EFFECT OF *ERCC1, XRCC1* AND *GSTP1* POLYMORPHISMS ON CLINICAL RESPONSE AND ADVERSE EVENTS OF PLATINUM-BASED CHEMOTHERAPY IN EPITHELIAL OVARIAN CANCER PATIENTS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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ผลของภาวะพหุสัณฐานของยีน *ERCC1, XRCC1* และ *GSTP1* ต่อการตอบสนองต่อการรักษาและการเกิดเหตุการณ์ไม่พึงประสงค์จากเคมีบำบัดสูตรที่มียากลุ่มแพลทินัมในผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว

น.ส.ศลิษา ลิบลับ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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ศลิษา ลิบลับ : ผลของภาวะพหุสัณฐานของยีน *ERCC1, XRCC1* และ *GSTP1* ต่อการตอบสนองต่อการรักษาและการเกิดเหตุการณ์ไม่พึงประสงค์จากเคมีบำบัดสูตรที่มียากลุ่มแพลทินัมในผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว. (EFFECT OF *ERCC1, XRCC1* AND *GSTP1* POLYMORPHISMS ON CLINICAL RESPONSE AND ADVERSE EVENTS OF PLATINUM-BASED CHEMOTHERAPY IN EPITHELIAL OVARIAN CANCER PATIENTS) อ.ที่ปรึกษาหลัก : ผศ. ภญ. ดร.ณัฏฐดา อารีเปี่ยม, อ.ที่ปรึกษาร่วม : ผศ. นพ.อภิชัย วสุรัตน์

ยาเคมีบำบัดกลุ่มแพลทินัมเป็นยาหลักที่ใช้เป็นอันดับหนึ่งในการรักษาโรคมะเร็งรังไข่ชนิดเยื่อบุผิว และพบว่าเภสัชพันธุศาสตร์เป็นปัจจัยหนึ่งที่อาจส่งผลต่อการตอบสนองต่อการรักษาและความเป็นพิษจากการรักษาด้วยเคมีบำบัดกลุ่มนี้ การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของภาวะพหุสัณฐานของยีน *ERCC1*, *XRCC1* และ *GSTP1* ต่อภาวะการตอบสนองต่อการรักษาและภาวะการเกิดเหตุการณ์ไม่พึงประสงค์จากเคมีบำบัดสูตรที่มียากลุ่มแพลทินัมในผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิวชาวไทย มีผู้เข้าร่วมการวิจัยทั้งหมด 52 ราย เป็นผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นมะเร็งรังไข่ชนิดเยื่อบุผิวระยะท้าย ที่ได้รับการรักษาด้วยเคมีบำบัดสูตรที่มียากลุ่มแพลทินัมเป็นส่วนประกอบ โดยผู้ป่วยมีอายุเฉลี่ยอยู่ที่ 55.7 ปี พบความชุกของยีน *ERCC1* (C>A, rs3212986), *XRCC1* (A>G, rs25487) และ *GSTP* (A>G, rs1695) ร้อยละ 35.6, 28.9 และ 10.6 ตามลำดับ ไม่พบความสัมพันธ์ของภาวะพหุสัณฐานของทั้งยีนทั้งสามต่อการตอบสนองต่อการรักษาด้วยเคมีบำบัด อย่างไรก็ตามผู้ป่วยที่มีการแปรผันของยีน *ERCC1 C8092A* ที่มีลักษณะจีโนไทป์แบบ A/A มีความเสี่ยงต่อการเกิดภาวะดื้อต่อยากลุ่มแพลทินัมมากกว่าจีโนไทป์ลักษณะอื่น (ร้อยละ 75 และ ร้อยละ 16.7, P = 0.046) นอกจากนี้ภาวะพหุสัณฐานของยีน *GSTP1* มีความสัมพันธ์กับการเกิดภาวะโลหิตจางความรุนแรงระดับ 2 โดยพบว่าผู้ที่มีภาวะพหุสัณฐานของยีนแบบ A/G มีความเสี่ยงต่อการเกิดภาวะโลหิตจางมากกว่าผู้ที่มีภาวะพหุสัณฐานของยีนแบบปกติ (A/A) อย่างมีนัยสำคัญทางสถิติ (ร้อยละ 81.8 และ ร้อยละ 46.3, P = 0.036) จากการศึกษานี้พบว่าภาวะพหุสัณฐานของยีน *ERCC1* และ *GSTP1* อาจเป็นดัชนีวัดทางชีวภาพที่มีประโยชน์ในการทำนายผลของการรักษาและการเกิดพิษจากเคมีบำบัดสูตรที่มียากลุ่มแพลทินัมในผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิวชาวไทย

สาขาวิชา เภสัชกรรมคลินิก ลายมือชื่อนิสิต .....

ปีการศึกษา 2561 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5976117633 : MAJOR CLINICAL PHARMACY
KEYWORD: ERCC1, XRCC1, GSTP1, EPITHELIAL OVARIAN CANCER

Salisa Liblab : EFFECT OF *ERCC1, XRCC1* AND *GSTP1* POLYMORPHISMS ON CLINICAL RESPONSE AND ADVERSE EVENTS OF PLATINUM-BASED CHEMOTHERAPY IN EPITHELIAL OVARIAN CANCER PATIENTS. Advisor: Asst. Prof. Nutthada Areepium, Ph.D.,Asst. Prof. Apichai Vasuratna, M.D.

Platinum-based chemotherapy is the first line chemotherapy regimen for epithelial ovarian cancer (EOC). Pharmacogenomics is one factor that might affect its efficacy and toxicity. This cohort study was aimed to investigate the association between *ERCC1*, *XRCC1* and *GSTP1* polymorphisms on clinical responses and adverse events related to platinum-based chemotherapy in Thai EOC patients. Fifty-two patients with advanced EOC were enrolled in this cohort study. The mean age was 55.7 years old. Genotyping analysis of *ERCC1* (C>A, rs3212986), *XRCC1* (A>G, rs25487) and *GSTP* (A>G, rs1695) were performed which allele frequencies were found at 35.6%, 28.9% and 10.6%, respectively. Neither of them associated with clinical responses. However, patients with homozygous variant type (A/A) of *ERCC1 C8092A* had higher risk of platinum-resistance (75% vs 16.7%, *P* = 0.046). In addition, the significant association of *GSTP1* polymorphism and grade 2 anemia was found. Patients with A/G genotype of *GSTP1* had higher rate of grade 2 anemia than those with wild type (81.8% vs 46.3%, *P* = 0.036). Genetic polymorphisms of *ERCC1* and *GSTP1* might be advantageous biomarkers to predicting clinical response and toxicity of platinum-based chemotherapy in Thai EOC patients.

Field of Study: Clinical Pharmacy
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Advisor's Signature .....
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Salisa Liblab

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Chapter 1

Introduction

1.1 Background and Rationale

Epithelial ovarian cancer (EOC) is the major histology of ovarian malignancies that exist around 90% [1]. It was the 7th most common cancer and the 8th most common cause of cancer death in women which reported in world cancer statistics. Because of the location, it is difficult to diagnose, most patients have been diagnosed at advanced stage that is stage III or IV with 5-year survival rate lower than 45% [2]. The National Health and Security Office, Thailand suggested that epithelial ovarian cancer should be treated by platinum-based chemotherapy, which is cisplatin or carboplatin, similar to the United states' National Comprehensive Cancer Network (NCCN) [1, 3].

Platinum compounds are alkylating agents that damage cancer cell by inhibition of DNA inter-strand and intra-strand crosslink including to DNA protein crosslink [4]. These mechanisms are associated with many genes such as *ERCC1*, a gene in Nucleotide excision repair (NER) system, which remove DNA lesion from platinum compounds or *XRCC1*, a gene in Base excision repair (BER) system, which remove base in DNA single strand lesion. Both genes are excise and repair platinum compound adduct at inter- and intra-strand of DNA. Moreover, detoxification pathway in cell cytoplasm associated with the glutathione S-transferase protein which encoded by *GSTP1.* The conjugation between platinum compounds and *GSTP1* resulted in depleted cytotoxic activity. Previous studies reported that the polymorphisms of these genes had been associated with clinical response and adverse events from platinum-based chemotherapy [5, 6].

The association between genetic polymorphisms of *ERCC1, XRCC1* and *GSTP1* with progression free survival (PFS) and overall survival (OS) in patients with epithelial

ovarian cancer in varies ethnicities such as American, European or Asian were reported in several studies [4, 7-10]. However, the results from prior studies had been inconsistent. For example, Krunin AV, et al. reported in Russian patients that changing of allelic status of *GSPT1 Ile105Val* from A to G, correlated with longer of PFS compared with wild type (P=0.002) [4]. In contrast, the study in South Korean of Kim HS, et al. showed no significant association between *GSTP1 Ile105Val* and PFS or OS, but in patients with allelic status changing from A to G had significantly around 3.08 times higher risk of hematological toxicities than another group (95%CI, 1.12-8.43). *ERCC1 C8092A* polymorphisms was associated with reduction of OS in patients with A allele (P=0.042) [9]. On the other hand, Steffensen KD et al reported the correlation between *ERCC1 C8092A* polymorphisms in epithelial ovarian cancer based on Denmark population. No association between genetic polymorphisms and PFS or OS were found [11]. Moreover, some studies were analyzed in the same population resulted in conflicting data, for example, the studies about correlation between *XRCC1 Arg399Gln* and PFS or OS in Chinese patients with epithelial ovarian cancer which reported different outcomes [12, 13].

Results from prior studies cannot clearly explain the relationship between genetic polymorphisms of *ERCC1, XRCC1* and *GSTP1* and chemotherapy treatment responses. Because of that reason, the aim of this research was to investigate the association of *ERCC1, XRCC1* and *GSTP1* polymorphisms with clinical treatment responses and adverse events from platinum-based chemotherapy in Thai patients who have epithelial ovarian cancer.

1.2 Research question

Are there any differences in clinical response and adverse events among epithelial ovarian cancer patients who were treated with platinum-based chemotherapy in various ERCC1, XRCC1, and GSTP1 genotypes?

1.3 Objectives

1. To investigate the impact of *ERCC1 XRCC1* and *GSTP1* polymorphisms in clinical responses from platinum chemotherapy in Thai epithelial ovarian cancer patients.
2. To investigate the impact of *ERCC1 XRCC1* and *GSTP1* polymorphisms in adverse events from platinum chemotherapy in Thai epithelial ovarian cancer patients.

1.4 Hypothesis

1. Genetic polymorphism of *ERCC1 C8092A* is associated with clinical responses and adverse events from platinum chemotherapy in patients with epithelial ovarian cancer.
2. Genetic polymorphism of *XRCC1 Arg399Gln* is associated with clinical responses and adverse events from platinum chemotherapy in patients with epithelial ovarian cancer.
3. Genetic polymorphism of *GSTP1 Ile105Val* is associated with clinical responses and adverse events from platinum chemotherapy in patients with epithelial ovarian cancer.

1.5 Conceptual framework

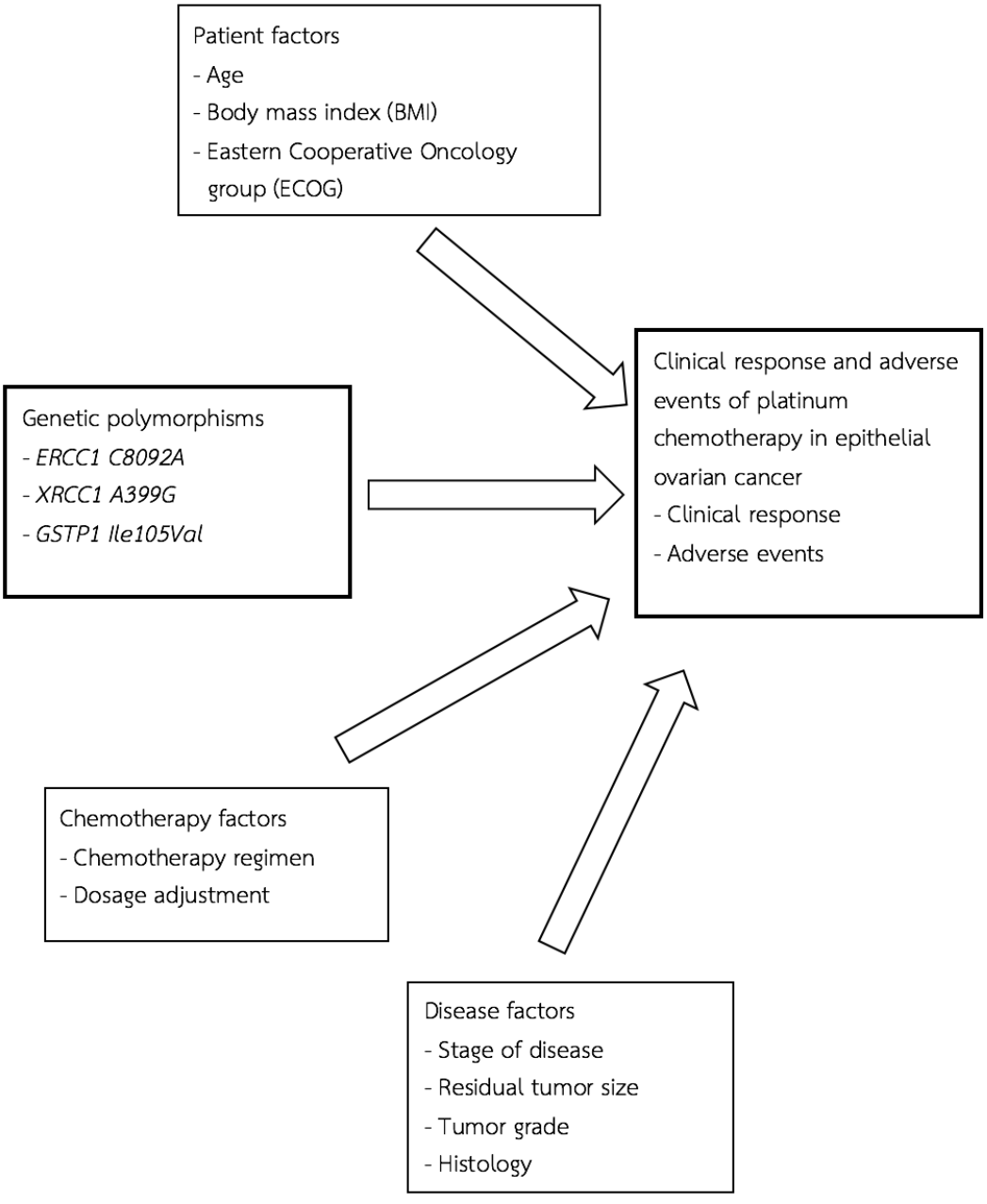

Figure 1 Conceptual framework

1.6 Operational definition

1. Patients are patients with epithelial ovarian cancer stage III or IV who were treated with platinum-based chemotherapy at King Chulalongkorn Memorial Hospital (KCMH).
2. *ERCC1* polymorphism is variation of *C8092A* in the nucleotide sequences of position 45409478 on chromosome 19 (dbSNP no. rs3212986). The mutation of glutamine, which encode with C, to lysine which encode with A. Three genotypes are CC, CA and AA.
3. *XRCC1* polymorphism is variation of *A399G* in the nucleotide sequence of position 43551574 on chromosome 19 (dbSNP no. rs25487). The mutation of arginine, which encode with G, to glutamine which encode with A. Three genotypes are GG, GA and AA.
4. *GSTP1* polymorphism is variation of *Ile105Val* in the nucleotide sequence of position 67585218 on chromosome 11 (dbSNP no. rs1695). The mutation of isoleucine, which encode with A, to valine which encode with G. Three genotypes are AA, AG and GG.
5. Clinical responses were determined in objective tumor response, which were classified by Response Evaluation Criteria in Solid Tumor or RECIST version 1.1 Clinical responses were evaluated by gynecologist after patients received platinum-based chemotherapy at least 3 cycles.
6. Clinical benefit was evaluated by tumor response and clinical symptom. Patients who had CR or PR or SD were classified as benefit and patients who had PD were classified as non-benefit.
7. Recurrent was determined in patients who had progression of disease which evaluated in computed tomography (CT). The evaluation was assessed after

patients completely received 6 cycles of platinum-based chemotherapy. Following time were in 1, 3, 6 months and every year.

8. Platinum resistance was identified as patients who have been changed chemotherapy regimen during treatment or recurrent within 6 months after completing a platinum-based regimen.
9. Adverse events were any unfavorable unintended signs or symptoms including abnormal laboratory or physical change that might be caused by platinum-based chemotherapy occur after the first cycle of chemotherapy treatment. Severity of adverse events were assessed according to Common Terminology Criteria for Adverse Events version 4.03 (CTCAE) at every cycle of chemotherapy. [14].
10. Grade 1 or 2 of adverse events was considered as mild, and grade 3 or 4 of adverse events was considered as severe.

1.7 Benefits from the study

Identify the effect of *ERCC1 XRCC1* and *GSTP1* polymorphisms on clinical response and adverse events in Thai epithelial ovarian cancer patients receiving platinum-based chemotherapy cancer.

Chapter 2

Literature review

2.1 Epithelial Ovarian cancer (EOC)

EOC is the most frequent cause of ovarian cancer. It is almost two-thirds of all ovarian tumors and about 90% of ovarian in Western World [2]. In Thailand, the statistics in 1990-2001 were revealed that 85% of ovarian cancer was EOC. EOC is outstandingly a disease in women at perimenopausal and postmenopausal period, around 80% to 90% occur at the age after 40 and the peak of incidence present at age 60 [15, 16]. A stage of disease at diagnosis is one of the most important prognosis factors, a five-year survival for patients with early stage (I and II) were about 70% to 93%. Whereas for advanced stage (III and IV), a five-year survival rate were reduced to only 25% to 37% [17].

