

P062

Effect of White Cell Concentration on Autologous Stem Cell Leukapheresis Products Stored Overnight Prior to Cryopreservation

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Aim

There is some uncertainty whether high white cell concentration in a leukapheresis stem cell product stored overnight prior to cryopreservation has a deleterious effect on the viability of stem cells. This study was performed to assess the effect of white cell concentration on the viability of autologous stem cell leukapheresis product stored overnight prior to cryopreservation.

Method

Peripheral blood stem cells were stored overnight at 4-6°C in the stem cell collection bag and processed next day. CD34 cell counts were performed on a fresh specimen representative of collection bag kept at room temperature within 24 hours. The effect of harvest white cell count (WCC) at various cut points was studied by comparison of viable CD34 cell counts (BMT network NSW method) on fresh leukapheresis product with viable CD34 cell counts on thawed samples. Analysis was performed using Stata 10 software.

Results

Thirty-one leukapheresis products were stored overnight at 4-6°C with median WCC of 254.4 x10⁹/L (range 50.4-708) and median storage duration of 19 hours (range 9-24). Regression analysis demonstrated a linear relationship between log of fresh CD34 cell count and log of thawed CD34 cell count. The log of cell concentration adjusted for the log of fresh CD34 cell count was borderline significant in predicting the thawed viable CD34 cell counts (t=1.91, p=0.066). ANCOVA analysis at white cell concentration cut points of >200 x 10⁹/L (n=21), >250 x 10⁹/L (n=16) and >300 x 10⁹/L (n=8) demonstrated no significant effect of white cell concentration on thawed viable CD34 cell counts at lower WCC (WCC >200 p=0.36, WCC >250 p=0.36) but was borderline for higher WCC > 300 (p=0.056).

Conclusion

Our results indicate that higher white cell concentration does not have a definite significant effect on the autologous stem cell leukapheresis products stored overnight up to 24 hours at 4-6°C. However further study with larger numbers is required to exclude this possibility.

No conflict of interest to disclose

P063**Outcomes and Management of Hodgkin Lymphoma Patients in Western Australia Following Autologous Stem Cell Transplantation****Clare Harma¹, Julian Cooney¹, Andrew McQuillan², Brad Augustson³**¹ *Haematology Dept, Royal Perth Hospital,* ² *Haematology Dept, Fremantle Hospital,*³ *Haematology Dept, Sir Charles Gairdiner Hospital***Aim**

To review patient outcomes and management of patients following autologous stem cell transplantation for Hodgkin Lymphoma in Western Australia.

Method

Case review of patients in Western Australia treated with autologous transplantation for Hodgkin Lymphoma, and a literature review- Despite advances in the use of combination chemotherapy, radiation therapy, imaging techniques and patient care, many patients undergoing treatment for Hodgkin lymphoma have relapsed from their disease and required therapy with autologous transplantation. Subsequent relapses occur post autograft, and the optimal management at this stage is difficult and unclear. Salvage therapy is challenging, and options include further chemotherapy including novel agents, radiation therapy and allogeneic transplantation (utilising PET to confirm remission prior to non-myeloablative conditioning).

Result

We will report follow up of patients in Western Australia treated with autologous stem cell transplantation, including the relapse rate, overall survival and late salvage therapies. Various treatments were given to those relapsing including aggressive chemotherapy, palliative chemotherapy and radiotherapy. Four patients progressed to allogeneic transplantation, and one was treated with Panobinostat.

Conclusion

Autologous transplantation is successful in many patients in obtaining long-term disease control. Relapse post autograft for Hodgkin lymphoma is a challenging area, and treatment options include novel therapeutic agents and also allogeneic transplantation.

No conflict of interest to disclose

P064

Successful Cord Blood Transplant in Philadelphia Positive Acute Lymphoblastic Leukaemia (ALL)

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Aim

To review management options in Ph+ve ALL, including cord transplantation.

Method

Literature review and case report- Philadelphia positive (Ph+) ALL, when treated with chemotherapy alone has a five year overall survival estimated to be between 10-20%. Results with chemotherapy in combination with tyrosine kinase inhibitors have provided limited improvements. When available, matched sibling or matched unrelated donor haemopoietic stem cell transplantation (HSCT) may provide the best long term disease control. Partially matched donors and cord blood sources of stem cells are being increasingly used in haematology. There are limited case reports to guide optimal management.

