Haematopoietic Stem Cell Transplantation

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Case Study

- 49 M from Aberdeen
- Known Hypo MDS
- Pancytopenia
- Treated with CSA
- failed Immunosuppressive therapy

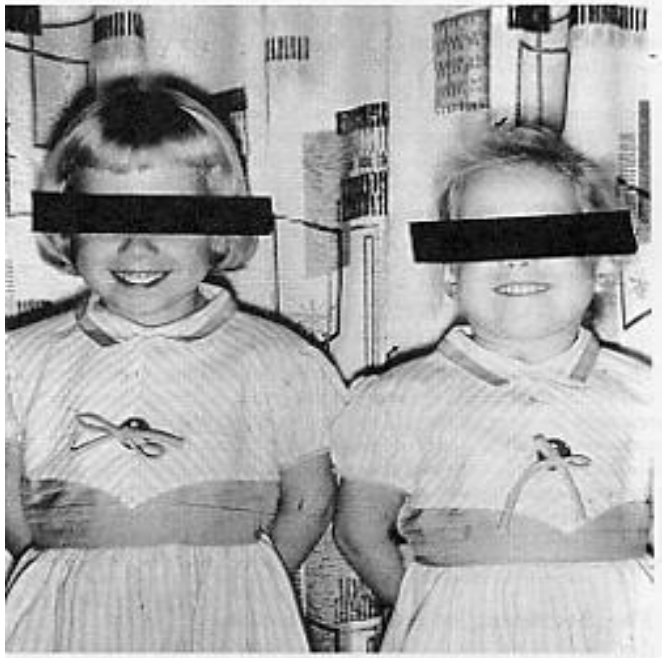
- Allograft planned

HSCT- evolution

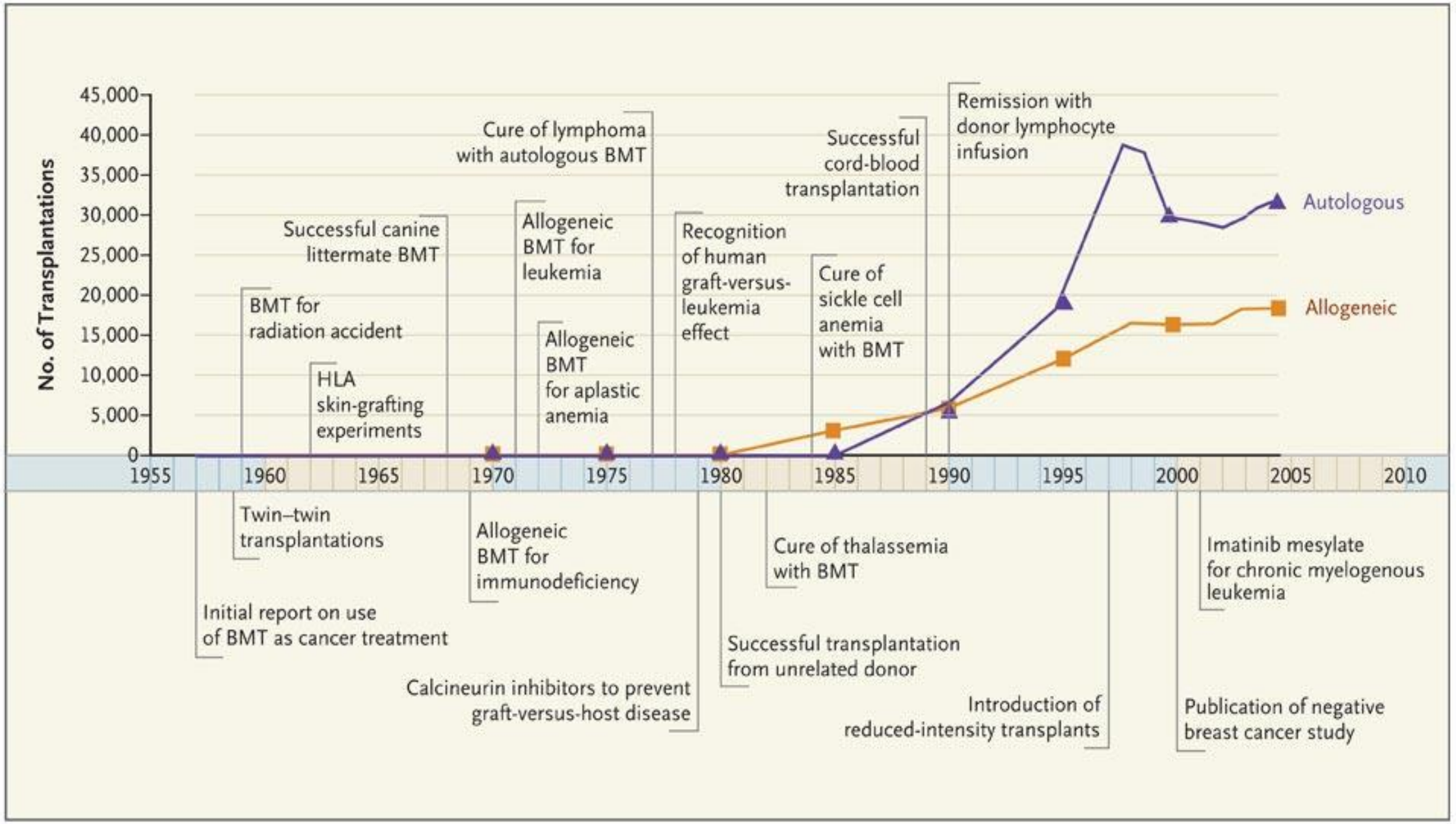
- 1957: marrow safely infused intravenously
- 1958: reports of successful identical twin transplants

Beginning of the Modern Era of HSCT: the end of 1960

- 1969: Cyclophosphamide added to radiation
- 1970: bone marrow harvests perfected to obtain stem cells
- 1989: peripheral blood stem cells harvested
- 1990: first successful cord blood transplant
- 1996: first non-ablative transplant



Thomas et al J Clin Invest 1959


No. of Transplantations
45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
1955
1960
1965
1970
1975
1980
1985
1990
1995
2000
2005
2010
Autologous
Allogeneic
Initial report on use of BMT as cancer treatment
Twin–twin transplantations
BMT for radiation accident
HLA skin-grafting experiments
Successful canine littermate BMT
Allogeneic BMT for immunodeficiency
Allogeneic BMT for aplastic anemia
Cure of lymphoma with autologous BMT
Calcineurin inhibitors to prevent graft-versus-host disease
Recognition of human graft-versus-leukemia effect
Successful transplantation from unrelated donor
Cure of thalassemia with BMT
Cure of sickle cell anemia with BMT
Successful cord-blood transplantation
Introduction of reduced-intensity transplants
Remission with donor lymphocyte infusion
Imatinib mesylate for chronic myelogenous leukemia
Publication of negative breast cancer study

Indications for Stem Cell Transplants

- Cancer:
  - **Leukemia**
  - **Myelodysplasia**
  - **Lymphoma**
  - **Multiple myeloma**
  - Breast cancer
  - Testicular cancer
  - Ovarian cancer
  - Brain tumors
  - Pediatric tumors
  - Sarcomas
  - Kidney cancers

- Non Cancers:
  - **Bone**
  - **Autoimmune diseases**
    - Rheumatoid arthritis
      - Juvenile and adult
    - Multiple Sclerosis
    - Scleroderma
    - Systemic Lupus
  - Immune deficiency
  - Metabolic disorders
  - **Sickle cell anemia**
  - **Thalassemia**

Aims of Transplantation

- Restore Haematopoiesis in marrow failure states
- Replace a diseased marrow by health donor marrow
- As a ‘rescue’ to reconstitute haematopoiesis following marrow-ablative chemo-radiotherapy
- As a mean for treating genetic disorders

Types of Transplant

Two main types based on source of stem cells

- **Autologous**: no immunologic conflict
  - Stem cell infusion as “rescue” from high dose chemo
    - “marrow lethal dose”

- **Allogeneic**: Minor HLA disparity
  - Related
  - Unrelated
  - Cord blood

- **Syngeneic**

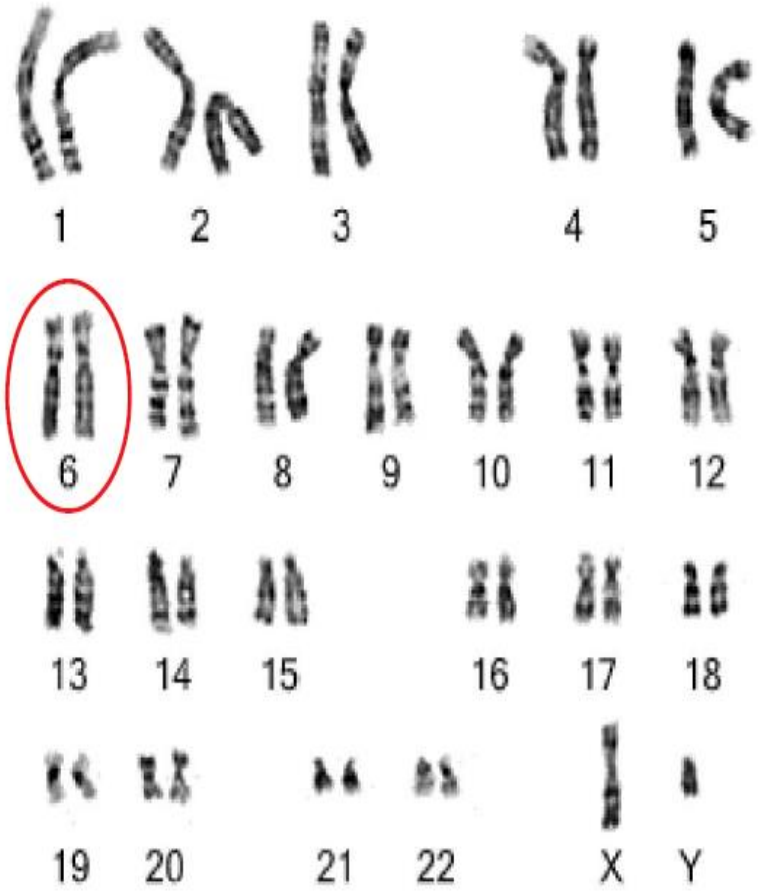
CASE STUDY contd.

