

Riverside Theatre
HSANZ: Molecular Basis of Haematological Disorders
Sponsored by Celgene

0900-1030
0900

Molecular-based Risk Stratification of Multiple Myeloma: Are We There Yet?

John D Shaughnessy, Jr. and Bart Barlogie

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High-risk multiple myeloma (HRMM) is routinely defined by laboratory parameters alone or in combination in the Durie-Salmon and, more recently, the ISS staging systems. The Bartl grade, a cell morphology-based staging system, has seen limited use. The presence of abnormal cytogenetics, high BrdU labeling index, interphase FISH abnormalities and flow cytometric measures have also been used. A molecular-based classification and risk stratification of MM may improve the definition of HRMM. Global gene expression profiling (GEP) with of CD138-selected plasma cells followed by unsupervised hierarchical cluster analysis revealed that MM comprises a spectrum of seven distinct reproducible subtypes. A validated molecular classification schema has been defined as follows: (**MS** = t(4;14); **MF** = t(14;16) or t(14;20); **CD-1** = t(11;14) or t(6;14) and **CD-2** = t(11;14) or t(6;14) with high *CD20* and/or *VPREB3*), hyperdiploidy (**HY** = high *DDK1*, *FRZB*, *NCAM1*, *TNFSF10*), low bone disease (**LB** = NF- κ B signature, high *CCND2*, *CST6* and *IL6R*) and proliferation (**PR** = high *MIK67*, *CCNB1*, *CCNB2*, *TOP2A*, and *TYMS*).

Correlating GEP with outcome in two independent cohorts permitted the identification of a high-risk signature (UAMS 17-gene model), present in approximately 13% of newly diagnosed disease. GEP and high-resolution comparative genomic hybridization in 92 cases confirmed that the altered expression of the 17 genes in the model is driven by 1q gains and 1p losses. This high-risk signature is evident in a subset of all 7 molecular subtypes and negatively influences outcome. For example, low-risk MS disease fares much better than high-risk MS disease. We recently reported that the addition of bortezomib to TT3 has significantly improved outcome in low-risk MS disease, thereby demonstrating the value of GEP in evaluating benefits of new treatments that might be otherwise masked. When subjected to multivariate analysis including the International Staging System (ISS) and a gene expression-based proliferation index (GEP PI), the UAMS 17-gene model remained a significant predictor of outcome.

Mulligan and colleagues developed outcome classifiers for relapsed disease treated with single agent bortezomib or high dose dexamethasone improved upon the risk stratification provided by the ISS. These predictive models showed some specificity for bortezomib. Using U133A data from newly diagnosed disease treated with ASCT, the Mayo clinic group validated the UAMS 17-gene model, but also showed that the t(4;14) translocation remained a significant adverse variable. The IFM recently reported on a 15-gene model of high-risk (IFM 15-gene model) related to cell proliferation. Multivariate showed that the UAMS 17-gene model was significant in all datasets while the IFM 15-model was significant in a limited number. This difference might be attributed to the dependence of the IFM model to cell proliferation.

GEP on 71 paired diagnostic and relapse samples indicate that the UAMS 17-gene model score increases in 80% of the cases and a low-risk to high-risk conversion in 14 of 24 (58%) severely impacted post-relapse survival. Expression of *TP53* is a surrogate for 17p13 deletion and *TP53* expression below a specific threshold seen in approximately 10% of newly diagnosed disease, imparts a poor prognosis in low-, but not high-risk defined by the UAMS 17-gene model. In conclusion, while the majority of patients with MM can anticipate long-term disease control, approximately 25% of patients with molecularly defined HRMM do not benefit from current approaches.

Riverside Theatre
HSANZ: Molecular Basis of Haematological Disorders
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0900-1030
0945

Molecular Pathogenesis of the Myeloproliferative Disorders

Anthony Green

University of Cambridge, Department of Haematology, Cambridge Institute for Medical Research, Cambridge, United Kingdom

The human myeloproliferative disorders represent a spectrum of clonal haematological malignancies, with three main members: polycythaemia vera (PV), essential thrombocythaemia (ET) and idiopathic myelofibrosis (IMF). For over quarter of a century it has been realised that these diseases reflect transformation of a multipotent haematopoietic stem cell, but the identity of underlying target gene(s) remained elusive.

This changed dramatically in 2005 when we and others demonstrated that a single acquired V617F mutation in JAK2 is present in virtually all patients with PV and in approximately half those with either ET or IMF. The mutation is present in a variable proportion of granulocytes, alters a highly conserved valine present in the negative regulatory JH2 domain, and dysregulates kinase activity. Retroviral and transgenic studies have shown that the mutation produces cytokine-independence in cell lines and an MPD phenotype in mice.

Our subsequent results suggest that V617F-positive ET and PV form a phenotypic continuum, that homozygosity for this mutation plays a key role in the PV phenotype and that V617F-negative ET and V617F-positive ET represent distinct disorders. More recently we have made the unexpected discovery that leukaemic transformation is associated with loss of the JAK2 V617F mutation and we have identified a cluster of new JAK2 mutations which define a previously unrecognised myeloproliferative syndrome. These data, together with those of other groups, are laying the foundation for new approaches to the diagnosis, classification and therapy of the myeloproliferative disorders.

Meeting Room 4
ANZSBT: Critical Bleeding
Sponsored by CSL

0900-1030
0900

Massive Transfusion and Coagulopathy: Pathophysiology and Clinical Implications

Jean-François Hardy

Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, Québec, Canada

Coagulopathy associated with massive transfusion (MT) remains an important clinical problem. In this presentation, we review the literature in an attempt to identify the causes of coagulopathy in massively transfused, adult and previously hemostatically competent patients and to differentiate between the elective surgical and the emergency settings.

In patients undergoing elective surgery, tissue trauma is controlled, normothermia is maintained, hypovolemia and shock are avoided, monitoring of hemostasis is ongoing and blood products are available in a timely fashion. A decrease in fibrinogen concentration is observed initially while thrombocytopenia is a late occurrence. Critically low levels of coagulation factors were seldom reported when whole blood was in common use. With the use of packed red blood cells (PRBC), dilution or consumption of coagulation factors has become a significant issue requiring specific treatment with, primarily, fresh frozen plasma (FFP). Platelet transfusions are seldom required in this context.

In the emergency setting (e.g. trauma, ruptured abdominal aortic aneurysm), tissue trauma, shock, tissue anoxia and hypothermia contribute to the development of microvascular bleeding. The exact cause of microvascular bleeding remains unknown. Massive consumption of coagulation factors and platelets, disseminated intravascular coagulation, anticoagulation by activated protein C and hyperfibrinolysis are suggested mechanisms. Several recent observations (chart and database reviews) suggest that the proactive administration of large volumes of platelets and FFP improve coagulation, decrease hemorrhage and improve survival in massively bleeding trauma patients. We can only speculate that, in this specific context, the benefits of early and aggressive platelet and coagulation factor replacement are related to the ongoing consumption coagulopathy at the time of surgery.

Coagulopathy associated with MT is an intricate, multifactorial and multicellular event. Appropriate management strategies should consider the patient's situation (elective vs. urgent surgery) and, whenever possible, the results of diagnostic laboratory tests of hemostasis (Can J Anaesth 2006;53:S40-S58).

Meeting Room 4
ANZSBT: Critical Bleeding
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0900-1030
0945

Critical Bleeding: Retrieval Service Perspective

Stephen Langford

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Meeting Room 4
ANZSBT: Critical Bleeding
Sponsored by CSL

0900-1030
1005

Critical Bleeding: Trauma Service Perspective

Sudhakar Rao

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Modern management of critically injured people relies on a efficient and effective trauma system. The evidence for trauma systems as opposed to individual trauma centres is compelling. An organised and properly resourced trauma system is the foundation on which other medical and pharmacological treatments of the critically ill trauma patient can be effective. Conversely, advances in surgical and medical care of injuries, (e.g. haemostasis, fluid resuscitation, critical care) will not the expected outcomes in the absence of an organised trauma system.

Trauma Care in Western Australia is multidisciplinary. The trauma service has expanded from a traditional focus of surgeons and now encompasses all surgical, medical specialities and all therapists (including clinical psychology). The outcome measures from trauma care now include more than just mortality and surgical morbidity.

Meeting Room 2/3
ASTH: Hormones, Thrombophilia and Thrombosis

0900-1030
0900

Female Hormones and Venous Thrombosis

FR Rosendaal

Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

Widespread use of female hormones began in the 1960s with the availability of oral contraceptives. The first thrombotic side effect of oral contraceptives was reported in 1961. Since first licensing in 1959, the oestrogen dose has been reduced, to either 50 µg or 30 µg ethinyloestradiol, or even less. The progestogen content has also changed over time, but here it concerned the chemical composition. Second generation progestogens include norgestrel and levonorgestrel, of which levonorgestrel is still widely used. Third generation progestogens are desogestrel and gestodene. Two other progestins are the anti-androgen cyproteronacetate and drospirenone, which is an anti-mineralocorticoid.

The most recent studies still show 2- to 6-fold increased risks of venous thrombosis caused by oral contraceptives. The absolute risk of venous thrombosis in women of reproductive age is low, and in oral contraceptive users it becomes two to three per 10 000 per year. The risk of venous thrombosis is highest during the first year of use. However, the risk does not accumulate with prolonged use. The higher the dose of oestrogen, the higher the risk of thrombosis.

A series of studies have confirmed an additional two-fold higher risk for third generation progestogens brands. Recently, it has been shown that oral contraceptives that contain cyproteronacetate confer a substantially increased risk of venous thrombosis, similar to that of third generation oral contraceptives. The safety of drospirenone is still unclear.

Obesity increases the risk of thrombosis about 2-fold, but obese women using oral contraceptives have a more than 20-fold increased risk. In familial thrombophilia oral contraceptives greatly enhance the risk of thrombosis in carriers of one of these defects.

The oestrogens in oral hormonal replacement therapy preparations are usually conjugated oestrogens retrieved from pregnant mare urine, or micronised oestradiol. The progestogen mostly used in combination preparation is medroxyprogesterone acetate (MPA). Besides oral administration, the hormones can also be administered transdermally by patches and subcutaneously.

From 1996 onwards, studies have established a clearly increased risk of venous thrombosis for users of oral hormonal replacement therapy, with a two- to fourfold increased risk compared to non-users.

As is the case for oral contraceptives, the risk of venous thrombosis is higher shortly after therapy has started, and in those with prothrombotic abnormalities.

Meeting Room 2/3

ASTH: Hormones, Thrombophilia and Thrombosis

0900-1030

0930

Update on the Protein C Anticoagulation Pathway

David Lane

Department of Haematology, Imperial College London, UK

Protein C is part of an important anticoagulant mechanism that down regulates blood coagulation. It is activated on the endothelial cell surface by thrombin/thrombomodulin complex. Activated protein C (APC), together with its cofactor protein S, proteolytically inactivates factor Va and factor VIIIa. Optimum activation of protein C to APC requires presentation of protein C to its activation complex. On large blood vessels this is achieved by specific interaction of protein C with its receptor, EPCR, an interaction mediated via its Gla domain. A number of specificity issues surrounding activation of protein C and the function of APC have been clarified recently:

By what mechanism is EPCR selectively expressed in large blood vessel endothelial cells?

How does protein C specifically recognise its receptor, EPCR?

How does APC specifically recognise protein S?

Protein S has been under the shadow of protein C for many years, perhaps reflecting the difficulty in its assay and complexity surrounding clinical manifestation of thrombotic problems arising from its deficiency. Exploring an area long been confused by methodological issues, the protein C independent functions of protein S, Hackeng and colleagues have now suggested that a key function of protein S is to enhance the inhibitory effect of TFPI against the tissue factor/factor Xa complex. If they are correct, this will indicate a bifunctional role for protein S, as a cofactor for TFPI at low tissue factor concentration and as a cofactor to APC at high tissue factor concentrations.

Protein C is also attracting increasing interest in the therapeutic context. APC is unique amongst the natural anticoagulant proteins in that it has been shown to be effective in reducing mortality of patients with severe sepsis. John Griffin and coworkers have raised an interesting question: does its effectiveness arise from its anticoagulant property or is another function responsible? A number of studies have shown that APC also has anti-inflammatory/cytoprotective properties that are mediated through cellular positioning of APC by its interaction with EPCR, subsequent cleavage of endothelial cell protease receptors and modulation of cytoprotective signalling pathways. Intriguingly, by selective mutation of its protease domain exosite loops, it has been shown that mutant forms of APC can be produced that have greatly diminished anticoagulant function but normal cytoprotective functions in vitro. It may be that such non anticoagulant functions are also the basis of beneficial effects of APC in tPA induced bleeding in small animal stroke models. APC mutants with selective cytoprotective function hold the promise of improved therapeutic agents.

Meeting Room 2/3
ASTH: Hormones, Thrombophilia and Thrombosis

0900-1030
1000

Protein C & S Gene Mutations and Regulation by Hormones

Quintin Hughes, Ross Baker

Centre for Thrombosis and Haemophilia, Royal Perth Hospital, Murdoch University, Australia.

Activated Protein C (aPC) inactivation of the procoagulants factor Va and factor VIIIa is enhanced by binding of the cofactor Protein S (PS). Approximately 60% of circulating PS is bound to C4b-binding protein (C4b-BP) leaving a remaining 40% 'free PS' fraction. Until recently it was thought that only the free form was available for aPC cofactor activity. However, recent evidence suggests that C4b-BP bound PS is able to enhance aPC proteolytic inactivation of factor Va. A separate study found that free PS exhibits cofactor activity for tissue factor pathway inhibitor (TFPI), stimulating TFPI inhibition of factor Xa. These recent findings in addition to what we already know about PS highlight its importance as a regulator of thrombin generation.

Expression of PS is affected by oestrogen and the many progestin isotypes that are both used in combined oral contraceptive formulations (COCs). We recently identified a progesterone response element (PRE) in the 5' untranslated region of the PROS1 gene that encodes PS, demonstrating differential expression in response to various progestins. This finding explains the observed differences in PS levels seen between users of 2nd and 3rd generation COCs that contain levonorgestrel and gestodene or desogestrel, respectively. Progestogenic upregulation represents a counterbalance to the downregulatory effect oestrogen has on PS production. The oestrogenic site responsible for Protein S upregulation is currently being investigated in both the 5' and 3' untranslated regions of the PROS1 gene.

Recent studies have suggested that acquired aPC resistance may be in part, a function of oestrogen driven acquired PS deficiency. Therefore, a better understanding of the exact process involved in the regulation of PS by oestrogen may provide a better insight into the acquisition of aPC resistance and the propensity of hormones to be associated with venous thrombosis.

Meeting Room 1
Nurses: Quality of Life

0900-1030
0900

Home Chemotherapy

Julie Wilkes
Royal Perth Hospital, Perth, WA, Australia

Abstract not received at time of going to print

Meeting Room 1
Nurses: Quality of Life

0900-1030
0930

QOL – Concepts and Measurement: The Patient’s Perspective

Anne Williams

Western Australian Centre for Cancer and Palliative Care, Curtin University of Technology; Centre for Nursing Research and SolarisCare Centre, Sir Charles Gairdner Hospital

This presentation explores the concept of QOL from the perspective of patients and describe a program of research in this area. The current program is based on a substantive theory “Optimising Personal Control to Facilitate Emotional Comfort” which was developed initially through a grounded theory study which investigated the experience of being a patient hospitalised in Western Australia. In that study patients were found to perceive that when they felt emotionally comfortable, healing was facilitated. A central component of emotional comfort was having a sense of personal control over their situation or environment during hospitalisation. Emotional comfort could be influenced by a number of factors such as the interpersonal interactions experienced, characteristics relating to the patient, and aspects of the hospital environment. For the purpose of this presentation the characteristics of those interpersonal interactions from healthcare staff that facilitated the state of emotional comfort will be described in terms of how they related to the patient’s perceived Level of Security, Level of Knowing, and Level of Personal Value.

An instrument to measure the interpersonal interactions experienced by patients during hospitalisation has been developed as part of this program of work. The instrument named, ‘Patient Evaluation of Emotional Care during Hospitalisation’ (PEECH) was tested through a survey of 132 patients. Encouraging reliability and validity estimates have been obtained through this preliminary psychometric testing and further directions for research have been identified.

Meeting Room 1
Nurses: Quality of Life

0900-1030
1000

What Can Qualitative Research Do for Me?

Moira Stephens

Centre for Values, Ethics and the Law in Medicine, University of Sydney, Sydney, NSW, Australia

Descartes said in 1637 “ For it seemed to me that I should find more truth in the reasonings of which a man makes with regard to matters which touch him closely..... than in the reasonings of a man of learning in his study, whose speculations remain without effect” (Tr. Wollaston 1960). This was arguably, one of the earliest comments on the strength and validity of empirical qualitative research!

This session will introduce and discuss a variety of ways of undertaking qualitative research and explore the differences in their perspectives. It will discuss ideas about methodology and rigour in qualitative research and how qualitative research is ideally situated to answer many questions and complexities evident in health care.

No conflict of interest to disclose

Meeting Room 7
BMTSAA Session

0900-1030
0900

From Stem Cells to Blood Cells

Louise Winteringham

The Western Australian Institute for Medical Research and The Centre for Medical Research The University of Western Australia, Perth, Australia

Haemopoiesis is the stepwise maturation of cells to form all the different cellular components of the blood. Haemopoiesis starts from multipotent, self renewing haemopoietic stem cells (HSC) which differentiate into multipotent progenitors that are unable to self renew but are able to differentiate. These multipotent progenitor cells become progressively lineage restricted to finally form all the mature differentiated cells of the blood. When this process fails leukemia can develop. Most often leukemias are treated with high doses of chemotherapy and radiation which target the rapidly dividing cancer cells. These therapies unfortunately also target rapidly dividing progenitor cells so bone marrow transplantation is often used to repopulate the bone marrow. However, in a number of patients the leukemia recurs. Chemotherapy and radiation fail to target the quiescent or very slow dividing HSC and research over the past 10 years has demonstrated that a small portion of these cells have the ability to reinitiate leukemia. These cells are referred to as cancer stem cells (CSC) or leukemia initiating cells (LIC). It is still not clear whether LIC arise from mutations in HSC that confer a greater ability to self renew or mutations in progenitor cells that cause "regression" and reactivation of stem cell programs. Regardless of their origin it has become clear that current treatments for leukemia do not target these LIC and this is likely to be, at least in part, the reason for relapse of patients following treatment. Current research is focused on identifying molecules that might contribute to the tumorigenic capacity of LIC, *vis.* deregulated proliferation and an inhibited ability to differentiate, and establishing which of these molecules will provide appropriate targets for consideration in future therapeutic strategies.

Meeting Room 7
BMTSAA Session

0900-1030
0940

Improved Functional Recovery After Transplantation of Human Bone Marrow Stromal Stem Cells (hBMSCs) From Spinal Cord Injured Patients Into the Acute and Chronic Injured Rat Spinal Cord

Stuart Hodgetts¹, Paul Simmons², David Haylock³ & Giles W Plant¹

¹Reds Spinal Cord Research Laboratory, UWA, Perth, Western Australia

²Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center, Houston, USA

³Australian Stem Cell Centre, Melbourne, Australia

Multipotent hBMSCs from spinal cord injury (SCI) patients were used to stimulate sparing and regeneration of descending neural pathways following moderate contusive SCI. hBMSCs transduced with a retrovirus encoding GFP (hBMSC^{GFP}) were transplanted into immunologically deficient (Nude) rat hosts subjected to a moderate SCI (10g,12.5mm) using an NYU impactor device. The therapeutic potential of hBMSC^{GFP} was assessed both behaviourally (recovery of function) and anatomically using immunohistochemistry. In both *acute* and *chronic* SCI studies, hBMSC^{GFP} initially survive well in the injured host spinal cord (SC) 1wk after transplantation, induce axonal growth, produce growth promoting molecules and co-exist with host glial cells such as astrocytes and Schwann cells within the lesion site. At 1wk post-transplantation, immunostained SC sections show the presence of RT97⁺/ -III tubulin⁺ axons, GFAP⁺ astrocytes, and p75⁺ Schwann cells intermingled with hBMSCs. S100⁺ profiles were seen to be in close proximity to transplanted hBMSC^{GFP} and hBMSCs were shown to produce large quantities of laminin & fibronectin *in vivo*. Immunostaining for RT97, GFAP, p75 remains high in and around the lesion site up to 4wks post-transplantation. Virtually no donor hBMSCs were present in the lesion site after 8wks, coinciding with a large increase in ED1⁺ macrophages within the lesion and both rostral and caudal SC tissue. Rats transplanted with hBMSCs showed a reduction in the size of the injury site, compared to controls. Extensive behavioural analysis of hBMSC^{GFP}-transplanted nude rats showed a marked improvement in open field BBB scoring (15 ± 0.5^{SEM}) c.f. controls (13 ± 0.7^{SEM}) 8wks post transplantation. Additional detailed computer generated functional analysis (Catwalk gait system) was also performed. These results provide new evidence on the use of hBMSCs for the repair of the chronic injured mammalian SC.

Riverside Theatre
HSANZ: Acute Leukaemia

1100-1200
1100

Unravelling the Complexity of Chromosome Abnormalities in Acute Myeloid Leukaemia

Lynda Campbell

Victorian Cancer Cytogenetics Service, St Vincent's Hospital Melbourne, Victoria, Australia

Chromosome abnormalities are found in 55-60% acute myeloid leukaemias and aid in morphological classification and prediction of outcome. Approximately 20% of successfully karyotyped AML cases contain "complex" chromosome abnormalities. Complexity is defined as either 3 or more or 5 or more abnormalities, depending on the study and invariably denotes a poor prognosis. However, simple counting of abnormalities may be misleading. A study of hyperdiploid AMLs showed that patients with ≥ 3 extra chromosomes but without structural abnormalities were better classified in an intermediate prognostic group (Luquet *et al*, 2008).

It is assumed that complex karyotypes reflect genomic instability. Yet there are patterns to be discerned within most complex karyotypes and it seems likely that these patterns represent an accumulation of critical events in the development of AML, as is observed in solid tumours such as colon cancer.

The most frequent abnormalities observed in AML, apart from the standard balanced translocations, are unbalanced rearrangements: gains or losses of whole chromosomes or deletions of part of chromosomes, such as 5q, 7q, 17p and 20q. Monosomies of chromosomes 5, 7, 17 and 20 are also frequently observed and both loss of the whole chromosome and loss of part of one arm should result in a similar genetic outcome: effectively removing a tumour suppressor gene (TSG) from the commonly deleted region of the chromosome, for example TP53 on 17p13.

However, there is increasing evidence that true monosomy of chromosomes 5, 17 and 20 does not exist and that two copies of fragments of these chromosomes are always found if looked for using FISH. These retained segments possibly reflect a second oncogenic effect of chromosomal deletion – indicating the location of oncogenes that are activated or up-regulated by the removal of an adjacent TSG.

No conflict of interest to disclose

Riverside Theatre
HSANZ: Acute Leukaemia

1100-1200
1130

New Strategies in Acute Promyelocytic Leukaemia

Martin Tallman

*Northwestern University Feinberg School of Medicine, Robert H. Lurie
Comprehensive Cancer Center, Chicago, IL, USA*

Since the initial description in 1957, the natural history of acute promyelocytic leukemia (APL) has changed from one characterized by high early mortality to one distinguished by a high cure rate. With routine administration of all-trans retinoic acid (ATRA) combined with chemotherapy in the early 1990's and arsenic trioxide (ATO) in the late 1990's, cure is now achieved in the majority of both newly diagnosed and relapsed patients. ATRA with anthracycline-based chemotherapy for induction and consolidation followed by ATRA plus low-dose chemotherapy maintenance is currently the standard of care for newly diagnosed patients. Early institution of ATRA and aggressive blood product support are critical to reduce induction mortality, reported to be 10% among patients entered on clinical trials, but certainly higher when all patients are considered. The relapse rate among high-risk patients is approximately 20-30% and new strategies include administration of intensified anthracyclines, intermediate- or high-dose ara-C in either induction or consolidation, or ATO as early consolidation. Central nervous system (CNS) prophylaxis for such patients and those with relapsed disease may be important to prevent subsequent extramedullary relapse. Given the excellent outcome for most patients with current therapy and potential short-and long-term toxicities, other new therapeutic strategies have focused on minimizing chemotherapy. Recent studies suggest that patients who are molecularly negative after intensive consolidation may not benefit from maintenance therapy. Most exciting is the combination of ATRA and ATO, given with minimal chemotherapy only for leukocytosis, which is a very effective new strategy for patients who are unable to tolerate anthracyclines or older adults and soon may replace conventional therapy for many, if not most, patients. A subset of patients with very low-risk disease may be cured with ATO alone. Patients with relapsed disease do well with ATO with CNS prophylaxis followed by autologous hematopoietic stem cell transplantation.

Meeting Room 4
ANZSBT: Landmark Studies

1100-1200
1100

Landmark Studies that Have Changed the Practice of Transfusion Medicine: The Top 10 RCTs

Morris A Blajchman

McMaster University and Canadian Blood Services, Canada

Evidence-based medicine (EBM) is a term coined in the late 1980s to describe the process of systematically finding, appraising, and using the available contemporary research findings as the basis for medical/scientific decision making. The process of EBM requires the application of formal rules of evidence in the evaluation of the available medical/scientific literature. The use of EBM by the medical/scientific community implies that every patient, or biomedical system, be managed using of the best evidence available at a particular point of time. Medical/scientific evidence ranges from **level 1** (the best evidence), which are mainly large randomized control trials (RCTs), to **level 5** (the poorest evidence) which comprises mainly anecdotal and other types of poor quality observational evidence. Undocumented expert opinion is thus also considered level 5 evidence! Level 1 evidence represents data from RCTs that are sufficiently large to give clear cut results as well as meta-analyses of all the available large RCTs on a topic. Over the past twenty years there have been approximately 2000 RCTs that have been done to evaluate various transfusion medicine interventions that can be used for the treatment of patients or to choose tools to assess the safety or other aspects of the blood supply. The foci of these randomized control trials have ranged widely from: (1) studying the efficacy of different platelet preparations for the treatment of patients at risk for thrombocytopenic bleeding; (2) the evaluation of leukoreduction in preventing transfusion-related immunomodulatory and other effects; (3) evaluating transfusion triggers for administering RBCs, platelets or plasma; (4) assessing the efficacy of various infectious disease markers in preventing transfusion-transmitted infections; (5) evaluating the role of various bioactive agents in reducing allogeneic transfusion requirements; or (6) studying the effects of allogeneic RBC transfusions on the post-operative length of stay in a hospital or intensive care unit. During this presentation, Dr. Blajchman will present his top ten list of Transfusion Medicine RCTs, ranking them in reverse order according to their quality and clinical impact.

References

1. Sackett DL. Rules of evidence and clinical recommendations on the use of antithrombotic agents. *Chest* 1989; 95 (Supl. 2): 2S-4S.
2. Hébert PC, Wells G, Blajchman MA *et al.* A multicenter randomized controlled trial of transfusion requirements in critical care. *N Engl J Med* 1999; 340: 409-417.
3. Blajchman MA. Landmark studies that have changed the practice of transfusion medicine. *Transfusion* 2005; 45: 1523-1530.

Meeting Room 2/3
ASTH: Anticoagulation

1100-1200
1100

The Future of New Antithrombotics

Sanjeev Chunilal

North Shore Hospital, Waitemata Health, Auckland, New Zealand

Abstract not received at time of going to print

Meeting Room 2/3
ASTH: Anticoagulation

1100-1200
1130

Warfarin Reversal: How Long Do We Have To Wait To Get It Right?

Hatem H Salem

Australian Centre for Blood Diseases, Alfred Hospital and Monash University, Victoria

Although Warfarin was first approved for human use in 1954, we continue to evolve our understanding of the drug and how best to use it and reverse its effects. Prevention of thromboembolic events consequent to atrial fibrillation remains the main indication for the long term use of this drug. While millions around the world are candidates for anticoagulant therapy for this indication, many are denied treatment with numerous studies attesting to the underutilisation of Warfarin in this target population. The main reason for the under prescribing of the drug is related to the risk of bleeding and the need for regular close monitoring. While it is true that bleeding often occurs when the INR is within the therapeutic range, an INR above the therapeutic range is well recognised as a risk factor for bleeding. This is particularly true in the elderly, those receiving concomitant anti-platelet therapy, or those at risk of falls. In many of these patients, reversing the effects of Warfarin is recommended. Patients on warfarin with active bleeding present another indication for warfarin reversal while in others urgent reversal may be required because of emergency surgery. In many other patients receiving Warfarin reversal may be required for a planned surgical or diagnostic procedure. In 2004, the Australasian Society of Haemostasis and Thrombosis published its consensus guidelines for Warfarin reversal. The group stressed the importance of a thorough evaluation of the patient with respect for the potential of bleeding versus the risk of recurrence of thrombosis. Guidelines were presented for managing Warfarin reversal under different scenarios. Unfortunately, and despite best efforts, Warfarin reversal continues to be carried out in a sub-optimal way with many patients requiring urgent reversal receiving either high dose vitamin K alone and or large volume of fresh frozen plasma. Those lucky enough to receive PCC often are administered an inadequate dose of the product.

In my presentation, I will review the various indications for Warfarin reversal highlighting the optimal way to manage these patients. The presentation will emphasize the use of PCC as an efficient and safe method in patients who require urgent Warfarin reversal.

Meeting Room 1
Nurses: Apheresis Update

1100-1200
1100

Immunoadsorption for ABO Incompatible Renal Transplants

Jo-Anne Moodie

North West Dialysis Service, Royal Melbourne Hospital, Vic, Australia

Aim

To describe the North West Dialysis Service (NWDS) experience of performing Immunoadsorption for ABO incompatible renal transplants.

Background

Transplantation from ABO incompatible donors has been avoided in Australia because of a high risk of severe irreversible rejection, the need for splenectomy and powerful immunosuppression. Immunoadsorption of either anti A or B antibodies (Glycorex Glycosorb columns) avoids rejection mediated by anti-blood group antibodies without depletion of other proteins or the requirement for replacement fluid [1]. In 2005 the first use of this technology in Australia was undertaken at the Royal Melbourne Hospital to enable Australia's first ABO incompatible renal transplant.

Method

Since December 2005, 5 patients with ABO incompatibility have been treated by NWDS using the Glycosorb ABO Column. The treatment involves antigen specific adsorption of ABO antibodies, using the conventional Cobe Spectra plasma exchange machine and lines, with the addition of the appropriate column. Treatments are performed pre and post transplantation in conjunction with conventional plasma exchange and immunosuppression, to halt rebound of antibodies. Titre levels are taken pre and post treatment to monitor effectiveness and suitability to undergo transplant.

Results

Experience with immunoadsorption indicates removal and thus reduction of ABO antibodies. Titre levels were reduced by as much as 3 dilutions. All grafts are still functioning, and no incidence of rejection has occurred, after a minimum of 12 months follow up. The use of columns also reduces the side effects experienced during regular plasma exchange, such as decreased fibrinogen levels.

Conclusion

A number of patients with end-stage kidney disease are potentially suitable for living related and unrelated transplantation, if it were not for blood group incompatibility. The use of immunoadsorption columns reduces titre levels and is a safe adjunct to treatment without adverse effects. Performing ABO incompatible transplants at NWDS provides an opportunity to patients that otherwise may not be able to undergo a transplant.

Meeting Room 1
Nurses: Apheresis Update

1100-1200
1130

On the Road to Accreditation With a Little Help From our Friends

Susan Price, Andrew McCutchan and Joanne Kanakis

Townsville Cancer Centre, The Townsville Hospital

The Townsville Health district provides care to over 600,000 people. The Townsville Cancer Centre is the only service north of Brisbane which has an Apheresis/Bone Marrow Transplant Unit. Since 1996 The Townsville Cancer Centre at the Townsville Hospital has been performing Therapeutic Apheresis procedures and Haemopoietic Progenitor Cell (HPC) collections.

During 2004-2005 TGA developed a position statement that stated all HPC's will now be required to meet licensing requirements using the Foundation for the Accreditation of Cellular Therapy (FACT) Standards. After several meetings with identified stakeholders, it was agreed that the National Pathology Accreditation Advisory Council (NPAAC) would develop the Australian Standard based on FACT quality principles. Since the first draft was released in 2006 we have slowly been transforming our service with the goal of becoming an accredited facility. We've has great assistance from fellow Qld Health and non Qld Health facilities.

In this presentation, the Apheresis Coordinator, Bone Marrow Transplant Coordinator and Quality Manager give an insight to where we started on the accreditation journey, how far we have come and where we are planning to go.

Riverside Theatre

1200-1300

Barry Firkin Oration

Evidence-based Medicine: Wish-fulfilment or Search for Excellence?

Alex Gallus

Flinders Medical Centre, Bedford Park, SA, Australia

Abstract not available

Riverside Theatre
Combined HSANZ / BMTSAA/ Nurses: Bone Marrow Transplantation

1400-1530
1400

Timing of Transplantation and Choice of Conditioning in MDS and MPD

H Joachim Deeg^{1,2} and Bart Scott^{1,2}

¹Fred Hutchinson Cancer Research Center and ²University of Washington School of Medicine, Seattle, WA, USA

Both myelodysplastic syndromes (MDS) and myeloproliferative disorders (MPD) are clonal marrow diseases, potentially curable by hematopoietic stem cell transplantation (HCT). The optimum timing, however, and the best conditioning strategy have remained controversial, and the availability of a growing number of non-transplant modalities is raising additional questions. Factors to be considered include disease stage, prognosis with or without non-HCT therapy, patient age, and comorbid conditions. Is the patient a risk taker? Is quality of life the primary objective? With MDS, generally patients in IPSS risk groups intermediate-2 and high, and select patients with intermediate-1 risk should be considered for transplantation early in their course; the remaining patients are likely to benefit from more conservative management initially. Transfusion dependence, marrow fibrosis and phenotypic aberrancies of marrow cells may be additional reasons for earlier transplantation. Patients who present with transformation to acute leukemia should probably receive 'debulking' chemotherapy before undergoing HCT. Among patients with less advanced/low risk MDS (<5% marrow myeloblasts, generally IPSS risk groups low and intermediate-1), the 3-year survival probability is 65% to 75% with HLA-matched related and unrelated donors. Among patients with more advanced/high-risk disease (≥5% marrow blasts, IPSS risk groups intermediate-2 and high), the probability of post-transplant relapse ranges from 10%-40%, and, as a result, relapse-free survival is inferior. The karyotype is the strongest risk factor for relapse. Patients with MPD (other than CML) have generally come to HCT because of progressive myelofibrosis and peripheral blood cytopenias or transformation to acute leukemia. Post-HCT survival in remission may be as high as 75% in the first case (relapse is infrequent), but only 30–35% in the second. Both conventional and reduced-intensity conditioning (RIC) have been used successfully. RIC allows for a decrease in non-relapse mortality and for application of HCT even in patients 60–70 years of age. However, in patients with either MDS or MPD, relapse rates after RIC tend to be higher than with conventional regimens. "Re-intensification" of RIC may overcome this problem. Graft-versus-host-disease, acute and chronic, has remained a frequent and challenging problem for all patients. The impact of epigenetic modification or JAK2 inhibitors on transplant decision and outcome remains to be determined.

Riverside Theatre
Combined HSANZ / BMTSAA/ Nurses: Bone Marrow Transplantation

1400-1530
1445

Therapeutic Applications of Mesenchymal Stromal Cells in Haemopoietic Stem Cell Transplantation

Ian Lewis

Division of Haematology, SA Pathology, Adelaide SA, Australia

In addition to haemopoietic stem cells (HSC), bone marrow (BM) also contains Mesenchymal Stromal Cells (MSC), which can differentiate into multiple mesodermal lineages including bone, cartilage, muscle and fat. In the laboratory these cells are isolated from the adherent layer of BM and appear as fibroblast like cells which can be induced to differentiate into bone, cartilage, muscle and other tissues. This property has led to the suggestion that MSC may have a role in tissue repair. BM MSC support haemopoiesis and may have an important role in promoting HSC engraftment particularly following cord blood transplantation (CBT). In a NOD/SCID mouse model we compared engraftment rates of double-unit CBT with MSC co-transplantation. We show at equivalent cell dose, single and double unit CBT lead to similar engraftment, suggesting the enhanced engraftment seen with double unit CBT reflects a cell dose effect. MSC co-transplantation enhanced engraftment of both single and double unit CBT and may be a potential strategy to be explored in the clinic.

MSC have also been shown to have unique immunomodulatory properties. They possess both immunogenic and immunosuppressive properties. This property has been exploited in the treatment of severe acute graft versus host disease (aGVHD), a life threatening immunological complication of allogeneic bone marrow transplantation, with encouraging preliminary results. The Royal Adelaide Hospital has participated in an international multi-centre study to evaluate the effectiveness of MSC infusion in severe steroid refractory aGVHD. Of 55 patients treated, 30 patients had a complete response and 9 showed improvement. Complete responders had lower transplantation-related mortality 1 year after infusion than did patients with partial or no response (37% vs 72%; $p=0.002$) and higher overall survival 2 years after haemopoietic-stem-cell transplantation (53% vs 16%; $p=0.018$). In conclusion, MSC infusion is a promising modality in the treatment of steroid resistant aGVHD.

Meeting Room 4
ANZSBT: Molecular Advances in Transfusion Medicine

1400-1530
1400

Experience Using DNA-based Assays to Predict Blood Group Antigens

Marion Reid

New York Blood Center, New York, NY, USA

We have over a decade of experience using PCR-based laboratory developed tests (LDTs) to predict a blood group in two main areas. (1) In the prenatal setting: we test DNA from a fetus at risk for hemolytic disease of the newborn for *RHD*, *RHCE*_c*, *KEL1/KEL2*, and other blood groups for which the molecular basis is known. Regardless of the implicated antibody(ies), we test for *RHD* to preempt a request for D-negative blood for an intrauterine transfusion. (2) In the transfusion setting: we test DNA from a patient who has been recently transfused, whose RBCs are positive in the direct antiglobulin test, to distinguish allo from auto antibodies, and to predict the presence of a weakly expressed antigen when a patient is unlikely to be immunized if transfused with antigen-positive RBC products. For patients and donors, we use PCR-based LDTs to predict the blood type when an antisera is in short supply or is weakly reactive, to resolve typing discrepancies between reagents of the same apparent specificity, to identify the molecular basis of unusual serological results and new antigens, and to type stored DNA from donors and a transplant patient after an antibody arises. We also analyze our in-house antibody identification panel for selected antigens. We have considerable experience using a beadchip platform and are currently using it to test donors to increase our antigen-negative inventory and to find donors whose RBCs lack a high-prevalence antigen. Having access to a larger number of antigen-negative donors, albeit a prediction requiring serological confirmation, should improve patient care in regard to transfusion therapy. The high-throughput technologies would make it feasible to match donor to the type of a patient, especially for RH alleles in patients with sickle cell disease. The extent to which this becomes a reality may be dictated by cost.

Meeting Room 4**ANZSBT: Molecular Advances in Transfusion Medicine****1400-1530****1445****Platelet Antigens****Paul Metcalfe***National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK*

To date 24 platelet-specific alloantigens have been defined by immune sera of which 12 are grouped in 6 bi-allelic systems (HPA-1, -2, -3, -4, -5 & -15). For the remaining 12, alloantibodies against the thetical but not the anti-thetical antigen have been observed. The molecular basis of 23 of the 24 serologically defined antigens has been resolved and these have been designated as Human Platelet Antigens (HPA). In all but one of the 23, the difference between self and non-self is defined by a single amino acid substitution, caused by a single nucleotide polymorphism (SNP) in the gene encoding the relevant membrane glycoprotein. The exception is HPA-14bw which is the result of an in-frame single codon deletion in glycoprotein IIIa. The HPA nomenclature system was adopted in 1990 to overcome problems with the previous nomenclature and was revised again in 2003.

For the 6 bi-allelic HPA systems, SNP typing has become routine and a variety of methods are in use. Several large scale studies have provided reliable information on allele frequencies and some have indicated significant differences between populations.

Meeting Room 2/3
ASTH: Von Willebrand Disease

1400-1530
1400

Molecular Aspects of vWD

David Lillicrap

Dept. Pathology & Molecular Medicine, Queen's University, Kingston, Canada

Von Willebrand disease (vWD) is the most common inherited bleeding disorder characterized in humans with a prevalence of symptomatic disease of approximately 1 in 1,000. The mucocutaneous bleeding problems that manifest in vWD are due to quantitative and/or qualitative defects in the multiligand adhesive protein von Willebrand factor (vWF).

The gene that encodes vWF is located on the short arm of chromosome 12. The gene spans 178 kb of genomic DNA and comprises 52 exons ranging in size from 40 bp (exon 50) to 1.3 kb (exon 28). Complicating the genetic analysis of vWD, there is a partial vWF pseudogene on chromosome 22 that duplicates exons 23-34 of the chromosome 12 gene with 3% sequence variation. In addition to its large size and genomic complexity, the vWF gene sequence is also highly polymorphic in nature with >80 non-synonymous single nucleotide polymorphisms in the coding region.

The three types of vWD represent either quantitative (types 1 and 3) or qualitative (type 2) traits affecting vWF hemostatic function. Type 3 vWD is the least frequent form of the disease with a prevalence of between 0.5 and 6 per million. This vWD variant is inherited as a recessive trait with transmitting parents showing no clinical or laboratory evidence of the disorder. The genetic basis of type 3 disease involves a heterogeneous collection of null mutations ranging from large vWF gene deletions to a group of missense mutations that presumably prevent secretion of the protein from its cell of synthesis.

There are four subclasses of type 2 vWD: 2A, 2B, 2M and 2N. These variants interfere with vWF's ability to interact with platelets and factor VIII, respectively. In the vast majority of cases, the genetic basis of these conditions involves missense mutations in the vWF gene.

Finally, the genetic pathology of type 1 vWD, which comprises ~80% of vWD cases, is now beginning to be characterized and appears to represent a miscellany of mutation types both at the vWF locus and at other genetic loci.

Meeting Room 2/3
ASTH: Von Willebrand Disease

1400-1530
1440

Testing for vWD

Simon Brown

Haemophilia Centre, Royal Children's Hospital, Brisbane; Queensland Blood Management Program, Queensland Health, Brisbane; Pathology Queensland, Royal Brisbane and Women's Hospital, Brisbane, Queensland

Despite being the most prevalent inherited bleeding disorder, von Willebrand disease (vWD) continues to pose problems with respect to its accurate diagnosis and sub classification. The problems faced by clinicians and laboratories particularly relate to the diagnosis of a partial quantitative deficiency of von Willebrand factor (vWF). The classification of vWD has undergone a number of modifications since the immunological characterisation of vWF and factor VIII, and the description of the utility of Ristocetin in vWD diagnosis in the early 1970's. Cloning of the vWF gene in the 1980's has lead to a detailed understanding of the molecular defects underlying type 2 vWD. This molecular knowledge has contributed to a re-evaluation of laboratory tests for vWD, in particular laboratory tests of vWF function. However, two large international studies that aimed to investigate the utility of molecular testing in the diagnosis of type 1 vWD have demonstrated the complex nature of a partial quantitative deficiency of vWF (pqd vWF). To date there is no single diagnostic test for a pqd vWF. The limitations of current laboratory tests need to be considered along with the pre-analytical variables and clinical situation when approaching the diagnosis of vWD.

Meeting 2/3
ASTH: Von Willebrand Disease

1400-1530
1505

Treatment of von Willebrand Disease

Scott Dunkley

Royal Prince Alfred Hospital, Camperdown, NSW, Australia

Issues surrounding the treatment of vWD are always highly contentious with regard to the need for therapy, the intensity of therapy and the type of therapy required. The flip side of this of course is that many patients, particularly those with type 1 vWD, do well even when subjected to a bleeding challenge such as surgery. Many persons with borderline VWF levels range may best be labelled as such rather than vWD and therapy individualised. In addition, therapy has the potential to cause harm and the spectre of blood borne disease transmission hangs over us particularly in the absence of recombinant therapy. It is appropriate to consider venous thrombosis prophylaxis in patients with vWD at times of risk including during therapy for surgical procedures.

A recent Australian retrospective study reinforced the efficacy and safety of DDAVP in mild to moderate type 1 vWD. However, in type 2 and 3 vWD, and with major surgical procedures, VWF containing factor concentrates are generally required for adequate haemostasis. The recently completed prospective Australian surgical 'Biostate' study has shed some light on dosing regimes required for surgical haemostasis and the differences compared to haemophilia A. Controversy exists on whether FVIII or VWF:RCo levels should be used to monitor such therapy and important insights have been gained from this study. Further, we will soon be faced with a choice of factor concentrates with differing FVIII to VWF:RCo ratios that may be selected depending on the specific clinical circumstance. Children with type 3 vWD are now all considered for primary prophylaxis. Finally, AHCDO have recently developed guidelines for the management of pregnancy in persons with vWD and inherited bleeding disorders and this also will be reviewed.

Riverside Theatre
HSANZ: Chronic Lymphocytic Leukaemia
Sponsored by Roche

1600-1730
1600

The Cell of Origin in Chronic Lymphocytic Leukemia

Nicholas Chiorazzi

The Feinstein Institute for Medical Research, Manhasset, New York, USA

While it has been relatively easy to determine the cell of origin of certain of the B-cell lymphoproliferative disorders (e.g. follicular cell and marginal zone lymphomas), for chronic lymphocytic leukemia (CLL) the issue is still a matter of debate. This is also complicated by the divergent clinical courses of patients whose leukemic clones differ in the presence or absence of IgV_H gene mutations, “mutated CLL” (M-CLL) and “unmutated CLL” (U-CLL), respectively. In this presentation, we will discuss the molecular, cellular, and phenotypic features of CLL cells and relate these to the known human and murine B-cell subsets.

