CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research Questions and Objectives

3.1.1 Primary Research Question

Does high-dose therapy (HDT) and autologous peripheral blood progenitor cell transplantation (PBPCT) administered after three cycles of CHOP improve the therapeutic outcome in term of increasing the rate of complete response by $\geq$ 50% as compared to the standard conventional CHOP chemotherapy in adult patients newly diagnosed as poor-risk aggressive NHL (high and high-intermediate risk groups by age-adjusted international index, histology subtypes of F-H by the International Working Formulation) ?.

3.1.2 Secondary Research Question

3.1.2.1 Is the incidence of life-threatening toxicities i.e., the febrile neutropenia, in patients who received HDT and PBPCT greater than in patients who received CHOP therapy?

3.1.2.2 What are the most significant factors that determine the complete remission in the patients?

3.2 Research Objectives

3.2.1 To determine the therapeutic potential of up-front HDT and PBPCT in term of increasing the complete remission rate in patients with poor risk aggressive NHL.

3.2.2 To compare the toxicities of HDT and PBPCT with CHOP chemotherapy.

3.2.3 To determine clinical variables that significantly correlate with the probability of achieving complete remission.

3.3 Research Hypothesis

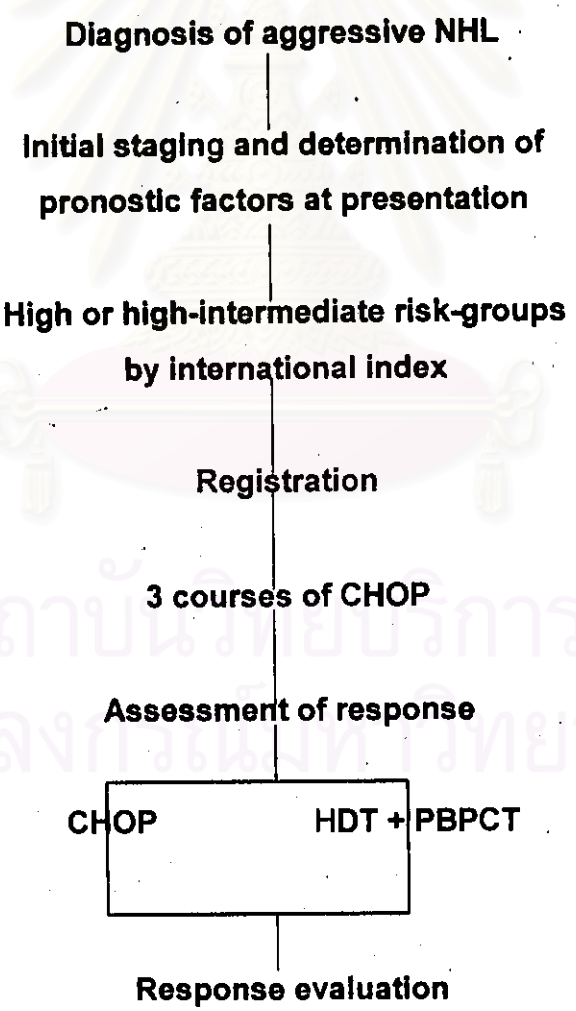
As evidence, both in vitro and in vivo, has shown that aggressive NHL is a highly dose-responsive tumor, administration of high-dose chemoradiotherapy (5-10 times higher than the conventional dose) with hemopoietic stem cell support as an up-front therapy before overt drug resistance developed in the patients should result in an improved rate of tumor response over what has been achieved with the current standard conventional chemotherapy treatment.