Most patients presented with advanced stage because ovarian cancer is asymptomatic until the disease has metastasized. The symptoms of EOC are not specific such as pelvic or abdominal pain, bloating, digestive disturbances and urinary symptoms, which may present for a few weeks. From that reasons, EOC has the highest fatality-to-case-ratio in gynecological cancer [1, 16, 18]. The screening program for EOC in the general population is not routine implemented because no clinical data support. EOC can be detected by pelvic examination, ultrasound of the pelvis and measurement of the level of tumor marker which is not sensitive enough for detection in the early stage. Cancer antigen 125 (CA-125) is a biological tumor variable which is associated with EOC. CA-125 is used to diagnose and monitor the prognosis of disease with reference value of 30 U/ml [17, 18].

Histological subtypes of EOC can be classified as 4 main types, including serous, endometrioid, mucinous and clear cell. However, most patients, around 70%, have serous tumor which is respond to chemotherapy around 80% but the disease

has poor prognosis [1, 15]. Mucinous or clear cell type were associated with lower progression-free survival (PFS) or overall survival (OS) compared with serous carcinomas [19]. In addition, epithelial tumors are sub-classified by histological grading that described as; Gx is grade cannot be assessed, grade (G) 1 is well differentiated, G2 is moderately differentiated and G3 is poorly differentiated. Histological grading is important because it can predict the disease prognosis. Five-year survival for G1 in early stage is 87%, while in advanced stage is 38%. For G2 and G3, the 5-year survival rate are 70% and 64% in early stage, whereas in advanced stage the rate was decrease as 25% and 19%, respectively [17, 18].

Staging of EOC is classified primarily as stage I to IV by using the FIGO system (International Federation of Gynecology and Obstetrics) as described [20].

Table 1 TNM Classification and FIGO staging system for ovarian cancer

FIGO	TNM	Description
I	**T1**	**Tumor confined to ovaries**
IA	T1a	Tumor limited to 1 ovary (capsule intact); no tumor on ovarian surface; no malignant cells in ascites or peritoneal washings
IB	T1b	Tumor limited to both ovaries (capsule intact); no tumor on ovarian surface; no malignant cells in ascites or peritoneal washings
IC		Tumor limited to 1 or both ovaries, with any of the following:
IC1	T1c1	Surgical spill
IC2	T1c2	Capsule ruptured before surgery or tumor on ovarian surface

Table 1 TNM Classification and FIGO staging system for ovarian cancer (continue)

FIGO	TNM	Description
IC3	T1c3	Malignant cells in the ascites or peritoneal washing
II	**T2**	**Tumor involves 1 or both ovaries with pelvic extension (below pelvic brim) or primary peritoneal cancer**
IIA	T2a	Extension and/or implants on uterus and/or ovaries
IIB	T2b	Extension to other pelvic intraperitoneal tissues
III	**T1/T2-N1**	**Tumor involves 1 or both ovaries or primary peritoneal cancer, with cytological or histological confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes**
IIIA1(i)		Metastasis up to 10 mm in greatest dimension
IIIA1(i)		Metastasis more than 10 mm in greatest dimension
IIIA2	T3a2-N0/N1	Microscopic extra-pelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes
IIIB	T3b-N0/N1	Macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes

Table 1 TNM Classification and FIGO staging system for ovarian cancer (continue)

FIGO	TNM	Description
IIIC	T3c-N0/N1	Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (include extension of tumor to capsule of liver or spleen without parenchymal involvement of either organ)
IV	**Any T, any N, M1**	**Distant metastasis excluding peritoneal metastases**
IVA		Pleural effusion with positive cytology
IVB		Parenchymal metastases and metastases to extra-abdominal organ (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

From the United states' National Comprehensive Cancer Network (NCCN) guideline, platinum-based chemotherapy regimens were recommended for EOC patients at stage II-IV, which is cisplatin 75-100 mg/m^2 through intravenous or intraperitoneal combines with paclitaxel 135 mg/m^2, this regimen repeat every 3 weeks for totally 6 cycles. On the other hand, carboplatin is recommended for intravenous regimen as target area under the curve (AUC) of 5-6 mg×ml/min combines with paclitaxel 135 mg/m^2, repeated every 3 weeks for a totally 6 cycles. Furthermore, carboplatin can be combined with other anticancer drugs such as docetaxel or liposomal doxorubicin [1].

2.2 Platinum-based drugs

Platinum drugs are alkylating agents that have cytotoxic property which inhibit cell growth by non-specific cell cycle proliferation. There are three platinum drugs that wildly use in cancer treatment such as cisplatin, carboplatin and oxaliplatin. The antitumor activities of platinum compounds are activated from complexing with DNA in cell. Mechanisms of action of platinum drugs are bind to DNA and produce intra- and inter-structural crosslinks and formation of DNA adducts. Bulky lesions of DNA adducted change conformation of DNA may affect to replication and inhibition of DNA synthesis which activate the apoptotic pathway then resulting in cell death. Cisplatin and carboplatin are the same complexations of platinum-DNA intra-strand crosslinks because carboplatin active form is identical to cisplatin, whereas oxaliplatin is different and may account in its spectrum of activity. However, platinum drugs, such as cisplatin and carboplatin, are constituted in chemotherapy treatment regimen of many type of solid malignancies, including head and neck, testicular, lung, colorectal and are the backbone for treatment of advanced epithelial ovarian cancer as well as other gynecologic cancers [21-24].

Cisplatin and carboplatin are mainly indicated in the primary therapy regimen for advanced stage EOC. For intravenous infusion regimen, carboplatin is recommended for combination with other chemotherapy agents at dose of target AUC 5-6 mg×ml/min, infusion over 1 hour, repeatedly every 3 weeks for 6-8 cycles, whereas cisplatin is prescribed at 75-100 mg/m^2, infusion over 6-8 hours [1, 25]. However, cisplatin is not only indicated for cytotoxic activity for many solid tumor but also affects kidney leading to nephrotoxicity, peripheral nerve leading to neurotoxicity and the inner ear causing ototoxicity, while carboplatin is a second-generation platinum compound developed for reducing the side effects of cisplatin. Carboplatin does not show nephrotoxic and neurotoxic properties but it manifests

side effect concern about bone marrow suppression and frequency of receiving carboplatin leading to reversible thrombocytopenia [5].

Cisplatin and carboplatin are neural inorganic substances and must be activated in cell by aquation reactions that consist with the sequential replacement of *cis*-chloro (cisplatin) or *cis*-diammine (carboplatin) with water molecules. This reaction occurs spontaneously in cytoplasm. The mono- or bi-aquated cisplatin form generate the interaction with endogenous nucleophiles, such as glutathione (GSH), methionine, metallothionein and protein. Furthermore, aquated cisplatin bind DNA with nucleophilic N7-sites of purine base and form protein-cisplatin-DNA complex that have been indicated as cisplatin cytotoxicity by platinum induce DNA lesion as inter- and intra- strand crosslink [5, 26].

2.3 Metabolic pathway of platinum compounds

The metabolic pathway of platinum compounds was shown in figure 2. In summary, platinum drug is delivered to intracellular by a transmembrane protein copper transporter 1 (*CTR1*) or *SLC31A1*, which was believed an important transporter role in uptake of platinum drug. In cytoplasm aquated platinum binds to cytoplasmic nucleophilic species including glutathione (GSH) and other cysteine-rich proteins. This complexation may imply the cytoplasmic effect of platinum drugs because of depletion of antioxidant and establishment of oxidative stress. However, nucleophilic species have diverse function as detoxification of electrophilic substrates both endogenous and exogenous substrates including platinum drugs. On the other hand, there are copper-transporting P-type adenosine triphosphates *ATP7A* and *ATP7B* have been indicated in efflux transporter which reduce platinum accumulation in cell. Moreover, other pumps that related to export platinum drugs are ABC ATPase like multidrug resistance protein 1 (*MRP1*), *MRP2* or *ABCC2* play a

role in detoxification of platinum drugs by delivered platinum out of cytoplasm [5, 27].

Platinum-DNA adducts are found in nucleus, while recognition of inter- and intra-strand DNA adducts induce production of an apoptotic signal and lead to cell death. The major lesions of platinum are primarily removed form DNA by nucleotide excision repair (NER) system after detected by mismatch repair (MMR) system. The NER genes recognize and repair bulky DNA damage because of platinum adducted, whereas the base excision repair (BER) genes recognize and repair of base lesion at DNA single-strand breaks cause by alkylating agents. One of the most important gene consist in the NER pathway is *ERCC1*, which perform the rate-limiting step of incision damaged DNA strand. Similarly, *XRCC* is the BER gene which assists in repairing process by recruitment and forming protein complex at the single-strand breaks. After the inducting process, non-repairable DNA induces to activate the apoptotic multi-branched signaling cascade that causes cell death [23, 27, 28].

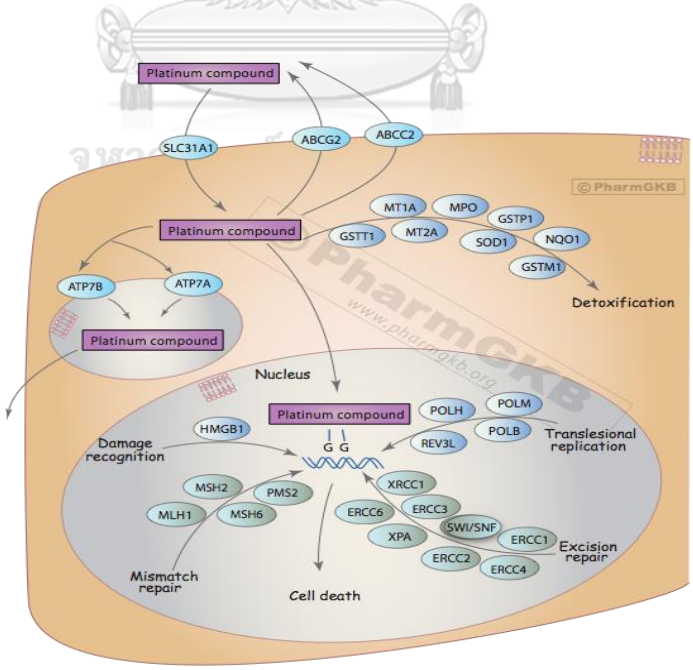

Figure 2 Metabolic pathway of platinum compounds [29]

2.4 Mechanism of platinum compounds resistance

Although, highly effective platinum drugs were indicated into the first-line chemotherapy regimen in many solid tumors such as colorectal, lung, prostate including gynecological cancers but platinum often lead to therapeutic cure rate with partial responses or disease stabilization. Because of genes mutation, there are multiple intrinsically resistance mechanisms to platinum-based chemotherapy. Therefore, active form of carboplatin is cisplatin, it was seemed that the resistance mechanism of carboplatin is similarly to cisplatin. In cytoplasm, there are several important mechanisms that lead to reduction of intracellular platinum accumulation. Firstly, *CTR1*, the major copper influx transporter, previous in-vitro study revealed the association between effect of loss *CTR1* function on platinum drugs uptake lead to reduce cellular platinum accumulation and their cytotoxicity [30]. Moreover, the overexpression of copper efflux transporter *ATP7B* was related to poor prognosis disease outcome than other patients with *ATP7B*-negative in ovarian carcinoma, who was treated by cisplatin-based regimen [31]. In addition, there are limited documentation about glutathione-S-transferase (*GST*) family such as *GSTM1, GSTT1* and *GSTP1* which involved in the detoxification of anticancer drugs including platinum with their mechanism is catalyzing the conjugation of glutathione moiety with electrophilic portion in platinum compounds, in order to increasing platinum excretion [32]. In patients with advanced gastric cancer were treated with fluorouracil (5-FU) and cisplatin, the differences in overall response rate was shown in patient who with genetic polymorphisms. *GSPTP1 105 Valine/Valine* carriers had a response rate of 67% compare with *GSPTP1 105* with at least one Isoleucine carrier who had a response rate only 21% [33].

In the step of on-target resistance, *ERCC1* gene performs one of the most important genes in platinum resistance. The expression of *ERCC1* had an effect on

clinical responses in the patients with serous epithelial ovarian cancer who were treated with standard first-line combination platinum and paclitaxel. This study revealed that shorter PFS and OS in patients with *ERCC1* polymorphism compare with another [34]. There is not only epithelial ovarian cancer that exhibited the association of *ERCC1* expression and platinum chemotherapy responses, but also in advanced non-small cell lung cancer and advanced gastric cancer as well. The data was shown in similar direction [35, 36]. Furthermore, the ability of repair platinum damaging DNA also depend on base excision repair enzyme; *XRCC1* which had shown significant correlation of overexpression and median OS and higher proportion of cell death in head and neck squamous carcinoma or non-small cell lung cancer patients. *XRCC1* overexpression lead to lower proportion of cell death and shorter OS [37, 38].

2.5 Genetic polymorphisms and platinum resistance in EOC

Single nucleotide polymorphisms (SNPs) are the most common genetic variation in human genome, which occur in 1 per 300 nucleotides throughout a person's DNA. Each SNP exhibits in a difference single DNA building block, that may have some effects on health and developing of disease [39]. The *ERCC1 C8092A* polymorphisms which effect on mRNA stability were associated with clinical outcomes in patients with EOC. There are many studies that justified the relationship between *ERCC1 C8092A* polymorphisms with disease progression and OS along with toxicities of platinum-based chemotherapy.

Krivak TC, et al. studied the association of *ERCC1* polymorphisms on disease progression and survival in advanced EOC patients who were treated with combination of cisplatin and paclitaxel. The data was collected from 233 patients with 91.9% of white, 3.0% of black and 5.2% of other races at the median of age was 57-year old. The genotypes distribution at *C8092A* was 56.2% of C/C, 36.9% of C/A and 6.9% of A/A, with the significant increasing risk of disease progression in patients

with at least A allele compared with C/C genotype (hazard ratio [HR] = 1.44; 95% CI, 1.06 to 1.94; P = 0.018), while median PFS and OS were shorter in C/A or A/A genotype versus C/C genotype at 6 months and 17 months, respectively [10].

Kim HS, et al. also reported interesting association between genetic polymorphisms and clinical outcomes in Korean EOC patients treated with platinum compounds. In 118 patients with all stages of EOC, it was found that *ERCC1 C8092A* polymorphisms associated with shorter PFS and OS. Patients with C/C genotype had significant longer PFS and OS than the others (median PFS, 37 vs 12 months; p<0.01 and mean OS, 147 VS 63 months; p=0.042). They also reported that *GSTP1 Ile105Val* was associated with higher rate of hematological toxicity which grade 3 or 4 in patients with A/A genotype than A/G or G/G genotype (78.7% and 54.2%, p=0.015) [9].

In Russian population of Khrunin AV, et al. found the relationship between *GSTP1*, *XRCC1* and *ERCC1* polymorphisms with efficacy and toxicity of cisplatin-based chemotherapy in 104 patients with stage I to IV ovarian cancer. Majority of participants were stage III and IV, overall responses rate was 83%, which consisted of 44% in complete responses and 39% in partial responses. However, the analysis of disease prognosis, partial responders were combined with non-responders, there were no correlation with genotypes and the outcomes. While *GSTP1 Ile105Val* was associated with PFS. Patients with homozygous Ile/Ile genotype had significant longer median PFS than Ile/Val and Val/Val (15 months compared with 8.5 and 8.0 months; P<0.001, respectively). Moreover, *XRCC1 Arg399Gln* was correlated with more grade 3 or 4 neutropenia, this severity occurred 3.02 times in patients with Arg/Arg compared with Arg/Gln or Gln/Gln (95% CI, 1.33-6.88). In term of nephrotoxicity, patients with heterozygous C/A genotype of *ERCC1 C8092A* had higher prevalence of this toxic with odd ratio 2.51 compared to the homozygous variants (P=0.009) [4].

Steffensen KD, et al. also studied the impacts of *ERCC1 C8092A* polymorphisms in EOC all stages with platinum resistance and survival. In 202 Danish patients who received first-line combination chemotherapy with carboplatin (AUC5) and paclitaxel (175 mg/m^2), every 3 weeks at least 4 cycles. The clinical response was evaluated after completed chemotherapy, 59% of patients had complete response and 32% had partial response whereas 8% of patients did not response to platinum-based chemotherapy. There was no correlation of any SNPs with first-line therapy response. As well as PFS and OS, even if A/A genotype carriers tended to had poorer PFS compared with C/C or C/A genotype but it did not reach statistically significance (9.5, 14.1 and 19.6 months, respectively). Patients with A/A had shorter median OS as 35.7 months compared with 42.9 and 40.7 months of C/C and C/A carriers, respectively but also not statistically significant [11].

Moxley KM, et al. had also studied the role of *ERCC1 C8092A* in predicting platinum-sensitivity, the study was analyzed in many races in USA, which included 83% of Caucasian, 7% of African-American, 3% of Native-American, 1% of Asia and 6% of Hispanic. The association between *ERCC1 C8092A* genotypes with shorter survival was evaluated in 106 patients with advanced stage EOC. There had nearly significant diffrenceds in median PFS among genotypes A/A, A/C and C/C (25.6, 9.6 and 9.5; P = 0.06). Similar to median OS which were 45.3, 33.5 and 31.7 in A/A, A/C and C/C, respectively (P = 0.06) [40].

Furthermore, non-bulky lesion of DNA damaged by platinum was excised by BER gene, the important one is *XRCC1*. In Chinese, Cheng CX, et al. studied in 310 EOC stage I-IV patients. They found that *XRCC1 Arg399Gln* polymorphisms associated with the risk of dead after treatment with platinum-based chemotherapy. Data was shown that 32% death rate in patients who carrying Arg/Arg, while death rate was lower in Gln/Gln group at 25.1%. From this study, *XRCC1 399* Arg/Arg patients had

lower survival rate compared with patients with Gln/Gln genotype with 1.69-fold (HR = 1.69; 95%CI = 1.07-2.78) [41].

Following the previous study, Li K and Li W included 335 all stage EOC Chinese patients received platinum-based chemotherapy after surgery to investigated the association between polymorphisms of *XRCC1* and survival. In patients who carrying Gln/Gln genotype had 0.44-fold risk of death than patients who carrying Arg/Arg (HR = 0.44; 95%CI, 0.28-0.91) [13].

