CORD BLOOD STEM CELLS: ROLE IN PEDIATRIC TUMORS AND POSSIBLE METHODS OF EX VIVO EXPANSION

A Thesis

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By

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ABSTRACT

Introduction: Expanding stem cell precursors using different culture media and supporting endothelial cells has been shown in previous studies to result in grafts that are capable of hematopoiesis in myeloablated host. The use of xenogenic endothelium or cell lines is not applicable to humans without concern. **Patients and methods:** We investigated the feasibility of ex vivo expansion of cord blood stem cells by co-culturing with autologus HUVEC or placenta in the presence of 3 or 5 growth factor cytokine cocktails. We compared the fold increase in expansion to the control arm of the study; the cord stem cells in liquid cultures with added cytokine cocktail. HPC, CD34+ cells, placenta and HUVEC were all isolated from the same umbilical cord. **Results:** Total cells, CD34 and CFU were significantly increased when cultured with the HUVEC or placenta compared to the liquid cultures. In the liquid cultures, five growth factor cytokine cocktails lead to an expansion that was significantly more than that observed using three cytokines only but less than the co culture arms. **Conclusion:** The use of autologus derived HUVEC or placenta provides an exciting and potentially clinically applicable approach to expand the umbilical cord stem

cells for multiple uses in both the pediatric and adult patient, superior to the cytokine supplemented liquid cultures.

Key Words: CD34; cord blood; hematopoietic stem cells; placental co cultures; HUVEC; ex vivo expansion

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LIST OF ABBREVIATIONS

aGVHD	**Acute Graft Versus Host Disease**
ALL	**Acute Lymphoblastic leukemia**
AML	Acute Myeloid leukemia
BMT	**Bone Marrow Transplant**
CB	**Cord Blood**
CBB	**Cord Blood Bank**
cGVHD	**Chronic Graft Versus Host Disease**
CI	**Cumulative Incidence**
CR	**Complete remission**
CMl	**Chronic Myeloid Leukemia**
DMSO	Dimethyl sulphoxide
EBMT	**European Bone Marrow Transplant**
FL	**FLT-3 Ligand**
G-CSF	**Granulocyte Colony Stimulating factor**
GM-CSF	**Granulocyte-MacrophageColony Stimulating factor**
GVHD	**Graft versus Host Disease**
GVL	**Graft Versus leukemia**
HLA	**Human leukocyte antigen**
HPC	**Hematopoeitic progenitor cells**
HSCT	**Hematopoeitic Stem Cell Transplant**
HUCBC	**Human Umbilical Cord Blood Cells**
IBMTR	**International Bone Marrow Transplant Registry**

IL-1	**Interleukin-1**
IL-3	**Interleukin-3**
IL-6	**Interleukin-6**
JCML	**Juvenille Chronic Myeloid Leukemia**
LFS	**Leukemia free survival**
LTC-IC	**Long term culture initiating cells**
LTRC	**Long Term Repopulating Cells**
MGDF	**Megakaryocyte growth and differentiation factor.**
MNC	**Mononuclear cells**
MSC	**Mesenchymal Stem cells**
NK-cells	**Natural Killer cells**
NMA	**Non-myeloablative**
NOD	**Non obese diabetic**
PBSC	**Peripheral Blood Stem Cell**
PTEN	**Phosphatase and tensin homolog**
SCF	**Stem Cell Factor**
SCID	**Severe Combined Immunodeficiency**
Th-1	**T-helper cells 1**
TNC	**Total Nucleated Cells**
TPO	**Thrombopoeitin**
TRM	**Transplant Related Mortality**
UCBT	**Umbilical Cord Blood Transplant**
URD	**Unrelated Donor**
CFU-E	**Colony forming unit- erythroid**
BFU-E	**Burst forming unit-erythroid**

CFU-GM	**Colony forming unit-granulocyte, macrophage**
CFU-GEMM	**Colony forming unit-granulocyte, erythroid, macrophage, megakaryocyte**

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INTRODUCTION

AND

AIM OF THE WORK

Umbilical cord blood is a promising source of hematopoiteic stem cells (HSC) for allogenic transplantation. An increasing number of patients are alive today because they were fortunate enough to be beneficiaries of this relatively new clinical treatment; cord blood transplantation. ***Knudtzon, 1974*** was the first to demonstrate the presence of hematopoietic progenitor cells in umbilical cord blood (UCB). It was not until later in October 1988, when a child with Fanconi anemia was the recipient of an HLA-matched cord blood transplant from his newborn sister. The recipient is alive, well, and cured of the hematological manifestation of Fanconi anemia; and is thus the longest survivor of cord blood transplantation ***(Gluckman et al., 1989)***.

Since that first transplant, cord blood stem cell transplants have been successfully performed on patients, mostly children with acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), juvenile chronic myelogenous leukemia (JCML), chronic myelogenous leukemia (CML), neuroblastoma,

non-Hodgkin's lymphoma, severe aplastic anemia, gaucher's disease, Hunter syndrome, Hurler syndrome, thalassemia, Wiskott-Aldrich syndrome, and x-linked lymphoproliferative

Briefly, Hurler syndrome is a severe form of Mucopolysaccaridoses (MPS) type I, while Hunter syndrome is type II MPS. While Wiskott-Aldrich syndrome is an x-linked congenital disorder characterized by thrombocytopenia, infections, malignancy among other manifestations, for which the only curative treatment is bone marrow transplantation.

In 1992, in an attempt to more quickly discern the true risks and benefits of this novel stem cell source, the international cord blood registry was established as a repository of clinical data on the outcomes observed in patients who received umbilical cord blood.

In 1995, ***Eurocord*** was developed, serving as both a registry and a forum for the development of cooperative group studies within the European community.

The objectives of the USA International Cord Blood Institute and Eurocord, is to determine the rate of durable engraftment, acute and chronic GVHD, relapse, and survival after the transplantations in patients with malignant, and non-malignant disorders.