



Haematopoietic Chimerism Analysis after Allogeneic Stem Cell Transplantation

Dr Ros Ganderton, Ms Kate Parratt, Dr Debbie Richardson,
Dr Kim Orchard and Dr Liz Hodges

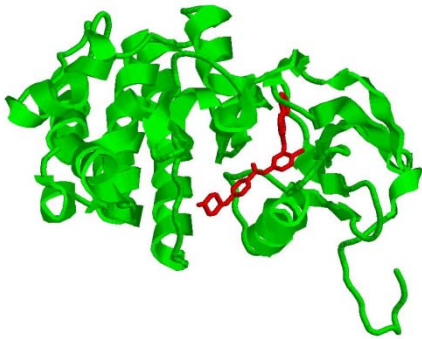
Departments of Molecular Pathology and Haematology
Southampton University Hospitals NHS Trust,
Southampton, United Kingdom

Haematopoietic Chimerism Analysis after Allogeneic Stem Cell Transplantation

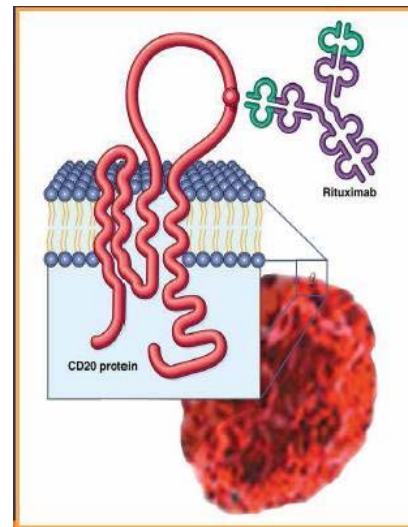
- The role of Stem Cell Transplants in haematological disease.
- The importance of monitoring the success of donor engraftment.
- The value of molecular monitoring.
- The improvement of molecular monitoring.

Leukaemia prognosis has improved through better treatment

- Existing regimes of Chemotherapy have been tailored to individual patients for improved patient management.
- Effective ‘magic bullet’ drugs have been developed to target specific diseases *eg.* Chronic Myeloid Leukaemia (CML).



Tyrosine kinase inhibitors targeting the oncogenic fusion protein in CML



Engineered monoclonal antibodies target surface markers on cancer cells

Stem Cell Transplants represent the only curative treatment for many leukaemia's

- Stem Cell Transplants are predominantly used as part of the treatment for certain types of malignant diseases which involve the bone marrow (leukaemia, lymphoma or myeloma).
- Stem Cell Transplants are also the only curative option where:
 - There is failure of the bone marrow to produce blood cells without obvious cause (aplastic anaemia).
 - There is an otherwise incurable inherited disease (severe forms of thalassaemia and immune deficiencies).

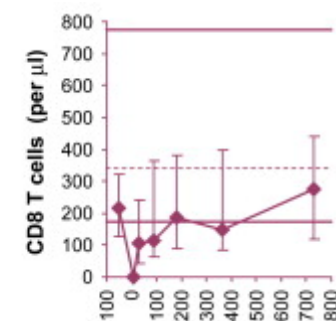
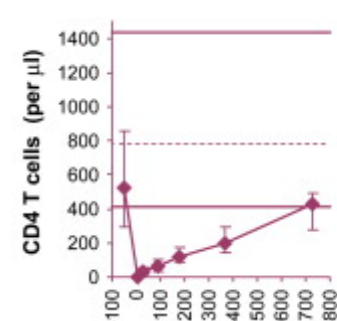
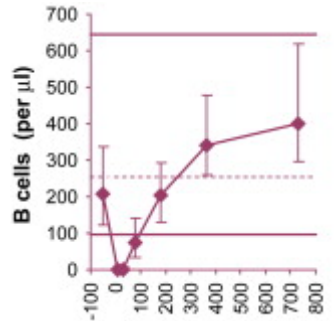
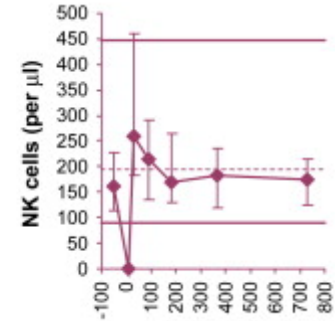
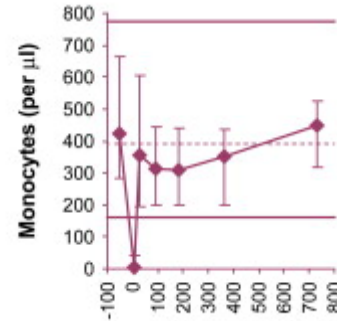
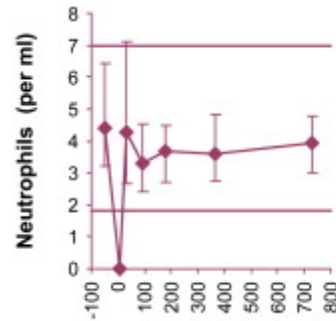
Monitoring engraftment is a key part of post-transplant treatment

