Role of Transfusion Medicine Specialist in Pediatric HSCT

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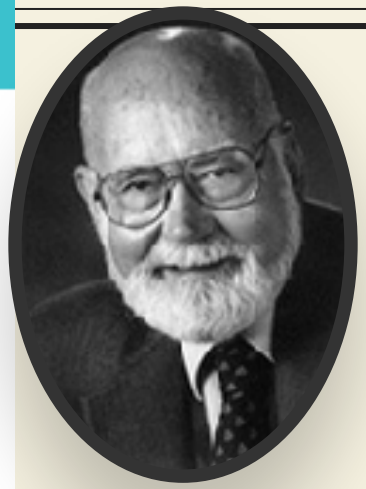
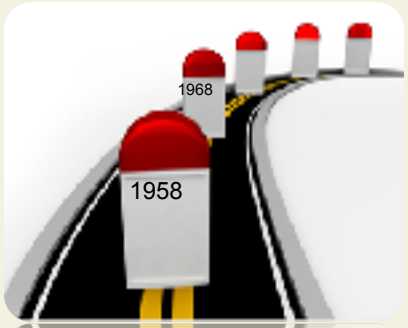
1956: THE FIRST SUCCESSFUL BONE MARROW TRANSPLANTATION

In 1956, the first successful bone marrow transplant was performed by Dr E. Donnall Thomas in Cooperstown, New York. This milestone involved identical twins, with bone marrow taken from the healthy twin, and given to the other, who had leukaemia.

This ground-breaking treatment paved the way for a life-saving therapy that is now standard for patients with blood cell disorders, such as leukaemia, sickle cell anaemia and inherited immune system disorders.



Over 50 years have passed since Dr. E Donall Thomas in Cooperstown, New York made headlines by performing the first hematopoietic stem-cell transplantation (HSCT). This milestone involved identical twins, with bone marrow from the healthy twin, and given to the other, who had leukemia.



1958: French immunologist Dr Jean Dausset identifies human leukocyte antigens (HLA).

1968: First bone marrow transplant for non-cancer treatment. US immunologist Dr Robert Good uses a bone marrow transplant to treat an 8-year-old boy with severe combined immunodeficiency syndrome (SCID). The donor is an HLA-matched sister.

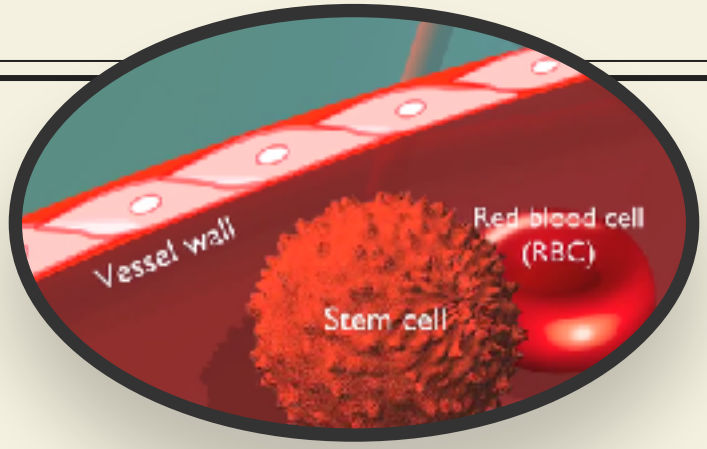
1970s: Development of apheresis by IBM engineer George Judson invented the technology & used leukapheresis techniques treat his son requiring repeated infusion of granulocytes

Other important milestones were:

- Discovery of hematopoeitic GM colony stimulating growth factor by Dr. Donal Metcalf from Australia.
- Dr. Curt Cavin discovered the CD 34 antigen present on population of MNCs

TRANSFUSION MEDICINE & CELLUAR THERAPY

–“Hematopoeitic Stem Cell Transplantation”



