ผลของภาวะพหุสัณฐานของยีน *ERCC1 GSTP1* และ *CTR1* ต่อการตอบสนองต่อการรักษาและการเกิดพิษจากการใช้ยาเคมีบำบัดกลุ่มแพลทินัมในผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กระยะลุกลาม

นางสาวศิริลักษณ์ คำภิโร

จุฬาลงกรณ์มหาวิทยาลัย
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บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
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ปีการศึกษา 2558
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EFFECTS OF *ERCC1, GSTP1* AND *CTR1* POLYMORPHISMS ON THE TREATMENT RESPONSES AND TOXICITIES OF PLATINUM-BASED CHEMOTHERAPY IN ADVANCED NON-SMALL CELL LUNG CANCER PATIENTS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	EFFECTS OF *ERCC1, GSTP1* AND *CTR1* POLYMORPHISMS ON THE TREATMENT RESPONSES AND TOXICITIES OF PLATINUM-BASED CHEMOTHERAPY IN ADVANCED NON-SMALL CELL LUNG CANCER PATIENTS
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ศิริลักษณ์ คำภิโร : ผลของภาวะพหุสัณฐานของยีน *ERCC1 GSTP1* และ *CTR1* ต่อการตอบสนองต่อการรักษาและการเกิดพิษจากการใช้ยาเคมีบำบัดกลุ่มแพลทินัมในผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กระยะลุกลาม (EFFECTS OF *ERCC1, GSTP1* AND *CTR1* POLYMORPHISMS ON THE TREATMENT RESPONSES AND TOXICITIES OF PLATINUM-BASED CHEMOTHERAPY IN ADVANCED NON-SMALL CELL LUNG CANCER PATIENTS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ภญ. ดร.ณัฏฐดา อารีเปี่ยม, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. นพ. ดร.วิโรจน์ ศรีอุฬารพงศ์, 96 หน้า.

ภาวะพหุสัณฐานของยีนที่เกี่ยวกับกระบวนการออกฤทธิ์ของแพลทินัมอาจส่งผลต่อการรักษาในผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กในระยะลุกลามที่ได้รับการรักษาด้วยยาในกลุ่มแพลทินัม การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของภาวะพหุสัณฐานของยีน *ERCC1 GSTP1* และ *CTR1* ต่อการตอบสนองต่อการรักษาและการเกิดพิษจากการใช้ยาเคมีบำบัดกลุ่มแพลทินัมในผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กในระยะลุกลาม ทำการศึกษาในผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กในระยะลุกลาม จำนวน 74 ราย ณ แผนกผู้ป่วยนอก โรงพยาบาลจุฬาลงกรณ์ กรุงเทพมหานคร พบความชุกของภาวะพหุสัณฐานของยีน *ERCC1* rs11615 ร้อยละ 27.7 ยีน *GSTP1* rs1695 ร้อยละ 25 และยีน *CTR1* rs12686377 ร้อยละ 22.3 ตามลำดับ ผลการศึกษาไม่พบความสัมพันธ์ระหว่างผลการตอบสนองต่อการรักษากับภาวะพหุสัณฐานของยีน *ERCC1 GSTP1* และ *CTR1* (P = 0.05) อย่างไรก็ตามพบว่ายีนเหล่านี้สัมพันธ์กับอาการไม่พึงประสงค์บางประการ ผู้ป่วยที่มีภาวะพหุสัณฐานของยีน *GSTP1* (AG หรือ GG) มีอุบัติการณ์การเกิดภาวะเม็ดเลือดขาวชนิดนิวโทรฟิลต่ำสูงกว่าผู้ป่วยที่มียีนแบบปกติ (AA) 2.8 เท่า (95% CI, 1.033–7.614, P = 0.03) ขณะที่ผู้ป่วยที่มียีน *CTR1* แบบปกติ (GG) มีอุบัติการณ์การเกิดน้ำหนักตัวลดลงมากกว่าผู้ป่วยที่มีภาวะพหุสัณฐานของยีนนี้ (GT หรือ TT) (26.3% vs. 0%, P = 0.01) ผลการศึกษาแสดงให้เห็นว่าควรพิจารณาภาวะพหุสัณฐานของยีนดังกล่าวในการเลือกสูตรยาเคมีบำบัดเพื่อรักษาผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กในระยะลุกลาม โดยเฉพาะอย่างยิ่งในการคาดการณ์อาการไม่พึงประสงค์จากการรักษา

ภาควิชา	เภสัชกรรมปฏิบัติ	ลายมือชื่อนิสิต	....
สาขาวิชา	เภสัชกรรมคลินิก	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
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KEYWORDS: ERCC1 / GSTP1 / CTR1 / PLATINUM-BASED REGIMEN / NSCLC

SIRILUK KUMPIRO: EFFECTS OF *ERCC1, GSTP1* AND *CTR1* POLYMORPHISMS ON THE TREATMENT RESPONSES AND TOXICITIES OF PLATINUM-BASED CHEMOTHERAPY IN ADVANCED NON-SMALL CELL LUNG CANCER PATIENTS. ADVISOR: NUTTHADA AREEPIUM, Ph.D., CO-ADVISOR: ASSOC. PROF. VIROTE SRIURANPONG, Ph.D., 96 pp.

The genetic polymorphisms involved in platinums' action appear to impact on clinical outcomes in patients treated with platinum-based regimens. Objectives of this study were to investigate the effects of *ERCC1*, *GSTP1* and *CTR1* polymorphisms on treatment response and toxicity in advanced NSCLC patients treated with platinum-based chemotherapy. This prospective cohort study enrolled 74 advanced NSCLC participants received treatment at out-patient department of King Chulalongkorn Memorial Hospital, Bangkok. The polymorphisms of *ERCC1* rs11615, *GSTP1* rs1695 and *CTR1* rs12686377 genes were 27.7%, 25% and 22.3%, respectively. There was no association between genetic polymorphisms and treatment response ($P > 0.05$). However, we found some genetic polymorphisms were related to some toxicity. Patients with *GSTP1* polymorphism (AG and GG) had approximate 2.8-fold higher incidence of neutropenia than a AA genotype (95% CI, 1.033–7.614), similar to *CTR1* polymorphism which was associated with weight loss, patients carrying homozygous wild type GG had higher incidence of weight loss (26.3% vs. 0%) compared to whom with genetic polymorphism (GT and TT). This study indicated that genetic polymorphisms should be considered in the selection of proper chemotherapy regimen in advanced NSCLC patients especially for prediction of adverse effects from the treatment.

Department:	Pharmacy Practice	Student's Signature	....
Field of Study:	Clinical Pharmacy	Advisor's Signature	....
Academic Year:	2015	Co-Advisor's Signature	....

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จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

CONTENTS

Page

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Page

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

LIST OF TABLE

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

LIST OF FIGURES

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER I

INTRODUCTION

Background and rationale

Lung cancer is the most common cancer related to mortality. Each year 1.59 million people die from lung cancer, accounting for 12.5% of all cancer deaths in South East Asia.[1] Histological classification has been identified types of lung cancer. There are two main forms of the disease, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Approximately 85 % of all patients represent NSCLC.[2, 3] At the time of diagnosis, patients usually appeared with stage IIIB – IV, locally advanced or metastasis disease.[4, 5]

The National Comprehensive Cancer Network (NCCN) recommended doublet chemotherapy regimens for locally advanced or metastatic NSCLC, of which platinum-based regimen is the first line therapy.[6] Platinum-based regimen shows several benefit to prolong survival, to improves clinical symptoms, and to increases quality of life. Platinum agents such as cisplatin, carboplatin, and oxaliplatin prohibit proliferating cells by disturbing DNA synthesis, converting cell into apoptosis.[7] Cisplatin—the most commonly used platinum for NSCLC—and others platinum agents have similar mechanism of action.[8] Furthermore, platinum agents induce cytotoxicity that not only disturbs tumor cells but also affects normal cells with active DNA replication. Cisplatin induces DNA damage by acting on monoadduct, inter and intra-strand cross links, disturbing DNA helix and inhibiting DNA replication that kill tumor cells death. Cytotoxicity and adverse effects of cisplatin are resulted from the oxidative damage on cell membrane and changes of energy metabolism. Patients who received platinum-based regimen usually had adverse drug events (AEs) such as nausea, vomiting, bone marrow suppression, nephrotoxic effect and neurotoxicity.[9] Although cisplatin enhances response rate, median survival rate, and time to progression, but the five-year survival rate is still less than 20%. Several

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Chulalongkorn University

factors influence platinum agents resistance; in particular, genetic factor appears as an interesting issue.[10]

Strong evidences support that the genetic factor affects disease progression and patient response to cisplatin therapy. Genetic polymorphisms may be one of prognosis markers for cisplatin response and overall survival of NSCLC patients. Cisplatin resistance has been elucidated by three different pathways involving in cellular mechanism to decrease platinum accumulation in cytoplasm, increase intracellular detoxification, and increase DNA repair capacity.[10-12]

Nucleotide excision repair (NER), a DNA repair system, displays an important role in DNA repairing by removing the damage. Several publications emphasize NER protein levels may change cisplatin responses, especially by excision repair cross-complementation group 1 gene (*ERCC1*). *ERCC1* manage interstrand and intrastrand cross-links caused by cisplatin therapy by forming complex with xeroderma pigmentosum complementation group F (*XPF*) then eliminating the damage lesion.[13] Previous studies showed that polymorphisms in *ERCC1* were related to cisplatin response and survival outcome, and probably are affected by differences of individual protein activity. For example, two SNPs, wild type rs11615 C/C and variant homozygous rs3212986 A/A, in *ERCC1* had shown significant enhancing in response and survival than other harboring genotypes.[14-19]

The glutathione *S*-transferase (*GST*) family involved in detoxification of cisplatin by catalyzing platinum-glutathione conjugates in phase II metabolizing process. This conjugation has increased solubility and can be excreted through ATP-binding cassette.[10] *GSTP1* is the abundant *GST* isoform in lung tissues. These SNPs in *GSTP1* rs1695 and rs1138272 were found to associate with different clinical outcome and toxicities. For instance, *GSTP*1 rs1695, patients who had variant genotypes (A/G or G/G) were more likely to respond to cisplatin treatment than wild type.[20-23] A number of studies revealed correlation variant genotypes and beneficial clinical outcome in patients receiving cisplatin.

The noticeable contributing factors to platinum response are platinum between cellular uptake and accumulation. A reduce in the intracellular accumulation and a decrease of platinum in cellular diminish cytotoxic effect, resulting in platinum resistance. *In vivo* and *in vitro* studies indicated that copper transporter 1 gene (*CTR1*) played important role in cisplatin influx mechanism. This gene establishes protein human copper transporter 1 (hCTR1), which related to copper transportation pathway through intracellular, and platinum agent expected to be found in cellular in a similar condition as copper transportation.[10] Moreover, some studies explained an association between *CTR1* polymorphism, three SNPs at rs7851395, rs12686377 and rs10981694, and cisplatin response and toxicity.[24-26]

Although pharmacogenetics have shown different genes that can affect toxicity and clinical outcome, a number of trials reported inconsistent results of the association between genotype polymorphisms and platinum response and overall survival in NSCLC patients. Additional information is needed for more precise tools in treatment selection. Therefore, a prospective study to explore association between the 3 SNPs located in 3 major genes *ERCC1*, *GSTP1* and *CTR1* polymorphism with treatment response and toxicity of platinum-based chemotherapy in patients with advanced NSCLC is needed.

Objective of the research

To investigate the effect of *ERCC1*, *GSTP1* and *CTR1* polymorphisms on treatment response and toxicity in advanced NSCLC patients treated with platinum-based chemotherapy.

Research question

1. Do *ERCC1* polymorphisms affect treatment response and toxicity in advanced NSCLC patients treated with platinum-based chemotherapy?
2. Do *GSTP1* polymorphisms affect treatment response and toxicity in advanced NSCLC patients treated with platinum-based chemotherapy?
3. Do *CTR1* polymorphisms affect treatment response and toxicity in advanced NSCLC patients treated with platinum-based chemotherapy?

Statement of hypothesis

1. There are differences in treatment response and adverse events among different genotypes of *ERCC1* rs11615.
2. There are differences in treatment response and adverse events among different genotypes of *GSTP1* rs1695.
3. There are differences in treatment response and adverse events among different genotypes of *CTR1* rs12686377.

Scope of the research

In this study, participants with advanced NSCLC receiving treatment with platinum-based chemotherapy were enrolled from King Chulalongkorn Memorial Hospital (KCMH).

Conceptual framework

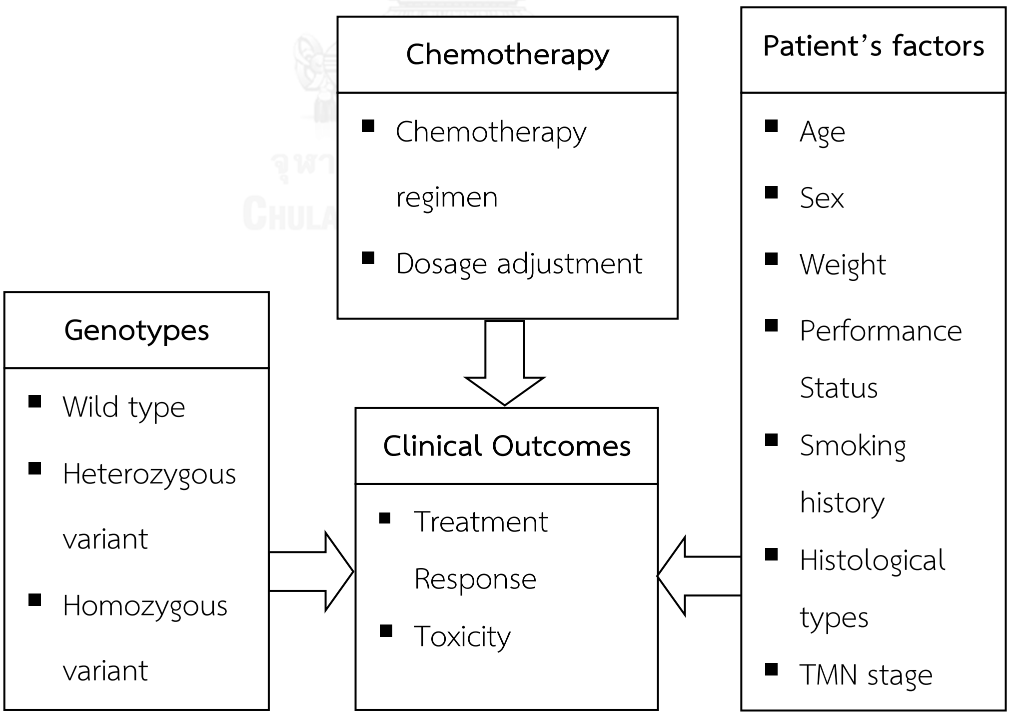

Figure 1 Conceptual framework

Operational definition

1. Participants were advanced NSCLC treated patients with platinum-based chemotherapy.
2. Advanced NSCLC was pathologically diagnosed of stage IIIB – IV NSCLC.
3. Platinum-based regimen was an antineoplastic regimen which contains cisplatin or carboplatin.
4. Second hand smoker was to define non-smoker who involuntarily or passively inhaled smoke from cigarette by family member.
5. Clinical outcomes were responses to chemotherapy and adverse events.
6. Response to chemotherapy was the effect of platinum chemotherapy, that the effects were defined as treatment response and disease control rate.
7. Treatment response was to evaluate participant outcome according to response evaluation criteria in solid tumors (RECIST) version 1.1 as follow complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD).
8. Overall response (OR) was to define treatment response of the participants as 2 groups, response and non-response group.
9. Response group was to define participants who had CR and PR from platinum-based regimen.
10. Non-response group was to define participants who had SD and PD from platinum-based regimen.
11. Disease control rate (DCR) was to define treatment response of the participants into progression group and non-progression group.
12. Progression group was to define participants who had PD from platinum-based regimen.
13. Non-progression group was to define participants who had CR, PR and SD from platinum-based regimen.
14. Adverse events (AEs) were toxicities resulting from platinum-regimen in each cycle, classified by Common Terminology Criteria for Adverse Events (CTCAE)

version 4.03 including emetic effect, hematological toxicity, nephrotoxicity and neurotoxicity were defined as grade 1-4.

15. Toxicity was participants who received any grade of toxicities from chemotherapy.
16. Grade of toxicities was the highest grade of toxicities from total cycles of chemotherapy.
17. Mild toxicity was participants with toxicity in grade 0-2.
18. Severe toxicity was participants with toxicity in grade 3-4.
19. Excision repair cross-complementation group 1 gene (*ERCC1*) polymorphism was genetic polymorphism at chromosome19–rs11615.
20. Glutathione *S*-transferase 1 gene (*GST1*) polymorphism was genetic polymorphism at chromosome 11–rs1695.
21. Copper transporter 1 gene (*CTR1*) polymorphism was genetic polymorphism at chromosome9–rs12686377.

Expected benefit and application of the research

Findings from this study may lead to better understanding in factors associated with platinum treatment response and tools for treatment selection in patient with advanced NSCLC.

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Chulalongkorn University

Limitation of the research

1. This study was limited to the length of follow up which cannot be observed long-term survival or delayed toxicity.
2. This trial was investigated in only one center, hence small population.

CHAPTER II

Literature Reviews

Lung cancer

Lung cancer is the most common cancer-related mortality. Each year 1.59 million people die from lung cancer, accounting for 12.5% of all cancer deaths in South East Asia. In Thailand, it is the second most common cause of cancer-related mortality.[1]

As for respiratory system, the lungs are organs involving in gas exchange. Lung cancer means irregular cell growth in tissue of the lungs. Clinical symptoms are present as cough, fatigue, shortness of breath, and chest pain.[2] A number of factors increase risks for lung cancer such as cigarettes and second-hand smoking, air pollution, occupation exposure, and family history of lung cancer, but smoking tobacco is the primary risk factor.[6] Histological classification has identified types of lung cancer as two main forms of the disease, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Approximately 85% of all patients represent NSCLC.[2, 3]

NSCLC is classified into two major types, squamous cell carcinoma and non-squamous cell carcinoma. Non-squamous cell carcinoma contains adenocarcinoma, large cell carcinoma, and other cell types of which adenocarcinoma is the most common type of lung cancers. In addition, type and stage of lung cancer influence how to plan for effective treatment. The stage is classified by the tumor node metastasis (TMN) staging system that NCCN refers to American Joint Committee on Cancer (AJCC) 7th edition.[27] [Appendix A] Regarding to stage of the disease, patients with NSCLC early stage, stage I, II, and IIIA have longer survival period than those with advanced stage, but majority of patients at the time of diagnosis appeared with stage IIIB – IV, locally advanced or metastasis disease.[4, 5]

Surgery, radiation, and chemotherapy are recommended to treat patients with NSCLC. These treatments can be used either separately or as a combination.

Concerning early stage, a surgery to remove some parts or the entire lung provides the best chance for cure. Both radiation and chemotherapy are treatments to reduce the size of tumors before surgery, or to remove any lung cancer cells that may still be in the body after surgery. It also reduces the risk of recurrence and improves survival.[6] In terms of advanced NSCLC, doublet chemotherapy regimens are recommended for first line treatment.

Patients' performance status is one important factor for treatment planning. The Eastern Cooperative Oncology Group (ECOG) developed ECOG performance status (PS) scale to explain the status of symptoms and functions regarding their ability to care for themselves, daily activity, and physical ability (walking, working, etc.).[28] The table shown below presents ECOG scores and the description of each score.