- Sibling Donors HLA typed
- 1 sibling ‘Fully’ Matched

Principles of Allogeneic Transplantation

- **Human leukocyte antigens (HLA): major determinants of histocompatibility between donor and recipient**

- **These antigens are cell-surface glycoproteins encoded by a series of closely linked genes located on the short arm of chromosome 6 (p21)**

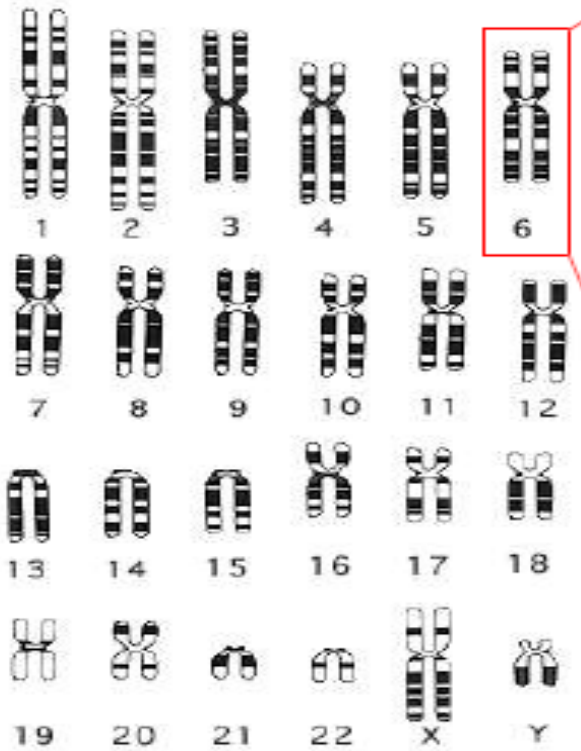


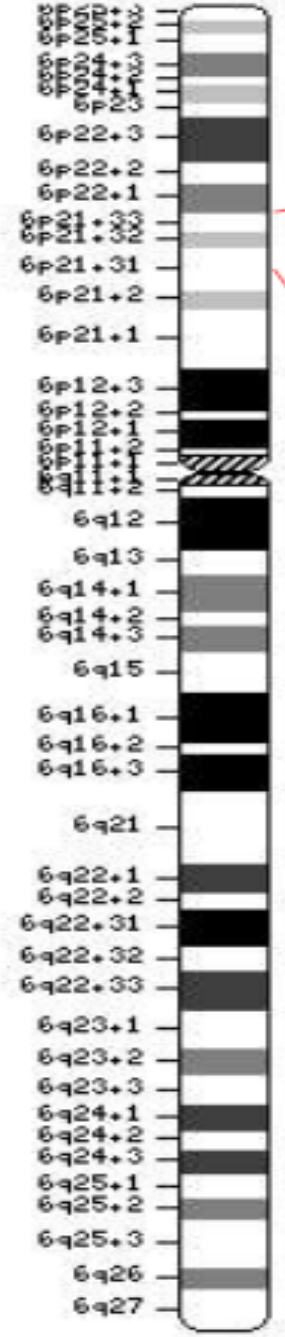
HLA SYSTEM

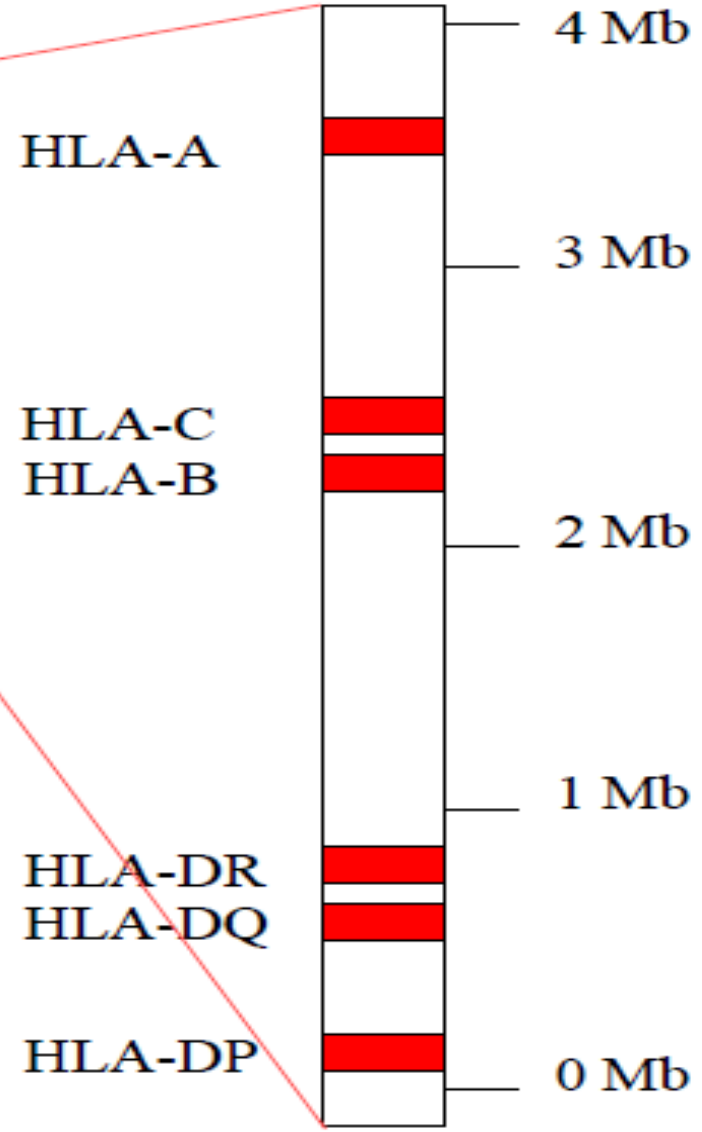
Two distinct types of HLA genes:

- 1) Class I: HLA-A, -B, -C genes
- 2) Class II: HLA-DR, -DQ, -DP genes

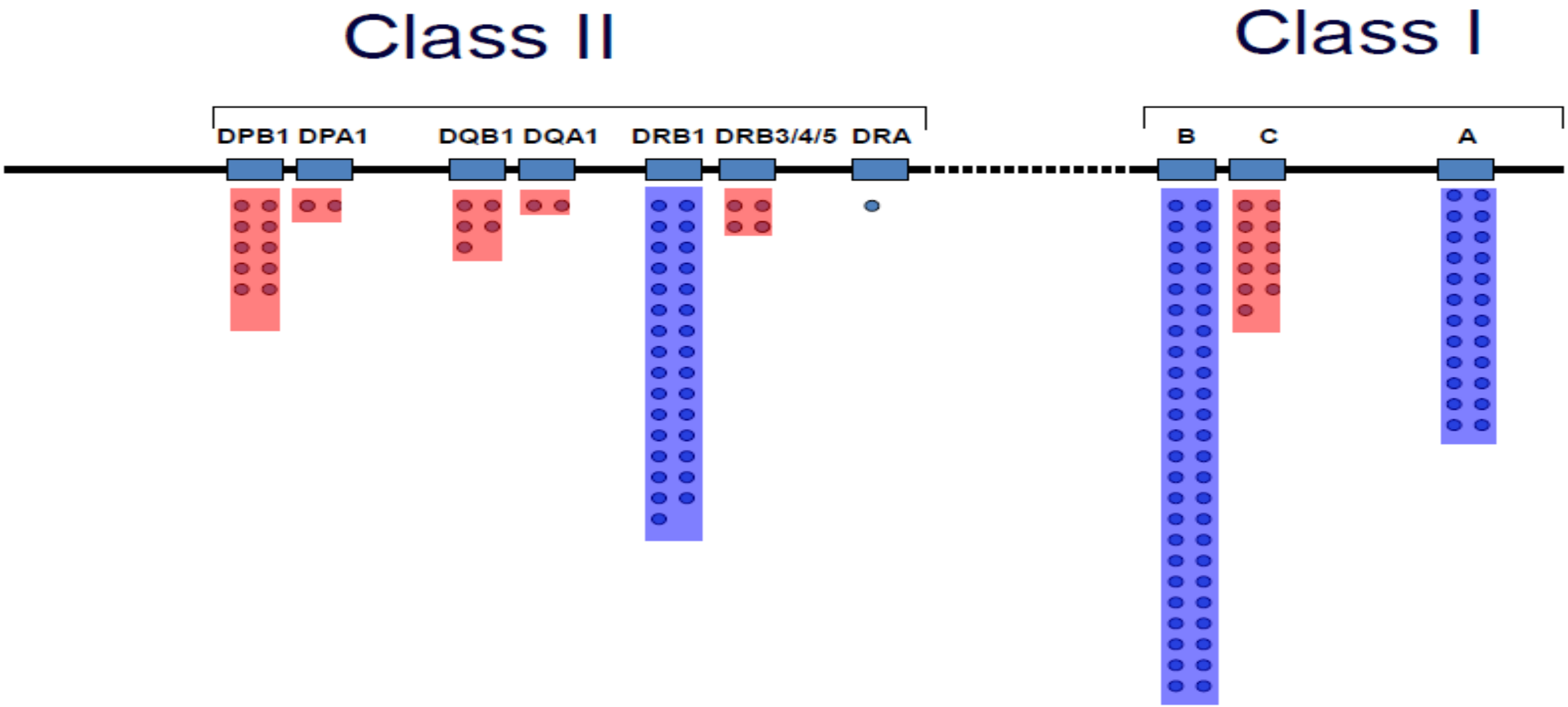
Chromosome 6
6p21.3



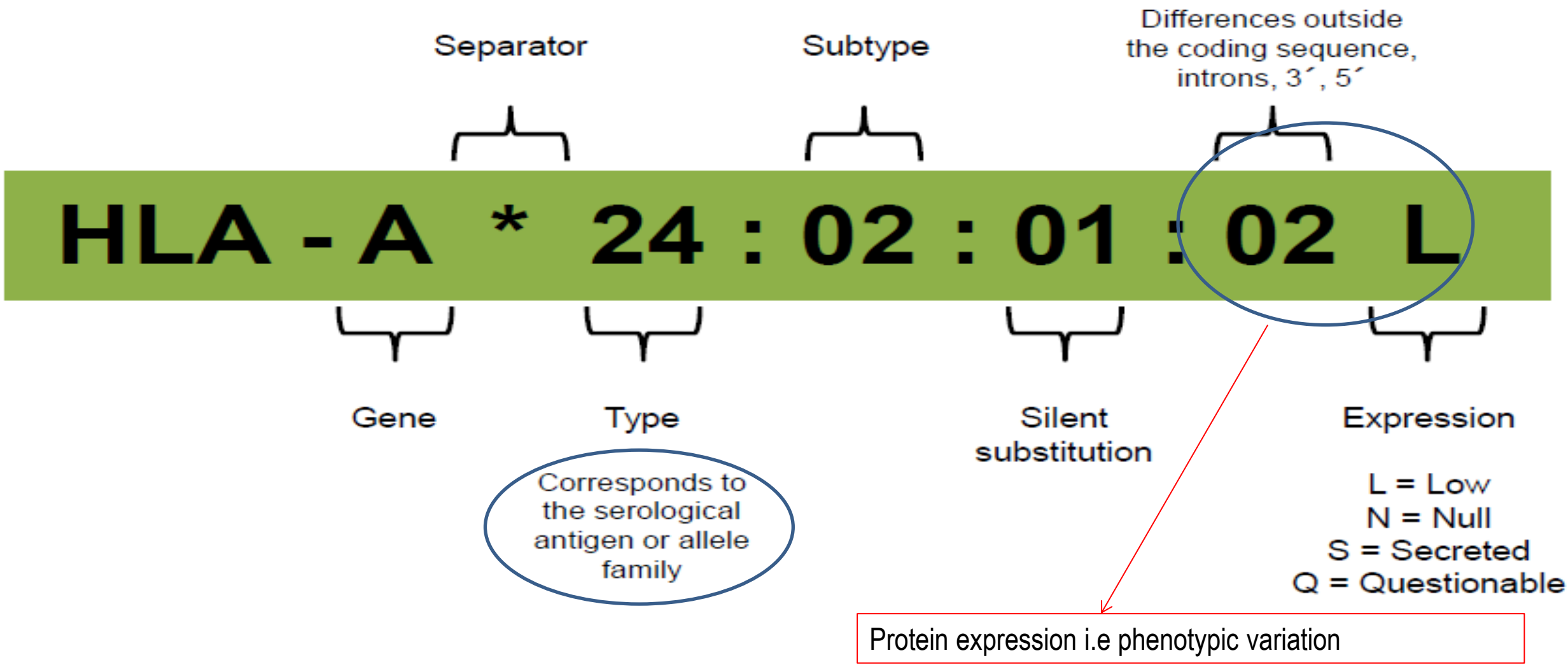




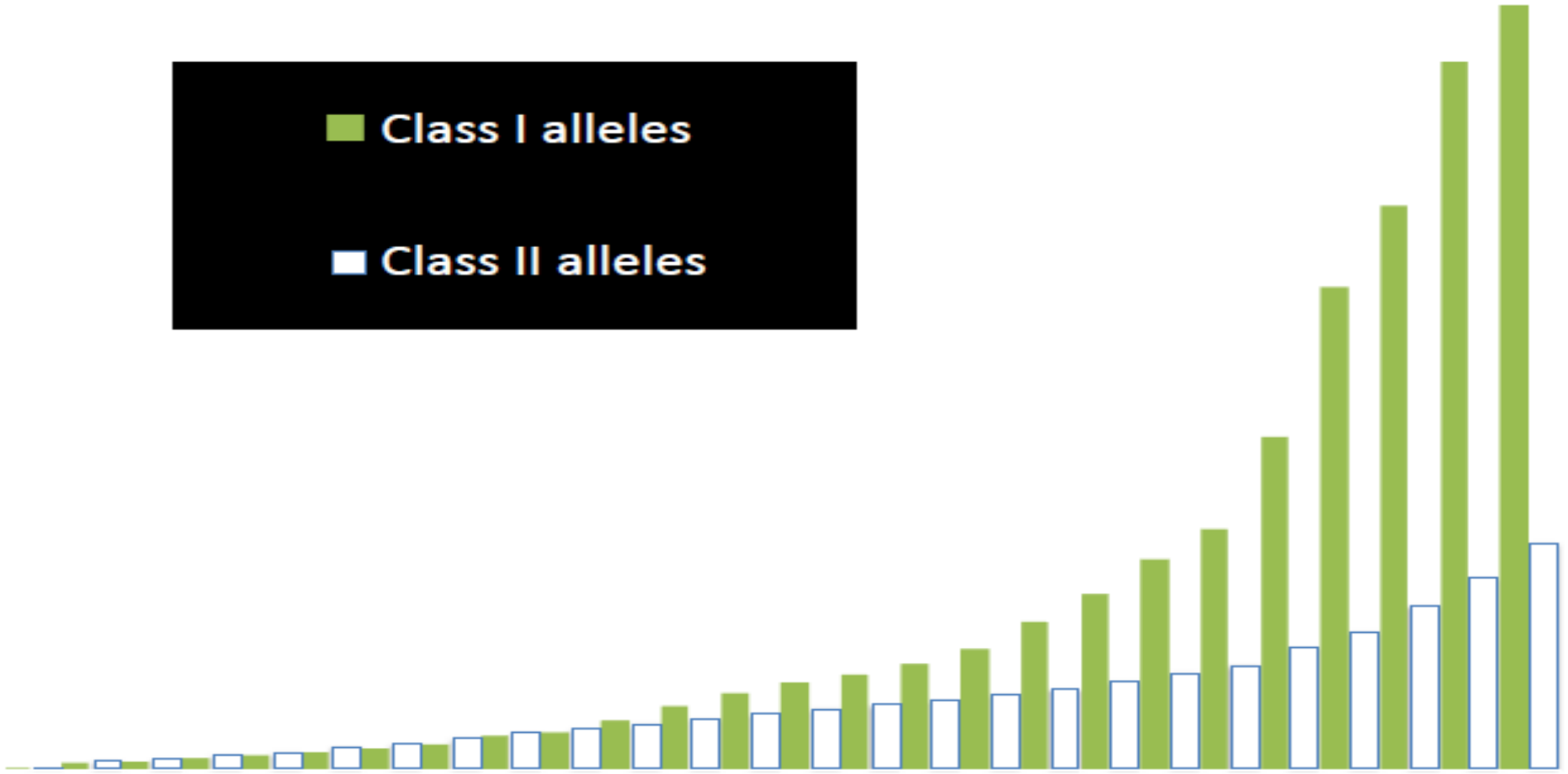
HLA Polymorphism



HLA Nomenclature



HLA Alleles 1987-2014



HLA Typing

Serology:

- Use Antibodies to type for HLA molecules on viable cells

DNA based

- Sequence Specific Oligonucleotide Probing (SSOP)
- Sequence Specific Priming (SSP)
- Sequence Based Typing (SBT)
- Next Generation whole gene sequencing (new!)

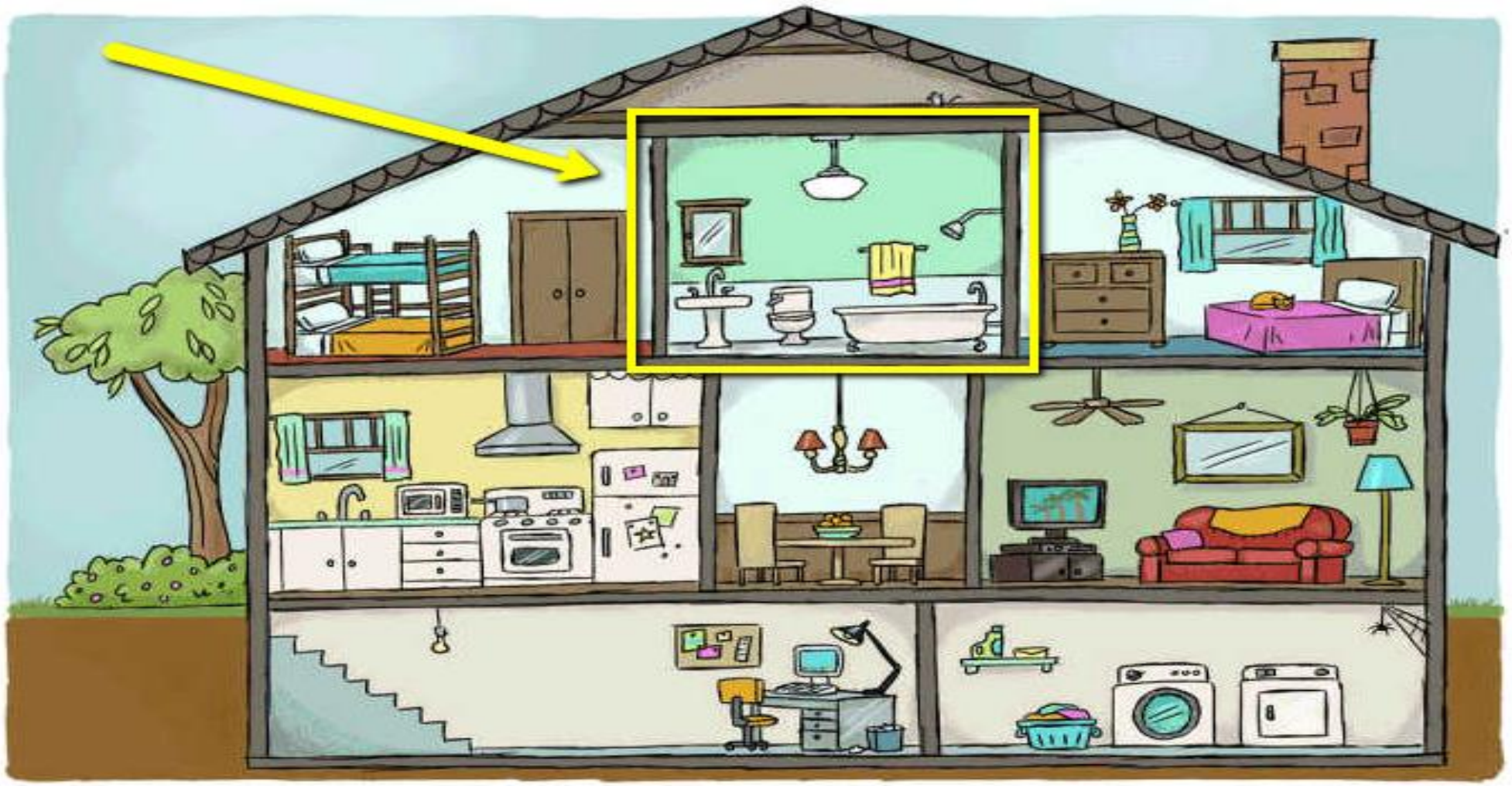
Low resolution Typing :HLA A-A* 02

Medium Resolution:

HLA-A*0201/09/43N/66/78/88/89/90/91+

High Resolution: HLA –A*0201/02/09

‘NGS’ resolution: HLA A* 24:01:03:03.



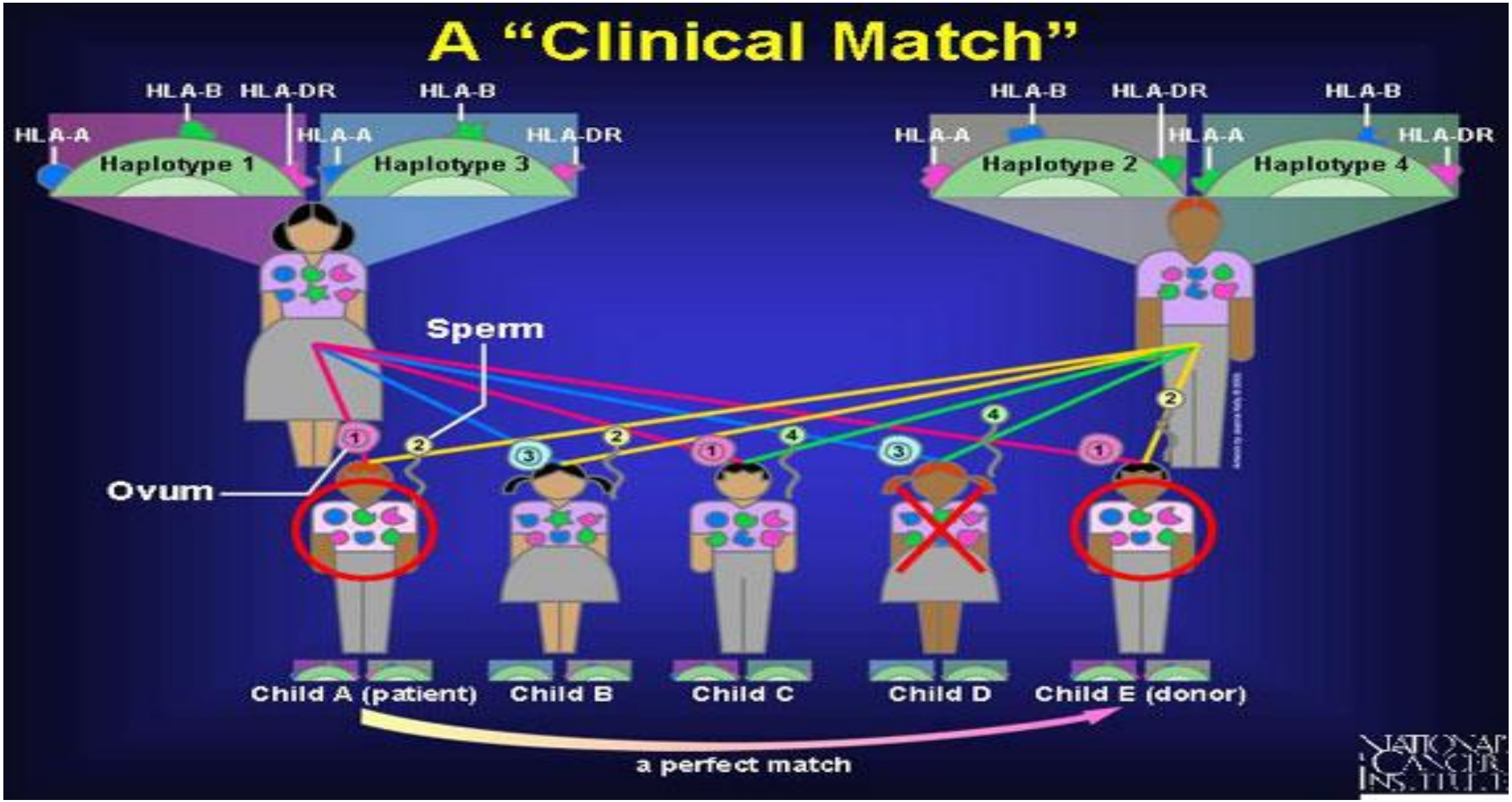
A typical ‘next gen’ HLA report

HLA Typing Results:

				Method
CLASS I	HLA-A	*24:03:01:01.	*33:03:01.	5
	HLA-B	*58:01:01:01.	*18:01:01:02.	5
	HLA-C	*03:02:02:01.	*07:01:01:01.	5
CLASS II	HLA-DRB1	*03:01.	*12:01/10.	4,5
	HLA-DRB3	*02:02/12/28/29N/34.		4
	HLA-DRB4			4
	HLA-DRB5			4
	HLA-DQA1	Not tested.		
	HLA-DQB1	*02:01:01.	*03:01:01.	5
	HLA-DPB1	*04:01:01.	*23:01:01.	5

Date HLA Tests Completed: 10/11/2016

Note: Where two 'strings' of alleles are given as a result, not all combinations between the two strings are always possible.
~ Other ambiguous HLA results may have not been excluded. If required, please contact the laboratory for more details.
Methods Utilised: 1 = Serology, 2 = PCR-SSO (polymerase Chain Reaction Sequence Specific Oligonucleotides), 3 = PCR-SSP (Polymerase Chain Reaction Sequence Specific Primers), 4 = Direct sequencing of PCR product, 5 = Third Generation Sequencing. For an up-to-date list of alleles covered by HLA typing methods, please contact the laboratory.