Based on several considerations, the normal human B-cell equivalent to a CLL cell likely [1] expresses CD5, constitutively or after stimulation, as well as other markers (CD23 and CD27) indicative of activation *in vivo*, [2] resides primarily in solid lymphoid tissues, and [3] expresses characteristic BCR structural features, i.e., unmutated IgV_H genes coding for polyreactive BCRs/mAbs or somatically mutated IgV_H genes coding for oligo/mono-reactive BCRs/mAbs. In addition, it is likely that selection and drive by either autoantigens or foreign antigens or a combination of both influences the “choice” of which normal B cells or sublineage is promoted into leukemic transformation.

These parameters suggest that U-CLL and M-CLL derive from distinct normal B-cell precursors. However, gene expression profiling suggests that the two CLL subgroups do not differ from each other in a large number of differentially expressed genes. Therefore, the most parsimonious scenario is that all CLL cases, regardless of IgV_H gene subgroup, derive from marginal zone B cells, which can express unmutated or mutated Ig V genes coding for polyreactive or mono/oligoreactive Igs. On the other hand, if one considers that the similarity in the expression phenotypes of U-CLL and M-CLL reflects a common transformation process (not a common ancestral lineage), then the derivation of U-CLL from two distinct B-cell subsets is plausible (e.g., U-CLL from the human equivalent of murine B-1 cells and M-CLL from MZ B cells, which could have developed mutations at an extra-follicular site). The possibility that M-CLL derive from follicular B cells that developed IgV_H mutations in classical GCs cannot be excluded, especially if such cells subsequently migrated and took up residence in MZ.

Riverside Theatre
HSANZ: Chronic Lymphocytic Leukaemia
Sponsored by Roche

1600-1730
1635

The Yin and Yang of Therapeutic Research in CLL

Michael J Keating & William Plunkett

University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Translational research is frequently misunderstood, it implying that identifying pathways and targets will lead to successful therapy. The frequent ineffectiveness of these agents in the clinic is frustrating. Combination therapy emphasized agents with non-overlapping toxicity and different modes of action. However many clinical studies combine agents with no mechanism-based strategy, ending up as being A + B versus B + C with minor differences in outcome and subtle differences in toxicity.

The ability to obtain CLL cells from patients prior to treatment allows exploration of different mechanisms. When clinicians who understand disease work with basic scientists who understand the pharmacology/pharmacodynamics, studies often succeed. The interaction of agents with synergistic outcome is paramount. In CLL, fludarabine generated great enthusiasm. So far we still do not know how this agent works or the target. The alkylating agents are also effective and Dr Plunkett and colleagues demonstrated that fludarabine would enhance the cytotoxicity of alkylating agents leading to the development of the fludarabine/cyclophosphamide combination now established as the best combination chemotherapy program available in CLL.

In parallel studies the interaction of fludarabine (F) and cytosine arabinoside (ara-C) with platinum analogs has been informative in the development of combination therapies. The interaction between oxaliplatin (O), fludarabine, cytosine arabinoside, and rituximab (R) has been dissected. The addition of F + ara-C (A) has increased cell killing by oxaliplatin in vitro and in vivo and led to a very effective regimen (OFAR) in refractory CLL and Richter's syndrome. New agents such as flavopiridol and SNS 032 are cell cycle checkpoint inhibitors, but their interaction with RNA polymerase and down-regulation of anti-apoptotic proteins may well be a more important mechanism which can be measured in the leukemic cells and targeted and an appropriate dose selected. Nelarabine is an agent with marked activity in T-cell leukemias including T-cell prolymphocytic leukemia and surprisingly in B-cell CLL. High ara-G triphosphate levels, the active drug formed after administration of nelarabine correlate with response. Fludarabine and presumably other purine analogs enhance the formation of ara-GTP and should improve the effectiveness of these regimens. A close working relationship between the clinicians and pharmacologists emphasizes the strengths of each individual so that the synergism of the relationships leads to more effective mechanism-based therapy.

Riverside Theatre
HSANZ: Chronic Lymphocytic Leukaemia
Sponsored by Roche

1600-1730
1710

BH3 Mimetic Antagonists of BCL-2 – Potential New Therapy for CLL and Lymphoproliferative Diseases

Andrew Roberts^{1,2,3}, Kylie Mason^{1,2}, Seong Lin Khaw^{1,3}, David CS Huang^{1,3}

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³*The University of Melbourne, Parkville, Victoria, Australia*

CLL is an archetypical example of how failure of apoptosis is central to the development and phenotype of malignancy. The anti-apoptotic protein, BCL-2, is uniformly overexpressed by B-CLL cells, leading to a pathological accumulation of these cells. Further, as in other B cell lymphoproliferative diseases and other cancers, overexpression of BCL-2 results in resistance to cytotoxic agents. For these reasons, a promising avenue to therapy for incurable lymphoid tumours is to directly target BCL-2 and related pro-survival proteins. One approach is to mimic their physiological antagonists, the BH3-only proteins. In previous work, we had determined that cell death induced by BH3 mimetics such as ABT737 was dependent upon the pro-apoptotic proteins Bax or Bak, providing evidence of exquisite specificity of action. More recently, we have found that ABT 737 is highly active in vivo against murine lymphomas, and efficiently induces killing in vitro of primary cells from chronic lymphocytic leukaemia patients, including those refractory to fludarabine and other cytotoxic agents. Further, ABT737 synergises with conventional cytotoxic agents, both in vitro and in vivo in these preclinical settings. The orally available BH3 mimetic, ABT263, which like ABT737 inhibits the function of both BCL-2 and BCL-xL, commenced clinical trials in late 2006. While the initial Phase I studies remain ongoing, preliminary data indicate significant single agent activity (Wilson et al, ASH 2007; Roberts et al ASCO 2008) in CLL.

Meeting Room 4
ANZSBT: Transfusion Risks

1600-1730
1600

Anaphylaxis: Diagnosis and Management

Simon Brown

Emergency Medicine, Royal Perth Hospital, University of Western Australia & Centre for Clinical Research in Emergency Medicine, Western Australian Institute for Medical Research

Anaphylaxis is a systemic reaction affecting multiple organ systems characterised by vasodilation (generalized erythema), extravasation (angioedema, urticaria, airway oedema) and smooth muscle contraction (bronchospasm, cramping abdominal and pelvic pains). In severe reactions these processes cause severe upper and/or lower airway obstruction, hypoxaemia and/or hypotension due to mixed hypovolaemic-distributive (and possibly also cardiogenic) shock. Anaphylaxis may be allergic (requiring prior sensitization and initiation of a specific immune response) or non-allergic. Allergic causes may be generally classified as IgE mediated or non-IgE mediated. Anaphylaxis in childhood is caused most often by food, with bronchospasm being more common, and there is usually a background of atopy and asthma. Poorly controlled asthma is the main risk factor for death in this age group. Venom and drug-induced anaphylaxis is more common in adults, in whom hypotension is more likely to occur. Age >35 and previous severe reactions are the main risk factors for hypotension and death.

Although many episodes are easy to diagnose by the combination of characteristic skin features with other organ effects, this is not always the case and a workable clinical definition of anaphylaxis and useful biomarkers of the condition have been elusive. Diagnosis can be difficult, with skin features being absent in up to 20%. Anaphylaxis must be considered as a differential for any acute onset respiratory distress, bronchospasm, hypotension or cardiac arrest. At post-mortem, diagnostic features are present in only 50% of those diagnosed clinically as anaphylaxis. Serial measurement of mast cell tryptase is more sensitive and specific than a single measurement. Other mediator assays are available in research settings.

The cornerstones of initial management are the supine position, intramuscular adrenaline into the lateral thigh (0.01 mg/kg up to 0.5 mg), intravenous fluid resuscitation, support of the airway and ventilation, and supplementary oxygen. If response is inadequate, the next step is an intravenous infusion of adrenaline. For severe &/or refractory bronchospasm, additional bronchodilator treatment with continuous salbutamol and corticosteroids are used. Nebulised adrenaline may be a useful adjunct for upper airway obstruction. For hypotension that is refractory to treatment consider more aggressive volume resuscitation, selective vasopressors, atropine (for bradycardia), inotropes that bypass the beta adrenoreceptor, and bedside echocardiographic assessment. Observe for at least 4 hours after the resolution of all symptoms (longer/overnight for severe reactions with hypotension and/or hypoxia). Refer to an allergist to assist with diagnosis as to the most likely trigger, allergen avoidance measures, risk assessment, preparation of an action plan and education on the use of self-injectable adrenaline.

Management guidelines continue to be opinion- and consensus-based, with retrospective studies accounting for the vast majority of clinical research papers on the topic. The clinical spectrum of anaphylaxis including major disease subgroups requires clarification, and validated scoring systems and outcome measures are needed to enable good quality prospective observational studies and randomised controlled trials.

Meeting Room 4
ANZSBT: Transfusion Risks

1600-1730
1625

Transfusion-related Anaphylaxis: Investigations and Management

Krishna G Badami

New Zealand Blood Service, Christchurch, New Zealand

Anaphylaxis is an IgE-mediated, severe, systemic, allergic reaction. Features include hives, facial swelling, stridor, wheeze, breathlessness, hypotension, tachycardia, nausea, abdominal cramps & diarrhoea & severe anxiety.

Following transfusion, localised, minor reactions (e.g. urticaria or wheeze) are common but anaphylactic reactions are rare (1:20000-40000 transfusions). Anaphylactic, and possibly also milder, reactions are ultimately caused by mast cell degranulation. This can result from IgE cross-linked by a re-encountered foreign plasma protein antigen on the mast cell surface as well as directly acting agents such as complement intermediates (C3a, C5a), leukotrienes and opioids.

Transfusion-related allergic reactions are more common with predominantly plasma containing components such as FFP and platelets but the specific trigger mostly remains undetermined. Exposure to IgA in IgA-deficient patients with IgE anti-IgA is a well-recognised, though infrequently-found cause. Other plasma proteins (e.g. haptoglobin in Japanese) may be responsible. In addition, non-transfusion-related drug & food allergens and the passive transfer of donor-derived IgE should be considered.

Clinical features overlap those occurring with other serious transfusion-related adverse events such as TRALI, TACO, HTR & bacterial sepsis but there are differentiating features. The diagnosis of anaphylaxis is essentially clinical. IgA (remote from transfusion), anti-IgA and paired serum mast cell tryptase measurements may be helpful. The predictive value of IgA and anti-IgA testing in the absence of previous reactions is low and while the specificity of raised mast cell tryptase is high, levels may be normal in anaphylaxis.

In addition to stopping the transfusion, prompt treatment with antihistamines, iv fluids, oxygen, bronchodilators, adrenaline, and steroids is required. To prevent future reactions, pharmacological & other non-transfusion alternatives, premedication, autologous donations, components from IgA-deficient donors or washed cellular components and low-IgA products should be considered.

Meeting Room 4
ANZSBT: Transfusion Risks

1600-1730
1700

Transfusion Transmissible Viral Infections

CR Seed

Australian Red Cross Blood Service

The Australian Red Cross Blood Service collects over one million homologous blood donations every year from voluntary non-remunerated blood donors. Each donation carries the potential to transmit a variety of blood borne viruses including Hepatitis B & C, human immunodeficiency virus (HIV), human T lymphotropic virus (HTLV), Human Cytomegalovirus (CMV) and dengue virus (DENV). The so called 'safety tripod' consisting of careful donor selection, state-of-the-art testing and pathogen inactivation minimises, but does not entirely eliminate the risk of transfusion transmission. However, the level of risk reduction achieved for the principal viruses over the past three decades has been remarkable. For example the risk of Hepatitis C, which was assessed as 1-2% in Australia in the late 1980's is currently estimated to be in excess of four orders of magnitude lower at approximately 1 in 3 million. This risk reduction has been principally driven by the initial implementation and subsequent improvement in viral screening tests, the most recent of which was the addition in 2000 of HIV and HCV RNA to the existing antibody based testing.

Despite this success there remains an ongoing threat from established, as well as emerging viruses particularly where an appropriate screening test is unavailable. An example is the threat of DENV which now appears to be transfusion transmissible and although not endemic in Australia, causes regular outbreaks in Northern Queensland. In the absence of a suitable high throughput screening test the risk to the blood supply is currently mitigated by additional donor selection measures implemented at the commencement of an outbreak. Donors who reside in, or have visited the affected area are identified by additional 'supplementary' donor questions. Those identified are temporarily restricted from donating fresh blood components although plasma for further fractionation which is subject to dedicated viral inactivation procedures shown to adequately inactivate DENV, is permissible. Although this strategy is effective in managing the risk, it results in a loss of valuable blood components.

One future option to avoid such losses in the absence of a test is the application of physicochemical pathogen reduction techniques for cellular components which offer, through chemical means the ability to inactivate a range of viral agents. Despite the potential advantages of such a 'catch all' solution there remain several hurdles to implementation including; the lack of a single method applicable to all components, lack of universality for all infectious agents, concern over toxicity of residual chemicals and a perception of a lack of cost effectiveness.

Ultimately, protecting the blood supply from transfusion transmissible viruses requires continuous vigilance and the war is far from over!

Meeting Room 2/3
ASTH: How Do I Treat?

1600-1730
1600

Antiphospholipid Antibody Syndrome

Chris Ward

Northern Blood Research Centre, University of Sydney; Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, St Leonards, NSW Australia

Antiphospholipid antibodies (APL) are a heterogeneous and poorly-understood class of autoreactive antibodies that prolong clotting times *in vitro*, but are associated with an increased risk of arterial and venous thromboembolism *in vivo*. Advances in basic science have shown that the major ligand for APL is beta2-glycoprotein I (GPI), and that thrombogenic APL can induce cellular and procoagulant changes by binding to membrane-associated proteins such as GPI. A wide variety of laboratory methods can be used to test for APL, but their correlation with clinical parameters, such as the risk of a first or recurrent vascular event, remains poor. The updated criteria for the APL syndrome include arterial, venous and microvascular thromboembolism, or obstetric events in the presence of a persistent APL. This diagnosis can only be made retrospectively, and there are marked differences between the cohorts in the literature with primary autoimmune disorders and those who present first with thromboembolism. APL are common in the general population, often transient in response to infection, and most of these individuals will not develop a clinical problem.

Effective anticoagulation is a mainstay of therapy, and recent studies have shown that an increased intensity of warfarin therapy (INR >3.0) is not necessary in the majority of patients. The presence of a persistent lupus anticoagulant may be an indication for prolonged or indefinite warfarin after a first venous thromboembolism. Longerterm LMWH therapy may be used in patients who develop thrombosis on warfarin, and there may be a role for antiplatelet therapy in selected cases. Catastrophic APL syndrome is a rare situation where maximal anticoagulation must be combined with strong immunosuppression, to remove the pathogenic antibody. Patients with this syndrome of multiorgan failure have a high mortality despite optimal therapy. The importance of antiphospholipid antibodies in pregnancy is still uncertain – although there is an association with miscarriage and other adverse pregnancy outcomes, there are only limited studies to guide antenatal therapies. APL remain a challenge for pathologists and clinicians alike.

Meeting Room 2/3
ASTH: How Do I Treat?

1600-1730
1630

PE in Pregnancy

Claire McLintock
Auckland City Hospital, Auckland, New Zealand

1630

Abstract not available at time of going to print

Meeting Room 2/3
ASTH: How Do I Treat?

1600-1730
1700

Venous Thrombosis in Unusual Sites

Harry Gibbs

Dept of Vascular Medicine, Princess Alexandra Hospital QLD

More than 90% of all cases of deep vein thrombosis (DVT) occur in the lower extremities. Deep vein thrombosis does however occur in other sites. DVT of the upper extremity may occur spontaneously or with indwelling vascular devices, i.e. catheters or pacemakers. The majority of spontaneous upper extremity DVT occurs due to compression of the subclavian vein at the thoracic. Upper extremity DVT is associated with pulmonary embolism in about 30% of cases, although this is less commonly fatal than with lower extremity DVT. For this reason, however, it is usual practice to use anticoagulant therapy for three months. Subsequent annual recurrence rates of about 3% are lower than for lower extremity DVT. The post-thrombotic syndrome occurs less frequently with upper extremity DVT than with lower extremity DVT and patients can be reassured that post-thrombotic of a severity that interfere with quality of life are very rare. In spite of this, it has been suggested that more aggressive approaches to management are required including catheter-directed thrombolysis, which is usually followed by first rib resection. This approach confers considerable morbidity due to a significant increase in the risk of bleeding and the potential for nerve or pulmonary complications from surgery. This aggressive approach to upper extremity DVT should not be regarded as standard therapy and should be further evaluated in appropriate clinical trials. Renal vein thrombosis occurs in patients with the nephrotic syndrome and may lead to deterioration in renal function. Renal function often subsequently improves. Anticoagulant therapy is indicated in this situation. Renal vein thrombosis may also occur in the presence of a renal cell carcinoma where there is endovascular spread. In this situation a combination of tumour and thrombus may extend into the inferior vena cava and from there to the right atrium. This should be regarded as tumour rather than thrombus and treated accordingly. Mesenteric venous thrombosis has a wide spectrum of clinical presentation from asymptomatic to rapidly fatal extensive bowel infarction. Mesenteric venous thrombosis may be spontaneous or secondary to intra-abdominal abnormality such as sepsis, surgery or neoplasia. Spontaneous mesenteric venous thrombosis is commonly associated with thrombophilic states and in particular with myeloproliferative disorders. The reason for the association between myeloproliferative disorders and mesenteric venous thrombosis is poorly understood. Anticoagulant therapy is indicated for mesenteric venous thrombosis. Cerebral sinus thrombosis usually presents as headache and may be associated with cerebral infarction. There is a high incidence of haemorrhagic transformation of cerebral infarction due to cerebral sinus thrombosis but anticoagulant therapy should nonetheless be administered. The prothrombin gene mutation is particularly associated with cerebral sinus thrombosis and the risk greatest in women receiving the combined contraceptive pill, who have the prothrombin gene mutation.

Summary

Deep vein thromboses in unusual sites are generally treated in the same fashion as for lower extremity DVT. Aetiological differences, however, are often present and differ according to the site involved.

Meeting Room 1
Nurses: Non-malignant Haematology

1600-1730
1600

Iron Deficiency Anaemia (IDA) – A Neglected Diagnosis and Common Reason for Transfusion (and Over-transfusion) in Stable Patients

Kathryn Robinson^{1,2}, Magdalena Sobieraj-Teague², Nicola Hurst^{1,2}, Russell Hunt¹, Barbara Parker¹, Beverleigh Qusted^{1,2}, Trish Roberts¹, Tracey Roffey¹

1. *BloodSafe Program, Adelaide, South Australia*

2. *Australian Red Cross Blood Service, Adelaide, South Australia*

Aim

To examine the frequency of IDA in transfused patients.

Method

Retrospective audits were conducted on consecutive red cell transfusions in 6 teaching hospitals in Adelaide in May and October 2006. Data was entered into AuditmakerTM software. Iron deficiency was defined as definite if the ferritin level was <20 µg/L. If iron studies were not performed or showed a ferritin <100 µg/L and transferrin saturation <20%, then CBE and film comments were reviewed independently by 2 haematologists to determine if IDA was likely.

Results

232 transfused adult patients were reviewed (47% female, mean age 70 years, range 19-98). In 11/221 (5%) of transfusion episodes the patient had definite IDA and in 43/221 (19%) the patient was assessed as having likely IDA, with an overall rate of 24%. IDA was more frequent in transfused obstetric/gynaecology patients (5/12 -42%) than medical (29/124 -23%) and surgical patients (20/85 -24%). 191/232 (82%) patients were stable. The frequency of IDA was higher in stable (50/191 -26%) than unstable patients (4/41 -10%). Post transfusion Hb was > 100 g/L in 73% and > 110 g/L in 33% of episodes. Only 13% of iron deficient patients received a single unit transfusion.

Conclusions

Barriers to best practice need attention at a national level to ensure that iron (oral and intravenous) become the cornerstone of therapy to optimise patient outcomes, reduce inappropriate use of red cells and the associated economic costs.

No conflict of interest to disclose.

Meeting Room 1
Nurses: Non-malignant Haematology

1600-1730
1630

A Practical Approach to Haemophilia

Claire Bell

Haemophilia Centre of WA, Royal Perth Hospital, Perth, WA

The aim of the haemophilia treatment centre (HTC) nurse is to provide comprehensive care for patients with bleeding disorders. The HTC nurse is usually the first point of contact for patients with bleeding disorders. Our role is multi-dimensional and involves assisting in attaining a correct diagnosis, counselling and support, acute and peri-operative management of the bleeding disorder, assisting patients with travel arrangements and ensuring patients have an emergency treatment plan. HTC nurses are also responsible for ordering plasma/recombinant factor supplies and data entry. Education is also an important role, both staff education and community education. It is essential that HTC nurses work with the haemophilia community to ensure that the service provided meets their needs.

At the Royal Perth Hospital HTC we are constantly trying to improve the service provided. An example is our recently commenced nurse run haemophilia carrier clinic, which aims to encourage more haemophilia carriers to attend the HTC. This is the beginning of nurse run clinics at the Royal Perth Hospital HTC which are the result of the HTC nurse changing from a clinical nurse consultant to a nurse practitioner position.

Meeting Room 1
Nurses: Non-malignant Haematology

1600-1730
1650

A Practical Approach to Thrombosis

Karen Flounders

Royal Perth Hospital Perth WA Australia

The Thrombosis Unit, at Royal Perth Hospital, provides a seamless service for acute Venousthromboembolism (VTE) from initial presentation to long term management. The Unit provides assessment for the long-term management of thrombophilia, recurrent VTE and post thrombotic syndrome. The Thrombosis Unit also provides a unique service in Perioperative Ambulatory Anticoagulation. This service responds to referrals from a variety of multi-disciplinary teams to ensure the safe provision of health care in patients already taking anticoagulants who are undergoing a wide variety of surgical procedures. All patients presenting to the Emergency Department with VTE are seen in the Thrombosis Unit where their care is planned and coordinated usually without the need for an overnight stay in hospital. The Thrombosis Unit also takes part in clinical trials for new emerging anticoagulants. The role of the Clinical Nurse Consultant is to coordinate the acute and long term care of all patients with VTE so that they receive the best possible care available to us at the time. With the Clinical Nurse Consultant Role being developed into a Nurse Practitioner role it is hoped that the service provided will be even more seamless with the introduction of Nurse Prescribing and Nurse Led Clinics.

Meeting Room 1
Nurses: Non-malignant Haematology

1600-1730
1710

Establishing a Haemophilia Nursing Program

Jill Smith¹, Anne Gillham², Anne-Louise Cruickshank², Mirriam Mokwena²

¹ *Haemophilia Centre of Western Australia, Perth*

² *Haemophilia Nurses' Committee, South Africa*

Haemophilia is an inherited bleeding disorder with no cure. In haemophilia A clotting factor VIII is missing and in haemophilia B clotting factor IX is missing.. The disease is characterised in its severest form by spontaneous bleeding into joints, muscles and organs.

Bleeding after trauma or surgery is catastrophic and frequently fatal unless treated. Globally, 75 % of people with haemophilia have no access to treatment and die young or become severely crippled. Treatment is to replace the missing clotting factor.

In South Africa, there is sufficient replacement clotting factor but this treatment is not readily accessible to all. The Department of Health recognised that all people with haemophilia should receive treatment regardless of where they live and that to achieve this, training of registered nurses in haemophilia care needed to be prioritised.

In 2002, the first haemophilia nurses' training course was undertaken and has been run annually since. The course is run over five days and includes lectures and practical demonstrations. Course content includes overview of haemophilia and diagnosis, treatment, von Willebrand's Disorder, women and bleeding disorders and product safety. The participants meet people with haemophilia so they can collect information to use as a case history, which is presented to the rest of the group. An examination completes the course.

The course has been shown to be successful by evaluations from the participants, people with haemophilia and their families. The number of haemophilia treatment centres has increased from 4 to 8 and the number of haemophilia clinics across the nation has increased. The haemophilia database shows an increase in numbers of people with haemophilia registered, reflecting the fact that more people have access to haemophilia treatment centres. The South African Haemophilia Medical Advisory Committee strongly supports the continuation of the course.

NOTES

Meeting Room 6
HSANZ Free Communications 1
O01

0830-1000
0830

CD300f Triggering Modifies Myeloid Cell Function

Courtney Modra, **Derek Hart** and Georgina Clark
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CD300f, a member of the CD300 family of immunoregulatory molecules, is capable of signalling through association with both SHP-1 phosphatase and the p85 α subunit of phosphoinositide 3-kinase. On normal cells, CD300f is expressed on blood and bone marrow monocytes. CD300f is expressed on myeloid derived cell lines and Acute Myeloid Leukaemias (AMLs) and is an acknowledged candidate for antibody targeting of AML (Modra CJ et al. Blood 2006;108:225B-225B and Zhao X et al Blood 2007;110:531a-532a). We have generated a monoclonal antibody, MMRI-23, specific for the extracellular domain of CD300f. MMRI-23 immunoprecipitates a 57kd protein from the myeloid derived cell lines HEL, THP-1 and U937 and this protein binds to a polyclonal antibody to CD300f (LMIR3) in Western blot analysis. Binding of MMRI-23 to myeloid cells was blocked by the CD300f recombinant proteins or the polyclonal CD300f antibody. We studied the functional consequences of signalling induced by MMRI-23 crosslinking of normal monocytes and myeloid cell lines. MMRI-23 binding was not altered by activation of peripheral blood monocytes but crosslinking monocytes with MMRI-23 induced downregulation of CD14 and CD33. There was significant inhibition of IL-6 but not IL-1 β , IL-8 or TNF α secretion. As SHP-1 signaling is involved in cellular responses to chemokines, we tested the effect of crosslinking monocytes with MMRI-23 on chemotaxis. Crosslinking monocytes with MMRI-23 increased specific chemotaxis towards CCL12 (SDF-1). To understand the mechanism of this increased specificity we investigated the role of CD300f signalling on CXCR4 function. The effect of crosslinking did not induced CXCR4 upregulation but did induce localization of CD300f and CXCR4 to the lipid rafts in myeloid cell line, U937. Thus CD300f plays a significant role in the regulation of monocyte migration to CXCR4. As CD300f is upregulated on around 70% of AMLs, this regulation of homing has major implications for the treatment of AML.

No conflict of interest.

Meeting Room 6
HSANZ Free Communications 1
O02

0830-1000
0845

Comparison of In Vivo Migration Patterns of Different DC Preparations from a Single Donor Using a Novel PET Agent and Flow Cytometry on Excised Lymph Nodes

Maureen Loudovaris¹; Mick Thompson^{1,2}; Dominic Wall¹; Andreas Mackensen³; Jacques Bartholeyns⁴; Maria Ferrantini⁵; Laura Santodonato⁵; Thomas Felzmann⁶; Rodney Hicks²; Miles Prince¹; **Simon Harrison**¹

¹ Centre for Blood Cell Therapy, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia. ² Centre for Molecular Imaging, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia. ³ Haematology & Oncology, University Clinical Hospital of Erlangen, Erlangen. ⁴ idm Pharma S.A., Paris, France. ⁵ Istituto Superior di Sanita, Department of Virology, Roma, Italy. ⁶ Laboratory of Tumor Immunology, Children Cancer Research Institute, St. Anna Children's Hospital, Vienna, Austria

Aim

The objective of this study was to compare the in vivo distribution of 5 preparations of dendritic cells (DC) from a single donor after intradermal (ID) administration by PET and correlate with FACS analysis of excised lymph nodes.

Method

DC were generated from a single healthy donor apheresis product. The DC types were GM-CSF+IL-13 (IL-13 imDC), matured with FMKp+IFN γ (IL-13mDC), GM-CSF+IL-4 (IL-4 imDC) and matured (IL-4 mDC) and GM-CSF+IFN- α (IFN- α DC). DC were labelled with Yttrium-86 oxine (prepared on site). 5×10^5 cells in 50 μ L were injected into the footpad and ID into the volar aspect of the foreleg of Balb/c nude mice (n=5 per group). Images were acquired on a small animal PET (SAPET) (Mosaic, Philips Medical Systems) at 2, 24 and 45 hours post administration. Subjects were then euthanized and popliteal, axillary and inguinal (control) lymph nodes beds were removed. LN were processed into single cell suspensions, stained with anti-human HLA-DR and CD11c and analysed by flow cytometry. SAPET images were graded: 0 – no signal, 1 – marginal, 2 – definite nodal signal, 3 – strong signal. DC detection by FACS were graded: 0 – no detection, 1 – <1% DC, 2 – 1-2% DC, 3 – >2% DC

Results

Mean DC radiolabelling efficiency was 72% (54% - 88%). Using PET, DC were detected in 23/25 and 24/25 axillary and popliteal nodes, respectively. Flow analysis detected DC in 12/21 and 17/22 axillary and popliteal nodes, respectively. The mean grade score by PET and FACS were 1.68 ± 0.36 and 0.8 ± 0.32 for the axillary node, while the popliteal nodes resulted in 2.25 ± 0.6 and 1.2 ± 0.7 . Detection and scores associated with the different DC preparation and correlation of the two detection methods will be presented

Conclusion

This novel PET agent provides a robust, reproducible method for quantification of *in vivo* functional DC tracking to LN.

No conflict of interest to disclose

Meeting Room 6
HSANZ Free Communications 1
O03

0830-1000
0900

Depletion of Host CD122⁺ Cells Facilitates Widespread Skeletal Multiple Myeloma Engraftment in NOD/SCID Recipients

Lisa M Freeman¹, Alfred Lam², Eugene Petcu², Robert Smith², Ali Salajegheh², Peter Diamond³, Andrew Zannettino³, Andreas Evdokiou⁴, John Luff⁵, Dalia Khalil¹, Frank Vari¹, Laurence Catley¹, **Derek NJ Hart**¹, Slavica Vuckovic¹

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³ *Bone and Cancer Research Laboratories, Institute for Medical and Veterinary Science and the Hanson Institute, Adelaide, South Australia 5000, Australia.*

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⁵ *The Queensland Institute of Medical Research, Royal Brisbane Hospital, Herston, Queensland 4029, Australia.*

To facilitate human multiple myeloma (MM) engraftment into NOD/SCID recipients, mice were depleted of CD122⁺ cells (NK and myeloid cells) by antibody-mediated ablation prior to transplantation with the MM cell lines (RPMI8226, RPMI8226-TGL or U226). The MM engraftment, skeletal MM distribution, osteolysis, lambda chain paraprotein and associating disease symptoms in CD122⁺ cell-depleted and CD122⁺ cell-replete mice were compared. The CD122⁺ cell-depleted mice engrafted at a significantly higher frequency with human CD38⁺, CD56⁺, PCA-1⁺ and CD138⁺ cells. In the CD122⁺ cell-depleted mice, bioluminescence MM signal involved the whole mouse compared to limited imaging signal in the CD122⁺ cell-replete mice. The majority 88%-100% of CD122⁺ cell-depleted mice developed MM engraftment throughout the appendicular and axial skeletons with osteolysis and rare subcutaneous plasmacytomas (11% of mice). Serum paraprotein appeared earlier at 4-5 weeks post-transplant in CD122⁺ cell-depleted mice and continued to increase during the 12-13 weeks of analysis. The majority, 92% of CD122⁺ cell-depleted mice developed hind-limb paralysis and had a significantly shortened 45 day survival. Thus, depletion of CD122⁺ cells reduced resistance to the human MM and produced a new MM xenograft-NOD/SCID model that recapitulates the clinical manifestations of MM and eliminates the major limitations associated with the published MM xenograft-mouse model.

No conflict of interest

Meeting Room 6
HSANZ Free Communications 1
O04

0830-1000
0915

BMP-2 Enhances TGF- β 3 - Mediated Chondrogenic Differentiation of Human Bone Marrow Multipotent Mesenchymal Stromal Cells (BM MSCs) *in vitro*

Bojiang Shen¹, Aiqun Wei², Helen Tao¹, Ashish D Diwan² and David DF Ma¹

¹ Blood, Stem Cell and Cancer Research unit, St Vincent's Hospital Applied Biomedical Research, ²Orthopaedic Research Institute and Department of Orthopaedic Surgery, St George Hospital, ^{1,2}University of New South Wales, Sydney, Australia

Aims

BM MSCs are capable of differentiating into various mesodermal cell lineages. Transforming growth factor- β (TGF- β) has been shown to promote chondrocytic differentiation. Since nucleus pulposus (NP) cells of the intervertebral disc are chondrocyte-like cells, we reasoned that same principles of chondrogenic differentiation of MSCs applies to generating NP-like cells for the potential use in IVD repair. In recent years, BMPs have emerged as key regulators of stem cell commitment and BMP-2 plays an essential role in chondrocyte differentiation. We hypothesised that a combination of BMP-2 with TGF- β 3 in 3D culture is more effective than TGF- β 3 to induce NP chondrocytic differentiation of BM MSCs.

Methods

MSCs were isolated from BM specimens of haematologically normal donors following informed consent. *Ex vivo* expanded MSCs encapsulated in alginate beads were induced to differentiate in serum-free medium in the presence of BMP-2 and TGF- β 3. The expression of chondrocytic genes and proteins was analysed by real-time PCR, Western blot, histological and immunohistochemical assays.

Results

(1) The expression of chondrocytic markers, particularly aggrecan (ACAN) and type II collagen (COL2A1) was up-regulated at higher levels than TGF- β 3 alone. (2) Receptor-regulated Smad 8 was up-regulated and Smad 3 was down-regulated in response to TGF- β 3 BMP-2. Blocking BMP-2 by noggin completely suppressed BMP-2 enhanced chondrocytic gene and protein expression. (3) Inhibition of ERK1/2 signaling resulted in an increase in ACAN and COL2A1 gene expression, suggesting a negative regulatory role of this pathway.

Conclusion

BMP-2 enhances TGF- β 3 – mediated chondrogenesis of MSCs. The combination of BMP-2 and TGF- β 3 in alginate 3 D culture is superior to the differentiation method using TGF- β alone and confirming a crucial role of BMP-2-Smad signaling pathway in this process.

No conflict of interest to disclose

Meeting Room 6
HSANZ Free Communications 1
O05

0830-1000
0930

Interaction of Human Allogeneic Mesenchymal Stromal Cells with Normal B Cells and B Cells from Patients with Autoimmune Disorders

Lisa Kaminskis, Kath Shaw, Anna Cook¹, Marian Sturm, Richard Herrmann
Cell and Tissue Therapies WA, Royal Perth Hospital & ¹Curtin University of Technology, Perth, WA, Australia

Aims

Human mesenchymal stromal cells (MSC), derived from adult bone marrow, have immunosuppressive and immunoregulatory capabilities, as has been demonstrated by their ability to inhibit the function and growth of cells modulating the immune system both *in vitro* and *in vivo*. This has led, in the case of T cells, to their use as a cell therapy in graft versus host disease and Crohn's disease. Recent reports indicate that MSC have a similar effect on human B cells. This preliminary study examined the effect of MSC on normal B cells and on B cells from patients with two B cell driven autoimmune diseases, systemic lupus erythematosus (SLE) and immune thrombocytopenic purpura (ITP).

Methods

B cells were co-cultured with MSC and their effect on B cell proliferation, cell cycle and migration was examined. B cells were isolated from peripheral blood mononuclear cells of 11 healthy donors and 7 patients with autoimmune disease (3 SLE, 4 ITP), by immunomagnetic negative cell selection. MSC were obtained from healthy donor bone marrow by density gradient centrifugation, followed by plastic adhesion and culture expansion. MSC were characterised according to ISCT guidelines, including phenotype (CD73⁺, CD90⁺, CD105⁺, CD45⁻, CD34⁻, CD14⁻) and differentiation capability (adipogenic, chondrogenic, osteogenic).

Results

For healthy B cells following co-culture with MSC, both cell proliferation (n=11) and cell cycle progression (n=4) was significantly inhibited ($p<0.05$) and migration of B cells in response to chemokines (CXCL12, CXCL13) was also depressed (n=3). MSC appeared to inhibit proliferation of B cells from patients with autoimmune disease (n=7), but, due to the difficulty in standardising patient samples given their clinical complexity, the results did not achieve statistical significance.

Conclusions

These preliminary results suggest that MSC may have a role in inhibiting the proliferation of B cells driving some autoimmune disorders. Continuing recruitment of patients for this study is warranted.

No conflict of interest to disclose

Meeting Room 6
HSANZ Free Communications 1
O06

0830-1000
0945

Transcriptional Silencing of the Suppressor of Cytokine Signalling-1 (SOCS-1) by Aberrant Methylation of Exon 2 in Acute Myeloid Leukaemia and Human Haematopoietic Cell Lines

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²*Academic Unit of Haematology, School of Medicine, University of Sheffield, United Kingdom*

³*Royal Hallamshire Hospital, Sheffield, South Yorkshire, United Kingdom*

Aim

Hypermethylation of the suppressor of cytokine signalling-1 (SOCS-1) gene has been reported to cause inactivation in leukaemogenesis. However, there is an issue in the use of different locations of SOCS-1 gene being amplified that gave discrepant methylation results in acute myeloid leukaemia (AML). The aim of the study was to determine the correct region of SOCS-1 for methylation analysis.

Method

47 cases of AML entered into the Medical Research Council (MRC) AML X and XII trials (United Kingdom) were studied together with four haematopoietic cell lines. SOCS-1 methylation status was analysed at both different locations: five prime untranslated region (5'UTR) and exon 2. Methylation-specific PCR (MS-PCR) was employed to determine if aberrant methylation of either region of SOCS-1 is involved in the pathogenesis of AML. Demethylation using azacytidine and quantitative reverse transcription PCR (RQ-PCR) were used to analyse the relative expression of SOCS-1 in haematopoietic cell lines.

Result

SOCS-1 methylation was detected in 19/47 (40%) AML patients using primer pairs for exon 2 but none in 5'UTR. Ten patients showed complete methylation while the other nine exhibited partially methylation. 2/4 cell lines (ME-1 and Jurkat) showed complete methylation. Demethylation experiments showed that there was an increase in relative expression of SOCS-1 in methylated cell lines after the addition of azacytidine. RQ-PCR results showed that SOCS-1 exon 2 methylation is associated with the transcriptional silencing of SOCS-1 gene in haematopoietic cell lines.

Conclusion

In conclusion, aberrant methylation of exon 2 but not 5'UTR may lead to epigenetic silencing of SOCS-1 in AML. The findings contribute to the underlying pathogenesis and moreover for the possibility of targeted therapy for patients with AML.

No conflict of interest to disclose

Meeting Room 7
HSANZ Free Communications 2
O07

0830-1000
0830

Significant Variation in Absolute Lymphocyte Count (ALC) for the Diagnosis of Chronic Lymphocytic Leukaemia (CLL) with 2008 Guidelines

Christopher Mulligan, Miriam Thomas, **Stephen Mulligan**

Departments of Haematology and Flow Cytometry, Symbion Pathology, Sydney NSW Australia

Background

The diagnostic criteria for CLL have recently changed with the 2008 Guidelines (Hallek et al, Blood, June 15, 111:5446, 2008). Previous Guidelines recommended an ALC of $\geq 5.0 \times 10^9/L$ as the diagnostic threshold. In 2005, Marti et al. (BJHaem 130:325) proposed criteria for small B-cell clones and used the term 'Monoclonal B-Lymphocytosis' (MBL) but suggested a cut-off $< 5.0 \times 10^9/L$ B-lymphocytes (not lymphocytes, i.e. ALC). The converse of this criterion is the new definition for CLL.

Methods

We applied the new CLL Guidelines to patients presenting in our laboratory between 2000-2005 to assess the changed diagnostic criteria. A cohort of 322 patients had a typical CLL phenotype below the new threshold.

Results

Using 2008 Guidelines, there is significant variation in the ALC for a diagnosis of CLL. This ranges from $5.0 \times 10^9/L$ to over $10.5 \times 10^9/L$. The ALC is the sum of clonal B-CLL (CD19/CD5+) cells, residual normal B-cells (CD19+/CD5-), T-cells and NK-cells and all may vary between patients. Hence, changes in the ALC may be difficult to interpret unless flow cytometry is performed with the full blood count. 52% of patients now termed MBL are simply redefined from early CLL under previous criteria. In this patient cohort, using CD19 and CD20 to enumerate B-lymphocytes made a minimal difference in the definition of MBL versus CLL despite the often low expression of CD20 in CLL. Measurement Uncertainty is higher using a B-lymphocyte count as two measurands (ALC and percentage B-cells) are required. The new time requirement for the B-lymphocytosis of ≥ 3 months duration introduces some practical difficulties for diagnostic laboratories.

Conclusion

Significant variation in the ALC is seen using 2008 Guidelines for the diagnosis of CLL. More than half of those now termed MBL were previously diagnosed as CLL. The new B-lymphocyte definition for CLL may require change in reporting practice in some diagnostic laboratories.

No conflict of interest to disclose

Meeting Room 7
HSANZ Free Communications 2
O08

0830-1000
0845

Autoimmune Haemolytic Anaemia in Chronic Lymphocytic Leukaemia. Analysis of Patients Presenting in Sydney Northern Metropolitan Area

Ian Bilmon, Jane Freeman, Luke Coyle, Chris Arthur, **Stephen Mulligan**
Department of Haematology, Royal North Shore Hospital, Sydney, NSW, Australia

Aim

Autoimmune Haemolytic Anaemia (AIHA) is a well recognised complication of Chronic Lymphocytic Leukaemia (CLL). Recent data suggests that both AIHA and a positive Direct Antiglobulin Test (DAT) may be independent risk factors for disease progression. There is debate regarding risk of haemolysis with purine analogue therapy. We analysed the patient cohort presenting to our institution providing haematology services to the northern half of the Sydney metropolitan region.

Methods

We reviewed clinical records and pathology results of patients presenting with CLL to our institution recently with AIHA. 15 patients were identified from a current cohort of 365 patients with CLL (4.1%). These cases are summarised with respect to demographics, CLL clinical stage, treatment, and complications.

Results

There were 15 patients, with a median age of 71 (range 53-86) years, with 8 males and 7 females. The clinical stage of these patients was Binet Stage A - 6, Stage B - 3, and Binet stage C - 6. All had a positive DAT. The CLL was previously treated in 6 patients prior to the onset of AIHA. All were treated for their AIHA with steroids, 4 patients required second line treatment in addition for control of the AIHA including splenectomy, intravenous gammaglobulin, cyclosporin and rituximab. 9 patients (60%) required therapy for progression of their CLL. 3 patients are deceased, 1 due to haemolysis complicated by massive pulmonary embolus, 2 due to progressive CLL.

Conclusion

AIHA was identified in 4.1% of patients with CLL. All were treated with and most responded to steroids. Most presented with early stage disease, but 60% had subsequent progression of their CLL requiring therapy. Although AIHA is a well recognised complication of CLL, data in the literature maybe skewed by ascertainment bias to clinical trials and particular institutions. This series reflects patients with AIHA and CLL presenting to the principal haematology service for Sydney northern metropolitan area.

No conflict of interest to disclose

Meeting Room 7
HSANZ Free Communications 2
O09

0830-1000
0900

CLL MRD Flow Cytometric Testing Using the International Standardised Approach

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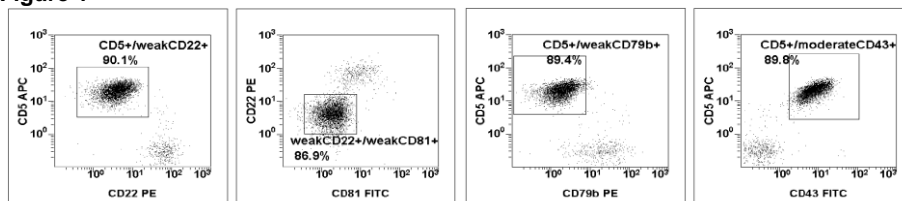
Aim

The emergence of potentially curative treatments for chronic lymphocytic leukaemia (CLL) has been accompanied by the development of molecular and flow cytometric (FC) techniques to detect minimal residual disease (MRD). FC has the advantages of wider availability, more rapid turnaround time and relative affordability. MRD testing by FC was recently standardised by an international consensus group (Rawstron et al). Our aim was to internally validate this method and incorporate for routine use in our diagnostic FC laboratory.

Method

Internal validation of a panel of surface markers based on the International Consensus method was performed including two important acquisition tubes: (1) CD81 FITC/CD22 PE/CD19 PerCP-Cy5.5/CD5 APC; and (2) CD43 FITC/CD79b PE/CD19 PerCPCy5.5/CD5 APC. Specificity and sensitivity were assessed using untreated CLL and normal healthy donor samples. The presence of CLL cells (Fig.1) is based on the differential staining patterns compared to normal B cells.

Figure 1



Results

Using this gating strategy, 100% specificity was demonstrated, - all five untreated CLL samples demonstrated >100 events in 4/4 boxes; all 10 normal donor samples demonstrated < 5 events in 4/4 boxes. Sensitivity was linear and demonstrated down to 0.01% (on 500,000 events). Additionally, 30 routine PB & BMA samples of untreated CLL patients were prospectively tested using the CLL MRD panel - 27 samples (PB n=20/22, BM n=7/8) demonstrated a typical CLL phenotype; 3 demonstrated an atypical phenotype. Nine patients treated with fludarabine-based regimens have undergone regular CLL MRD testing to date. Two patients initially demonstrating CLL MRD negativity by flow have subsequently demonstrated MRD positivity at low levels.

Conclusion

The International standardised approach for flow cytometric MRD monitoring in CLL is a rapid and sensitive method for determining MRD status in PB and BMA. Given occasional antigenic aberrancy a baseline MRD panel should be performed pre-treatment to improve reliability.

