

Minutes

XXXth Meeting of the Biomedical Excellence for Safer Transfusion Collaborative

Seattle, Washington
October 19-20, 2005

These minutes represent presentations and discussions at the BEST meeting on October 20 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. A memorial remembrance of Tibi Greenwalt was offered.

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.

The process for selecting the leadership for the next round of BEST was reviewed. At this meeting, Scientific Members and Manufacturer Members could vote for any of their group (except the current chair) for placement on the Nominating Committee. Each was given a ballot and instructed to circle three names to indicate their choices. The person receiving the most votes would serve as the chair. Members of the Nominating Committee who wished to be considered for nomination would remove themselves from the committee, but no additional selections would be required so long as at least 4 persons remained on the committee. The ballots were counted by Susan AuBuchon, and the results announced: Sunny Dzik, chair; Hermann Eichler; Michael Murphy; Larry Dumont; Lorna Williamson; Nancy Heddle; Wolfram Walker; Anneke Brand. The committee was to convene briefly at the end of the BEST meeting, and they will present one or more nominations at the next BEST meeting for the group to vote on.

Future meetings were reviewed. BEST XXXI will be March 17-18 in Barcelona. All were urged to review the material on the website and to make sure they had made reservations at the Hotel Condes by December 15, the date at which our block of rooms will be released. BEST XXXII will occur in Cape Town September 1-2, immediately before the ISBT Congress.

AuBuchon made a special presentation of a plaque to Gary Moroff who could not attend the previous meeting. The plaque recognized his many contributions to BEST and his excellent and vigorous leadership of the Cellular Therapy Team.

Paolo Rebullia also made a special presentation in which he claimed to have discovered that Dzik was a descendent of Pope Pius II!

The contents of the BEST website were reviewed. Presentations will again be posted on the website.

After the summarizations of the team meetings were offered, the BEST Puzzler was explained. The key was the recognition that the Schmiedeleut Hutterites had been studied by Dr. Elo Giblett of the Puget Sound Blood Center. Sherrill Slichter made arrangements for Dr. Giblett to attend the meeting, and all were pleased to greet her and thank her for coming. The Puzzler prize went to Adonis Stassinopoulos with "honorable mentions" to Leslie Rose, Silvano Wendel and Chris Prowse.

Report: Cellular Therapy Team

H. Eichler, Z. Szczepiorkowski

Participants

Team Members: Anneke Brand, Francine Décary, John Hess, Claes Högman, Riitta Kekomaki, Jeff McCullough, David McKenna, Gary Moroff, Derwood Pamphilon, Mark Popovsky, Paolo Rebulli, Jo-Anna Reems, Ronald Sacher, Adonis Stassinopoulos, Sam Wortham.

Guests: Micheal Creer, Locksley McGann, Herold Meryman, Ivan Rich (HemoGenix Inc.)

Ziggy Szczepiorkowski as the new co-team leader welcomed all participants of the team meeting and gave an overview of the agenda listing current cellular therapy team projects. On behalf of all team members, Hermann Eichler and Ziggy Szczepiorkowski with great appreciation acknowledged Gary Moroff's role, enthusiasm and devotion in building up the CT team from its inception. Co-team leaders bestowed, this time officially, the title of an Honorary Co-Leader on Dr. Moroff which he kindly accepted in Seattle, as he could not join us in Rome.

1. Administrative Issues and Update on Manuscripts (Hermann Eichler)

The BEST study #15 manuscript is still under final revision by Tsuneo Takahashi. Gary Moroff will soon get in contact with Dr. Takahashi to finalize the manuscript for submission to *Cytotherapy* by the end of November. 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 been accepted by *Transfusion* and is expected to be published within the next few months.

Starting from the Seattle meeting, all team members will be updated monthly by the team leaders using an Excel file listing the current status and scope of work of the ongoing CT projects.

2. Guest Presentation: Cryopreservation from a historical perspective (Harold Meryman)

The "educational" portion of the meeting was devoted to cryopreservation and its current understanding with a dose of historical data. Two speakers, Drs. Meryman and McGann, were invited to present their thoughts on this issue. Both of the speakers have tremendous depth of knowledge in cryopreservation which they shared freely with the group. Dr. Meryman (his autobiography can be found at *Transfus Med Rev.* 2005 Apr;19(2):167-71) devoted his presentation to an extremely important issue of the relationship between extra-cellular and intra-cellular environment during cooling. He presented the data from very instructive experiments performed in his laboratory several decades ago which showed quite clearly that each cell type has measurable osmotic limits. His presentation included a very interesting movie showing cell behavior under different osmotic conditions. Finally, he offered the following pearls of wisdom as simple rules: 1) determine the osmotic limits of the cell based on function; 2) take advantage of the full range of osmotic tolerance during addition or removal of cryoprotectant. (when some cryoprotectant is present in the sample, "isotonicity" is then defined as the current osmolality of the sample.); 3) never add a solution that exceeds an osmotic limit as some cells will always meet undiluted solution; and 4) use a cryoprotectant concentration sufficient to prevent the freezing out of enough water to exceed the hypertonic limit. The presentation was very well received and all slides are available on the BEST website.