- Stem cell transplants patients are vulnerable to infection after myeloablative therapy - they have none of their own immunity.
- Drug treatment and donor derived immune cells play a role in graft vs. host disease.
- It is critical to monitor immune reconstitution and engraftment.

Monitoring engraftment is a key part of post-transplant treatment

Monitoring the return of white blood cells in the peripheral blood –

The first signs of successful engraftment are indicated by the recurrence of neutrophils, monocytes and erythrocytes in the peripheral blood



Monitoring engraftment is a key part of post-transplant treatment

- Molecular Monitoring plays a key role.

PCR-based techniques provide the dual advantages of:

Sensitivity and **Specificity**.



- Change can be detected before evident morphologically.
- Small quantities of tissue can be used.
- Genetic differences between donor and recipient can be exploited to track the success of donor engraftment.

Monitoring donor 'chimerism' can be used to assess the success of engraftment

- After stem cell transplant, engraftment can be assessed by monitoring the proportions of donor and recipient cells in the blood.
- Determining the mix of cells, or 'chimerism', provides useful information for the scheduling of specific therapeutic interventions such as:
 - The withdrawal of immunosuppressive drugs.
 - Administration of donor lymphocyte infusions.



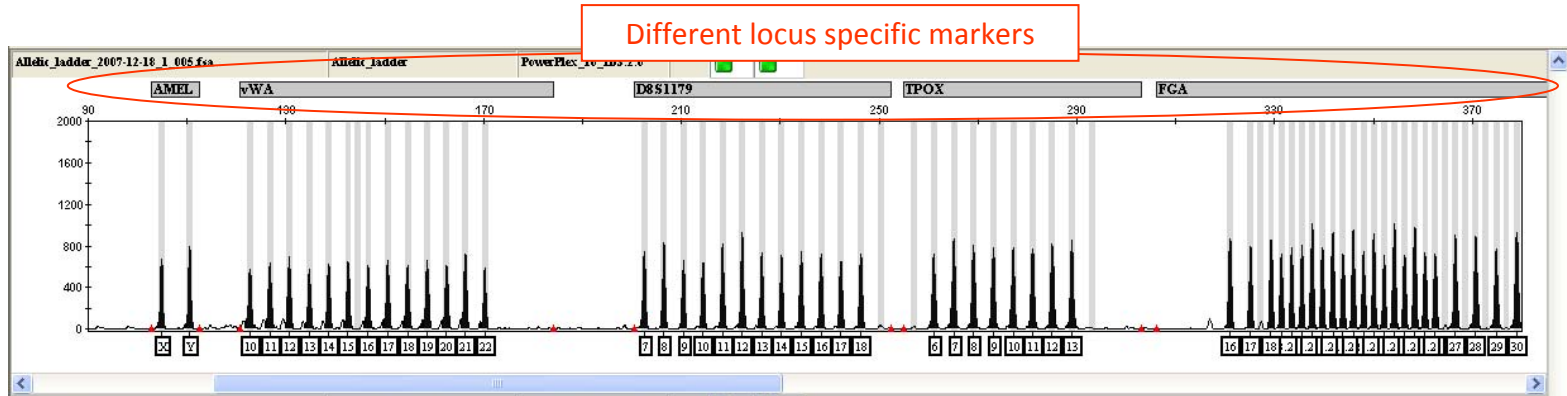
The chimera of Greek mythology was said to possess the head of a lion, the body of a goat and the hind part of a dragon. Despite these disparate components, the chimera was functional, able to spit fire and terrorize Asia Minor until its demise.