Result

We report the case of a 26yo female was diagnosed in December 2007 with Philadelphia positive ALL. She underwent induction with 3 cycles of Hyper CVAD and Imatinib. A suitable cord stem cell source of 3.14×10^7 TNC/kg (17×10^6 CD34) matched at 4 out of the 6 HLA loci was identified, with no other suitable donors found. In May 2008 she underwent a matched unrelated cord blood transplant, with TBI and Cyclophosphamide conditioning and she continued Imatinib. GVH prophylaxis was Cyclosporine and Mycophenolate. The patient developed early severe graft versus host disease affecting the skin liver and gut. This was treated with intravenous Methylprednisolone, Etanercept, Basiliximab and mesenchymal stem cells (per MSC trial) with good response.

59 days post-transplant, Variable Number of Tandem Repeats (VNTR) PCR revealed donor engraftment and ongoing remission. Cytogenetics confirmed engraftment using sex chromosomes on day 99. Post transplant she developed *Pseudomonas aeruginosa* mastoiditis and otitis media, which was successfully treated, and a later episode of HSV. She later weaned off all medications and is currently very well, returning to work and has ongoing molecular remission.

Conclusion

Thus, cord transplantation may be a worthwhile option to provide long-term disease control in Ph⁺ ALL.

No conflict of interest to disclose

P065**Transplantation of Cord Blood – Volume Determination and Filtration at the Time of Infusion****David Ford**¹, Simon Cooper¹, Annette Trickett²¹ *Prince of Wales and Sydney Children's Hospitals, Randwick, NSW*² *Blood and Marrow Transplant Network NSW, Darlinghurst, NSW*

Since 2004, the volume of thawed cord blood (CB) units from 3 national and 8 international CB banks was measured before clinical transplantation. The thawed CB unit volume was measured with a 60 or 30mL syringe at the bedside before infusion. Data from 39 CB units demonstrated that the thawed volume was a median of 91% (range 76 to 127%) of the stated volume provided by the CB bank. There were insufficient CB units to ascribe any consistent volume discrepancy with any particular CB bank.

Many transplant facilities prefer to filter cryopreserved stem cell products before infusion, however this has proved difficult if the CB volume is also to be measured. The recent introduction of the Pedi-Syringe™ filter, a 60mL syringe fitted with a 150µm in-line filter connected to a transfer bag spike, has allowed this process to be performed. A validation study of Pedi-Syringe™ filter, performed using CB units unsuitable for the Sydney CB Bank, was performed to determine nucleated and CD34 cell loss after filtration via a Pedi-Syringe. Six thawed CB units, ranging from 20 to 45 mL, yielded a post-filtration median nucleated cell recovery of 111% (range 96 – 144%). Viable CD34 analysis, performed on 3 of these CB units, indicated a median recovery of 109% (range 92 – 131%) compared to the pre-filtration data.

This study demonstrates that (i) the frozen CB volume stated by the issuing bank is not always accurate, and (ii) that filtration of thawed CB through a 150µm syringe filter provides a convenient way of measuring the CB volume at the time of infusion that is not associated with significant loss of nucleated or viable CD34+ cells.

This research was supported by GenesisBPS who provided samples for the validation study of the Pedi-Syringe™ filter. The company had no role in analysing the data or preparing the abstract

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P066

FLT-3 and NPM1 Mutation Screening Using Capillary Electrophoresis

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Screening for mutations in NPM1 and FLT3, have been shown to be beneficial in predicting outcome for patients with cytogenetically normal AML.

Studies have shown that FLT3 Internal Tandem Duplications (FLT3-ITD) mutant to wild type allelic ratio has prognostic benefit, as patients with a high percentage of mutant alleles have an increased relapse risk and decreased overall survival.

We have validated a protocol to detect this mutation using capillary electrophoresis and GeneScan analysis of PCR fragment length. The GeneScan electropherogram produces peaks with specific band sizes for each product detected and the allelic ratio can be determined from the area under the peaks for each PCR product.

Previously, the method used to detect this mutation was PCR with analysis of product bands by agarose gel electrophoresis. GeneScan analysis is a more sensitive method for detecting mutations (as low as 2% of total DNA). The method is less time consuming and allows the allelic ratio and length of the ITD to be determined with much greater accuracy than agarose gel analysis.

