

## Minutes

### XXXIst Meeting of the Biomedical Excellence for Safer Transfusion Collaborative

Barcelona, Spain  
March 17-18, 2006

*These minutes represent presentations and discussions at the BEST meeting on March 18 as well as a synopsis of the team meetings held immediately before. They include some detail and discussions that occurred only in the team meetings in order to provide all members with as much information as possible about past and planned studies. Additional details can be obtained from the leader of each team, who prepared the summary of the team's activity given below, often with assistance from presenters.*

Jim AuBuchon opened the meeting with a welcome to all attendees. First-time attendees and guests were introduced.

Since Scott Murphy was unable to attend the meeting, AuBuchon presented a brief summary of the financial status of BEST and asked for questions regarding the report in the book. He offered a breakdown of 2005 expenses by category, including:

Travel	\$173, 995
Meetings	59, 128
Books (materials+FedEx)	12, 949
Studies	46, 723
Web	15, 180
Legal	8, 983
Other	21, 099
Total	\$338, 057

This compares to income of \$266,961 consisting of dues (for 2005 plus arrears made up) of \$254,975 and interest income of \$11,986.

He noted that BEST has been formally granted federal recognition as a not-for-profit corporation and will be filing an annual Form 990 with the US government to detail its financial and programmatic status, as required by law. He noted with thanks that 5 MANUSCRIPTS had been published by BEST in 2005, a record number, and he thanked the authors for their diligence.

The process for selecting the leadership for the next round of BEST was reviewed. Sunny Dzik, chair of the Nominating Committee as selected in Seattle, reported that the committee offered the name of Lorna Williamson for the next chair of BEST. There were no nominations for the floor, and Dr. Williamson was elected unanimously. She then nominated a slate for the Executive Committee (Team Leaders) to commence after BEST XXXII to include:

Mike Murphy	Hermann Eichler
Sunny Dzik	Ziggy Szczepiorkowski
John Hess	Nancy Heddle
Pieter van der Meer	Larry Dumont

She noted that evolution of leadership remained important as did continuity from chair to chair so that not all the Executive Committee turned over at the same time. She thanked Hans Gulliksson for his willingness to step down from team leadership after 7 years of able leadership, and she noted that other teams were anticipating a mid-cycle evolution of leadership during her term. There were no nominations from the floor, and the slate was elected unanimously. (The new Executive Committee will meet after BEST XXXII to select the Associate Scientific and Scientific Members for the next term. Broad announcement of this "renovation" will be made to solicit applications for membership.)

Future meetings were reviewed. BEST XXXII will occur in Cape Town September 1-2 at the Cullinan Inn, immediately before the ISBT Congress. A brief discussion of the plan of the meeting was held, and the majority clearly wished the team meetings to be grouped as at present. Therefore, the meeting schedule for BEST XXXII will be:

<u>Friday afternoon</u>	<u>Saturday morning</u>	<u>Saturday afternoon</u>
<i>Team Meetings</i>	<i>Team Meetings</i>	<i>BEST Meeting</i>
Cellular Therapy	Conventional Components	
Clinical Studies	Transfusion Safety	

Attendees were advised to make their room arrangements through the ISBT Congress; there will NOT be a room block held specifically for BEST attendees.

BEST XXXIII will be planned by the new leadership for the Spring of 2007.

AuBuchon requested that any suggestions for the BEST website be forwarded to him for consideration.

Cees Smit Sibinga presented a brief overview of the Transfusion Medicine Institute that he heads under WHO auspices to improve transfusion knowledge and practice in developing countries. (See BEST Book for detailed information.)

Miguel Lozano presented the answers to the puzzler he had created for the meeting. The correct responses were:

1. Santiago Ramón y Cajal
2. Frederick Duran Jordà
3. Manuel Rodríguez Sánchez, “Manolete”

Bonus question: Petilla de Aragon, a part of the province of Navarra (despite its location in Aragon, due to medieval jurisdictions)

The winner was announced as Wolfgang Mayr; Sunny Dzik and Nancy Heddle were also noted as having answered all questions correctly. Others with the three primary questions correct were Amanda Thomson, David McKenna, Silvano Wendel, Ralph Vassallo and Chris Prowse. AuBuchon thanked Lozano for his work in constructing such an interesting puzzler and in facilitating local arrangements.

After the summarizations of the team meetings were offered, AuBuchon thanked all in attendance for helping make this – the biggest BEST meeting ever – such a successful one.

