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HSANZ Free Communications 9: Haematopoietic Stem Cell Transplantation II

Arena 1B

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New Malignancies Post Stem Cell Transplant: A Report from the ABMTRR

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Aim

To investigate the incidence and characteristics of new malignancies post haematopoietic stem cell transplant (HSCT) in Australia and New Zealand.

Methods

HSCT records were drawn from the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR). The population for this study was HSCT performed in Australia or New Zealand between 1992 and 2006, where the patient had not died before 1996. Patients selected for this study had developed a new malignancy post HSCT not related to the disease for which they were transplanted.

Results

Excluding basal and squamous cell skin carcinomas, a total of 198 new malignancies were reported among 11,224 HSCT performed between 1992 and 2006. From 3,943 allogeneic HSCT, 66 new malignancies were reported; and from 7,281 autologous HSCT, 132. The 5-year cumulative incidence of new malignancies was 1.8% post allogeneic and 1.7% post autologous HSCT, in comparison to a recent University of Minnesota study which reported a 5-year cumulative incidence of 3.5% for autologous and allogeneic HSCT combined. Of the malignancies that developed post allogeneic HSCT, the largest numbers were for melanoma (10, 15% of all reported), lymphoproliferative disorders (6, 9%) and adenocarcinoma (5, 8%). Among the malignancies that developed post autologous HSCT, the largest numbers were for myelodysplastic syndromes (15, 11% of all reported), AML (11, 8%), adenocarcinoma (10, 8%) and lung cancer (10, 8%). Survival probability at 5 years post diagnosis of the new malignancy was 60% for allogeneic and 22% for autologous HSCT recipients.

Conclusions

New malignancies post HSCT are an unusual but serious adverse event which may lead to poor survival outcome particularly among autologous recipients. The ABMTRR is a valuable national resource which provides accurate follow-up information on long-term transplant survivors.

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Allogeneic Transplantation in Acute Myeloid Leukaemia in Untreated First Relapse: A Single-centre Retrospective Outcome Analysis Over 10 Years

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Background

Allografting may offer the only chance of cure for patients with acute myeloid leukaemia (AML) in first relapse. There are limited data identifying factors effecting the outcome in these patients.

Method

A retrospective study of 54 consecutive patients from 1994 - 2006 who underwent allograft in untreated first relapse at RMH to identify prognostic factor(s) associated with event-free survival (EFS). Exploratory univariate analysis was performed using donor source, cytogenetics at initial diagnosis, age groups (<40 and ≥ 40), duration of first complete remission (CR1), bone marrow (BM) blast count at relapse and stem cell source.

Results

The median age was 37 years (range 18 - 69) with 31 sibling and 23 non-sibling matched allografts. Cytogenetics at diagnosis were favourable in 8 (15%), intermediate in 33 (61%), poor in 7 (13%) and no mitosis in 6 (10%) patients. 41% had normal cytogenetics. Median duration of CR1 was 8 months (range 1- 45) and median marrow aspirate blast count at relapse was 32% (range 1 - 94). The conditioning was predominantly myeloablative (94%). Source of stem cells was BM (n=25) and PBSC (n=29). Using Kaplan-Meier survival analysis, 10-year overall survival (OS) was 39% (median OS: 25 months). Median EFS was 14 months (range 1 - 161). Duration of CR1 was the only factor correlated with a better transplant outcome: CR1 ≥ 6 months (n=34) was associated with improved median EFS of 51 months compared with 3.5 months for CR1 <6 months (n=20) (p<0.0001). Progressive disease remained the major cause of death (64%) with early transplant-related mortality of 15%.

Conclusion

Duration of CR1 appears to be a prognostically significant factor in allograft outcome in first untreated relapse. Allografting is of little apparent benefit in early relapse.

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End of Life Care in Haematology Patients and Evaluation of a Haematology Quality of Dying Assessment (HQODA) Tool

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Aim

Identify factors impacting on quality of EOL, and evaluate a Haematology-Quality of Dying Assessment (HQODA) tool.

Methods

We retrospectively studied patients (pts) dying over 12 months managed by a single haematology unit. Data from the EOL period were extracted from medical records. To evaluate the HQODA, a random sample of 21 pts was studied more extensively. Five domains were analysed from change of focus to EOL care; pain: non pain symptoms; advanced care planning; peace/dignity; & duration of dying period. Each domain was scored 0-2, from worst to best, for a possible total HQODA score of 10, representing a very high quality of dying. Inter-Observer Reliability testing of the total HQODA score was by performing a kappa statistic to see if independent observers rated the same patients as above or below the median.

Results

The median total HQODA score was 7 (range 4-9). The most striking finding was that all patients had a brief duration of dying dimension, severe pain was uncommon (10%), but almost all (90%) patients had some degree of distress from nonpain symptoms, such as confusion & dyspnea. Complete advance care planning with documentation of wishes or a clearly designated surrogate decision maker was rare (24%). A large minority (24%) of deaths were considered unpeaceful or undignified. A kappa statistic of 0.58 ($p < 0.01$) indicated good reproducibility of the H-QODA for patients dying above or below the median value.

Conclusions

The EOL phase for haematology patients is short (< 1month) in virtually all cases. Therefore attempts to improve symptom management need to be preplanned or based on rapid response times. The HQODA tool reproducibly identified patients undergoing above or below average quality of dying experiences

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Microbiological Contamination of Autologous Peripheral Blood Stem Cell Product – Incidence and Clinical Outcome

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Aim

Microbiological screening for contamination is part of quality control of autologous peripheral blood stem cell (PBSC) manipulation. There is wide variation in the incidence of positive cultures between different laboratories and no consensus on the interventions required for contaminated PBSC product. The clinical significance of reinfusion is uncertain. This study aims to determine the incidence of microbiological contamination of PBSC product and to evaluate the clinical significance of transfusing contaminated PBSC products during autologous transplantation.

Method

A retrospective analysis of microbiological cultures performed in 606 consecutive autologous PBSC harvests from 278 patients, between 1995 and 2005 in The Canberra Hospital was undertaken. Medical records of patients who received contaminated PBSC harvests were reviewed to assess clinical outcomes.

Result

Eleven (1.26%) of the 873 cultures were positive, in eight (2.9%) patients. One patient had a clinical diagnosis of line related sepsis made on the day of collection. Four of the collections with positive cultures had them taken before and after cryopreservation and all were positive only in the post manipulation sample. Most positive cultures were due to Coagulase-negative *Staphylococcus* (10 isolates) and one was due to *Corynebacterium accolens*. Of the 11 contaminated PBSC products, 7 had been reinfused. Prophylactic antibiotics were administered when there was known coagulase-negative *Staphylococcus*. No clinical signs of infection followed infusion of any of the 7 contaminated products.

Conclusion

In our laboratory, the incidence of microbiological contamination of autologous PBSC product was low and usually due to skin commensals. Reinfusion of culture positive PBSC harvests with skin commensals is safe with antibiotic prophylaxis, although others have reinfused without antibiotic cover, also with apparent safety. These data have been used to develop a local policy to respond to culture positive harvests.

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HSCT Patient and Family Education Improves Respiratory Virus Infection Awareness and Influenza Vaccination

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Aim

Following haematopoietic stem cell transplantation (HSCT), respiratory virus (RV) infections have high morbidity and mortality. Patient and family awareness of methods to prevent this is unknown. This study aims to describe and improve HSCT patients' and their family/friends' awareness of:

1. the risk of RV infections following HSCT, and
2. effective methods of preventing RV infections (family vaccination, avoiding symptomatic contacts).

Methods

All patients preparing for HSCT at Westmead Hospital, Sydney, and their families, are invited to a bi-monthly education day including a session on RV infection. 186 attendees at 8 consecutive sessions were invited to complete self-administered pre/post questionnaires. 113 (61%) completed both questionnaires and were included (33 patients, 80 family/friends). Family vaccination was assessed at HSCT admission. Statistical analysis was performed using SPSS (chi-square test).

Results

Awareness increased that RVs cause respiratory illness (74% to 94%, $P < 0.0001$) and are common post-HSCT (87% to 95%, $P < 0.0001$). Awareness that influenza in HSCT recipients could be fatal or require ICU management increased from 67% to 90% ($P < 0.0001$), however 15% of patients and 8% of family/friends did not acknowledge this at follow-up ($P = 0.03$). The proportion of respondents listing at least one effective method of preventing influenza post-HSCT increased from 43% to 82% ($P < 0.0001$); listing vaccination increased from 12% to 61% ($P < 0.0001$). Belief that family vaccination reduces influenza risk post-HSCT increased in family/friends from 63% to 98% ($P < 0.0001$), but was unchanged (75% to 82%, $P = 0.1$) and significantly lower in patients ($P = 0.005$). On admission for HSCT, at least one family member had been immunised against influenza in 48/107 families. Vaccination was three-times as likely if the patient or family had attended an education day (60% v 23%, RR 2.7, $P < 0.0001$).

Conclusions

Pre-HSCT education increases awareness of RV illness, prevention methods, and uptake of family influenza vaccination. Education of family in addition to patients is essential.

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**Fludarabine/Melphalan Reduced-Intensity Allogeneic Stem Cell
Transplantation for Advanced Haematological Malignancies. A Single
Centre Experience of 37 Patients**

0945

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Aim

We report the outcomes of reduced-intensity allogeneic stem cell transplantation using Fludarabine (30mg/m²) and Melphalan (140 mg/m²) conditioning at our centre.

