TRANSCHYMAL-RM WITH CLINIMACS® SYSTEM FOR PRACTISING HEMATO-ONCOLOGISTS IN INDIA

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Technical Bulletin

Leukemias are blood cancers that begin in cells that normally as part of regular cell division develop into different types of blood cells. Leukemia usually starts in early forms of white blood cells, but some types start in other blood cell types as well. The classification of leukemia is based on whether the condition is acute and fast growing or chronic and slower growing; whether started in myeloid cells or lymphoid cells. Acute myeloid leukemia (AML) starts in the bone marrow and often moves into the circulating blood. It can spread to other parts of the system like lymph nodes, liver, spleen, central nervous system and testicles. AML nomenclature in medical literature is like: acute myelocytic leukemia, acute non-lymphocytic leukemia acute myelogenous leukemia, and acute granulocytic leukemia. The key treatment given for most types of AML is chemotherapy, sometimes along with a targeted therapy drug, followed by a stem cell transplant. Surgery and radiation therapy are not major options for AML.

Hematopoietic stem cells can be harvested either from aspirated bone marrow or mobilized peripheral blood (PB). Between bone marrow and mobilized peripheral blood, peripheral blood stem cell (PBSC) grafts contain more T cells and hematopoietic progenitor cells (CD34+) cells.

Fundamentally, a T cell is a type of lymphocyte that develops in the thymus gland and playing a central role in the human immune response causing graft rejection. T cells are different from other lymphocytes by the presence of a T-cell receptor on the cell surface and so can be segregated.

Hematopoietic stem cell transplantation (HCT) is the standard care and cure option for Acute Myeloid Leukemia (AML). Conversely, Graft-Versus-Host Disease (GVHD) after HCT is the devil event, leading to morbidity and mortality. It is medically a known fact mediations (either invivo or exvivo) that reduce the number of donor T cells in the graft will shrink the risk of GVHD. Ex vivo T-cell depletion (TCD) manual, semi-manual techniques, procedures followed are

heterogeneous with inconsistent degrees of depletion shown. These discrepancies have restricted the clinical practise for T-cell–depleted HCT addressing hematological malignancies till recent with associated severe GVHD.

The availability of clinical-grade magnetic bead columns for cell separation has made it efficient to separate and fractionate certain populations of cells (wanted and unwanted) with accuracy. Miltenyi's CliniMACS® System is based on magnetic cell sorting technology that allows the operator to perform clinical-scale magnetic enrichment of target cells, or depletion of unwanted cells, in a closed and sterile system. A phase II trial of HLA-identical sibling HCT with CD34-selected T-cell–depleted grafts in patients with early AML conducted by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), USA demonstrated that CliniMACS® mediated T-cell depletion technique provided steady 4- to 5-log reduction in T-cell content in all participating centers.

Here, we report our study related acquired sample (mobilized PB) specific input data before and after using CliniMACS® in India from 10 different hospitals in India performing haplo-identical HCT graft; Recorded log reduction values in T-cell content.

The objective in recording the specific line items in the table is to establish the standardized use of CliniMACS® in India by our group that offers the technology/service to practitioners while highlighting T cell depletion and CD34+ cell enrichment in the same process. The analyzed data gives clues on volumes (min-max) that can be collected, range of percentage of cell type population labelled, fractionated, log reduction values. The log reduction value is a measure of how thoroughly the process reduces the concentration of T cells in the process. It is defined as the common logarithm of the ratio of the levels of contamination before and after the process, so an increment of 1 corresponds to a reduction in concentration by a factor of 10.