## **Report: Cellular Therapy Team**

*H. Eichler, Z. Szczepiorkowski*

### **Participants**

Team Members: Anneke Brand, John Hess, Riitta Kekomaki, David McKenna, Derwood Pamphilon, Jo-Anna Reems, Ronald Sacher, Ziggy Szczepiorkowski, Cees Smit Sibinga.

Guests: Harold Meryman, Christina Celluzzi, Kurt Gunter, Stein Holme, Eckart Kaempgen, Eileen Selogie, Jan Simak, Tomo Yokomizo,

Ziggy Szczepiorkowski welcomed all participants of the team meeting and gave an overview of the agenda listing current cellular therapy team projects. Unfortunately, Hermann Eichler could not attend the meeting due to acute illness, and his input was missed during the meeting.

### **1. Administrative Issues and Update on Manuscripts (Ziggy Szczepiorkowski)**

The BEST study #15 manuscript has been accepted for publication in *Cytotherapy* after expedited submission by Gary Morroff and Hermann Eichler. The manuscript containing the results of BEST #19 entitled “Multiple laboratory comparison of in-vitro assay utilized to characterize hematopoietic cells in cord blood” has already

published in Transfusion and an editorial was written by Dr. Michael Creer (a guest during our last meeting in Seattle).

Note: The first part of the meeting was devoted to a review of functional testing of dendritic cells. Though, the titles of presentation were somewhat overlapping the topics were carefully chosen to illustrate differences and similarities between DC laboratories in the US and Europe in approaching this issue.

## **2. Guest Presentation: ‘In vitro testing for functional characteristics of dendritic cells’ (Dr. Eckart Kaempgen)**

Professor Eckart Kaempgen from University in Erlangen presented a very interesting overview of the current status of functional DC testing in his laboratory. In his presentation he divided the functional DC testing into four categories 1) uptake and processing of antigen; 2) cell migration; 3) cytokine production; and 4) priming of naïve T cells. He then proceeded with more in-depth analysis of each of these approaches. He illustrated basic conditions of each test and its applicability in clinical trials (see presentation posted on the BEST website). Additional tests, which are not purely functional, such as dendritic cell phenotype, morphology and yield, may play an important role as release criteria according to Prof. Kaempgen. As an example of such a test he introduced a concept of a wash out assay. In this assay the mature DCs are washed and kept for 24 hours in the cytokine free medium. The cell morphology is assessed at this point. The results of a comparison of different maturation media on the results of the “wash-out” assay were presented. There was striking difference in DCs which were matured with MCM-mimic (IL-1, IL-6, TNF-alpha and PGE2 – an approach advocated by research group from Erlangen) or TNF-alpha/PGE2 in comparison to other maturation stimuli (i.e. Poly-I:C, TNF- alpha, and CD-40L). In addition, the use of CD25 marker was advocated by Prof. Kaempgen to further phenotype the DCs. In summary the following were recommendations from Prof. Kaempgen for standard in vitro functional assays of DC used in clinical trials: 1. Basic DC function (migratory capacity (i.e. transwell assay), MLR, and antigen specific priming) 2. Clinical applications/QC (i.e. phenotype – CD25, CD83, CCR7; washout assay – evaluation of the DC yield after 24 hour incubation in the absence of cytokines). The presentation was very well organized and delivered. The speaker received a lot of questions and a very stimulating discussion followed. The summary of the presentation will be available on the BEST website shortly. This was an excellent introduction to the next talk with dealt in much greater detail with advantages and disadvantages of each assay under discussion.

## **3. Guest Presentation: ‘Dendritic Cell Function: Pros and Cons of Different Evaluation Methods’ (Dr. Christina Celluzzi)**

The second speaker of this “educational” portion of our meeting was Dr. Christina Celluzzi, Asst Prof from University of Maryland. Dr. Celluzzi’s laboratory participated in the BEST 28 study. Dr. Celluzzi had a difficult topic challenge in her presentation. She was asked to identify potential shortcomings of the different tests presented by Prof. Kaempgen and she has done an admirable job with this difficult topic. Her full presentation is available on the BEST website but, briefly, she introduced each assay with a very thorough discussion of technical aspects (a feature appreciated by those in the audience who are not familiar with some of the assays). Dr. Celluzzi discussed viability assays such as vital stain exclusion (e.g. trypan blue), 7-AAD, and annexin-propidium iodide with extensive discussion of pro and cons of using any of these assays to assess viability of DCs. In one of the final slides, Dr Celluzzi put together a summary table with all discussed assays and their advantages and disadvantages. In summary, Dr. Celluzzi suggested several assays, which can be fairly easily adapted to clinical practice despite their shortcomings, for assessment of T cell proliferation such as T cell cytokine release assay, ELISPOT (cytokine release), intracellular cytokine, peptide-MHC tetramer and cytotoxic T cell function.

