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AUTOLOGOUS BLOOD OF HIGH QUALITY FOR CANCER PATIENTS

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Immunomodulation compromises patients after transfusion, especially cancer patients, by an increased rate of tumor recurrence and postoperative infections. This immunosuppression is caused by the allogeneic barrier, and by blood storage at low temperature. We use intraoperative blood salvage as a source of fresh, autologous red blood cells (RBC) after elimination of contaminating cancer cells by blood irradiation. This application is based on the following studies: Demonstration of tumor cells in shed blood (1). We analyzed the blood shed during 108 cases of transfusion relevant oncological surgery for tumor cells, and tested for their functional potential. In 99 cases we detected tumor cells in a number ranging from 10 to 107. The cells proved viable, proliferating, invasive, and tumorigenic. The number of tumor cells in the shed blood turned out to be an independent predictor of survival time, recurrence rate and metastasis rate. The demonstration of these cells confirms the contraindication of intraoperative blood salvage in cancer surgery unless an efficient method of their elimination is available.

Demonstration of efficient elimination of tumor cells by blood irradiation (2). While leukocyte depletion filters showed only limited capacity to reduce the number of tumor cells in blood (3), sensitivity of cancer cells to irradiation is well established, and is rather uniform for oxygenized cells in suspension. From the known radiosensitivities a 12log reduction in proliferating cells can be expected from a 50 Gray irradiation. We experimentally tested the elimination rate by mixing to blood high numbers of cancer cells from tumor cell lines or from solid tumors, and demonstrated a at least 10log reduction by blood irradiation. No proliferating cell or cell with remaining DNA-metabolism could be demonstrated after a 50 Gray irradiation. This guarantees a probability of 99,95% of no single proliferating cell left even after a maximal contamination of the shed blood with 109 tumor cells, thus allowing safe retransfusion of this blood. Demonstration of high quality of salvaged and irradiated RBC . Hemolysis increased from 0.18% after cell salvage to 0.25% after blood irradiation (50Gy), and thus is of no clinical relevance, when compared to that found in the shed blood, or in stored blood. Similarily, potassium release in these fresh irradiated cells was low and did not increase for at least two hours of storage at room temperature. Cells showed normal osmotic resistance. Posttransfusion 24h recovery of RBC was slightly but significantly higher than even in the venous control, probably due to a selective loss of aged RBC during cell washing. In contrast to stored blood, where at the end of the accepted shelf life up to 25% of RBC are hemolysed and no longer available for oxygen transport, after intraoperative blood salvage and irradiation most cells stay in circulation with a normal half-life. With normal levels of 2,3DPG the RBC are able to release oxygen to the tissues without delay, in contrast to stored blood units, where Hb-values might be normalized without an equivalent functional effect. Meanwhile, we have used intraoperative blood salvage with blood irradiation in more than 500 oncologic procedures including liver transplantation and spinal metastasis (4). With these fresh, unstored, unrefrigerated, autologous washed RBC high quality blood is available for optimal hemotherapy in cancer patients, while the limited resources of banked blood can be saved for other patients. 1 Hansen E, Wolff N, Knuechel R, et al. Arch Surg 1994; 130:387 2 Hansen E, Knuechel R, Altmeppen J, et al. Transfusion 1999; 39:608 3 Hansen E, Altmeppen J, Hansen K, et al. Transfusion 1999; 39: S239. 4 Hansen E, Altmeppen J, Taeger K. Clin Anesth (Baillière) 1997; 11:335

CONVERTING WHOLE BLOOD DONORS TO AUTOMATED DOUBLE RED CELL DONORS - THE AUSTRALIAN EXPERIENCE.

