



# BMTSAA Newsletter

Easter Edition 2002

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## Editorial

Wishing our members a Happy New Year and with Easter almost upon us, it will not be long until this year has passed us by. In my absence, I would like to thank Annette and Co. for getting the last newsletter together, while I was having surgery. I thank many of you for your best wishes for me during at time. I would have liked to see this edition to have made it to you before this year. However my organisational skills were a bit lacking around that time, so that was not the case. However as it's only possible to get this newsletter to you with the help of others old man time has his day. We all know how hard it is to get things done once December hits us.

'The Grapevine' has some brief information about a tragic laboratory accident that is so relevant to most of our working conditions. I have not reported this fully as the news report does not really explain how this unfortunate accident occurred. I will report on this incident when the coroner's report becomes available.

*David Ford  
Editor*

## BMTSAA PRESIDENT'S REPORT 2001

What a year (& a bit!) between BMTSAA meetings! It certainly was a jam-packed time filled with some incredible highs & lows.

Whether at the actual venues or watching on TV, the Olympics were amazing. I have to admit that I was one of the people who felt completely 'non-plussed' about the whole thing prior to the occasion & was hoping to be out of Sydney at the time.

What a mistake that would have been. From my first experience of being part of it all, I was hooked & then went to every event that I could get tickets for. When no tickets were available, the next best thing was to view on the big screens around the city. Olympic fever then turned into Paralympic

fever, with the latter athletes producing even more inspiring performances.

On the flip side of the coin were the terrorist attacks on September 11<sup>th</sup>, and the associated repercussions. I think that we were all amazed that anyone would do such a thing and no doubt those images will be firmly imprinted in most people's minds for a very long time.

Interspaced with these significant events were many other happenings in the world, which were of variable importance to individual people. Behind all these things are a team of people who make it happen.

I would like to acknowledge and thank the team of people that work behind the scenes of the BMTSAA to make it happen:

- Gail Lazarro puts in a phenomenal amount of work as secretary. The latter role is clearly the linchpin of any society and Gail has generously filled this position since the foundation of the BMTSAA.
- Nancy Messino has managed to keep the financial affairs of the society in check since its initiation. Due to Nancy and others, the BMTSAA can now put on a yearly scientific meeting, pay the expenses of invited speakers, and give out a number of awards. All members are encouraged to apply for these awards which can only continue if they attract a good number of applicants.
- Dianne Tucker has played an active role in the BMTSAA since the start, but even more so during her period as president. Under her presidency the BMTSAA grew and became a more professional organisation.
- David Ford continues to do a sterling job as editor of the BMTSAA newsletter. I now have first-hand experience of how hard it is to put this newsletter together & have praise for all our editors who have devoted so much time to this task. A plea goes out to all members to contribute any useful / funny/ quirky information or gossip.
- Cheryl Hutchins has set a new standard for the quality of BMTSAA meetings. A tremendous amount of organisation would have gone into producing a meeting that ran as smoothly as the one in Brisbane.
- Scott Ragg for managing the website. All are encouraged to visit.
- Finally I would like to thank all members for their continued support, and the scientific meeting speakers for presenting their data.

Happy New Year!

*Annette Trickett  
President BMTSAA*

**THE BONE MARROW TRANSPLANT  
SCIENTISTS ASSOCIATION OF  
AUSTRALASIA**

**MINUTES OF THE ANNUAL GENERAL  
MEETING OF THE BONE MARROW  
TRANSPLANT SCIENTISTS' ASSOCIATION  
OF AUSTRALASIA HELD ON WEDNESDAY  
24<sup>TH</sup> OCTOBER 2001 AT THE BRISBANE  
CONVENTION CENTRE, SOUTH BANK,  
BRISBANE, AUSTRALIA AT 3.15 PM.**

**1. WELCOME TO MEMBERS**

The president, Annette Trickett welcomed members to the Annual General Meeting of The Bone Marrow Transplant Scientists Association of Australasia.

**2. PRESENT**

*Vicki Antonenas, Lisa Barrow, Mike Bell, Simon Bol, Sue Carnoutsos, Annabella Chang, Pam Dyson, Lisa Fava, Karen Grimmett, Helen Hanlin, Cheryl Hutchins, Kylie James, Gail Lazzaro, Josephine McAlonan, Luiza Mints-Kotowska, Heike Mumford, Steve Noga, Emanuel Raniolo, Robyn Rodwell, Rosemary Sparrow, Judy Stevens, Marian Sturm, Debra Taylor, Annette Trickett, Dianne Tucker, Emilia Varga, Dominic Wall, Lyanne Weston, Nicole Wiggins.*

**3. APOLOGIES**

Wade Blackwood, Judy Bosio, Jamie Case, David Ford, John Ivey, Kerrie Jones, Michael Lees, Nancy Messino, Scott Ragg, Alison Rice, Carole Smith, Boon Yap.

**4. REGISTRATION OF PROXIES**

Nicole Wiggins appointed by Beth Rees  
Nicole Wiggins appointed by Scott Ragg

**5. CONFIRMATION OF THE  
MINUTES OF THE ANNUAL GENERAL  
MEETING OF THE BONE MARROW  
TRANSPLANT SCIENTIST'S ASSOCIATION  
OF AUSTRALASIA HELD ON MONDAY 24<sup>TH</sup>  
JULY 2000 AT THE HYATT REGENCY  
HOTEL, PERTH, AUSTRALIA AT 8.30 AM.**

Dianne Tucker moved that the members accept the minutes as being true and correct. Seconded by Rosemary Sparrow. Carried.

**6. OUTGOING PRESIDENT'S  
ADDRESS**

Dianne Tucker addressed the meeting, formerly ending her role as President. Dianne thanked the members and council for their support during her 4 year term and extended good wishes to the incoming President Annette Trickett.

**7. PRESIDENT'S REPORT**

Annette Trickett presented her report for 2001. This report is to be published in the next edition of the Bone Marrow Transplant Scientists Association Newsletter.