Donor /Recipient chimerism can be assessed using simplified genetic fingerprinting

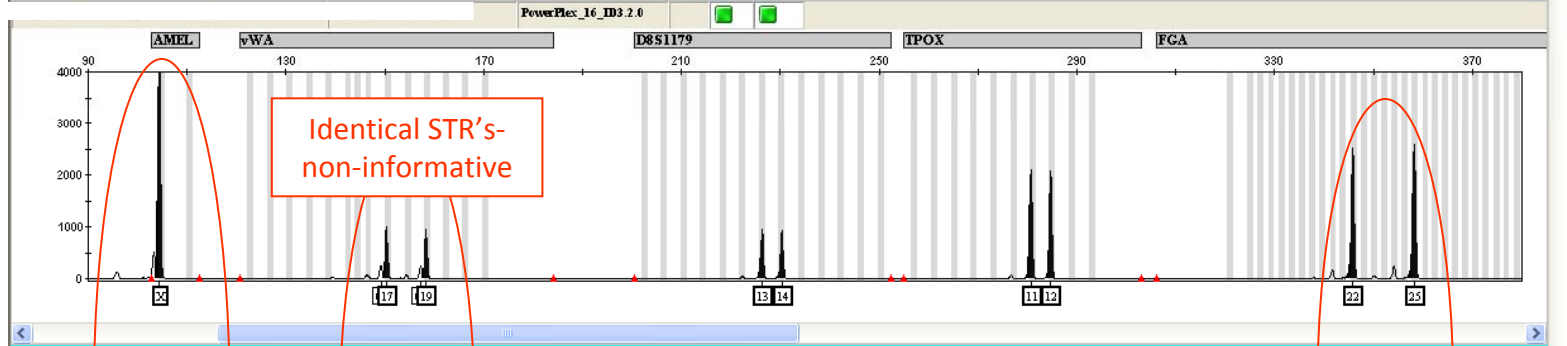
- Early methods of chimerism analysis included:
 - Cytogenetics
 - Isoenzyme analysis
 - Blood group phenotyping
 - Sex chromosome differentiation using fluorescence in situ hybridization (FISH)
- PCR-based methods rely on the amplification of highly polymorphic repetitive DNA sequences:
 - short tandem repeats (STR)
 - Variable number of tandem repeat (VNTR) sequences.

Short tandem repeats (STR's) are chosen to uniquely define the donor and recipient

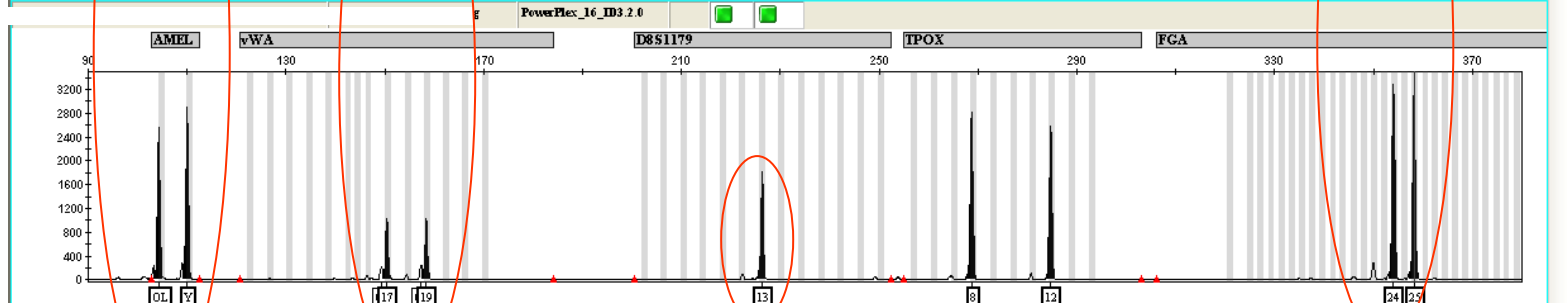
Allelic ladder showing all polymorphic variants