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The Australian Red Cross Blood Service - Victoria (ARCBS-VIC) provides approximately 7250 units of red blood cells (RBCs) per annum to around 160 patients undergoing treatment at the Medical therapy Unit at Monash Medical Centre (MMC) in Melbourne. The vast majority of these patients are being treated for homozygous beta thalassaemia. Approximately 4980 units or 69% of all RBCs required by these patients need to be filtered and washed to prevent serious transfusion reactions and to reduce patient morbidity. The current process of filtering and washing these RBCs is a manual process which is time consuming and costly. In addition, the process reduces the shelf-life of RBCs from 35 days to 24 hours, making the logistics of scheduling transfusion appointments and managing patient non-compliance extremely problematic. There is a real risk that RBCs once washed, if not required by the original patient, may need to be discarded once the 24-hour shelf life expires, at a high cost to ARCBS-VIC. It is now possible to automate the process of RBC collection using state-of-the-art apheresis technology developed by the Haemonetics® Corporation. The procedure, which takes approximately 30 minutes, provides the blood service with two units of filtered and washed cells, collected in a closed system and therefore with an expiry of 35 days. This product is revolutionising the treatment of this patient population by immediately reducing donor exposure by at least 50%, standardising product parameters such as absolute RBC volume and haematocrit, and by providing flexibility in scheduling regular three to four week transfusion appointments. A joint project between ARCBS-VIC, MMC and Haemonetics® took place in 1997/98 to investigate transfusion of two-unit RBC collections to patients with transfusion dependent thalassaemia. The study was conducted in two phases. The component validation phase demonstrated that plasma-reduced leukoreduced RBCs collected by the Haemonetics® MCS+ SDR protocol can be stored up to 35 days without any deleterious effect on the quality of the RBCs. All in-vitro parameters were well maintained within acceptable limits. The second phase involved collection of two-unit RBCs from 100 volunteer donors for the purpose of transfusion to a selected cohort of thalassaemia patients. This resulted in the transfusion of 192 units over 48 transfusion episodes without any significant adverse reactions being seen. Following the success of this project, a decision to implement a two-unit RBC collection program to support these patients was made, and the program commenced in late 1999. This paper presents an overview of the first six months of this program. Keeping in mind the unique culture of blood collection in Australia, it examines the logistics of setting up the program including the human and other resources necessary. It looks at the level of acceptance of the program by donors, staff of ARCBS-VIC, and patients and staff at MMC. In particular, the paper examines the local issues regarding the conversion of committed whole blood donors to an automated panel focussing on recruitment and retention strategies, the impact on both apheresis and whole blood collection areas and the numerous logistical problems that were encountered. The paper concludes with some recommendations for phase two of the program, when it is anticipated that two-unit RBC collections will become part of routine apheresis operations at ARCBS-VIC.

GENOTYPING FETAL DNA EXTRACTED FROM MATERNAL PLASMA

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Current methods for obtaining tissue for fetal genotyping include amniocentesis between 15-19 weeks gestation and chorion villus sampling (CVS) at 9-12 weeks gestation. Both techniques are invasive and there is a risk of fetal loss following either procedure, as well as a pronounced risk of the sensitization of the mother to paternally-derived fetal antigens. Rh haemolytic disease of the newborn (HDN) may affect RhD-positive babies when the RhD-negative mother has become sensitized to the fetal D antigen, and transfer of antibody across the placenta can lead to destruction of fetal red cells. Evidence of two-directional transplacental cell trafficking has accumulated and fetal lymphocytes have been identified in the maternal circulation. These cells are few in number, and there have been reports of the persistence of fetal lymphocytes in maternal blood and/or skin for many years. The possibility that residual fetal cells from previous pregnancies could contaminate the results for a current pregnancy has discouraged further attempts at isolation of fetal lymphocytes. Recent reports have indicated that fetal DNA may be extracted from maternal plasma however and identified using a polymerase chain reaction (PCR) technique. Fetal DNA, detected by the gene sequences corresponding to RhD or to SRY, has been reported to be present in the plasma throughout the pregnancy and is rapidly cleared from the plasma after delivery of the infant. We have modified this PCR technique and a preliminary investigation of the occurrence of fetal DNA in maternal plasma has been made. Serial plasma samples from 37 RhD-negative pregnant women, together with available postnatal samples, have been stored frozen and fetal DNA has been extracted from each series of samples. We can confirm that fetal DNA, as the gene sequence RhD, is detectable from at least 6 weeks gestation continuously throughout and shortly after the birth of a RhD-positive baby. Our series has been expanded to include 41 mothers of any blood group giving birth to male infants, so involving an investigation of the gene sequence SRY as it appeared in the maternal plasma before and after delivery. We have detected this sequence in maternal plasma taken as early as 3 weeks gestation. Most mothers cleared detectable fetal DNA from the circulation between 10-100 hours after delivery. These studies will be expanded to include gene sequences corresponding to other blood group antigens involved in HDN, such as Kell and the Rh genes E and c. Detection of the gene sequences for the blood group antigens Fya , Fyb, Jka and Jk b will be invaluable in identifying fetal DNA extracted from maternal plasma in the event of a negative result for the gene sequence of primary concern. It is hoped to further expand this testing to include RhD variant genes as well as platelet antigens of the HPA series, knowledge of which may be pertinent in cases of suspected neonatal alloimmune thrombocytopenia (NAIT).