3.4 Research Design

This is a randomized prospective controlled trial comparing the efficacy of HDT and PBPCT with conventional CHOP chemotherapy in patients with poor risk aggressive NHL. Initial staging and determination of prognostic factors will be performed on newly diagnosed aggressive NHL patients at the tirtiary care medical centers. Patients with high and high-intermediate risk groups according to the international prognostic index will be registered. A well-defined sampling frame is developed to recruit the patients so that selective bias is minimized and inference to target population is obtained. Standard CHOP chemotherapy will be administered to the patients. After third cycle of CHOP, tumor response will be assessed. Patients will then be stratified into groups according to the tumor response and initial

prognostic features and randomized to receive either HDT and PBPCT or continue CHOP therapy. Strategy for controlling possible cointerventions that might occur during the two-arm treatment is defined. Objective tumor response is assessed by a valid and reliable measurement tool after HDT with PBPCT and the last cycle of CHOP chemotherapy. Patients who showed progressive disease after randomization were considered as treatment failure and were taken off study for ethical reason.

Figure 1 Study Schema



3.5 The Sample

3.5.1 Target Population

Patients aged 15-55 years newly diagnosed as poor risk (high-intermediate and high risk groups by the age-adjusted international prognostic index) aggressive NHL (category F-H by the Working Formulation).

3.5.2 Sample Population

Patients diagnosed at Chulalongkorn and Pramongkutkloa Hospitals who meet the eligibility criteria were registered into the study. Since most patients diagnosed as having lymphoma will not be treated outside the tirtiary care centers, the patients recruited in our study could be representatives of the target population.

3.5.3 Eligibility Criteria

3.5.3.1 Inclusion Criteria

1. Patients must have a histologic diagnosis of one of the following histologic types according to the International Working Formulation (Table 3):

Diffuse mixed (IWF-F)

Diffuse large-cell (IWF-G)

Diffuse large-cell-immunoblastic (IWF-H)

All biopsy specimens including those diagnosed outside the participating centers will be reviewed by a hematopathologist at Department of Pathology.

2. Newly diagnosed, age 15-55 years.

3. Patients must have high-intermediate or high risk groups as defined by the age-adjusted international prognostic index .

Stage III/ IV (Ann Arbor Classification, Table 6)

Serum lactate dehydrogenase (LDH) > normal (> 450 u/l)

Performance status, ECOG grade 2-4 (Appendix A)

High-intermediate : 2 risk factors, High : 3 risk factors.

4. Complete work up for the following baseline evaluation and measurement :

Complete history and physical examination

Complete review of pathological specimen

Evaluation of the performance status (ECOG scores)

Evaluation of stage of disease and B symptoms

Complete blood count with differential count

Bone marrow aspiration and biopsy

Blood urea nitrogen (BUN), creatinine (Cr), liver function tests (LFTs), Uric acid, Albumin, Globulin

Serum LDH

Chest X-ray

Computerized study of abdomen

Electrocardiogram (ECG)

Serology for anti-HIV

5. Have measurable disease i.e., have a tumor mass objectively and reproducibly measurable in two perpendicular diameters by physical examination, radiography, sonography and computerized tomographic scan.

6. Patient's free inform consent.

3.5.3.2 Exclusion Criteria

1. Patients who have received prior antilymphoma treatment with chemotherapy or radiotherapy.

2. Serologic evidence of human immunodeficiency virus exposure.

3 Patients with history of impaired cardiac status, myocardial infarction within three months, angina pectoris requiring medication, cardiomegaly on examination or by chest radiograph.

4. Patients with serum creatinine $\geq$ 1.8 mg/dl, bilirulin $\geq$ 1.5 times upper limit of normal range, SGOT or SGPT $\geq$ 3 times upper limit of normal range, unless due to tumor involvement.

5. Patients with active uncontrolled infection, active non-malignant gastric or duodenal ulcer, uncontrolled diabetes mellitus or other severe medical conditions which would preclude aggressive cytotoxic chemotherapy.

6. Patients with history of malignant disease in the previous 5 years.

7. Pregnant or lactacting women.

8. Serious medical or psychiatric illness which prevent informed consent.

9. Patients who are likely to be lost to follow up (e.g., unwilling or difficult to return, cannot be contacted).

Most of the exclusion criterias are mandated by ethical considerations regarding conditions that chemotherapy might do more harm than good. Criteria 9 is selected to minimized compliance bias.