Recipient STR's



Donor STR's



Sex chromosomes

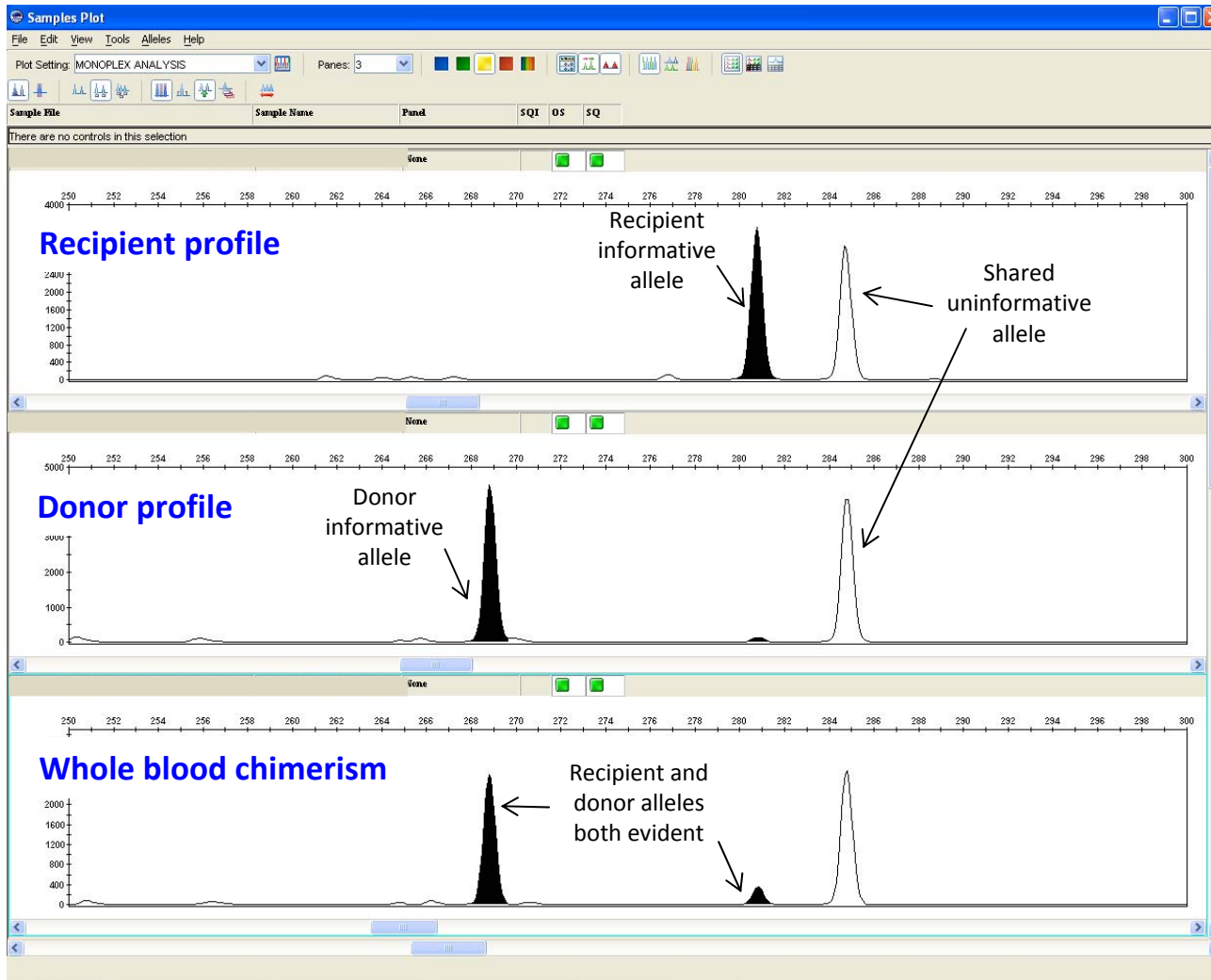
Homozygous marker

One Informative and one non-informative STR

Whole blood chimerism analysis

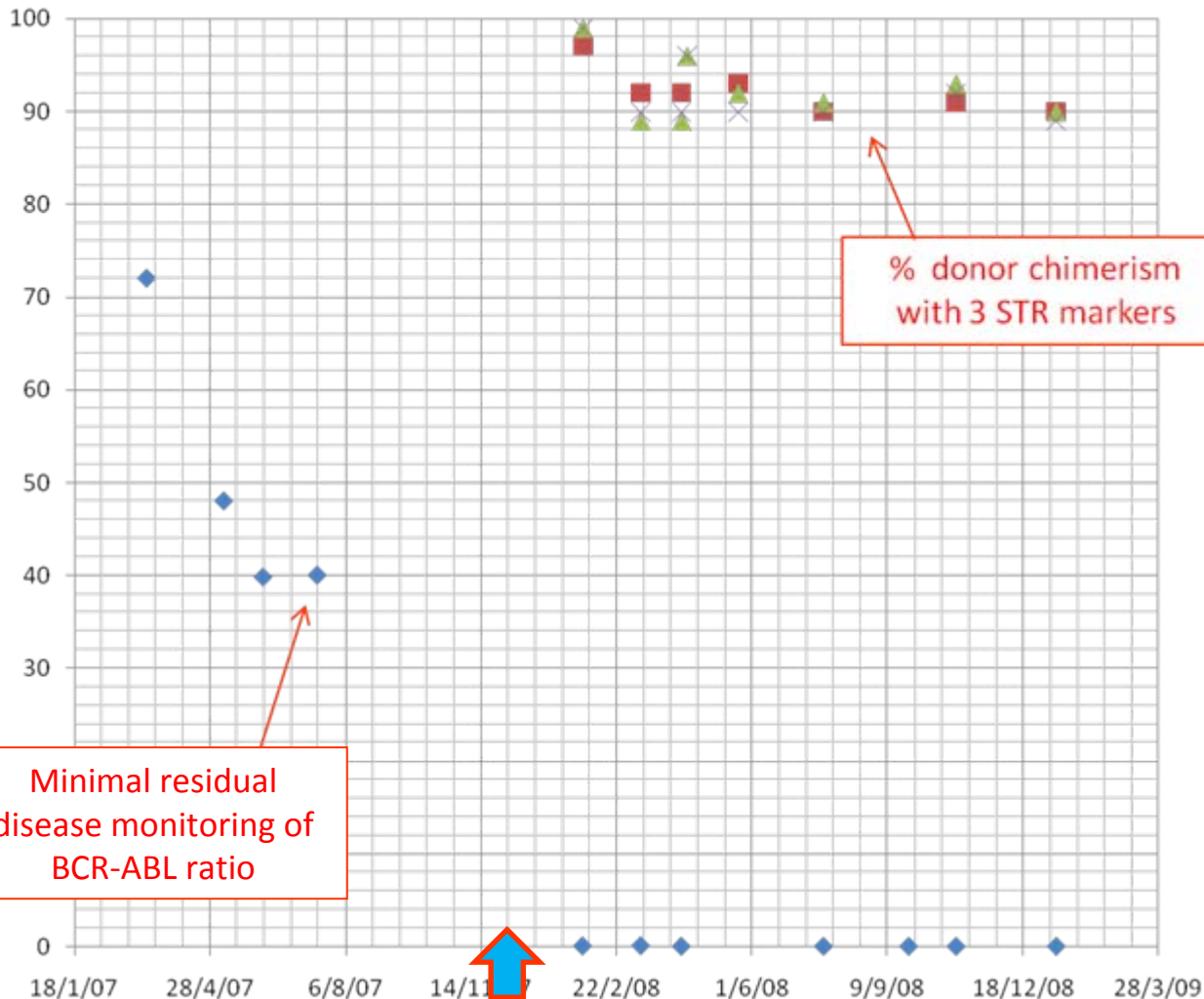
- Blood samples are taken at routine points post transplant, or where there is suspicion of relapse.
- DNA is extracted from EDTA blood sample using a magnetic purification method (Qiagen EZ1).
- PCR reactions using three STR markers are set up using a commercially available forensics kit (Promega PowerPlex16 Monoplex System).
- The differently sized fluorescent PCR products are detected and analysed on a capillary system genetic analyser (Applied Biosystems 3130xl).

Whole blood chimerism analysis



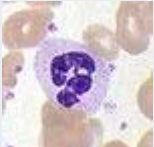



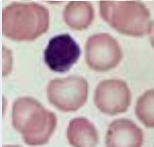
- An assessment of the proportions of donor and recipient chimerism can be calculated from the Capillary analyser output.