Table 1 ECOG performance status[28]

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

Treatment of advanced NSCLC

NCCN recommended doublet chemotherapy regimens for locally advanced or metastatic NSCLC, which platinum-based regimen is the first-line therapy. Platinum-based regimen shows several benefits— it prolongs survival, improves clinical symptom, and increases quality of life.[6] A meta-analysis of platinum-based regimens versus non-platinum-based shows increase in response rate of 62% (OR, 1.62; 95%CI = 1.46 to 1.8; $P < .0001$) and increase in one-year survival rate of 5% (34% v 29%; OR, 1.21; 95% CI, 1.09 to 1.35; $P = .0003$) in patients who received platinum-based regimens.[29]

Platinum-based regimens are a combination of platinum agents (eg. cisplatin, carboplatin) with taxane (eg, paclitaxel, docetaxel), vinorelbine, vinblastine, etoposide, pemetrexed, or gemcitabine. These regimens produce one-year survival rates of 30% to 40%, superior to a single agent. However, patients must have good PS—ECOG score 0-1 in order to gain benefit from doublet chemotherapy. For patients who have poor PS—ECOG score > 2, NCCN recommended a single-agent chemotherapy due to toxicity concerns.[6]

In Thailand, the Nation Health Security Office (NHSO) recommended chemotherapy for first-line drugs. This recommendation is for treatment of patient with advanced NSCLC, including the following:

1. ECOG score 0 – 1: platinum-based doublet regimen
2. ECOG score 0 – 1 and age more than 70 years: single agent chemotherapy
3. ECOG score 2: single or double chemotherapy depends on individual clinical symptoms.
4. Radiology and PS are the assessment of tumor response, and the goal is to treat for four to six cycles unless otherwise specified.

Chemotherapy regimens, including platinum-based doublets, are as follows:

1. Regimen PE = cisplatin* plus etoposide
2. Regimen CG = cisplatin* plus gemcitabine
3. Regimen CbPac = carboplatin plus placlitaxel

Note The replacement of cisplatin salvage therapy with carboplatin can be considered as a safe therapeutic strategy in patients who cannot continue to receive cisplatin due to chronic kidney disease, or severe nausea and vomit.

Chemotherapy regimens for advanced NSCLC patient are shown in Table 2. Docetaxel is recommended for second-line drug treatment in patients with advanced NSCLC who received first-line drug and had PS 0-1. The dosage recommended regimen of docetaxel is intravenous 60-75 mg/m^2 every 21 days for 4-6 cycles.

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Table 2 Chemotherapy regimens for advanced NSCLC patient

No.	Regimen	Medicine	Regimen	Dose ($mg/m^2/day$)	Total (mg/m^2)
1A	PE	cisplatin	80 mg/m^2 IV day 1	80	320-480
		etoposide	100 mg/m^2 IV day 1,2,3	100	1,200-1,800
1B	carboplatin/ etoposide	carboplatin	AUC 5-6 mg/ml/min in day 1	maximum total dose ≤ 750 mg	3,000-4,500
		etoposide	100 mg/m^2 IV day 1,2,3	100	1,200-1,800
2A	CG	cisplatin	80 mg/m^2 IV day 1	80	320-480
		gemcitabine	1,000 mg/m^2 IV day 1,8	1,000	8,000-12,000
2B	carboplatin/ gemcitabine	carboplatin	AUC 5-6 mg/ml/min in day 1	maximum total dose ≤ 750 mg	3,000-4,500
		gemcitabine	1,000 mg/m^2 IV day 1,8	1,000	8,000-12,000
3	CbPac	carboplatin	AUC 5-6 mg/ml/min in day 1	maximum total dose ≤ 750 mg	3,000-4,500
		paclitaxel	200 mg/m^2 IV day 1	200	800-1,200

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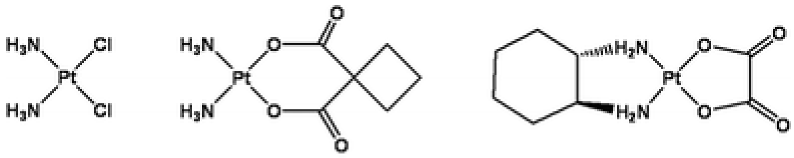
Platinum agents

Platinum agents cisplatin, carboplatin, and oxaliplatin, have been used for treatment of many types of solid tumor. In 1970, cisplatin was discovered as an inhibitor of growth in *Escherichia coli*, and carboplatin was the second generation analog. These agents which share the same mechanism of action, are completely cross-resistant, and destroy the structure of DNAs. Oxaliplatin is another analog, but its mechanism is difference from that of cisplatin.[9]



A B C

Figure 2 Chemical structure of platinum agents: [A] cisplatin, [B] carboplatin, [C] oxaliplatin[30]

Cisplatin enters into the cell by passive diffusion and active pathway such as copper transporter. Aquation and hydrolysis reactions perform aquated platinum complexes which convert platinum agents into a reactive form. These complexes forms are believed to bind to the nuclear DNA, react in the cytoplasm with thiol-containing molecules—methionine, metallothioneins (MT) and glutathione (GSH). Thus, only a portion of complex can bind to the nuclear DNA that urges cell to apoptosis process, causing cell death. [30]

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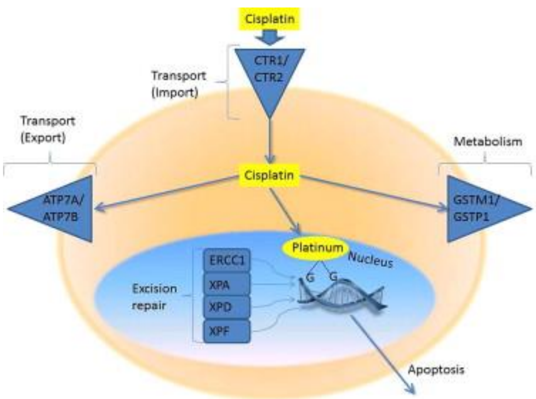

Figure 3 Cisplatins' mechanism of action[31]

Although the cisplatin motivates several signal transduction pathways, the treatment thought to establish the cytotoxic effect by forming crosslink on DNA. Cisplatin forms covalent bond to two adjacent guanines on the same strand of the nuclear DNA, interrupting replication. Cisplatin and carboplatin can form three different types of DNA lesions on purine bases: monoadducts, intrastrand crosslinks, and interstrand crosslinks, leading to various cellular responses including replication arrest, transcription inhibitor, cell-cycle arrest, DNA repair and apoptosis. Although oxaliplatin forms fewer crosslink-DNA protein bound than other platinum agents, it is can also break on DNA similarly to the other types of DNA adduct do.[8, 9, 31]

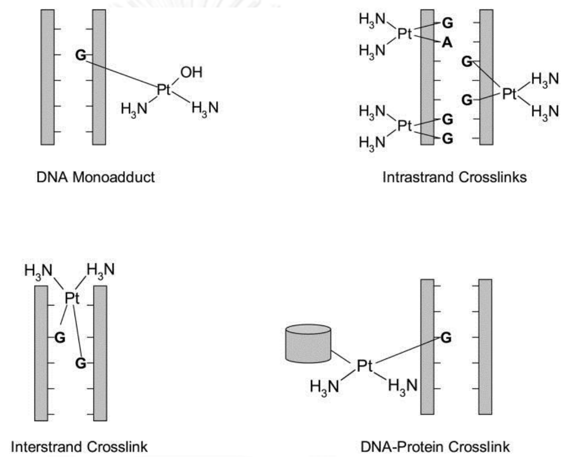

Figure 4 Platinum agents adduct on DNA[9]

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Cisplatin is associated with adverse events (AEs) on nervous system, the organ of Corti, and the kidney. Nephrotoxicity and peripheral neurotoxicity are the most common toxicities. Incidence of cisplatin-induced acute renal failure with the cumulative dose is varying from 14 to 100% of patients.[32, 33] Approximately half of patients have peripheral neurotoxicity, but the onset of toxicity is delayed until a cumulative dose is more than 300 mg/m^2.[34] In addition, nausea and vomit are the most common symptoms found in patients who receive cisplatin whereas both carboplatin and oxaliplatin cause less emetogenic effect.[9]

As for carboplatin, the major AE is myelosuppression involved in dose dependent, characterized by thrombocytopenia and neutropenia. Moreover, almost 12% of carboplatin-treated patients' develop hypersensitivity, but the use of cisplatin in patients who have developed hypersensitivity by carboplatin is not recommended because of the feasibility of fatal cross-hypersensitivity.[9, 32, 35] A study showed association between gene polymorphisms and cisplatin toxicities, found in *CTR1* rs10981694 patients carrying C allele who are less tolerant to ototoxicity (OR, 0.47; P < .01).[26]

Because of serious AEs involved in platinum agents, a third-generation drug was developed to avoid substantial toxicity by other standard platinum regimens. Although platinum-based regimens have higher response rates than third-generation drugs (-6%, 95% CI: -11% - 0%), there is non-statistic significant difference in 1-year survival rate (3%, 95% CI: -3% - 10%).[36, 37]

Treatment outcomes of cancer can be evaluated according to the response evaluation criteria in solid tumors (RECIST) version 1.1[38] which by determining the same method and technique to characterize each identified lesion, and imaging base evaluation at baseline and during follow-up as 4 types including:

Table 3 Treatment response[38]

Response	Definition
1. complete response (CR)	Disappearance of all target lesions
2. partial response (PR)	At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD
3. stable disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started
4. progressive disease (PD)	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

One of the major problems found in patients receiving platinum regimens is that tumors become resistant. Strong evidences also support that the genetic factor possibly affects disease progression and patient response to cisplatin therapy. Genetic polymorphisms may be one of prognosis markers for cisplatin response and overall survival of NSCLC patients. Three different pathways involved in cellular mechanism resistance are decreased platinum accumulation in cytoplasm, increased intracellular detoxification, and increased DNA repair capacity.[11]

A large number of research reported that polymorphisms in *ERCC1*, *GSTP1* and *CTR1* genes which have demonstrated various clinical response, are resulted from genetic variances. Literatures involve in genetic polymorphisms affecting clinical outcomes are gathered. These selected SNPs of three genes *ERCC1* rs11615, *GSTP1* rs1695, and *CTR1* rs12686377 that polymorphisms showed significant different tumor response and toxicity were reviewed

Platinum agents induce cell death by creating platinum-DNA adducts. Nucleotide excision repair (NER) is a DNA repair system that removes such DNA adducts. *ERCC1* gene in NER pathway is perhaps a major component which affects platinum-resistance through the process of elimination of the DNA lesion. Previous research revealed the relationship between genetic polymorphisms and a response of platinum chemotherapy in advanced NSCLC patients. A variety of genotypes had influences on platinum-response. Participants with C/C genotype of *ERCC1* C118T had better treatment outcomes from platinum therapy. Zhao et al. conducted a study in 192 Chinese patients and found that patients with C/C genotype had significant higher response rates than those with T/T genotype (OR = 0.27, 95%CI = 0.10 – 0.71, *P* = 0.003), while patients with T/T genotype showed a significant increase in risk of death compared to those with C/C genotype (HR = 2.38, 95%CI = 1.03 – 6.13, *P* = 0.04).[17] Cheng and colleagues also showed that C/C genotype patients had a 2.04-fold higher positively short-term response rates than those with combined C/T and T/T cases (OR = 2.041, 95%CI = 1.065 – 3.910, *P* = 0.032). Moreover, Cheng et al. found that C/C genotype improved the long-term survival as measured by median progression free survival (PFS) and overall survival (OS) rates in a group of 142 Chinese patients.[14] However, in a study of 187 Mongolians with advanced NSCLC stage IIIA-IV, polymorphisms were not associated with either tumor response or clinical outcomes.[39] Similar results from a study of Krawczyk et al. performed in 115 Caucasians found no significant response to platinum-based regimen, and a study of Mlak and colleagues in 62 Caucasians found that there was no statistically significant relationship between SNPs and the response to therapy or PFS.[40, 41] Krawczyk et al. also reported that results of 43 Caucasians with C/C and C/T genotypes had significantly higher median of PFS than T/T genotype (4 vs 0.3 months, HR = 0.438. 95%CI = 0.084 – 0.881, *P* = 0.03). Furthermore, C/C and C/T genotype patients had one-year overall survival longer than T/T genotypes patient, but there was no statistic significant difference (50% vs. 28.6%, *P* = 0.531).[42] However, inconsistent data was also reported as Sullivan et al. studied 162 Caucasians and found that patients with T/T genotype or those harboring T allele (T/T or T/C) showed significantly higher response rates (RR) than those with a C/C genotype (83.9% vs. 50.0%; OR = 0.11; 95% CI = 0.01–0.66; *P* = 0.015 in a recessive model). Otherwise, PFS, OS and toxicity were not significant differences.[19] Furthermore, Ren and colleagues studied 388 Chinese patients and found those patients with C/T or T/T elucidated longer median survival rates than those with C/C allele (18 vs. 13.8 months, *P* = 0.014). Moreover the study also showed that in the elder group (age ≥ 70 years) with C/C genotype had poorer prognosis than patients with harboring T allele (12.5 vs. 28.5; *P* = 0.022).[43] In addition, Li et al. found that the response rate of C/T and T/T was higher than that of C/C genotype, but there was no statistic significant difference (OR = 1.997, 95%CI = 0.778–5.062, *P* = 0.145; adjusted OR = 1.892, 95%CI = 0.728–4.915, *P* = 0.191).[44] In terms of toxicity, Powrozek et al. found no association between SNPs in *ERCC1* rs11615 and toxicity resulting from platinum therapy in 55 Caucasians.[15]

When platinum agents enter into cell cytoplasm, the aquated platinum complexes create more reactive specie to bind DNA. Conjugated with glutathione enhances solubility, that causing chemoresistance. *GSTP1* is the crucial isoform of GSTs family in lung tissues which responsible for detoxification of platinum agents regarding to phase II metabolism. A number of information demonstrated the association between tumor response and SNPs of *GSTP1* rs1695. ADA et al. reported that in 138 Turkish, *GSTP1* polymorphisms were related to survival.[45] Patients with variant genotypes (A/G and G/G genotype) had higher response rates to platinum therapy than wild type patients. Han et al. discovered in 325 Mongolian patients and found that the *GSTP1* polymorphism significantly associated with response rates to chemotherapy, patients with A/G and G/G genotypes exhibited higher response rates than patients carried A/A genotype (OR = 2.31, 95%CI = 1.35-3.95 and OR = 5.68, 95%CI = 1.61-30.46, respectively). In terms of survival rates, patients with G/G genotype had longer survival time compared to those with A/A genotype (54.50 vs 22.20 months, HR = 0.36, 95%CI = 0.11-0.98, P = 0.03).[21] Similar to the study in 116 Chinese patients by Zhou and colleagues which found that SNPs of *GSTP1* were significant associated with tumor response. Patients who carried at least one allele of G (A/G or G/G) were more responding than those with A/A genotype (OR = 3.961, 95%CI = 1.531-10.245, P = 0.05).[22] Sun et al. also noted that 113 of the Han Chinese patients harboring variant allele (A/G or G/G) had higher response rates to platinum chemotherapy than wild type (A/A) patient. After adjusting by logistic regression, the OR for response were 2.881, and the 95%CI were between 1.167 and 7.113 (P = 0.022).[23]

On the other hand, Deng and colleagues studied 97 Han Chinese patient, and found that those with A/A genotype had higher tumor response rates than A/G and G/G (OR = 4.302, 95%CI = 1.193-15.515, P = 0.026). Moreover, patients with A/A carriers had longer disease control rate (DCR) than those with other carriers, A/G and G/G (OR = 3.740, 95%CI = 1.238-11.298, P = 0.019).[20] Indeed, Pillot et al. informed that 42 Caucasian patients with A/A genotype had more responses to platinum-based regimen than patients with A/G and G/G, but the difference was not statistically significant.[46] Nonetheless, Booten and colleagues showed no significant relationships between different genotypes and response to chemotherapy in 433 Caucasians.[47]

Platinum agents enter into the cell by passive diffusion and active pathway. Regarding active pathway, human copper transporter 1 (hCTR1) is an essential protein for uptaking platinum agents into intracellular encoded by *CTR1*. Xu et al. reported that 282 Han Chinese patients with T/T genotype were more likely to be resistant to platinum-based chemotherapy (OR = 0.41, 95%CI = 0.21 – 0.78, P = 0.01). However, the difference in survival rates was not significant.[25]

These findings are inconsistent in terms of impacts of drug metabolizing enzyme polymorphisms on survival and toxicity. More information is needed to create more precise tool for polymorphisms influencing on tumor response and toxicity resulted from platinum-based chemotherapy.

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CHAPTER III
RESEARCH METHODOLOGY

This research was conducted during January 2016 to July 2016 at out-patient department, King Chulalongkorn Memorial Hospital (KCMH), Bangkok, Thailand.

PART I Methodology

1. Research design

A prospective cohort study was conducted. Participants' demographic data and blood samples were collected and then genotypic analysis was performed. Medical record review and patients interview were done to collect information of treatment responses and toxicity evaluation. The effects of *ERCC1* rs11615, *GSTP1* rs1695 and *CTR1* rs12686377 polymorphisms on treatment outcomes in advanced NSCLC patients treated with platinum-based chemotherapy were evaluated.

2. Patients

2.1 Population and sample

1. Population

   The patients who were pathologically diagnosed with advanced NSCLC in stage IIIB – IV at KCMH.

2. Sample

   Participants with stage IIIB and IV of NSCLC, treated with platinum-based chemotherapy during study period were recruited.

2.2 Inclusion criteria

1. Age ≥ 18 years old.
2. Participants who were diagnosed with histologically or cytologically documented advanced NSCLC stages of IIIB and IV disease.

3. Participants with Eastern Cooperative Oncology Group (ECOG, PS) status 0–2.
4. Participants who were planning to be treated with platinum–based chemotherapy for at least two cycles.

2.3 Exclusion criteria

1. Participants who were pregnant or lactating.
2. Participants who were received surgery or radiation for lung cancer treatments, or any chemotherapy regimen other than platinum-contained agents.
3. Participants who disagreed to sign informed consent.

2.4 Sample size calculation

To estimate numbers of participants in this study, previous studies were report the prevalence of responder, there were 0.44 and 0.78, while the relative risk were 0.28 and 0.59. These were reported by Sullivan and colleague and Krawczyk and team.[19, 42] Expected dropout rate around 20%, and given alpha-error as 0.05. As for precision, that allowable error as 0.1, so in this trial required number approximately 70 of participants.

The following term were defined as

n	=	sample size
Z_{α}	=	1.96
Z_{β}	=	1.28
P_1	=	the prevalence of responder
R	=	relative risk
E	=	allowable error

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

$$\tilde{p}=\frac{1}{2}\,(p_1)(1+R), \quad \tilde{q} = 1 - \tilde{p}$$

$$n = \frac{\{1.96\sqrt{2(0.44)(0.57)} + 1.28\sqrt{(0.61)\big(1 + 0.61 - (1 + 0.44^2)\big)}\}^2}{(0.61)(1 - 0.44)^2}$$

3. **Study protocol**

This prospective cohort study enrolled participants with advanced NSCLC who received platinum-based regimen for 6 months after Ethic Committee approved at KCMH.

1. Study protocol was approved by Institutional Review Board, Faculty of medicine, Chulalongkorn University, Bangkok, Thailand, IRB number 598/58.
2. Participants were selected according to inclusion and exclusion criteria.
3. Participants signed in the informed consent form at Wongwanich building 4th floor. Participants have the free choice, based on sufficient and timely information concerning the benefits and disadvantages of the project, of whether and how these activities occur, according to their systems of customary decision-making.
4. Baseline characteristics and clinical information were collected. The information was collected from participants' medical record as follow: age, sex, weight, performance status (PS), histological types and TMN stage. For smoking history and symptoms related to adverse events (AEs), participants were interviewed by investigator.
5. Before participants received platinum-based regimen, participants' baseline were collected from participants' medical record. As regarding laboratory tests, there were including complete blood count (CBC) and renal function tests. In terms of CBC, there were collected follow as: Red

blood cell (RBC), Hemoglobin (Hgb), Hematocrit (Hct), White blood cell (WBC), neutrophils, lymphocyte, monocyte and eosinophil. With regarding renal function tests, serum creatinine, blood urea nitrogen (BUN) and proteinuria were collected.