In contrast, Kang S, et al. found no association between *XRCC1 399* polymorphisms and disease progression or death of EOC patients. This study included 213 Chinese patients at stage I-IV. All patients were received first-line platinum-base chemotherapy regimen after surgery. There was no significant relationship between the wild-type homozygous compared with the variant homozygous and heterozygous genotypes [12].

Beeghly A, et al. studied the association between *GSTP1* polymorphisms and survival rate of EOC patients treated with platinum-based chemotherapy regimens. The conjugation of GST family enzymes with platinum compounds will increase solubilizing and excretion chemotherapeutic agents out of cell. This study conducted in 182 patients from Gynecological Oncology Unit in Italy. They included all stages of disease with the majority was stage III and IV. Responders were the patient who had complete response, while non-responders were the patients who had partial response, stable disease and disease progression. The relationship between *GSTP1* polymorphisms and response of treatment was not found, similar to disease progression or death [7].

Chapter 3

Methods

3.1 Research design

This study was prospective cohort study to assess the association between genetic polymorphisms with both clinical responses and adverse events from platinum-based chemotherapy in epithelial ovarian cancer patients.

3.2 Scope of research

All epithelial ovarian cancer patients were treated with platinum-based chemotherapy.

3.3 Population and sample

3.3.1 Population

Epithelial ovarian cancer patients stage III or IV were treated with platinum-based chemotherapy.

3.3.2 Sample

All epithelial ovarian cancer patients stage III or IV were treated with platinum-based chemotherapy at outpatient department King Chulalongkorn Memorial Hospital during May 2018 to September 2018.

Inclusion criteria

Patients included into this study must meet all criteria as listed below

1. Age over 18-year old
2. Patients who had histopathology or cytology confirmation for epithelial ovarian cancer stage III or IV.
3. Patients received intravenous platinum-based chemotherapy (cisplatin or carboplatin).
4. Patients received chemotherapy at least 1 cycle were determined on adverse events and at least 3 cycles were determined on clinical responses.

5. Patients had an Eastern Cooperative Oncology group (ECOG) performance status score as 0, 1 or 2.
6. Patients were able to communicate about their symptoms of present illness.
7. Patients were willing to participate in this research by signing inform consent.

Exclusion criteria

Patients were excluded from this study if

1. Patients who had additional treatments with chemotherapy.
2. Patients who withdraw of consent.

Sample size calculation

From previous study, Cheng et al. [41] suggested that effect of genetic polymorphisms *XRCC1 Arg399Gln* related to treatment response from platinum-based chemotherapy in epithelial ovarian cancer, relative risk (RR) in patients whom with Arg/Arg (wild type) is 1.67 times.

In this study, the sample size was calculated by G*power; statistical power analyses program, and set the alpha-error as 0.05 of two-sided = 1.96 and beta error of 0.1 = 1.28

P_1 = Proportion of treatment response in patients whom with wild type = 0.5

P_2 = Proportion of treatment response in patients whom with variant type = 0.3

The calculated sample size was around 24 patients per arm, thus required sample size was estimated as 48 patients. However, the target sample size that including 5% dropout was set at 51 patients [42].

3.4 Procedures

1. Patients who fulfill inclusion/exclusion criteria were asked to participate the study when they arrived to hospital.
2. Patients and/or legal guardians were informed about the objective and study protocol by the investigator. The investigator explained benefits/risks and answer the questions until patients fully understand. Patient was freely to decide whether to participate or not before an informed consent was signed.
3. The demographic and clinical data were collected from medical records.
4. Approximately 5 milliliters blood from venous blood vessel was collected from each participant by a registered nurse and stored in EDTA tubes.
5. Genomic DNA was extracted from blood sample by using Qiagen blood kit at laboratory, department of Medicine, Faculty of Medicine, Chulalongkorn University.
6. Variation in *ERCC1 XRCC1* and *GSTP1* were genotyping with real-time polymerase chain reaction (RT-PCR) system according to Taqman allelic discrimination assay.
7. Clinical responses were determined by gynecologist after patients received platinum chemotherapy at least 3 cycles. The data was collected from medical records.
8. Adverse events were assessed in every visit of drug administration by patient interview and laboratory results evaluation.
9. Statistical analyses were performed by using SPSS version 22.

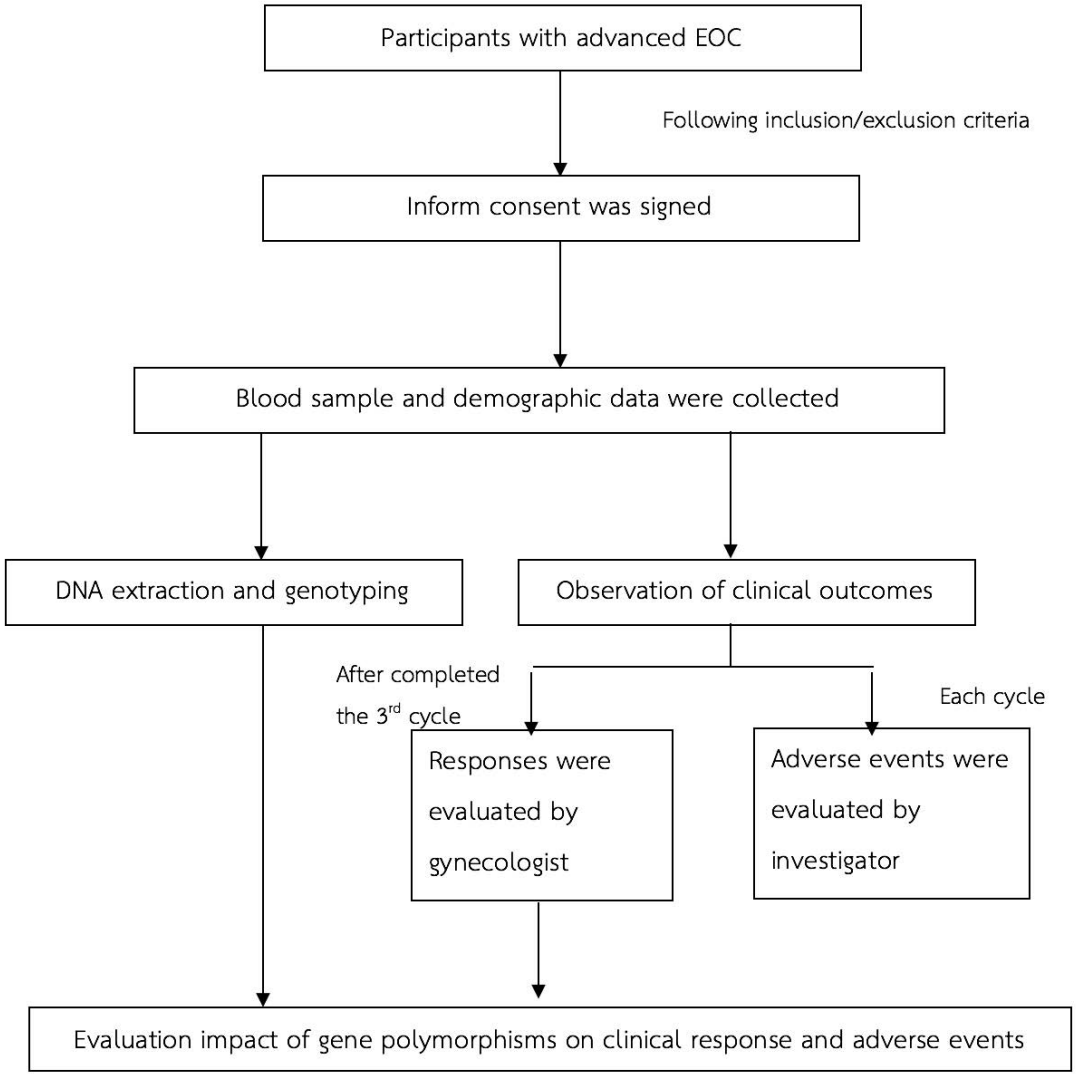

Figure 3 Research procedure

3.5 Buffy coat extraction

Blood sample was drawn approximately 5 milliliters and collected in vacutainer tube (purple-stopper) containing EDTA from patients before chemotherapy administration.

Buffy coat is a leucocyte-enriched fraction of whole blood which 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, the intermediate layer of 3

different fractions is buffy coat with concentrated leucocyte. Two-hundred microliters of the buffy coat was pipetted into 1.5 ml microcentrifuge tube and stored in a freezer at -80 °C until extracted DNA with the with the use of a QIAamp Blood Mini Kits (QAIGEN Gmbh, Hilden, Germany).

3.6 DNA extraction

The buffy coat was used for DNA extraction by ultilzing QIAamp® DNA Blood Mini Kit per following protocol;

1. Equilibrated buffy coat to room temperature
2. Pipetted 20 microliters QIAGEN protease into 1.5 ml microcentrifuge tube containing buffy coat 200 microliters.
3. Added 200 microliters of Buffy AL into the tube. Mix by vortex mixer for 15 seconds.
4. Incubated at 56 °C for 10 minutes.
5. Added 200 microliters of 100% ethanol to sample, then mixed again by vortex mixer for seconds.
6. Carefully applied the mixture to QIAamp mini spin column (in a 2-ml collection tube) without wetting the rim. Closed the cap, and centrifuged at 6000 x g (8000 rpm) for 1 minutes. Placed the QIAamp mini spin column in a 2-ml clean collection tube, and discarded the tube containing the filtrate.
7. Carefully opened the QIAamp Mini spin column and added 500 microliters buffer AW1 without wetting the rim. Closed the cap and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min.
8. Carefully opened the QIAamp Mini spin column and added 500 microliters buffer AW2 without wetting the rim. Closed the cap and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min.

9. Placed the QIAamp Mini spin column in a new 2 ml collection tube and discarded the old collection tube with the filtrate. Centrifuged at full speed for 1 min.
10. Placed the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube, and discarded the collection tube with the filtrate. Carefully opened the QIAamp Mini spin column and added 200 microliters of Buffer AE. Incubated at room temperature (15-25 °C) for 1 min.

After DNA extraction were done, Thermo Scientific NanoDrop 2000c spectrophotometer was use for measuring the concentration and purity of DNA samples.

3.7 *ERCC1*, *XRCC1* and *GSTP1* genotyping

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

1. *ERCC1* polymorphism (C→A), Assay ID: rs3212986
2. *XRCC1* polymorphism (G→A), Assay ID: rs25487
3. *GSTP1* polymorphism (A→G), Assay ID: rs1695

ERCC1, *XRCC1* and *GSTP1* polymorphisms were analyzed by the polymerase chain reaction (PRC)-restriction fragment length polymorphism method. The primers and probes were commercially available (Applied Biosystems Inc., Foster city, CA, USA). For *ERCC1* and *XRCC1*, the 20 microliters of reaction mixture containing 2 microliters of 20 nanogram genomic DNA, 0.5 microliters of 40x SNP genotyping assay, 10 microliters of 2x TaqMan Genotyping master mix, and 7.5 microliters of DNase-free water in 96-well plate. Amplification was performed by following program: 10 min at 95 °C, followed by 50 cycles of 15 seconds at 95 °C and 1 min at 60 °C. For *GSTP1*,

the 20 microliters of reaction mixture containing 2 microliters of 20 nanogram genomic DNA, 1 microliter of 20x SNP genotyping assay, 10 microliters of 2x TaqMan Genotyping master mix, and 7 microliters of DNase-free water in 96-well plate. Amplification was performed by following program: 30 seconds at 60 °C then 10 min at 95 °C, followed by 40 cycles of 15 seconds at 95 °C, 1 min at 60 °C and 30 seconds at 60 °C.

After PCR amplification, perform an endpoint plate read on a StepOnePlus Real time PRC System (Applied Biosystems 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 allele are in each sample.

3.8 Data analysis

The data were analyzed by SPSS program version 22.0 (SPSS. Co., Ltd, Bangkok Thailand)

Basic clinical characteristics of participants such as stage of disease, extent of disease, ECOG performance status score, comorbidity, chemotherapy regimen and previous therapy were presented in descriptive statistic as percentage. Continuous variable such as age, weight, tumor marker level and time to progression were presented in descriptive statistic as mean ± standard deviation (SD) if the information was normal distribution, or median and inter quartile range (IQR) or range if the information was not normal distribution.

Association of genetic polymorphisms with response and adverse events were evaluated by using the Chi-square test in 2x2 table, if one or more cells had an expected frequency of 5 or less, Fisher's exact test were used.

Table 2 Statistical analysis

Hypothesis	Variable	Statistic
1. *ERCC1* C8092A polymorphism is associated with clinical response and adverse events from platinum chemotherapy	Independent variable: genotypes (categorical) Dependent variable: - clinical response type (categorical) - adverse event grade (categorical)	Chi square test or Fisher's exact test
2. *XRCC1* Arg399Gln polymorphism is associated with clinical response and adverse events from platinum chemotherapy	Independent variable: genotypes (categorical) Dependent variable: - clinical response type (categorical) - adverse event grade (categorical)	Chi square test or Fisher's exact test
3. *GSTP1* Ile105Val polymorphism is associated with clinical response and adverse events from platinum chemotherapy	Independent variable: genotypes (categorical) Dependent variable: - clinical response type (categorical) - adverse event grade (categorical)	Chi square test or Fisher's exact test

3.9 Ethical consideration

This study was approved by Institutional Review Board of Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. The IRB approval number was 162/61.

The study was conducted in 3 basic ethical principles; Respect for Persons, Beneficence and Justice.

Respect for Persons: investigator must give sufficient information on which to decide whether or not to participate, including the objective, the study protocol, 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 was confidential and case report form was unable to identify patients.

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

3.10 Budget

Budgeting of this study was totally 161,000 bath. Research grant funds have been provided for this study by the 90th Anniversary Chulalongkorn University Fund (Ratchadapiseksomphot Endowment Fund).

Chapter 4

Results

4.1 Patients' characteristics

This study was cohort study, a total of 52 patients with advanced EOC treated with platinum-based chemotherapy were enrolled between May to September 2018 at obstetrics and gynecology department, King Chulalongkorn Memorial Hospital. The patients' characteristics were shown in Table 3.

The average age was 55.71±10.52 with ranging from 35 to 85 years old. The clinical data was collected from medical records; 10 patients were diagnosed in advanced stage without complete staging. Poorly differentiated serous adenocarcinoma histology was the most common found in 19 patients (36.54%). And 46 patients (88.46%) had surgery for tumor debulking. Four patients had mixed epithelial histological types. Three patients had serous adenocarcinoma and endometrioid adenocarcinoma. One patients had a mixed with endometrioid adenocarcinoma and clear cell carcinoma. Most of patients were in favorable performance status which ECOG score 0 and 1. Mainly chemotherapy regimen was carboplatin (AUC 5 mg×ml/min) combined with paclitaxel (175 mg/m^2), none of patients received cisplatin. Twenty-seven patients with recurrent disease previously treated with platinum based chemotherapy.

Table 3 Patients' characteristics

Characteristic	**N (%)**	
Total number of participants	52	
Age	55.71±10.52 (35,85)	
Characteristics	n	%
FIGO stage		
■ Stage III	30	57.69
■ Stage IV	12	23.08
■ N/A	10	19.23
Performance status		
■ ECOG score=0	30	57.69
■ ECOG score=1	19	36.54
■ ECOG score=2	3	5.77
Histological type		
■ Serous adenocarcinoma	28	53.85
■ Endometrioid adenocarcinoma	8	15.38
■ Clear cell carcinoma	5	9.62
■ Mucinous adenocarcinoma	1	1.92
■ Mixed	4	7.69
■ N/A	6	11.54
Tumor grading		
■ Well differentiated	4	7.69
■ Moderate differentiated	6	11.54
■ Poorly differentiated	26	50.00
■ Unidentified	6	11.54
■ N/A	10	19.23

Table 3 Patients' characteristics (Continue)

Characteristic	N	%
Debulking status		
■ Complete	16	30.77
■ Optimal	21	40.38
■ Suboptimal	9	17.31
■ No surgery	4	7.69
■ N/A	2	3.85
Chemotherapy regimen		
■ Single carboplatin	3	5.77
■ Carboplatin + Paclitaxel	49	94.23

Abbreviation: ECOG = Eastern Cooperative Oncology Group.

4.2 Genotypic distribution

Genotype frequencies were revealed in Table 4. *ERCC1* polymorphism, (C>A, rs3212986) was found as the wild type (C/C), heterozygous variant (C/A) and homozygous variant (A/A) at 48.08%, 32.69% and 19.23%, respectively. *XRCC1* polymorphism, (G>A, rs25487) was found as the wild type (G/G), heterozygous variant (G/A) and homozygous variant (A/A) at 53.85%, 36.54% and 9.61%, respectively. *GSTP1* polymorphism (A>G, rs1695) was found only 2 genotypes as wild type homozygous variant (A/A) and heterozygous variant (A/G) at 78.85% and 21.15%, respectively.

Table 4 Genotype frequency

Total number of participants = 52	N	%
ERCC1 C8092A genotype		
■ Homozygous wild genotype (C/C)	25	48.08
■ Heterozygous variant genotype (C/A)	17	32.69
■ Homozygous variant genotype (A/A)	10	19.23
XRCC1 A399G genotype		
■ Homozygous wild genotype (G/G)	28	53.85
■ Heterozygous variant genotype (G/A)	19	36.54
■ Homozygous variant genotype (A/A)	5	9.61
GSTP1 Ile105Val genotype		
■ Homozygous wild genotype (A/A)	41	78.85
■ Heterozygous variant genotype (A/G)	11	21.15
■ Homozygous variant genotype (G/G)	0	0

Hardy-Weinberg Equilibrium (HWE) was used to estimate expected wild type homozygous, expected heterozygous and expected variant type homozygous by mean of χ^2 test, which a P-value < 0.05 was required for statistical significant. HWE was the correlation of allele frequencies and genotype variation in populations.