Both presentations were opened for discussion which was very lively and added some new questions to the follow-up on BEST 28 study (see below).

## **4. BEST #28: Inter-laboratory Pilot Study on Quality Characteristics of Dendritic Cells; Summary of survey about immunological assays for functional DC testing (Ziggy Szczepiorkowski on behalf of Hermann Eichler)**

At the Seattle meeting, a preliminary draft of the manuscript presenting the data from the first experiment set was presented. This manuscript was further refined by Hermann based on the information obtained after the Seattle meeting. It was noted that inclusion of 7-AAD, a viability marker, in the analysis of antigen expression may have influenced the final proportion of cells positive for different antigens. Hermann and Ziggy decided to remove this variable and request from the participants a reanalysis of the data. The data sets were sent to Hermann for inclusion into the final manuscript. The new version of the manuscript had this information partially included and presented a much tighter data set.

In Hermann's absence, the team discussed the manuscript briefly. Additional discussion dealt with the follow-up on this study and the planned use of the frozen cells remaining in the participants' laboratories. The participants suggested a review of additional variables to be included in the second set of experiments such as time from thaw, viability and CD25. It was decided that the participants would discuss these issues during a conference call after this meeting. The manuscript should be submitted before the meeting in Cape Town.

### **5. Cord Blood Assay Study – Results with Frozen/Thawed Samples (BEST 30) (Anneke Brand)**

Dr. Anneke Brand presented an update on this project. Her presentation consisted of two parts. The first part was a discussion on the BEST 30.1 and its outcome. A draft manuscript as a letter to editor was forwarded to participants for comments prior to this meeting. Derwood offered to review the manuscript for content and language. After this review the manuscript will be sent to all participants for a final review and subsequently submitted to BMT as a letter to the editor. The second part of Anneke's presentation was devoted to a follow-up of BEST 30.1, referred to as BEST #30.2, which has a slightly modified protocol for a second send-around of frozen CB samples. The following centers received the samples and reported the results Helsinki, Leiden, Dartmouth, Mannheim,, Minneapolis, Seattle, Tokyo, and Montreal. In this send-out validated dry shippers from the participants' sites were utilized. This approach seemed to work well. The review of the preliminary results showed again significant variability between the participating sites (for details see the presentation on the BEST website). The discussion regarding the sources of the observed discrepancies between the centers ensued. It was concluded that a common SOP should be created to minimize the number of (un)controlled variables (e.g. dilutions, pipets, time intervals, reagents). The performance of electronic cell counters was also questioned and a suggestion was made to use more than one cell counter if the center has an access to more than one. The final suggestion was to compare gating strategies as it has been done in previous BEST studies. A conference call will be arranged after the Barcelona meeting to address interpretation of the data with a follow up during the Cape Town meeting.

### **6. Update on the HALO Assay (Ziggy Szczepiorkowski)**

In Seattle, Dr. Rich from Hemogenix presented a new assay which could be used to replace the currently used CFU assay. The study protocol has been prepared by Jo-Anna Reems and participated sites were selected. However, it was felt that a commitment to ongoing participation by Hemogenix was an important part of our undertaking this study. As this has not happened to this date, the Team Co-Chairs supported by the Chair have decided to withdraw this study. Ziggy announced that Jo-Anna continues to be interested in pursuing this study outside the BEST and all interested participants should communicate directly with her.

### **7. BEST #29: Release Criteria Utilized with Cellular Therapy Products (Ronald Sacher/Ziggy Szczepiorkowski)**

Dr. Szczepiorkowski presented the progress of the project. He presented a design of a web based questionnaire which was discussed by the team. Several changes and suggestions were offered to finalize the draft. Ms. Selogie felt that with the current design this survey should be fairly easy to implement in the web format. The team members stressed that it was critical that the survey cannot take more than 15-20 minutes to complete. It was also suggested that initially a paper copy of the survey should be distributed to the team members for evaluation and assessment. This will be a validation process for this survey. The pilot survey will have a "feel" of the web survey. Once this pilot study is done the next step would be to put it on the BEST web site to be accessible by members and non-members. The participation goal would be to obtain at least 75 responses. The plan is to have it ready for the ISBT Cape Town meeting so we can distribute the web link to interested participants. In addition a number of organizations will be approached to help to send out the link (e.g. AABB, FACT, JACIE, Cord Blood Forum etc). The full presentation is available on the BEST website.