**8. TREASURER'S REPORT**

Gail Lazzaro read the financial summary for 2000-2001 on behalf of the Treasurer. The key items were:-

- Commonwealth Bank Account Balance being \$4,711.76
- Acknowledgment of Sponsors to the end of June 2001:- AMRAD, AMGEN, Gambro, Nat Tech, Taylor Wharton & Baxter.

The Treasurer advised that copies of the report would be available in the next edition of the Bone Marrow Transplant Scientists Association Newsletter.

**9. MEMBERSHIP COMMITTEE  
REPORT**

There was no membership committee for the period July 2000 to August 2001. Membership applications had been reviewed by Council. Gail Lazzaro read the recommendations of Council. Annette Trickett moved that the recommendations be accepted. Seconded by Nicole Wiggins.

**10. RATIFICATION OF NEW  
MEMBERS**

The following applicants were ratified as members of the Bone Marrow Transplant Scientists Association of Australasia.

<i>Scientific Members</i>	
Marian Sturm	Royal Perth Hospital
Josephine McAlonan	Royal Brisbane Hospital
Emilia Varga	Royal Brisbane Hospital
Judy Bosio	ARCBS – NW (Perth)

**Associate Members**

Luiza Mints-Kotowska	Peter MacCallum Cancer Institute
Graeme Chapman	Becton Dickenson
Lia Kubala	Canterbury Health Laboratories, Christchurch
Heike Mumford	Royal Hobart Hospital
Janine Davies	Pathcentre, Perth

**11. BUSINESS ARISING FROM  
PREVIOUS MINUTES**

*(a) ISHAGE Affiliation*

Annette Trickett invited Steve Noga, President of ISHAGE to address the meeting. Steve advised that discussions were taking place with regard to the possibility of a special ISHAGE membership opportunity for BMTSAA members. The membership may incur a reduced fee and would include all membership privileges with the exception of a printed copy of Cytotherapy. An electronic copy would be accessible.

*(b) CD34+ Cell QAP*

Annabella Chang advised the members that the CD34<sup>+</sup> CellQAP had commenced as a module in the Royal College of Pathologists Haematology QAP. She advised that there had been some minor problems with couriers which had been overcome. Annabella advised that both she and Dr David Ma continue to provide the coordination, sample preparation, result evaluation and feedback of the QAP while the RCPA perform the administration. New software is currently being trialed for data collation and reporting purposes.

*(c) Change to the Constitution*

The Secretary read the following proposed amendments to The Constitution. The proposed amendments were voted upon and accepted unanimously.

The Motions:-

To amend rule 1.4(c) of the constitution to delete

- Nomination of Chairman for elections.
- Declaration of vacancies, call for nominations.
- Election of new Councillors and Office Bearers.

To amend rule 2.1(b) of the constitution to "Nominations must be registered with the Secretary not less than 60 days prior to the elections."

To amend rule 2.1(f) of the constitution to "Nominations will be circulated to all members not less than 30 days prior to the elections."

To amend rule 2.2 of the constitution to add 2.2(d)

"Election of Office Bearers and Councillors shall be conducted by postal vote."

Proposed by Gail Lazzaro  
Seconded by Dianne Tucker. Carried.

*(d) ABMDR Courier Guidelines*

Cheryl Hutchins advised that these guidelines had been completed and sent to a number of centres performing allogeneic bone marrow transplantation for review. Cheryl advised that she could be contacted at the Royal Brisbane Hospital for information or the opportunity to comment.

**12. APPOINTMENT OF AUDITOR**

Gail Lazzaro moved that the Treasurer's advice be sought regarding the appointment and that the recommendation be approved by council. Seconded by Cheryl Hutchins.

**13. COMMITTEE APPOINTMENTS**

The President advised that Pamela Dyson had agreed to represent The Association at preliminary planning meetings for the HSAZ/ASBT conference in 2002. Annette Trickett proposed that she continue as Chairman of the Organising Committee. Pam advised that both Colin Story and Emanuel Raniolo may be able to assist. The following organising committee was proposed for the ASM in Adelaide in 2002:-  
Pamela Dyson (Chair)  
Colin Story  
Emanuel Raniolo

**14. GENERAL BUSINESS**

*(a) BMTSAA 2002*  
Pamela Dyson announced that the dates for the HSAZ/ASBT Adelaide Meeting were 9<sup>th</sup> to 12<sup>th</sup> September 2002 and that a fully integrated BMTSAA was proposed. Past conflict with the HSAZ/ASBT program was raised. Several members requested a separate day back to back

with the HSANZ meeting. The President asked that this be investigated.

**(b) Baxter Grant**

Gail Lazzaro announced that Baxter had supported The Association with a \$5,000 grant. Council had agreed to use the funds for two travel grants of \$2,500 each. It was anticipated that the grants would be awarded in March for use before the end of the 2001 – 2002 financial year.

**(c) BMTSAA Merck Sharp & Dohme Investigator's Award**

Annette Trickett advised that Merck Sharp & Dohme (formerly AMRAD) had generously sponsored the Annual Scientific Meeting by way of an \$1,800 Investigator's Award. The President encouraged all members to submit abstracts for future meetings to be eligible for such an opportunity.

**(d) Stem Cell Technologies Colony Assay Workshop**

Dianne Tucker advised that discussions were taking place with representatives from StemCell Technologies and the Australian distributor "Chemicon" regarding the possibility of a training workshop to be conducted at the Royal Children's Hospital in Melbourne. She asked for interested members to register their names with either herself or Scott Ragg.