Allele frequencies equation $p + q = 1$

Genotype frequencies equation $p^2 + 2pq + q^2 = 1$

Allele frequencies of *ERCC1* polymorphism, (C>A, rs3212986) were presented at 35.58% whereas allele frequencies of *XRCC1* polymorphism, (G>A, rs25487) were presented at 28.88% and allele frequencies of *GSTP1* polymorphism (A>G, rs1695) were presented at 10.58%, the data was shown in Table 5

Table 5 Allele frequency

Total number of participants = 52						
Gene	Allele	52 x 2 N (%)	Genotype	N (%)	Predicted (HWE)	P-value
ERCC1	C	67 (64.42)	CC	25 (48.08)	21.58	0.037
			CA	17 (32.69)	23.84	
	A	37 (35.58)	AA	10 (19.23)	6.58	
XRCC1	G	75 (72.12)	GG	28 (53.85)	27.04	0.509
			GA	19 (36.54)	20.92	
	A	29 (28.88)	AA	5 (9.61)	4.04	
GSTP1	A	93 (89.42)	AA	41 (78.85)	41.58	0.394
			AG	11 (21.15)	9.84	
	G	11 (10.58)	GG	0 (0.00)	0.58	

4.3 Overall benefit

Clinical benefit was evaluated after completion of chemotherapy per RECIST criteria or at least as documented by physician in patients' medical record. From 52 patients which were included into this study, 48 patients completed 6 cycles of platinum-based chemotherapy and were evaluated for responses and 4 patients were still waiting for clinical responses evaluation. In the group which treatment responses were evaluated, we categorized them into 2 groups as good and poor responses. Good response was defined in a patient who the disease can be controlled or no worsening clinical symptoms while poor response was referred to patient who had disease progression or unfavorable clinical symptoms. Of the 48 patients, 3 patients (6.25%) were assigned to other chemotherapy regimen due to worsening symptoms or disease progression. Tumor debulking was significantly associated to clinical benefit (P=0.006). The other factors were not associated with clinical benefits.

Table 6 Association of factors and overall benefit

Total number of participants = 48					
Factor	n	Overall benefit		χ^2 value	P-value
		Good response (n=45, %)	Poor response (n=3, %)		
Age				1.366	0.283
■ < 60 years	31	30 (96.77)	1 (3.23)		
■ ≥ 60 years	17	15 (88.23)	2 (11.77)		
FIGO stage				1.565	0.567
■ Stage III	27	26 (96.30)	1 (3.70)		
■ Stage IV	13	12 (92.31)	1 (7.69)		
■ N/A	8	7 (87.50)	1 (12.50)		
Performance status				0.586	1.000
■ ECOG score=0	28	26 (29.86)	2 (7.14)		
■ ECOG score=1	17	16 (94.12)	1 (5.88)		
■ ECOG score=2	3	3 (100.00)	0 (0.00)		
Histological type				3.515	0.755
■ Serous adenocarcinoma	24	22 (91.67)	2 (8.33)		
■ Endometrioid adenocarcinoma	8	8 (100.00)	0 (0.00)		
■ Clear cell carcinoma	5	5 (100.00)	0 (0.00)		
■ Mucinous adenocarcinoma	1	1 (100.00)	0 (0.00)		
■ Mixed	4	4 (100.00)	0 (0.00)		
■ N/A	6	5 (––80.00)	1 (20.00)		

Table 6 Association of factors and overall benefit (continue)

Total number of participants = 48					
Factor	N	Overall benefit		χ^2 value	P-value
		Good response (N=45, %)	Poor response (N=3, %)		
Tumor grading				3.064	0.511
■ 1	4	4 (100.00)	0 (0.00)		
■ 2	5	4 (80.00)	1 (20.00)		
■ 3	23	22 (95.65)	1 (4.35)		
■ Unidentified	6	6 (100.00)	0 (0.00)		
■ N/A	10	9 (90.00)	1 (10.00)		
Debulking status				9.831	**0.006***
■ Complete	15	15 (100.00)	0 (0.00)		
■ Optimal	19	19 (100.00)	0 (0.00)		
■ Suboptimal	8	7 (87.50)	1 (12.50)		
■ No surgery	4	2 (50.00)	2 (50.00)		
■ N/A	2	2 (100.00)	0 (0.00)		
Chemotherapy regimen				0.213	1.000
■ Single carboplatin	3	3 (100.00)	0 (0.00)		
■ Carboplatin + Paclitaxel	45	42 (93.33)	3 (6.67)		

Our study also exhibited no association between overall benefit and *ERCC1*, *XRCC1* and *GSTP1* polymorphisms. The data was shown in Table 7.

Table 7 Association of genetic polymorphisms to overall benefit

Total number of patients = 48					
Gene	N	Overall benefit		χ^2 value	P-value
		Good response (N=45, %)	Poor response (N=3, %)		
ERCC1				3.356	0.132
CC	23	22 (95.65)	1 (4.35)		
CA	15	15 (100.00)	0 (0.00)		
AA	10	8 (80.00)	2 (20.00)		
XRCC1				3.041	0.204
GG	25	23 (92.00)	2 (8.00)		
GA	18	18 (100.00)	0 (0.00)		
AA	5	4 (80.00)	1 (20.00)		
GSTP1				0.303	0.512
AA	38	36 (94.74)	2 (5.26)		
AG	10	9 (90.00)	1 (10.00)		

4.4 Recurrent status

A total of 52 patients, there were 27 patients (51.92%) who had the history of receiving platinum-based chemotherapy for their EOC and had disease recurrence prior to enrollment of this study. Of the 27 cases, 7 patients (13.46%) had disease recurrence within 6 months and defined as platinum resistant cases. There were no significant differences in the recurrent status in the age, FIGO stage, histological type, performance status and chemotherapy regimen. The significant differences were found in 2 factors which were grading of tumor and debulking status (P=0.037 and P=0.007, respectively). The results showed that patients who had moderate

differentiated histological pattern had higher rate of disease recurrence similar to suboptimal debulking surgery.

Table 8 Association between factors and recurrent status

Total number of participants = 52					
Factor	N	Recurrent status		χ^2 value	P-value
		Not recurrent (N=25, %)	Recurrent (N=27, %)		
Age				1.156	0.282
■ < 60 years	33	14 (42.42)	19 (57.58)		
■ ≥ 60 years	19	11 (57.89)	8 (42.11)		
FIGO stage				3.644	0.165
■ Stage III	30	11 (36.67)	19 (63.33)		
■ Stage IV	14	9 (64.29)	5 (35.71)		
■ N/A	8	5 (62.50)	3 (37.50)		
Histological type				3.681	0.649
■ Serous adenocarcinoma	28	11 (39.29)	17 (60.71)		
■ Endometrioid adenocarcinoma	8	5 (62.50)	3 (37.50)		
■ Clear cell carcinoma	5	2 (40.00)	3 (60.00)		
■ Mucinous adenocarcinoma	1	1 (100.00)	0 (0.00)		
■ Mixed	4	2 (50.00)	2 (50.00)		
■ N/A	6	4 (66.67)	2 (33.33)		

Table 8 Association between factors and recurrent status (continue)

Total number of participants = 52					
Factor	N	Recurrent status		χ^2 value	P-value
		Not recurrent (N=25, %)	Recurrent (N=27, %)		
Tumor grading				9.489	**0.037***
■ 1	4	4 (100.00)	0 (0.00)		
■ 2	5	1 (20.00)	4 (80.00)		
■ 3	26	10 (38.46)	16 (61.54)		
■ Unidentified	6	2 (33.33)	4 (66.67)		
■ N/A	11	8 (72.73)	3 (27.27)		
Debulking status				12.214	**0.007***
■ Complete	17	13 (76.47)	4 (23.53)		
■ Optimal	20	6 (30.00)	14 (70.00)		
■ Suboptimal	9	2 (22.22)	7 (77.78)		
■ No surgery	4	2 (50.00)	2 (50.00)		
■ N/A	2	2 (100.00)	0 (0.00)		
Performance status				3.832	0.119
■ ECOG score=0	30	15 (50.00)	15 (50.00)		
■ ECOG score=1	19	7 (36.84)	12 (63.16)		
■ ECOG score=2	3	3 (100.00)	0 (0.00)		
Chemotherapy regimen				0.441	0.603
■ Single carboplatin	3	2 (66.67)	1 (33.33)		
■ Carboplatin + Paclitaxel	49	23 (46.94)	26 (53.06)		

Abbreviation: ECOG = Eastern Cooperative Oncology Group.

Patients who were recurrent within 6 months after completion of 6 cycles of platinum-based chemotherapy were considered as platinum resistance. Among 27 cases with disease recurrences, there were 7 patients (25.92%) identified as platinum resistance. However, we did not find any significant association between resistance status with any factors. The data was showed in table 9.

Table 9 Association between factors and resistance status

Total number of participants = 27					
Factor	N	Resistance status		χ^2 value	P-value
		Sensitive (N=20, %)	Resistance (N=7, %)		
Age				3.431	0.145
■ < 60 years	19	16 (84.21)	3 (15.79)		
■ ≥ 60 years	8	4 (50.00)	4 (50.00)		
FIGO stage				0.523	1.000
■ Stage III	19	14 (73.68)	5 (26.32)		
■ Stage IV	5	4 (80.00)	1 (20.00)		
■ N/A	3	2 (66.67)	1 (33.33)		
Histological type				3.002	0.590
■ Serous adenocarcinoma	17	13 (76.47)	4 (23.53)		
■ Endometrioid adenocarcinoma	3	3 (100.00)	0 (0.00)		
■ Clear cell carcinoma	3	2 (66.67)	1 (33.33)		
■ Mucinous adenocarcinoma	0	0 (0.00)	0 (0.00)		
■ Mixed	2	1 (50.00)	1 (50.00)		
■ N/A	2	1 (50.00)	1 (50.00)		

Table 9 Association between factors and resistance status (continue)

Total number of participants = 27					
Factor	N	Resistance status		χ^2 value	P-value
		Sensitive (N=20, %)	Resistance (N=7, %)		
Tumor grading				5.354	0.096
▪ 1	0	0 (0.00)	0 (0.00)		
▪ 2	4	2 (50.00)	2 (50.00)		
▪ 3	16	14 (87.50)	2 (12.50)		
▪ Unidentified	4	3 (75.00)	1 (25.00)		
▪ N/A	3	1 (33.33)	2 (66.67)		
Debulking status				5.752	0.089
▪ Complete	4	4 (100.00)	0 (0.00)		
▪ Optimal	14	10 (71.43)	4 (28.57)		
▪ Suboptimal	7	6 (85.71)	1 (14.29)		
▪ No surgery	2	0 (0.00)	2 (100.00)		
Performance status				0.010	1.000
▪ ECOG score=0	15	11 (73.33)	4 (26.67)		
▪ ECOG score=1	12	9 (75.00)	3 (25.00)		
Chemotherapy regimen				0.363	1.000
▪ Single carboplatin	1	1 (100.00)	0 (0.00)		
▪ Carboplatin + Paclitaxel	26	19 (73.08)	7 (26.92)		

Abbreviation: ECOG = Eastern Cooperative Oncology Group.

For platinum resistant cases, polymorphism of *ERCC1* seem to have impact. Patients with variant alleles had higher risk of platinum resistance. Patients with A/A genotypes had greater rate of resistance when compared with patients who had at

least one wild type C allele, C/C and C/A, (75.00% vs. 16.67%, P=0.046). However, we did not found any associations of *XRCC1* and *GSTP1* polymorphisms and disease resistance rate as shown in Table 10.

Table 10 Association of genetic polymorphisms to resistance status

Total number of participants = 27					
Gene	N	Resistance		χ^2 value	P-value
		Non-Resistance (N=20, %)	Resistance (N=7, %)		
ERCC1				5.130	0.108
CC	14	11 (78.57)	3 (21.43)		
CA	9	8 (88.89)	1 (11.11)		
AA	4	1 (25.00)	3 (75.00)		
ERCC1				5.615	**0.046***
CC+CA	18	15 (83.33)	3 (16.67)		
AA	4	1 (25.00)	3 (75.00)		
XRCC1				0.663	0.838
GG	14	11 (78.57)	3 (21.43)		
GA	9	6 (66.67)	3 (33.33)		
AA	4	3 (75.00)	1 (25.00)		
XRCC1				0.013	1.000
GG+AA	18	13 (72.22)	5 (27.78)		
AA	4	3 (75.00)	1 (25.00)		
GSTP1				0.034	1.000
AA	20	15 (75.00)	5 (25.00)		
AG	7	5 (71.43)	2 (28.57)		

4.5 Tumor Response

In the recurrent cases (n=27), 5 patients were waiting for clinical recurrence evaluation. As result, only 22 patients were evaluated for recurrence clinical

response as presented in Table 11. Moreover, 3 patients were treated with other regimens without platinum such as single gemcitabine, paclitaxel and liposomal-doxorubicin. Most of patients were responding to chemotherapy as complete or partial response. However, around 36% of patients had stable disease and disease progression.

Table 11 Tumor responses

Total number of participants = 22		
Response	N	%
Complete response (CR)	3	13.64
Partial response (PR)	11	50.00
Stable disease (SD)	3	13.64
Progressive disease (PD)	5	22.72

There was no difference in the clinical response rate among age, time to recurrence, stage, histological type, grading of tumor, debulking surgery status and ECOG score, (N=22). These factors were not associated with clinical response as revealed in Table 12.

Table 12 Association between factors and clinical responses

Total number of participants = 22					
Factor	N	Clinical response		χ^2 value	P-value
		CR+PR (N=14, %)	SD+PD (N=8, %)		
Age				0.187	1.000
▪ $<$ 60 years	15	10 (66.67)	5 (33.33)		
▪ $\geq$ 60 years	7	4 (57.14)	3 (42.86)		
Time to recurrent				3.274	0.137
▪ $<$ 6 months	6	2 (33.33)	4 (33.33)		
▪ $\geq$ 6 month	16	12 (75.00)	4 (25.00)		

Table 12 Association between factors and clinical response (continue)

Total number of participants = 22					
Factor	N	Clinical response		χ^2 value	P-value
		CR+PR (N=14, %)	SD+PD (N=8, %)		
FIGO stage				0.705	0.812
▪ Stage III	15	10 (66.67)	5 (33.33)		
▪ Stage IV	4	2 (50.00)	2 (50.00)		
▪ N/A	3	2 (66.67)	1 (33.33)		
Histological type				6.443	0.108
▪ Serous adenocarcinoma	14	11 (78.57)	3 (21.43)		
▪ Endometrioid adenocarcinoma	1	1 (100.00)	0 (0.00)		
▪ Clear cell carcinoma	3	1 (33.33)	2 (66.67)		
▪ Mucinous adenocarcinoma	0	0 (0.00)	0 (0.00)		
▪ Mixed	2	1 (50.00)	1 (50.00)		
▪ N/A	2	0 (0.00)	2 (100.00)		
Tumor grading				4.483	0.217
▪ 1	0	0 (0.00)	0 (0.00)		
▪ 2	3	2 (66.67)	1 (33.33)		
▪ 3	13	10 (76.92)	3 (23.08)		
▪ Unidentified	4	2 (50.00)	2 (50.00)		
▪ N/A	2	0 (0.00)	2 (100.00)		
Debulking status				3.843	0.270
▪ Complete	3	2 (66.67)	1 (33.33)		
▪ Optimal	12	9 (75.00)	3 (25.00)		
▪ Suboptimal	5	3 (60.00)	2 (40.00)		
▪ No surgery	2	0 (0.00)	2 (100.00)		

Table 12 Association between factors and clinical response (continue)

Total number of participants = 22					
Factor	N	Clinical response		χ^2 value	P-value
		CR+PR (N=14, %)	SD+PD (N=8, %)		
Performance status				0.321	0.675
▪ ECOG score=0	12	7 (58.33)	5 (41.67)		
▪ ECOG score=1	10	7 (70.00)	3 (30.00)		

Abbreviation: ECOG = Eastern Cooperative Oncology Group.

Most of recurrent patients were still received platinum-based chemotherapy, either combination or as a single agent. There were 3 patients received single paclitaxel, gemcitabine or liposomal-doxorubicin. Chemotherapy regimen were tended to associated with clinical responses (P=0.054). However, the association among single carboplatin or combination chemotherapy and non-platinum-based regimen were not found in clinical response. Furthermore, delay treatment or dose reduction of chemotherapy seemed not affected with clinical outcomes. Interestingly, 13 of 19 patients treated with carboplatin still had treatment benefit in term of complete or partial response. The data was presented in Table 13.

Table 13 Association between chemotherapy treatment and clinical response

Total number of participants = 22					
Factor	N	Clinical response		χ^2 value	P-value
		CR+PR (N=14,%)	SD+PD (N=8,%)		
Chemotherapy Regimen				6.621	0.054
■ Carboplatin	1	0 (0.00)	1 (100.00)		
■ Carboplatin + Paclitaxel	15	12 (80.00)	3 (20.00)		
■ Carboplatin + Lipo-doxorubicin	2	1 (50.00)	1 (50.00)		
■ Carboplatin + Gemcitabine	1	0 (0.00)	1 (100.00)		
■ Other	3	1 (33.33)	2 (66.67)		
Delay of treatment				0.187	1.000
■ Yes	15	10 (66.67)	5 (33.33)		
■ No	7	4 (57.14)	3 (42.86)		
Reduce dose				0.749	0.613
■ Yes	5	4 (80.00)	1 (20.00)		
■ No	17	10 (58.82)	7 (41.78)		

The impact of *ERCC1*, *XRCC1* and *GSTP1* polymorphisms and clinical responses were evaluated. The effect of *ERCC1* was existing nearly significance which homozygous variant type (A/A) had lower rate of response when compared with the genotypes composing of C wild type allele, C/C and C/A, (25.00% vs 72.22%, P=0.117). Moreover, patients with A/A genotype of *XRCC1* showed lower clinical response rate when compared with G/G and G/A (50.00% vs 66.67%, P=0.602). For *GSTP1* polymorphisms, the effect of variation was slightly different within genotypes, as describe in Table 14.