POLYMORPHISMS IN THE HFE GENE PROTECT AGAINST IRON DEFICIENCY ASSOCIATED WITH BLOOD DONATION

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Hereditary haemachromatosis (HH) is a disorder characterised by excessive accumulation of iron. 3 variant alleles of the HFE gene have been described which are associated with the disease; C282Y, H63D and S65C. Homozygosity for C282Y carries the greatest relative risk of HH, whilst the presence of H63D either in combination with C282Y or in homozygotes carries substantial increased risk. The high frequency of the variant alleles in the normal population suggests underlying positive selective pressure. The aim of this study was to test the hypothesis that a single HH associated allele affords protection against iron deficiency occurring as a result of blood loss. 397 donors attending the Pirie Street Blood Donation Centre were recruited to this study. Only those donors with an initial capillary Haemaglobin screen within prescribed donation limits were accepted. Non fasting venous blood samples were collected from all donors and assayed for serum iron, ferritin and transferrin to establish iron status. HFE genotype was determined by restriction fragment length polymorphism analysis. Donation history was determined from donor records. 130 males and 267 females participated in the study. Females under 50 years were over-represented because this group was considered to be at the greatest risk of iron deficiency. As expected, iron depletion was evident in females under 50 compared to the other groups. 24% of the women under 50 in this study were classified as iron deficient (ferritin less than 10ug/L). Serum ferritin levels in women under 50 were directly related to the number of donations in the preceding year. This trend was also evident in females over 50 and males. In women carrying a single HH associated allele, significantly higher serum ferritin levels were observed compared to those carrying only wild type alleles ($p=0.002$). This effect was lost when the number of donations exceeded 2 over the course of a year. No significant difference in ferritin levels was seen between males carrying a variant HFE allele and those carrying the wild type gene. The frequency of women carrying a single variant HFE allele was significantly lower in the iron deficient group than in the iron replete group. This data supports our hypothesis that the presence of a variant HFE allele provides protection against iron deficiency. The effect is most marked in women under fifty with moderate blood loss (less than 1000ml).

'US CLINICAL TRIAL DATA ON CHIRON TMA HIV-1/HCV ASSAY'

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Background: Direct detection of viral nucleic acid of HIV-1 and HCV can further enhance the safety of blood supply by identifying blood units donated during the pre-seroconversion infectious period. The Chiron Procleix™ TMA HIV-1/HCV Assay System is developed specifically for high throughput blood screening with simultaneous detection of HIV-1 and HCV RNA. This assay is manufactured by Gen-Probe Inc. Project: Under an Investigational New Drug (IND) application, the TMA assay was implemented in five blood center labs to screen over 70% of the US blood supplies. Donor plasma specimens are pooled into minipools comprising 128, 24 or 16 specimens for initial testing. Individual specimens composing a reactive pool are re-tested. A reactive specimen is further tested with the TMA Discriminatory Assays to determine the viral specific reactivity. HIV-1 and HCV serologic results on these specimens are collected and compared. Seronegative donors with positive TMA results are enrolled into a follow-up study. Results: The TMA assay has been successfully implemented for routine blood screening and caused no impact on blood supply and product release time. Since March/April 1999, nearly 8 million donations have been tested. The specificity of this assay is >99.4% for plasma pools and ?99.98% at the level of individual donor specimens. The TMA assay had identified three HCV antibody positive units that were missed (false negative) in serology testing. A total of 4 HIV-1 and 27 HCV seronegative window infectious units have been identified for the yield of 1 in 1,962,500 for HIV-1 and 1 in 290,740 for HCV. For one of the HCV RNA positive donations, the platelet unit was transfused and patient was infected. Conclusion: Chiron TMA HIV-1/HCV assay has been used for blood screening on over 70% of nation’s blood supply. It is shown that the TMA assay identifies additional infectious donations and detects failures in routine serology testing. Furthermore, nucleic acid testing results are useful for donor counseling.