**(e) CD 34 QAP Expert Panel**

Annabella Chang, Coordinator for the RCPA CD34<sup>+</sup> QAP, advised that the opinion of experts was required for a number of issues, the most urgent being to define acceptable limits for results submitted in the CD34 QAP. The BMTSAA council has agreed to the formation of an Expert Advisory Panel within the society, and to endorse the panel's decision. The following suggestions had been put to members in the most recent edition of the BMTSAA Newsletter along with an invitation to volunteer or nominate for the panel :-

- Members of the expert panel need to have strong laboratory and/or clinical background in blood and marrow transplantation and/or flow cytometry.
- Those with appropriate skills may volunteer, or be nominated, with the final panel selected by the Council of the BMTSAA in Brisbane, in October 2001.
- The number of people on the panel would depend on the number of nominees, but probably between 5 and 6.
- One member could represent, and report back to the BMTSAA.
- One member could represent, and report back to the AFCG.
- One member could be a clinician involved in stem cell transplantation.

Nominations already received were read. Annette Trickett recommended an expert in statistical analysis. Simon Bol suggested that expertise be sought outside the BMTSAA particularly for flow cytometry in general. Steve Noga and Dianne Tucker suggested Graeme Chapman and Frank

Batty respectively. Vicki Antonenas offered assistance. It was agreed that further nominations should be directed to Annabella and that Council would review them prior to making a decision

**15. OTHER BUSINESS**

Gail Lazzaro advised that concern had been raised with regard to E-mail addresses and security. Members were reminded that E-mail addresses were listed in the Directory of Members and that this information was readily accessible at the web site. Scott Ragg agreed to remove E-mail addresses on request. The meeting closed at 1645.

Laboratory has followed the Rubinstein dextran/albumin wash protocol for 9 cord blood units for unrelated cord blood transplantation in children with a median weight of 23.8 Kg and for 3 adult patients with a median weight of 53 Kg. Cryopreserved bone marrow and peripheral blood stem cells are typically thawed and infused immediately for transplantation without washing. In contrast, cord blood units are commonly washed before infusion. In this report, we compared the cell dose in the cord blood unit (provided by the cord blood bank from which the unit was processed and stored) to values obtained after thawing and washing of cord blood units for transplantation. Data summarized in the table.

Patient	Wt. (Kg)	Counts Pre-Freezing		Counts After Washing		TNC loss %
		TNC x10 <sup>7</sup> /Kg	CD34+cells x10 <sup>5</sup> /Kg	TNC x10 <sup>7</sup> /Kg	CD34+cells x10 <sup>5</sup> /Kg	
Adults	45	2.6	0.58	1.84	0.55	29.2
1						
2	63	3.4	NA	2.0	1.6	41.2
3	51	1.69	0.59	1.5	0.8	11.2
Children	43	2.87	NA	2.55	1.5	11.1
1						
2	18.5	7.29	2.3	5.2	2.1	28.7
3	24.5	4.1	NA	3.4	2.4	17.1
4	12.1	6.9	0.54	4.9	0.98	28.9
5	15.5	5.5	2.3	3.7	2.9	32.7
6	21.4	5.1	NA	3.4	NA	33
7	24.4	4.75	NA	2.8	1.6	41.1
8	40	1.43	0.43	1.0	NA	30
9	15	6.8	1.1	5.4	3.2	20.6

NA= not available

*Gail Lazzaro  
Secretary  
Annette Trickett  
President*

The median proportion of total nuclear cell lost during thawing/washing was 27.2% (range 11.1 to 41.2 %). The observed cell loss suggests that the current practice of washing cord blood units before infusion into patients, especially for larger patients, including adults, should be carefully re-evaluated, since cell dose in the cord blood unit is important for engraftment.

TOO  
**MEETING ABSTRACTS**



**EFFECT OF WASHING PROCEDURES ON UNRELATED CORD BLOOD UNITS FOR TRANSPLANTATION IN CHILDREN AND ADULTS: EXPERIENCE AT WESTMEAD HOSPITAL AND THE CHILDRENS HOSPITAL AT WESTMEAD**

*V Antonenas, KF Bradstock, M Hertzberg, M Bleakley\* and PJ Shaw\**

*The Blood and Marrow Transplant Unit, Westmead Hospital, Sydney and \*Oncology Unit, The Childrens Hospital at Westmead, Sydney, Australia*

For patients in need of allogeneic transplantation but lacking a suitable family donor or matched unrelated bone marrow donor, unrelated cord blood units represent an alternative source of stem cells. From 1997 to present, the Westmead BMT



**CD34 CELL SELECTION AT WESTMEAD HOSPITAL: REPORT ON TWO DEVICES**

*V Antonenas, KF Bradstock, B Kramer\*, G McCowage\*, PJ Shaw\**

*The Blood and Marrow Transplant Unit, Westmead Hospital, Sydney and \*Oncology Unit, The Childrens Hospital at Westmead, Sydney, Australia*

From November 1999 to May 2001, the Westmead BMT Laboratory has performed 16 CD34+ selections on bone marrow (BM) and peripheral blood stem cells (PBSC). We compared the performance characteristics of the two currently available CD34 selection devices: Nexell Isolex 300i (software version 2.5) and Miltenyi CliniMACS.

Device	Sample Type	Number of Selected Procedures	CD34 Purity (%)	CD34 Yield (%)	CD34/Kg x10 <sup>6</sup>	Log T Cell Depletion
Isolex 300i	Autologous PBSC	5	92.6 (70-99)	62.6 (50-80)	8.2 (2.4-18.7)	NT
	Allogeneic PBSC	2	93.5 (93-94)	54 (43-65)	4.5 (4.4-4.6)	4.1 (3.9-4.2)
CliniMAC S	Autologous PBSC	1	92	74	7.4	NT
	Allogeneic PBSC	1	90	69	2.4	3.9
	Matched-Unrelated BM	7	89.4 (82-93)	52.7 (43-65)	8.2 (0.5-25.5)	3.4 (3.0-3.6)

Results of the CD34+ selections are shown in the table; mean values (range).

Because the CD34 selection procedure on the Isolex can be affected by high red cell contamination, the matched-unrelated BM were processed on the CliniMACS. The purity for both devices was comparable and in regards to PBSC, the yield was higher with CliniMACS. Technically, both devices are easy to operate and the processing time is slightly shorter on the CliniMACS. The Isolex can handle products with higher white cell counts and higher T cell depletion was achieved with the Isolex.

