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CHOP 療程為基礎的化學治療在犬多中心型淋巴瘤的毒性反應以及腫瘤治療效果間的相關性

Correlation between Toxicity and Antitumor Efficacy of CHOP-based Chemotherapy in Canine Multicentric Lymphoma

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Abstract

This study was aimed at investigating the correlation between chemotherapy toxicity and antitumor efficacy in canine multicentric lymphoma. Medical records of 69 dogs with multicentric lymphoma received CHOP-based chemotherapy at National Taiwan University Veterinary Hospital were reviewed, and impact of bone marrow toxicity and GI toxicity on time to tumor progression (TTP), overall survival time (OST), and short-term tumor-killing effect was evaluated. Neutrophil nadir lower than 5000 /μl improved OST (P =0.045). Presence of GI signs, including anorexia, vomiting, and diarrhea, or presence of only vomiting improved TTP and OST (P =0.042, 0.007 for TTP; 0.023, <0.001 for OST). Presence of diarrhea of grade 3 or 4 decreased TTP and OST (P =0.034, 0.017). More than 10 years old was associated with less low-grade GI toxicities and less favorable outcome. Occurrence of neutropenia or GI toxicity after a treatment increased the like hood of effective treatment over ineffective treatment, implying a positive relationship between toxicity and short-term efficacy. The results of the study supported the concept of toxicity-adjusted dosing, but prospective trials are warranted to develop a sophisticated toxicity-adjusted dosing regimen.

Key words: chemotherapy, bone marrow toxicity, GI toxicity, lymphoma, CHOP, dogs

摘要

本研究目的為探討犬多中心型淋巴瘤的化療毒性以及療效間的相關性。六十九隻罹患多中心型淋巴瘤且在國立台灣大學附設動物醫院接受以 CHOP 療程為基礎的化學治療的犬隻的病歷被回顧，並且分析其中骨髓毒性以及消化道毒性對於疾病進展時間(Time to tumor progression, TTP)，總存活時間(Overall survival time, OST)，以及短期的腫瘤抑制效果的影響。嗜中性球低點小於 5000/μl 可增進 OST (P =0.045)。出現食慾不振、嘔吐、腹瀉其中一種消化道毒性或者僅出現嘔吐皆可增加 TTP 及 OST (P =0.042, 0.007 對於 TTP; 0.023, <0.001 對於 OST)。出現毒性分級三或四的腹瀉會降低 TTP 及 OST (P =0.034, 0.017)。年齡超過十歲較不容易出現毒性分級低的消化道毒性，同時也有較不理想的治療成果。在單次治療後出現嗜中性球低下或者消化道毒性，會增加該次治療成為有效治療而非無效治療的可能性，顯示毒性以及短期療效之間存在正向關係。本研究的結果支持依據毒性反應來調整藥物劑量的概念，但是必須靠前瞻性研究才能建立起完善的依據毒性做劑量調整的規範。

關鍵字：化學治療，骨髓毒性，消化道毒性，淋巴瘤，CHOP，犬

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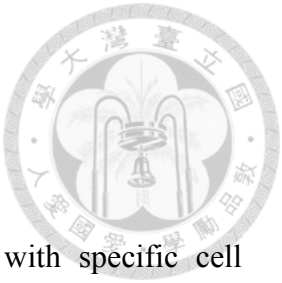


1 Introduction

Chemotherapeutic agents cause DNA damage or interfere with specific cell cycles, thus are most effective on rapid-dividing cells, such as tumor cells, gastrointestinal epithelium cells, and bone marrow cells. As a result, chemotherapy can induce both favorable antitumor responses and unfavorable adverse events.

Possible positive correlation between toxicity and antitumor effects has long been noticed in both human and veterinary oncology practice. In human medicine, the intensity of chemotherapy toxicity and efficacy have been both linked to pharmacodynamics in numerous studies with various chemotherapeutic agents and tumor types. There are also several clinical studies showing patients with hematological toxicity are prone to have better treatment outcomes. However, relevant clinical analysis in veterinary medicine is little. As more and more evidence suggested that body surface area-based dosing, the traditional way to dose chemotherapeutic agents, poorly adapts to inter-patient variation, toxicity-adjusted dosing may be a supplemental method to improve dosing accuracy, and more investigation is needed before this approach can be practically performed.

This study is aimed at retrospectively analyzing the correlation between chemotherapy toxicity and efficacy in canine multicentric lymphoma, focusing on gastrointestinal and bone marrow impacts, in both long-term aspect and short-term aspect, utilizing the medical records of the National Taiwan University Veterinary Hospital.

2. Literature Review

2.1 Canine multicentric lymphoma and CHOP-based chemotherapy

Lymphoma is the most common hematopoietic neoplasm of dogs. Eighty-four percent of dogs with lymphoma developed the multicentric form, which is usually characterized by the presence of superficial lymphadenopathy. Without treatment, most dogs with lymphoma will die of their disease in 4 to 6 weeks after diagnosis, although significant variability exists (Vail et al., 2013).

Over the last 30 years, the standard of care for dogs with multicentric lymphoma has evolved from single-agent chemotherapy protocols to combination chemotherapy protocols. The duration of protocols also changed from indefinite to 6 months or less. The standard of care combination protocols are now generally recognized as "CHOP-based" protocols, consisting of cyclophosphamide, hydroxyl-daunorubicin (doxorubicin), Oncovin (vincristine), and prednisone. There are many variations of this particular combination of drugs. Variations from this protocol include differences in the order of drug administration, addition of L-asparaginase or methotrexate to the protocol, slight differences in drug doses, and increased or decreased protocol duration (Chun, 2009). Currently randomized prospective evidence does not strongly recommend one

protocol over the other as long as the basic CHOP components are present. CHOP-based chemotherapy induces remission in approximately 80%~95% of dogs, with overall median survival times of 10 to 12 months. Approximately 20% to 25% of treated dogs will be alive after initiation of these protocols (Vail et al., 2013).

Generally, CHOP-based chemotherapy protocols were well tolerated by dogs with lymphoma. In a clinical trial using a 6-month, maintenance free, CHOP-based protocol, 22 in 53 (41.5%) dogs requiring a treatment delay or dose modification due to bone marrow or gastrointestinal toxicities, but only 5 (9.4%) dogs needing hospitalization (Garret et al., 2002).

Many factors have been shown to influence treatment response and survival of canine lymphoma. The well-established negative prognostic factors included WHO clinical stage V, WHO clinical substage b, T-cell phenotype, presence of anemia at diagnosis, hypercalcemia, and prolonged steroid pre-treatment (Vail et al., 1996; Khanna et al., 1998; Marconato et al., 2011; Jagielski et al., 2002).

2.2 Correlation between toxicity and antitumor efficacy of chemotherapy

2.2.1 Related research in human medicine

Most studies focusing on both chemotherapy toxicity and antitumor efficacy investigated the issue of how to dose chemotherapeutic agents precisely. To achieve maximum tumor-killing effect, the dose should be as high as possible. But concurrent chemotherapy toxicity sets the limit of dose escalation, since severe toxicity can compromise life quality or even cause mortalities. In fact, all chemotherapeutic agents are characterized by a narrow therapeutic window and significant variability in therapeutic and toxic effects. Current body surface area (BSA)-based dosing regime fails to adapt to interpatient or intrapatient pharmacodynamic variability, which leads to unstandardized systemic anticancer drug exposure, despite under the same dose of the same drug (Hon et al., 1998; Gao et al., 2008).

Hon et al. summarized a substantial amount of studies demonstrating the relationship between systemic exposure and both efficacy and toxicity (Hon et al., 1998). Systemic exposure of certain drug was measured by various pharmacokinetic parameters in different studies, such as systemic clearance, steady-state plasma concentration (Cp_{SS}), and area under the concentration–time curve (AUC). Some of the studies linked systemic exposure to antitumor efficacy, evaluated by response to treatment, overall survival time, or disease-free survival time, according to different

study designs, whereas some of the studies linked systemic exposure to toxicity, including hematological toxicity, gastrointestinal toxicity, and ototoxicity. The majority of studies focused on hematological toxicity. All of the studies showed a similar result: the higher systemic exposure, the higher efficacy or toxicity.

The relationship between systemic exposure and both efficacy and toxicity was investigated most extensively for 5FU, a widely used chemotherapeutic agent. AUC of 5FU was highly correlated with hematological and gastrointestinal toxicities in patients with head and neck cancer. An AUC threshold value of 30000 μg/L · h was highly predictive of toxicity (Thyss et al., 1986). The half-cycle and full-cycle AUC ($AUC_{0-3days}$ and $AUC_{0–5days}$) were also higher in toxic than in nontoxic cycles (Santini et al., 1989). Patients with an average AUC per cycle for all 3 cycles >29 000 μg/L · h exhibited longer survival (Milano et al., 1994). Note the optimal threshold AUC for survival being very close to the maximum tolerated AUC for toxicity.

Apart from systemic exposure, some clinical studies directly investigated the relationship between hematological toxicity and efficacy. Gao et al summarized several studies associating neutropenia during chemotherapy with increased survival in patients with several different tumors (Gao et al., 2008). For instance, three studies of

node-positive early breast cancer treated with either cyclophosphamide, methotrexate and fluorouracil or cyclophosphamide, doxorubicin, and ftorafur demonstrated that low leukocyte nadir after chemotherapy can lead to increased distant disease-free survival or overall survival (Poikonen et al., 1999; Saarto et al., 1997; Colleoni et al., 1998).

Studies directly linked non-hematological toxicity with efficacy was sparse. One study of advanced hepatocellular carcinoma treated with sorafenib showed that development of grade 3 to 4 diarrhea was associated with increased overall survival (Koschny et al., 2013). However, sorafenib is a target therapy drug, not a traditional anticancer drug, thus not fully corresponded to the scope of the current study. Three studies demonstrated that the occurrence of hand-foot syndrome (HFS), a particular presentation of skin toxicity, was associated with better outcome in colorectal cancer patients treated with capecitabine, with or without other chemotherapeutic agents (Stintzing et al., 2011; Hofheinz et al., 2012; Twelves et al., 2012). Interestingly, in one of the three studies, gastrointestinal toxicity and diarrhea were significantly more common in patients with HFS but not often co-incident with hematological toxicities (Hofheinz et al., 2012).

Human oncology studies establishing relationship between toxicity and efficacy are summarized in **Table 1**.

Some studies investigating correlation between toxicity and efficacy explained the connection by interpatient pharmacodynamic variations (Stintzing et al., 2011; Rankin et al., 1992; Mayers et al., 2001; Cameron et al., 2003; Di Maio et al., 2005): The response of cancer cells to chemotherapy depends on a sufficient amount of active drug reaching the target. These factors also apply to healthy cells. The availability of active drug at tumor cells or healthy cells is affected by pharmacokinetic factors (ie, the metabolism, distribution, and catabolism) of drugs, which produce a similar effect in tumor cells and healthy cells. In addition, some studies also proposed a view of interpatient genetic variations (Di Maio et al., 2005): The sensitivity of tumor cells and healthy cells is affected, by genetic predisposition, which can similarly affect both cell types on the same patient, but is also modified by tumor-specific acquired resistance.

2.2.2 Related research in veterinary medicine

To the author's knowledge, only two studies in veterinary medicine have established a positive correlation between chemotherapy toxicity and antitumor efficacy. The first study retrospectively investigated the impact of chemotherapeutic dose intensity and hematologic toxicity on first remission duration in dogs with lymphoma treated with a chemoradiotherapy protocol (Vaughan et al., 2007). The study result showed that development of grade III or IV neutropenia during chemotherapy was associated with prolonged first remission duration. The second study implemented a dose-intense CHOP-based chemotherapy protocol for canine lymphoma, and found that dogs required dose reductions and treatment delays had significantly longer time to tumor progression and lymphoma-specific survival times (Sorenmo et al., 2010).

Some other veterinary clinical studies of various cancers included the occurrence of toxicity in prognostic value analysis, but none have found a relationship between toxicity and outcome. Four studies of canine lymphoma treated with CHOP-based chemotherapy showed that toxicity had no influence on either disease-free interval or overall survival (Simon et al., 2006; Keller et al., 1993; Zemann et al., 1998; Garrett et al., 2002). The definition of occurrence of toxicity differed from one study to another. One study regarded toxicity of any grade, according to the Veterinary Co-Operative

Oncology Group's common terminology criteria for adverse events v1.0 (VCOG-CTCAE v1.0), as occurrence of toxicity, and recorded number of neutropenic episodes and degree and number of gastrointestinal toxicosis episodes for evaluation (Simon et al., 2006). Two studies regarded toxicities that caused treatment change as occurrence of toxicity (Keller et al., 1993; Garrett et al., 2002), whereas the other study defined occurrence of toxicity as neutrophil less than 1000 /μl or hospitalization for GI adverse events (Zemann et al., 1998). As we can see, the criteria for toxicity were relatively loose in the first study, and stricter in the three latter studies. These differences could affect the result of analysis. There are also one study of canine appendicular osteosarcoma and two studies of canine urinary bladder transitional carcinoma finding that hematological and gastrointestinal toxicity not correlated with outcome (Bacon et al., 2008; Chun et al., 1997; Marconato et al., 2011). Except one transitional carcinoma study defined neutropenia as neutrophil less than 2000 /μl, the other two studies utilized loose toxicity criteria by including toxicities of all grade, according to VCOG- CTCAE v1.0, into analysis.

Selected veterinary studies investigating relationship between toxicity and efficacy are summarized in **Table 2**.

3. Aim

The purpose of this study was to investigate the correlation between chemotherapy toxicity and antitumor efficacy in canine multicentric lymphoma, in either long-term or short-term aspect.

4. Materials and Methods

4.1 Patient selection

Medical records of dogs with multicentric lymphoma between January 2000 and December 2014 at The National Taiwan University Veterinary Hospital were reviewed retrospectively. Dogs that were cytologically or histologically diagnosed with multicentric high-grade lymphoma and received CHOP-based chemotherapy for the treatment of lymphoma without any chemotherapy prior to CHOP were included in the present study. Dogs that failed to finish at least the first two cycles of CHOP due to reasons other than lymphoma-related death (ie, tumor progression or severe chemotherapy toxicity) were excluded from the study.

4.2 Chemotherapy protocol

This study utilized a 6-month, maintenance-free, modified version of the University of Wisconsin (UW)-Madison chemotherapy protocol (UW-25), which is provided in **Table 3 (**Garrett et al., 2002**).** Dose reductions of 20% to 30% and treatment delays for 3 days to 7 days would be performed if neutrophil less than 2000 to

3000 /μl or severe gastrointestinal toxicity, depending on the clinician's preferences and the patient's whole body status.

Because of the retrospective nature of the study, protocol adjustments were frequently seen for the included patients. The standard UW-25 protocol administered one treatment of vincristine, followed by cyclophosphamide, and again vincristine, and then doxorubicin; so one full cycle of UW-25 can be abbreviated as VCVA (ie, V for vincristine, C for cyclophosphamide, and A for doxorubicin). But in this study, dogs were sometimes treated by a "VCVCA" protocol, or even "VCVCVCA". The other frequent protocol adjustments were prolonged treatment intervals and dose reductions without actual occurrence of hematological or gastrointestinal toxicity. In addition, there were many premature treatment cessations, by which many patients did not receive four cycles of chemotherapy. All these protocol adjustments were attributed to practical requirements, such as client compliance, economic considerations, patient age, and clinician's preferences.

The included dogs were treated by 5 different veterinarians, respectively, among which there was a senior veterinarian being the mentor of all the other 4 veterinarians, leading to a similar treatment approach for all dogs.

For dogs that went out of remission or became resistant to CHOP protocol, various rescue drugs were offered, including L-asparginase, lomustine, dacarbazine, actinomycin D, and DMAC protocol (ie, dexamethasone, melphalan, actinomycin D, and cytosine arabinoside).

4.3 Diagnosis and staging

Of the 69 dogs included in the study, the diagnosis of lymphoma was made by biopsy of lymph node in 4 dogs, by aspiration cytology of lymph node in 65 dogs.

All dogs were clinically staged at diagnosis by means of a modification of the World Health Organization (WHO) 5-stage criteria for canine lymphoma (Owen, 1980). Dogs were assigned to stage V if neutropenia or circulating lymphoblasts were detected in peripheral blood. Dogs were assigned to stage IV if no evidence of stage V disease and hepatomegaly or splenomegaly noted in radiography or ultrasonography, or heterogeneous texture of liver or spleen noted in ultrasonography. Because of lack of a standard staging manner of all dogs, some dogs with more advanced lymphoma (stage V or IV) could be classified as less advanced stage.

4.4 Response and toxicity assessment

Overall response to treatment was classified as complete remission (CR), partial remission (PR), and no response (NR). The status at which a patient had the least tumor burden during treatment was taken for evaluation. Complete remission was defined as disappearance of all target lesions, and any pathological lymph nodes must have reduction in short axis to <10 mm. Partial remission was defined as at least a 30% decrease in the sum of diameters of target lesions. Responses other than complete remission and partial remission was defined as no response.

Besides overall response to treatment, this study documented detailed toxicity and response during the treatment course. For each treatment, the following data was recorded: drug, time from diagnosis, gastrointestinal toxicity, hematological toxicity, response to that treatment, and whether dose reduction or not.

Gastrointestinal toxicities recorded included anorexia, vomiting, and diarrhea, and graded based on the Veterinary Co-operative Oncology Group common terminology criteria for adverse events v1.0 (VCOG-CTCAE v1.0). Because anorexia of grade 1 was difficult to be identified from medical records, none of it was documented. For

treatments that medical records were too obscure to tell the occurrence of GI toxicity, or actual cause of GI adverse event could not be determined, the corresponding GI toxicity to that treatment would be assigned as unavailable, and excluded from analysis.

Hematological toxicities recorded included only neutropenia, and graded according to VCOG-CTCAE v1.0, except that the criteria of grade 1 neutropenia was adjusted to meet the needs of the study and further sub-graded to grade 1.0 and grade 1.1, as provided in **Table 4**. The presence of neutropenia after a treatment would be assigned as unavailable and excluded from analysis if blood work was not performed in two weeks after vincristine, cyclophosphamide, and chlorambucil, or four weeks after doxorubicin, mitoxantrone, lomustine, dacarbazine, and actinomycin D.

Response to a single treatment was classified as complete remission (CR), partial remission (PR), stable disease (SD), progressive disease (PD), and uncertain response. Definitions of complete remission and partial remission were similar to that for overall response evaluation mentioned above. Progressive disease was defined as at least a 20% increase in the sum of diameters of target lesions, or occurrence of new target lesions, such as lymphoblasts in peripheral blood. Responses with neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD were classified as stable disease.

Responses not clearly recorded in the medical records were assigned as uncertain response.

4.5 Prognostic factors

Two endpoints for response were evaluated for prognostic significance. Time to tumor progression (TTP) was defined as the period of time (in days) from diagnosis to progressive disease or relapse. Overall survival time (OST) was defined as the period of time (in days) from diagnosis to death. Patients who lost follow-up were censored on the last day of contact. Patients still in remission or alive at the end of the study were censored on the last day of data collection (2014/5/30) for TTP or OST analysis, respectively.

The clinical factors evaluated for potential prognostic significance included: sex and neuter status, age (grouped as ≤5 y/o, 6~10 y/o, and ≥11 y/o), body weight (<15 kg or not), immunophenotype of neoplastic cells if available, WHO clinical stage, WHO clinical substage, presence of hypercalcemia at diagnosis, presence of anemia at diagnosis, pre-treatment with steroid, response to treatment (complete remission, partial

remission, or no response), and time to finish the first two CHOP cycles (<80 days or not).

4.6 Long-term analysis

Long-term analysis investigated the relationship between toxicity and overall outcome, using time to tumor progression (TTP) and overall survival time (OST) as endpoints.

Chemotherapy toxicity could happen with different presentation forms (ie, neutropenia, anorexia, vomiting, and diarrhea), after different drugs, at different time during the protocol, with various severities (ie, grade 1 to 4), and at various frequencies. By shifting these variables, the criteria of occurrence of toxicity also changed. For instance, defining toxicity of any grade after any drugs at any time during the protocol as occurrence of toxicity is a loose criterion, whereas defining toxicity of higher than grade 3 happened more than 3 times during the first two cycles as occurrence of toxicity is a very strict criterion. Setting appropriate criteria might be crucial to establish a relationship between toxicity and efficacy.

To thoroughly examine the influences of these variables on the appropriateness of toxicity criteria, this study developed a 5-digit coding system, as provided in **Table 5**. The first digit represents different forms of toxicity: 1 stands for neutropenia; 2 stands for anorexia; 3 stands for vomiting; 4 stands for diarrhea; 5 stands for combined GI signs, consisting of anorexia, vomiting, and diarrhea. The second digit represents different timing in the protocol: 1 stands for full course; 2 stands for the first two cycles. The third digit represents different drugs: 1 stands for all drugs, 2 stands for vincristine; 3 stands for cyclophosphamide; 5 stands for doxorubicin. The fourth digit represents various toxicity grades: 1 stands for taking toxicity of all grade as occurrence of toxicity; 3 stands for taking only toxicity of high grade as occurrence of toxicity; 4 stands for dividing patients into no toxicity, low-grade toxicity and high-grade toxicity, with slightly different threshold between neutropenia and the other GI signs, as shown in **Table 5**. Toxicity grade for a patient was assigned as the highest grade along all treatments under consideration. The fifth digit represents various frequencies: 0 stands for toxicity occurrence at least once; 3 stands for more than twice; 5 stands for more than four times.

Each 5-digit code corresponds to a particular toxicity criterion, and patients were

grouped according to these criteria. For example, code 11110 divides patients into two groups: Patients who experienced at least once neutropenia of any grade at any time after any drug, and patients who did not. Another example, code 31540 divides patients into three groups: Patients who experienced at least once vomiting of grade 1 or 2 at any time after doxorubicin, patients who experienced at least once vomiting of grade 3 or 4 at any time after doxorubicin, and patients who did not experience vomiting at any time after doxorubicin. Total 360 (5 x 2 x 4 x 3 x 3 =360) groupings were made. After excluding groupings dividing patients into two groups with less than 5 patients in one group or dividing patients into three groups with less than 5 patients in two groups, 154 groupings remained. Kaplan-Meier curves of TTP and OST were plotted for the 154 groupings to detect outcome differences.