NPM1 mutations indicate a better prognosis in the absence of FLT3-ITD mutations. Our previous method of mutation detection involved sequencing the NPM1 gene and analysing the sequence for mutations. We have now validated a protocol using fragment length analysis with capillary electrophoresis and GeneScan software. One set of primers are used to screen for the most common NPM1 mutations. This method is faster than the multi step sequencing protocol, but still retains the same level of accuracy and sensitivity in detecting mutations.

No conflict of interest to be disclosed

P067**Clinical Implication of Genetic Mutation Testing for FLT3 and NPM1 in AML Patients**

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A common mutation in patients with AML occurs in the *FLT3* and *NPM1* genes. AML patient mutations in *FLT3* are either Internal Tandem Duplication (ITD) seen in up to 30% or D835, characterized with a missense mutation, in approximately 7%. Approximately 25% have *NPM1* mutations.

Royal Adelaide Hospital AML patients were tested for *FLT3* -ITD/ D835 at diagnosis (95) and at relapse (16). The diagnostic method for detection of the *FLT3* - ITD and D835 mutations, is based on PCR amplification of the region of usual occurrence. Testing for the D835 mutation requires digestion of the PCR product with *EcoRV* while ITD mutations are determined by estimating the size difference of the PCR product on agarose gel. Mutations in *NPM1* are detected via sequencing of the PCR product of exon 12.

111 AML patients were studied: all were tested for ITD yielding 34 positives (31%) and 104 patients for D835 with 8 positive (8%). 84 patients were tested for *NPM1* mutations with 16 positives (19%). 12 patients were *NPM1/ FLT3* positive and 4 were *NPM1* positive/ *FLT3* negative. 58 of 99 patients had a normal karyotype.

Identification of the above mutations is useful for determining appropriate therapeutic options in normal karyotype AML. *NPM1* positive/ *FLT3* negative are the most favorable prognostic factor, with a high complete remission (CR) rate. Generally *FLT3* mutations are less favorable tending to respond better to allogeneic stem cell transplantation. In such patients transplantation should be considered in first remission because of the increased likelihood of relapse. These patients may be eligible for *FLT3* inhibitor clinical trials. *NPM1/FLT3* positive patients are considered to have an intermediate prognosis and CR after chemotherapy is likely to be achieved, although sibling transplants may be considered.

No conflict of interest to disclose

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P068

Does -80°C Storage of Haemopoietic Progenitor Cells-Apheresis Product Affect Haemopoietic Recovery?

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Introduction

Haemopoietic progenitor cells-apheresis (HPC-A) transplantation is widely performed to support high-dose chemotherapy for treatment of haematologic and solid tumours. In the autologous setting, HPC-A products are routinely cryopreserved and reinfused at a later date. Evidence of cross-contamination of Hepatitis B in stem cell product stored submerged in liquid nitrogen (LN₂) led to the recommendation of using vapour-phase storage for HPC-A products (Tedder et al). The Centre for Blood Cell Therapies (CBCT) at Peter Mac quarantines HPC-A products derived from sero-positive patients in a -80°C mechanical freezer. This however, does not conform to the Therapeutic Goods Administration requirement of storing HPC-A product at or below -140°C as per the British Pharmacopeia.

Methods

In this retrospective study, we reviewed post-transplantation haemopoietic recovery data for patients collected from 2001 onwards, whose product was stored at -80°C prior to transplantation, in order to validate our recommendation that products stored at -80°C are infused within 6 months of collection. Haemopoietic recovery was measured as time taken for neutrophil count to reach 0.5 x 10⁹/L and platelets to reach 20 x 10⁹/L unsupported.

Results

To date, 15 patients have been transplanted using cryopreserved HPC-A product stored at -80°C for between 20 days and 50 months (median 75 days). Haemopoietic recovery was achieved in all patients. The time to haemopoietic recovery following transplantation of -80°C product was compared to the median annual data for infusion with products stored in vapour-phase LN₂. Time to neutrophil recovery was unchanged at 10 days (p=0.3356); time to platelet recovery was slightly delayed at 13 days (p=0.0015) compared with 11 days.

Conclusion

Our study has shown that HPC-A stored at -80°C for up to 50 months can achieve haemopoietic recovery thus confirming that storage up to 6 months is appropriate. The potential for longer-term storage at -80°C warrants further investigation.