Table 14 Association of genetic polymorphisms and clinical responses

Total number of participants = 22					
Gene	N	Clinical response		χ^2 value	P-value
		CR+PR (N=14, %)	SD+PD (N=8, %)		
ERCC1				3.265	0.172
CC	12	8 (66.67)	4 (33.33)		
CA	6	5 (83.33)	1 (16.67)		
AA	4	1 (25.00)	3 (75.00)		
ERCC1				3.154	0.117
CC+CA	18	13 (72.22)	5 (27.78)		
AA	4	1 (25.00)	3 (75.00)		
XRCC1				0.606	1.000
GG	9	6 (66.67)	3 (33.33)		
GA	9	6 (66.67)	3 (33.33)		
AA	4	2 (50.00)	2 (50.00)		
XRCC1				0.393	0.602
GG+GA	18	12 (66.67)	6 (33.33)		
AA	4	2 (50.00)	2 (50.00)		
GSTP1				0.269	1.000
AA	15	9 (60.00)	6 (40.00)		
AG	7	5 (71.42)	2 (28.57)		

In term of clinical benefit, for *ERCC1* polymorphism, patients with at least one wild type C allele (C/C, C/A) had higher rate of clinical benefit (83.33% vs 50.00%, P=0.210). Likewise, *XRCC1*, homozygous variant genotype (A/A) had lower rate of clinical benefit when compared with other genotypes (P=0.210). Although, the effect of variant genotype of *ERCC1* and *XRCC1* showed poorer clinical benefit, these associations were not different at statistic level. For *GSTP1* polymorphism, clinical

benefit rates were higher in patients with variant allele G compare to whom with homozygous wild type, (73.33% vs 85.71%, P=1.000). The results were described in Table 15.

Table 15 Association of genetic polymorphisms and clinical benefit

Total number of participants = 22					
Gene	N	Clinical benefit		χ^2 value	P-value
		CR+PR+SD (N=17, %)	PD (N=5,%)		
ERCC1				3.218	0.246
CC	12	9 (75.00)	3 (25.00)		
CA	6	6 (100.00)	0 (0.00)		
AA	4	2 (50.00)	2 (50.00)		
ERCC1				2.071	0.210
CC+CA	18	15 (83.33)	3 (16.67)		
AA	4	2 (50.00)	2 (50.00)		
XRCC1				2.326	0.344
GG	9	8 (88.89)	1 (11.11)		
GA	9	7 (77.78)	2 (22.22)		
AA	4	2 (50.00)	2 (50.00)		
XRCC1				2.071	0.210
GG+GA	18	15 (83.33)	3 (16.67)		
AA	4	2 (50.00)	2 (50.00)		
GSTP1				0.417	1.000
AA	15	11 (73.33)	4 (26.67)		
AG	7	6 (85.71)	1 (14.29)		

4.6 Genetic polymorphisms and adverse events

The most common hematologic adverse event occurred in this study was anemia (51 cases, 98.08%), followed by neutropenia (37 cases, 71.15%) and thrombocytopenia (20 cases, 38.46%). The most severe adverse events from every cycle after received chemotherapy was used for evaluation the association of the events and polymorphisms. Incidences of adverse events were presented in Table 16.

Table 16 Overall toxicities

Adverse events (N=52)	Grade 0 (N, %)	Grade 1 (N, %)	Grade 2 (N, %)	Grade 3 (N, %)	Grade 4 (N, %)
Anemia	1 (1.92)	23 (44.23)	28 (53.85)	0 (0.00)	0 (0.00)
Neutropenia	15 (28.85)	12 (23.08)	14 (26.92)	9 (17.30)	2 (3.85)
Thrombocytopenia	32 (61.54)	14 (26.93)	4 (7.69)	1 (1.92)	1 (1.92)
Peripheral neuropathy	29 (55.77)	16 (30.77)	7 (13.46)	0 (0.00)	0 (0.00)

4.6.1 Anemia

For anemia, the highest severity which found in this study was at grade 2. Patients, who had age at least 60 year-old, had more risk of grade 2 anemia than other (OR=5.769; 95%CI =1.564-21.283; P=0.009). The other factors such as stage of disease, histological type, tumor grading, debulking status, performance status or chemotherapy regimen were not associated with anemia. Genetic *ERCC1* and *XRCC1* polymorphisms were not associated with anemia. While patients with variant genotype (A/G) of *GSTP1* had higher risk of grade 2 anemia than wild type (A/A) which presented in 81.82% compared with 46.34% (OR=5.2; 95%CI: 1.000-27.146; P=0.036), as presented in Table 17.

Table 17 Association of genetic polymorphisms and anemia

Total number of participants = 52					
Gene	N	Anemia (grade)		χ^2 value	P-value
		Grade 0,1 (N=24, %)	Grade 2 (N=28, %)		
ERCC1				0.988	0.610
CC	25	11 (44.00)	14 (56.00)		
CA	17	7 (41.18)	10 (58.82)		
AA	10	6 (60.00)	4 (40.00)		
XRCC1				2.400	0.356
GG	28	12 (42.86)	16 (57.14)		
GA	19	8 (42.10)	11 (57.90)		
AA	5	4 (80.00)	1 (20.00)		
GSTP1				4.393	**0.036***
AA	41	22 (53.66)	19 (46.34)		
AG	11	2 (18.18)	9 (81.82)		

4.6.2 Neutropenia

There were 15 patients who did not have neutropenia (28.28%) while 11 patients had grade 3 or 4 neutropenia (21.15%). The patients with heterozygous variant genotype (A/C) of ERCC1 had higher risk of grade 3 or 4 neutropenia compared with C/C (29.41% vs 24.00%). None of patients with homozygous variant genotype (A/A) had severe neutropenia. The association of *ERCC1* polymorphism and neutropenia was marginally significant (P=0.058). The data was shown in Table 18. When neutropenia was categorized into 2 groups as mild or severe, *XRCC1* and *GSTP1* polymorphisms were not associated with this event, as shown in Table 19.

Table 18 Association of genetic polymorphisms and grading distribution of neutropenia

Total number of participants = 52						
Gene	N	Neutropenia (grade)			χ^2 value	P-value
		Grade 0 (N= 15, %)	Grade 1+2 (N=26, %)	Grade 3+4 (N= 11, %)		
ERCC1					8.705	0.058
CC	25	10 (40.00)	9 (36.00)	6 (24.00)		
CA	17	4 (23.53)	8 (47.06)	5 (29.41)		
AA	10	1 (10.00)	9 (90.00)	0 (0.00)		
XRCC1					3.652	0.445
GG	28	6 (21.43)	14 (50.00)	8 (28.57)		
GA	19	8 (42.10)	9 (47.37)	2 (10.53)		
AA	5	1 (20.00)	3 (60.00)	1 (20.00)		
GSTP1					1.267	0.555
AA	41	11 (26.83)	22 (53.66)	8 (19.51)		
AG	11	4 (36.36)	4 (36.36)	3 (27.28)		

Table 19 Association of genetic polymorphisms and severity of neutropenia

Total number of participants = 52					
Gene	N	Neutropenia (severity)		χ^2 value	P-value
		Mild (N=41, %)	Severe (N=11, %)		
ERCC1				3.522	0.159
CC	25	19 (76.00)	6 (24.00)		
CA	17	12 (70.59)	5 (29.41)		
AA	10	10 (100.00)	0 (0.00)		

Table 19 Association of genetic polymorphisms and severity of neutropenia (continue)

Total number of participants = 52					
Gene	N	Neutropenia (severity)		χ^2 value	P-value
		Mild (N=41, %)	Severe (N=11, %)		
XRCC1				2.219	0.278
GG	28	20 (71.43)	8 (28.57)		
GA	19	17 (89.47)	2 (10.53)		
AA	5	4 (80.00)	1 (20.00)		
GSTP1				0.313	0.681
AA	41	33 (80.49)	8 (19.51)		
AG	11	8 (72.73)	3 (27.27)		

4.6.3 Thrombocytopenia

Grade 3 or 4 thrombocytopenia was occurred at 3.85%. For *ERCC1*, patients with A/C genotype had the highest rate of grade 3 or 4 thrombocytopenia but none of patients with homozygous variant genotype (A/A) had grade 3 or 4 thrombocytopenia. However, the difference was not significant, as described in Table 20 and 21. Patients with heterozygous genotype (G/A) of *XRCC1* had more risk of severe thrombocytopenia compared with other genotypes. While *GSTP1* polymorphism showed trend of more severe thrombocytopenia risk in variant type compared with wild type (9.09% vs 2.44%).

Table 20 Association of genetic polymorphisms and grading distribution of thrombocytopenia

Total number of participants = 52						
Gene	N	Thrombocytopenia (grade)			χ^2 value	P-value
		Grade 0 (N=31, %)	Grade 1+2 (N=18, %)	Grade 3+4 (N=2, %)		
ERCC1					3.808	0.399
CC	25	17 (68.00)	7 (28.00)	1 (4.00)		
CA	17	11 (64.71)	5 (29.41)	1 (5.88)		
AA	10	4 (40.00)	6 (60.00)	0 (0.00)		
XRCC1					4.364	0.361
GG	28	14 (50.00)	13 (46.43)	1 (3.57)		
GA	19	14 (73.69)	4 (21.05)	1 (5.26)		
AA	5	4 (80.00)	1 (20.00)	0 (0.00)		
GSTP1					4.991	0.072
AA	41	23 (56.10)	17 (41.46)	1 (2.44)		
AG	11	9 (81.82)	1 (9.09)	1 (9.09)		

Table 21 Association of genetic polymorphisms and severity of thrombocytopenia

Total number of participants = 52					
Gene	N	Thrombocytopenia (severity)		χ^2 value	P-value
		Mild (N= 50, %)	Severe (N=2, %)		
ERCC1				0.791	1.000
CC	25	24 (96.00)	1 (4.00)		
CA	17	16 (94.12)	1 (5.88)		
AA	10	10 (100.00)	0 (0.00)		

Table 21 Association of genetic polymorphisms and severity of thrombocytopenia (continue)

Total number of participants = 52					
Gene	N	Thrombocytopenia (severity)		χ^2 value	P-value
		Mild (N= 50, %)	Severe (N=2, %)		
XRCC1				0.811	1.000
GG	28	27 (96.43)	1 (3.57)		
GA	19	18 (94.74)	1 (5.26)		
AA	5	5 (100.00)	0 (0.00)		
GSTP1				1.038	0.382
AA	41	40 (97.56)	1 (2.44)		
AG	11	10 (90.91)	1 (9.09)		

4.6.4 Peripheral neuropathy

Grade 2 of peripheral neuropathy was the highest severity which found in this study. Of a total of 52 patients, 29 patients did not have peripheral neuropathy (55.77%), while 7 patients had grade 2 (13.46%). Patients with *ERCC1* polymorphism (C/A, A/A) had lower risk of grade 2 of peripheral neuropathy (N=3/27; 11.11%) when compared with wild type (C/C). Conflicting pattern was found in polymorphism of XRCC1 polymorphism. Patients with homozygous variant of XRCC1 had highest risk of grade 2 peripheral neuropathy. However, there was no significant association of *ERCC1* and *XRCC1* polymorphisms and peripheral neuropathy. For *GSTP1*, none of patients with A/G had grade 2 of peripheral neuropathy, while A/A genotypes presented 17.07%. Homozygous wild type carriers had greater risk of grade 2 peripheral neuropathy than heterozygous variant type but the relationship was insignificant. The data was shown in Table 22.

Table 22 Association of genetic polymorphisms and peripheral neuropathy

Total number of participants = 52					
Gene	N	Peripheral neuropathy (grade)		χ^2 value	P-value
		Grade 0,1 (N=45, %)	Grade 2 (N= 7, %)		
ERCC1				0.323	1.000
CC	25	21 (84.00)	4 (16.00)		
CA	17	15 (88.24)	2 (11.76)		
AA	10	9 (90.00)	1 (10.00)		
XRCC1				0.757	0.852
GG	28	24 (85.71)	4 (14.29)		
GA	19	17 (89.47)	2 (10.53)		
AA	5	4 (80.00)	1 (20.00)		
GSTP1				2.710	0.322
AA	41	34 (82.93)	7 (17.07)		
AG	11	11 (100.00)	0 (0.00)		

Chapter 5
Discussion

The primary objective of this cohort study was to investigate the impact of *ERCC1*, *XRCC1* and *GSTP1* polymorphisms on clinical responses and adverse events from platinum-based chemotherapy in Thai EOC patients. There were few studies reporting the prevalence of *XRCC1 A399G* and *GSTP1 Ile105Val* polymorphisms in Thais [43, 44]. No study has reported the prevalence of *ERCC1 C8092A* polymorphisms in Thais.

5.1 Prevalence of *ERCC1*, *XRCC1* and *GSTP1* polymorphisms

The prevalence of *ERCC1* of C→A (rs3212986) was found around 24-29% in Caucasians and Europeans [10, 11]. In Asians, the study in Korean population reported variant allele frequency as 25.9% [9]. For our study in EOC patients, the frequency of variant allele was found higher as 35.6%. However, *ERCC1* genotype distributions were significant departed from the HWE (P=0.037) with small sample size might be the reason of this disequilibrium.

For *XRCC1 A399G* of A→G (rs25487), the report of variant allele frequency in Thai cervical cancer patients was 22% [43]. The data was quite similar to this study which found variant allele frequency as 28.9%. In other Asian population, variant allele frequency in EOC patients was found as 24.6% in Korean and 38.7% in Chinese [9, 41].

For genetic polymorphism of *GSTP1 Ile105Val,* in our study the variant allele frequency was found at 10.6% which only heterozygous variant genotype (A/G) was found. Another report in Thai population in non-small cell lung cancer patients, variant allele frequency were reported as 26.1% which consisted in 5% of

homozygous variant genotype (G/G) in equating between gender [44]. Variant allele frequency in Chinese EOC patients was shown rather higher than ours as 29.3% [45]. On the other hand, in Korean EOC patients showed alike frequency of variant allele as this study at 11.4% [9].

5.2 Impact of ERCC1, XRCC1 and GSTP1 polymorphisms in clinical responses

The previous publications reported that *ERCC1*, *XRCC1* and *GSTP1* polymorphisms contributed to various responses to platinum-based chemotherapy in EOC patients. We hypothesized that genetic polymorphisms were associated with treatment responses. The results of patients with recurrent disease (n=22) were indicated that patients with homozygous variant genotype either *ERCC1* or *XRCC1* had inferior clinical responses than others (25.00% vs 72.22 %; P=0.117, 50.00% vs 66.67%; P=0.602, respectively). However, the significant differences in treatment responses among genotypes of these interesting genes could not be found in this study due to limited number of patients.

5.2.1 Impact of *ERCC1* polymorphism in clinical responses

Several prior studies were determined the effect of *ERCC1* polymorphism on platinum-based treatment benefits as progression-free survival and overall survival. One study in Caucasians population revealed that homozygous wild type C/C genotype had shorter median PFS and OS than variant genotypes (median PFS 9.5 months vs 11.9 months; P=0.05, median OS 31.7 months vs 36.5 months; P=0.02, respectively) [40]. On the contrary, another study in Caucasians presented that C/A or A/A genotypes had shorter median OS than C/C genotype [10]. It was found in Korean population that C/C genotype had longer PFS and OS than C/A or A/A genotype, significantly (median PFS 37 months vs 12 months; P<0.01, mean OS 149

months vs 63 months; P=0.042, respectively) [9]. Therefore, it cannot be concluded that carrying variant allele gaining any risk or benefit in term of treatment outcomes.

The PFS or OS were not use as outcome indicators in our study. Disease responses such as CR, PR, SD, or PD were used instead due to limited time for patient follow-up. The RECIST criteria [46] were used to evaluate tumor response which assessed clinical response as 2 groups of CR and PR compared with nonresponders who identified as SD and PD. In the Danish population, a total of 157 EOC patients, the homozygous C/C common genotype showed higher percentage in SD+PD group when compared among 3 genotypes. However, there was no correlation between *ERCC1* polymorphisms and clinical response (P=0.53) [11]. In contrast with this study, the homozygous A/A minor genotype was reported as higher rate of SD and PD when compared with others (P=0.172).

Platinum resistance was defined as a patient who had to change chemotherapy regimen during treatment or recurrent within 6 months after completing a platinum-based regimen [17]. The previous study reported that A/A genotype showed higher percentage of resistance than C/C or C/A (60% vs 33% and 40%, respectively; P=0.36) [11]. The result was correlated with this study, A/A genotype showed statistically significant higher rate of platinum resistance than C/C and C/A (75.00% vs 16.767%, P=0.046).

5.2.2 Impact of *XRCC1* polymorphism in clinical responses

In cervical cancer patients received platinum-based chemotherapy, *XRCC1 A399G* polymorphism was associated with treatment response. Patients with wild type G/G had better responses (CR+PR) compared with G/A or A/A, significantly (65.5% vs 31.0% and 3.4%, respectively; P=0.0015) [47]. The data was similar with the other study which revealed that the treatment responses were different among

genotypes of *XRCC1*. Patients who carried G/G genotype had higher rate of good response (CR+PR) than G/A or A/A (90.0% vs 0% (0/2) and 83.33%; P=0.002) [8].

From previous studies in cervical cancer, *XRCC1* were presented as the important factor to predicted platinum-based chemotherapy response. Many studies in Chinese EOC patients were investigated the association of *XRCC1* polymorphism and survival rate. There were 2 studies in 2012 had the conflicting results, one revealed that G/G genotype increased risk of death compared with A/A (HR=1.69, 95%CI=1.07-2.78) [41]. While the another showed the opposite that patients with A/A genotype had increased risk of death than G/G (HR=1.98, 95%CI=1.09-3.93) [48]. Li Kai and Li Wusheng [13] as well reported that A/A genotype had lower risk of death than G/G (HR=0.44, 95%CI=0.28-0.91). In contrast, with others which presented no significant association between *XRCC1* polymorphism and survival rate [12, 45].

