APPLICATION OF FLOW CYTOMETRY IN BONE MARROW TRANSPLANTATION

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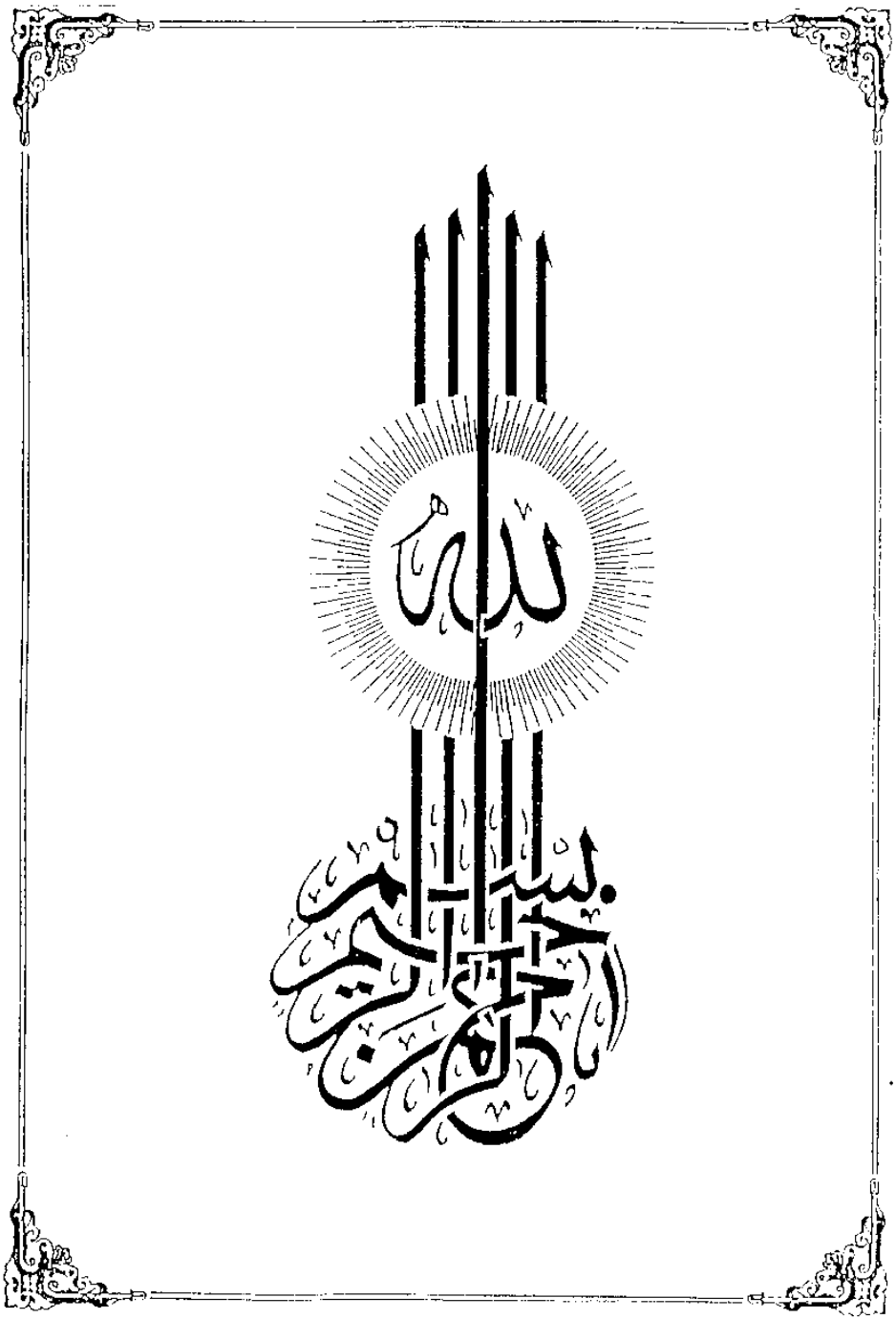

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ABSTRACT

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The main purpose of this essay is to evaluate the role of flow cytometry in the field of bone marrow transplant. The FCM's use in all the stages of BMT is evaluated and its role as a research tool in animal studies is also mentioned.

CONTENTS

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LIST OF ABBREVIATIONS

ABMT	Autologous bone marrow transplantation.
ALL	Acute lymphoblastic leukemia.
AML	Acute myelogenous leukemia.
ANLL	Acute non-lymphoblastic leukemia.
Ara-C	Cytosine arabinoside.
BCP	B-cells precursors.
BEAM	High-dose BCNV,Etoposide,aracytine,and Melphalan.
BM	Bone marrow.
BMT	Bone marrow transplantation.
Brd Vrd	Bromodeoxyuridine.
CCRF-CEM	Human leukemic lymphoblastosis.
CDCA	Complement dependent cytotoxicity assay.
CEM/VLB	Vinblastine resistant.
CML	Chronic myelogenous leukemia.
CMV	Cytomegalovirus.
CSA	Cyclosprine A.
EBV	Epstein-Barr virus.
EC	Endothelial cells.
EPICS	Electronically programmable individual cell sorte.
FACS	Fluorescence-activated cell sorting.
FCM	Flow cytometry.
FITC	Fluorescein isothiocyanate.
FS	Forward scatter.
G-CSF	Granulocyte colony-stimulating factor.
GVHD	Graft versus host disease.
GVL	Graft versus leukaemia.
HLA	Human leucocytic antigen.
HSC	Hematopoietic stem cell.
HSCs	Hematopoietic stem cells.
ICAM1	Intracellular adhesion molecule 1.
IF	Immunofluorescence.