Integration of Cellular Therapy into Transfusion Medicine

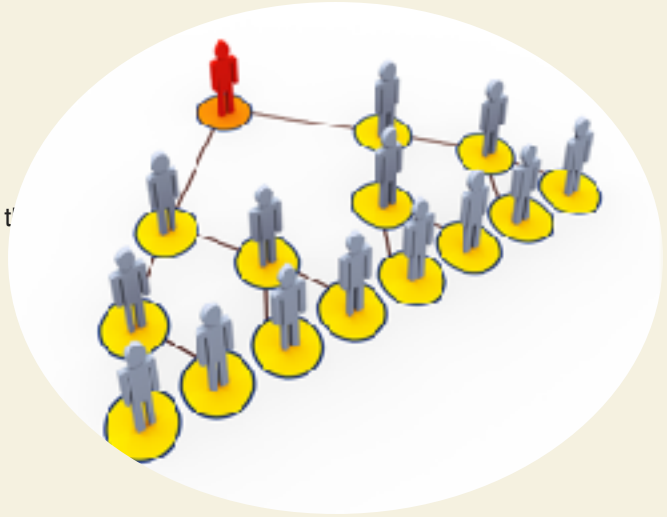
Transfusion Medicine Practitioners have grown from being mere observers in the field of cellular therapy CT to becoming active participants and leaders.

TM nee blood banking had always been focused on end stage blood cells i.e. RBCs, platelets & granulocytes has expanded horizon to include dividing cells with potential for regeneration – Stem cells.

Blood banks and transfusion services are well versed in processes & regulations governing

- Collection
- Processing
- Labeling
- Storage
- Quality control of blood components.

Hematopoietic stem cells are the ultimate blood component, and it stands to reason t
role in functioning of any HSCT transplant program.



“A well-organized transfusion service is a 'conditio sine qua non' of a well-structured successful transplant program”

WELL- ORGANIZED TRANSFUSION SERVICE

- HLA testing lab
- Facility for CD34 stem cell enumeration :
- Collection Facility including an Apheresis Unit: PBSC & TPE
- Stem cell processing & storage facility
- Transfusion Service: 24 hour availability blood/blood components
- Blood Irradiation Facility
- Extracorporeal photopheresis (desirable)

Stem cells

Source & Indications

HSCT:

- Pre-transplantation phase: pre-procedure workup
- Collection phase:
    - Harvest procedure
    - Evaluation and Quality control
    - Product processing
    - Storage with or without Cryopreservation

Transplant Phase

    - Thawing and Infusion

Unrelated donor registry

Special considerations in pediatric patients/donors

Our data

Future

STEM CELLS

Population of undifferentiated cells which are able

- to divide for indefinite period
- to self renew
- to generate a functional progeny of highly specialized cells

STEM CELL SOURCES

- Bone Marrow
- Blood
- Umbilical Cord
- Fetal Liver

POTENTIAL STEM CELL SOURCES

- **Autologous stem cells:** Patients receive their own stem cells.
- **Syngeneic Donor :** Patients receive stem cells from their identical twin.
- **Allogeneic HSCT:** Patients receive stem cells from someone other than the patient or an identical twin.
    - HLA-matched related donors
    - HLA-matched unrelated donors (MUD)
    - Haploidentical related donors
- **Umbilical cord blood**

INDICATIONS

- Hematological diseases
  - Benign : Thalassemia, Aplastic Anemia etc
  - Malignant : Leukemia Lymphoma etc
- Myeloma
- Immune deficiency disorders
  - Pediatric and Adult
- Neurological Disease (eg: MS)
- Autoimmune diseases
- Inborn errors of metabolism

GOAL

- Restore hematopoiesis in marrow failure states
- Replace a diseased marrow by a healthy
- donor marrow
- As a “rescue” to reconstitute hematopoiesis following marrow ablative chemo-radiotherapy
- As a mean for treating genetic disorders

PRE-TRANSPLANT PHASE

PRINCIPLES OF ALLOGENEIC HSCT

MATCHED DONOR TRANSPLANT:

Related Donor

- One Sibling: 25 % chance of full match
- Two Siblings: 44 % chance of full match
- Three siblings: 58% chance of full match

Unrelated Donor

- In case HLA matched donor is not found in the family then a donor can be searched from different donor registries.