No conflict of interest to disclose

Meeting Room 7
HSANZ Free Communications 2
O10

0830-1000
0915

Susceptibility to Chronic Lymphocytic Leukaemia in a Large Multi-generational Family

Stephen Fuller¹, Elli Papaemmanuil², Leah McKinnon¹, Emily Webb², Gabrielle Sellick², Lan Phuong Dao-Ung¹, Kristen Skarratt¹, Dale-Mari Crowther², Richard Houlston², James Wiley¹

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2. *Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, SM2 5NG, UK.*

Aims

Epidemiological and case reports of families have provided evidence that a subset of chronic lymphocytic leukaemia (CLL) is a consequence of an inherited genetic predisposition. These families can be powerful means for identifying disease-causing genes through genetic linkage-based analyses. The aim of this study was to search for predisposition genes in the largest reported family to date with 11 members from three generations affected by CLL. Unaffected family members were studied for the presence of a monoclonal B-cell lymphocytosis (MBL) population. Concordance for *IgVH* usage was analysed in MBL and CLL subjects.

Methods

A genome-wide linkage search was undertaken using the Genechip Mapping 10K 2.0 Xba Array containing ~10,200 SNP markers (Affymetrix Inc., Santa Clara CA, USA). Parametric and non-parametric multipoint linkage analyses were performed using the GENEHUNTER and ALLEGRO programs. Peripheral blood was collected for B-cell immunophenotyping by 4-color flow cytometry. *IgVH* gene usage and mutation analysis was conducted using ABI3730xl sequencers in conjunction with Genescan and Chromas software (Applied Biosystems, Foster City, USA).

Results

We found maximal linkage to loci at 14q24 and 14q31 (non-parametric linkage statistic 2.24, $P=0.03$). Near to 14q31 are two oncogenes *TCL1A* and *TCL1B* and at 14q24 is the tumour suppressor *ZFP36L1*. Five of 44 unaffected family members had MBL, significantly higher than expected based on a population prevalence of 2.5% ($p=0.005$).

Conclusions

This large family segregating CLL supports the role of inherited predisposition. Linkage analysis has not revealed a strong candidate locus however suggestive linkage to 14q24 and 14q31 has identified genes that may harbour disease-causing variations. A significant over-representation of MBL in unaffected members supports MBL being a marker of carrier status. In CLL and MBL cases there was no correlation in *IgVH* usage that argues against exposure to a single super-antigen in disease development.

No conflict of interest to disclose

Meeting Room 7
HSANZ Free Communications 2
O11

0830-1000
0930

CCL2 and CXCL2 Enhance Long-term Survival of Primary Chronic Lymphocytic Leukaemia Cells *in vitro*

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Aim

To investigate the *in vitro* culture conditions that would allow the long-term culture of primary CLL cells.

Method

Blood and/or bone marrow was collected, after informed consent, from patients with treated and untreated CLL. Diagnosis of CLL was made according to NCI criteria. Purified mononuclear cells were cultured at various cell density in RPMI 1640 medium, supplemented with 10% heat inactivated FCS. Human cytokine array was performed on supernatant of CLL cells which had been cultured in complete media for 7 days using the ChemiArray system (Human Cytokine Antibody Array III, Chemicon, Temecula, CA).

Results

By increasing the initial seeding cell density to high levels ($\geq 5 \times 10^7$ /ml) CLL cells can be cultured out to approximately 100 days without stromal cells from another source. Decreasing the seeding cell densities or using CD5⁺/19⁺ selected cells resulted in reduced cell survival. The surviving cells belonged to the malignant clone and were all CD5⁺/19⁺ and were mostly quiescent, with only 3.5% of the CLL B cells actively dividing. Two novel soluble factors, the chemokines CCL2 (MCP-1) and CXCL2 (GRO β) were identified in the culture media, which appear to enhance the survival of CLL B cells. Addition of these chemokines resulted in improved *in vitro* survival of CLL B cells, while blocking these growth factors with specific antibodies resulted in a loss of survival.

Conclusion

This study demonstrates for the first time the establishment of a long term culture system for CLL without additional stromal cell support. Furthermore, two novel cytokines appear to enhance survival of CLL cells *in vitro*. The long term culture model and these chemokines will allow us to gain insight into factors modulating B-CLL survival and may lead to targeted therapy as well as serve as an appropriate model to test new therapies.

No conflict of interest to disclose

Meeting Room 7
HSANZ Free Communications 2
O12

0830-1000
0945

Immunoglobulin G (IgG) Subclass Deficiency in Chronic Lymphocytic Leukaemia

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Aim

Hypogammaglobulinaemia is a common complication of Chronic Lymphocytic Leukaemia (CLL) with an incidence that varies considerably in reported literature. Immunoglobulin replacement therapy has documented benefit for patients with hypogammaglobulinaemia, CLL and recurrent episodes of infection. Very little data exists on IgG subclasses in CLL.

Methods

We measured IgG subclasses in a cohort of patients with CLL representing a variety of disease stages, treatment status and history of infection to analyse the implications of IgG subclass deficiency in the disease.

Results

There were 55 patients analysed with 32 males and 23 females with mean age 66.7 (range 21-89) years. Distribution by Binet Stage was A - 35, B - 17, C - 3. 14 patients had received chemotherapy. Low immunoglobulin levels were as follows: IgG <6.0g/L - 13; IgA <0.69g/L - 18; IgM <0.5g/L - 23 patients. IgG subclass deficiency were as follows: IgG1 <4.0g/L-16; IgG2 <1.3g/L-10; IgG3 <0.4g/L-22; IgG4 <0.06g/L-13, with a total of 33/55 patients (60%) having a deficiency of at least one IgG subclass. Recurrent infection was seen in 13 patients (23.6%) of whom 9 were previously treated and 4 had infection but no treatment (and 4 had treatment but no infection). Among the 13 patients with recurrent infection, 4 had total IgG ≤6.0g/L. Of 9 patients with infection and total IgG >6.0g/L, there was 1 IgG1 <3.0g/L, 1 IgG2 <1.2g/L, and 3 IgG3 <0.4g/L in 5 different patients (ie 5/9 [55%] had an IgG subclass deficiency with normal total IgG. There were 6 of 55 patients with a paraprotein, 3 IgG (all IgG1) and 3 IgM. 3 had polyclonal hypergammaglobulinaemia. There were 9 patients with a positive Coombs' test (DAT) including 4 of 6 with a paraprotein (3 IgG, 1 IgM) and 5 had low IgG and only 2 with normal total IgG.

Conclusion

Preliminary analysis shows that IgG subclass deficiency is common in a cohort of 55 patients with CLL, especially Stage B and C disease. IgG subclass deficiency (60%) is more common than low total IgG (23.6%) and the rate of infection. Abnormal immunoglobulin levels appear associated with a positive DAT. Paraproteins were seen in 11% of patients.

No conflict of interest to disclose

Riverside Theatre
HSANZ Free Communications 3
O13

0830-1000
0830

Acute Myeloid Leukaemia (AML) in Western Australia (WA) 1991-2005: A Retrospective Population-based Study of 830 Patients Regarding Epidemiology, Cytogenetics, Treatment and Outcome

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1: Royal Perth Hospital. 2: Sir Charles Gairdner Hospital, 3: Fremantle Hospital, 4: WA Cancer Registry

Aim

To collect epidemiological, prognostic, treatment and outcome data on all adults diagnosed with AML in WA between 1991 and 2005.

Methods

Patients were identified utilizing the Western Australia Cancer Registry, cytogenetic databases, and hospital inpatient discharge diagnoses. Data was retrospectively collected regarding age, karyotype at diagnosis, antecedent malignancy or haematologic disease, induction therapy, enrolment in clinical trials, transplantation and outcome. Survival was estimated using the Kaplan-Meier method and Breslow log-rank test used to compare curves. Cox proportional hazard model was used to analyse factors associated with overall survival. Pearson Chi-Square and Fisher's Exact Test were used for categorical comparisons.

Results

A total of 989 patients were diagnosed in the study period, of whom 830 had detailed information collected. In these patients the median age was 65 years. 92.4% attended three tertiary referral hospitals. 41% of cases represented secondary AML. 86% of cases had cytogenetic analysis. The most frequent karyotypes observed were normal (38.3%), complex with >5 anomalies (10.6%) and t(15;17) (8.1%). 62.7% of analyzed cases received intensive induction while 33.7% were palliated. Less than 15% of patients were enrolled in clinical trials. Overall 16.3% received an autologous or allogeneic transplant. The median overall survival was 5 months. In patients treated intensively the CR rate was 56.9% and median OS 12 months. Age, secondary disease and karyotype were significantly predictive of CR, remission duration and overall survival.

Conclusion

This is the first large population based study of AML in Australia. Age distribution, survival and remission rates are comparable to International population-based studies. The high median age of patients was reflected in the rate of secondary AML and trial eligibility. These findings highlight the need for prospective data collection.

This research was supported by the Leukaemia Foundation. The company had no role in analysing the data or preparing the abstract.

Riverside Theatre
HSANZ Free Communications 3
O14

0830-1000
0845

Acute Promyelocytic Leukaemia: A 10-year Experience in a Single Institution

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Aims

To analyze the haematologic profile and outcomes of patients treated for acute promyelocytic leukaemia (APL) in Singapore General Hospital (SGH) over a ten-year period.

Methods

Retrospective review of clinical data of all APL patients diagnosed and treated from March 1996 to December 2006.

Results

During the 10-year period, 48 patients were diagnosed with APL - 26 males and 22 females. Mean age was 41.3 years (range 16 to 67). The most common presentations were bleeding (25%) and fever (21%). Patients were categorized into 3 risk groups based on their presenting white cell and platelet counts – low (27%), intermediate (48%), high risk (25%). At diagnosis, 43 patients (90%) were positive for the PML-RAR α mutation. 47 patients had cytogenetic analysis, of which 8% were normal, 62% had a translocation between chromosomes 15 and 17 [t(15;17)] alone and 30% patients had t(15;17) plus other cytogenetic abnormalities. Treatment was based on our APL protocol of induction therapy with idarubicin and all-trans-retinoic acid (ATRA) followed by consolidation with idarubicin for 2 cycles and maintenance with 6-mercaptopurine, methotrexate and ATRA. 46 patients received induction therapy while 2 died before therapy could be instituted. The most common adverse events following induction were neutropaenia (54%) and bleeding (28%). At the end of induction therapy, 58% had achieved molecular remission. 41 patients received consolidation therapy and molecular remission was achieved in a further 30%. Maintenance therapy was given to 85% of patients. Six patients suffered a relapse, of whom 4 were of intermediate risk.

Conclusion

Using our APL treatment protocol, 88% of patients achieved molecular remission by the end of consolidation therapy. Six patients (13%) suffered relapse; 4/6 achieved a second complete remission with chemotherapy regimens that included arsenic trioxide, one patient died while another was lost to follow up.

No conflict of interest to disclose

Riverside Theatre
HSANZ Free Communications 3
O15

0830-1000
0900

Outcomes of Treatment of Adult Acute Lymphoblastic Leukemia with Hyper-CVAD in Brisbane

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Aim

Hyper-CVAD remains a commonly used induction chemotherapy for acute lymphoblastic leukemia (ALL) in Australia, with excellent reported outcomes and acceptable levels of toxicity. Questions remain regarding the use of allogeneic bone marrow transplantation and outcome for patients with advanced age. We aim to report our outcomes using this regimen with reference to previously published experience.

Method

Consecutive patients with newly diagnosed precursor B- or T-cell ALL treated with Hyper-CVAD at the Royal Brisbane and Womens Hospital and Princess Alexandra Hospitals from 1995 to 2007 were retrospectively identified. Allogeneic bone marrow transplantation was recommended for any patient deemed at high risk of relapse (eg Philadelphia chromosome positive) or if a matched sibling donor was available.

Results

Sixty four patients were identified from institutional databases with a median age of 30 years (range 14-76). The complete remission rate was 87%, with an early induction mortality rate of 5%. Median overall and disease free survival was 42 and 28 months respectively. Age (< 30) was most predictive of improved overall survival ($p=0.0041$). Allogeneic bone marrow transplantation in first complete remission was performed in 12 patients, of which 10 remain alive and disease free at a median follow up of 24 months. A statistically significant improvement in overall survival could not be demonstrated for allografted patients, although there was a trend toward improved progression free survival ($p=0.051$). Seven subjects were over the age of 60, of which five achieved complete remission (85%). Median survival in this age group was shorter at 18 months.

Conclusion

Local outcomes using Hyper-CVAD for acute lymphoblastic leukemia are comparable to previously published international experience. The role of allogeneic bone marrow transplantation remains controversial, however appropriate selection of patients at high risk of relapse with standard maintenance therapy can result in prolonged disease free survival.

No conflict of interest to disclose

Riverside Theatre
HSANZ Free Communications 3
O16

0830-1000
0915

Ciprofloxacin Prophylaxis During HyperCVAD Chemotherapy at Home. A Retrospective Cohort Analysis

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Background

The Home Cancer Care Service (HCCS) administers chemotherapy at home for patients from Royal Perth (RPH) and Sir Charles Gairdner Hospital (SCGH). Following highly myelosuppressive chemotherapy patients from RPH are prescribed ciprofloxacin when neutropenic (defined as ANC < 1 x 10⁹/L). Patients from SCGH receive no prophylaxis.

Aim

To retrospectively review all patients referred for HyperCVAD chemotherapy during the period 1/02-6/08 with respect to; the rates of NCI CTCAE Grade 3/4 febrile neutropenia (FN) and neutropenic infection (NI), organisms isolated and days of hospitalisation.

Method

Patients who were followed up post HyperCVAD chemotherapy at home were identified by internal records. Patients' notes and iSOFT Clinical Manager were used to obtain relevant data. The chi-squared test with continuity correction was used to compare rates of FN and NI, and the Wilcoxon two sample test was used for days of hospitalisation.

Results

52 patients were followed-up post HyperCVAD, 24 from RPH (NHL 6, ALL 18) and 28 from SCGH (NHL 18, ALL 10). The results are shown in Table 1.

	Hyper CVAD A arm		Hyper CVAD B arm	
	RPH	SCGH	RPH	SCGH
Number of cycles	56	70	52	75
Number of episodes of G3/4 FN	2 (3.6%)	3 (4.3%) NS	13 (25%)	24 (32%) NS
Number of episodes of G3/4 NI	3 (5.4%)	2 (2.9%) NS	12 (23%)	21 (28%) NS
Gram-positive organisms	4	2	10	12
Gram-negative organisms	0	0	1	16
Other infections	0	0	3	0
Days of hospitalisation for FN & NI	27	23 NS	159	279 NS

Table 1: Episodes of FN, NI, organisms grown and days of hospitalisation

Conclusion

There was no statistically significant difference between the rates of FN, NI or days of hospitalisation in those patients who received ciprofloxacin prophylaxis and those who did not. However, there was a trend towards lower rates of FN, NI and days of hospitalisation in the B arm with prophylaxis. Importantly, there were a greater number of episodes of gram-negative sepsis in the B arm without the use of ciprofloxacin prophylaxis (16 versus 1), which resulted in 2 episodes of septic shock. Ciprofloxacin prophylaxis during HyperCVAD chemotherapy at home is recommended to prevent clinically significant gram-negative sepsis.

No conflict of interest to disclose

Riverside Theatre
HSANZ Free Communications 3
O17

0830-1000
0930

Case Report: The Eradication of Monosomy 7 in Therapy-Related Myelodysplastic Syndrome/Acute Myeloid Leukaemia with LBH589

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LBH589 (Novartis) is a potent histone deacetylase inhibitor (HDACi) currently in clinical development. Preliminary data suggests biological activity in a range of malignant haematological disorders including acute myeloid leukaemia (AML). We report the case of a 64 yo man with therapy-related AML with a prolonged response to only a brief course of treatment with LBH589 treatment. The patient was initially diagnosed with Burkitt-like lymphoma in 2000 and achieved a CR with CHOP chemotherapy. Subsequent relapse in October 2002 was salvaged with CODOX-M/IVAC and a LACE conditioned ASCT. Secondary myelodysplasia characterised by macrocytosis and neutropenia was diagnosed in January 2006. BM biopsy revealed dysplastic erythropoiesis and granulopoiesis. No excess of blasts was evident. Cytogenetic analysis demonstrated monosomy 7. Transformation to AML was evident by September 2006 at which stage he declined AML induction therapy. He subsequently enrolled in a Phase I/II trial of oral LBH589. He commenced treatment with oral LBH589 in February 2007 at a dose of 40mg three days per week and rapidly cleared his circulating blasts. After receiving only 12 doses of LBH589 he withdrew from study with Grade 4 fatigue. He remained aleukaemic but required ongoing transfusional support for the next 12 months. BM biopsy in January 2008 revealed 12% blasts with no evidence of monosomy 7 in 187 interphase cells. A HLA identical unrelated donor was identified and the patient underwent RIC allogeneic SCT on 29 May 2008. He was discharged to home on Day 35.

This case illustrates the efficacy of histone deacetylase inhibitors in the treatment of therapy-related MDS/AML. Further studies are awaited to help clarify its use in this situation, either alone or in combination with other chemotherapy agents.

This research was supported by Novartis Corporation. The company had no role in analysing the data or preparing the abstract.

Riverside Theatre
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O18

0830-1000
0945

Eculizumab for the Treatment of Patients with Paroxysmal Nocturnal Haemoglobinuria (PNH): The Australian Experience

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Aim

In PNH, erythrocytes lack the GPI-anchored terminal complement inhibitor CD59 resulting in chronic haemolysis. Eculizumab is a terminal complement inhibitor that has been evaluated in 187 patients in two phase III studies and an extension study in North America, Europe and Australia where it was shown to reduce haemolysis, transfusion requirements and thrombotic events and to improve fatigue and quality of life. We report the results of the Australian cohort in these trials and compare outcomes to the entire cohort.

Methods

Eculizumab was administered to 14 Australian patients at 5 institutions. Dosage was 600mg IV every 7±2 days for 4 doses followed 7±2 days later by a 900mg dose followed by 900mg every 14±2 days during maintenance. Haemolysis, transfusions and FACIT-fatigue scores were measured before and during treatment.

Results

The median age of the Australian cohort was 34 years (range 19-65) and duration of disease was 5.85 years (range 1.5-19). Duration of treatment ranged from 1.5 to 2.5 years. Intravascular haemolysis, as assessed by lactate dehydrogenase, was reduced by 87% 3235-321 IU/L) resulting in the reduction of transfusions from 7.5 units/patient (median) before therapy to 0 units (median) during the last 6 months of treatment ($p=0.0005$). Fatigue improved rapidly and significantly and this was maintained through to the last 6 months of therapy (FACIT-fatigue score increased a median of 8.0 where a change of ≥ 3 is considered clinically meaningful). No drug-related serious adverse event was reported. There were no differences in these outcomes in the Australian population when compared to the entire cohort.

Conclusions

Eculizumab was well tolerated and highly efficacious in this group of Australian PNH patients. The benefits of eculizumab were rapid and sustained throughout the treatment period and were similar to those observed in the entire PNH study population.

JS has acted as a consultant for Alexion Pharmaceuticals Inc and H-AK is an employee of Alexion Pharmaceuticals.

Meeting Room 8
HSANZ Free Communications 4
O19

0830-1000
0830

Autologous Stem Cell Transplantation (auto-SCT) in Older Patients with Multiple Myeloma (MM) and Relapsed Diffuse Large B-cell Lymphoma (DLBCL). A Single Centre Study

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Aim

To examine survival outcomes in older pts undergoing auto-SCT for MM and relapsed DLBCL and to identify factors predicting outcomes in the elderly.

Method

A retrospective outcome analysis including all adults with MM and relapsed DLBCL who underwent auto-SCT over a 5 yr period at our institution was performed. Survival estimates for pts aged <60 (group 1) were compared to those ≥60 yrs (group 2). A number of variables, including remission status and the Sorror Comorbidity Index (HCT-CI) were assessed for their utility in predicting survival outcomes.

Results

Between 1/03 and 12/07, 59 pts in group1 and 62 pts in group 2 were identified. Median follow-up 27 months. The day 100 non-relapse mortality (NRM) was 4.1% overall. Day 100 and 1 yr NRM was 1.7% and 1.7% in group1 vs 6.5% and 8.1% in group 2 ($p=0.4$). 3 yr overall survival (OS) was 71% for all pts with a trend towards poorer OS in group 2 (74% vs 65%, $p=0.06$). Overall 3 year disease-free survival (DFS) was 57% with significantly improved DFS in group1 (76% vs 39%, $p<0.005$) regardless of diagnosis. In group 2 remission status at auto-SCT predicted improved DFS ($p=0.02$) and HCT-CI score ≥ 2 was associated with a trend towards poorer OS ($p=0.05$).

Conclusion

Auto-SCT in older pts with MM and DLBCL is associated with similar NRM and OS but poorer DFS when compared to younger pts. Remission status is an important predictor of DFS in the elderly. The HCT-CI may be a useful predictor of OS in older pts undergoing auto-SCT. Prospective trials analysing other age-associated variables such as nutritional, social and frailty indices may be of use in identifying other more sensitive and specific predictors of survival in this growing demographic.

No conflict of interest to state

Meeting Room 8
HSANZ Free Communications 4
O20

0830-1000
0845

Reduction in Emesis with Addition of Aprepitant to Standard Anti-Emetic Therapy in Patients Undergoing High Dose Chemotherapy and Stem Cell Transplantation

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Aim

To determine whether addition of Aprepitant to standard antiemetic therapy is effective in reducing emesis in patients undergoing high dose conditioning chemotherapy and stem cell transplantation.

Methods

A prospective study was performed from September 2005 to June 2008 in 40 consecutive patients undergoing high dose chemotherapy and autologous (n=39) and syngeneic (n=1) stem cell transplantation for predominantly haematological malignancy. The first cohort of 20 patients received standard anti-emetic therapy and Aprepitant 125mg on day 1 of conditioning therapy and subsequently 80mg daily until 48 hours post completion of therapy and the second cohort of 20 patients received standard anti-emetic therapy only. The 2 groups were compared with respect to age, conditioning regimen, emesis incidence, percentage days of emesis and total number of emesis episodes.

Results

Twenty-three males and 17 females were equally distributed between the 2 groups. The Aprepitant group had non-significant lower median age 57.5 (range 19-78) years and more patients undergoing BEAM (n=10) and less high dose Melphalan (n=6) conditioning therapy compared to the non-Aprepitant group with median age 63 (range 18-72) years and BEAM and Melphalan conditioning in 6 and 10 patients respectively. Other conditioning regimens included Busulfan/Cyclophosphamide (n=5), Busulfan/Melphalan/Velcade, Thiotepa/Busulfan/Cyclophosphamide and Carboplatin/Etoposide/Melphalan (n=1 each). Emesis occurred in 4 patients (20%) in the Aprepitant group versus 11 patients (55%) in the non-Aprepitant group, p=0.02. Mean days of emesis was 5% in the Aprepitant group versus 30% in the non-Aprepitant group, p=0.005. There was no difference in number of emesis episodes with median 4.5 (range 1-5) episodes in the Aprepitant group versus 5 (range 1-12) in the non-Aprepitant group. No patient receiving Aprepitant and conditioned with Melphalan or Busulfan/Cyclophosphamide developed emesis but the differences between the groups were non-significant.

Conclusion

Aprepitant reduces emesis incidence in patients undergoing high dose conditioning chemotherapy and stem cell transplantation.

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Meeting Room 8
HSANZ Free Communications 4
O21

0830-1000
0900

Neurological Involvement by Systemic Diffuse Large B Cell Non-Hodgkin Lymphoma (DLBCL) Has an Extremely Poor Outcome Following High Dose Chemotherapy and Autologous Stem Cell Rescue (ASCT): Germinal Centre (GC) Phenotype May Have a Better Disease Free Survival

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Background

ASCT is an established therapy for relapsed/refractory DLBCL improving disease free and overall survival. Neurological involvement by DLBCL presents a unique situation due to the poor CNS penetration of most chemotherapeutic agents. Here we report our experience in twelve such patients.

Material and method

Twelve patients had CNS involvement by DLBCL at the time of relapse. Median age was 51 years. Prior to ASCT, eight patients were treated with high dose methotrexate ($> 3 \text{ g/m}^2$), 4 patients had cranial radiotherapy and one underwent surgical debulking. Seven patients were in complete remission (CR), four in partial remission (PR) and one had untreated relapse at the time of ASCT. The conditioning regimens were LACE in 6 patients, busulfan/melphalan in 3, BEAM in 2 and Stanford BCNU in 1.

Results

Eleven patients have relapsed post ASCT, between one and 66 months post ASCT (median of 4.5 months). Six patients had isolated neurological relapse and four had systemic relapse only and one had both CNS and systemic relapse. No patient with neuronal only relapse could be salvaged by further therapy. Two patients with systemic relapse are salvaged by further therapy. The EFS and OS for the patients in CR at the time of ASCT with GC phenotype and non-GC phenotype (by immunohistochemistry) were 36 vs 3.5 months and 40 vs 7 months, respectively.

Conclusion

CNS involvement at the time of DLBCL relapse is associated with subsequent high relapse rates following salvage ASCT, even when performed in CR. For those undergoing ASCT in CR, a GC phenotype appears to convey a superior DFS and OS.

The authors have no conflict of interest to disclose

Meeting Room 8
HSANZ Free Communications 4
O22

0830-1000
0915

Quality of Life Assessment of Patients with Multiple Myeloma After Autologous Stem Cell Transplantation (ASCT)

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Introduction

There are very few published studies that focus specifically on quality of life issues in patients with multiple myeloma after autologous stem cell transplantation (ASCT).

Methods

The trial was available to all patients diagnosed with multiple myeloma at the Launceston General Hospital over a 36 month period. Of the twenty recruited patients with multiple myeloma undergoing ASCT (140mg/m² Melphalan for each transplant), 17 were eligible for assessment of quality of life with median age of 49 years (range 37-70 years). A full patient profile was collected including demographic and medical data and risk factors for multiple myeloma. Assessment of quality of life was made using The European Organization for Research and Treatment of Cancer (EORTC) Quality of Life QLQ-C30 questionnaire, conducted via interviews.

Results

Preliminary analysis shows that the mean Global Health Measure to be 3.44 (1=very poor, 7=excellent), and a mean Global Quality of Life of 3.61. About 50% of patients have experienced moderate fatigue, mild dyspnoea and mild physical impairment after transplant. The social and emotional functions were moderately affected. Cognitive function was mild-moderately impaired after transplant. The majority of patients experienced mild-moderate nausea and vomiting with mild gastrointestinal upset in form of either constipation or diarrhoea. Interestingly more than half of the patients reported moderate-severe financial difficulties during the course of treatment. Less than half of patients suffered from some degree of insomnia.

Conclusion

In summary, probably a sole focus on patient-survival does not sufficiently provide indication regarding the tolerability and appropriateness of a proposed intervention on the patient's perceived quality of life. As physicians or healthcare providers, our primary concern should be toward the patient-welfare as well as survival. Therefore we should employ the tools of quality of life assurance in conjunction with overall survival in order to deliver the best possible patient outcomes.

The authors confirm that there is no conflict of interest in relation to this research.

Meeting Room 8
HSANZ Free Communications 4
O23

0830-1000
0930

Oral CID Chemotherapy Followed by Autologous Stem Cell Transplant (ASCT) is an Effective Therapy in the Initial Management of Multiple Myeloma

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Aim

CID (Cyclophosphamide, Idarubicin and Dexamethasone. *Hematol J.* 2004;5:216) is a convenient oral regimen with no neurotoxic agents. We aimed to study the efficacy of CID followed by ASCT in the initial management of unselected patients with multiple myeloma.

Method

A retrospective analysis was performed on all potentially transplant-eligible patients treated with this regimen at the Princess Alexandra Hospital during the period from Feb 1997 to May 2007. Responses were assessed according to IMWG criteria.

Result

79 patients were identified: 54% were male; median age at diagnosis 59yrs (range, 32-74); median follow-up 41 months (range 11-128). 39%, 32% and 29% of patients had ISS Stage I, II and III disease, respectively. 64% of patients received 4 cycles of CID, with 26% receiving fewer and 9% receiving more than 4 cycles. Pre-autograft response rate (CR+PR) was 46% rising to 53% for those receiving at least 4 cycles of CID. 90% of patients aged \leq 65yrs (53/59) progressed to ASCT whereas 60% (12/20) aged >65yrs proceeded to ASCT. Reasons for not progressing to ASCT were: early death n=2; failed stem cell collection n=3; unfit for ASCT n=7 (<65yrs n=3, >65yrs n=4); and other n=2. The median time to autograft from diagnosis was 5 months. Day 100 TRM was 3%. The response post-ASCT was 94% including CR 32%, nCR 8%, vgPR 17% and PR 37%. For the entire 79 patients, this translated into a median EFS of 24 months and OS of 79 months. Initial response to CID did not influence EFS or OS. Post-ASCT achievement of CR strongly predicted both EFS ($p=0.001$) and OS ($p=0.01$).

Conclusion

CID chemotherapy in combination with ASCT is an effective myeloma regimen. Due to its oral nature and lack of neurotoxic agents, CID would be an attractive regimen to combine with newer agents such as lenalidomide or bortezomib.

No conflict of interest to disclose

Meeting Room 8
HSANZ Free Communications 4
O24

0830-1000
0945

¹³¹I-Rituximab BEAM Conditioning in Autologous Stem Cell Transplantation for Aggressive Non-Hodgkin's Lymphoma

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3. The University of Western Australia, Perth WA

Aim

High-dose chemotherapy with autologous stem cell transplant (ASCT) is used in aggressive NHL with overall survival (OS) 26-46% and progression free survival (PFS) 34-60%. The addition of radioimmunotherapy to standard conditioning demonstrates improved OS 81-88% and PFS 70-72%. This study assesses the efficacy of in-house prepared ¹³¹I-rituximab with BEAM conditioning in patients with relapsed or high risk B-cell NHL.

Methods

Individualised dosimetry was planned at day -19 to deliver whole body radiation dose of 0.75Gy ¹³¹I-rituximab on day -12. BEAM chemotherapy was administered and stem cells were given with a median dose of 2.65x10⁶/kg (range 2.2-6.4).

Eight patients received treatment: 3 Mantle Cell, 1 MALT, 4 DLBC (one transformed follicular). Pre-transplant, 6 with relapsed disease were in complete remission and one in partial remission. One patient with mantle cell received treatment as consolidation in first remission.

Results

Median time to neutrophil engraftment was 12 days (range 9-25). Median time to platelet engraftment was 20 days (range 11-61). One patient had delayed platelet engraftment due to HHV6 reactivation. One patient had graft suppression due to CMV infection and gancyclovir therapy requiring a second stem cell infusion. Two patients experienced transient late neutropenia.

Median follow up is 24 months (range 2-70). Seven patients are alive and disease free (OS 88%, PFS 88%). One patient with DLBC relapsed 10 months post-treatment and died at 29 months. Median PFS is 14 months (range 2-70).

Conclusion

¹³¹I-rituximab plus BEAM conditioning is low-cost and safe with comparable efficacy to commercially available radioimmunoconjugates.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Presidential Symposium
O25

0830-1000
0830

Clinical Evaluation of Non-invasive Prenatal *RHD* Genotyping of the Fetus

Catherine Hyland¹, Glenn Gardener², Helen Davies¹, Glenda Millard¹, Minna Ahvenainen¹, Christopher Ward³, Jonathan Morris³, Robert Flower¹, Jonathan Hyett⁴

1 Australian Red Cross Blood Service. 2 Mater Health Services. 3 PALMS Royal North Shore Hospital. 4 Royal Prince Alfred Hospital

Introduction

This study examines the accuracy of a non-invasive test to predict fetal *RHD* gene status within an Australian obstetric population.

Methods

Study design is a prospective cohort study: 130 RhD negative women provided venous blood samples when presenting for routine antenatal care in 2 obstetric hospitals. This included 17 RhD isoimmunised women. Free fetal DNA (ffDNA) was extracted from plasma and three regions of the *RHD* gene amplified. Test design is based on an algorithm for testing multiple replicates of multiple exons.

Primary outcome measure is a comparison of the predicted fetal *RHD* status and the infant's RhD serotype. Secondary analysis reviews results of the male linked-*SRY* gene, and if negative, the hypermethylated *RASSF1A* gene assay, as controls to confirm the presence of fetal DNA.

Results

When *RHD* status was assigned all predictions were correct (126/126) for 88 positives and 38 negatives. No result was assigned for four cases including one where a fetal *RHD* gene variant was identified and one where the mother had an *RHD* gene variant. Fetal *SRY* status was correctly assigned in 123 of 126 cases. Amongst the 38 *RHD* negatives all *SRY* assignments were correct. The *RASSF1A* test indicated fetal DNA was present in the fourteen cases assessed as both *RHD* and *SRY* negative as a safeguard against a false negative result.

Conclusions

This study establishes a reliable non-invasive test to assess fetal *RHD* status. Testing of multiple exon regions is necessary to guard against *RHD* variants. The next phase will complete a larger scale population study, including paired samples at different gestations, to increase the power of the study.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Presidential Symposium
O26

0830-1000
0845

Development of an On-line “Ordering and Receipting Blood System” (ORBS)

Geoff Simon

Queensland Blood Management Program, Queensland Health

Aim

ORBS is a web based ordering and receipting system that is being designed, developed and implemented by the Queensland Blood Management Program in conjunction with stakeholders. Its primary use will be to facilitate electronic ordering and receipting of blood and blood products in both public and private sectors, replacing the current manual fax based systems.

Method

Business process analysis has underpinned the development of web based software using contemporary programming methodologies.

Stakeholders directly involved with the ORBS project through participation in the monthly ORBS Working Party include the Australian Red Cross Blood Service, the National Blood Authority, Pathology Queensland (representing public sector), Queensland Medical Laboratory and Sullivan Nicolaides Pathology (representing private sector) and the Queensland Blood Management Program. Other stakeholders including Mater Pathology, CSL and other suppliers are kept informed and provide input via the ORBS Consultative Group which meets quarterly.

Result

Pilot software for web based ordering of fresh blood products went live between the Gold Coast Hospital and ARCBS in January 2008, and the electronic receipting of products commenced trial at the pilot site in May. Development of software for the on-line ordering of manufactured blood products is currently under way.

Conclusion

It is hoped that the pilot will be expanded to include private sector laboratories during 3rd quarter 2008, and that a state wide roll out across all private and public pathology laboratories in Queensland will commence later in the year.

Significant interest has been expressed by the National Blood Authority, ARCBS and other States and Territories in the potential for ORBS to contribute to improvement in a range of governance, planning, operational, financial and clinical issues.

No conflict of interest to declare

Meeting Room 4
ANZSBT Presidential Symposium
O27

0830-1000
0900

A Pilot Study on the Incidence of HLA class I, class II and HNA Antibodies in Blood Donors

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Background

Transfusion-Related Acute Lung Injury (TRALI) is a severe complication of transfusion where antibodies to Human Neutrophil Antigen (HNA), Human Leukocyte Antigen (HLA) class I and class II have been implicated. As multiparous female donors are more likely to develop leukocyte antibodies, many blood services have begun using male predominant clinical Fresh Frozen Plasma to avoid these leukocyte antibodies.

Aim

This was a pilot study to gather evidence to determine the frequency of HLA and HNA antibodies in an Australian apheresis donor population

Method

De-identified serum samples from 296 random donors were collected and screened using Luminex LSM kits for HLA class I and II antibodies, and granulocyte agglutination test and immunofluorescence test for HNA antibodies. Gender was recorded.

Results

	Female	Male	Total
n	80	216	296
Antibodies			
HLA class I	21 (26.3%)*	25 (11.6%)+	46 (15.5)#
HLA class II	3 (3.8%)*	0	3 (1.0)#
HLA class I & II	9 (11.5%)*	0	9 (3.0)#
HNA	1 (1.3%)*	4 (1.9%)+	5 (2.0)#
HNA & HLA class I & II	1 (1.3%)*	0	1 (0.3)#
Column Total	35 (43.8%)*	29 (13.4%)+	64 (21.6)#

* = % of female; + = % of male; # = % of total

HLA antibodies were detected in 59 donors (19.9%) and HNA antibodies in 6 donors (2.0%).

Conclusion

The novel Australian data from this pilot study indicates that (i) the incidence of HLA antibodies in male donors was higher than expected and (ii) more scientific evidence is required to direct strategies to maximise the use of each blood donation while minimising the risk of TRALI complications.

Acknowledgement

Thanks to the team at the Victorian Transplantation and Immunogenetics Laboratory in Melbourne for performing the HLA testing, and Genghis Lopez for performing the HNA testing.

No conflict of interest to disclose

Meeting Room 4
ANZSB Presidential Symposium
O28

0830-1000
0915

Acute Coagulopathy in Trauma

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Aim

To determine the incidence of trauma associated coagulopathy and its outcome.

Methods

Trauma, epidemiological, pathology and transfusion databases were electronically linked into a single database. Demographic, pre-hospital, laboratory data on admission and 24 hour transfusion data were evaluated.

Results

From the trauma database 6519 patients were identified between 1998 and 2006. Only eight hundred and fourteen patients (13%) had an initial coagulation screen taken in the emergency room within the first hour of admission (median time of 15 minutes). The incidence of coagulopathy was 11% (89/814) and was present in 8 % of patients with an ISS of 16-24, in 22 % of patents with an ISS of 25-50 and in 55% of patients with an ISS of >50. Overall mortality in the coagulopathy group was 27% (24/89) and of those 63% (15/24), there was an early mortality within first 24-48 hours of injury. Mortality increased with injury severity and was higher with in patients with coagulopathy across all ISS severity grades. 73% of patients with coagulopathy received massive transfusion compared to 8% in the non-coagulopathy group.

Conclusion

Acute traumatic coagulopathy is common and is associated with severity of the injury, need for massive transfusion and high risk of mortality. A routine coagulation screen (INR/PT, APTT, fibrinogen and platelets) on admission is an important laboratory test for injured patients.

No conflict of interest to disclose

Meeting Room 4
ANZSB Presidential Symposium
O29

0830-1000
0930

Microparticles in Stored Red Cell Concentrates: A Proteomic Investigation

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Aim

Microparticles accumulate in stored cellular blood products, including red cell concentrates (RCCs). The protein composition of red blood cell (RBC) microparticles may give insight into the effects of storage of RCCs and ageing of RBCs. In this study we quantified RBC-derived microparticles in RCCs throughout the 42 day storage period and used proteomic techniques to identify proteins that are enriched in microparticles compared with the truly soluble proteins in matched RCC supernatant.

Methods

The number of glycophorin A-positive microparticles (ie. RBC-derived) present in leucoreduced RCC supernatant were quantified throughout storage using a flow cytometry absolute counting assay ($n \geq 3$). Supernatant from Day 42-stored RCCs was ultracentrifuged (100,000g, 24 hours) to isolate the microparticle fraction. Proteins from microparticle-free supernatant and washed microparticles were analysed using two-dimensional electrophoresis. Proteins of interest were identified using tandem mass spectrometry.

Results

The number of glycophorin A-positive microparticles was low up to day 28 and increased several fold up to Day 42 of storage. Microparticles in RCCs were found to be enriched in haemoglobin and immunoglobulins compared with the corresponding supernatant. Preliminary results suggest that microparticles in buffy coat poor RCCs are enriched in a number of proteins including neutrophil-derived proteins such as S100-A9 protein, compared to leucofiltered RCCs.

Conclusion

Microparticles are generated in leucodepleted RCCs, predominantly in the last two weeks of storage. A number of proteins were enriched in the microparticles generated in buffy coat poor RCCs, compared with leucofiltered RCC. These results suggest that leucofiltration may influence the composition of microparticles in RCC but does not prevent accumulation during RCC storage. The biological significance of microparticle accumulation during RCC storage and the effects on RBC function and efficacy require further investigation.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 1
O30

0830-1000
0830

Pregnancy Outcome in Women with Mechanical Heart Valves Treated with Enoxaparin

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Background

Pregnancy for women with mechanical heart valves presents a challenging dilemma. Warfarin is the preferred option for maternal health, but for the fetus anticoagulation that does not cross the placenta, such as enoxaparin, is preferable.

Aim

To determine the efficacy and safety of enoxaparin in women with mechanical heart valves during pregnancy.

Methods

Pregnancy outcome data were collated on a prospective (1997-2008) cohort of women with mechanical heart valves managed with enoxaparin \pm aspirin. Women were classified as: Group 1 enoxaparin commenced by 6w gestation and continued through pregnancy; Group 2 enoxaparin commenced 7-19w; Group 3 warfarin \rightarrow enoxaparin (6-14w) \rightarrow warfarin or warfarin \rightarrow enoxaparin \geq 34w

Results. 47 pregnancies in 31 women.

	Gp1 (n=21)*	Gp2 (n=14)*	Gp3 (n=12)*
Antenatal thrombotic complications**	0	4	1
Antenatal haemorrhagic complications†	6	2	0
No maternal complications	15	7	11

* pregnancies continuing after 12w Gp1 n=14, Gp2 n=12, Gp3 n=11 **TIA, CVA, valve thrombosis

† antepartum hemorrhage, placental abruption, haematoma, haematemesis.

There were no thrombotic complications in Gp1 and four in Gp2 (one on enoxaparin, 2 on enoxaparin started after no anticoagulation for months and one on sub-therapeutic unfractionated heparin). One woman in Gp3 had a CVA at 13w on sub-therapeutic enoxaparin. The thromboembolic rate was 1 per 80 enoxaparin-prescribed months. Among pregnancies continuing after 12w, all 14 (100%) in Gp1 resulted in live births, in Gp2 there was 1 perinatal death following spontaneous premature birth and 10 (92%) live births, and in Gp3 there were 3 late pregnancy losses, one neonatal death attributable to warfarin and 8 (67%) healthy live babies.

Conclusion

Successful pregnancy can be achieved with therapeutic-dose enoxaparin, with enoxaparin-attributable thrombotic complications in 4.4% of pregnancies and haemorrhagic complications in 17.8%.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 1
O31

0830-1000
0845

Risk Assessment for Peri-operative Management of Patients on Warfarin Therapy

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Background

Around 10% of patients per year on warfarin therapy require temporary interruption of anticoagulant therapy for surgical procedures. The management of these patients is challenging because of the balance between the risks of a thrombo-embolic event (TE) with interruption of warfarin therapy versus the risk of excessive peri-operative haemorrhage with anticoagulation.

Aim

To evaluate the outcomes of a standardised preoperative TE/bleeding risk assessment in consecutive patients referred for peri-procedural anticoagulant management at a large teaching hospital.

Methods

735 patients were consecutively assessed for TE and peri-operative bleeding risk according to predefined criteria. 4 risk profiles were established using the combination of high/low risk categories for TE and bleeding. Patients were then managed by a predefined protocol according to risk and included therapeutic anticoagulation with IV heparin or low molecular weight heparin (LMWH), post procedure prophylactic dose anticoagulation with LMWH or cessation of warfarin only without bridging anticoagulation. Patients were then observed concerning the success of reversal of anticoagulation (INR<1.5) time to return to therapeutic post procedure (INR >2) and post procedure TE/ bleeding events up to day 30.

Results

The patients were prescribed warfarin for previous VTE (37%), atrial fibrillation (34%), mechanical heart valve(s) (20%) and other indications (9%). Around half of the patients were judged to be at high risk for TE of which 33% also were high risk for peri-procedural haemorrhage. In contrast, those at low risk of TE, had a higher proportion of patients at high risk for peri-procedural haemorrhage (66%). 55% of patients required full anticoagulation with either LMWH or intravenous heparin. Warfarin was successfully reversed in almost all patients on the day of procedure (99%) and the return to therapeutic INR was achieved by day 7 after reintroduction of warfarin in 90% of patients. 10 bleeding events were recorded by day 30 (1.4%) of which 4 were major bleeds (3 in breast surgery). 2 cerebrovascular events (0.25%) and no VTE were observed.

Conclusion

Peri-procedural risk review for TE and bleeding is complex and is assisted by a standardised assessment protocol involving both patient and clinician agreement. Patients at low risk for TE are more often referred because of concerns about bleeding and no or at most prophylactic dose LMWH is recommended. Breast surgery should be regarded as high risk for bleeding and a more conservative approach is advocated.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 1
O32

0830-1000
0900

Antithrombin Deficiency in Pregnancy: Thromboprophylaxis and Venous Thromboembolism (VTE) Incidence in 16 Pregnancies

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² *University of Otago Medical School, Wellington, New Zealand*

Aim

Few data are available to guide management of the rare but highly thrombogenic condition of congenital antithrombin deficiency in pregnancy and post-partum. The largest previous case series reports 9 pregnancies in 8 women. Our study correlates thromboprophylactic approaches and VTE incidence in 16 pregnancies in 7 AT deficient women.

Methods

AT deficient women were identified retrospectively from the Wellington Hospital laboratory database. Clinical details were obtained from hospital records. Data were collected on AT level, concurrent thrombophilic defects (protein C, protein S, FV Leiden, PT20210A, lupus anticoagulant), prior VTE, age at conception, thromboprophylaxis, VTE occurrence and pregnancy outcome.

Results

We identified 16 pregnancies in 7 women. Median AT level (functional) was 58% (51 to 64). 4 women had complete thrombophilia screens. None had concurrent thrombophilic defects. Median age at conception was 24.5 (19-37) yrs. 8 DVTs (2 complicated by PE) were diagnosed in 5 women in pregnancy or within 3 months post-partum. 2/4 women with no prior VTE experienced pregnancy-related VTE. 3 early pregnancy losses occurred. There were no maternal deaths.