## **8. Summary of questionnaire on future activities of BEST Cellular Therapy Team (Ziggy Szczepiorkowski)**

At the Seattle meeting, the group reviewed the preliminary results of the survey on new projects. Ideas and suggestions were summarized in a PowerPoint presentation posted on the BEST website. Prior to the meeting in Barcelona the results were distributed and this time an additional scoring sheet was distributed as well. The team members were encouraged to score the level of enthusiasm and willingness to participate in several projects (see the BEST book for details). The summary of the votes will be presented by email after the Barcelona Meeting. The results will be discussed in Cape Town and new projects will be advised based on this list. There were also suggestions from the team members regarding additional projects. The following suggestions were submitted: 1) a survey on transportation and storage of cellular therapy products (Derwood) and 2) a study of transient warming effect (Jo-Anna). Both Derwood and Jo-Anna will submit their suggestions in writing to Hermann and Ziggy after the Barcelona Meeting.

It was noted that the meeting was well attended with lively discussion and least 17 attendees. The project plan will be submitted to the team members by email.

### **Report: Clinical Studies Team**

*L. Williamson, N Heddle*

Lorna Williamson welcomed members and guests to the session, which aimed to cover possible studies in red cells, plasma and platelets.

The session started with a presentation from the first guest speaker, Dr Önder Arslan, University of Ankara, Ankara, Turkey, on 'The use of the haemoglobin content of red cell units to guide prescribing'. He described a protocol which had been developed using in-house blood bank software to try to provide the optimum amount of haemoglobin to meet the patient's requirements, while keeping the number of donations issued to a minimum. The protocol required knowledge of the donor's haemoglobin level, the patient's actual and target haemoglobin, and the patient's weight. In 67% of patients this had reduced a 3-unit request to 2, or a 2-unit request to 1. However, the number of 'matches' fell as the patient's weight increased, with few matches possible in patients >75kg. Points made in discussion included (1) the suggestion that the Hb of the processed red cells would also be useful, especially as the units had been processed manually (2) uncertainty as to how effective this strategy would be in populations which were heavier (3) uncertainty as to how often clinicians were clear what target Hb they were trying to achieve (4) whether the same approach could be taken for platelets. Tor Hervig agreed to conduct a survey to find out how often the relevant parameters would be available in practice as a first step.

This was followed by a thought-provoking presentation from Andreas Greinacher entitled 'Provision of blood for the unknown patient'. He presented data on the planned use of RhD pos blood in emergency situations/massive transfusion as a means of preserving precious RhD neg stock. He showed that in this setting, alloimmunisation occurred at a rate of 1.5%, compared with 47% in elective surgery. This was totally accounted for by non-survivors in the massive transfusion group, and had led to a local policy of the 'emergency box' containing only RhD positive red cells. Even more intriguingly, he presented data showing that overall survival of RhD pos emergency patients was significantly better than RhD neg patients transfused for elective surgery, raising the possibility of immune modulation mediated by the RhD mis-matched red cells. Discussion points included (1) a strong consensus towards the provision of RhD neg emergency red cells for women of child-bearing potential (2) interest in understanding what provision was made amongst BEST members for emergency situations eg provision of platelets/FFP as routine. Miguel Lorenzo agreed to organise a survey in the first instance.

The first speaker in the plasma section was Dr Vanessa King of NovoNordisk, who described a new haemostasis research facility designed to accommodate collaborative research with academic partners. One specific area of interest was in how to monitor the effects of haemostatic proteins. BEST members agreed to consider whether there were any ideas for generalisable studies which would add to knowledge in this area.

Dana Devine continued the plasma theme with a talk on 'Quality monitoring and indications for FFP'. Following up on an idea first raised at a previous BEST meeting by Rebecca Cardigan, she requested that members considered whether there were studies which could be done to find more relevant quality monitoring markers for FFP than factor VIII, which everyone agreed was inappropriate. Conversely, assay of minor but potentially important constituents of cryoprecipitate such as factor XIII and vWf was not required. In the USA, no coagulation factor measurements in FFP are required at all. At the same time, there was a need for studies to better define appropriate indications for FFP prescription. Williamson drew members' attention to recent publications by Dzik's team on the lack of correlation between haemostatic abnormalities and bleeding, and by Simon Stanworth on the lack of evidence for benefit of FFP in any clinical setting. Stanworth and Alan Timmouth were about to embark on a study of FFP prescription. In discussion, it was pointed out that countries such as France in which there were licenced concentrates of fibrinogen and factor XIII used very little FFP. The impact of near-patient coagulation testing also needed to be considered. It was agreed that members who had ideas for quality monitoring studies should contact Dana Devine, and that members should look at the proposed Timmouth/Stanworth FFP study provided in the BEST book with a view to joining this study.