Sufficient CD34+ cells can be isolated from either device for transplantation with successful neutrophil and platelet engraftment.

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### HIGH POTENCY DENDRITIC CELLS FROM PERIPHERAL BLOOD STEM CELL HARVESTS AS POTENTIAL CELLULAR VECTORS FOR IDIOTYPE VACCINATION

L. Barrow, R Brown, A Murray, B Pope, J Gibson, D Joshua  
Institute of Haematology, Royal Prince Alfred Hospital, Camperdown, NSW

*Ex vivo* priming of PBSC harvests is a common strategy used for idiotypic vaccination of patients with myeloma. However a range of different antigen presenting cells are present in these products and the majority of the cells have low antigen presenting potency. Failure to use high potency dendritic cells (DC) may be a major cause of the poor response to idiotypic vaccination. We have therefore developed a simple *ex vivo* strategy based on semi-purified DC and have performed a series of *in vitro* experiments to provide a scientific basis for a future clinical trial. Mononuclear preparations of peripheral blood (n=13) contained 0.1-0.9% high potency DC (CMRF44+, CD19-, CD14-). There was no difference in the number of DC in PBSC mobilised with G-CSF (mean 0.28%, n=7) when compared with GM-CSF (mean 0.24%, n=6) and the leucapheresis collection itself did not concentrate DC. In longitudinal studies (n=10), the peak DC count (day 12 post PBSC harvest) did not correlate with the peak CD34+ cell count or white cell count. A simple affinity purification of DC resulted in an average 63.0 fold purification of DC. The final DC enriched suspensions contained a mean of 18.8% DC from normal blood and 9.9% DC from patients with myeloma. Analysis of DC subsets demonstrated that the number of CD11c+ DC was not significantly different in PBSC

harvests from patients with myeloma (81.3% of DC, n=6), blood samples from patients with myeloma (93.0% of DC, n=11) and normal controls (92.1% of DC, n=5). The percentage of CD123<sup>hi</sup> DC, by contrast, was higher in PBSC from myeloma patients (22.6%) than peripheral blood samples from patients with myeloma (7.0%, p=0.01). Incubation of DC with huCD40LT resulted in a more than 2-fold increase in CD80 expression. Incubation of DC with ovalbumin-FITC demonstrated the antigen uptake potential of these cells. Our studies have shown that it is possible to produce an enriched population of high potency DC using a simple affinity separation technique. The cells take up antigen and respond to different stimuli as would be required for idiotypic vaccination strategies.

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### VALIDATION OF THE CONDITIONS FOR USE OF AN IN-HOUSE CLONOGENIC ASSAY CONTROL

Judy Bosio (1), Hilary M<sup>c</sup>Kibbin (2), Gail Lazzaro (1)

1. Australian Red Cross Blood Service – North West, Perth, WA. 2. King Edward Memorial Hospital, Subiaco, WA.

Cord blood is recognised as a valuable source of haemopoietic stem cells for transplantation in the treatment of a number of malignant and genetic diseases. A major limitation in its use is the total nucleated cell dose or more specifically the number of stem and progenitor cells required to achieve sustained engraftment.

Colony assays of clonogenic haemopoietic cells provide an indication of engraftment potential. The number of variables in the assay system have historically hindered both inter and intra-laboratory standardisation. With the commercial availability of standardised culture medium complete with cytokines, the culture conditions and the operator have become primary areas of concern with respect to quality control. We have established and validated the conditions for use of an in-house CFU-GM control to monitor these variables.

A donation of cord blood was collected into CPD-A anticoagulant, processed and cryopreserved within 24 hours. Buffy coat cells were diluted in Gentrin 40 and cryopreserved in 10% DMSO at a final cell concentration of 4.1x10<sup>6</sup>/ml. Cells were dispensed in 1ml aliquots by two operators maintaining stringent control of the temperature of vials during preparation for cryopreservation. The samples were frozen in a controlled rate freezer and stored in the vapour phase of liquid nitrogen.

Control samples were rapidly thawed, mixed by vortexing and immediately diluted for plating in a cold solution of saline/Albumex 4 (1:1). Cells were cultured in triplicate in Methocult HCC4534 at a plating concentration of 5x10<sup>4</sup> cells per plate. Plates were incubated at 37°C in 7% CO<sub>2</sub> and read daily between day 7 and 21. Results of three separate assays showed 65% variability in the colony numbers at day 14. Subsequent experiments (n=2), utilising controlled thaw conditions (42°C ± 1°C), demonstrated reproducible assay results over the entire culture period (range 452 – 470 CFU-GM per 15 x 10<sup>4</sup> nucleated cells). These experiments

also indicated the optimal culture time for this laboratory (11 - 15 days).

In separate experiments, samples were thawed at 42°C over increasing periods of time. The control samples were cultured as described above and were read at 14 days. CFU-GM numbers were preserved in samples thawed for up to 2 minutes after which time a dramatic decrease (80%) was observed.

A CFU-GM control for in-house use provides a valuable tool for monitoring culture conditions and operator performance for both laboratory quality control requirements and training purposes. The validation identified thaw temperature (42°C ± 1°C) and thaw time (< 2 mins) as critical conditions for use and furthermore defined the time limits for reading culture assays for our laboratory (11 – 15 days).

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### SINGLE VERSUS DUAL PLATFORM CD34 ANALYSIS : PRACTICAL CONSIDERATIONS RELATING TO A CHANGE IN LABORATORY PRACTICE.

Carnoutsos SA, Kubala LM, Haring LF, McKenzie JL.

Specialist Haematology and Haematology Research Laboratory, Canterbury Health Laboratories, Christchurch Hospital, Christchurch, New Zealand.