4.7 Short-term analysis

Short-term analysis investigated the relationship between toxicity and efficacy caused by the same single treatment, using the toxicity and response records for each treatment.

Neutropenia of more than grade 1.1 was considered as occurrence of toxicity. Anorexia, vomiting and diarrhea were evaluated together as combined GI signs, and toxicity grade of a treatment was assigned as the highest grade within the three. GI toxicity of any grade was regarded as occurrence of toxicity.

Treatments were categorized as effective treatment, ineffective treatment, and uncertain treatment based on tumor response. Effective treatments cause partial remission or the very first complete remission in a row of complete remissions. Ineffective treatments lead to progressive disease. Treatments with uncertain response, stable disease, and subsequent complete remission after the initial complete remission were defined as uncertain treatments.

Whether occurrence of toxicity had impact on the like hood of effective treatment, ineffective treatment, and uncertain treatment was examined. Treatments were analyzed either all together, or separately according to drugs, including vincristine, cyclophosphamide, and doxorubicin.

4.8 Statistics

Differences in outcome (TTP and OST) according to potential prognostic factors (sex and neuter status, age, body weight, immunophenotype, WHO clinical stage and substage, presence of hypercalcemia at diagnosis, presence of anemia at diagnosis, pre-treatment with steroid, response to treatment, and time to finish the first two CHOP cycles) were assessed by the Kaplan-Meier log-rank test. Factors with a P value <0.05 were regarded as significant prognostic factors.

For long-term analysis, differences in outcome (TTP and OST) according to the 154 groupings were assessed by the Kaplan-Meier log-rank test. For groupings with P value <0.05, chia-square test was performed to examine the correlation between grouping and significant prognostic factors. Multivariate Cox regression analysis was used to evaluate selected groupings with a P value <0.05 and significant prognostic factors for their independent association with TTP and OST.

For short-term analysis, chia-square test was performed to investigate the association between occurrence of toxicity and response. Odds ratio of the odds of effective treatment to ineffective treatment was calculated.

Statistical significance was defined as $P < 0.05$. All analyses were performed using SPSS statistical software Version 18.

5. Results

5.1 Patient characteristics

Total 69 dogs were included in this study. Medical records of 175 dogs with lymphoma were reviewed, and 106 dogs was excluded due to not receiving CHOP protocol, dropping out of CHOP protocol before the end of the first two cycles, or incomplete medical record.

≤5 y/o, 6~10 y/o, and ≥11 y/o

Thirty-six dogs were male (24, 34.8%, neutered and 12, 17.4%, intact) and 33 dogs were female (10, 14.5%, neutered and 23, 33.3%, intact). There were 49 purebred dogs: Golden Retriever (n=15) was the most common breed represented. Other breeds were Beagle (n=5), Chihuahua (n=4), Maltese (n=3), Schnauzer (n=3), Shi Tzu (n=3), Bull terrier (n=2), English Cocker Spaniel (n=2), Corgi (n=2), Labrador Retriever (n=2), Pomeranian (n=2), Yorkshire Terrier (n=2), Dachshund (n=1), Pug (n=1), Rottweiler (n=1), and Bichon Frise (n=1). Nineteen dogs were mixed breed dogs. Breed was unknown for 1 dog. The mean age was 7.5 years (range, 2–14 years), with 21 (30.4%) dogs ≤5 years old, 34 (49.3%) dogs within 5 to 10 years old, and 14 (20.3%) dogs ≥11

years old. The mean body weight was 18.8 kg (range, 1.9–57.1 kg), with 33 (47.8%) dogs <15 kg and 36 (52.2%) dogs >15 kg. The immunophenotype of the lymphoma cells were B-cell type in 22 (32%) dogs, T-cell type in 2 (3%) dogs, and not determined in 45 (65%) dogs. By WHO clinical staging standards, 33 (48%) dogs were in stage III, 23 (33%) dogs were in stage IV, and 13 (19%) dogs were in stage V. Forty-four (64%) dogs were in substage a and the 25 (36%) remaining dogs were in substage b. Two (3%) dogs had hypercalcemia at diagnosis. Twenty-five (36%) dogs were anemic at diagnosis. Eight (12%) dogs received steroids prior to CHOP protocol.

5.2 Response and toxicity

Forty-six dogs achieved CR, and 19 dogs achieved PR. No response to treatment was observed in 4 dogs. Total response rate (CR + PR) was 94%. Median time to tumor progression (TTP) was 185 days (range, 16-831 days). Median overall survival time (OST) was 282 days (range, 32-841 days). Kaplan-Meier curves of TTP and OST are shown in **Figure 1~2**.

Forty-eighty (69.6%) dogs were dead due to lymphoma. Five (7.2%) dogs were euthanized due to lymphoma progression. Two (2.9%) dogs were dead after seizure and

lymphoma involvement was highly suspected. Nine (13%) dogs lost follow-up, with median and mean follow-up time 518 and 795 days, respectively. Three (4.3%) dogs were still alive at the end of the study, with follow-up time 1318, 1745, and 2358 days, respectively.

Twenty-four dogs (35%) finished the first two cycles less than 80 days, whereas 40 (58%) dogs more than 80 days. Five (7%) dogs were dead due to lymphoma or severe chemotherapy toxicity before finishing the first two cycles.

Toxicity profile was presented as the number of patients experienced certain type of toxicity of certain grade, as listed in **Table 6~7**. The protocol was generally well tolerated. Anorexia, vomiting, and diarrhea of grade 4, which necessitated hospitalization, occurred in only 0 (0%), 1 (1%), and 2 (3%) dogs, respectively. Neutropenia of more than grade 1.1, which necessitated treatment delay, occurred in 47 (50%) dogs, whereas grade 2 and 3 neutropenia occurred in only 10 (15%) dogs. 2 (3%) dogs were dead due to chemotherapy toxicity.

5.3 Prognostic factors

Age, WHO clinical stage, presence of anemia at diagnosis, response to treatment, and time to finish the first two CHOP cycles were identified as significant for TTP. Age, WHO clinical stage, WHO clinical substage, presence of anemia at diagnosis, response to treatment, and time to finish the first two CHOP cycles were identified as significant for OST. More than 10 years old was related to worse outcome. WHO clinical stage III, WHO clinical substage a, absence of anemia at diagnosis, and finishing the first two cycles >80 days were associated with better outcome. Patients in the three categories of response to treatment exhibited different outcome, with CR being the best and NR being the worst. Median TTP and OST and P values according to above factors are provided in **Table 8**. Kaplan-Meier curves for TTP and OST of above factors are demonstrated in **Figure 3~14**.

Immunophenotype was available for only 24 (35%) dogs. Presence of hypercalcemia at diagnosis, and pre-treatment with steroid were only noted in 2 (3%) and 8 (12%) dogs. These three factors were excluded from analysis due to low case numbers.

5.4 Long-term analysis

In the 154 groupings, statistical significance was found in 9 and 15 groupings for TTP and OST, respectively. All P values for TTP and OST of the 154 groupings are provided in **Table 9**. To best illustrate these results, some groupings were selected and compared in order to elucidate the influences of the five variables (form of toxicity, timing during the protocol, drug, toxicity grade, frequency) on the correlation between toxicity and efficacy.

Form of toxicity and toxicity grade

Table 10 included P values for 15 groupings which set timing during the protocol as full course, drugs as all drugs, and frequency as at least once (ie, first, second and fifth digit of coding system fixed to 1). In other words, these 15 groupings focused on only form of toxicity and toxicity grade, neglecting the other three variables. The continued part of **Table 10** is composed of groupings dividing patients into three groups, and the exact P values between each group are provided in **Table 11**.

Based on results of grouping 11110, patients who experienced neutropenia of more than grade 1.0 (ie, neutrophil <5000 μl) had longer OST (P =0.045) than patients who

did not experience any episode of neutropenia, whereas there was no difference in TTP (P =0.094). If shifting definition of occurrence of toxicity to neutropenia of more than grade 2 (ie, neutrophil <1500 μl), as in grouping 11130, differences could be only observed in the Kaplan-Meier curve for OST (**Figure 16**), but no statistical significance was detected in either TTP (P =0.151) or OST (P =0.063). If dividing patients into no toxicity, low-grade toxicity (grade 1.0 and 1.1) and high-grade toxicity (grade 2 and 3), as in grouping 11140, statistical difference was found in OST (P =0.044), with actually only low-grade toxicity group superior to no toxicity group (P =0.039) and high-grade toxicity group superior to no toxicity group (P =0.005), but no significant differences in low-toxicity group and high-toxicity group (P =0.055). The Kaplan-Meier curves for OST for the three neutropenia groupings were demonstrated in **Figure 15~17**.

Groupings with first digit assigned as 2 examined anorexia and efficacy. The three anorexia groupings (21110, 21130, and 21140) in **Table 10** were all statistical insignificant. In fact, no statistical significance was found in any anorexia groupings in the study.

Groupings with first digit assigned as 3, 4, and 5 focused on vomiting, diarrhea and combined GI signs. In groupings 31110, 41110, and 51110, toxicity of any grade was

considered as occurrence of toxicity, and statistical significance was found in vomiting and combined GI signs, but not diarrhea, for TTP (P =0.042 and 0.007) and OST (P =0.023 and <0.001). If shifting definition of occurrence of toxicity to toxicity of more than grade 3, as in groupings 31130, 41130, and 51130, differences were only detected in diarrhea groupings for both TTP (P =0.034) and OST (P =0.017), and in contrary to the study's hypothesis of toxicity improving efficacy, patients who experienced diarrhea of more than grade 3 exhibited poorer outcome. The Kaplan-Meier curves of the above groupings with P value <0.05 were shown in **Figure 18~23**. After dividing patients into no toxicity, low-grade toxicity (grade 1 and 2) and high-grade toxicity (grade 3 and 4), as in grouping 31140, 41140, and 51140, statistical significance was found in diarrhea and combined GI signs for TTP (P =0.015 and 0.017) and OST (P =0.004 and 0.001), and in vomiting for OST (P =0.04). Looking into the Kaplan-Meier curves, as shown in **Figure 24~29** for these groupings, one can tell that low-toxicity groups performed better than no toxicity groups, but high-toxicity groups diminished the favorable prognostic value of toxicity, leading to similar or worse outcome comparing to no toxicity or low-grade toxicity groups. Detailed intergroup P values for those groupings are provided in **Table 11**. Patients with high-grade vomiting or combined GI signs had

similar outcome to patients with low-grade vomiting or combined GI signs, although poorer outcome in high-grade toxicity group could be subjectively observed in the Kaplan-Meier's curves; Patients with high-grade diarrhea had poorer outcome than both patients with low-grade diarrhea and no diarrhea.

Timing during the protocol

No groupings focusing on only toxicities appeared in the first two cycles of the protocol (ie, the second digit assigned as 2) showed statistical significance.

Drugs

Among groupings that focusing on particular drug (ie, the third digit assigned as 2, 3, or 5), statistical significance was found for TTP and OST in grouping 11310 (P =0.042 and 0.019), 41230 (P =0.022 and 0.015), and 41240 (P =0.031 and 0.014), and for only OST in grouping 51210 (P=0.03). The results of grouping 11310 suggested that patients who experienced neutropenia of more than grade 1.0 after cyclophosphamide had better outcome. The results of grouping 41230 and 41240, which defined occurrence of toxicity as diarrhea of more than grade 3 after vincristine or divided patients into 3 groups according to diarrhea grading after vincristine, were similar to the

results of diarrhea groupings in the previous section: diarrhea of high grade was associated with poorer outcome. The results of grouping 51210 suggested that patients who experienced anorexia, diarrhea, or vomiting of more than grade 1 after vincristine had longer OST, but not TTP. The Kaplan-Meier curves for these four groupings with P value <0.05 were provided in **Figure 30~36**.

Frequency

Among groupings that examined if frequency of toxicity is a determinant (ie, the fifth digit assigned as 3 or 5), statistical significance was found for OST in grouping 31113 (P =0.015) and 31143 (P =0.014), and for TTP and OST in grouping 41143 (P =0.045 and 0.019). The results of these three groupings were similar to their non-frequency-adjusting counterparts (ie, 31110 for 31113, 31140 for 31143, and 41140 for 41143): In grouping 31113, patients who experienced vomiting of any grade more than three times exhibited better outcome, as in grouping 31130; In groupings 31143 and 41143, patients who experienced vomiting or diarrhea of grade 1 or 2 more than twice had better outcome than patients who did not experience vomiting or diarrhea more than twice, but patients who experienced vomiting or diarrhea of grade 3 or 4 more than twice had similar or poorer outcome comparing to no toxicity group and

low-grade toxicity group, as in grouping 31140 and 41140. **Table 12** listed P values of the three above-mentioned groupings and their no frequency-adjusting counterparts. **Figure 37~40** showed the Kaplan-Meier curves with P value <0.05.

For all grouping with P value <0.05, excluding drug-specific groupings (ie, the third digit assigned as 2, 3, or 5) and frequency-adjusting groupings (ie, the fifth digit assigned as 3 or 5), chia-square test was performed to examine the correlation between grouping and significant prognostic factors. Age, response to treatment and anemia were found to be associated with some of these groupings: Age was correlated to grouping 31110, 31140, 41140, 51110, and 51140 (P =0.015, 0.016, 0.016, 0.028, and 0.009); Response to treatment was correlated to grouping 11110, 11130, 31110, 41110, 51110, and 51140 (P =0.004, <0.001, 0.006, 0.001, < 0.001, and 0.001); Anemia was correlated only to grouping 11310 (P =0.022). P values and percentages of the above comparisons, are listed in **Table 13~15**. As demonstrated in **Table 13~14,** more than 10 years old was associated with lower percentage of toxicity in grouping 31110, and lower percentage of low-grade toxicity in grouping 31140, 41140, and 51140, whereas CR was related to higher percentage of toxicity in grouping 11110, 31110, and 51110, higher percentage of low-grade toxicity in grouping 41140 and 51140, and higher

percentage of both low-grade and high-grade toxicity in grouping 11140. These results indicated that the connections found was plausible: More than 10 years old, a negative prognostic factor, was with less patients in the favorable toxicity group; CR, a positive prognostic factor, was with more patients in the favorable toxicity group.

In multivariate analysis, all groupings with P value <0.05, excluding drug-specific groupings and frequency-adjusting groupings, and all significant prognostic factors were included for Cox regression. For both TTP and OST, WHO clinical stage, time to finish the first two cycles of the protocol, and grouping 11140 (ie, neutropenia, dividing into no toxicity, low-grade toxicity and high-grade toxicity groups) remained statistical significance (P <0.001, =0.011, and 0.008 for TTP; P <0.001, = 0.002, and 0.001 for OST).

5.5 Short-term analysis

In short-term analysis for neutropenia, statistical differences were detected in comparisons including treatment with all drugs (P =0.008) and with only vincristine (P =0.013). Odds ratio of the odds of effective treatment to ineffective treatment were 0.33

and 0.23, respectively, demonstrating that with occurrence of neutropenia, effective treatment was more likely to happen than ineffective treatment.

In short-term analysis for combined GI signs, statistical differences were detected in comparisons including treatment with all drugs, with vincristine, or with cyclophosphamide (P <0.001, <0.001, and =0.003). Odds ratio of the odds of effective treatment to ineffective treatment were 0.53, 0.56, and 0.42, respectively, demonstrating that with occurrence of GI toxicity, effective treatment was more likely to happen than ineffective treatment.

Table 16 listed all the P values and odds ratios in the short-term analysis.

6. Discussion

6.1 Patient characteristics, overall response, and toxicity profile

The patient characteristics of this study resembled clinical experiences at the author's hospital, with stage III and substage a the most common, anemia sometimes observed, and hypercalcemia very rare. Besides the low incidence of hypercalcemia, the patient characteristics were also comparable to many lymphoma studies (Zemann et al., 1998; Hosoya et al., 2007; Garrett et al., 2002; Moore et al., 2001; Chun et al., 2000; Simon et al., 2006).

The response rate of this study was 94%, coincident with 80-95% by other CHOP-based protocols, where as median OST was 282 days, similar to or slightly less than the survival time of 10-12 months by other CHOP-based protocols (Vail et al., 2013).

The toxicity profile was comparable to the other study using the same 6-month, maintenance free, CHOP-based protocol as this study (Garret et al., 2002). In this study, only 2 (3%) dogs developed GI sign of grade 4 and were hospitalized, and treatment delay consequent to neutropenia occurred in 47 (50%) dogs. In the study by Garret et al.,

5 (9.4%) dogs needed hospitalization and 53 (41.5%) dogs required treatment delays. In addition, the toxicity profile also resembled clinical experiences at the author's hospital.

6.2 Prognostic factors

The previously proved prognostic factors, such as WHO clinical stage, WHO clinical substage, anemia, and response to treatment were also established as of prognostic significance in this study. The other two prognostic factors in this study, age and time to finish the first two cycles of the protocol, were somehow inconsistent findings comparing to previous studies.

The result for WHO clinical stage was slightly different from the well-confirmed connection of stage V disease and poor outcome. Instead, better outcome was associated with stage III, superior to stage IV and V. This deviation could due to lack of standardized staging tests for each patient, thus falsely assigning patients to lower stages. Particularly, because no bone marrow aspiration was performed in any patients in this study, many stage V diseases could be underestimated as stage IV.

It is a common clinical observation at the author's hospital that patients with very

young age (ie, < 2 y/o) were prone to have poor outcome. But only few studies have proposed that older patients were more likely to have poorer outcome (Zemann et al., 1998; Hosoya et al., 2007; Myers et al., 1997). The majority of lymphoma studies showed that age was not a significant prognostic factor (Valerius et al., 1997; Hahn et al., 1994; Keller et al., 1993; Kiupel et al., 1999; Price et al.,1991; MacEwen et al., 1987; Greenlee et al., 1990; Garrett et al., 2002; Simon et al., 2006; Moore et al., 2001).

Finishing the first two cycles of the protocol >80 days was associated with better outcome. This finding was in contrast to some theories that supported high-intensity chemotherapy (Sorenmo et al., 2010). Finishing the first two cycles >80 days could be caused by toxicities and consequent treatment delays, but the chia-square tests performed in long-term analysis did not find any correlation between toxicity and time to finish the first two cycles. More aggressive treatment could also be implemented for more advanced disease, but hard to be verified from the current data.

6.3 Long-term analysis

6.3.1 Neutropenia and efficacy

Based on the findings of this study, setting a threshold of neutrophil <5000 /μl could differentiate patients from having longer OST to shorter OST (P =0.043), whereas a threshold of neutrophil <1500 /μl had weaker power of differentiation (P =0.063). Setting an appropriate neutropenia threshold could be crucial to finding a connection between neutropenia and efficacy. In the other two veterinary studies establishing a relationship between toxicity and efficacy, thresholds of neutropenia were set as <1000 /μl (Vaughan et al., 2007) and <1500 /μl (Sorenmo et al., 2010), respectively. It would be informative to know how the results of the two previous studies would change by shifting threshold to 5000 /μl.

The result that neutrophil <1500 /μl was a weaker threshold than neutrophil <5000 /μl might suggest that high-grade neutropenia is not more favorable than low-grade. This was similar to the conclusion of a human lung cancer study (Di Maio et al., 2005) that the presence, but not severity, of chemotherapy-induced neutropenia were prognostic for increased survival. However, in the Kaplan-Meier curve for OST of patients dividing into no toxicity, low-grade toxicity, and high-grade toxicity (**Figure 17**), the trend of high-grade toxicity performing the best and no toxicity performing the worst could be observed, while statistical significance was only detected between no

toxicity group and low-grade toxicity group (P= 0.039), and no toxicity group and high-grade toxicity group (P=0.005), but not low-grade toxicity group and high-grade toxicity group (P =0.055), with a P-value slightly exceeded 0.05. These findings provided weak evidence of severe toxicity ensuring even better outcome, and strictly speaking left the question unanswered. Nevertheless, since no neutropenia-related illness or mortality occurred in this study, and theoretically severity of neutropenia parallels systemic exposure and thus tumor-killing effect, high-grade toxicity leading to even better outcome could be a plausible result.

Another study of human breast cancer (Cameron et al., 2003) showed that only moderate neutropenia (grade 1~3, neutrophil ranged from lower normal limit to 500 /μl) was associated with increased survival, while grade 4 (neutropenia <500 /μl) neutropenia produced similar outcome as no neutropenia. It should be noted that grade 4 neutropenia never occurred in this study, and the range of moderate neutropenia of the human breast cancer study was identical to low-grade plus high-grade neutropenia in this study, so that the results of the two studies were actually comparable.

6.3.2 GI toxicity and efficacy

Vomiting and combined GI signs were found to be strong determinants for better outcome, while diarrhea was a weak determinant and anorexia was not a determinant. To the author's knowledge, this is the first report in the veterinary literature establishing the correlation between GI toxicity and antitumor efficacy. The inconsistence between anorexia and other GI signs could be explained by the fact that the actual causes of anorexia are difficult to decide, so that episodes of anorexia not secondary to chemotherapy may be easily misinterpreted as chemotherapy toxicities. The intense negative impact of high-grade diarrhea on outcome, as illustrated in the following, made diarrhea a weaker determinant than vomiting and combined GI signs.

The strongest statistical significance (ie., $P<0.001$) of all groupings was found in two combined GI signs groupings, grouping 51110 and 511140. The result could suggest that evaluating all GI signs together was a better method to assess toxicity. As the same injuries to GI tract could present as various clinical signs, assessing different GI signs separately could fail to demonstrate true significance.