Table 1. VTE occurrence in relation to prophylaxis

	Prophylaxis	Pregnancies	VTE (%)
Antepartum	None	8	3 (37.5%)
	Low dose LMWH/UFH	7	1 (14.3%)
	Warfarin	1	0
Intrapartum	Antithrombin concentrate	1	0
Post-partum	None	6	2 (33.3%)
	Warfarin to INR 2.5	10	2 (20%)

Conclusion

In our series, without prophylaxis VTE occurred in 37.5% pregnancies and 33.3% post-partum periods. Antepartum prophylaxis with low dose heparin was associated with a VTE rate of 14% and postpartum prophylaxis with warfarin with a VTE rate of 20%. These data support consideration of more intensive antepartum thromboprophylaxis in AT deficient women. Post-partum warfarin with target INR 2.5 does not reliably protect against VTE in this high risk group.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 1
O33

0830-1000
0915

The Use of a Prothrombin-Complex Concentrate (Prothrombinex-VF[®]) in Medical and Surgical Patients – A Review of a Single Institution's Experience

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Aim

Prothrombin-complex concentrates are used for factor replacement in deficient patients, primarily in warfarin-induced coagulopathy. This study aims to characterise the use of a prothrombin-complex concentrate, Prothrombinex-VF[®] (CSL Bioplasma) (PTX), in a tertiary hospital setting.

Methods

The transfusion medicine laboratory computer database identified all uses of PTX since its introduction into routine use from December 2005 to May 2008.

Results

There were 73 patients treated with PTX, with 79 patient episodes. Median age was 58 years (16-88). Total number of ampoules of PTX used was 58 in 2006, 204 in 2007, 94 in the 1st quarter of 2008 and a predicted total of 336 for 2008. Mean dose was 4.2 ampoules/patient or 29 IU/kg. Mean use of concurrent FFP was 2.7 Units/patient (0-14). Cardiothoracic surgery (CTS) accounted for 40 patient episodes and included: heart transplant (15), LVAD insertion (10), heart valve replacement +/- CABG (6), aortic root replacement (3), lung transplant (4), heart-lung transplant (1) and lung biopsy (1). 11 out of 40 CTS patients receiving PTX intra-operatively were not on warfarin and the INR was <2.0 prior to procedure. In non-CTS (39 patient episodes), 21 were actively bleeding (intracerebral, abdominal, pulmonary, vascular or limb). Bleeding was absent in 18, and PTX was used to reverse coagulopathy prior to an invasive procedure. 20 out of 39 non-CTS patients were not on warfarin and were coagulopathic (INR>2.0) from another cause. 14 (19%) patients died during the inpatient admission. There were 3 episodes (4%) of thrombosis following PTX use.

Conclusions

There appears to be increasing use of PTX in recent years. 31/73 (42%) of patients treated with PTX were not on warfarin, despite only limited published data to support its efficacy outside warfarin-reversal. Larger clinical trials are needed to assess the clinical effectiveness and adverse event rate of PTX use.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 1
O34

0830-1000
0930

Survival in Patients with Malignancy and Venous Thromboembolism Varies Significantly with Tumour Subtype and Thrombotic Load

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Aim

Although the association between malignancy and venous thromboembolism (VTE) is firmly established, less is known about the time course and impact on survival that VTE has among different malignant subtypes. We aimed to estimate survival in a cohort of patients with known malignancy presenting with VTE.

Method

A cohort of 558 consecutive patients with VTE and known malignancy was identified from the Thrombosis Unit registry at Auckland City Hospital between January 14, 1997 and October 10, 2006. All events were confirmed by standard imaging procedures. The date and site (upper limb, lower limb, ilio caval/abdominal or pulmonary embolus) of VTE, diagnosis date and type of malignancy were recorded. Kaplan-Meier and log-rank (Mantel-Cox) analysis was applied. The mean follow up was 21.4 months.

Result

Overall, mean survival from VTE was 13.5 months with 6 month, one, two and five year survivals of 64%, 53%, 43% and 33% respectively. Survival was longest for haematological malignancy at 44.4 months followed by prostate, bowel, breast (metastatic breast), lung and pancreatic malignancy at 29.4, 27.4, 15.5(6.2), 2.4, and 1.9 months respectively. Corresponding survivals at one to five years and survival from cancer diagnosis for the different malignancies was also determined. Mean survival varied with thrombotic load from 31.1 months for upper limb/jugular DVT reducing to only 10.1 months for ilio caval/abdominal DVT but this did not reach statistical significance.

Conclusion

This experience of 53% one year survival after VTE in cancer patients is longer than early large registry studies but similar to recent reports. Survival is critically determined by tumour type and correlates with tumour burden, at least in women with breast cancer. There is also a trend toward reduced survival in those with a higher thrombotic load.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 1
O35

0830-1000
0945

Warfarin Reversal, for Elective Surgical Procedures, Using Low Dose Intravenous Vitamin K: Impact on Vitamin K-Dependent Factor Levels

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The optimal management strategy for temporary warfarin reversal prior to elective surgery has not yet been established. Standardisation of management has been difficult - due to a number of different strategies available and the variable risk in both thromboembolism (TE) and bleeding. Current guidelines recommend discontinuing warfarin 4-5 days prior (+/- bridging anticoagulation), aiming for an INR <1.5. An alternative method involves administering low dose intravenous vitamin K (vit K IV) the day prior to the procedure.

Last year we presented safety and efficacy data on the use of vit K IV, for short term reversal of warfarin, in patients undergoing elective surgery. The results demonstrated that this method was rapid, safe and successfully lowered the INR to within an acceptable range for surgery to proceed according to schedule. There was no excess of bleeding or TE complications during follow-up, delayed discharge or apparent resistance to warfarin stabilisation after surgery. The protocol was simple and convenient for both the patients and the healthcare institution and is now used as standard practice at this tertiary hospital.

As a secondary objective we assessed the effect of therapeutic warfarin and the subsequent reversal using vit K₁ IV, on vit K-dependent coagulation factor levels. Factors II and X showed the greatest reduction as a consequence of warfarin therapy. Although there was variation in the absolute levels of individual factors, there was a relatively uniform pattern of depletion with warfarin and increment following vit K₁, correlating with increase and decrease in INR respectively. Apart from FX, all mean factor levels incremented to within normal range following vit K₁, with factor IX and VII being higher than II and X. Furthermore, extrapolation of the data suggest that an acceptable INR <1.5, correlates with factor levels of at least greater than 30%.

No conflict of interest to be disclosed

Meeting Room 1
Nursing Free Communications 1
O36

0830-1000
0830

Got the Protocol – How to Achieve Compliance? E-learning Lessons as a Tool to Improve Compliance to Clinical Protocols.

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Background

Intrathecal chemotherapy can result in serious adverse events. No intrathecal policy existed at St George Hospital, Sydney, up until 2003 when a patient inadvertently received intrathecal vincristine. Recommendations following this event prompted the development and instigation of stringent institutional guidelines regarding all matters related to prescribing intrathecal chemotherapy with an accompanying educational session for all new medical officers and nurses employed within Cancer Services. However compliance was haphazard and remained poor, with clinical incident reports relating to deviations from the policy occurring regularly. Thus alternatives to the paper policy were sort.

Objectives

- To reduce errors in intrathecal chemotherapy prescribing
- To improve compliance in adherence to the policies regarding intrathecal chemotherapy
- Develop a methodology that was workable in the clinical setting

Method

An e-learning lesson to reach these objectives was developed by a physician and several Registered Nurses in an attempt to overcome this situation and improve compliance through Cancer Solutions™. Cancer Solutions™ <http://moodle.educan.com.au> is an integrated electronic learning management system that was developed for all clinical staff employed in the Comprehensive Cancer Service at St. George Hospital.

Results

Since its implementation all medical staff who prescribe intrathecal chemotherapy are mandated by the institution to undertake the lesson and attain a 100% grade prior to prescribing intrathecal treatments. Since this inception no prescribing errors have been reported to date, and compliance to the policy has being almost complete. These results demonstrate that the lesson has been successfully instigated into the clinical setting.

Conclusion

The success of this e-lesson has encouraged staff at St George Hospital to adapt its application to other hospital policies in order to improve compliance in other important clinical practices. Currently this methodology is being developed and trailed at St George Hospital using the Neutropenic Sepsis policy and preliminary results to date are encouraging.

No conflict of interest to disclose

Meeting Room 1
Nursing Free Communications 1
O37

0830-1000
0845

Changing Practice - Making It Safer

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During the administration of cytotoxic drugs via a newly implemented needless system, staff identified the risk of aerosols when they observed a visible leakage of drugs when disconnecting IV direct bolus injection syringes. Staff sought a control measure that would reduce the hazard associated with cytotoxic aerosols. Solutions considered included: a) change of practice, b) policy review and c) equipment.

Review of clinical practice techniques and reflecting upon policy, aerosols continued to be problematic on occasion. It was identified that the needless system may need to be substituted for a less hazardous system. Following a review of the literature and investigating equipment solutions, the PhaSeal System from Carmel Pharma was identified as providing a closed system that would enhance the safety of cytotoxic IV direct bolus injection administration route. Studies have shown that the PhaSeal System when compared with other devices confined the drug thus preventing the risk of exposure to cytotoxic aerosols.

PhaSeal was implemented in a trial capacity following extensive education and staff support. During the trial phase, staff visually noted the presence of solution in the Injector Luer Chamber. Concerns related to patients not receiving their required dose were raised. Collaboration with the pharmacists, Nurse Unit Manager, Nurse Educators and clinicians determined the maximum volume to be 0.8 mls and not of significance.

The trial phase concluded with the PhaSeal Injector Luer and Connector Luer Lock devices being implemented as standard equipment for the administration of cytotoxic IV direct bolus injections. This added control measure has been well integrated into the unit with staff identifying the risk of occupational exposure to cytotoxic drugs during administration as significantly reduced.

No conflict of interest to disclose

Meeting Room 1
Nursing Free Communications 1
O38

0830-1000
0900

To Enteral Feed or Not

Wendy Jar

South Island Bone Marrow Transplant Unit, Canterbury District Health Board, Christchurch, New Zealand

Background

The South Island Bone Marrow Transplant Unit is a 15 bedded unit in Christchurch, New Zealand that treats patients with haematological diseases. In 2007 6 people had allogeneic transplants. The question of whether to routinely enteral feed this group of patients was raised.

Method

An audit of 9 patients who had allogeneic transplants was undertaken. Apart from age, gender, diagnosis; the following data was collected: length of stay, pre and post transplant weight plus weight 3 and 6 months post transplant, days of mucositis, diagnosis of GvHD and grade, whether they had TPN, enteral feeding or not, dietician input throughout their admission and whether nausea and vomiting was an issue for these patients.

Results

The results were varied. All patients experienced nausea and vomiting with conditioning despite being on regular antiemetics. Most people experienced a weight loss of up to 10% during the admission. From the data available no one had reached their pre transplant weight 6 months after the transplant. The degree and grade of mucositis and GvHD varied.

Conclusion

The dietician will assess this patient group pre transplant admission. The dietician will identify at risk patients. The possibility of enteral feeding will be raised with all patients. The aim is for enteral feeding to be planned, not a last minute process. Enteral feeding is to remain a case by case clinical decision. Guidelines have been developed regarding the insertion of either a nasogastric or nasojejunal tube including tube type and size and where they get placed.

Other issues identified were:

Is weight an accurate measure of nutritional status?

How long should the dietician follow up post transplant?

Is the antiemetic regime around conditioning therapy sufficient?

No conflict of interest to disclose

Meeting Room 1
Nursing Free Communications 1
O39

0830-1000
0915

Cryotherapy to Prevent Oral Mucositis in Patients Receiving High Dose Melphalan for Autologous Stem Cell Transplantation

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¹Haematology Care Centre, Sir Charles Gairdner Hospital, Nedlands, WA, Australia. ²PathWest Laboratory Medicine, Nedlands, WA, Australia.

Aim

To assess the efficacy of cryotherapy in reducing the incidence of oral mucositis in patients receiving high dose melphalan for autologous stem cell transplantation.

Method

In 2006 we introduced cryotherapy as standard of care in patients undergoing autologous stem cell transplantation with high-dose melphalan (200mg/m²) for myeloma and amyloidosis or BEAM (melphalan 140mg/m²) for non-Hodgkin lymphoma and Hodgkin disease. Taking into consideration the purported half-life of melphalan and the logistics of out-patient stem cell transplantation, we asked patients to suck ice blocks for 30 minutes prior to, during administration and for 2 hours after melphalan. Fifty consecutive patients undergoing stem cell transplantation for myeloma, amyloidosis and lymphoma at our institution were prospectively evaluated. They were assessed daily from day 0 to day 11 by trained nurses using the WHO Oral Mucositis Scale. The incidence and grade of OM were recorded. The results were compared to a control group comprising 30 patients transplanted for myeloma and lymphoma before the introduction of cryotherapy.

Results

Age, sex, diagnosis and conditioning therapy were similar in the two groups. The intervention was well tolerated. The results regarding the incidence of OM are shown in figure 1. There was a reduction in the incidence of OM in the group receiving cryotherapy (70% vs 16%). The benefit was seen in patients transplanted for myeloma (68% vs 4%) and lymphoma (80% vs 22%). This reduction in OM was associated with a reduction in the need for opioid analgesia (47% vs 8%).

Conclusion

Our findings confirm that cryotherapy is a highly effective method to prevent oral mucositis in patients receiving high-dose melphalan. This simple intervention can result in a marked improvement in one of the worst symptoms associated with autologous stem cell transplantation.

No conflict of interest to disclose

Meeting Room 1
Nursing Free Communications 1
O40

0830-1000
0930

The Assessment of a Senior Haematology Nurse Performing Unsedated Bone Marrow Biopsies in a Large Tertiary Cancer Centre

Simon Kuzyl

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Aim

To implement a Nurse initiated bone marrow aspirate and trephine (BMAT) policy and procedure at RPAH.

Background

Physicians have traditionally performed BMAT. However, increasing demands on health care resources has reduced the time available to perform minor medical procedures. Specialist trained nurses are performing BMAT at institutions throughout the world.

Method

A nursing policy and procedure was researched, developed and approved by the key stakeholders. Training of the Haematology CNS was undertaken by the Haematology Registrar and consisted of successful completion of 10 supervised and 10 independent BMAT.

Key Performance Indices

- Number of successful versus unsuccessful BMAT;
- Quality of slide preparation and specimens;
- Patient satisfaction and pain surveys.

Results

- Between Sep 2007 to Feb 2008, 41 BMAT were performed (10 newly diagnosed and 31 for re-staging).
- Successful BMAT in 40 out of 41. In the single unsuccessful BMAT material was not obtained after two attempts and the Haematology Registrar completed the procedure.
- 21 patients completed patient satisfaction surveys. Of the 20 surveys not completed, 12 patients were of non- English speaking background (NESB), 5 had previously completed the survey and 3 were confused.
- Pain/discomfort was reported as none or minimal through to mild or moderate in 19 of the 21 surveys. 2 patients described the pain/discomfort as the worst possible.

**Note: BMAT performed by Medical Officers have previously not been measured, therefore, comparisons with a nurse led service is unavailable.*

Conclusion

- All the Bone Marrow Biopsies attended to by the CNS at RPAH resulted in satisfactory outcomes for the patient, demonstrated by the successful diagnosis and re-staging of haematological malignancies. Evidence is validated from the satisfaction of the Department of Haematology and patients on whom these procedures were performed.
- This ward- based service will continue with ongoing reviews of the KPI's and offers an alternative provider of BMAT.

No conflict of interest to disclose

Meeting Room 1
Nursing Free Communications 1
O41

0830-1000
0945

AMD3100 for Patients Failing Haemopoietic Stem Cell (HSC) Mobilisation – The Illawarra Experience

Fran Owen, Julie Ryan, Peter Presgrave, Kim Cartwright, Pauline Warburton
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Aim

Failure to mobilise sufficient HSC limits the ability to deliver optimal treatment in several haematological malignancies. AMD3100 (Genzyme) is a novel HSC mobilising agent that inhibits SDF-1/CXCR4 interaction. In combination with G-CSF, it has been shown to mobilise CD34+ HSC in patients with NHL, Hodgkin's Lymphoma (HL) and Multiple Myeloma (MM) who have previously failed mobilisation. Six patients in Wollongong have received AMD3100 through the AnorMED (Genzyme Corp.) CUP.

Method

6 patients (ages 24-57) had failed 7 previous attempts to collect sufficient HSC. Diagnoses were MM – 2, relapsed NHL – 2, relapsed HD - 2. Prior mobilisation regimens were high dose Cyclo + G-CSF and ICE + G-CSF. 4 had been heavily pretreated and 1 had received a prior HSC transplant. G-CSF 10mcg/kg/BD sc. was given for 4 days, with AMD3100 240mcg/kg/D sc. given 10-11 hours prior to first anticipated apheresis. AMD3100/G-CSF was continued until the final apheresis.

Result

Patients had a median of 3 apheresis procedures (range 2-4). A median cell dose of 2.3×10^6 CD34+ (range 1.5-5.1) was obtained. The median PB CD34 count was 10.7 prior to AMD3100 and 18.35 after. 1 patient required 2 mobilisations with AMD3100 and 1 patient failed to mobilise. Prior mobilisation attempts had produced a median PB CD34 count of 5.7. There was 1 report of diarrhoea and abdominal cramping and 1 report of stinging at the injection site. 3/5 patients have proceeded to transplantation. Median time to engraftment was 12 days.

Conclusion

The use of AMD3100 at Wollongong Hospital has enabled 5/6 patients to mobilise sufficient CD34+ cells to proceed to autologous transplant. Three have been transplanted successfully to date.

No conflict of interest to disclose

Meeting Room 9
Nursing Free Communications 2
O42

0830-1000
0830

Telephone Triage in Cancer Nursing

Kirstin Bubner

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Telephone based health advice is growing in acceptance as a cost effective strategy to help reduce pressure on the health care systems. Providing advice for cancer patients can be challenging for the nurse when dealing with a broad range of diseases, treatments and side effects. In order for a telephone based triage system to be effective, the nurse must have an adequate knowledge base combined with excellent assessment and communication skills.

With the complex needs of cancer patients and more treatments being moved to the outpatient setting the number of after-hours calls has increased. These calls are being directed to our inpatient ward where staff are often unfamiliar with these patients and their individual care.

Our project looked at the redevelopment of our current telephone triage system and the design of a flow chart to be used as a learning tool to assist staff when managing these calls. We evaluated the results from past forms over a five year period, performed a literature review including investigation of the legal implications involved. In discussion with colleagues in haematology/medical oncology units around Australia the majority did not have a system currently in place.

We were able to develop a new form that has been reviewed by many departments in our hospital including the risk manager and forms committee. The form we created will not only be used in the Cancer Centre but be used across the whole hospital and will be filed in the patient's case notes. The flow chart that was designed highlights the need for clinical expertise in the cancer setting and gives a step by step guide if the staff member feels incapable of handling the call.

The future direction of this project is to introduce the form into current practice and conduct regular audits and evaluations to maintain a high quality service for our patients. Education will be provided to staff when the forms are introduced and at regular intervals.

With many treatments being moved to the outpatient setting along with the complex ongoing needs of patients the number of after hour's calls has increased.

No conflict of interest to disclose

Meeting Room 9
Nursing Free Communications 2
O43

0830-1000
0845

Evaluation of the Leukaemia Foundation Disease Specific DVD Series Developed to Facilitate Education and Support Programmes to Empower Patients and their Families Living with Leukaemia, Lymphoma, Myeloma and Related Blood Disorders

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¹ Leukaemia Foundation Melbourne, Victoria. ²Leukaemia Foundation Southern Tasmania. ³Leukaemia Foundation Northern Tasmania. ⁴Leukaemia Foundation Hume, Victoria. ⁵Leukaemia Foundation Metropolitan Melbourne/Grampians, Victoria. ⁶ Leukaemia Foundation Barwon SW, Victoria. ⁷Leukaemia Foundation Metropolitan Melbourne, Victoria. ⁸ Leukaemia Foundation Gippsland, Victoria. ⁹ Leukaemia Foundation Metropolitan Melbourne, Victoria

Aim

The Leukaemia Foundation has developed a series of six disease specific DVD's that were launched nationally in June 2008 with every hospital that treat haematological malignancies around Australia receiving a complementary set! The aim of this presentation is to evaluate the effectiveness of this educational resource by reporting on Phase 1 of the evaluation study among health professionals particularly in rural and remote settings as access to haematologists for presenting educational seminars for patients and families is limited.

Method

Phase 1: Two months following distribution of the educational DVD's, (August 2008) evaluation of this new resource will be conducted using a questionnaire directed to health professionals of the 154 treating centers nationally that received a set. The questionnaire with reply paid envelope will be hand delivered by Leukaemia Foundation Support Services staff.

Phase 2: A questionnaire to evaluate and explore the quality of life outcomes experienced by patients and their families that viewed the DVD relevant to their diagnosis will be conducted. The questionnaire will be distributed in October 2008.

Results

Data collected from the Phase 1 questionnaire completed by survey recipients will be analyzed and presented at the HAA conference, including discussion of the benefits to patient support and identifying any areas of unmet needs. The questionnaire also invites the survey recipients to suggest/recommend topics for additional DVD production that will increase patient support outcomes for people living with blood cancers.

Conclusion

Internal pilot use of the DVD's in Victoria and Tasmania clearly indicate outstanding success. This DVD project has enabled rural and remote Leukaemia Foundation Support Services staff to facilitate numerous Education and Support Programmes with ease providing patients and their carer's access to expert specialist information.

No conflict of interest to disclose

Meeting Room 9
Nursing Free Communications 2
O44

0830-1000
0900

“You can talk about it and people understand.” Telephone Support Groups: Psychosocial support for Myeloma Patients in New South Wales

Tracy King¹, Felix Ratcliff ². **Contributors:** John Friedsam ², Paula Vallentine ² Gill Batt²
The Myeloma Foundation of Australia Inc, Camperdown, NSW 2050; ² The Cancer Council NSW, Woolloomooloo NSW 2011

Background

Psychosocial support options for Multiple Myeloma patients are currently extremely limited in NSW, particularly for rural and regional residents. An incurable haematological malignancy, Multiple Myeloma is characterized by bone marrow compromise, destructive bone lesions, immune dysfunction and renal impairment. Despite life expectancy improvements from 3-5 years to 5-7 years, patients continue to experience a difficult and prolonged illness requiring ongoing clinical care and psychosocial support. Over the past five years, the Cancer Council NSW (CCN) has successfully run Telephone Support Groups (TSGs) enabling geographically and physically isolated people affected by cancer to participate in professionally led support groups. This mode of program delivery is ideally suited for this marginalised patient group.

Objectives

- To improve and increase equity of access of psychosocial support for Multiple Myeloma patients in NSW.
- To reduce the emotional, physical and practical challenges for this marginalised patient population.
- To enhance service delivery through a strategic partnership between Myeloma Foundation of Australia (MFA) and CCN.

Method

CCN and MFA partnered in May 2007 to establish a TSG for Multiple Myeloma patients. Groups were offered bi-monthly for one hour for 3-7 participants, using the Mutual Aid Model and led by two trained facilitators. Participants were recruited using multiple strategies: seminars, newsletter advertising and client-initiated and Health Professional referrals.

Results

Interest levels and participation rates developed rapidly following the group's inception and marketing. High demand necessitated the scheduling of a second group. Retention rates and qualitative feedback indicated participants found TSGs extremely valuable, highly supportive and convenient model of accessing psychosocial support.

Conclusion

Valuable insights have been gained into the psychosocial needs of this patient group. TSGs have proved to be a highly effective mode of support for Multiple Myeloma patients. Importantly, this strategic partnership between MFA and CCN strengthened inter-agency relationships, proved highly successful, cost-effective and filled a significant gap in service provision for this underserved marginalised group.

No conflict of interest to disclose

Meeting Room 9
Nursing Free Communications 2
O45

0830-1000
0915

Myelodysplastic Syndromes (MDS) – The Value of Transfusion Plans and Transfusing at Higher Haemoglobins

Jan Chapple

Oncology Outpatient Department, St. Vincent's Public Hospital, Fitzroy, Victoria, Australia

Background

Myelodysplastic Syndromes (MDS) are a group of diseases that affect the production of normal blood cells in the bone marrow and can contribute to potential serious morbidity and mortality. It is well known that MDS can have a significant impact on quality of life. The median age of people diagnosed with MDS is 65-70 years of age and most will die of their disease. Anaemia is observed in 90% of MDS sufferers and is usually the main cause of symptoms and often requiring transfusion support. This ageing population often have other co-morbidities and limited treatment options, highlighting the importance of supportive care.

Aim

The aim of this project was to assess and individualize care of patients with Myelodysplastic Syndromes (MDS) in the outpatient setting by determining their unmet needs and improving patient outcomes

Methods

Commencing January 2007 all MDS patients attending SVHM oncology department underwent a nursing assessment that included a psychosocial profile, brief risk screen and patient evaluation of their symptoms using the M.D. Anderson Symptom Inventory (MDASI) which was then repeated at subsequent visits over a 3 month time frame. Following the assessment and risk screening, referrals were made to members of the multidisciplinary team and community services.

Outcomes

This presentation reports on findings and evaluation of screening and its impact on patient management and quality of life

No conflict of interest to disclose

Meeting Room 9
Nursing Free Communications 2
O46

0830-1000
0930

Nursing Time Required to Administer a Red Cell Transfusion – Does the Allocated Time Accurately Reflect Reality?

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1. Australian Red Cross Blood Service (ARCBS), Southbank, Vic, Australia
2. BloodSafe Program, Flinders Medical Centre, Bedford Park, SA, Australia
3. SA Pathology, Flinders Medical Centre (FMC), Bedford Park, SA, Australia
4. Peter Mac Callum Cancer Centre (PMCC), East Melbourne, Vic, Australia

Background

The most common risk related to blood transfusion today is human error, with “incorrect blood component transfused” accounting for a large proportion of serious events from haemovigilance system reports. Transfusion administration is complex and involves many important steps. As part of process mapping in the “cost of transfusion” study, nursing care time allocated for transfusion is being prospectively studied using real time recording of nursing interventions.

Aims

To examine the methods used to calculate nursing time required for each step for administration of red cells and compare those times with administration times currently in use at two different hospitals.

Method

Process maps outlining each step for red cell administration were used as a template for timing nursing care required. Each step was timed for multiple patients in various clinical settings (e.g. inpatient, outpatient) to determine average times. Times related to “units of care” were extracted from the FMC computer nursing care planning system (Excelcare) for inpatients receiving red cells (no outpatient timings included). At PMCC inpatient care calculations are based on nurse: patient ratios of 1:5 with no further breakdown of time for procedures.

Results

Allocated Excelcare times vary from 11-27 mins per unit for inpatient collection & administration of red cells. Outpatient timings undertaken at each centre indicate variations of 30-34 minutes (including patients requiring additional precautions) for a single unit and 34-45 minutes for 2 units.

Conclusion

Time for red cell administration has been considered for inpatients at FMC in planning systems however no time is allocated for outpatients at either centre. Limited literature is available to guide nursing time allocation for red cell transfusion. Considering the real clinical risks related to transfusion, determining nursing time required for administration is important and data from the cost of transfusion study may inform realistic time allocation.

No conflict of interest to disclose

Meeting Room 9
Nursing Free Communications 2
O47

0830-1000
0945

Twenty Units of Blood and Counting....

Emma Brownrigg¹, Debbie Carr², Amanda Castles³, Michael Copeman⁴, **Kim Creighton⁵**, Catherine Demasi⁶, Julie Domanski⁷, Susan Harrison⁸, Cherry Hodges⁹, Joanne Kanakis¹⁰, Sharyn Kelleher¹¹, Joanna McCosker-Rees¹², Rachel Prall¹³, Dawn Richardson¹⁴, Patricia Ryan¹⁵, Craig Swan⁴, Susan Terry¹⁶, Julie Tovey¹⁷, **Rosita Van Kuilenburg¹⁸**, Melissa Wills¹⁹, Cassandra Slader⁴

1. Mater Children's Hospital, QLD 2. Calvary Mater Hospital, NSW 3. Ringwood Private Hospital, VIC. 4. Novartis Pharmaceuticals Australia Pty Ltd 5. Gold Coast Hospital, QLD. 6. Royal Adelaide Hospital, SA 7. Royal Darwin Hospital, NT 8. Concord Hospital, NSW. 9. Clinipath, WA. 10. Townsville Hospital, QLD 11. Royal Prince Alfred Hospital, NSW 12. Royal Children's Hospital, QLD 13. Royal Hobart Hospital, TAS. 14. Launceston General Hospital, TAS. 15. Liverpool Hospital, NSW. 16. Cairns Hospital, QLD. 17. Fremantle Hospital, WA. 18. Princess Alexandra Hospital, QLD. 19. St Vincent's Hospital Melbourne, VIC.

Iron overload is seen in patients who have received multiple packed red cell transfusion. Generally, patients who have received >20 transfused units will be at risk. Currently in Australia there is no universal method of tracking the number of transfusions a patient has received, and a cumulative figure requires manual calculation. Therefore, identification of at-risk patients is not straightforward.

Aim

Firstly, to review the primary diagnosis of transfused patients in haematology day units. Secondly, to quantify number of transfusions received and serum ferritin levels of transfused patients. Thirdly, to review the use of iron chelation in these patients and document potential reasons for non-chelation including co-morbidities and concomitant medications.

Method

Retrospective multi-centre medical record audit of outpatient transfusions during the 12-week index period (12 consecutive weeks from HREC/institution approval) were reviewed.

Results

To date, 237 patients, aged 0-95 have been reviewed from 10/20 centres. Common underlying conditions necessitating transfusion were: MDS (35%), chemotherapy-induced anaemia (21%) and thalassaemia (14%). The medical record of 26% patients indicated that transfusions had also been given elsewhere. In total, 119 had received >20units, 58 (49%) prescribed chelation therapy. Fourteen patients who had received >20units had no documented serum ferritin. Patients receiving iron chelation were younger (median 42 cf.73 years, $p=0.0004$), had received more transfusions (median 60 cf.18, $p<0.0001$) and more units (median 187 cf.37, $p<0.0001$). There was no difference in number of concurrent medical conditions ($p=0.73$) or concomitant medications ($p=0.14$).

Conclusion

Patients who have received multiple packed cell transfusions are not monitored for iron overload. Chelation may be 'underused'. The development and implementation of a universal tracking tool for packed red cell transfusion may be one means to prevent iron overload toxicities.

This research was supported by Novartis Pharmaceuticals Australia Pty Ltd. The company assisted the participating centres with the data analysis and abstract preparation.

Riverside Theatre
HSANZ: New Insights into Old Problems

1030-1130
1030

Hereditary Haemochromatosis Update

John K Olynyk

School of Medicine & Pharmacology, University of Western Australia, Fremantle Hospital Campus, Department of Gastroenterology, Fremantle Hospital, Western Australia

Hfe-related hereditary hemochromatosis is a common disorder of iron overload occurring in individuals homozygous for the C282Y *HFE* gene mutation. It can be a progressive and fatal condition. Early detection and phlebotomy prior to the onset of cirrhosis can reduce morbidity and normalize life expectancy. It is readily identified through biochemical testing for iron overload using serum transferrin saturation and genetic testing for C282Y homozygosity. Recent advances in the understanding of the regulation of iron transport and natural history of disease evolution have substantially improved our knowledge of this disorder. Hepcidin is the key stores regulator for iron status within the body. When the HFE protein undergoes the C282Y homozygous mutation, impaired protein trafficking to the cell membrane results in impaired signaling of iron status with resultant reduced production of hepcidin and inappropriate increased iron absorption. There is a large range of phenotypic expression of hereditary Haemochromatosis, with up to 28% of men and 1% of women developing iron-overload related disease by the age of 65. The underlying modifiers of genetic and phenotypic expression are unclear.

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Riverside Theatre
HSANZ: New Insights Into Old Problems

1030-1130
1100

The Role of DKK1-mediated Suppression of Wnt/ β -catenin in Multiple Myeloma

John D Shaughnessy, Jr

Myeloma Institute for Research & Therapy, UANS, Little Rock, AR, USA

Multiple myeloma (MM) has a unique and absolute requirement for the bone marrow microenvironment for its growth and survival. The molecular rules governing this requirement are now being elucidated and emerging data suggests that tumor cell-induced perturbation of the Wnt signaling pathway within the bone marrow milieu may be central to this relationship. Most cases of MM are thought to evolve through a precursor stage, monoclonal gammopathy of undetermined significance (MGUS), at a low frequency of 1-2% per year. While sharing virtually all genetic abnormalities with MGUS, a clinically distinguishing feature of MM is osteolytic bone disease that develops at sites of focal tumor growth detected by MRI much earlier in the disease course. We have shown that MM plasma cells, but not those from the healthy donors or patients with the benign plasma cell dyscrasia, MGUS synthesize and secrete dickkopf-1 (DKK1), a soluble inhibitor of Wnt signaling and that this molecule can suppress osteoblastogenesis and promote osteoclastogenesis. The molecular basis of *DKK1* activation in MM cells is not known but studies from our group suggest that thalidomide and lenalidomide induce *DKK1* expression through reactive oxygen species and JNK, suggesting that endogenous ROS may activate DKK1 in therapy naïve disease.

In addition to its role in MM, DKK1 appears to play important roles in normal bone development and other bone pathologies. A working model of how DKK1 might play a master regulatory role in lytic bone disease and tumor progression in MM is emerging. Tumor-derived DKK1 suppresses nuclear Wnt/ β -catenin signaling in MM cells driving β -catenin interaction with cadherin cell adhesion complexes enhancing cell adhesion and focal tumor growth. High local concentrations of DKK1 and FRZB suppress Wnt-dependent differentiation of mesenchymal stem cells (MSC) into osteoblasts (OB) in the local environment surrounding the focal lesion. DKK1-mediated suppression of Wnt signaling in MSC indirectly enhances osteoclastogenesis by promoting synthesis of RANK ligand (RANKL) and suppressing osteoprotegerin (OPG), a RANKL decoy. Moreover, DKK1-mediated suppression of Wnt in MSC maintains high-level synthesis of IL-6, a potent MM growth factor. Preclinical studies suggest that neutralizing DKK1 and/or enhancing Wnt/ β -catenin signaling in the bone may have clinical utility in the treatment of MM.

Meeting Room 4
ANZSBT: Cardiac and Obstetric Transfusion Challenges

1030-1130
1030

Cardiac Surgery Transfusion Issues

Jean-François Hardy

Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, Québec, Canada

Hemorrhage associated with cardiac surgery (CS) remains an important clinical problem. Numerous strategies have been recommended to decrease transfusion of allogeneic blood products (ABP) associated with CS. Considerations other than transmission of viral disease mandate that transfusion of ABP be kept to a minimum; e.g. allogeneic transfusions are immunosuppressive and associated with an increased risk of postoperative infection in patients undergoing myocardial revascularization. Strategies to reduce transfusion of ABP in adult CS will be reviewed. Some are relatively benign, but others carry their own risks that must be weighed against those involved when transfusing ABP.

Antiplatelet agents are associated with increased postoperative bleeding and should be discontinued, if possible, prior to elective surgery. However, this may not always be possible, nor advisable (because of the risk of thrombosis), especially when surgery is urgent.

Recombinant human erythropoietin (EPO) has been shown to decrease exposure to ABP in patients undergoing elective CS. Nevertheless, doubts persist regarding possible thrombotic adverse events associated with EPO in these patients. Also, it appears that EPO is not cost effective in cardiac surgery.

Prior to the BART trial, the antifibrinolytic aprotinin was believed to be the best available agent to reduce transfusions associated with CS. Several meta-analyses suggested that aprotinin also decreased the risk of stroke and repeat surgery for massive bleeding, and saved lives. However, since 2006, observational studies suggested an association between aprotinin and cardiovascular and cerebrovascular complications, renal failure and mortality. BART demonstrated a modest reduction of massive bleeding in patients receiving aprotinin compared to tranexamic acid (TA) or ϵ -aminocaproic acid (EACA) but, more importantly, an increased risk of death (from a cardiac cause; all other rates of adverse events were similar) in the aprotinin group (NEJM 2008;358:2319-31). The only antifibrinolytic agent still commonly available today is TA.

Finally, there is little evidence to support the use of recombinant activated factor VII in CS.

Meeting Room 4
ANZSBT: Cardiac and Obstetric Transfusion Challenges

1030-1130
1100

Obstetric Haemorrhage

Andrew Barr

King Edward Memorial Hospital for Women and Royal Perth Hospital, Perth, Western Australia

Severe post partum haemorrhage accounts for approximately 20% of maternal deaths, the most common aetiology being uterine atony due to inadequate myometrial contraction post-delivery. Other causes of post partum haemorrhage include genital tract laceration, retained products of conception and disseminated intravascular coagulation (DIC) either alone or in conjunction with one of the above.

Traditional definitions of post partum haemorrhage as blood loss of greater than 500ml in 24 hours post delivery and of massive haemorrhage as blood loss exceeding 10 units in 24 hours are no longer relevant in the obstetric field since obstetric haemorrhage is categorised by extremely rapid blood loss reflecting the uterine flow in late pregnancy of up to 600ml/minute.

During pregnancy, placental abruption, haemolysis and low platelets (HELLP) syndrome, sepsis and amniotic fluid embolism may all be complicated by DIC and massive blood loss.

A massive transfusion protocol is essential to streamline and standardise haematological and transfusion response to massive haemorrhage. Whereas the emphasis in recent years has been on crystalloids as initial volume replacement followed by red cells with coagulation factor replacement with fresh frozen plasma, cryoprecipitate and platelets geared to coagulation profile results, there has recently been a shift towards earlier and more aggressive blood component replacement.

Recently, recombinant factor VIIa (Novoseven) has been reported to be effective not only in controlling refractory life threatening massive haemorrhage, thereby reducing maternal mortality but enabling uterine preservation in some patients where hysterectomy may otherwise have been obligatory. Intraoperative red cell salvage procedures are now emerging as a valuable adjuvant to management of obstetric blood loss.

Meeting Rooms 2/3
ASTH: Venous Thrombosis

1030-1130
1030

Epidemiology of Venous Thrombosis

FR Rosendaal

Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

The incidence of a first venous thrombosis is 2 per 1000 individuals per year. Around two-thirds manifests as deep vein thrombosis of the leg, and one third as pulmonary embolism. Around five percent of venous thromboses prove fatal, with deaths predominantly among the elderly and patients with severe underlying disease, notably cancer. The recurrence rate of thrombosis is high at around five percent per year. Individuals from families with inherited thrombophilia tend to develop thrombosis at a young age, and to have frequent recurrences.

Venous thrombosis is a multicausal disease, which occurs when several risk factors are present simultaneously in a particular combination. This often concerns the simultaneous presence of long-term risk factors, e.g. genetic defects, and short term acquired factors. Some of the acquired risk factors are very strong, causing thrombosis in several percent of those afflicted, which implies a relative risk of 50 or higher. These are orthopaedic, neurosurgical and major abdominal interventions, major trauma with multiple fractures, central venous catheters and metastasised cancer, particularly adenocarcinomas. Moderate risk factors are antiphospholipid antibody syndrome, puerperium, prolonged bedrest and non-metastasised cancers, while pregnancy, oral contraceptive use, hormone replacement therapy, obesity and long-haul travel constitute mild risk factors, with a 2- to 5-fold increased risk.

Heterozygous antithrombin deficiency and homozygous factor V Leiden are the strongest genetic risk factors, increasing the risk of thrombosis 20- to 50-fold. Heterozygous protein C and protein S deficiencies are moderate contributors to risk, with a relative risk of 10. Other genetic factors that are associated with venous thrombosis are either mild and increase the risk 2- to 5-fold, as is the case for factor V Leiden, prothrombin 20210A and non-O blood groups. Mildly increased risks are also present for abnormalities in the coagulation system of which the origin is unclear, such as elevated levels of procoagulant factors (fibrinogen, II, vWF, VIII, IX, X, XI) and anti-fibrinolytic factors (TAFI), and low levels of anticoagulant factors (TFPI).

After a first event of venous thrombosis patients have a high risk to again experience thrombosis between 3 and 10 percent per year. Up to half of the recurrences after a first thrombosis in the leg, occur in the other leg, indicating that systemic changes rather than residual local damage are associated with rethrombosis.

Meeting Room 2/3
ASTH: Venous Thrombosis

1030-1130
1100

New Strategies for Predicting VTE Recurrence and Optimizing Treatment Duration

Timothy A Brighton

Haematology SEALS Prince of Wales Hospital Randwick & University of NSW Kensington, Sydney Australia

Recurrent vein thrombosis, either Deep Vein Thrombosis (DVT) or Pulmonary Embolism (PE), accounts for 10-20% of all cases of vein thrombosis. As with first cases of vein thrombosis, recurrent thrombosis is associated with significant morbidity and a defined mortality. Recurrent thrombosis may occur in the context of reversible factors (such as surgery, leg injury, acute illness with immobility, pregnancy and childbirth) and many of these cases should be preventable with appropriate prophylaxis. A smaller proportion of patients experience recurrent unprovoked thrombosis and are considered to have a "thrombophilia". Generally this "thrombophilia" is not measurable or predicted by laboratory assays. Patients with recurrent unprovoked vein thrombosis generally receive life-long anticoagulation. There are opportunities to reduce the occurrence of recurrent unprovoked thrombosis by extending the duration of anticoagulation after the first episode of unprovoked thrombosis. Clinical studies demonstrate that while on treatment extended duration anticoagulation is effective, but comes at a significant cost with increased major/fatal bleeding and considerable inconvenience to the patient. There are no established strategies for optimising treatment duration. Currently clinicians attempt to individualise therapy based on approximate estimates or risks of recurrence and of bleeding. Ideally some way of stratifying risk of recurrence after first unprovoked thrombosis could enable better selection of patients for observation or extended anticoagulation. Recent studies have found measuring plasma D-dimer and/or imaging for residual vein thrombosis may be promising.

Meeting Room 1
Nurses: New Approaches

1030-1130
1030

Nutritional Challenges in Haematology Patients

Wayne Epton

Royal Perth Hospital, Perth, Western Australia

Haematology patients face many challenges during their medical journey. The nutritional challenge is one that can be often overlooked, and is almost always underestimated. Most haematology patients are well nourished at time of diagnosis, however their nutritional status can deteriorate rapidly. Chemotherapy and radiation therapy can induce gastrointestinal toxicity resulting in problems such as anorexia, early satiety, changes in taste and smell, nausea, vomiting, diarrhoea and alimentary mucositis. Patients develop aversions to hospital food, which is compounded by the need to implement restrictions to minimise food borne illness during times of neutropenia. The hospital diet in conjunction with these problems greatly reduces oral intake and absorptive capacity exacerbating a decline in nutritional status. Malnutrition in oncology patients has been associated with reduced quality of life and tolerance to treatment, increased morbidity and worsened prognosis. Malnutrition prior to transplantation has been shown to be a negative prognostic factor for outcome post transplant with better nourished patients having a shorter time to engraftment. Practice guidelines for the nutritional support of patients undergoing bone marrow transplant recommend that nutritional assessment should be conducted routinely before transplant with artificial nutritional support commenced accordingly. There is debate on the best route of artificial nutrition. Traditionally the preferred route has been Total Parenteral Nutrition due to gastrointestinal dysfunction caused by treatment and complications of transplantation such as graft versus host disease. However, recent research has indicated that Enteral nutrition may be safely administered in these patients. Nutrition research is being conducted into specific immuno-modulating nutrients and their role in reducing the inflammatory response post transplantation. Antioxidants, omega-3 fatty acids and glutamine are all being examined to help modulate the immune system and reduce the side effects of transplantation.

Meeting Room 1
Nurses: New Approaches

1030-1130
1100

Nurse Practitioners – The Western Australian Experience

Susan Hyde

Sir Charles Gairdner Hospital, Perth, Western Australia

In November 2002, the *Nurses Amendment Bill 2002* was introduced into the Western Australian Legislative Assembly and then to the Legislative Council. Legislative changes allowing nurse practitioners in Western Australia to practice in designated areas came into effect on 9 April 2003.

The legislative changes, including amendments to seven Acts and one regulation, address education, registration and the development of a structural framework to allow nurse practitioners to practice in designated areas. Area designation is achieved following submission and approval of a business case and clinical protocol to the Director General of Health.

The legislation allows registered nurse practitioners working in designated areas to prescribe Schedule 1 and 4 medications, order routine pathology and diagnostic imaging tests.

This presentation will describe the role expansion from haematology clinical nurse consultant to registration and area designation of one of the first nurse practitioners in Western Australia.

The expanding role of the clinical nurse consultant involves the acquisition of new practice and skills, including knowledge and skills legitimising role autonomy within areas of practice that overlap traditional boundaries of medical practice. This model of care is based on the contribution of the multi-disciplinary team in the delivery of patient centred care.

Riverside Theatre
HSANZ: Carl de Gruchy Oration

1130-1230

Haemophilia, History and Hope

Alison Street

Haemostasis, Thrombosis & Haemophilia Unit, The Alfred Hospital, Melbourne, Victoria, Australia

People with haemophilia, alive today, reflect a remarkable history of the medical and social revolutions of our times. They form a resilient community who, in the last fifty years, have experienced dependence on astute clinical observation and demanding early generation coagulation testing, for diagnosis of their condition and limited supplies of coagulation factor concentrate which provided the yin-yang of improved length and quality of life with attendant transmission of viral infection to carry as subsequent medical and psychosocial burdens.

Since the sequencing of the F IX gene in 1981 and F VIII gene in 1984, the genetic revolution has delivered plentiful and safe product. Patients with haemophilia in Australia are now well-supported by government funding to receive treatment. Their biggest risk is development of inhibitors, secondary to product infusion. In our geographic region, however, 75% of patients are without diagnosis and/or sufficient product for care. The World Federation of Haemophilia, an international organisation with national member organisations in 113 countries, is committed to delivering hope and "treatment for all" even beyond developed countries. Many volunteers are participants in programs supporting these activities.