Historically the immunophenotyping laboratory has required the enumeration of CD34+ cells in mobilised peripheral blood and apheresis products to be performed as soon as possible after collection of the sample. Often this has resulted in out-of-hours analysis and therefore an increase in overtime hours. The study aimed to assess the feasibility of performing CD34 analysis using the Stem-Kit™ CD34+ HPC Enumeration Kit on samples kept at 4°C overnight and compare this to the dual platform analysis performed on the fresh product. Previous studies in our laboratory have shown the unsuitability of the dual platform analysis on stored samples due to cell clumping phenomena. The study also addressed the issues of potential employee cost savings and the effect of increasing the result turnaround time to the clinician and patient. Ten apheresis products and six mobilised peripheral blood samples were examined. The results indicate that the single platform CD34 analysis method compared favourably with the dual platform and was suitable for use on stored samples. Delayed reporting of the single platform CD34 result did not impact on patient care with regard to G-CSF administration

when a target value of  $2.0 - 5.0 \times 10^6$  CD34/kg was used.

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### THE RCPA CD34<sup>+</sup> CELL QAP: REPORT OF THE FIRST SURVEY.

**Annabella Chang** (1) & David DF Ma (2)  
 (1) Dept. Haematology, PALMS. Royal North Shore Hospital. Sydney.  
 (2) Dept. Haematology & Stem Cell Transplantation. St. Vincent's Hospital. Sydney.

A CD34<sup>+</sup> cell QAP has been established to ensure accurate and reliable CD34<sup>+</sup> stem cell results for clinical decisions in the management of haemopoietic stem cell transplant patients, and for the accreditation of transplant laboratories. The first survey conducted in 2001 consisted of a mobilised peripheral blood (PB) and a peripheral blood stem cell harvest (PBSC). Data on instruments, reagents, methodology and results were collected from 36 centres.

The ISHAGE protocol was used by 29 centres, (23 dual, 6 single platform) with 7 centres using other methods (5 dual, 2 single platform) The distribution of the values obtained were summarised by boxplots (see table). The stem cell dose was calculated for a 76 kg patient.

Parameter	No. of results	Median	50% of results within	Smallest observed value not outlyer	Largest observed value not outlyer	No. with outlying values *	No. with extreme values #
PB wcc x 10 <sup>6</sup> /ml	34	8.5	7.8 – 9.2	6.2	10.4	2	1
PB % CD34	36	0.17	0.15 – 0.21	0.07	0.25	4	0
PB CD34/ul	35	14	12.4 – 19.4	3.3	28	3	2
PBSC wcc x 10 <sup>6</sup> /ml	34	33.8	32.2 – 35.7	7.3	39.9	1	1
PBSC %CD34	36	0.82	0.72 – 0.92	0.61	1.1	2	1
PBSC CD34/ul	35	286	253 – 320	216	393	2	5
PBSC CD34 x 10 <sup>6</sup> /m <sup>2</sup>	35	8.3	7.4 – 9.3	6.3	11.4	2	5

The submitted results showed clerical errors, miscalculations, or errors in the use of units. After correction of these errors, there were no longer any extreme values. In the clinical setting, these results could give rise to different decisions as to whether or not this patient is sufficiently mobilised to initiate leukapheresis, and the number of transplants available from this PBSC.

Although centres may have established their own internal ranges and correlations with their clinical data, CD34<sup>+</sup> cell results need to be comparable between institutions in order to compare data, or for collaboration in multi-centre studies. While the boxplot shows the distribution of data, acceptable limits over different ranges needs to be defined for the CD34<sup>+</sup> QAP to be clinically relevant. These acceptable limits will be established in consultation with transplant centres.

\* Outliers are values that are between 1.5 and 3 box-lengths from the upper and lower edge of the box.

# Extremes are values that are more than 3 box-lengths from the upper and lower edge of the box.

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### STEM CELL PROCESSING IN THE TWENTY FIRST CENTURY: IMPLEMENTATION OF THE AUSTRALIAN CODE OF GOOD

### MANUFACTURING PRACTICE FOR HUMAN BLOOD AND TISSUE.

**P. G. Dyson**, S. Niutta, P. Harrison, I. Lewis, L.B. To.  
 Division of Haematology, Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia.

Human tissue products and extracts have been available for transplantation, implantation, or injection, as banked or non-viable human tissues or extracts, and cell or tissue-based viable products, for a number of years. We now have available for transplantation tissue-based products that have been altered physically, chemically or genetically. Appropriate regulation of products manufactured from human tissue is critical to ensure that such highly manipulated cell products are consistently produced to a standard that ensures that they are fit for their intended use and pose no risk to the recipient due to inadequate safety quality or efficacy. Current Good Manufacturing Practice (cGMP) regulations have been designed and implemented to ensure that these quality objectives are met.

To ensure that haemopoietic stem cell products produced for transplantation are of the highest standards we have developed a cGMP quality system with associated clean room facility for the processing of human cell-based products. As well

as facility design, planning of the Therapeutic Product Facility has included other key elements of GMP including adequate documentation, production controls, quality assurance, validation, equipment, personnel management, and environmental monitoring.

There were a number of challenges to facility development. While the TGA administer GMP they provide no input into facility planning so the development of a facility to meet regulatory requirements drew heavily on experience from the pharmaceutical industry. Provision of adequate funding in the setting of a public hospital was a major consideration in facility design as was siting of the facility in the context of existing personnel and workpractices. The project implementation team was drawn from a number of disciplines within the institution a wide range of skills being required to address the many aspects of this complex project. To ensure that the completed facility complies with all the relevant Australian standards a consultant engineer was engaged to assist with project validation. Extensive documentation was prepared which formed the basis for personnel education along with training in principles and practise of GMP. The modification of existing work patterns to ensure optimal personnel, material, and process flow within the facility has presented a major challenge. Another continuing challenge will be ensuring that the application of GMP regulations to stem cell

processing does not inhibit research of new technologies or their clinical implementation.