We had access to CliniMACS® processed 37 cases between 2015 and 2018. The table below captured peripheral blood volume, apheresis data points, labelled cells population percentage, T cell, CD3+ cells percentage:

Tabel-1

PBSC (Apheresis)																				
S.No	Donor Weight (kg)	Vol (mL)	WBC conc/ul	Total WBC x10^9	TCR α/β				TCR g/D				B cells				CD34		NK cells
					/ul	Total TCR α/b	Total TCR α/β (10^9)	%	/ul	Total TCR g/d	Total TCR g/d (10^9)	%	/uL	Total B cells	Total B cells (10^9)	%	/ul	%	
Mean	72.03	267	247662	54.77	37132	8767253541	8.77	16.43	1590	381052568	0.38	0.70	12229.46	3329855541	3.33	5.93	2653	1.24	0
SD	13.99	132	108525	17.65	35481	6361993990	6.362	12.63	2328	463545260	0.46	0.83	11960.85	3478130721	3.48	5.92	1858	1.08	0

Original labelled and before depleting T cells												
Vol (mL)	WBC conc /ul	Total WBC x10^9	TCR α/β		TCR g/D				B cells (CD19)		CD34		NK cells
			/ul	%	/ul	TCRg/d (10^9)	TCR g/d (10^9)	%	/uL	%	/ul	%	
153.9	308797.3	48	113805.3784	38.11	1984.89	313143297	0.31	0.63	11696.9	3.49	3267	0.98	5406.7
24.45	98419.023	17	399864.0539	140.96	2515.56	399563855	0.40	0.80	9651.72	2.55	2482	0.70	8398.1

Final target cells (depleted) obtained from CliniMACS® ready to apply clinically															
Vol (mL)	WBC conc /ul	Total WBC x10^9	TCR α/β			TCR g/D				B Cells			CD34		NK cells
			/ul	Total TCR ab	Total TCR ab (10^9)	%	/ul	Total TCR g/d	Total TCR g/d (10^9)	%	/uL	Total B cells	Total B (10^9)	%	/ul	%	
402.6	96043	38.2	9.22	3358290	0.03	0.02	635.49	262203837.8	0.26	0.98	7.4	2980759	0.00	0.01	1352	1.91	
61.61	119373.1	44.8	38.4	13463398.36	0.13	0.07	725.64	309338060	0.31	1.39	5.5	2136496	0.00	0.01	791.3	1.43	

Log Reduction		TCR g/D Recovery %	CD 34 Recovery %
TCRa/b	CD19		
-4.54	-3.2	71.99	71.37
1.16	0.9	81.56	37.96

WBC: WBC or White blood cells or leukocytes are the cells of the immune system involved in protecting the body against both infectious disease and foreign particles. All white blood cells are produced and derived from stem cells in the bone marrow known as hematopoietic stem cells.

TCR α/β, TCR g/D:

The T-cell receptor (TCR) is a molecule found on the surface of T cells or T lymphocytes that is responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules. The TCR is composed of two different protein chains (heterodimer). In humans in 95% of T cells, the TCR consists of an alpha (α) chain and a beta (β) chain, whereas in 5% of T cells the TCR consists of gamma and delta (γ/δ) chains. This ratio changes during ontogeny and in diseased states (eg: Leukemia).

B cells: B cells or B lymphocytes are a type of white blood cell - small lymphocyte subtype. They work in the humoral immunity component of the adaptive immune system by secreting antibodies.

CD34+ cells: Hematopoietic stem cells (HSC) are multipotential, can self-renew and produce mature blood cells, such as erythrocytes, leukocytes, platelets, and lymphocytes in the healthy human body. CD34 is a marker of human HSC, and

all colony-forming activity of human bone marrow (BM) cells is originated from the CD34+ fraction.