Method

Thirty-seven patients with advanced haematological malignancies underwent Sibling (full match)- 51 %; Mismatch Sibling/Other Family – 11%, Cord- 3% and Matched Unrelated Donor – 35% transplantation. Graft vs Host Disease (GVHD) prophylaxis consisted of Cyclosporine A, Methotrexate and Prednisone (with the addition of ATG for matched unrelated donors). The median patient age was 50 years (range, 29 – 64 years). Twenty-two had previous failed autologous transplantation, and 1 had previous failed allogeneic transplantation. Only 4 were in complete remission at time of transplantation. Disease at time of transplant was: 6 primary acute leukaemia, 3 secondary acute leukaemia, 3 secondary MDS, 1 primary MDS, 11 multiple myeloma, 12 NHL and 1 myelofibrosis.

Results

At a median follow-up of 21 months (range, 0.2 – 84 months), 12 (32 %) remain alive. Median survival was 17 months (range 0.2 – 68 months). Sustained donor engraftment (neutrophils) occurred in 33 (89 %) patients. Eight (22 %) patients relapsed. GVHD occurred in 24 individuals; 42 % had acute GVHD (50% had \geq Grade 3 severity) and 55% had chronic GVHD (44% moderate to severe). Ten of the 12 survivors had mild to moderate chronic GVHD. The day 100 transplant-related mortality (TRM) was 32 %, with the overall (all cause) mortality at 1 year of 51%. GVHD accounted for 6 (24%) deaths, primary disease 7 (28%) and sepsis 12 (48%).

Conclusion

The combination of Fludarabine/Melphalan as a reduced intensity conditioning regime for allogeneic transplantation in a group of high-risk patients with advanced disease can lead to prolonged disease control in up to one third of patients, but at the cost of high TRM.

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Does the Murine Erythroleukaemia Model correlate with De Novo and Secondary Erythroleukaemia regarding P53 and C-Kit Mutations?

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Introduction

The murine erythroleukaemia model, using the Friend retrovirus provides important molecular information and highlights the multistep molecular pathogenesis of acute myeloid leukaemia (AML). Both P53 and C-Kit mutations are common abnormalities in this model, giving leukaemic cells both a survival and proliferative advantage. Post-therapy response assessment by morphology in erythroleukaemia present difficulties and demonstrates a need to identify better disease monitoring methods.

Aim

To study both de-novo acute erythroleukaemia and secondary AML patients with an "erythroleukaemia morphological phenotype" for:

- P53 and c-kit mutations.
- P53 mutation correlation by high resolution melting (HRM) and immunohistochemistry (IHC) at diagnosis.

Assess the usefulness of P53 IHC as a marker for minimal residual disease

Methods

Patients were identified through the Pathology database from 1998 - 2007. DNA was extracted from archival marrow aspirates. P53 mutation testing, encompassing exons 5-8 was performed using HRM. C-Kit testing for the D816V mutations was performed using a competitive PCR blocker methodology.

Results

Six de-novo erythroleukaemia patients were evaluated, median age 60 (52-95), 4 males. P53 mutations were seen in 2/5 tested, P53 IHC was positive in 1 mutation positive case. C-Kit mutations were seen in 1/5 patients. At diagnosis 2 had complex karyotypic anomalies, 3 normal/intermediate prognosis karyotype and 1 had no mitoses. Median DFS was 22 weeks. 9 secondary AML patients with an erythroleukaemia morphological phenotype were included. Median age 66 (22-95), 7 males. HRM P53 positivity was seen in 6/7 cases examined; all these demonstrated IHC P53 positivity. C-Kit mutations were seen in 4/7 cases tested, 3 of which had a concomitant P53 mutation. Five had a complex karyotype, 2 normal and 2 had no mitoses. Median DFS was 5 weeks. Four P53 IHC positive case at diagnosis demonstrated 5-15% residual P53 positivity post induction, although reported as morphological complete remission.

Conclusion

P53 and C-Kit mutations were seen predominantly in the secondary erythroleukaemia cases. P53 mutation positive cases correlated with IHC and a complex karyotype and DFS was poor. P53 IHC may be useful for minimal residual disease assessment,

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PKC epsilon Signaling in Ph+ ALL – A Novel Role in Reduced Sensitivity to Imatinib

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Aim

Philadelphia positive (Ph+) ALL is less responsive than CML to imatinib when used as a single agent, with frequent occurrence of relapse and ~30% primary resistance. To identify the molecular mechanism of this reduced sensitivity, we used gene expression profiling (GEP) of leukaemic cells from patients receiving imatinib to identify candidate genes that are pivotal in bcr/abl signalling and imatinib resistance.

Method & Result

Blast cells were isolated from Ph+/CD10+/CD19+ ALL patients before treatment and on consecutive days after administration of imatinib, and their gene expression interrogated by GEP microarrays. Over 400 genes with ≥ 1.5 -fold up- or down-regulation were identified in treated cells compared to pre-treatment. One of 6 selected genes of interest is the epsilon isoform of Protein Kinase C (PKC ϵ), known to play important roles in the positive regulation of survival, proliferation or cell adhesion, or resistance to drug induced cell death when expressed at high levels in various cancers. The expression of PKC ϵ was >20 fold higher in Ph+ ALL cells (patients and cell line: SUP-B15) than Ph+ CML cells (patients and cell line: K562). This correlated well with the degree of imatinib response as 50% of SUP-B15 cells compared to 90% of K562 cells underwent apoptosis. In SUP-B15 cells, PKC ϵ was progressively down regulated as imatinib induced apoptosis increased in a dose dependent manner, suggesting that PKC ϵ is important for pro-survival signalling. Thus, the over expression of PKC ϵ in K562 cells appeared to reduce their sensitivity to imatinib; whilst treatment of SUPB15 cells with PKC inhibitors reduced cell survival. The selective inhibition of PKC ϵ was evaluated by siRNA knockdown.

Conclusion

In conclusion, our experimental data suggest that high expression of PKC ϵ in Ph+ ALL compared to CML resulting in reduced sensitivity to imatinib, and that PKC ϵ down regulation is necessary for apoptosis.

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0900

NO-Aspirin is a Potent Mediator of Apoptosis in B-Cell Progenitor Acute Lymphoblastic Leukaemia (ALL) Cells

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Although patients with acute lymphoblastic leukaemia frequently achieve complete remission, disease relapse is common and difficult to treat, necessitating new therapeutic strategies for the treatment of ALL. NO-aspirin is a novel drug which has recently been shown to have enhanced efficacy against a number of solid tumors. We hypothesized that NO-aspirin would also effectively kill leukaemic cells. Overnight treatment with NO-aspirin attenuated growth of the pre-B ALL cell lines Nalm6, Reh and LK63, with IC₅₀s of 2, 3 and 6.5 micron respectively. The reduction in cell growth was not due to cell cycle arrest, as measured by cell cycle analysis but was the consequence of apoptosis. Caspase-3 activation was detected as early as 3h, peaked at 6h and was sustained for up to 12h following drug treatment. The pan-caspase inhibitor Z-VAD-FMK completely prevented Caspase activation and completely abrogated NO-aspirin induced cell death, confirming that apoptosis was the sole form of cell death. NO-aspirin also resulted in increased levels of reactive oxygen species (ROS). N-acetylcysteine (NAC), a free radical scavenger significantly inhibited NO-aspirin mediated apoptosis, suggesting that NO-aspirin mediates cell death at least partially via production of ROS in ALL cells. A time course analysis of leukaemic cells treated with NO-aspirin revealed phosphorylation of p38MAPK and JNK proteins. Further quantitative evaluation of genes involved in apoptosis revealed that Mcl-1, TNFR and Bcl-2 were down-regulated while Survivin remained unchanged in drug treated Nalm6 cells. Experiments are currently underway to examine the role of NF-κB signalling in NO-aspirin induced cell death in leukaemic cells. Our results suggest that NO-aspirin induces apoptosis in leukaemic cells via production of reactive oxygen species and activation of MAPK signalling pathways. It is possible that NO-aspirin could be used in combination with conventional drugs used to treat ALL with minimal additional toxicity.

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O100 **0915**
Analysis of Retroviral Integration Sites in Experimentally-Induced Murine B Cell Lymphomas

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Aim and Background

The pathways that are important for mature B cell survival, growth and proliferation are poorly understood, but are likely to be critical in the pathogenesis of B cell lymphoproliferative disorders. We have identified the retroviral integration sites in a set of 25 murine B cell lymphomas in order to clarify how known pathways are integrated and to illuminate new pathways.

Method

Donor murine splenic B cells containing a homozygous p53 mutation were transduced with a green fluorescent protein (GFP)-labelled c-myc-containing retrovirus. Most transduced cells underwent premature apoptosis after re-injection into recipient mice, but a small percentage of cells proliferated excessively, forming a widely disseminated B cell lymphoma. It is proposed that the retroviral integration sites were critical for the dysregulated cell growth. The integration sites of the retrovirus within the mouse genome have thus been determined for each tumour by Splinkerette Polymerase Chain Reaction (PCR), DNA sequencing and correlation with the mouse genome on the Ensembl and NCBI (National Center for Biotechnology Information) databases.

Results

Within experiments (same donor, different recipients) different lymphoma samples show both common and unique retroviral insertion sites. Intriguingly, common insertion sites have also been identified in lymphomas which developed from different donors and recipients, in experiments performed months apart.

Conclusion

It is proposed that in the mice developing B cell lymphoproliferative disorders the retrovirus has been integrated into the mouse genome at a critical site which enhances the activity of a proto-oncogene or reduces the activity of a tumour suppressor gene, or that the integration site facilitates markedly increased c-myc expression. This method therefore has great potential to identify critical steps in lymphomagenesis, and thus potential therapeutic targets. Further experiments are in progress.

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Novel Heritable Mutation of the Transcription Factor RUNX1 as a Cause of Autosomal Dominant Familial Platelet Disorder with Predisposition to Acute Myeloid Leukaemia (FPD/AML)

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Aim

To identify the causative heritable mutation in a family with autosomal dominant familial platelet disorder with predisposition to acute myeloid leukaemia (FPD/AML).