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### AN IMPROVED METHOD FOR THE RECOVERY OF TOTAL NUCLEATED CELLS IN CORD BLOOD

**Gail Lazzaro**, (1), Judy Bosio (1), Hilary M<sup>C</sup> Kibbin (2)  
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 2. King Edward Memorial Hospital, Subiaco, WA.

A significant proportion of the cost of cord blood banking lies in the resources required for the distribution of information to donors, obtaining informed consent, interview, collection and processing of cord blood donations which do not translate into bankable units. A collaborative cord blood pilot program undertaken by the Australian Red Cross Blood Service and King Edward Memorial Hospital identified a 19% loss of bankable units at the processing stage. The aim of this study was to improve the total nucleated cell recovery in buffy coat preparations thereby increasing the potential for banking success and cost efficiency.

Cord blood was collected from delivered placentae into CPDA anticoagulant and processed within 24 hours. Cord blood was transferred into a Stemcare (Fresenius HemoCare) cord blood processing set. The set was aligned with a full saline bag prop and secured with a centrifugation support (BagRap). The assembly was centrifuged at 1000g for 20 minutes with slow acceleration and no break. Buffy coat cells were separated using a Baxter Optipress II (back plate: standard, force: 10, buffy coat volume: 40 mls and buffy coat level: 6.8). Whole cord blood, buffy coat, plasma, and red cell fractions were tested for the total number of nucleated cells, CFU-GM and CD34+ cells.

11 cord blood donations were processed. In all but one haemolysed donation, the adopted processing assembly and centrifugation method facilitated a clean undisturbed interface. In this exception, 42% of total nucleated cells (TNC) were detected in the red cell fraction as a result of the inability of the Optipress II to recognise the interface. The TNC recovery in the buffy coat was 49.6%. In one case, a genuine flow restriction during the Optipress II program resulted in a TNC recovery of 7.4% with 88% of cells detected in the red cell fraction. In the remaining 9 procedures the mean TNC recovery was 87.9% (range 76.2 - 95.7%). When compared to TNC yields obtained during the pilot program (mean recovery: 70.8%, range: (61.1 - 80.6%), these results represent a 17.1% increase in TNC recovery.

This processing technique consistently produces a high yield of nucleated cells in buffy coat derived from cord blood. Separation is achieved in a closed system without additives, thereby preserving sterility and the integrity of red cell and plasma fractions required for future testing. The requirement for washing procedures associated with reagents that are not approved for human infusion (eg hydroxyethyl starch) is averted. This method has significantly improved the processing outcomes in our laboratory and will translate into a

higher success rate of achieving bankable units and ultimately cost efficiency.



## ENGRAFTMENT FOLLOWING

### REINFUSION OF CD34<sup>+</sup> STEM CELLS ISOLATED BY CLINIMACS

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Stem cell isolation techniques allow T cell reduction of allogeneic grafts, with the possibility of modulating graft versus host effect, and tumour cell reduction of autologous grafts. However, there are some concerns over delayed or reduced engraftment capacity of CD34<sup>+</sup> selected grafts.

Since 1999, our centre has isolated CD34<sup>+</sup> stem cells from 45 PBSC harvests of 31 patients (11 allogeneic, 20 autologous), using the CliniMACS system. Mean purity of selected harvests was 91.3 ± 1.0% (SEM), while recovery was 70.0 ± 1.8%. Of the selected harvests, 7 allogeneic and 11 autologous grafts have been reinfused. Allogeneic grafts contained a mean of 5.6 ± 0.9 x 10<sup>6</sup>/kg CD34<sup>+</sup> stem cells (range 2.1 -8.2), and all but one patient reached neutrophil engraftment (≥1.0 x 10<sup>9</sup> /L) by day 16 (mean 13.2 ± 0.8 days). The patient that failed to engraft was a relapsed AML with myelodysplastic syndrome. Autologous grafts contained a mean of 3.1 ± 0.3 x 10<sup>6</sup>/kg CD34<sup>+</sup> stem cells (range 2.0-5.0) and mean neutrophil engraftment was reached by 17.2 ± 2.3 days (range 10-29). Four of the patients took over 20 days and included those who had been heavily pretreated, had myelodysplastic syndrome or were in relapse. If these are excluded, then mean neutrophil engraftment was reached by 12.1 ± 0.9 days (range 10-16). Neutrophil engraftment at our centre for unselected grafts is 13.9 ± 0.4 days (n =54).

CD34<sup>+</sup> stem cell isolation does not significantly prolong neutrophil engraftment for either allogeneic or autologous transplants.



### HLA IDENTICAL SIBLING BONE MARROW TRANSPLANT-CYTOKINES, CELLS AND GRAFT VERSUS HOST DISEASE

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Activated T lymphocytes in the donor release cytokines that contribute to the cytokine storm that in turn up-regulates MHC expression. With increased MHC expression there is increased recognition of donor/recipient differences by alloreactive T-cells in the donor graft. These reactive T-cells proliferate and secrete cytokines, in particular, Interleukin -2 (IL-2). The cytokines released by the T-cells activate more donor T-cells and other mononuclear cells that in turn induce the secretion of Interleukin-1 (IL-1) and Tumour Necrosis Factor alpha (TNF $\alpha$ ). The resulting cycle of cytokine release manifests itself clinically as GvHD. By analysing donor T-cells, their specific subsets and the cytokines they produce, in response to recipient antigenic stimulation, the possibility of predicting those recipients of HLA-identical sibling

marrow that might develop GvHD after transplantation was investigated.

Ninety-six recipients received bone marrow transplants from HLA identical sibling donors as treatment for their haematological disorders from 6 different transplant centres. Peripheral Blood Mononuclear Cells (PBMC) for both recipient and donor were cryopreserved and stored in liquid nitrogen. Recipients used in this study all received bone marrow transplants from January 1990 to June 2000 and received Methotrexate and Cyclosporin as prophylaxis for GvHD. Prior to transplant (or prior to the knowledge of transplant outcome) the frequency of IL-2, IL-4, IL-10 and INF $\gamma$  releasing T effector cells were determined in the donors when stimulated by their respective HLA identical sibling recipients. The CTLL-2 bioassay was used to detect IL-2 production while Elispot technology was used to detect the other cytokines. Cytokines frequencies were also monitored when donor and recipient cell populations were cultured with assay media or with an HLA 3<sup>rd</sup> party disparate cells.