3. Guest Presentation: Cryopreservation: what we know and future directions (Locksley McGann)

The second speaker of this "educational" portion of our meeting was Dr. Locksley McGann (read his CV at <http://myprofile.cos.com/mcgann197>) who devoted his professional career to studying different aspects of cryopreservation. In his very eloquent, extremely well illustrated and entertaining presentation (see the BEST website) he outlined tremendous complexity of cryopreservation and the impact of low temperatures on living cells. He argued, similarly to Dr. Meryman, that each cell type should be considered as a different entity when one designs cryopreservation protocols. Dr. McGann cleverly introduced the topic by comparing slow and fast cooling. He noted that slow cooling leads to ice crystal formation with decreased cell size while fast cooling leads to small ice crystals with intracellular freezing. Our current knowledge is based on empirical data gathered over several decades. He felt that cryopreservation is a highly complex process with multiple variables in the need of study. In practice, one needs to go beyond just a rate of cooling which is only one of several critical variables. To study this complex process he introduced a model predicting how cells would behave under

different cryopreservation conditions. This model has demonstrated that a more accurate description of the concentrative properties of the cytoplasm is required. And finally he discussed, albeit briefly, the possibility that the accumulated knowledge about cryopreservation may lead us to DMSO free systems.

Both presentations were filled with extremely useful information which we hope will result in a new BEST study.

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

Dr. Anneke Brand presented an update on this project. The aim of this study is to understand why Cord Blood Banks report a wide range for post-thaw recovery parameters estimating the quality of their cryopreservation procedures. In a first part of this study, referred as #30.1, each of three cord blood specimens was sent-out to 9 centers in Nov/Dec 2004. There was a fairly good correlation between centers in measuring total nucleated cells, but significant differences in reported differentials as well as the CD34 content. In addition, this study yielded only incomplete results due to a transient warming event of the samples during delivery (no growth of CFU's). However, Dr. Brand drafted a manuscript containing results of 30.1 entitled "Evaluation of the variation in in vitro assay results with identical frozen/thawed samples of cord blood hematopoietic stem cell products". This draft will be forwarded to all participants by e-mail for providing comments and suggestions to Dr. Brand by the end of November.

As a follow-up of BEST 30.1, a slightly modified protocol was drafted for a second send-around of frozen CB samples since the Rome meeting, referred to as #30.2. These CB specimens were already sent out or will soon be delivered to overall 12 centers (Helsinki, Leiden, Dartmouth, Cincinnati, Mannheim, Baltimore, Minneapolis, Seattle, Tokyo, St. Louis, Québec, London) using liquid nitrogen shippers (dry shippers) for optimized temperature conditions. Study results should be reported to Anneke Brand as soon as possible to integrate data into the draft manuscript. The plan is to complete the draft manuscript including data from #30.2 before the Barcelona meeting.

5. BEST #32: Mesenchymal stem cells – Evaluation of culture conditions (Jo-Anna Reems)

Jo-Anna Reems gave a short update of the planned project. In Rome she presented the results of a pilot study performed in two labs on the expansion of mesenchymal stem cells isolated from frozen/thawed bone marrow. After discussion the group then decided to start BEST study #32 with the objective to determine optimal culture conditions for MSC expansion. The plan was to finalize the draft study protocol presented by Dr. Reems at the Rome meeting. As the protocol was crafted and near completion it became clear that this MSC project might not be realized as quickly as other team projects. The main reason is lack of assay standardization between participating sites. The team therefore decided to postpone the start of #32 until the sites gained more experience in this field. Therefore, the MSC study has been placed on hold at this time.

6. Guest Presentation: The HALO Assay (Ivan Rich, Chairman & CEO HemoGenix Inc.)

Dr. Rich informed the CT team about the newly developed HALO assay (Hematopoietic / Hematotoxicity Assay via Luminescence Output) designed to perform quality control of hematopoietic stem cell products (for detailed information: www.hemogenix.com). The presentation is also posted on the BEST website. Compared to the classical colony-forming assay, the HALO assay detects cell proliferation instead of cell differentiation, and it is designed to give results in a shorter period of time (7 days vs. 14 days). Most team members performing the CFU assay were highly interested in acquiring more experience in this new detection system of cell proliferation. Dr. Rich offered to support a multi-center BEST study for generating data on the possible impact of the HALO assay for evaluation of quality parameters of hematopoietic cells.

7. BEST #36: Evaluation of the HALO assay (Jo-Anna Reems)

Dr. Reems presented a proposal for a new commercial assay to evaluate HPC function, and the CT team decided to start a study project to evaluate this HALO assay. Dr. Reems will lead a group of team members (Anneke Brand, Paolo Rebullà, Ronald Sacher) for drafting a study protocol until the next team meeting in Barcelona. The

following sites/team members indicated their interest in participating in the study: Seattle (Reems), Leiden (Brand), Milan (Rebulla), Cincinnati (Sacher), Dartmouth (Szczepiorkowski), and Bristol (Pamphilon).

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

At the Rome meeting preliminary data of study #28 were presented by Dr. Eichler, and prior to the Seattle meeting a first manuscript was drafted. The draft was handed out during the team meeting but will also be submitted as electronic version for review and addition of missing data/information to all participants of this study. Discussing the results on QC assays of DC samples 1-3 tested in study #28 it became clear that evaluation of DC viability by 7-AAD as well as quantification of antigen expression resulted in high variability between sites. The follow-up plan is to obtain completed information from each site, to repeat the study using an additional frozen/thawed sample of mature DCs when indicated (e.g. Bristol), and to re-analyze flow data files of antigen expression measurements. This was already performed on files from three participating labs (NIH, Dartmouth, St.Paul).