In contrast to neutropenia, high-grade GI toxicity was proved not to produce even better outcome. High-grade vomiting and combined GI signs led to shorter TTP or OST than low-grade toxicity, and high-grade diarrhea led to shorter TTP and OST outcome than both no diarrhea and low-grade diarrhea. These findings illustrated the intrinsic difference between bone marrow toxicity and GI toxicity: GI toxicity is related to more morbidity and mortality, and overall body condition would be compromised following severe GI clinical signs, thus prevent favorable outcome.

6.3.3 Timing of toxicity during the protocol and efficacy

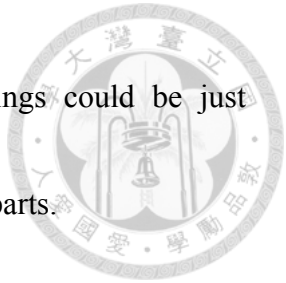
Tumor cells, but not normal cells, are recognized as capable of developing resistance to chemotherapeutic agents gradually along treatments (Gupte et al., 2013). According to this theory, a drug at certain dose could cause sufficient systemic exposure and thus toxicity and antitumor efficacy after the initial treatments, but as resistance developed, only toxicity remained without concurrent efficacy. However, this scenario did not take place in this study, because timing of toxicity was not proved to be significant for outcome. An identical result was also found in a human colorectal cancer

study (Hofheinz et al., 2012), showing that patients developing skin toxicity during the first two cycles of treatment had no better outcome than patients with late skin toxicity. In fact, several patients in this study exhibited frequent toxicity after initial treatments, but gradually no toxicity in the remaining protocol. This change could be the consequences of disease stabilization, but it was uncertain whether resistance to chemotherapy of normal cells did exist and played a role.

6.3.4 Toxicity after different drugs and efficacy

In current literature, the principles of toxicity enhancing efficacy are mostly believed to be associated with either interpatient pharmacodynamics variations or common sensitivity of neoplastic and normal cells to various drugs. Consequently, it was not anticipated that toxicity by a particular drug would have more significance than by other drugs. In this study, only 4 drug-specified groupings had P values <0.05. Three of the 4 groupings were focused on vincristine, and all had non-drug-specified counterparts with P value <0.05. Considering that vincristine was the most commonly

administered drug, the findings in these 3 drug-specified groupings could be just reflecting the significance found in the 3 non-drug-specified counterparts.



6.3.5 Frequency of toxicity and efficacy

By examining the influence of frequency of toxicity on the relationship between toxicity and efficacy, this study tried to answer two questions: Firstly, whether frequency was a determinant for better outcome, in other words, whether only single episode of certain toxicity was sufficient to improve survival; Secondly, if single episode of certain toxicity ensures favorable outcome, whether more episodes ensure even longer survival.

Since the no groupings changed from insignificant to significant after adding frequency conditions, a conclusion could be derived that single episode of toxicity could be sufficient. In the three frequency-adjusting groupings with P values <0.05, no survival benefit was observed over their non-frequency-adjusting counterparts. However, because setting higher frequency leads to fewer patients in the toxicity groups, sampling bias could be amplified, and this limitation leaves the seconds question not

elucidated. Theoretically, if occurrence of toxicity equals effective tumor killing, then frequent toxicity equals multiple effective tumor killings. It is straightforward that multiple effective tumor killings are beneficial to patients with gross disease, but for patients in remission, whether multiple effective tumor killings are advantageous or redundant was hard to determine.

6.3.6 Age and GI toxicity

In the chia-square tests performed in long-term analysis in the study, older patients were observed to have less low-grade GI toxicity. Baum et al demonstrated that the number of proliferating cells in canine intestine epithelium decreased during aging, but only weak correlation was found (Baum et al., 2007). It was speculated that due to lower proliferation rate of GI mucosa cells of older patients, they were more resistant to chemotherapy. However, only less low-grade GI toxicity, but not less GI toxicity of all grades was found in older patients, so that the above explanation is still far from satisfactory.

6.3.7 Confounding factors

More than 10 years old was associated less low-grade GI toxicity and poorer outcome. If more than 10 years old was a solid negative prognostic factor for canine lymphoma, then the result of low-grade GI toxicity producing better outcome could be just caused by confounding, with age being the confounding factor. Under this circumstance, the established relationship between GI toxicity and efficacy would be less credible. However, since more than 10 years old is not a solid negative prognostic factor for canine lymphoma, GI toxicity could also be the confounding factor, creating a spurious connection between age and outcome. The actual causal relationship could not be determined in this study.

CR was associated with more bone marrow and GI toxicity and better outcome. Since the impact of response to treatment on survival is rather straightforward, and in some studies response to treatment were also chosen as endpoints, it was not a concern if response to treatment was a confounding factor of toxicity and outcome.

6.3.8 Multivariate analysis

Among all the groupings with P <0.05, only grouping 11110 (ie, neutrophil < 5000 /μl at least once) retained significance in multivariate analysis. This could suggest that neutropenia was a stronger prognostic factor than GI toxicity. But due to the low case number of this study, the result of Cox regression was less plausible.

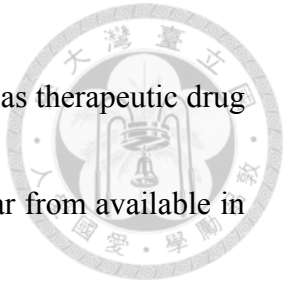
6.4 Short-term analysis

Clinical observations of relationship between toxicity and efficacy mostly involved short-term efficacy, which means that with occurrence of toxicity, stronger tumor-killing effect would be anticipated. The purpose of short-term analysis was to verify the clinical impressions, and may offer some guidance for clinical decisions, as failure to achieve remission after a treatment might be the consequence of absence of toxicity. In this study, short-term analysis demonstrated that occurrence of toxicity actually increased the like hood of effective treatment over ineffective treatment. However, the intrinsic limitation of short-term analysis lies in that if a patient was in remission or having stable disease, it could be chemotherapy suppressing the tumor or the tumor simply not yet relapsing into gross disease. Hence, many treatments were

assigned as uncertain treatments, and despite the above result, it was uncertain treatments that accounted for the largest proportion of all treatments in nearly every toxicity status, which could diminish the usefulness of the findings.

6.5 Toxicity-adjusted dosing

The primary goal of investigating correlation between toxicity and efficacy was to develop a more appropriate dosing system, since absence of toxicity implying underdosing and could cause less favorable treatment outcome. Many studies demonstrating a positive relationship between toxicity and efficacy mentioned that a prospective dose escalation trial could further strengthen the relationship (Mayers et al., 2001; Stintzing et al., 2011; Vaughan et al., 2007), or even advocated clinical applications of increasing traditional doses given lacking of toxicity (Cameron et al., 2003; Carpenter et al., 1982; Di Maio et al., 2005; Saarto et al., 1997; Sorenmo et al., 2010). Gao et al (Gao et al., 2008) named this dosing strategy as toxicity-adjusted dosing (TAD), and regarded it as convenient and practical and can be supplemental to traditional body surface area-based dosing to approach maximum tolerated dose.



Innovative individual dosing methods in human oncology, such as therapeutic drug monitoring (TDM) and glomerular filtration rate-based dosing, are far from available in veterinary practice. Toxicity-adjusted dosing could be the most feasible method to improve the current dosing system for veterinary oncology patients. The results of this study not only supported but also offered guidance to this strategy: Achieving neutrophil nadir to lower than 5000 /μl could be set as a goal of treatment, whereas dose modifications according to GI toxicity could be risky, as high-grade GI toxicity did not produce better outcome, and high-grade diarrhea even produced poorer outcome. Nevertheless, increasing dose due to absence of neutropenia might also cause more GI toxicity, adding uncertainty to this approach, and it was hard to decide if adjusting dose based on GI toxicity was truly a suboptimal approach since low-grade GI toxicity actually increased survival. Due to the doubts discussed above, implementing prospective, controlled clinical trials would assist to develop a more sophisticated toxicity-adjusted dosing algorithm for canine lymphoma. In addition, similar studies focusing on other malignancies could also prompt generalizations of toxicity-adjusted dosing strategy to other tumors.

6.6 Limitations

The retrospective nature of this study caused the primary limitations. Above all, inadequate blood sampling frequency was a major issue when assessing neutropenia. Ideally, blood sampling should be done on the 7^{th} day after vincristine, cyclophosphamide, and chlorambucil and at the 14^{th} day after doxorubicin. However, this was not always accomplished even in the first two cycles of the protocol and rarely accomplished beyond the first two cycles. In this study, blood sampling 7 to 14 days after vincristine, cyclophosphamide, and chlorambucil, and 14 to 28 days after doxorubicin was regarded as acceptable for assessing bone marrow toxicity. The true incidence of neutropenia could be underestimated under the loose criterion. In addition, because it is a common practice that vincristine injection followed by oral cyclophosphamide at home, skipping one hospital visit, the true incidence of vincristine-induced neutropenia could be underestimated more than other drugs.

Detecting GI toxicities from medical records was somehow a minor issue compared to inadequate blood sampling frequency, as with complete history taking, all GI signs after last visit would be documented, which accounted for most of the scenarios. However, failure to perform complete history taking still happened occasionally, and

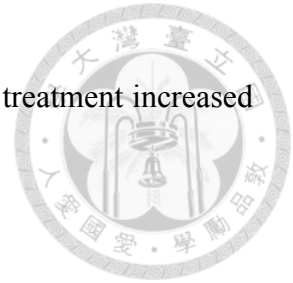
under those circumstances grading of GI toxicities was not accurate enough. Correctly assigning GI signs as GI toxicities secondary to chemotherapy on the basis of medical records was also another challenge in the study, since the true causes of GI signs are sometimes not easy to obtain even in clinical practice. This is an inevitable obstacle for all studies evaluating chemotherapy toxicity.

Lastly, frequent protocol variations among patients and low case number are also limitations of the study. Uneven treatment course, an extra variable of comparisons between patients, decreased the credibility of this study. Although a case number of 69 dogs was moderate in veterinary oncology studies, more case included would decrease the sampling bias and improve reliability of the study.

7. Conclusion

In long-term analysis, a positive correlation between toxicity and efficacy was found for some forms of toxicity: Neutrophil nadir lower than 5000 /μl improved OST; Vomiting or combined GI signs of any grade improved TTP and OST. High-grade toxicity was not proved to be of more benefits than low-grade toxicity: Patients with neutrophil nadir lower than 1500 /μl had similar survival to patients with Neutrophil nadir of 1500 ~ 5000 /μl, although improved OST in high-grade toxicity group could be subjectively observed in the Kaplan-Meier's curve; Patients with high-grade vomiting or combined GI signs had similar outcome to patients with low-grade vomiting or combined GI signs, although poorer outcome in high-grade toxicity group could be subjectively observed in the Kaplan-Meier's curves; Patients with high-grade diarrhea had poorer outcome than both patients with low-grade diarrhea and no diarrhea. No drug was found to induce stronger relationship between toxicity and efficacy. Frequency and timing of toxicity were not determinants for relationship between toxicity and efficacy. More than 10 years old was associated with less low-grade GI toxicity and decreased survival, but the causal connections could not be determined. In

short-term analysis, occurrence of neutropenia or GI toxicity after a treatment increased the like hood of effective treatment over ineffective treatment.



To the author's knowledge, this is the first report demonstrating the positive correlation between GI toxicity and outcome, and also the first report analyzing the relationship between toxicity and short-term efficacy. The results of the study supported the concept of toxicity-adjusted dosing, but prospective trials are warranted to develop a sophisticated toxicity-adjusted dosing regimen.

8. References

Bacon NJ, Ehrhart NP, Dernell WS, et al. Use of alternating administration of carboplatin and doxorubicin in dogs with microscopic metastases after amputation for appendicular osteosarcoma: 50 cases (1999-2006). J Am Vet Med Assoc 232: 1504-1510, 2008.

Baum B, Meneses F, Kleinschmidt S, et al. Age-related histomorphologic changes in the canine gastrointestinal tract: a histologic and immunohistologic study. World J Gastroenterol 13: 152-157, 2007.

Cameron DA, Massie C, Kerr G, et al. Moderate neutropenia with adjuvant CMF confers improved survival in early breast cancer. Br J Cancer 89: 1837-1842, 2003.

Chun R. Lymphoma: Which Chemotherapy Protocol and Why? Top Companion Anim Med 24: 157-162, 2009.

Chun R, Garrett LD, Vail DM. Evaluation of a high-dose chemotherapy protocol with no maintenance therapy for dogs with lymphoma. J Vet Intern Med 14: 120-124, 2000.

Chun R, Knapp DW, Widmer WR, et al. Phase II clinical trial of carboplatin in canine transitional cell carcinoma of the urinary bladder. J Vet Intern Med 11: 279-283, 1997.

Colleoni M, Price K, Castiglione-Gertsch M, et al. Dose-response effect of adjuvant cyclophosphamide, methotrexate, 5-fluorouracil (CMF) in node-positive breast cancer. International Breast Cancer Study Group. Eur J Cancer 11: 1693-1700, 1998.

Cortes EP, Holland JF, Wang JJ, et al. Amputation and adriamycin in primary osteosarcoma. N Engl J Med 291: 998-1000, 1974.

Di Maio M, Gridelli C, Gallo C, et al. Chemotherapy-induced neutropenia and treatment efficacy in advanced non-small-cell lung cancer: a pooled analysis of three randomised trials. Lancet Oncol 6: 669-677, 2005.

Garrett LD, Thamm DH, Chun R, et al. Evaluation of a 6-month chemotherapy protocol with no maintenance therapy for dogs with lymphoma. J Vet Intern Med 16: 704-709, 2002.

Gao B, Klumpen HJ, Gurney H. Dose calculation of anticancer drugs. Expert Opin Drug Metab Toxicol 4: 1307-1319, 2008.

Greenlee PG, Filippa DA, Quimby FW, et al. Lymphomas in dogs. A morphologic, immunologic, and clinical study. Cancer 66: 480-490, 1990.

Gupte M, Tuck AN, Sharma VP, et al. Major differences between tumor and normal human cell fates after exposure to chemotherapeutic monofunctional alkylator. PLoS One 8: e74071, 2013.

Hahn KA, Richardson RC, Hahn EA, et al. Diagnostic and prognostic importance of chromosomal aberrations identified in 61 dogs with lymphosarcoma. Vet Pathol 31: 528-540, 1994.

Hofheinz RD, Heinemann V, von Weikersthal LF, et al. Capecitabine-associated hand-foot-skin reaction is an independent clinical predictor of improved survival in patients with colorectal cancer. Br J Cancer 107: 1678-1683, 2012.

Hon YY, Evans WE. Making TDM work to optimize cancer chemotherapy: a multidisciplinary team approach. Clin Chem 44: 388-400, 1998.

Horwich A, Sleijfer DT, Fossa SD, et al. Randomized trial of bleomycin, etoposide, and cisplatin compared with bleomycin, etoposide, and carboplatin in good-prognosis metastatic nonseminomatous germ cell cancer: a Multiinstitutional Medical Research Council/European Organization for Research and Treatment of Cancer Trial. J Clin Oncol 15: 1844-1852, 1997.

Hosoya K, Kisseberth WC, Lord LK, et al. Comparison of COAP and UW-19 protocols for dogs with multicentric lymphoma. J Vet Intern Med 21: 1355-1363, 2007.

Jagielski D, Lechowski R, Hoffmann-Jagielska M, et al. A retrospective study of the incidence and prognostic factors of multicentric lymphoma in dogs(1998-2000). J Vet Med A Physiol Pathol Clin Med 49: 419-424, 2002.

Keller ET, MacEwen EG, Rosenthal RC, et al. Evaluation of prognostic factors and sequential combination chemotherapy with doxorubicin for canine lymphoma. J Vet Intern Med 7: 289-295, 1993.

Khanna C, Lund EM, Redic KA, et al. Randomized controlled trial of doxorubicin versus dactinomycin in a multiagent protocol for treatment of dogs with malignant lymphoma. J Am Vet Med Assoc 213: 985-990, 1998.

Kiupel M, Teske E, Bostock D. Prognostic factors for treated canine malignant lymphoma. Vet Pathol 36: 292-300, 1999.

Koschny R, Gotthardt D, Koehler C, et al. Diarrhea is a positive outcome predic- tor for sorafenib treatment of advanced hepatocellular carcinoma. Oncology 84: 6-13, 2013.

MacEwen EG, Hayes AA, Matus RE, et al. Evaluation of some prognostic factors for advanced multicentric lymphosarcoma in the dog: 147 cases (1978-1981). J Am Vet Med Assoc 190: 564-568, 1987.

Marconato L, Stefanello D, Valenti P, et al. Predictors of long-term survival in dogs with high-grade multicentric lymphoma. J Am Vet Med Assoc 238: 480-485, 2011.

Marconato L, Zini E, Lindner D, et al. Toxic effects and antitumor response of gemcitabine in combination with piroxicam treatment in dogs with transitional cell carcinoma of the urinary bladder. J Am Vet Med Assoc 238: 1004-1010, 2011.

Mayers C, Panzarella T, Tannock IF. Analysis of the prognostic effects of inclusion in a clinical trial and of myelosuppression on survival after adjuvant chemotherapy for breast carcinoma. Cancer 91: 2246-2257, 2001.

Milano G, Etienne MC, Rene´e N, et al. Relationship between fluorouracil systemic exposure and tumor response and patient survival. J Clin Oncol 12: 1291–1295, 1994.

Moore AS, Cotter SM, Rand WM, et al. Evaluation of a discontinuous treatment protocol (VELCAP-S) for canine lymphoma. J Vet Intern Med 15: 348-354, 2001.

Myers NC 3rd, Moore AS, Rand WM, et al. Evaluation of a multidrug chemotherapy protocol (ACOPA II) in dogs with lymphoma. J Vet Intern Med 11: 333-339, 1997.

Owen L. TNM classification of tumors in domestic animals. Geneva: WHO: 46–47, 1980.

Poikonen P, Saarto T, Lundin J, et al. Leucocyte nadir as a marker for chemotherapy efficacy in node-positive breast cancer treated with adjuvant CMF. Br J Cancer 11: 1763-1766, 1999.

Price GS, Page RL, Fischer BM, et al. Efficacy and toxicity of doxorubicin/cyclophosphamide maintenance therapy in dogs with multicentric lymphosarcoma. J Vet Intern Med 5: 259-262, 1991.

Rankin EM, Mill L, Kaye SB, et al. A randomised study comparing standard dose carboplatin with chlorambucil and carboplatin in advanced ovarian cancer. Br J Cancer 65: 275-281, 1992.

Saarto T, Blomqvist C, Rissanen P, et al. Haematological toxicity: a marker of adjuvant chemotherapy efficacy in stage II and III breast cancer. Br J Cancer 75: 301-305, 1997.

Santini J, Milano G, Thyss A, et al. 5-FU therapeutic monitoring with dose adjustment leads to an improved therapeutic index in head and neck cancer. Br J Cancer 59: 287–290, 1989.

Simon D, Nolte I, Eberle N, et al. Treatment of dogs with lymphoma using a 12-week, maintenance-free combination chemotherapy protocol. J Vet Intern Med 20: 948-954, 2006.

Sorenmo K, Overley B, Krick E, et al. Outcome and toxicity associated with a dose-intensified, maintenance-free CHOP-based chemotherapy protocol in canine lymphoma: 130 cases. Vet Comp Oncol 8: 196-208, 2010.

Stintzing S, Fischer von Weikersthal L, Vehling-Kaiser U, et al. Correlation of capecitabine-induced skin toxicity with treatment efficacy in patients with metastatic colorectal cancer: results from the German AIO KRK-0104 trial. Br J Cancer 105: 206-211, 2011.

Thyss A, Milano G, Rene´e N, et al. Clinical pharmacokinetic study of 5-FU in continuous 5-day infusions for head and neck cancer. Cancer Chemother Pharmacol 16: 64-66, 1986.

Twelves C, Scheithauer W, McKendrick J, et al. Capecitabine versus 5-fluorouracil/folinic acid as adjuvant therapy for stage III colon cancer: final results from the X-ACT trial with analysis by age and preliminary evidence of a pharmacodynamic marker of efficacy. Ann Oncol 23: 1190-1197, 2012.

Vail DM, Kisseberth WC, Obradovich JE, et al. Assessment of potential doubling time (Tpot), argyrophilic nucleolar organizer regions (AgNOR), and proliferating cell nuclear antigen (PCNA) as predictors of therapy response in canine non-Hodgkin's lymphoma. Exp Hematol 24: 807-815, 1996.

Valerius KD, Ogilvie GK, Mallinckrodt CH, et al. Doxorubicin alone or in combination with asparaginase, followed by cyclophosphamide, vincristine, and prednisone for treatment of multicentric lymphoma in dogs: 121 cases (1987-1995). J Am Vet Med Assoc 210: 512-516, 1997.

Vaughan A, Johnson JL and Williams LE. Impact of chemotherapeutic dose intensity and hematologic toxicity on first progression free survival durationin dogs with

lymphoma treated with a chemoradiotherapy protocol. Journal of Veterinary Internal Medicine 21: 1332–1339, 2007.

Vial DM, Pinkerton ME, Young KM. Hematopoietic tumors. In: Withrow SJ, Vail DM, Page RL, ed. Withrow and MacEwen's small animal clinical oncology. 5th ed. Elsevier, Maryland Heights, 619-621, 2013.

Veterinary Cooperative Oncology Group (VCOG). Veterinary Cooperative Oncology Group - Common Terminology Criteria for Adverse Events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.0. Vet Comp Oncol 2: 195-213, 2004.

Zemann BI, Moore AS, Rand WM, et al. A combination che- motherapy protocol (VELCAP-L) for dogs with lymphoma. J Vet Intern Med 12: 465-470, 1998

Table 1 (Revised from Hon et al., 1998)

Human oncology studies establishing relationship between toxicity and efficacy.

