, Bangkok 10330, Thailand, Tel 662-256-4493

Approval of Documents related to Study Protocol

The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, has approved/acknowledged the following study which is to be carried out in compliance with the International guidelines for human research protection as Declaration of Helsinki, The Belmont Report, CIOMS Guidelines and International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

Study Title : ASSOCIATION OF *RRM1, ERCC1* AND *CTR1* POLYMORPHISMS WITH RESPONSE AND TOXICITIES OF GEMCITABINE-PLATINUM CHEMOTHERAPY IN PATIENTS WITH UNRESECTABLE CHOLANGIOCARCINOMA

Study Code : -

Principal Investigator : Mr. Skolchart Pongmaneratanakul

Affiliation of PI : Faculty of Pharmaceutical Sciences, Chulalongkorn University

Document Reviewed :
1. Protocol version 2 Date 22/01/59

Signature ..... Signature .....
(Emeritus Professor Tada Sueblinvong MD) (Assistant Professor Prapapan Rajatapiti MD,PhD)
Chairperson Member and Secretary
The Institutional Review Board The Institutional Review Board

Date of Approval : August 4, 2016

Approval granted is subject to the following conditions (see back of this Certificate)

All approved investigators must comply with the following conditions:

1. Strictly conduct the research as required by the protocol;
2. Use only the information sheet, consent form (and recruitment materials, if any), interview outlines and/or questionnaires bearing the Institutional Review Board's seal of approval ; and return one copy of such documents of the first subject recruited to the Institutional Review Board (IRB) for record keeping;
3. Report to the Institutional Review Board any serious adverse event or any changes in the research activity within five working days;
4. Provide reports to the Institutional Review Board concerning the progress of the research upon the specified period of time or when requested;
5. If the study cannot be finished within the expiring date of the approval on the certificate, the investigator is obliged to reapply for approval at least one month before the date of expiration.
6. All the above approved documents expire on the same date of the previously approved protocol (Protocol Number.....599/18.....)

หมายเลขโครงการ 599/58

คณะกรรมการพิจารณาจริยธรรมการวิจัย
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
1873 ถ.พระราม 4 เขตปทุมวัน กรุงเทพฯ 10330 โทร. 0-2256-4493

หนังสือรับรองเอกสารที่เกี่ยวข้องกับโครงการวิจัย

คณะกรรมการพิจารณาจริยธรรมการวิจัย คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ดำเนินการให้การรับรอง/รับทราบ เอกสารที่เกี่ยวข้องกับโครงการวิจัยตามแนวทางหลักจริยธรรมการวิจัยในคนที่เป็นมาตรฐานสากลได้แก่ Declaration of Helsinki, The Belmont Report, CIOMS Guidelines และ International Conference on Harmonization in Good Clinical Practice หรือ ICH-GCP

ชื่อโครงการ : ความสัมพันธ์ของภาวะพหุสัณฐานของยีน *RRM1 ERCC1* และ *CTR1* กับการตอบสนองต่อการรักษาและการเกิดพิษจากยาเคมีบำบัดเจมไซตาบีนและแพลทินัมในผู้ป่วยมะเร็งท่อน้ำดีที่ไม่สามารถผ่าตัดได้

เลขที่โครงการวิจัย : -

ผู้วิจัยหลัก : นายสกลชาติ พงษ์มณีรัตนกุล

สังกัดหน่วยงาน : คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

เอกสารที่ได้รับการทบทวน :

1. Protocol version 2 Date 22/01/59

ลงนาม .....
(ศาสตราจารย์กิตติคุณแพทย์หญิงธาดา สืบหลินวงศ์)
ประธาน
คณะกรรมการพิจารณาจริยธรรมการวิจัย

ลงนาม.....
(ผู้ช่วยศาสตราจารย์ ดร.พญ.ประภาพรรณ รัชตะปีติ)
กรรมการและเลขานุการ
คณะกรรมการพิจารณาจริยธรรมการวิจัย

วันที่รับรอง: 4 สิงหาคม 2559

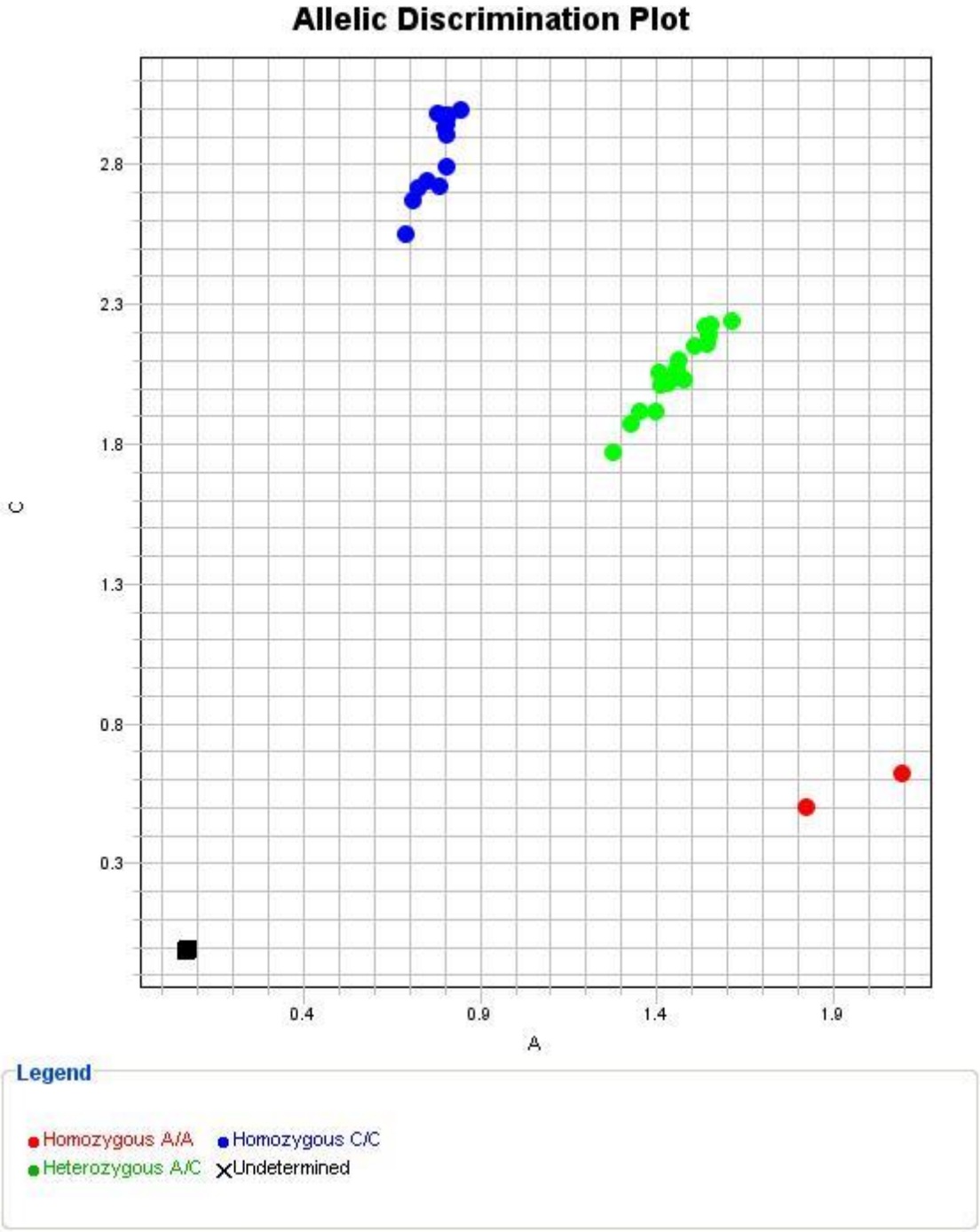
ทั้งนี้ การรับรองนี้มีเงื่อนไขดังที่ระบุไว้ด้านหลังทุกข้อ (ดูด้านหลังของเอกสารรับรองโครงการวิจัย)

นักวิจัยทุกท่านที่ผ่านการรับรองจริยธรรมการวิจัยต้องปฏิบัติดังต่อไปนี้

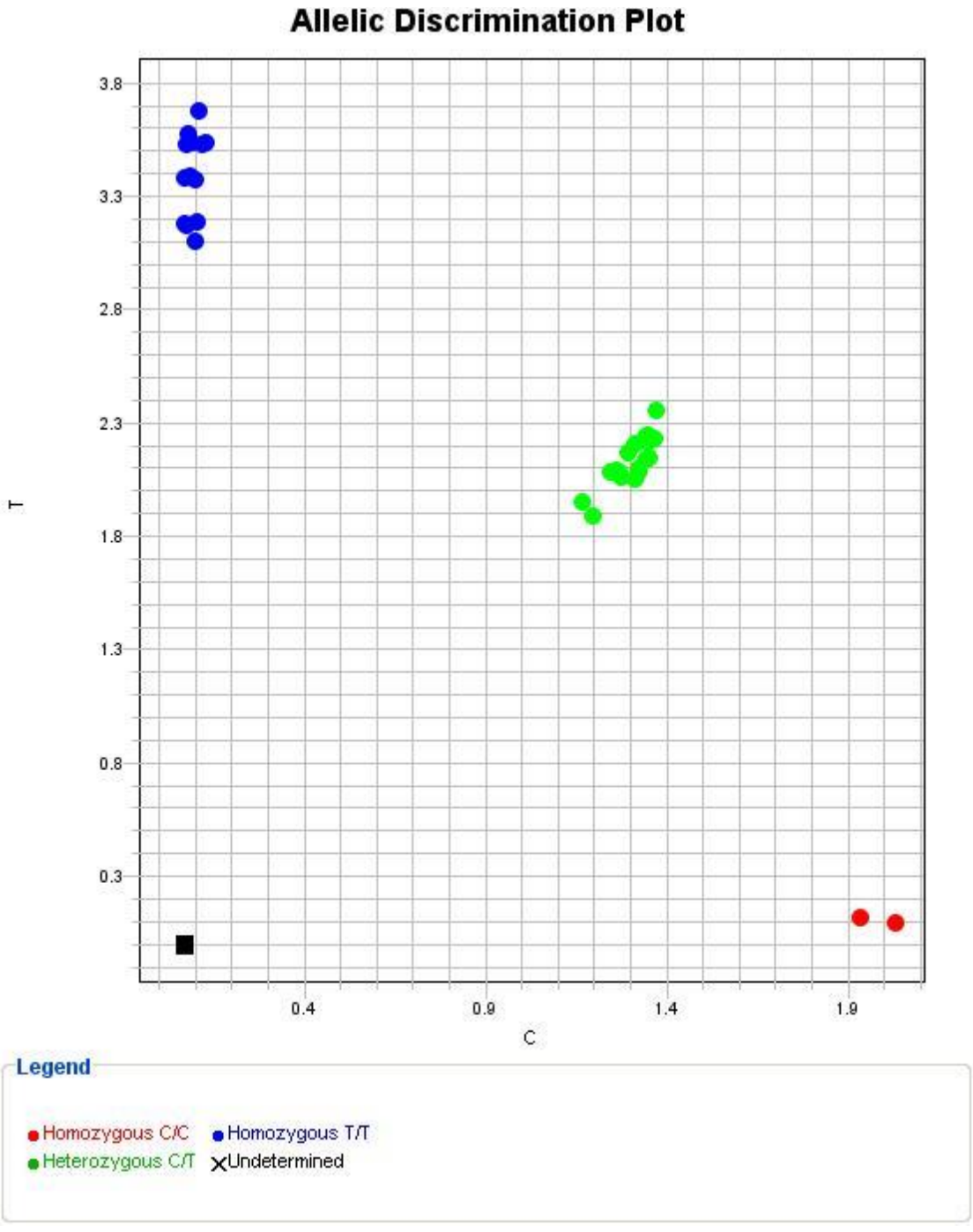
1. ดำเนินการวิจัยตามที่ระบุไว้ในโครงร่างการวิจัยอย่างเคร่งครัด
2. ใช้เอกสารแนะนำอาสาสมัคร ใบยินยอม (และเอกสารเชิญเข้าร่วมวิจัยหรือใบโฆษณาถ้ามี) แบบสัมภาษณ์ และหรือ แบบสอบถาม เฉพาะที่มีตราประทับของคณะกรรมการพิจารณาจริยธรรมเท่านั้น และส่งสำเนาเอกสารดังกล่าวที่ใช้กับผู้เข้าร่วมวิจัยจริงรายแรกมาที่ฝ่ายวิจัย คณะแพทยศาสตร์ เพื่อเก็บไว้เป็นหลักฐาน
3. รายงานเหตุการณ์ไม่พึงประสงค์ร้ายแรงที่เกิดขึ้นหรือการเปลี่ยนแปลงกิจกรรมวิจัยใดๆ ต่อคณะกรรมการพิจารณาจริยธรรมการวิจัย ภายใน 5 วันทำการ
4. ส่งรายงานความก้าวหน้าต่อคณะกรรมการพิจารณาจริยธรรมการวิจัย ตามเวลาที่กำหนดหรือเมื่อได้รับการร้องขอ
5. หากการวิจัยไม่สามารถดำเนินการเสร็จสิ้นภายในกำหนด ผู้วิจัยต้องยื่นขออนุมัติใหม่ก่อน อย่างน้อย 1 เดือน
6. เอกสารทุกฉบับที่ได้รับการรับรองครั้งนี้ หมดอายุตามอายุของโครงร่างการวิจัยที่ได้รับการรับรองก่อนหน้านี้ (หมายเลขโครงการ...699/58.....)