DETECTION OF ANTIBODIES TO PEPTIDES DEFINING MILTENBERGER ANTIGENS IN AN ELISA IN WHICH STREPTAVIDIN IS USED TO CAPTURE THE PEPTIDE ANTIGEN

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In ethnic groups from southern China and SE Asia, antibodies of Miltenberger specificities comprise in the vicinity of 60% of the clinically significant antibodies detected. We report a simple ELISA in which biotinylated-peptides, captured by streptavidin conjugated to the plastic surface, are the target for detection of antibodies to these red cell antigens. Binding of antibodies (IgG) was quantified by measurement of absorbence following incubation with HRP-protein-G and substrate. Peptide epitopes representing the Mi(a) (MNS 7), Mur (MNS 10) and Hil (MNS 20) Miltenberger antigens from the exon 3 region of the various glycophorins were synthesised in a biotinylated form and then applied directly to streptavidin or neutravidin coated plastic wells. Wells coated with streptavidin or neutravidin at similar levels with an unrelated (viral) peptide were used as controls. The sensitivity of the test at various levels of streptavidin and neutravidin were compared. For streptavidin, high levels of background absorbency, consistent with previous reports for systems of this type, were observed. For positive sera, ratios of activity (test/control) were between 2 and 10. Antibody to the various antigens were detected in twelve of nineteen sera that agglutinated Mi(a) positive red cells, with anti-Mur the most common (12) and anti-Hil the least common (2). The specific activity was removed by absorption with s-positive Mi(a) positive cells but not s-positive Mi(a) negative cells, confirming that this test is specific. In three of seven anti-S sera, antibody activity was detected to the Mur peptide and in one case to the Mi(a) peptide as well. This activity was not removed by absorption with S-positive cells (which removed all anti-S activity) or s-positive Mi(a) positive cells. Various sera with anti-M, anti-N or other activities were also tested but no activity was detected in the ELISA. Because of the activity detected in the anti-S sera, the test in its current form cannot be used to screen for anti-Mi activity. However, the ELISA can be used to quantify the relative level of antibody to the various Mi epitopes and thus provide a basis for evaluation of the relative clinical significance of sera in which there is anti-Mur alone in comparison to sera containing high levels of activity to all three Mi epitopes.

EFFECT OF DISEASE STAGE AND FUNCTIONAL STATUS ON THE TRANSFUSION TRIGGER AND NUMBER OF UNITS TRANSFUSED IN HIV PATIENTS.