Cytokine producing T lymphocyte precursors, were analysed for statistical relevance to development of acute GvHD after transplant. Chi<sup>2</sup> analysis was performed using Fischer's Exact Test. For IL-2 detection the HTLP assay, the two-sided P value is <0.0001, considered extremely significant. IL-10 production was negatively correlated with the post transplant development of severe acute GvHD. The two sided p value was 0.0048 and considered very significant. 15-20% of all donors in this study group failed to produce IL-4 or INF $\gamma$  when stimulated by their sibling recipients and were therefore not assessable. When the recipient versus 3<sup>rd</sup> party HLA disparate response is analysed on the basis of transplant outcome those recipients that went on to develop GvHD grade 0-I were compared with those that developed GvHD grade II-IV after transplant. Welch's approximate t test found that the difference between these two groups was extremely significant with a two tailed p value = 0.008

Through this study a better understanding should be gained of the cytokine and cellular profile of those recipients of HLA identical sibling bone marrow that will probably develop acute (grade II-IV) graft-versus-host disease.

## TOO Conference Reports



More than 600 delegates from Australasia together with international guests and invited speakers attended the Joint Annual Scientific Meetings of the Haematology Society of Australia and New Zealand (HSANZ) and the Australasian Society of Blood Transfusion in Brisbane, Queensland from 21 to 24 October 2001. As well as bringing together the disciplines of haematology and blood transfusion, the meeting provided of forum for four satellite groups, The Australasian Society of Thrombosis and Haemostasis, The Australasian Leukaemia and Lymphoma Study Group, The Australian and New Zealand Apheresis Association (ANZAA) and the

Bone Marrow Transplant Scientist's Association of Australasia (BMTSAA).

As in previous years, new and improved strategies for the treatment of haematological malignancy provided the framework for the HSANZ program. This year, "good news" in the form of the signal transduction inhibitor STI571 (Glivec®) for the treatment of CML featured prominently in presentations from both keynote and national speakers including Dr Brian Druker, Dr Moshe Talpaz and Dr Christopher Arthur.

The ANZAA program had a strong transplantation content. Along with haemopoietic stem cell transplant topics, the program included sessions related to GMP, novel cell therapies and a focus on allogeneic stem cell donation. Of particular interest was a presentation by Dr John Bashford on the duty of care to allogeneic stem cell donors. Potential medical, practical and ethical complications were discussed including coercion, a difficult issue especially for related donors. Management teams for donors clearly separate from recipients would constitute a major change for some Australian transplant centres.

The BMTSAA held a one day scientific meeting on 25<sup>th</sup> October which included laboratory focussed free communications, bone marrow courier and cryogenic safety forums. The Association was fortunate to have Dr John Gribben and Dr Gordon Keller accept invitations to give keynote addresses providing highlights of the meeting. Dr John Gribben who had presented extensively for HSANZ, changed focus from his work with immunotherapy and the treatment of B cell malignancies along with the detection of MRD and implications for long term outcome to discuss "The Induction of Anergy in Haploidentical Transplants using CTLA-4-Ig". Dr Gordon Keller provided an excellent overview of embryonic stem cell research, its potential in cell replacement therapies and the legal and regulatory restraints imposed in the United States. His talk concluded with interactive discussion relating to the more provocative ethical questions of embryonic stem cell research and the "non-plasticity" of haemopoietic stem cells

*Gail Lazzaro*

This report will appear in the ISHAGE Telegraph



### ASH CONFERENCE 2001; ORLANDO, FLORIDA

This year's ASH conference was held from December 7<sup>th</sup> - 11<sup>th</sup> in the massive convention centre in Orlando. The major attractions of this town, other than the convention centre, are theme parks and discount shopping malls. There seem to be endless numbers of both.

According to some of the ASH veterans, the numbers of delegates at this year's meeting were well below average but attendances still topped the 10,000 mark. On most days there were 20 simultaneous sessions and 800 posters, which meant that evenings were spent reading abstracts and working out the agenda for the next day. It would be impossible to do a full meeting report, hence I have just covered a few of the highlights.

Carl June from the University of Pennsylvania presented data on polyclonal activated T cell

adoptive transfers. This group theorises that adoptive T cellular immunotherapy can restore effective anti-tumour responses if appropriate tumour immunity is lacking. There are various potential forms of adoptive T cell therapy:

- Polyclonal T cells to restore T cell function, Th1/2 switch, or in vivo immunisation
- Antigen-specific T cells for cell based vaccines: cancer, HIV, etc.
- Allogeneic T cells for DLI
- Gene modified T cells for anti-viral & anti-tumour effects.

Polyclonal T cell expansion is achieved using anti-CD3/CD28 beads which act as an artificial dendritic cell. Functional & homing properties of the T cells are maintained after expansion. The ex vivo culture conditions can be manipulated to preferentially expand Th1 or Th2 cells. For Th1 cells, IL-2, IL-12 and anti-IL-4 are added to the culture medium. For Th2 cells, IL-2 and IL-4 is added.