Appendix F

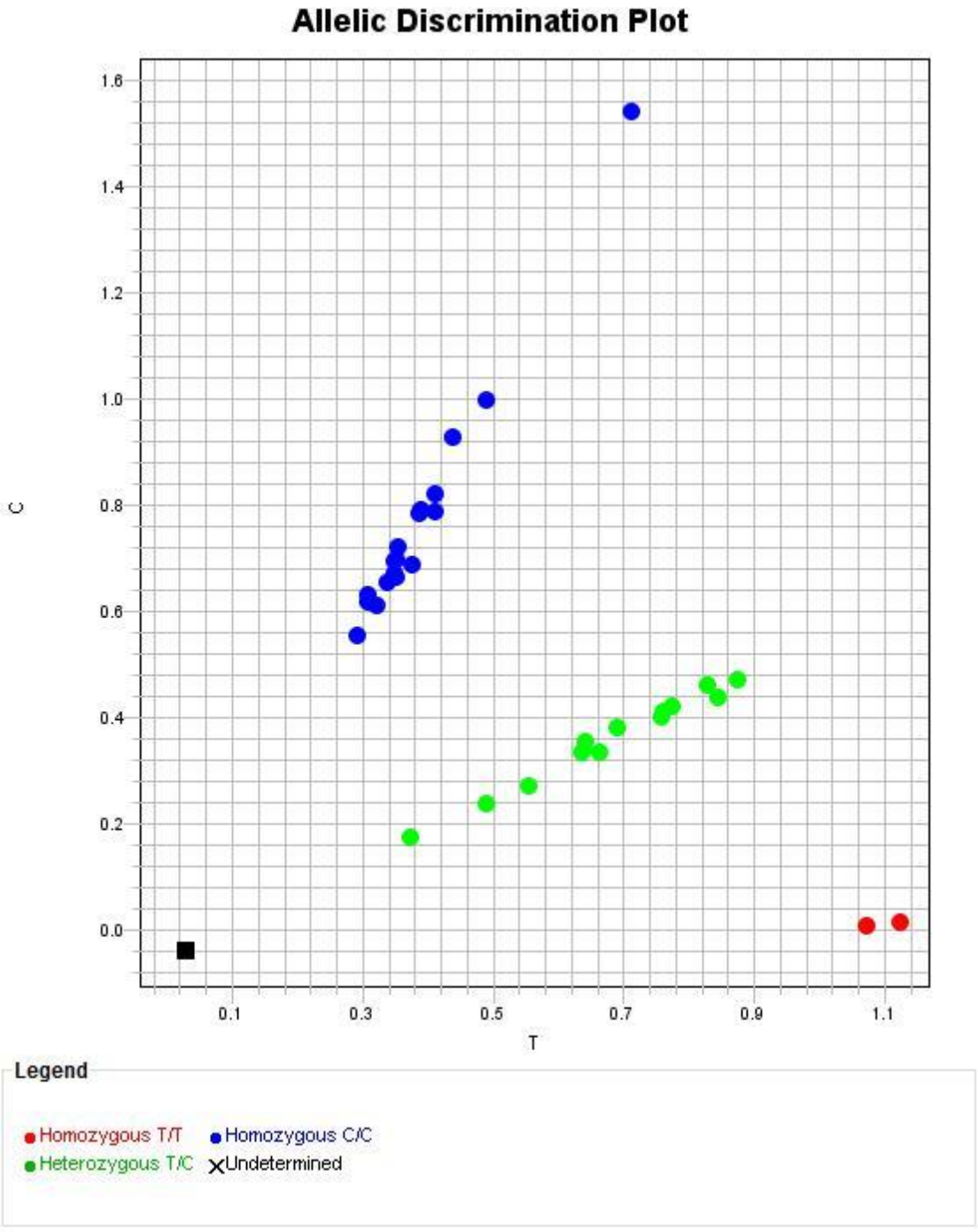
Allelic Discrimination Plot (SNP Assay: rs12806698)



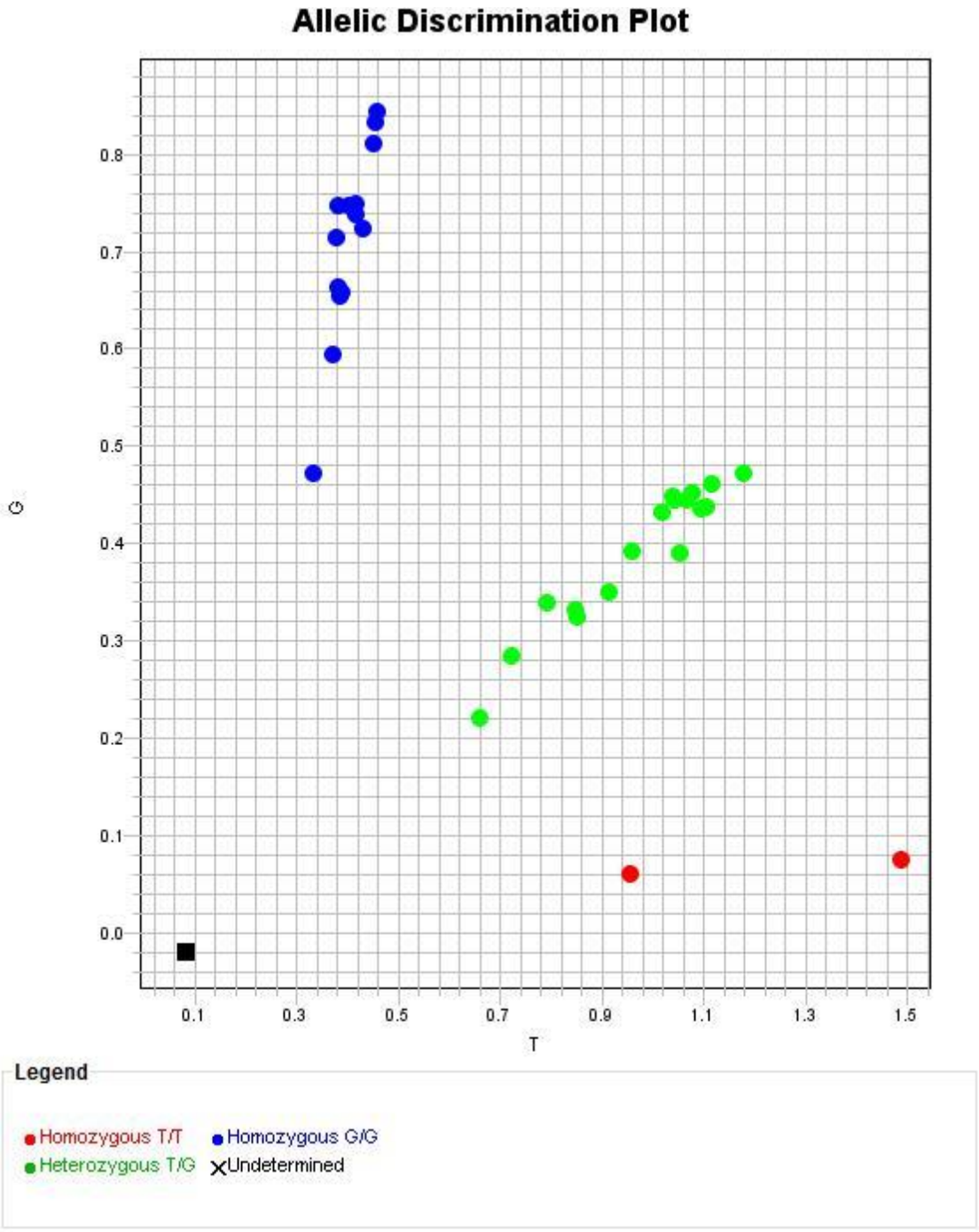
Allelic Discrimination Plot (SNP Assay: rs11030918)



Allelic Discrimination Plot (SNP Assay: rs11615)



Allelic Discrimination Plot (SNP Assay: rs12686377)



Appendix G

Patients' characteristic and response rate

Characteristic	N (%)	Responders (%)	Non-responder (%)	P-value χ^2 test
No. of patients	31	3 (9.7)	28 (90.3)	
Gender				
Male	12 (38.7)	2 (16.7)	10 (83.3)	0.543*
Female	19 (61.3)	1 (5.3)	18 (94.7)	
Age (year)				
<65	17 (54.8)	0 (0.0)	17 (100.0)	0.081*
>=65	14 (45.2)	3 (21.4)	11 (78.6)	
Extent of disease				
Locally advanced	8 (25.8)	1 (12.5)	7 (87.5)	0.616
Metastatic	16 (51.6)	2 (12.5)	14 (87.5)	
Mixed	7 (22.6)	0 (0.0)	7 (100.0)	
Primary tumor site				
Cholangiocarcinoma	23 (74.2)	2 (8.7)	21 (91.3)	0.466
Ampulla	4 (12.9)	1 (25.0)	3 (75.0)	
Gallbladder	4 (12.9)	0 (0.0)	4 (100.0)	
Performance status				
0	4 (12.9)	0 (0.0)	4 (100.0)	0.727
1	26 (83.9)	3 (11.5)	23 (88.5)	
2	1 (3.2)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	20 (64.5)	1 (5.0)	19 (95.0)	0.485
Gemcitabine/Carboplatin	6 (19.4)	1 (16.7)	5 (83.3)	
Gemcitabine	5 (16.1)	1 (20.0)	4 (80.0)	

*-Fisher's exact test

Patients' characteristic and tumor control rate

Characteristic	N (%)	Tumor control (%)	Progressive disease (%)	P-value χ^2 test
No. of patients	31	22 (71.0)	9 (29.0)	
Gender				
Male	12 (38.7)	8 (66.7)	4 (33.3)	0.704*
Female	19 (61.3)	14 (73.7)	5 (26.3)	
Age (year)				
<65	17 (54.8)	11 (64.7)	6 (35.3)	0.456*
>=65	14 (45.2)	11 (78.6)	3 (21.4)	
Extent of disease				
Locally advanced	8 (25.8)	5 (62.5)	3 (37.5)	0.417
Metastatic	16 (51.6)	13 (81.3)	3 (18.8)	
Mixed	7 (22.6)	4 (57.1)	3 (42.9)	
Primary tumor site				
Cholangiocarcinoma	23 (74.2)	17 (73.9)	6 (26.1)	0.612
Ampulla	4 (12.9)	2 (50.0)	2 (50.0)	
Gallbladder	4 (12.9)	3 (75.0)	1 (25.0)	
Performance status				
0	4 (12.9)	3 (75.0)	1 (25.0)	0.787
1	26 (83.9)	18 (69.2)	8 (30.8)	
2	1 (3.2)	1 (100.0)	0 (0.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	20 (64.5)	13 (65.0)	7 (35.0)	0.213
Gemcitabine/Carboplatin	6 (19.4)	6 (100.0)	0 (0.0)	
Gemcitabine	5 (16.1)	3 (60.0)	2 (40.0)	

*-Fisher's exact test

Patients' characteristic and hematologic toxicities

Patients' characteristic and severity of anemia

Characteristic	N (%)	Grade 1-2 (%)	Grade 3-4 (%)	P-value χ^2 test
No. of patients	33	25 (75.8)	8 (24.2)	
Gender				
Male	14 (42.4)	10 (71.4)	4 (28.6)	0.695*
Female	19 (57.6)	15 (78.9)	4 (21.1)	
Age (year)				
<65	19 (57.6)	16 (84.2)	3 (15.8)	0.238*
>=65	14 (42.4)	9 (63.4)	5 (35.7)	
Extent of disease				
Locally advanced	10 (30.3)	6 (60.0)	4 (40.0)	0.369
Metastatic	16 (48.5)	13 (81.3)	3 (18.8)	
Mixed	7 (21.2)	6 (85.7)	1 (14.3)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	18 (72.0)	7 (28.0)	0.479
Ampulla	4 (12.1)	3 (75.0)	1 (25.0)	
Gallbladder	4 (12.1)	4 (100.0)	0 (0.0)	
Performance status				
0	4 (12.1)	3 (75.0)	1 (25.0)	0.197
1	28 (84.8)	22 (78.6)	6 (21.4)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	17 (77.3)	5 (22.7)	0.841
Gemcitabine/Carboplatin	6 (18.2)	4 (66.7)	2 (33.3)	
Gemcitabine	5 (15.2)	4 (80.0)	1 (20.0)	

*-Fisher's exact test

Patients' characteristic and risk of leukopenia

Characteristic	N (%)	Grade 0 (%)	Grade 1-4 (%)	P-value χ^2 test
No. of patients	33	7 (21.2)	26 (78.8)	
Gender				
Male	14 (42.4)	3 (21.4)	11 (78.6)	1.000*
Female	19 (57.6)	4 (21.1)	15 (78.9)	
Age (year)				
<65	19 (57.6)	5 (26.3)	14 (73.7)	0.670*
>=65	14 (42.4)	2 (14.3)	12 (85.7)	
Extent of disease				
Locally advanced	10 (30.3)	3 (30.0)	7 (70.0)	0.493
Metastatic	16 (48.5)	2 (12.5)	14 (87.5)	
Mixed	7 (21.2)	2 (28.6)	5 (71.4)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	5 (20.0)	20 (80.0)	0.214
Ampulla	4 (12.1)	0 (0.0)	4 (100.0)	
Gallbladder	4 (12.1)	2 (50.0)	2 (50.)	
Performance status				
0	4 (12.1)	0 (0.0)	4 (100.0)	0.452
1	28 (84.8)	7 (25.0)	21 (75.0)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	7 (31.8)	15 (68.2)	0.108
Gemcitabine/Carboplatin	6 (18.2)	0 (0.0)	6 (100.0)	
Gemcitabine	5 (15.2)	0 (0.0)	5 (100.0)	

*-Fisher's exact test

Patients' characteristic and severity of leukopenia

Characteristic	N (%)	Grade 1-2 (%)	Grade 3-4 (%)	P-value χ^2 test
No. of patients	26	23 (88.5)	3 (11.5)	
Gender				
Male	11 (42.3)	8 (72.7)	3 (27.3)	0.063*
Female	15 (57.7)	15 (100.0)	0 (0.0)	
Age (year)				
<65	14 (53.8)	13 (92.9)	1 (7.1)	0.580*
>=65	12 (46.2)	10 (83.3)	2 (16.7)	
Extent of disease				
Locally advanced	7 (26.9)	5 (71.4)	2 (28.6)	0.125
Metastatic	14 (53.8)	14 (100.0)	0 (0.0)	
Mixed	5 (19.2)	4 (80.0)	1 (20.0)	
Primary tumor site				
Cholangiocarcinoma	20 (76.9)	18 (90.0)	2 (10.0)	0.601
Ampulla	4 (15.4)	3 (75.0)	1 (25.0)	
Gallbladder	2 (7.7)	2 (100.0)	0 (0.0)	
Performance status				
0	4 (15.4)	4 (100.0)	0 (0.0)	0.668
1	21 (80.8)	18 (85.7)	3 (14.3)	
2	1 (3.8)	1 (100.0)	0 (0.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	15 (57.7)	13 (86.7)	2 (13.3)	0.554
Gemcitabine/Carboplatin	6 (23.1)	6 (100.0)	0 (0.0)	
Gemcitabine	5 (19.2)	4 (80.0)	1 (20.0)	