In addition, Hermann Eichler presented the summary of a survey about immunological assays already established in labs participating BEST #28. Sites responding to the questionnaire were St. Paul, Dartmouth, Baltimore, Bristol, Leiden, and Mannheim. Information provided by responding sites is summarized as an attachment to the minutes. Focusing on the capacity of DCs to stimulate for cytotoxic T lymphocyte response, the intracellular cytokine assay testing for Th1/Th2 T cell response seems to be established in most labs. It was decided to discuss the issue of immunological assays for functional DC testing during the next meeting in Barcelona by inviting expert speakers in this field. Thereafter, the next steps for this team project should be discussed.

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

Dr. Szczepiorkowski presented the history and current status of this project. He noted that there was a delay in putting together a manuscript as the data are very limited and scattered. The majority of information comes from the governmental and regulatory sources rather than from well designed research studies. This will be a potential area of interest for the Cellular Therapy Team. In his summary Ziggy noted that 1) a pilot questionnaire was distributed and analyzed in 2003/2004; 2) the CT team decided in Rome to pursue two subprojects. Project A – A review article: The Acceptance and Release Criteria for Cellular Therapy Products: A review of the literature, evidence and regulatory guidelines in the US and worldwide and Project B – A web based questionnaire to collect the data on current practice; and 3) the final drafts of both will be discussed in Barcelona. Ziggy presented the first draft of the proposed Web-based questionnaire which was discussed by the team with a few very valuable suggestions for improvement. The questionnaire will be presented to our Web master in order to review its feasibility and cost as proposed. The general outlined of the questionnaire will be 1) information about the respondent; 2) information about the accreditation status; 3) information about general policies; 4) information about acceptance criteria; 5) information about release criteria general; 6) information about release criteria per product type; and 7) questions regarding specific testing methods. The team agreed that generally the answers will require mostly yes/no with only limited number of questions requiring a number and only a very few narrative responses. The participants agreed that the questionnaire cannot take more than 15-20 minutes to be successfully implemented with a reasonable response rate.

10. Summary of questionnaire on future activities of BEST Cellular Therapy Team (Hermann Eichler)

At Rome the group decided to administer a survey to gather information on general interests and additional study ideas from the team members. Prior to the Seattle meeting a questionnaire was distributed by the chairs containing three questions (On what kind of activities should the CT team focus in future studies? What kind of cells/material should further be included in CT studies? What kind of other tests/assays or cell manipulation methods should further be studied?). Five group members responded to the questionnaire (David McKenna, Paolo Rebulla, Ronald Sacher, Ziggy Szczepiorkowski, Hermann Eichler). Ideas and suggestions are

summarized in a PowerPoint presentation posted on the BEST website. One topic of the Barcelona meeting will be to set up a strategic plan listing possible future CT projects that can be transferred to collaborative studies.

It was noted that the meeting was very well attended with lively discussion and least 21 attendees. The meeting was adjourned at noon.

Report: Clinical Studies Team

L. Williamson, N Heddle

1. Preamble.

Scott Murphy joined the meeting by telephone link- a BEST first. The team sends him all BEST wishes for his restoration to full health.

AuBuchon presented Williamson with a book (100 First Words!) in recognition of her promotion to Reader in Transfusion Medicine in the University of Cambridge. Williamson thanked all who had provided citations and invited BEST friends to sign the book.

2. Status of extended storage platelets in the USA- Jim AuBuchon.

AuBuchon updated the meeting on progress with FDA, who had clarified that they regarded extended storage as a 'significant' change to the current situation, thus requiring volunteer recovery and survival data only (a 'minor' change would require in vitro data only, while a 'major' one would require a clinical trial. Bags so far approved by FDA for extended platelet storage were Pall, for whole blood derived platelets, and Gambro and Baxter, for apheresis.

With regard to bacterial testing, FDA had stated that they were prepared to accept a residual risk of <1:10,000, with the lower bound of the 95% CI as 1 in 5,000. To prove this would ideally require a comparison of day 1 and day 8 test results in 50,000 units, and would cost in the region of \$9m. A pragmatic 'post-marketing surveillance' approach was therefore being taken, in which screening data were compared with actual results from outdate cultures.

Current application of BacTAlert to Gambro platelets required a 24-36 hour hold pre-sampling. Both aerobic and anaerobic cultures were being performed, although in 10⁶ cultures in UK, no anaerobes had been detected. However, the anaerobic bottle possibly offered earlier detection of aerobes as well, while the doubling of the sample volume offered increased sensitivity. Culture studies in the literature had suggested positivity rates of around 1 in 3000 at sampling and 1 in 2000-12,000 at outdate (Rock et al, Transfusion 2004;44:37-42; Larsen et al Vox Sang 2005;88:93097).

Culture of PRP platelets was greatly facilitated by pre-storage pooling being permitted, since only 1 culture/pool was needed instead of 4-6 individual ones. However, it was noted that many hospitals were still using surrogate methods (glucose or pH dipstick, swirling), with very low sensitivity. The implication of this is that although PRP platelets currently represent only 25% of all platelets supplied in USA, they could account for 75% of the residual risk.