6. Blood sampling were processed for DNA extraction and genotyping. For the isolated of DNA from peripheral blood lymphocytes, Qiagen blood mini kit (Qiagen, German) were using by the manufacture's protocol. Analysis genotypes of *ERCC1* rs11615, *GSTP1* rs1695 and *CTR1* rs12686377 were performed by using real time polymerase chain reaction restriction (qPCR): Taqman® assay. The specimens were stored for 1 year after the study completion date. After that, the specimens would be destroyed by facilities; the facilities must provide certification of destruction of each individual sample, or a batch of sample as a whole, in writing to the principle investigation.
7. At visit 2–7 of chemotherapy, participants were evaluated for AEs according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. [Appendix B] Adverse events of chemotherapy of participants with irregular baseline characteristics were evaluated when changing the grade.
8. At visit 3–7, participants were evaluated for treatment response following to response evaluation criteria in solid tumors (RECIST) version 1.1 into 4 types including: complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Then, participants were defined as 2 groups follow as: "responders" who with CR and PR and "non - responders" who with SD and PD.
9. Genotypes of *ERCC1*, *GSTP1* and *CTR1* genes were analyzed related to demographic, treatment response and toxicity resulting from chemotherapy. Statistical analysis of the results performed using the computer software SPSS version 22.

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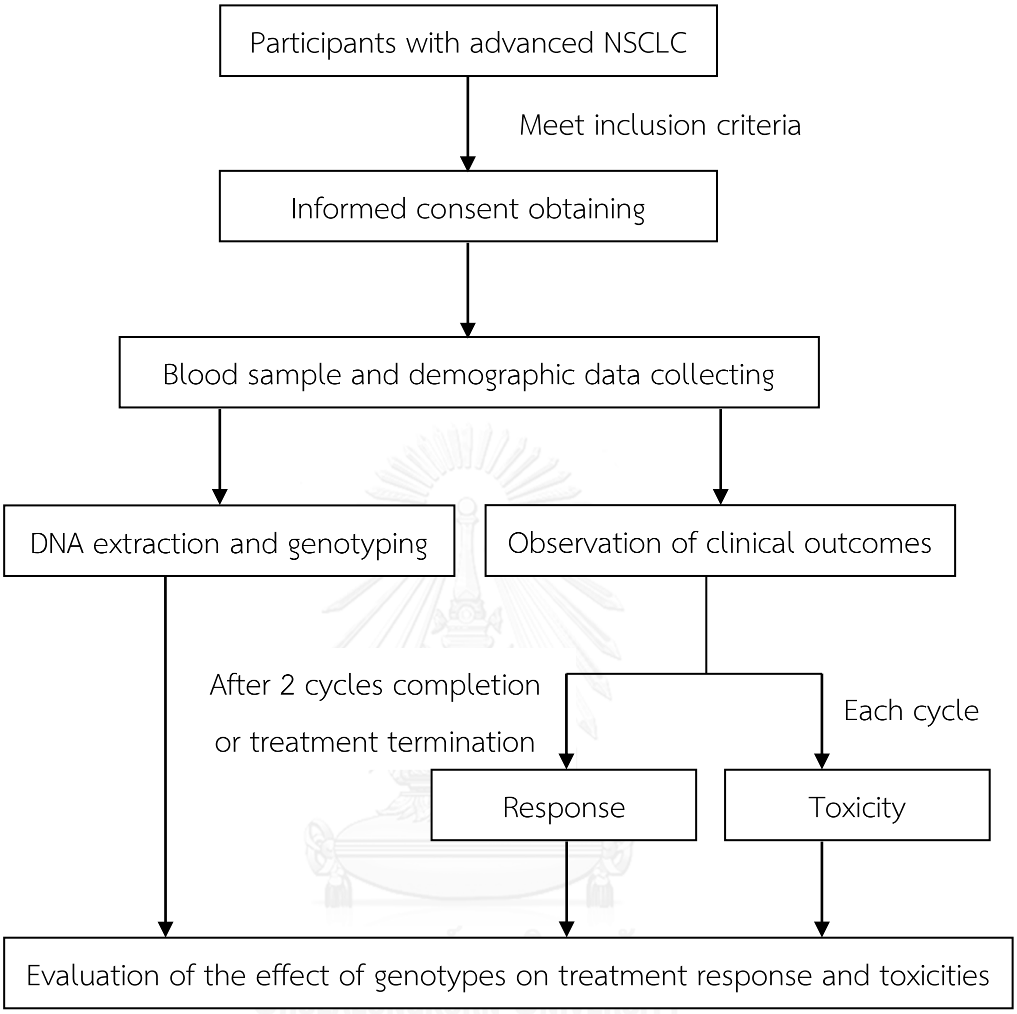

Figure 5 Procedure of methodology

4. Bio-analysis

4.1 Plasma extraction

1. EDTA tube contained 5 ml of blood sample.
2. Blood tube was centrifuged for 10 minutes at 2500 x *g* at room temperature (15–25 °C).
3. This gave three layers: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; concentrated erythrocytes in the bottom layer.
4. Carefully aspirated the buffy coat and pooled into a centrifuge tube and stored at –20 °C.

4.2 DNA extraction

1. Equilibrate samples to room temperature (15–25°C).
2. Pipet 20 microliter QIAGEN Protease (or proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.
3. Added 200 microliter sample to the microcentrifuge tube. Used up to 200 microliter whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 106 lymphocytes in 200 microliter PBS.
4. Added 200 microliter Buffer AL to the sample. Mix by pulse-vortexing for 15 s.
5. Incubated at 56°C for 10 min.
6. Briefly centrifuged the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
7. Added 200 microliter ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuged the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
8. Carefully applied the mixture from step 6 to the 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 min. Placed the

QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discarded the tube containing the filtrate.

9. Carefully opened the QIAamp Mini spin column and added 500 microliter Buffer AW1 without wetting the rim. Closed the cap and centrifuged at 6000 x *g* (8000 rpm) for 1 min. Placed the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discarded the collection tube containing the filtrate.
10. Carefully opened the QIAamp Mini spin column and added 500 microliter Buffer AW2 without wetting the rim. Closed the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
    Recommended: Placed the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discarded the old collection tube with the filtrate. Centrifuged at full speed for 1 min.
11. Placed the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discarded the collection tube containing the filtrate. Carefully opened the QIAamp Mini spin column and added 200 microliter Buffer AE or distilled water. Incubated at room temperature (15–25°C) for 1 min, and then centrifuged at 6000 x *g* (8000 rpm) for 1 min.

4.3 Genotyping

1. Created and set up a sequence detector plate document.
2. Prepared the reaction plate using reaction mix per well plate following:
    - 10 microlitre of 2x TaqMan Universal PCR Master Mix
    - 6.5 microlitre of DNase-free water
    - 3 microlitre of 30 nanogram genomic DNA sample
    - 0.5 microlitr of 40x Primer and TaqMan Probe (FAM) dye mix
3. Ran the plate on an ABI PRISM® Sequence Detection System or Real-Time PCR System using the following thermal cycling conditions: Amplification by polymerase chain reaction for 10 minute at 95° followed by 40 cycles of 15 seconds at 95°C and 1 min at 60°C.

4. Generated a standard curve to quantify the amount of DNA in each sample.

After PCR amplification, performed an endpoint plate read on a Realtime PCR instrument. Using the fluorescence measurements made during the plate read, the SDS software plots R_n values based on the fluorescence signals from each well, and then determined which alleles are in each sample.

5. **Tools**

1. Data collecting sheet [Appendix C]
2. Information sheet [Appendix D]
3. Consent form [Appendix E]

6. **Data collection**

Table 4 Time schedule for information collecting

Time	Data
Visit 1	Demographics; age, sex, weight, height, body surface area (BSA), performance status (PS), smoking history, histological types, chemotherapy treatment regimen and TMN stage at entry
Visit 2 – 7	Adverse events resulting from chemotherapy; emetic effect, hematological toxicity, nephrotoxicity and neurotoxicity
Visit 3 – 7	Response to chemotherapy

PART II **Data analysis and statistics**

Statistical analysis of the results was performed using the computer software SPSS version 22 (SPSS. Co., Ltd., Bangkok, Thailand). All tests were 2 – sides and difference was considered as statistic significant when *P* value was less than 0.05.

1. **Demographic**

The baseline characteristics were performed using mean ± standard deviation (SD) for continuous variables and number of participants (%) for category variables.

2. **Clinical outcomes**

The clinical information on toxicity and response was compared across genotype by performed χ^2 or Fisher exact test for categorical variables. In terms of OR and DCR were expressed as number of participants (%).

Table 5 Statistic method for analyzing data

Hypothesis	Variable (*)		Statistic
1. There are differences in treatment response among different genotypes of *ERCC1* rs11615, *GSTP1* rs1695, and *CTR1* rs12686377.	***Independent variables*** *(category data)*		χ^2 or Fisher exact test
	wild type	(0)	
	variant heterozygous	(1)	
	variant homozygous	(2)	
	Dependent variables *(category data)*		
	responders	(0)	
	non – responders	(1)	
2. There are differences in adverse events among different genotypes of *ERCC1* rs11615, *GSTP1* rs1695, and *CTR1* rs12686377.	***Independent variables*** *(category data)*		χ^2 or Fisher exact test
	wild type	(0)	
	variant heterozygous	(1)	
	variant homozygous	(2)	
	Dependent variables *(category data)*		
	not found	(0)	
	grade 1	(1)	
	grade 2	(2)	
	grade 3	(3)	
	grade 4	(4)	

(*) The number used instead the variables in statistical analysis.

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PART III Ethical consideration

In this study, all participants will sign an informed consent form for their medical information and blood sample collection. This study will be submitted for approval by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University and will perform in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Respect for person

Researcher will clearly described the research protocol, involving in benefit and harm that participants may entail. Participants must be completely independent to give informed consent and to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Data or material identification must be confidential disclosure in study report and publication.

Beneficence/ non – beneficence

Participants will not receive any direct benefit, but in the future more information may be enhance efficacy and safety in patients who treated with platinum – base regimen. In this study, participants may have pain and bruise while blood sample collected. Participants will not earn any payment, but this trial supports 300 Baths for travelling expenses.

Justice

In this study, researchers will concern the equality of age, sex and race. Inclusion and exclusion criteria will be explicit. There are several widely accepted formulations of just ways to distribute burdens and benefits. Each formulation mentions some relevant property on the basis of which burdens and benefits should be distributed. These formulations are (1) to each person an equal share, (2) to each person according to individual need, (3) to each person according to individual effort, (4) to each person according to societal contribution, and (5) to each person according to merit.

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CHAPTER IV

RESULTS

PART I Patient characteristics

In this study, 74 participants with advanced NSCLC who met inclusion criteria were enrolled from KCMH during January to July 2016. Five cases were excluded due to alteration of performance status contributed to the modification of chemotherapy regimens in three participants. One of participant was referred to other hospital, and another one participant was dead prior to receive the treatment. Thus, present study had 69 participants for evaluation of tumor response, and two participants had chronic kidney disease (CKD) which could not evaluate for hematological toxicity.

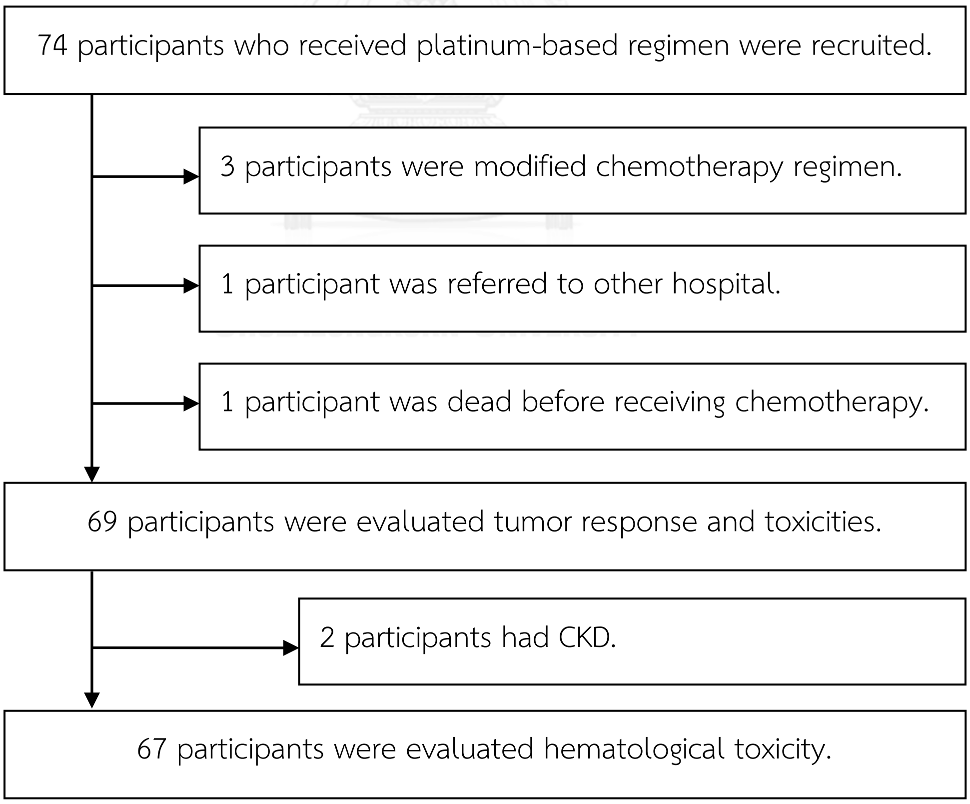

Figure 6 Participant enrollment

Seventy-four participants were included. The proportion of male and female was nearly equal. Thirty-four participants (45.9%) were male and 40 participants (54.1%) were female, their mean age was 61.45 ± 11.57 years, as shown in Table 6.

Table 6 Participants characteristics

Total number of participants	**74**	
Age (mean ± SD)	61.45 ± 11.57	
Age range (min, max)	28, 87	
Characteristic	N	%
Gender		
Male	34	45.9
Female	40	54.1

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When genetic polymorphism at *ERCC1* rs11615, *GSTP1* rs1695 and *CTR1* rs12686377 were analyzed the genotypic frequency of *ERCC1* rs11615 as CC, CT and TT were 52.7%, 39.2%, and 8.1%, respectively. As for *GSTP1* rs1695, the frequency of genotype was found as AA, AG and GG at 55.4%, 39.2% and 5.4%, respectively. Regarding to *CTR1* rs12686377, genotypic frequency was found as GG, GT, and TT at 62.2%, 31.1% and 6.8%, as shown in table 7.

Table 7 Genotype frequency

Total number of participants	**74**		
Gene	Genotype	N	%
ERCC1	CC	39	52.7
	CT	29	39.2
	TT	6	8.1
GSTP1	AA	41	55.4
	AG	29	39.2
	GG	4	5.4
CTR1	GG	46	62.2
	GT	23	31.1
	TT	5	6.8

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Allele frequency of polymorphism at *ERCC1* rs11615, *GSTP1* rs1695 and *CTR1* rs12686377, *ERCC1* polymorphism C→T, *GSTP1* polymorphism A→G and polymorphism G→T were 27.7%, 25%, and 22.3%, respectively, as shown in Table 8.

Table 8 Allele frequency

Total number of participants	**74**						
Gene	Allele	74 x 2 allele		Genotype	N	%	Predicted
		N	%				(HWE)
ERCC1	C	107	72.3	CC	39	52.7	38.7
				CT	29	39.2	29.6
	T	41	27.7	TT	6	8.1	5.7
GSTP1	A	111	75.0	AA	41	55.4	41.6
				AG	29	39.2	27.8
	G	37	25.0	GG	4	5.4	4.6
CTR1	G	115	77.7	GG	46	62.2	44.7
				GT	23	31.1	25.6
	T	33	22.3	TT	5	6.8	3.7

As mention earlier, only 69 participants were evaluated for association between polymorphism and tumor response. Thirty-two participants, or 46.4% were male and 37 participants (53.6%) were female, average mean age were 61.86 ± 11.76 years old. Their average mean body weight was 54.23 ± 9.65 kilograms and average mean body surface area was 1.54 ± 0.17 m^2. As regard complete blood count baseline, average hemoglobin was 12.05 ± 1.97 g/dL, average neutrophil was 5,934.74 ± 3,585.26 cell/mm^3 and average platelet was 317.33 ± 114.85 x 10^3 cell/mm^3. More than half of them or 39 participants (56.5%) were 4 cycles of chemotherapy. With regarding to smoking status, nearly half of all or 28 participants (40.1%) had smoker. As for histology, almost of them or 61 participants (88.4%) had adenocarcinoma. Almost all of participants, or 58 participants (84.1%) had clinical stage IV. Nearly all of participants were in good performance status represented by the ECOG score 0 in 10 participants or 14.5% and ECOG score 1 (56 participants or 81.2%). In terms of chemotherapy regimen, the participants received carboplatin with gemcitabine, paclitaxel, pemetrexed and single agent carboplatin were 31 (44.9%), 24 (34.8%), 9 (13%), and 5 (7.2%), respectively. Characteristic of 69 cases are shown in Table 9-11.

Table 9 Participants characteristics

Total number of participants 69		
Characteristic	**Mean ± SD**	**min, max**
Before received chemotherapy		
Age (year)	61.86 ± 11.76	28, 87
Body weight (kilogram)	54.23 ± 9.65	34, 74
Body surface area (m^2)	1.54 ± 0.17	1.17, 1.91
Hemoglobin (g/dL)	12.05 ± 1.97	7.2, 17.00
Neutrophil (cell/mm^3)	5,934.74 ± 3,585.26	1,400, 20,420
Platelet (x 10^3 cell/mm^3)	317.33 ± 114.85	118, 698
Serum creatinine (mg/dL)	0.84 ± 0.32	0.38, 1.84

Table 10 Number of chemotherapy cycle

Total number of participants 69		
Characteristic	N	%
Number of chemotherapy cycle		
2	7	10.1
3	6	8.7
4	39	56.5
5	9	13.0
6	8	11.6

Table 11 Participants characteristics

Total number of participants	**69**	
Characteristic	N	%
Gender		
Male	32	46.4
Female	37	53.6
Smoking Status		
Smoker	28	40.1
Non smoker	26	37.7
Second hand smoker	15	21.7
Histology		
Adenocarcinoma	61	88.4
Squamous cell carcinoma	4	5.8
Not specified	2	2.9
Other	2	2.9
Clinical Stage		
IIIB	10	14.5
IV	58	84.1
Other	1	1.4
Performance status		
ECOG score = 0	10	14.5
ECOG score = 1	56	81.2
ECOG score = 2	3	4.3
Chemotherapy Regimen		
Carboplatin/Gemcitabine	31	44.9
Carboplatin/Paclitaxel	24	34.8
Carboplatin/Pemetrexed	9	13.0
Single agent carboplatin	5	7.2

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Genetic polymorphisms of 69 cases were reported in Table 12. Genotypic frequency at *ERCC1* rs11615 was CC, CT and TT 50.7%, 40.6%, and 8.7%, respectively. As for *GSTP1* rs1695, the frequency of genotype was found as AA, AG and GG 53.6%, 40.6% and 5.8%, respectively. With regarding to *CTR1* rs12686377, genotypic frequency was found as GG, GT, and TT 59.4%, 33.3% and 7.2%.