3.5.4 Sample Size Estimation

The primary outcome of interest is the proportion of patients who achieve a complete remission following conventional CHOP and HDT. This outcome was selected because cure cannot be obtained without a prior attainment of complete remission. From the previous studies, the expected rate of complete remission after CHOP therapy (pc) for poor-risk patients was 50%[5]. The complete remission rate of HDT administered at the early phase of treatment as reported from phase II uncontrolled trials is 80-95%[14,16]. In addition, previous economic appraisal on cost-effectiveness analysis of up-

front HDT with PBPCT in Chulalongkorn Hospital found that to be a more cost-effective therapy than CHOP, HDT and PBPCT must obtain a CR rate of $\geq$ 80%[136]. In order to be clinical significance, our study is therefore proposed to detect an absolute increase complete remission rate by at least 60% for patients who receive the HDT and PBPCT. By using the formula calculating sample sizes for comparing two proportions,

$$n = [z_{\alpha}\{2pc(1- pc)\}^{1/2} - z_{\beta}\{pt(1- pt) + pc(1 - pc)\}^{1/2} /(pt - pc)]^2$$

n = number of sample in each group

z_{α} = 1.645, the one-tailed z value of 0.05

z_{β} = - 0.84, the lower one-tailed separating the lower 20% from the upper 80%

pc = 0.50

pt = 0.80

The estimated sample size is 32 for each group. For coverage of patients who die or have a progressive disease during the first three cycles of CHOP (5%), and patients who will be lost to follow-up (5%), total number of patients needed to be registered is 70.

3.6 Experimental Maneuver

3.6.1 Sampling of the Population

To minimize selection bias, every consecutive patient newly diagnosed as NHL at the participating centers during the study period were screened and those who met the eligibility criteria were enrolled. Recruitment of the patients involved both direct contact at the outpatient department or at the hospital wards so that there were no admission rate bias. Identification of patients were also obtained from interdepartmental consultation.

3.6.2 Randomization

After the third cycle of CHOP treatment, patients will be assessed for tumor response. Patients will then be stratified into groups according to the following parameters which are likely to affect treatment outcome, i.e. age ($\leq$ 45 vs. > 45 years), the risk-group (high vs. high-intermediate) and tumor response (complete response vs partial response vs stable disease vs progressive disease). Within each strata, patients will then be randomized in a block of 4-6 to receive CHOP or HDT with PBPCT, using the random number table. This stratified randomization is performed to ensure homogeneity of patients enrolled into the two treatment arms, thus avoiding procedure bias. To minimizing selection bias, a randomization list is generated in advance of recruiting the first patient by a biostatistician and is kept at the secretary's office at the hematology department. The investigating clinician had no knowledge of this list.

3.6.3 Intervention

3.6.3.1 Treatment Administration

3.6.3.1.1 CHOP chemotherapy

	Dose	Day of cycles
Vincristine (O)	1.4 mg/m^2 IV (max 2 mg)	day 1
Cyclophosphamide (C)	750 mg/m^2 IV	day 1
Doxorubicin (H)	50 mg/m^2 IV	day 1
Prednisolone (P)	75 mg P.O.	day 1-5

Repeat every 21 days x 8 cycles

Patients were prescribed allopurinol 300 mg/day for the first 28 days of treatment. Patients were monitored closely over the 24 hours following the initial dose of chemotherapy to detect and treat adverse effects of rapid tumor lysis.

Safety parameters and dose-modification

CBC, BUN, Cr and LFTs are measured on day 1 of each cycle.

The following dose modifications are based on day 1 values of each cycle.