- Chimerism value calculated from peak height and area.
- Only qualitative or semi-quantitative assessment.

Tracking donor engraftment with whole blood chimerism analysis



- Patient refractory to conventional treatment for CML.
- Post transplant: Chimerism analysis monitors efficacy of engraftment. Minimal residual disease monitoring confirms treatment of primary disease.

Whole blood chimerism analysis has a high discrimination rate but a limited sensitivity

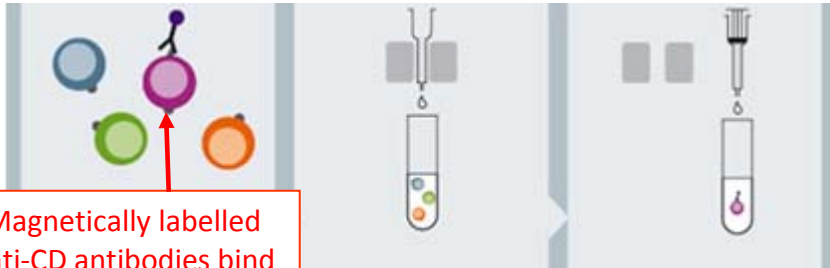
White cell type		Approx. % in adults
Neutrophils		54-62%
Eosinophils		1-6%
Basophils		<1%
Monocytes		2-8%
Lymphocytes		25-33%

- The whole blood analysis has a moderate sensitivity of 1-5%.
- If blood components are sorted in to their specific lineages the sensitivity can increase 10-100 fold.
- Effective enrichment of specific populations of cells, like B cells, T cells or early stem cells from the background of mature granulocytes.