No conflict of interest to disclose

P069**Implementing an Effective HPC-A Collection Efficiency Monitoring System for Each Cell Separator****Sushil Narayan***Stem Cell Transplantation Programme, PA Hospital, Brisbane*

An effective HPC-A collection efficiency (**CE**) monitoring system for Cell Separators (**CS**) is essential to minimize procedure related risks to patients and maximizes cost effectiveness. This monitoring system should form part of the CS/Collection process validation and is a quality management system requirement. Recognising the limitations of CE measurement, the benefit of on-going quarterly CE monitoring verifies that each CS in-use is performing satisfactorily and problems are detected much earlier.

Validation of HPC-A collection procedure as part of commissioning 2 CS showed that median CE differed significantly (actual difference of > 20% for a quarter, n = 25, P value = 0.0273). Had the facility continued overall CE monitoring as done historically, the difference in CE between the 2 CS would not have been detected in a timely manner. As a result of CE monitoring, investigation and corrective action, the CS's preventive maintenance schedule has been updated by the supplier and there is now no difference in CE between the two CS under observation. The facility has implemented quarterly monitoring of CE for each CS as part of its KPI & Clinical Indicators programme.

We have also implemented a review process for CE < 35% using a set review template. This has resulted in changes to HPC-A collection procedures such as CS setting changes by the operator when harvesting patients with high WBC. Improved CE monitoring has worked well for our facility and may offer other facilities an early warning system for lower CE and poor performing CS. It has enhanced communications between apheresis staff, processing lab and the supplier. Additional benefits include improving the skills of the apheresis staff in instrument adjustment to improve harvest quality and consistency,

*No conflict of interest to disclose***A301**

P070

Safety and Efficacy of Autografts Containing Reduced Numbers (<math> < 2 \times 10^6 / \text{kg}</math>) of CD34⁺ Blood Stem Cells: A Comparison Study

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Aim

Whilst a minimum infusion of $2 \times 10^6 / \text{kg}$ CD34⁺ blood stem cells for an autologous BMT is generally regarded as safe practice, there is a clinical tendency to require up to $5 \times 10^6 / \text{kg}$ for an autograft on the supposition of enhanced patient safety. This study aimed to compare the efficacy and safety of autografts containing $< 2 \times 10^6 / \text{kg}$ CD34⁺ cells with autografts composed of higher CD34⁺ doses.

Methods

150 consecutive PBSC first autografts for haematological malignancies between 1998 and 2008 were divided into 3 groups based on the dosage of CD34⁺ cells. Engraftment kinetics and survival curves were assessed using Log Rank (Mantel-Cox) test, with all other factors scrutinised using Students unpaired t-test.

Results

	<math> < 2 \times 10^6 / \text{kg}</math>	2 - 5 x 10 ⁶ /kg	>5 x 10 ⁶ /kg
n	29	101	20
Median CD34⁺/kg	1.57 * [#]	2.68 * [^]	6.81 ^{^#}
Engraftment	29/29	101/101	20/20
Days Neut >0.5	11 * [#]	10 *	10 [#]
Days Neut >1.0	11 * [#]	11 *	10 [#]
Days Plat >20	18 * [#]	13 *	11 [#]
Alive @ Day + 30	27/29	100/101	20/20
Alive @ Day + 100	23/29 *	96/101*	19/20
No of Admitted Days	13 [#]	9	6 [#]

* [^] [#] significant difference ($p < 0.05$) between these groups

All patients achieved myeloid engraftment after reinfusion. Neutrophil engraftment with $< 2 \times 10^6 / \text{kg}$ CD34⁺ cells was delayed by up to one day compared to the higher doses, a statistically significant but clinically irrelevant finding. Platelet engraftment was delayed by up to a week in the low dose population, indicating that these patients may require additional blood product support. Importantly, transplant related mortality (Day +30) was not significantly different between the groups, however, the tendency of the low dose group to have more admitted days may be indicative of increased morbidity. Medical record review revealed the significant difference in Day +100 survival was due to progressive disease, rather than the autograft *per se*.