There was a limit study in treatment response, the study in Korean EOC patients did not find the association between *XRCC1* polymorphism and platinum-based chemotherapy response [9]. Similarly, the study in Russia showed no significant relationship with *XRCC1* polymorphism and tumor response [4]. As same as ours which found no association of *XRCC1* polymorphism and clinical responses. However, A/A genotype had higher percentage of poorly response than G/G and G/A as 50.00% vs 33.33% (P=0.602). For clinical benefit investigation, A/A genotype also had greater rate of PD than G/G and G/A (50.00% vs 16.67%). Even though, there was no significant at the statistical level (P=0.210).

5.2.3 Impact of *GSTP1* polymorphism in clinical responses

GSTP1 plays vital role in conjugation with platinum drugs and increases solubility that resulting in more excreted via efflux pumps out of the cell [49]. This activity might be an essential part of free platinum drugs accessible for interaction

with DNA [50]. Beeghly A. et al. [7] studied the association of *GSTP1* polymorphism of platinum-based treatment response and survival in EOC patients in Italy. Neither each of 3 genotypes nor combination variant genotypes (A/G or G/G) had been found in the association of treatment response and overall survival. In contrast, the study in Russian found the association of *GSTP1 Ile105Val* polymorphism of progression-free survival with strongly significant (P=0.002). The result showed that patients with A/A genotype had longer median PFS than patients with one or two G allele (15 months vs 8.0 and 8.5 months) [4]. In Asian population studies, Yoshihama T et al. [51] found similar association in Japanese EOC patients. *GSTP1* A/A carriers had longer PFS and OS compared with A/G or G/G genotypes (P<0.001 and P=0.0012). Conversely, the study in Chinese EOC patients was similar to our study. They also found only 2 genotypes of *GSTP1* as A/A and A/G which no significant correlation to risk of death (P=0.65). Variation of this gene was associated with better rate of clinical responses (71.42% vs 60.00%, P=1.000).

5.3 Impact of *ERCC1*, *XRCC1* and *GSTP1* polymorphisms on adverse events

The most common adverse events related to platinum-based chemotherapy were hematological toxicities and peripheral neuropathy. The severities were defined following by Common Terminology Criteria for Adverse Events version 4.03 (CTCAE) as 1 to 5. Recent publications discovered that *ERCC1*, *XRCC1* and *GSTP1* polymorphisms were associated with toxicities of platinum-based chemotherapy. This was the first study investigated the relationship of these genetic polymorphisms and adverse events from platinum-based in Thai EOC patients.

5.3.1 Impact of *ERCC1* polymorphism on adverse events

We found the highest severity of anemia as grade 2 and did not correlate with *ERCC1* polymorphism. Similar to previous studies, *ERCC1* was not a significant

factor to predicted grade 3 or 4 hematotoxicities (P>0.05) [4, 9]. In addition, peripheral neuropathy was associated with *ERCC1 C8092A* in Korean population which C/C genotype that showed higher rate of severe neuropathy than C/A or A/A genotypes (8.5% vs 25%, P=0.019) [9]. Our study, the highest grade of peripheral neuropathy was only grade 2. The result was correlated to the prior study that C/C genotype had more severe neuropathy than others (C/A and A/A) as 16.00% vs 11.76% and 10.00%, respectively.

5.3.2 Impact of *XRCC1* polymorphism on adverse events

The data of relationship between *XRCC1* polymorphism and toxicity were limited. There was a significant correlation between *XRCC1 A399G* and severe neutropenia in Russians' studies. The relation was observed that homozygous common genotype (G/G) had greater 3.02-fold of grade 3 or 4 neutropenia than the other with at least one A allele (G/A or A/A) which presented 71.4% compared with 45.3% (P=0.026) [4]. Even though, in this study was not found the statistically significant correlation of adverse events. The result was similar to prior study which G/G genotype carriers had more percentage of severe neutropenia than G/A or A/A (28.57% vs 10.53 and 20.00%, respectively). For other hematological toxicities, neither of them associated with *XRCC1* polymorphism at the statistically significant level.

5.3.3 Impact of *GSTP1* polymorphism in adverse events

Several prior studies reported the association of *GSTP1 Ile105Val* polymorphism and hematotoxicity. Kim HS et al. [9] found that patients with homozygous A/A genotype presented higher rate of severe hematological toxicity as grade 3 or 4 of anemia, leukopenia, neutropenia and thrombocytopenia than patients with A/G or G/G (78.7% vs 54.2%, respectively, P=0.015). In addition,

Yoshihama T et al. [51] were presented according result that A/A wild type had higher risk of severe hematotoxicity which grade 4 of neutropenia, grade $\geq$ 3 thrombocytopenia and anemia, than other with at least one G variant allele (OR=5.71, P<0.001). In contrast with this study, variant genotype (A/G) had significantly higher risk of grade 2 anemia than A/A wild type (81.82% vs 46.34%, P=0.036). While the other association of hematotoxicity was not found at statistically significant level, but variant genotype also showed higher rate of severe toxicity. None of previous study found the relationship of *GSTP1* polymorphism and peripheral neuropathy [4, 9]. Our study also found no statistically differences between 2 genotypes of *GSTP1* in grade 2 of peripheral neuropathy. However, none of the patients with A/G genotype had grade 2 of neuropathy (0 of 11) compared with 17.07% of homozygous A/A genotype (7 of 41). Despite, the result of genetic polymorphism of this study disagreed with prior studies but these results were following through the theory that variation of this gene will decrease detoxification ability of platinum drugs which may lead to higher level of cytotoxic substance in cell and culminate in higher risk of toxicity [52].

5.4 Conclusion

Findings from this study explained possible source of variation in the treatment outcomes of platinum in Thai EOC patients. Prevalence of *ERCC1*, *XRCC1* and *GSTP1* were 35.6%, 28.9% and 10.6%, respectively which were relatively high compared with other studies. Some interesting potential associations between *ERCC1*, *XRCC1* and *GSTP1* polymorphisms and treatment outcomes in Thai EOC patients were noticed. *ERCC1* polymorphism were associated with platinum resistance in recurrent patients (n=27). In patients with homozygous variant genotype had more risk of platinum-resistance (P=0.046). Moreover, the effect genetic polymorphism of *ERCC1* possibly associated with clinical responses. Patients with at

least one C major allele tend to have a better clinical response. In term of adverse events, *GSTP1* polymorphism was associated with grade 2 of anemia. The limitations of our study were the small sample size and short follow-up period, leading to low statistical power and reduced possibility to detect the true effect. Moreover, the response evaluation was not following by RECIST in clinical practice. Normally, the gynecologists were followed patients by clinical symptoms or tumor marker. In addition, time to follow up in this study was not equal in each patient. Future studies with larger sample size or survival analysis may present more significant impact of these genetic polymorphisms on treatment outcomes and toxicities.

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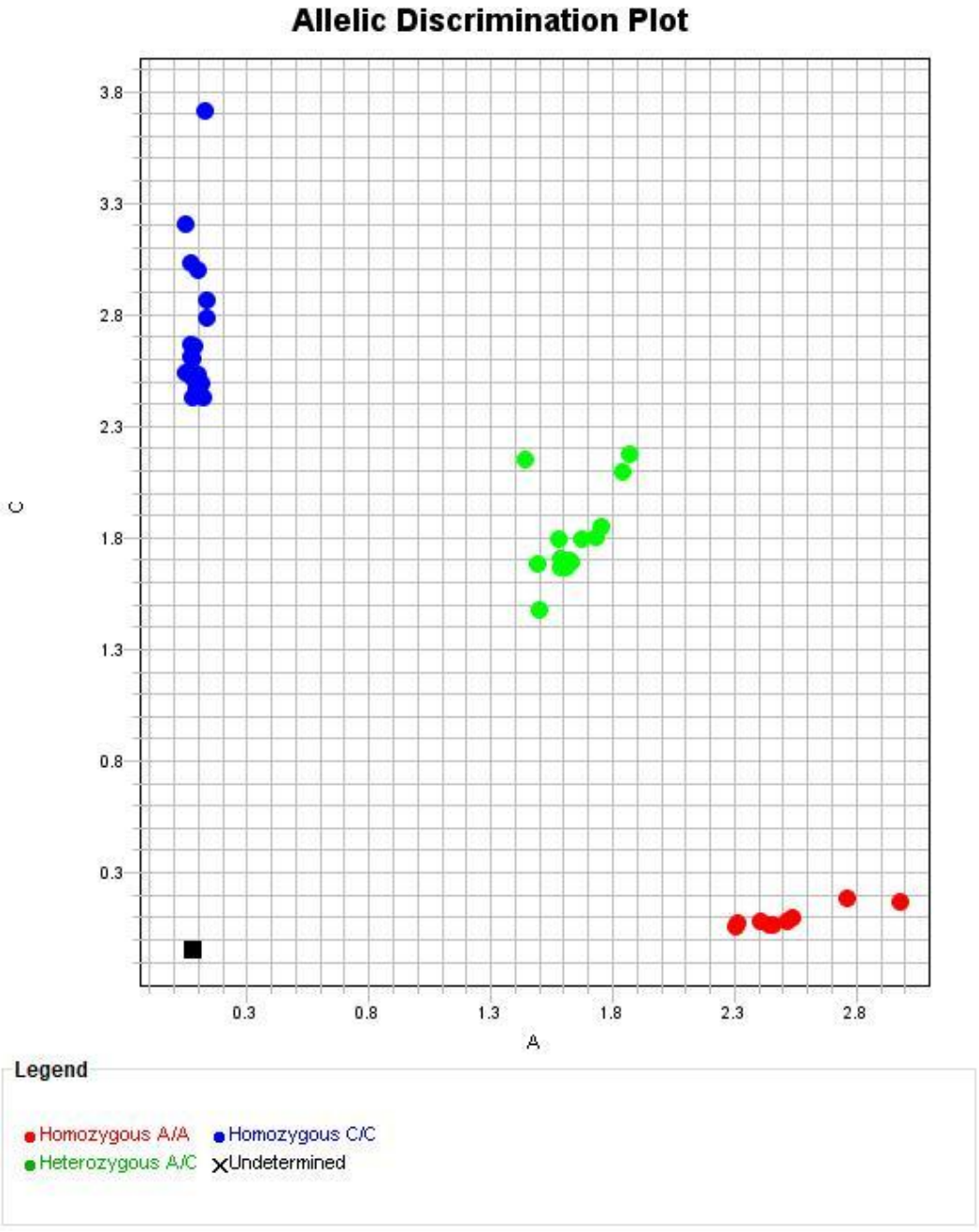
APPENDIX

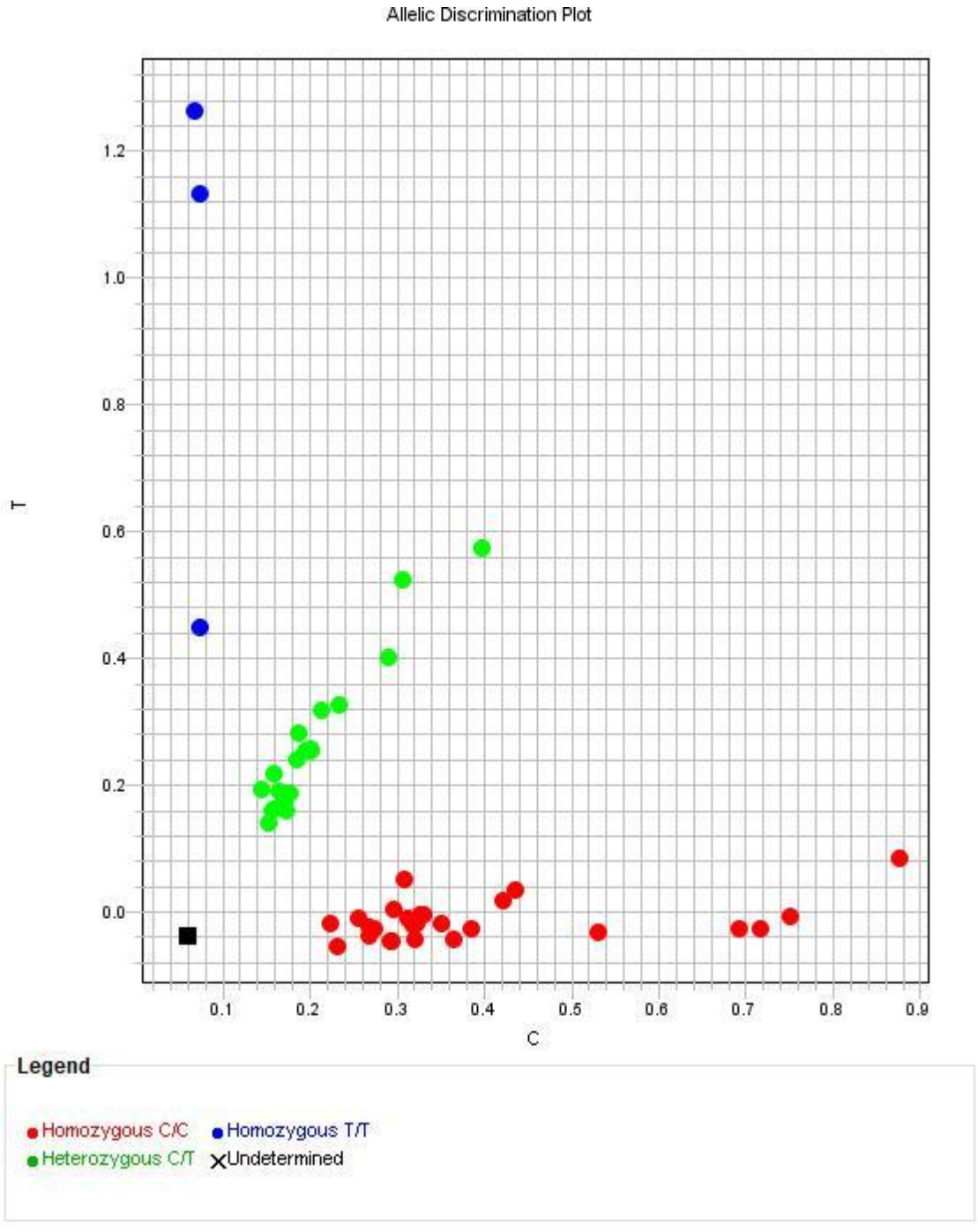
APPENDIX A

Allelic discrimination plot

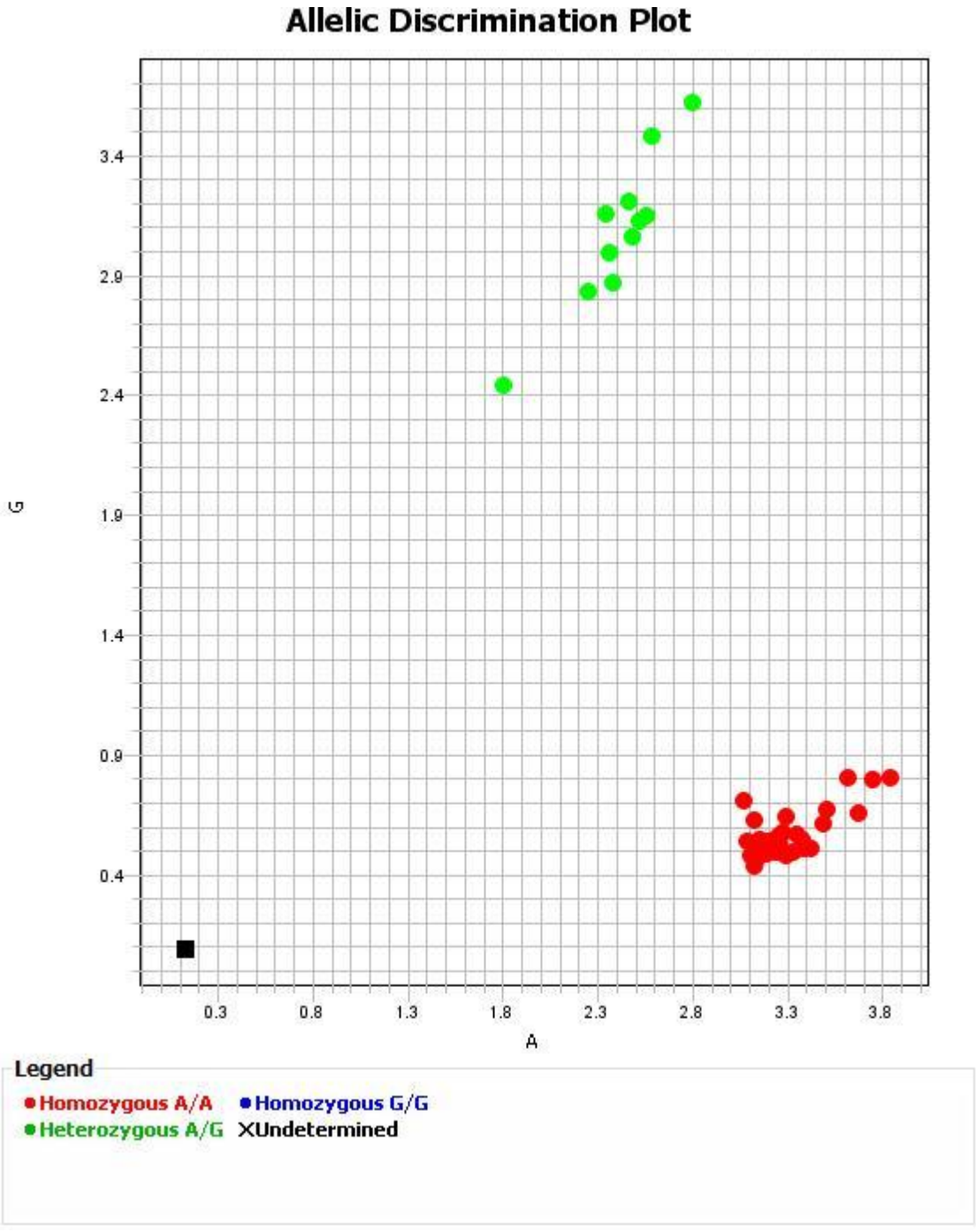
ERCC1 rs3212986 Allelic discrimination plot



XRCC1 rs25487 Allelic discrimination plot



GSTP1 rs1695 Allelic discrimination plot



APPENDIX B

Data collecting form

Data collecting form

Number of patient..... Age at diagnosis.....