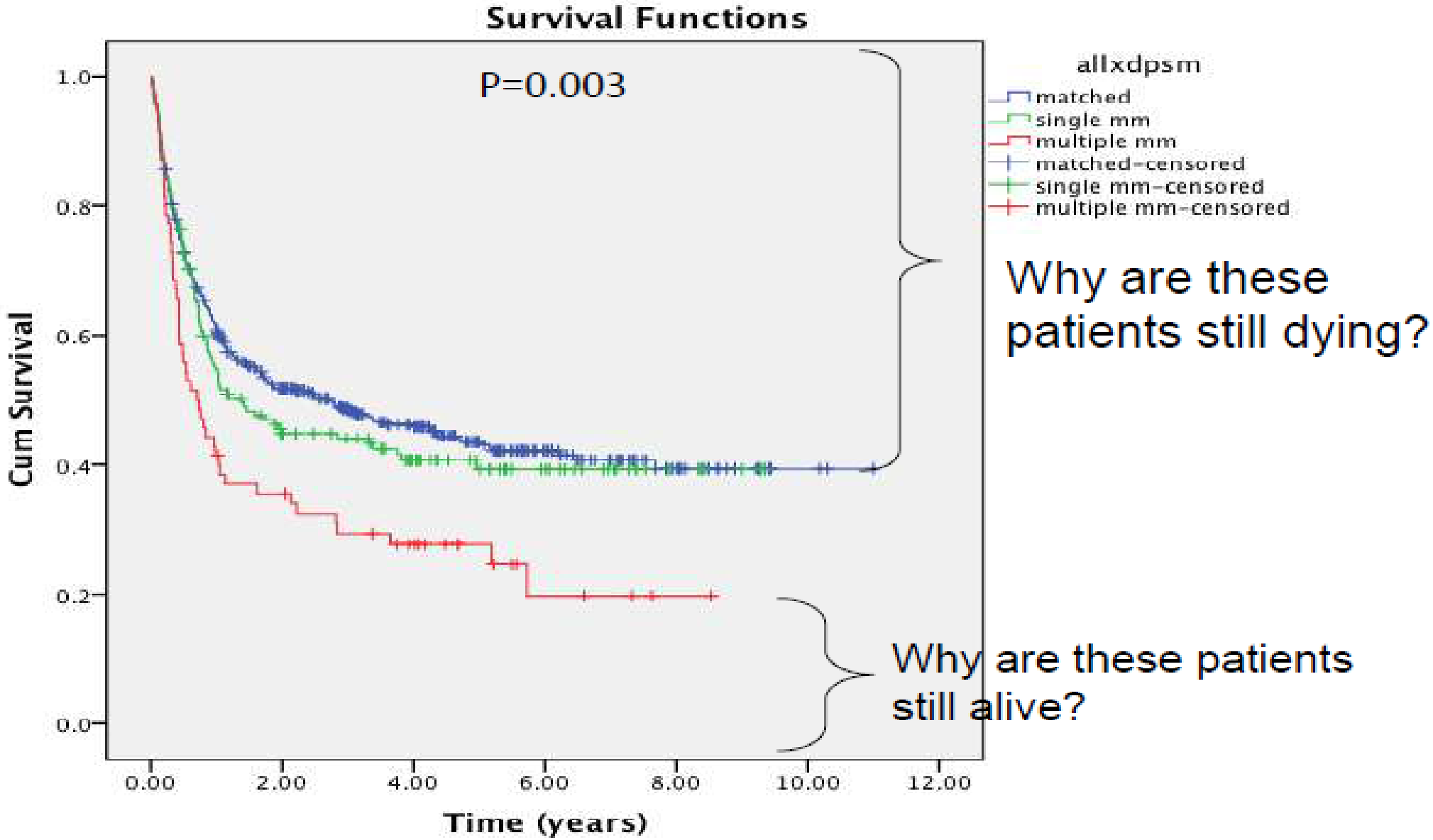

A "Clinical Match"
HLA-B
HLA-DR
HLA-A
Haplotype 1
HLA-B
HLA-A
HLA-DR
Haplotype 3
HLA-B
HLA-DR
HLA-A
Haplotype 2
HLA-B
HLA-A
HLA-DR
Haplotype 4
Sperm
Ovum
Child A (patient)
Child B
Child C
Child D
Child E (donor)
a perfect match

Optimizing donor MHC match is one of the two biggest factors in determining complication /survival rate

Increasing MHC mismatches increases –

- acute graft versus host disease
- chronic graft versus host disease
- infectious complications
- hematologic complications
- Improves graft versus leukaemia

Survival depends on HLA typing?



Why are these patients still dying?

Why are these patients still alive?

N = 727

Factors influencing the outcome of Allo-HSCT

- **Disease factors**
  - stage
- **Patient - related factors**
  - Age
- **Donor - related factors**
  - Histopompatibility (HLA) (*NIMA/KIR ligand status?*)
  - Sex
  - Viral status (**CMV positivity**)
  - ABO status
- **Peri-transplant factors**
  - Conditioning
  - GVHD prevention
  - Stem cell source and content
- **Post-transplant factors**
  - GVHD

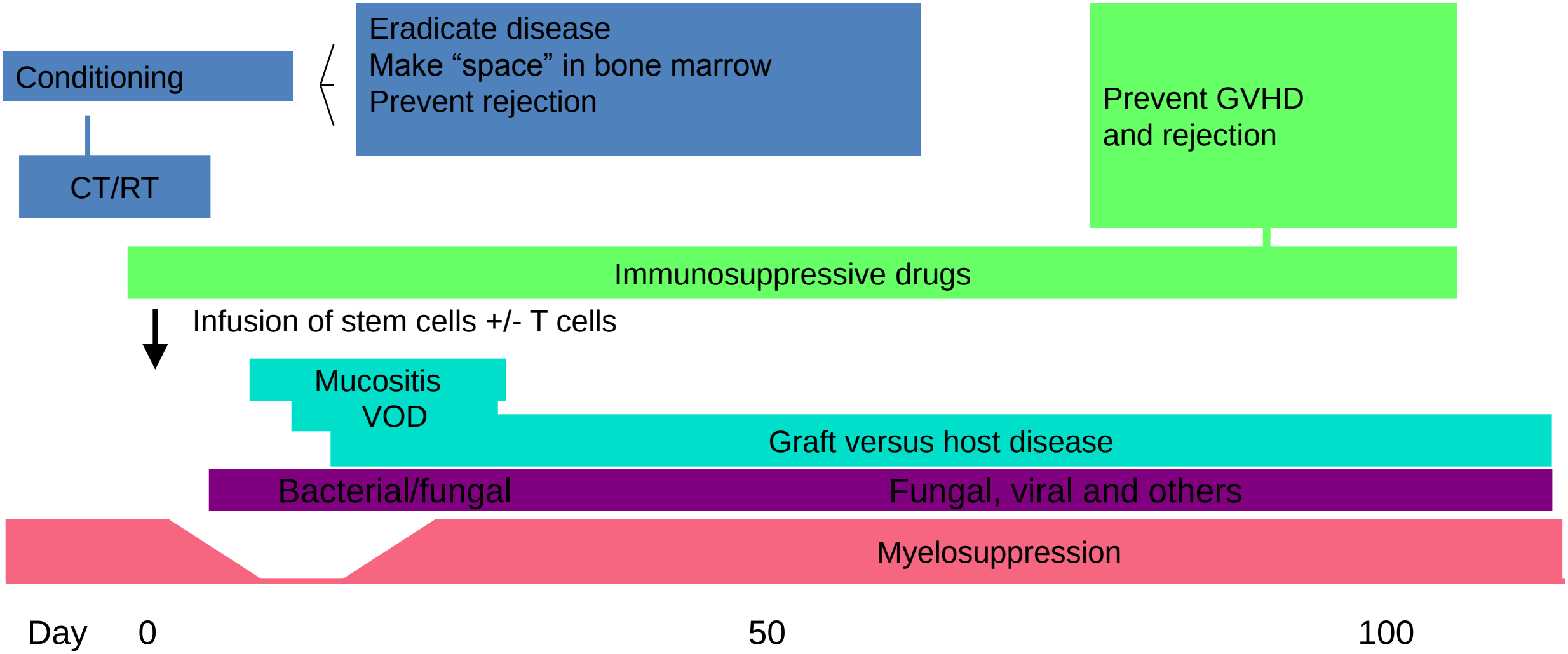
Steps in a Transplant:

- **Determine patient eligibility**
- Identify a donor
- Collect marrow stem cells
- Prepare the patient
- Transplant the stem cells
- Post transplant care

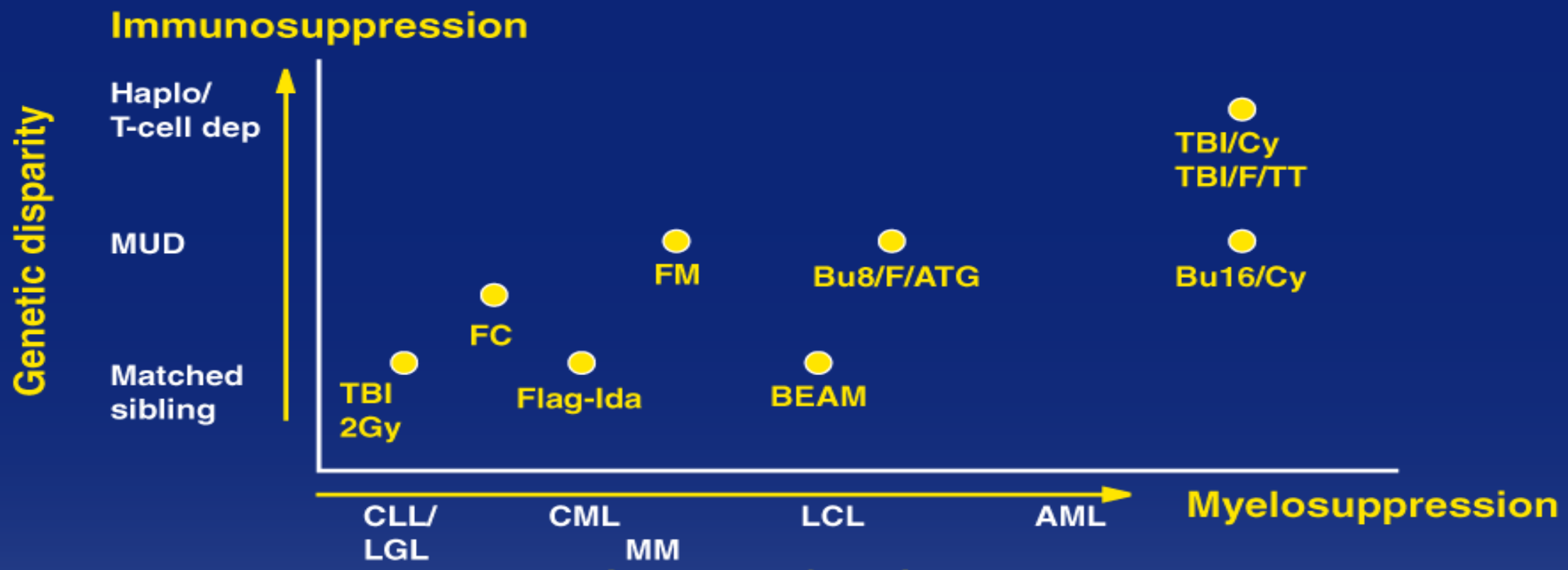
Case Study Contd

- Donor identified– Sibling in this case
- Major ABO mismatch O+ve/ A+ve (IgG Anti A titres- 1024)
- FCC-RIC conditioned Allograft (Note patient age>40yrs)