Conclusions

Successful and safe engraftment occurs after infusion with low numbers ($< 2 \times 10^6 / \text{kg}$) of CD34⁺ cells. Clinicians should anticipate slightly delayed neutrophil and platelet engraftment. There is no evidence to support the notion of high dose ($> 5 \times 10^6 / \text{kg}$) CD34⁺ cells as enhancing patient recovery and safety when compared to infusions between $2-5 \times 10^6 / \text{kg}$.

There is no conflict of interest to declare

P071**Allogeneic HSCT with Reduced Intensity Conditioning with Fludarabine and 2 Gy TBI Is Safe and Provides an Adequate Donor Engraftment in Patients with Acute Myeloid Leukaemia and Multiple Myeloma**

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Patients with AML older than 56 years have a very poor outcome. Most patients with multiple myeloma (MM) would die by about 5 – 7 years following diagnosis. Allogeneic HSCT offers a chance for cure, however is associated with significant toxicity. Success using fludarabine and 2 Gy TBI as a regimen in preparation for an allogeneic HSCT is reported. We report our experience in using this regimen in patients with AML (n=5) and MM (n=5).

Patients

All patients were conditioned with fludarabine 30 mg/m² from day -4 to -2 and 2 Gy TBI on day 0. Cyclosporin was commenced at 6.25 mg/kg PO BD from day -3 and mycophenolate mofetil at 15 mg/kg BD from day 0.

Five patients with AML (median age 60 years, M=4/F=1) in CR1 underwent allogeneic HSCT. Three received grafts from unrelated donors and two from siblings.

Five patients with MM (4 ISS stage III, 1 stage II, M=5/F=0, median age 51 years), after undergoing cytoreductive melphalan 200 mg/m² autologous stem cell transplantation underwent allogeneic stem cell transplantation as per above.

Results

All patients had >90% donor T cell chimerism by day +30. No day 30 or day 100 mortality was seen.

AML patients: No patients dropped platelet count below 20 x 10⁹/l and neutrophils below 0.2 x 10⁹/l or required platelet transfusion. No grade III-IV AGVHD was seen. At 6 months, two patients have relapsed.

MM patients: Two patients dropped neutrophils to <0.2 x 10⁹/l. No patient required platelet transfusions or developed grade III-IV AGVHD. One patient developed limited oral chronic GVHD. All except one are in ongoing CR1.

Conclusion

The Fludarabine/2 Gy TBI conditioned allogeneic HSCT is safe with no day 30 and day 100 mortality, requires minimal transfusion support, is associated with modest cytopenias and GVHD and the entire protocol can be delivered as an outpatient resulting in major pharmaco-economic benefit.

No conflict of interest to disclose

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P072

Mesenchymal Stromal Cell Culture : Improvements in Methodology

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Clinical studies have indicated that ex-vivo expanded mesenchymal stromal cells (MSC) may play a role in reducing the effects of graft versus host disease. Alternative media and cell factories were tested using normal bone marrow with a view to improving methodology. DMEM- low media supplemented with L-glutamine (DME-L) and DMEM-low glucose with Glutamax (DME-G) were trialed to determine the more effective media. (Glutamax is a stabilised form of L-glutamine).

Cells were cultured in two T175 flasks: one containing DME-L and the second DME-G. Both culture medias were supplemented with 10% FCS and 1% penicillin/streptomycin.

Superior results were obtained for DME-G when compared to DME-L. DME-G cultured cells achieved confluency within 18 days while DME-L media failed to achieve confluency in 26 days.

Flow cytometric analysis showed very low levels of mesenchymal markers for the cells cultured in DME-L compared to DME-G. Low levels of haematological markers were observed for cells cultured in both media types.

Clinical scale culture of MSC requires multiple flasks, considerable incubator space and is very labour intensive. A Nunc two layer cell factory was tested in parallel with T175 single layer flasks.

The cell factory with a surface area of 1264cm², was set up with 47.9x10⁶ mononuclear cells. 350 mls of DME-G + 10% FCS /1% penicillin /streptomycin was used.

A total of 14.14x 10⁶ MSC were obtained from the first passage. After three passages, the potential number of MSC was 84.31x 10⁶, a potential expansion of 5.96 from the initial passage.

We conclude that the use of cell factories is viable in the culture of MSC for transplant.