Underlying disease.....

Current medication.....

FIGO stage ☐ Stage III ☐ Stage IV

☐ Recurrent (1) Yes (2) No

Histological type

☐ Serous adenocarcinoma ☐ Endometrioid adenocarcinoma

☐ Clear cell carcinoma ☐ Mucinous adenocarcinoma ☐ Other

Tumor grading ☐ 1 ☐ 2 ☐ 3 ☐ Unidentified

Previous cancer treatment ☐ Radiation (1) Yes (2) No

☐ Chemotherapy (1) Platinum based

(2) Non-platinum based

(3) No previous chemotherapy

Debulking status ☐ Optimal ☐ Suboptimal ☐ No surgery

Chemotherapy regimen.....

Chemotherapy response ☐ Complete response (CR) ☐ Partial Response (PR)

☐ Stable disease (SD) ☐ Progression disease (PD)

Platinum sensitivity ☐ Platinum-sensitive ☐ Platinum-resistance

Genotyping

- *ERCC1 8092* polymorphisms

  ☐ C/C ☐ C/A ☐ A/A

- *XRCC1 399* polymorphisms

  ☐ A/A ☐ A/G ☐ G/G

- *GSTP1 105* polymorphisms

  ☐ A/A ☐ A/G ☐ G/G

Adverse events collecting data sheet

Cycle Date	Normal range	1	2	3	4	5	6
Platinum (Dose)							
Weight							
Height							
BMI							
BSA (m^2)							
ECOG PS							
Tumor marker							
CA-125	0-35 units/ml						
Hematology							
Hgb	12-15 g/dl						
Hct	36-45 g/dl						
WBC	4.5-11.0 $x10^3$ g/dl						
Neutrophil	40.0-70.9 $x10^3$ g/dl						
ANC	>1,500						
Platelet	150-450 x 10^3 /ul						
Liver Function							
SGOT/AST	5-40 mg/dk						
SGPT/ALT	7-56 mg/dl						
ALP	40-120mg/dl						
Kidney Function							
Creatinine	0.5-1.0mg/dl						
BUN	7-20 mg/dl						

Adverse events grade

Cycle Date	1	2	3	4	5	6
Anemia						
Neutropenia						
Thrombocytopenia						
Kidney injury						
SGOT/AST ↑						
SGPT/ALT ↑						
ALP ↑						
Nausea						
Vomiting						
Diarrhea						
Constipation						
Oral mucositis						
PN						
Fatigue						
Insomnia						
Weight loss						
Other						

APPENDIX C

Classified grading of adverse events

Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03

Adverse Events	Grade	Meaning
Blood and lymphatic system		
Anemia	1	Hemoglobin (Hgb) < LLN - 10.0 g/dl
	2	Hgb < 10.0 - 8.0 g/dl
	3	Hgb < 8.0 – 6.5 mg/dl; transfusion indicated
	4	Hgb < 6.5 mg/dl; life-threatening consequences (urgent intervention indicated)
Neutropenia	1	Neutrophils < LLN – 1500/mm^3
	2	Neutrophils < 1500 - 1000/mm^3
	3	Neutrophils < 1000 – 500/mm^3
	4	Neutrophils < 500/mm^3
Thrombocytopenia	1	Platelet < LLN – 75,000/mm^3
	2	Platelet < 75,000 – 50,000/mm^3
	3	Platelet < 50,000 – 25,000/mm^3
	4	Platelet < 25,000/mm^3
Renal system		
Acute kidney injury	1	Creatinine level increase of > 0.3 mg/dl; Creatinine 1.5 - 2.0 x above baseline
	2	Creatinine 2-3 x above baseline
	3	Creatinine > 3 x above baseline or Creatinine > 4.o mg/dl; hospitalization indicated
	4	Life-threatening consequences; dialysis indicated

Adverse Events	Grade	Meaning
Liver		
Increase aspartate aminotransferase (AST)	1	> ULN - 3.0 x ULN
	2	> 3.0 - 5.0 x ULN
	3	> 5.0 – 20.0 x ULN
	4	> 20.0 x ULN
Increase alanine aminotransferase (ALT)	1	> ULN - 3.0 x ULN
	2	> 3.0 - 5.0 x ULN
	3	> 5.0 – 20.0 x ULN
	4	> 20.0 x ULN
Increase alkaline phosphatase (ALP)	1	> ULN – 2.5 x ULN
	2	> 2.5 – 5.0 x ULN
	3	> 5.0 – 20.0 x ULN
	4	> 20.0 x ULN
Gastrointestinal system		
Nausea	1	Loss of appetite without alteration in eating habits
	2	Oral intake decrease without weight loss, dehydration or malnutrition
	3	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated
	4	-
Vomiting	1	1-2 episodes (separated by 5 minutes) in 24 hrs
	2	3-5 episodes (separated by 5 minutes) in 24 hrs

Adverse Events	Grade	Meaning
Vomiting (continue)	3	≥6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN, or hospitalization indicated
	4	life-threatening consequences (urgent intervention indicated)
Constipation	1	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema
	2	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental ADL
	3	Obstipation with manual evacuation indicated; limiting self-care ADL
	4	life-threatening consequences (urgent intervention indicated)
Diarrhea	1	Increase of < 4 stools/ day over baseline; mild increase in ostomy output compare to baseline
	2	Increase of 4-6 stools/ day over baseline; moderate increase in ostomy output compare to baseline
	3	Increase of ≥ 7 stools/ day over baseline; hospitalization indicated; severe increase in ostomy output compare to baseline (limiting self-care ADL)
	4	life-threatening consequences (urgent intervention indicated)

Adverse Events	Grade	Meaning
Mucositis Oral	1	Asymptomatic or mild symptoms; intervention not indicated
	2	Moderate pain; not interfering with oral intake; modified diet indicated
	3	Severe pain; interfering with oral intake
	4	life-threatening consequences (urgent intervention indicated)
Nervous system		
Peripheral motor neuropathy	1	Asymptomatic; clinical or diagnosis observation only (intervention not indicated)
	2	Moderate symptoms; limiting instrumental ADL
	3	Severe symptoms; limiting self-are ADL (assistive device indicated)
	4	life-threatening consequences (urgent intervention indicated)
Peripheral sensory neuropathy	1	Asymptomatic; clinical or diagnosis observation only (intervention not indicated)
	2	Moderate symptoms; limiting instrumental ADL
	3	Severe symptoms; limiting self-are ADL (assistive device indicated)
	4	life-threatening consequences (urgent intervention indicated)
Miscellaneous		
Weight loss	1	5 to < 10% from baseline; intervention not indicated

Adverse Events	Grade	Meaning
Weight loss (continue)	2	10 to < 20% from baseline; nutritional support indicated
	3	≥ 20% from baseline; tube feeding or TPN indicated
	4	-
Fatigue	1	Fatigue relieved by rest; limiting instrumental ADL
	2	Fatigue not relieved by rest; limiting instrumental ADL
	3	Fatigue relieved by rest; limiting self-care ADL
	4	-
Insomnia	1	Mild difficultly falling asleep, staying asleep or waking up early
	2	Moderate difficultly falling asleep, staying asleep or waking up early
	3	Severe difficultly in falling asleep, staying asleep or waking up early
	4	-

APPENDIX D

RECIST criteria

Response Evaluation Criteria in Solid tumor (RECIST) criteria version 1.1

1. Complete response (CR): Disappearance of all target lesions. Any pathological lymph nodes must have reduction in short axis < 10 mm.

2. Partial response (PR): At least a 30% decrease in sum of diameters of target lesions, taking as reference the baseline sum diameters.

3. Progression disease (PD): At least 20% decrease in sum of diameters of target lesions. In addition to the relative increase of 20 percent, 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 to quality for Partial response or Progression disease, compare to the sum of the smallest diameters at baseline.

APPENDIX E

Ethical Approval

COA No. 413/2018

IRB No. 162/61

INSTITUTIONAL REVIEW BOARD

Faculty of Medicine, Chulalongkorn University

1873 Rama 4 Road, Patumwan, Bangkok 10330, Thailand, Tel 662-256-4493

Certificate of Approval

The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, has approved 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 Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

Study Title : EFFECT OF ERCC1, XRCC1 AND GSTP1 POLYMORPHISMS ON CLINICAL RESPONSE AND ADVERSE EVENTS OF PLATINUM-BASED CHEMOTHERAPY IN EPITHELIAL OVARIAN CANCER PATIENTS.

Study Code : -

Principal Investigator : Miss Salisa Liblab

Affiliation of PI : Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Review Method : Expedited

Continuing Report : At least once annually or submit the final report if finished.

Document Reviewed :

1. Research Proposal Version 2.0 Date 29 April 2018
2. Protocol Synopsis Version 2.0 Date 29 April 2018
3. Information sheet for research participant Version 2.0 Date 29 April 2018
4. Informed consent for participating volunteers Version 2.0 Date 29 April 2018

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

5. Data collecting form Version 1.0 Date 21 March 2018
6. Budget Version 1.0 Date 21 March 2018
7. Curriculum Vitae and GCP Training
   - Miss Salisa Liblab
   - Assist.Prof. Apichai Vasuratna, M.D.
   - Natacha Phoolcharoen, M.D.
   - Assist.Prof. Nutthada Areepium, Ph.D.

Signature	Signature .....
(Emeritus Professor Tada Sueblinvong MD)	(Associate Professor Supeecha Wittayalertpanya)
Chairperson	Member and Assistant Secretary, Acting Secretary
The Institutional Review Board	The Institutional Review Board

Date of Approval : May 3, 2018
Approval Expire Date : May 2, 2019

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

APPENDIX F

Information sheet

เอกสารชี้แจงข้อมูลคำอธิบายสำหรับผู้เข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย

ผลของภาวะพหุสัณฐานของยีน *ERCC1, XRCC1* และ *GSTP1* ต่อการตอบสนองต่อการรักษาและการเกิดเหตุการณ์ไม่พึงประสงค์จากเคมีบำบัดสูตรที่มียากลุ่มแพลทินัมในผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว

ผู้สนับสนุนการวิจัย ทุน 90 ปีจุฬาลงกรณ์มหาวิทยาลัย

ผู้วิจัยหลัก

ชื่อ เภสัชกรหญิงศลิษา ลิบลับ

ที่อยู่สถานศึกษาของผู้วิจัย ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 10330

เบอร์โทรศัพท์ติดต่อ 24 ชั่วโมง 081-476-4939

อาจารย์ที่ปรึกษาวิทยานิพนธ์

ชื่อ ผู้ช่วยศาสตราจารย์ เภสัชกรหญิง ดร.ณัฏฐดา อารีเปี่ยม

ที่อยู่ที่ทำงานของผู้วิจัย ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 10330

เบอร์โทรศัพท์ที่ทำงาน 02-218-8403

เบอร์โทรศัพท์ติดต่อ 24 ชั่วโมง 081-622-2858

อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม

ชื่อ ผู้ช่วยศาสตราจารย์ นายแพทย์อภิชัย วสุรัตน์

ที่อยู่ที่ทำงานของผู้วิจัย ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 10330

เบอร์โทรศัพท์ที่ทำงาน 02-256-4000

เบอร์โทรศัพท์ติดต่อ 24 ชั่วโมง 061-702-4914

ชื่อ อาจารย์ แพทย์หญิงณัฐชา พูลเจริญ

ที่อยู่ที่ทำงานของผู้วิจัย ภาควิชาสูติศาสตร์-นรีเวชวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 10330

เบอร์โทรศัพท์ที่ทำงาน 02-256-4000

เบอร์โทรศัพท์ติดต่อ 24 ชั่วโมง 061-702-4914

เรียน ผู้เข้าร่วมโครงการวิจัยทุกท่าน

ท่านได้รับเชิญให้เข้าร่วมในโครงการวิจัยนี้ เนื่องจากท่านเป็นผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว ก่อนที่ท่านจะตัดสินใจเข้าร่วมในการศึกษาวิจัยดังกล่าว ขอให้ท่านอ่านเอกสารฉบับนี้อย่างถี่ถ้วน เพื่อให้ท่านได้ทราบถึงเหตุผลและรายละเอียดของการศึกษาวิจัยในครั้งนี้ หากท่านมีข้อสงสัยใด ๆ เพิ่มเติม กรุณาซักถามจากผู้ทำวิจัย ซึ่งจะเป็นผู้สามารถตอบคำถามและให้ความกระจ่างแก่ท่านได้

ท่านสามารถขอคำแนะนำในการเข้าร่วมโครงการวิจัยนี้จากครอบครัว เพื่อน หรือแพทย์ประจำตัวของท่านได้ ท่านมีเวลาอย่างเพียงพอในการตัดสินใจโดยอิสระ ถ้าท่านตัดสินใจแล้วว่าจะเข้าร่วมในโครงการวิจัยนี้ ขอให้ท่านลงนามในเอกสารแสดงความยินยอมของโครงการวิจัยนี้ และท่านจะได้รับสำเนาใบที่ยินยอมที่เก็บไว้ 1 ฉบับ

<u>เหตุผลความเป็นมา</u>

โรคมะเร็งรังไข่ชนิดเยื่อบุผิว คือ โรคที่เซลล์เยื่อบุผิวของรังไข่มีการเจริญเติบโตที่ผิดปกติ โดยทั่วไปโรคนี้มักไม่แสดงอาการ ผู้ป่วยส่วนใหญ่จะได้รับการวินิจฉัยว่าเป็นโรคมะเร็งรังไข่ชนิดเยื่อบุผิวเมื่อโรคมีการลุกลามเข้าสู่ระยะท้าย คือ ระยะที่ 3 หรือ 4 ซึ่งผู้ป่วยจะได้รับการรักษาด้วยการให้เคมีบำบัดที่มียากลุ่มแพลทินัม คือ ซิสพลาตินหรือคาร์โบพลาติน เป็นยาหลัก แต่ผลการตอบสนองก็มีความแตกต่างกันในแต่ละบุคคลและยังให้ผลที่ไม่น่าพอใจ ได้มีผลการศึกษาในก่อนหน้านี้พบว่า ภาวะพหุสัณฐานของยีนหรือความหลากหลายทางพันธุกรรมมีความสัมพันธ์กับการตอบสนองต่อเคมีบำบัด จึงนำมาสู่การศึกษาผลของความหลากหลายทางพันธุกรรมของยีนอีอาร์ซีซีวัน (*ERCC1*) เอ็กซ์อาร์ซีซีวัน (*XRCC1*) และจีเอสทีพีวัน (*GSTP1*) ต่อการตอบสนองต่อการรักษาและการเกิดเหตุการณ์ไม่พึงประสงค์จากยากลุ่มแพลทินัมในผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว

<u>วัตถุประสงค์ของการวิจัย</u>

วัตถุประสงค์จากการวิจัยในครั้งนี้ คือ เพื่อศึกษาผลของความหลากหลายทางพันธุกรรมของยีนอีอาร์ซีซีวัน (*ERCC1*) เอ็กซ์อาร์ซีซีวัน (*XRCC1*) และจีเอสทีพีวัน (*GSTP1*) ต่อการตอบสนองต่อการรักษาและการเกิดเหตุการณ์ไม่พึงประสงค์จากยากลุ่มแพลทินัมในผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว จำนวนผู้เข้าร่วมวิจัย คือ 136 คน

<u>วิธีการที่เกี่ยวข้องกับการวิจัย</u>

หลังจากท่านให้ความยินยอมที่จะเข้าร่วมในโครงการวิจัยนี้ ท่านจะได้รับการตรวจและรักษาตามปกติ โดยแพทย์จะเป็นผู้คัดกรองว่าท่านควรได้รับการรักษาด้วยเคมีบำบัดสูตรที่มียากลุ่มแพลทินัมเป็นองค์ประกอบ

สำหรับงานวิจัยครั้งนี้ท่านจะได้รับการเจาะเลือดทางหลอดเลือดดำปริมาณ 5 ซีซี (หนึ่งช้อนชา) หนึ่งครั้งก่อนการให้ยาเคมีบำบัดโดยพยาบาล ณ หน่วยให้ยาเคมีบำบัดผู้ป่วยนอก ตึกว่องวานิช โรงพยาบาลจุฬาลงกรณ์ หลังจากนั้นเลือดของท่านจะถูกนำไปสกัดแยกดีเอ็นเอ (DNA) ณ ห้องปฏิบัติการโมเลกุล คณะแพทยศาสตร์ โรงพยาบาลจุฬาลงกรณ์ และนำไปวิเคราะห์หาลักษณะความหลากหลายทางพันธุกรรมของยีนอีอาร์ซีซีวัน (*ERCC1*) เอ็กซ์อาร์ซีซีวัน (*XRCC1*) และจีเอสทีพีวัน (*GSTP1*) ณ ห้องปฏิบัติการ บริษัทยี

นพลัส จำกัด 240/56, 240/58 อาคารโยธยาทาวเวอร์ ถนนรัชดาภิเษก แขวงห้วยขวาง เขตห้วยขวาง กทม. 10310

หลังจากนั้นเมื่อท่านมาพบแพทย์ตามรอบการรับยา ท่านจะได้รับการติดตามการเกิดเหตุการณ์ไม่พึงประสงค์จากเคมีบำบัดโดยผู้วิจัยจะเป็นผู้สัมภาษณ์ท่านทั้งหมด 6 ครั้ง ตามรอบการรับยาร่วมกับมีการเก็บข้อมูลจากเวชระเบียน จากนั้นข้อมูลที่ได้จะนำไปวิเคราะห์ทางสถิติ ท่านควรจะต้องอยู่ในโครงการวิจัย 5 เดือนหรือจนกว่าจะมีผลประเมินการรักษา