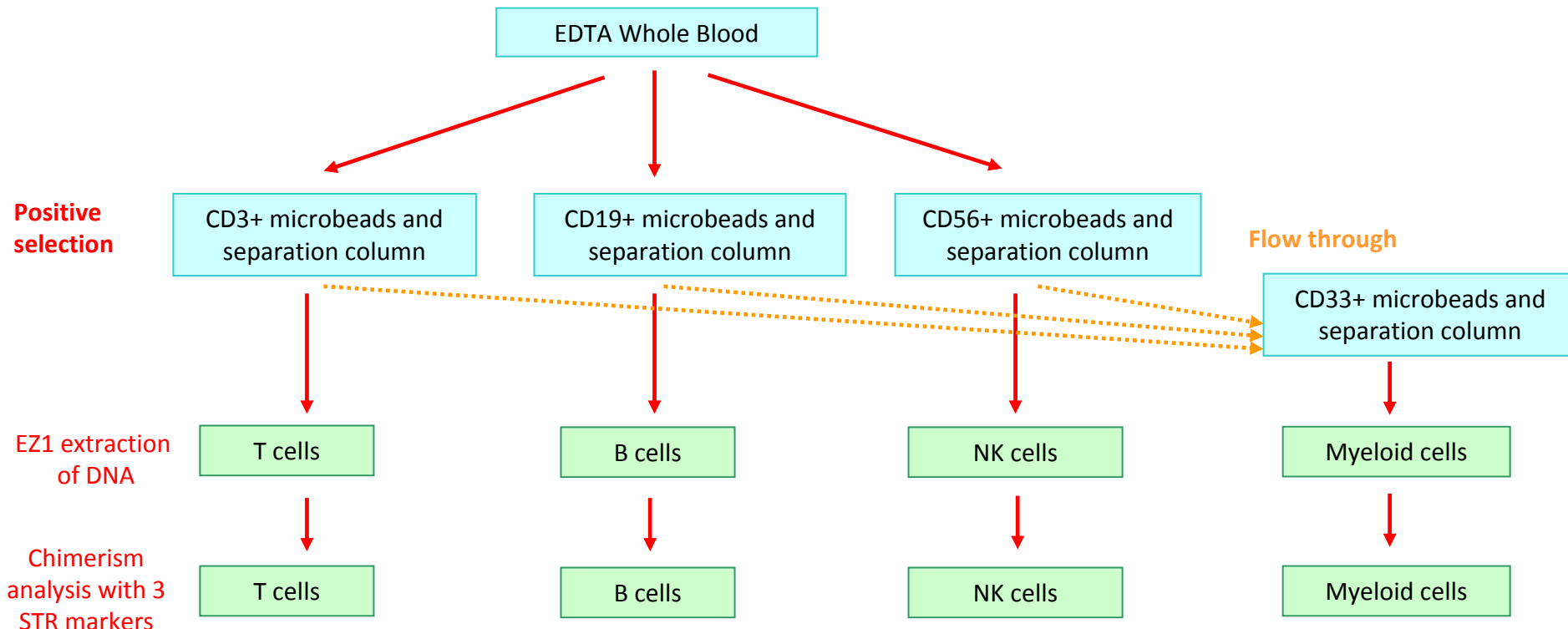
Lymphocyte class	Proportions
CD4+ 'Helper' T cells	46% (28-59%)
CD 8+ 'Cytotoxic' T cells	19% (13-32%)
B cells	23% (18-47%)
NK cells	7% (2-13%)

Lineage-specific chimerism analysis



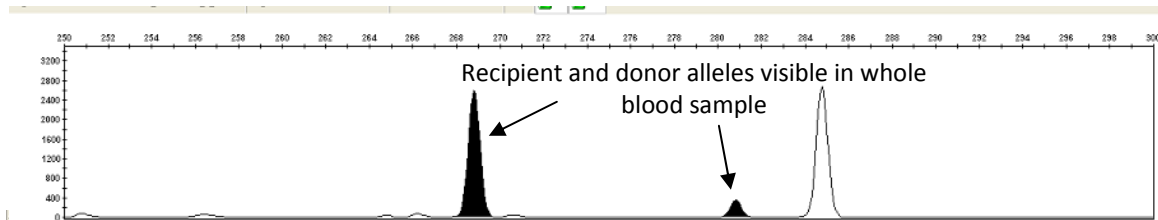
Magnetically labelled anti-CD antibodies bind specific cells