Table 12 Genotype frequency

Total number of participants	**69**		
Gene	Genotype	N	%
ERCC1	CC	35	50.7
	CT	28	40.6
	TT	6	8.7
GSTP1	AA	37	53.6
	AG	28	40.6
	GG	4	5.8
CTR1	GG	41	59.4
	GT	23	33.3
	TT	5	7.2

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Treatment responses were presented in Table 13. None of participants had complete response (CR), whereas 9 participants (13%) had partial response (PR) to platinum-based chemotherapy. 87% of participants did not response to the chemotherapy, 42 participants (60.9%) had stable disease (SD), and 18 participants (26.1%) had progressive disease (PD).

Table 13 Tumor response

Total number of participants	**69**	
Response	N	%
PR	9	13.0
SD	42	60.9
PD	18	26.1

There was no difference in response rate in each chemotherapy regimens, as shown in Table 14. Gender, smoking status, histology, clinical stage and performance status, number of cycles, age, body weight and body surface area, were not associated with overall response, as shown in Table 15-16.

Table 14 Association between chemotherapy regimens and overall response

Total number of participants	**69**				
Regimen	N	Overall response		Overall response	*P* value
		CR + PR (%)	CR + PR (%)		
Carboplatin/Gemcitabine	31	2 (6.5)	29 (93.5)	5.776	0.123
Carboplatin/Paclitaxel	24	6 (25.0)	18 (75.0)		
Carboplatin/Pemetrexed	9	0 (0)	9 (100)		
Single agent carboplatin	5	1 (20.0)	4 (80.0)		

Table 15 Association between patients' characteristic and overall response

Total number of participants 69					
Factor	N	Overall response		χ^2 value	*P* value
		CR + PR (%)	SD + PD (%)		
Gender				0.708	0.400
Male	32	3 (9.4)	29 (90.6)		
Female	37	6 (16.2)	31 (83.8)		
Smoking Status				3.732	0.155
Smoker	26	5 (19.2)	21 (80.8)		
Non smoker	28	1 (3.6)	27 (96.4)		
Second hand smoker	15	3 (20.0)	12 (80.0)		
Histology				3.835	0.147
Adenocarcinoma	61	6 (9.8)	55 (90.2)		
Squamous cell carcinoma	4	0 (0)	4 (100)		
Other	2	1 (50.0)	1 (50.0)		
Clinical Stage				0.625	0.732
IIIB	10	2 (20.0)	8 (80.0)		
IV	58	7 (12.1)	51 (87.9)		
Other	1	0 (0)	1 (100)		
Performance status				1.185	0.553
ECOG score = 0	10	1 (10.0)	9 (90.0)		
ECOG score = 1	56	7 (12.9)	49 (87.5)		
ECOG score = 2	3	1 (33.3)	2 (66.7)		
Number of cycles				3.176	0.529
2	7	0 (0)	7 (100)		
3	6	0 (0)	6 (100)		
4	39	6 (15.4)	33 (84.6)		
5	9	1 (11.1)	8 (88.9)		
6	8	2 (25.0)	6 (75.0)		

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Table 16 Association between age, body composition and overall response

Total number of participants 69					
Factor	N	Overall response		χ^2 value	*P* value
		CR + PR (%)	SD + PD (%)		
Age				0.708	0.400
< 62 year	32	3 (9.4)	29 (90.6)		
≥ 62 year	37	6 (16.2)	31 (83.8)		
Body weight				0.097	0.756
< 54 kg	34	4 (11.8)	30 (88.2)		
≥ 54 kg	35	5 (14.3)	30 (85.7)		
Body surface area				0.562	0.453
< 1.54 m^2	31	3 (9.7)	28 (90.3)		
≥ 1.54 m^2	38	6 (15.8)	32 (84.2)		

As regard to toxicities, most frequent adverse event found in this study was anemia (69.6%). Follow by nausea and vomit (58%) and neutropenia (44.9%), other adverse events were shown in Table 17.

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Table 17 Toxicities

Total number of participants 69					
Toxicities	All grades	Grade			
	N (%)	I	II	III	IV
Anemia	48 (69.6)	17 (35.4)	19 (39.6)	12 (25.0)	
Nausea and vomit	40 (58.0)	15 (37.5)	21 (52.5)	4 (10.0)	
Neutropenia	32 (47.8)	11 (34.4)	11 (24.4)	10 (31.3)	
Peripheral neuropathy	21 (30.4)	14 (66.7)	6 (28.6)	1 (4.8)	
Thrombocytopenia	17 (24.5)	13 (76.5)	1 (5.9)	2 (11.8)	1 (5.9)
Weight loss	11 (15.9)	8 (72.7)	3 (27.3)		

According to the toxicity outcome, no statistical significant difference was observed between toxicities and chemotherapy regimens. Also, confounders involving in gender, smoking status, histology, clinical stage and performance status was not associated with toxicities. These findings were similar the response to chemotherapy outcome.

PART II Association between genetic polymorphisms and treatment responses

For *ERCC1*, participants with homozygous wild type (CC) had higher rate of overall response (17.1% vs. 10.7% and 0%) when compared with other genotypes. While opposite pattern was discovered in *GSTP1*, participants with homozygous variant type (GG) tended to have higher response rate. Similar pattern was found in *CTR1* polymorphism, higher response rate were found in variant genotype (GT). However, there was no statistical association between genetic polymorphisms with treatment response either in overall response rate or disease control rate as elaborated in Table 18 and 19.

Table 18 Association between genetic polymorphisms and overall responses

Total number of participants 69						
Gene	Genotype	N	Overall response		χ^2 value	*P* value
			CR + PR (%)	SD + PD (%)		
ERCC1	CC	35	6 (17.1)	29 (82.9)	1.553	0.460
	CT	28	3 (10.7)	25 (89.3)		
	TT	6	0 (0)	6 (100)		
GSTP1	AA	37	5 (13.5)	32 (86.5)	0.645	0.724
	AG	28	3 (10.7)	25 (89.8)		
	GG	4	1 (25.0)	3 (75.0)		
CTR1	GG	41	5 (12.2)	36 (87.8)	1.159	0.560
	GT	23	4 (17.4)	19 (82.6)		
	TT	5	0 (0)	5 (100)		

Table 19 Association between genetic polymorphism and disease control rate

Total number of participants	**69**					
Gene	Genotype	N	Disease control rate		χ^2 value	*P* value
			PR + SD (%)	PD (%)		
ERCC1	CC	35	28 (80.0)	7 (20.0)	2.542	0.281
	CT	28	20 (71.4)	8 (28.6)		
	TT	6	3 (50.0)	3 (50.0)		
GSTP1	AA	37	28 (75.7)	9 (24.3)	0.152	0.927
	AG	28	20 (71.4)	8 (28.6)		
	GG	4	3 (75.0)	1 (25.0)		
CTR1	GG	41	29 (70.7)	12 (29.3)	0.537	0.765
	GT	23	18 (78.3)	5 (21.7)		
	TT	5	4 (80.0)	1 (20.0)		

As in this study, majority of patients (N = 31) received carboplatin and gemcitabine regimen. This subgroup was analyzed to explore the association between genetic polymorphisms and between treatment responses. it could be seen that participants with allele C of *ERCC1* had more disease control rate than participants with allele T (*P* = 0.029), patients with harboring T at *ERCC1* rs11615 was a 6.5-fold higher poor prognosis than patients with CC genotypes, 95% confidence interval (CI) had between 1.09 and 38.63. In contrast, there was no relationship between disease control rate and SNPs in *GSTP1* (*P* = 0.88) and *CTR1* (*P* = 0.50), as shown in Table 20.

Table 20 Association between genetic polymorphism and disease control rate in patient who received carboplatin/gemcitabine regimen

Total number of participants	31							
Gene	Genotype	N	Disease control rate		*P* value	Odds	95% CI	
			PR + SD (%)	PD (%)		Ratio	Lower	Upper
ERCC1	CC	15	13 (86.7)	2 (13.3)	0.029*	1		
	CT + TT	16	8 (50.0)	8 (50.0)		6.5	1.09	38.63
GSTP1	AA	13	9 (69.2)	4 (30.8)	0.88	1		
	AG + GG	18	12 (66.7)	6 (33.3)		1.125	0.243	5.207
CTR1	GG	19	12 (63.2)	7 (36.8)	0.50	1		
	GT + TT	12	9 (75.0)	3 (25.0)		0.571	0.115	2.845

The second most common used chemotherapy regimen in this study was carboplatin and paclitaxel, 24 participants were given this regimen. However, the association between genetic polymorphisms was not seen when subgroup analysis was performing as described in table 21.

Table 21 Association between genetic polymorphism and disease control rate in patient treated with carboplatin/paclitaxel regimen

Total number of participants	24							
Gene	Genotype	N	Disease control rate		*P* value	Odds	95% CI	
			PR + SD (%)	PD (%)		Ratio	Lower	Upper
ERCC1	CC	12	8 (66.7)	4 (33.3)	0.653	1		
	CT + TT	12	9 (75.0)	3 (25.0)		0.667	0.113	3.930
GSTP1	AA	14	9 (64.3)	5 (35.7)	0.404	1		
	AG + GG	10	8 (80.0)	2 (20.0)		0.450	0.068	2.998
CTR1	GG	15	10 (66.7)	5 (33.3)	0.562	1		
	GT + TT	9	7 (77.8)	2 (2.2)		0.571	0.085	3.883

When considering combined genotype (homozygous wild type and variant genotype) of each gene, it could be seen that there was not statistically significantly related to each genotype of three genes and disease control rate, as shown in Table 22 – 24.

Table 22 Association between genetic polymorphism and disease control rate in combined genotype (*ERCC1* and *GSTP1*)

Total number of participants	**69**							
allele	**allele**	N	Disease control rate		*P*	Odds	95% CI	
ERCC1	***GSTP1***		PR + SD (%)	PD (%)	value	Ratio	Lower	Upper
CC	AA	17	15 (88.2)	2 (11.8)	0.237	1		
	AG + GG	18	13 (72.2)	5 (27.8)		2.885	0.477	17.454
CT + TT	AA	20	13 (65.0)	7 (35.0)	0.693	1		
	AG + GG	14	10 (71.4)	4 (28.6)		0.743	0.169	3.262

Table 23 Association between genetic polymorphism and disease control rate in combined genotype (*ERCC1* and *CTR1*)

Total number of participants	**69**							
allele	**allele**	N	Disease control rate		*P*	Odds	95% CI	
ERCC1	***CTR1***		PR + SD (%)	PD (%)	value	Ratio	Lower	Upper
CC	GG	23	18 (78.3)	5 (21.7)	0.722	1		
	GT + TT	12	10 (83.3)	2 (16.7)		0.720	0.177	4.412
CT + TT	GG	18	11 (61.1)	7 (38.9)	0.388	1		
	GT + TT	16	12 (75.0)	4 (25.0)		0.524	0.120	2.292

Table 24 Association between genetic polymorphism and disease control rate in combined genotype (*GSTP1* and *CTR1*)

Total number of participants	**69**							
allele	**allele**	N	Disease control rate		*P* value	Odds	95% CI	
GSTP1	***CTR1***		PR + SD (%)	PD (%)		Ratio	Lower	Upper
AA	GG	21	15 (71.4)	6 (28.6)	0.490	1		
	GT + TT	16	13 (81.3)	3 (18.8)		0.588	0.120	2.780
GG	GG	20	14 (70.0)	6 (30.0)	0.761	1		
	GT + TT	12	9 (75.0)	3 (25.0)		0.778	0.154	3.927

PART III Effect of genetic polymorphisms and toxicities

The association between genetic polymorphisms and toxicities were presented in Table 25 – 30. According to the results, the polymorphisms were negatively correlated with nausea and vomiting, peripheral neuropathy, anemia and thrombocytopenia. On the contrary, *GSTP1* polymorphism was statistically significant related to neutropenia (*P* = 0.03), patients with variant genotypes had approximately 2.8-fold higher neutropenia than a AA genotype (95% CI, 1.033 – 7.614). Similarly, *CTR1* rs12686377 was found to be association with weight loss (*P* = 0.01).

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Table 25 Association between genetic polymorphism and anemia

Total number of participants 67									
Gene	Genotype	N	Anemia		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
ERCC1	CC	34	13 (38.2)	21 (61.8)	3.323	0.190	1		
	CT	27	5 (18.5)	22 (81.5)					
	TT	6	1 (16.7)	5 (83.3)					
	CC + TT	33	6 (18.2)	27 (81.8)	3.315	0.069	2.786	0.906	8.563
GSTP1	AA	38	10 (26.3)	28 (73.7)	2.380	0.304	1		
	AG	25	9 (36.0)	16 (64.0)					
	GG	4	0 (0)	4 (100)					
	AG + GG	29	9 (31.0)	20 (60.0)	0.180	0.671	0.794	0.273	2.309
CTR1	GG	41	13 (31.7)	28 (68.3)	0.759	0.684	1		
	GT	23	5 (21.7)	18 (78.3)					
	TT	3	1 (33.3)	2 (66.7)					
	GT + TT	26	6 (23.1)	20 (76.9)	0.583	0.445	1.548	0.503	4.766

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Table 26 Association between genetic polymorphism and neutropenia

Total number of participants	**67**								
Gene	Genotype	N	Neutropenia		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
ERCC1	CC	34	17 (50.0)	17 (50.0)	0.199	0.905	1		
	CT	27	15 (55.6)	12 (44.4)					
	TT	6	3 (50.0)	3 (50.0)					
	CT + TT	33	18 (54.5)	15 (45.5)	0.139	0.710	0.833	0.319	2.176
GSTP1	AA	38	24 (63.2)	14 (36.8)	6.751	0.034*	1		
	AG	25	8 (32.0)	17 (68.0)					
	GG	4	3 (75.0)	1 (25.0)					
	AG + GG	29	11 (37.9)	18 (62.1)	4.195	0.041*	2.805*	1.033	7.614
CTR1	GG	41	22 (53.7)	19 (46.3)	0.463	0.793	1		
	GT	23	12 (52.2)	11 (47.8)					
	TT	3	1 (33.3)	2 (66.7)					
	GT + TT	26	13 (50.0)	13 (50.0)	0.085	0.770	1.158	0.433	3.097

Table 27 Association between genetic polymorphism and thrombocytopenia

Total number of participants 67									
Gene	Genotype	N	Thrombo-cytopenia		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
ERCC1	CC	34	22 (64.7)	12 (35.3)	3.259	0.196	1		
	CT	27	20 (74.1)	7 (25.9)					
	TT	6	6 (100)	0 (0)					
	CT + TT	33	26 (78.8)	7 (21.2)	1.635	0.201	0.494	0.166	1.470
GSTP1	AA	38	25 (65.8)	13 (34.2)	1.522	0.467	1		
	AG	25	20 (80.0)	5 (20.0)					
	GG	4	3 (75.0)	1 (25.0)					
	AG + GG	29	23 (79.3)	6 (20.7)	1.480	0.244	0.502	0.164	1.539
CTR1	GG	41	29 (70.7)	12 (29.3)	2.680	0.262	1		
	GT	23	18 (78.3)	5 (21.7)					
	TT	3	1 (33.3)	2 (66.7)					
	GT + TT	26	19 (73.1)	7 (26.9)	0.043	0.836	0.890	0.297	2.667

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Table 28 Association between genetic polymorphism and nausea and vomit

Total number of participants 69

Gene	Genotype	N	Nausea and vomit No (%)	Nausea and vomit Yes (%)	χ^2 value	*P* value	Odds Ratio	95% CI Lower	95% CI Upper
ERCC1	CC	35	13 (37.1)	22 (62.9)	1.846	0.397	1		
	CT	28	12 (42.9)	16 (57.1)					
	TT	6	4 (66.7)	2 (33.3)					
	CT + TT	34	16 (47.1)	18 (52.9)	0.696	0.404	0.665	0.254	1.738
GSTP1	AA	38	18 (47.4)	20 (52.6)	1.197	0.550	1		
	AG	27	10 (37.0)	17 (63.0)					
	GG	4	1 (25.0)	3 (75.0)					
	AG + GG	31	11 (35.5)	20 (65.4)	0.990	0.320	1.636	0.618	4.330
CTR1	GG	41	16 (39.0)	25 (61.0)	0.478	0.788	1		
	GT	23	11 (47.8)	12 (52.2)					
	TT	5	2 (40.0)	3 (60.0)					
	GT + TT	28	13 (46.4)	15 (53.6)	0.374	0.541	0.738	0.279	1.952

Table 29 Association between genetic polymorphism and peripheral neuropathy

Total number of participants 69

Gene	Genotype	N	Peripheral neuropathy		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
ERCC1	CC	35	28 (80.0)	7 (20.0)	3.921	0.141	1		
	CT	28	17 (60.7)	11 (39.3)					
	TT	6	3 (50.0)	3 (50.0)					
	CT + TT	34	20 (58.8)	14 (41.2)	3.653	0.056	2.800	0.957	8.192
GSTP1	AA	38	25 (65.8)	13 (34.2)	0.571	0.752	1		
	AG	27	20 (74.1)	7 (25.9)					
	GG	4	3 (75.0)	1 (25.0)					
	AG + GG	31	23 (74.2)	8 (25.8)	0.570	0.450	0.669	0.235	1.906
CTR1	GG	41	30 (73.2)	11 (26.8)	4.287	0.117	1		
	GT	23	13 (56.5)	10 (43.5)					
	TT	5	5 (100)	0 (0)					
	GT + TT	28	18 (64.3)	10 (35.7)	0.620	0.431	1.515	0.537	4.273

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Table 30 Association between genetic polymorphism and weight loss

Total number of participants 69									
Gene	Genotype	N	Weight loss		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
ERCC1	CC	35	28 (80.0)	7 (20.0)	1.003	0.605	1		
	CT	28	25 (89.3)	3 (10.7)					
	TT	6	5 (83.3)	1 (16.7)					
	CT + TT	34	30 (88.2)	4 (11.8)	0.873	0.350	0.533	0.141	2.021
GSTP1	AA	38	34 (89.5)	4 (10.5)	4.428	0.109	1		
	AG	27	22 (81.5)	5 (18.5)					
	GG	4	2 (50.0)	2 (50.0)					
	AG + GG	31	24 (77.4)	7 (22.6)	1.851	0.174	2.470	0.652	9.421
CTR1	GG	41	30 (73.2)	11 (26.8)	8.937	0.011*	1		
	GT	23	23 (100)	0 (0)					
	TT	5	5 (100)	0 (0)					
	GT+ TT	28	28 (100)	0 (0)	8.937	0.003*	N/A	N/A	N/A

As for severe toxicity, only *ERCC1* polymorphism was related to severe neutropenia (*P* = 0.045), as shown in Table 31. When analyzing participants with thrombocytopenia at visit 2-3, *GSTP1* was statistically significantly associated with severe thrombocytopenia (*P* = 0.04), as shown in Table 32. No significant difference was observed between *CTR1* polymorphism and any severe toxicity.