Hematologic toxicities

ANC	and/or	PLTS	% full doses
$\geq 1.5 \times 10^9/l$		$\geq 100 \times 10^9/l$	100% all drugs
$1\text{-}1.49 \times 10^9/l$		$75\text{-}99 \times 10^9/l$	50% C/H, 100% O/P
$< 1 \times 10^9/l$		$< 75 \times 10^9/l$	Defer cycle 1 week

If patients had biopsy-proven lymphomatous invasion of bone marrow, full doses chemotherapy may be given for cycle 1 and 2 regardless of the peripheral blood count. Adjustment of the drug was carried out for the subsequent cycles.

Hepatic toxicity

Bilirubin(mg/dl)	and/or	SGOT	% H,O	% C,P
< 1.5		< 50	100%	100%
1.5 - 3.0		50 - 150	50%	100%
3.1 - 5.0		151 - 300	25%	100%
> 5.0		> 300	0%	100%

Nephrotoxicity

Serum creatinine	C
< 2.0 mg/dl	100%
≥ 2.0 mg/dl	50%

Steroid toxicity

Discontinue prednisolone for active peptic ulcer disease, uncontrolled diabetes, serious infection or steroid psychosis.

Cardiac toxicity

Frequent physical examination and EKG monitoring are neccessary. The maximum cumulative dose of doxorubicin allowed is 550 mg/m^2. A reduction in the QRS wave voltage is considered as specifically predictive of cardiotoxicity. If this occurs, the drug should not be given to the patient.

Neurotoxicity

Vincristine dose should be reduced by 50% if muscle strength decreased by 50% or if persistent, moderately severe paresthesias or constipation develop. For more severe toxicity, vincristine should be discontinued.

3.6.3.1.2 High-dose therapy and PBPCT

A. ESHAP chemotherapy

Patients who has complete remission will receive ESHAP chemotherapy for 2 courses, the first course is started at week four after the third cycle of CHOP. Patients who attain partial remission, stable or

progressive disease received ESHAP 4 courses started at four week after third cycle of CHOP.

	Dose	Day of cycle
Etoposide (E)	40 mg/m^2	day 1-4
Cisplatin (P)	25 mg/m^2	day 1-4
Cytarabine (A)	2 gm/m^2	day 5
Methylprednisolone (S)	500 mg IV	day 1-4

Repeat every 21 days x 2 cycles.

Patients were prescribed allopurinol 300 mg/ day for the first 28 days of treatment. Patients were monitored closely over the 24 hours following the initial dose of chemotherapy to detect and treat adverse effects of rapid tumor lysis. Patients received Dextrose 0.45% Normal Saline 3 lit/day and mannitol 25-50 gm/day during cisplatin infusion.

Dose adjustments were made in accordance with myelosuppression developed in the prior cycle of chemotherapy. Serum creatinine was measured on day 1 of each cycle.

Event	A	E	P
Severe neutropenia < 200/cumm	1 g/ m^2 x 2	20mg/m^2	25mg/ m^2
Thrombocytopenia < 20,000/cumm	1 g/m^2 x 2	20mg/m^2	25mg/ m^2
Sepsis associated with neutropenia	0.5 g/m^2 x 1	10mg/m^2	25mg/m^2
Serum creatinine 1.5-2.0 mg/ml			18mg/m^2
2.1-3.0 mg/ml			12mg/m^2
> 3.0 mg/ml			Omit

B. Stem cell mobilization and collection

Neupogen 10 ug/kg is given sc. daily for 7 days starting on day 21 post last course of ESHAP. Double lumen sialastic catheter was placed prior to stem cell apheresis. Stem cell collection was performed on day 5-7 of Neupogen therapy and cryopreserved at vapor phase of liquid nitrogen.

C. Conditioning regimen

After stem cell collection, patients will receive fractionated total body irradiation 1,200 rads for three days and cyclophosphamide 60 mg/kg for 2 days. Stem cell products were infused at 48 hours post last dose of cyclophosphamide.

3.6.3.1.3 Concomittant Therapy

Patients in the two treatment arms received the same supportive care in order to avoid the procedure bias.

Acetaminophen may be given to alleviate fever or pain.

For antiemetics, metoclopramide or ondasetron were given.