*-Fisher's exact test

Patients' characteristic and risk of neutropenia

Characteristic	N (%)	Grade 0 (%)	Grade 1-4 (%)	P-value χ^2 test
No. of patients	33	13 (39.4)	20 (60.6)	
Gender				
Male	14 (42.4)	6 (42.9)	8 (57.1)	0.727*
Female	19 (57.6)	7 (36.8)	12 (63.2)	
Age (year)				
<65	19 (57.6)	8 (42.1)	11 (57.9)	0.710
>=65	14 (42.4)	5 (35.7)	9 (64.3)	
Extent of disease				
Locally advanced	10 (30.3)	4 (40.0)	6 (60.0)	0.504
Metastatic	16 (48.5)	5 (31.3)	11 (68.8)	
Mixed	7 (21.2)	4 (57.1)	3 (42.9)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	11 (44.0)	14 (56.0)	0.222
Ampulla	4 (12.1)	0 (0.0)	4 (100.0)	
Gallbladder	4 (12.1)	2 (50.0)	2 (50.0)	
Performance status				
0	4 (12.1)	1 (25.0)	3 (75.0)	0.566
1	28 (84.8)	12 (42.9)	16 (57.1)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	10 (45.5)	12 (54.5)	0.544
Gemcitabine/Carboplatin	6 (18.2)	2 (33.3)	4 (66.7)	
Gemcitabine	5 (15.2)	1 (20.0)	4 (80.0)	

*-Fisher's exact test

Patients' characteristic and severity of neutropenia

Characteristic	N (%)	Grade 1-2 (%)	Grade 3-4 (%)	P-value χ^2 test
No. of patients	20	9 (45.0)	11 (55.0)	
Gender				
Male	8 (40.0)	2 (25.0)	6 (75.0)	0.197*
Female	12 (60.0)	7 (58.3)	5 (41.7)	
Age (year)				
<65	11 (55.0)	6 (54.5)	5 (45.5)	0.406*
>=65	9 (45.0)	3 (33.3)	6 (66.7)	
Extent of disease				
Locally advanced	6 (30.0)	1 (16.7)	5 (83.3)	0.161
Metastatic	11 (55.0)	7 (63.6)	4 (36.4)	
Mixed	3 (15.0)	1 (33.3)	2 (66.7)	
Primary tumor site				
Cholangiocarcinoma	14 (70.0)	6 (42.9)	8 (57.1)	0.210
Ampulla	4 (20.0)	1 (25.0)	3 (75.0)	
Gallbladder	2 (10.0)	2 (100.0)	0 (0.0)	
Performance status				
0	3 (15.0)	1 (33.3)	2 (66.7)	0.497
1	16 (80.0)	7 (43.8)	9 (56.3)	
2	1 (5.0)	1 (100.0)	0 (0.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	12 (60.0)	5 (41.7)	7 (58.3)	0.340
Gemcitabine/Carboplatin	4 (20.0)	3 (75.0)	1 (25.0)	
Gemcitabine	4 (20.0)	1 (25.0)	3 (75.0)	

*-Fisher's exact test

Patients' characteristic and risk of thrombocytopenia

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	20 (60.6)	13 (39.4)	
Gender				
Male	14 (42.4)	9 (64.3)	5 (35.7)	0.710
Female	19 (57.6)	11 (57.9)	8 (42.1)	
Age (year)				
<65	19 (57.6)	14 (73.7)	5 (26.3)	0.073
>=65	14 (42.4)	6 (42.9)	8 (57.1)	
Extent of disease				
Locally advanced	10 (30.3)	6 (60.0)	4 (40.0)	0.970
Metastatic	16 (48.5)	10 (62.5)	6 (37.5)	
Mixed	7 (21.2)	4 (57.1)	3 (42.9)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	14 (56.0)	11 (44.0)	0.222
Ampulla	4 (12.1)	2 (50.0)	2 (50.0)	
Gallbladder	4 (12.1)	4 (100.0)	0 (0.0)	
Performance status				
0	4 (12.1)	2 (50.0)	2 (50.0)	0.658
1	28 (84.8)	17 (60.7)	11 (39.3)	
2	1 (3.0)	1 (100.0)	0 (0.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	16 (72.7)	6 (27.3)	0.045*
Gemcitabine/Carboplatin	6 (18.2)	1 (16.7)	5 (83.3)	
Gemcitabine	5 (15.2)	3 (60.0)	2 (40.0)	

*-P-value of < 0.05 was considered statistically significant.

Patients' characteristic and severity of thrombocytopenia

Characteristic	N (%)	Grade 1-2 (%)	Grade 3 (%)	P-value χ^2 test
No. of patients	13	12 (92.3)	1 (7.7)	
Gender				
Male	5 (38.5)	5 (100.0)	0 (0.0)	1.000*
Female	8 (61.5)	7 (87.5)	1 (12.5)	
Age (year)				
<65	5 (38.5)	5 (100.0)	0 (0.0)	1.000*
>=65	8 (61.5)	7 (87.5)	1 (12.5)	
Extent of disease				
Locally advanced	4 (30.8)	4 (100.0)	0 (0.0)	0.164
Metastatic	6 (46.2)	6 (100.0)	0 (0.0)	
Mixed	3 (23.1)	2 (66.7)	1 (33.3)	
Primary tumor site				
Cholangiocarcinoma	11 (84.6)	10 (90.0)	1 (9.1)	1.000
Ampulla	2 (15.4)	2 (100.0)	0 (0.0)	
Gallbladder	-	-	-	
Performance status				
0	2 (15.4)	2 (100.0)	0 (0.0)	1.000
1	11 (84.6)	10 (90.0)	1 (9.1)	
2	-	-	-	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	6 (46.2)	5 (83.3)	1 (16.7)	0.532
Gemcitabine/Carboplatin	5 (38.5)	5 (100.0)	0 (0.0)	
Gemcitabine	2 (15.4)	2 (100.0)	0 (0.0)	

*-Fisher's exact test

Patients' characteristic and non-hematologic toxicities

Patients' characteristic and risk of increased AST

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	11 (33.3)	22 (66.7)	
Gender				
Male	14 (42.4)	2 (14.3)	12 (85.6)	0.067*
Female	19 (57.6)	9 (47.4)	10 (52.6)	
Age (year)				
<65	19 (57.6)	7 (36.8)	12 (63.2)	0.719*
>=65	14 (42.4)	4 (28.6)	10 (71.4)	
Extent of disease				
Locally advanced	10 (30.3)	3 (30.0)	7 (70.0)	0.884
Metastatic	16 (48.5)	6 (37.5)	10 (62.5)	
Mixed	7 (21.2)	2 (28.6)	5 (71.4)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	8 (32.0)	17 (68.0)	0.076
Ampulla	4 (12.1)	0 (0.0)	4 (100.0)	
Gallbladder	4 (12.1)	3 (75.0)	1 (25.0)	
Performance status				
0	4 (12.1)	1 (25.0)	3 (75.0)	0.706
1	28 (84.8)	10 (35.7)	18 (64.3)	
2	1 (3.0)	0 (0.0)	1 (66.7)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	11 (50.0)	11 (50.0)	0.016**
Gemcitabine/Carboplatin	6 (18.2)	0 (0.0)	6 (100.0)	
Gemcitabine	5 (15.2)	0 (0.0)	5 (100.0)	

*-Fisher's exact test

**-P-value of < 0.05 was considered statistically significant.

Patients' characteristic and risk of increased ALT

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	15 (45.5)	18 (54.5)	
Gender				
Male	14 (42.4)	5 (35.7)	9 (64.3)	0.482
Female	19 (57.6)	10 (52.6)	9 (47.4)	
Age (year)				
<65	19 (57.6)	10 (52.6)	9 (47.4)	0.482
>=65	14 (42.4)	5 (35.7)	9 (64.3)	
Extent of disease				
Locally advanced	10 (30.3)	3 (30.0)	7 (70.0)	0.477
Metastatic	16 (48.5)	8 (50.0)	8 (50.0)	
Mixed	7 (21.2)	4 (57.1)	3 (42.9)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	12 (48.0)	13 (52.0)	0.090
Ampulla	4 (12.1)	0 (0.0)	4 (100.0)	
Gallbladder	4 (12.1)	3 (75.0)	1 (25.0)	
Performance status				
0	4 (12.1)	3 (75.0)	1 (25.0)	0.313
1	28 (84.8)	12 (42.9)	16 (57.1)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	14 (63.6)	8 (36.4)	0.011*
Gemcitabine/Carboplatin	6 (18.2)	1 (16.7)	5 (83.3)	
Gemcitabine	5 (15.2)	0 (0.0)	5 (100.0)	

*-P-value of < 0.05 was considered statistically significant.

Patients' characteristic and risk of increased ALP

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	10 (30.3)	23 (69.7)	
Gender				
Male	14 (42.4)	2 (14.3)	12 (85.7)	0.131*
Female	19 (57.6)	8 (42.1)	11 (57.9)	
Age (year)				
<65	19 (57.6)	5 (26.3)	14 (73.7)	0.707
>=65	14 (42.4)	5 (35.7)	9 (64.3)	
Extent of disease				
Locally advanced	10 (30.3)	2 (20.0)	8 (80.0)	0.256
Metastatic	16 (48.5)	7 (43.8)	9 (56.3)	
Mixed	7 (21.2)	1 (14.3)	6 (85.7)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	7 (28.0)	18 (72.0)	0.653
Ampulla	4 (12.1)	1 (25.0)	3 (75.0)	
Gallbladder	4 (12.1)	2 (50.0)	2 (50.0)	
Performance status				
0	4 (12.1)	1 (25.0)	3 (75.0)	0.766
1	28 (84.8)	9 (32.1)	19 (67.9)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	8 (36.4)	14 (63.6)	0.559
Gemcitabine/Carboplatin	6 (18.2)	1 (16.7)	5 (83.3)	
Gemcitabine	5 (15.2)	1 (20.0)	4 (80.0)	

*-Fisher's exact test

Patient's characteristic and increase TB

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	22 (66.7)	11 (33.3)	
Gender				
Male	14 (42.4)	7 (50.0)	7 (50.0)	0.136*
Female	19 (57.6)	15 (78.9)	4 (21.1)	
Age (year)				
<65	19 (57.6)	13 (68.4)	6 (31.6)	1.000
>=65	14 (42.4)	9 (64.3)	5 (35.7)	
Extent of disease				
Locally advanced	10 (30.3)	6 (60.0)	4 (40.0)	0.480
Metastatic	16 (48.5)	10 (62.5)	6 (37.5)	
Mixed	7 (21.2)	6 (85.7)	1 (14.3)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	17 (68.0)	8 (32.0)	0.724
Ampulla	4 (12.1)	2 (50.0)	2 (50.0)	
Gallbladder	4 (12.1)	3 (75.0)	1 (25.0)	
Performance status				
0	4 (12.1)	4 (100.0)	0 (0.0)	0.229
1	28 (84.8)	17 (60.7)	11 (39.3)	
2	1 (3.0)	1 (100.0)	0 (0.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	17 (77.3)	5 (22.7)	0.122
Gemcitabine/Carboplatin	6 (18.2)	2 (33.3)	4 (66.7)	
Gemcitabine	5 (15.2)	3 (60.0)	2 (40.0)	

*-Fisher's exact test

Patient's characteristic and increase Cr

Characteristic	N (%)	Grade 0 (%)	Grade 1 (%)	P-value χ^2 test
No. of patients	33	26 (78.8)	7 (21.2)	
Gender				
Male	14 (42.4)	8 (57.1)	6 (42.9)	0.026[1,2]
Female	19 (57.6)	18 (94.7)	1 (5.3)	
Age (year)				
<65	19 (57.6)	16 (84.2)	3 (15.8)	0.422[1]
>=65	14 (42.4)	10 (71.4)	4 (28.6)	
Extent of disease				
Locally advanced	10 (30.3)	9 (90.0)	1 (10.0)	0.383
Metastatic	16 (48.5)	11 (68.8)	5 (31.3)	
Mixed	7 (21.2)	6 (85.7)	1 (14.3)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	18 (72.0)	7 (28.0)	0241
Ampulla	4 (12.1)	4 (100.0)	0 (0.0)	
Gallbladder	4 (12.1)	4 (100.0)	0 (0.0)	
Performance status				
0	4 (12.1)	3 (75.0)	1 (25.0)	0.859
1	28 (84.8)	22 (78.6)	6 (21.4)	
2	1 (3.0)	1 (100.0)	0 (0.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	18 (81.8)	4 (18.2)	0.108
Gemcitabine/Carboplatin	6 (18.2)	3 (50.0)	3 (50.0)	
Gemcitabine	5 (15.2)	5 (100.0)	0 (0.0)	

1-P-value of < 0.05 was considered statistically significant.

2-Fisher's exact test

Patients' characteristic and risk of nausea

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	17 (51.5)	16 (48.5)	
Gender				
Male	14 (42.4)	8 (57.1)	6 (42.9)	0.579
Female	19 (57.6)	9 (47.4)	10 (52.6)	
Age (year)				
<65	19 (57.6)	10 (52.6)	9 (47.4)	1.000
>=65	14 (42.4)	7 (50.0)	7 (50.0)	
Extent of disease				
Locally advanced	10 (30.3)	6 (60.0)	4 (40.0)	0.683
Metastatic	16 (48.5)	7 (43.8)	9 (56.3)	
Mixed	7 (21.2)	4 (57.1)	3 (42.9)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	14 (56.0)	11 (44.0)	0.514
Ampulla	4 (12.1)	2 (50.0)	2 (50.0)	
Gallbladder	4 (12.1)	1 (25.0)	3 (75.0)	
Performance status				
0	4 (12.1)	3 (75.0)	1 (25.0)	0.373
1	28 (84.8)	14 (50.0)	14 (50.0	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	11 (50.0)	11 (50.0)	0.919
Gemcitabine/Carboplatin	6 (18.2)	3 (50.0)	3 (50.0)	
Gemcitabine	5 (15.2)	3 (60.0)	2 (40.0)	

Patients' characteristic and risk of fatigue

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	19 (57.6)	14 (42.4)	
Gender				
Male	14 (42.4)	6 (42.9)	8 (57.1)	0.142
Female	19 (57.6)	13 (68.4)	6 (31.6)	
Age (year)				
<65	19 (57.6)	14 (73.7)	5 (26.3)	0.029*
>=65	14 (42.4)	5 (35.7)	9 (64.3)	
Extent of disease				
Locally advanced	10 (30.3)	5 (50.0)	5 (50.0)	0.672
Metastatic	16 (48.5)	9 (56.3)	7 (43.8)	
Mixed	7 (21.2)	5 (71.4)	2 (28.6)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	13 (52.0)	12 (48.0)	0.519
Ampulla	4 (12.1)	3 (75.0)	1 (25.0)	
Gallbladder	4 (12.1)	3 (75.0)	1 (25.0)	
Performance status				
0	4 (12.1)	2 (50.0)	2 (50.0)	0.457
1	28 (84.8)	17 (60.7)	11 (39.3)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	14 (63.6)	8 (36.4)	0.065
Gemcitabine/Carboplatin	6 (18.2)	1 (16.7)	5 (83.3)	
Gemcitabine	5 (15.2)	4 (80.0)	1 (20.0)	

*-P-value of < 0.05 was considered statistically significant.