Table 31 Association between genetic polymorphism and severe neutropenia

Total number of participants 67								
ERCC1	N	neutropenia		χ^2 value	*P* value	Odds Ratio	95% CI	
		Grade 0-2 (%)	Grade 3-4 (%)				Lower	Upper
CC	34	26 (76.5)	8 (23.5)	4.025	0.045*	1		
CT + TT	33	31 (93.9)	2 (6.1)			0.210	0.041	1.075

Table 32 Association between genetic polymorphism and severe thrombocytopenia at visit 2-3

Total number of participants 11								
GSTP1	N	Thrombocytopenia		χ^2 value	*P* value	Odds Ratio	95% CI	
		Grade 1-2 (%)	Grade 3-4 (%)				Lower	Upper
AA	7	7 (100)	0 (0)	11.00	0.004*	N/A	N/A	N/A
AG	3	3 (100)	0 (0)					
GG	1	0 (0)	1 (100)					

Toxicities at visit 2-3 shown in Table 33-34, *GSTP1* polymorphisms were significantly correlated with neutropenia (*P* = 0.048), and weight loss (*P* = 0.007), participants with variant allele genotype of *GSTP1* rs1695 (AG + GG) presented 8.88-fold higher weight loss than whom with homozygous wild type (AA) (*P* = 0.021, 95%CI = 1.007-78.317) Moreover, SNPs in *CTR1* was found to be associated with weight loss, homozygous wild type of *CTR1* (GG) had higher incidence weight loss than variant genotypes (GT + TT) (*P* = 0.021, 95%CI = 0.722-0.953). Whereas there was no relationship between *ERCC1* polymorphism and toxicities at visit 2-3.

Table 33 Association between genetic polymorphism and neutropenia at visit 2-3

Total number of participants	**67**								
Gene	Genotype	N	Neutropenia		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
GSTP1	AA	38	29 (76.3)	9 (23.7)	6.085	0.048*	1		
	AG	25	13 (52.0)	12 (48.0)					
	GG	4	4 (100)	0 (0)					
	AG + GG	29	17 (58.6)	12 (41.4)	2.393	0.122	2.275	0.795	6.509

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Table 34 Association between genetic polymorphism and weight loss at visit 2-3

Total number of participants 69									
Gene	Genotype	N	Weight loss		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
GSTP1	AA	38	37 (97.4)	1 (2.6)	9.969	0.007*	1		
	AG	27	23 (85.2)	4 (14.8)					
	GG	4	2 (50.0)	2 (50)					
	AG + GG	31	25 (80.6)	6 (19.4)	5.238	0.022*	8.880*	1.007	78.317
CTR1	GG	41	34 (73.2)	7 (26.8)	5.320	0.070	1		
	GT	23	23 (100)	0 (0)					
	TT	5	5 (100)	0 (0)					
	GT+ TT	28	28 (100)	0 (0)	5.320	0.021*	0.829*	0.722	0.953

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Combined genotypes and toxicities were analyzed, as shown in Table 35 and 36. Concerning genotype of *ERCC1* and *GSTP1*, participants with CC genotype of *ERCC1* and AG + GG genotype of *GSTP1* associated with high of risk of neutropenia (*P* = 0.039). Participants with this haplotype were 4.4-fold higher incidence of neutropenia than a CC genotype of *ERCC1* and AA genotype of *GSTP1* (95%CI = 1.041-18.599). In terms of weight loss, Both CC and CT + TT genotypes of *ERCC1* and either GG or GT + TT genotype of *CTR1* showed relationship with weight loss.

Table 35 Association between genetic polymorphism and neutropenia in combined genotype (*ERCC1* and *GSTP1*)

Total number of participants	**67**							
allele	**allele**	N	Neutropenia		*P*	Odds	95% CI	
ERCC1	***GSTP1***		No (%)	Yes (%)	value	Ratio	Lower	Upper
CC	AA	18	12 (66.7)	6 (33.3)	0.039*	1		
	AG + GG	16	5 (31.3)	11 (68.8)		4.400*	1.041	18.599
CT + TT	AA	20	12 (60.0)	8 (40.0)	0.435	1		
	AG + GG	13	6 (46.2)	7 (53.8)		1.750	0.427	7.171

Table 36 Association between genetic polymorphism and weight loss in combined genotype (*ERCC1* and *CTR1*)

Total number of participants	**69**							
allele	**allele**	N	Weight loss		*P* value	Odds	95% CI	
ERCC1	***CTR1***		No (%)	Yes (%)		Ratio	Lower	Upper
CC	GG	23	16 (69.6)	7 (30.4)	0.033*	1		
	GT + TT	12	12 (100)	0 (0)		N/A	N/A	N/A
CT + TT	GG	18	14 (77.8)	4 (22.2)	0.045*	1		
	GT + TT	16	16 (100)	0 (0)		N/A	N/A	N/A

As for genotypes of *GSTP1* and *CTR1* gene was shown in Table 37, participants with AG + GG genotype of *GSTP1* gene and GT + TT of *CTR1* genes was found to be correlated with weight loss (*P* = 0.017). No other significant difference in toxicities with other genotypes was observed.

Table 37 Association between genetic polymorphism and weight loss in combined genotype (*GSTP1* and *CTR1*)

Total number of participants 69								
allele	**allele**	N	Weight loss		*P* value	Odds	95% CI	
GSTP1	***CTR1***		No (%)	Yes (%)		Ratio	Lower	Upper
AA	GG	22	18 (81.8)	4 (18.2)	0.071	1		
	GT + TT	16	16 (100)	0 (0)		N/A	N/A	N/A
AG + GG	GG	19	12 (63.2)	7 (36.8)	0.017*	1		
	GT + TT	12	12 (100)	0 (0)		N/A	N/A	N/A

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The Table 38 showed the association between genetic polymorphism and toxicities in participant who received carboplatin/gemcitabine, the results indicated that participants carrying at least one T allele in *CTR1* gene was statistically significantly related to higher incidence of nausea and vomit (P = 0.014).

Table 38 Association between genetic polymorphism and nausea and vomit in subgroup analysis of carboplatin/gemcitabine

Total number of participants 31									
Gene	Allele	N	Nausea and vomit		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
CTR1	GG	19	2 (10.5)	17 (89.5)	5.985	0.014*	1		
	GT + TT	12	6 (50.0)	6 (50.0)			0.118	0.018	0.749

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Subgroup analysis in carboplatin/paclitaxel regimen was performed was shown in Table 39 – 40. Allele C of *ERCC1* was found to be associated with anemia (*P* = 0.041). Alike, allele G of *CTR1* was correlated with weight loss (*P* = 0.028).

Table 39 Association between genetic polymorphism and anemia in subgroup analysis of carboplatin/paclitaxel

Total number of participants 24									
Gene	Allele	N	Anemia		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
ERCC1	CC	12	8 (66.7)	4 (33.3)	4.196	0.041*	1		
	CT + TT	12	3 (25.0)	9 (75.0)			6.000	1.018	35.374

Table 40 Association between genetic polymorphism and weight loss in subgroup analysis of carboplatin/paclitaxel

Total number of participants 24									
Gene	Allele	N	Weight loss		χ^2 value	*P* value	Odds Ratio	95% CI	
			No (%)	Yes (%)				Lower	Upper
CTR1	GG	15	9 (60.0)	6 (40.0)	4.8	0.028*	N/A		
	GT + TT	9	9 (100)	0 (0)					

The participants treated with carboplatin/pemetrexed were presented in Table 41. Incidence of nausea and vomit symptoms was higher in participants with allele A of *GSTP1* (*P* = 0.018) and allele G of *CTR1* (*P* = 0.016).

Table 41 Association between genetic polymorphism and nausea and vomit in subgroup analysis of carboplatin/pemetrexed

Total number of participants	**9**							
Gene	Allele	N	Nausea and vomit		χ^2 value	*P* value	Exact test	
			No (%)	Yes (%)			2 sided	1 sided
GSTP1	AA	6	5 (83.3)	1 (16.7)	5.625	0.018*	0.048*	0.048*
	AG + GG	3	0 (0)	3 (100)				
CTR1	GG	4	4 (100)	0 (0)	5.760	0.016*	0.048*	0.040*
	GT + TT	5	1 (20.0)	4 (80.0)				

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CHAPTER V
DISCUSSION AND CONCLUSION

PART I Prevalence of genetic polymorphisms

1. Prevalence of *ERCC1* rs11615

The prevalence of allele C→T was found approximately 30-40% in Asian population. Ren and colleague demonstrated that allele T frequency in Chinese patient was made up 40.29% of total.[43] Likewise, the frequency of allele T was accounted for 32.89% of Mongolian advanced NSCLC.[39] As regards Caucasian, allele C was minor allele in *ERCC1* at rs11615. The frequency of allele T was reported by Mlak and coworker, allele C was contributed 66.13% to NSCLC patients in Poland, as same as Sullivan and team revealed 76.1% of allele C in Spain population.[19, 41] In Thailand, the data were alike other Asian population. Kaewbubpa and colleague presented that the allele frequency in Thai NSCLC patients was found 45% of total[48], but our results was found that lower prevalence of allele C→T which accounted for 27.7%.

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2. Prevalence of *GSTP1* rs1695

Previous publications reported the prevalence of allele A→ G in Asian was around 14% - 32% of total. [20, 21, 23] Two studies similarly showed frequency of allele G in Thailand. Khansakorn and coworker showed that the prevalence of allele G was 23.92% to general population, alike Pongteerat and team reported that allele frequency of G was responsible for 19% of breast cancer patients.[49, 50] Allele frequency founded in our study in advanced NSCLC patients was accounted for 25% which was close to previous studies and lower prevalence while compared with Caucasian population which found approximately 35%. [46, 47]

3. Prevalence of *CTR1* rs12686377

Only one study reported the prevalence of *CTR1* at rs12686377, Chinese NSCLC patients was account for 46.3% of allele G→T.[25] According to our results, the frequency of allele G was found 22.3% which was less than the earlier research.

PART II Effect of polymorphisms and treatment responses to platinum-based chemotherapy

Previous publications reported that *ERCC1*, *GSTP1* and *CTR1* polymorphisms contributed to various responses and toxicities to platinum-based chemotherapy in NSCLC patient. Our study was the first to investigate the effect of *GSTP1* rs1695 and *CTR1* rs12686377 and treatment response in Thailand. We hypothesized that genetic polymorphisms were associated with differences in treatment responses. However, the significant differences in treatment responses among genotypes of *ERCC1*, *GSTP1* and *CTR1* could not be seen in this study.

1. Effect of *ERCC1* polymorphism and treatment response

ERCC1 performs repairing the DNA damage lesion which is influence by platinum therapy. Inconsistence with the association between chemotherapy responses and genetic polymorphisms were found in previous studies. Our negative findings paralleled with Du and colleague which noted that SNPs of *ERCC1* was not related to disease control rate (63.7% vs. 72.9%, P = 0.220, OR = 0.655, 95%CI = 0.332-1.290) as same as Huangs' study represented that there was no association between genetic polymorphism at rs11615 and overall response (53.61% vs. 46.39%, P = 0.16, OR = 0.66, 95%CI = 0.36-1.23).[18, 39] Although not statistically significant, our study revealed similar direction of treatment response (17.1% vs. 10.7% vs. 0%, χ^2 = 1.553, P = 0.460) concordant with result of Cheng and their team which discovered that patient with a CC genotype more likely to get better response to platinum-based chemotherapy than those another genotypes (35% vs. 12%, χ^2 = 4.284, P = 0.038) and patients with C/C genotype was found to have a 2.04-fold higher responses, involving in complete response and partial response (P = 0.032, 95%CI = 1.065-3.910), as same as Su and colleague discovered that a CC genotype appeared to be more responders when compared to patient with T allele (CT + TT) (76.9% vs. 23.1%, OR = 3.91, P = 0.012).[14, 51] While patients with T allele appeared to be higher response rate to platinum therapy than who had C allele in Zhaos' study which reported the difference of response to chemotherapy was 71.84% vs. 28.16% (P = 0.001, OR = 0.50, 95%CI = 0.32-0.78) similar to results of Sullivan and coworker which reported that the difference was 83.9% vs. 50% (P= 0.015, OR = 0.11, 95%CI = 0.01-0.66).[17, 19]

Interestingly, our subgroup analysis demonstrated that participants with allele C of *ERCC1* who received carboplatin and gemcitabine seemingly had better disease control rate than participants with allele T (86.7% vs. 50%, P = 0.029), patients with harboring T at *ERCC1* rs11615 likely to be a 6.5-fold poorer prognosis than patients with CC genotypes (95%CI = 1.09-38.63).

2. Effect of *GSTP1* polymorphism and treatment response

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GSTP1 gene is the most abundant isoform in lung tissues. That gene involves in detoxification of platinum agents by phase II metabolizing process. We found that genetic polymorphism was not related to response to platinum-based regimen (13.5% vs. 10.7% vs. 25.0%, χ^2 = 0.645, P = 0.724). This finding was consistent with Pillots' (0% vs. 26%, P = 0.057) and Bootens' (P = 0.93) reports. They noted that polymorphism of *GSTP1* at rs1695 was not significantly associated with responses to platinum-based chemotherapy.[46, 47] However, these results were opposite with previous studies. Their studies findings were represented that patients carrying at least one variant allele correlated with a higher response rates, in terms of CR and PR when compared with homozygous wild type, which were conducted by Sun and colleague (P = 0.010, OR = 3.030, 95%CI = 1.282-7.194), Zhou and team (χ^2 =

8.013, *P* = 0.005, OR = 3.961, 95%CI = 1.531-10.245) and Han and coworker (38.6% vs. 50.44% vs. 10.96%, *P* < 0.05, OR = 2.32; 95%CI = 1.35-3.95 and 5.68; 95%CI = 1.61-30.46).[21-23] Our finding was contradictory, we found that homozygous wild type AA had higher response to the treatment.

3. Effect of *CTR1* polymorphism and treatment response

Platinum agents enter the cell by hCTR1 protein which is encoded by *CTR1* gene. Our finding showed that polymorphism was not related to differences in both overall response (12.2% vs. 17.4% vs. 0%, χ^2 = 1.159, *P* = 0.560) and disease control rate (70.7% vs 78.3% vs 80%, χ^2 = 0.537, *P* = 0.765) which was opposite to the results from Xu and team which reported that *CTR1* rs12686377 probably associated with platinum chemotherapeutic response in NSCLC patients (13% vs. 29% vs. 7%, *P* = 0.01).[25]

PART III Effect of polymorphisms and toxicities of platinum-based chemotherapy

Recent publications discovered that polymorphisms in SNPs of *ERCC1* rs11615, *GSTP1* rs1695, and *CTR1* rs12686377 were related to toxicities of platinum-based regimen. This was the first study in Thailand which examined whether polymorphisms of the three genes in advanced NSCLC patient was correlated with the risk of toxicities from platinum-based regimen or not.

1. Effect of *ERCC1* polymorphism and toxicities

A number of studies revealed that no association of *ERCC1* polymorphism and toxicities in patient treated with platinum-based regimen. KimCurran and colleague indicated that *ERCC1* was not statistically significantly correlated with toxicities in Chinese population (*P* > 0.05).[52] In the same way, no significant difference was observed between the risk of toxicities from chemotherapy and polymorphic genotypes of rs11615, Chen and team (*P* > 0.005).[53] Our findings were consistent with these reports (*P* > 0.005).

2. Effect of *GSTP1* polymorphism and toxicities

Few publications demonstrated the correlation with toxicities and SNPs of *GSTP1* in NSCLC patients. There were discrepancies in previous studies. Pillot and colleague indicated that polymorphism in *GSTP1* were not related to toxicities after platinum-based chemotherapy ($P > 0.05$).[46] While other publications were revealed that genetic polymorphism tended to associated with hematological toxicities. Deng and team noted significant difference with *GSTP1* rs1695 in anemia ($P = 0.046$), but inconsistent with our study, we did not find an association between *GSTP1* polymorphism and anemia. These results may be due to different incidence of anemia in two studies (70% vs. 10%).[20] Whereas Booten and coworker reported that grade of neutropenia was significant difference in rs1695 genotypes of *GSTP1* gene ($P = 0.020$). That result was consistent with current study.[47] We found the relationship between neutropenia and *GSTP1* polymorphism ($\chi^2 = 6.751$, $P = 0.034$). Patients carrying at least one variant allele were at approximately 2.805-fold higher risk of neutropenia than those with homozygous wild type ($P = 0.041$, 95%CI = 1.033-7.614).

3. Effect of *CTR1* polymorphism and toxicities

We did not found the association between *CTR1* rs12686377 and other toxicities including nausea and vomiting, peripheral neuropathy, anemia, neutropenia and thrombocytopenia. Interestingly, we found that weight lost was associated with *CTR1* rs12686377 polymorphism. Proportion of patients with weight lost was higher in whom with GG genotype. Previous researches reported that decreasing more than 5% of body weight was increased in the relative risk of death.[54] Moreover, the response to chemotherapy was more likely found in patients who had no change in body weight.[55] Although several factors appeared to have an effect on body weight, but our findings revealed that polymorphism of rs12686377 at *CTR1* gene was a one of determining factors for weight loss in advanced NSCLC patient treated with platinum-based chemotherapy (26.8% vs. 0%, χ^2 = 8.937, *P* = 0.003). After considering baseline characteristics, we found performance status was also associated with weight loss (*P* = 0.022). In addition, patients with ECOG score = 1, weight loss showed a relationship with polymorphic *CTR1* rs12686377 gene (*P* = 0.31), homozygous wild type (GG) had higher incidence weight loss when compared with variant genotype (GT + TT) (26.5% vs. 0%, χ^2 = 6.939, *P* = 0.008, OR = 0.735, 95%CI = 0.601-0.900).

Sample size in our study was relatively small (n = 69). Although genetic polymorphisms of these three genes found in this study were quite common but number of patients in each genotypes might be too small to generate statistically differences in term of treatment responses. However, even with small number of patients, impacts of genetic polymorphisms on some toxicities such as weight loss or neutropenia can be observed in our study which indicated the importance of genetic polymorphisms. Further study with larger sample size is needed to gain more informative conclusion regarding to association between treatment outcomes and these genetic polymorphisms.

Conclusion

Results from this study indicated that genetic polymorphisms of *ERCC1* rs11615, *GSTP1* rs1695 and *CTR1* rs12686377 in Thai were not uncommon and possibly explained the variation of platinum-based treatment response. Prevalence of polymorphisms of *ERCC1* rs11615, *GSTP1* rs1695 and *CTR1* rs12686377genes were 27.7%, 25% and 22.3%, respectively. When only one genetic polymorphism was taken into account, association with treatment response cannot be seen, while toxicity in term of neutropenia and weight loss were significantly associated with *GSTP1* and *CTR1* polymorphisms. Subgroup analysis in patients received carboplatin and gemcitabine (n = 31) were found association between treatment response and genetic polymorphisms. Disease control rate in patients with homozygous wild type *ERCC1* (CC) was higher than in whom with variant genes (86.7% vs 50%, *P* = 0.029). This finding supported the importance of *ERCC1* polymorphisms. When combined effects of genetic polymorphisms were considered, we could not see the significant differences regarding to treatment response. However, the differences in toxicities were strongly associated with genetic polymorphisms. Neutropenia and weight loss which could lead to dose reduction or unfavorable prognosis were associated with *GSTP1* and *CTR1* polymorphisms. These adverse effects influenced to dose reduction which can lead to treatment failure. Therefore, genetic polymorphism should be a one of factors to consider in the selection of proper chemotherapy regimen in advanced NSCLC patients.

Limitation

Due to a relatively small sample size of this study caused a small number of patients in each genotype which accounted for under power of statistic testing. Moreover, most of patients received combination chemotherapy which the effect of combined drug such as gemcitabine, paclitaxel and pemetrexed can lessen the impacts of genetic polymorphisms. Future studies with larger sample size and more homogeneous treatment pattern should be conducted to elucidate the significance of these genetic polymorphisms on treatment response and toxicity prior to summarize whether these genetic polymorphisms impact on treatment outcomes or not.

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REFERENCES

1. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet].Lyon, France: International Agency for Research on Cancer; 2013 [Internet]. [cited 24/05/2026].

2. Travis WD BE, Muller-Hermelink HK, et al. Pathology and Genetics: Tumours of the Lung, Pleura, Thymus and Heart. 2004.

3. Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clinic proceedings. 2008;83(5):584-94.