The chance to find donors may be better for more homogenous racial groups.

HAPLOIDENTICAL HSCT

Allogeneic HSCT using one HLA haplotype matched first-degree relative donor (haploidentical donor) is alternative treatment for patients with hematologic malignancies/diseases who lack HLA-matched related or unrelated donor.

HAPLOIDENTICAL HSCT

Main limitations of this treatment were high rate of graft failure (GF) and graft-versus-host disease (GVHD), due to intense alloreactive reactions to the major HLA mismatch between recipient and the donor.

Methods to tackle graft failure: use of “megadoses” of hematopoietic stem cells (over 10 million CD34+ cells/kg with a very low T cell content) (1 $\times 10^4$ CD3+ cells/kg)

HAPOIDENTICAL HSCT

Despite using T cell low mega dose approx 10–20% still developed Graft Failure

- Graft rejection following haplo SCT is generally attributed to cytolytic host versus- graft reaction mediated by host T and/or NK-cells surviving conditioning regimen.
- Preformed donor-specific anti- HLA antibodies (DSAs) present at the time of transplant have been reported in 24% of HSCT recipients

ROLE IN PRETRANPLANT PHASE

- HLA typing of recipient and presumptive donors
- Performing plasma exchange procedures in recipients with
  - Donor Specific Antibodies (DSA) in haploidentical HSCT
  - ABO mismatch bone marrow transplants with high isohemagglutinin titres

COLLECTION PHASE (PROCEDURE)

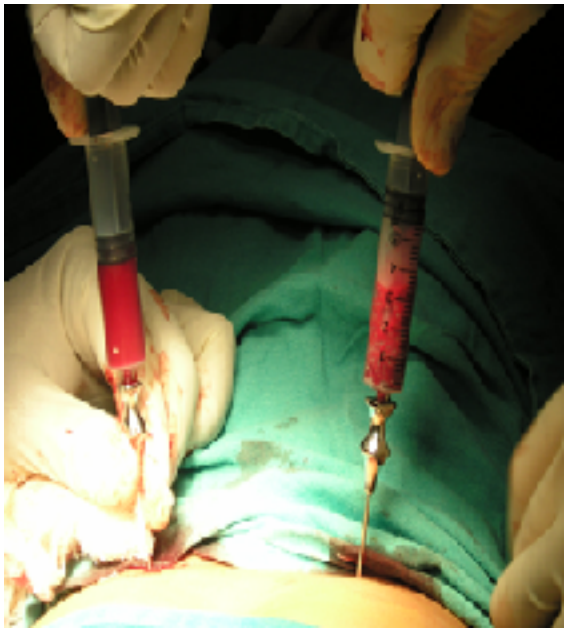
PROCEDURE

Most blood stem cells reside in the bone marrow and a small number are present in the bloodstream

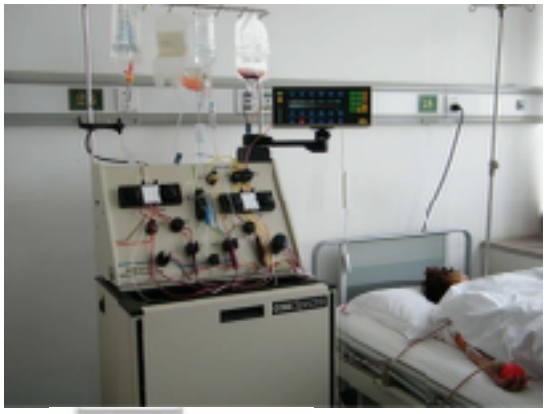
- Harvested from Bone Marrow- With or without G-CSF
- Stem Cells in the blood can be increased and subsequently harvested after G-CSF mobilization