Patients' characteristic and risk of constipation

Characteristic	N (%)	Grade 0 (%)	Grade 1-2 (%)	P-value χ^2 test
No. of patients	33	25 (75.8)	8 (24.2)	
Gender				
Male	14 (42.4)	11 (78.6)	3 (21.4)	1.000*
Female	19 (57.6)	14 (73.7)	5 (26.3)	
Age (year)				
<65	19 (57.6)	13 (68.4)	6 (31.6)	0.416*
>=65	14 (42.4)	12 (85.7)	2 (14.2)	
Extent of disease				
Locally advanced	10 (30.3)	9 (90.0)	1 (10.0)	0.297
Metastatic	16 (48.5)	12 (75.0)	4 (25.0)	
Mixed	7 (21.2)	4 (57.1)	3 (42.9)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	19 (76.0)	6 (24.0)	0.998
Ampulla	4 (12.1)	3 (75.0)	1 (25.0)	
Gallbladder	4 (12.1)	3 (75.0)	1 (25.0)	
Performance status				
0	4 (12.1)	4 (100.0)	0 (0.0)	0.390
1	28 (84.8)	20 (71.4)	8 (28.6)	
2	1 (3.0)	1 (100.0)	0 (0.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	17 (77.3)	5 (22.7)	0.641
Gemcitabine/Carboplatin	6 (18.2)	5 (83.3)	1 (16.7)	
Gemcitabine	5 (15.2)	3 (60.0)	2 (40.0)	

*-Fisher's exact test

Patients' characteristic and risk of vomiting

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	25 (75.8)	8 (24.2)	
Gender				
Male	14 (42.4)	11 (78.6)	3 (21.4)	1.000*
Female	19 (57.6)	14 (73.7)	5 (26.3)	
Age (year)				
<65	19 (57.6)	16 (84.2)	3 (15.8)	0.238*
>=65	14 (42.4)	9 (64.3)	5 (35.7)	
Extent of disease				
Locally advanced	10 (30.3)	8 (80.0)	2 (20.0)	0.431
Metastatic	16 (48.5)	13 (81.3)	3 (18.8)	
Mixed	7 (21.2)	4 (57.1)	3 (42.9)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	19 (76.0)	6 (24.0)	0.998
Ampulla	4 (12.1)	3 (75.0)	1 (25.0)	
Gallbladder	4 (12.1)	3 (75.0)	1 (25.0)	
Performance status				
0	4 (12.1)	4 (100.0)	0 (0.0)	0.110
1	28 (84.8)	21 (75.0)	7 (25.0)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	18 (81.8)	4 (18.2)	0.265
Gemcitabine/Carboplatin	6 (18.2)	3 (50.0)	3 (50.0)	
Gemcitabine	5 (15.2)	4 (80.0)	1 (20.0)	

*-Fisher's exact test

Patients' characteristic and risk of weight loss

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	26 (78.8)	7 (21.2)	
Gender				
Male	14 (42.4)	9 (64.3)	5 (35.7)	0.106*
Female	19 (57.6)	17 (89.5)	2 (10.5)	
Age (year)				
<65	19 (57.6)	16 (84.2)	3 (15.8)	0.422*
>=65	14 (42.4)	10 (71.4)	4 (28.6)	
Extent of disease				
Locally advanced	10 (30.3)	6 (60.0)	4 (40.0)	0.106
Metastatic	16 (48.5)	15 (93.8)	1 (6.3)	
Mixed	7 (21.2)	5 (71.4)	2 (28.6)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	19 (76.0)	6 (24.0)	0.541
Ampulla	4 (12.1)	3 (75.0)	1 (25.0)	
Gallbladder	4 (12.1)	4 (100.0)	0 (0.0)	
Performance status				
0	4 (12.1)	4 (100.0)	0 (0.0)	0.091
1	28 (84.8)	22 (78.6)	6 (21.4)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	17 (77.3)	5 (22.7)	0.947
Gemcitabine/Carboplatin	6 (18.2)	5 (83.3)	1 (16.7)	
Gemcitabine	5 (15.2)	4 (80.0)	1 (20.0)	

*-Fisher's exact test

Patients' characteristic and risk of diarrhea

Characteristic	N (%)	Grade 0 (%)	Grade 1-3 (%)	P-value χ^2 test
No. of patients	33	29 (87.9)	4 (12.1)	
Gender				
Male	14 (42.4)	13 (92.9)	1 (7.1)	0.620*
Female	19 (57.6)	16 (84.2)	3 (15.8)	
Age (year)				
<65	19 (57.6)	17 (89.5)	2 (10.5)	1.000*
>=65	14 (42.4)	12 (85.7)	2 (14.3)	
Extent of disease				
Locally advanced	10 (30.3)	8 (80.0)	2 (20.0)	0.461
Metastatic	16 (48.5)	14 (87.5)	2 (12.5)	
Mixed	7 (21.2)	7 (100.0)	0 (0.00	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	22 (88.0)	3 (12.0)	0.556
Ampulla	4 (12.1)	3 (75.0)	1 (25.0)	
Gallbladder	4 (12.1)	4 (100.0)	0 (0.0)	
Performance status				
0	4 (12.1)	3 (75.0)	1 (25.0)	0.014**
1	28 (84.8)	26 (92.9)	2 (7.1)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	20 (90.9)	2 (9.1)	0.742
Gemcitabine/Carboplatin	6 (18.2)	5 (83.3)	1 (16.7)	
Gemcitabine	5 (15.2)	4 (80.0)	1 (20.0)	

*-Fisher's exact test

**-P-value of < 0.05 was considered statistically significant.

Patients' characteristic and risk of maculo-papular rash

Characteristic	N (%)	Grade 0 (%)	Grade 3 (%)	P-value χ^2 test
No. of patients	33	30 (90.9)	3 (9.1)	
Gender				
Male	14 (42.4)	14 (100.0)	0 (0.0)	0.244*
Female	19 (57.6)	16 (84.2)	3 (15.8)	
Age (year)				
<65	19 (57.6)	16 (84.2)	3 (15.8)	0.244*
>=65	14 (42.4)	14 (100.0)	0 (0.0)	
Extent of disease				
Locally advanced	10 (30.3)	10 (100.0)	0 (0.0)	0.483
Metastatic	16 (48.5)	14 (87.5)	2 (12.5)	
Mixed	7 (21.2)	6 (85.7)	1 (14.3)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	22 (88.0)	3 (12.0)	0.590
Ampulla	4 (12.1)	4 (100.0)	0 (0.0)	
Gallbladder	4 (12.1)	4 (100.0)	0 (0.0)	
Performance status				
0	4 (12.1)	2 (50.0)	2 (50.0)	0.010**
1	28 (84.8)	27 (96.4)	1 (3.6)	
2	1 (3.0)	1 (100.0)	0 (0.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	21 (95.5)	1 (4.5)	0.430
Gemcitabine/Carboplatin	6 (18.2)	5 (83.3)	1 (16.7)	
Gemcitabine	5 (15.2)	4 (80.0)	1 (20.0)	

*-Fisher's exact test

**-P-value of < 0.05 was considered statistically significant.

Patients' characteristic and risk of insomnia

Characteristic	N (%)	Grade 0 (%)	Grade 1-4 (%)	P-value χ^2 test
No. of patients	33	29 (87.9)	4 (12.1)	
Gender				
Male	14 (42.4)	12 (85.7)	2 (14.3)	1.000*
Female	19 (57.6)	17 (89.5)	2 (10.5)	
Age (year)				
<65	19 (57.6)	16 (84.2)	3 (15.8)	0.620*
>=65	14 (42.4)	13 (92.9)	1 (7.1)	
Extent of disease				
Locally advanced	10 (30.3)	10 (100.0)	0 (0.0)	0.355
Metastatic	16 (48.5)	13 (81.3)	3 (18.8)	
Mixed	7 (21.2)	6 (85.7)	1 (14.3)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	22 (88.0)	3 (12.0)	0.556
Ampulla	4 (12.1)	3 (75.0)	1 (25.0)	
Gallbladder	4 (12.1)	4 (100.0)	0 (0.0)	
Performance status				
0	4 (12.1)	2 (50.0)	2 (50.0)	0.046**
1	28 (84.8)	26 (92.9)	2 (7.1)	
2	1 (3.0)	1 (100.0)	0 (0.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	19 (86.4)	3 913.6)	0.558
Gemcitabine/Carboplatin	6 (18.2)	6 (100.0)	0 (0.0)	
Gemcitabine	5 (15.2)	4 (80.0)	1 (20.0)	

*-Fisher's exact test

**-P-value of < 0.05 was considered statistically significant.

Patients' characteristic and risk of peripheral sensory neuropathy

Characteristic	N (%)	Grade 0 (%)	Grade 1-4 (%)	P-value χ^2 test
No. of patients	33	30 (90.9)	3 (9.1)	
Gender				
Male	14 (42.4)	14 (100.0)	0 (0.0)	0.244*
Female	19 (57.6)	16 (84.2)	3 (15.8)	
Age (year)				
<65	19 (57.6)	16 (84.2)	3 (15.8)	0.244*
>=65	14 (42.4)	14 (100.0)	0 (0.0)	
Extent of disease				
Locally advanced	10 (30.3)	8 (80.0)	2 (20.0)	0.317
Metastatic	16 (48.5)	15 (93.8)	1 (6.3)	
Mixed	7 (21.2)	7 (100.0)	0 (0.0)	
Primary tumor site				
Cholangiocarcinoma	25 (75.8)	22 (88.0)	3 (12.0)	0.590
Ampulla	4 (12.1)	4 (100.0)	0 (0.0)	
Gallbladder	4 (12.1)	4 (100.0)	0 (0.0)	
Performance status				
0	4 (12.1)	3 (75.0)	1 (25.0)	0.002**
1	28 (84.8)	27 (96.4)	1 (3.6)	
2	1 (3.0)	0 (0.0)	1 (100.0)	
Chemotherapeutic regimens				
Gemcitabine/Cisplatin	22 (66.7)	19 (86.4)	3 (13.6)	0.438
Gemcitabine/Carboplatin	6 (18.2)	6 (100.0)	0 (0.0)	
Gemcitabine	5 (15.2)	5 (100.0)	0 (0.0)	

*-Fisher's exact test

**-P-value of < 0.05 was considered statistically significant.

Performance status and risk of toxicities.

	Performance status		
Toxicity	0	1-2	P-value
Diarrhea			
Yes	1 (25.0)	3 (75.0)	0.420
No	3 (10.3)	26 (89.7)	
Maculo-papula rash			
Yes	2 (66.7)	1 (33.3)	0.033 [1,2]
No	2 (6.7)	28 (93.3)	
Insomnia			
Yes	2 (50.0)	2 (50.0)	0.062
No	2 (6.9)	27 (93.1)	
Peripheral sensory neuropathy			
Yes	1 (33.3)	2 (66.7)	0.330
No	3 (10.0)	27 (90.0	

1-Fisher's exact test

2-P-value of < 0.05 was considered statistically significant.

RRM1 polymorphisms and response rate

Genotype N=31	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RRM1 (-) 37			
AC	2 (11.8)	15 (88.2)	1.000
CC+AA	1 (7.1)	13 (92.9)	
RRM1 (-) 524			
CT	2 (11.8)	15 (88.2)	1.000
CT+TT	1 (7.1)	13 (92.9)	

RRM1 polymorphisms and tumor control rate

Genotype N=31	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RRM1 (-) 37			
AC	12 (70.6)	5 (29.4)	1.000
CC+AA	10 (71.4)	4 (28.6)	
RRM1 (-) 524			
CT	12 (70.6)	5 (29.4)	1.000
CT+TT	10 (71.4)	4 (28.6)	

RRM1 Polymorphisms and severity of anemia

Genotype N=33	Grade 1-2 (%)	Grade 3-4 (%)	χ^2 test	P-value
Anemia				
RRM1 (-) 37				
CC	10 (76.9)	3 (23.1)	0.772	0.680
AC	13 (72.2)	5 (27.8)		
AA	2 (100.0)	0 (0.0)		
CC	10 (76.9)	3 (23.1)	-	1.000*
AC+AA	15 (75.0)	5 (25.0)		
RRM1 (-) 524				
TT	10 (76.9)	3 (23.1)	0.772	0.680
CT	13 (72.2)	5 (27.8)		
CC	2 (100.0)	0 (0.0)		
TT	10 (76.9)	3 (23.1)	-	1.000*
CT+CC	15 (75.0)	5 (25.0)		

*-Fisher's exact test

RRM1 Polymorphisms and risk of leukopenia

Genotype N=26	Grade 0 (%)	Grade 1-4 (%)	χ^2 test	P-value
Leukopenia				
RRM1 (-) 37				
CC	2 (15.4)	11 (84.6)	1.267	0.531
AC	4 (22.20	14 (77.8)		
AA	1 (50.0)	1 (50.0)		
CC	2 (15.4)	11 (84.6)	-	0.676*
AC+AA	5 (25.0)	15 (75.0)		
RRM1 (-) 524				
TT	2 (15.4)	11 (84.6)	1.267	0.531
CT	4 (22.20	14 (77.8)		
CC	1 (50.0)	1 (50.0)		
TT	2 (15.4)	11 (84.6)	-	0.676*
CT+CC	5 (25.0)	15 (75.0)		