Allogeneic stem cell transplantation



Intensity of preparative regimens



Standard dose chemotherapy w/o BMT

Needs BMT

Maximally tolerated high dose

Flag-Ida
FC
PFA

FM

BEAM

Bu8/F/ATG

Bu16/Cy
Cy/TBI

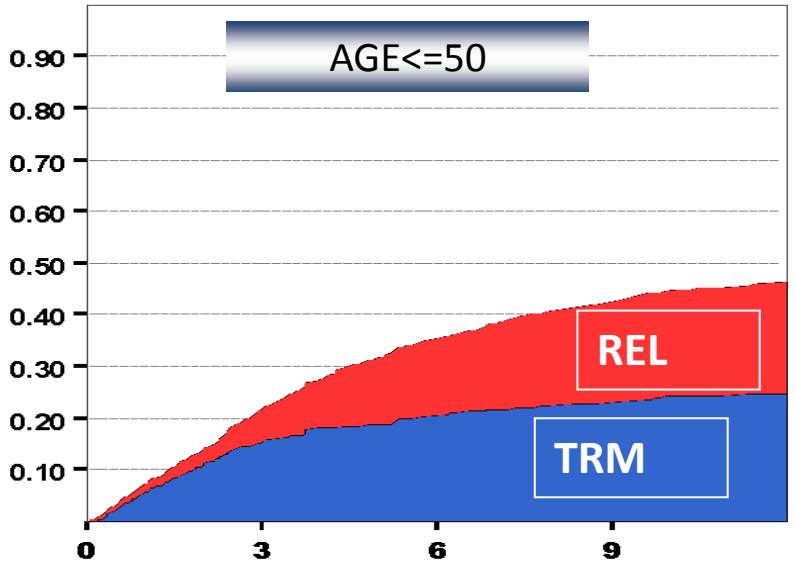
LIST OF CONDITIONING REGIMENS USED AT KCH

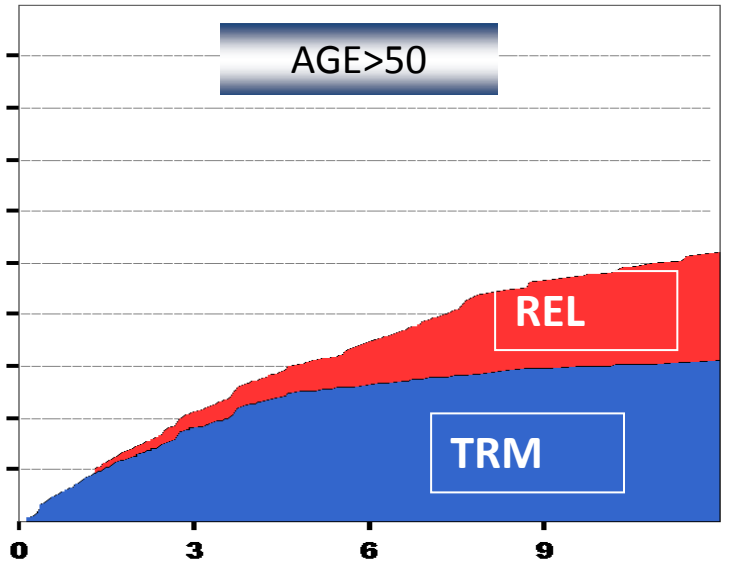
STANDARD MyeloAblative	REDUCED INTENSITY (Non-MA)
FB4C (for myeloid)	FB2C (or FBC)-- (for myeloid)
FB4ATG (for myeloid)	FB2ATG (or FBATG)-- (for myeloid)
BEAM-ATG (for Lymphoma)	FLAMSA-Bu2—for Myeloid
BEAM Campath (for Lymphoma)	FCC or FCC-TBI (<6gy) for AA
Bu4-Cyclo- Campath (AML/MDS)	Flu-ATG for graft failure
Cyclo-TBI campath (for ALL/Lymphoma)	FMC for lymphoma
Cyclo TBI methotrexate (for ALL/lymphoma)	FMATG for lymphoma
Cyclo-Campath or Cyclo ATG for AA	Flu/Cy/TBI (double cord-Minnesota or National cord protocol)
HAPLO MA—Cy/TBI-Cd34cells	HAPLO RIC- Flu/Cyclo/TBI
High dose melphalan (HDM200) auto	Mel 140 Auto
Cyclo/ATG (for MS/autoimmune)	BCNU-Thiotepa auto

Combined Outcomes (NRM and Relapse) Cumulative Incidences

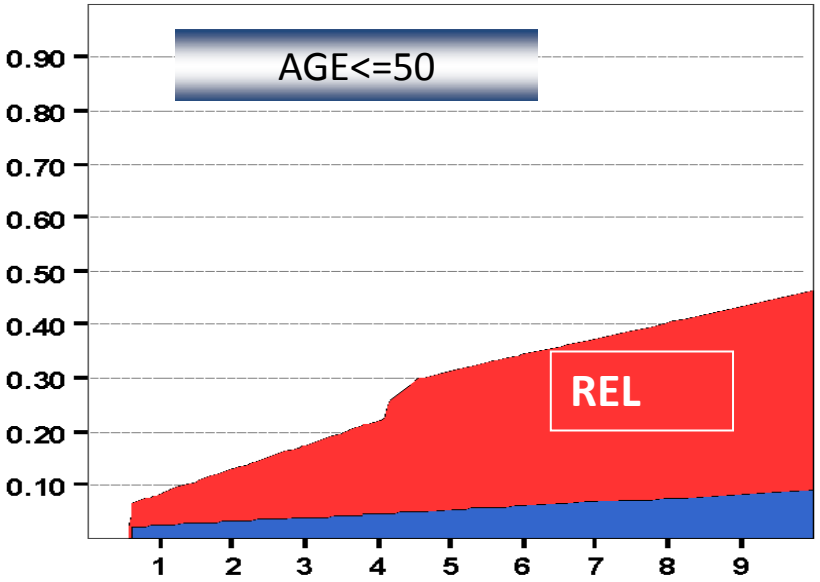
Effect of Age and Transplant Group

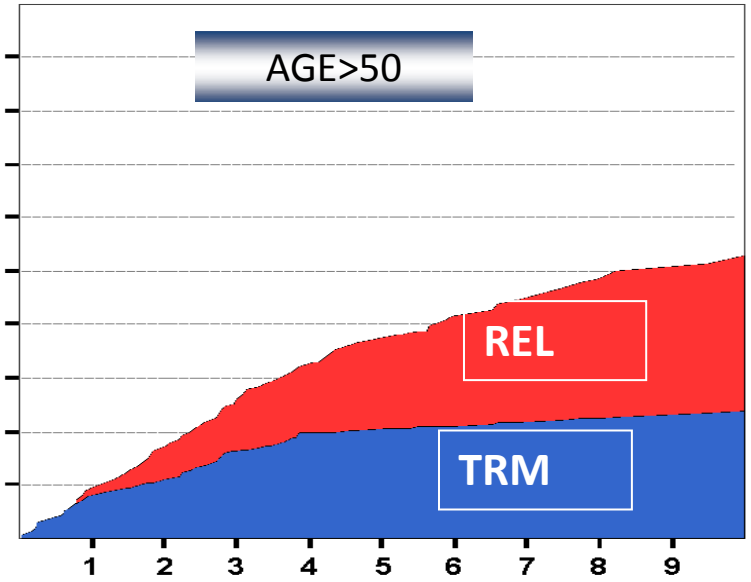
Standard





RIC





EBMT 2004

Haemopoietic Stem Cell Transplantation - Major

Early (Day 0 – 100)

- Graft failure
- Acute GVHD
- Infections
- PRCA if major ABO mismatch
- Acute organ toxicity
  - Cardiac / Kidneys
  - Hepatic VOD
  - Mucositis
  - Lung (*Interstitial Pneumonitis, Alveolar Haemorrhage)*

Late (Beyond day 100)

- Disease relapse
- Chronic GVHD
- Infections
- PRCA if major ABO mismatch
- Late effects of conditioning
  - Endocrine / infertility
  - Cardiac toxicity
  - Pulmonary toxicity
  - Secondary malignancies

ABO COMPATIBILITY

DONOR

RECIPIENT

Blood Group	O	A	B	AB
O	Compatible	Major	Major	Major
A	Minor	Compatible	Major and minor	Major
B	Minor	Major and minor	Compatible	Major
AB	Minor	Minor	Minor	Compatible

Choice of blood transfusion in allogeneic marrow transplant

Major ABO incompatibility	Minor ABO incompatibility
Recipient has antibody against donor RBC	Donor has antibody against recipient RBC