Method

Confirmation of family pedigree, enrolment into ethics committee approved Australian Familial Haematological Cancer Study, procurement of genomic DNA from pedigree members, and genetic analysis by sequencing of RUNX1 and CEBPA genes.

Results

The proband initially presented aged 50 with mild thrombocytopaenia initially diagnosed as ITP when bone marrow examination (including cytogenetics analysis) was normal. Three years later she was referred with severe progressive thrombocytopaenia unresponsive to high dose steroids and IVIg together with mild anaemia and neutropaenia. Marrow examination revealed subtle dysplasia with monosomy 7 in 12/20 metaphases. A diagnosis of myelodysplastic syndrome (MDS) was made. Three months later (Feb 2007), she progressed to AML. The proband's mother had had mild thrombocytopaenia with subsequent MDS (aged 70) and died of AML two years later. The proband's only sibling and nephew have mild thrombocytopaenia without features of MDS. The proband entered cytogenetic remission following AML therapy, continues to show dysplastic features, has received 2 cycles of consolidation therapy and is awaiting unrelated donor allogeneic stem cell transplant. Sequencing of PCR products from exons of the RUNX1 gene identified a heterozygous mutation in the proband's constitutional DNA: c.958C>T in exon 7, in the transactivation domain, causing a nonsense mutation, p.Arg293X (sequence variation for RUNX1 classified according to GenBank Accession No. NC_000021). The sibling has the same mutation and other studies in relatives are underway.

Conclusion

This study has identified a novel RUNX1 mutation responsible for FPD/AML in this family. Clinicians should be aware of this rare inherited disorder and the availability of genetic testing. People with mutations may be asymptomatic, and genetic testing in such families is essential before considering bone marrow transplantation from a living related donor.

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O102 **0945** **A Novel Dendritic Cell-Based Strategy for the Discovery of Leukaemic Antigen for Immunotherapy**

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Aim

Acute lymphoblastic leukaemia (ALL) is the most common malignancy in children. While current treatment strategies for ALL result in good therapeutic outcomes, the prognosis for patients with relapsed leukaemia is poor. The use of immunotherapy to prolong remission in these patients is critically dependent on the identification of new tumour-associated antigens (TAA). We aim to identify new TAA by harnessing a novel strategy utilising *E. coli* expressing an ALL-specific gene library and a pore-forming listeriolysin (LLO), which can efficiently deliver TAA to the MHC class I presentation pathway on dendritic cells (DC) for recognition by ALL-specific cytotoxic T lymphocytes (CTL).

Methods

We have optimised a screening assay in 96-well plate format using *E. coli*/LLO expressing a model antigen, influenza matrix protein (FMP). The *E. coli* were loaded onto DC and incubated with a CTL clone specific for FMP. Antigen presentation was detected by IFN- γ secretion by the CTL clone. ALL-specific CTL were generated from cord blood using ALL-RNA-loaded CD34⁺DC as stimulators.

Results

Using the FMP model, we have optimised *E. coli*, DC and T cell numbers and their ratios relative to each other and determined the sensitivity of the high-throughput screening assay. We have generated polyclonal anti-leukaemic CTL cultures that lyse ALL xenograft and cell line targets, but not autologous or allogeneic mononuclear cells. Using an IFN- γ secretion assay and flow cytometry sorting, we have established 111 individual CD8⁺ clones, 15 of which appear ALL-specific. An ALL-specific gene library will now be expressed in *E. coli*/LLO and presented on MHC class I molecules to ALL-specific CTL for the identification of new leukaemic TAA.

Conclusions

The *E. coli*/LLO system is a novel strategy that will enable the screening of thousands of leukaemic proteins simultaneously to rapidly identify new ALL targets for immunotherapy and provide options for relapsing patients following transplantation.

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Escalated Dose BEACOPP is Highly Myelosuppressive but Safely Deliverable Outside the Context of a Clinical Trial: The Peter Mac Experience

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Introduction

In order to assess tolerability of BEACOPP in a local non-trial setting, ten consecutive patients with Hodgkin Lymphoma (HL) treated with Escalated BEACOPP (EB) at Peter MacCallum Cancer Centre were reviewed.

Patient Characteristics

EB was offered due to stage IV disease at presentation (9), the presence of extranodal disease (9) or failure to respond to ABVD(1). One patient changed to EB after change in histological diagnosis. The median age was 26(19-45). 2 had bulky disease. The median Hasenclever score was 3.5 (2-5). One patient received pre-planned consolidation RT. All received peg-GCSF.

Results

One patient received 8 cycles of EB, 6 patients received 6 cycles of EB, 1 received EB for 4 cycles followed by Standard BEACOPP (SB) for 4 cycles, 1 received 4 cycles of EB and 2 of SB. There were no delays in chemotherapy

In 57 evaluable cycles the median platelet nadir was 52(7-339), ANC 0.16(0-5.26), Hb 89 (62-134). 15% of cycles required platelet support, 40% required PRBC. There were six admissions for febrile neutropenia (3 nights median duration). One outpatient developed a PE post therapy, one developed grade 2 peripheral neuropathy. Two attempts were made to mobilise stem cells after cycle 3, both successfully.

Bleomycin was omitted mid therapy in 7 cases for asymptomatic decrease in DLCO on routinely measured RFTs (post cycle 3 or 4). In 1 patient bleomycin was omitted from the start. One patient developed symptomatic pneumonitis another developed pulmonary emboli. 6 of 7 who experienced a decrease in DLCO through treatment returned to near baseline.

All patients achieved a metabolic CR by PET, in 8 of 10 after 2 cycles of EB. At a median follow up of 26months (9-40), all patients remain in clinical CR. There are no cases of secondary myelodysplasia.

Conclusion

EB is deliverable outside the trial context, with acceptable haematological toxicity and rate of hospital admissions.

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O104 **0845**

How Useful are Mid-Treatment PET Scans in Predicting Outcome to Chemotherapy in Newly Diagnosed Lymphoma?

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Aims

We aimed to review the utility of mid-cycle PET scanning in predicting outcome in newly diagnosed lymphoma patients treated within our clinical group.

Methods

After a PET service opened at our institution in January 2006, outcomes of all newly diagnosed lymphoma patients who had PET scans performed pre, during (mid-cycle) and post-chemotherapy +/- radiotherapy were retrospectively reviewed. For the purpose of this analysis, negative mid-cycle PET scans were considered true-negative if progressive disease (PD) did not develop within 3mths of end of primary therapy, and false-negative if PD did develop during and / or within 3mths of primary therapy. Positive mid-cycle PET scans were considered true-positive if residual / PD was confirmed clinically or on CT scanning during subsequent follow-up, and false-positive if no PD occurred clinically and on other radiological testing during follow-up.

Results

Between January 2006 and June 2007, a total of 42 newly diagnosed lymphoma patients had completed their primary therapy and had mid-cycle PET scans performed. Histological diagnoses included 14 DLBCL, 10 FL, 10 HL and 8 other NHL subtypes. Within the limited follow-up duration thus far, overall 5 patients (12%) had false-negative, 4 (9%) false-positive, 25 (60%) true-negative and 8 (19%) true-positive mid-cycle scans. Diagnoses of patients with false-negative mid-cycle scans included NSHL 1, NLPHL 1, DLBCL 1, FL (G3a) 1 and BL 1. 3/25 (12%) of patients with true-negative mid cycle scans have suffered PD thus far, at 3+, 5 and 6mths post completion of therapy.

Conclusions

Our experience suggests that mid-cycle PET scans in newly diagnosed lymphoma are associated with a significant incidence of false-negative / false-positive results. As such results of mid-cycle PET scans should be interpreted with caution, and should not currently be used to direct subsequent lymphoma therapy outside of defined clinical trials.

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A Prospective Study of the Prognostic Impact of ^{18}F -FDG-PET and Molecular Response in patients with Relapsed Indolent Non-Hodgkin Lymphoma following Iodine-131-Rituximab Radio-immunotherapy

Mark Bishton¹, Rod Hicks¹, Miles Prince¹, Max Wolf¹, John F Seymour^{1,2}

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Aim

PET has excellent sensitivity and specificity in indolent NHL, although the impact of PET response on outcome following radio-immunotherapy (RIT) is unknown. In follicular NHL, high molecular response rates for BCL-2 re-arrangements occurs following first-line treatment with RIT, although outcomes following molecular remission post RIT at relapse are unknown. We present a prospective study of PET and molecular responses in relapsed indolent NHL following ^{131}I -rituximab in PMCC patients on this previously reported multi-centre study.

Methods

PET-CT scans of the chest, abdomen, and pelvis were performed at baseline, 3, 6, and 12 months after RIT. Patients with follicular lymphoma had bone marrow and peripheral blood analysed for Bcl-2 rearrangement at baseline, and 3, 6, and 12 months if positive. Three month response status was correlated with EFS.

Results

29 pts [follicular (24), small lymphocytic (2), marginal zone (3)] had a response rate of 83%. At 3-months, 15/27 (56%) evaluable patients achieved complete metabolic response (CMR). 3-month PET status predicted outcome with median time to progression (TTP) 15 months for CMR (n = 15), 11 months for PMR (n = 7) and three months for non-responders (n=5) (P=0.001). CT response status at 3-month did not display as clear a separation of categories, with median TTP of 24 months for CR (n=9), 12 months for PR (n=10) and six months for non-responders (n=9) (P=0.0023). 5/11 patients with Bcl-2 re-arrangements became molecularly negative with TTP of 24 months, and six remained positive with TTP of 6 months (P=0.0025).

Conclusion

^{131}I -rituximab induces metabolic and molecular responses in relapsed indolent NHL. Although CT CR predicts TTP, lack of PET response conveys the most robust prediction of lack of treatment benefit. PET provides statistically superior prognostic stratification, although both identify responders from poor-responders. Patients with Bcl-2 re-arrangements at diagnosis who become PCR negative have prolonged TTP.