Drugs	Tumor type	Toxicity	Effect	Reference
Doxorubicin	Osteosarcoma	Low leukocyte nadir	Relapse rate ↓	Cortes et al., 1974
CMF	Node-positive early breast cancer	Low leukocyte nadir	DDFS ↑	Poikonen et al., 1999
CAFt	Node-positive early breast cancer	Low leukocyte nadir	OS ↑ DDFS ↑	Saarto et al., 1997
CMF	Node-positive early breast cancer	Low leukocyte nadir	OS ↑ DDFS ↑	Colleoni et al., 1998
Miscellaneous	Advanced non-small-cell lung cancer	Neutropenia (Grade 1~4)	OS ↑	Di Maio et al., 2005
Carboplatin ± chlorambucil	Advanced ovarian cancer	Low leukocyte nadir	PFS ↑	Rankin et al., 1992
BEP or CEB	Germ cell tumor	Low leukocyte nadir	Relapse rate ↓	Horwich et al., 1997
Sorafenib	Advanced hepatocellular carcinoma	Diarrhea (Grade 3~4)	OS ↑	Koschny et al., 2012
Capecitabine	Colorectal cancer	Hand-foot-skin reaction (HFSR)	OS ↑ DFS ↑	Stintzing et al., 2011 Hofheinz et al., 2012

BEP: Cisplatin + etoposide + bleomycin; CAFt: Cyclophosphamide + doxorubicin + oral ftorafur; CEB: Carboplatin + etoposide + bleomycin; CMF: Cyclophosphamide + methotrexate + fluorouracil; DDFS: Distant disease-free survival; PFS: Progression-free surival; DFS: Disease-free survival; OS: Overall survival.

Table 2

Selected veterinary studies investigating relationship between toxicity and efficacy.

Drugs	Tumor type	Case No.	Toxicity	Effect	Reference
CHOP + RT	LSA	62	- Neutropenia > Grade 3	Remission duration ↑	Vaughan et al., 2007
CHOP	LSA	130	- Tx delays or dose reductions	OS ↑ TTP ↑	Sorenmo et al., 2010
CHOP	LSA	77	- Neutropenia > Grade 1 - No. and grading of GI toxicity	No effect on 1st DFI	Simon et al., 2006
CHOP	LSA	55	Tx change due to toxicity	No effect on OS and TTP	Keller et al., 1993
CHOP	LSA	58	- Neutrophil <1000 /μL - Hospitalized for GI	No effect on CR and TTP	Zemann et al., 1998
Doxorubicin Carboplatin	Append-icular OSA	50	- BM suppression - GI toxicity	No effect on OS and DFI	Bacon et al., 2008
Carboplatin	TCC of UB	14	- BM suppression - GI toxicity	No effect on OS	Chun et al., 1997
Gemcitabine	TCC of UB	38	- BM suppression - GI toxicity	No effect on OS	Marconato et al., 2011

RT: Radiotherapy; LSA: Lymphoma; TCC: Transitional cell carcinoma; Tx: Treatment; BM: Bone marrow; OS: overall survival; CR: Complete remission; TTP: Time to tumor progression; DFI: Disease-free interval

Table 3

The 6-month, maintenance-free, modified version of the University of Wisconsin (UW)-Madison chemotherapy protocol (UW-25) utilized in this study. (Garrett et al., 2002)

Protocol	week															
	1	2	3	4	6	7	8	9	11	13	15	17	19	21	23	25
Vincristine (0.7 mg/m^2 IV)	•		•		•		•		•		•		•		•	
Cyclophosphamide (200~250 mg/m^2 PO or IV)		•				•				•				•		
Doxorubicin (30 mg/m^2 IV)				•				•				•				•
Prednisolone	2 mg/kg PO q24h x 7d, then 1.5 mg/kg PO q24h x 7d, then 1.0 mg/ kg PO q24h x 7d, then 0.5 mg/ kg PO q24h x 7d, then stop															

Table 4

Modified neutropenia grading system based on VCOG-CTCAE v1.0.

Grade 1		Grade 2	Grade 3	Grade 4
Grade 1.0 3000-5000/μL	**Grade 1.1** 1500-3000/μL	1000–1499/ μL	500–999 /μL	<500 /μL

Table 5

Five-digit coding system for long-term analysis.

<table>
<tr><th colspan="2">Digit</th><th colspan="6">Definitions of code</th></tr>
<tr><td>1st digit</td><td>Form of toxicity</td><td colspan="6">1 =Neutropenia
2 =Anorexia
3 =Vomiting
4 =Diarrhea
5 =GI (Anorexia +Vomiting +Diarrhea)</td></tr>
<tr><td>2nd digit</td><td>Timing</td><td colspan="6">1 =Full course
2 =Cycle 1~2</td></tr>
<tr><td>3rd digit</td><td>Drugs</td><td colspan="6">1 =All drugs
2 =Vincristine
3 =Cyclophosphamide
5 =Doxorubicin</td></tr>
<tr><td rowspan="5">4th digit</td><td rowspan="5">Toxicity grade</td><td colspan="3">Neutropenia</td><td colspan="3">Anorexia; Vomiting; Diarrhea; GI</td></tr>
<tr><td>No toxicity</td><td colspan="2">Toxicity</td><td>No toxicity</td><td colspan="2">Toxicity</td></tr>
<tr><td>1= None</td><td colspan="2">Grade 1~3</td><td>1= None</td><td colspan="2">Grade 1~4</td></tr>
<tr><td>3= None~ Grade1.1</td><td colspan="2">Grade 2~3</td><td>3= None~ Grade 2</td><td colspan="2">Grade 3~4</td></tr>
<tr><td>No toxicity</td><td>Low -grade</td><td>High -grade</td><td>No toxicity</td><td>Low -grade</td><td>High -grade</td></tr>
<tr><td colspan="2">4= None</td><td>Grade 1.0~1.1</td><td>Grade 2~3</td><td>4= None</td><td>Grade 1~2</td><td>Grade 3~4</td></tr>
<tr><td>5th digit</td><td>Frequency</td><td colspan="6">0 =at least once
3 =more than twice
5 =more than four times</td></tr>
</table>

Table 6

Neutropenia profile presented as the number and percentage of patients experienced certain type of toxicity of certain grade.

	Neutropenia grade									
	1.0~3		1.0		1.1		2		3	
	No.	%	No.	%	No.	%	No.	%	No.	%
All cycles	57	83%	47	68%	37	54%	6	9%	4	6%
Cycle 1~2	53	77%	34	49%	31	45%	5	7%	3	4%
Vincristine	47	68%	31	45%	28	41%	4	6%	3	4%
Cyclophosphamide	28	41%	28	41%	9	13%	3	4%	1	1%
Doxorubicin	9	13%	5	7%	2	3%	1	1%	0	0%

No grade 4 neutropenia was documented.

Table 7

GI toxicity profile presented as the number and percentage of patients experienced certain type of toxicity of certain grade.

	Anorexia grade					
	2~3		2		3	
	No.	%	No.	%	No.	%
All cycles	43	62%	30	43%	30	43%
Cycle 1~2	33	48%	22	32%	21	30%
Vincristine	30	43%	22	32%	14	20%
Cyclophosphamide	16	23%	11	16%	7	10%
Doxorubicin	22	32%	8	12%	14	20%

No grade 1 and grade 4 anorexia was documented.

Table 7 cont'd

	Vomiting grade									
	1~4		1		2		3		4	
	No.	%	No.	%	No.	%	No.	%	No.	%
All cycles	53	77%	36	52%	25	36%	21	30%	1	1%
Cycle 1~2	45	65%	25	36%	19	28%	14	20%	1	1%
Vincristine	32	46%	19	28%	13	19%	11	16%	0	0%
Cyclophosphamide	21	30%	14	20%	8	12%	2	3%	0	0%
Doxorubicin	25	36%	9	13%	5	7%	12	17%	1	1%

Table 7 cont'd

	Diarrhea grade									
	1~4		1		2		3		4	
	No.	%	No.	%	No.	%	No.	%	No.	%
All cycles	47	68%	38	55%	20	29%	12	17%	2	3%
Cycle 1~2	39	57%	28	41%	12	17%	10	14%	1	1%
Vincristine	37	54%	28	41%	16	23%	6	9%	0	0%
Cyclophosphamide	27	39%	17	25%	1	1%	1	1%	0	0%
Doxorubicin	25	36%	7	10%	9	13%	7	10%	2	3%

Table 8

Median TTP and OST and P values for prognostic factor analysis.

	Age				Sex				
	Median (days)			P value	Median (days)				P value
	≤5 y/o	6~10 y/o	≥11 y/o		M	Mc	F	Fsp	
N	21	34	14		24	12	10	23	
TTP	175	323	122	**<0.001**	273	116	155	196	0.225
OST	292	413	145	**<0.001**	339	224	240	240	0.486
	BW				Anemia				
	Median (days)			P value	Median (days)				P value
	<15 kg		>15 kg		Presence		Absence		
N	33		36		25		44		
TTP	264		175	0.56	126		289		**0.001**
OST	355		240	0.3	224		371		**0.001**
	Stage				Substage				
	Median (days)			P value	Median (days)				P value
	III	IV	V		a		b		
N	33	23	13		44		25		
TTP	320	163	120	**0.003**	264		149		0.06
OST	371	243	240	**0.011**	355		178		**0.017**
	Response				T_c1c2				
	Median (days)			P value	Median (days)				P value
	CR	PR	NR		<80 days		>80 days		
N	46	19	4		24		40		
TTP	273	130	21	**<0.001**	163		289		**0.007**
OST	355	164	81	**<0.001**	232		357		**0.006**

N: Numbers of patients in each status; TTP: Time to tumor progression; OST: Overall survival time; T_c1c2: Time to finish the first two cycles of the protocol.

P values <0.05 were printed in bold type.

Table 9
P values for TTP and OST of the 154 groupings in long-term analysis

Grouping	11110	111113	11115	11130	11140	11143	21110	21113	21115
TTP	0.094	0.218	0.759	0.151	0.130	0.430	0.734	0.438	0.787
OST	**0.045**	0.121	0.451	0.063	**0.044**	0.098	0.936	0.229	0.899
Grouping	21130	21140	21143	31110	31113	31115	31130	31133	31140
TTP	0.582	0.859	0.111	**0.042**	0.065	0.886	0.308	0.151	0.053
OST	0.596	0.822	0.089	**0.023**	**0.015**	0.365	0.447	0.149	**0.040**
Grouping	31143	41110	41113	41115	41130	41140	41143	41145	51110
TTP	0.056	0.167	0.142	0.900	**0.034**	**0.015**	**0.045**	0.864	**0.007**
OST	**0.014**	0.091	0.057	0.392	**0.017**	**0.004**	**0.019**	0.415	**<0.001**
Grouping	51113	51115	51130	51133	51140	51143	11210	11213	11230
TTP	0.435	0.442	0.384	0.560	**0.006**	0.185	0.930	0.811	0.486
OST	0.223	0.167	0.447	0.622	**<0.001**	0.051	0.835	0.568	0.234
Grouping	11240	11243	11310	11313	11330	11340	11343	11510	12110
TTP	0.783	0.856	**0.042**	0.328	0.296	0.142	0.751	0.306	0.422
OST	0.491	0.527	**0.019**	0.129	0.351	0.059	0.336	0.285	0.279
Grouping	12113	12130	12140	12143	12210	12230	12240	12310	12340
TTP	0.536	0.559	0.661	0.444	0.669	0.961	0.911	0.323	0.547
OST	0.455	0.277	0.381	0.447	0.807	0.515	0.735	0.290	0.536
Grouping	12510	21210	21213	21230	21240	21310	21330	21340	21510
TTP	0.714	0.609	0.469	0.904	0.856	0.547	0.504	0.445	0.334
OST	0.813	0.461	0.530	0.820	0.581	0.342	0.941	0.550	0.542
Grouping	21530	21540	31210	31213	31230	31240	31310	31340	31510
TTP	0.233	0.477	0.213	0.366	0.837	0.436	0.474	0.464	0.356
OST	0.463	0.755	0.090	0.841	0.997	0.187	0.199	0.304	0.276
Grouping	31530	31540	41210	41213	41230	41240	41243	41310	41340
TTP	0.259	0.093	0.418	0.575	**0.022**	**0.031**	0.570	0.578	0.379
OST	0.538	0.168	0.256	0.507	**0.015**	**0.014**	0.507	0.079	0.193
Grouping	41510	41530	41540	51210	51213	51215	51223	51230	51240
TTP	0.860	0.226	0.445	0.112	0.199	0.359	0.108	0.357	0.275
OST	0.139	0.159	0.335	**0.030**	0.073	0.135	0.137	0.565	0.094

TTP: Time to tumor progression; OST: Overall survival time.

P values <0.05 were printed in bold type.

Table 9 cont'd

Grouping	51243	51310	51313	51330	51340	51343	51510	51530	51540
TTP	0.622	0.185	0.636	0.504	0.247	0.596	0.786	0.282	0.527
OST	0.319	0.065	0.305	0.941	0.168	0.268	0.989	0.498	0.727
Grouping	22110	22113	22130	22140	22210	22230	22240	22310	22330
TTP	0.461	0.697	0.384	0.667	0.773	0.725	0.810	0.723	0.779
OST	0.632	0.780	0.617	0.865	0.591	0.675	0.639	0.895	0.690
Grouping	22340	22510	22530	22540	32110	32113	32130	32140	32210
TTP	0.760	0.283	0.153	0.353	0.216	0.652	0.629	0.626	0.417
OST	0.814	0.527	0.548	0.799	0.149	0.243	0.956	0.681	0.205
Grouping	32230	32240	32310	32340	32510	32530	32540	42110	42113
TTP	0.629	0.715	0.945	0.443	0.431	0.130	0.075	0.676	0.734
OST	0.749	0.435	0.597	0.495	0.274	0.521	0.195	0.572	0.806
Grouping	42130	42140	42143	42210	42230	42240	42310	42510	42530
TTP	0.108	0.184	0.631	0.649	0.076	0.206	0.568	0.946	0.155
OST	0.084	0.126	0.297	0.721	0.055	0.557	0.672	0.978	0.131
Grouping	42540	52110	52113	52115	52130	52140	52143	52145	52210
TTP	0.273	0.485	0.927	0.741	0.632	0.592	0.522	0.931	0.456
OST	0.249	0.268	0.826	0.335	0.928	0.484	0.303	0.438	0.295
Grouping	52213	52230	52240	52243	52310	52330	52340	52510	52530
TTP	0.779	0.548	0.706	0.086	0.500	0.779	0.675	0.953	0.284
OST	0.671	0.628	0.569	0.731	0.341	0.690	0.467	0.700	0.742
Grouping	52540								
TTP	0.284								
OST	0.775								

TTP: Time to tumor progression; OST: Overall survival time.

P values <0.05 were printed in bold type.

Table 10

Median TTP and OST and P values for 15 groupings regardless of factors of timing, drug, and frequency (ie, second, third, and fifth digit of coding system fixed to 1).

		Grouping	Median (days)		P	Grouping	Median (days)		P
			No toxicity	*Toxicity*			*No toxicity*	*Toxicity*	
			n=13	*n=56*			*n=58*	*n=11*	
Neutropenia	***TTP***	11110	130	215	0.094	11130	175	320	0.151
	OST		227	324	**0.045**		250	734	0.063
			n=26	*n=43*			*n=39*	*n=30*	
Anorexia	***TTP***	21110	185	175	0.734	21130	185	211	0.582
	OST		282	289	0.936		282	289	0.596
			n=18	*n=51*			*n=47*	*n=22*	
Vomiting	***TTP***	31110	149	239	**0.042**	31130	185	156	0.308
	OST		227	339	**0.023**		282	289	0.447
			n=22	*n=47*			*n=55*	*n=14*	
Diarrhea	***TTP***	41110	149	215	0.167	41130	211	156	**0.034**
	OST		234	338	0.091		324	202	**0.017**
			n=10	*n=59*			*n=35*	*n=34*	
GI	***TTP***	51110	73	215	**0.007**	51130	185	178	0.384
	OST		128	338	**<0.001**		282	289	0.447

TTP: Time to tumor progression; OST: Overall survival time.

Grouping 11110: No toxicity =No neutropenia; Toxicity =Neutropenia grade 1.0~3, at least once. Grouping 21110: No toxicity =No anorexia; Toxicity =Anorexia grade 1~4, at least once. Grouping 31110: No toxicity =No vomiting; Toxicity =Vomiting grade 1~4, at least once. Grouping 41110: No toxicity =No diarrhea; Toxicity =Diarrhea grade 1~4, at least once. Grouping 51110: No toxicity =No GI signs; Toxicity =GI signs grade 1~4, at least once. Grouping 11130: No toxicity =No neutropenia or neutropenia grade 1.0~1.1; Toxicity =Neutropenia grade 2~3, at least once. Grouping 21130: No toxicity =No anorexia or anorexia grade 1~2; Toxicity =Anorexia grade 3~4, at least once. Grouping 31130: No toxicity =No vomiting or vomiting grade 1~2; Toxicity = Vomiting grade 3~4, at least once. Grouping 41130: No toxicity =No diarrhea or diarrhea grade 1~2; Toxicity = Diarrhea grade 3~4, at least once. Grouping 51130: No toxicity =No GI sign or GI signs grade 1~2; Toxicity = GI signs grade 3~4, at least once.

P values <0.05 were printed in bold type.

Figure 10 cont'd

		Grouping	Median (days)			P
			No toxicity	*Low-grade*	*High-grade*	
			n=13	*n=45*	*n=11*	
Neutropenia	***TTP***	11140	130	196	320	0.13
	OST		227	280	734	**0.044**
			n=26	*n=13*	*n=30*	
Anorexia	***TTP***	21140	185	160	211	0.859
	OST		282	324	289	0.822
			n=17	*n=30*	*n=22*	
Vomiting	***TTP***	31140	178	299	156	0.053
	OST		234	357	289	**0.04**
			n=22	*n=33*	*n=14*	
Diarrhea	***TTP***	41140	149	299	156	**0.015**
	OST		234	357	202	**0.004**
			n=10	*n=25*	*n=34*	
GI	***TTP***	51140	73	326	178	**0.017**
	OST		128	357	289	**0.001**

TTP: Time to tumor progression; OST: Overall survival time.

Grouping 11140: No toxicity =No neutropenia; Low-grade toxicity =Neutropenia grade 1.0~1.1, at least once; High-grade toxicity = Neutropenia grade 2~3, at least once. Grouping 21140: No toxicity =No anorexia; Low-grade toxicity =Anorexia grade 1~2, at least once; High-grade toxicity =Anorexia grade 3~4, at least once. Grouping 31140: No toxicity =No vomiting; Low-grade toxicity =Vomiting grade 1~2, at least once; High-grade toxicity =Vomiting grade 3~4, at least once. Grouping 41140: No toxicity =No diarrhea; Low-grade toxicity =Diarrhea grade 1~2, at least once; High-grade toxicity =Diarrhea grade 3~4, at least once. Grouping 51140: No toxicity =No GI signs; Low-grade toxicity =GI signs grade 1~2, at least once; High-grade toxicity =GI signs grade 3~4, at least once.

P values <0.05 were printed in bold type.

Table 11

Intergroup P values for the 4 groupings dividing patients into three groups (ie., no toxicity group, low-grade toxicity group, and high-grade toxicity group) in Table 10.

	Grouping	No toxicity and Low-grade	No toxicity and High-grade	Low-grade and High-grade
TTP	***11140***	0.073	**0.038**	0.169
	31140	**0.02**	0.248	0.074
	41140	**0.025**	0.947	**0.007**
	51140	**0.004**	**0.031**	0.116
OST	***11140***	**0.039**	**0.005**	0.055
	31140	**0.005**	0.273	0.113
	41140	**0.005**	0.655	**0.002**
	51140	**<0.001**	**0.007**	0.074

TTP: Time to tumor progression; OST: Overall survival time.

Grouping 11140: No toxicity =No neutropenia; Low-grade toxicity =Neutropenia grade 1.0~1.1, at least once; High-grade toxicity = Neutropenia grade 2~3, at least once. Grouping 31140: No toxicity =No vomiting; Low-grade toxicity =Vomiting grade 1~2, at least once; High-grade toxicity =Vomiting grade 3~4, at least once. Grouping 41140: No toxicity =No diarrhea; Low-grade toxicity =Diarrhea grade 1~2, at least once; High-grade toxicity =Diarrhea grade 3~4, at least once. Grouping 51140: No toxicity =No GI signs; Low-grade toxicity =GI signs grade 1~2, at least once; High-grade toxicity =GI signs grade 3~4, at least once.

P values <0.05 were printed in bold type.

Table 12

Median TTP and OST and P values for the 3 frequency-adjusted groupings with P <0.05 and their non-frequency-adjusted counterparts.

Grouping		Median (days)		P
		No toxicity	*Toxicity*	
		n=18	*n=51*	
31110	***TTP***	149	239	**0.042**
	OST	227	339	**0.023**
		n=47	*n=22*	
31113	***TTP***	175	299	0.065
	OST	234	404	**0.015**

Grouping 31110: No toxicity =No vomiting; Toxicity =Vomiting grade 1~4, at least once. Grouping 31113: No toxicity =No vomiting or vomiting ≦2 times; Toxicity =Vomiting grade 1~4, >2 times

Table 12 cont'd

Grouping		Median (days)			P
		No toxicity	*Low-grade*	*High-grade*	
		n=17	*n=30*	*n=22*	
31140	***TTP***	178	299	156	0.053
	OST	234	357	289	**0.04**
		n=49	*n=17*	*n=3*	
31143	***TTP***	163	320	831	0.056
	OST	234	478	841	**0.014**
		n=22	*n=33*	*n=14*	
41140	***TTP***	149	299	156	**0.015**
	OST	234	357	202	**0.004**
		n=47	*n=21*	*n=1*	
41143	***TTP***	175	264	114	**0.045**
	OST	240	357	164	**0.019**

Grouping 31140: No toxicity =No vomiting; Low-grade toxicity =Vomiting grade 1~2, at least once; High-grade toxicity =Vomiting grade 3~4, at least once. Grouping 31143: No toxicity =No vomiting or vomiting ≦2 times; Low-grade toxicity =Vomiting grade 1~2, >2 times; High-grade toxicity =Vomiting grade 3~4, >2 times. Grouping 41140: No toxicity =No diarrhea; Low-grade toxicity = Diarrhea grade 1~2, at least once; High-grade toxicity = Diarrhea grade 3~4, at least once. Grouping 41143: No toxicity =No Diarrhea or diarrhea ≦2 times; Low-grade toxicity = Diarrhea grade 1~2, >2 times; High-grade toxicity = Diarrhea grade 3~4, >2 times.