IL-2'aTLs	IL-2 activated T lymphocytes.
K 562	Human leukemic erythroblasts.
K 562/Dox	Doxorubicin resistant.
LFA1	Lymphocyte function associated antigen 1.
LPC	Leukaemic progenitor cells.
M-CSF	Macrophage colony-stimulating factors.
MDR	Multi-drug resistant.
MLC	Mixed lymphocyte culture.
MNC	Mononuclear cells.
MoAb	Monoclonal antibodies.
MoAb +C'	Monoclonal antibodies + complement.
MRD	Minimal residual disease.
NAb	Natural antibodies.
NHL	Non, Hodgkin's lymphoma.
NK-cells	Natural killer cells.
PBMC	Peripheral blood mononuclear cells.
PBSC	Peripheral blood stem cells.
PBSCT	Peripheral blood stem cell transplantation.
PCR	Polymerase chain reaction.
PI	Propidium iodide.
PMTs	Photo multiplier tubes.
RI	Index of resistance.
RMI	Reticulocyte maturity index.
SCID	Severe combined immune deficiency.
TBI	Total body irradiation.
TCD	T-cell depleted.
TCR	T-cell receptor.
TdT	Terminal deoxynucleotidyl transferase.
VNTR	Variable number of tandem repeats.
VOD	Veno-occlusive disease.
WBI	Whole body irradiation.
X-RITC	Rhodamine Iodo-tetra thiocyanate.

INTRODUCTION

and aim of work

INTRODUCTION

Bone marrow transplantation (BMT) is the treatment of choice for children and adults with haematological malignancies, severe aplastic anemia and some hereditary diseases (**Johnson and Pochedly, 1990**).

In the field of BMT, flow cytometry (FCM) has many clinical and research applications, including evaluation of the donor's bone marrow (BM) before transplant, and recipient's peripheral blood and BM after transplant. Pretransplant, FCM is used to evaluate donor-recipient compatibility by HLA typing, BMT cross match and mixed lymphocyte culture (**Garovoy et al., 1983**). Moreover, flow cytometric serological HLA-A, B, C, DR and DQ typing, using recombinant IL-2 activated T-lymphocytes, is applicable in pediatric candidates for allogenic BMT (**Kubo et al., 1991**).

Depletion of T-cells in donor's BM, which might cause graft versus host disease (GVHD) in the recipient, can also be monitored for FCM (**Hiruma et al., 1992**). Moreover, quantitative minimal residual disease (MRD) assays are used to analyze remission BM samples for MRD detection and evaluation of the efficacy of ex-vivo BM purging (**Aihara et al., 1991; Uckun et al., 1992b**). Meanwhile, fluorescence-activated cell sorting (FACS) has been applied to searching, enrichment and isolation of haematopoietic stem cells for pure stem cell transplantation (**Baines et al., 1988; Siena et al., 1991**).

FCM can be used to enumerate reticulocytes, and to provide a reticulocyte maturity index (RMI) (**Davis and Bigelow,1989a**). The RMI, independent of mere reticulocyte

enumeration, is the earliest indicator of marrow engraftment and is used in monitoring BM engraftment post-transplant. The RMI can identify 3 patterns of BM engraftment (early, delayed and failed) (**Davis et al., 1989b; Davis and Bigelow, 1989b, 1991**).

FCM is also applied to study the developmental hematopoietic hierarchy during early engraftment of autologous (**Uckun et al., 1992a**) versus allogeneic (**Leino et al., 1991**) BMT. The effect of cytokines administration (after autologous BMT) (**Nemunaitis et al., 1991**) and donor-type natural killer cell infusion (after allogeneic BMT) (**Murphy et al.,1992**)on the augmentation of haematopoietic engraftment and immune reconstitution, can be monitored by FCM.

Finally, early after allogeneic BMT, the detection of mixed chimerism can be done by a simple flow cytometry method, using labeled monoclonal antibodies against specific MHC antigens (**Leenaerts et al., 1990**). Moreover, the polymerase chain reaction (PCR) and other in situ hybridization techniques can detect mixed chimerism in flow sorted cell populations. Also, FCM is used in the study of post-transplant immune dysfunction following T-cell depleted and non-depleted allografts (**Duncombe et al., 1992**), in monitoring the development of GVHD, and in the early detection of mechanisms of graft failure. Meanwhile, flow cytometric animal studies on antigen-specific tolerance across of xenogeneic barrier are currently done (**Ildstad et al., 1991**).

AIM OF THE WORK:

The aim of this work is to review the applications of flow cytometric studies in the field of BMT, evaluating their diagnostic, prognostic and therapeutic utility.