ผู้วิจัยจะไม่แจ้งผลการตรวจความหลากหลายทางพันธุกรรม จนกว่าจะมีหลักฐานชัดเจนว่ามีความสัมพันธ์กับการตอบสนองต่อการรักษาหรือการเกิดเหตุการณ์ไม่พึงประสงค์จากเคมีบำบัดกลุ่มแพลทินัม

<u>ความรับผิดชอบของอาสาสมัครผู้เข้าร่วมในโครงการวิจัย</u>

ขอให้ท่านแจ้งผู้ทำวิจัยในกรณีที่พบอาการผิดปกติต่าง ๆ เกิดขึ้น หรืออาการอื่น ๆ ที่พบร่วมด้วยระหว่างที่อยู่ในโครงการวิจัย ถ้ามีการการเปลี่ยนแปลงเกี่ยวกับสุขภาพของท่าน ขอให้ท่านรายงานให้ผู้ทำวิจัยทราบโดยเร็ว

<u>ความเสี่ยงที่อาจได้รับ</u>

เนื่องจากการวิจัยครั้งนี้เป็นการศึกษาปัจจัยในระดับยีนจึงมีความจำเป็นในการเจาะเลือดจำนวน 5 มิลลิลิตร (หนึ่งช้อนชา) เพื่อนำไปวิเคราะห์ จึงมีความเสี่ยงเพียงเล็กน้อย เช่น ท่านอาจรู้สึกเจ็บบริเวณที่ถูกเจาะเลือด

<u>ความเสี่ยงที่ได้รับจากการเจาะเลือด</u>

ท่านมีโอกาสที่จะเกิดอาการเจ็บ เลือดออก ช้ำจากการเจาะเลือด อาการบวมบริเวณที่เจาะเลือด หรือหน้ามืด นอกจากนี้โอกาสที่จะเกิดการติดเชื้อบริเวณที่เจาะเลือดพบได้น้อยมาก

<u>ความเสี่ยงที่ไม่ทราบแน่นอน</u>

ท่านอาจเกิดอาการข้างเคียง หรือความไม่สบาย ซึ่งอาการข้างเคียงเหล่านี้เป็นอาการที่ไม่เคยพบมาก่อน เพื่อความปลอดภัยของท่าน ควรแจ้งผู้ทำวิจัยทันทีเมื่อเกิดความผิดปกติใด ๆ เกิดขึ้น

หากท่านมีข้อสงสัยใด ๆ เกี่ยวกับความเสี่ยงที่อาจได้รับจากการเข้าร่วมโครงการวิจัย ท่านสามารถสอบถามจากผู้ทำวิจัยได้ตลอดเวลา

หากมีการค้นพบข้อมูลใหม่ ๆ ที่อาจมีผลต่อความปลอดภัยของท่านในระหว่างที่ท่านเข้าร่วมในโครงการวิจัย ผู้วิจัยจะแจ้งให้ท่านทราบทันที

<u>การพบแพทย์นอกตารางนัดหมายในกรณีที่เกิดอาการข้างเคียง</u>

หากมีอาการข้างเคียงใด ๆ เกิดขึ้นกับท่าน ขอให้ท่านรีบมาพบแพทย์ที่สถานพยาบาลทันที ถึงแม้ว่าจะอยู่นอกตารางการนัดหมาย เพื่อแพทย์จะได้ประเมินอาการข้างเคียงของท่านและให้การรักษาที่เหมาะสมทันที หากอาการดังกล่าวเป็นผลจากการเข้าร่วมในโครงการวิจัย ท่านจะไม่เสียค่าใช้จ่าย

ประโยชน์ที่อาจได้รับ

ท่านจะไม่ได้รับประโยชน์โดยตรงจากการวิจัยครั้งนี้ แต่ข้อมูลที่ได้จากการวิจัยสามารถเป็นแนวทางในการพิจารณาการรักษาผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิวด้วยสูตรเคมีบำบัดที่มียากลุ่มแพลทินัมเป็นองค์ประกอบ ตามความหลากหลายของยีนอีอาร์ซีซีวัน (*ERCC1*) เอ็กซ์อาร์ซีซีวัน (*XRCC1*) และจีเอสทีพีวัน (*GSTP1*) ให้เหมาะสมในผู้ป่วยแต่ละรายได้ในอนาคต

วิธีการและรูปแบบการรักษาอื่น ๆ ซึ่งมีอยู่สำหรับอาสาสมัคร

ท่านไม่จำเป็นต้องเข้าร่วมโครงการวิจัยนี้เพื่อประโยชน์ในการรักษาโรคที่ท่านเป็นอยู่ เนื่องจากมีแนวทางการรักษาอื่น ๆ หลายแบบสำหรับรักษาโรคของท่านได้ ดังนั้น จึงควรปรึกษาแนวทางการรักษาวิธีอื่น ๆ กับแพทย์ผู้ทำการรักษาให้ท่านก่อนตัดสินใจเข้าร่วมในการวิจัย

ข้อปฏิบัติของท่านขณะที่เข้าร่วมในโครงการวิจัย

ขอให้ท่านปฏิบัติดังนี้

- ขอให้ท่านให้ข้อมูลทางการแพทย์ของท่านทั้งในอดีต และปัจจุบัน แก่ผู้ทำวิจัยด้วยความสัตย์จริง
- ขอให้ท่านแจ้งให้ผู้ทำวิจัยทราบความผิดปกติที่เกิดขึ้นระหว่างที่ท่านเข้าร่วมในโครงการวิจัย
- ขอให้ท่านงดการใช้ยาอื่นนอกเหนือจากที่ผู้ทำวิจัยจัดให้ รวมถึงการรักษาอื่น ๆ เช่น การรักษาด้วยสมุนไพร การซื้อยาจากร้านขายยา
- ขอให้ท่านแจ้งให้ผู้ทำวิจัยทราบทันที หากท่านได้รับยาอื่นนอกเหนือจากยาที่ใช้ในการศึกษาตลอดระยะเวลาที่ท่านอยู่ในโครงการวิจัย
- ขอให้ท่านนำยาที่ใช้ในการศึกษาของท่านทั้งหมดที่เหลือจากการรับประทานมาให้ผู้ทำวิจัยทุกครั้งที่นัดหมายให้มาพบ

อันตรายที่อาจเกิดขึ้นจากการเข้าร่วมในโครงการวิจัยและความรับผิดชอบของผู้ทำวิจัย

หากพบอันตรายที่เกิดขึ้นจากการวิจัย ท่านจะได้รับการรักษาที่เหมาะสมทันที ผู้วิจัยยินดีจะรับผิดชอบค่าใช้จ่ายในการรักษาพยาบาลของท่าน และการลงนามในเอกสารให้ความยินยอมไม่ได้หมายความว่าท่านได้สละสิทธิ์ทางกฎหมายตามปกติที่ท่านพึงมี

ค่าตอบแทนสำหรับผู้เข้าร่วมวิจัย

ท่านจะไม่ได้รับเงินค่าตอบแทนจากการเข้าร่วมในการวิจัย แต่ท่านจะได้รับค่าเดินทางรวมทั้งหมด 300 บาทต่อคนในวันที่เจาะเลือด

การปกป้องรักษาข้อมูลความลับของอาสาสมัคร

ข้อมูลที่อาจนำไปสู่การเปิดเผยตัวท่าน จะได้รับการปกปิดและจะไม่เปิดเผยแก่สาธารณชนในกรณีที่ผลการวิจัยได้รับการตีพิมพ์ ชื่อและที่อยู่ของท่านจะต้องได้รับการปกปิดอยู่เสมอ โดยจะใช้เฉพาะรหัสประจำโครงการวิจัยของท่าน

จากการลงนามยินยอมของท่าน ผู้ทำวิจัย และผู้สนับสนุนการวิจัย คณะกรรมการจริยธรรมการวิจัย ผู้ตรวจสอบการวิจัย และหน่วยงานควบคุมระเบียบกฎหมาย สามารถเข้าไปตรวจสอบบันทึกข้อมูลทาง

การแพทย์ของท่านได้แม้จะสิ้นสุดโครงการวิจัยแล้วก็ตาม โดยไม่ละเมิดสิทธิของท่านในการรักษาความลับเกินขอบเขตที่กฎหมายและระเบียบกฎหมายอนุญาตไว้

จากการลงนามยินยอมของท่าน ผู้ทำวิจัยสามารถบอกรายละเอียดของท่านที่เกี่ยวกับการเข้าร่วมโครงการวิจัยนี้ให้แก่แพทย์ผู้รักษาท่านได้

<u>การยกเลิกการให้ความยินยอม</u>

หากท่านต้องการยกเลิกการให้ความยินยอมดังกล่าว ท่านสามารถติดต่อผู้วิจัยได้ที่เบอร์โทรศัพท์ 081-476-4939 ตลอด 24 ชั่วโมง หรือสามารถแจ้งหรือเขียนบันทึกขอยกเลิกการให้คำยินยอม โดยส่งไปที่ เภสัชกรหญิงศลิษา ลิบลับ ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 254 ถนนพญาไท เขตปทุมวัน กรุงเทพมหานคร 10330

หากท่านขอยกเลิกการให้คำยินยอมหลังจากที่ท่านได้เข้าร่วมโครงการวิจัยแล้ว ข้อมูลส่วนตัวของท่านจะไม่ถูกบันทึกเพิ่มเติม อย่างไรก็ตามข้อมูลอื่น ๆ ของท่านอาจถูกนำมาใช้เพื่อประเมินผลการวิจัย และท่านจะไม่สามารถกลับมาเข้าร่วมในโครงการนี้ได้อีก ทั้งนี้เนื่องจากข้อมูลของท่านที่จำเป็นสำหรับใช้เพื่อการวิจัยไม่ได้ถูกบันทึก

<u>การจัดการเก็บตัวอย่างชีวภาพที่เหลือ</u>

ขอเก็บตัวอย่างเลือดสำหรับตรวจซ้ำ เพื่อยืนยันความถูกต้องของผลการทดลองเป็นระยะเวลา 1 ปี หลังจากนั้นตัวอย่างเลือดจะถูกทำลายตามวิธีมาตรฐาน (เผาด้วยระบบไร้ควัน) ทันที

<u>สิทธิ์ของผู้เข้าร่วมในโครงการวิจัย</u>

ในฐานะที่ท่านเป็นผู้เข้าร่วมในโครงการวิจัย ท่านจะมีสิทธิ์ดังต่อไปนี้

1. ท่านจะได้รับทราบถึงลักษณะและวัตถุประสงค์ของการวิจัยในครั้งนี้
2. ท่านจะได้รับการอธิบายเกี่ยวกับระเบียบวิธีการของการวิจัยทางการแพทย์ รวมทั้งยาและอุปกรณ์ที่ใช้ในการวิจัยครั้งนี้
3. ท่านจะได้รับการอธิบายถึงความเสี่ยงและความไม่สบายที่จะได้รับจากการวิจัย
4. ท่านจะได้รับการอธิบายถึงประโยชน์ที่ท่านอาจจะได้รับจากการวิจัย
5. ท่านจะได้รับการเปิดเผยถึงทางเลือกในการรักษาด้วยวิธีอื่น ยา หรืออุปกรณ์ ซึ่งมีผลดีต่อท่าน รวมทั้งประโยชน์และความเสี่ยงที่ท่านอาจได้รับ
6. ท่านจะได้รับทราบแนวทางในการรักษา ในกรณีที่พบโรคแทรกซ้อนภายหลังการเข้าร่วมในโครงการวิจัย
7. ท่านจะมีโอกาสได้ซักถามเกี่ยวกับงานวิจัยหรือขั้นตอนที่เกี่ยวข้องกับงานวิจัย
8. ท่านจะได้รับทราบว่าการยินยอมเข้าร่วมในโครงการวิจัยนี้ ท่านสามารถขอถอนตัวจากโครงการเมื่อไรก็ได้ โดยผู้เข้าร่วมในโครงการวิจัยสามารถขอถอนตัวจากโครงการโดยไม่ได้รับผลกระทบใด ๆ ทั้งสิ้น
9. ท่านจะได้รับเอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในโครงการวิจัยและสำเนาเอกสารใบยินยอมที่มีทั้งลายเซ็นและวันที่

10. ท่านมีสิทธิ์ในการตัดสินใจว่าจะเข้าร่วมในโครงการวิจัยหรือไม่ก็ได้ โดยปราศจากการใช้อิทธิพลบังคับข่มขู่ หรือการหลอกลวง

หากท่านไม่ได้รับการชดเชยอันควรต่อการบาดเจ็บหรือเจ็บป่วยที่เกิดขึ้นโดยตรงจากการวิจัย หรือท่านไม่ได้รับการปฏิบัติตามที่ปรากฏในเอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในการวิจัย ท่านสามารถร้องเรียนได้ที่ สำนักงานคณะกรรมการจริยธรรมการวิจัย คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ตึกอานันทมหิดลชั้น 3 โรงพยาบาลจุฬาลงกรณ์ ถนนพระราม 4 ปทุมวัน กรุงเทพฯ 10330 โทรศัพท์/โทรสาร 0-2256-4493 ในเวลาราชการ หรือ e-mail : medchulairb@chula.ac.th

การลงนามในเอกสารให้ความยินยอม ไม่ได้หมายความว่าท่านได้สละสิทธิ์ทางกฎหมายตามปกติที่ท่านพึงมี

ขอขอบคุณในการให้ความร่วมมือของท่านมา ณ ที่นี้

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APPENDIX G

Consent Form

เอกสารแสดงความยินยอนเข้าร่วมโครงการสำหรับอาสาสมัคร

การวิจัยเรื่อง ผลของภาวะพหุสัณฐานของยีน *ERCC1, XRCC1* และ *GSTP1* ต่อการตอบสนองต่อการรักษาและการเกิดเหตุการณ์ไม่พึงประสงค์จากเคมีบำบัดสูตรที่มียากลุ่มแพลทินัมในผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว

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

ข้าพเจ้า นาง/ นางสาว.....

ที่อยู่.....

ได้อ่านรายละเอียดจากเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัยวิจัยที่แนบมาฉบับวันที่..... และข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยโดยสมัครใจ

ข้าพเจ้าได้รับสำเนาเอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนาม และวันที่ พร้อมด้วยเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัย ทั้งนี้ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย ระยะเวลาของการทำวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัย และแนวทางรักษาโดยวิธีอื่นอย่างละเอียด ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัยจนมีความเข้าใจอย่างดีแล้ว โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้ารับทราบจากผู้วิจัยว่าหากเกิดอันตรายใด ๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่เสียค่าใช้จ่าย

ข้าพเจ้ามีสิทธิที่จะบอกเลิกเข้าร่วมในโครงการวิจัยเมื่อใดก็ได้ โดยไม่จำเป็นต้องแจ้งเหตุผล และการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคหรือสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะเมื่อได้รับการยินยอมจากข้าพเจ้าเท่านั้น คณะกรรมการพิจารณาจริยธรรมการวิจัยในคนอาจได้รับอนุญาตให้เข้ามาตรวจและประมวลข้อมูลของข้าพเจ้า ทั้งนี้จะต้องกระทำไปเพื่อวัตถุประสงค์เพื่อตรวจสอบความถูกต้องของข้อมูลเท่านั้น โดยการตกลงที่จะเข้าร่วมการศึกษานี้ข้าพเจ้าได้ให้คำยินยอมที่จะให้มีการตรวจสอบข้อมูลประวัติทางการแพทย์ของข้าพเจ้าได้

ผู้วิจัยรับรองว่าจะไม่มีการเก็บข้อมูลใด ๆ เพิ่มเติม หลังจากที่ข้าพเจ้าขอยกเลิกการเข้าร่วมโครงการวิจัยและต้องการให้ทำลายเอกสารและ/หรือ ตัวอย่างที่ใช้ตรวจสอบทั้งหมดที่สามารถสืบค้นถึงตัวข้าพเจ้าได้

ข้าพเจ้าเข้าใจว่า ข้าพเจ้ามีสิทธิ์ที่จะตรวจสอบหรือแก้ไขข้อมูลส่วนตัวของข้าพเจ้าและสามารถยกเลิกการให้สิทธิในการใช้ข้อมูลส่วนตัวของข้าพเจ้าได้ โดยต้องแจ้งให้ผู้วิจัยรับทราบ

ข้าพเจ้าได้ตระหนักว่าข้อมูลในการวิจัยรวมถึงข้อมูลทางการแพทย์ของข้าพเจ้าที่ไม่มีการเปิดเผยชื่อจะผ่านกระบวนการต่าง ๆ เช่น การเก็บข้อมูล การบันทึกข้อมูลในแบบบันทึกและในคอมพิวเตอร์ การตรวจสอบ การวิเคราะห์ และการรายงานข้อมูลเพื่อวัตถุประสงค์ทางวิชาการ รวมทั้งการใช้ข้อมูลทางการแพทย์ในอนาคตหรือการวิจัยทางด้านเภสัชภัณฑ์ เท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความเต็มใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

.....ลงนามผู้ให้ความยินยอม

(.....) ชื่อผู้ยินยอมตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

การจัดการกับตัวอย่างทางชีวภาพ

☑ มีและขอเก็บตัวอย่างชีวภาพที่เหลือไว้เป็นระยะเวลา 1 ปี เพื่อการวิจัยในอนาคต

ข้าพเจ้าได้อธิบายถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการไม่พึงประสงค์หรือความเสี่ยงที่อาจเกิดขึ้นจากการวิจัย หรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียดให้ผู้เข้าร่วมในโครงการวิจัยตามนามข้างต้นได้ทราบและมีความเข้าใจดีแล้ว พร้อมลงนามลงในเอกสารแสดงความยินยอมด้วยความเต็มใจ

.....ลงนามผู้ทำวิจัย

(.....) ชื่อผู้ทำวิจัย ตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

.....ลงนามพยาน

(.....) ชื่อพยาน ตัวบรรจง

วันที่ .....เดือน.....พ.ศ.....

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