Riverside Theatre
HSANZ/ASTH: Combined Presidential Symposium
O48

1330-1500
1330

Sequential Treatment with Rituximab and CHOP Chemotherapy in B-cell Post-transplant Lymphoproliferative Disease (PTLD) – Will Risk Stratified Sequential Treatment Become the New Standard in Therapy?

Ralf Trappe¹, Sylvain Choquet², Stephan Oertel¹, Veronique Leblond², Tor Ekman³, Monica Sender³, **Peter Mollee**⁴, John Moore¹², Petra Reinke¹, Hans Lehmkuhl⁵, Manfred Hummel⁵, Sven Jonas¹, Ruth Neuhaus¹, Gilles Salles⁶, Franck Morschhauser⁷, Arnaud Jaccard⁸, Daan Dierickx⁹, Thierry Lamy¹⁰, Bernd Dörken¹, Ioannis Anagnostopoulos¹, Martine Raphael¹¹ & Hanno Riess¹ for the German, French, Swedish, Australian & Belgian Study Group on PTLD

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Aim

This trial aimed to investigate the efficacy and safety of sequential treatment with rituximab and CHOP-21 in patients with PTLD unresponsive to reduction of immunosuppression

Methods

In this multicenter phase II trial, patients were treated sequentially with rituximab at days 1, 8, 15 and 22 (4xR) followed by four cycles of CHOP-21 combined with G-CSF support starting 4 weeks after the last dose of rituximab (sequential treatment, **ST**). Following interim analysis a protocol amendment was introduced in October 2006. As treatment response to 4xR was shown to predict overall survival, risk stratification according to response to rituximab was introduced. In the amended protocol patients with a CR after 4xR receive 4 additional cycles of rituximab while all others will receive four cycles of R-CHOP-21 instead of CHOP-21 (risk stratified sequential treatment, **RSST**).

Results

86 patients are reported: median follow up 24.6mo(ST, n=69) and 6.4mo(RSST, n=17). Median age was 54(ST) and 60(RSST). The overall response rate of **ST** was 88% (CR 65%, PR 23%). 73.7% and 62.0% of patients included were without disease progression at one and two years, respectively(Fig 1). Following ST, 33% had WHO °3/4 infections and there were ten early deaths (17%). With **RSST** the overall response rate was 92% and 85% achieved CR. 92.9% of patients included were without disease progression at one year with no relapses so far (Fig 1). 5/15 patients (33%) had WHO °3/4 infections and there were two early deaths (16%).

Conclusions

In this largest prospective study in PTLD sequential treatment with rituximab and CHOP-21 + G-CSF is well tolerated and highly effective. As compared to rituximab monotherapy more patients achieve a CR with sequential treatment and time to progression (TTP) is very much prolonged. Our first prospective data evaluating risk stratified sequential treatment suggest even superior CR rates and further increased TTP with a similar toxicity level.

This research was supported by Roche. The company had no role in analysing the data or preparing the abstract.

Riverside Theatre
HSANZ/ASTH: Combined Presidential Symposium
O49

1330-1500
1345

Thalidomide Consolidation Further Improves the Progression Free Survival (PFS) of Multiple Myeloma (MM) Patients That Have Already Achieved a Complete (CR) or Very Good Partial Response (VGPR) Following a Single ASCT Procedure

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Aim

Thalidomide has been demonstrated to be effective in the treatment of both newly diagnosed and relapsed MM. However, the role of thalidomide in the post-ASCT context remains unclear. This study assessed whether the addition of thalidomide consolidation to maintenance prednisolone therapy following ASCT would improve the durability of responses achieved and overall survival.

Methods

Between January 2002 and March 2005, 269 patients with newly diagnosed MM who achieved disease stabilisation or better with conventional induction chemotherapy, received a single high-dose melphalan conditioned ASCT. Post-ASCT, 129 patients were randomly assigned to receive indefinite prednisolone maintenance therapy (control group) and 114 to receive the same maintenance therapy in addition to 12 months of thalidomide consolidation (thalidomide group). The primary study endpoints were progression-free survival (PFS) and OS. The secondary endpoint was tolerability.

Results

After a median follow-up of 3 years, the post-randomisation 3-year PFS rates were 42% and 23% ($p<0.0001$) and the OS rates 86% and 75% ($p=0.004$) in the thalidomide and control groups, respectively. Importantly, thalidomide consolidation led to improvement in PFS irrespective of post-ASCT response at the time of randomisation with median PFS of 931 days versus 789 days ($p=0.05$) for CR/VGPR patients, and 910 days versus 504 days ($p<0.001$) for no CR/VGPR patients, in the thalidomide and control arms, respectively. There was no difference in survival between the thalidomide and control groups 12 months after disease progression (79% versus 77%, respectively, $p=0.244$). Neurological toxicities were more common in the thalidomide arm but there were no differences between arms for thrombo-embolic events.

Conclusion

Consolidation therapy with 12 months of thalidomide combined with prednisolone maintenance prolongs PFS irrespective of post-ASCT response. Furthermore, thalidomide consolidation therapy did not adversely impact on survival in the subsequent salvage setting. Thalidomide consolidation should be offered to all MM patients following a single ASCT procedure.

This research was supported by Novartis Australia Pty Ltd. Amgen Australia Pty Ltd and Pharmion Australia Pty Ltd. The companies had no role in analysing the data or preparing the abstract.

Riverside Theatre
HSANZ/ASTH: Combined Presidential Symposium
O50

1330-1500
1400

Adoptively Transferred CMV Specific T Cells Exhibit Multiple Effector Functions Associated with Protective Immunity Including the Concurrent Production of Multiple Cytokines and Cytolytic Activity

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² Westmead Hospital, Sydney, Australia. ³ Westmead Millennium Institute, Sydney, Australia. ⁴ University of Sydney, Sydney, Australia

Introduction

Donor derived CMV specific T cells were generated for prophylactic infusion into haemopoietic stem cell transplant recipients as part of a phase I/II clinical trial aiming to reduce the incidence of CMV reactivation. CMV specific T cells were expanded by co-culturing with dendritic cells transfected with Ad5F35pp65, a recombinant adenovirus promoting the presentation of epitopes derived from the entire CMV antigen pp65.

Results and Discussion

An assay measuring antigen specific production of interferon- γ , IL-2, TNF- α and MIP-1 β by intracellular cytokine flow cytometry was established to quantify the frequency of T cells with specificity towards CMV and to assess the quality of these responses by simultaneous production of multiple inflammatory cytokines.

The proportion of T cells producing at least one cytokine in response to CMV pp65 following *ex vivo* expansion was greatly enriched (mean 59%, 19.1-90%) compared to the starting population (0.5-1.5%). T cell responses directed towards the adenovirus hexon protein were also detected in all cultures, accounting for 0.65 to 9% of the T cell response. Greater than 90% of CMV specific CD8 T cell responders simultaneously produced two or three cytokines, predominantly interferon- γ , TNF- α and MIP-1 β . IL-2 producing T cells were less frequent ranging from 0.5-40%. However, IL-2 producing cells were the most multi-functional or the highest quality, consistently producing all four cytokines examined. We have previously demonstrated CMV specific interferon- γ producing CD8⁺ T cells mobilize CD107, a marker of degranulation and cytotoxic activity. Taken together, this study demonstrates *ex vivo* expanded CMV specific T cells generated for this clinical trial are highly multi-functional with the capacity to lyse infected cells and simultaneously produce multiple inflammatory cytokines. In total, 13 CMV specific T cell products have now been infused in to transplant recipients and we aim to correlate these functional profiles with immune reconstitution *in vivo*.

No conflict of interest to disclose

Riverside Theatre
HSANZ/ASTH: Combined Presidential Symposium
O51

1330-1500
1415

The Level of Glycolytic Metabolism of AML Blasts as a Predictor of Drug Sensitivity and Clinical Prognosis

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Aim

To determine whether the extent of glycolytic metabolism of AML blasts from clinical samples is indicative of drug sensitivity and AML prognosis.

Method

Leukemic blasts were isolated from tissue banked bone marrows (BM) samples from 22 AML patients. For each BM, we determined the level of glycolytic metabolism by the % FCCP-inhibition of reduction of the water-soluble tetrazolium dye, WST-1/PMS at the cell surface. In addition, we assessed sensitivity (by annexin V/propidium iodide double staining) to 1 μ M all-trans retinoic acid (ATRA), 2 μ M arsenic trioxide (ATO), combined 1 μ M ATRA+2 μ M ATO, and 10 μ M phenoxodiol.

Results

AML blasts formed two distinct groups based on the level of glycolytic metabolism: a moderately glycolytic group and a highly glycolytic group ($p < 0.001$). Highly glycolytic blasts were more resistant to combined ATRA and ATO treatment compared with moderately glycolytic blasts ($p = 0.25$) but not to ATRA or ATO treatment alone nor to treatment with phenoxodiol, a redox-active isoflavene currently in clinical trials for solid cancers. AML blasts from 7 out of 10 BM samples taken at diagnosis were highly glycolytic, whereas blasts from 10 out of 12 BM taken at relapse were moderately glycolytic. The level of glycolytic metabolism did not change from diagnosis to relapse in three available diagnosis/relapse pairs of patients with $>80\%$ blasts in both samples. At the time of analysis, 8 out of 10 patients with highly glycolytic AML blasts were still alive whereas 11 out of 12 patients with moderately glycolytic AML blasts had died.

Conclusion

Our results suggest that the extent of glycolytic metabolism, as measured by % FCCP-inhibition of dye reduction, is a stable metabolic characteristic of AML blasts and may be of prognostic value.

No conflict of interest to disclose

Riverside Theatre
HSANZ/ASTH: Combined Presidential Symposium
O52

1330-1500
1430

Increasing Shear Rates Cause Shedding of Platelet Glycoprotein (GPVI)

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Glycoprotein (GPVI), which binds collagen, and GPIb-IX-V, which binds von Willebrand factor (vWF) and other ligands, form a unique adhesion-signalling complex on human platelets. Following vascular damage or disease, engagement of GPVI/GPIb-IX-V leads to $\alpha_{IIb}\beta_3$ -dependent thrombus formation. We previously showed ligand binding to GPVI leads to metalloproteinase-dependent ectodomain shedding, generating an ~55-kDa soluble GPVI fragment and an ~10-kDa remnant fragment that remained membrane-associated.

Aim

To determine whether shear force was sufficient to induce shedding of GPVI.

Methods

Human platelet-rich plasma or washed platelets were subjected to increasing shear rates in a cone-plate viscometer and then levels of intact and cleaved GPVI were examined by western blot using anti-GPVI antibodies raised against either the GPVI ectodomain (recognising intact and ~55-kDa soluble GPVI) or the GPVI cytoplasmic tail (recognising intact and ~10-kDa remnant GPVI).

Results

Increasing platelet aggregation was observed in platelet suspensions subjected to shear rates from 300 s^{-1} up to 3000 s^{-1} for 5 minutes and >90% aggregation was achieved using a shear rate of $10,000\text{ s}^{-1}$. Aggregation was blocked by inclusion of $10\text{ }\mu\text{g/ml}$ function blocking anti- $\alpha_{IIb}\beta_3$ (CRC64). Increasing shear rates also induced a loss of full length GPVI and the appearance of the ~55-kDa soluble GPVI ectodomain and increasing levels of the ~10-kDa GPVI remnant on platelets, and 5 to 7-fold increase in soluble GPVI in plasma by ELISA. Proteolysis of GPVI was blocked by the metalloproteinase inhibitor, GM6001, implying that shearing of platelets was sufficient to cause activation of platelet metalloproteinases, in the absence of GPVI ligands. Preliminary data further suggested that blockade of $\alpha_{IIb}\beta_3$, GPIb α or vWF minimally affects shear-induced shedding of GPVI.

Conclusions

Together, these results suggest GPVI shedding is triggered in shear-activated platelets, with potential implications for the stability of a forming thrombus at arterial shear rates.

No conflict of interest to disclose.

Riverside Theatre
HSANZ/ASTH: Combined Presidential Symposium
O53

1330-1500
1445

Warfarin Anticoagulation Can Be Effectively Reversed with Prothrombinex®-Vf without the Need for Fresh Frozen Plasma

Hatem H Salem and Lesley Poulton

Australian Centre for Blood Diseases and Eastern Clinical Research Unit, Central and Eastern Clinical Schools, Faculty of Medicine, Monash University, Victoria

Introduction

Anticoagulation with Warfarin is increasingly used for the treatment and prevention of thromboembolic events. Many of these patients require urgent reversal because of emergency surgery, active bleeding or a high risk of bleeding. In 2004, the ASTH published a consensus guideline for Warfarin reversal. The working group recommended that for immediate Warfarin reversal, Prothrombin Complex Concentrate (PCC) should be administered in combination with Fresh Frozen Plasma (FFP). The formulation of the guidelines was based on the fact that PCCs afford immediate correction of the effects of Warfarin, and that Prothrombinex-VF, the only PCC approved in Australia and New Zealand for Warfarin reversal at the time of the guidelines, is a concentrate containing factors II, IX and X, and low levels of factor VII. Although not tested in a clinical study, the working group deemed the adjunct use of FFP essential as a source of factor VII. This study addresses the need to for FFP as an adjunct to Prothrombinex-VF in Warfarin reversal.

Study design and patients

This study included patients in need of urgent Warfarin reversal (n=19) as well as patients on Warfarin who were at high risk of thrombosis and needed elective surgery (n=6). The study plan was to obtain a baseline INR and for the reversal to be performed using Prothrombinex-VF at a dose of 25-40 U/kg BW and an INR obtained thirty minutes after the completion of the infusion. If Warfarin reversal was inadequate, patients would receive two to three units of fresh frozen plasma with a repeat INR thirty minutes later. Treatment-related complications were noted throughout the period of hospitalization, and until their discharge.

Results

A total of 29 Warfarin reversals in 25 patients were carried out. Indications for reversal included the need for urgent surgery in 11, marked prolongation of the INR and a perceived risk of bleeding in 3, active bleeding in 6, and a high risk of thrombosis with a need for a planned surgical procedure in 9 patients. The pre-treatment INR varied from 1.8-10. Following the administration of Prothrombinex-VF the INR corrected fully (0.9-1.1) in 18, and was below 1.5 in 8 patients. In three patients with very high INR, the infusion resulted in considerable correction of the reading (1.6 in one and 1.7 in the other two). None of the patients required infusion of fresh frozen plasma. No bleeding was noted during or after surgery, and in the 6 patients with active bleeding the problem settled.

Conclusion

Prothrombinex-VF effectively reverses the effects of Warfarin enabling rapid control of bleeding and the conduct of safe surgery. Adjunct infusion of fresh frozen plasma is not required.

This research was supported by an unrestricted educational grant from CSL. The company had no role in analyzing the data or preparing the abstract.

Meeting Room 4
ANZSBT: Contingency Planning

1330-1500
1330

National Blood Supply Contingency Plan (NBSCP)

Stephanie Gunn¹, Julie Bland¹; Joanne Pink²; Ashley Eccles³ and Karen Botting³
¹. *National Blood Authority, Canberra, ACT Australia.* ². *Australian Red Cross Blood Service (ARCBS), Melbourne, VIC, Australia.* ³. *Department of Human Services, Melbourne, VIC, Australia*

Background

Under the *National Blood Authority Act 2003* and the National Blood Agreement signed by all Governments, the NBA is required to establish contingency and mitigation measures to ensure the security of supply of blood and blood products. The NBA asked key stakeholders to assess the likelihood and potential impact of the effect of possible scenarios on supply and demand, which was consistent with risk standards. Possible scenarios, available alternative product arrangements, and the effectiveness of strategies to reduce risk were analysed.

The Plan

An agreed contingency arrangement was endorsed by all governments. It describes:

- supply and demand risks;
- appropriate mitigation strategies;
- triggers and response to key product shortages;
- integration with broader health emergency management arrangements; and
- roles and responsibilities.

Response

The response involves three levels of accountability. Nationally, the NBA gathers and communicates information between governments and suppliers. Operationally, suppliers will manage activities around collection, manufacture, distribution and interface with the clinical community. Clinically, governments do not pre-determine the treatment of patients requiring blood and blood products. The assessment of patient requirements in the context of individual needs and the capacity of their facilities to provide that treatment is extremely important.

Institutions are encouraged to establish Emergency Blood Management Plans (EBMP) that:

- provide effective integration between hospitals and pathology services;
- articulate how the institution will coordinate the blood and blood product related crisis, including regular reporting on stock levels,
- include a process to vet all requests for products against a prioritisation schedule and identify alternative treatments and therapies, where applicable.

Meeting Room 4
ANZSBT: Contingency Planning

1330-1500
1350

1350

Australia's National Haemovigilance Project

Simon Towler¹; Jennifer Roberts²; Stephanie Gunn²; Ray Carty²
1 Haemovigilance Project Working Group, 2 National Blood Authority

Background

Under the National Blood Agreement, the National Blood Authority (NBA) must "facilitate the development of national data systems for safety and quality...". Described is the approach the NBA has taken in accessing and reporting blood-related adverse event information.

Aim

To develop a national haemovigilance reporting framework in Australia.

Methods

A national haemovigilance project working group (HPWG) was established. The HPWG agreed and defined a national minimum dataset of serious adverse events which was endorsed by the Jurisdictional Blood Committee. Jurisdictions were asked to report their state based transfusion-safety reporting capabilities and provide data from their existing reporting systems. A report, describing the contributions of each jurisdiction was published.

Results

All jurisdictions reported active transfusion-safety programs of varying sophistication. Four submitted blood-related incident data. Two provided validated data from existing transfusion-specific reporting systems. Approximately 65% of more than 600 reported incidents related to procedural errors, predominantly near-patient misidentification. Reports of transfusion reactions were also dominant.

Conclusions

The following policy recommendations were made:

1. Establishment of an enduring haemovigilance program to realise improvements in transfusion safety and quality through improved collaboration and standardisation of national reporting;
2. Improved data collection and quality through enhanced validation, improved recognition of TRALI and better collaboration with holders of other national quality data sets;
3. Improved procedures and processes with an emphasis on minimising overnight transfusions, proficiency training and competency testing, particularly in near-patient activities, procedural audits and active adoption of universal specimen labelling and patient identification protocols; and
4. Promotion of stronger awareness of and appropriate adoption of patient blood management through government and clinical sector collaboration to reduce unnecessary exposure to blood and associated risks.

Acknowledgements

The Haemovigilance Project Working Group and all jurisdictions are gratefully acknowledged.

Meeting Room 4
ANZSBT: Contingency Planning

1330-1500
1420

Protecting the Blood Supply From Emerging Pathogens: The Role of Pathogen Inactivation

Morris A Blajchman

McMaster University and Canadian Blood Services, Canada

At the present time it is widely considered that the blood supply is the safest state it has ever been. This widely-held view by both transfusion medicine professionals and the lay public is important particularly in light of the transfusion-related epidemics of HIV and HCV of the 1980s and 1990s. Nonetheless, adverse effects, both infectious and non-infectious of allogeneic blood transfusions continue to occur, some with very serious outcomes, including death. Apropos of the latter, approximately 100 transfusion-related deaths are reported annually in the United States, the vast majority of which are not due to transfusion-transmitted infections (TTIs). Less than 10% are due to TTIs! There are, nonetheless, widely held concerns that new microbial agents could emerge to threaten the blood supply to cause significant morbidity and mortality to allogeneic transfusion recipients. One of the major forms of TTIs occur in recipients of platelets that may be contaminated with bacteria. These events are being observed even though, since March 2004, platelet components in North America and elsewhere have been screened for the presence of bacteria. Deaths due to bacterial sepsis thus continue to be reported worldwide, both because RBC units are not screened for bacteria and because the available methods for screening platelets for bacteria are suboptimal. The latter are being screened at 24 hours post platelet preparation, a time that the small numbers of organisms present in a platelet unit might not be detected. Finally, over the past ten years, various other microbiological agents have emerged as potential threats to the blood supply. These include: Chagas Disease, malaria, vCJD, dengue virus, etc. At the present time, most strategies that have been implemented for preventing TTIs have been primarily reactive. Recently however, pathogen inactivation (PI) technologies, that can be considered as representing a proactive approach to TTIs, have become available. Such technologies exist for the treatment of plasma and platelets, but not yet for RBCs, to prevent TTIs. Pathogen inactivated plasma and platelets have begun to be used widely, particularly in Europe. A consensus conference dealing with the implementation of pathogen inactivation was held in Toronto in March 2007. The Consensus Panel recommended the implementation of pathogen inactivation technology "when a feasible and safe method to inactivate a broad spectrum of infectious agents is available". This statement signaled a fundamental change to the existing safety paradigm for Transfusion Medicine, one that encourages a proactive strategy against existing and emerging pathogens. During his presentation, Dr. Blajchman will provide a status report on the available PI technologies as well as summarizing the proceedings of the Canadian Consensus Conference on PI held in 2007.

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Meeting Room 1
Nurses: Transplantation

1330-1500
1330

Graft-versus-host Disease: Prevention, Treatment, Acute, Chronic, Still a problem

Jeff Szer

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Graft-versus-host disease (GVHD) is an enigmatic problem seen after allogeneic haemopoietic stem cell transplantation (HSCT). The enigma is twofold: while risk factors for the development of GVHD are well established (donor-host HLA disparity, stem cell source, quality of prophylaxis, recipient age) it is still difficult or impossible to predict with any certainty those patients likely to develop life-threatening disease and there remains the dichotomy between the adverse consequences of GVHD and the disease controlling or eradicating effects of the graft-versus-host immunological reaction.

Acute GVHD is traditionally described as occurring during the first 3 months after HSCT. Target organs include the skin, liver and gastrointestinal tract and the well-characterised cytokine storm is fundamental to these damaging effects. Prophylactic regimens include a wide variety of immunosuppressive drugs delivered before during and immediately after the transplant and experimental techniques such as extracorporeal photopheresis (ECP). Central to all of these is the degree of HLA matching of the HSCT donor. Despite this and with all other factors being apparently equal, aging patients develop more GVHD than their younger counterparts. Reduced intensity conditioning transplants have not had a major impact on GVHD risk but have changed the traditionally accepted time course, with delayed onset of otherwise typical acute GVHD.

Chronic GVHD is defined as occurring beyond 3 months after HSCT and is a protean disease resembling many autoimmune conditions but generally sparing the kidneys. The use of peripheral blood cells as a stem cell source is generally reported as increasing the risk and severity of chronic GVHD but is associated with a reduced relapse risk for patients transplanted for advanced malignancy. An increasing problem in recent years is sclerodermatous chronic GVHD. This form of the condition may produce disabling consequences and is a major therapeutic challenge. Chronic progressive lung disease as a consequence of chronic GVHD is in the same category.

Newer immunosuppressive drugs such as pentostatin, mycophenolate, sirolimus and others (including the newly characterised iMids) are being investigated both as preventive and therapeutic interventions. Experimental agents including ECP and infusions of mesenchymal stromal cells are the subject of active investigation to improve responses and outcomes. Umbilical cord blood cells in adults may reduce the risk of GVHD while rHu-G-CSF-stimulated bone marrow cells may increase the GVL effect without increasing GVHD.

There is still much to learn about GVHD, a unique man-made disease with life-threatening and life-saving consequences.

Meeting Room 1
Nurses: Transplantation

1330-1500
1400

ALL – Classification and Treatment in Young Adults

Sarah Steed

Royal Perth Hospital, Perth, WA, Australia

Abstract not received at time of going to print

Meeting Room 1
Nurses: Transplantation

1330-1500
1430

HEADSS UP: Recognising and Responding to the Needs of Young People Receiving Treatment for Haematological Malignancies

Meg Plaster

Princess Margaret Hospital, Perth, WA, Australia

The challenges, psychological and social turbulence that mark the unique stage of life known as adolescence are critical hallmarks of development from childhood to adulthood. Adolescence is a period of life that is difficult to define chronologically, and in modern western societies extends much longer than in generations gone by; well beyond the teenage years for most. Key developmental tasks include social identification, formation of a sexual identity, emancipation from parents/carers, progression towards financial independence and development of a vocational/professional direction. A malignant diagnosis and its sequelae during this stage of life present unique challenges to the young person's achievement of many of these tasks, with the potential for significant ongoing physical and psychosocial implications over the remainder of the individual's productive life.

Many young people with cancer share common needs that are significantly determined by their developmental age and life stage. A sound understanding of these developmental tasks is necessary for health care professionals to plan, and provide care and support to young people receiving treatment for cancer, and to anticipate the hurdles that might affect the treatment journey. A comprehensive, age-appropriate psychosocial and needs assessment, such as the HEADSS assessment, is an important part of understanding the individual to plan care. Patient-centred care with some flexibility and negotiation is essential to avoid delays to treatment, improve adherence and to help target those at risk of immediate and longer term sequelae that would benefit from additional support from the age-appropriate services being developed around the country. Treatment modalities and disease outcomes between different haematological malignancies vary and the way in which services are currently provided requires some examination to determine the most suitable models of age-appropriate care for this population. Each of these settings presents different challenges to the young person's ability to adapt to and cope with the disease and treatment. The nuances of providing care in different settings presents diverse and often insidious challenges for the multidisciplinary team of health care professionals supporting the young person through diagnosis, treatment and beyond. Comprehensive, age-appropriate assessment is fundamental to recognising and responding to the unique needs of this population and can be universally undertaken regardless of treatment setting or model of care.

This paper will explore the unique challenges faced by young people that are further complicated by a haematological malignancy and the necessary treatment and provide strategies to assess and work with young people for improved outcomes.

Riverside Theatre
HSANZ: Lymphoma

1530-1630
1530

Should We Further Centralise Haematology Services?

Simon Rule

Chair BCSH Haem Onc Committee, UK

In the UK, the British Committee for Standards in Haematology (BCSH) produced guidelines on the provision of facilities for patients with haematological malignancies and severe bone marrow failure in 1995. This laid down the minimum requirements needed to deal with 4 levels of complexity, from simple chemotherapy through to allogeneic transplantation. These have provided the basis for the organisation of services around the country. Since then, a number of important developments have taken place in the provision of care for patients with cancer in general and for those with haematological malignancies in particular. These include legal changes to the hours worked by junior doctors, the loss of specific haematology junior staff cover at night, working patterns of consultants, some recent very complex trial regimens for leukaemia and aggressive lymphoma and a general trend towards centralisation of cancer services. An extensive consultation process has been taken with all haematologists with the vast majority in favour of new definitions being developed. This talk will outline the on-going discussions, the proposed levels and how they are defined.

Riverside Theatre
HSANZ: Lymphoma

1530-1630
1600

Lymphomatoid Granulomatosis (LYG) and Other EBV Lymphoproliferative Disorders (LPD)

WH Wilson

National Cancer Institute, Bethesda, Maryland, United States

EBV LPD's occur in immuno-compromised settings and include senial, MTX-associated, HIV-associated, post-transplant-associated and LYG LPD. Immune dysfunction is likely permissive of EBV+ B cell expansion and progressive genetic abnormalities. LYG is a model of EBV dysregulation. It is an angiocentric-destructive process with EBV+ B-cells and reactive T-cells. LYG is graded I-II (rare-moderate large EBV+ B-cells) (usually polyclonal or oligoclonal) and III (numerous large EBV+ B-cells (usually monoclonal). Historically, steroids and/or chemotherapy have a 14 mos median survival. We are investigating Interferon- α (I- α) for grade I/II and DA-EPOCH +/-Rituximab for grade III LYG. Characteristics of 40 pts are: male 65%; median age (range) 46 (18-67) and median ECOG P.S. 1 (0-3). Disease sites include lung 98%, CNS 30%, kidney 23%, skin 23%, liver 20% and nodes 8%. LYG grades are I-28%, II-20% and III-52%. Prior treatment was none-30%, chemotherapy+/- R-35%, and steroids alone-30%. In grade I/II, I- α begins at 7.5 million IUs TIW and escalates as tolerated until disease regression and continued 1 yr after CR. Of 27 pts, 56% are in CR at a median of 52 mos (3-153). In 12 pts who progressed on I- α , grade III was found in 9. Thus, in 19 pts with only grade I/II, 84% achieved sustained CR with I- α . In 11 evaluable pts with CNS disease, 64% achieved remission with I- α alone. 15 pts completed DA-EPOCH \pm R with 40% CR. At 46 mos median F/U, OS and PFS are 69% and 82%, respectively. Median EBV VL are 18 copies/ 10^5 genome equivalents (0-22727)(normal<200). In 12 CR pts, CD8 cells increased a mean of (131 \pm 44) ($p^2=0.013$) but not CD4 cells (65 \pm 75) with treatment. I- α produces sustained remissions in grade I/II LYG, and is effective in CNS LYG.

Meeting Room 4
ANZSBT: Solid Organ Transplantation

1530-1630
1530

Renal Transplantation: HLA, ABO and Other Challenges

Shlomo Cohney

Department of Nephrology, Royal Melbourne Hospital, Melbourne, Victoria, Australia

Renal Transplantation is the treatment of choice for End Stage Kidney Disease, adding decades of life to patients with renal failure. Cellular rejection occurs in only 10% of patients, and is rarely a cause of graft loss. In contrast, rejection due to anti-HLA antibodies remains a significant cause of graft loss. These antibodies and antibodies directed against ABO blood group antigens have remained a barrier to live donor transplantation in 25% to 35% of cases. The development of diagnostic criteria and a management strategy for antibody mediated rejection (AbMR) has reduced early graft loss, with the use of IVIG being pivotal. The ability to overcome AbMR renewed interest in overcoming antibodies pre-transplantation. A positive cross match (due to donor specific anti-HLA antibodies) has previously been an absolute contraindication to transplantation, but protocols using plasmapheresis and IVIG have led to success rates of 85% to 95%.

The absence of cadaveric renal transplantation in Japan necessitated the development of ABO blood group incompatible transplantation (ABOi) twenty years ago. However, it included splenectomy and immunosuppression greater than that used in "compatible" transplantation, and was accompanied by an excess of early graft loss. More recently, ABOi programmes began in the United States and Sweden, splenectomy being replaced by the use of Rituximab, and results in all centres improved. Sweden introduced the use of blood group antigen specific immunoadsorption columns (Glycorex, Glycosorb), avoiding the complications associated with plasmapheresis.

Since December 2005, 35 patients have successfully undergone ABOi at Royal Melbourne Hospital (RMH) with the protocol being refined so that patients receive immunosuppression virtually identical to that administered to "compatible" transplants with the exception of antibody removal. Patient and graft survival stand at 100% with a median follow up of 14 months. The advent of ABOi has enabled a doubling of renal transplant recipients at RMH despite a fall in transplant numbers elsewhere in Australia. Propagation of ABOi throughout Australia should significantly increase transplant activity, reduce transplant waiting times and increase longevity for all Australians with ESKD.

ABSTRACTS - Monday 20



Meeting Room 4
ANZSBT: Solid Organ Transplantation

1530-1630
1600

Solid Organ Transplantation: Transfusion Laboratory Considerations

Chris Hogan

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Australia*

Abstract not received at time of going to print

Meeting Room 2/3
ASTH: Challenges in Haemophilia

1530-1630
1530

Gene Transfer to Cure Haemophilia

David Lillicrap

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Haemophilia remains a prime candidate for the successful application of somatic cell gene transfer. As a recessive monogenic disease in which minor increments of clotting factor levels significantly reduce the risk of spontaneous bleeding the potential benefits of FVIII and FIX gene transfer are substantial.

Over the past two decades, major advances have been made in the development of gene transfer technologies. Hundreds of hemophilic mice have now been treated successfully with a variety of gene transfer protocols to generate expression of therapeutic levels of clotting factor for many months. In addition, approximately 100 hemophilic dogs have also been treated effectively with gene transfer, with some animals now being followed for more than 6 years since the transgene administration. Thus, therapeutic successes in both small and large animal models of haemophilia are now achievable with several transgene delivery systems.

To date, 43 human haemophiliacs have been treated in six small clinical studies. These protocols have involved three different viral vectors (adenovirus, AAV and retrovirus) and, in one trial, the use of ex vivo electroporation of autologous fibroblasts. Although no serious adverse events have been documented in these studies, only very minor (2-4%) increments of clotting factor levels have been documented for very brief periods of time (several days). The one exception to this pattern has been the result obtained in one patient treated in the most recent AAV "liver" trial in which a peak FIX level of 12% was obtained, and therapeutic FIX levels were maintained for 6 weeks. In this case, extinction of FIX expression was coincident with CD8+ T cell-mediated cytotoxicity against transduced hepatocytes.

Future haemophilia gene transfer studies will attempt to minimize host immune responses to the vector and transgene product through the incorporation of transient immunosuppression and the use of vector serotypes to which pre-existing immunity is less prevalent.

Meeting Room 2/3
ASTH: Challenges in Haemophilia

1530-1630
1600

Why Do Inhibitors Develop in Haemophilia?

Mark P Smith

Canterbury District Health Board, Christchurch, New Zealand

Inhibitor formation results from vaccination, in the course of therapy, of the haemophilia A patient with FVIII, 10 – 15% of patients acquiring lasting immune memory for FVIII. When viewed as a vaccination strategy, FVIII seems poorly immunogenic, with factors optimising immune recognition including the patient's *F8* gene mutation, the patient's immune response genes and environmental parameters. FVIII epitope mapping describes the technique of determining substructures on the FVIII molecule recognised by the immune response. Scandella pioneered this technique, demonstrating that some human anti-FVIII antibodies recognize a common C2 domain epitope which overlaps the phospholipid-binding site. More recently research has focused on the association between epitope specificity and Immune Tolerance Induction (ITI) outcome. An analysis of inhibitor patients found C2 light chain specificity in all of 11 patients studied, regardless of ITI outcome, whereas A2 specificity occurred only in several cases of failed or partial ITI. This is the first indication that the diversity of the immune response, reflected in epitope specificity, may be a predictor of what will happen to immune memory during ITI. Immune response can also be measured by the diversity of variable region immunoglobulin genes recruited in the inhibitor response. Use of these methods in study of congenital haemophilia may give further insight into the biology of FVIII inhibitors at crucial time points, including at inhibitor diagnosis and during tolerance induction.

A fundamental and likely outcome of research into the biology of the immune response to FVIII will be identification of key predictive parameters that will allow planning of optimal ITI strategies as well as more cost efficient utilisation of resource for tolerisation.

Meeting Room 1
Nurses: Cord Blood

1530-1630
1530

The Role of the Cord Blood Bank Midwife ...

Jenny Forrester

BMDI Cord Blood Bank, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria 3052 and the Angliss Hospital, Ferntree Gully, Victoria

The BMDI Cord Blood Bank (BMDI CBB) in Melbourne is a collaborative project between the Bone Marrow Donor Institute, Murdoch Children's Research Institute and the Royal Children's Hospital. There are three public CBB's in Australia, together forming the AusCord network of CBBs.

Umbilical cord blood, traditionally discarded, is rich in haematopoietic stem cells and can be used as an alternative to bone marrow for the treatment of leukaemia and other serious blood disorders. It is easily and painlessly obtained.

Cord Blood (CB) donation to the public CBB network is a voluntary, informed consented procedure performed by specially trained BMDI CBB collection staff, all of whom are both registered nurses and midwives. The collection staff are involved in all facets of CB donation including recruitment, enrolment, medical assessment according to a strict criteria, obtaining consent, collection of both CB and maternal blood and a later six month follow-up. This process is aimed at minimising the risk of transmission of infectious and genetic diseases, ensuring that the safety of the donor is not compromised and to produce a product of high quality to maximise the rate of a successful transplant outcome.

The collection of CB by specially trained CBB staff midwives results in significantly higher CB volumes and cell counts, regardless of mode of delivery, when compared to non-staff midwives. The median volume of CB collected for banking is 99 ml (range 56 – 239 ml, n = 773 CBU). To date, more than 8,028 donations have been banked at the BMDI CBB and over 200 patients have received transplants from our CBB in eleven years of Cord Blood banking.

Meeting Room 1
Nurses: Cord Blood

1530-1630
1600

The Expanding Role of Cord Blood Transplantation

Ken Bradstock

Blood and Marrow Transplant Service, Westmead Hospital, Westmead, NSW, Australia 2145

Around 30% of patients with hematologic malignancies or bone marrow failure disorders requiring an allogeneic hemopoietic cell transplant do not have an identifiable adult donor suitably matched for major histocompatibility antigens. Owing to the greater degree of mismatching permissible, the use of banked unrelated umbilical cord blood has emerged as a feasible option for these patients. However, the major factor limiting the wide application of unrelated cord transplantation, particularly in adults, is the low number of stem cells present in banked cord blood units, relative to the size of the recipient. Data from transplants using single unrelated cord blood units has shown clear relationships between both nucleated cell doses and the degree of HLA-mismatching, and a number of transplant outcome parameters, including rates of neutrophil and platelet engraftment, transplant-related mortality, survival and disease-free survival. Algorithms linking the interaction between cell dose and HLA-matching with transplant outcome have been proposed, to assist with cord unit selection, but significant unresolved issues remain. This talk will present data on unrelated cord blood transplants for adults at a single Australian centre, highlighting the above issues, and discuss strategies for improving outcome, including double cord unit transplantation, co-transplantation of facilitator cells or third party hemopoietic stem cells, ex vivo cord unit stem cell expansion, and the use of reduced intensity conditioning therapy. These measures have the potential to reduce the toxicity and risks of cord blood transplantation, allowing more patients with serious hematologic disorders to benefit from allogeneic transplantation.

No conflicts of interest

Meeting Room 6
HSANZ: Masterclass

1730-1830

CLL, Where Have We Come From and Where Are We Going

Michael J Keating

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The alkylating agents were the standard of care up until the mid-1980s. The discovery of the activity of purine analogs such as fludarabine and cladribine led to more effective and potent therapy without curative potential. More recently it is obvious that the combinations of purine analogs and alkylating agents have led to even more effective treatment of patients with CLL with higher response rates and longer progression-free survival (PFS). The monoclonal antibodies, rituximab and alemtuzumab add to the armamentarium. The combination of rituximab (R) with either fludarabine (F) or fludarabine and cyclophosphamide (FC) has led to programs which routinely get complete remissions in the range of 50 – 70%. Remissions achieved with chemoimmunotherapy are prolonged and patients experience very good health during this time. The major role of alemtuzumab to-date has been in the management of minimal residual disease. Our ability to identify residual CLL cells by flow cytometry or PCR techniques has led to clinical trials with intravenous and eventually subcutaneous alemtuzumab. The subcutaneous route is not associated with remissions of the same duration as the I.V.

The chemoimmunotherapy strategies have allowed us to identify the role of new prognostic factors in outcome. While it is now established that FISH cytogenetics, mutation status, ZAP-70, and CD38 expression are important in predicting probability of CLL progressing and overall survival, the relevance of these prognostic factors with different chemotherapy programs is ill defined. Recent analysis has identified clearly that patients with 17p deletion on FISH who progress and need therapy have an inferior outcome to other patients. Also it is apparent that the mutation status of the immunoglobulin gene (IgVH) is the best predictor of probability of remission duration on FCR programs.

The development of non-ablative stem cell transplant emerged from CLL studies. It is now apparent that NST can be applied to patients up to 75 years of age and possibly beyond. This is associated with curative potential and the potency of the graft versus leukemia effect challenges us to develop more effective immunotherapeutic strategies in the management of this disease. New drugs of many classes are emerging with promise in CLL. The eventual role of these agents in management of this disease should lead to enhancing the curative potential in CLL.

Meeting Room 7
HSANZ: Masterclass

1730-1830

Conditioning Therapy for Allogeneic Hematopoietic Cell Transplantation

H Joachim Deeg^{1,2} and Bart Scott^{1,2}

¹Fred Hutchinson Cancer Research Center and ¹University of Washington School of Medicine, Seattle, WA, USA

The objective of conditioning for transplantation differs between diseases. In nonmalignant inherited or acquired marrow or immunological disorders, the intent is to provide functional stem cells that can replace or complement lymphohematopoietic function in the patient. While engraftment of donor cells is essential, persistence of part of the host's cells may be acceptable. Therefore, the least toxic regimens have been used that allow for engraftment and immunologic and hematopoietic reconstitution. The ideal of establishing mixed chimerism without graft-vs.-host disease has been achieved in the minority of cases. Most patients, however, are being transplanted for clonal malignant disorders of the lympho-hematopoietic system. Therefore, the intent is to ablate marrow function in the host and replace it by donor-derived cells. This is true even in the so-called "nonmyeloablative" transplant strategy, a more appropriate term for which is "reduced-intensity" conditioning. In the interest of truly advancing the field, it might be best to recognize an entire spectrum of conditioning regimens using, for example, only 200 cGy of total body irradiation (TBI) or regimens combining high-dose TBI with chemotherapy agents. Our goal always is to minimize toxicity/mortality and optimize efficacy, that is, prevent occurrence of the underlying disorder. The immunosuppressive drug, fludarabine, has found increasing application, combined with TBI, melphalan, or other compounds. Ongoing work suggests that rather than focusing narrowly on a transplant conditioning regimen, one should view the entire "package," including possible pre-transplant chemo- or immunotherapy, and post-transplant modifications, be it in the form of immunosuppressive drugs, antibodies, modification of regulatory T cells, infusion of NK cells, or other means. To improve the success rate, it may not be necessary to develop entirely new modalities, but rather to combine available strategies in the most effective way. Newly developed agents, such as tyrosine kinase inhibitors, epigenetic modulators, and others, may prove to be important adjuvants in this setting.

Meeting Room 4
ANZSBT: Masterclass

1730-1830

Age of Red Cells for Transfusion: Is Fresh Best?

Panel:

Morris Blajchman, Canada

Jean-François Hardy, Canada

Age of Red Cells: Is Fresh Best?

Jean-François Hardy

Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, Québec, Canada

Red blood cells (RBC) are used to compensate hemoglobin losses and maintain oxygen transport and delivery. Despite their benefits, RBC may be harmful in some clinical situations and the "storage lesions" of banked blood, in addition to the accumulation of biological by-products in the transfused units, have been incriminated. The storage lesion is characterized by a multitude of biochemical and biomechanical changes and may lead to tissue hypoxia by various mechanisms: abnormality of oxygen-Hb dissociation and decreased unloading of oxygen in the tissues; loss of the RBC's normal shape and deformability, leading to inability of the RBC to traverse the microcirculation; increased adhesion to the vascular endothelium, thereby reducing microvascular flow. These changes alone may be insufficient to cause harm but, in severely ill patients, they may exacerbate preexisting microcirculatory dysfunction (such as in sepsis) and further impair oxygen delivery. A number of retrospective studies (including very recent data in cardiac surgery patients - NEJM 2008;358:1229-39) have documented an association between prolonged storage of RBC and an increase in mortality, pneumonia, serious infections, multi-organ failure and length of stay (Transfusion 2006;46:2014-27). On the other hand, 3 small prospective randomized trials have been unable to demonstrate a benefit from the use of fresh blood. In 104 infants, the use of fresh whole blood in heart surgery had no advantage over reconstituted blood (NEJM 2004;351:1635-44). Transfusion of RBC ≥ 20 days old had no adverse effect on gastric tonometry in 22 euvoletic, anemic, critically ill patients compared to RBC ≤ 5 days (Crit Care Med 2004;32:364-71). Finally, clinical outcomes were similar in a pilot study evaluating the clinical effects of prolonged storage of red cells in 57 critically ill and cardiac surgical patients (Anesth Analg 2005;100:1433-8). Large clinical trials on this controversial issue are, therefore, warranted. Demonstration that fresh RBC are best would, no doubt, have major implications on blood procurement services.

ABSTRACTS - Monday 20



Monday 20 October
ASTH: Masterclass

1730-1830

Publishing Without Perishing

David Lane, UK

NOTES:

Meeting Room 1
Nurses: Workshop

1730-1830

Venous Access Devices: "What is the role of the Nurse?"

Cathy Smyth & Barbara O'Callaghan

CNC IV Therapy SCGH

With more treatments being offered intravenously there is an increased need for central venous access. This places more responsibility on the nurse in caring for and helping to avoid complications associated with long term access.

This presentation explores how we as nurses can minimize the risk of complications associated with these devices.

It also explores various issues surrounding the insertion of these devices by specialist teams, Who should make the decisions regarding insertion, type of device and when they should be inserted. Are doctors or nurses better armed to make these decisions? Is there really more than one way to care for a device?

ABSTRACTS - Monday 20



NOTES

Riverside Theatre
HSANZ: Normal and Malignant Haemopoiesis

0830-1000
0830

Management of MPD in the JAK2 Era

Anthony Green

University of Cambridge, Department of Haematology, Cambridge Institute for Medical Research, Cambridge, United Kingdom

The myeloproliferative disorders (MPDs) have long been something of a Cinderella subject within clinical haematology. This situation reflected the lack of robust diagnostic tools together with the absence of randomised data on which to base management decisions. Recent molecular insights and randomised clinical studies have combined to shed much needed light on the management of these fascinating diseases.

The discovery of JAK2 mutations has not only illuminated the pathogenesis of the MPDs, but has also provided a powerful diagnostic tool. Allele specific PRC for the V617F mutation has greatly simplified the diagnosis of polycythaemia vera and has also provided a positive diagnostic test for approximately 50% of patients with essential thrombocythemia or idiopathic myelofibrosis. In addition tests for JAK2 exon 12 and MPL mutations are now becoming available. These advances are dramatically altering diagnostic practice but they are also having a more profound effect. Previously unrecognised sub-categories of the MPDs have been revealed, and these insights are rendering current terminologies obsolete and are resulting in an improved understanding of the relationship between different MPDs.