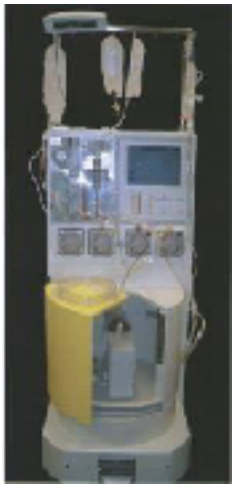
PBSCs are easier to collect than bone marrow stem cells

BONE MARROW HARVESTING



BONE MARROW COLLECTION





APHERESIS MACHINE

ABO BLOOD GROUP ASSOCIATION

- ABO blood group system has independent inheritance from human leukocyte antigen (HLA)
- ABO incompatibility occurs in approximately 25– 50 % HLA matched allogeneic HSCT
- Though ABO and Rh compatibility are not required for the successful outcome of BMT
- Complications due to ABO mismatch:
- Antibodies and antigens present in the graft and recipient blood, vascular & lymphatic endothelium, perivascular connective tissue, bile duct epithelium

COMPLICATION DUE TO ABO MISMATCH

Incompatibility	Consequence	Cause
ABO major	Acute hemolysis	Infusion of incompatible red cells
	Delayed granulocyte and platelet engraftments	• Loss of hematopoeitic cells during processing to remove red cells. • Expression of ABO antigens on granulocytes and platelets
	Delayed red cell engraftment	Host anti-donor isohemagglutinin
	Pure red cell aplasia	Persistence of anti-donor isohaemgglutinin
ABO minor	Acute hemolysis	Donor plasma with high isohaemgglutinin titers
	Delayed hemolytic reaction	Passenger lymphocytes producing anti-host isohemagglutinin

RISK AMERIOLATION

Risk of intravascular hemolysis

- Lower in peripheral blood stem cells (PBSC). Vol. of red cells is approx.10 to 40 ml.
- More in bone marrow. Vol. of red cells is >100 ml
- Maximum 40 ml

Risk Amelioration

- Reduction of isohemagglutinin in the recipient: by Plasmapheresis or transfusion of donor specific plasma
- Minimizing red cell contamination: Red cell depletion of allograft

ERYTHROCYTE DEPLETION AND/OR VOLUME REDUCTION

Methods

1. Use of continuous flow cell-separator such as Cobe Spectra/ Sepax Biosafe

- Final red cell volume < 10 ml/kg reduction 80- 90%
- CD34+ recovery around 80-90%
- Costly

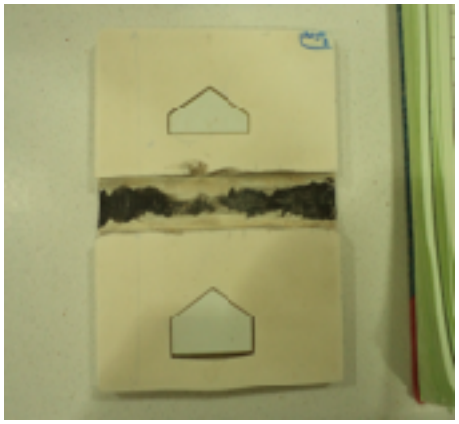
2. Sedimentation Techniques : Using HES /Percoll /Ficoll :

- RBCs reduction 70-80%
- CD34+ recovery around 50-80%.
- Cheap

ERYTHROCYTE DEPLETION AND/OR VOLUME REDUCTION- OUR CENTRE

3. Centrifugation: Using automated component extractor, marrow harvest is separated into plasma, buffy coat and red cells