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Various clinical and laboratory data influence the decision to transfuse red blood cells (RBCs) and influence the number of units to transfuse. We examined whether the hemoglobin level before first transfusion in HIV infected patients and the number of transfused units had any relationship to quality of life (QOL), physical functioning or HIV disease stage. Methods. The multi-center Viral Activation Transfusion Study (VATS) enrolled HIV+ patients with anemia who required RBC transfusion (Busch et al, 1996). 428 patients were included in the analysis, which was limited to the first transfusion episode (all RBC units given in the first two days). The QOL instrument, developed specifically for HIV infected patients (Bozette et al, 1995), was self-administered prior to transfusion. It includes several subscales and a summary Perceived Health Index scale. The Karnofsky Score, a measure of physical functioning, is completed by a health care worker. Data were analyzed by descriptive statistics, nonparametric Spearman correlation, multiple regression (for hemoglobin) and generalized linear regression models (for number of units). Results. The patient population was 80% male and 59% white. The mean(±sd) age was 38.3(±8.3) years, Karnofsky score 72.7(±12.7) and hemoglobin 72(±13) g/L. 68% had CD4+ cell count <50 and 39% had plasma HIV-1 RNA ³100,000 copies/mL. The hemoglobin level was inversely correlated with Karnofsky score (correlation r=-.21, p<.001), Perceived Health Index (r=-.11, p<.05) and CD4 count (r=-.19, p<.001). Hemoglobin level was also associated with a number of QOL subscales. In multivariate analysis, Karnofsky score (p=.003) and CD4 count (p=.001) remained significant; Perceived Health Index was not (p=.87). Subjects received between 1 and 6 RBC units, with 90% receiving 2 or 3 units. The number of units transfused was most strongly associated with hemoglobin level (r=-.43, p<.001). The mean hemoglobin was 75.5 g/L for patients receiving 2 units and 68.3 g/L for those receiving 3 units. The number of units transfused was also correlated with CD4 count (r=.18, p<.001) and Karnofsky score (r=.14, p<.001) in unadjusted analysis. In multivariate analysis, hemoglobin was associated with the number of units transfused (p<.001) but not with Karnofsky score (p=.84) or CD4 count (p=.17). Conclusions. In this group of end-stage HIV+ patients, lower Karnofsky scores and CD4 counts prompted transfusion at higher hemoglobin levels. However, after controlling for hemoglobin level, the number of units transfused was not associated with Karnofsky score or CD4 level. Self-perceived QOL was not associated with the transfusion trigger or with the number of units transfused after accounting for these other factors. HIV stage and functional status influenced the decision to transfuse at a particular hemoglobin level but did not influence the number of RBCs to transfuse.

MODULATION OF MONOCYTE ACTIVATION BY HUMAN IMMUNOGLOBULIN G AND HUMAN IMMUNOGLOBULIN A

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Immunoglobulins modulate monocyte activation via Fc receptors present on the cell surface. There are three main classes of Fcγ receptors, FcγRI, II and III which are all constitutively expressed on monocytes and macrophages. IgG binding to these receptors causes monocyte activation which is characterised by phagocytosis, endocytosis, ADCC and release of inflammatory mediators such as TNF, IL-1 and IL-6. Interaction of IgA with its receptor FcαRI also triggers metabolic activation in monocytes however IgA may have an additional down-regulatory function preventing an exaggerated and harmful inflammatory response. *In vitro* experiments have shown that human serum IgA down-regulates the release of inflammatory cytokines TNF and IL-6 from human monocytes activated with LPS.[1] The anti-inflammatory properties of IgA may also be due to its potent induction of IL-1 receptor antagonist which can inhibit IL-1 activity.[2] Commercially available IVIG preparations such as Intragam (CSL Ltd.) contain predominantly IgG but also small amounts of IgA. An IgA-depleted preparation, Intragam P (CSL Ltd.) is also available. In these experiments, Intragam and Intragam P were compared in their ability to modulate LPS-induced TNF production by monocytes in a mixed lymphocyte culture. Monocytic IL-10 production was also measured, as monocyte activation may be accompanied by negative feedback inhibition by this anti-inflammatory cytokine. Ficoll-separated PBMCs from healthy donors, were cultured in non-adherent conditions in 10% FCS/ MEM for 4 and 24 hours at 10^6 cells/ml. The cells were treated with 0, 1 or 5 mg/ml Intragam or Intragam P +/- 100 ng/ml LPS +/- 250IU IFNγ in the presence of protein transport inhibitors, monensin or brefeldin A. The cells were harvested and washed and monocytes were labelled with CD14. The cells were fixed and permeabilized and intracellular cytokines were labelled with anti-human TNF antibody (Mab11), anti-human IL-6 antibody (IC206P) and anti-human IL-10 antibody (JES3-19F1). Monocytic intracellular cytokine levels were measured by flow cytometry. Results showed no difference between Intragam and Intragam P in the ability to modulate intracellular TNF, IL-6 or IL-10 production in LPS/ IFNγ - stimulated human monocytes. This result may be due to the amount of IgA present in Intragam being insufficient to provoke a response in this system.