When there is major ABO incompatibility

Recipient	Donor	Red cell
A	O	O
A	B	O
A	AB	O or A
B	O	O
B	A	O
B	AB	O or B

Recipient	Donor	Red cell
AB	O	O
AB	B	O or B
AB	A	O or A
O	A	O
O	B	O
O	AB	O

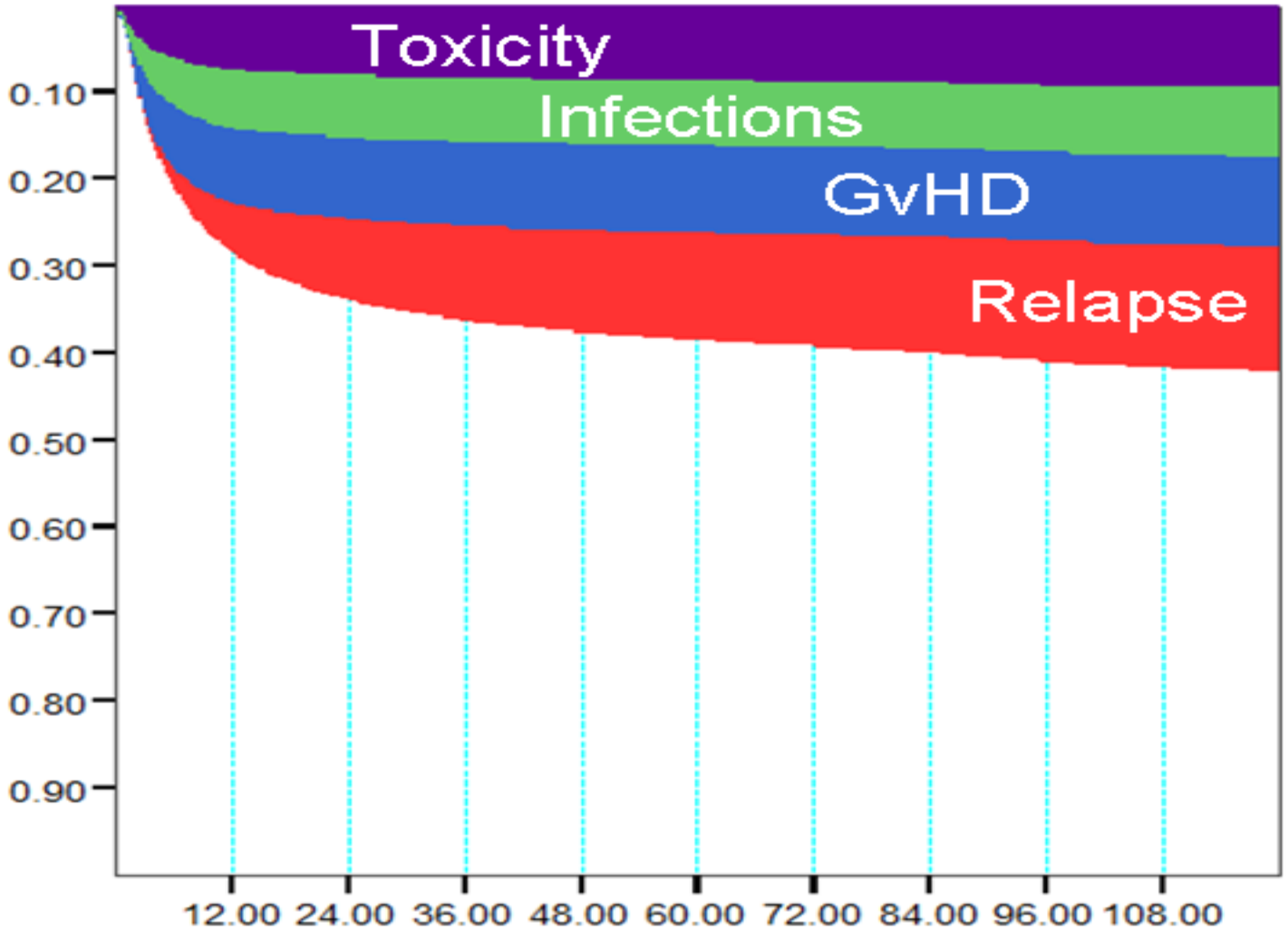
Case Study Contd

- Day 0- June 2012
- Plasma exchanges performed at D-1 and Day 0
- Patient engrafted at Day 14 post BMT
- Sept 2012– Anti A titres remain 1:1024
- Developed PRCA post BMT in Sept 2012 (~D 100)

Case Study cont.

- Completed Rituximab x 4 in Oct 2012 and again in Feb 2014
- IVIG in Feb 2013
- Anti-A titres dropped to 1:256 April 2014– still ongoing chronic haemolysis (DAT neg)
- But remained mixed CD3 donor chimerism and transfusion dependent
- DONOR LYMPHOCYTE Infusion x4 (June'14- Dec'14)
- Developed Acute GVHD

Principle Causes of Failure in HSCT



Toxicity
Infection
GvHD/Rejection

Relapse

Allogeneic HSCT

Graft-versus Malignancy Effect

- Lower incidence of leukemic relapse in patients who get acute or chronic GVHD
- Higher relapse rates in syngeneic vs. allogeneic BMT
- High relapse rates in T cell depleted BMT
- Cytogenetic remission induced after post BMT relapse of CML by infusion of donor leukocytes

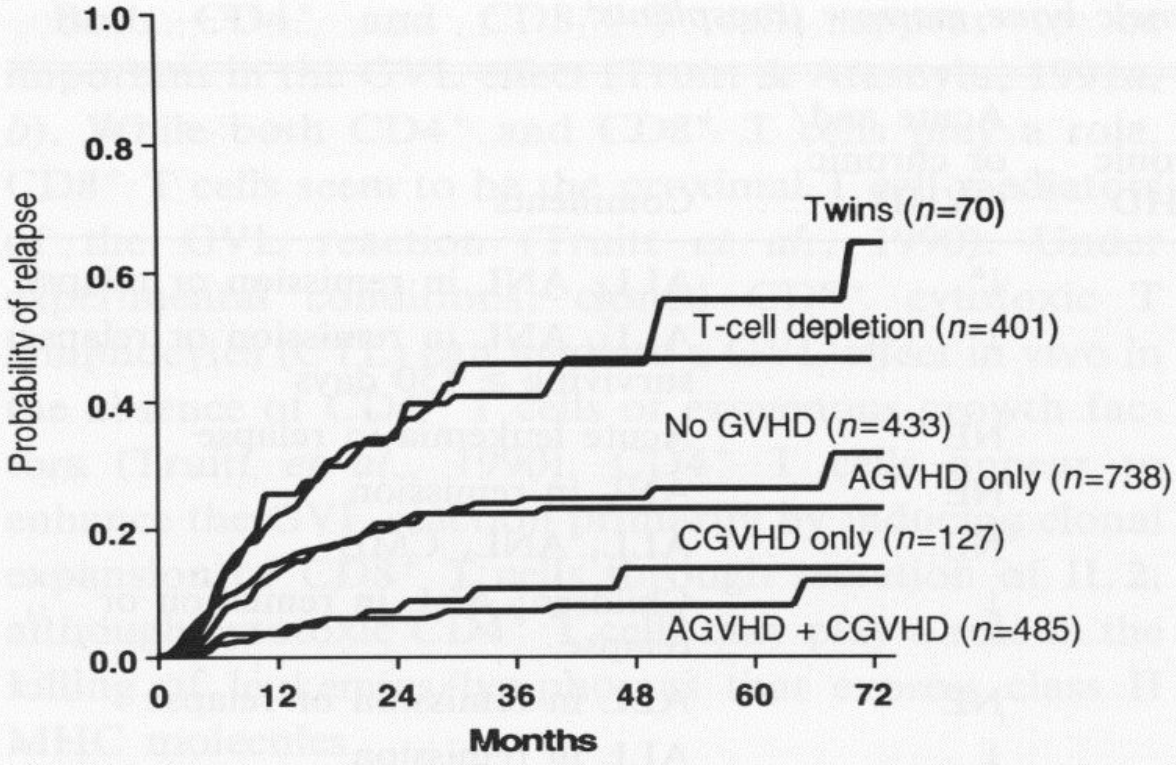


Fig. 88.2. Actuarial probability of relapse after bone marrow transplantation for early leukemia according to type of graft and development of graft-versus-host disease. (From Horowitz *et al.*, 1990.)

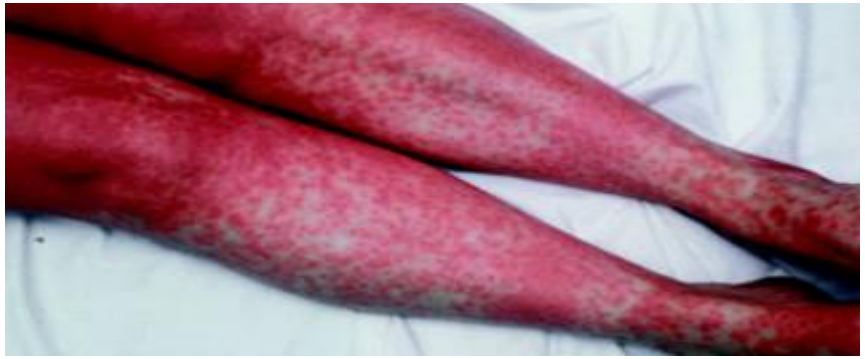
Graft versus Host disease

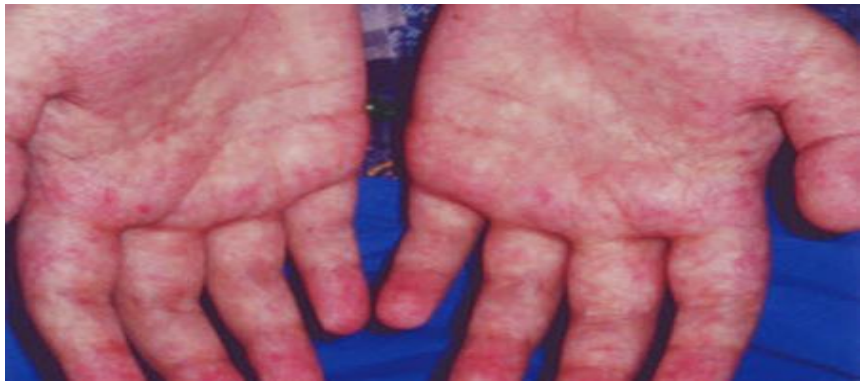
Factors influencing GvHD

- HLA Typing Match- DRB1mm
- Minor histocompatibility antigens- HA8
- Donor and host factors other than HLA type
  - T cell depletion in vivo or ex-vivo
  - Age
  - Donor parity/sex mismatch
  - CMV positivity?
  - Poor drug compliance
- NK- KIR ligand mismatch (KIR2L⟷interaction with HLA class 1 Ag)
- Source of Graft and use of GCSF post
- Conditioning regimens (T deplete, T replete)

Acute Graft-vs-Host disease

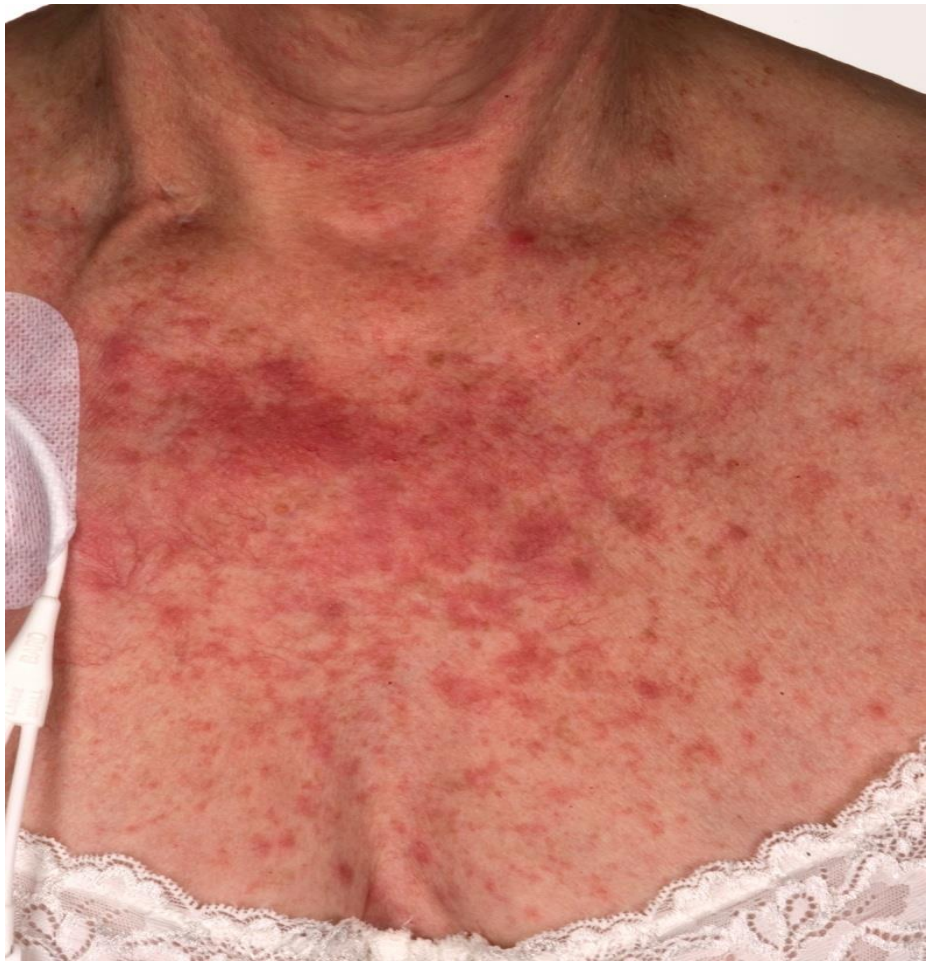
- Primarily affects the Skin, Gastrointestinal tract, and the Liver.