4. Sangha R, Price J, Butts CA. Adjuvant therapy in non-small cell lung cancer: current and future directions. The oncologist. 2010;15(8):862-72.

5. Ho C, Tong KM, Ramsden K, Ionescu DN, Laskin J. Histologic classification of non-small-cell lung cancer over time: reducing the rates of not-otherwise-specified. Current oncology. 2015;22(3):e164-70.

6. National Comprehensive Cancer Network. Non-small cell lung Cancer, Washington, USA; 4,2016 [Internet]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf.

7. Rinaldi M, Cauchi C, Gridelli C. First line chemotherapy in advanced or metastatic NSCLC. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2006;17 Suppl 5:v64-7.

8. Maccio A, Madeddu C. Cisplatin : an old drug with a newfound efficacy -- from mechanisms of action to cytotoxicity. Expert opinion on pharmacotherapy. 2013;14(13):1839-57.

9. Rabik CA, Dolan ME. Molecular mechanisms of resistance and toxicity associated with platinating agents. Cancer treatment reviews. 2007;33(1):9-23.

10. Rose MC, Kostyanovskaya E, Huang RS. Pharmacogenomics of cisplatin sensitivity in non-small cell lung cancer. Genomics, proteomics & bioinformatics. 2014;12(5):198-209.

11. Seve P, Dumontet C. Chemoresistance in non-small cell lung cancer. Current medicinal chemistry Anti-cancer agents. 2005;5(1):73-88.
12. Schettino C, Bareschino MA, Maione P, Rossi A, Ciardiello F, Gridelli C. The potential role of pharmacogenomic and genomic in the adjuvant treatment of early stage non small cell lung cancer. Current genomics. 2008;9(4):252-62.
13. Bowden NA. Nucleotide excision repair: why is it not used to predict response to platinum-based chemotherapy? Cancer letters. 2014;346(2):163-71.
14. Cheng J, Ha M, Wang Y, Sun J, Chen J, Wang Y, et al. A C118T polymorphism of ERCC1 and response to cisplatin chemotherapy in patients with late-stage non-small cell lung cancer. Journal of cancer research and clinical oncology. 2012;138(2):231-8.
15. Powrozek T, Mlak R, Krawczyk P, Homa I, Ciesielka M, Koziol P, et al. The relationship between polymorphisms of genes regulating DNA repair or cell division and the toxicity of platinum and vinorelbine chemotherapy in advanced NSCLC patients. Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico. 2015.
16. Shi Z-H, Shi G-Y, Liu L-G. Polymorphisms in ERCC1 and XPF gene and response to chemotherapy and overall survival of non-small cell lung cancer. International Journal of Clinical and Experimental Pathology. 2015;8(3):3132-7.
17. Zhao X, Zhang Z, Yuan Y, Yuan X. Polymorphisms in ERCC1 gene could predict clinical outcome of platinum-based chemotherapy for non-small cell lung cancer patients. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(8):8335-41.
18. Du Y, Su T, Zhao L, Tan X, Chang W, Zhang H, et al. Associations of polymorphisms in DNA repair genes and MDR1 gene with chemotherapy response and survival of non-small cell lung cancer. PloS one. 2014;9(6):e99843.
19. Sullivan I, Salazar J, Majem M, Pallares C, Del Rio E, Paez D, et al. Pharmacogenetics of the DNA repair pathways in advanced non-small cell lung cancer patients treated with platinum-based chemotherapy. Cancer letters. 2014;353(2):160-6.

20. Deng JH, Deng J, Shi DH, Ouyang XN, Niu PG. Clinical outcome of cisplatin-based chemotherapy is associated with the polymorphisms of GSTP1 and XRCC1 in advanced non-small cell lung cancer patients. Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico. 2015;17(9):720-6.

21. Han B, Guo Z, Ma Y, Kang S, Wang Y, Wei Q, et al. Association of GSTP1 and XRCC1 gene polymorphisms with clinical outcome of advanced non-small cell lung cancer patients with cisplatin-based chemotherapy. Int J Clin Exp Pathol. 2015;8(4):4113-9.

22. Zhou F, Yu Z, Jiang T, Lv H, Yao R, Liang J. Genetic polymorphisms of GSTP1 and XRCC1: prediction of clinical outcome of platinum-based chemotherapy in advanced non-small cell lung cancer (NSCLC) patients. Swiss medical weekly. 2011;141:w13275.

23. Sun N, Sun X, Chen B, Cheng H, Feng J, Cheng L, et al. MRP2 and GSTP1 polymorphisms and chemotherapy response in advanced non-small cell lung cancer. Cancer chemotherapy and pharmacology. 2010;65(3):437-46.

24. Kim ES, Tang X, Peterson DR, Kilari D, Chow CW, Fujimoto J, et al. Copper transporter CTR1 expression and tissue platinum concentration in non-small cell lung cancer. Lung Cancer. 2014;85(1):88-93.

25. Xu X, Duan L, Zhou B, Ma R, Zhou H, Liu Z. Genetic polymorphism of copper transporter protein 1 is related to platinum resistance in Chinese non-small cell lung carcinoma patients. Clinical and experimental pharmacology & physiology. 2012;39(9):786-92.

26. Xu X, Ren H, Zhou B, Zhao Y, Yuan R, Ma R, et al. Prediction of copper transport protein 1 (CTR1) genotype on severe cisplatin induced toxicity in non-small cell lung cancer (NSCLC) patients. Lung Cancer. 2012;77(2):438-42.

27. Edge SB BD, Compton CC, Fritz AG, Greene FL, Trotti A,editors. AJCC cancer staging manual (7th ed). New York: NY: Springer; 2010.

28. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. American journal of clinical oncology. 1982;5(6):649-55.

29. D'Addario G, Pintilie M, Leighl NB, Feld R, Cerny T, Shepherd FA. Platinum-based versus non-platinum-based chemotherapy in advanced non-small-cell lung cancer: a meta-analysis of the published literature. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(13):2926-36.

30. Gibson D. The mechanism of action of platinum anticancer agents-what do we really know about it? Dalton Transactions. 2009(48):10681-9.

31. Rose MC, Kostyanovskaya E, Huang RS. Pharmacogenomics of Cisplatin Sensitivity in Non-small Cell Lung Cancer. Genomics, Proteomics & Bioinformatics. 2014;12(5):198-209.

32. Hartmann JT, Lipp HP. Toxicity of platinum compounds. Expert opinion on pharmacotherapy. 2003;4(6):889-901.

33. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of Cisplatin Nephrotoxicity. Toxins. 2010;2(11):2490-518.

34. Amptoulach S, Tsavaris N. Neurotoxicity Caused by the Treatment with Platinum Analogues. Chemotherapy Research and Practice. 2011;2011:5.

35. Dizon DS, Sabbatini PJ, Aghajanian C, Hensley ML, Spriggs DR. Analysis of patients with epithelial ovarian cancer or fallopian tube carcinoma retreated with cisplatin after the development of a carboplatin allergy. Gynecologic oncology. 2002;84(3):378-82.

36. Grossi F, Aita M, Defferrari C, Rosetti F, Brianti A, Fasola G, et al. Impact of third-generation drugs on the activity of first-line chemotherapy in advanced non-small cell lung cancer: a meta-analytical approach. The oncologist. 2009;14(5):497-510.

37. Baggstrom MQ, Stinchcombe TE, Fried DB, Poole C, Hensing TA, Socinski MA. Third-Generation Chemotherapy Agents in the Treatment of Advanced Non-small Cell Lung Cancer: A Meta-Analysis. Journal of Thoracic Oncology. 2007;2(9):845-53.

38. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). European journal of cancer (Oxford, England : 1990). 2009;45(2):228-47.

39. Huang SJ, Wang YF, Jin ZY, Sun JY, Guo ZL. Role of ERCC1 variants in response to chemotherapy and clinical outcome of advanced non-small cell lung cancer.

จุฬาลงกรณ์มหาวิทยาลัย

Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2014;35(5):4023-9.

40. Krawczyk P, Kucharczyk T, Kowalski DM, Powrozek T, Ramlau R, Kalinka-Warzocha E, et al. Polymorphisms in TS, MTHFR and ERCC1 genes as predictive markers in first-line platinum and pemetrexed therapy in NSCLC patients. Journal of cancer research and clinical oncology. 2014;140(12):2047-57.

41. Mlak R, Krawczyk P, Ramlau R, Kalinka-Warzocha E, Wasylecka-Morawiec M, Wojas-Krawczyk K, et al. Predictive value of ERCC1 and RRM1 gene single-nucleotide polymorphisms for first-line platinum- and gemcitabine-based chemotherapy in non-small cell lung cancer patients. Oncology reports. 2013;30(5):2385-98.

42. Krawczyk P, Wojas-Krawczyk K, Mlak R, Kucharczyk T, Biernacka B, Milanowski J. Predictive value of ERCC1 single-nucleotide polymorphism in patients receiving platinum-based chemotherapy for locally-advanced and advanced non-small cell lung cancer--a pilot study. Folia histochemica et cytobiologica / Polish Academy of Sciences, Polish Histochemical and Cytochemical Society. 2012;50(1):80-6.

43. Ren S, Zhou S, Wu F, Zhang L, Li X, Zhang J, et al. Association between polymorphisms of DNA repair genes and survival of advanced NSCLC patients treated with platinum-based chemotherapy. Lung Cancer. 2012;75(1):102-9.

44. Li D, Zhou Q, Liu Y, Yang Y, Li Q. DNA repair gene polymorphism associated with sensitivity of lung cancer to therapy. Medical oncology (Northwood, London, England). 2012;29(3):1622-8.

45. Ada AO, S CK, Hancer F, Bilgen S, Suzen SH, Alpar S, et al. CYP and GST polymorphisms and survival in advanced non-small cell lung cancer patients. Neoplasma. 2010;57(6):512-21.

46. Pillot GA, Read WL, Hennenfent KL, Marsh S, Gao F, Viswanathan A, et al. A phase II study of irinotecan and carboplatin in advanced non-small cell lung cancer with pharmacogenomic analysis: final report. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2006;1(9):972-8.

47. Booton R, Ward T, Heighway J, Ashcroft L, Morris J, Thatcher N. Glutathione-S-transferase P1 isoenzyme polymorphisms, platinum-based chemotherapy, and non-

small cell lung cancer. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2006;1(7):679-83.
48. Walenee Kaewbubpa VS, Nutthada Areepium. ERCC1 (C118T) polymorphism in advanced non-small-cell lung cancer patients undergoing platinum-based chemotherapy. THAI CANCER JOURNAL. 2015;35(4):170-5.
49. Nitchaphat Khansakorn WW, Prapin Tharnpoophasiam, Bunlue Hengprasith, Lerson Suwannathon, Krittaya Pethchpoung, Krongtong Yoovathaworn,, Suwannee Chanprasertyothin TS, Sming Kaojarern, Piyamit Sritara and Jintana Sirivarasai. Impact of GSTM1, GSTT1, GSTP1 polymorphism and environmental lead exposure on oxidative stress biomarkers. Scientific Research and Essays 2011;6(31):6540-7.
50. Tanett Pongteerat Pr, Pimkanya Morgong, Pensri Saelee. Glutathione-S-transferase P1 Gene Polymorphism at Ile105Val and Ala114Val in Patients with Breast Cancer. THAI CANCER JOURNAL. 2014;34(1):26-33.
51. Su D, Ma S, Liu P, Jiang Z, Lv W, Zhang Y, et al. Genetic polymorphisms and treatment response in advanced non-small cell lung cancer. Lung Cancer. 2007;56(2):281-8.
52. KimCurran V, Zhou C, Schmid-Bindert G, Shengxiang R, Zhou S, Zhang L, et al. Lack of correlation between ERCC1 (C8092A) single nucleotide polymorphism and efficacy/toxicity of platinum based chemotherapy in Chinese patients with advanced non-small cell lung cancer. Advances in medical sciences. 2011;56(1):30-8.
53. Chen S, Huo X, Lin Y, Ban H, Lin Y, Li W, et al. Association of MDR1 and ERCC1 polymorphisms with response and toxicity to cisplatin-based chemotherapy in non-small-cell lung cancer patients. International journal of hygiene and environmental health. 2010;213(2):140-5.
54. Soria JC, Mauguen A, Reck M, Sandler AB, Saijo N, Johnson DH, et al. Systematic review and meta-analysis of randomised, phase II/III trials adding bevacizumab to platinum-based chemotherapy as first-line treatment in patients with advanced non-small-cell lung cancer. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2013;24(1):20-30.
55. Staal-van den Brekel AJ, Schols AM, Dentener MA, ten Velde GP, Buurman WA, Wouters EF. Metabolism in patients with small cell lung carcinoma compared

with patients with non-small cell lung carcinoma and healthy controls. Thorax. 1997;52(4):338-41.

จุฬาลงกรณ์มหาวิทยาลัย
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APPENDIX

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APPENDIX A

TMN staging of lung cancer

American Joint Committee on Cancer

Lung Cancer Staging

7th EDITION

Definitions

Primary Tumor (T)

TX Primary tumor cannot be assessed, or tumor proven by the presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy

T0 No evidence of primary tumor

Tis Carcinoma in situ

T1 Tumor 3 cm or less in greatest dimension, surrounded by lung or visceral pleura, without bronchoscopic evidence of invasion more proximal than the lobar bronchus (for example, not in the main bronchus)[1]

T1a Tumor 2 cm or less in greatest dimension

T1b Tumor more than 2 cm but 3 cm or less in greatest dimension

T2 Tumor more than 3 cm but 7 cm or less or tumor with any of the following features (T2 tumors with these features are classified T2a if 5 cm or less): involves main bronchus, 2 cm or more distal to the carina; invades visceral pleura (PL1 or PL2); associated with atelectasis or obstructive pneumonitis that extends to the hilar region but does not involve the entire lung

T2a Tumor more than 3 cm but 5 cm or less in greatest dimension

T2b Tumor more than 5 cm but 7 cm or less in greatest dimension

T3 Tumor more than 7 cm or one that directly invades any of the following: parietal pleural (PL3), chest wall (including superior sulcus tumors), diaphragm, phrenic nerve, mediastinal pleura, parietal pericardium; or tumor in the main bronchus less than 2 cm distal to the carina[1] but without involvement of the carina; or associated atelectasis or obstructive pneumonitis of the entire lung or separate tumor nodule(s) in the same lobe

T4 Tumor of any size that invades any of the following: mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, esophagus, vertebral body, carina, separate tumor nodule(s) in a different ipsilateral lobe

Distant Metastasis (M)

M0 No distant metastasis

M1 Distant metastasis

M1a Separate tumor nodule(s) in a contralateral lobe, tumor with pleural nodules or malignant pleural (or pericardial) effusion[2]

M1b Distant metastasis (in extrathoracic organs)

Notes

[1] The uncommon superficial spreading tumor of any size with its invasive component limited to the bronchial wall, which may extend proximally to the main bronchus, is also classified as T1a.

[2] Most pleural (and pericardial) effusions with lung cancer are due to tumor. In a few patients, however, multiple cytopathologic examinations of pleural (pericardial) fluid are negative for tumor, and the fluid is nonbloody and is not an exudate. Where these elements and clinical judgment dictate that the effusion is not related to the tumor, the effusion should be excluded as a staging element and the patient should be classified as M0.

ANATOMIC STAGE/PROGNOSTIC GROUPS			
Occult Carcinoma	TX	N0	M0
Stage 0	Tis	N0	M0
Stage IA	T1a	N0	M0
	T1b	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T2b	N0	M0
	T1a	N1	M0
	T1b	N1	M0
	T2a	N1	M0
Stage IIB	T2b	N1	M0
	T3	N0	M0
Stage IIIA	T1a	N2	M0
	T1b	N2	M0
	T2a	N2	M0
	T2b	N2	M0
	T3	N1	M0
	T3	N2	M0
	T4	N0	M0
	T4	N1	M0
Stage IIIB	T1a	N3	M0
	T1b	N3	M0
	T2a	N3	M0
	T2b	N3	M0
	T3	N3	M0
	T4	N2	M0
	T4	N3	M0
Stage IV	Any T	Any N	M1a
	Any T	Any N	M1b

Financial support for AJCC 7th Edition Staging Posters provided by the American Cancer Society

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APPENDIX B

Classified grade of toxicity

Table Classified grade of toxicity

Adverse event	Grade	Meaning
Nausea	1	Loss of appetite without alteration in eating habits
	2	Oral intake decreased without significant weight loss, dehydration or malnutrition
	3	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated
	4	-
Vomit	1	1 - 2 episodes (separated by 5 minutes) in 24 hrs.
	2	3 - 5 episodes (separated by 5 minutes) in 24 hrs.
	3	≥ 6 episodes (separated by 5 minutes) in 24 hrs.; tube feeding, TPN or hospitalization indicated
	4	Life-threatening consequences; urgent intervention indicated
Weight loss	1	5 to <10% from baseline; intervention not indicated
	2	10 - <20% from baseline; nutritional support indicated
	3	≥ 20% from baseline; tube feeding or TPN indicated
	4	-

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Table Classified grade of toxicity

Adverse event	Grade	Meaning
Neutropenia	1	1,800 – 1,500 cell/mm3
	2	< 1,500 – 1,000 cell/mm3
	3	< 1,000 – 500 cell/mm3
	4	< 500 cell/mm3
Anemia	1	12 – 10 g/dL
	2	<10.0 - 8.0 g/dL
	3	Hgb <8.0 g/dL; transfusion indicated
	4	Life-threatening consequences; urgent intervention indicated
Thrombocytopenia	1	150,00 – 75,000 cell/mm3
	2	<75,000 – 50,000 cell/mm3
	3	< 50,000 – 25,000 cell/mm3
	4	< 25,000 cell/mm3
Peripheral neuropathy	1	Asymptomatic; clinical or diagnostic observations only; intervention not indicated
	2	Moderate symptoms; limiting instrumental ADL
	3	Severe symptoms; limiting self care ADL; assistive device indicated
	4	Life-threatening consequences; urgent intervention indicated

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APPENDIX C
Data collecting sheet

Table 1. Demographic data

No.	Variables	Categories	Data
1	age (years)	cont.	
2.	sex	(0) = male (1) = female	
3.	smoking history	(0) = non smoker (1) = smoker (2) = second hand smoker	
4.	histological types	(0) = adenocarcinoma (1) = squamous cell carcinoma (2) = undifferentiated large cell carcinoma (3) = other types	
5.	family history	(0) = unknown (1) = no (2) = yes	

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Table 2. Participants' status								
Variables	Unit	Before treatment	cycle					
			1	2	3	4	5	6
1. height	cm.							
2. weight	kg.							
3. body surface area	m^2							
4. performance status	0 - 2							
5. TMN staging								

Table 3. Underlying and current diseases		
No.	Disease (ICD10)	Treatment
1		
2		
3		
4		
5		

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Table 4. Herbs and supplements		
No.	List	Dosage
1		
2		
3		
4		
5		

Table 5. Previous cancer treatment			
Treatment	Categories	Date	Data
1. Radiation	(0) = no (1) = yes		
2. Surgery	(0) = no (1) = yes		

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Table 6. Current chemotherapy regimen								
Medication	List	Data	Dosage (mg)					
			C.1	C.2	C.3	C.4	C.5	C.6
1. Platinum	(0) = cisplatin (1) = carboplatin (2) = oxaliplatin							
2. Other CTX.	(0) = etoposide (1) = gemcitabine (2) = placitaxel (3) = other							

Table 7. Clinical outcomes								
Variables	status	visit						
		1	2	3	4	5	6
1. treatment response	(0) = CR (1) = PR (2) = SD (3) = PD						
2. toxicity	(0) = grade 1						
2.1 emetic effect	(1) = grade 2						
2.2 hematological	(2) = grade 3						
2.3 nephrotoxicity	(3) = grade 4						
2.4 neurotoxicity	(4) = grade 5						
3. CEA							