*-Fisher's exact test

RRM1 Polymorphisms and severity of leukopenia

Genotype N=26	Grade 1-2 (%)	Grade 3-4 (%)	χ^2 test	P-value
Leukopenia				
RRM1 (-) 37				
CC	8 (72.7)	3 (27.3)	4.625	0.099
AC	14 (100.0)	0 (0.0)		
AA	1 (100.0)	0 (0.0)		
CC	8 (72.7)	3 (27.3)	-	0.063*
AC+AA	15 (100.0)	0 (0.0)		
RRM1 (-) 524				
TT	8 (72.7)	3 (27.3)	4.625	0.099
CT	14 (100.0)	0 (0.0)		
CC	1 (100.0)	0 (0.0)		
TT	8 (72.7)	3 (27.3)	-	0.063*
CT+CC	15 (100.0)	0 (0.0)		

*-Fisher's exact test

RRM1 polymorphisms and risk of neutropenia

Genotype N=33	Grade 0 (%)	Grade 1-4 (%)	χ^2 test	P-value
Neutropenia				
RRM1 (-) 37				
CC	7 (53.8)	6 (46.2)	2.249	0.325
AC	5 (27.8)	13 (72.2)		
AA	1 (50.0)	1 (50.0)		
CC	7 (53.8)	6 (46.2)	1.877	0.276
AC+AA	6 (30.0)	14 (70.0)		
RRM1 (-) 524				
TT	7 (53.8)	6 (46.2)	2.249	0.325
CT	5 (27.8)	13 (72.2)		
CC	1 (50.0)	1 (50.0)		
TT	7 (53.8)	6 (46.2)	1.877	0.276
CT+CC	6 (30.0)	14 (70.0)		

RRM1 polymorphisms and severity of neutropenia

Genotype N=20	Grade 1-2 (%)	Grade 3-4 (%)	χ^2 test	P-value
Neutropenia				
RRM1 (-) 37				
CC	3 (50.0)	3 (50.0)	1.6507	0.471
AC	5 (38.5)	8 (61.5)		
AA	1 (100.0)	0 (0.0)		
CC	3 (50.0)	3 (50.0)	-	1.000*
AC+AA	6 (42.9)	8 (57.1)		
RRM1 (-) 524				
TT	3 (50.0)	3 (50.0)	1.6507	0.471
CT	5 (38.5)	8 (61.5)		
CC	1 (100.0)	0 (0.0)		
TT	3 (50.0)	3 (50.0)	-	1.000*
CT+CC	6 (42.9)	8 (57.1)		

*-Fisher's exact test

RRM1 polymorphism and risk of thrombocytopenia

Genotype N=33	Grade 0 (%)	Grade 1-4 (%)	χ^2 test	P-value
Thrombocytopenia				
RRM1 (-) 37				
CC	7 (53.8)	6 (46.2)	1.551	0.461
AC	11 (61.1)	7 (38.9)		
AA	2 (100.0)	0 (46.2)		
CC	7 (53.8)	6 (46.2)	0.411	0.717
AC+AA	13 (65.0)	7 (35.0)		
RRM1 (-) 524				
TT	7 (53.8)	6 (46.2)	1.551	0.461
CT	11 (61.1)	7 (38.9)		
CC	2 (100.0)	0 (46.2)		
TT	7 (53.8)	6 (46.2)	0.411	0.717
CT+CC	13 (65.0)	7 (35.0)		

RRM1 polymorphism and severity of thrombocytopenia

Genotype N=13	Grade 1-2 (%)	Grade 3-4 (%)	χ^2 test	P-value
Thrombocytopenia				
RRM1 (-) 37				
CC	6 (100.0)	0 (0.0)	0.929	1.000
AC	6 (85.7)	1 (14.3)		
AA	0 (0.0)	0 (0.0)		
CC	6 (100.0)	0 (0.0)	-	1.000*
AC+AA	6 (85.7)	1 (14.3)		
RRM1 (-) 524				
TT	6 (100.0)	0 (0.0)	0.929	1.000
CT	6 (85.7)	1 (14.3)		
CC	0 (0.0)	0 (0.0)		
TT	6 (100.0)	0 (0.0)	-	1.000*
CT+CC	6 (85.7)	1 (14.3)		

*-Fisher's exact test

RRM1 (-) 37 polymorphism and risk of hematologic toxicities at cycle 3 and 4

N=30	Grade	CC (%) n=13	AC+AA (%) n=17	P-value (Fisher's exact test)
Anemia	1-4	13 (44.8)	16 (55.2)	1.000
	0	0 (0.0)	1 (100.0)	
Leukopenia	1-4	9 (37.5)	15 (62.5)	0.360
	0	4 (66.7)	2 (33.3)	
Neutropenia	1-4	4 (26.7)	11 (73.3)	0.139
	0	9 (60.0)	6 (40.0)	
Thrombocytopenia	1-4	4 (57.1)	3 (42.9)	0.666
	0	9 (39.1)	14 (60.9)	

RRM1 (-) 37 polymorphism and severity of hematologic toxicities at cycle 3 and 4

	Grade	CC (%)	AC+AA (%)	P-value (Fisher's exact test)
Anemia	1-2	8 (36.4)	14 (63.6)	0.192
(n=29)	3-4	5 (71.4)	2 (28.6)	
Leukopenia	1-2	7 (33.3)	14 (66.7)	0.533
(n=24)	3-4	2 (66.7)	1 (33.3)	
Neutropenia	1-2	2 (22.2)	7 (77.8)	1.000
(n=15)	3-4	2 (33.3)	4 (66.7)	
Thrombocytopenia	1-2	4 (57.1)	3 (42.9)	-
(n=7)	3-4	-	-	

RRM1 (-) 524 polymorphism and risk of hematologic toxicities at cycle 3 and 4

N=30	Grade	TT (%) n=13	CT+CC (%) n=17	P-value (Fisher's exact test)
Anemia	1-4	13 (44.8)	16 (55.2)	1.000
	0	0 (0.0)	1 (100.0)	
Leukopenia	1-4	9 (37.5)	15 (62.5)	0.360
	0	4 (66.7)	2 (33.3)	
Neutropenia	1-4	4 (26.7)	11 (73.3)	0.139
	0	9 (60.0)	6 (40.0)	
Thrombocytopenia	1-4	4 (57.1)	3 (42.9)	0.666
	0	9 (39.1)	14 (60.9)	

RRM1 (-) 524 polymorphism and severity of hematologic toxicities at cycle 3 and 4

	Grade	TT (%)	CT+CC (%)	P-value (Fisher's exact test)
Anemia	1-2	8 (36.4)	14 (63.6)	0.192
(n=29)	3-4	5 (71.4)	2 (28.6)	
Leukopenia	1-2	7 (33.3)	14 (66.7)	0.533
(n=24)	3-4	2 (66.7)	1 (33.3)	
Neutropenia	1-2	2 (22.2)	7 (77.8)	1.000
(n=15)	3-4	2 (33.3)	4 (66.7)	
Thrombocytopenia	1-2	4 (57.1)	3 (42.9)	-
(n=7)	3-4	-	-	

RRM1 (-) 37 polymorphism and non-hematologic toxicities

N=33	Grade	CC (%)	AC+AA (%)	P-value (Fisher's exact test)
Liver function test		n=13	n=20	
Increased AST	1-4	9 (40.9)	13 (59.1)	1.000
	0	4 (36.4)	7 (63.6)	
Increased ALT	1-4	9 (50.0)	9 (50.0)	0.284
	0	4 (26.7)	11 (73.3)	
Increased ALP	1-4	9 (39.1)	14 (60.9)	1.000
	0	4 (40.0)	6 (60.0)	
Increased Bilirubin	1-4	4 (36.4)	7 (63.6)	1.000
	0	9 (40.9)	13 (59.1)	
Increased Cr	1-4	2 (28.6)	5 (71.4)	0.676
	0	11 (42.3)	15 (57.7)	
Nausea	1-4	6 (42.8)	8 (63.2)	1.000*
	0	7 (42.9)	12 (63.2)	
Fatigue	1-4	6 (37.5)	10 (62.5)	1.000*
	0	7 (41.2)	10 (62.5)	
Constipation	1-4	3 (37.5)	5 (62.5)	1.000
	0	10 (40.0)	15 (60.0)	
Vomiting	1-4	3 (37.5)	5 (62.5)	1.000
	0	10 (40.0)	15 (60.0)	
Weight loss	1-4	3 (42.9)	4 (57.1)	1.000
	0	10 (38.5)	16 (61.5)	
Diarrhea	1-4	2 (50.0)	2 (50.0)	1.000
	0	11 (37.9)	18 (62.1)	
Maculo-papular rash	1-4	1 (33.3)	2 (66.7)	1.000
	0	12 (40.0)	18 (60.0)	
Insomnia	1-4	1 (25.0)	3 (75.0)	1.000
	0	12 (41.1)	17 (58.6)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	0.261
	0	13 (43.3)	17 (56.7)	

*-Chi-square test

RRM1 (-) 524 polymorphism and non-hematologic toxicities

N=33	Grade	TT (%)	CT+CC (%)	P-value (Fisher's exact test)
Liver function test		n=13	n=20	
Increased AST	1-4	9 (40.9)	13 (59.1)	1.000
	0	4 (36.4)	7 (63.6)	
Increased ALT	1-4	9 (50.0)	9 (50.0)	0.284
	0	4 (26.7)	11 (73.3)	
Increased ALP	1-4	9 (39.1)	14 (60.9)	1.000
	0	4 (40.0)	6 (60.0)	
Increased Bilirubin	1-4	4 (36.4)	7 (63.6)	1.000
	0	9 (40.9)	13 (59.1)	
Increased Cr	1-4	2 (28.6)	5 (71.4)	0.676
	0	11 (42.3)	15 (57.7)	
Nausea	1-4	6 (42.8)	8 (63.2)	1.000*
	0	7 (42.9)	12 (63.2)	
Fatigue	1-4	6 (37.5)	10 (62.5)	1.000*
	0	7 (41.2)	10 (62.5)	
Constipation	1-4	3 (37.5)	5 (62.5)	1.000
	0	10 (40.0)	15 (60.0)	
Vomiting	1-4	3 (37.5)	5 (62.5)	1.000
	0	10 (40.0)	15 (60.0)	
Weight loss	1-4	3 (42.9)	4 (57.1)	1.000
	0	10 (38.5)	16 (61.5)	
Diarrhea	1-4	2 (50.0)	2 (50.0)	1.000
	0	11 (37.9)	18 (62.1)	
Maculo-papular rash	1-4	1 (33.3)	2 (66.7)	1.000
	0	12 (40.0)	18 (60.0)	
Insomnia	1-4	1 (25.0)	3 (75.0)	1.000
	0	12 (41.1)	17 (58.6)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	0.261
	0	13 (43.3)	17 (56.7)	

*-Chi-square test

ERCC1 polymorphism and risk of hematologic toxicities at cycle 3 and 4

N=30	Grade	CC (%) n=17	TC+TT (%) n=17	P-value (Fisher's exact test)
Anemia	1-4	17 (58.6)	12 (41.4)	0.433
	0	0 (0.0)	1 (100.0)	
Leukopenia	1-4	13 (54.2)	11 (45.8)	0.672
	0	4 (66.7)	2 (33.3)	
Neutropenia	1-4	7 (46.7)	8 (53.3)	0.462
	0	10 (66.7)	5 (33.3)	
Thrombocytopenia	1-4	6 (85.7)	1 (14.3)	0.104
	0	11 (47.8)	12 (52.2)	

ERCC1 polymorphism and severity of hematologic toxicities at cycle 3 and 4

	Grade	CC (%)	TC+TT (%)	P-value (Fisher's exact test)
Anemia	1-2	12 (54.5)	10 (45.5)	0.665
(n=29)	3-4	5 (71.4)	2 (28.6)	
Leukopenia	1-2	11 (52.4)	10 (47.6)	1.000
(n=24)	3-4	2 (66.7)	1 (33.3)	
Neutropenia	1-2	3 (33.3)	6 (66.7)	0.315
(n=15)	3-4	4 (66.7)	2 (33.3)	
Thrombocytopenia	1-2	6 (85.7)	1 (14.3)	-
(n=7)	3-4	-	-	

ERCC1 polymorphism and non-hematologic toxicities

N=28	Grade	CC (%)	CT+TT (%)	P-value (Fisher's exact test)
Liver function test		n=15	n=13	
Increased AST	1-4	9 (52.9)	8 (47.1)	0.934*
	0	6 (54.5)	5 (45.5)	
Increased ALT	1-4	6 (46.2)	7 (53.8)	0.464*
	0	9 (60.0)	6 (40.0)	
Increased ALP	1-4	12 (52.2)	11 (47.8)	0.722
	0	6 (60.0)	4 (40.0)	
Increased Bilirubin	1-4	4 (44.4)	5 (55.6)	0.689
	0	11 (57.9)	8 (42.1)	
Increased Cr	1-4	3 (42.9)	4 (57.1)	0.670
	0	12 (57.1)	9 (42.9)	
Nausea	1-4	9 (64.3)	5 (35.1)	0.256*
	0	6 (42.9)	8 (57.1)	
Fatigue	1-4	7 (53.8)	6 (46.2)	0.978*
	0	8 (53.3)	7 (46.7)	
Constipation	1-4	2 (33.3)	4 (66.7)	0.372
	0	13 (59.1)	9 (40.9)	
Vomiting	1-4	6 (85.7)	1 (14.3)	0.084
	0	9 (42.9)	12 (57.1)	
Weight loss	1-4	2 (33.3)	4 (66.7)	0.372
	0	13 (59.1)	9 (40.9)	
Diarrhea	1-4	1 (33.3)	2 (66.7)	0.583
	0	14 (56.0)	11 (44.0)	
Maculo-papular rash	1-4	2 (100.0)	0 (0.0)	0.484
	0	13 (50.0)	13 (50.0)	
Insomnia	1-4	1 (33.3)	2 (66.7)	0.583
	0	14 (56.0)	11 (44.0)	
Peripheral sensory neuropathy	1-4	1 (33.3)	2 (66.7)	0.583
	0	14 (56.0)	11 (44.0)	

*-Chi- square test

CTR1 polymorphism and risk of hematologic toxicities at cycle 3 and 4

N=30	Grade	GG (%) n=13	GT+TT (%) n=17	P-value (Fisher's exact test)
Anemia	1-4	12 (41.4)	17 (58.6)	1.000
	0	0 (0.0)	1 (100.0	
Leukopenia	1-4	9 (37.5)	15 (62.5)	0.660
	0	3 (50.0)	3 (50.0)	
Neutropenia	1-4	4 (26.7)	11 (73.3)	0.264
	0	8 (53.3)	7 (46.7)	
Thrombocytopenia	1-4	2 (28.6)	5 (71.4)	0.669
	0	10 (43.5)	13 (56.5)	