Several randomised studies have refined our approach to management over the past five years. The ECLAP study showed that aspirin is beneficial in patients with polycythaemia vera. The PT-1 high risk study demonstrated that patients receiving hydroxyurea do better than those receiving anagrelide. Detailed follow up of the PT-1 cohort is shedding light on the natural history of essential thrombocythemia, the relationship of essential thrombocythemia and polycythaemia vera and is also questioning the utility of the WHO classification together with the existence of pre-fibrotic myelofibrosis so far the molecular advances have not dramatically affected management options for individual patients, but JAK2 inhibitors and other targeted therapies are already in phase I studies, and the results are eagerly awaited.

Riverside Theatre
HSANZ: Normal and Malignant Haemopoiesis

0830-1000
0910

Mutagenesis Screens for Genetic Dissection of Haematopoiesis

Warren Alexander

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Haematopoiesis is an exquisitely controlled process that coordinates the precise maintenance of normal numbers of multiple lineages of functionally distinct blood cells as well as reserving sufficient latent capacity for rapid cell production in times of acute need, such as infection or bleeding. A detailed understanding of the molecular regulation of hematopoiesis provides insights not only into healthy blood cell production and function but also the processes that go awry in leukaemias and inflammatory diseases. We have established large-scale mutagenesis screens in mice to comprehensively dissect the molecular regulation of blood cell production. Initially we have focussed on production of blood platelets, the small anuclear cells crucial for blood clotting and several alleles that alter circulating platelet number in wild-type mice have been isolated and characterised. In a separate screen we have used mice that lack c-Mpl, the receptor for thrombopoietin (TPO). Because *Mpl*^{-/-} mice have compromised hematopoietic stem cell capacity and defective megakaryocytopoiesis, this screen is a suppressor screen for isolation of mutations that ameliorate thrombocytopenia or overcome stem cell deficiency. Since compensatory mechanisms operate in *Mpl*^{-/-} mice to allow normal production of mature circulating blood cells of non-platelet lineages, this screen is also sensitised to allow detection of subtle mutations in stem cells or these other mature lineages. A number of mutant pedigrees have emerged including dominantly inherited mutations in the transcriptional regulators c-Myb/p300 and the epigenetic regulator Suz12 that significantly increase platelet counts in *Mpl*^{-/-} mice. Alleles regulating stem cell activity, including a mutation in the *Erg* gene, which is associated with leukaemia and other cancers in humans, have allowed novel insights into hematopoietic stem cell activity.

Riverside Theatre
HSANZS: Normal and Malignant Haemopoiesis

0830-1000
-935

Molecular Monitoring in Clinical Practice for Chronic Myeloid Leukaemia

Susan Branford

Institute of Medical and Veterinary Science, Adelaide, South Australia

The hallmark of chronic myeloid leukaemia (CML) is the Philadelphia chromosome which is a reciprocal translocation between the BCR gene on chromosome 22 and the ABL gene on chromosome 9 resulting in a chimeric BCR-ABL gene. The quantitation of the resultant BCR-ABL transcripts is now routinely used to monitor response to kinase inhibitor drugs. These drugs bind within the BCR-ABL kinase domain and inhibit the kinase activity. The initial drug (Imatinib) was spectacularly successful at reducing the leukaemic burden but resistance developed in a small number of patients. The major mechanism of resistance is mutation within the BCR-ABL kinase domain that leads to interference of inhibitor binding and reactivation of the kinase activity. The role of molecular monitoring for patients with CML is multifaceted. Milestone measurements up to 18 months of first line imatinib therapy are prognostic and provide warning signals of suboptimal response. Serial measurement for patients with a complete cytogenetic response determine ongoing treatment efficacy or signal pending relapse. A rising level of BCR-ABL is a trigger for kinase domain mutation analysis. The characterization of BCR-ABL inhibitor resistant mutations is important to direct therapeutic intervention because it is now apparent that each resistant mutation functions as a distinct protein with unique biological properties that may confer a gain or loss of function. The benefit to patients of regular molecular analysis is a reassurance of ongoing response using the most sensitive of techniques or a potential improvement in outcome for those where relapse is indicated early. However, despite the obvious benefits of molecular analysis the measurement techniques may not be quite ready for acceptance into the routine clinical monitoring practices of all clinicians. The challenge is to now standardize and simplify the method so that it can be readily incorporated into routine laboratory testing procedures.

Meeting Room 4
ANZSBT Free Communications 1
O54

0830-1000
0830

The Use of Intravenous Immunoglobulin (IVIg) in Haematological Conditions in Australia

Peta Dennington¹, Julianne Lefante², Anne McNae², David Lynn Aston², David Jones³
for the ARCBS Transfusion Medicine team
Australian Red Cross Blood Service, Sydney¹, Perth², Adelaide³, Australia.

Background

ARCBS provided IVIg according to Australian Health Ministers' Advisory Council Guidelines (June 2000) until 2 March 2008, and subsequently according to the *Criteria for the Clinical Use of Intravenous Immunoglobulin in Australia* (National Blood Authority, 2008). A six month period was allowed for the transition of existing patients to the new Criteria. In the 12 months to March 2008, some 2,046,336 grams of IVIg was issued of which 28.5% was for haematological indications in 2680 patients.

Aim

To analyse and monitor IVIg usage trends for both IVIg replacement and immunomodulatory therapy in haematological diseases before and during the transition period. This will provide a baseline for comparison after full implementation of the new Criteria.

Methods

Patient and product data (diagnoses, treatment episodes, doses etc) for IVIg issues for haematological diagnoses from March 2007 to February 2008, and then March 2008 onwards, was extracted from the ARCBS national IVIg database (S.T.A.R.S.) and analysed.

Results

In the last year under the previous guidelines, 70 % of IVIg issued for haematological indications was used for replacement therapy and 30 % for immunomodulation. Within the replacement therapy group, treatment for immunodeficiency secondary to B-cell malignancies accounted for the majority of use (CLL 28 %, myeloma 16 %, NHL and other conditions 14 %), followed by a range of other diseases or medical therapies causing life-threatening infections and hypogammaglobulinaemia (10 %). For immunomodulation, the main uses were ITP (18 %), other autoimmune cytopenias (2 %), mismatched HSCTs (4 %) and FMAIT / NAIT (3.5 %).

Conclusion

Haematological conditions account for a substantial proportion of all IVIg use in Australia and these data provide a baseline for monitoring trends in future use. Data from the transition period will be analysed in September 2008 for presentation at the October 2008 meeting.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 1
O55

0830-1000
0845

Database Linkage to Establish the Frequency of Underlying Iron Deficiency Anaemia (IDA) in Transfused Patients

Kathryn Robinson^{1, 2}, Romi Sinha^{1, 2}, Magdalena Sobieraj-Teague^{1, 2}, David Roxby³

1. SA BloodSafe Program, Adelaide, South Australia, Australia

2. Australian Red Cross Blood Service, Adelaide, South Australia, Australia

3. SA Pathology, Flinders Medical Centre, Adelaide, South Australia, Australia

Aim

To establish the frequency of IDA in transfused patients using database linkage.

Methods

Using linked electronic laboratory and hospital databases, all patients receiving red cell transfusion in 2006 at two major hospitals were reviewed to establish the likelihood of IDA based on iron studies and red cell indices. IDA was defined as unequivocal where the serum ferritin was <20ug/L and likely where the serum ferritin was between 20 and 100ug/L, with a transferrin saturation <20% and suggestive red cell indices or blood film features.

Results

The databases identified a total of 2260 transfused patients for review. 27% (598/2260) had iron studies performed within the preceding 4 weeks. 23% (138/598) of these had unequivocal IDA (serum ferritin of <20ug/L) representing 6% (138/2260) of all transfused patients in 2006. Of the group that had iron studies performed 21% (127/598) had a serum ferritin between 20 and 100ug/L and of those 29% (37/127) had likely IDA.

Conclusion

Data linkage is a useful tool to identify patients receiving red cell transfusion with underlying IDA that can be used to monitor improvements in practice related to appropriate use of blood and utilisation of iron therapies, and direct further targeted medical record audit. There is also the potential to link the data to electronic pharmacy records to determine the number of patients receiving iron therapy.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 1
O56

0830-1000
0900

A Review of Alloimmune Neonatal Cases (ANN) from 2003-2008

Penny Hassell¹, Bronwyn Williams², Yoke Lin Fung¹

1. Australian Red Cross Blood Service, QLD. 2. Royal Brisbane Hospitals

To review all cases of alloimmune neonatal neutropenia (ANN) referred to Australian Red Cross Blood Service - QLD (ARCBS-QLD) between 2003- 2008. Referred ANN cases were investigated serologically by Granulocyte Immunofluorescence Test (GIFT) and Granulocyte Agglutination Test (GAT), and where possible the haematological data and clinical details were reviewed. A total of the 36 suspected cases of ANN were investigated serologically and 18 (50%) cases had a detectable neutrophil reactive antibody

Table 1. Antibodies detected

Antibody Type	Number (% of Total)	Antibody specificity		
		HNA-1a	HNA-1b	HNA-2a
HNA only	2 (11%)	2		
HNA & HLA	6 (33%)	3	2	1
HLA only	6 (33%)			
Maternal Auto Ab	1 (5%)	Broad reactivity		
Undefined Specificity	3 (17%)			
Total	18 (100%)			

Table 2. Haematological and clinical details from 12 confirmed ANN cases.

Ab Specificity	Manifestations	Day	WCC ($\times 10^9/L$)	ANC ($\times 10^9/L$)
HNA-1a & HLA	septic screen /minor skin lesion	0	6.4	0
HNA-2a & HLA	well at birth, omphalitis day 5	0	6.5	0.12
Undefined	well, Foetal maternal haemorrhage pre delivery	0	4.6	0.18
HNA-1b & HLA	Caesarean section , intrauterine growth retardation, previous intrauterine foetal death/well	0	7.0	0.24
HNA-1a & HLA	Prem - well	0/11	7.7	2.16/0.09
HLA only	34wk prem/well	0/5	5.7	1.79/0.85
HLA only	Twin 1 Prem abcess FTT Twin 2, small , well	3	1.6	0.05
HLA only	29 week prem, not unwell	0	2.9	0.78
Undefined	none, low BSL		11.3	0.97
HLA only	Mucopolysaccharidosis type 6	Wk 7	7.8	1.26
HNA-1a & HLA	Silent abruption pre delivery	0	5.4	0
Maternal AutoAb	History of ANN	0/2	16.5/12.7	3.95/1.8

Serological confirmation of ANN is important as it supports the clinical diagnosis of ANN and consequently directs appropriate treatment.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 1
O57

0830-1000
0915

Establishment of New National Registries for Thrombotic Thrombocytopenic Purpura and Neonatal Alloimmune Thrombocytopenia: Sharing Information to Build Knowledge

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Background

Thrombotic thrombocytopenic purpura (TTP) and neonatal alloimmune thrombocytopenia (NAIT) are uncommon, but associated with significant morbidity and mortality. Resource implications of treatment, including specialised blood product provision, are substantial and increasing. In NAIT, questions exist regarding the role of screening, early intervention and optimal management. In TTP, the application of diagnostic tests, management of relapsed or refractory disease, and role of emerging therapies remain undefined. No national data exist regarding incidence, current clinical practice, complications and outcomes in these conditions, while even major centres see relatively few cases. Lack of data has led to widespread variation in practice, while disease rarity hampers definitive clinical trials.

Methods and results

Clinical registries provide a means to aggregate clinical experience and provide data to inform management. Two new registries have been established for these diseases to determine incidence and natural history, explore factors influencing clinical outcomes, inform patient management and inspire further research. Monash University's Department of Epidemiology and Preventive Medicine has extensive registry experience and an established methodology and registry infrastructure. The Australian Red Cross Blood Service provides clinical and laboratory transfusion expertise and specialised blood products. An independent Steering Committee with clinical expert representation monitors each registry.

Patients are identified and registered by the treating clinician. Secure web-based data entry and a standardised data dictionary enable authorised users to report demographics, presentation, laboratory parameters, management (including complications) and outcomes to the registry databases.

Sharing information with participating clinicians and hospitals is a high priority. Regular reports regarding accrual and outcomes, including analyses of national, state and local incidence and management, will be provided by each registry.

Conclusions

These two new national registries for TTP and NAIT should enable a better understanding of epidemiology, current management and resource utilisation, inform clinical practice and provide a platform for future clinical trials.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 1
O58

0830-1000
0930

Fresh Frozen Plasma Usage in Six New Zealand Hospitals

Richard Charlewood¹; Rachel Donegan¹; Christopher Corkery²; Liz Thrift³; Fiona King⁴; Angela Wright⁵; **Suzi Rishworth**⁶; Jim Faed⁶; Susanta Ghosh²
New Zealand Blood Service: ¹ Auckland, New Zealand; ² Hamilton, Waikato, New Zealand; ³ Palmerston North, Manawatu, New Zealand; ⁴ Wellington, New Zealand; ⁵ Christchurch, Canterbury, New Zealand; ⁶ Dunedin, Otago, New Zealand

Aim

A steady increase in the use of fresh frozen plasma (FFP) has been seen internationally. This audit investigated the appropriateness of FFP usage within six hospitals across New Zealand, using NHMRC/ASBT guidelines as the reference.

Method

Six Transfusion Nurse Specialists audited a minimum of 50 episodes of FFP per site. An episode being defined as each time the participating blood bank issued one or more unit of FFP to a patient. Data collected during each episode included demographic data, time of transfusion, number of units administered, prescribed rate and actual duration of the transfusion, the number of other components or Prothrombinex® - HT administered in the previous 12 hours, clinical diagnosis, indication for transfusion, rate of bleeding, cardiac status, recent warfarin therapy, relevant co-morbidities and coagulation and blood count results. Upon completion of the data collection, two Transfusion Medicine Specialists assessed the appropriateness of each episode, based upon the NHMRC/ASBT guideline, the principles of managing bleeding patients, and considering Prothrombinex® - HT as an alternative to FFP.

Results

335 episodes, involving 867 units of transfused FFP were collected. 79% were assessed as appropriate or probably appropriate. 50% of the episodes revealed under-dosing. 18% of episodes were for warfarin reversal, with Prothrombinex® - HT use minimal. Those patients who received FFP for mildly abnormal INR results showed an average of 0.1 fall in INR, despite a therapeutic dose of FFP. 98.7% of episodes (excluding thrombotic thrombocytopenia purpura and specific coagulation factor proteins) had coagulation results obtained 6.7 hours before transfusion. FFP was prescribed and transfused in under an hour in 90% and 88% of the episodes, respectively.

Conclusions

The appropriateness of FFP use and the appropriate dose findings compared well with other countries. The high level of under-dosing however is certainly a concern. The infrequent use of Prothrombinex® - HT suggest the Australian Society of Thrombosis and Haemostasis (ASTH) warfarin reversal guidelines have not been embedded into practice. Administering FFP for mildly elevated INR yielded poor results. Areas where practice could be improved were identified, and education to address this was recommended.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 1
O59

0830-1000
0945

Audit of Cryoprecipitate Use in Two Sydney Teaching Hospitals: Combining Retrospective and Prospective Approaches

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Aim

To document the reasons behind the increasing demand for cryoprecipitate.

Methods

In one hospital, a prospective audit studied 20 consecutive orders of cryoprecipitate. In another hospital, retrospective audit retrieved six months of issues of cryoprecipitate accompanied by full blood counts and coagulation studies.

Results

The prospective audit included 17 patients given 20 doses of cryoprecipitate over 1 month. The retrospective audit covered 738 units of cryoprecipitate issued to 61 patients, 97 doses given over 6 months. Patients received total doses of 4 to 30 units. Cryoprecipitate was used by a range of clinicians with fibrinogen levels regularly monitored in the liver transplant service.

The audits demonstrated that cryoprecipitate use was often not aligned with current NHMRC clinical practice guidelines. The transfusion of cryoprecipitate occurred without testing the fibrinogen level and when the fibrinogen level is measured to be above 1g/L.

During the prospective audit, 2 of the 20 orders noted the fibrinogen as less than 1.0g/L at the time of the order. Most orders went to cardiac theatre, ICU, or for active resuscitation. Cardiac ICU and cardiac theatres comprised 45% of issues and general ICU 40% with 90% of doses for bleeding patients. Use in a massive transfusion protocol was 20% of issues.

Within the retrospective audit 67 doses were given with the fibrinogen level above 1.0g/L and with no result below 1.0g/L within 24 hours of the use of cryoprecipitate. A further 19 doses were issued without a fibrinogen level within twenty four hours of dosing.

Conclusion

Cryoprecipitate orders were frequently made in situations outside recommended NHMRC Clinical Practice Guidelines, the specific reasons for this not identified by this audit. Closer review of orders for cryoprecipitate by haematologists and the laboratory staff prior to product issue may be required along with increased laboratory monitoring of fibrinogen levels.

No conflict of interest to disclose

Meeting Room 3
ANZSBT Free Communications 2
O60

0830-1000
0830

The Age of Red Blood Cells (RBCs) at the Time of Blood Donation and Storage Influence the Generation of Microparticles Positive for Glycophorin A (GPA), CD55 and CD59

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Circulating RBCs have a life span of 120 days; however, the influence of the age of the RBC at the time of blood donation on the RBC storage effects is not well understood.

Aim

In this study the influence of RBC age upon the generation of microparticles in separated young and old stored RBCs was investigated. In particular, the detection and quantitation of microparticles positive for the complement regulatory molecules (CRMs), CD55, CD59 and for GPA were investigated over the RBC storage period.

Method

Leukocyte-filtered RBCs were prepared using standard blood bank procedures. RBC units were separated into young and old RBCs by density centrifugation and stored at 2-8°C. Cell free supernatant samples were collected from stored RBCs on day 1, 14, 28 and 42. Expression of CRMs (CD55, CD59) and GPA on microparticles was determined by flow cytometry (FCM) using an absolute count assay with TruCount beads (BD Biosciences).

Results

Old RBCs showed significant accumulation of GPA-positive microparticles over the storage period ($p=0.009$), whereas no significant accumulation was evident for young RBCs. Significant differences were detected between old and young RBC supernatants at day 28 ($p=0.049$) and day 42 ($p=0.027$). CD59 and CD55-positive microparticles accumulated in the RBC supernatant in both young and old RBCs, but showed a different pattern of accumulation compared to GPA-positive microparticles.

Conclusion

These results suggest that the age of RBCs at the time of donation influences the subsequent storage related effects. Increased generation of GPA-positive microparticles occurs in stored old RBCs compared to young RBCs. These results are consistent with the changes observed with GPA, CD59 and CD55 expression on the cell surface of young and old stored RBCs. The effects of RBC age and storage on the efficacy of RBC transfusion require further investigation.

No conflict of interest to disclose

Meeting Room 3
ANZSBT Free Communications 2
O61

0830-1000
0845

RhD Genotyping of Foetal DNA Extracted from Maternal Plasma, Goettingen Germany

Mary Zmijarevic

Royal Melbourne Hospital, Victoria, Australia

Aim

RhD genotyping is widely practised in Europe. A SAFE (special non-invasive advances in foetal and neonatal evaluation) network has been established in Europe looking into techniques of obtaining foetal DNA from maternal plasma and testing the foetus for various genes. In my visit to the molecular transfusion medicine laboratory in the University of Goettingen, Germany, my aim was to gain experience in the technique of genotyping of foetal DNA extracted from maternal plasma for the Rhesus and Kell gene, with particular focus on the RhD gene.

Method

Maternal plasma is collected in EDTA and sent to the transfusion laboratory for processing as soon as possible. Samples are centrifuged and plasma separated and aliquoted, along with the buffy coat sample. The RhD phenotype of the mother is checked. Plasma samples are frozen for storage until processing.

DNA is extracted from the plasma by an automated analyser. Once the DNA is extracted, a PCR reaction is performed on exons 5 and 7 along with a control (β globin) using the real time PCR analyser.

Results

Positive results for the RhD gene show amplification of exons 5 and 7 along with the β globin gene. Negative results for the RhD gene do not show amplification of exons 5 and 7, but show amplification of the β globin gene.

Conclusion

Extracting foetal DNA from maternal plasma is a non-invasive method of genotyping. The foetus may be genotyped for the Rh D, C, c, E and Kell gene. This information would be useful in the management of allo-immunised pregnant women and also may play a future role in the management of Rhlg.

This research was supported by the ANZSBT. The Society had no role in the analysing the data or preparing the abstract.

Meeting Room 3
ANZSBT Free Communications 2
O62

0830-1000
0900

Prospective Determination of Blood Group Frequency of a Multiethnic Patient Population in Western Sydney

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Aims

To determine the ABO, Rh, Kell, Kidd, Duffy and S blood group frequencies in blood group samples analysed by the transfusion laboratory of the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital and compare these to data collected by the Australian Red Cross Blood Service (ARCBS) and published European blood group norms.

Methods

Additional serological reagents were added to the automated blood group analyser's reagent panel. Samples were from patients referred for routine group and hold testing for the first time. These results will be compared to phenotyping data collected on blood donors by ARCBS

Results

Samples were collected on 1007 patients. ABO, Rh and Kell testing was performed on all samples and further testing on 560. ABO frequencies were A 36%, B 16%, O 41%, AB 6%. These frequencies are significantly different to previous ARCBS data published in the ARCBS transfusion manual 2003. RhD negativity was 9% versus 16% for Caucasian populations. The frequency of K antigen positive samples was lower than would be expected in a Northern European population (5.5% vs 8%) while the frequency of Fy(a+b-) was 35% vs 20% in Europe. Comparative data in the current ARCBS blood donor panel is being analysed.

Conclusions

The blood group antigen frequency in patients undergoing blood group testing at ICPMR differs from previous ARCBS data and European populations, reflecting underlying population demographic differences. Many of the ethnic groups represented in Western Sydney have different antigenic frequencies for many of the commonly recognized blood groups. Strategies to provide compatible red cells for these patients need to include ongoing recruitment of eligible blood donors with the appropriate red cell antigen phenotypes.

This research was supported in part by CSL Limited and Ortho Diagnostics. The companies had no role in analysing the data or preparing the abstract

Meeting Room 3
ANZSBT Free Communications 2
O63

0830-1000
0915

Development and Validation of Antibody Screening Cells Specifically Designed for Asian Populations – The First Example of the Addition of Peptide Antigens to Human Red Cells using KODE™ Technology

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Background

Alloantibodies to antigens displayed on variant glycoporphins are common in many Asian populations and have been shown to cause both transfusion reactions and haemolytic disease of the foetus and newborn. The detection of these antibodies is problematic as commercially available panels for antibody screening do not include the antigens necessary for detection of these antibodies. The use of naturally-occurring phenotype-positive cells is also problematic because not all antibodies detected are clinically significant, the specific epitope reactivity cannot be identified, and the rbc types required for full epitope analysis are of very limited availability.

Aim

To use KODE™ technology to add peptide epitopes to screening cells and to evaluate their stability and suitability for use in the detection of alloantibodies. The peptides investigated represented a series of defined variant MNS (Miltnerberger) epitopes. These were used for the detection, identification and investigation of clinically significant alloantibodies, particularly alloantibodies found in Asian populations.

Methods

Candidate carrier molecules were constructed and peptides attached. The epitope specificity of antisera with generic anti-Mi(a) reactivity were characterized by ELISA. The candidate constructs were inserted into red cells and reactions in routine antiglobulin test platforms were investigated. The stability of the transformed cells was also investigated.

Results

There was no abnormal lysis of RBC expressing KODE™-peptide antigens and the expression of these peptides on the transformed cells was stable with time. Detection of antibodies to other rbc antigens by IAT was the same for transformed and untransformed cells. Antibodies to variant MNS (Miltnerberger) epitopes that have been reported to be clinically significant were detected for almost all serums for which a previous ELISA specificity had been defined.

Conclusion

Various different KODE™ systems are suitable for addition of carbohydrate or protein antigens to human red blood cells. Under optimal conditions there is no change in the expression of other rbc blood group antigens. KODE™ technology allows for sensitive detection of clinically significant antibodies to vMNS antigens well as identification of their specific epitope reactivity.

No conflict of interest to disclose

Meeting Room 3
ANZSBT Free Communications 2
O64

0830-1000
0930

The Value of Routine Process Controls in Improving Safety in Immunohaematology Testing – 3 Years Experience

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² CSL Bioplasma Immunohaematology, Parkville, Australia

Background

Control products that are formatted and tested like patient samples have been commercially available in Australia for 3 years. These controls allow immunohaematology laboratories to test reagents for pre-purchase approval, control routine immunohaematology grouping and antibody tests on manual and automated testing platforms and perhaps most importantly allow continual assessment of staff competency.

Methods

Case studies were recorded from laboratories where control product use identified laboratory errors and allowed correction of the problem and prevention of error reoccurrence.

Results

The routine use of process controls was shown to detect a range of laboratory technical errors, reagent and system failures and result misinterpretation. Some laboratories were found to be using poorly performing tests and reagents that appeared satisfactory when in-house QC material was used. A range of technical errors were detected in immunohaematology instrumentation such as poor diluent, inaccurate fluidics, poor temperature control and test substrate failure. Many of these errors would not have been detected without the use of a routine process control. Perhaps most importantly, the routine use of a high quality externally produced QC product has been shown to identify transcription and transposition errors thus allowing staff training and system improvements to minimise the potential for these most dangerous errors.

Conclusion

The use of a high quality routine process control allows immunohaematology laboratories to implement Quality Control and Quality Assurance systems that have been available to other diagnostic disciplines such as biochemistry for decades. Test, staff and instrument performance can be constantly monitored, errors and poor performance detected and laboratory safety continuously improved.

No conflict of interest to disclose

Meeting Room 3
ANZSBT Free Communications 2
O65

0830-1000
0945

Contact Activation Markers Factor XIIa, Prothrombin Fragments F1 and 2, C3a and sC5b-9 in Buffy Coat Pooled Platelets Stored in SSP+

Suzanne Candy, Jason Carlesso, Christine Robinson, Kathleen Doherty
Australian Red Cross Blood Service, Adelaide, South Australia

Aim

Markers, such as Factor XIIa, Prothrombin fragments F1 and 2 (PF1&2), Complement components C3a and sC5b-9 (soluble Terminal Complement Complex), at high levels may cause platelet activation and adverse reactions in transfusion recipients. This study assessed levels of selected contact activation markers in supernatants of buffy coat pooled platelets stored in the platelet additive solution SSP+.

Method

Buffy coat pooled platelets (n=17) resuspended in SSP+ on day 1 were stored at 20-24°C with agitation for 8 days. Samples were taken at day 1, 6 and 8 and supernatants tested for concentration or functional changes in activation markers by ELISA and platelet P Selectin levels were determined by flow cytometry.

Results

Levels of PF1&2 (pre and post filtration at day 1) were $6.83 \pm 0.0.94$ (S.D) U/L and 59.53 ± 5.64 pmol/L respectively decreasing to 50.32 ± 1.49 pmol/L at day 8. Factor XIIa increased to 9.26 ± 1.49 at day 5, decreased to 8.21 ± 0.42 at day 6, and remained stable until day 8. Factor XIIa and PF1&2 were below levels observed in normal plasma. C3a and sC5b-9, however, increased across the storage period to levels of 507.00 ± 129.55 nmol/l and 295.02 ± 45.26 nmol/L respectively. Final levels of C3a and sC5b-9, were below those reported to cause serious adverse effects. The final levels of C3a was below those previously reported for buffy coat platelets suspended in plasma but levels of sC5b-9 were similar. P Selectin results indicated low levels of platelet activation 3.16 ± 0.80 % (day 8).

Conclusion

Levels of the activation markers tested in buffy coat pooled platelets in SSP+ are below or equivalent to those reported for the component suspended in plasma. The use of SSP+ platelet additive solution reduces the levels of some contact activation markers in platelet supernatant providing improved quality and safety.

No conflict of interest to disclose

Meeting Room 2/3
ASTH: New Understanding of Blood Coagulation

0830-1000
0830

The Central Role of Thrombin in Haemostasis

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Following vascular injury, blood loss is controlled by the mechanisms of haemostasis. During this process, the serine proteinase, thrombin, is generated both locally and rapidly at sites of vessel damage. It plays a pivotal role in clot promotion and inhibition, cell signalling, as well as additional processes that influence fibrinolysis and inflammation. These functions involve numerous cleavage reactions, which must be tightly coordinated. Failure to do so can lead to either bleeding or thrombosis. The crystal structures of thrombin, in combination with biochemical analyses of thrombin mutants, have provided insight into the ways in which thrombin functions, and how its different activities are modulated. Many of the interactions of thrombin are facilitated by exosites on its surface that bind to its substrates and/or cofactors. The use of cofactors not only extends the range of thrombin specificity, but also enhances its catalytic efficiency for different substrates. This explains a paradox; i.e. thrombin is a specific proteinase, and yet one that has multiple, and sometimes opposing substrate reactions. In this presentation, we describe the context in which thrombin acts during haemostasis and explain the roles that its exosites and cofactors play in directing thrombin function. Thereafter, we develop the concept of cofactor competition as a means by which the activities of thrombin are controlled.

Meeting Room 2/3
ASTH: New Understanding of Blood Coagulation

0830-1000
0910

A Factor XII Dependent Coagulation Pathway Feeds Back From Thrombin Generation and Requires Platelet Activation

Jonas Emsley,¹ Jing Jing,² Michael C Berndt³ and Robert K Andrews²

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Background

Factors XII (FXII) and factor XI (FXI) of the intrinsic coagulation pathway are not necessary for haemostasis, but have been implicated in pathological thrombus formation at arterial shear rates in mice.

Aim

In this study, we examine whether the mechanism for FXII and FXI activation in human plasma involves the contact system, by measuring thrombin generation and FXIIa activity in re-calcified human citrated plasma in the absence of exogenous tissue factor.

Methods and Results

These studies revealed a FXII-dependent feedback pathway, which followed the initiation phase of coagulation and was dependent on the presence of platelets. Using FXII- or FXI-deficient plasma, we confirmed that thrombin generation in this assay was strongly dependent on both coagulation factors (rescued by re-constituting with purified FXII or FXI, respectively), and required cofactor high molecular-weight kininogen (HK), shown using HK-deficient plasma. Parallel studies monitoring activated FXII (FXIIa) revealed that at all platelet concentrations the appearance of FXIIa followed thrombin generation, and was inhibited by hirudin. Inhibition by hirudin was at least partially overcome by adding platelet thrombin receptor-agonist peptide (TRAP), suggesting thrombin-dependent FXII activation involved a requirement for thrombin activating platelets. FXII has been previously shown to bind GPIIb/IIIa (the major-ligand-binding subunit of the GPIIb/IIIa-V complex). To test the role of GPIIb/IIIa in FXII-dependent thrombin generation, platelets were pretreated with the cobra venom metalloproteinase, Nk, that cleaves GPIIb/IIIa and removes the ligand-binding domain, or by anti-FXII antibody (B7C9), previously shown to block the FXII-GPIIb/IIIa interaction. Either Nk treatment in the presence of Ca²⁺, but not EDTA, or B7C9, significantly inhibited thrombin generation.

Conclusion

Together, the results suggest a FXII-dependent feedback pathway involved in the amplification of coagulation in human plasma, which requires activated platelets and GPIIb/IIIa, but does not resemble the consolidation pathway where thrombin feeds back directly to activate FXI.

No conflict of interest to disclose.

Meeting Room 2/3
ASTH: New Understanding of Blood Coagulation

0830-1000
0935

Protease-activated Receptors as Targets for Novel Anti-platelet Agents

Justin Hamilton

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Platelets are essential components of the arterial thrombi which cause heart attacks and most strokes. Consequently, substantial research has been directed at defining the mechanisms by which platelets contribute to a growing thrombus since interfering with such mechanisms may afford effective antithrombotic approaches. Thrombin is a potent platelet activator, and we have shown that mice whose platelets do not respond to thrombin (PAR4^{-/-} mice) are protected against experimental models of thrombosis yet do not exhibit spontaneous bleeding. Specifically, we observed protection against tissue factor-induced pulmonary embolism, ferric chloride-induced thrombosis in mesenteric arterioles, and laser-induced injury of cremasteric arterioles. Importantly, PAR4^{-/-} mice showed no evidence of haemorrhage following the trauma of birth and exhibited no spontaneous bleeding throughout adult life, suggesting that thrombin-induced platelet activation via PARs may be important for platelet activation during thrombosis but not during physiological haemostasis. In addition, we have examined platelet activation and fibrin formation concurrently in forming thrombi and shown that, despite marked reductions in platelet accumulation and activation in thrombi of PAR4^{-/-} mice when compared with thrombi formed in control mice, no differences in the kinetics or quantity of fibrin accumulation were observed, suggesting that interfering with PAR-mediated platelet activation does not affect thrombin-dependent coagulation. These studies suggest that thrombin-induced platelet activation, mediated by PARs, is an important mechanism behind thrombus formation and that PAR antagonists may provide novel anti-platelet therapy with less bleeding than occurs with existing anticoagulants.

Riverside Theatre
HSANZ Free Communications 5
O66

1030-1130
1030

Retreatment with Rituximab +/- Chemotherapy in 178 Patients with Relapsed and Refractory B-cell Lymphomas: A Single Institution Study

Anna Johnston, Bertrand Coiffier
Centre Hospitalier Lyon-Sud, Lyon, France

Aim

To investigate the efficacy of retreatment with rituximab +/- chemotherapy in patients with relapsed or refractory B-cell lymphomas. To compare the progression-free survival (PFS) and overall survival (OS) of these patients with a group of historical controls treated with chemotherapy.

Methods

This was a retrospective single-institution case-control study which included all patients retreated with rituximab +/- chemotherapy for relapsed or refractory B-cell lymphomas at Centre Hospitalier Lyon-Sud since the 1st January 1999. Outcome measures were response rate, PFS from diagnosis (PFS0), PFS from relapse (PFS1) and OS. Controls were a group of chemotherapy-treated patients and were matched with cases for age and histological type of B-cell lymphoma.

Results

178 patients were included of which 28.7 % had diffuse large B-cell lymphoma and 28.1% had follicular lymphoma. The overall response rate for the second treatment was 66.3% (37.6% CR /29% PR). The median PFS0 was 13.2 months. The median PFS1 was 18 months. The 5 year OS was 57%. The PFS0 of the patient group was inferior to that of the control group, at 3 years 91% of the patients had relapsed compared to 78% of the control group ($p<0.001$). There were no significant differences between the patient and control groups for PFS1. The overall survival from relapse was superior for the patient group, with 54% of patients alive at 3 years compared to 38% of controls ($p=0.002$).

Conclusion

Retreatment with rituximab +/- chemotherapy in patients with relapsed and refractory B-cell lymphomas is efficacious with a longer PFS1 than PFS0. This historical comparison suggests relapse occurring after rituximab-containing therapy may be more aggressive. However, rituximab-containing salvage therapy permitted an identical progression free-survival. The OS from relapse of the rituximab-retreated group was superior although it is uncertain if this is due to rituximab or better subsequent salvage. Randomized data are needed.

This research was supported by Roche. The company had no role in analysing the data or preparing the abstract.

A143

Riverside Theatre
HSANZ Free Communications 5
O67

1030-1130
1045

Fludarabine based Combinations are Highly Effective as First-Line or Salvage Treatment in Patients with Waldenström Macroglobulinemia

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Aim

Treatment with single agent rituximab (R), alkylators or purine analogues is safe and moderately effective as first-line treatment for patients (pts) with Waldenström Macroglobulinemia (WM). However, efficacy in relapsed/refractory WM is unsatisfactory. We analysed if combinations of fludarabine (F) with alkylators and/or R were safe and more effective.

Material and Methods

26 episodes of intravenous F-combination therapy were administered to 24 pts from 12/94 - 06/08: FC (F 25mg/m² d1-3, cyclophosphamide (C) 250mg/m² d1-3; n=7); FCR (FC+R 375mg/m² d1; n=15); FM (F+mitoxantrone (M) 10mg/m² d1; n=3); FR, n=1).

Patient characteristics

Median age 57yrs [range; 36-89], 84% male, 6 pts were previously untreated (25%), 18 pre-treated pts had a median number of 2 [1-7] prior treatments, prior single-agent F in 5 pts (21%), 9 pts (37.5%) were alkylator-refractory, median time from diagnosis 22 Mo [0-153], baseline paraprotein (PP) 31g/L [7-64].

Results

A total of 96 cycles were administered, median 4 [2-6] per pt. Grade ≥3 neutropenia and infections complicated 20% and 3% of cycles, respectively, none of which were life-threatening. However, three heavily pre-treated pts subsequently developed secondary AML/MDS (1 fatal) at 52, 61 and 99 Mo. post-treatment. Of 23 evaluable pts, 18 (78%) had an objective response (1 CR/17 PR), with a median PP reduction of 93% [71-100%]. The median time-to-progression for all pts was 57 Mo. With a median follow-up of surviving pts of 63 Mo. the 5- and 10-year-actuarial survival rates are 85 ± 8%, and 68 ± 13%, respectively. With a median follow-up of 50 Mo. [12-96] all 6 previously untreated pts remain alive and progression-free.

Conclusion

F-combination therapy is highly active in WM, both, untreated and alkylator-refractory, leading to high response rates and prolonged remissions. However, possible contribution to the cumulative risk of treatment-related MDS/AML in heavily pre-treated pts is a potential concern.

No conflict of interest to disclose

Riverside Theatre
HSANZ Free Communications 5
O68

1030-1130
1100

Radioimmunotherapy of Newly Diagnosed, Advanced, Low-Grade Lymphoma with ^{131}I -Rituximab: The Initial Study

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Introduction

Radioimmunotherapy (RIT) with ^{131}I -labeled tositumomab has previously been shown to be safe and effective first-line therapy for low-grade Non Hodgkin lymphoma (NHL), with a CR of 75% and five-year PFS of 59%. We report a physician sponsored multicentre phase II trial of ^{131}I -rituximab RIT in previously untreated, advanced, low-grade NHL. This was performed using in-house immunoconjugation of ^{131}I to rituximab.

Methods

Eligibility criteria comprise CD20 +ve low-grade NHL, ECOG status 0-2 and adequate haematopoietic function (neutrophils $> 1.0 \times 10^9/\text{L}$, platelets $> 70 \times 10^9/\text{L}$, Hb $> 100\text{g/L}$). Administered therapeutic activities of ^{131}I -rituximab limited the whole body absorbed radiation dose to 0.75 Gy.

Results

To date 29 patients have been enrolled. The diseases consist of follicular NHL (n= 26), lymphoplasmacytoid lymphoma (n=2) and low-grade NHL-not specified (n=1). Disease assessment by PET-CT scan at three months showed an ORR of 100 % with a CR of 73 %. Haematological toxicity was minimal with a mean neutrophil nadir of $1.39 \times 10^9/\text{L}$ at week 7 and a mean platelet nadir of $89 \times 10^9/\text{L}$ at week 4. One patient experienced grade 4 haematological toxicity requiring one prophylactic platelet transfusion. There were no other toxicities. Eight patients were assessable at 12 months; one patient required treatment for progressive disease. R-CHOP-14 chemotherapy was given with CR on PET-CT at 12 months. Two of three patients in PR at three months had reduced disease at 12 months. Four remained in CR.

Conclusion

^{131}I -rituximab radioimmunotherapy is a low cost, safe and effective first-line therapy for advanced low grade lymphoma.

No conflict of interest

Riverside Theatre
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O69

1030-1130
1100

Complete Remission on ^{18}F FDG-PET prior to Autologous Stem Cell Transplantation Predicts Superior Event Free Survival of Patients with Relapsed or Refractory Hodgkin Lymphoma

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Aim

Response status on ^{18}F FDG-PET (PET) early during first-line chemotherapy of Hodgkin Lymphoma (HL) is highly predictive of event free survival (EFS). In patients (pts) with relapsed/refractory HL, however, the predictive value of PET prior to high-dose chemotherapy and autologous stem cell transplantation (ASCT) is less well established.

Methods

A retrospective analysis was undertaken of PET-remission status, clinical characteristics and outcome for 48 consecutive patients (pts) with refractory or relapsed HL who underwent a PET scan after salvage chemotherapy and before ASCT between 1998 and 2008.

Results

The median age of pts at the time of ASCT was 38 [18-61] years, 23/48 (48%) of pts were male. The median follow-up of surviving pts was 38 [3-109] months. Following salvage chemotherapy, 23/48 (48%) and 25/48 (52%) of pts were PET-negative and PET-positive, respectively. At the time of analysis, neither the median EFS nor OS was reached in either of the groups. However, EFS for PET-negative pts was significantly longer than for those with persistent PET-avid disease ($p=0.0458$, Gehan-Breslow-Wilcoxon test). There was a trend for improved OS for PET-negative vs. PET-positive pts that did not reach statistical significance ($p=0.1173$). An analysis of known clinical predictive factors revealed that female sex and duration of first remission of ≥ 12 months predicted superior EFS ($p=0.0094$ and 0.0441 , respectively). Other characteristics, e.g. presence of B-symptoms or extranodal disease at the time of relapse, age \geq or < 38 years, type of salvage or conditioning regimen or treatment centre did not influence EFS, and none of the parameters impacted on OS. Female sex was the only positive predictive factor for obtaining a PET-remission post salvage therapy.

Conclusion

Our data show that achievement of a PET-negative remission after salvage therapy predicts superior EFS to ASCT for patients with relapsed or refractory HL.

No conflict of interest to disclose

Meeting Room 6
HSANZ Free Communications 6
070

1030-1130
1030

Patient-Specific IC50 Assays in Imatinib-Resistant CML Patients are Variable and May be Clinically Relevant

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Aim

In CML patients failing imatinib the choice of second-line tyrosine kinase inhibitor (TKI) is governed by trial availability or drug access. *In-vitro* modelling of common kinase domain mutants may provide some guidance, however approximately 40% of patients relapse without a mutation, 24% have rare and 22% multiple mutations. We aimed to assess the predictive value of the patient specific IC50 (PS-IC50), in imatinib-resistant patients (IM-R) treated with second line TKI's.

Methods

Blood was collected from 16 IM-R patients, prior to second line therapy. PS-IC50 were determined in-vitro by assessing the reduction in p-CrkI.

Results

The median PS-IC50^{nilotinib} was 78nM (n=12;R:35-250nM). There was a marked difference in IC50^{nilotinib} between patients achieving, and those not achieving 1 and 2 log reductions in BCR-ABL on nilotinib. Four patients progressed, and there was a significant difference in PS-IC50^{nilotinib} between these 4 and those who did not progress $p < 0.001$. Five patients had baseline mutations. Four mutations had been assessed by *in-vitro* modeling (M-IC50), demonstrating low M-IC50 (<75nM) predictive of a good response. The PS-IC50^{nilotinib} ranged from 66-250nM (median 180nM). Two patients achieved a >1 log reduction (66 and 120nM) and only the patient with a PS-IC50 of 66nM achieved > 2 log reduction. Four patients were treated with dasatinib. Two had no detectable mutations (PS-IC50=1.5 and 3.3nM). Both achieved >3 log reductions in BCR-ABL on dasatinib. One patient had a Y253F mutation (M-IC50:3.1nM: PS-IC50=3.9nM) and achieved a 4 log reduction on dasatinib. The remaining patient had a Y253H mutation (M-IC50: 1.4nM PS-IC50=6nM). This patient failed to achieve >1.5 log reduction with continued persistence of the mutant clone.

Conclusion

This dataset suggests the PS-IC50, developed for *de novo* patients, is applicable to IM-R patients. It provides a measure of intrinsic sensitivity to TKI induced kinase inhibition, and may provide a more accurate predictor of subsequent response or progression than published M-IC50. Further assessment in IM-R patients is warranted.

This research was supported by Novartis Pharmaceuticals. The company had no role in analysing the data or preparing the abstract.

A147

Meeting Room 6
HSANZ Free Communications 6
O71

1030-1130
1045

High Rate of Sustained Complete Molecular Response of Chronic Myeloid Leukaemia After Withdrawal of Imatinib: Progress of the ALLG CML8 Study

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2. Australasian Leukaemia & Lymphoma Group

3. Novartis Pharmaceuticals, Australia

There is a progressive reduction in BCR-ABL mRNA levels over time in imatinib-treated chronic myeloid leukaemia (CML) patients. After 5 years 40-50% will have stable undetectable BCR-ABL by sensitive RT-PCR methods ('complete molecular response', CMR). CML patients in CMR for ≥ 2 years were monitored closely in the ALLG CML8 study to assess the stability of CMR after imatinib withdrawal. Patients are enrolled in two cohorts – de novo imatinib (NEW) and imatinib after prior interferon therapy (IFN). Both cohorts continue to accrue.

Aim

To estimate the rate of sustained CMR after imatinib withdrawal in CML patients.

Methods

To date 13 patients are enrolled in the IFN cohort, and 5 patients in the NEW cohort. PCR monitoring was done monthly for the first year or until molecular relapse. Molecular relapse was defined as loss of major molecular response (MMR) or any two consecutive positive results. Imatinib was resumed at molecular relapse.

Results

Molecular relapse has occurred in 3 patients in the IFN cohort; 2 patients in the NEW cohort. Median time to relapse was 97 days (range 2-4.5 months). The 5 patients with molecular relapse were in CMR (n=4) or MMR (n=1) at last follow-up. No patient has experienced haematological relapse. No ABL kinase domain mutations were detected. Median duration of follow-up in the NEW cohort is <6 months. In the IFN cohort the estimated rate of sustained CMR was 75% with median follow-up 12 months (range 1-22).

Conclusions

No patient has lost CMR more than 5 months after imatinib withdrawal, indicating a dichotomy between early relapse and sustained remission. Among patients treated with imatinib after prior interferon 8 of 11 patients with more than 6 months of follow-up remain in CMR. Data on de novo imatinib-treated patients are awaited. All patients with recurrent BCR-ABL have responded to resumption of imatinib treatment.

This research was supported by Novartis Pharmaceuticals. The company had no role in analysing the data, but did review the abstract.