1.Wolf *et.al.* (1994) Blood **83**:1278-1288.
2.Wolf *et.al.* (1996) Clin Exp Immunol **105**:537-543

INFLAMMATORY MEDIATORS DETECTED IN POOLED BUFFY COAT PLATELET PRODUCTS PREPARED WITH PLASMA AND T-SOL ADDITIVE SOLUTION.

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The traditional platelet product issued by transfusion services has undergone many modifications in recent years. These changes have conveyed many benefits to transfused recipients, particularly in the reduction of adverse reaction rate as a result of lowered leucocyte content. Furthermore, the introduction of T-Sol as platelet suspension media has the added benefit of saving plasma and potentially overcoming further transfusion reactions arising from plastic activated complement and non-compatible proteins by diluting residual plasma.

In this study, cytokine (IL-6 and IL-8) content, specific plasma proteins (IgA, IgG and IgM) and the complement component C3a have been targeted as markers for potential adverse reactions. Twelve samples of Buffy Coat Platelet Concentrates (BC PCs) prepared with plasma and 12 prepared with T-Sol were analysed on days 1 and 5 of storage. Results showed that the amount of total protein present in T-Sol PCs (16.4±2 g/L) was consistently one third from that present in plasma PCs (52±2 g/L). The same ratio was found in the content of potentially non-compatible proteins (IgA, IgG and IgM). Furthermore, there was a significant difference in the content of complement component C3a in plasma BC PCs as compared to T-Sol PCs. Results are listed below:

PC Type	C3a (ng/mL) Day 1	C3a (ng/mL) Day 5
Plasma	2180±3	8978±4
T-Sol	253±2	397±4
p-value	< 0.0001	<0.0001

Finally, no significant difference was found in the level of cytokines (IL-6 and IL-8) present in both types of platelet products. This level was minimal in both cases (< 10 ng/mL for IL-6 and < 80 ng/mL for IL-8).

These results demonstrate that BC PCs prepared with T-Sol additive solution may offer an improved option for blood transfusion recipients by reducing exposure to immunoreactive plasma proteins

EVIDENCE THAT TREATMENT OF MATERNAL AUTOIMMUNE NEUTROPENIA WITH G-CSF PREVENTS PASSIVELY ACQUIRED NEONATAL NEUTROPENIA

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Clinical History: A pregnant woman with autoimmune neutropenia (AIN) had previously delivered one infant with severe neutropenia due to the placental transfer of maternal neutrophil reactive autoantibodies. Prophylactic G-CSF for the AIN was ceased prior to her pregnancy and recommenced at 30/40. We followed her from 16/40 monitoring her anti-neutrophil autoantibody levels. Method: Maternal serum samples were collected during gestation and post delivery. These were tested in doubling dilutions against neutrophils from 2 random donors and a constant volunteer donor, the patient’s husband and after delivery, against cord neutrophils. Testing was performed by granulocyte immunofluorescence test (GIFT), granulocyte agglutination test (GAT) and the monoclonal antigen immobilisation of granulocyte antigens (MAIGA) test. Results: The autoantibody had broad specificity. The titre peaked at 26/40 and between sampling times the titre varied by one tube. Upon recommencing G-CSF the neutrophil count rose while the titre fell. A healthy infant was delivered at 38/40 weeks with a neutrophil count of 3.95 X 10 9/L with no detectable neutrophil antibodies. Conclusions: G-CSF produced a fall in the anti-neutrophil antibody titre, with a corresponding rise in the neutrophil count. There was good correlation between antibody titres against the cord and husbands neutrophils. Although the validity of monitoring titres of anti-neutrophil autoantibodies in pregnancy has not been thoroughly examined, the antibody titres provided a useful guide for the clinical management of this case.