For relieve of gastric pain, antacids, sucrafate or ranitidine were given.

Hospitalization was indicated for patients who develop post chemotherapy febrile neutropenia (body temperature (BT) $\geq$ 38.5^{0}C and absolute neutrophil count is $\leq$ 500/cumm). A third-generation cephalosporin or Ciprofoxacin and aminoglycoside were used as initial empiric antibiotic coverage. Empirical intravenous Amphotericin B was added if fever does not subside in 5 days.

Platelet transfusions were given to maintain the platelet count $\geq$ 20 x10^9/l.

Involved field radiotherapy was indicated at three weeks post eight cycle of CHOP and HDT for patients who have bulky disease (diameter of mass larger than 10 cm) at presentation.

3.7 Measurement

3.7.1 Outcome Variables

3.7.1.1 Tumor response is defined by the following operational definition.

Complete Remission

Disappearance of all measurable or evaluable disease, signs or symptoms related to the tumor for at least 4 weeks, during which no new lesions appear.

Partial remission

When compared with pretreatment measurements, a reduction $\geq$ 50% in the sum of the product of two perpendicular diameters of all measurable lesions lasting for at least 4 weeks, during which no new lesions appear and no existing lesion is enlarged. Disappearance of constitutional symptoms if initially present.

Stable disease

A < 50% reduction and < 25% increase in the sum of the products of the perpendicular diameters of all measurable lesions, and the appearance of no new lesions.

Progressive disease

An increase in the product of two perpendicular diameters of a measured lesion by $\geq$ 25% over the size present at entry or for patients who respond, the size at the time of maximum regression and/or the appearance of new areas of malignant disease.

A deterioration in performance status or increasing symptoms does not constitute progression; however, their appearance should warrant a new evaluation for extent of disease.

Patients who did not achieve complete remission were considered as "treatment failure".

3.7.1.2 The most clinically important toxicity to be considered was the febrile neutropenia defined as BT $\geq$ 38.0^0 C in association with the absolute neutrophil count $\leq$ 0.5 x 10^9/l occurred after chemotherapy. The toxicity events were monitored and recorded by the investigator during study period.

3.7.2 Procedures for assessment of tumor response

The following procedures were used for baseline assessment of tumor burden (Table 9). The procedures were repeated at three weeks post third cycle of CHOP to assess tumor response (complete response, partial response, stable disease and progressive disease). The primary outcome i.e., state of complete remission was assessed at four weeks after eight cycle of CHOP and HDT with PBPCT, except for patients who received adjunctive radiotherapy for bulky disease, assessment were performed at four weeks post radiation.

Table 9 Procedures performed at baseline and at assessment tumor response

	At Staging	Three weeks after 3rd cycle of CHOP, Four weeks after 8th cycle of CHOP and HDT
History and physical exam	X	X
Performance status	X	X
Tumor measurement (sum of two perpendicular diameters at the largest mass)	X	X
CBC, LFT, BUN, Cr	X	X
Bone marrow biopsy/aspirate	X	E
Chest X-ray*	X	E
Abdominal/pelvic CT scan*	X	E

Bone scan, lumbar puncture, barium study of bowels,chest CT or tissue biopsy will be performed as clinically indicated.

E : If abnormal at time of initial staging or clinically indicated.

* Radiologists who are blinded to treatment assessed the results of the study. Since these procedures were measured by a number of assessors, interobserver consistency of these assessment were carried out.

3.7.3 Predictive variables

The following patient characteristics were considered as clinical significant variables used to determine the probability to attaining complete remission after treatment. These variables were recorded in the data record form and are defined by the following operational definitions.

3.7.3.1 Age

Defined as age in years as recorded in the identification card.

3.7.3.2 Prognostic index

Defined as "high" and "high-intermediate" by the international age-adjusted index.

3.7.3.3 Tumor respose before randomization

Complete remission, partial remission, stable disease or progressive disease.