Wednesday 17 October
HSANZ Free Communications 11: Lymphoma

0830-1000
Meeting Room 8

O106 **0915** **Long-Term Outcome of 75 Patients with Advanced-Stage Cutaneous T Cell Lymphoma (CTCL)**

Miles Prince,¹ Suzanne Arulogun,^{1,3} Kirsten Herbert,¹ Chris Baker,⁴ Odette Blewitt,¹ Peter Foley,⁴ Lee-Mei Yap,⁴ Gail Ryan² and Chris McCormack⁴

Departments of ¹Haematology and ²Radiation Oncology, Peter MacCallum Cancer Centre, ³Monash University, ⁴Department of Dermatology, St Vincent's Hospital, Melbourne, Victoria, Australia

Background

Cutaneous T cell lymphoma (CTCL) is a rare disease, and only a small number of long-term follow-up analyses worldwide have been performed on large cohorts of patients. Although mainly an indolent disease, a proportion of patients present with or progress to advanced CTCL (stages IIB-IVB), and the morbidity and mortality is significant among this group.

Aims

All patients treated at our institutions with advanced-stage CTCL were studied to obtain information on long-term survival outcomes in advanced-stage CTCL, including erythrodermic CTCL (erythrodermic mycosis fungoides and Sezary syndrome) and large cell transformation (LCT).

Methods

Analysis of the Victorian cutaneous lymphoma database, containing 370 patients with CTCL, was undertaken. A multi-centre, 26-year, retrospective cohort analysis was performed of 75 patients (20%) with advanced-stage MF/SS, including 15 patients (4%) with LCT and 34 patients (9%) with erythroderma. Survival data were analysed using Kaplan-Meier survival analysis.

Results

Large cell transformation developed in 15 patients who presented with T2 MF (n=7), T3 MF (n=5) and T4 MF (n=3). Seven patients had LCT when first diagnosed with MF. Of the remaining 8 patients, median time from diagnosis of MF/SS to transformation was 21 months (1 to 348 months). Two-year survival rate from time of transformation was 55%. Median overall survival (OS) from diagnosis of LCT was 27 months. Thirty-four patients presented with or developed erythrodermic MF/SS. Of these, 27 patients (79%) demonstrated a malignant clone in the peripheral blood (Sezary syndrome). Patients with erythrodermic MF and Sezary syndrome had a 2-year survival rate of 100% and 64%, respectively; median OS was 60 months and 49 months, respectively.

Conclusions

Large cell transformation is not an infrequent occurrence in advanced-stage MF, and indicates a relatively poor prognosis and a need for aggressive therapy. Survival in Sezary syndrome is worse than in erythrodermic MF.

Wednesday 17 October

HSANZ Free Communications 11: Lymphoma

0830-1000

Meeting Room 8

O107

0930

Extracorporeal Photopheresis for Advanced Cutaneous T Cell Lymphoma (CTCL): Our Experience at Peter MacCallum/St Vincent's Hospital

Miles Prince,¹ Suzanne Arulogun,^{1,3} Kirsten Herbert,¹ Chris Baker,⁴ Odette Blewitt,¹ Peter Foley,⁴ Lee-Mei Yap,⁴ Gail Ryan² and Chris McCormack⁴

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Background

Numerous treatment options for mycosis fungoides (MF) and Sezary syndrome (SS) have emerged over the past two decades. Studies have reported that extracorporeal photopheresis is effective in producing clinical and symptomatic responses in patients with erythrodermic MF and SS, and prolongs survival in some patients with advanced disease.

Aims

Patients with MF or SS treated with photopheresis from January 2002 to May 2007 at our centre were analysed for rates of response. The efficacy of a modified treatment protocol (described below) was compared with results from previous reports using the conventional protocol.

Methods

A single-centre, five-year retrospective cohort analysis was performed on 13 patients who received photopheresis treatment. Survival data were analysed using Kaplan-Meier survival analysis.

Results

Thirteen patients were treated with photopheresis consistently for 2 or more months (median 16 months, 3 to 64 months) with a modified treatment protocol of one treatment of photopheresis weekly for 6 sessions, fortnightly for 6 sessions then monthly. Twelve patients presented with erythrodermic disease and 12 patients demonstrated a circulating malignant T cell clone. All patients presented with stage III disease, except one who had stage IVA disease. Ten patients were concomitantly treated with one or more conventional therapies. The overall response rate was 62%, with 2 patients (15%) achieving a complete response, 3 patients (23%) achieving a partial response and 3 patients achieving a minor response. Median time to progression among the non-responders (3 patients with stable disease and 2 with progressive disease) was 19 months (2.5 to 28 months). Median progression-free survival was 21 months. Photopheresis was well tolerated by all patients.

Conclusions

Response rates and survival analysis compare favourably with those of previous studies. The modified treatment protocol used at this institution does not appear to produce different response rates compared with the conventional protocol.

Wednesday 17 October
HSANZ Free Communications I I: Lymphoma

0830-1000
Meeting Room 8

O108 **0945** **A Multicentre Phase II Trial of an Outpatient-Based Stratified Salvage Approach for Advanced Lymphoma**

Sant-Rayn Pasricha¹, Andrew Grigg², John Catalano³, Michael Leahy⁴, Craig Underhill⁵, Chris Arthur⁶, James D'Rozario⁷, Ray Lowenthal⁸, Kerry Taylor⁹, Andrew Spencer¹.

¹Alfred Hospital, Melbourne, Victoria, Australia; ²Royal Melbourne Hospital, Melbourne, Victoria, Australia; ³Frankston Hospital, Frankston, Victoria, Australia; ⁴Fremantle Hospital, Fremantle, Western Australia, Australia; ⁵Border Medical Oncology, Wadonga, Victoria, Australia; ⁶Royal North Shore Hospital, New South Wales, Sydney, Australia; ⁷Canberra Hospital, Canberra, ACT, Australia; ⁸Royal Hobart Hospital, Hobart, Tasmania, Australia; ⁹The Mater Adult Hospital, Brisbane, Queensland Australia

Introduction

We have previously demonstrated utility with vinorelbine, gemcitabine and filgrastim (VGF) for advanced lymphoma. We now report a larger multicentre study of a stratified approach utilizing this regimen, with/ without ifosfamide (F-GIV).

Method

Patients were stratified into Group 1 (G1-first relapse, follicular NHL >12 months, other NHL >6 months); Group 2 (G2-primary refractory, early relapse, or > first relapse); Group 3 (G3-relapse post-ASCT with PFS > 6 months). G1 and G3 received VGF (Vinorelbine 25mg/m², Gemcitabine 1000mg/m² D1 and 8, Pegfilgrastim 6mg D9), G2 received F-GIV (VGF with ifosfamide 3000mg/m² D1). Patients were restaged at 2 cycles. Those with progressive disease exited the trial. Responsive patients (>50% reduction in disease and negative functional imaging) received 2 further cycles of the same therapy, the remainder escalated to F-GIV (G1 and G3) or IVAC (G2) (inpatient ifosfamide, etoposide, cytarabine with Pegfilgrastim).

Result

Ninety patients were accrued between December 2002 and December 2004 (G1=26; G2 =52, 27 primary refractory; G3=12). Median age 58y (range 17-78). Diagnoses were DLBCL=40, FL=19, HL=17, PTCL=6, others=8. G1 and G2 received 79 and 140 cycles of VGF and F-GIV, respectively, with grades 3/4 neutropenia or thrombocytopenia in 24% and 18% (VGF) and 68% and 55% (F-GIV) of cycles. G3 received 28 and 8 cycles of VGF and F-GIV respectively, with no unexpected toxicities. Escalation from VGF to F-GIV or F-GIV to IVAC improved response in 5/12 and 4/10 cases respectively. 29 patients proceeded to ASCT. After a median follow-up of 40 months, estimated 4 year OS is 34% with group specific OS of G1 49%, G2 28% and G3 31%. Subtype-specific survival at 4 years was 27% (DLBCL), 29% (FL), 69% (HL) and 33% (PTCL).

Conclusion

VGF and F-GIV can be safely administered in an ambulatory care setting and show significant activity against a variety of advanced lymphomas.

Wednesday 17 October

HSANZ Free Communications 12: Myeloma II

0830-1000

Meeting Room 9

O109

0830

The Presence of T Cell Clones in Patients with Multiple Myeloma is Increased After Thalidomide Maintenance Therapy (ALLG-MM6) and is Associated with an Improved Survival

Ross Brown¹, Andrew Spencer², Nola Kennedy², M Dolotin¹, Karieshma Kabani¹, P Joy Ho¹, Daniel M Sze¹, John Gibson¹, Doug Joshua¹

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We have previously reported that clones of CD8+ CD57+ CD28- perforin+ T cells are present in 59% of patients with multiple myeloma (MM) and are associated with a prolonged survival. This study aimed to confirm this observation with a different patient cohort and to investigate a possible relationship between the generation of T cell clones and the immunomodulatory agent, thalidomide. ALLG-MM6 is a randomised trial of maintenance therapy \pm thalidomide (max 200mg/day) post autologous stem cell transplant in 243 patients. Patients were >18 y, non-progressive, <12 months prior therapy, ECOG <3 and serum creatinine <2 mg/dl. At 2y median follow-up both progression free survival (PFS) and overall survival (OS) were significantly prolonged in the thalidomide arm ($p=0.0005$ and $p=0.021$ respectively) (Blood 2006; 108:22a). A total of 221 blood samples from 120 of these patients (104 pre-transplant and 117 after maintenance) were available for analysis of TCR V β expression by a 4-colour flow cytometry (Beta Mark). Controls were 42 age-matched normals. Clonality of TCR V β expansions was verified in 6 samples by CDR3 length analysis and direct sequencing. 93% of all clones were CD8+ and all 24 TCR V β families studied were represented. T cell clones were detected in 48% of patients pre-transplant, 68% after 8 months maintenance (76% in thalidomide and 60% in control arm) and 57% after 12 months maintenance. The incidence of patients with multiple clones was significantly greater after thalidomide (49%) compared to the control arm (23%) ($\chi^2=6.8$; $p=0.01$). There was a trend for patients to develop new T cell clones after thalidomide (52%) compared with the control arm (40%). The presence of T cell clones regardless of therapy was associated with a longer PFS (median = 32.1 vs 24.1 months; $\chi^2=4.2$; $p=0.04$). Median PFS in the thalidomide arm was 40.1 months for patients with clones and 28.3 months for patients without clones while in the no thalidomide arm, median PFS was 15.9 months without and 21.3 months with T cell clones ($\chi^2=9.5$; $p=0.002$). These observations confirm the prognostic significance of circulating T cell clones and demonstrate their enhanced impact after thalidomide therapy.