3. Extended storage of PRP and apheresis platelets. Stein Holme/Sherrill Slichter.

Holme reviewed the literature of volunteer recovery and survival studies of PRP and apheresis platelets. Older studies (not using the BEST protocol) had not shown up any major differences between the 2 types of product, nor had 2 recent separate studies of the 2 products in the AuBuchon lab several months apart. However, Blajchman reminded the group that a paired study in his lab had shown a clear advantage of apheresis platelets. Holme proposed a laboratory study to try to understand which variables between apheresis and PRP platelets might account for any differences observed (bag type, anti-coagulant, donor physiology, platelet sub-populations etc). A lively discussion followed, in which Heddle reminded the group that volunteer recovery and survival studies were themselves a surrogate to predict what might happen when platelets are transfused into patients.

Since both PRP and apheresis platelets clearly worked to prevent and treat bleeding, it was concluded that such an in vitro study was not high priority at the present time.

Slichter updated her previous data showing that while recovery and survival (R&S) of apheresis platelets (Gambro and Haemonetics) meet the 50%/66% criteria till day 8, poor survival data precluded longer storage. For PRP platelets, recovery fell off rapidly after day 7, while survival gradually fell from day 5 onwards, becoming unacceptable after day 6. However, in the TRAP trial, platelet transfusions were given every 1.8 days, so the absolute survivals of 6.1 and 4.3 days for apheresis and PRP platelets respectively might still be perfectly adequate for patients. Slichter had also noted that 1 and 24 hour increments, and hence days to next transfusion, all fell with each successive platelet transfusions given (Slichter et al, Blood 2005;105:4106).

In the second part of her presentation, Slichter explored the assumption underpinning the requirement to compare R&S of stored platelets with that of a fresh sample- namely that there is a clear relationship in a given donor between the behaviour of fresh and stored platelets. Comparison of fresh and stored R&S values for Haemonetics platelets showed some correlation, though this was dependent on the inclusion or exclusion of some outlying samples. Possible reasons for the absence of such a relationship included (1) different processing of fresh and stored samples (2) the presence of a different platelet population in the fresh sample, since it would be taken 8 days after an apheresis collection. Therefore to remove these variables, Slichter had compared day 1 and day 8 R&S data of apheresis platelets. There was a good correlation of recovery between day 1 and day 8 Gambro platelets (but not Haemonetics), but no correlation of survival for either type. For PRP platelets, there was good correlation of recovery but not survival. The implications of these findings were: (1) should there be absolute minimum values for R&S of stored platelets? (2) should the requirements be different for apheresis and PRP platelets?

4. Recovery and survival studies of extended storage buffy coat platelets. Lorna Williamson.

Williamson's lab had developed a method for preparing platelets from a single volunteer buffy coat (BC), and had validated the product in vitro against standard buffy coat derived pooled platelets. She was now undertaking R&S studies of autologous single unit BC platelets stored in Pall ELX bags in plasma. She was proposing to extend these studies to one or more platelet additive solutions, depending on interest (so far expressed by AuBuchon and Slichter). Possible solutions were Composol (NPBI) and modified PAS3 (marketed as SSP+ by Macopharma), since both of these were licenced in Europe. Neither had an FDA licence, making studies in the USA more difficult, but the electrolyte solution Plasmalyte was licenced in USA, and was similar in composition to Composol. It was agreed that Williamson, Slichter and AuBuchon would have a further conference call soon to take this forward.

5. Update on SToP trial and International Study Consolidating Databases. Nancy Heddle.

SToP Study: N Heddle gave an update on the SToP platelet dose study. Recruitment currently stands at 46 patients. There are currently 5 centers enrolling patients (Hamilton, Ottawa, Bergen, Dartmouth and Cedars Sinai in LA). Toronto will be coming on board soon and Dr. Carey in Cincinnati is also interested in participating. When all sites are enrolling patients we anticipate that we will enrol 240 patients/ year; hence, the study is expected to take 2 years to complete. The group is also considering enrolling some auto transplant patients. The Data Safety Monitoring Board is functioning to monitor patient safety. All bleeding events need to be adjudicated and Dr. Aubuchon suggested that the Scientific Members of BEST take on this role. N Heddle will contact the Scientific Members about this role.

International Study on Platelet Transfusions: The manuscript arising from the consolidation of data from the TRAP study, the Italian Platelet transfusion trigger study and the Canadian febrile reaction studies has been submitted and has now been accepted for publication. One of the reviewers raised some questions about the effectiveness of platelet transfusions in patients with morning platelet counts under $5 \times 10^9/L$ and the risk factors for bleeding in these patients. The group had a discussion about possible reasons for this lack of response and the discussion was aimed at stimulating ideas for possible

6. Tracking of transfused platelets using HLA differences between donor and patient. Lorna Williamson and Anneke Brand.

Williamson reported that her preliminary studies using a monoclonal antibody to HLA-A2 to differentiate donor and patient platelets post-transfusion had now been published and would be placed on the BEST website (Hughes DL, Evans G, Metcalfe P, Goodall AH, Williamson LM. Tracking and characterisation of transfused platelets by two colour, whole blood flow cytometry. *Br J Haematol* 2005;130:791-4). She was now proposing to perform a direct comparison of this method with radiolabelling to assess R&S of BC pooled platelets in patients, using the COST programme to handle events from the flow cytometer. During discussion, several helpful methodological suggestions were made:

- (1) since interpretation of data might be confounded by adsorption of soluble HLA from recipient plasma to donor platelets, perhaps the study should be restricted to transfusion of HLA-A2 positive platelets into HLA-A2 negative recipients OR that HPA-1 differences between donor and patient should be used instead of HLA
- (2) To ensure that the antibody detected was binding through the F(Ab) end and not via Fc, Andreas Greinacher suggested that the Fc gamma RII receptor on platelets should be blocked using a monoclonal antibody
- (3) Chris Prowse reported that he had used COST to analyse flow cytometry data generated by transfusing human platelets into rabbits, and offered help, which is gratefully received.

Brand reported progress in generating monoclonal antibodies to different HLA specificities using Bioreactor technology, and their usefulness in tracking different populations of transfused platelets. Antibodies to fifteen specificities had been generated, covering 98% of donors. Some specificities seemed much more useful than others, apparently due to differences in antibody avidity. So far, 7 antibodies had proved useful and 3 had not. AuBuchon posed the challenging question of what the proponents of this technology thought it might be used for. Both Brand and Williamson regarded it as a research tool to help understand the behaviour of different types of platelet product. Brand also saw it as a way of investigating individual patients eg with platelet refractoriness. These developments will be taken further, and Slichter and AuBuchon expressed interest in being involved.

Report: Transfusion Safety Team

S. Dzik, M. Murphy

1. Welcome new Associate Scientific Member

The team welcomed Dr Simon Stanworth (UK) as a new Associate Scientific Member. We look forward to his participation and contributions to the meeting.

2. Bacterial Detection Survey. C Prowse and S Wendel

The bacterial detection survey study is closed. The data obtained from the survey need to be entered into an electronic format (C Prowse to do) for statistical analysis (S Wendel to do). A manuscript based on the analysis will then be prepared, shared with the group, and submitted for publication.

Goal for Barcelona: Have manuscript submitted for publication. The team can address the reviewers' comments in Barcelona.

3. Statistical Process Control of Blood Sample Collection. N Beckman

Dr Beckman gave an update on progress with this study. After consultation with BEST manufacturing consultants and with external consultants, Neil has developed a SPC model that can be adapted to monitoring errors in the blood sample collection and labeling process. This work will build nicely on previous BEST work (COBS study) that documented substantial error rates in sample collection. The goal of the project is to present a useful and easy-to-use tool whereby any transfusion service can use the SPC technique to monitor its process. Neil has applied his model to error rates at Queen Elizabeth Hospital, Birmingham in the UK and a preliminary draft manuscript has been prepared.

There was much enthusiasm in the team for this approach. Several members have existing data sets and “error logs” in their hospitals that can be used to demonstrate that the model Neil has developed is robust and widely adaptable. The following members offered to test this by applying their data to the SPC model:

Anneke Brand, The Netherlands
Sunny Dzik, USA
Nancy Heddle, Canada
Tor Hervig, Norway
Ira Shulman, USA
Ziggy Szczepiorkowski, USA
Silvano Wendel, Brazil

Goal for Barcelona:

- a) Neil will distribute instructions and tools to the above participants and receive back their SPC graphics.
- b) Neil, Mike, Sunny and others will review the above results with SPC consultants to finalize the model for SPC monitoring based on empiric results obtained.
- c) Neil, Mike, Sunny and others will revise current manuscript and present final draft pre-submission manuscript to the team in Barcelona.

4. Prevention of Bedside Errors (PROBE-TM). M Murphy

The PROBE-TM project tests whether or not a simple intervention will have an effect on the safety procedure done at the time of bedside transfusion. The intervention is a label applied to cover the blood container ports. The label instructs the user to check the patient’s wristband. Using direct observation audits of nurses giving blood transfusion, the study examines baseline performance of the bedside check, examines the performance immediately after implementation of the label warning, and examines performance several weeks after implementation of the label warning. A “test” ward and a “control” ward are enrolled in each hospital using a randomized cluster design. Pre-study statistical considerations were used to determine the proper sample size.

Dr Murphy gave an update on current progress of this active protocol. 15 sites have agreed to participate and all data is collected for 12 of these. The final 3 sites are expected to complete data collection by December 2005 and the study should be closed by years end. The project required multiple IRB approvals at participating sites. The trial is registered at <http://www.clinicaltrials.gov>.

Goals for Barcelona

Complete the study and analyze results for presentation to the BEST group. A draft manuscript will be prepared in time for Barcelona if time permits.

5. Guest Lecture. Dr Jasna Skodlar, Zagreb, Croatia

The Transfusion Safety team welcomed Dr Jasna Skodlar (Croatia) who presented her experience using a World Health Organization Basic Information Sheet to assess clinical and laboratory circumstances at the time of blood transfusion. The form is completed by the treating physician before and after a transfusion episode (TE). A TE is defined as a 24 hour period during which one or more transfusions were given. The form was assessed in 5 hospitals at various times during a 6 month period. Participation was voluntary and Dr Skodlar estimated that approximately one half of physicians completed forms. Compliance with filling-in the requested information varied among physicians and range from 80% to 20%. Over 3,000 forms were collected and analysed.