No conflict of interest to disclose

P073

Exploring the Long Term Impact of Haemopoietic Stem Cell Transplantation on Quality Of Life in Order to Better Inform Patient Care

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Aim

The Alfred has established a Late Effects Clinic (LEC) to assist in the management of long term survivors of haemopoietic stem cell transplantation. This study aims to highlight which long term effects on quality of life are prevalent amongst our patients in order to better target resources to meet their needs.

Method

The FACT-BMT quality of life questionnaire was administered to patients attending the LEC. This questionnaire has 50 questions that cover physical, social/family, emotional and functional domains as well as transplant specific symptoms.

Results

Thirty eight surveys were evaluated. Twenty three respondents had received an allograft and 15 an autograft. The median age was 55 years with an average of 5 years since transplantation (range 2-10). Overall quality of life was good with a mean FACT-BMT score of 119 (possible score 0-148).

Variables (min-max possible score)	Mean scores (range)
Physical well being (0-28)	23.2 (14-28)
Social well being (0-28)	23.7 (12-28)
Emotional well being (0-24)	20.6 (14-24)
Functional well being (0-28)	21.1 (8-28)
BMT well being module (0-40)	30.8 (220-39)
Overall FACT-BMT	119 (77-148)

The most common physical problems included lack of energy (82%), fatigue (72%), difficulty sleeping (29%), frequent infections (28%), blurry eyesight (26%), shortness of breath (24%) and skin problems (24%). Cognition was also impaired with 44% having significant difficulty with memory and 40% difficulty concentrating. Social/family well being was high with most feeling close to and supported by family and friends. Sexual health concerns were common with half of respondents indicating low satisfaction with their sex life and twenty percent were worried about fertility. Staff at the LEC clinic have been specifically trained in sexual health to tackle this issue. Emotional well being was also maintained with low scores for anxiety/depression and high scores for hope and confidence in staff. Some respondents worried that their condition would worsen (47%). Despite some respondents experiencing significant difficulties, none expressed strong regret at having undergone transplantation.

Conclusions

While overall quality of life is good amongst long term survivors of transplantation, there are areas that are impacted in the long term. Use of a quality of life tool can assist in highlighting areas of need to focus resource development and provision.

No conflict of interest to disclose

P074

Immune Reconstitution Post Haemopoietic Stem Cell Transplantation: A Comparison of Myeloablative and Reduced Intensity Conditioning Approaches

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Aim

To examine in detail the kinetics of immune reconstitution post allogeneic stem cell transplantation and compare the effects of myeloablative (MAB) and reduced intensity conditioning (RIC) approaches.

Method

Peripheral blood was collected pre transplant, D14, D30, then monthly to 6 months and 3 monthly thereafter. Flow cytometry was used to phenotype T, B and NK cells in detail.

Results

Thirty five patients have been enrolled with median follow up of 150 days (range 30-360). Twelve patients received myeloablative conditioning (MAB), 12 reduced intensity conditioning with fludarabine/melphalan/Campath (RIC-FMC) and 11 very reduced intensity conditioning with 2GyTBI +/- fludarabine (RIC-2GyTBI).

Early post transplant, the absolute number of NK cells fell, however they formed the majority of peripheral blood lymphocytes for MAB and RIC-FMC patients. In contrast, RIC-2GyTBI patient NK cell numbers remained steady. B cells were markedly depleted and recovery delayed until at least 9-12months.

T cell numbers showed recovery by D90-120, with RIC-2GyTBI patients reconstituting earliest. CD8⁺ subsets recovered before CD4⁺ (D90-120 vs D270-D360) for all groups. RIC-2GyTBI patients had more CD4⁺ cells than RIC-FMC or MAB patients and in particular CD4⁺CD45RA⁺ cells (naïve phenotype) showed higher numbers and appear to normalise by 12 months. RIC-FMC patients in contrast remained profoundly CD4⁺CD45RA⁺ cytopenic at 12 months.

RIC-FMC patients had the lowest T regulatory cells and most delayed recovery (D120). T regulatory cells were higher for the RIC-2GyTBI group and normalised earlier (D60). NKT cells are detectable but absolute numbers remained low at 12 months post transplant and longer follow up is warranted.

Conclusions

These data shed light on the complex nature of immune reconstitution post allogeneic stem cell transplantation. The conditioning received appears to have an impact on the kinetics of some lymphocyte subset reconstitution. Further studies into the impact on thymic function, chimerism and clinical outcomes are ongoing.

No conflict of interest to disclose