Table 13

Numbers and percentages of patients in different toxicity status of different age groups, grouping 31110, 41140, 51110, and 51140.

Age / Grouping	≤5 y/o		5~10 y/o		≥11 y/o		P value
	No.	%	No.	%	No.	%	
31110							
No toxicity	7	33.3%	4	11.8%	7	50.0%	**0.015**
Toxicity	14	66.7%	30	88.2%	7	50.0%	
31140							
No toxicity	6	28.6%	4	11.8%	7	50.0%	
Low-grade	12	57.1%	15	44.1%	3	21.4%	**0.016**
High-grade	3	14.3%	15	44.1%	4	28.6%	
41140							
No toxicity	7	33.3%	7	20.6%	8	57.1%	
Low-grade	13	61.9%	18	52.9%	2	14.3%	**0.016**
High-grade	1	4.8%	9	26.5%	4	28.6%	
51110							
No toxicity	3	14.3%	2	5.9%	5	35.7%	**0.028**
Toxicity	18	38.1%	32	94.1%	9	64.3%	
51140							
No toxicity	3	14.3%	2	5.9%	5	35.7%	
Low-grade	12	57.1%	10	29.4%	3	21.4%	**0.009**
High-grade	6	28.6%	22	64.7%	6	42.9%	

Grouping 31110: No toxicity =No vomiting; Toxicity =Vomiting grade 1~4, at least once. Grouping 31140: No toxicity =No vomiting; Low-grade toxicity =Vomiting grade 1~2, at least once; High-grade toxicity =Vomiting grade 3~4, at least once. Grouping 41140: No toxicity =No diarrhea; Low-grade toxicity =Diarrhea grade 1~2, at least once; High-grade toxicity =Diarrhea grade 3~4, at least once. Grouping 51110: No toxicity =No GI signs; Toxicity =GI signs grade 1~4, at least once. Grouping 51140: No toxicity =No GI signs; Low-grade toxicity =GI signs grade 1~2, at least once; High-grade toxicity =GI signs grade 3~4, at least once.

P values <0.05 were printed in bold type.

Table 14

Numbers and percentages of patients in different toxicity status of different response groups, grouping 11110, 11140, 31110, 41140, 51110, and 51140.

Response / Grouping	NR		PR		CR		P value
	No.	%	No.	%	No.	%	
11110							
No toxicity	3	75.0%	5	26.3%	5	10.9%	**0.004**
Toxicity	1	25.0%	14	73.7%	41	89.1%	
11140							
No toxicity	3	75.0%	5	26.3%	5	10.9%	
Low-grade	1	25.0%	12	63.2%	32	69.6%	**<0.001**
High-grade	0	0.0%	2	10.5%	9	19.6%	
31110							
No toxicity	3	75.0%	13	68.4%	24	52.2%	**0.006**
Toxicity	1	25.0%	6	31.6%	22	47.8%	
41140							
No toxicity	3	75.0%	11	57.9%	8	17.4%	
Low-grade	0	0.0%	3	15.8%	30	65.2%	**0.001**
High-grade	1	25.0%	5	26.3%	8	17.4%	
51110							
No toxicity	1	25.0%	8	42.1%	1	2.2%	**<0.001**
Toxicity	3	75.0%	11	57.9%	45	97.8%	
51140							
No toxicity	1	25.0%	8	42.1%	1	2.2%	
Low-grade	1	25.0%	3	15.8%	21	45.7%	**0.001**
High-grade	2	50.0%	8	42.1%	24	52.2%	

Table 14 cont'd

Grouping 11110: No toxicity =No neutropenia; Toxicity =Neutropenia grade 1.0~3, at least once. Grouping 11140: No toxicity =No neutropenia; Low-grade toxicity =Neutropenia grade 1.0~1.1, at least once; High-grade toxicity = Neutropenia grade 2~3, at least once. Grouping 31110: No toxicity =No vomiting; Toxicity =Vomiting grade 1~4, at least once. Grouping 41140: No toxicity =No diarrhea; Low-grade toxicity =Diarrhea grade 1~2, at least once; High-grade toxicity =Diarrhea grade 3~4, at least once. Grouping 51110: No toxicity =No GI signs; Toxicity =GI signs grade 1~4, at least once. Grouping 51140: No toxicity =No GI signs; Low-grade toxicity =GI signs grade 1~2, at least once; High-grade toxicity =GI signs grade 3~4, at least once.

P values <0.05 were printed in bold type.

Table 15

Numbers and percentages of patients in different toxicity status in different anemia status in grouping 11310.

Anemia / Grouping	Absence		Presence		P value
	No.	%	No.	%	
11310					
No toxicity	21	47.7%	19	76.0%	**0.022**
Toxicity	23	52.3%	6	24.0%	

Grouping 11310: No toxicity: No neutropenia after cyclophosphamide; Toxicity: Neutropenia grade 1.0~3 after cyclophosphamide, at least once.

P values <0.05 were printed in bold type.

Table 16
Results of short-term analysis.

Tx type / Toxicity status	No. of Effective Txs	No. of Ineffective Txs	No. of Uncertain Txs	P	Odds ratio	95% CI
GI toxicity						
All drugs						
No toxicity	192	225	793	**<0.001**	0.53	0.36~0.78
Grade 1~4	87	54	137			
Vincristine						
No toxicity	101	79	382	**<0.001**	0.56	0.30~1.01
Grade 1~4	46	20	62			
Cyclophosphamide						
No toxicity	31	70	207	**0.003**	0.42	0.18~0.94
Grade 1~4	16	15	32			
Doxorubicin						
No toxicity	22	18	78	**0.053**	0.54	0.19~1.54
Grade 1~4	18	8	25			
Neutropenia						
All drugs						
No toxicity or grade 1.0	226	216	554	**0.008**	0.33	0.15~0.76
Grade 1.1~3	25	8	31			
Vincristine						
No toxicity or grade 1.0	116	68	198	**0.013**	0.23	0.07~0.81
Grade 1.1~3	22	3	18			

Txs: Treatments. P values <0.05 were printed in bold type.

Figure 1
Kaplan-Meier's curve for TTP of all patients; Median: 185 days.

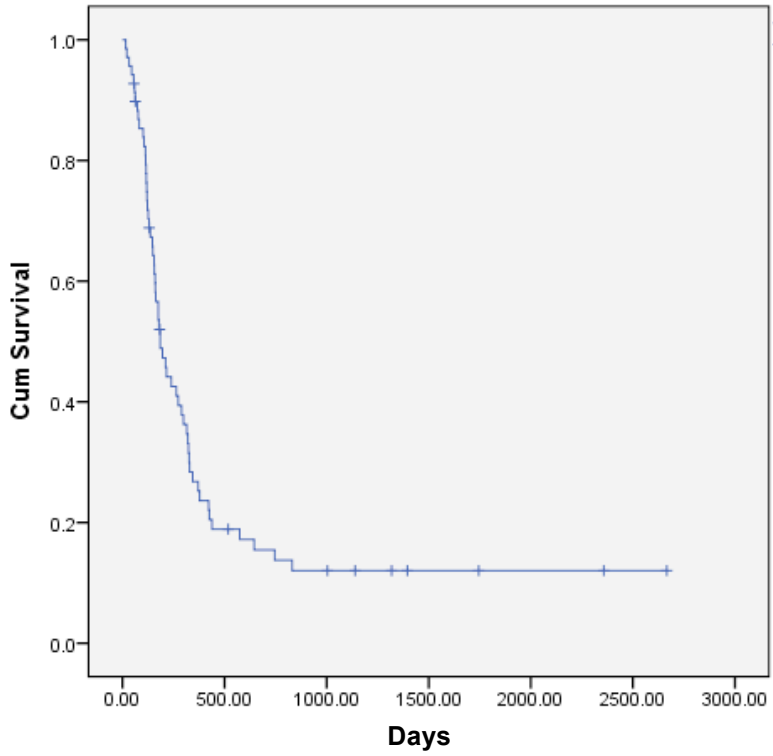


Figure 2
Kaplan-Meier's curve for OST of all patients; Median: 282days.

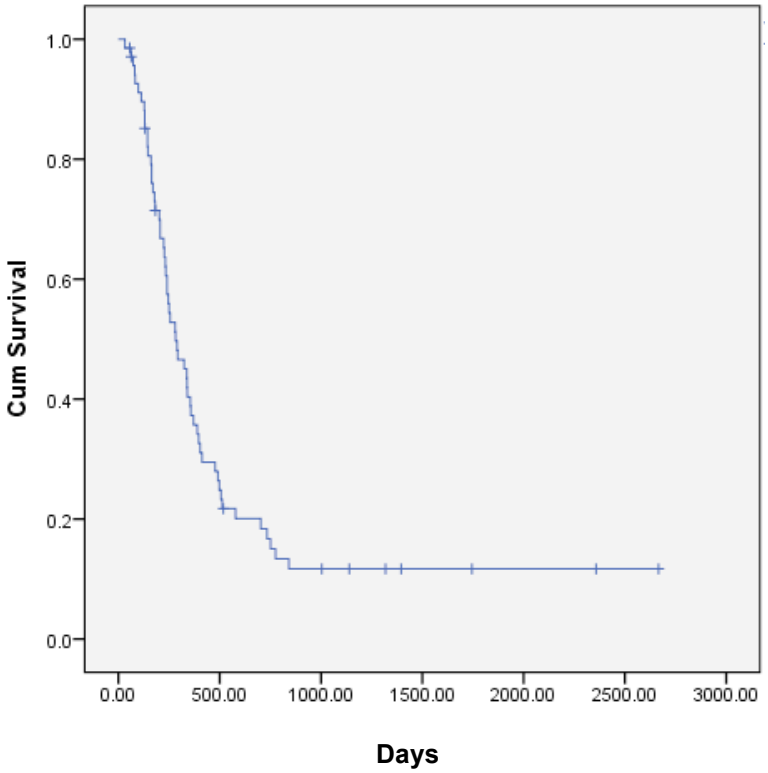
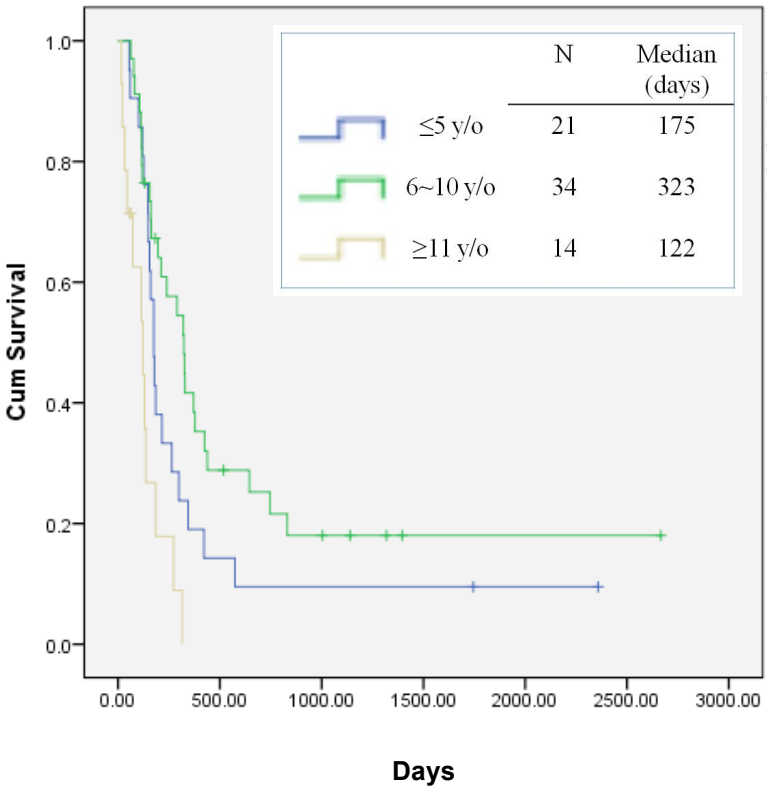
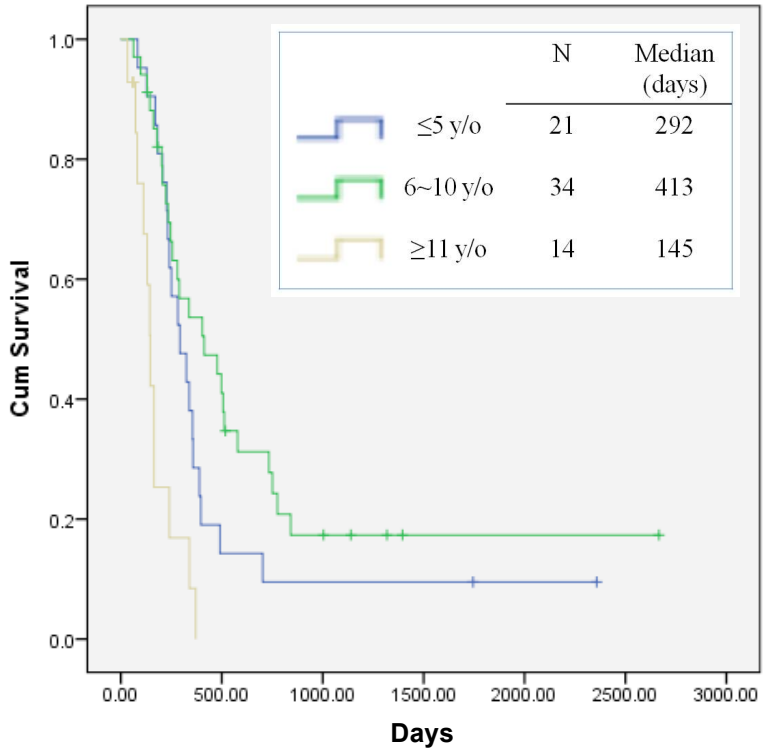


Figure 3
Kaplan-Meier's curve for TTP, age.



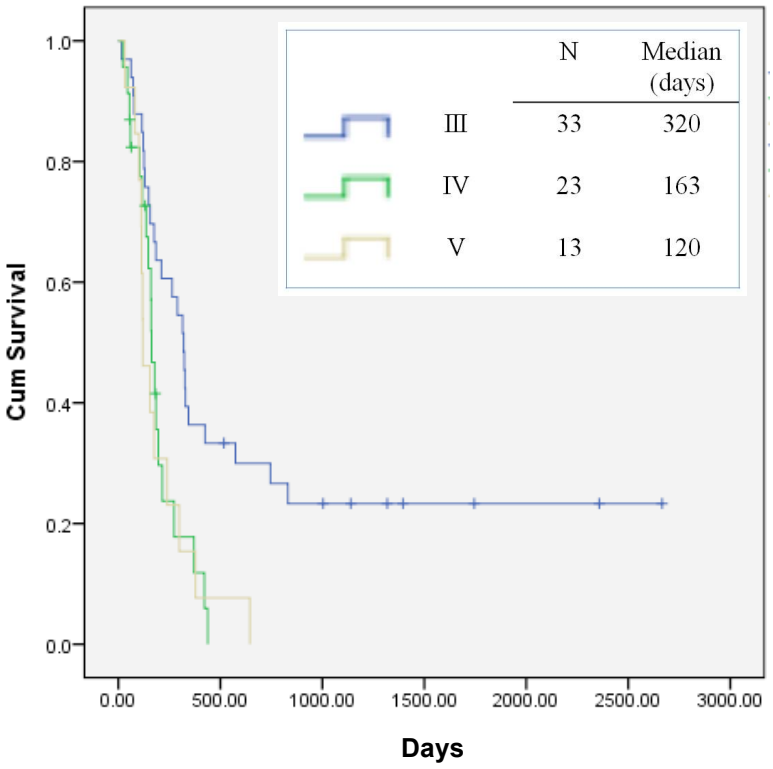
P <0.001

Figure 4 Kaplan-Meier's curve for OST, age.



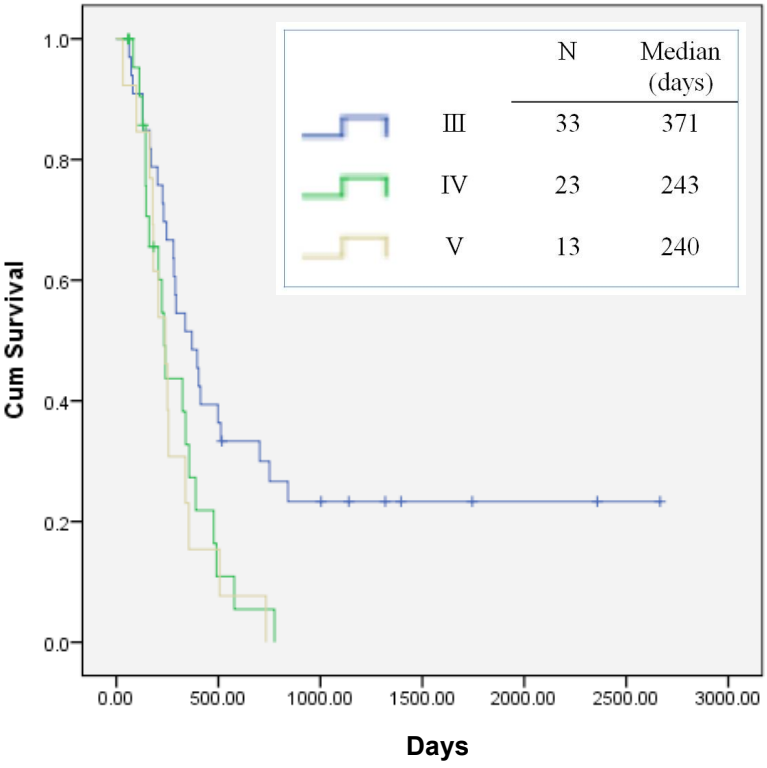
P <0.001

Figure 5
Kaplan-Meier's curve for TTP, WHO clinical stage



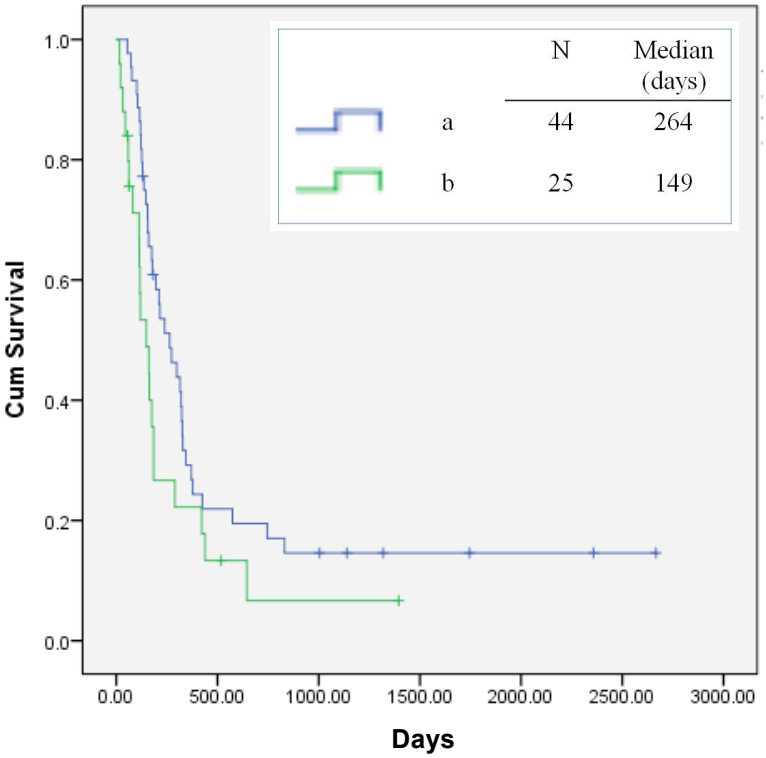
P =0.003

Figure 6
Kaplan-Meier's curve for OST, WHO clinical stage.