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DATE………………………………… CHEMOTHERAPY CYCLE …………………

1. Weight…………………kg
2. BSA……………………m^2
3. PS score…………………
4. Neurologic sign………………………………………
5. Nausea…………………………………………………
6. Vomiting………………………………………times

Table 8. Laboratory tests

List	Reference	unit	value
1. Hemoglobin (Hgb)	≥ 10	g/dL	
2. Hematocrit (Hct)	37 - 47	%	
3. WBC	≥ 3,000	cell/mL	
4. PMN	50 - 60	%	
5. ANC	≥ 1,500	cell/mL	
6. Platelet	≥ 50,000	cell/mL	
7. Serum Creatinine	0.5 - 1.3	mg/dL	
8. CrCl	≥ 50	ml/min	
9. CEA	2 - 5	ng/mL	
10. BUN	10 - 20	mg/dL	

Table 9. Medications

No.	Medication	Unit	Dosage
1			
2			
3			
4			
5			
6			
7			
8			

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APPENDIX D
Information sheet

เอกสารข้อมูลคำอธิบายสำหรับผู้เข้าร่วมในโครงการวิจัย

ชื่อโครงการวิจัย ผลของภาวะพหุสัณฐานของยีน *ERCC1 GSTP1* และ *CTR1* ต่อการตอบสนองต่อการรักษาและการเกิดพิษจากการใช้ยาเคมีบำบัดในกลุ่มแพลทินัมในผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กระยะสามบีถึงสี่

ผู้ทำวิจัย เภสัชกรหญิงศิริลักษณ์ คำภิโร นิสิตระดับปริญญาโท ภาควิชาเภสัชกรรมปฏิบัติ สาขาวิชาเภสัชกรรมคลินิก จุฬาลงกรณ์มหาวิทยาลัย

สถานที่วิจัย โรงพยาบาลจุฬาลงกรณ์

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เรียน ผู้เข้าร่วมโครงการวิจัยทุกท่าน

ท่านได้รับเชิญให้เข้าร่วมในโครงการวิจัยนี้เนื่องจากท่านเป็นผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กระยะสามบีถึงสี่ ก่อนที่ท่านจะตัดสินใจเข้าร่วมในการศึกษาวิจัยดังกล่าว ขอให้ท่านอ่านเอกสารฉบับนี้อย่างถี่ถ้วน เพื่อให้ท่านได้ทราบถึงเหตุผลและรายละเอียดของการศึกษาวิจัยในครั้งนี้ หากท่านมีข้อสงสัยใดๆ เพิ่มเติม กรุณาซักถามจากผู้ทำวิจัย หรือแพทย์ผู้ร่วมทำวิจัยซึ่งจะเป็นผู้สามารถตอบคำถามและให้ความกระจ่างแก่ท่านได้

ท่านสามารถขอคำแนะนำในการเข้าร่วมโครงการวิจัยนี้จากครอบครัว เพื่อน หรือแพทย์ประจำตัวของท่านได้ ท่านมีเวลาอย่างเพียงพอในการตัดสินใจโดยอิสระ ถ้าท่านตัดสินใจแล้วว่าจะเข้า

ร่วมในโครงการวิจัยนี้ ขอให้ท่านลงนามในเอกสารแสดงความยินยอมของโครงการวิจัยนี้ ท่านจะได้รับสำเนาใบยินยอมที่เก็บไว้ 1 ฉบับ

เหตุผลความเป็นมา

ผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กระยะสามบีถึงสี่ ได้รับการรักษาด้วยยาเคมีบำบัดสูตรที่มีแพลตินัมเป็นสูตรแรกในการรักษา ยาที่อยู่ในกลุ่มแพลตินัมเป็นองค์ประกอบ ได้แก่ ซิสพลาตินและคาร์โบพลาติน พบว่าผู้ป่วยตอบสนองต่อยากลุ่มนี้ พบได้ร้อยละ 30 ถึง 40 แต่ในทางตรงข้ามก็เกิดเหตุการณ์ไม่พึงประสงค์จากการใช้ยาเคมีบำบัดกลุ่มนี้ได้บ่อย และตอบสนองต่อยากลุ่มแพลตินัมได้น้อยนั้น สาเหตุอาจมาจากความแตกต่างทางพันธุกรรมของแต่ละบุคคล ซึ่งรบกวนการทำงานของยาในกลุ่มแพลตตินัม ทั้งกระบวนการการนำยาเข้าสู่เซลล์ที่เกี่ยวข้องกับยีนซีทีอาร์วัน กระบวนการกำจัดยาออกจากเซลล์ที่เกี่ยวข้องกับยีนจีเอสทีพีวัน และการซ่อมแซมสายดีเอ็นเอที่เกิดความเสียหายจากยาในกลุ่มแพลตตินัมที่เกี่ยวข้องกับยีนอีอาร์ซีซีวัน ซึ่งความหลากหลายทางพันธุกรรมของยีนที่แตกต่างกันในแต่ละบุคคล อาจจะส่งผลต่อการตอบสนองต่อยาที่แตกต่างกัน ดังนั้นผู้วิจัยจึงสนใจที่จะศึกษาเกี่ยวกับความหลากหลายทางพันธุกรรมของยีนอีอาร์ซีซีวัน จีเอสทีพีวัน และยีนซีทีอาร์วันต่อการตอบสนองของยากลุ่มแพลตตินัมและเหตุการณ์ไม่พึงประสงค์ในผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กระยะสามบีถึงสี่ ณ โรงพยาบาลจุฬาลงกรณ์ เพื่อเป็นประโยชน์ต่อในการพิจารณาการรักษาผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กระยะสามบีถึงสี่ด้วยยาเคมีบำบัดที่เหมาะสมของผู้ป่วยแต่ละรายได้ในอนาคต

วัตถุประสงค์ของการวิจัย

วัตถุประสงค์จากการวิจัยในครั้งนี้คือเพื่อเปรียบเทียบระหว่างอัตราการตอบสนองต่อยาเคมีบำบัดและเหตุการณ์ไม่พึงประสงค์ของผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กระยะสามบีถึงสี่ ซึ่งรักษาด้วยสูตรแพลตตินัมกับความหลากหลายทางพันธุกรรมของยีนอีอาร์ซีซีวัน (*ERCC1*) ยีนจีเอสทีพีวัน (*GSTP1*) และยีนซีทีอาร์วัน (*CTR1*) มีจำนวนผู้เข้าร่วมในโครงการวิจัย คือ 70 คน

วิธีการที่เกี่ยวข้องกับการวิจัย

หลังจากท่านให้ความยินยอมที่จะเข้าร่วมในโครงการวิจัยนี้ ท่านจะได้รับตรวจและรักษาตามปกติ โดยแพทย์จะเป็นผู้คัดกรองว่าท่านมีคุณสมบัติที่เหมาะสมที่จะเข้าร่วมในการวิจัย

สำหรับงานวิจัยครั้งนี้ท่านจะได้รับการเจาะเลือดทางหลอดเลือดดำปริมาณ 5-10 ซีซี (หนึ่งถึงสองช้อนชา) จำนวน 1 ครั้ง เพื่อนำไปวิเคราะห์หาความหลากหลายทางพันธุกรรมของยีนอีอาร์ซีซีวัน (*ERCC1*) ยีนซีทีอาร์วัน (*CTR1*) และ ยีนจีเอสทีพีวัน (*GSTP1*) หลังจากนั้นเมื่อท่านมาพบแพทย์ตามรอบการรับยาจะได้รับการติดตามเหตุการณ์ไม่พึงประสงค์จากยาเคมีบำบัดทุกรอบการรับยา และจะมีการเก็บข้อมูลค่าความสมบูรณ์ของเม็ดเลือดและค่าการทำงานของไตจากเวชระเบียนของท่าน

ความรับผิดชอบของอาสาสมัครผู้เข้าร่วมในโครงการวิจัย

เพื่อให้งานวิจัยนี้ประสบความสำเร็จ ผู้ทำวิจัยใคร่ขอความความร่วมมือจากท่าน โดยจะขอให้ท่านปฏิบัติตามคำแนะนำของผู้ทำวิจัยอย่างเคร่งครัด รวมทั้งแจ้งอาการผิดปกติต่าง ๆ ที่เกิดขึ้นกับท่านระหว่างที่ท่านเข้าร่วมในโครงการวิจัยให้ผู้ทำวิจัยได้รับทราบ

ความเสี่ยงที่ได้รับจากการเจาะเลือด

ท่านมีโอกาสที่จะเกิดอาการเจ็บ เลือดออก ช้ำจากการเจาะเลือด อาการบวมบริเวณที่เจาะเลือดหรือหน้ามืด และโอกาสที่จะเกิดการติดเชื้อบริเวณที่เจาะเลือดพบได้น้อยมาก

ความเสี่ยงที่ไม่ทราบแน่นอน

ท่านอาจเกิดอาการข้างเคียง หรือความไม่สบาย ซึ่งอาการข้างเคียงเหล่านี้เป็นอาการที่ไม่เคยพบมาก่อน เพื่อความปลอดภัยของท่าน ควรแจ้งผู้ทำวิจัยให้ทราบทันทีเมื่อเกิดความผิดปกติใดๆ เกิดขึ้น

หากท่านมีข้อสงสัยใดๆ เกี่ยวกับความเสี่ยงที่อาจได้รับจากการเข้าร่วมในโครงการวิจัย ท่านสามารถสอบถามจากผู้ทำวิจัยได้ตลอดเวลา

ประโยชน์ที่อาจได้รับ

ท่านจะไม่ได้รับประโยชน์ใด ๆ จากการเข้าร่วมวิจัยครั้งนี้ แต่ข้อมูลที่ได้จากการวิจัยสามารถเป็นแนวทางในการพิจารณาการรักษาผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็กระยะสามบีถึงสี่ ด้วยยาเคมีบำบัดกลุ่มแพลตตินัมตามความหลากหลายทางพันธุกรรมของยีนอีอาร์ซีซีวัน (*ERCC1*) ยีนซีทีอาร์วัน (*CTR1*) และ ยีนจีเอสทีพีวัน (*GSTP1*) ให้เหมาะสมในผู้ป่วยแต่ละรายได้ในอนาคต

อันตรายที่อาจเกิดขึ้นจากการเข้าร่วมในโครงการวิจัยและความรับผิดชอบของผู้ทำวิจัย

หากพบอันตรายที่เกิดขึ้นจากการวิจัย ท่านจะได้รับการรักษาอย่างเหมาะสมทันที โดยผู้วิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่าย หากเกิดอันตรายจากการวิจัย และการลงนามในเอกสารให้ความยินยอมไม่ได้หมายความว่าท่านได้สละสิทธิ์ทางกฎหมายตามปกติที่ท่านพึงมี

ค่าตอบแทนสำหรับผู้เข้าร่วมวิจัย

ท่านจะไม่ได้รับเงินค่าตอบแทนจากการเข้าร่วมในการวิจัย แต่ท่านจะได้รับค่าเดินทางเป็นเงิน 300 บาท และจะจ่ายในวันที่เจาะเลือด

การเข้าร่วมและการสิ้นสุดการเข้าร่วมโครงการวิจัย

การเข้าร่วมในโครงการวิจัยครั้งนี้เป็นไปโดยความสมัครใจ หากท่านไม่สมัครใจจะเข้าร่วมการศึกษาแล้ว ท่านสามารถถอนตัวได้ตลอดเวลา การขอถอนตัวออกจากโครงการวิจัยจะไม่มีผลต่อการดูแลรักษาโรคของท่านแต่อย่างใด

การปกป้องรักษาข้อมูลความลับของอาสาสมัคร

ข้อมูลที่อาจนำไปสู่การเปิดเผยตัวท่าน จะได้รับการปกปิดและจะไม่เปิดเผยแก่สาธารณชน ในกรณีที่ผลการวิจัยได้รับการตีพิมพ์ ชื่อและที่อยู่ของท่านจะต้องได้รับการปกปิดอยู่เสมอ โดยจะใช้เฉพาะรหัสประจำโครงการวิจัยของท่าน และจะไม่มีการแจ้งผลการตรวจยืน จนกว่าจะมีหลักฐานชัดเจนว่ามีความสัมพันธ์กับการตอบสนองต่อการรักษาและอาการไม่พึงประสงค์

จากการลงนามยินยอมของท่านผู้ทำวิจัย หากท่านต้องการยกเลิกการให้สิทธิ์ดังกล่าว ท่านสามารถแจ้ง หรือเขียนบันทึกขอยกเลิกการให้คำยินยอม โดยส่งไปที่ เภสัชกรหญิงศิริลักษณ์ คำภิโร ที่อยู่ ภาควิชาเภสัชกรรมปฏิบัติ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 10330 หากท่านขอยกเลิกการให้คำยินยอมหลังจากที่ท่านได้เข้าร่วมโครงการวิจัยแล้ว ข้อมูลส่วนตัวของท่านจะไม่ถูกบันทึกเพิ่มเติม อย่างไรก็ตามข้อมูลอื่น ๆ ของท่านอาจถูกนำมาใช้เพื่อประเมินผลการวิจัย และท่านจะไม่สามารถกลับมาเข้าร่วมในโครงการนี้ได้อีก ทั้งนี้เนื่องจากข้อมูลของท่านที่จำเป็นสำหรับใช้เพื่อการวิจัยไม่ได้ถูกบันทึก

จากการลงนามยินยอมของท่านผู้ทำวิจัยสามารถบอกรายละเอียดของท่านที่เกี่ยวกับการเข้าร่วมโครงการวิจัยนี้ให้แก่แพทย์ผู้รักษาท่านได้

การจัดการกับตัวอย่างเลือดที่เหลือ

ตัวอย่างเลือดจากอาสาสมัคร ที่เหลือจากการวิจัย ผู้วิจัยขอเก็บตัวอย่างสำหรับตรวจซ้ำ เพื่อยืนยันความถูกต้องของผลการทดลองเป็นระยะเวลา 1 ปี หลังจากนั้นจะถูกทำลายตามวิธีมาตรฐานทันที

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

ขอขอบคุณในการร่วมมือของท่านมา ณ ที่นี้

APPENDIX E
Consent form

เอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัย

การวิจัยเรื่อง ผลของภาวะพหุสัณฐานของยีน *ERCC1 GSTP1* และ *CTR1* ต่อการตอบสนองต่อการรักษาและการเกิดพิษจากการใช้ยาเคมีบำบัดในกลุ่มแพลทินัมในผู้ป่วยมะเร็งปอดชนิดไม่ใช่เซลล์เล็ก ระยะสามบีถึงสี่

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

ข้าพเจ้า นาย/นาง/นางสาว...

ที่อยู่...

ได้อ่านรายละเอียดจากเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัยวิจัยที่แนบมาฉบับวันที่.............. และข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยโดยสมัครใจ

ข้าพเจ้าได้รับสำเนาเอกสารแสดงความยินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนาม และ วันที่ พร้อมด้วยเอกสารข้อมูลสำหรับผู้เข้าร่วมโครงการวิจัย ทั้งนี้ก่อนที่จะลงนามในใบยินยอมเข้าร่วมการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย ระยะเวลาของการทำวิจัย วิธีการวิจัย อันตราย หรืออาการที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัย และแนวทางรักษาโดยวิธีอื่นอย่างละเอียด ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัยจนมีความเข้าใจอย่างดีแล้ว โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้ารับทราบจากผู้วิจัยว่าหากเกิดอันตรายใด ๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่เสียค่าใช้จ่าย

ข้าพเจ้ามีสิทธิที่จะบอกเลิกเข้าร่วมในโครงการวิจัยเมื่อใดก็ได้ โดยไม่จำเป็นต้องแจ้งเหตุผล และการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคหรือสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะเมื่อได้รับการยินยอมจากข้าพเจ้าเท่านั้น บุคคลอื่นในนามของบริษัทผู้สนับสนุนการวิจัย คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน อาจได้รับอนุญาตให้เข้ามาตรวจและประมวลข้อมูลของข้าพเจ้า ทั้งนี้จะต้องกระทำไปเพื่อวัตถุประสงค์เพื่อตรวจสอบความถูกต้องของข้อมูลเท่านั้น โดยการตกลงที่จะ

เข้าร่วมการศึกษานี้ข้าพเจ้าได้ให้คำยินยอมที่จะให้มีการตรวจสอบข้อมูลประวัติทางการแพทย์ของข้าพเจ้าได้

ผู้วิจัยรับรองว่าจะไม่มีการเก็บข้อมูลใด ๆ เพิ่มเติม หลังจากที่ข้าพเจ้าขอยกเลิกการเข้าร่วมโครงการ วิจัยและต้องการให้ทำลายเอกสารและตัวอย่างที่ใช้ตรวจสอบทั้งหมดที่สามารถสืบค้นถึงตัวข้าพเจ้าได้

ข้าพเจ้าเข้าใจว่า ข้าพเจ้ามีสิทธิ์ที่จะตรวจสอบหรือแก้ไขข้อมูลส่วนตัวของข้าพเจ้าและสามารถยกเลิกการให้สิทธิในการใช้ข้อมูลส่วนตัวของข้าพเจ้าได้ โดยต้องแจ้งให้ผู้วิจัยรับทราบ

ข้าพเจ้าได้ตระหนักว่าข้อมูลในการวิจัยรวมถึงข้อมูลทางการแพทย์ของข้าพเจ้าที่ไม่มีการเปิดเผยชื่อ จะผ่านกระบวนการต่าง ๆ เช่น การเก็บข้อมูล การบันทึกข้อมูลในแบบบันทึกและในคอมพิวเตอร์ การตรวจสอบ การวิเคราะห์ และการรายงานข้อมูลเพื่อวัตถุประสงค์ทางวิชาการ รวมทั้งการใช้ข้อมูลทางการแพทย์ในอนาคต เท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความเต็มใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

....ลงนามผู้ให้ความยินยอม

(...) ชื่อผู้ยินยอมตัวบรรจง

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

การจัดการกับตัวอย่างทางชีวภาพ

☐ ไม่มีตัวอย่างชีวภาพ

☐ มีแต่ไม่มีการขอเก็บ

☑ มีและขอเก็บตัวอย่างชีวภาพที่เหลือไว้เพื่อการวิจัยในอนาคต

ข้าพเจ้า ☐ ยินยอม

☐ ไม่ยินยอม

ให้เก็บตัวอย่างชีวภาพที่เหลือไว้เพื่อการวิจัยในอนาคต

....ลงนามผู้ให้ความยินยอม

(...) ชื่อผู้ยินยอมตัวบรรจง

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

ข้าพเจ้าได้อธิบายถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตราย หรืออาการไม่พึงประสงค์หรือความเสี่ยงที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด ให้ผู้เข้าร่วมในโครงการวิจัยตามนามข้างต้นได้ทราบและมีความเข้าใจดีแล้ว พร้อมลงนามลงในเอกสารแสดงความยินยอมด้วยความเต็มใจ

....ลงนามผู้ทำวิจัย

(...........นางสาว..ศิริลักษณ์..คำภิโร.............................) ชื่อผู้ทำวิจัย ตัวบรรจง

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

....ลงนามพยาน

(...) ชื่อพยาน ตัวบรรจง

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

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APPENDIX F

Incidence of toxicity in each visit

Table Incidence of toxicity in each visit

Toxicity	Visit	N	%
Anemia	1	0	0
	2	31	44.30
	3	33	47.83
	4	38	61.29
	5	27	48.21
	6	9	52.94
	7	3	37.50
Neutropenia	1	0	0
	2	19	27.54
	3	9	13.04
	4	13	20.97
	5	7	12.50
	6	3	17.65
	7	1	12.50
Thrombocytopenia	1	0	0
	2	6	8.70
	3	7	10.15
	4	6	9.68
	5	1	1.79
	6	3	17.65
	7	0	0