The separate leukocyte lineages are isolated by cell separation using AutoMACS[®] immuno-magnetic separation technology

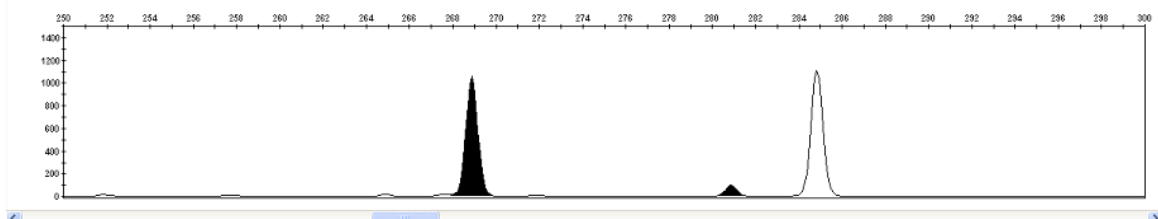


Lineage-specific chimerism analysis

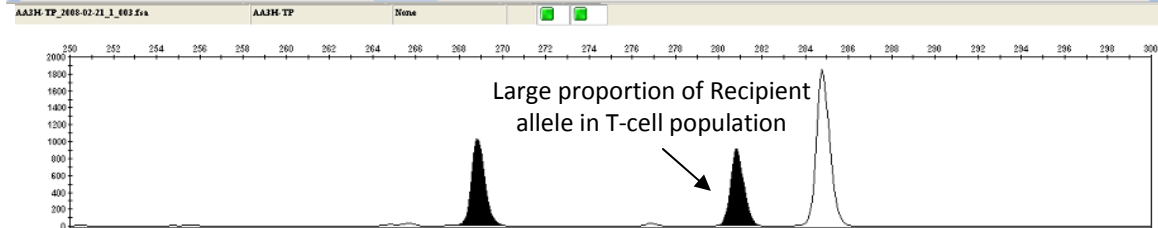
Whole blood chimerism



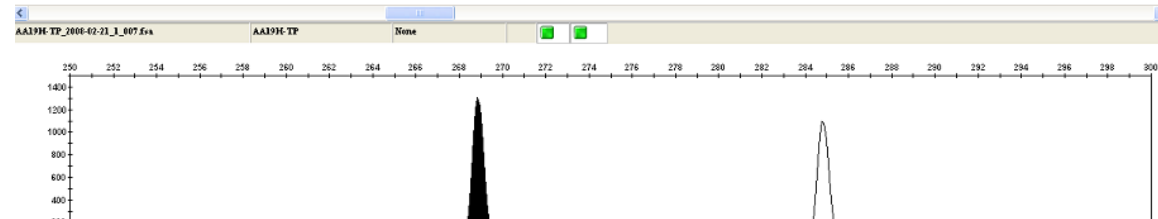
CD33 Myeloid fraction



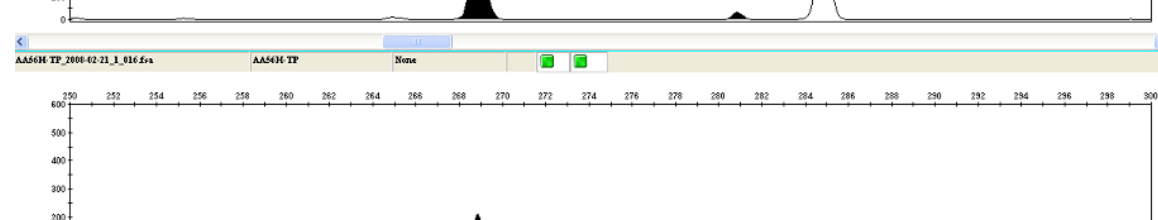
CD3 T-cell fraction



CD19 B-cell fraction



CD56 NK fraction



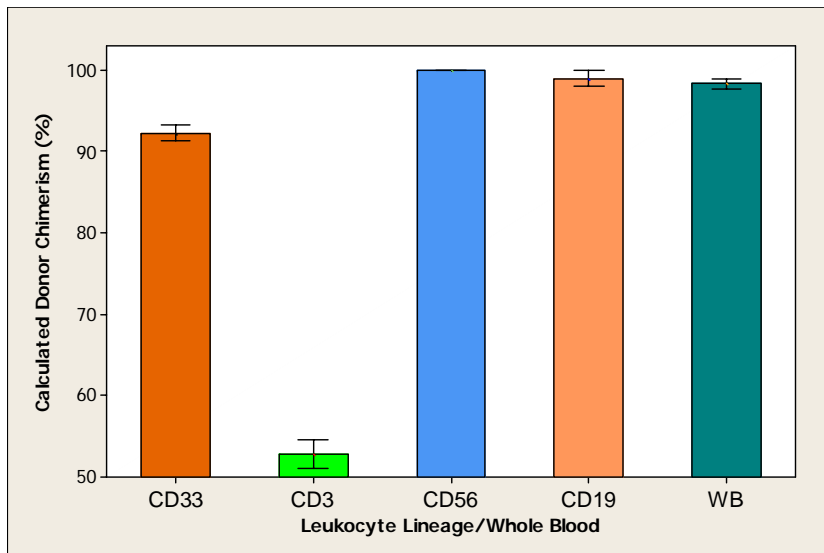
Lineage-specific chimerism analysis

- Lineage-specific chimerism analysis represents a valuable adjunct to whole blood studies.
- Due to increased sensitivity, it can be used to reveal mixed chimerism in specific leukocyte populations that are masked in the whole blood analysis.
- It can provide information about reoccurrence of the original clonal disease.
- Information about the relative proportions of donor and recipient T-cells is important in understanding the dynamics of engraftment and predicting graft vs leukaemia and graft vs host effects.

Lineage-specific chimerism analysis: a small scale study

- Extended validation tests using control samples indicated chimerism could be accurately assessed in the difference leukocyte lineages.
- Of eight patients studied, two patients displayed full donor chimerism in both the whole blood and lineage-specific analysis.
- Three patients with whole blood donor chimerism with less than 100% showed more pronounced fluctuations in lineage-specific donor chimerism.

Lineage specific chimerism analysis: an interesting case study



- Patient received a stem cell transplant after refractory chronic myeloid leukaemia (CML).
- Consistently showed whole blood donor chimerism of 90-95%.
- In a snap shot lineage specific analysis, patient displayed a similar level of donor chimerism in the myeloid fraction, but a more significant decrease in CD3 (T-cell) fraction.
- Result gives useful information as to whether the chimeric state is due to a recurrence of the original myeloid (CD33) clone.

AND