CTR1 polymorphism and severity of hematologic toxicities at cycle 3 and 4

	Grade	GG (%)	GT+TT (%)	P-value (Fisher's exact test)
Anemia	1-2	11 (50.0)	11 (50.0)	0.187
(n=29)	3-4	1 (14.3)	6 (85.7)	
Leukopenia	1-2	9 (42.9)	12 (57.1)	0.266
(n=24)	3-4	0 (0.0)	3 (100.0)	
Neutropenia	1-2	3 (33.3)	6 (66.7)	0.604
(n=15)	3-4	1 (16.7)	5 (83.3)	
Thrombocytopenia	1-2	2 (28.6)	5 (71.4)	-
(n=7)	3-4	-	-	

CTR1 polymorphism and non-hematologic toxicities

N=28	Grade	GG (%)	GT+TT (%)	P-value (Fisher's exact test)
Liver function test		n=10	n=18	
Increased AST	1-4	5 (29.4)	12 (70.6)	0.444[1]
	0	5 (45.5)	6 (54.5)	
Increased ALT	1-4	3 (23.1)	10 (76.9)	0.254
	0	7 (46.7)	8 (53.3)	
Increased ALP	1-4	4 (21.1)	15 (78.9)	0.035[2]
	0	6 (66.7)	3 (33.3)	
Increased Bilirubin	1-4	3 (33.3)	6 (66.7)	1.000
	0	7 (36.8)	12 (63.2)	
Increased Cr	1-4	3 (42.9)	4 (57.1)	0.674
	0	7 (33.3)	14 (66.7)	
Nausea	1-4	6 (42.9)	8 (57.1)	0.695
	0	4 (28.6)	10 (71.4)	
Fatigue	1-4	6 (46.2)	7 (53.8)	0.433
	0	4 (26.7)	11 (73.3)	
Constipation	1-4	3 (50.0)	3 (50.0)	0.634
	0	7 (31.8)	15 (68.2)	
Vomiting	1-4	2 (28.6)	5 (71.4)	1.000
	0	8 (38.1)	13 (61.9)	
Weight loss	1-4	0 (0.0)	6 (100.0)	0.062
	0	10 (45.5)	12 (54.5)	
Diarrhea	1-4	0 (0.0)	3 (100.0)	0.533
	0	10 (40.0)	15 (60.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	0.524
	0	10 (38.5)	16 (61.5)	
Insomnia	1-4	1 (33.3)	2 (66.7)	1.000
	0	9 (36.0)	16 (64.0)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	0.533
	0	10 (40.0)	15 (60.0)	

1–Chi-square test, 2-P-value of <0.05 was considered statistically significant.

Combination of *RRM* and *ERCC1* polymorphism and non-hematologic toxicities

N=28	Grade	RR37AC-RR524CT/CC (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=8	n=20	
Increased AST	1-4	5 (29.4)	12 (70.6)	1.000
	0	3 (27.3)	8 (72.7)	
Increased ALT	1-4	2 (15.4)	11 (84.6)	0.221
	0	6 (40.0)	9 (60.0)	
Increase ALP	1-4	5 (26.3)	14 (73.7)	1.000
	0	3 (33.3)	6 (66.7)	
Increased Bilirubin	1-4	1 (11.1)	8 (88.9)	0.214
	0	7 (36.8)	12 (63.2)	
Increased Cr	1-4	1 (14.3)	6 (85.7)	0.663
	0	7 (33.3)	14 (66.7)	
Nausea	1-4	5 (35.7)	9 (64.3)	0.678
	0	3 (21.4)	11 (78.6)	
Fatigue	1-4	3 (23.1)	10 (76.9)	0.686
	0	5 (33.3)	10 (66.7)	
Constipation	1-4	2 (33.3)	4 (66.7)	1.000
	0	6 (27.3)	16 (72.7)	
Vomiting	1-4	4 (57.1)	3 (42.9)	0.142
	0	4 (19.0)	17 (81.0)	
Weight loss	1-4	1 (16.7)	5 (83.3)	0.640
	0	7 (31.8)	15 (68.2)	
Diarrhea	1-4	1 (33.3)	2 (66.7)	1.000
	0	7 (28.0)	18 (72.0)	
Maculo-papular rash	1-4	2 (100.0)	0 (0.0)	0.074
	0	6 (23.1)	20 (76.9)	
Insomnia	1-4	1 (33.3)	2 (66.7)	1.000
	0	7 (28.0)	18 (72.0)	
Peripheral sensory neuropathy	1-4	1 (33.3)	2 (66.7)	1.000
	0	7 (28.0)	18 (72.0)	

Combination of *RRM1* and *ERCC1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RR37CC-RR524TT/CC	0 (0.0)	5 (100.0)	1.000
The others	2 (9.5)	19 (90.5)	

Combination of *RRM1* and *ERCC1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RR37CC-RR524TT/CC	4 (80.0)	1 (20.0)	1.000
The others	15 (71.4)	6 (28.6)	

Combination of *RRM1* and *ERCC1* polymorphisms and risk of hematologic toxicities

N=28	Grade	RR37CC-RR524TT/CC (%) n=5	The others (%) n=23	P-value (Fisher's exact test)
Anemia	1-4	5 (17.9)	23 (82.1)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	4 (19.0)	17 (81.0)	1.000
	0	1 (14.3)	6 (85.7)	
Neutropenia	1-4	2 (12.5)	14 (87.5)	0.624
	0	3 (25.0)	9 (75.0)	
Thrombocytopenia	1-4	2 (18.2)	9 (81.8)	1.000
	0	3 (17.6)	14 (82.4)	

Combination of *RRM1* and *ERCC1* polymorphisms and severity of hematologic toxicities

	Grade	RR37CC-RR524TT/CC (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	4 (19.0)	17 (81.0)	1.000
	3-4	1 (14.3)	6 (85.7)	
Leukopenia (n=21)	1-2	3 (15.8)	16 (84.2)	0.352
	3-4	1 (50.0)	1 (50.0)	
Neutropenia (n=16)	1-2	1 (12.5)	7 (87.5)	1.000
	3-4	1 (12.5)	7 (87.5)	
Thrombocytopenia (n=11)	1-2	2 (20.0)	8 (80.0)	1.000
	3-4	0 (0.0)	1 (100.0)	

Combination of *RRM1* and *ERCC1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	RR37CC-RR524TT/CC (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=5	n=23	
Increased AST	1-4	4 (23.5)	13 (76.5)	0.619
	0	1 (9.1)	10 (90.9)	
Increased ALT	1-4	4 (30.8)	9 (69.2)	0.153
	0	1 (6.7)	14 (93.3)	
Increased ALP	1-4	4 (21.1)	15 (78.9)	1.000
	0	1 (11.1)	8 (88.9)	
Increased Bilirubin	1-4	3 (33.3)	6 (66.7)	0.290
	0	2 (10.5)	17 (89.5)	
Increased Cr	1-4	2 (28.6)	5 (71.4)	0.574
	0	3 (14.3)	18 (85.7)	
Nausea	1-4	3 (21.4)	11 (78.6)	1.000
	0	2 (14.3)	12 (85.7)	
Fatigue	1-4	3 (23.1)	10 (76.9)	0.639
	0	2 (13.3)	13 (86.7)	
Constipation	1-4	0 (0.0)	6 (100.0)	0.553
	0	5 (22.7)	17 (77.3)	
Vomiting	1-4	2 (28.6)	5 (71.4)	0.574
	0	3 (14.3)	18 (85.7)	
Weight loss	1-4	1 (16.7)	5 (83.3)	1.000
	0	4 (18.2)	18 (81.5)	
Diarrhea	1-4	0 (0.0)	3 (100.0)	1.000
	0	5 (20.0)	20 (80.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	5 (19.2)	21 (80.8)	
Insomnia	1-4	0 (0.0)	3 (100.0)	1.000
	0	5 (20.0)	20 (80.0)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	1.000
	0	5 (20.0)	20 (80.0)	

Combination of *RRM1* and *ERCC1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RR37CC-RR524TT/CT	0 (0.0)	3 (100.0)	1.000
The others	2 (8.7)	21 (91.3)	

Combination of *RRM1* and *ERCC1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RR37CC-RR524TT/CT	2 (66.7)	1 (33.3)	1.000
The others	17 (73.9)	7 (26.1)	

Combination of *RRM1* and *ERCC1* polymorphisms and risk of hematologic toxicities

N=28	Grade	RR37CC-RR524TT/CT (%) n=3	The others (%) n=25	P-value (Fisher's exact test)
Anemia	1-4	3 (10.7)	25 (89.3)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	3 (14.3)	18 (85.7)	0.551
	0	0 (0.0)	7 (100.0)	
Neutropenia	1-4	2 (12.5)	14 (87.5)	1.000
	0	1 (8.3)	11 (91.7)	
Thrombocytopenia	1-4	2 (18.2)	9 (81.8)	0.543
	0	1 (5.9)	16 (94.1)	

Combination of *RRM1* and *ERCC1* polymorphisms and severity of hematologic toxicities

	Grade	RR37CC-RR524TT/CT (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	2 (9.5)	19 (90.5)	1.000
	3-4	1 (14.3)	6 (85.7)	
Leukopenia (n=21)	1-2	2 (10.5)	17 (89.5)	0.271
	3-4	1 (50.0)	1 (50.0)	
Neutropenia (n=16)	1-2	1 (12.5)	7 (87.5)	1.000
	3-4	1 (12.5)	7 (87.5)	
Thrombocytopenia (n=11)	1-2	2 (20.0)	8 (80.0)	1.000
	3-4	0 (0.0)	1 (100.0)	

Combination of *RRM1* and *ERCC1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	RR37CC-RR524TT/CT (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=3	n=25	
Increased AST	1-4	2 (9.1)	15 (90.9)	1.000
	0	1 (11.8)	10 (88.2)	
Increased ALT	1-4	2 (15.4)	11 (84.6)	0.583
	0	1 (6.7)	14 (93.3)	
Increased ALP	1-4	2 (10.5)	17 (89.5)	1.000
	0	1 (11.1)	8 (88.9)	
Increased Bilirubin	1-4	1 (11.1)	8 (88.9)	1.000
	0	2 (10.5)	17 (89.5)	
Increased Cr	1-4	0 (0.0)	7 (100.)	0.551
	0	3 (14.3)	18 (85.7)	
Nausea	1-4	1 (7.1)	13 (92.9)	1.000
	0	2 (14.3)	12 (85.7)	
Fatigue	1-4	2 (15.4)	11 (84.6)	0.583
	0	1 (6.7)	14 (93.3)	
Constipation	1-4	1 (16.7)	5 (83.3)	0.530
	0	2 (9.1)	20 (90.0)	
Vomiting	1-4	0 (0.0)	7 (100.0)	0.551
	0	3 (14.3)	18 (85.7)	
Weight loss	1-4	1 (16.7)	5 (83.3)	0.530
	0	2 (9.1)	20 (90.0)	
Diarrhea	1-4	1 (33.3)	2 (66.7)	0.298
	0	2 (8.0)	23 (92.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	3 (11.5)	23 (88.5)	
Insomnia	1-4	0 (0.0)	3 (100.0)	1.000
	0	3 (12.0)	22 (88.0)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	1.000
	0	3 (12.0)	22 (88.0)	

Combination of *RRM1* and *ERCC1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/CT	0 (0.0)	7 (100.0)	1.000
The others	2 (10.5)	17 (89.5)	

Combination of RRM1 and *ERCC1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/CT	4 (57.1)	3 (42.9)	0.340
The others	15 (78.9)	4 (21.1)	

Combination of *RRM1* and *ERCC1* polymorphisms and risk of hematologic toxicities

N=28	Grade	RR37AC-RR524CT/CT (%) n=7	The others (%) n=21	P-value (Fisher's exact test)
Anemia	1-4	7 (25.0)	21 (75.0)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	6 (28.6)	15 (71.4)	0.639
	0	1 (14.3)	6 (85.7)	
Neutropenia	1-4	5 (31.3)	11 (68.8)	0.662
	0	2 (16.7)	10 (83.3)	
Thrombocytopenia	1-4	1 (9.1)	10 (90.9)	0.191
	0	6 (35.3)	11 (64.7)	

Combination of *RRM1* and *ERCC1* polymorphisms and severity of hematologic toxicities

	Grade	RR37AC-RR524CT/CT (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	5 (23.8)	16 (76.2)	1.000
	3-4	2 (28.6)	5 (71.4)	
Leukopenia (n=21)	1-2	6 (31.6)	13 (68.4)	1.000
	3-4	0 (0.0)	2 (100.0)	
Neutropenia (n=16)	1-2	3 (37.5)	5 (62.5)	1.000
	3-4	2 (25.0)	6 (75.0)	
Thrombocytopenia (n=11)	1-2	1 (16.7)	5 (83.3)	1.000
	3-4	0 (0.0)	5 (100.0)	

Combination of *RRM1* and *ERCC1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	RR37AC-RR524CT/CT (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=7	n=21	
Increased AST	1-4	5 (29.4)	12 (70.6)	0.668
	0	2 (18.2)	9 (81.8)	
Increased ALT	1-4	4 (30.8)	9 (69.2)	0.670
	0	3 (20.0)	12 (80.0)	
Increased ALP	1-4	5 (26.3)	14 (73.7)	1.000
	0	2 (22.2)	7 (77.8)	
Increased Bilirubin	1-4	2 (22.2)	7 (77.8)	1.000
	0	5 (26.3)	14 (73.7)	
Increased Cr	1-4	3 (42.9)	4 (57.1)	0.318
	0	4 (19.0)	17 (81.0)	
Nausea	1-4	2 (14.3)	12 (85.7)	0.385
	0	5 (35.7)	9 (64.3)	
Fatigue	1-4	3 (23.1)	10 (76.9)	1.000
	0	4 (26.7)	11 (73.3)	
Constipation	1-4	2 (33.3)	4 (66.7)	0.622
	0	5 (22.7)	17 (77.3)	
Vomiting	1-4	1 (14.3)	6 (85.7)	0.639
	0	6 (28.6)	15 (71.4)	
Weight loss	1-4	3 (50.0)	3 (50.0)	0.144
	0	4 (18.2)	18 (81.8)	
Diarrhea	1-4	1 (33.3)	2 (66.7)	1.000
	0	6 (24.0)	19 (76.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	7 (26.9)	19 (73.1)	
Insomnia	1-4	1 (33.3)	2 (66.7)	1.000
	0	6 (24.0)	19 (76.0)	
Peripheral sensory neuropathy	1-4	2 (66.7)	1 (33.3)	0.145
	0	5 (20.0)	20 (80.0)	

