CHAPTER I

INTRODUCTION

Background and rationale

Allogeneic hematopoietic stem cell (HSC) transplantation has been used successfully in the treatment of hematological diseases, neoplasias, severe congenital immunodeficiency states and metabolic disorders[1-4]. Historically, the bone marrow (BM) has represented the main source of HSCs in pediatric and adult individuals, but is limited by several important factors. For instance, in many case, a suitable donor is hardly found because using HLA-match ralated sibling donors in bone marrow transplant has been that only 30% to 40% of potential recipients in need of such therapy have an HLA-matched related family donor[5].

This difficulty has led to a search for alternative source of HSCs for use in human transplantations. Two sources of human HSCs have been identified: peripheral blood (PB) and cord blood (CB). Initial studies of using matched related allogeneic PB stem cell suggested that the risk of graft rejection and developing graft-versus-host disease (GVHD) are similar as compared with matched related allogeneic BM stem cell[6-8]. Furthurmore, PB stem cell collection by administration of G-CSF which can mobilize significant numbers of stem cells into blood circulation sometimes result poor mobilization, and more importantly, may be due to splenic rupture during G-CSF application[9]. Umbilical cord blood (UCB) have become attractive source of HSCs for allogeneic HSC transplantation[2,10-12]. Cord blood hematopoietic stem cell grafts have been successfully used as grafts for pediatric recipients who lack matched sibling donors[2-5]. One major advantage of using CB in comparison with PB or BM might be the reduced incidence of acute GVHD caused by CB grafts[13]. Although CB is easily available and has been shown to contain a large number of HSCs, reconstitution of hematopoiesis after intensive chemotherapy and grafting of CB cells in older children and even more in adult is slower compared to the use of PB or BM grafts[14]. This prolong period of aplasia results in increased risk for infections and bleeding in patients. The limited potential for rapid hematological recovery after

transplantation of CB cells is probably due to a limited number of HSC contained in one cord blood harvest or number of HSC transplanted per kg/w, and may be abrogated by *ex vivo* expansion of the required numbers of stem and progenitor cells.

Research Questions

Which culture condition will be the best to expand and/or maintain *human cord blood hematopoietic stem / progenitor cells?*

Objectives

To find the best culture condition to expand and/or maintain human cord blood hematopoietic stem / progenitor cells.

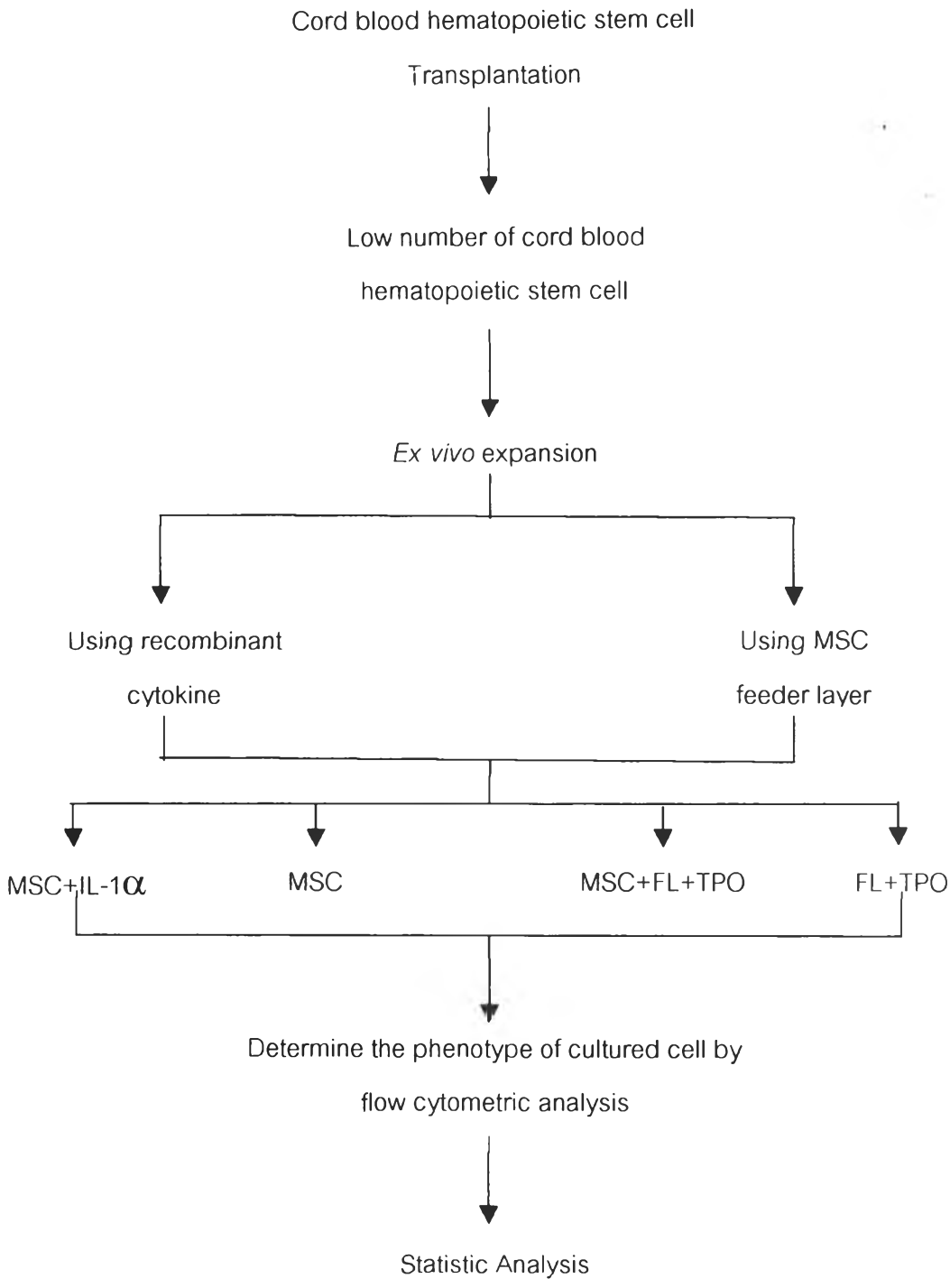
Hypothesis

MSC-base condition could exhance the ability to expand and/or *maintain human cord blood hematopoietic stem/progenitor cells in ex vivo* culture.

Assumption

The subjects in this study was cord blood collected immediately after delivery at the end of full-term preagnancies of healthy mother at Ramathibodi hospital, Bangkok. Informed consent of the mother was obtained.

Conceptual framework



Operational Definition

A plus or minus symbol use to identification the expression of the surface molecules. For example, CD34+CD38- cells are cells which have CD34 molecule but not CD38 molecule on their surface. Furthermore a "dim" or "bright" are using to identify the degree of low or high expressing surface molecule, respectively.

Expected Benefit

The result from this study may uses to be the basic knowleage for further studies about CB HSCs transplantation.

Research Methodology

1. Sample Collection : Heparinized human BM was obtained by aspiration from the posterior iliac crest of hematologically normal donors who had given informed consent. UCB was collected immediately after delivery at the end of full-term pregnancies in a sterile blood bag containing the anticoagulant CPDA-1.
2. MSCs culturing
3. Process of study
    a. CB Mononuclear cells isolation
    b. CB $CD133^+$ cell enrichment
    c. *Ex vivo* culture of enriched CB $CD133^+$ cell
    d. Flow cytometry analysis
    e. Morphology analysis
4. Data collection and analysis