3.7.3.4 The treatment arm

CHOP versus high-dose therapy with PBPCT

3.8 Consideration of Confounding Factors

3.8.1 Selection Bias

To minimize selection bias, every consecutive patient newly diagnosed as NHL at the participating centers during the study period were screened by the investigator and those who met the eligibility criteria were enrolled. In addition, all biopsy specimens including those diagnosed outside the participating centers were reviewed by one hematopathologist so that the diagnosis of eligible subtypes of NHL is confirmed.

3.8.2 Assessment Bias

Due to the pattern of therapeutic administration, this study cannot be conducted in a blinded fashion. The assessment of outcome therefore is very important. Radiologists who are blinded to patient treatment interpretated the radiological findings. In order to verify the reliability of the interpretation, another radiologist interrprete the same radiologic study files and the degree of agreement between the two observers was computed. An intraclass correlation of > 0.5 is regarded as a criteria for reliability acceptance.

3.8.3 Co-intervention

As one of the major causes of treatment-failure related to both treatment arms is life-threatening febrile neutropenia, therefore, the rationale of treatment of this complication particularly types of antibiotics in both treatment arm should be the same. This has been mentioned in Section 3.4.3.1.3. In addition, indication for platelet transfusions and other supportive treatment such as, the administration of antiemetics have been stated.

3.8.4 Compliance

Outcome bias might occur if the proportion of patients who are loss follow-up is greater than 20%. Patients who are likely to loss follow-up is therefore not enrolled into the study.

3.9 Data Collection

The data of the study was collected and recorded in the special data record forms. Patient's baseline characteristics were recorded at time of registration by the investigator. Sources of data are the interview, physical examination, laboratory and special investigations. Auditing and updating the data were performed every two weeks. Emphasis was placed on minimizing missing data, error of data collection and records.

For data entry into computer program, every variable was given a name that will identify its field in the data set. Every possible value was then coded with a number so that it can be entered into the computer, for example, 1 is the permitted response for "yes", 2 for "no", 9 or 99 for "missing" and 8 or 98 for "cannot evaluate". The coding instruction is also printed on the record form.

3.10 Data Analysis

3.10.1 Demographic Data

Each variable recorded was examined first for its frequency distribution so that the outlying values were checked and the central tendency and

pattern of spread were determined. Baseline demographic data is presented as the following:

Age	Mean ± SD (Range)
Histological types	Percentage
Stage	Percentage
Performance status	Percentage
Serum LDH > 450 u/l	Percentage
Prognostic risk-group	Percentage

Patient's characteristics in the two treatment arms were compared by appropriate statistics:

Age	Mean ± SD	t-test
Histological types	Percentage	Fisher's exact
Stage	Percentage	Fisher's exact
Performance status	Percentage	Fisher's exact
Serum LDH > 450 u/l	Percentage	Fisher's exact
Prognostic risk-group	Percentage	Fisher's exact

3.10.2 Statistical analysis of the primary outcome

The analytic approach of the study was based on the intention-to-treat basis, since the full value of randomization will be preserved and control over baseline confounders is maximized. Rate of complete response (CR) was calculated as number of patients who achieve CR in that treatment arm divided by the total number of patients enrolled for that treatment although some might be lost to follow-up, have progressive diseases or die before outcome assessment. Reliability for this response rate was calculated for the confidence interval for population CR rate.

$$95\%\ CI \text{ for } pCR = pCR \pm 1.96SE(pCR)$$

$$\text{where } SE(pCR) = [\, pCR(1 - pCR)/\, n]^{1/2}$$

Comparison of response rates for the two treatment arms were computed by the Fisher's exact test and the 95% CI for the true difference between the two proportion (δ).

$$95\%CI \text{ for } \delta = (\, pt - pc) \pm 1.96SE(pt - pc)$$

$$\text{where } SE(pt\text{-}pc) = [\, pt(\, 1 - pt)/nt + pc(1 - pc)/\, nc]^{1/2}$$

Comparison of other tumor responses i.e., the rate of progressive disease, partial response and stable disease were computed by the Fisher's exact and the 95% CI for the true difference between the two proportion (δ).

