Department of Clinical Haematology
Oxford BMT Programme

The Diagnosis and Management of Primary and Secondary Graft Failure after Haemopoietic Stem Cell Transplant

Background

Post-transplant engraftment is defined by a sustained neutrophil count > $0.5x10^9$/l. The day of engraftment is the first day of 3 consecutive days with a neutrophil count greater than $0.5x10^9$/l. Engraftment typically occurs 20-25 days after bone marrow infusion in allogeneic sibling BMT, 15-20 days after peripheral blood stem cell infusion and 23-35 days after unrelated cord blood infusion (UCBT). Adequate erythroid and megakaryocytic engraftment usually follows but in some instances, more commonly in VUD (volunteer unrelated donor) patients, platelet and red blood cell support may be required for a prolonged period post-transplant. Failure of engraftment occurs in 5 to 10% of VUD BMT patients and approximately 1% of sibling BMT patients, but 15 to 20% after UCBT.

The frequency of graft failure is increased in both groups by the presence of HLA mismatch, low stem cell dose, T-cell depletion and reduced intensity conditioning regimens.

Term Definition

Neutrophil Recovery	First of 3 successive days with an absolute neutrophil count (ANC) of 0.5 x 10^9/L after post-transplantation nadir.
Platelet Recovery	First of 3 consecutive days with a platelet count of 20 x 10^9/L in the absence of platelet transfusion for 7 consecutive days.
Graft Rejection	Immune-mediated process where the recipient's immune system attacks the donor cells, preventing engraftment.
Primary Graft Failure	Lack of achievement of ANC 0.5 x 10^9/L by day +30 for PBSC or unstimulated BM, and by day +42 for UCB, with associated pancytopenia.
Secondary Graft Failure	Decline in haematopoietic function (involving haemoglobin, platelets, or neutrophils) after initial recovery, necessitating blood products or growth factor support.

Poor Graft Function	Frequent dependence on blood and/or platelet transfusions and/or growth factor support in the absence of other causes like disease relapse, drugs, or infections.
Donor Chimerism	Full chimerism: >95% donor cells; Mixed/Partial chimerism: 5-95% donor cells; Absent chimerism: <5% donor cells, for both myeloid and lymphoid lineages.

Primary Graft Failure

This is defined by the absence of any haematological function of the graft, and is characterised by failure to achieve a neutrophil count > 0.5 x 10^9/L within 28 days of stem cell infusion (recognising that the neutrophil count may be > 0.5 x 10^9/L in the first few days after stem cell infusion reflecting residual host hematopoiesis).

Secondary Graft Failure

This is defined as graft failure after evidence of donor engraftment. After initial evidence of neutrophil recovery the count falls below 0.5x10^9/l. This is almost always accompanied by significant thrombocytopenia (platelets < 30x10^9/l) and anaemia. It is important, although sometimes difficult, to exclude all other causes of pancytopenia, e.g. infections (parvovirus, CMV, HHV-6), drug toxicity, GvHD and hypersplenism).

Diagnosis of Graft Failure

A lack of a sustained engraftment as defined above. Usually, in graft failure the neutrophil count will be < 0.1 x 10^9/L. Assessment of primary graft failure will usually be carried out around day +25 to +35. Graft function should be assessed by a bone marrow aspirate and trephine biopsy. Samples of bone marrow should be sent for cytogenetic analysis and / or Fluorescent In-Situ Hybridisation (FISH) which is particularly useful in patients with a sex mismatched transplant or where there is a molecular marker such as BCR-ABL.
FISH is a rapid technique and can be performed on interphase cells (i.e. cell division is not a requirement).
A bone marrow and/or peripheral blood sample should be sent to the Molecular Haematology Laboratory for chimerism studies. Chimerism analysis can be performed by Short Tandem Repeat (STR) analysis (sensitivity of 1-5%). Lineage specific chimerism, i.e. STR analysis performed on DNA of sorted peripheral blood T cells or disease specific cell subsets such as CD34 is more sensitive then whole blood chimerism. In particular, a decrease in donor T-cells specific chimerism is highly predictive of pending graft failure

whereas an increase in host-derived CD34 expressing cells can predict pending relapse of a stem cell disorder.
For whole blood chimerism, 4+ml of EDTA PB should be sent.
For T-cell specific chimerism, 8-20 ml of EDTA PB
For other lineages: send 4+ ml of BM aspirate.
As a general rule the larger the volume of the sample size provided to the laboratory the higher the probability of success of the assay, particularly if sorting of cell subsets is required.

Management of Primary Graft Failure

Patients with neutrophils < 0.1 x 10^9/L and no evidence of haemopoiesis on the marrow trephine should be considered for an urgent second transplant using the same or an alternative donor (PBSC/BM/Cord). The conditioning to be used should be discussed with the responsible consultant. Autologous cells can be reinfused as a rescue if available.
The morbidity and mortality in patients with graft failure is high. G-CSF can be prescribed to try and increase the neutrophil count although there is no evidence base to support this.

Secondary Graft Failure

Although very rare after an HLA-identical sibling allo-SCT, secondary graft failure occurs in up to 5-10% of patients undergoing VUD SCT. Potentially reversible causes of graft failure should be considered and sought.
Bacterial sepsis can cause severe but often transient bone marrow suppression.
Viral infections such as CMV should be considered.
Drug toxicity (e.g. co-trimoxazole) may be a contributor and poor graft function can be seen in patients with graft versus host disease and hypersplenism.
If secondary graft failure is severe and sustained assessment of marrow function should be carried out as for primary graft failure above.

Management of Secondary Graft Failure

A second allograft from the original donor, if performed early, can be effective in restoring marrow function. Chimerism studies may help determine whether further immunosuppressive conditioning is required (i.e. it may not be necessary if there is predominantly donor lymphopoiesis). If there is co-existent GvHD or a high- risk of developing severe GVHD, the new stem cell product may require T-cell depletion. PBSC, rather than bone marrow stem cells, are usually requested since they appear to engraft earlier.

Check for the presence of donor specific anti HLA antibodies in the patient

Audit
These processes are subject to the OxBMT audit programme.

Authors
Tim Littlewood, BMT Programme Director, Version 1, 2010

Circulation
NSSG Haematology Website

Reference
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Review

Name	Revision	Date	Version	Review date
Prof V. Rocha Professor of Haematology	Minor amendments	Jan 2013	1.1	Jan 2015
Dr Littlewood, Haematology Consultant Dr Angela Hamblin, Haematology Specialist Registrar	Minor amendments, clarity Laboratory information	Feb 2017	1.2	Feb 2019
Dr James Davies, Haematology Consultant	Minor amendments	Oct 2020	1.3	Oct 2022
Dr Andy Peniket, Haematology Consultant	Addition of definitions	Oct 2024	1.4	Oct 2026