Wednesday 17 October
HSANZ Free Communications 12: Myeloma II

0830-1000
Meeting Room 9

O110

0845

XBP-1 Levels Predict Sensitivity of Myelomas to Proteasome Inhibitor-Bortezomib

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². Institute of Haematology, Royal Prince Alfred Hospital, Sydney, NSW, Australia

Proteasome inhibitors (PI) are remarkably effective in relapsed and refractory myeloma but the origin of this peculiar sensitivity remains unclear. Myeloma is dependent on the unfolded protein response (UPR) and its regulator, transcription factor *XBP-1*. PI perturbs the unfolded protein response (UPR). We therefore hypothesize that the dependence on the UPR and *XBP-1* mediates sensitivity to PI.

Aims

1. Correlate Bortezomib sensitivity with *XBP-1* *in vitro* and in myeloma patients.
2. Check the effect of manipulating *XBP-1* on Bortezomib sensitivity.
3. Develop Bortezomib-resistant myeloma cell lines to ascertain the effects on *XBP-1* and the UPR.

Methods

1. Sensitivity to Bortezomib was measured *in vitro* and *XBP-1* mRNA levels and its isoforms measured by a two-step quantitative QPCR assay, in 6 myeloma cell lines and 17 other cancer cell lines.
2. Marrow biopsies from Bortezomib-treated myeloma patients were analysed for *XBP-1* expression. Myeloma cells (CD38 hi, CD14 lo, kappa or lambda light chain +ve) were purified by flow cytometry.
3. Manipulation of *XBP-1* levels in myeloma cell lines by shRNA-mediated knockdown and overexpression by retroviral transduction.
3. Analysis of intracellular light chain production by immunoblotting and flow cytometry.
4. Development of Bortezomib-resistant myeloma lines by adaptation to long-term exposure.

Results

There is a strong inverse correlation in myeloma cell lines between total or unspliced *XBP-1* with Bortezomib sensitivity ($r = -0.9$) but not in other cancer cell lines. Overexpression and knockdown of *XBP-1* had little effect on Bortezomib sensitivity. Bortezomib-resistant myeloma cell lines show marked downregulation of *XBP-1* and light chain production.

Conclusions

XBP-1 is a marker of Bortezomib sensitivity and its clinical utility is being tested now. Sensitivity to PI is related to the dependence on the UPR for active immunoglobulin synthesis, reflected in the level of *XBP-1*.

Wednesday 17 October

HSANZ Free Communications 12: Myeloma II

0830-1000

Meeting Room 9

O111

0900

CYT997 Induces Apoptosis in Human Multiple Myeloma Cell Lines

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Aim

CYT997 inhibits tubulin polymerisation and has been found to inhibit a wide variety of cancer cells with IC50s in the low nanomolar range. We determined the impact of CYT997 on Human Myeloma Cell Lines (KMS-12PE, LP-1, NCI-H929, RPMI-8226 and U266) and primary tumour samples.

Method

A panel of Human Myeloma Cell Lines (HMCLs) were utilised to evaluate the effects of CYT997 (10 to 1000 nM). Cell proliferation via MTS assay was assessed after 24 and 48 hours. Annexin-V/Propidium Iodide (PI) staining and cell cycle analysis were used to study the profile of the cells after treatment with 50 nM CYT997. CYT997 was also tested against the HMCL RPMI-8226 when grown in co-culture with HS5 stromal cells (an *in vitro* assay to simulate the bone marrow microenvironment). Cells were treated with 100 nM CYT997 and RPMI-8226 cell death determined by PI staining after 48 hours. Primary samples from patients diagnosed with multiple myeloma were treated with various concentrations of CYT997 (50 – 1000 nM) and apoptosis was measured by Apo 2.7.

Results

MTS assays demonstrated a time and dose response to CYT997. At concentrations of 50 nM CYT997 a decrease of 20 – 40% cell proliferation was observed, when used at 100 nM a 45 – 90% decrease was noted after 48 hours. Annexin-V/PI staining further confirmed that a single 50 nM dose of CYT997 induced apoptosis in 15 – 40% of cells after 48 hours. Cell cycling analysis demonstrated that CYT997 caused an accumulation of cells in the G₂M phase after 24 hours in the KMS-12PE, NCI-H929, and U266 cell lines. When RPMI-8226 cells grown in co-culture with HS5 cells were treated with 100 nM CYT997 apoptosis was induced in 25% of myeloma cells. CYT997 was found to be effective in primary tumour samples at 100 nM; killing 10 – 50% (mean 32%, n=4) of tumour cells after 48 hours. A combination study with Velcade showed enhanced cytotoxicity.

Conclusion

CYT997 exhibits anti-tumour activity against both myeloma cell lines and primary myeloma tumours from patients with advanced disease. Further evaluation of CYT997 as a potential therapeutic agent for myeloma is justified.

Wednesday 17 October
HSANZ Free Communications 12: Myeloma II

0830-1000
Meeting Room 9

O112 **0915**
Cytogenetic Findings in Patients with Multiple Myeloma: Our Experience in Western Australia

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¹ *Department of Haematology, Royal Perth Hospital, WA, Australia*

² *Department of Haematology, Fremantle Hospital, WA, Australia*

Aim

We reviewed cytogenetic abnormalities of patients with myeloma in Western Australia. Specifically we investigated the prevalence and prognostic value of genetic changes in patients who proceeded to transplantation, and assessed the concordance of our results with those reported in the literature. In addition, we evaluated the outcome of cases of hyperdiploid myeloma, and assess if this is modulated by the presence of co-existent chromosome 13 abnormalities.

Method

We performed a retrospective analysis of the cytogenetic database in Western Australia, reviewing medical records of patients diagnosed with myeloma over the last 10 years. Clinical and laboratory data specific for prognostication according to WHO were collected and patients assigned to stages according to the International Myeloma Staging System. Data on therapy utilised were collected, with responses evaluated and duration of remission if achieved recorded.

Conventional cytogenetics were performed on 26 patients until 2000. FISH analysis with a 13q probe was commenced in 1998 with further probes added over time.

Results of cytogenetics were then correlated with clinical and laboratory data and evaluated with survival.

Results

Data were available on 309 patients, of which 132 proceeded to transplantation. With the use of the myeloma staging system, 53% of our patients demonstrated Stage I disease. The abnormality rate by conventional cytogenetics was 35%. By FISH, hyperdiploidy was the commonest abnormality (49%), followed by 13q deletions (47%), IgH translocations (33%) and p53 deletions (8%). Co-existent hyperdiploidy and chromosome 13 deletions occurred in 10%.

Analysis of the prognostic impact of individual chromosome abnormality reveals a significantly increased serum B2 microglobulin with chromosome 13 deletion, and a negative association between t(4;14) and paraprotein level. The presence of hyperdiploidy in cases of 13q deletions appears to confer a survival advantage.

Conclusion

Our study demonstrated the negative prognostic impact of 13q deletions and positive association of hyperdiploidy and t(11;14) with survival. Furthermore, we have shown that hyperdiploidy modulates the survival of patients with 13q deletions.

Wednesday 17 October

HSANZ Free Communications 12: Myeloma II

0830-1000

Meeting Room 9

O113

0930

The Role of Serum Free Light Chain Assessment in the Diagnosis and Management of Monoclonal Gammopathies – A Review and Report on its Assessment at Sir Charles Gairdner Hospital, Nedlands, Western Australia

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Aim

The serum free light chain (SFLC) assay is a relatively new test, with reported increased sensitivity compared to electrophoretic techniques for light chains. The assay is specific for unbound κ and λ light chains, does not detect intact Ig and is not dependant on renal function as with urine techniques. SFLC assessment is currently used to diagnose and monitor response to therapy in light chain secreting monoclonal gammopathies and may be able to replace urine electrophoresis – hence obviating the need for cumbersome 24 hour urine collections. Given the reported potential advantages of the SFLC assay, in combination with serum protein electrophoresis (SPEP), we undertook a comparison of the sensitivity of this combination to our current institutional practice of SPEP and urine protein electrophoresis (UPEP) \pm immunofixation (IFE). It is anticipated that approximately 300-400 (monoclonal gammopathy) undiagnosed samples will be processed in a period of 1 year. Here we present an interim analysis of the first 2 months of data collected.

Method

Undiagnosed hospital samples with a clinical history of possible monoclonal gammopathy were included. Samples without a direct request for SFLC had the SFLC assay added on review of the routine requests for SPEP or UPEP. Quantitation of SFLC was by nephelometry using the FREELITE[®] Human Kappa and Lambda Free kits (The Binding Site, Birmingham) on a Dade Behring BNII Nephelometer. Serum and urine electrophoresis was performed on the SPIFE 3000[™] (Helena Laboratories) with scanning densitometry using the included QuickScan 2000 program. IFE was performed using Helena Laboratories anti-sera against IgG, IgA, IgM, κ and λ with protein fixative. In-patient notes for all cases were obtained and reviewed to determine diagnosis where possible. In instances where an abnormality was only found on SFLC assessment (and not by the routine screening), the referring physician was notified.