PATENTABILITY IN BIOTECHNOLOGY INVENTIONS

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Biotechnology Patents covers a wide range of subject matter, ranging from microorganisms to complex biotechnological processes. Many biotechnological inventions involve the isolation and characterisation and use of genes and proteins derived from plants, animals, yeast, bacteria and viruses and there is much overlap with the pharmaceutical industry. More recently, advances in biotechnology have hit the headlines, such as patent claim for the human genome, genetherapy, genetically engineered mice, crops with herbicide, disease or pest resistance, and the cloning of animals (such as Dolly the sheep). In this area there is not only the development of new technology to consider but also the legal, moral, ethical and environmental issues that comes with it. Although theoritically, the patent laws should apply to biotechnology like any other technology, in practice this is not always easy. Despite the recent redrafting of the Patents Act (Cth) in 1990, advances in biotechnology in recent years, especially in the area of genetic engineering, have developed and evolved so rapidly that it has resulted in difficulties when applying and interpreting the law. To make matters worse, legislation with respect to patents in each country varies vastly in provisions such as banning on cloning, varying levels of ‘manner of manufacture’ and prohibition on patenting inventions that are contrary to morality and public order. With the advent of patentable life forms and gene sequences, moral issues are being brought into light and this is a unique issue for biotechnology patents. While moral, ethical and environmental aspects are still debated, the legal framework of intellectual property struggles to cope with modern biotechnology research outcomes. This paper discusses the scope of biotechnological patent rights and associated issues that need to be considered by researchers.

COLLECTION OF LARGE NUMBERS OF AUTOLOGOUS PLATELETS VIA THE COBE SPECTRA V-6 FOR USE IN PATIENTS UNDERGOING HIGH-DOSE CHEMOTHERAPY AND STEM CELL TRANSPLANTATION.

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Thrombopoietin (rhTPO) is a naturally occurring, glycosylated polypeptide that stimulates the differentiation of bone marrow stem cells into megakaryocytes and ultimately platelets. We have recently administered rhTPO (Genentech, Inc, CA USA) at the Mater Hospital under a randomised dose finding clinical trial to induce thrombocytosis and facilitate collection of large numbers of platelets for cyropreservation and later administer as required in autologous transplantation. The aim of the study is to explore the feasibility and the efficacy of autologous platelet (PLT) support with two dose schedules of rhTPO administered to patients undergoing high dose chemotherapy and peripheral blood transplant. rhTPO 1.2g/kg IV bolus (4 patients, day 1 and 4) and 2.4g/kg IV bolus (3 patients, day 1) was administered upon platelet recovery (>100 x 109/L) after peripheral blood stem cell collection or just prior to autologous transplant in one patient who had previously collected peripheral blood stem cells in 1996. Platelet counts and adverse events are monitored daily after rhTPO administration and collected via the COBE SPECTRA V-6 once platelet count reaches >600 x 109/L or on day 12 after first rhTPO IV injection if the platelet count has not reached 600 x 109/L. Seven patients have been collected so far with a median age of 58yrs (range 50-70), all patients had received chemotherapy prior to mobilisation of peripheral blood stem cells. All patients had a Vascath central venous access device inserted in the outpatient clinic. Two patients developed an infection requiring the Vascath to be removed prior to platelet collection resulting in peripheral apheresis access. We utilised the platelet collection mode over 120 minutes. Once parameters have been set the target volume is reduced via the increasing the concentration of cells/microlitre collected. This reduces the amount of plasma platelets are suspended in and therefore reduces the final volume to assist in cyropreservation of small volumes. The concentration is initially increased from 1,400,000 to 2,700,000 cells/microlitre and raised further if possible during the procedure if platelet clumping is not hazardous. The blood inlet/anticoagulant ratio is raised as soon as possible to maximise collections and is raised intermittently. To minimise adverse events such as hypotension an IV fluid bolus is given during the procedure if the final volume collected >500mLs.

Patient No	Plt Count Day 1 of rhTPO x 10^9/L	Plt Count Day 1 of Apheresis x 10^9/L	Total Plt Yield x 10^{11}	Number of Apheresis
1	100	715	42.33	3
2	113	374	17.81	4
3	215	405	21.14	4
4	143	604	26.04	3
5	127	713	34.26	2
6	100	369	19.31	4
7	181	623	25.97	2

Collection of autologous platelets has been possible in this setting and patient accrual continues. Details of planning patients for multiple platelet apheresis with the use of rhTPO, platelet collection parameters, further results and adverse events associated with apheresis will be presented.