- The low donor CD3 value could also provide evidence of the success of the engraftment - reduced T-cell chimerism is strongly associated with graft failure.

Chimerism analysis – future developments

- Sensitivity can be improved to 0.1% -0.0001% with lineage specific analysis; advantage offset by increased workload and need for specialist equipment.
- This level of sensitivity can also be achieved with a chimerism method based on single nucleotide polymorphisms (SNP's) analysis by real time PCR.
- Early reports have indicated SNP-PCR is superior to STR-PCR in predicting relapse.
- Although the sensitivity of donor chimerism is enhanced, the discriminative capabilities of SNP-PCR may be more limited.
- With mixed chimerism, it may be important to use SNP qPCR in conjunction with STR-PCR to maximize predictive capabilities for transplant complications.

Haematopoietic Chimerism analysis – conclusions

- Mixed chimerism by STR PCR analysis in transplants performed for malignant disease can:
 - Signal the reappearance of malignant cells.
 - Give a measure of the efficiency of engraftment.
- Lineage-specific chimerism analysis is more sensitive and can:
 - Reveal mixed chimerism in specific leukocyte populations that are masked in whole blood analysis.
 - Give information about the relative proportions of donor and recipient T cells helping to understand the dynamics of engraftment.
- Current technologies can't always distinguish between:
the absence of engraftment or delayed engraftment
OR,
disease relapse and delayed engraftment.
SO,
timely use of lineage-specific chimerism and more sensitive qPCR based analysis methods could offer improved therapeutic benefits of stem cell transplants.

Thanks to:

- Kate Parratt, Department of Haematology, Southampton University Hospitals NHS Trust
- Department of Molecular Pathology, Southampton University Hospitals NHS Trust
Dr Shaoli Zhang and Dr Liz Hodges.
- Bone Marrow Transplant Unit, Southampton University Hospitals NHS Trust
Dr Kim Orchard, Dr Deborah Richardson, Dr Kate Hill and Sister Joan Newman.
- Research project
funding from :