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Table Incidence of toxicity in each visit

Toxicity	Visit	N	%
Nausea and vomit	1	0	0
	2	20	28.99
	3	16	23.19
	4	13	30.65
	5	9	16.07
	6	4	23.53
	7	2	25.0
Peripheral neuropathy	1	0	0
	2	8	11.59
	3	12	17.39
	4	14	22.58
	5	8	14.28
	6	3	17.65
	7	0	0
Weight loss	1	0	0
	2	5	7.25
	3	2	2.90
	4	4	6.45
	5	1	1.79
	6	0	0
	7	0	0

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Table Incidence of hematological toxicity each visit

Toxicity	Visit	Grade (%)			
		I	II	III	IV
Anemia	1	0 (0)	0 (0)	0 (0)	0 (0)
	2	17 (24.64)	9 (13.04)	5 (7.25)	0 (0)
	3	17 (24.64)	15 (21.74)	1 (1.45)	0 (0)
	4	15 (24.19)	18 (29.03)	5 (8.06)	0 (0)
	5	10 (17.86)	13 (23.21)	4 (7.14)	0 (0)
	6	1 (5.88)	6 (35.29)	2 (11.76)	0 (0)
	7	2 (25.0)	1 (12.50)	0 (0)	0 (0)
Neutropenia	1	0 (0)	0 (0)	0 (0)	0 (0)
	2	5 (7.24)	8 (11.59)	6 (8.70)	0 (0)
	3	3 (4.35)	4 (5.80)	2 (2.90)	0 (0)
	4	6 (9.68)	4 (6.45)	3 (4.84)	0 (0)
	5	2 (3.57)	4 (7.14)	1 (1.79)	0 (0)
	6	1 (5.88)	1 (5.88)	1 (5.88)	0 (0)
	7	0 (0)	1 (12.5)	0 (0)	0 (0)
Thrombocytopenia	1	0 (0)	0 (0)	0 (0)	0 (0)
	2	6 (8.70)	0 (0)	0 (0)	0 (0)
	3	6 (8.70)	1 (1.45)	0 (0)	0 (0)
	4	5 (8.06)	1 (1.61)	0 (0)	0 (0)
	5	1 (1.79)	0 (0)	0 (0)	0 (0)
	6	1 (5.88)	0 (0)	1 (5.88)	1 (5.88)
	7	0 (0)	0 (0)	0 (0)	0 (0)

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Table Incidence of non-hematological toxicity in each visit

Toxicity	Visit	Grade (%)			
		I	II	III	IV
Nausea and vomit	1	0 (0)	0 (0)	0 (0)	0 (0)
	2	9 (13.04)	9 (13.04)	2 (2.90)	0 (0)
	3	6 (8.70)	8 (11.59)	2 (2.90)	0 (0)
	4	5 (8.06)	7 (11.29)	1 (1.61)	0 (0)
	5	4 (7.14)	5 (8.93)	0 (0)	0 (0)
	6	2 (11.76)	2 (11.76)	0 (0)	0 (0)
	7	1 (12.5)	1 (12.5)	0 (0)	0 (0)
Peripheral neuropathy	1	0 (0)	0 (0)	0 (0)	0 (0)
	2	7 (10.14)	1 (1.45)	0 (0)	0 (0)
	3	9 (13.04)	2 (2.90)	1 (1.45)	0 (0)
	4	12 (19.35)	1 (1.61)	1 (1.61)	0 (0)
	5	6 (10.71)	2 (3.57)	0 (0)	0 (0)
	6	2 (11.76)	1 (35.19)	0 (0)	0 (0)
	7	0 (0)	0 (0)	0 (0)	0 (0)
Weight loss	1	0 (0)	0 (0)	0 (0)	0 (0)
	2	4 (5.80)	1 (1.45)	0 (0)	0 (0)
	3	1 (1.45)	1 (1.45)	0 (0)	0 (0)
	4	4 (6.45)	0 (0)	0 (0)	0 (0)
	5	0 (0)	1 (1.79)	0 (0)	0 (0)
	6	0 (0)	0 (0)	0 (0)	0 (0)
	7	0 (0)	0 (0)	0 (0)	0 (0)

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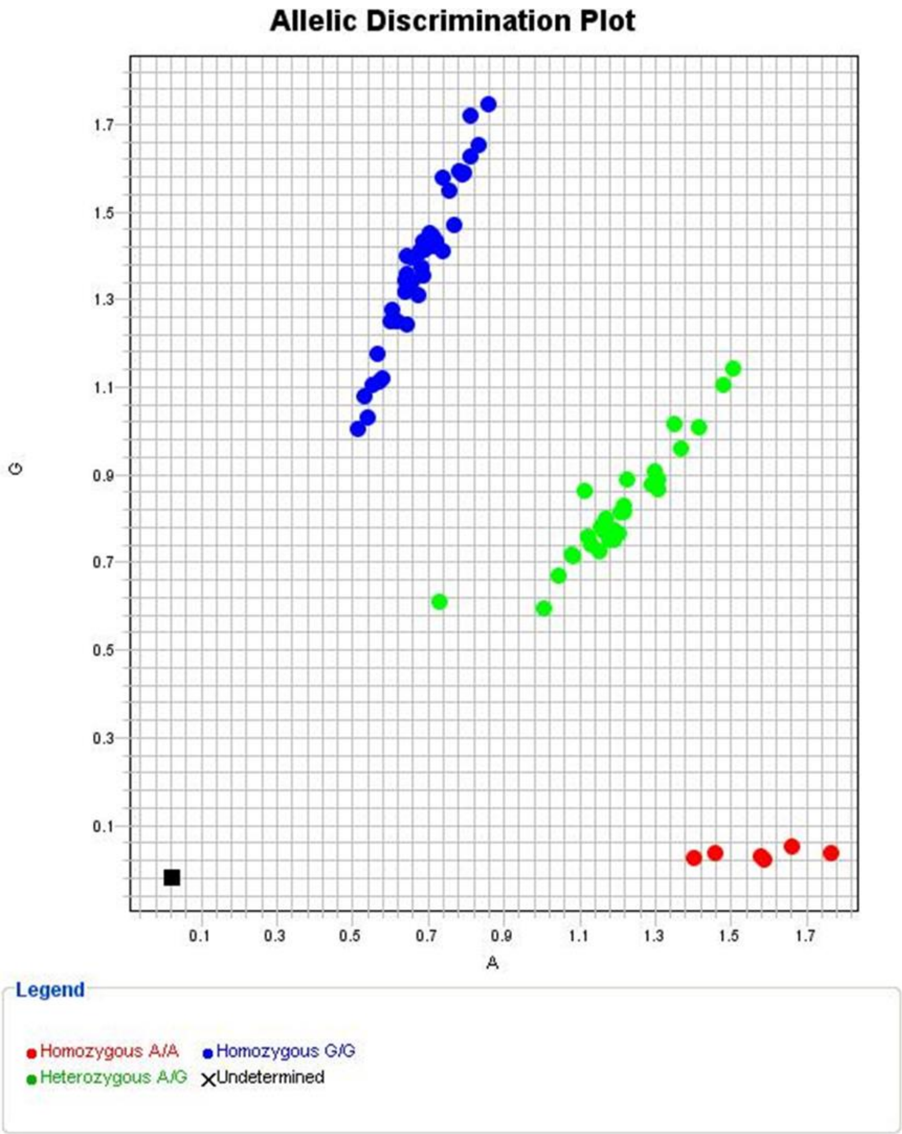
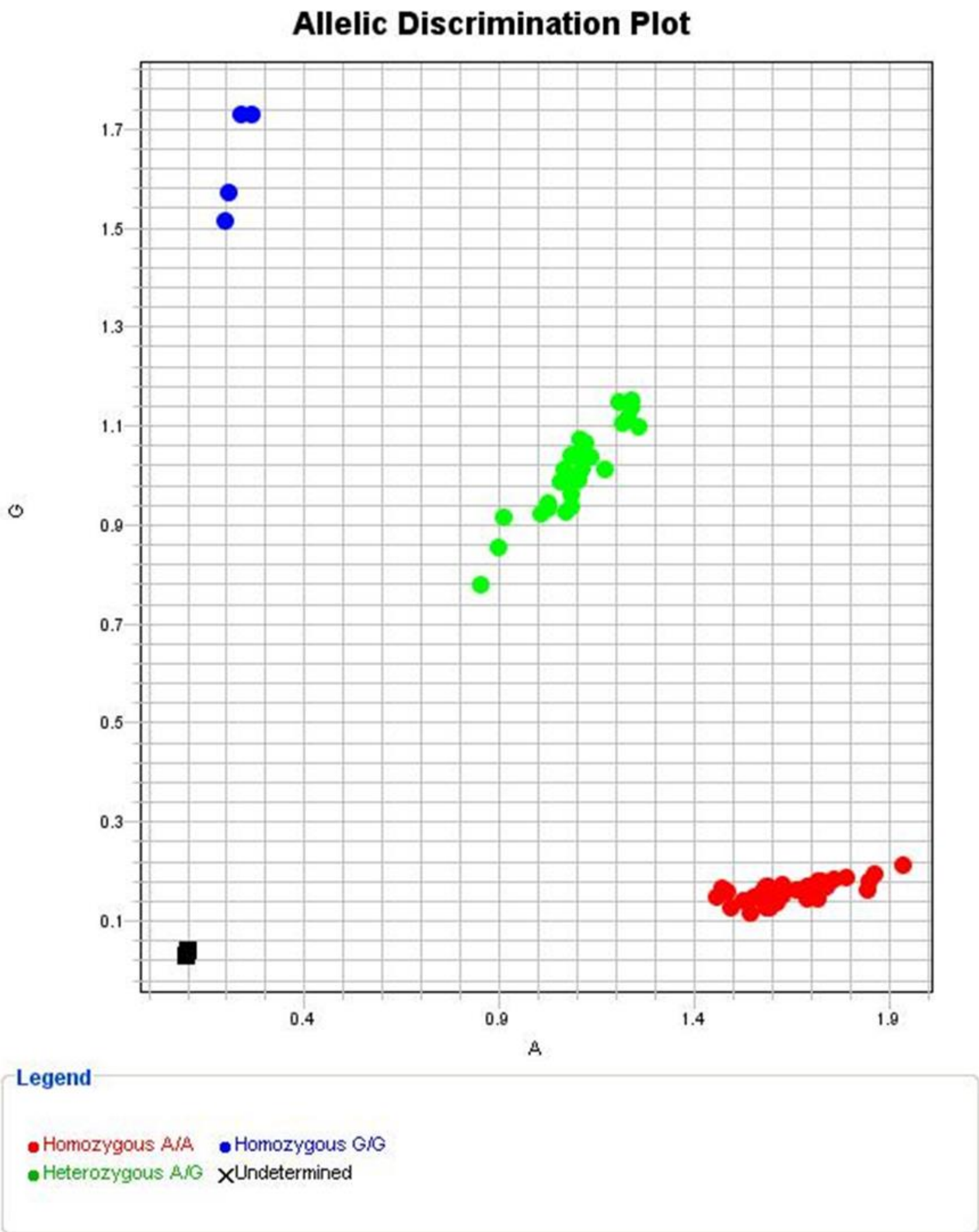
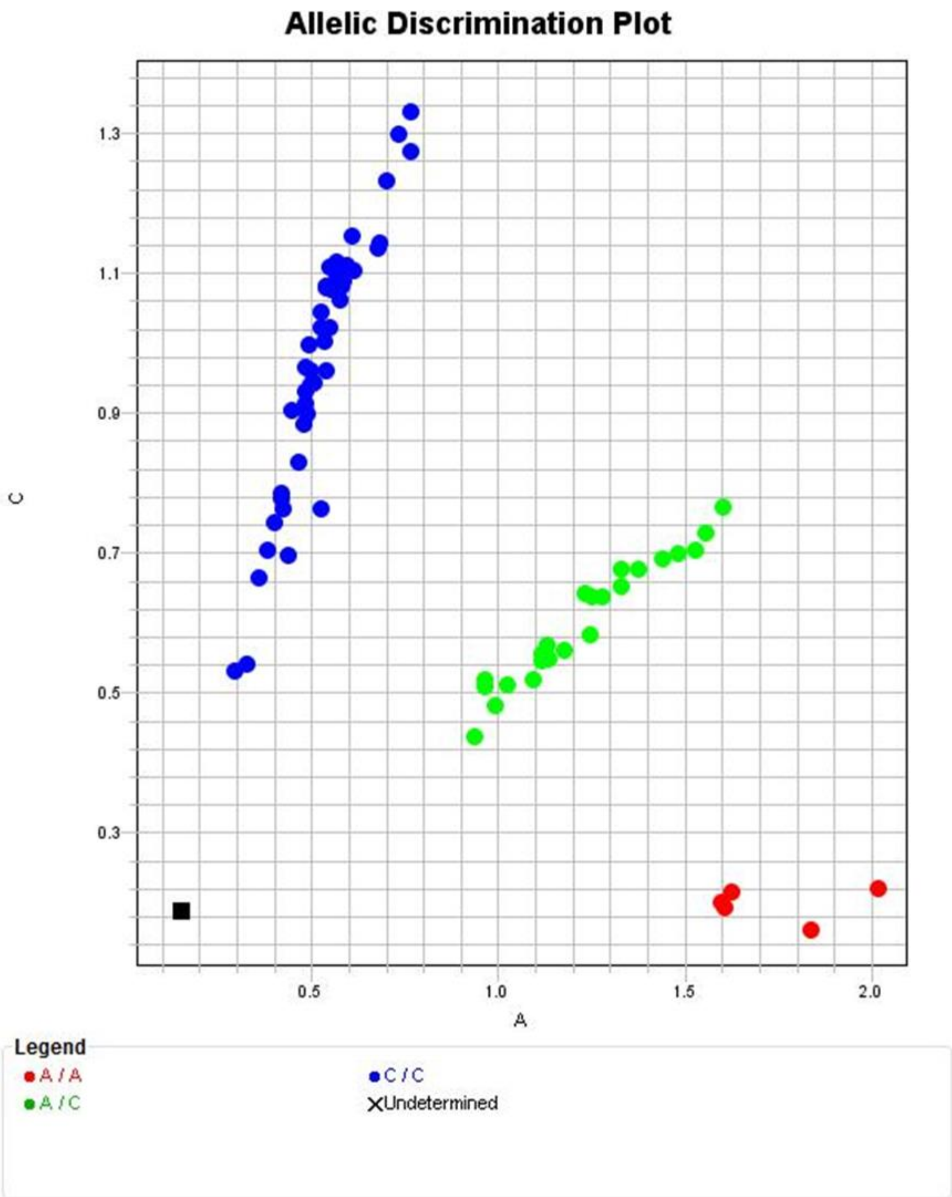
APPENDIX G

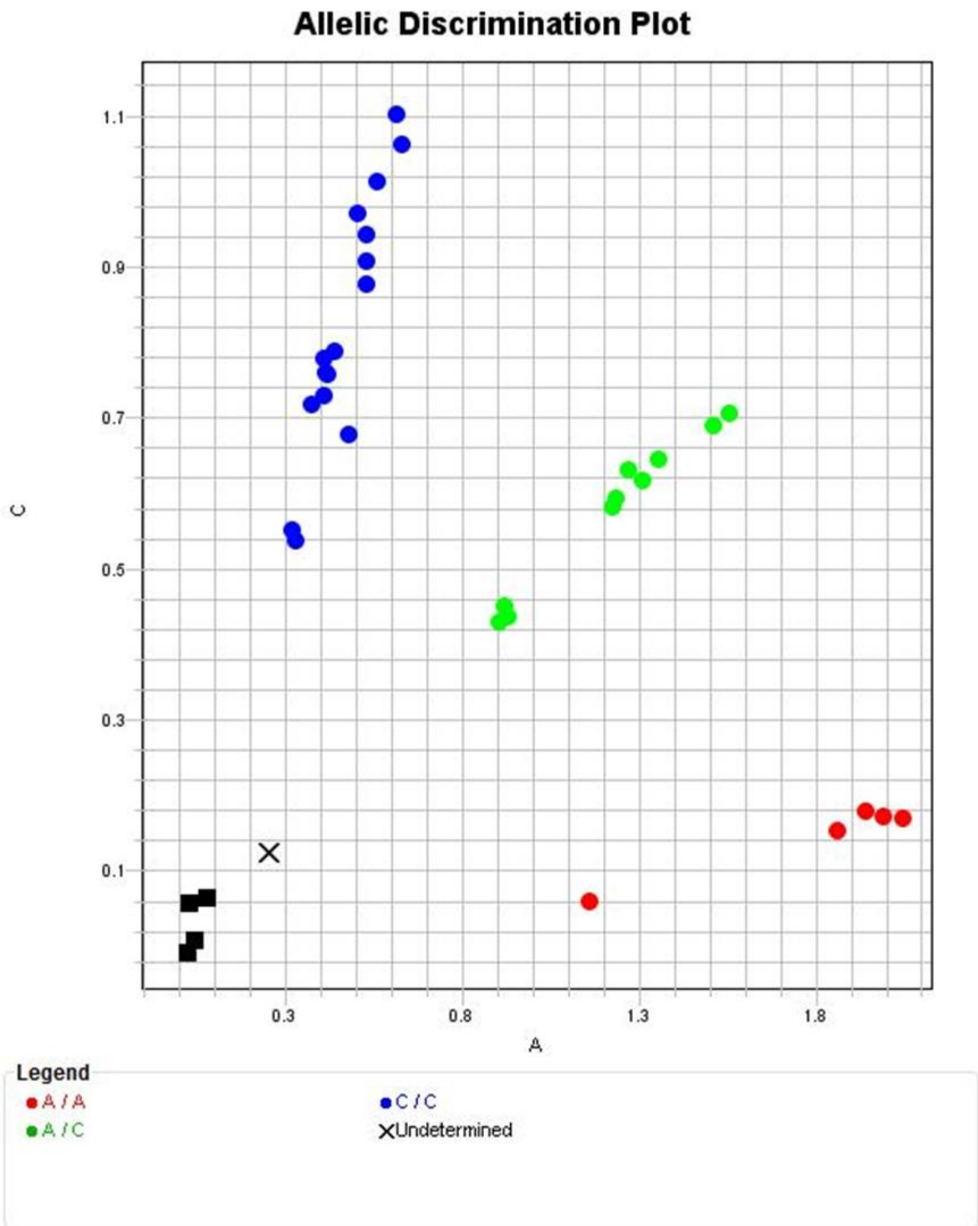
Allelic discrimination plot





Allelic Discrimination Plot
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
G
0.4
0.9
1.4
1.9
A
Legend
Homozygous A/A
Homozygous G/G
Heterozygous A/G
Undetermined


Allelic Discrimination Plot
1.3
1.1
0.9
0.7
0.5
0.3
C
0.5
1.0
1.5
2.0
A
Legend
A / A
C / C
A / C
Undetermined


Allelic Discrimination Plot
1.1
0.9
0.7
0.5
0.3
0.1
C
0.3
0.8
1.3
1.8
A
Legend
A / A
C / C
A / C
Undetermined

VITA

Siriluk Kumpiro was born in Nakornratchasima, Thailand on 24th April 1986. She graduated from Suranaree Wittaya School in Nakornratchasima in March 2004, and from Khon Kaen University at Khon Kaen with a Bachelor degree of Pharmaceutical Science in March 2009. After receiving her education and at the present, she has work as a pharmacist in Maharat Nakornratchasima Hospital. She admitted to Faculty of Pharmaceutical science, Department of clinical pharmacy, Chulalongkorn University in August 2014.

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