Meeting Room 6
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072

1030-1130
1100

The IC50 Assay is Predictive of Molecular Response and Indicative of Optimal Dose in *de-novo* CML Patients

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²Department of Medicine. University of Adelaide. Adelaide AUSTRALIA

Aim

There is significant interpatient variability in the IC50^{imatinib}, a measure of patient intrinsic sensitivity to imatinib induced kinase inhibition. Furthermore, the IC50^{imatinib} is predictive of optimal and suboptimal response (n=60)¹. In an expanded patient pool (n=116) we evaluate the IC50 as a predictor of response, and dose selection.

Methods

Samples were obtained from *de novo* CML patients enrolled to either TIDEL or TOPS trials. The IC50 performed as described¹.

Results

Previously the IC50^{imatinib} was divided about the median value (0.6µM) into low and high, and a significantly greater proportion of patients with low IC50^{imatinib} achieved MMR by 12 months. In this expanded cohort, we confirm this finding (low IC50-65% achieve MMR vs high IC50-39%;*p*=0.014). Dividing the IC50's into quartiles demonstrates the IC50^{imatinib} is a continuous variable with a greater proportion of patients in the lower quartile achieving MMR (67% vs 32%;*p*=0.042). Addressing dose, we demonstrate that no patient with IC50>0.95µM achieves MMR on 400mg (*p*=0.018 when compared to all other groups). At 600mg there is a difference between patients with IC50<0.7µM and those >0.95µM (*p*<0.05). In contrast, at 800mg the effect of IC50^{imatinib} is overcome. Failure to achieve CCyR by 12 months is a suboptimal imatinib response. Assessing the molecular equivalent (2 log reduction) we demonstrate a greater proportion of patients with IC50^{imatinib}>0.7µM fail to achieve a 2 log reduction when treated with 400mg (IC50<0.7µM-11%:>0.7µM-33%;*p*=0.034), and 600mg (IC50<0.7µM-12%:>0.7µM-22%;*p*=0.036). There is no significant difference in the 800mg patient cohort (IC50<0.7µM-7%:>0.7µM-14%;*p*=0.79).

Conclusion

This analysis confirms the IC50^{imatinib} is predictive of optimal and suboptimal imatinib response. Furthermore, patients with an IC50^{imatinib} <0.7µM are more likely to respond well to doses of 400mg imatinib. There is no statistically relevant outcome benefit for these patients receiving higher doses. In contrast patients with higher IC50^{imatinib} (>0.7µM) may benefit from higher dosing regimens (*p*=0.012).

1. White D et al *Blood*. 2005;106:2520-2526.

This research was supported by Novartis Pharmaceuticals. The company had no role in analysing the data or preparing the abstract

Meeting Room 6
HSANZ Free Communications 6
073

1030-1130
1115

Targeted JAK2 Inhibitors for Myeloproliferative Diseases

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The recent identification of the JAK2V617F mutation has convincingly and directly linked the JAK2 tyrosine kinase to the molecular pathogenesis of MPDs (myeloproliferative disorders). The JAKV617F mutation has been detected in more than >90% of polycythemia vera (PV) patients and ~50% of patients with essential thrombocythemia or idiopathic myelofibrosis. The JAK2V617F mutation results in constitutive activation of JAK2. Specific inhibition of JAK2 therefore represents an attractive approach to develop a highly targeted therapy for MPDs. We have undertaken a program to discover potent and selective inhibitors of JAK2 for use in the treatment of patients with MPDs which has culminated in the identification of CYT387, a potent and selective inhibitor of JAK2 in vitro and in vivo. CYT387 is a novel small molecule inhibitor of JAK1 and JAK2 with good potency and selectivity over all kinases assayed including JAK3. The compound shows good cellular activity in a panel of JAK2-dependent cell lines and in downstream signaling events. In MPD patient-derived samples the compound inhibits erythropoietin-induced STAT5 phosphorylation (IC₅₀ ~300nM) and selectively inhibits V617F mutant colony formation (IC₅₀ 1-3uM). CYT387 has excellent pharmacokinetic properties and has been shown efficacy in an in vivo model of PV. Taken together, the data clearly demonstrates that CYT387 is a promising candidate for the treatment of V617F-positive MPD's, and formal preclinical studies are now well underway with clinical trials planned for early 2009.

This research was supported by Cytopia Research Pty. Ltd. CYT387 is a proprietary compound under development as an internal research program. The abstract has been prepared and is presented by Cytopia Research and Emmanuelle Fantino is an employee of the company. External collaborators research has been supported by Cytopia Research PTY LTD, however the company had no control over experimental design, data analysis or interpretation.

A150

Meeting Room 7
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O74

1030-1130
1030

Prophylactic Infusion of Donor Derived CMV-Specific Cytotoxic T Lymphocytes in Haemopoietic Stem Cell Transplant (HSCT) Recipients

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Background

Cytomegalovirus (CMV) remains an important cause of morbidity and mortality post HSCT. We report the results of an ongoing phase I clinical trial of CMV specific cytotoxic T lymphocytes (CTL) in HSCT recipients.

Aim

To assess the safety and efficacy of prophylactic infusion of CMV CTL in patients receiving HSCT.

Methods

CMV CTL were generated using dendritic cells transfected with an adenoviral vector containing the pp65 gene. CTL were infused on or after day 28 post transplant and immune responses to the CMV antigens pp65 and IE-1 were monitored by ELISPOT assay.

Results

12 HSCT recipients were infused and monitored for up to 453 days post transplant. There were no immediate infusion related adverse events. All patients demonstrated CMV immune reconstitution. Rapid sustained post-infusion recovery of predominantly pp65 directed immunity was seen in 8/12 participants. Although T cell activity against adenovirus could be detected in cultured products, no reconstitution of adenovirus specific immunity could be detected post infusion. The rate of CMV reactivation was lower in trial participants than in historical controls (33% vs 52% p=ns). More importantly no trial participant required specific anti-viral therapy compared with 28/56 historical controls. Peak CMV DNA titre was also lower in the 4 trial patients with reactivation than in controls (669 vs 4394 copies/ml). Rates of GVHD, infection and death were not increased.

Conclusion

Use of CMV-specific CTL is safe post-HSCT and may control CMV reactivation preventing the need for ganciclovir or foscarnet therapy.

No conflict of interest to disclose

Meeting Room 7
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O75

1030-1130
1045

T Cell Control of Monoclonal Gammopathies: Further Evidence from Waldenstrom's Macroglobulinaemia

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Aim

The presence of expanded CD8+ CD57+ TCR V β -restricted T cell clones in the blood of patients with multiple myeloma confers a favourable prognosis. The clonality of these TCR V β expansions has previously been verified by CDR3 length analysis and direct sequencing. For patients with Waldenstrom's Macroglobulinaemia (WM), clinical evidence suggests that T cells play an important role in maintaining the stability of the disease as there is an increased rate of transformation to aggressive lymphoma after the use of T cell suppressive agents such as Cladribine and Fludarabine. We postulate that these anti-tumour agents also effect cytotoxic T cell clones and lead to the loss of the host's immunological control of the disease.

Method

TCR V β -restricted cytotoxic T cell expansions were analysed by 4 colour flow cytometry using a BetaMark (Beckman Coulter) assay. T regulatory cells (Tregs) were assayed as CD4+, CD25^{hi}+, CD127- cells. Sorting was performed on a FACS ARIA (BD) and the Affymetrix 133plus microarray was outsourced.

Results

CD8+ CD57+ TCR V β -restricted cytotoxic T cell expansions were present in 74% of patients with WM (n=19) and 48% of myeloma (n=221). Expansions covered the spectrum of the TCR V β repertoire. The lack of CD8+ CD57+ TCR V β -restricted cytotoxic T-cell expansions in specific patients correlates with nucleoside analogue therapy ($\chi^2=13.8$; $p<0.001$). Treg cells have been reported to be increased in cancer patients. The mean % Tregs (CD4+ CD25^{hi}+ CD127-) in the WM patients (7.0 ± 1.8) was not different from normal (7.0 ± 2.1) although there was a significantly lower number of absolute Tregs in WM patients ($3.2\pm2.4 \times 10^7/L$) (normal= $5.1\pm2.1 \times 10^7/L$). Trogocytosis involving CD80 and CD86 expression on T cells occurs in 30% of patients with myeloma (n=45) and has been suggested to be one mechanism of tumour escape. In contrast there was no detectable trogocytosis in the blood samples from 11 patients with WM ($\chi^2=4.1$; $p<0.05$). Cytotoxic T-cell clones in WM were flow-sorted into CD57+ and CD57-clones, and assayed by microarray for gene expression. Three of the six most significantly overexpressed genes in the CD57+ population (Affymetrix 133plus microarray) were related to T cell cytotoxicity and included perforin1, granzyme B and H.

Conclusion

These findings are consistent with the concept of T cells controlling disease in WM.

No conflict of interest to disclose

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O76

1030-1130
1100

Mechanisms Underlying the Selective Impairment of EBNA1-specific Effector T-cells Observed in PTLD

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Aim

Immunosurveillance by cytotoxic T lymphocytes (CTL) plays a critical role in the detection and killing of lymphoma, whereas the ability to evade recognition by CTL is thought to promote cancer survival. In EBV-driven PTLD, evasion is believed to be simply the result of iatrogenic immunosuppression leading to an absence of EBV-specific cellular surveillance. If this were true, EBV-specific immunity would be “globally” depressed; conversely if lymphoma-specific mechanisms operate this would result in selective impairment against only those EBV-antigens expressed within the diseased lymph node. Although EBV-latent antigen expression in PTLD is variable, EBV nuclear antigen 1 (EBNA1) is universally expressed. Recent evidence suggests that although poorly immunogenic, EBNA1 may offer a viable antigenic target for the treatment of PTLD. A deeper understanding of the mechanisms utilized by the lymphoma cell to evade EBNA1 is needed to optimize immunotherapeutic strategies.

Methods

To this end CD8+ CTL, capable of both proliferation and interferon- gamma (IFN-gamma) and/or CD107 granule release, were studied in 12 PTLD patients. Blood was taken prior to therapy and results compared with 19 healthy EBV-seropositive controls. T-cells were expanded using peptide-panels (17mer overlapping by 10) directed against the EBV antigens EBNA1 or the lytic protein BZLF1 (the latter is *not* expressed in PTLD) in a 14 day culture and assayed by flow cytometry.

Results

Strikingly, we observed a four-to-five fold reduction in both IFN-gamma and CD107 releasing EBNA1-specific CTL in PTLD patients as compared to healthy controls. By contrast, BZLF1-specific CTL were unimpaired relative to controls, consistent with a lymphoma specific inhibition mediated within the tumour microenvironment. Of note, direct visualization using EBNA1-specific class I pentamers on peptide-specific T-cell lines showed strong expression of the T-cell inhibitory receptor PD1 (in contrast to BZLF1-specific T-cells) and concomitant expression of its ligands PDL1 & 2 on EBV-transformed cell lines and 16 spontaneously outgrown primary patient samples.

Conclusions

These results demonstrate for the first-time a selective impairment of immunity against a highly-relevant tumour-associated antigen and suggest that EBNA1-specific CTL are under the inhibitory influence of PDL1 & 2 expressed by malignant B-cells. Strategies to reverse this impairment are being developed.

The authors declare no conflict of interest

A153

Meeting Room 7
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O77

1030-1130
1115

Gene-modified CD8+ T Cells Undergo Functional Polarization to Effector and Central Memory Cells in Response to Antigen Exposure

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Aim

Adoptive transfer (AT) of autologous genetically-redirectioned T cells has considerable potential as cancer immunotherapy. Persistence of AT T cells, critical for tumour control, requires the development (in vitro or in vivo) of a memory T cell subset. We investigated the generation of memory T cell subsets in chimeric T cell receptor-expressing T cells prior to, and after exposure to cognate antigen.

Method

Gene-modified T cells (LeY-T) expressed a chimeric receptor comprising a single chain variable fragment (scFv) which binds Lewis Y (LeY) antigen, linked to intracellular signaling domains (CD3 zeta and CD28). The T cell functional status was assessed by phenotype, homeostatic cytokine-induced proliferation, and response to target antigen at baseline, seven and 30 days after re-exposure to Lewis antigen expressing cell lines (K562 or OVCAR-3).

Results

CD8+ LeY-T cells have an effector memory (EM) phenotype (CD45RA-/CCR7-/CD28+/perforin^{hi} and variable CD27 expression) directly from in vitro culture. Cells express IL-15R beta and the common gamma chain and proliferate in response to IL-15 and in a limited fashion to IL-7. At baseline CD8+ LeY-T cells respond to LeY by proliferating and secreting IFN-gamma but not IL-2. At seven and then 30 days after exposure to LeY antigen, the LeY-T cells had polarised into an EM or central memory (CM) (CD45RA-/CCR7+/CD28+/perforin^{lo}) functional phenotype. In addition, seven days after Lewis antigen exposure, LeY-T cells retain the capacity to proliferate in response to Lewis antigen and to secrete IFN-gamma, but do not secrete IL-2.

Conclusion

LeY-T cells have an effector memory functional status direct from in vitro culture. After exposure to Lewis antigen in vitro, LeY T cells polarize to either EM or CM cells. These results suggest that the LeY T cells have the potential to form long-term memory populations in vivo after adoptive transfer.

No conflict of interest to disclose

Meeting Room 8
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078

1030-1130
1030

Efficacy of Salvage Therapy for Acute Myeloid Leukaemia Relapsing After Allogeneic Stem Cell is Dependant on the Induction of Graft-versus-host Disease

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Introduction

Acute myeloid leukaemia relapsing after allogeneic stem cell transplant (SCT) has a very poor prognosis. The choice of salvage therapy remains unclear. We report the outcome of 38 patients relapsing after allogeneic transplant.

Methods

We analysed the outcome of all patients relapsing after allogeneic transplant between 2000 and 2007. Type of salvage therapy, presence of GVHD and duration of remission post initial SCT were correlated with duration of progression free survival (PFS) after salvage therapy.

Results

Of 116 allogeneic transplants for AML, 38 patients (31%) relapsed. The initial transplant was undertaken in complete remission (CR) 1 (n=14), CR>1 (n=4), untreated relapsed (n=13), progressive disease (n=7). The median time from initial SCT to relapse was 131 days (27-702). Six patients refused salvage therapy. In addition to immunosuppression withdrawal, 32 received salvage therapy including second SCT (n=4), chemotherapy followed by donor lymphocyte infusion (DLI) (n=10), chemotherapy alone (n=8), targeted therapy (tyrosine kinase inhibitor, arsenic, gemtuzamab, idarubicin) and interferon alone (n=11). Median OS after salvage was 154 days (range 24-1804). Those obtaining a further CR (n=15) after salvage therapy had a median PFS of 379 days (range 36-1804) compared to 84 days (range 24-295) in 17 without a further CR (p = 0.0002). Patients developing graft-versus-host disease (GVHD) (n = 22) had a median PFS of 240 days (range 24-1804) compared to 69.5 days (range 28-1179) in patients without GVHD (n = 10) (p = 0.01). Seven patients with long-term disease control experienced extramedullary relapse including bone, breast, skin and myocardial/pericardial infiltration.

Conclusions

These data emphasise the poor prognosis of AML relapsing after alloSCT. Salvage therapies that induced the onset of GVHD were associated with some instances of durable disease control, but with a high risk of extramedullary disease progression.

No conflict of interest to disclose

A155

Meeting Room 8
HSANZ Free Communications 8
079

1030-1130
1045

Prospective Study of Capsule Endoscopy for the Diagnosis of Gastro-intestinal Graft-versus-host Disease

Hazizi Othman¹, **David Ritchie**², Paul Tescher¹, Andrew Grigg², Jeff Szer², Andrew Roberts², David Curtis², Simon Durrant³, Mark Appleyard⁴, Gregor Brown¹, Finlay Macrae¹

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Background

Mucosal biopsy obtained during standard endoscopy (SE) is the gold standard for the diagnosis of Gastrointestinal (GI) Graft-versus-Host Disease (GVHD). SE has limited access to the small bowel. Capsule endoscopy (CE) allows the visualization of the entire bowel, providing data on the extent of GI GVHD.

Aim

To prospectively assess the utility of CE to confirm the presence and/or extent of GI GVHD.

Method

Open labelled multi-centred single arm study of CE in recipients of SCT who were planned for SE for investigation of GI GVHD. CE was allowed to be performed either before or after SE. CE data were reviewed by two independent, blinded gastroenterologists. Diagnostic findings on CE findings were compared to those obtained by SE+ biopsy.

Results

Planned enrolment = 40 patients. To date, 19 pts have been enrolled. 17 pts had assessable SE+biopsy and CE investigations, 1 pt had capsule retention and 1 pt had CE only (due to thrombocytopenia). CE provided interpretable data in 18 pts. SE+biopsy confirmed GI GVHD in 13/18 pts, of whom 11/12 (92%) had concurring findings on CE. 10/11 pts showed small bowel changes on CE inaccessible by SE, thus establishing the true extent of GI involvement by GVHD. A SE+biopsy diagnosis of colonic GVHD was not supported by CE in 1 pt. Of the remaining 5 pts with negative SE+biopsy findings, 4 showed no evidence of GVHD on CE, and in 1 patient, CE established a diagnosis of GI GVHD in the small bowel despite negative findings on upper GI SE+biopsy. In 1 pt unable to undertake SE, CE also confirmed the presence of GVHD changes.

Conclusion

CE is feasible in the assessment of GI GVHD. It demonstrates high positive and negative predicative values for the diagnosis or exclusion of GI GVHD and may better determine the extent of GI involvement.

The authors have no conflict of interest

Meeting Room 8
HSANZ Free Communications 8
O80

1030-1130
1100

Reconstitution of Regulatory T Cells following Allogeneic Stem Cell Transplantation and Association with Graft-versus-host Disease

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Background

Naturally occurring regulatory T cells (Treg) are actively involved in the control of peripheral immunity. A number of animal studies have demonstrated the critical role of these cells in the outcome of allogeneic hematopoietic stem cell transplantation (HCT). In these models, Treg can exert a potent suppressive effect and prevent graft-versus-host disease (GvHD).

Methods

Using flow cytometry to detect cells co-expressing CD4⁺CD25^{Hi} with CD127^{Lo}, we measured Treg in the blood of normal donors as well as 22 patients undergoing allogeneic HCT. Blood samples from patients were obtained twice weekly following transplantation to day 100.

Results

The percentage of CD4⁺ cells within the lymphoid population post HCT was significantly lower (median 27.8% range 8.5 – 44.4%) than that observed in normal individuals (median 40% range 31.8 – 61.4%) ($p=0.001$). In normal donors, Treg make up a median of 2.9% of the total lymphoid population (range 2.1 – 5.5%). Treg reconstitute promptly after HCT and could be identified within 7 days of transplant. From 6 weeks post-transplant, the percentage of Treg within the lymphoid compartment is significantly reduced compared with normals (HCT recipients median 1.0%, range 0.03 – 5.8%, $p<0.0001$). A low percentage of Treg was significantly associated with development of both grade I ($n=6$) and grades II-IV ($n=5$) aGVHD ($p=0.009$ and $p<0.0001$ respectively). The absolute number of Tregs/ul was also significantly lower in patients with aGVHD grades II-IV (median 3.76/ul range 0.2 – 230/ul) compared with patients with only mild or no GVHD (median 16.18/ul range 1.4 – 117/ul) ($p=0.0024$).

Conclusion

Treg reconstitute rapidly post HCT. A reduction in the percentage of Treg within the lymphoid population is significantly associated with development of aGVHD, raising the possibility that the administration of exogenously cultured Treg may have value in preventing or treating severe aGVHD.

No conflict of interest to declare

Meeting Room 8
HSANZ Free Communications 8
O81

1030-1130
1115

The Effect of Immunosuppressive Drugs on the Expansion and Function of Antigen Specific T Cells

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Westmead Millennium Institute, University of Sydney¹; Sydney Cellular Therapies Laboratory, Westmead Hospital, Australia²

Aim

To determine the effect of immunosuppressive drugs commonly used in stem cell and solid organ transplantation on antigen specific T cell expansion and responses.

Method

CMV pp65 peptide (NLVPMVATV) specific T cells were cultured in vitro for 1 week in the presence and absence of cyclosporine, tacrolimus, dexamethasone, sirolimus or mycophenolic acid. Cultures were then analysed for cell number and response to antigen challenge by intracellular cytokine secretion.

Result

Control cultures expanded from 10×10^6 total cells containing an average of 64.7% NLV-tetramer⁺ cells to a mean of 26.5×10^6 cells containing 72.3% NLV-tetramer⁺ cells. Sirolimus, dexamethasone and a combination of dexamethasone and cyclosporine A significantly impeded both total and antigen specific T-cell expansion (mean NLV-tetramer⁺ number 19.4×10^6 for control v 7.9, 8.4 and 8.2×10^6 for immunosuppressives at middle concentration respectively, $p=0.02$, 0.04, 0.03). In contrast, tacrolimus, alone or in combination with dexamethasone, was most effective in reducing the number of cells producing interferon-gamma after antigen challenge and did so without affecting T-cell expansion (mean number of interferon gamma producing cells 12.8×10^6 for control v 2.6 and 1.3×10^6 for immunosuppressives, $p=0.0003$, 0.0003). This effect was not observed with cyclosporine A. None of the immunosuppressives affected apoptosis, cell death, or expression of memory markers.

Conclusion

Sirolimus and dexamethasone impede antigen specific T cell expansion while tacrolimus reduces the secretion of interferon-gamma in response to antigen without affecting T cell expansion. These data may guide the use of immunosuppressives for the prevention and treatment of transplantation associated antigen driven T-cell responses including graft rejection and graft versus host disease.

No conflict of interest to disclose

A158

Meeting Room 2/3
ASTH Free Communications 2
O82

1030-1130
1030

Reduced Fibrinolysis and Increased Fibrin Generation are Found in Patients with the Antiphospholipid Syndrome (APLS)

Jennifer Curnow^{1,3}, Marie-Christine Morel-Kopp^{1,2}, Ninfa Rojas¹, Margaret Aboud¹ and Christopher Ward^{1,2}.

1 Northern Blood Research Centre, University of Sydney. 2 Department of Haematology, Royal North Shore Hospital, Sydney NSW. 3 Department of Haematology, Concord Repatriation General Hospital, Sydney, NSW, Australia

Aim

We have previously demonstrated hypercoagulability utilizing a global haemostatic assay, the Overall Haemostatic Potential (OHP) in a heterogenous population of patients with a demonstrable lupus anticoagulant. Our aim in this study was to determine whether the OHP assay demonstrated a persistent hypercoagulable state in a well-defined prospective population with APLS and whether the assay was able to predict the occurrence of thrombotic complications.

Methods

Blood was collected on three occasions, three months apart from 54 patients with APLS between May 2005 and November 2007. Clinical data were collected including history of prior and subsequent thrombotic events. Two control groups consisted of 200 healthy blood donors and 20 patients with autoimmune disorders, but no history of thrombosis. Assays performed were PT, INR, APTT, FVIIIc, antiphospholipid antibodies (LAC, ACLA, B2GP1) and the OHP assay which utilizes thrombin (0.03 IU/ml) and rt-PA (350 ng/ml) to trigger fibrin generation and fibrinolysis, respectively, in platelet poor plasma. Statistical analysis involved calculation of means, SD and T-tests utilizing SPSS v16.0.

Results

50% APLS patients were male, compared with 10% of the autoimmune control group. APLS had been diagnosed on the basis of persistent antiphospholipid antibodies and at least one thrombotic event (range 1-6 events/pt): VTE (n=46 patients), ATE (n=6) or recurrent late miscarriage (n=2). VTEs were predominantly unprovoked: 49/66. Samples from APLS patients on anticoagulation (OAC, n=35) were analysed separately. OHP results were stable over the 6 month duration of tests (Paired T-tests, p: NS). APLS patients had significantly shorter PT, higher fibrinogen, increased fibrin generation parameters and reduced fibrinolysis parameters (p<0.001) compared with healthy donors. The autoimmune control group also showed hypercoagulable OHP parameters compared with healthy donors (p<0.001), with assay results comparable to those in APLS patients, not on OAC. Only one patient had recurrent DVT during the study.

Conclusions

The OHP assay identifies a hypercoagulable state in APLS patients with reduced fibrinolysis and increased fibrin generation, even when anticoagulated. More prolonged follow up will be necessary to determine whether the assay predicts recurrent thrombotic events.

No conflict of interest to disclose

A159

Meeting Room 2/3
ASTH Free Communications 2
O83

1030-1130
1045

Endogenous Thrombin Potential (ETP) as a Novel Method for Characterization of Procoagulant Snake Venoms

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Aim

Snake venom induced consumption coagulopathy (VICC) results from venomous snakes worldwide with associated morbidity and mortality from bleeding. However, the mechanisms responsible for snake toxins triggering human coagulation remain poorly understood. Here we use endogenous thrombin potential (ETP) to further characterise the relative potency, calcium and factor dependence of venoms from five important Australian snakes.

Methods

Snake venoms of interest were used as the coagulation triggering agents in a modification of the traditional ETP method. Thrombin generation in defibrinated plasma was determined by cleavage of the thrombin-specific chromogenic substrate, Unitrate®. All reactions were performed on an automatic biochemistry analyzer (Cobas Mira, Roche) where optical density at 405nm was recorded every 20 seconds over a 20 minute incubation at 37°C. ETP dose-response curves were generated in the presence and absence of calcium, and factor deficient plasmas were used to investigate the dependence of venom-induced thrombin generation on individual clotting factors.

Results

All five procoagulant Australasian snake venoms initiated thrombin generation in the absence and presence of calcium, but only *Pseudonaja textilis* (Brown snake) venom demonstrated a large increase in ETP with the addition of calcium. Venom potency ranged from the least potent *Oxyuranus scutellatus* (Taipan) venom to intermediate *Notechis scutatus* (Tiger snake) and *Hoplocephalus stephensii* (Stephen's-Banded Snake) to most potent *Tropidechis carinatus* (Rough-scale snake) and *P.textilis*. ETPs for *P.textilis* and *O.scutellatus* venoms were reduced in the absence of prothrombin, while ETPs for *N.scutatus*, *T.carinatus* and *H.stephensii* venoms were severely reduced with prothrombin and factor V deficient plasmas.

Conclusions

This study provides new information on potency of Australian procoagulant venoms. Contrary to previous studies using clotting tests and prothrombin substrates, only *P.textilis* venom appears to be calcium dependent. ETP is likely to be a useful assay to understand the mechanisms of other procoagulant venoms.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 2
O84

1030-1130
1100

Global Assessment of Protein S Deficiency by ProC Global and Calibrated Automated Thrombin Generation Assays

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Aim

The ProC Global (PCG) and the Calibrated Automated Thrombin Generation (CAT) assays were used to evaluate a cohort of individuals at risk of impaired haemostasis due to low protein S levels.

Method

Plasma samples were obtained by a standardised protocol from 38 healthy donors and a group of 18 females either on the oral contraceptive pill (OCP) (n=13), pregnant (n=4), or protein S deficient (n=1). The samples were analysed by ProC Global and CAT assays. The CAT test system was used to determine the Endogenous Thrombin Potential (ETP) and the percentage of thrombomodulin-induced inhibition of ETP (TI-ETP). Free protein S antigen (PSAg), protein C and antithrombin III (ATIII) levels were also determined.

Results

Reference ranges were established for ETP and TI-ETP. ETP was raised in 8/18 (44%) of individuals in the study cohort and 5/13 (38%) of the OCP group. Reduced TI-ETP was found in 7/18 (39%) and 5/13 (38%). PCG normalised ratio was reduced in 4/18 (22%) and 2/13 (15%). All low PCG samples had a low TI-ETP. Low PSAg levels were found in 7/18 (39%) and 2/13 (15%) of the respective groups. Comparison of global assays with natural anticoagulant levels in all donors showed that ETP correlated best with ATIII ($r = -0.48$) and TI-ETP with PSAg ($r = 0.44$). PCG was weakly correlated with PSAg ($r = 0.15$).

Conclusions

Thrombin generation was raised in the study group. The incidence of elevated ETP or reduced TI-ETP was approximately 2-fold greater than that of reduced PCG normalised ratio. The inclusion of thrombomodulin in the CAT assay may assist in the investigation of protein S deficiency.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 2
O85

1030-1130
1115

Plasma Fibronectin Levels are Elevated in Patients With Venous Thromboembolism

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Aim

Recent evidence suggests that atherothrombosis and venous thrombosis (VTE) share common risk factors. As various clinical states including arterial disease are associated with elevated plasma fibronectin levels we assessed the hypothesis that high plasma fibronectin levels are associated with VTE.

Method

Plasma fibronectin levels were measured by ELISA assay in a well defined VTE and control cohort, the Scripps Venous Thrombosis Registry consisting of 113 VTE cases and 113 age and sex matched controls

Result

Significantly higher mean fibronectin concentrations were observed in VTE cases compared to controls (127% vs 103%, $p < 0.0001$); the difference was greater for idiopathic VTE cases compared to secondary VTE cases (133% vs 120%, respectively). The odds ratio (OR) for association of VTE (using a cut-off of $>90\%$ of the control values), were 9.37 (95%CI 2.73-32.2; $p < 0.001$) and this OR remained significant after adjustment for sex, age, BMI, factor V Leiden and prothrombin nt20210A (OR 7.60, 95%CI 2.14-27.0; $p = 0.002$). In particular, the OR was statistically significant for idiopathic VTE before and after these statistical adjustments. The OR for association of VTE of plasma fibronectin levels above the 90th percentile for the male cohort remained significant before and after statistical adjustments however was not significant in females.

Conclusion

Our results extend the potential association between the biomarkers and risk factors between atherothrombosis and VTE and suggest that elevated plasma fibronectin levels are associated with VTE, in particular in males.

No conflict of interest to disclose

Riverside Theatre
ANZSBT: Ruth Sanger Oration

1030-1130

External Quality Assurance in Transfusion in Australia

John Rowell

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Transfusion has changed from the technical profession of determining blood groups, identifying a myriad of antibodies and cross-matching blood in the 1960s to a highly automated laboratory providing a wide range of products and advice with emphasis on appropriate use of blood and blood products. Initially External Quality Assurance (EQA) programs provided by the Royal College of Pathologists of Australasia (RCPA) were voluntary, concentrating on methodology, but aimed at improvement of performance of laboratories. Results of EQA were manually recorded, analysed and returned to laboratories with initial participation of approx 30%. Over time the EQA program has adapted to changing laboratory methodology and practice, assessing clerical functions, adherence to guidelines, surveys of practice and complications of transfusion. Significant challenges remain if EQA exercises are to assess individual laboratory performance. In many laboratories, computerization is integral to result checking and provision of appropriate blood. Automation is becoming more frequent – even in medium sized laboratories. Laboratories and staff maybe involved in education and liaison with clinical staff concerning appropriate use of blood. The evolution of EQA in Australia will be discussed to highlight achievements and remaining challenges to assess laboratory performance.

Riverside Theatre
HSANZ: Lymphoproliferative Disease

1330-1500
1330

Role of the B-cell Receptor in Chronic Lymphocytic Leukemia

Nicholas Chiorazzi

The Feinstein Institute for Medical Research, Manhasset, New York, USA

The development and maturation of normal B lymphocytes requires the successful rearrangement and recombination of several immunoglobulin (Ig) variable (V) region gene segments (V[D]J) of the H and L chain loci. This recombination can involve multiple genes from each locus (V_H: 44, D: 27, J_H: 6 and V_L: V_K-46, V_λ-36, and J_L: J_K-5, V_λ-7). In addition, during the recombination process, nucleotide changes can occur at gene segment junctions. Therefore, the likelihood that the primary amino acid V_HDJ_H/V_LJ_L sequences of two B lymphocytes are identical approximates 1×10^{-12} . In a series of studies that will be discussed in this presentation, it has been documented that the B cells that overgrow in chronic lymphocytic leukemia (CLL) can have remarkably similar B-cell receptor (BCR) amino acid sequences at a frequency approaching 1×10^{-2} . These non-random BCR amino acid structures arise from the use and association of Ig V_H, D, and J_H segments that differ from those seen in normal human B cells.

Furthermore, these biased BCR structures differ in frequency and features between the two subgroups of CLL patients that can be identified based on the presence (M-CLL) or absence (U-CLL) of significant numbers of V_H gene mutations, who follow very different clinical courses (indolent vs. aggressive, respectively). This suggests that the specific antigens that select and drive CLL cell precursors and full-fledged CLL and/or the ability of these antigens to trigger the cells through the BCR may differ between U-CLL and M-CLL. Indeed, data indicate that the level of antigenic specificity differs between these two CLL subgroups, with U-CLL BCRs binding multiple (auto)antigens (polyreactive) and M-CLL BCRs binding a more restricted number of (auto)antigens (mono/oligoreactive). Furthermore, BCRs of U-CLL patients are more likely to transmit a signal when crosslinked than those of M-CLL patients.

Collectively, these findings suggest that CLL develops from a limited set of B-lymphocytes of defined BCR structure and antigen-binding properties and imply that selection of B cells with such structures represents an important promoting factor in the evolution of the leukemic cell. In addition, the differential capacities of U-CLL vs. M-CLL clones to be activated by BCR engagement suggests that this process may be linked to the divergent clinical courses of these two subsets of patients.

Riverside Theatre
HSANZ: Lymphoproliferative Disease

1330-1500

1415

Molecular Biomarkers in Diffuse Large B-cell Lymphomas

WH Wilson

National Cancer Institute, Bethesda, Maryland, United States

Molecular profiling can measure the expression of thousands of genes in parallel and link biology to a genetic expression signature. The application of this technology to lymphoma is providing insights into unique molecular signatures of distinct types of B-cell malignancies. It can relate lymphoid neoplasms to normal stages in B-cell development and physiology, and may provide a new way to classify lymphomas, predict clinical outcome and identify molecular targets for treatment. Molecular profiling of DLBCL indicates it can be divided into three subtypes derived from a germinal center B cell, termed a GC B cell-like (GCB), activated post-germinal center B cell, termed activated B cell-like (ABC), and primary mediastinal B-cell lymphoma (PMBL), derived from a thymic B-cell. Genes associated with GCB DLBCL included known markers of germinal center differentiation such as CD10 and the *bcl-6* gene, which may be translocated or mutated in DLBCL, as well as numerous new genes. In contrast, most genes that defined ABC DLBCL were not expressed by normal GC B cells, but instead were induced during in vitro activation of peripheral B cells such as cyclin D2 and CD44. These results suggest that the GCB and ABC DLBCL subtypes are derived from B cells at different stages of differentiation and are pathogenetically distinct. If this new taxonomy defines true DLBCL subtypes, it should have clinical prognostic value. Because all biopsies were untreated patients receiving doxorubicin-based chemotherapy, it was possible to correlate survival and the DLBCL subtype. This analysis revealed a statistically significant difference in overall survival at 5-years of 59% in GCB and 31% in ABC subtypes of DLBCL. This clinical difference in outcome has also been shown to apply to rituximab based treatments. A molecular prognostic analysis identified high proliferation, ABC subtype, and low lymph node signature as adverse biological features.

Meeting Room 4
ANZSBT: Platelet Alloimmunisation

1330-1500
1330

Platelet Alloimmunisation

Paul Metcalfe

National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK

Alloantibodies against Human Platelet Antigens (HPA) are involved in feto-maternal alloimmune thrombocytopenia (FMAIT), platelet refractoriness (PR) and post-transfusion purpura (PTP). Anti-HPA-1a is the most common antibody involved in FMAIT and PTP cases, but is rarely involved in PR. A wide range of techniques have been developed for platelet antibody detection over the last 25 years but only a small number of techniques have proved suitable for reliable routine use. The use of at least two techniques is essential in maximising the detection HPA antibodies. However, quality exercises have demonstrated that there is still considerable inter-laboratory variation in the sensitivity of antibody detection tests and there is a clear need for standardisation in HPA antibody detection. Quantitation of anti-HPA-1a in International Units is now possible following the adoption of an International Standard and the relevance of antibody titre in FMAIT can now be investigated in multi-centre studies.

Meeting Room 4
ANZSBT: Platelet Alloimmunisation

1330-1500
1415

Clinical Management of Foetal and Neonatal Alloimmune Thrombocytopenia

James Bussel

Weill Cornell Medical College, Division of Pediatric Hematology Oncology, New York, USA

Alloimmune thrombocytopenia is the platelet equivalent of RH incompatibility or hemolytic disease of the fetus and newborn. Classically, the mother is HPA-1a negative (HPA1b1b) and the father is HPA1a positive (75% of the time HPA1a1a); the nomenclature used to be PIA1 or Zwa. If sufficiently stimulated and genetically predisposed, the mother makes IgG anti-HPA-1a and this IgG antibody crosses the placenta and attacks fetal platelets (? and megakaryocytes). There is no routine screening and the diagnosis is therefore suspected clinically if the fetus/newborn has an intraparenchymal ICH, a platelet count < 50,000/ul at birth, or is unexplained. Treatment of the severely thrombocytopenic neonate (< 30,000/ul unless there are factors such as ICH or asphyxia which warrant increasing the count) consists of random platelets with or without concomitant IVIG; studies suggest matched platelets should be required relatively infrequently but are optimal if handled correctly and available. Ultrasound, CT or MR of the head is mandatory if there is significant thrombocytopenia (<50,000/ul). The next pregnancy (if affected) will tend to be more severely affected and treatment can be provided to the mother, the exact protocol for which depends upon the history of the previous affected sibling. As an oversimplification, if the previous fetus has had an in utero or perinatal intracranial hemorrhage (ICH), treatment should start at 12 weeks of gestation with IVIG 1 gm/kg/infusion administered twice each week. If there was not an ICH, then treatment could begin at 20-30 weeks with IVIG 1 gm/kg/week and prednisone 0.5 mg/kg/day. Currently fetal sampling under platelet cover is recommended to determine response at 20-26 weeks in the higher risk setting, and at approximately 34 weeks in fetuses without a previous sibling hemorrhage. Sampling is intended not only to inform delivery but in particular to allow intensification of treatment in those not responding (well) to the initial treatment. Intensification consists of adding treatment to go to IVIG 2 gm/kg/week (in 2 infusions) and prednisone 1 mg/kg/day; serial fetal platelet transfusions should virtually never be required.

Meeting Room 2/3
ASTH: Arterial Thrombosis

1330-1500
1330

Thrombosis in AV Fistula

Ashley Irish

Royal Perth Hospital, Perth, WA, Australia

Abstract not received at time of going to print

Meeting Room 2/3
ASTH: Arterial Thrombosis

1330-1500
1400

What the Hell is tPA Doing In My Brain?

Andre Samson, Rachael Borg, Be'eri Niego, Tang Yongqing and Robert L Medcalf
Australian Centre for Blood Diseases, Monash University, Melbourne, Victoria, Australia

A major function of *tissue-type plasminogen activator* (tPA) is to convert extracellular plasminogen into the active protease plasmin. For tPA to generate plasmin in the blood, both tPA and plasminogen must first bind to fibrin. This co-factor activity of fibrin facilitates intravascular tPA-mediated plasmin generation and is essential for thrombolysis.

tPA-mediated plasmin generation is also important to brain function (e.g. memory formation) and dysfunction (e.g. excitotoxicity). Unlike the blood, however, the brain is devoid of fibrin. Hence, the co-factors responsible for tPA-mediated plasmin generation in the brain remain largely unknown. In this context, we find that injured cells act as a potent "fibrin-like" co-factor for tPA-mediated plasmin formation in the brain.

Specifically, we show that tPA strongly binds to brain cells that have been injured both *in vitro* and *in vivo*. We also demonstrate that injured brain cells bind plasminogen and, in turn, significantly accelerate tPA-mediated plasmin formation. Interestingly, the binding of tPA to injured cells spatially and temporally correlates with the appearance of protein aggregates. Hence, we hypothesize that tPA binds to protein aggregates that are formed during the cell death process. Lastly, whilst the binding of plasminogen to injured cells is completely blocked by the anti-fibrinolytic agent, tranexamic acid, tPA-binding is only weakly inhibited by tranexamic acid. This fact is further evidenced by the inability of the truncated tPA molecule, Reteplase, to bind to injured brain cells. Thus, it may be possible to inhibit tPA-mediated plasmin formation within the injured brain whilst leaving intravascular fibrinolysis unperturbed. Such an approach would be especially desirable during thrombolytic therapy in ischaemic stroke patients; a situation where intravascular plasmin activity is beneficial and intracerebral plasmin activity is harmful. Beyond stroke, our observations also have important ramifications for other disease states where tPA-mediated plasmin formation is key.

No conflict of interest to disclose

Meeting Room 2/3
ASTH: Arterial Thrombosis

1330-1500
1430

Aspirin and Clopidogrel Resistance - What Does It Mean?

Jamie Rankin

Royal Perth Hospital, Perth, WA, Australia

Abstract not received at time of going to print

Riverside Theatre
HSANZ: Clinical BMT

1530-1630
1530

Post-Transplant Malignancies: Risk Factors Related to Patient, Donor and Transplant Regimen

H Joachim Deeg

Fred Hutchinson Cancer Research Center and University of Washington School of Medicine, Seattle, WA, USA

One of the adverse delayed effects after transplantation is the development of new malignancies. Three patterns have been observed. 1) Post-transplant lymphoproliferative disorders (PTLD), most of which occur within 6–9 months of transplantation; 2) myelodysplasia (MDS) / acute myeloid leukemia (AML), generally within a few years after transplantation; 3) solid cancers / sarcomas, the incidence increasing for decades after transplantation. PTLD generally develop in donor B cells and are EBV+. Histo-incompatibility, T-cell depletion, and severe immunosuppression for GVHD are the major risk factors. Hodgkin disease has been observed as well. MDS/AML develops in several settings. In autologous stem cell recipients, damage inflicted upon the microenvironment and exposure of stem cells to cytotoxic therapy before transplantation are relevant. Prior exposure to alkylating agents, the use of TBI for conditioning, and possibly peripheral blood rather than marrow stem cells increase the risk. In the allogeneic setting, donor and host-derived malignancies occur. Only in a proportion of donor-derived cases was the malignancy detected in the donor. In the remaining patients, it appears to have developed de novo in the transplant recipient. Most new malignancies are solid tumors, in particular, tumors of the skin (basal cell, squamous cell or melanoma). The next most frequent malignancy is breast carcinoma, the incidence increasing significantly by 15 to 20 years after transplantation; only single high-dose TBI was identified as a risk factor. TBI of any dose was a risk factor for basal cell, but not for squamous cell carcinoma; the latter one being more frequent with chronic GVHD. The effect of irradiation was particularly significant in younger patients. Of note, more recent birth cohorts were at higher risk of developing basal cell carcinomas than earlier birth cohorts. Studies correlating gene polymorphisms (e.g. xenobiotic pathways) with tumor development are in their infancy. Also, it is not clear to what extent the genetic makeup of a patient, associated with the original disease, contributes to the post-transplant malignancy. Life-long monitoring of patients is recommended.

Riverside Theatre
HSANZ: Clinical BMT

1530-1630
1600

Oral Complications of Bone Marrow Transplantation

Ian Hewson

Royal Melbourne Hospital, Melbourne, Victoria, Australia

Oral complications can adversely affect the outcome of bone marrow transplant (BMT) patients, good oral health is essential prior to the patient commencing their conditioning. The most common and debilitating oral complication of BMT is oral mucositis. Severe Oral mucositis (WHO grade 3-4) is associated with significant pain, impairing the patients quality of life, when patients are neutropenic there is a significant risk of sepsis it can also compromise methotrexate GVHD prophylaxis.

Severe oral mucositis (OM) significantly increases the use of narcotic analgesia, TPN and total hospital costs, OM is the most debilitating patient reported side effect of BMT conditioning.

Xerostomia, taste dysfunction are acute and chronic side effects of BMT, profound xerostomia can lead to rampant dental caries with rapid destruction of oral hard tissues. With allogeneic BMT the risk of oral GVHD is between 70 – 80% of those patients who develop GVHD, oral GVHD can vary from an asymptomatic stria on the buccal mucosa to widespread erosions of the non keratinized mucosa causing significant pain and compromising the patients oral intake.

Continuing immunosuppression can lead to viral, bacterial or fungal oral infections and occasionally oral malignancy, especially oral SCC.

Meeting Room 4
ANZSBT: Rare Blood

1530-1630
1530

Provision of Rare Blood

Marion Reid

New York Blood Center, New York, NY, USA

Provision of rare blood to a patient who needs it can be accomplished by a combination of testing by hemagglutination and by DNA-based methods. A rare donor can be defined as one in 200 or one that is hard to find when a patient needs it! A donor is classified as being rare for two reasons: (i) because of an absence of a combination of antigens or (ii) an absence of a high-prevalence antigen. Patients who are most likely to be chronically-transfused and therefore, need rare blood include those with certain inherited diseases (sickle cell disease, thalassemia, Diamond Blackfan anemia) or with acquired conditions (autoimmune hemolytic anemia, to support a patient during certain medical treatments). Alloantibodies can develop after transfusion or pregnancy because of differences between antigens on recipient and donor or fetal RBCs. The more antibodies a patient makes, the harder it is to find appropriate antigen-negative donors. To prevent a transfusion reaction, a patient with clinically-relevant antibodies should, for the rest of her/his life, be transfused with antigen-negative RBC components. Use of precisely-matched blood will require a large inventory of antigen-negative donor blood. Antigen-negative donors are found from the following sources: patients (allogeneic, when return to health and eligible, or autologous if ineligible to donate and rare enough), siblings of patient, donors identified by antibodies in their serum, donors found by deliberate screening, and siblings of a donor. Rare donors can be found by screening by hemagglutination or by using a microarray platform followed by confirmation by hemagglutination (either with a reagent if it is available, and/or crossmatching using a hemagglutination method that is optimal for the antibody/antigen combination in question). Microarray platforms use computerized recording and interpretation of results, and have the potential for direct down-loading of interpretations to a donor data base.