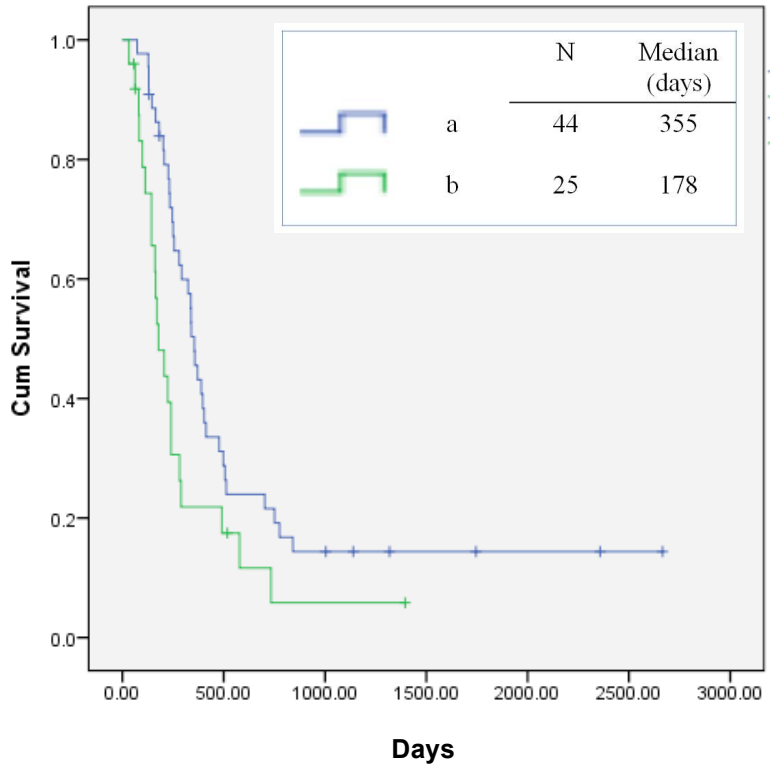
P =0.011

Figure 7
Kaplan-Meier's curve for TTP, WHO clinical substage



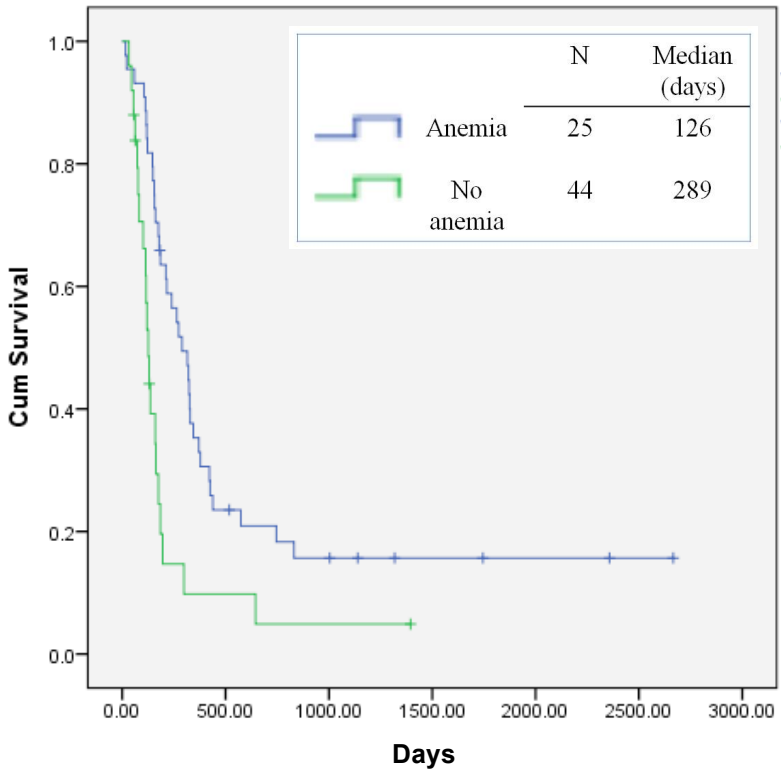
P =0.06

Figure 8
Kaplan-Meier's curve for OST, WHO clinical substage



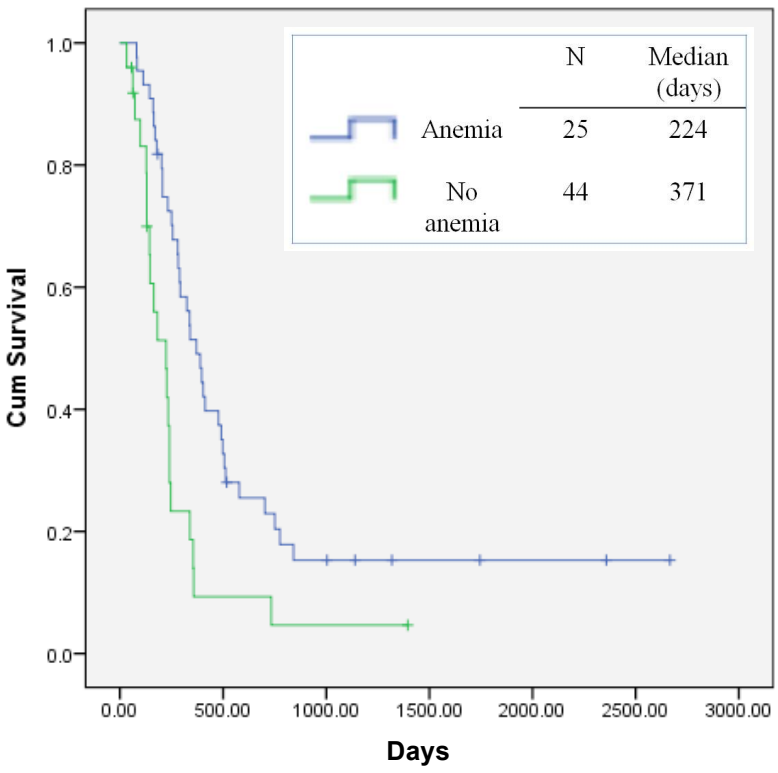
P =0.017

Figure 9
Kaplan-Meier's curve for TTP, anemia



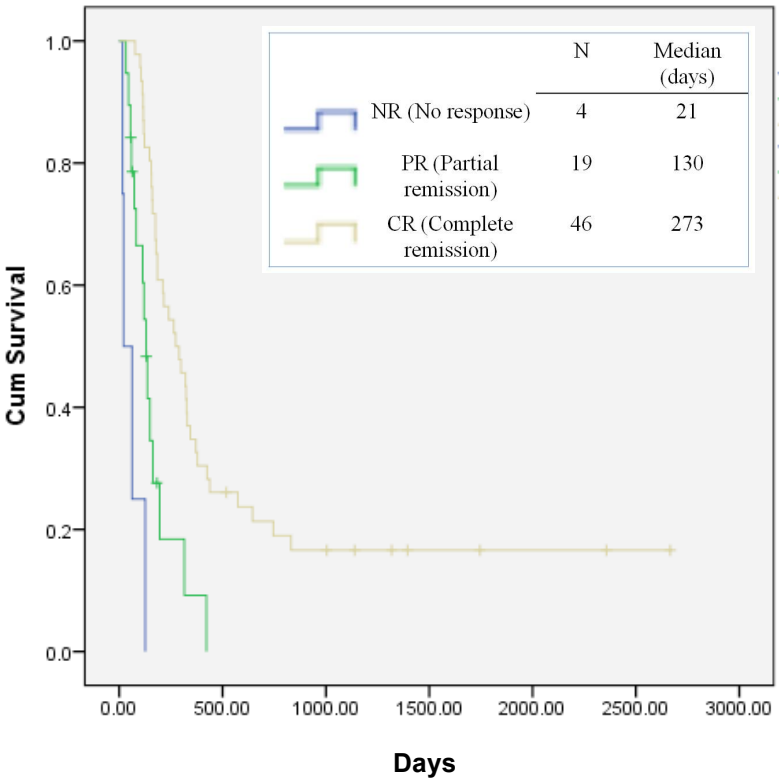
P=0.001

Figure 10
Kaplan-Meier's curve for OST, anemia



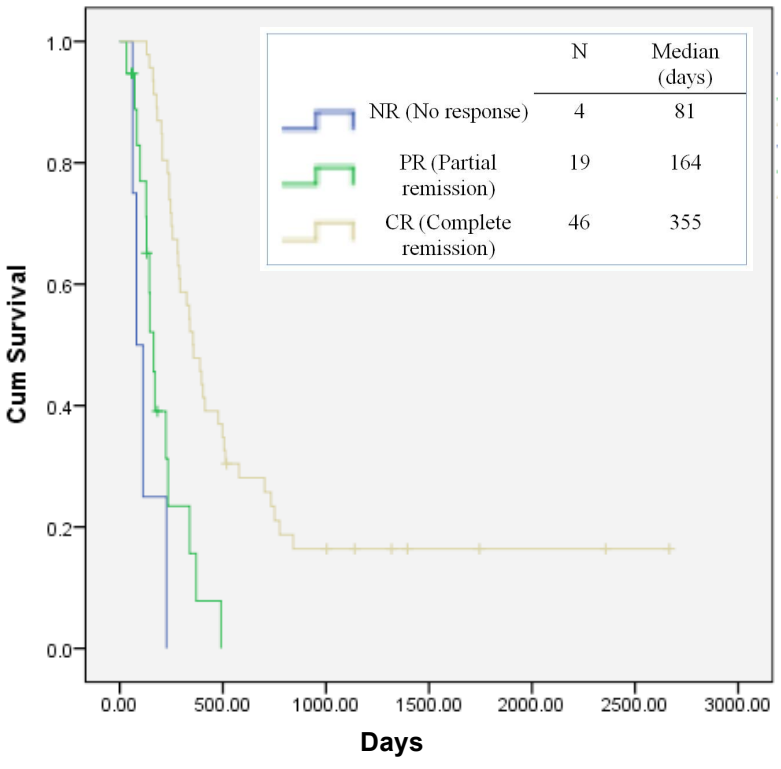
P=0.001

Figure 11
Kaplan-Meier's curve for TTP, response



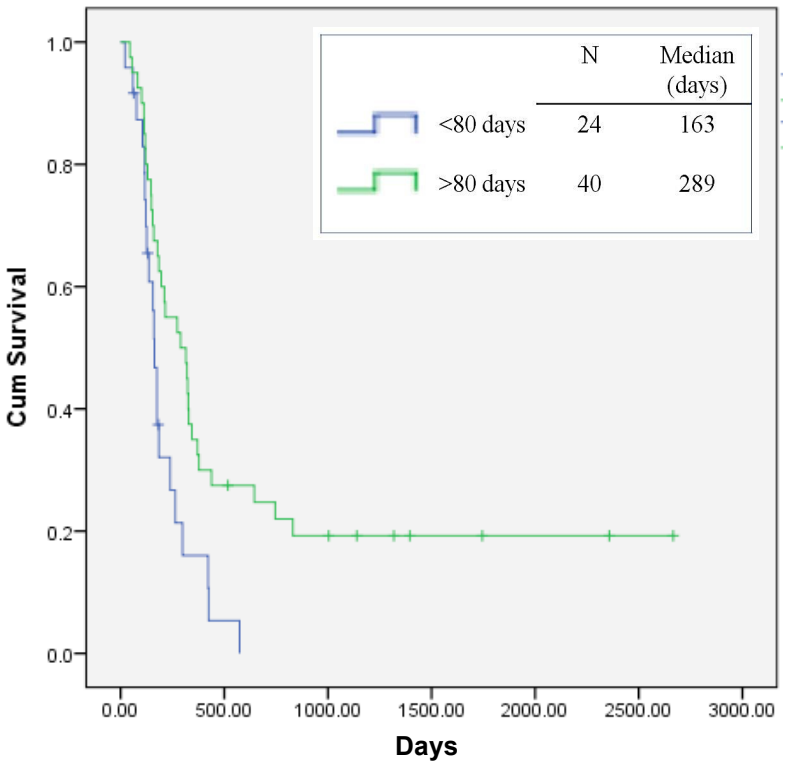
P <0.001

Figure 12
Kaplan-Meier's curve for OST, response



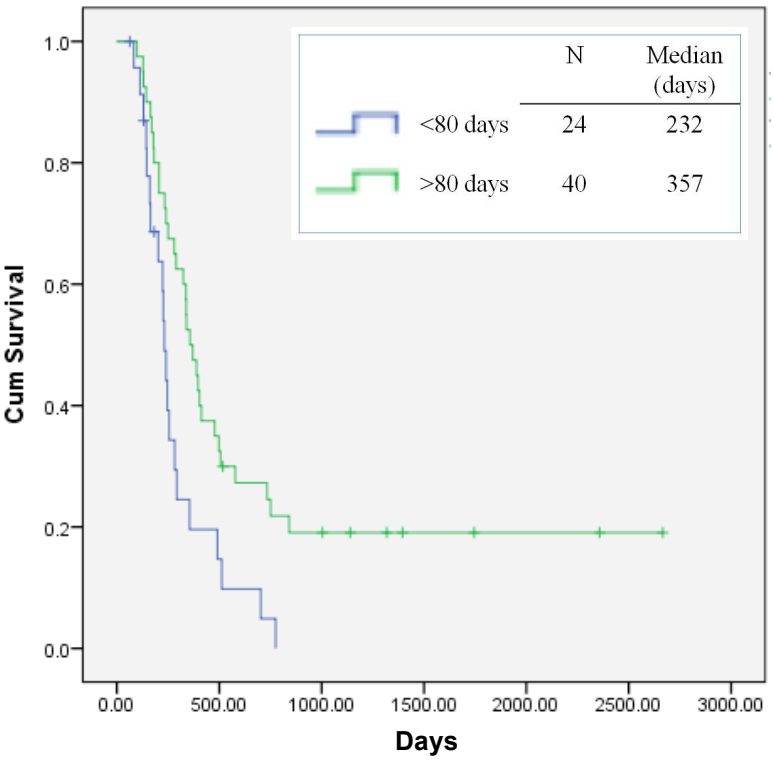
P <0.001

Figure 13
Kaplan-Meier's curve for TTP, time to finish the first two cycles



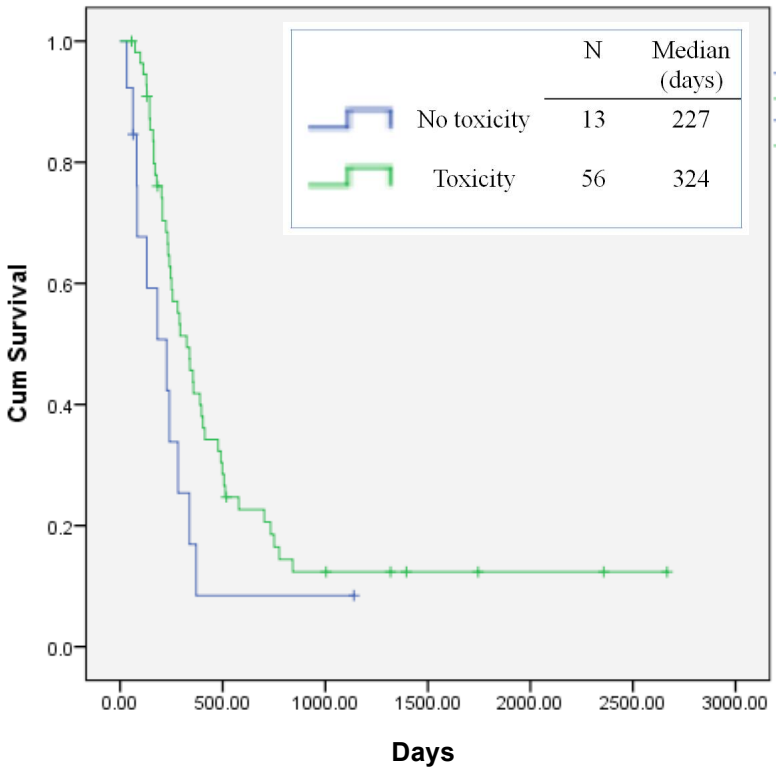
P =0.007

Figure 14
Kaplan-Meier's curve for OST, time to finish the first two cycles



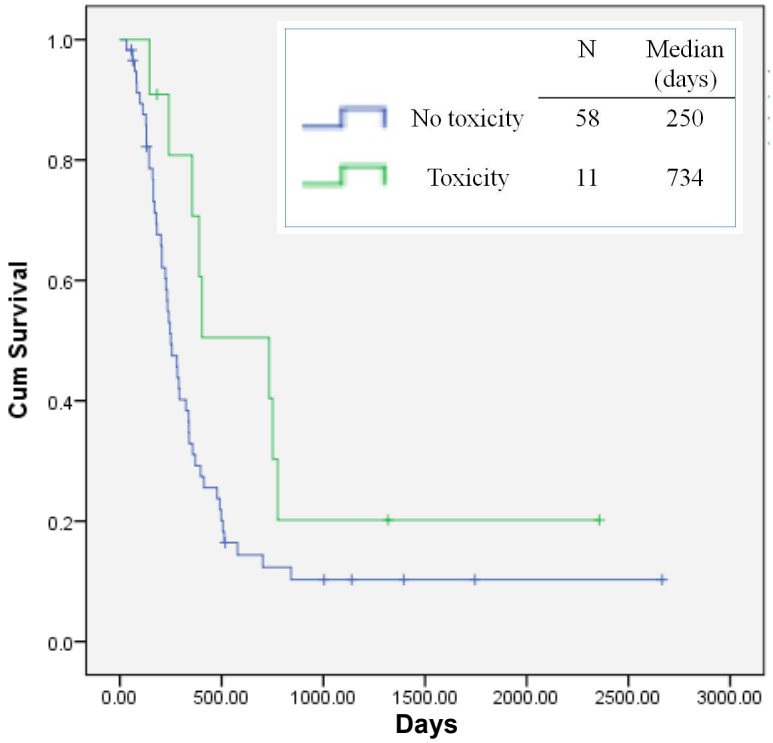
P =0.006

Figure 15
Kaplan-Meier's curve for OST, grouping 11110
No toxicity: No neutropenia
Toxicity: Neutropenia grade 1.0~3, at least once



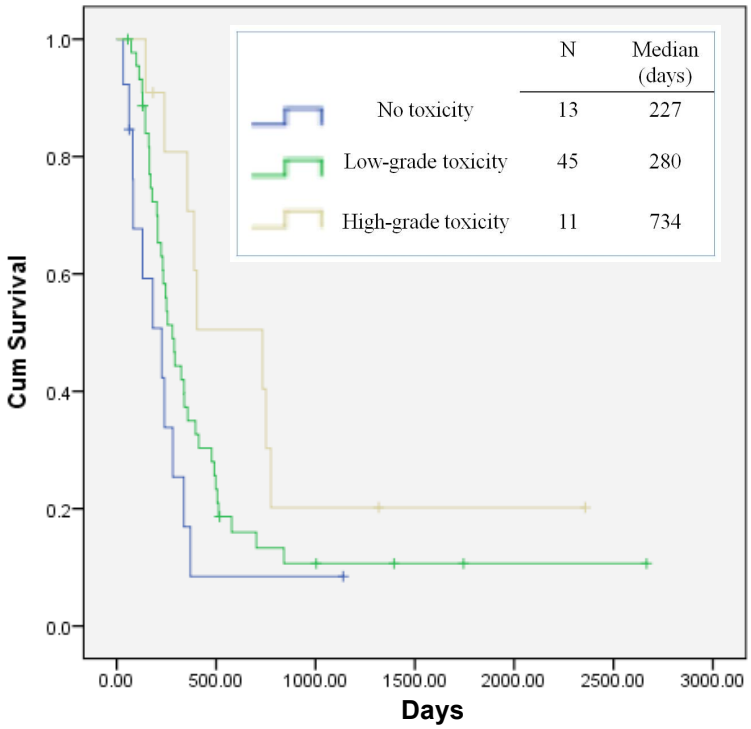
P=0.045

Figure 16
Kaplan-Meier's curve for OST of grouping 11130
No toxicity: No neutropenia or neutropenia grade 1.0~1.1
Toxicity: Neutropenia grade 2~3, at least once



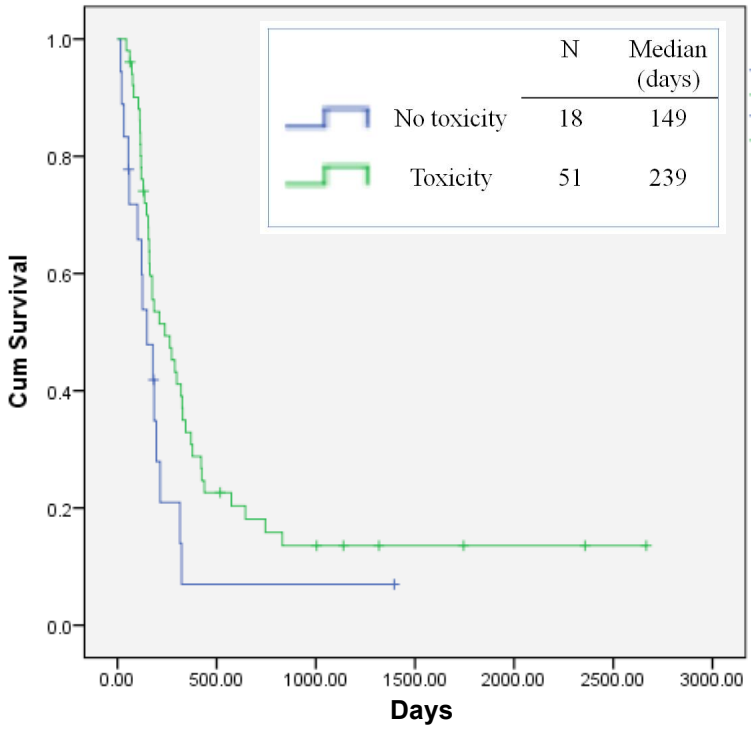
P=0.063

Figure 17
Kaplan-Meier's curve for OST of grouping 11140
No toxicity: No neutropenia
Low-grade toxicity: Neutropenia grade 1.0~1.1, at least once;
High-grade toxicity: Neutropenia grade 2~3, at least once



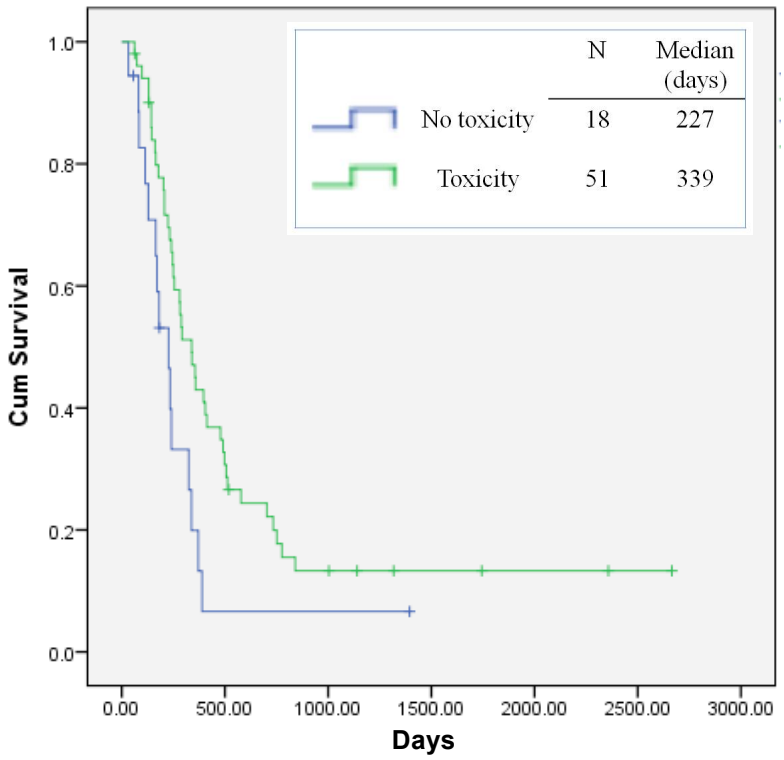
P =0.044

Figure 18
Kaplan-Meier's curve for TTP of grouping 31110
No toxicity: No vomiting
Toxicity: vomiting grade 1~4, at least once