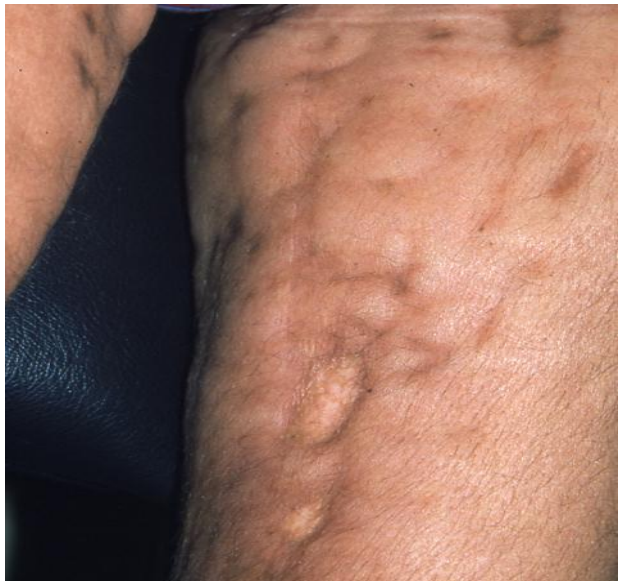


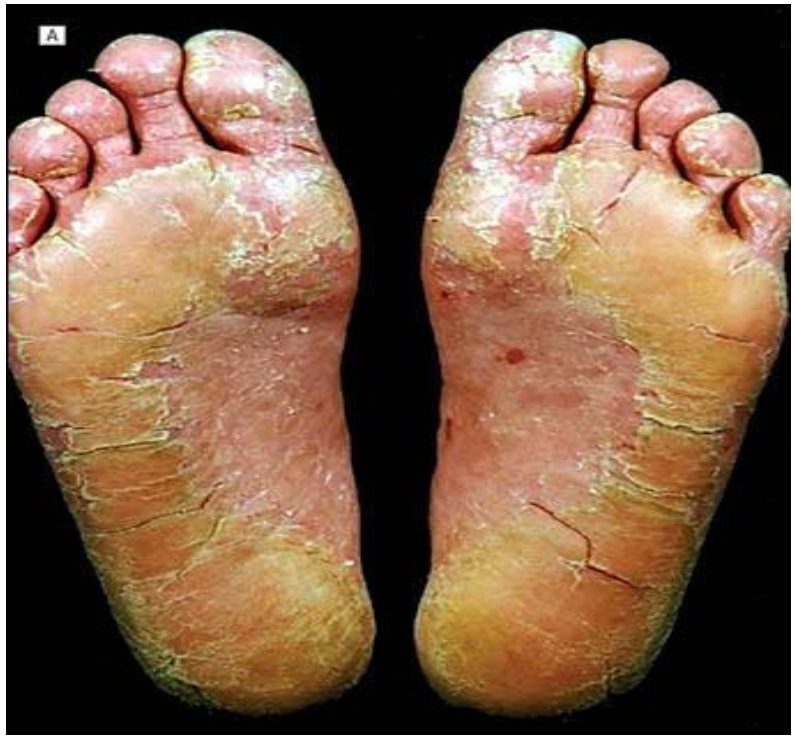


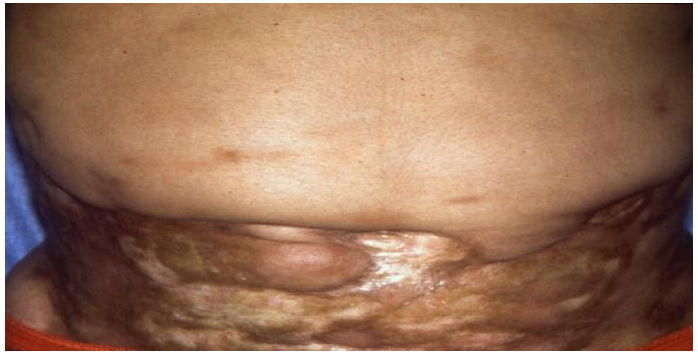
- Severity: Clinical grading (grade I – IV)
- Prophylactic regimens: Cyclosporine/Methotrexate, Tacrolimus/MTX
- More Common in T-replete conditioning
- Treatment: Steroids. Steroid-resistant cases have poor prognosis

Chronic Graft versus host disease









Case Study contd

- GVHD controlled with systemic steroids.
- 2 months later again developed further mixed donor Chimerism (never achieved full donor chimerism)
- Remained Transfusion dependent secondary to ongoing Haemolysis.

<u>Problem:</u>

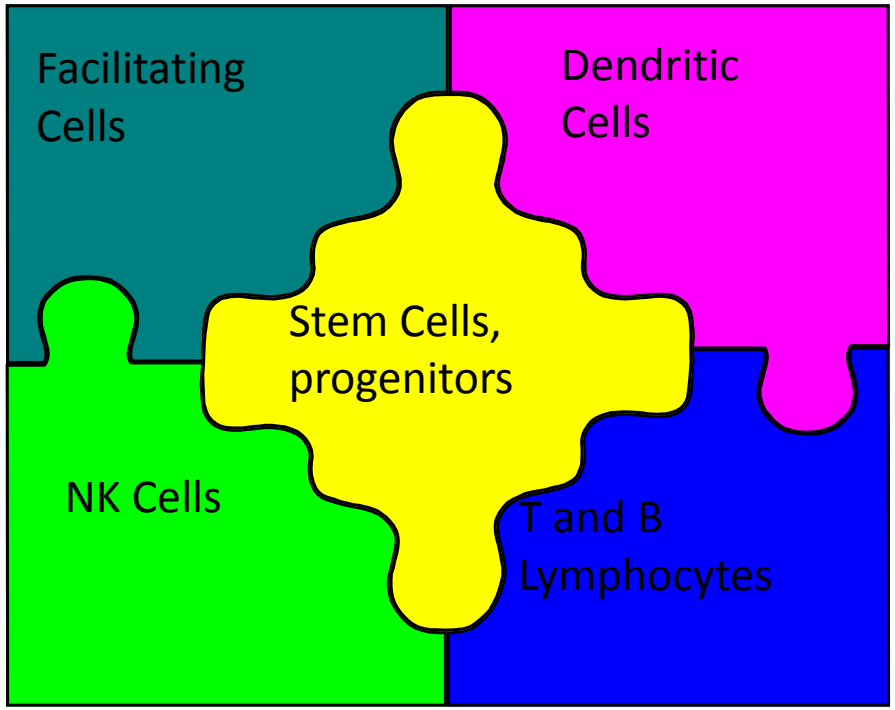
- Donor CD3 low and despite boosted by DLI and GvHD- Donor lymphocytes failed to switch off host ABO antibodies

Case study contd.

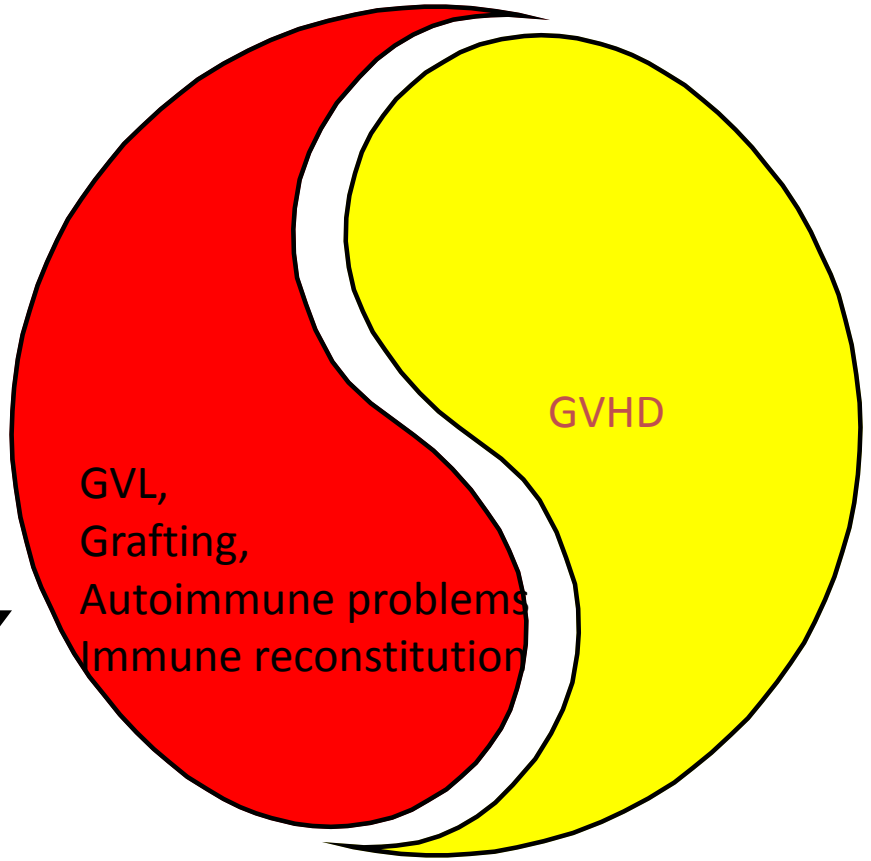
- In view of ongoing PRCA and major symptom burden:-
- 2nd Allograft in Feb 2016– UNRELATED DONOR; ABO MATCHED (CMV and Gender mismatched)
- Chimerism Sept'16:
  - Unfractionated PB 93% (DNR1:0%/ DNR2:98%);
  - CD3=98% (DNR1: 88% /DNR2:11%),
  - CD15=97% (DNR1:0% /DNR2:97%)
- But patient now developed AUTOIMMUNE HAEMOLYSIS (DAT pos)