After coffee, the session focussed on platelets, starting with an 'Update on the StoP study' from Nancy Heddle. So far 69 patients had been enrolled, and Toronto will start soon. In particular, she reported on the web-based adjudication system for bleeding events, based on the WHO grading system. This was currently being piloted by several BEST members, and helpful comments had been received, which will be incorporated into further iterations of the system.

The rest of the system was devoted several presentations on the theme of 'Platelet radiolabelling studies- getting from raw data to results'. Firstly, Ralph Vassallo reported on a 'send-around' study in which 8 labs had entered raw gamma counter data into their local spread-sheets, all of which were stated to be based on BEST recommended calculations. Despite this, there was considerable variation in results, suggesting the need for improved standardisation in this area. During discussion, there was strong support for a 'BEST spreadsheet' to be available through the website. As an extension of this, it was agreed that BEST could also help new radiolabelling sites validate their use of this standard spreadsheet by providing datasets on request. Ralph Vallasso agreed to lead on taking these suggestions forward.

The next topic was the ongoing issue of the value of 'fresh v stored' comparisons, and whether they were preferable to the creation of absolute standards for platelet recovery (as was required for red cells) and survival. In preparation for this discussion, Jim AuBuchon and Sherrill Slichter had drafted a manuscript outlining the issues and inviting written comments and opinions on the key issues. Doug Bolgiano, Biostatistician at Puget Sound Blood Center, presented an analysis of sources of variance, performed by combining data from many R&S studies. For recovery, interdonor variability accounted for a large part of the variance, but this was not true for survival, where storage time and radiolabel were the biggest contributors to variance. Inter-laboratory differences accounted for only 3-4%. This suggested that a fresh:stored comparison might be useful for recovery but NOT survival. Sherrill Slichter extended the argument by showing data demonstrating a very strong correlation between fresh and stored recoveries, and postulating that this was also of no value, since fresh values could almost be 'back-predicted' from stored ones! A very lively debate followed, including discussion of which option was better in reducing volunteer exposure to radiation- this depended on whether the sample size reduction by use of paired samples outweighed the need to use 2 labels on individual volunteers (but sample size may have to remain large if survival values show no fresh:stored correlation). In addition, it was unknown whether the strong correlation between fresh and stored recovery values would hold true for future platelet production systems. The consensus amongst attendees at this session therefore seemed to be in favour of retaining the fresh:stored comparison for the time being.

The last presentation of the morning was by Gary Elfring, a computer consultant invited by Ray Goodrich. He proposed that we switch to SAS for calculation of recovery and survival values, given that COST runs on a old version of Windows, and that new laboratories were not always able to run it easily. This would have the additional value of allowing the data manipulation currently done on the separate spreadsheet to be performed with SAS also. There was broad agreement in principle for pursuing this approach, and it was agreed that further discussions should take place between Jim AuBuchon, Ralph Vassallo and Gary Elfring on this issue.

The session ran into the lunchbreak, but it was generally agreed that the morning had been particularly wide-ranging and productive.

## **Report: Transfusion Safety Team**

*S. Dzik, M. Murphy*

Dr Michael Murphy hosted the team meeting. Dr Murphy congratulated the group on their efforts noting that this meeting marked the first time that the Team had active projects which addressed each of the 3 main sectors of the hospital-based transfusion process:

- \* pre-transfusion testing
- \* decision to transfuse
- \* bedside administration of blood.

### **1. Bacterial Detection Survey: C Prowse & S Wendel**

A reminder summary of the bacterial detection survey was presented by Dr Wendel who has converted the raw data into a database format suitable for queries. Discussion of the data centered on:

- \* analysis of the current data set when it is complete, and consider preparation of a publication depending on the quality of the data
- \* potential for repeated surveys to examine evolution of techniques and practices over time

*Next steps prior to Cape Town:*

Silvano and Chris to:-

1. complete the data collection and analysis, circulate it to participants, and review whether publication is appropriate,
2. devise revised survey tool for application to a smaller group of transfusion services on a regular basis.

### **2. Statistical Process Control (SPC): Neil Beckman**

Neil Beckman updated the group on the SPC project. Following on the Collection of Blood Samples (COBS) study, the SPC project seeks to offer blood banks a user friendly validated tool for monitoring the performance of their sample labeling process. Real data obtained from hospitals in the UK, Canada (Nancy Heddle) and USA (Sunny Dzik) were plotted using control charts and these charts used with local boundary limits for the plotting of additional data.