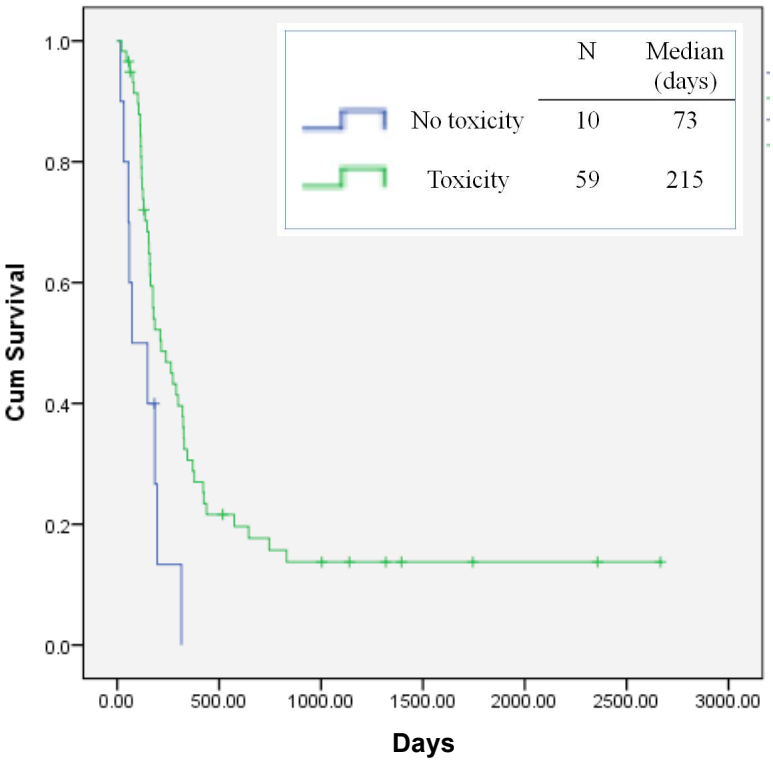
P=0.042

Figure 19
Kaplan-Meier's curve for OST of grouping 31110
No toxicity: No vomiting
Toxicity: vomiting grade 1~4, at least once



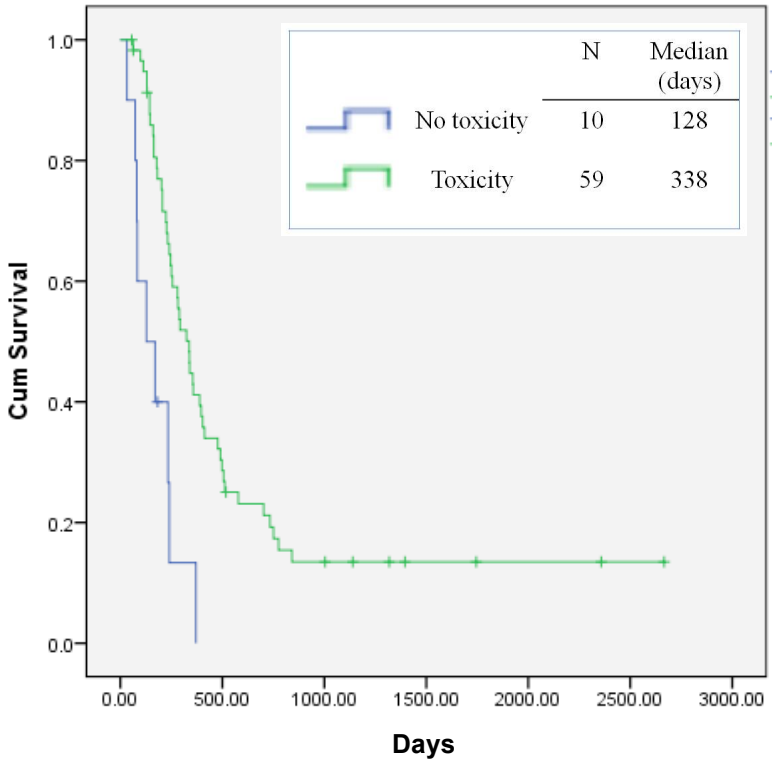
P=0.023

Figure 20
Kaplan-Meier's curve for TTP of grouping 51110
No toxicity: No GI signs
Toxicity: GI signs grade 1~4, at least once



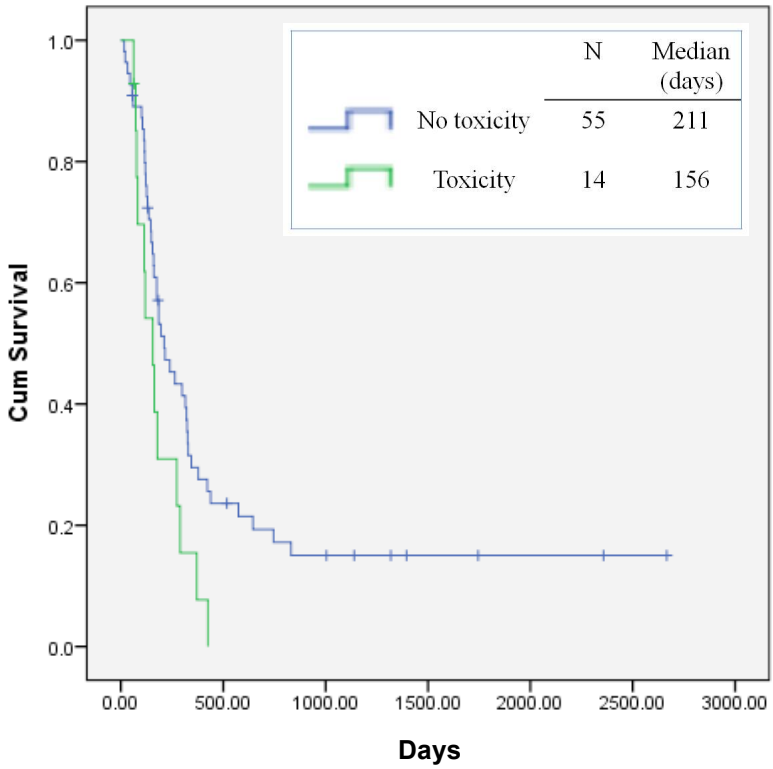
P=0.007

Figure 21
Kaplan-Meier's curve for OST of grouping 51110
No toxicity: No GI signs
Toxicity: GI signs grade 1~4, at least once



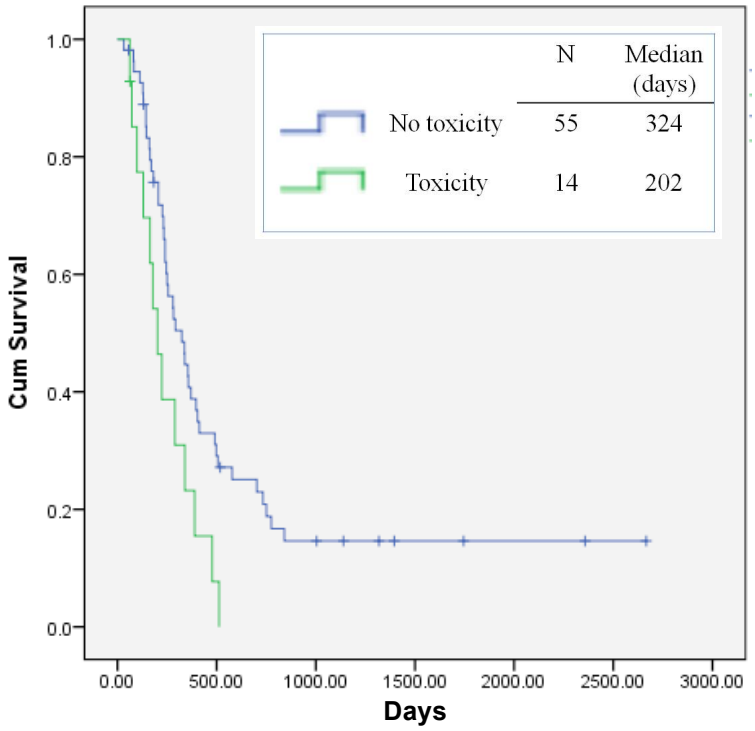
P <0.001

Figure 22
Kaplan-Meier's curve for TTP of grouping 41130
No toxicity: No diarrhea or diarrhea grade 1~2
Toxicity: Diarrhea grade 3~4, at least once



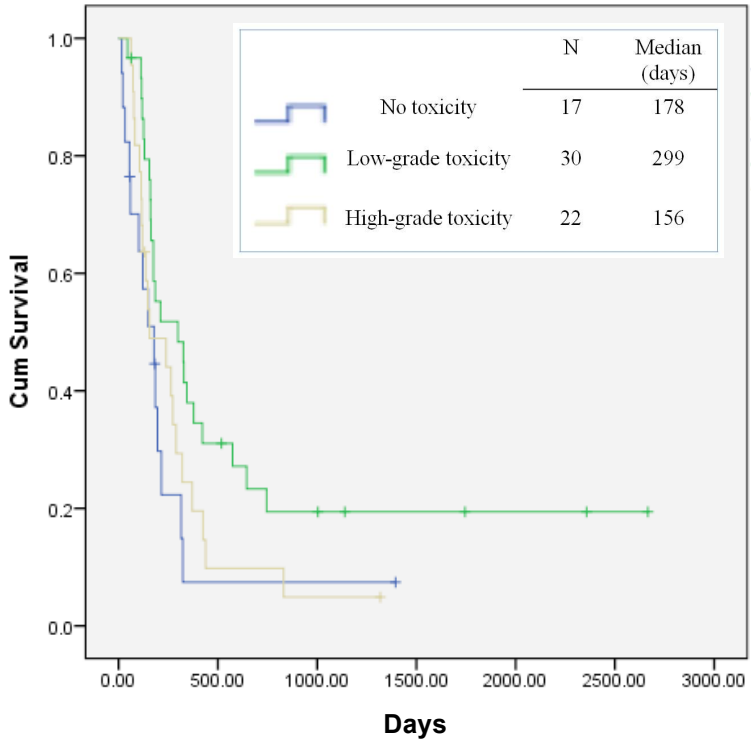
P =0.034

Figure 23
Kaplan-Meier's curve for OST of grouping 41130
No toxicity: No diarrhea or diarrhea grade 1~2
Toxicity: Diarrhea grade 3~4, at least once



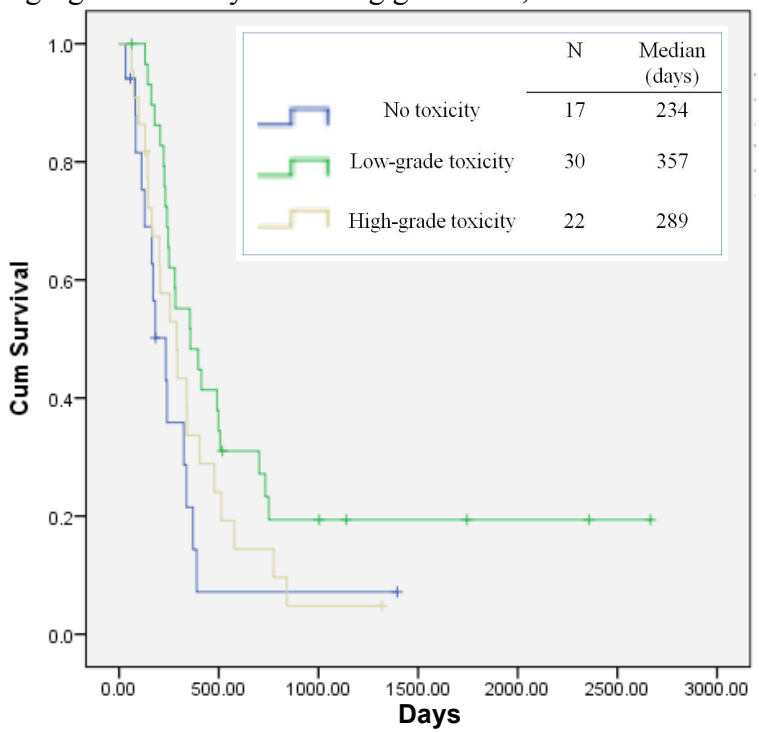
P =0.017

Figure 24
Kaplan-Meier's curve for TTP of grouping 31140
No toxicity: No vomiting
Low-grade toxicity: Vomiting grade 1~2, at least once
High-grade toxicity: Vomiting grade 3~4, at least once



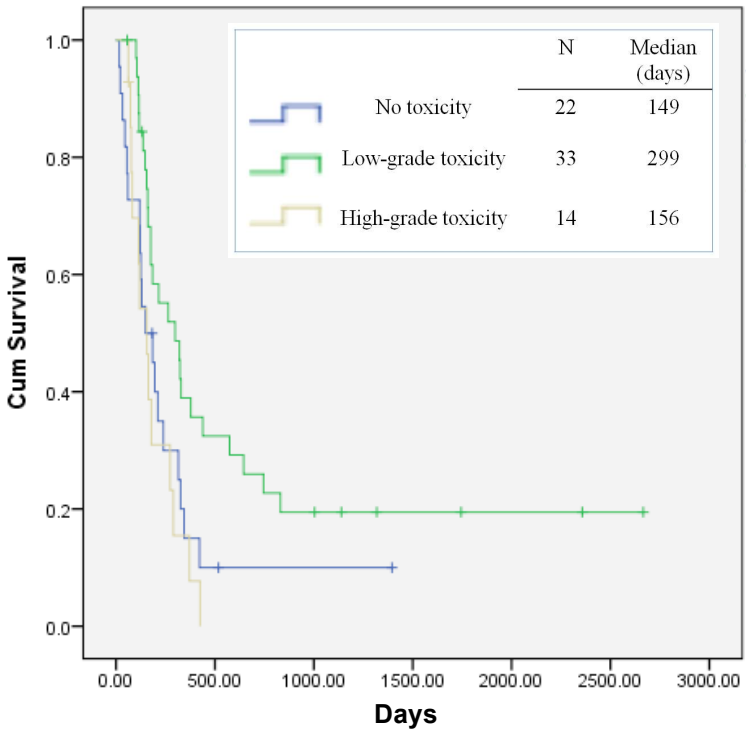
P =0.053

Figure 25
Kaplan-Meier's curve for OST of grouping 31140
No toxicity: No vomiting
Low-grade toxicity: Vomiting grade 1~2, at least once
High-grade toxicity: Vomiting grade 3~4, at least once



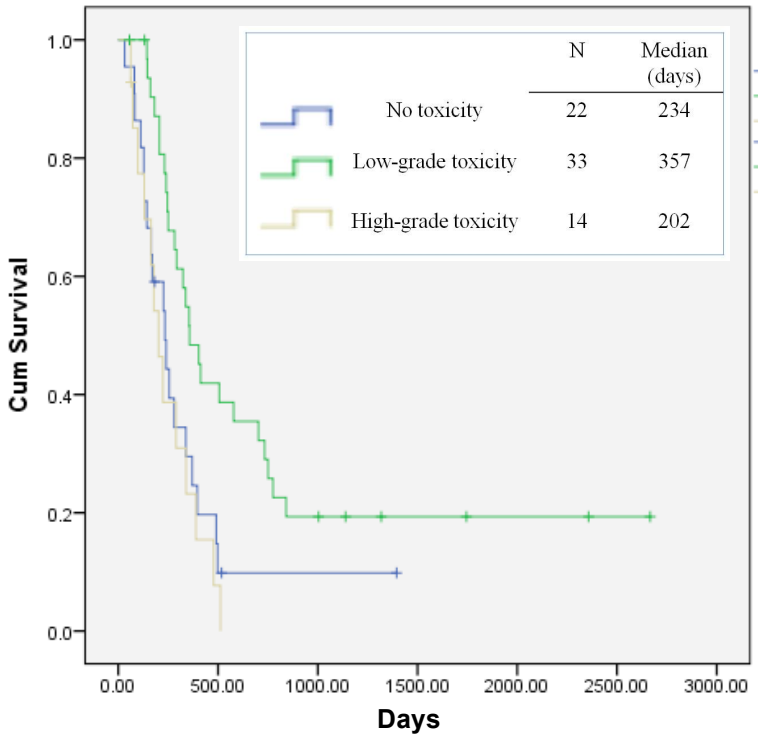
P =0.040

Figure 26
Kaplan-Meier's curve for TTP of grouping 41140
No toxicity: No diarrhea
Low-grade toxicity: Diarrhea grade 1~2, at least once
High-grade toxicity: Diarrhea grade 3~4, at least once



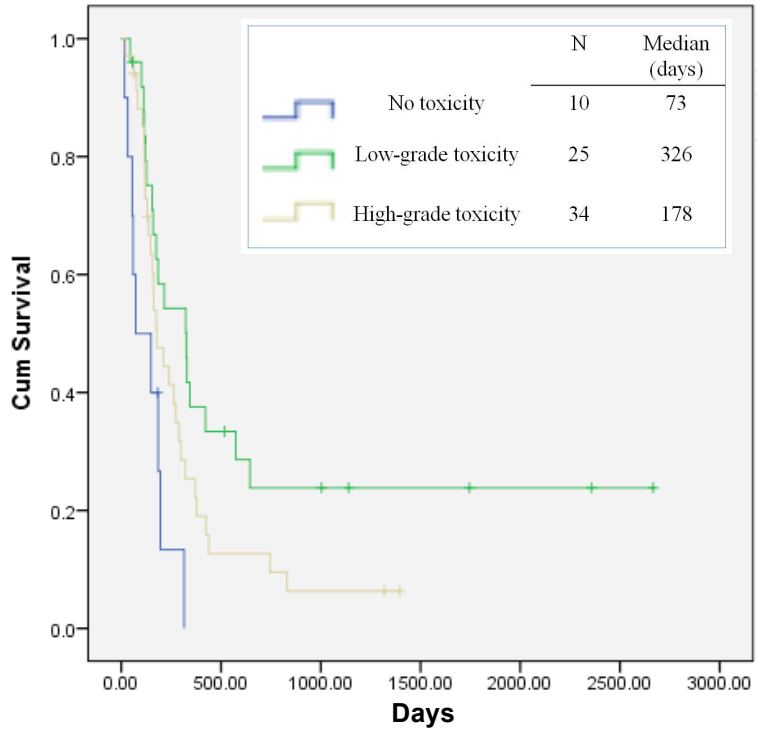
P =0.015

Figure 27
Kaplan-Meier's curve for OST of grouping 41140
No toxicity: No Diarrhea
Low-grade toxicity: Diarrhea grade 1~2, at least once
High-grade toxicity: Diarrhea grade 3~4, at least once



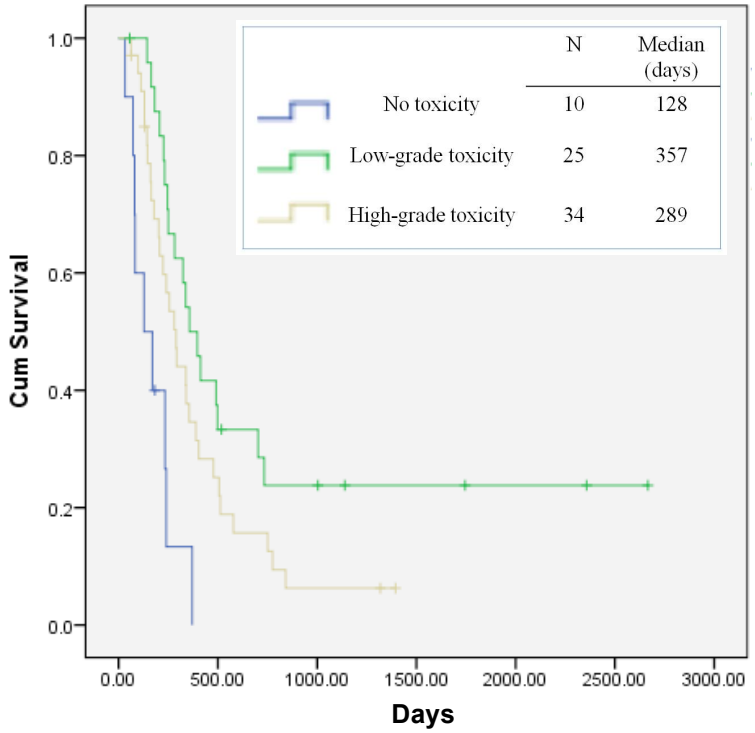
P =0.004

Figure 28
Kaplan-Meier's curve for TTP of grouping 51140
No toxicity: No GI signs
Low-grade toxicity: GI signs grade 1~2, at least once
High-grade toxicity: GI signs grade 3~4, at least once