The data provided a rich source of information on current transfusion practices in Croatia. Dr Skodlar summarized the most important findings for the group and a copy of her presentation is available on the web.

The team thanked Dr Skodlar for her presentation. In the question and answer period, Dr Skodlar noted that a substantial investment was needed to persuade physicians to fill out forms for each transfusion. A strong relationship with the treating physicians was one key to the success of her assessment. Team leaders will review the potential for use of a manual form for future BEST studies. It was noted, however, that many sites have substantial computerization of their data and that use of the WHO form might not gain wide acceptance.

6. Update on machine-readable technology for transfusion safety:

Drs Murphy (UK) and Dzik (USA) gave updates on current work in their institutions on the implementation of machine-readable technology to promote transfusion safety.

Sunny presented an update on the use of passive radiofrequency chips (smart tags) applied to patient wristbands and to blood bag labels (at crossmatch). In Boston, he performed a pilot project designed to perform the bedside check in Operating Rooms. The system employs RFID readers built into the form pads of the OR Table. These readers detect and read the wristband when the patient is placed on the OR Table and communicate the patient identification data to bedside computers. The bedside computers can also read the RFID tag on blood bags and compare patient data with blood bag data and report whether or not the blood matches the intended recipient. The project remains under development and is not ready yet for any form of multicenter validation.

Mike updated the team on work in Oxford designed to implement bar code technology for transfusion safety. The goal of the program is an end-to-end control of information. Patients are given wristbands with eye-readable, one-dimensional bar code, and two-dimension bar code information. A hand held digital assistant can scan the patient wristband and print specimen labels at the bedside. Later, at the time of transfusion, the same hand held device can be used to match data scanned from the blood bag and scanned from the patient wristband to prevent mis-transfusion events. The hand held computer is docked into a receiver that is networked to the blood bank system. Thus, positive identification of which units were actually administered can be reported back to the transfusion service.

Mike also showed preliminary information on the use of a “self-service” remote blood refrigerator that uses bar code information to help prevent mis-transfusion. This locked refrigerator can be opened by presentation of a bar-coded “pickup slip”. The user then removes the unit and rescans the unit so that the system can check and report back to the user that the correct unit was removed from the refrigerator.

7. Assessing the Decision-to-Transfuse. A Timmouth & S Stanworth

Drs Timmouth (Canada) and Stanworth (UK) introduced a new project to the team.

BEST has actively investigated the process of sample collection and labeling leading to pre-transfusion testing and is currently studying the process of bedside administration of blood. Between these two processes lies the physician “decision-to-transfuse.” This decision is perhaps the single most critical step that determines how blood therapies are used. The goal of this project is to explore opportunities for BEST to investigate this step in transfusion care.

Simon began with an overview of the issues that surround the decision to transfuse and noted that methods used to monitor (and assess) the decision-to-transfuse are integrally related to methods used to intervene (and influence physician decision-making). The goal of such interventions has traditionally been to reduce unnecessary transfusions, but increasingly the goal has re-focused on promoting the optimum (most appropriate) use of blood therapies addressing concerns regarding not only over-transfusion but also inadequate transfusion.

Monitoring techniques vary widely and include both retrospective and concurrent monitoring. Paper systems and computerized systems are used. Some programs compare local decisions to algorithms and other programs gather usage data. Algorithms include both published guidelines and locally prepared guidelines. Algorithms vary from simple assessments against one or a few laboratory tests to more complicated assessments that consider more clinical features. Data collection varies from large patient groups to individual physicians. Information obtained from monitoring is used differently. Some programs may simply refer findings to hospital committees while other programs may use results to provide feedback to individual physicians.

Alan then discussed two published reviews on the topic of blood use assessment. These reviews have suggested that nearly all published accounts demonstrate improvement in blood utilization when interventions are applied. It is not clear from the published literature whether multiple interventions are superior to single interventions. The existing literature has weaknesses due to lack of control groups and the likely strong publication bias that favors publication of “positive” studies.

In the discussion that followed, it was clear that this was a largely unexplored area of Transfusion Medicine despite its obvious importance. It was noted that circumstances may differ at teaching hospitals and 'private' hospitals. Because practicing physicians are the "study subjects", study designs need to be different from traditional patient-based studies and the opportunity of "cross-contamination" between "test subjects" and "control subjects" exists. Finally, assessment of only transfusions actually given overlooks the need to assess those decisions NOT to transfuse blood components.

Given the lack of full information on techniques used to monitor and influence the decision to transfuse, the team felt that a survey of current practices was the appropriate next step. The goal of such a survey would be to determine not only what was currently being done, but also to identify common practices amongst BEST members that could be used as the foundation for future BEST studies.

Goals for Barcelona:

- a) Drs Tinmouth and Stanworth will identify a core-group of BEST participants and using conference calls will begin to develop a survey for BEST members. The survey will focus on the assessment of the decision-to-transfuse (monitoring) but will also include questions regarding HOW monitoring data is used at each institution. (A full survey on forms of physician intervention was felt to be not as useful at this time.)
- b) The survey will be "tested" for ease of use and validity prior to dissemination to the whole group.
- c) The developed and de-bugged survey (ready for distribution to BEST members) will be presented at Barcelona.