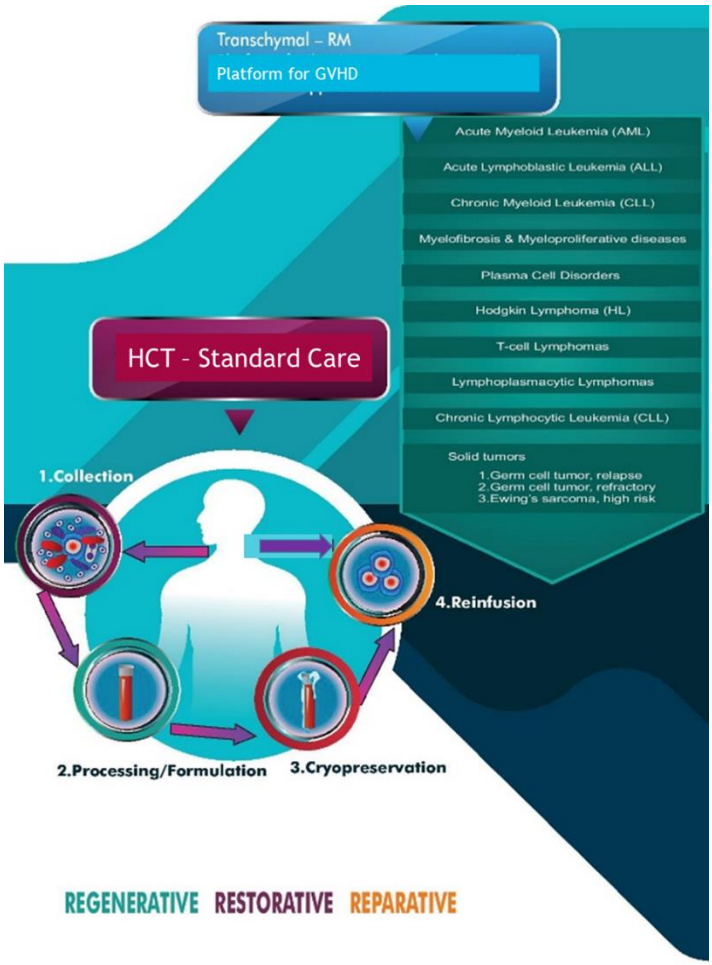
NK cells: Natural killer cells or NK cells are a type of cytotoxic lymphocyte crucial to the innate immune system. Immune cells detect the major histocompatibility complex (MHC) presented on infected cell surfaces, triggering cell death.

A detailed data analysis documents a mean volume of 266.81 ml while stressing on the difficulty with maintaining uniformity in the initial volume of peripheral blood collection. The varying log reduction values with T cells of the exvivo CliniMACS® produce (-2.3994 to -6.4090) are the result of varying donor related parameters (including initial volume of the sample collected) assuming the limitations with machines (Apheresis and CliniMACS®). A mean of 71.365% of CD34 recovery in the final produce ready to apply clinically has been correlated to successful allogenic HCT from this sample size.

Introducing TRANSCHYMAL-RM for planned HCT with log reduction values not in the range of 4 to 5.

TRANSCHYMAL-RM is a platform technology of formulating stem cells before clinical application to reduce GVHD. Best suited for Stem cells harvested/processed in closed systems. Formulation does not involve manipulation, culture in the labs. Can be integrated as a clinical tool in Point Of Care/Standard Care offered to hematological malignancies to reduce GVHD with no side effects.

Bone Marrow Transplantations:	Immunotherapies given as standard care:	Peripheral blood Stem Cell Transplantations :
Diamond-Blackfan anemia Essential thrombocytosis Ewing sarcoma Fanconi anemia Germ cell ovarian cancer Hemophagocytic lymphohistiocytosis Juvenile myelomonocytic leukemia Medulloblastoma Myelofibrosis Neuroblastoma Polycythemia vera Testicular cancer Thalassemia Other rare diseases	Adoptive Immunotherapy: Chimeric Antigen Receptor (CAR) T-cell therapy gives patients large amounts of T cells that are all genetically engineered to find and fight the cancer Tumor infiltrating lymphocyte (TIL) therapy uses a patient's T cells that are collected from a piece of surgically-removed tumor While these cells may recognize the cancer, there are too few of them to succeed. The number of these cells is increased substantially in the lab and then given back to the patient Endogenous T-cell (ETC) therapy uses T cells from a patient's blood. From this diverse pool of T cells, only those that may recognize signatures specific to the cancer are selected. The number of these specific T cells is increased substantially and then given back to the patient	Leukemia, Lymphoma, Myeloma



TRANSCHYMAL-RM can be applied to the blood related disorders where HCT is offered as Standard Care