Much discussion ensued on the best use of the SPC approach. To summarize:

\* Needs more examples from other BEST members

\* *It was agreed that entering real data in a retrospective fashion was adequate to demonstrate the monitoring tool.*

\* After discussion of how many different types of errors to enumerate or to combine into groups, it was suggested that a simple approach would track two control charts:

1. mis-labeled samples (using a strict, clear definition based on LOCAL rules)
2. mis-collected (WBIT) samples

\* The control chart of mis-labeled samples may prove to be particular usefulness to each facility as an “early monitor” of process failure.

\* The WBIT control chart may prove to be of particular value in national standard setting.

\* The idea of combining data sets from different hospitals into a group control chart should be explored for low-frequency events, like WBIT events.

\* The statistical basis for the creation of the Upper Control Limit should be explored.

\* The lower control limit should be dropped.

\* The software should be made more intuitive and simple to use.

\* The software could be posted on the members section of the BEST web site.

*Next steps prior to Cape Town:*

1. Neil Beckman to address basis of UCL and to simplify software to 2 groups of charts.

2. Neil, Sunny, and Mike to solicit data from additional BEST members for validation of the simplified software. Volunteers were: Neil, Sunny, Nancy, Ziggy, Ira, Tor, Andreas, Cees, and Silvano.
3. A draft manuscript based on the above results would be presented in Cape Town.
4. The issue of using the technique to establish a national performance standard would be put off as a separate effort from the manuscript above.

### **3. PROBE- TM: Mike Murphy and Angela Casbard**

Mike Murphy introduced a final presentation of the goal of PROBE-TM, the Prevention of Bedside Errors in Transfusion Medicine. This was a randomized, cluster-design trial that investigated the value of a simple label intervention designed to alert transfusionists to the importance of checking the patients's ID bracelet at the moment of transfusion. At each participating facility, one care-unit was randomized to receive the intervention and one to serve as a control. A baseline observational audit was followed by the introduction of the labels (or control = no label), followed by a 2 week observational audit, followed by a period of continued use of the label (or control), followed by a follow up observational audit. The endpoint was based on whether or not the nurses correctly performed all elements of the basic bedside check. Mike introduced Angela Casbard (UK) to present the preliminary statistical analysis of the results. The study found clear evidence that the introduction of the auxillary label failed to improve the performance of the bedside check. This was true when the data were analyzed according to the primary endpoint or when analyzed according to a more limited endpoint of whether or not the nurses simply looked at the wristband.

The was good discussion of the results of this study and agreement that the methodology would prove useful for future studies of the bedside transfusion process.

Nancy Heddle suggested that a follow up study could be a qualitative focus-group research study involving the participating nurses at PROBE-TM sites. A 3 step plan was suggested: 1) to perform a structured interview regarding the bedside check; 2) to review the results of the study with the nurses; and 3) to elicit nurses opinions on how the process could be improved.

#### *Next Steps prior to Cape Town:*

1. Mike Murphy to lead the group of participants in submission of the manuscript of PROBE-TM to Transfusion.
2. The team to consider by conference call the follow up project suggested by Nancy.

### **4. Assessing the decision to transfusion: Simon Stanworth and Alan Tinmouth**

Simon Stanworth and Alan Tinmouth presented a DRAFT survey instrument designed to explore among BEST members the methods by which local hospitals connected to BEST collect information on the appropriateness of transfusion in the hospital. The group completed the survey and then reviewed each question with an aim to clarify the survey. The use of tables was encouraged to capture information on RBCs, FFP, Plts, IVIgG, and r7a. Additional data collection on Albumin was suggested.

#### *Next steps prior to Cape Town:*

1. Drs Tinmouth and Stanworth to revise survey instrument and redistribute it to the core group for completion.
2. A conference call(s) would be set up to review the revised survey.
3. After revision was completed, the survey would be converted to an electronic format and posted on the members-only section of the BEST web site
4. The general membership of BEST would be invited at Cape Town to participate in the completion of the survey.

Dr Murphy closed the session with an open invitation to any BEST member to contact him or Sunny Dzik in order to discuss projects related to the overall theme of proper use of blood transfusion and transfusion safety.

## Report: Conventional Components Team

*H. Gulliksson, J. Hess*

Presentations from the team meeting are available at the BEST Collaborative Home Page, "Members only, Meeting presentations, Barcelona, Spain; March 2006, Conventional Components". Please notice that these presentations are the sole property of the creator(s) of the slide set and are not for reproduction or distribution.

John Hess welcomed the team, opened the meeting of the Conventional Components Team and chaired the first part of the team meeting focusing on red cells.