Report: Conventional Components Team

H. Gulliksson, J. Hess

Hans Gulliksson welcomed the team and opened the meeting of the Conventional Components Team by presenting the agenda. Presentations from the team meeting are available at the BEST Collaborative Home Page, "Members only, Meeting presentations, Seattle, WA, USA; October 2005, 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, focusing on red cells ("thinking about better RBC storage"), chaired the first part of the team meeting.

RBC Section Presentations:

1. Alan Tinmouth gave a brief overview and update of his 2001 Review "The clinical consequences of the red cell storage lesion," in *Transfusion Medicine Reviews* 15:91-107. He pointed out that there are many reasons to worry about the safety of older RBCs including loss of efficacy with decreased RBC recovery and reduced oxygen off-loading. There are also reasons to worry about the safety of stored RBC including elevated potassium, biologically active lipids that cause TRALI, and prothrombotic microvesicles. Never the less, there are few prospective studies of the safety and efficacy of older RBC and their results are equivocal. Thus, two studies that have looked at the effect of older cells on gastric pH have yielded contradictory results. Several recent studies that report a relationship between the age of blood and multiple organ failure and death yield inconsistent results in the various subgroups and are deeply confounded by the relationship between injury severity and the amount of blood given, and between the amount of blood issued and the actions of the blood bank to insure that the oldest cells are issued.
2. Chris Prowse reviewed the advantages and disadvantages of trying to set a standard dose of hemoglobin for RBC concentrates. He reviewed the range of hemoglobin content in RBC units obtained by various methods of unit preparation and processing and derived from several donor populations. Despite some variability, RBC units generally contain about 50 g of hemoglobin. On the other hand, patients vary in

size by more than 2.5 orders of magnitude from less than a kilogram to more than 200 kg. There is a general desire for standard blood products, but the ability to accurately measure clinical increments in hemoglobin concentration is probably not capable of resolving the differences in hemoglobin dose with different RBC units.

3. John Hess reviewed the safety of constituents in RBC additive solutions. Constituents that are already part of anticoagulant and additive systems include water, citrate, phosphate, glucose, saline, adenine, mannitol, protons, DEHP, and guanosine. Potential additional agents include bicarbonate, trehalose, Trolox, tirilizad, erythropoietin. With the exception of salts such as bicarbonate that are already widely used in medicine, most of the additional agents would require extensive testing for safety.
4. Claes Högman presented data from a clinical trial using CP2D and an alkaline additive solution that did not contain glucose, that RBC could be stored for 4 weeks with high DPG but lower ATP.

RBC Section Projects:

1. BEST XXVI is now published as Hess JR, Kagen LR, van der Meer PF, Simon T, Cardigan R, Greenwalt TJ, AuBuchon JP, Brand A, Lockwood W, Zanella A, Adamson J, Snyder E, Taylor HL, Moroff G, Hogman C. Inter-laboratory comparison of measuring red cell ATP, DPG, and haemolysis. Vox Sang 2005; 89:44-48.
2. John Hess will lead a review of RBC radiolabeling to develop a common BEST protocol and supporting data
3. Chris Prowse and John Hess review the data about the increment in hemoglobin following administration of a unit of RBC to decide if BEST needs to study the issue further.

Platelet Section Presentations:

1. BEST #23 - In vitro pH Effects on In vivo Recovery and Survival of Platelets.

Larry Dumont presented the results of a study that examined the hypothesis that in vivo recovery of autologous, radiolabeled platelets is not affected by in vitro pH_{22°C} > 7.4. The objective was to consolidate and report the effects of in vitro pH for preparation methods that have been cleared for commercial marketing and sales. Supporting corporations were Baxter, Gambro, Haemonetics, Hemosystems, Navigant, and Terumo. Participating laboratories were ARC in Norfolk and Philadelphia, Dartmouth-Hitchcock, Hoxworth, Puget Sound and Yale. From outside USA, McMaster (Canada) and Bloemfontein (South Africa) participated. In the test of hypothesis, no correlation between pH and recovery/survival was found. On the other hand, strong correlation was noted between pH and “method/bag combination” and “time of storage”, respectively. Dumont concluded that pH is not a predictor of in vivo outcome based on the present data for platelets stored for 5-9 days in plasma with a final pH at 22°C in the range 6.32 to 7.90. He also concluded that these data do not lend support to regulations, rules and standards that set an upper limit on pH during in vitro storage. Draft was presented in the book.

2. Regarding the BEST #24 study, “Effect of two days’ shipping on platelet function and biochemical analyses”, preparation of a manuscript is in progress.
3. BEST #35 - Platelet quality control questionnaire/standardization.

Pieter van der Meer gave a brief overview of the status of this project. A platelet fixation study for sending around would involve the development of human platelet fixation methods based on either

formaldehyde or CytoChex or alternatively unfixed platelets at 4°C. An additional study to compare different analyzers in the same laboratory revealed maximal differences on platelet count between equipment for fresh platelet samples of 25%, for fixed platelet control of 10%, and for whole blood control, 10%. The effect of different diluents (plasma, Isoton, PBS) on platelet count was also 10%. Other approaches discussed by Pieter van der Meer included a “Gold standard” counting method based on ICH guidelines. The previously discussed “send around” will take place in the Netherlands in October 2005, and for participating BEST laboratories preliminarily in January 2006.