Most tumour antigens are normal self-antigens. Although resting T cells do not generally respond to autologous tumour, it is postulated that CD3/28 activated T cells may respond. A phase I clinical trial in patients with NHL (refractory to a doxorubicin-based regimen, or relapsed disease) has started. The patients are apheresed to obtain T cells for polyclonal Th1 CD3/28 expansion, then PBSC are mobilised with Cy, VP16, GM-CSF & G-CSF and  $\geq 5 \times 10^6$  CD34/kg are collected. CD34 cells are selected using the Isoplex. Patients are given an autologous PBSCTx after BEAC regimen, with GM-CSF starting at d+1 until ANC  $> 5 \times 10^9/L$ . The expanded Th1 CD4+ and CD8+ cells are reinfused on d+12. To date, 17 patients have been treated with escalating doses of T cells: 3 pts @  $5 \times 10^6/kg$ , 12 pts @  $18 \times 10^6/kg$ , and 2 pts @  $117 \times 10^6/kg$ . Maximal clinical response was CR in 5 pts, PR in 5 and stable disease in 7. There were 10 deaths: 9 progressive disease, 1 toxicity. The treatment is safe & feasible (but more toxicity is observed compared to a similar trial in HIV patients, probably due to the amount of chemotherapy). Infusion is associated with lymphocytosis, fever, fatigue, and Th1 cytokine secretion. This treatment reverses the Th1 cytokine activation deficits observed in vitro. In future studies this group aims to use labelled T cells to study trafficking, and vaccination with autologous tumour to improve tumour-specific response.

Another clinical trial aims to Th1 activate allogeneic DLI for patients following mini allo Tx or relapse following standard BMT. The current protocol gives the initial DLI as standard (non-activated) cells then the activated DLI is given for repeat infusions. 6 patients have been treated: no acute GvHD in 1 pt, grade I in 3 pts & grade II in 2 pts. There were 4 pts evaluable for chronic GvHD: limited disease seen in 1 pt, extensive in 1 pt. 3 pts achieved a CR (CLL & ALL).

Data in a mouse model has demonstrated that infusion of donor Th2 cells with the BMT can prevent GvHD. Hence a clinical trial has been initiated to determine whether activated Th2 cells can prevent GvHD following mini allo SCTx for leukaemia or lymphoma.

There were 2 posters that addressed the potential to increase the number of cells available in cord blood by combining 2 or more units for transplant. J.Gryn & colleagues from Pittsburgh have infused multiple unmatched UCBTx into adult patients. To date, 9 patients with refractory

haematological malignancies have been given allo UCBTx using 67 units of HLA- and ABO-unmatched CB. All units were sex mismatched with recipient. Conditioning consisted of fludarabine / melphalan / ATG or cyclophosphamide / TBI / ATG. Cyclosporin was given for 30-60 days. 3 patients engrafted: all grafts were from a single CB unit unrelated to the recipient HLA type. GvHD  $<$  grade 2 was seen in all evaluable patients. Two patients are still alive.

JN Barker from Minneapolis reported UCBTx in adults using 2 partially matched units. Patients with high-risk or advanced haematological malignancy lacking other suitable donors were eligible. 12 patients aged 22-60 were conditioned with either cyclophosphamide / TBI or non-myeloablative busulphan / fludarabine / 200 cGy TBI. CB units were 1-2 antigen mismatched to recipient and each other. Total nucleated cells infused =  $2.3-6.2 \times 10^7/kg$ . ANC  $> 500$  was achieved in 12-28 days and was sustained in 11/12. 45% of patients had engraftment with both units at day 21, but engraftment with a single unit was seen in 82% of patients at day 40 (usually with the more cellular unit). Acute GvHD grade II-IV in 60% of patients, or grade III-IV in 10%. A survival plateau of 39% was seen at 3 months.

Ron Gress from the NIH in Bethesda gave an overview of their work on T cell recovery after HSCTx. The following table summarises the recovery and timing of CD4+ T cells after transplant in an adult population.

	PreTx	Post	6/12	12/12	24/12	36/12	48/12
Thymic size:	+	-	++	+++	++	++	+
TREC	++	-	+	++	+++	+++	+++
CD4 T cells	+++	+	+	+	++	++	++
CD4 CD45RA $\beta$ diversity	+++	+	+	++	++	+++	+++
CD4 CD45RO $\beta$ diversity	+++	+	++	++	++	+++	+++

Although it was originally assumed that adults were incapable of generating new T cells, there is now a substantial body of data that refutes this. Early CD4 regeneration is predominantly via peripheral expansion of mature T cells but de novo cell production (as assessed by TREC analysis) can be observed at 6 months posttransplant in most patients. Emergence of TRECs coincide with increased numbers of CD45RA+ cells.

IL-7 enhances T cell regeneration by both de novo (thymic dependent) & peripheral expansion. In the IL-7 transgenic mouse, IL-7 is expressed at similar level to the developing thymus leading to increased levels of CD4- CD8- precursor T cells in the thymus. It is unclear whether IL-7 has a role in lineage commitment & proliferation of these cells.

*The TGFB transgenic mouse produces almost exclusively CD8 cells hence this cytokine appears to have a negative role in CD4 proliferation.*

IL-15 stimulates conversion of CD8+ CD28+ T cells to CD28- cells. A similar phenotype switch is seen in peripherally expanded cells and hence it is postulated that IL-15 may play a role in peripheral expansion. IL-15 also sustains CD8+ cells in vitro. IL-15 levels are substantially increased in patients during the early post transplant period (0-3 months) and moderately increased thereafter.

Annette Trickett



## The Grapevine

*Laboratory death caused by asphyxiation of nitrogen.*

Last December there was an incident at the Australian Animal Health Laboratory in Geelong. The medical technologist involved was highly regarded for his methodical and conscientious approach. He worked in the laboratory's microbiological security unit. It is believed that he entered the LN storage area where a leak had developed in the piping system and was immediately rendered unconscious because both the ventilation and alarm systems failed. The true cause of the accident has to be established. Third hand information indicates that the alarm monitor and ventilation failure caused this misadventure. See full report, 'The Age' Saturday 22 December 2001.

David Ford

## Off the Web

For some entertainment have a look at this chat session from American ABC. Ninety people in America have been cryogenically frozen, hoping to be brought back to life one day when medicine can cure them. To the companies that charge at least \$28,000 to freeze them, they're optimistically called "patients." [http://more.abcnews.go.com/sections/community/2020/chat\\_cryonics0208.html](http://more.abcnews.go.com/sections/community/2020/chat_cryonics0208.html)

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