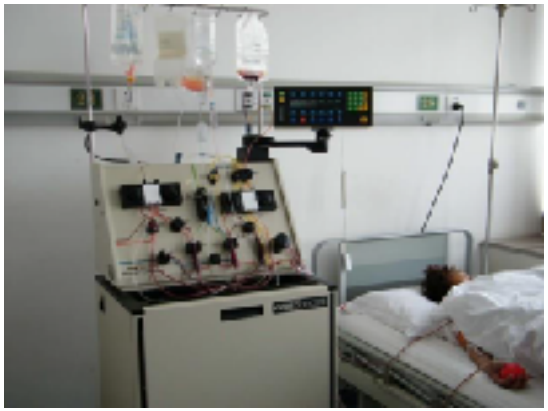
- Final red cell volume 85-90%
- CD34+ recovery around 80-90%
- Cheap

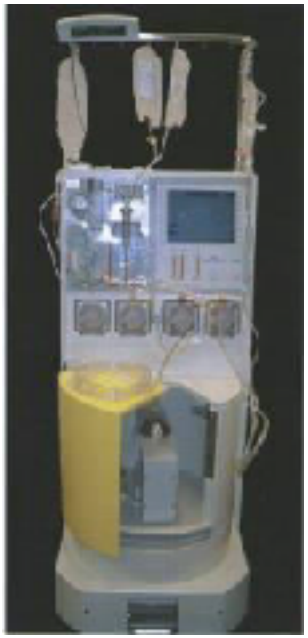


Modified Backplate

Red Cell Reduction of Bone Marrow using Optipress II

PBSC





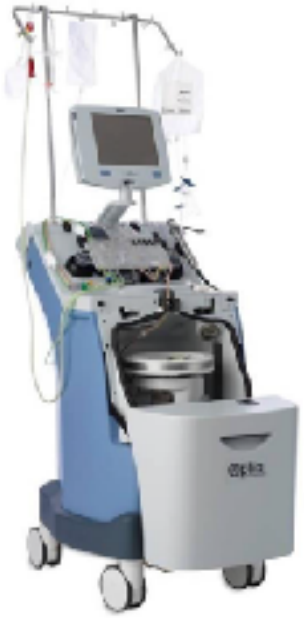


Table 33-3. Instrument Settings for Peripheral Blood Progenitor Cell Collections

Instrument	RBC Content (%)	Flow Rate (mL/min)	Anticoagulant to Whole Blood Ratio	Cycle Volume (mL)	Number of Cycles
Spectra	1 to 3	60 to 150	1:12 to 1:15	Continuous	N/A
Amicus	6 to 8	40 to 75	1:12 to 1:15	1000 to 1400	7 to 14
COM.TEC	6 to 8	40 to 60	1:10 to 1:14	300 to 500	25 to 40

PBSC

- Large volume leukapheresis using cell separators
- G-CSF Mobilized stem cells from the peripheral blood.
- 3-5 times the patient/donor's blood volume is processed
- Apheresis is generally continued till the following doses (per Kg body weight of the patient) are collected.
  - Autologous: > 2 x 10^6/kg (more dose required if cryopreserved)
  - Matched HSCT >2 x 10^6/kg
  - Haploidentical HSCT >8-10 x 10^6/kg

VENOUS ACCESS

- Peripheral access: Anticubital vein for 16-18 gauge needle for draw and at least 22 gauge for return. The eligible donor must have prominent veins on both the arms.
- Autologous: Specially designed large-bore, double-lumen catheters are used.

COMPLICATIONS OF G-CSF & APHERESIS

G-CSF

- G-CSF induced bone pain – 80%
- Allergy/ anaphylaxis to g-GCSF (1 in 300)
- Low platelet count, anemia
- Excessive white cell drive – possibility of splenic rupture (?1 in 4000)
- Cardiovascular events due to plaque inflammation