### **RBC Section Presentations:**

1. Jan Simak, PhD, of the Laboratory of Cellular Hematology at the Center for Biologics Evaluation and Research of the U.S. Food and Drug Administration opened the meeting with a talk on "Cellular Membrane Microparticles in Blood and Blood Products: Potentially pathogenic agents and biomarkers." He described the development of flow cytometric methods to identify microparticles derived from RBC, granulocytes, platelets, and endothelial cells, and their use in the evaluation of human disease states and cellular blood products. He showed that increased concentrations of platelet and granulocyte derived microparticles were found in patients with paroxysmal nocturnal hemoglobinuria and sickle cell anemia, and that concentrations of endothelial derived microparticles correlate with the volume of injury in patients with ischemic stroke. Finally, he showed that microparticles occurring in stored apheresis platelet concentrates and incubated with tissue factor promote coagulation while the same particles exposed to endothelial cell cultures lead to the expression of ICAM-1. Dr. Simak has allowed the posting of his presentation on the BEST website and is seeking collaborators for advancing this work.
2. Jerard Seghatchian followed with a presentation entitled "Biologic Response Modifiers, Microparticles in Blood and Non-viral/nonhaemolytic transfusion hazards." He described studies using stored blood products, specifically platelet concentrates, in which WBC and platelet derived cytokines were increased after 5 days of storage and increased phosphatidyl serine exposure measured by Annexin V binding was strongly correlated with microvesicle concentrations. Moreover, simple assays of pro- and anti-coagulant activity could be produced using chromogenic substrates for thrombin and protein C activity. Leukoreduction only partially prevented the passage of WBC derived microvesicles into platelet concentrates so that HLA and ICAM-3 still appear in leucoreduced products. Dr. Seghatchian suggested studies to compare the effects of methods of production on proinflammatory and procoagulant potential of stored blood products.
3. Hans Gulliksson showed data on the loss of RBC pH, gain of ATP, loss of 2,3,DPG, and increase in hemolysis associated with an overnight room temperature hold of WB collected in Baxter and Terumo bags before preparation of blood components. No negative effects on platelets prepared from WB after storage overnight were seen. He wondered if the loss of 2,3-DPG might be reduced or prevented with changes in storage solutions. John Hess explained that the pH dependence of the diphosphoglycerate mutase/diphosphoglycerate phosphatase enzyme caused the results seen, and that it would be difficult to change the results with a modification of the RBC storage solutions. However, alkaline storage solutions do provide longer storage and some of the time could be used as warm storage. Pieter van der Meer then showed how the Amsterdam Sanquin group has used butane-diol plates to achieve rapid cooling to 22 degrees and minimized the warm storage lesion.
4. John Hess followed with a discussion of inconsistencies he found in measures of double label RBC recovery he made when he worked in the U.S. Army laboratory and those made at Hoxworth in conjunction with Dr. Tibor Greenwalt. This was followed with a discussion of the new FDA criteria for measuring RBC recovery with specific attention to the requirement for a "standard deviation of less than 9%" and a "95% one-sided lower limit of confidence of greater than 70%." John Hess reviewed several historic product licensure trials to show how the standard might fail products that have been approved in the past. Jim AuBuchon gave a review of recent data for presentation to the FDA a high priority under

the designation of the BEST #37 study. Letters were passed out to manufacturers to get their permission to collate data from recent RBC storage studies they sponsored to support discussions with the FDA about revision of the definition.

5. Finally, Chris Prowse presented a short review of methods of measuring RBC rheology. The group considered that none of the methods were sufficiently standard or sensitive to justify study at this time, but that more effort was needed on the measures of the adequacy of storage.

#### **Platelet Section Presentations:**

1. Larry Dumont concluded the BEST #24 study, "Interruption of agitation of platelet concentrates – Effects of shipping". This study is an extension of other studies conducted by BEST members on the effects of interruption of agitation (Hunter S et al, Transfusion 2001;41:809-814 and Van der Meer et al, Vox Sang 2005;88:227-234). The objective was to determine the effects of true platelet shipping on metabolic and in vitro functional parameters for platelets in plasma. Based on Hunter's results,  $pH_{22^{\circ}C}$  should also be maintained at greater than or equal to 6.5 at the end of the storage period. Six (6) laboratories were participating and totally 94 products were included in the study. Matched platelet products were prepared from primary pools at either low ( $1000 \times 10^9$  plt/L) or high concentrations ( $2000 \times 10^9$  plt/L) of platelets. Platelet products were shipped beginning day 3 of storage. Concurrent, paired controls were maintained in the laboratory under standard conditions with continuous agitation. Various in vitro platelet characteristics were determined prior to and following shipment. Results indicated that swirling was generally well maintained. There was also a trend to lower ESC, lower HSR and higher p-selectin levels, although not statistically significant. At day seven, 5 products had  $pH_{22^{\circ}C} < 6.5$  and 24 products  $pH_{22^{\circ}C} > 7.4$ . Preliminary conclusions suggest: 1. Platelets held without agitation upregulated glycolysis resulting in accumulation of lactate and  $CO_2$  and reduced pH; 2. Glycolysis rate returned to control values following re-establishment of agitation; 3. This effect was enhanced at high platelet concentrations ( $p=0.02$ ); 4. There were no significant effects on ESC, HSR, P-selectin as a function of shipping; 5. pH of platelets were maintained for shipments up to 24 hours. Preparation of a manuscript is in progress.