4. Storage of Platelets at +4°C (from the Rome BEST Conventional Components Team meeting, continued)

Dirk de Korte presented the results of an in vitro study involving platelet storage at +4°C. This study included apheresis platelets stored in plasma and buffy-coat-derived platelet concentrates stored in platelet additive solution (Composol). In a paired study, test units were stored at +4°C, either without agitation or with agitation (platelets stored in plasma only). Reference platelets were stored at room temperature using conventional methodology. Agitation of platelets stored in plasma at +4°C resulted in “precipitates” of platelet concentrates. The results also showed preservation of glycolysis at +4°C but at a lower rate (ratio 1/3), decrease in platelet count and mitochondrial activity and increased percentage of cells positive to CD-62P. De Korte concluded that the results suggested lower platelet quality compared to the storage at room temperature, at least during the first days of storage.

Hans Gulliksson summarized data from a paired study, in which conventional in vitro parameters were used for comparison of test platelets from pooled buffy coat stored in PAS-II (T-Sol) at +4°C without agitation with reference platelets stored in the same environment at room temperature on a flat bed agitator. Incubation at 37°C of samples for 30 min was used in parallel to restore discoid platelet shape. The results suggested maintenance of platelet glycolysis but at a lower rate than at room temperature (cf. de Korte). The discoid shape was rapidly lost. On the other hand, ATP, the major energy carrier was “surprisingly” well preserved and the results did not indicate release of content from alpha-granules or disintegration of platelets. The manuscript is submitted to Transfusion.

Chris Prowse presented data from preliminary studies on cold storage of human platelets using accepted in vitro and in vivo models based on the “Stossel/Hoffmeister approach”. Platelets from pooled buffy coat were used in a study with a four-arm design including: 1) storage at 22°C in plasma with agitation (primary reference); 2) storage at 4°C in plasma with agitation; 3) storage at 4°C in plasma without agitation; 4) storage at 4°C in plasma without agitation and with UDP-Galactose added. Compared with the primary reference (storage at 22°C in plasma with agitation), all other preparations showed lower HSR, platelet count, pH, and ATP and loss of swirling. Glucose consumption, release of RANTES and presence of soluble P-selectin was lower as well. Higher levels were seen for surface P-selectin. Poor in vivo recovery and survival was found for all preparations except for the primary reference in in vivo platelet recovery and survival studies in rabbits by flow cytometry for human platelets.

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

October 20, 2005

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

AuBuchon opened the meeting with a review of financial and organizational matters. The progress toward attainment of 501(c)(3) (not-for-profit) status with the US Internal Revenue Service was reviewed, and successful conclusion was anticipated in the near future after responding to questions that had been posed on review of the initial application.

AuBuchon then led a review of financial matters since S. Murphy could not be in attendance. He distributed an updated financial statement that included distribution of team expenses. The potential for expenditures beyond the usual annual limit for each team (\$25,000) was discussed and accepted, particularly for next year with increased travel expenses for Associate Scientific Members. The level of dues were discussed, and it was unanimously agreed that the dues for 2006 should remain at \$15,000 for each Manufacturer Member.

The group reviewed the plans for a transition to the new leadership of BEST in the next year and the manner in which Associate/Scientific Membership would be decided. AuBuchon will prepare a review of meeting attendance, and team leaders will also consider levels of activity within their teams in order to prepare to select a membership that will be as active and productive as possible at the next "renovation".

The policies of BEST were reviewed and were not changed. It was agreed that guests should attend meetings by BEST's invitation to accomplish a specific purpose rather than to allow self-invitation.

The website content was reviewed, and all remained very pleased with it. Team leaders requested the webmaster's email so that they could post items directly.

Dzik provided a summary of the brief meeting of the Chair Nominating Committee that had met immediately before the Executive Committee. He explained that they would correspond via e-mail and meet via conference call to consider candidates.

The Executive Committee then reviewed the results of this BEST meeting and the format for future meetings. Several teams felt that they had insufficient time to accomplish all that they wished. After considering several alternatives, it was agreed that teams needing additional time to review data or proposed protocols in detail would set up "pre-meetings" prior to their scheduled team meeting times. Space for these should be coordinated through S. AuBuchon. Minutes and presentations would be posted on the website as after previous meetings.

Upcoming meetings of BEST were discussed. At the Barcelona meeting, the schedule for team meetings will be:

Friday afternoon, March 17: Conventional Components and Transfusion Safety

Saturday morning, March 18: Cellular Therapy and Clinical Studies

The Executive Committee discussed the potential contents of various team meetings and the BEST meeting and agreed that literature critiques were probably best undertaken in other forums; analyses, if offered, should be focused on positive attributes that would help direct BEST studies.

The preliminary arrangements for the Cape Town meeting (September 1-2, 2006) were reviewed.

The group gave preliminary consideration to BEST XXXIII. Several locations were considered with Amsterdam (for an April, 2007 meeting) being the most probable. Other possibilities included Rio de Janeiro, Berlin, Warsaw and Dublin. The new leadership of BEST, when selected, will plan for this meeting.

There being no further business to conduct, the meeting was adjourned at 11:30 am.