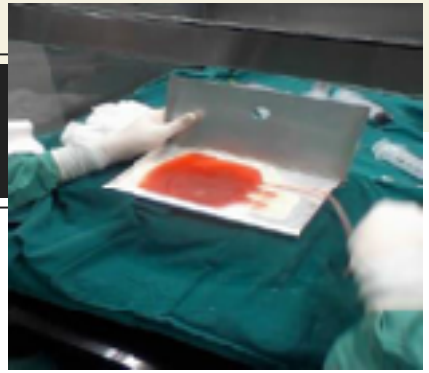
Procedure associated complications

- Access site bruising/pain
- Induced vasovagal complications
- Shifts in blood volume with resultant cardiovascular changes
- Hypocalcaemia due to citrate anticoagulant
- Iatrogenic hypercalcemia due to supplementation
- Most serious adverse events during apheresis are related to the use of venous catheters. This includes thrombosis, infection, bleeding or pneumothorax

CRYOPRESRVATION

- It is a process where cells are preserved for longer periods by cooling to low sub-zero temperatures
- Cryoprotectant like DMSO are added. DMSO prevents ice crystal formation, prevents formation of toxic solute concentrations that can result from cell dehydration and stabilizes the
- Cell membrane in order to prevent damage during thawing.

CRYOPRESRVATION



Controlled rate freezing

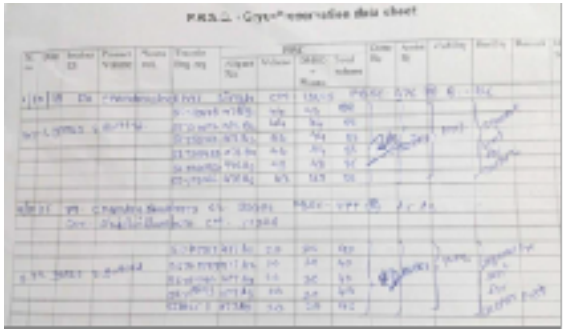
- – 1-2°C/min to -30 °C, then 2-10 °C/min to -196 °C

Uncontrolled rate freezing

- -70 to -80 °C

Mechanical freezer (<-80 °C) or in liquid nitrogen freezer(-196 °C)

All freezers and liquid nitrogen tanks containing HPC should be regularly monitored and have alarm systems



HPC PROCESSING:
POSITIVE/NEGATIVE SELECTION

Isolex300i to isolate stem cells

CliniMACS

QUALITY CONTROL OF PBSC PRODUCT

- CD34/CD3 enumeration: by flowcytometry after collection and post cryopreservation after thawing
- Viability : Trypan Blue dye exclusion test or 7AAD
- Sterility testing: Blood culture done after collection and post cryopreservation after thawing

TRANSPLANTATION PHASE

SPECIAL TRANSFUSION REQUIREMENTS

Special Blood Requirement

- Irradiated
- Leukocyte-Reduced
- Crossmatched platelets
- Granulocytes incase of antibiotic resistant life-threatening infections

BLOOD SELECTION WHEN RECIPIENT/DONOR ARE NOT ABO IDENTICAL

Patient ABO	Donor ABO	RBC	FFP 1st Choice plt	2nd Choice plt
O	A B AB	O O O	A,AB B,AB AB	B,O A,O A,B,O
A	O B AB	O O A,O	A,AB AB AB	B,O B,A,O A,B,O
B	O A AB	O O B,O	B,AB AB AB	A,O A,B,O B,A,O
AB	O A B	O A,O B,O	AB AB AB	A,B,O A,B,O A,B,O

POST-TRANSPLANTATION PHASE

REFRACTORY GVHD

Allogeneic hematopoietic stem cell transplantation acute GVHD occurs in 20–50% of patients, and chronic GVHD occurs in 30–50% of engrafted survivors.

GVHD is usually treated with steroids and immunosuppressive drugs.

Extracorporeal photopheresis (ECP) has also been proven highly effective in steroid/immunosuppressive non- responsive GVHD patients.

“Children are not little adults.”

ECV

- Before embarking on a procedure the ECV for each instrument must be clear
- ECV varies from equipment to equipment
- It may be different for different procedures in the same equipment

  Eg. ECV for leukapheresis on Cobe spectra= 285ml and 185ml for Amicus.
- ECV represents larger fraction of TBV in a child than adult, resulting greater volume shift.