Results

Between the 1st March and 30th April, 2007, 73 cases were identified - 19% (14/73) had a paraprotein detectable by SPEP and 21% (15/73) an abnormal κ to λ ratio (κ/λ) by SFLC assessment (normal = 0.26 – 1.65). In only 20 cases (27%), a urine specimen was also obtained, of which 35% (7/20) had a detectable urine M-protein. 19/73 (26%) of cases had an abnormal SFLC/SPEP result compared to 14/73 (19%) using the standard (UPEP/SPEP). The additional cases were mainly had an abnormal κ/λ only - 2 cases with B-cell lymphoma and 3 with unrelated disorders. There were no cases in which the UPEP detected an abnormality without either the SPEP or SFLC assay being abnormal. In fact, both the SPEP and SFLC assay were abnormal in all UPEP positive cases. Three cases of MGUS were identified by SPEP or UPEP, but not SFLC assessment.

Conclusion

Our results indicate at least non-inferiority using SPEP in combination with SFLC (with no cases of monoclonal gammopathy missed) when compared to SPEP and UPEP. Three cases of MGUS were missed by SFLC compared to SPEP, which is roughly in line with expectations. It must be borne in mind that UPEP was not performed in all cases. The time to review cases meant that adding UPEP when a SFLC abnormality is detected is not feasible, as many patients would have undergone treatment in the intervening period. Nevertheless, using these figures would be a better indicator of “real-life” use of these assessments. Overall, the results are in line with reports in the literature. They are limited by the small numbers currently available for evaluation but with accrual over the coming months, firmer conclusions may become possible. An update of these results will be presented.

Wednesday 17 October
HSANZ Free Communications 12: Myeloma II

0830-1000
Meeting Room 9

O114 **0945**
Prolonged Progression Free Survival in Patients with Relapsed Multiple Myeloma (MM) Treated with Thalidomide is Not Related to Depth of Response

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Aim

To provide an update on long-term survival from two multicentre phase II trials using thalidomide +/- IFN α 2B (MM-thal) and combination celecoxib-thalidomide (Cel-thal) in relapsed or refractory MM, and to identify predictors of progression-free survival (PFS) beyond 24 months (m).

Method

In 1998 and 2001, two prospective multicentre phase-II trials in relapsed or refractory MM were performed to assess efficacy of thalidomide +/- IFN α 2B, and combination celecoxib-thalidomide, respectively. Both were previously reported (Blood 2003;102:69; Clin Can Res 2005;11:5504). The analysis of PFS has been updated using the Kaplan-Meier method. Baseline characteristics were compared between patients having PFS \geq 24m and $<$ 24m using Fisher's exact test or the Cochran-Armitage test, to identify predictors of long-term disease control.

Result

Median follow-up for the MM-thal (n=75) and Cel-thal (n=66) trials was 73m and 47m, respectively. Median PFS in the MM-thal trial was 5.5m, with estimated PFS of 13% at 2 years (95% CI:7-23%), and 5% at 5 years (95% CI:2-13%). In the cel-thal trial, median PFS was 6.8m, with estimated PFS of 26% at 2 years (95% CI:17-38%) and 16% at 5 years (95% CI:9-27%). Overall, 19% (27/141) of patients (10 MM-thal, 17 Cel-thal) had PFS beyond 24m. 15% were complete responders, 70% partial responders, with 15% having stable disease. The most significant predictors for prolonged PFS \geq 24m were β_2 M \leq 3mg/l (p=0.0004), stage \leq 2 disease (p=0.0014), and non-refractory disease to previous therapy (p=0.039). Bone marrow infiltrate following thalidomide did not predict for outcome.

Conclusion

Thalidomide, and in particular combination celecoxib-thalidomide has substantial activity in relapsed MM with prolonged PFS beyond 24m in approximately 19% of patients. The strongest predictor of prolonged PFS is β_2 M. The depth of response to thalidomide had little influence on predicting remission duration.

Wednesday 17 October
ANZSBT Symposium: Haemovigilance

0830-1000
Central Room A

0830

Haemovigilance – the New Zealand Experience

Peter Flanagan

New Zealand Blood Service, Auckland, New Zealand

New Zealand Blood Service formally implemented its haemovigilance programme in May 2005 following a successful pilot programme. The scheme utilises principles contained in the Council of Europe Guide and is mirrored on pre-existing schemes in the United Kingdom (SHOT) and the Republic of Ireland. Haemovigilance is part of a range of activities that together aim to raise awareness of blood transfusion issues and risks and to improve the overall practice of blood transfusion.

Transfusion Safety Officers have been identified in each major hospital. This is a voluntary role that provides a link between the hospital and the national haemovigilance office. Standard reports are completed for all reported adverse events. Approximately 34 reports are received each month and the available data indicate that all New Zealand hospitals are actively participating in the scheme. Annual reports are produced and are available at www.nzblood.co.nz.

A total of 824 reports were received between 1 May 2005 and 31 May 2007. The majority of these involved either mild to moderate allergic reactions (32%) or non febrile haemolytic transfusion reactions (46%). Severe respiratory complications accounted for 43 (5%) of reports, of these 24 involved cases of TRALI and 19 cases of circulatory overload. 4 acute haemolytic reactions were reported along with 20 cases of delayed haemolytic reaction. 19 severe allergic/anaphylactic type reactions were reported.

A total of 39 cases of incorrect blood component transfused were reported plus a further 4 equipment related and 3 component related incidents. No cases of transfusion transmitted infection nor bacterial sepsis were reported during the period.

The NZBS haemovigilance programme appears to be working well and has the support of local hospitals. The data have the ability to inform on priority areas for improvement and to assess the efficacy of interventions aimed at quality improvement.

Wednesday 17 October
ANZSBT Symposium: Haemovigilance

0830-1000
Central Room A

0900

Australian National Experience

Chris Hogan

Abstract not received at time of going to print

Wednesday 17 October
ANZSBT Symposium: Haemovigilance

0830-1000
Central Room A

0930

The BeST Stirrers. STIR - A Haemovigilance System for Victoria

Lisa Stevenson^{1,4}, David Beilby⁴, Karen Botting^{1,4}, Julie Domanski⁴, Chris Hogan⁴, Geoff Magrin⁴, Ellen Maxwell⁴, Richard Rogers⁴, Carole Smith⁴, Neil Waters^{2,4}, Deane Wilks⁴, Erica Wood^{2,4}, Larry McNicol^{1,3}

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Background

A defined objective of the Victorian Better Safer Transfusion (BeST) program is to respond to sentinel events and key transfusion adverse events referred to the program. The BeST Serious Transfusion Incident Reporting (STIR) system was developed for health services in Victoria to report centrally. It was designed by a working group of BeST and piloted in 2006. In 2007 the system has expanded statewide in Victoria and now includes several Tasmanian hospitals.

Results

The system captures ten clearly defined serious transfusion incidents relating to pre-transfusion samples and the transfusion of fresh blood components, i.e. red cells, platelets, fresh frozen plasma and cryoprecipitate. Near misses are defined within the system. The system is a two-layer process: notification of the incident to BeST and a follow-up investigation process by the health service. Forms are provided by STIR to guide the process. The initial forms were paper based and then to an electronic form online and a web based program our ultimate goal. The data are then collated, reviewed by the STIR expert group and the aggregate data are then reported widely to health services with recommendations for improvements in transfusion practice.

STIR Pilot

The pilot was conducted between July and October 2006. Nine Victorian health services participated with six of them reporting incidents during the pilot. Reporting was agreed to be retrospective until January 2006 to ensure the system was well tested. It was estimated by the pilot health services that approximately 40,000 units of red blood cells were transfused during this period, with 29 incidents involving red cells reported. Near miss reports accounted for 43 percent of the data, demonstrating that a majority of incidents were due to procedural problems or system issues that have the potential to cause serious harm.

Conclusion

As a result of STIR data collection and the subsequent hospital-based review process, health services have already made recommendations to improve transfusion practices. In addition, aggregated statewide data will highlight the most important areas for transfusion improvement, thereby making best use of limited resources.

Wednesday 17 October
ASTH Free Communications 3: Platelets/Microparticles

0830-1000
Central Room C

O115 **0830**
The Association of Depression with Platelet activation: Evidence for a Treatment Effect

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Increasing evidence suggests that depression is an independent risk factor for cardiovascular disease (CVD), although the mechanism is not well understood. A prothrombotic state has been linked with depression, however the majority of studies examining this association have been cross-sectional. We hypothesized that markers of platelet activation would be increased in depressed compared to non-depressed individuals, and that treatment of depression would reduce activation.

We studied 104 depressed outpatients (69% females) and 45 control subjects (60% females) (aged 20-64 years) all without a history of CVD. Level of depression was assessed using the Montgomery Asberg Depression Rating Scale (MADRS). The depressed subjects were treated with combinations of medication and psychotherapy. Markers of cardiovascular risk including platelet activation (using flow cytometry) and von Willebrand factor (vWF) plasma levels were assessed at baseline and at 6 months following treatment of depression. Statistical analyses were carried out using SPSS.

Compared to controls, depressed subjects had increased *in vivo* platelet activation, as evidenced by higher platelet numbers expressing P-selectin (CD62p)($p=0.002$) and CD63 ($p=0.023$), and increased circulating platelet-monocyte aggregates ($p<0.001$). Since ABO blood group influences vWF plasma levels, we divided the subjects into O and non-O subgroups. vWF was increased in non-O depressed subjects compared to non-O controls (p 0.011) but there was no difference between patients and controls with blood group O. After six months of treatment for depression, there was a significant reduction of platelet activation markers and vWF (in non-O patients).