3.10.3 Statistical analysis of the secondary outcomes

Rates of febrile neutropenia and death in the two treatment groups were compared using the Fiher's exact test and the 95% CI of the difference in the same manner as in the primary outcome.

3.10.4 Statistical analysis of the predictive variables

For analysis of factors significantly associated with complete response, predictive variables that were analysed were age, prognostic risk-group, tumor status at the time of randomization and type of treatment. The stepwise logistic regression was used to determine the significant predictive factors.

All statistical analysis were approach by the STATA version 3.1 program.

3.11 Ethical Consideration

There are a possible risk of life-threatening complications in both treatment arms. Full meticulous safety precautions were provided to minimize the risks and maximize the benefit.

The study was conducted in full conformance with the principles of the Declaration of Helsinki and with the local laws and regulations concerning clinical trials. All patients were informed of the aims, methods, benefits and potential hazards of the trial. Informed consent preferably in the written form must be obtained from each patient participating in the trial, after explanation of the study. This consent must be obtained before any trial specific procedure are performed on the patient.

It was made completely clear to patients that they are free to refuse to participate in the trial, or withdraw their consent at any time and for any reason, without incurring any penalty or withholding of treatment on the part of the investigator. All the data obtained from the study are kept confidentially.

3.12 Limitations

Because of the pattern of treatment administration, the study design cannot be conducted in a double-blinded manner. Therefore co-intervention might occur during the study period. To partially solve the problem, the protocol has specified and standardized the intervention clearly particularly on the concomittant therapy. In addition, investigations assessing primary outcome variable are measured by assessors (radiologist) who have no knowledge of the study group.

3.13 Expected Benefits and Applications

With current standard chemotherapy regimen, patients identified as having poor-risk aggressive NHL still have an unfavorable prognosis. Only 30% of these patients survive at 5-year post-treatment. This study research will provide knowledge whether HDT and PBPCT can improve the therapeutic outcome when administered at the early phase of treatment compared to standard treatment. The results of the study can lead to important changes in the clinical practice in one of the most prevalent hematologic malignancies.

3.14 Obstacles and Strategies to Solve the Problems

3.14.1 Limitation of hospitalization beds : Because hospitalization is needed during HDT and PBPCT and when patients develop febrile neutropenia, shortage of available hospital bed might occur. Special space in the research ward and in the bone marrow transplant unit will be arranged for patients under the trial.

3.14.2 Completeness of follow-up : Completeness of follow-up is very important in this study. Outcome bias can occur if the proportion of patients lost to follow-up is not small eg, greater than 20% especially if there is a differential rate between treatment arms. To minimize this problem, patients

were informed clearly of the importance of the follow-up at the outset of the study. Those who are difficult to follow-up will be excluded from entry into the study.The name, address and telephone number of the patients and one or two close acquaintances were recorded. Regular contact is made by mail or telephone. In addition, 5% of the calculated sample size has been added for coverage of patients who will be lost to follow-up. However, should a significant loss follow-up patients occur, baseline demographic data especially the prognostic features will be analysed between those who are followed and those who are lost.

3.14.3 Missing and inaccurate data : This problem can bias the conclusion if it affects a significant proportion of the measurements. The research assistant was trained for data collection procedure and certified a clear understanding of operative definitions of recorded data. Principal investigator edited what was written on the forms, checking the completeness and appropiateness of data entries while subjects were still in the clinic. Frequency distributions and cross-tabulations of important variables werel be checked at regular intervals for assessing completeness and quality of data.

3.15 Administration and Time Schedule

The first two months of the study was spent on training the research team and submission for ethics committee approval. Operation definitions of recruitment, measurement procedure, data collection and quality control system were explained. Program implementation was performed in the third month up to the last three months when data analysis and report writing were carried out.