Stem Cell Grafts are Complex

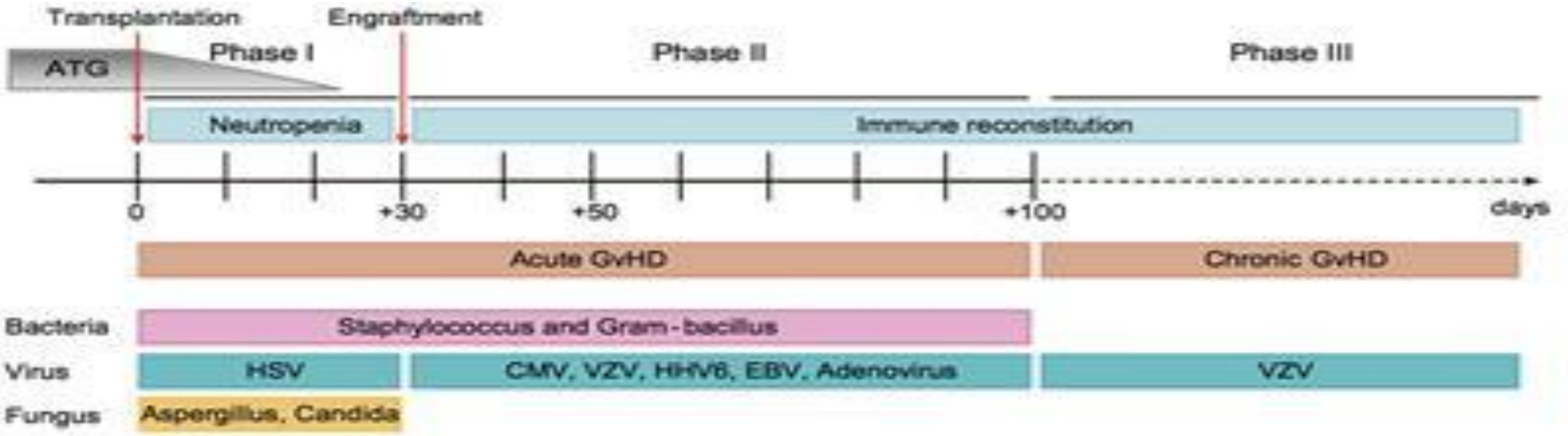
Stem cell graft components

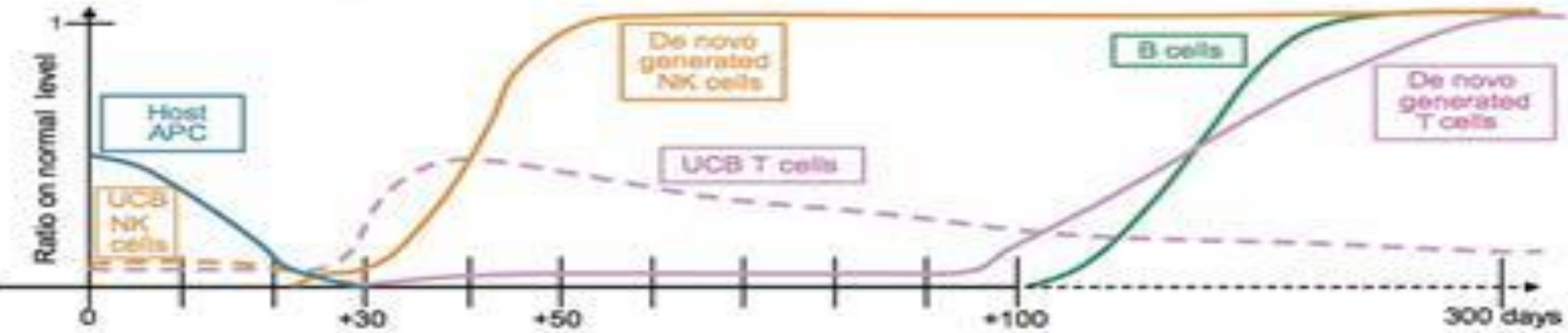


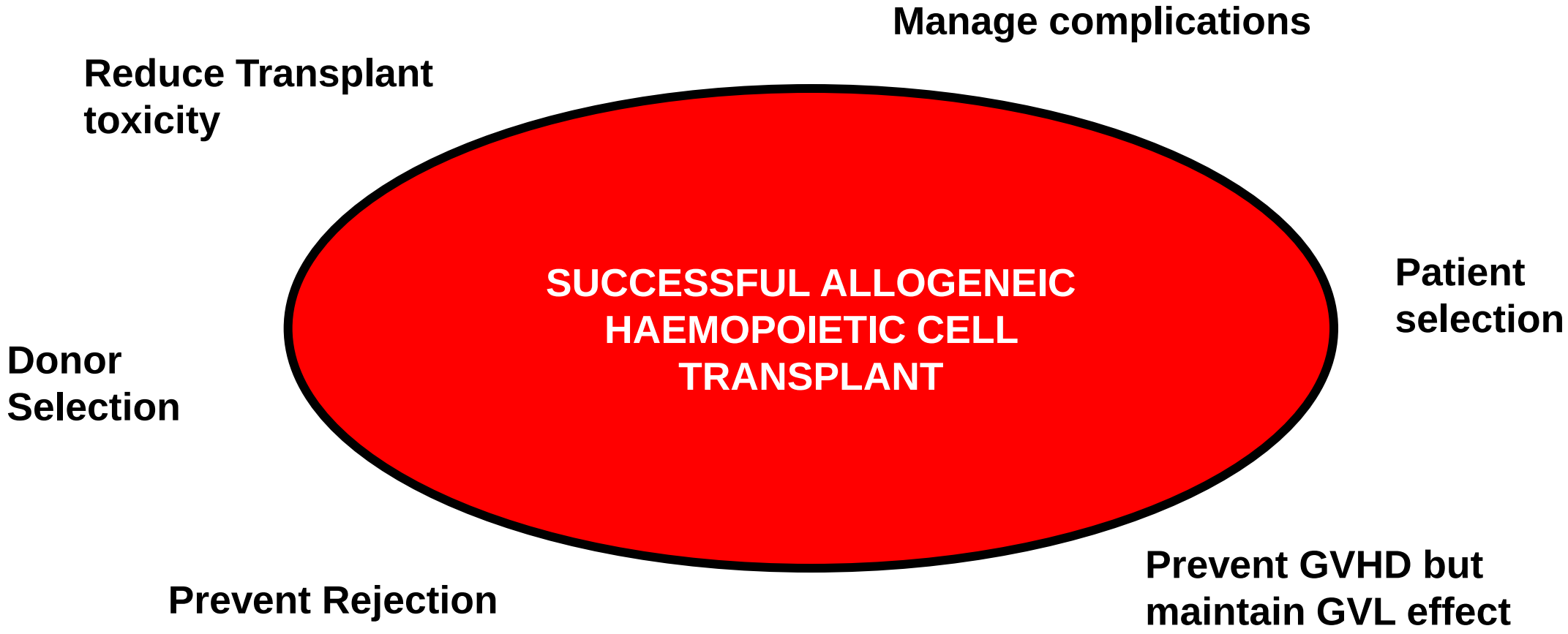
T Lymphocyte functions



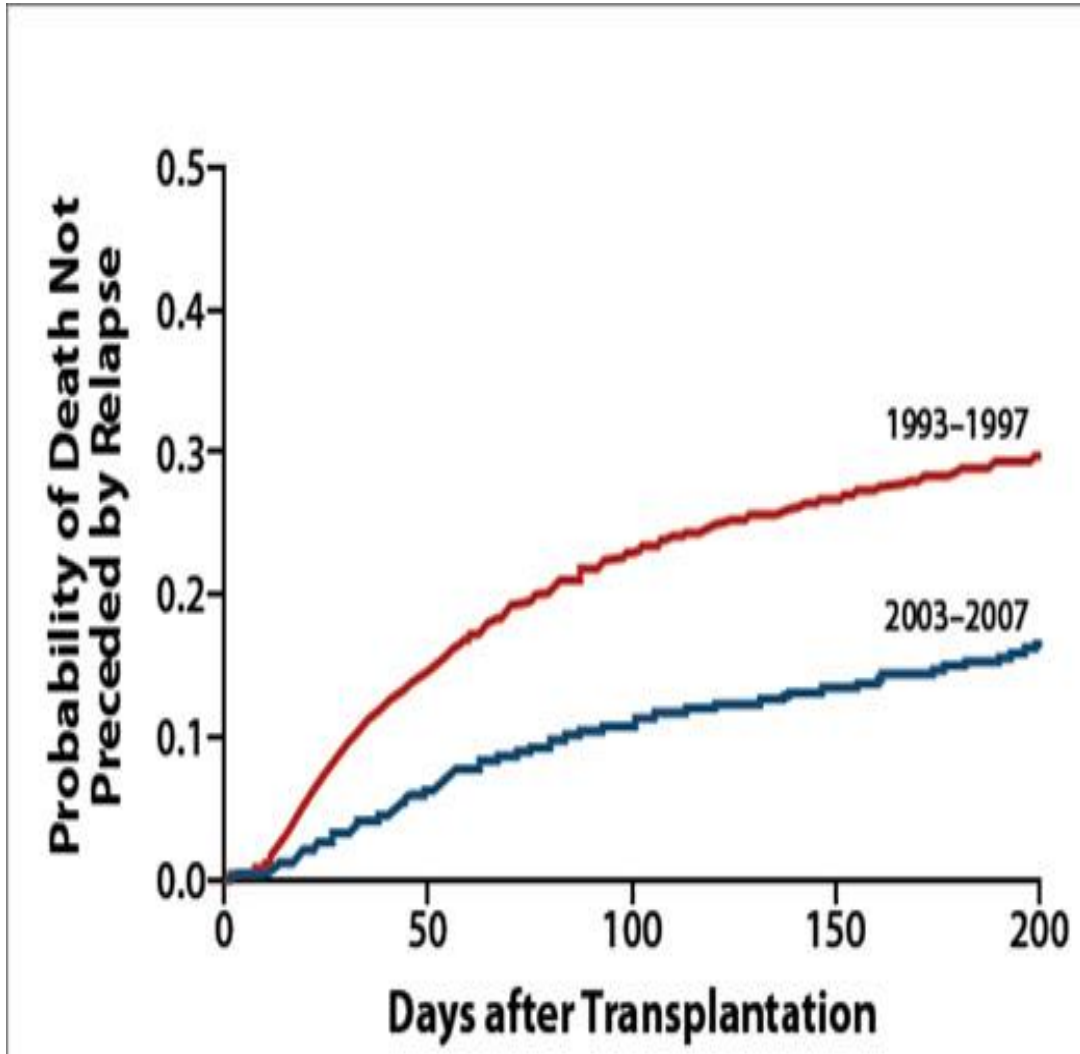
Immune reconstitution

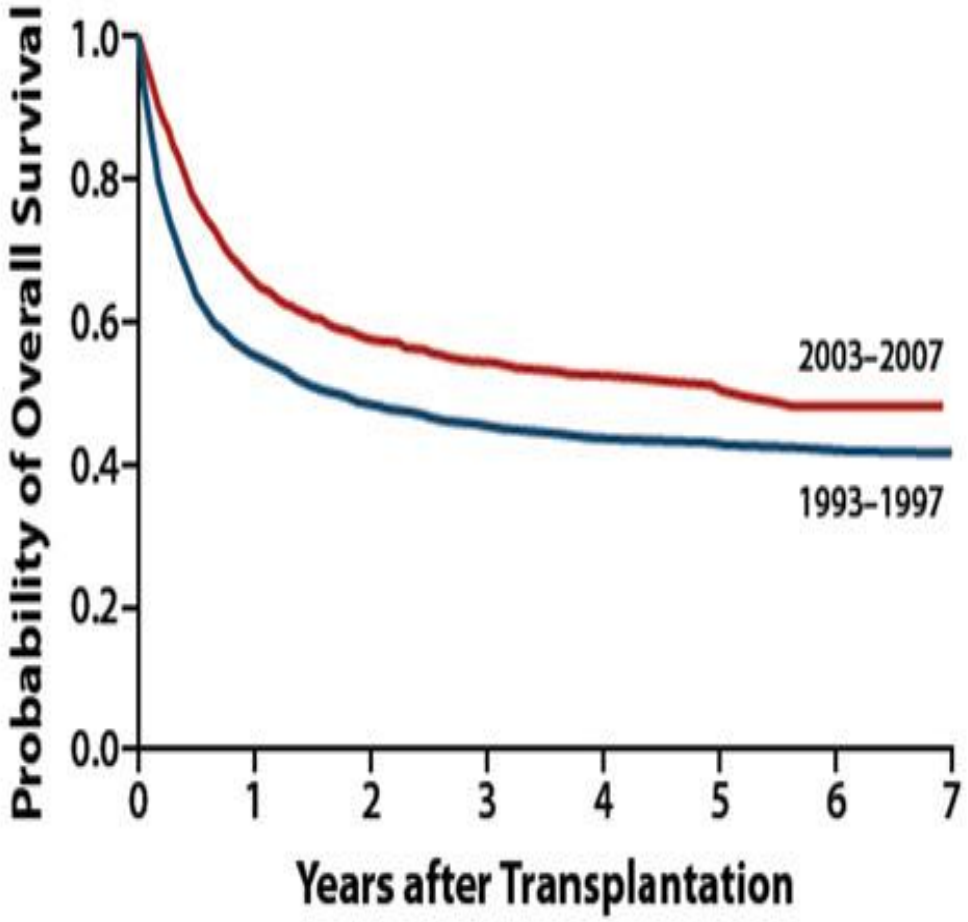





Manage complications
Reduce Transplant toxicity
SUCCESSFUL ALLOGENEIC HAEMOPOIETIC CELL TRANSPLANT
Patient selection
Donor Selection
Prevent Rejection
Prevent GVHD but maintain GVL effect

Future Focus





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Questions?