Combination of *RRM1* and *CTR1* polymorphisms and risk of hematologic toxicities

N=28	Grade	RR37AC-RR524CT/GG (%) n=5	The others (%) n=23	P-value (Fisher's exact test)
Anemia	1-4	5 (17.9)	23 (82.1)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	3 (14.3)	18 (85.7)	0.574
	0	2 (28.6)	5 (71.4)	
Neutropenia	1-4	2 (12.5)	14 (87.5)	0.624
	0	3 (25.0)	9 (75.0)	
Thrombocytopenia	1-4	1 (9.1)	10 (90.9)	0.619
	0	4 (23.5)	13 (76.5)	

Combination of *RRM1* and *CTR1* polymorphisms and severity of hematologic toxicities

	Grade	RR37AC-RR524CT/GG (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	5 (23.8)	16 (76.2)	0.290
	3-4	0 (0.0)	7 (100.0)	
Leukopenia (n=21)	1-2	3 (15.8)	16 (84.2)	1.000
	3-4	0 (0.0)	2 (100.0)	
Neutropenia (n=16)	1-2	1 (12.5)	7 (87.5)	1.000
	3-4	1 (12.5)	7 (87.5)	
Thrombocytopenia (n=11)	1-2	0 (0.0)	6 (100.0)	0.455
	3-4	1 (20.0)	4 (80.0)	

Combination of *RRM1* and *CTR1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	RR37AC-RR524CT/GG (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=5	n=23	
Increased AST	1-4	4 (23.5)	13 (76.5)	0.619
	0	1 (9.1)	10 (90.9)	
Increased ALT	1-4	2 (15.4)	11 (84.6)	1.000
	0	3 (20.0)	12 (80.0)	
Increased ALP	1-4	2 (10.5)	17 (89.5)	0.290
	0	3 (33.3)	6 (66.7)	
Increased Bilirubin	1-4	2 (22.2)	7 (77.8)	1.000
	0	3 (15.8)	16 (84.2)	
Increased Cr	1-4	3 (42.9)	4 (57.1)	0.082
	0	2 (9.5)	19 (90.5)	
Nausea	1-4	3 (21.4)	11 (78.6)	1.000
	0	2 (14.3)	12 (85.7)	
Fatigue	1-4	3 (23.1)	10 (76.9)	0.639
	0	2 (13.3)	13 (86.7)	
Constipation	1-4	2 (33.3)	4 (66.7)	0.285
	0	3 (13.6)	19 (86.4)	
Vomiting	1-4	1 (14.3)	6 (85.7)	1.000
	0	4 (19.0)	17 (81.0)	
Weight loss	1-4	0 (0.0)	6 (100.0)	0.553
	0	5 (22.7)	17 (77.3)	
Diarrhea	1-4	0 (0.0)	3 (100.0)	1.000
	0	5 (20.0)	20 (80.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	5 (19.2)	21 (80.8)	
Insomnia	1-4	1 (33.3)	2 (66.7)	0.459
	0	4 (16.0)	21 (84.0)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	1.000
	0	5 (20.0)	20 (80.0)	

Combination of *RRM1* and *CTR1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/GT	0 (0.0)	9 (100.0)	0.529
The others	2 (11.8)	15 (88.2)	

Combination of *RRM1* and *CTR1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RR37AC-RR524CT/GT	8 (88.9)	1 (11.1)	0.357
The others	11 (64.7)	6 (35.3)	

Combination of *RRM1* and *CTR1* polymorphisms and risk of hematologic toxicities

N=28	Grade	RR37AC-RR524CT/GT (%) n=9	The others (%) n=19	P-value (Fisher's exact test)
Anemia	1-4	9 (32.1)	19 (67.9)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	8 (38.1)	13 (61.9)	0.371
	0	1 (14.3)	6 (85.7)	
Neutropenia	1-4	8 (50.0)	8 (50.0)	0.039*
	0	1 (8.3)	11 (91.7)	
Thrombocytopenia	1-4	4 (36.4)	7 (63.6)	1.000
	0	5 (29.4)	12 (70.6)	

* P-value of <0.05 was considered statistically significant

Combination of *RRM1* and *CTR1* polymorphisms and severity of hematologic toxicities

	Grade	RR37AC-RR524CT/GT (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	5 (23.8)	16 (76.2)	0.165
	3-4	4 (57.1)	3 (42.9)	
Leukopenia (n=21)	1-2	8 (42.1)	11 (57.9)	0.505
	3-4	0 (0.0)	2 (100.0)	
Neutropenia (n=16)	1-2	4 (50.0)	4 (50.0)	1.000
	3-4	4 (50.0)	4 (50.0)	
Thrombocytopenia (n=11)	1-2	3 (50.0)	3 (50.0)	0.545
	3-4	1 (20.0)	4 (80.0)	

Combination of *RRM1* and *CTR1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	RR37AC-RR524CT/GT (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=9	n=19	
Increased AST	1-4	7 (41.2)	10 (58.8)	0.249
	0	2 (18.2)	9 (81.8)	
Increased ALT	1-4	5 (38.5)	8 (61.5)	0.689
	0	4 (26.7)	11 (73.3)	
Increased ALP	1-4	7 (36.8)	12 (63.2)	0.670
	0	2 (22.2)	7 (77.8)	
Increased Bilirubin	1-4	2 (22.2)	7 (77.8)	0.670
	0	7 (36.8)	12 (63.2)	
Increased Cr	1-4	1 (14.3)	6 (85.7)	0.371
	0	8 (38.1)	13 (61.9)	
Nausea	1-4	4 (28.6)	10 (71.4)	1.000
	0	5 (35.7)	9 (64.3)	
Fatigue	1-4	4 (30.8)	9 (69.2)	1.000
	0	5 (33.3)	10 (66.7)	
Constipation	1-4	1 (16.7)	5 (83.3)	0.630
	0	8 (36.4)	14 (63.6)	
Vomiting	1-4	3 (42.9)	4 (57.1)	0.646
	0	6 (28.6)	15 (71.4)	
Weight loss	1-4	4 (66.7)	2 (33.3)	0.064
	0	5 (22.7)	17 (77.3)	
Diarrhea	1-4	2 (66.7)	1 (33.3)	0.234
	0	7 (28.0)	18 (72.0)	
Maculo-papular rash	1-4	2 (100.0)	0 (0.0)	0.095
	0	7 (26.9)	19 (73.1)	
Insomnia	1-4	1 (33.3)	2 (66.7)	1.000
	0	8 (32.0)	17 (68.0)	
Peripheral sensory neuropathy	1-4	3 (100.0)	0 (0.0)	0.026*
	0	6 (24.0)	19 (76.0)	

* P-value of <0.05 was considered statistically significant.

Combination of *RRM1* and *CTR1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RR37CC-RR524TT/GG	1 (25.0)	3 (75.0)	0.289
The others	1 (4.5)	21 (95.5)	

Combination of *RRM1* and *CTR1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RR37CC-RR524TT/GG	3 (75.0)	1 (25.0)	1.000
The others	16 (72.7)	6 (27.3)	

Combination of *RRM1* and *CTR1* polymorphisms and risk of hematologic toxicities

N=28	Grade	RR37CC-RR524TT/GG (%) n=4	The others (%) n=24	P-value (Fisher's exact test)
Anemia	1-4	4 (14.3)	24 (85.7)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	3 (14.3)	18 (85.7)	1.000
	0	1 (14.3)	6 (85.7)	
Neutropenia	1-4	1 (6.3)	15 (93.8)	0.285
	0	3 (25.0)	9 (75.0)	
Thrombocytopenia	1-4	1 (9.1)	10 (90.9)	1.000
	0	3 (17.6)	14 (82.4)	

Combination of *RRM1* and *CTR1* polymorphisms and severity of hematologic toxicities

	Grade	RR37CC-RR524TT/GG (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	3 (14.3)	18 (85.7)	1.000
	3-4	1 (14.3)	6 (85.7)	
Leukopenia (n=21)	1-2	3 (15.8)	16 (84.2)	1.000
	3-4	0 (0.0)	2 (100.0)	
Neutropenia (n=16)	1-2	1 (12.5)	7 (87.5)	1.000
	3-4	0 (0.0)	8 (100.0)	
Thrombocytopenia (n=11)	1-2	1 (10.0)	9 (90.0)	1.000
	3-4	0 (0.0)	1 (100.0)	

Combination of *RRM1* and *CTR1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	RR37CC-RR524TT/GG (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=4	n=24	
Increased AST	1-4	1 (5.9)	16 (94.1)	0.269
	0	3 (27.3)	8 (72.7)	
Increased ALT	1-4	1 (7.7)	12 (92.3)	0.600
	0	3 (20.0)	12 (80.0)	
Increased ALP	1-4	1 (5.3)	18 (94.7)	0.084
	0	3 (33.3)	6 (66.7)	
Increased Bilirubin	1-4	1 (11.1)	8 (88.9)	1.000
	0	3 (15.8)	16 (84.2)	
Increased Cr	1-4	0 (0.0)	7 (100.0)	0.545
	0	4 (19.0)	17 (81.0)	
Nausea	1-4	3 (21.4)	11 (78.6)	0.596
	0	1 (7.1)	13 (92.9)	
Fatigue	1-4	3 (23.1)	10 (76.9)	0.311
	0	1 (6.7)	14 (93.3)	
Constipation	1-4	1 (16.7)	5 (83.3)	1.000
	0	3 (13.6)	19 (86.4)	
Vomiting	1-4	1 (14.3)	6 (85.7)	1.000
	0	3 (14.3)	18 (85.7)	
Weight loss	1-4	0 (0.0)	6 (100.0)	0.549
	0	4 (18.2)	18 (81.8)	
Diarrhea	1-4	0 (0.0)	3 (100.0)	1.000
	0	4 (16.0)	21 (84.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	4 (15.4)	22 (84.6)	
Insomnia	1-4	0 (0.0)	3 (100.0)	1.000
	0	4 (16.0)	21 (84.0)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	1.000
	0	4 (16.0)	21 (84.0)	

Combination of *RRM1* and *CTR1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
RR37CC-RR524TT/GT	0 (0.0)	6 (100.0)	1.000
The others	2 (10.0)	18 (90.0)	

Combination of *RRM1* and *CTR1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
RR37CC-RR524TT/GT	4 (66.7)	2 (33.3)	1.000
The others	15 (75.0)	5 (25.0)	

Combination of *RRM1* and *CTR1* polymorphisms and risk of hematologic toxicities

N=28	Grade	RR37CC-RR524TT/GT (%) n=9	The others (%) n=19	P-value (Fisher's exact test)
Anemia	1-4	9 (32.1)	19 (67.9)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	5 (23.8)	16 (76.2)	1.000
	0	1 (14.3)	6 (85.7)	
Neutropenia	1-4	3 (18.8)	13 (81.3)	1.000
	0	3 (25.0)	9 (75.0)	
Thrombocytopenia	1-4	3 (27.3)	8 (72.7)	0.653
	0	3 (17.6)	14 (82.4)	

Combination of *RRM1* and *CTR1* polymorphisms and severity of hematologic toxicities

	Grade	RR37CC-RR524TT/GT (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	4 (19.0)	17 (81.0)	0.622
	3-4	2 (28.6)	5 (71.4)	
Leukopenia (n=21)	1-2	3 (15.8)	16 (84.2)	0.048*
	3-4	2 (100.0)	0 (0.0)	
Neutropenia (n=16)	1-2	1 (12.5)	7 (87.5)	1.000
	3-4	2 (25.0)	6 (75.0)	
Thrombocytopenia (n=11)	1-2	3 (30.0)	7 (70.0)	1.000
	3-4	0 (0.0)	1 (100.0)	

* P-value of <0.05 was considered statistically significant.

Combination of *RRM1* and *CTR1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	RR37CC-RR524TT/GT (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=6	n=22	
Increased AST	1-4	5 (29.4)	12 (70.6)	0.355
	0	1 (9.1)	10 (90.9)	
Increased ALT	1-4	5 (38.5)	8 (61.5)	0.069
	0	1 (6.7)	14 (93.3)	
Increased ALP	1-4	6 (31.6)	1 (68.4)	0.136
	0	0 (0.0)	9 (100.0)	
Increased Bilirubin	1-4	3 (33.3)	6 (66.7)	0.352
	0	3 (15.8)	16 (84.2)	
Increased Cr	1-4	2 (28.6)	5 (71.4)	0.622
	0	4 (19.0)	17 (81.0)	
Nausea	1-4	2 (14.3)	12 (85.7)	0.648
	0	4 (28.6)	10 (71.4)	
Fatigue	1-4	3 (23.1)	10 (76.9)	1.000
	0	3 (20.0)	12 (80.0)	
Constipation	1-4	0 (0.0)	6 (100.0)	0.289
	0	6 (27.3)	16 (72.7)	
Vomiting	1-4	1 (14.3)	6 (85.7)	1.000
	0	5 (23.8)	16 (76.2)	
Weight loss	1-4	2 (33.3)	4 (66.7)	0.581
	0	4 (18.2)	18 (81.8)	
Diarrhea	1-4	1 (33.3)	2 (66.7)	0.530
	0	5 (20.0)	20 (80.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	6 (23.1)	20 (76.9)	
Insomnia	1-4	0 (0.00)	3 (100.0)	1.000
	0	6 (24.0)	19 (76.0)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	1.000
	0	6 (24.0)	19 (76.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and risk of hematologic toxicities

N=28	Grade	CC/GG (%) n=4	The others (%) n=24	P-value (Fisher's exact test)
Anemia	1-4	9 (32.1)	19 (67.9)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	2 (9.5)	19 (90.5)	0.253
	0	2 (28.6)	5 (71.4)	
Neutropenia	1-4	1 (6.3)	15 (93.8)	0.285
	0	3 (25.0)	9 (75.0)	
Thrombocytopenia	1-4	2 (18.2)	9 (81.8)	1.000
	0	2 (11.8)	15 (88.2)	

Combination of *ERCC1* and *CTR1* polymorphisms and severity of hematologic toxicities