2. BEST #35 - Platelet quality control questionnaire/standardization.

Pieter van der Meer gave a brief overview of the status of this project. In February, a platelet send around was organized, and in total 94 analyzers in 63 labs (in 14 countries) participated. Eight samples with varying platelet numbers were shipped to the centers and were counted in six-fold. The three upper levels had to be diluted. The results showed that, depending on the analyzer, CVs between analyzers could be as low as 2%, and as high as 14%, indicating that the imprecision was mostly machine-dependent. An analysis of the deviation of particular analyzer types from the overall observed values indicated that the deviation of the platelet counts might be as high as -40% to +50%. One particular machine type gave low results throughout the study. Since this machine was clustered to a particular country, the effect of shipping was determined. In fact, the results showed that shipping had very little effect on the final outcomes. The possibility was considered that this particular machine may have suffered from the method of fixation (high mean platelet volumes). Dr. Devine suggested that this analyzer may actually give low results when counting platelet concentrates. Van der Meer will report to the participating centers in April, and hoped to have a draft manuscript in the September book. Further, a draft guideline for counting platelets on hematology analyzers was printed in the book, and the input of the members was requested. Participation was solicited from the members to actually perform the protocol. This will enable to set feasible requirements to the parameters in the guideline.

Gulliksson then adjourned the meeting after thanking the members for excellent contributions.

### **Conclusion**

At the conclusion of the meeting, AuBuchon thanked all for their contributions and participation and adjourned the meeting.

## **Minutes of the Meeting of the Executive Committee**

March 18, 2006

The Executive Committee convened immediately after the BEST XXXI meeting. Present were J. AuBuchon (chair), W. Dzik, H. Gulliksson, N. Heddle, J. Hess, Z. Szczepiorkowski, L. Williamson and S. AuBuchon (staff).

Williamson offered comments regarding the transition plan toward the next chairmanship, and the group discussed possible means of recruiting and evaluating applications for membership. The potential for developing alternative meeting structures was also considered.

The financial and administrative structures of BEST were reviewed. AuBuchon requested feedback regarding the contents of the Form 990 draft that he distributed. The group decided to respond to the requirement for public availability of the form by providing written copies if requested rather than posting the form on the website. The financial impact of invited guests was noted to be significant, but the importance of having the expertise of guests was also noted.

For the next meeting of BEST, it was decided that the focus of team presentations would be on new studies. This will leave sufficient time to invite local guests to the next meeting, possibly requesting them to offer an introduction to blood services in their country. It would also leave time for the new chair to identify issues of importance to her.

Potential new manufacturer members of BEST were discussed, and contact with them was divided amongst the group. The possibility of a dues increase for 2007 was considered given that the dues level has remained unchanged since the inception of BEST.

The potential for invitation to attendance or membership by "patient advocacy" groups was considered. However, this was felt beyond the purpose of BEST.

The potential for listing links to other sites on the BEST website were considered. However, it was felt these were not necessary as all BEST members were very familiar with the available sites, and little was to be gained by reciprocal listings.

The policies of BEST were reviewed and revised. Specifically, the mechanism for assigning study numbers was established as well as clearance for submission of a manuscript as a "BEST publication". Both will require review and acceptance by the Executive Committee.

The deadline for submission of team minutes was set as April 20.

Preliminary discussions were held regarding future meetings. Williamson will begin to develop plans for a meeting in the spring of 2007, possibly in Amsterdam. A meeting in the fall of 2007 in conjunction with the AABB Annual Meeting was considered. Williamson will discuss with Manufacturer Members their feelings about leaving the meeting in Anaheim (site of the AABB meeting) versus locating it in Los Angeles or another nearby venue.

There being no further business to conduct, the meeting was adjourned at 7:00 pm.