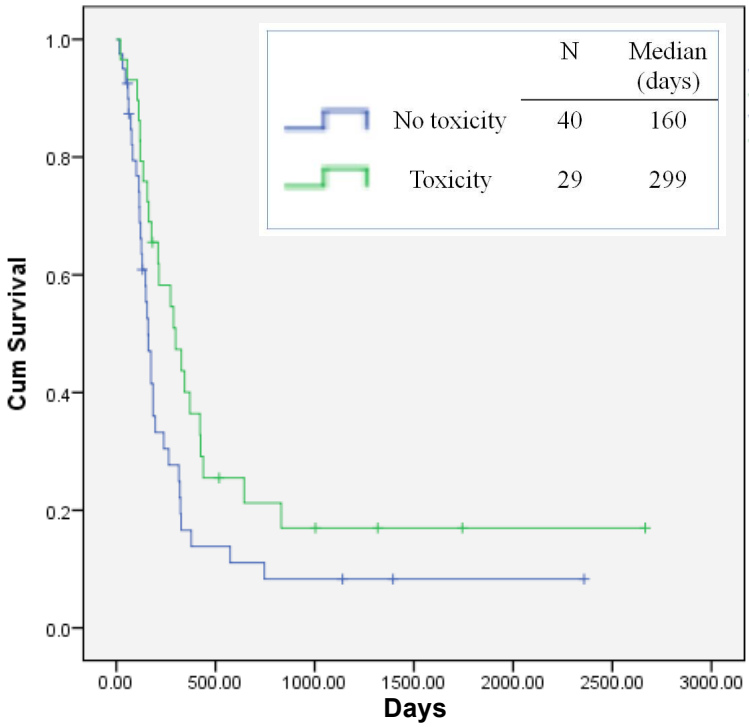
P =0.006

Figure 29
Kaplan-Meier's curve for OST of grouping 51140
No toxicity: No GI signs
Low-grade toxicity: GI signs grade 1~2, at least once
High-grade toxicity: GI signs grade 3~4, at least once



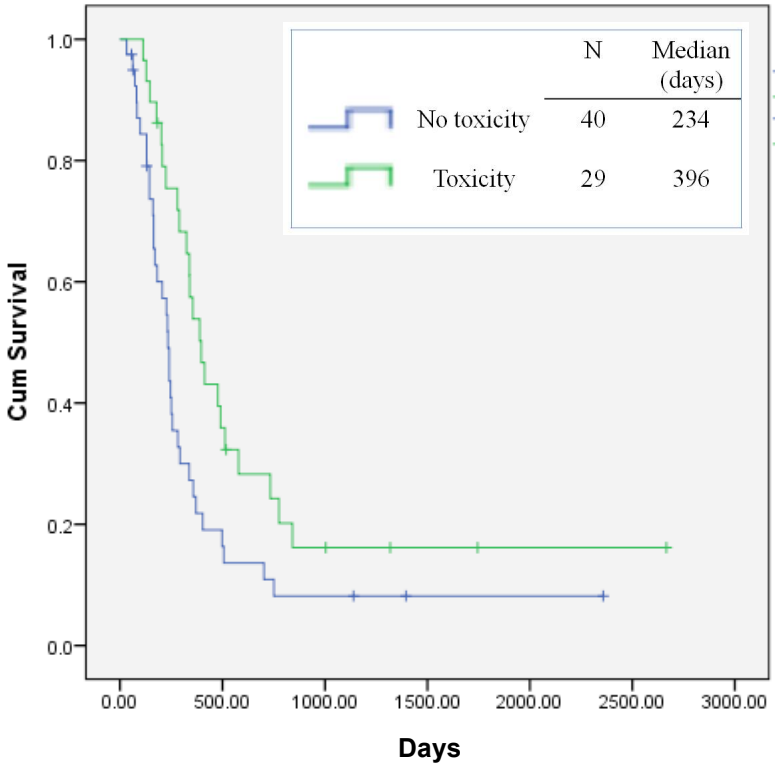
P <0.001

Figure 30
Kaplan-Meier's curve for TTP of grouping 11310
No toxicity: No neutropenia after cyclophosphamide
Toxicity: Neutropenia grade 1.0~3 after cyclophosphamide, at least once



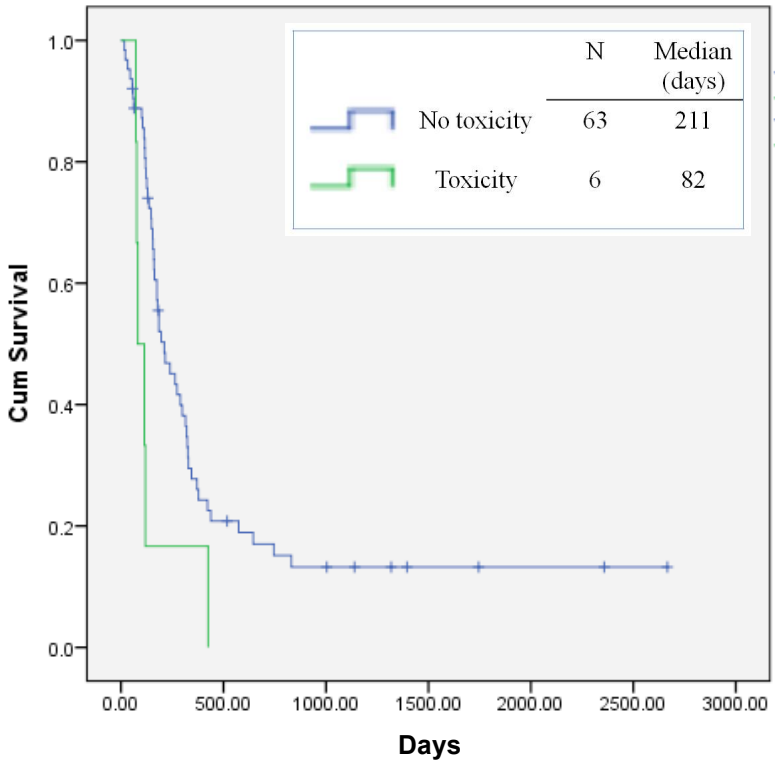
P =0.042

Figure 31
Kaplan-Meier's curve for OST of grouping 11310
No toxicity: No neutropenia after cyclophosphamide
Toxicity: Neutropenia grade 1.0~3 after cyclophosphamide, at least once



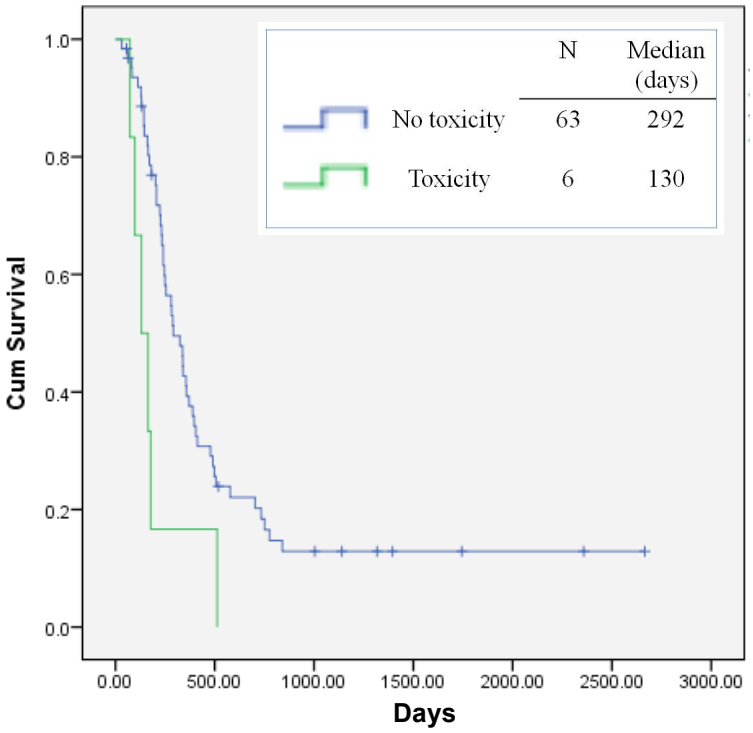
P =0.019

Figure 32
Kaplan-Meier's curve for TTP of grouping 41230
No toxicity: No diarrhea or diarrhea grade 1~2 after vincristine
Toxicity: Diarrhea grade 3~4 after vincristine, at least once



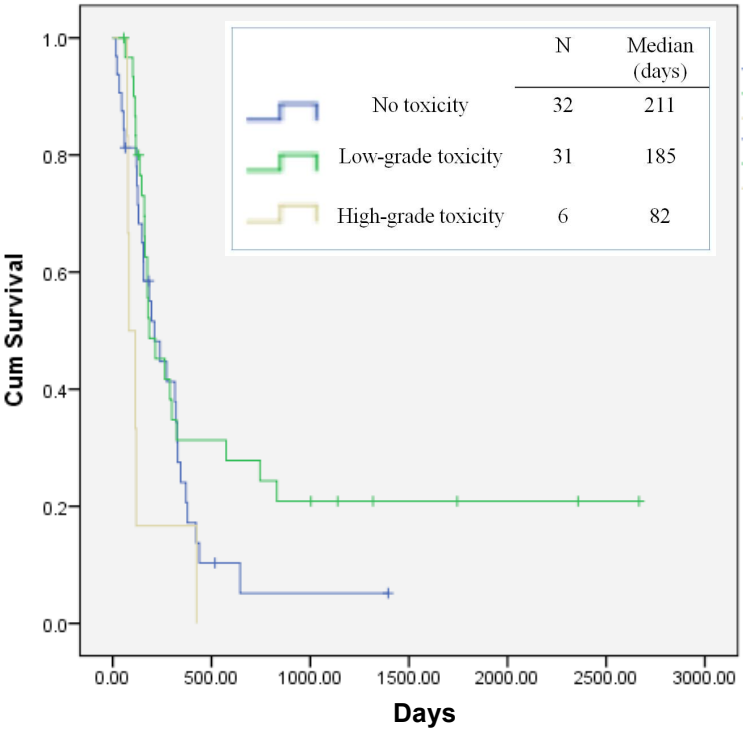
P =0.022

Figure 33
Kaplan-Meier's curve for OST of grouping 41230
No toxicity: No diarrhea or diarrhea grade 1~2 after vincristine
Toxicity: Diarrhea grade 3~4 after vincristine, at least once



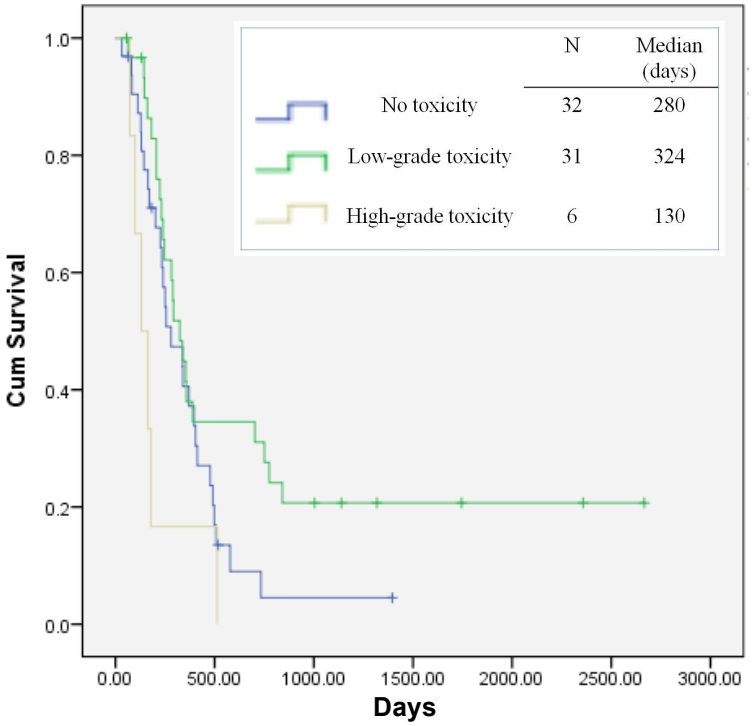
P =0.015

Figure 34
Kaplan-Meier's curve for TTP of grouping 41240
No toxicity: No diarrhea after vincristine
Low-grade toxicity: Diarrhea grade 1~2 after vincristine, at least once
High-grade toxicity: Diarrhea grade 3~4 after vincristine, at least once



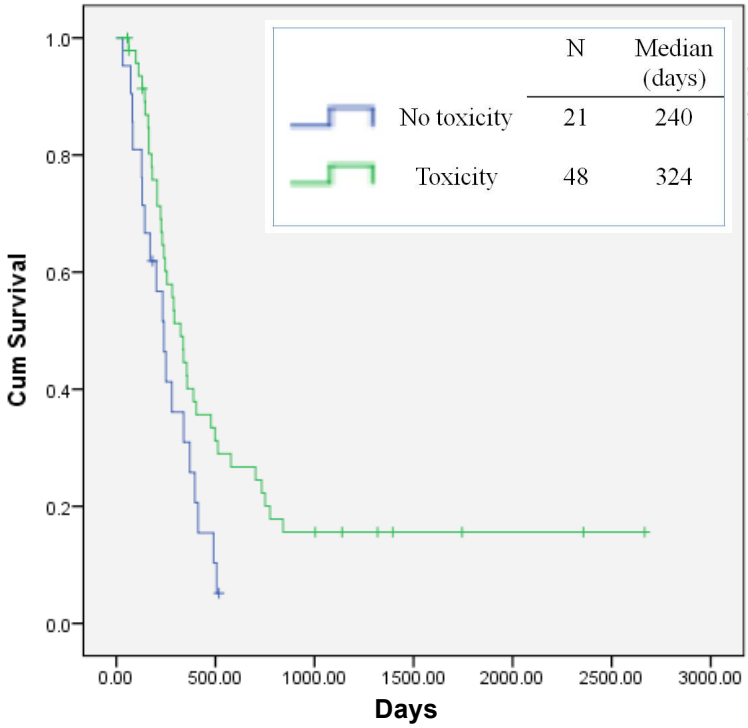
P =0.031

Figure 35
Kaplan-Meier's curve for OST of grouping 41240
No toxicity: No diarrhea after vincristine
Low-grade toxicity: Diarrhea grade 1~2 after vincristine, at least once
High-grade toxicity: Diarrhea grade 3~4 after vincristine, at least once



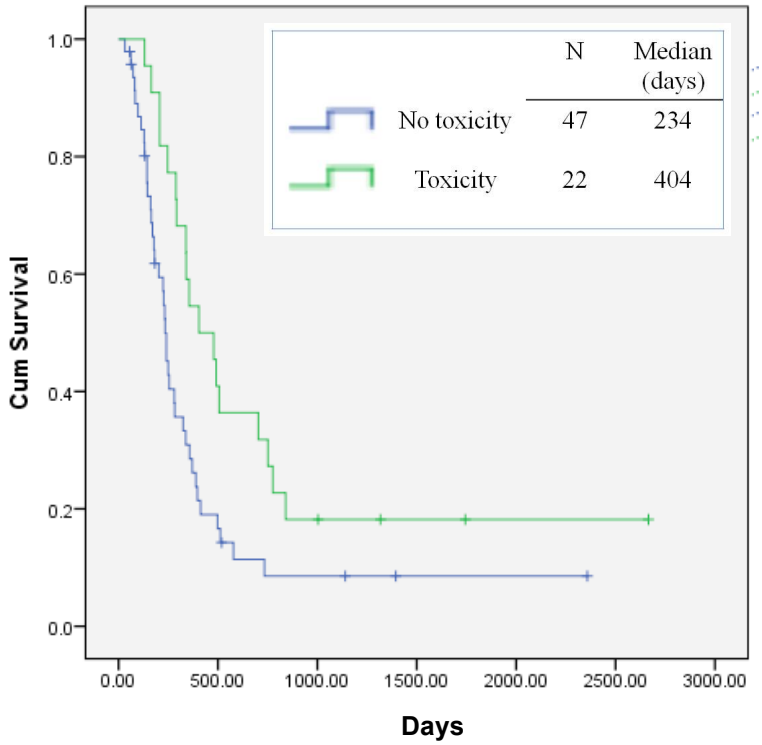
P =0.014

Figure 36
Kaplan-Meier's curve for OST of grouping 51210
No toxicity: No GI signs after vincristine
Toxicity: GI signs grade 1~4 after vincristine, at least once



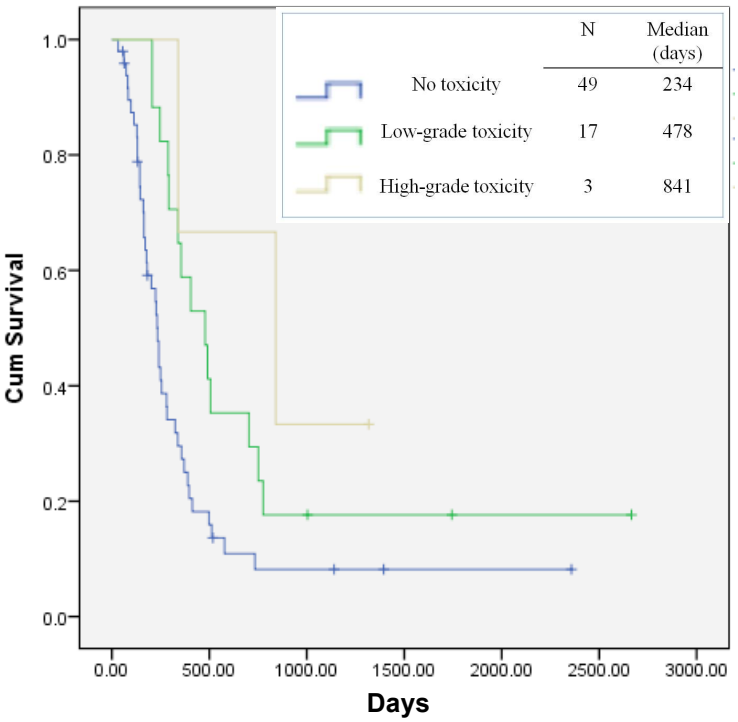
P =0.030

Figure 37
Kaplan-Meier's curve for OST of grouping 31113
No toxicity: No vomiting or vomiting ≦2 times
Toxicity: Vomiting grade 1~4, >2 times



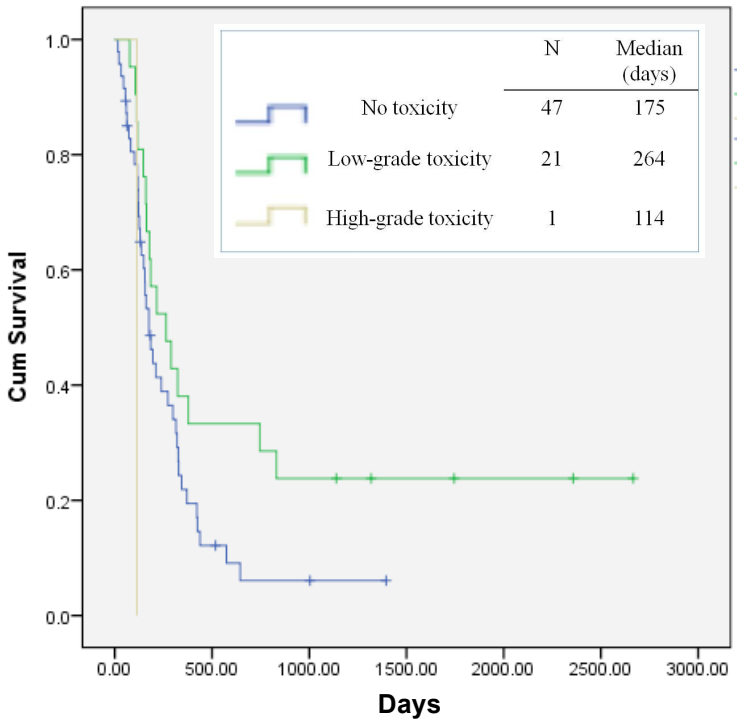
P =0.015

Figure 38
Kaplan-Meier's curve for OST of grouping 31143
No toxicity: No vomiting or vomiting ≦2 times
Low-grade toxicity: Vomiting grade 1~2, >2 times
High-grade toxicity: Vomiting grade 3~4, >2 times



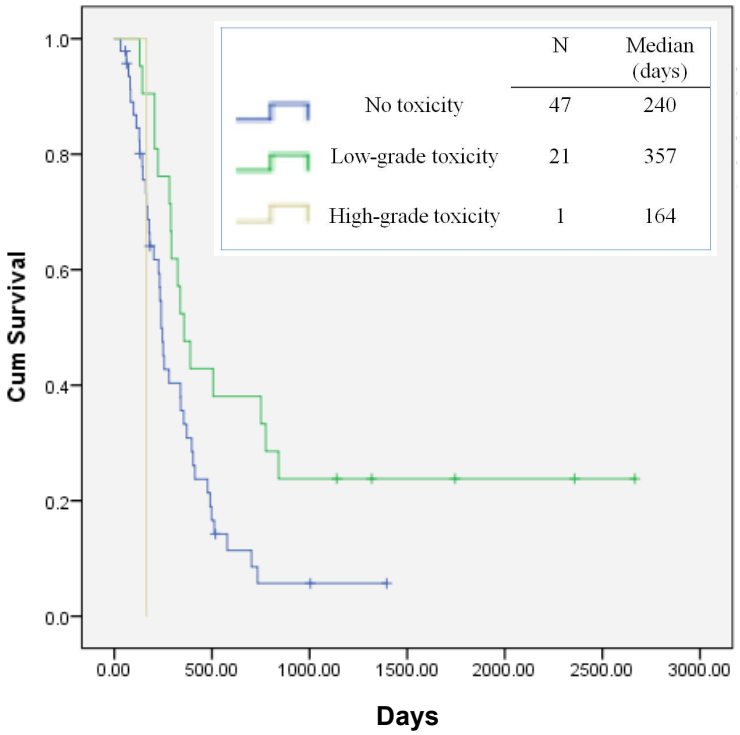
P =0.014

Figure 39
Kaplan-Meier's curve for TTP of grouping 41143
No toxicity: No diarrhea or diarrhea ≦2 times
Low-grade toxicity: Diarrhea grade 1~2, >2 times
High-grade toxicity: Diarrhea grade 3~4, >2 times



P =0.045

Figure 40
Kaplan-Meier's curve for OST of grouping 41143
No toxicity: No diarrhea or diarrhea ≦2 times
Low-grade toxicity: Diarrhea grade 1~2, >2 times
High-grade toxicity: Diarrhea grade 3~4, >2 times



P =0.019