In conclusion, platelet activation markers and vWF are elevated in depression, and are lower after antidepressive treatment. These data suggest that prothrombotic changes associated with depression may contribute to the observed increase in CVD, and that treatment can reduce CVD risk.

Wednesday 17 October

ASTH Free Communications 3: Platelets/Microparticles

0830-1000

Central Room C

O116

0845

Dietary Inhibition of Platelet Function and Thrombin Generation

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Cardiovascular disease is the major cause of morbidity and mortality in developed countries. Population studies have suggested lower rates of cardiovascular disease in countries with a "Mediterranean" diet, with a high proportion of vegetables in the diet. It has been proposed that components of the Mediterranean diet, including garlic and tomatoes, can affect platelet function.

The aim of our study was to measure the *ex vivo* effects of vegetable juice consumption on platelet function and coagulation.

Twenty volunteers agreed to drink 250 ml of a standardised vegetable juice daily for 3 weeks. Volunteers were asked to avoid medication with known anti-platelet effects and garlic. Citrated blood was collected at baseline and after 3 weeks of vegetable juice. Investigations included PFA-100, thromboelastography (TEG), platelet aggregation in platelet-rich plasma (PRP), ADP-induced platelet activation measured by flow cytometry and thrombin generation in both PRP and PPP (platelet-poor plasma), measured by calibrated automated thrombogram (CAT).

PFA-100, TEG and CAT in PRP showed no difference between pre- and post-treatment samples. Platelet aggregation induced by ADP was strongly reduced at 3 weeks (% light transmission 74.7 vs 58.5, $p=0.003$ and slope 88 vs 75, $p<0.001$). A lesser degree of inhibition was noted with aggregation induced by collagen, arachidonic acid and adrenalin. Following ADP-induced platelet activation, P-selectin exposure (mean fluorescence intensity 77 vs 68, $p=0.006$) and CD63 exposure (% positive platelets 26.3 vs 21.4, $p=0.036$) were significantly reduced at 3 weeks, as well as Pac-1 antibody binding (% positive platelets 64.7 vs 52.5, $p=0.028$). The CAT in PPP showed an increased lagtime (2.87 vs 3.19, $p=0.001$) and reduction in the endogenous thrombin potential (1591 vs 1506, $p=0.027$).

In this pilot study, the daily consumption of vegetable juice significantly inhibited platelet function and thrombin generation. These effects may contribute to the observed effect of diet on cardiovascular risk.

O117 **0900**

Phospholipid-dependent Procoagulant Activity of Platelet-derived Microparticles is Restricted to the Annexin V Binding Subpopulation

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Aim

It has recently been considered that some populations of microparticles may fail to bind Annexin V. The aim of this work was to compare antigenic characteristics and phospholipid-dependent procoagulant activity (PL-PCA) of Annexin V positive and negative subpopulations of platelet-derived microparticles.

Method

Platelet-derived microparticles were obtained from unstimulated and stimulated samples. Microparticles were characterised by flow cytometry and PL-PCA measured by XACT. Electron microscopy was performed on the Annexin V negative subpopulation obtained by magnetic cell sorting.

Results

In unstimulated platelet poor plasma, 70% of CD41+ events less than 0.82µm failed to bind Annexin V. Varying assays constituents (calcium and Annexin V concentration / buffer type) did not alter Annexin V binding. The proportion of microparticles that bound Annexin V was dependent upon type of agonist used. There was no significant difference in CD41, CD61, CD42a, CD62p and CD63 expression between Annexin V positive and negative microparticles. However, CD42b expression was significantly decreased in the Annexin V positive subpopulation. Electron microscopy of Annexin V negative microparticles confirmed these events as vesicles. A significant correlation between Annexin V binding and PL-PCA was found ($p < 0.01$) and Annexin V binding inhibited greater than 90% of PL-PCA, suggesting that Annexin V binding was a true reflection of procoagulant activity.

Conclusions

The presence of Annexin V negative microparticles was confirmed by flow cytometry and electron microscopy, however phospholipid-dependent procoagulant activity appears to be limited to those microparticles which bind Annexin V. Annexin V negative microparticles may exert procoagulant or functional activity through other mechanisms such as p-selectin, tissue factor or via other cellular interactions.

Wednesday 17 October

ASTH Free Communications 3: Platelets/Microparticles

0830-1000

Central Room C

O118

0915

Microparticles in Heterozygote Factor V Leiden Subjects

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Introduction

Patients with Factor V Leiden(FVL) mutation, a heritable thrombophilic state, show considerable heterogeneity in deep vein thrombosis events. Microparticles(MP) are $<1\mu\text{m}$ membrane bound particles shed from cell surface have recently been described to be important in thrombosis. The association of microparticles with thrombotic events in patients with FVL is not known.

Aim

To study platelet, endothelial, leukocyte and factor V bearing microparticles in heterozygotes for Factor V Leiden mutation.

Methods

Forty four patients with heterozygote Factor V Leiden mutation and 50 controls were studied. The levels of platelet, endothelial and leukocyte microparticles were enumerated using a flow cytometry based technique on a BD FACS Canto flow cytometer using an established protocol. The antibodies for CD41 to identify platelet MP, CD62 for endothelial MP and CD45 for leukocyte MP were used. Factor V bearing microparticles were also enumerated using an antibody to Factor V.

Results

The mean platelet MP in healthy controls was 1206.9 ± 927.7 MP/ μL compared to 1638.9 ± 1043.4 MP/ μL in FVL subjects. The mean endothelial microparticles in controls was 366.9 ± 108.6 MP/ μL compared to 507.4 ± 261.1 MP/ μL in FVL subjects. The MP with surface expression of Factor V were enumerated at 92.9 ± 56.0 MP/ μL in controls and 140.3 ± 61.7 MP/ μL in FVL subjects. Thus, mean values for platelet and endothelial microparticles were significantly higher in FVL subjects compared to controls. Factor V bearing microparticles were also higher in FVL patients compared to normal controls. Leukocyte microparticles were also elevated in the FVL group. Analysis in a small subgroup of FVL subjects with thrombosis ($n=14, 32\%$) did not show any significant differences in MP levels compared to those without thrombosis ($n=30, 68\%$).

Conclusions

Platelet, endothelial and leukocyte microparticles are significantly higher in the circulating plasma of subjects with FVL mutation. Activation of platelets, endothelium and higher Factor V on surface of MP, which may be a protective mechanism for catalytic degradation, may be important contributors to thrombosis in these patients.

O119

0930

Soluble CD36 Circulates in Normal Plasma on the Membranes of Microparticles Derived From Platelets and Monocytes

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Background and Aim

CD36 is an integral membrane glycoprotein expressed on the surface of platelets, monocytes, endothelial and smooth muscle cells. It is involved in the uptake of long chain fatty acids and oxidized lipoproteins and its altered expression has been associated with atherosclerosis, platelet aggregation, and insulin resistance. Recently it was reported that soluble CD36 could be detected in normal plasma and was increased in diabetes. The aim of the present study was to determine the form and source of this circulating CD36.

Methods

Platelets were isolated from normal peripheral blood and the resultant platelet poor plasma (PPP) subjected to ultracentrifugation at 100,000g to produce a pellet (P100). Density gradient ultracentrifugation was used to fractionate the PPP into VLDL, LDL and HDL. Western blotting and flow cytometry were then used to detect CD36 in the various fractions.

Results

Western blotting revealed that the CD36 molecule present in the platelet, PPP and P100 preparations was a ~85kDa protein that is typical of membrane bound CD36. Pre-treatment with PNGase-F to cleave N-linked glycosylated residues resulted in similar band shifts, suggesting that the CD36 present in PPP was identical to that on the surface of platelets, and thus unlikely to be a cleaved product. Isolation of lipoprotein fractions did not enhance detection of CD36, indicating that it does not exist in plasma bound to these ligands.

Immunoblotting for glycoprotein IIb revealed the presence of this platelet antigen in both platelet lysates and P100 fractions. Flow cytometric analysis of PPP confirmed that CD36 was present in a small but variable proportion of cellular derived microparticles (<1µm in diameter) which mainly expressed antigens characteristic of platelets (CD41a) or monocytes (CD45/14).

Conclusion

Soluble CD36 in plasma is not a cleaved protein but rather membrane-bound on the surface of microparticles derived mainly from platelets and monocytes.

Wednesday 17 October
ALLG Symposium: Lymphoma

1100-1230
Arena 1B

1100

Predicting Clinical Outcomes from Tumor Genotyping and Phenotyping

Ronald Levy

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Idiotypic vaccines can be produced by a variety of technologies, including mammalian cells, insect cells, tobacco plants, bacteria, naked DNA and cell-free protein expression. All of these methods result in vaccines that work in preclinical animal models. The results will be compared and contrasted.

Gene expression profiling has become a popular method for subclassification of lymphomas and other malignancies. Many lessons have been learned about the biology of lymphomas. Using this type of data to predict clinical outcomes has been difficult because of a lack of prospective trials and because of the difficulty of reducing this technology to a practical, reproducible method. On the other hand, candidate genes and proteins have been identified and tests are being produced to measure them quantitatively and reproducibly in tumor tissues. In particular, a test involving the measurement of six genes on material derived from archival paraffin embedded tissues seems promising, as does the use of a novel antibody against the LMO2 antigen, expressed in normal germinal center B cells.

Wednesday 17 October
ALLG Symposium: Lymphoma

1100-1230
Arena 1B

1145

The Changing Landscape in the Treatment of Follicular Lymphoma: 2007 and Beyond

Myron C Czuczman

Abstract not received at time of going to print

Dr Myron Czuczman sponsored by Roche Products

Wednesday 17 October
ANZSBT Symposium: Bacterial contamination

1100-1230
Central Room A

1100

Residual Risk After Blanket Bacterial Culture Testing of Platelets

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American Red Cross Blood Services, Washington, DC, USA

The American Red Cross initiated system-wide bacterial testing of all apheresis platelet (PLT) collections in March 2004, yet continues to receive reports of septic reactions after transfusion of screened components.