TBV

- TBV varies with body considerations:
  - Greater in males than in females of same weight
  - Overestimates in obese and underestimates in muscular patients
- TBV should calculated using age based estimate of blood volume in ml/kg
- Apheresis machines use complex formulae to calculate TBV using gender, height and weight in adults.
- Significant discrepancies are noted in children when same are calculated using Nadler formula

TBV

All versions of Spectra & Com.Tec: calculations on blood volume in children are discrepant

New version of Optia & Amicus will not calculate blood volume in children <25 kg

In addition, boys less than 10 to 12 years or weight less than 30 kg have smaller blood volume than that predict by Spectra formula.

SPECIAL CONSIDERATIONS IN PEDIATRIC PATIENTS/DONORS

- For patient/donors weighing less than 25 kgs the apheresis kits need to be primed with blood.
- Larger volume needs to be processed.
- Venous access can be challenging. Central or femoral lines preferred in <10 yrs age group
- Pediatric donors are minors and screening for medical history is done through parents
- Hypocalcemia incidence more in pediatric donors
    - May not exhibit symptoms of parasthesia or if they do, cannot inform operator
    - Often present with abdominal pain, emesis, pallor and/or hypotension

TOTAL BLOOD VOLUME (TBV)

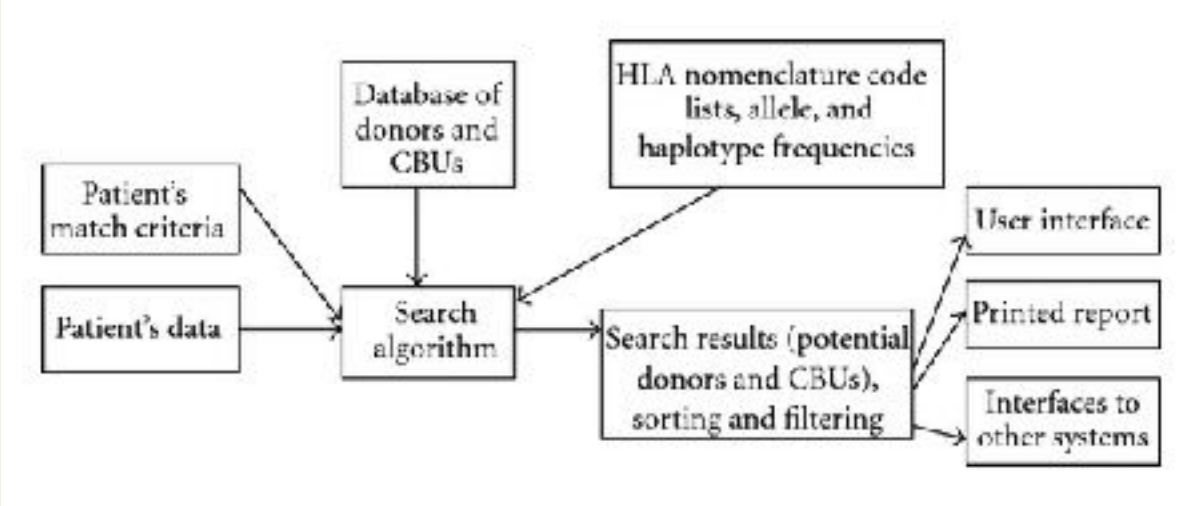
- Consequently calculated TBV must always be verified against manual weight based estimates for children esp < 30 kgs
- In pre-pubertal boys if female is entered as gender calculations become more accurate.