	Grade	CC/GG (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	3 (14.3)	18 (85.7)	1.000
	3-4	1 (14.3)	6 (85.7)	
Leukopenia (n=21)	1-2	2 (10.5)	17 (89.5)	1.000
	3-4	0 (0.0)	2 (100.0)	
Neutropenia (n=16)	1-2	0 (0.0)	8 (100.0)	1.000
	3-4	1 (12.5)	7 (87.5)	
Thrombocytopenia (n=11)	1-2	1 (16.7)	5 (83.3)	1.000
	3-4	1 (20.0)	4 (80.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	CC/GG (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=4	n=24	
Increased AST	1-4	1 (5.9)	16 (94.1)	0.269
	0	3 (27.1)	8 (72.7)	
Increased ALT	1-4	0 (0.0)	13 (100.0)	0.102
	0	4 (26.7)	11 (73.3)	
Increased ALP	1-4	1 (5.3)	18 (94.7)	0.084
	0	3 (33.3)	6 (66.7)	
Increased Bilirubin	1-4	0 (0.0)	9 (100.0)	0.273
	0	4 (21.1)	15 (78.9)	
Increased Cr	1-4	1 (14.3)	6 (85.7)	1.000
	0	3 (14.3)	18 (85.7)	
Nausea	1-4	3 (21.4)	11 (78.6)	0.596
	0	1 (7.1)	13 (92.9)	
Fatigue	1-4	3 (23.1)	10 (76.90	0.3111
	0	1 (6.7)	14 (93.3)	
Constipation	1-4	1 (16.7)	5 (83.3)	1.000
	0	3 (13.6)	19 (86.4)	
Vomiting	1-4	2 (28.6)	5 (71.4)	0.253
	0	2 (9.5)	19 (90.5)	
Weight loss	1-4	0 (0.0)	6 (100.0)	0.549
	0	4 (18.2)	18 (81.8)	
Diarrhea	1-4	0 (0.0)	3 (100.0)	1.000
	0	4 (16.0)	21 (84.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	4 (15.4)	22 (84.6)	
Insomnia	1-4	0 (0.0)	3 (100.0)	1.000
	0	4 (16.0)	21 (84.0)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	1.000
	0	4 (16.0)	21 (84.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
CC/GT	0 (0.0)	10 (100.0)	0.508
The others	2 (12.5)	14 (87.5)	

Combination of *ERCC1* and *CTR1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
CC/GT	8 (80.0)	2 (20.0)	0.668
The others	11 (68.8)	5 (31.3)	

Combination of *ERCC1* and *CTR1* polymorphisms and risk of hematologic toxicities

N=28	Grade	CC/GT (%) n=10	The others (%) n=18	P-value (Fisher's exact test)
Anemia	1-4	10 (35.7)	18 (64.3)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	8 (38.1)	13 (62.9)	1.000
	0	2 (28.6)	5 (71.4)	
Neutropenia	1-4	6 (37.5)	10 (62.5)	1.000
	0	4 (33.3)	8 (66.7)	
Thrombocytopenia	1-4	5 (45.5)	6 (54.5)	1.000
	0	5 (29.4)	12 (70.6)	

Combination of *ERCC1* and *CTR1* polymorphisms and severity of hematologic toxicities

	Grade	CC/GT (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	7 (33.3)	14 (66.7)	0.674
	3-4	3 (42.9)	4 (57.1)	
Leukopenia (n=21)	1-2	7 (36.8)	12 (63.2)	1.000
	3-4	1 (50.0)	1 (50.0)	
Neutropenia (n=16)	1-2	3 (37.5)	5 (62.5)	1.000
	3-4	3 (37.5)	5 (62.5)	
Thrombocytopenia (n=11)	1-2	3 (50.0)	3 (50.0)	1.000
	3-4	2 (40.0)	3 (60.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	CC/GT (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=10	n=18	
Increased AST	1-4	8 (47.1)	9 (52.9)	0.226
	0	2 (18.2)	9 (81.8)	
Increased ALT	1-4	6 (46.2)	7 (53.8)	0.433
	0	4 (26.7)	11 (73.3)	
Increased ALP	1-4	9 (47.4)	10 (52.6)	0.098
	0	1 (11.1)	8 (88.9)	
Increased Bilirubin	1-4	4 (44.4)	5 (55.6)	0.677
	0	6 (31.6)	13 (68.4)	
Increased Cr	1-4	2 (28.6)	5 (71.4)	1.000
	0	8 (38.1)	13 (61.9)	
Nausea	1-4	5 (35.7)	9 (64.3)	1.000
	0	5 (35.7)	9 (64.3)	
Fatigue	1-4	4 (30.8)	9 (69.2)	0.705
	0	6 (40.0)	9 (60.0)	
Constipation	1-4	0 (0.0)	6 (100.0)	0.062
	0	10 (45.5)	12 (54.5)	
Vomiting	1-4	3 (42.9)	4 (57.1)	0.674
	0	7 (33.3)	14 (66.7)	
Weight loss	1-4	2 (33.3)	4 (66.7)	1.000
	0	8 (36.4)	14 (63.6)	
Diarrhea	1-4	1 (33.3)	2 (66.7)	1.000
	0	9 (36.0)	16 (64.0)	
Maculo-papular rash	1-4	2 (100.0)	0 (0.0)	0.119
	0	8 (30.8)	18 (69.2)	
Insomnia	1-4	1 (33.3)	2 (66.7)	1.000
	0	9 (36.0)	16 (64.0)	
Peripheral sensory neuropathy	1-4	1 (33.3)	2 (66.7)	1.000
	0	9 (36.0)	16 (64.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
CT/GG	0 (0.0)	5 (100.0)	1.000
The others	2 (9.5)	19 (90.5)	

Combination of *ERCC1* and *CTR1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
CT/GG	2 (40.0)	3 (60.0)	0.281
The others	17 (81.0)	4 (19.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and risk of hematologic toxicities

N=28	Grade	CT/GG (%) n=6	The others (%) n=22	P-value (Fisher's exact test)
Anemia	1-4	6 (21.4)	22 (78.6)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	4 (19.0)	17 (81.0)	0.622
	0	2 (28.6)	5 (71.4)	
Neutropenia	1-4	2 (12.5)	14 (87.5)	0.354
	0	4 (33.3)	8 (66.7)	
Thrombocytopenia	1-4	1 (9.1)	10 (90.0)	0.355
	0	5 (29.4)	12 (70.6)	

Combination of *ERCC1* and *CTR1* polymorphisms and severity of hematologic toxicities

	Grade	CT/GG (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	6 (28.6)	15 (71.4)	0.288
	3-4	0 (0.0)	7 (100.0)	
Leukopenia (n=21)	1-2	4 (21.1)	15 (78.9)	1.000
	3-4	0 (0.0)	2 (100.0)	
Neutropenia (n=16)	1-2	2 (25.0)	6 (75.0)	0.467
	3-4	0 (0.0)	8 (100.0)	
Thrombocytopenia (n=11)	1-2	1 (16.7)	5 (83.3)	1.000
	3-4	0 (0.0)	5 (100.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	CT/GG (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=6	n=22	
Increased AST	1-4	4 (23.5)	13 (76.5)	1.000
	0	2 (18.2)	9 (81.8)	
Increased ALT	1-4	3 (23.1)	10 (76.9)	1.000
	0	3 (20.0)	12 (80.0)	
Increased ALP	1-4	3 (15.8)	16 (84.2)	0.352
	0	3 (33.3)	6 (66.7)	
Increased Bilirubin	1-4	3 (33.3)	6 (66.7)	0.252
	0	3 (15.8)	16 (84.2)	
Increased Cr	1-4	2 (28.6)	5 (71.4)	0.622
	0	4 (19.0)	17 (81.0)	
Nausea	1-4	3 (21.4)	11 (78.6)	1.000
	0	3 (21.4)	11 (78.6)	
Fatigue	1-4	3 (23.1)	10 (76.9)	1.000
	0	3 (20.0)	12 (80.0)	
Constipation	1-4	2 (33.3)	4 (66.7)	0.581
	0	4 (18.2)	18 (81.8)	
Vomiting	1-4	0 (0.0)	7 (100.0)	0.288
	0	6 (28.6)	15 (71.4)	
Weight loss	1-4	0 (0.0)	6 (100.0)	0.289
	0	6 (27.3)	16 (72.7)	
Diarrhea	1-4	0 (0.0)	3 (100.0)	1.000
	0	6 (24.0)	19 (76.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	6 (23.1)	20 (76.9)	
Insomnia	1-4	1 (33.3)	2 (66.7)	0.530
	0	5 (20.0)	20 (80.0)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	1.000
	0	6 (24.0)	19 (76.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and response rate

Genotype N=26	Responder (%)	Non-responder (%)	P-value (Fisher's exact test)
CT/GT	0 (0.0)	6 (100.0)	1.000
The others	2 (10.0)	18 (90.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and tumor control rate

Genotype N=26	Tumor control (%)	Progressive disease (%)	P-value (Fisher's exact test)
CT/GT	5 (83.3)	1 (16.7)	1.000
The others	14 (70.0)	6 (30.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and risk of hematologic toxicities

N=28	Grade	CT/GT (%) n=6	The others (%) n=22	P-value (Fisher's exact test)
Anemia	1-4	6 (21.4)	22 (78.6)	-
	0	0 (0.0)	0 (0.0)	
Leukopenia	1-4	6 (28.6)	15 (71.4)	0.288
	0	0 (0.0)	7 (100.0)	
Neutropenia	1-4	6 (37.5)	10 (62.5)	0.024*
	0	0 (0.0)	12 (100.0)	
Thrombocytopenia	1-4	2 (18.2)	9 (81.8)	1.000
	0	4 (23.5)	13 (76.5)	

* P-value of <0.05 was considered statistically significant.

Combination of *ERCC1* and *CTR1* polymorphisms and severity of hematologic toxicities

	Grade	CT/GT (%)	The others (%)	P-value (Fisher's exact test)
Anemia (n=28)	1-2	3 (14.3)	18 (85.7)	0.144
	3-4	3 (42.9)	4 (57.1)	
Leukopenia (n=21)	1-2	5 (26.3)	14 (73.7)	0.500
	3-4	1 (50.0)	1 (50.0)	
Neutropenia (n=16)	1-2	3 (37.5)	5 (62.5)	1.000
	3-4	3 (37.5)	5 (62.5)	
Thrombocytopenia (n=11)	1-2	1 (16.7)	5 (83.3)	1.000
	3-4	1 (20.0)	4 (80.0)	

Combination of *ERCC1* and *CTR1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	CT/GT (%)	The others (%)	P-value (Fisher's exact test)
Liver function test		n=6	n=22	
Increased AST	1-4	4 (23.5)	13 (76.5)	1.000
	0	2 (18.2)	9 (81.8)	
Increased ALT	1-4	4 (30.8)	9 (69.2)	0.371
	0	2 (13.3)	13 (86.7)	
Increased ALP	1-4	5 (26.3)	14 (73.7)	0.630
	0	1 (11.1)	8 (88.9)	
Increased Bilirubin	1-4	1 (11.1)	8 (88.9)	0.630
	0	5 (26.3)	14 (73.7)	
Increased Cr	1-4	2 (28.6)	5 (71.4)	0.622
	0	4 (19.0)	17 (81.0)	
Nausea	1-4	2 (14.3)	12 (85.7)	0.648
	0	4 (28.6)	10 (71.4)	
Fatigue	1-4	3 (23.1)	10 (76.9)	1.000
	0	3 (20.0)	12 (80.0)	
Constipation	1-4	2 (33.3)	4 (66.7)	0.581
	0	4 (18.2)	18 (81.8)	
Vomiting	1-4	1 (14.3)	6 (85.7)	1.000
	0	5 (23.8)	16 (76.2)	
Weight loss	1-4	4 (66.7)	2 (33.3)	0.010*
	0	2 (9.1)	20 (90.9)	
Diarrhea	1-4	2 (66.7)	1 (33.3)	0.107
	0	4 (16.0)	21 (84.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	6 (23.1)	20 (76.9)	
Insomnia	1-4	1 (33.3)	2 (66.7)	0.530
	0	5 (20.0)	20 (80.0)	
Peripheral sensory neuropathy	1-4	2 (66.7)	1 (33.3)	0.107
	0	4 (16.0)	21 (84.0)	

* P-value of <0.05 was considered statistically significant.

Combination of *RRM1*, *ERCC1*, and *CTR1* polymorphisms and risk of non-hematologic toxicities

N=28	Grade	RR37AC-RR524CT/CC/GG (%) n=2	The others (%) n=26	P-value (Fisher's exact test)
Increased AST	1-4	1 (5.9)	16 (94.1)	1.000
	0	1 (9.1)	10 (90.9)	
Increased ALT	1-4	0 (0.0)	13 (100.0)	0.484
	0	2 (13.3)	13 (86.7)	
Increased ALP	1-4	1 (5.3)	18 (94.7)	1.000
	0	1 (11.1)	8 (88.9)	
Increased Bilirubin	1-4	0 (0.0)	9 (100.0)	1.000
	0	2 (10.5)	17 (89.5)	
Increased Cr	1-4	1 (14.3)	6 (85.7)	0.444
	0	1 (4.8)	20 (95.2)	
Nausea	1-4	1 (7.1)	13 (92.9)	1.000
	0	1 (7.1)	13 (92.9)	
Fatigue	1-4	1 (7.7)	12 (92.3)	1.000
	0	1 (6.7)	14 (93.3)	
Constipation	1-4	1 (16.7)	5 (83.3)	0.389
	0	1 (4.5)	21 (95.5)	
Vomiting	1-4	1 (14.3)	6 (85.7)	0.444
	0	1 (4.8)	20 (95.2)	
Weight loss	1-4	0 (0.0)	6 (100.0)	1.000
	0	2 (9.1)	20 (90.9)	
Diarrhea	1-4	0 (0.0)	3 (100.0)	1.000
	0	2 (8.0)	23 (92.0)	
Maculo-papular rash	1-4	0 (0.0)	2 (100.0)	1.000
	0	2 (7.7)	24 (92.3)	
Insomnia	1-4	0 (0.0)	3 (100.0)	1.000
	0	2 (8.0)	23 (92.0)	
Peripheral sensory neuropathy	1-4	0 (0.0)	3 (100.0)	1.000
	0	2 (8.0)	23 (92.0)	

VITA

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