Study Design and Methods

The rates of confirmed bacterial contamination of apheresis PLT collections detected by prospective quality control (QC) testing, and by surveillance of reported septic reactions to screened-negative apheresis PLTs were determined. The mechanism of QC test failure leading to septic reactions was modeled by determining the effect of concentration on the probability of sampling one or more viable bacteria in an 8-mL sample of a 300-mL product.

Results

Between March 1, 2004, and May 31, 2006, bacterial culture testing was performed on 1,004,206 donations; of these, 186 (1:5,399) had confirmed positive culture results. A significantly higher rate of confirmed-positive bacterial cultures was seen with products collected utilizing two-arm collection procedures compared to one-arm procedures (22.7 vs. 11.9 per 105 donations; odds ratio [OR], 1.9; 95% confidence interval [CI], 1.4-2.7). During this period, 20 septic transfusion reactions were reported, including 3 fatalities (1:498,711 fatalities per distributed component). The frequency of septic reactions was 4.7-fold higher for collections utilizing two-arm procedures (1:41,173; 95% CI, 1:25,000-1:66,667) compared to collections from one-arm procedures (1:193,305; 95% CI, 1:52,632-1:500,000; OR, 4.7; 95% CI, 1.2-18.4); most septic reactions (16 of 20) were due to *Staphylococcus* spp. and occurred on Day 5 (13 of 20) after collection. These data, and modeling based on published studies, suggests a significant rate of false negative cultures due to low bacterial concentrations at the time of product sampling.

Conclusion

PLT contamination with bacteria that evade detection by QC culture remains a significant residual transfusion risk, in particular for older PLTs and skin-commensal bacteria in components collected by two-arm apheresis procedures during the study period.

Wednesday 17 October
ANZSBT Symposium: Bacterial contamination

1100-1230
Central Room A

1130

Bacterial Contamination: Regulator View

Richard Pembrey
Therapeutic Goods Administration, Canberra, ACT, Australia

Presenting the process of oversight, development, interpretation and monitoring of mandated standards and the code of Good Manufacturing Practice illustrate the factors influencing the view of the measures that the regulator will both encourage and require manufacturers to adopt to reduce the risk of bacterial contamination of blood products. The role of the regulator of a therapeutic product is concerned with the safety, quality and efficacy of the lawful supply of blood components and products for the treatment of patients. This is exercised through the granting of a manufacturing licence to blood banks which have been shown at audit to comply with the Australian Code of Good Manufacturing Practice for Human Blood and Tissue (2000) and the standard mandated in a Therapeutic Goods Order, the Council of Europe Guide to the preparation, use and quality assurance of blood components. In addition, the manufacturing blood bank is required to comply with recall procedures that involve notification of "defects" in manufacture and the withdrawal of faulty products from supply.

The regulator through these mechanisms oversees and monitors manufacturing practices and systems that seek to minimise the risk of bacterially contaminated blood products during manufacture and the timely withdrawal of products that may be contaminated from the supply chain from the manufacturer to the patient.

Wednesday 17 October

ANZSBT Symposium: Bacterial contamination

1100-1230

Central Room A

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ARCBS Implementation of Bacterial Contamination Screening (BCS) of Platelets

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Australian Red Cross Blood Service

Worldwide, bacterial contamination of platelets is recognised as the most significant residual infectious risk of blood transfusion in developed countries. ARCBS has seen progressive reductions in bacterial risk over the last few years with improvements in skin decontamination and the introduction in diversion pouches on all blood bags. ARCBS now has the opportunity to achieve best practice in terms of limiting and detecting bacterial contamination of platelet components since Australian governments approved implementation for 100% platelets from April 2008.

Bacterial screening is a culture based test that has an incubation step that continues for the entire shelf life of the product, hence a component could flag positive at any stage during incubation. All components will be issued as "negative to date". It is important to note that different types of bacteria have replication times and the time to detection will also depend on the amount of bacteria present in the initial sample. ARCBS is maximising the probability of detecting low levels of bacteria by using an anaerobic as well as an aerobic blood culture bottle for each sample and high end sample volumes (7mL per bottle) which have been shown to increase sensitivity.

All platelets will be held for a minimum period of 24 hours **post collection** before sampling to allow enough time for any bacteria present to proliferate to detectable levels. The status of the samples will be monitored 24 hours a day, 7 days a week to ensure that immediate follow up is performed as soon as a sample flags positive. ARCBS will communicate with clinicians if a component has been transfused and is subsequently found to have flagged as initially or confirmed positive.

The timeframes and impact of implementation will be discussed.

Wednesday 17 October
ASTH Symposium: Thrombocytopenia

1100-1230
Central Room C

1100

New Insights to the Regulation of Platelet Survival

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Although haematologists know a great deal about the regulation of blood cell production and function, relatively little is known about how blood cells senesce and what regulates their survival in the absence of pathology. For platelets, it has been well recognised that consumption, either by activation or through phagocytosis, is a major mediator of reduced platelet life span and underpins many thrombocytopenias. It is also well established that in health a person's platelet count varies little and that this homeostasis is under polygenic control. Which genes regulate platelet life span and by what process senescent platelets are destroyed have remained mysteries. Since platelets lack a nucleus, it has been assumed that the clearance of senescent platelets is a platelet extrinsic function of organs such as the spleen, and indirect evidence for this is apparent in anyone undergoing a splenectomy. We now have strong evidence that during health, the regulation of platelet life span is tightly regulated by the intracellular protein products of two genes, *BCL-x_L* and *BAK* (Mason et al, Cell 128:1173-86, 2007), and that this is a platelet intrinsic phenomenon. The anti-apoptotic protein, *BCL-x_L*, is the key mediator of platelet survival. Loss-of-function mutations in the gene encoding *Bcl-x_L* or its pharmacological inhibition decrease platelet half-life and cause thrombocytopenia in a dose-dependent manner. The major downstream effector responsible for mediating platelet death is pro-apoptotic *Bak*. Deletion of *Bak*, and to a lesser extent, its relative *Bax*, can extend platelet life span and reverse the effects of *Bcl-x_L* antagonism both *in vitro* and *in vivo*. *Bcl-x_L* and *Bak* constitute the major components of a molecular clock that determines platelet life span: as platelets age, degradation of *Bcl-x_L* triggers *Bak*-mediated apoptosis and clearance from the circulation. In concert with other processes (eg activation) that may dominate during disease, platelet apoptosis is an important physiological process that maintains platelet homeostasis during health, and may be amenable to manipulation for therapeutic gain.

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Inherited and Acquired Thrombocytopenia

Alan T Nurden and Paquita Nurden

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Defects in platelet production or accelerated destruction result in insufficient numbers to assure hemostasis and result in bleeding. Platelets are formed in the bone marrow where mature MKs migrate to the endothelial lining of the vascular sinus where a molecular switch results in proplatelet formation and the production of platelets in large numbers by a budding process. Genetic defects in transcription factors may give rise to thrombocytopenia associated with erythrocyte abnormalities (*GATA-1*), skeletal, psychomotor and/or cardiac defects (*HOXA11*, *FLI-1*), acute myelogenous leukemia (*RUNX-1*) or marrow failure (*c-MPL*). Giant platelets and macrothrombocytopenia (MT) result from a defective GPIb-IX/cytoskeletal interaction. We have shown MT in a variant form of type 2B von Willebrand disease suggesting that abnormal GPIb-mediated signalling can affect MK maturation and platelet production. Another of our studies has identified giant platelets in patients with periventricular heterotopia and X-linked filamin A mutations. Patients with *MYH9* mutations and altered nonmuscular myosin heavy chain function combine MT with various combinations of neutrophil inclusions, sensorineural hearing loss, glomerular nephritis and cataracts. In the X-linked Wiskott Aldrich syndrome (WAS), immunodeficiency, eczema, lymphoma and small platelets are associated with a deficiency of WASp, a cytoskeletal and signalling protein. In the mostly acquired DiGeorge syndrome hemizygous deletion at 22q11.2 (including the gene encoding GPIIb/IIIa) is accompanied by MT in about 20% of patients; a finding that underlines how complex haplotypes, modifier genes and/or environmental factors can influence the platelet phenotype. In acquired disease, immune-related thrombocytopenia can be autoimmune in nature (idiopathic thrombocytopenic purpura), result from incompatible alloantigen systems (post-transfusion purpura or neonatal alloimmune thrombocytopenia) or develop from drug-dependent antibodies (quinine/quinidine, heparin, abciximab...). A fall in platelet count can be associated with bacterial or viral infections, accompany major illnesses such as cancer and leukemia and their treatment, and be associated with diminished megakaryocytopoiesis and/or accelerated apoptosis.

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ASTH Symposium: Thrombocytopenia

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1200

ITP in 2007 – An Update

Timothy Andrew Brighton
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The purpose of this overview will be to provide an update for clinicians on all aspects of ITP. The diagnosis of ITP is largely a clinical one and remains a diagnosis of exclusion. Platelet antibody testing in practical terms adds little to the diagnostic algorithm and will be discussed. A complete understanding of the pathogenesis of ITP remains elusive. While the autoimmune paradigm is a generally accepted, the mechanism(s) of the variable clinical phenotype is unexplained. Factors other than autoantibodies, including macrophage function and cytokines, maybe relevant. Recent epidemiological studies of ITP patients suggest ITP is not a disease of young women but increasingly a disease of older adults and equally distributed with respect to gender. The therapy of ITP remains a challenging area. The older age group has more frequent major bleeding at platelet counts that would not usually warrant treatment in younger adults. There is renewed clinical trial interest in ITP with novel therapies, and these studies will be discussed.