Simple formula for plasma calculation

PV=TBV x(1-HCt) or TBV-RCV

RCV=TBV xHCT

UNRELATED DONOR REGISTRY



Basic concept of the donor search algorithm

Ref: David Steiner, “Computer Algorithms in the Search for Unrelated Stem Cell Donors,” Bone Marrow Research, vol. 2012, Article ID 175419, 7 pages, 2012. doi:10.1155/2012/175419

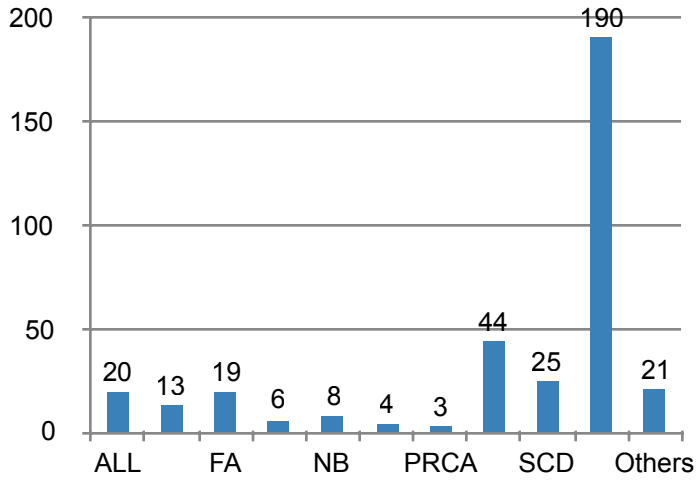
“Self learning is the best form of learning.”

INFRASTRUCTURE

OUR EXPERIENCE-PEDIATRIC HSCT

Diagnosis	Numbers
ALL	20
AML	13
FA	19
HD	6
NB	8
NHL	4
PRCA	3
SAA	**44**
SCD	25
TM	**190**
Others	21
Grand Total	**353**

Total Transplants	806

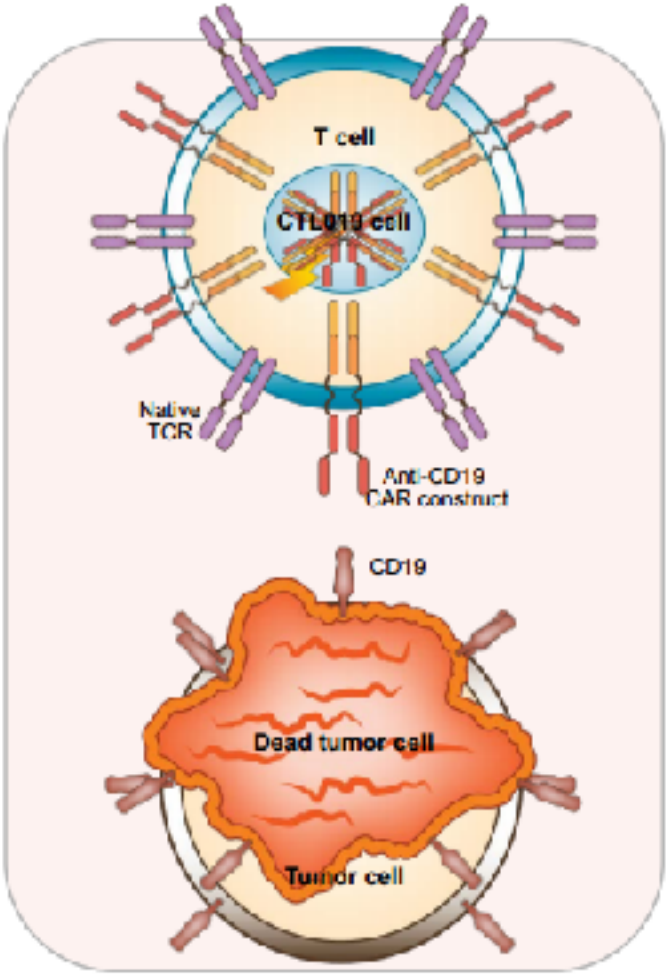


Overall Survival	81.25%

FUTURE

CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELLS

CAR-T therapy takes advantage of the cytotoxic potential of T cells, thereby killing tumor cells in an antigen-dependent manner




Thank You