Meeting Room 4
ANZSBT: Rare Blood

1530-1630
1605

Rare Blood: The Australian Perspective

Brian Fisher¹, Tanya Brown²

¹ Production Services Management, Australian Red Cross Blood Service, Perth, WA, Australia. ² Red Cell Reference Laboratory, Australian Red Cross Blood Service, Perth, WA, Australia.

The Australian Red Cross Blood Service (ARCBS) supplies approximately 790,000 red cell components (RC) each year. The vast majority of these are used within Australia uneventfully for recipients whose plasma is free from irregular blood group antibodies. At times the ARCBS is required to supply rare blood when the intended recipient has a clinically significant antibody directed against a red cell antigen that is common in the population, or a mixture of antibodies that individually would not present difficulty in supply but in combination result in there being only a small number of compatible Australian donors.

Once the need for rare blood has been established the ARCBS is able to search in real-time the Australia-wide RC inventory database held on the National Blood Management System (NBMS) to ascertain if any suitable units are present in either normal or frozen inventory. When suitable RC are located they are transported via the ARCBS network to the recipient's location, and if insufficient suitable RC are found the NBMS donor database is then searched to identify compatible donors who are invited to donate. In the event of an unsuccessful national donor search, including the intended recipient's family, ARCBS is able to escalate the search to other countries in the region and international rare donor registries. Australia also plays a role in supplying rare blood on the international blood exchange program and in 2007/8 sent RC with O_h(Bombay), Vel- and Rh_{null} phenotypes to assist overseas patients

Changing patient demographics due to Australia's immigration policy is resulting in novel demands for rare blood with requests for Jk(a-b-), Fy(a-b-) and U- becoming more common.

Standard phenotype testing in all 5 ARCBS testing sites is routine but screening for rarer phenotypes occurs when specific resources become available. New challenges to the blood service as a result of the increasing ethnic diversity in the population will be met by a combination of strategies. These include initiatives to gain a better understanding of the cultural, societal and systemic barriers to ethnic minorities donating blood, and rare phenotype detection using molecular techniques which allow bulk screening without the need for difficult to obtain and frequently weak antisera.

No conflict of interest to disclose.

Meeting Room 2/3

ASTH: Coagulation Issues in Snakes and Children

1530-1630

1530

Snake Bite Coagulopathy: Antivenom, FFP or Nothing At All

GK Isbister

Calvary Mater Newcastle Hospital, Newcastle & Menzies School of Health Research, Charles Darwin University, Darwin

Coagulopathy is a major effect of many snake venoms and an important clinical syndrome associated with snake bite worldwide. The most common clinical manifestation is a venom induced consumption coagulopathy (VICC) due to procoagulant toxins in the venom. VICC is a particular problem in envenoming by Australian elapid snakes and accounts for almost 80% of severe snake envenoming in Australia. Australian elapids, including brown snakes (*Pseudonaja*), tiger snakes and related genera (*Notechis*, *Tropidechis*, *Hoplocephalus*) and taipans (*Oxyuranus*) all contain potent prothrombin activators which result in VICC characterized by fibrinogen, factor V and factor VIII deficiencies. Antivenom has been the major treatment for snake envenoming for decades and significant resources are put into maintaining antivenoms in Australian hospitals. Until recently, information on dosing, effectiveness and safety has been limited to anecdotal reports and small observational studies. Recent results from the Australian Snakebite Project (ASP) have begun to address these issues. In vitro and clinical studies have shown that only 1 to 2 vials are required to bind all venom in patient sera from brown snake envenoming, and similar small doses are required for other snakes. However, recovery from VICC takes many hours and is dependent on the synthesis of new clotting factors and decisions regarding further antivenom administration should wait for at least 6 hours and probably 12 hours after antivenom. A recently developed systems model of the coagulation pathway predicted that the procoagulant component of the venom had a short duration of action (< 1 hour) and antivenom neutralization must occur immediately venom enters the circulation to reduce recovery time of VICC. Assessing the effectiveness of antivenom in patients with VICC is difficult in a clinical trial so a time to event analysis of recovery of VICC was used to explore the effectiveness of antivenom. This study showed that neither the dose nor timing of antivenom affected the time to recovery, raising questions about the effectiveness of antivenom. In addition, the study demonstrated that only the early administration (< 4 hours after antivenom) of fresh frozen plasma decreased the time to recovery. Future studies will need to continue to explore this to determine the most effective and safest approach to the treatment of VICC.

Meeting Room 2/3
ASTH: Coagulation Issues in Snakes and Children

1530-1630
1600

Anticoagulation in Children

Paul Monagle

Department of Haematology, Royal Children's Hospital Melbourne & Department of Paediatrics, University of Melbourne, Vic, Australia

Thromboembolic disease in neonates and children has been labeled as a new epidemic within tertiary paediatric hospitals. As survival rates for major congenital heart disease, childhood cancer and other major medical and surgical conditions improve, the frequency of secondary complications such as thromboembolic disease is rapidly increasing. Thus the use of anticoagulant drugs in neonates and children is also increasing dramatically. Despite this there are many barriers to the safe and effective use of these drugs in children, and evidence suggests mere extrapolation of adult based anticoagulant guidelines is inappropriate. The different epidemiology of thromboembolic disease and patient related comorbidities in children compared to adults, the impact of developmental haemostasis, and the difficulties associated with drug delivery and monitoring in paediatric populations all have considerable impact on the use of these drugs in children compared to adults. Our understanding of these age related issues is the subject of much current research which is challenging our conventional thinking in many ways. This presentation will provide an update on the current status of our knowledge in this field, describe some of the current research and anticipated future directions and discuss currently accepted best practice models for delivering anticoagulation therapy in children, as well as assessing the value/role of new anticoagulant drugs in children as they become available on the market.

Meeting Room 7
HSANZ: Masterclass

1630-1730

How To Create a Multidisciplinary Translational Cancer Research Program: The Myeloma Institute for Research and Therapy as a Model

John D Shaughnessy, Jr, USA

NOTES:

Meeting Room 6
HSANZ: Masterclass

1630-1730

Management of Acute Lymphoblastic Leukemia

Martin S Tallman

Northwestern University Feinberg School of Medicine, Robert H. Lurie Comprehensive Cancer Center, Chicago, IL, USA

Historically, therapy for adults with acute lymphoblastic leukemia (ALL) has been based on successful strategies in pediatric populations and includes induction, consolidation, intensification, central nervous system (CNS) prophylaxis and maintenance. Immunophenotyping reveals early pre-B accounts for 55% percent of adult ALL, pre-B 15%, mature B 5%, and T-lineage 25%. Many cytogenetic and molecular subtypes reflect heterogeneity and include TEL/AML1 which is uncommon, but associated with a favorable prognosis, and BCR-ABL and MLL/AF4 which are more common, but confer an unfavorable prognosis. Other favorable prognostic factors among standard-risk patients include age < 35 years, white count < 30,000/uL for B-lineage and < 100,000/uL for T-lineage and complete remission (CR) within four weeks, although a recent large intergroup study has not confirmed the latter. Multiagent chemotherapy in adults yields a CR rate of 80-90%, but long-term leukemia-free-survival of only 35-40%, in contrast to the excellent outcomes in childhood ALL. Adolescent and young adults may fare better when treated on pediatric protocols. Maintenance therapy is important except in mature B-lineage and Philadelphia chromosome positive (Ph pos) ALL. CNS radiation is probably not necessary when systemic high-dose methotrexate or ara-C and aggressive intrathecal methotrexate are administered. The role of allogeneic hematopoietic stem cell transplantation (HSCT) is evolving. The UKALLXII/ECOG2993 intergroup trial showed a clear benefit among standard-risk, but not high-risk patients and no role for autologous HCST compared to chemotherapy. While the outcome of older adults remains poor, remarkable improvement in overall survival among patients with Ph pos ALL with the combination of Imatinib or Dasatinib and intensive chemotherapy represents a major success. Most such patients in CR undergone HSCT, although this issue now needs revisiting. New agents such as Nelarabine for T-lineage, Rituximab and Alemtuzumab for B-lineage, clofarabine, pegylated L-asparaginase, gamma secretase inhibitors and liposomal vincristine are in clinical trials and may find important roles.

Meeting Room 8
ANZSBT: Masterclass

1630-1730

Complex Cases: Red Cell and Platelet Alloimmunisation

Panel:

Prof James Bussel, USA

Dr Paul Metcalfe, UK

Dr Marion Reid, USA

NOTES

Meeting Room 3
ASTH: Masterclass

1630-1730

Diagnosis and Management of Mild Bleeding Disorders

David Lillicrap

Dept. Pathology & Molecular Medicine, Queen's University, Kingston, Canada

Most clinically encountered mild bleeding disorders represent problems with the primary hemostatic mechanism and involve either quantitative or qualitative defects affecting von Willebrand factor (vWF) or platelets. In addition, mild to moderate deficiencies of many of the procoagulant clotting factors can also present in this fashion.

Of prime importance in the assessment and diagnosis of mild bleeding problems is an objective quantitative evaluation of clinical bleeding. While this can be done through the attainment of a routine clinical history by an experienced hematologist, there is some reason to believe that a standardized bleeding questionnaire may facilitate identification of these cases and enable appropriate determination of the bleeding severity. This in turn will impact the extent to which laboratory testing will be pursued to establish a diagnosis.

In most instances, routine screening studies for mild bleeding disorders are not informative. Evaluation of primary hemostasis through performance of a bleeding time or PFA analysis is relatively sensitive to vWF and/or platelet defects but many clinicians will proceed directly to more specific forms of testing if the clinical history is positive. Evidence of chronic blood loss may be apparent in the CBC and occasionally, examination of the blood smear may reveal platelet abnormalities. Finally, most single clotting factor deficiencies need to be <35% to become apparent in screening tests like the PTT and PT. Thus, if the history is suggestive of a plasma clotting factor deficiency, single factor assays should be performed.

"Second phase" studies for these disorders should include specific testing for von Willebrand disease and platelet functional defects. During these studies it is important to rule out interference from drug effects and to assay vWF components at least twice to take into account the temporal variability of vWF and FVIII levels.

Further investigation for far less frequent conditions should rely upon the strength of the bleeding history and other factors such as a family history of bleeding.

Meeting Room 6
HSANZ Free Communications 9
O86

0830-1000
0830

Synergistic Interactions Between DNA-damaging Agents and HDACi's Induce Upregulation of BCL6 in the Promyelocytic Leukaemia Cell Line, HL-60

Faith Kwa^{1,2}, Miroslav Kapuscinski¹, Merrole Cole-Sinclair³

¹The Alfred, Melbourne, Victoria, Australia; ²The University of Melbourne, Parkville, Victoria, Australia; ³St Vincent's Hospital, Fitzroy, Victoria, Australia

Aims

To investigate the effects of histone deacetylase inhibitors (HDACi's) sodium butyrate (NaBu) and Trichostatin A (TSA) on 1) the sensitivity of HL-60 cells to chlorambucil (CLB) and fludarabine (Flu) and 2) expression of genes involved in chromatin remodelling (DNA methyltransferase 1, DNMT1; histone acetyltransferase 1, HAT1; histone deacetylase 7A, HDAC7A; Vitamin D receptor, VDR; B cell lymphoma 6, BCL6; and survivin).

Methods

The null p53-mutant cell line HL-60, a model for chemo-resistant leukaemia cells, was treated with drug combinations (CLB+NaBu/TSA and Flu+NaBu/TSA). Cell viability was determined by tetrazolium salt-based proliferation assays. Apoptosis was analysed by flow cytometry [(annexin-V FITC and propidium iodide)]. Gene expression was measured by qRT-PCR. Drug interaction analysis was performed using the Additive Model [$\text{Observed}_{\text{viable}}/\text{Expected}_{\text{viable}}$ (O/E) ≤ 0.8 (synergistic); $0.8 < \text{O/E} < 1.2$ (additive); $\text{O/E} \geq 1.2$ (antagonistic)]. Statistical analyses of 4 independent experiments included ANOVA and pooled t-test ($p < 0.05$). All experimental work was performed at The Alfred.

Results

2 μ M CLB+2 μ M TSA, 5 μ M CLB+0.2mM NaBu and 1 μ M Flu+1 μ M TSA were the most efficient combinations in promoting synergistic cell death (all p values < 0.001 , all $\text{O/E} < 0.8$) but NaBu antagonised Flu cytotoxicity (all $\text{O/E} > 1.2$). Flow cytometry analyses on day 3 demonstrated that all 3 combinations had synergistic effects on apoptosis with 1 μ M Flu+1 μ M TSA showing the greatest synergy (all p values < 0.05 , all $\text{O/E} < 0.8$). qRT-PCR results revealed small (< 2 -fold) but significant ($p < 0.05$) changes in DNMT1, HAT1, HDAC7A, VDR and survivin expression. BCL6 was induced by NaBu/TSA alone or in combination with CLB/Flu where the largest increase (19.7-fold) was seen in 5 μ M CLB+0.2mM NaBu-treated cells ($p < 0.001$).

Conclusion

The expression of chromatin remodelling enzymes, VDR, and survivin does not play a major role in augmented cytotoxic responses to combinations of DNA-damaging agents and HDACi's in HL-60 cells but may involve BCL6 upregulation via HDACi-induced p21 expression.

No conflict of interest to disclose

Meeting Room 6
HSANZ Free Communications 9
O87

0830-1000
0845

Rapid Isolation of DNA Breakpoints in Leukaemia by Bottleneck PCR

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¹ Flinders University and Medical Centre, Adelaide, South Australia, Australia

² Monoquant, Adelaide, Australia

Aim

To isolate DNA breakpoints in chronic myeloid leukaemia (CML) and acute promyelocytic leukaemia (APML), as a first step for monitoring MRD using DNA

Methods

PCR primers were designed to cover the breakpoint site in CML and APML. For CML 6 forward primers covered a 3kb BCR and 282 reverse primers covered a 140kb ABL region, while for APML 6 forward primers covered a 3kb PML and 34 reverse primers covered 16kb RARa region. Primers were positioned approximately 500bp apart to minimise product size and ensure efficient amplification. Patient DNA from diagnosis was amplified in six PCRs, each containing one of the forward primers and all of the reverse primers. First round products were diluted and subjected to 2-4 rounds of a novel PCR technique we have termed bottleneck PCR. Due to the high number of individual primers in a reaction, the amplification of non-specific products, primarily reverse to reverse, is inevitable. The principle of bottleneck PCR is to use tagged reverse primers and adjust conditions of the PCR so as to ensure that the reverse primer or primers hybridises inefficiently. This modification results in selection against amplification of products resulting from reverse-reverse priming. Following several rounds of bottleneck, products were resolved by gel electrophoresis, with patient specific products sequenced to identify breaks.

Results

To date, breakpoints have been isolated and sequenced in 28/29 (97%) CML patients and in 2/2 APML patients. Further studies are ongoing.

Conclusion

The combination of multiplex and bottleneck PCR is a simple and a rapid strategy for sequencing the breakpoint in CML and APML, and may have application for other translocations. Sequence data from patients have been used to establish a DNA based quantitative PCR system to measure the level of MRD in CML patients.

This research was supported by Monoquant. The company had no role in analysing the data or preparing the abstract.

Meeting Room 6
HSANZ Free Communications 9
O88

0830-1000
0900

Dipeptidyl Peptidase Expression in Chronic Lymphocytic Leukaemia (B-CLL)

Melanie Sulda^{1,2}, Peter Macardle³, Rachel Hall¹, Catherine Abbott², Bryone Kuss¹

¹. Department of Haematology and Genetic Pathology, Flinders Medical Centre (FMC), Adelaide, South Australia (SA)

². School of Biological Sciences, Flinders University, Adelaide, SA

³. Department of Immunology, FMC, Adelaide, SA

Aim

The dipeptidyl peptidases (DPs) DPIV/CD26, DP8, DP9 and fibroblast activation protein (FAP) are a related group of serine proteases. Non-selective DP inhibition with ValboporoPro/Talabostat is reported to cause apoptotic cell death in indolent CLL *in vitro*. Hence the aims were to:

1. Characterise mRNA and protein expression of known enzymes with DP activity in B-CLL lymphocytes
2. Compare DP expression to prognostic markers in B-CLL
3. Determine whether the DPIV/8/9 inhibitor p32/98 causes apoptosis in B-CLL and normal lymphocytes

Method

DP expression was assessed in sorted CD5⁺/CD19⁺ B-CLL cells by real-time qRT-PCR or by flow cytometry. Prognostic potential was assessed by comparing DP expression to IgVH mutational status, CD38, ZAP-70 expression and clinical features.

Results

DP8, DP9, DP11 and PEP (prolyl endopeptidase) were constitutively expressed in B-CLL. DP8 expression was significantly higher in B-CLL, than any other DP. Contrary to published data, CD26 expression was only detected in 4/40 patients. FAP mRNA and protein were not expressed. DP expression did not correlate with any prognostic or clinical indicators. Selective inhibition of DP activity by p32/98 at 100µM final did not cause apoptosis of B-CLL or normal lymphocytes.

Conclusion

Total rather than selective DP inhibition may be required to promote cell death. Due to the broad expression of DPs *in vivo*, this is unlikely to be tumour specific. CD26 expression is infrequent in CLL and not prognostically linked. The expression of DP8 in normal CD5⁺/CD19⁺ B-lymphocytes is currently being investigated for the significance of DP8 expression in B-CLL.

No conflicts of interest to disclose

Meeting Room 6
HSANZ Free Communications 9
O89

0830-1000
0915

The BH3 Mimetic Compound ABT-737, Synergises with Standard Chemotherapy to Induce Apoptosis in Chronic Lymphocytic Leukaemia (CLL)

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¹ Walter and Eliza Hall Institute of Medical Research, Parkville, Vic, Australia.

² Department of Clinical Haematology and Bone Marrow Transplantation, The Royal Melbourne Hospital, Parkville, Vic, Australia

Aim

Overexpression of Bcl-2 is universal in CLL and is associated with chemo-resistance. Targeting Bcl-2 with compounds that mimic its physiological antagonists (i.e., the BH3-only proteins) may have a role in treatment of CLL. ABT-737 is one such BH3 mimetic that shows potent *in vitro* cytotoxicity in CLL samples. We evaluated the relationship of established clinical prognostic factors and individual Bcl-2 family protein levels on *in vitro* sensitivity to ABT-737 in primary CLL cells. Additionally, synergistic combinations of ABT-737 with other anti-leukaemia agents were identified.

Methods

Circulating CLL cells from 30 patients were assessed *ex vivo* for sensitivity to ABT-737 alone or in combination with cytotoxic drugs. Cell lysates were analysed and quantified by Western blot for the level of expression of Bcl-2 family proteins. These data were correlated with the single agent and combination results.

Results

ABT-737 is efficacious ($LC_{50} < 100\text{nM}$) as a single agent in most (21/30) primary CLL samples, independent of response to prior therapy or prognostic markers (CD38, p53 status). Some CLL samples are sensitized by standard cytotoxic agents (dexamethasone, etoposide, fludarabine and mafosfamide) to killing by ABT-737. The synergistic response was not predicted by response to either ABT-737 or the cytotoxic drug as a single agent. Surprisingly, there was no direct correlation between the levels of Bcl-2 family proteins and cytotoxicity LC_{50} .

Conclusion

We have identified that a minority of CLL samples are relatively insensitive *in vitro* to Bcl-2 antagonism by ABT-737 as a single agent, but these are not readily predictable based on clinical features. Combination with a second anti-leukaemia agent may result in synergistic killing. Clinical trials are in progress with an orally bioavailable BH3 mimetic, ABT-263, that has a similar spectrum of activity and these results suggest a potential role for future combination therapy in this disease.

This research was supported by Abbott and Genentech. The company had no role in analysing the data or preparing the abstract.

Meeting Room 6
HSANZ Free Communications 9
O90

0830-1000
0930

Combining RAD001 (Everolimus) with Bortezomib Induces Synergistic Killing in Precursor-B Acute Lymphoblastic Leukemia

Philip Saunders¹, Kenneth Bradstock², Linda Bendall¹

¹University of Sydney, Westmead Institute for Cancer Research, Westmead Millennium Institute, Sydney, NSW, Australia. ²Department of Haematology, Westmead Hospital, Sydney, NSW, Australia

Five year survival for patients with relapsed pre-B ALL is less than 10%, requiring novel approaches to therapy. Bortezomib has been shown to enhance chemosensitivity in pre-B ALL and demonstrates efficacy in relapsed disease. We hypothesized that RAD001 would enhance the sensitivity of the pre-B ALL cells to proteasome inhibition.

Combining RAD001 (>2 μ M) with Bortezomib (10nM) in vitro significantly ($p < 0.05$) enhanced pre-B ALL cell kill, greater than the additive effect of individual therapies. Intracellular flow cytometry demonstrated up regulation of, bax, bim, puma and cleaved caspase 3, in the absence of a p53 response. This data indicates that enhanced killing is independent of p53.

We observed bortezomib to be a potent activator of caspase 8 and sought to determine if RAD001 induced synergy through up regulation of death receptor expression. We saw no increase in surface expression of DR4 or DR5 by RAD001, Bortezomib, or the combination of both agents.

Bortezomib is reported to induce apoptosis at G2/M via down regulation of NF κ B and BCL2. We sought to examine the cell cycle impact of combining RAD001 with Bortezomib. We observed Bortezomib alone and Bortezomib in combination with RAD001 (>2 μ M) resulted in an increased proportion of pre-B ALL cells in S phase and G2/M, relative to control. We hypothesize that combining RAD001 with Bortezomib enhances apoptosis signal induction at G2/M, providing a rationale for synergy.

Combining RAD001 with Bortezomib induces synergistic killing in pre-B ALL, independent of p53. Enhanced killing is associated with caspase activation and cell cycle accumulation in G2/M. We believe our data indicates combining RAD001 with Bortezomib has the potential to enhance responses for patients with pre-B ALL.

Acknowledgements

University of Sydney Postgraduate Award
Millennium Foundation Polypedal Scholarship
Cancer Institute NSW and NHMRC Project Grant 352326
Novartis supply of RAD001 for research use.
Novartis had no role in analyzing the data or preparing the abstract

Meeting Room 6
HSANZ Free Communications 9
O91

0830-1000
0945

Dose Escalated RAD001 (Everolimus) Enhances Chemosensitivity in Precursor-B Acute Lymphoblastic Leukemia, through a JNK Dependent Impairment of Cell Cycle Arrest, in Response to DNA Damage or Microtubule Disruption

Philip Saunders¹, Kenneth Bradstock², Linda Bendall¹

¹*University of Sydney, Westmead Institute for Cancer Research, Westmead Millennium Institute, Sydney.* ²*Department of Haematology, Westmead Hospital, Sydney*

Five year survival for patients with relapsed pre-B ALL remains dismal, requiring novel approaches to therapy. We evaluated the potential of mTOR inhibitor RAD001 to enhance chemosensitivity in pre-B ALL.

Combining 16µM RAD001 with DNA damage or vincristine in vitro, induced caspase-dependent synergistic killing ($p < 0.05$) of pre-B ALL cells. We observed 16µM RAD001 suppressed p53, markedly attenuating p21 responses. Lentiviral siRNA knock down of p53 in Nalm6 cells significantly increased ($p < 0.05$) cell death by vincristine relative to luciferase knockdown cells with an intact p53 response, indicating suppression of p53 enhances chemosensitivity.

Intracellular flow cytometry revealed combining 16 µM RAD001 with DNA damage or vincristine activated the JNK pathway and c-Jun. c-Jun is reported to suppress the p53 and p21 promoters and prolong the half-life of p53 analogue, p73. Concordantly, we observed up regulation of p73, puma, bax, bim and cleaved caspase 3, indicating a p53 independent pathway to cell death.

We hypothesized that 16µM RAD001 enhances chemosensitivity through altered cell cycle regulation. 1.5µM RAD001 inhibited pRb, Ki67 and PCNA expression, increasing G0/1 cell cycle arrest in response to DNA damage or vincristine. In contrast, 16µM RAD001 increased pRb, cyclin D1, Ki67, CDC2 and PCNA expression. Enhanced DNA content, BrdU uptake and PCNA expression indicate cell cycle progression in response to DNA damage or vincristine when combined with 16 µM RAD001. We observed JNK inhibition reduced PCNA expression at G0/1 and G2 in pre-B ALL cells exposed to DNA damage and G2/M with vincristine, indicating impairment of cell cycle arrest by 16µM RAD001 is JNK dependent.

RAD001 (16µM) enhances chemosensitivity independent of p53, associated with JNK dependent impairment of cell cycle arrest, in response to DNA damage or microtubule disruption. Our data indicates dose escalated RAD001 has the potential to enhance chemosensitivity for patients with pre-B ALL.

Acknowledgements

University of Sydney Postgraduate Award
Millennium Foundation Polypedal Scholarship
Cancer Institute NSW and NHMRC Project Grant 352326
Novartis supply of RAD001 for research use.
Novartis had no role in analyzing the data or preparing the abstract

Riverside Theatre
HSANZ Free Communications 10
O92

0830-1000
0830

A New Generation IAP Inhibitor (IAPi) Induces Apoptosis of Human Myeloma Cells and Synergises with Conventional and Novel Anti-Myeloma Therapeutics

Tiffany Khong and **Andrew Spencer**
Alfred Hospital, Melbourne, Victoria, Australia

Aim

To evaluate a novel IAP (Inhibitor of Apoptosis Protein) inhibitor (IAPi) as a potential therapeutic for multiple myeloma (MM)

Methods

Dose responsiveness to IAPi (5 to 50 μ M for 24 to 72 hours) was determined via MTS assays of 9 genetically heterogeneous human myeloma cell lines (HMCL). Immunoblotting of caspases 3 and 7, PARP, ICAD, CAD and ROCK-1, with or without, specific caspase inhibition or siRNA knock-down of caspases 3 and 7 was performed to characterise the mechanism(s) of cell killing. Co-culture experiments with IL6, IGF-1 or the human stromal cell line HS5 (n = 3 for each) were used to quantify potential cytokine-mediated abrogation of IAPi anti-MM activity. Finally, the anti-MM activity of IAPi in combination with other therapeutics was investigated against both HMCL and primary MM samples.

Results

IAPi demonstrated IC_{50s} of 25 to 50 μ M against all 9 HMCL at 72 hours. Immunoblotting following IAPi treatment with and without caspase inhibitors or siRNA knock-down was consistent with activation of both the intrinsic and extrinsic apoptotic pathways. Rapid falls in ICAD and CAD plus cleavage of ROCK-1 and PARP confirmed IAPi-induced apoptosis via caspase 3 and 7. Surprisingly, HS-5 co-culture or contemporaneous addition of IL-6 or IGF-1 to IAPi treated cells showed enhanced MM cell apoptosis (median 1.2, 1.1 and 1.3 fold increase, respectively) compared to IAPi only treated controls. Combining IAPi with conventional cytotoxics, an anti-TRAIL agonistic antibody or a novel HSP90 inhibitor all demonstrated synergistic killing of MM cells with combination indices (CalcuSyn) of less than 1.

Conclusion

IAPi induces down-regulation of ICAD/CAD and ROCK-1/PARP cleavage via caspase 3 and 7 dependent processes. Furthermore, IAPi retains anti-MM activity in the context of relevant exogenous growth factor exposure and induces synergistic killing of MM cells when combined with conventional and novel anti-MM agents. IAPi represents a potentially novel therapeutic approach to MM.
This research was supported by Novartis Corporation. The company had no role in analysing the data or preparing the abstract

Riverside Theatre
HSANZ Free Communications 10
O93

0830-1000
0845

Clinical and Immunohistochemical Features Associated with a Response to Bortezomib in Patients with Multiple Myeloma

Mark Dawson¹, Stephen Opat¹, Yamna Taouk¹, Mark Donovan¹, Michele Zammit¹, Katherine Monaghan¹, Noemi Horvath², Andrew Roberts³, Miles Prince⁴, Mark Hertzberg⁵, Catriona McLean¹ and **Andrew Spencer¹**

¹ Alfred Hospital, Melbourne, Victoria, Australia. ² Royal Adelaide Hospital Cancer Centre, Adelaide, South Australia, Australia. ³ Royal Melbourne Hospital, Melbourne, Victoria, Australia. ⁴ Peter MacCallum Cancer Center, Melbourne, Victoria, Australia. ⁵ Westmead Hospital, Sydney, New South Wales, Australia

Aim

To identify clinical parameters and/or protein expression characteristics that are predictive of response to bortezomib therapy for relapsed or refractory multiple myeloma (MM).

Methods

We analysed the baseline clinical parameters and profiled the baseline MM cell expression of a range of immunohistochemical markers of cell cycle, apoptosis and angiogenesis (CD138, CyclinD1, Bcl2, Bcl-XL, p53, p16^{INK4A}, p21^{CIP/WAF1}, relA, VEGF_{R1} and FGFR3) in pre-treatment trephines from a cohort (n=90) of relapsed MM patients recruited to one of two prospective multicentre trials of bortezomib salvage therapy. Univariate analyses were performed using the Student's t-test, Wilcoxon rank sum test or Fisher's exact test. Multivariate analysis was performed using multiple linear regression. Progression free survival (PFS) and overall survival (OS) were assessed using the Kaplan-Meier method.

Results

Response (CR or PR) to bortezomib was associated with a previous history of CR to alternative anti-MM treatment. Patients who expressed cyclin-D1 were more likely to respond (67% vs 47%; expression vs no expression, respectively; p = 0.08). In contrast, patients who expressed p16^{INK4A}, cytoplasmic p53 or high Bcl2 had poor responses (22% vs 57%, 42% vs 69% and 48% vs 74%; expression vs no expression, respectively; with p = 0.05, p = 0.01 and p = 0.01, respectively). A high likelihood of response (89%) was seen with p16^{INK4A} negative/Cyclin-D1 positive tumours. Conversely p53 positive/Cyclin-D1 negativity was associated with very low response rates (14%). Patients who did or did not express FGFR3 responded equally well to bortezomib. Patients who achieved a response to bortezomib and those patients who expressed cyclin-D1 demonstrated a significant OS advantage (p=0.0004 and p=0.05, respectively) whereas FGFR3 had no impact on survival.

Conclusion

Baseline clinical parameters and selective immunohistochemical markers can be used to identify patients that are most likely to achieve a meaningful clinical response to bortezomib salvage therapy.

This research was supported in part by the Roche Pharmaceuticals HOTT Fellowship. The company had no role in analysing the data or preparing the abstract.

Riverside Theatre
HSANZ Free Communications 10
O94

0830-1000
0900

Targeting Lewis Y-positive Multiple Myeloma and Acute Myeloid Leukaemia with Gene-modified T cells demonstrating Memory Phenotype

Stefan Peinert^{1,2}, David S Ritchie^{1,2}, Dirk Hönemann^{1,2}, Simon J Harrison^{1,2}, Preethi Guru², Jennifer A Westwood², Michael H Kershaw², Amanda Shin², Mark J Smyth², Joseph A Trapani², Andrew M Scott³, Fiona E Smyth³, Glenn A Cartwright³, Barbara E Power⁴, Phillip K Darcy², David Westerman¹, Peter Gambell¹, Paul Neeson², H Miles Prince^{1,2}

¹ Department of Hematology and Medical Oncology, Peter MacCallum Cancer Centre, East Melbourne, Vic, Australia. ² Cancer Immunology Program, Peter MacCallum Cancer Centre, East Melbourne, Vic, Australia. ³ Ludwig Institute for Cancer Research, Austin Hospital, Heidelberg, Vic, Australia. ⁴ CSIRO Health Sciences and Nutrition, Parkville, Vic, Australia

Aim/Background

Haematologic malignancies such as AML and MM are amenable to immunotherapy as evidenced by the immune mediated allogeneic graft versus leukaemia/myeloma effect. Studies of adoptive immunotherapy with gene modified T cells have shown clinical activity in solid tumours. Thus, our aim was to generate gene-modified clinical-grade T cells directed against malignancies expressing the carbohydrate antigen Lewis^Y (Le^Y). Moreover, we aimed to produce cells that possessed T cell memory, an attribute considered essential for *in vivo* T cell persistence and effective killing of tumour cell targets.

Methods/Results

We identified various cell lines that expressed Le^Y. Furthermore, 27% and 43% of primary MM and AML bone marrow samples were Le^Y-positive, respectively. We manufactured a novel retroviral vector construct resulting in efficient transduction of PBMC-derived T cells with resultant high expression of a single-chain anti-Le^Y chimeric T cell receptor. Using a GMP-conform protocol we achieved up to >100-fold expansion of T cells at the end of the culture (day 12). Anti-Le^Y T cells lysed Le^Y-positive tumour cells *in vitro* while sparing Le^Y-negative control tumour cells and Le^Y expressing neutrophils (low - moderate Le^Y expression). Detailed analysis of end-of-expansion T cells revealed similar transduction rates in CD4 and CD8 T cell subsets. Furthermore, T cells showed low expression levels of CD45RA and CCR7 and active proliferation in response to IL-2 and IL-15, suggesting an effector memory phenotype. Co-culture with Le^Y expressing tumour cells resulted in further proliferation and IFN- γ production of anti Le^Y T cells. We have developed a first in human phase I trial for patients with Le^Y-positive MM or AML.

Conclusion

Le^Y is a promising and immunologically relevant target for T cell immunotherapy and our product is likely to lead to *in vivo* persistence of anti-Le^Y T cells, an outcome which will be specifically addressed in our upcoming study.

No conflict of interest to disclose

Riverside Theatre
HSANZ Free Communications 10
O95

0830-1000
0915

In Vitro Efficacy of Agonistic Antibody to TRAIL-R1 (Mapatumumab) and low dose Bortezomib in Multiple Myeloma

Karen Chen¹, Tsin Tai¹, Amanda Shin¹, Joanne Davis¹, Miles Prince^{1,2}, David Ritchie^{1,2}, Paul Neeson¹

¹ Haematology Immunology Translational Research Laboratory, Cancer Immunology Research Program and the ² Haematology Department, Peter MacCallum Cancer Centre, Melbourne, Australia.

Aim

The proteasome inhibitor Bortezomib (Bz) is a potent inducer of plasma cell apoptosis via its suppressive effects on NF- κ B. Bz is also associated with thrombocytopenia and inhibition of dendritic cell (DC) function, which may limit the induction of autologous T cell responses to myeloma antigens. In order to limit these effects of Bz we evaluated whether the novel agonistic antibody that targets TRAIL-R1 Mapatumumab (Mp) would allow significant downward titration of Bz dose whilst still inducing plasma cell death.

Method

Cultured human plasma cell lines (RPMI8226, U266, LP-1, NCI-H929, OPM-2 and JJN3) were treated with Mp and/or Bz for 24 or 48 hours. Cells were stained with annexin V-FITC and the viability dye 7-AAD and analyzed on the BD LSRII flow cytometer. In addition, the myeloma cell surface expression of Tumour Necrosis Factor Apoptosis-Inducing Ligand Receptors (TRAIL-R) 1 and 2 were assessed and correlated with Mp sensitivity.

Results

All cell lines were sensitive to 10nM Bz monotherapy. In contrast 4/6 cell lines were sensitive to Mp alone, RPMI8226 at 0.06ug/ml, U266 and OPM-2 at 1ug/ml each and LP-1 at 10ug/ml. When non-apoptosis inducing Bz doses were used in combination with titrated doses of Mp (from 0.01 to 50ug/ml) apoptosis of RPMI8226, U266 and OPM-2 was enhanced. In contrast, LP-1, JJN3 and NCI-H929 had no additional killing beyond that found by Mp alone. TRAIL-R 1/2 were expressed by RPMI8226 at a higher level than U266, correlating with enhanced sensitivity to combination Mp/Bz therapy.

Conclusion

Bortezomib is a potent anti-plasma cell therapy. The effective dose of Bz may be reduced significantly when used in combination with Mp suggesting that this combination therapy may be more efficacious in selected patients. These results emphasize the need for clinical studies to explore dose modification of Bz, which may in-turn reduce clinical side-effects and enhance endogenous immune responses.

This research was supported by Human Genome Sciences Inc. The company examined this abstract prior to submission

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Safety and Efficacy of Bortezomib Combined with the Deacetylase Inhibitor Romidepsin in Patients with Relapsed or Refractory Multiple Myeloma: Interim Results of a Phase I/II Trial

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Introduction

There are substantial pre-clinical data demonstrating synergistic activity of proteasome inhibitors and deacetylase inhibitors (DACi) in multiple myeloma (MM). This is the first clinical trial combining these two classes of drugs.

Methods

This is an ongoing open label single-centre single-arm phase I/II dose escalation trial of bortezomib, dexamethasone and romidepsin in patients with relapsed or refractory MM. All patients received bortezomib (1.3mg/m²; d1, 4, 8, 11) with dexamethasone (20mg d1, 2, 4, 5, 8, 9, 11, 12). The romidepsin dose escalation commenced at 8mg/m² IV d1, 8, 15 every 28d and involved an initial accelerated dose escalation phase, with intra-patient dose escalation of romidepsin to 10, 12 and 14mg/m². Response rates were assessed according to M-protein response criteria, CR documented to EMBT criteria.

Results

To date, 20 patients have entered the study with 16 evaluable for response and toxicity. Median number of prior therapies = 2 (2-5). Most patients had previously taken potential neurotoxic medications; vincristine (3), thalidomide (4), bortezomib (1). No DLTs were demonstrated at 8mg (n=1) or 10mg (n=3) of romidepsin. At 12mg, 3 episodes of Grade 4 thrombocytopenia and one episode of febrile neutropenia occurred. The maximum tolerated dose was declared as 10mg romidepsin. 10/15 patients have enrolled in phase II. Other toxicities include: Grade 3: fatigue (n=1), neutropaenia (n=1), sepsis (n=1); Grade 2: peripheral neuropathy (n=3), nausea (n=1), diarrhoea (n=1). Three patients required bortezomib dose reduction due to peripheral neuropathy. As of July 2008 the median number of treatment cycles delivered was 3 (1-8, N=20); Maintenance cycles was 7 (3-14, n=7). 5 patients have progressed. The overall response rate is 14/16 (87.5%), 4 CR, 6 PR and 4 MR.

Conclusion

These interim results demonstrate an extremely encouraging response rate, some durable responses and acceptable toxicity of a proteasome inhibitor-DACi combination in this patient group.

This research was supported by Gloucester Pharmaceuticals and Janssen-Cilag. The company had no role in analysing the data or preparing the abstract

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Bone Turnover, Bone Mineral Density and other Characteristics in Post-Transplant Myeloma Patients

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Aim

Given the frequency of bony complications in myeloma and controversies regarding optimal use of bisphosphonates, we investigate bone mineral density and biochemical markers of turnover in transplant patients. We investigated for associations with bisphosphonate use, presence of osteonecrosis of the jaw and myeloma disease characteristics.

Method

We identified all patients alive following previous autologous transplantation for myeloma at a single centre- Royal Perth Hospital, and reviewed clinical and laboratory data including cytogenetics. All patients received a survey questionnaire. Dental problems and use of bisphosphonates were recorded. Bone density, urine and blood metabolic markers were performed. Clinical records were reviewed including laboratory data and cytogenetics.

Results

54 patients were identified and contacted. Age range was 41-71 years, (46% males) and time since initial transplant 24-3621days. Two patients had received second autografts and one a later sibling allograft. The cytogenetics and laboratory characteristics were recorded. Bone turnover was found to be variable in this heterogeneous group of patients. While all had received autologous transplantation, there were significant time differences from the time of diagnosis, variations in steroid therapy and the use of bisphosphonates.

While a previous case of osteonecrosis of the jaw (ONJ) occurred in a patient having marked elevation in bone density and also a long bone fracture after receiving prolonged intravenous bisphosphonate, another patient with ONJ was found to have reduced bone density. Statistical and final full data will be available at the meeting.

Conclusion

Bone turnover and mineral density studies may assist in the management of patients with myeloma, including optimisation of bisphosphonate use. Varying results were identified in the studied post-transplant group of patients, who had received different duration and type of bisphosphonate and other therapies. Prospective studies from the time of myeloma diagnosis are planned in attempts to optimise future clinical care.

No conflict of interest to disclose

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No Bones About It: Determining the Optimal Aspiration Volume During Bone Marrow Harvest

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Background

The use of bone marrow (BM) as a stem cell source is increasing once again due to the lower rate of chronic GVHD compared with peripheral blood stem cells. There is no generally accepted technique for harvesting BM.

Method

BM was collected from the posterior iliac crests (PIC) in 2 separate bags: 10ml aspirates from the left and 20 ml aspirates from the right. Samples taken at the start and after 100, 150, 200, 250, & 500 ml were analyzed for TNC, CD34⁺ and CD3⁺ cell counts.

Results

Total numbers of harvests included in the study were four. The CD34 and CD3 cell number (mean \pm SEM $\times 10^6$) at the start of harvest were 5.8 ± 0.05 , 65.8 ± 12.0 respectively for 10 ml aspirates and 9.1 ± 0.5 , 106.9 ± 22.1 respectively for the 20ml aspirates. There is a rapid fall in the yield of CD34⁺ cells obtained with increasing harvest volume (19 and 25% of the initial number after 250 ml for 10 and 20 ml aspirates respectively; 14 and 11% respectively after 500 ml). In contrast the CD3⁺ cell numbers fall more slowly (43 and 50% after 250 ml, 45 and 38% after 500 ml). By the time 500 ml has been aspirated, there is no difference in the total number of CD34⁺ cells obtained from a 10 ml versus a 20 ml aspirate of bone marrow. The ratio of CD3 to CD34 was increasing both in 10 and 20 ml aspirates, indicating an increasing amount of PB contamination in the aspirates as the harvest volume increases

Conclusion

CD34⁺ cell yields fall rapidly when BM is harvested along the PIC. Using additional areas such as the anterior iliac crests or sternum or a second harvest may be preferable to a large volume PIC harvest for optimizing CD34⁺ stem cell collection. After 500 ml of BM has been harvested, 20 ml BM aspirates do not increase CD34⁺ cell numbers and 10 ml aspirates should be taken to minimize unnecessary blood loss and reduce T cell contamination.

No conflict of interest to declare

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Higher Incidence of CMV Reactivation Following Fludarabine Based Reduced Intensity Conditioning Transplants – A Retrospective Comparison with Myeloablative Transplants

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Limited data exist on the incidence of Cytomegalovirus (CMV) reactivation following fludarabine based reduced intensity conditioning (RIC) transplants. A retrospective cohort study of RIC and myeloablative transplants (MAT), performed between January 2002 and May 2008, was done to compare rates and risk factors for CMV reactivation. Pre-transplant ganciclovir was given to sero-positive recipients while all patients received Valaciclovir as herpes prophylaxis. Weekly monitoring for CMV was done using a qualitative PCR; if positive, quantification was done using either pp65 antigenemia or quantitative PCR (COBAS R). One hundred RIC transplants (27 unrelated) and 155 MAT (60 unrelated) were performed during the study period. The median age of RIC transplants (65 Males; 35 Females) was 52 years (range: 18 - 65). By CMV serostatus, 71 were high risk (D+/R+ or D-/R+), 15 intermediate (D+/R-) and 14 low risk (D-/R-). Acute graft versus host disease (GVHD) was observed in 44% of RIC and 64% of MAT. CMV reactivation was seen in 43 patients at a median of 48 days (range: 24 days – 64 months). In high risk patients, reactivation was significantly higher with RIC (61.4%) than MAT (41.6%; $p = 0.01$) and remained so even after adjusting for acute GVHD (adjusted OR 2.90, 95% CI: 1.49-5.68, $p=0.001$). Type of donor (sibling vs MUD) or use of ATG during conditioning did not influence reactivation. Of the 15 RIC transplants that received Campath, 10 had CMV reactivation. Analysis of patients with high risk serostatus who did not receive Campath ($n=166$) showed reactivation in 57% RIC compared to 42% MAT ($p=0.06$). Overall CMV disease was seen in 9% following RIC and 2.5% following MAT. The higher incidence of CMV reactivation in fludarabine based RIC transplants highlights the need for improved prophylactic and preemptive strategies including use of CMV specific CTL's in this vulnerable patient population.

No conflict of interest to disclose

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A Retrospective Comparison of the Outcome of Single Versus Double Unrelated Umbilical Cord Blood Units for Allogeneic Transplantation In Adults with Advanced Hematological Malignancies

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Unrelated umbilical cord blood (CB) has emerged as an alternative stem cell source for allogeneic transplantation for patients with hematologic malignancies, but in adults is limited by the low number of stem cells present in banked CB units. One strategy to overcome this problem is to transplant multiple CB units into each recipient, thereby increasing the stem cell dose, and potentially increasing the rate of engraftment and thereby reducing transplant mortality. We have compared the outcomes of transplants using 2 CB units in adults with poor prognosis hematologic diseases with those in a similar patient cohort who received transplants with single CB units. Eleven patients, median age 27 years and median weight 69 kg, received transplants of 2 partially-matched unrelated CB units after myeloablative conditioning therapy at Westmead Hospital, and the results were compared with an historical cohort of 9 patients undergoing single unit CB transplant at the same centre. Neutrophil recovery to 0.5×10^9 /L was seen by median day 32 (18-53), and platelet recovery to 50×10^9 /L by day 91 (56-381). These results were not significantly different from those reported in patients receiving single CB transplants. Acute graft versus host disease grades II-IV was seen in 4 patients, but no chronic graft versus host disease occurred. Transplant-related complications were responsible for the deaths of 5 patients in the first 3 months post-transplant, while 2 patients died of relapse of their hematologic malignancy. Four patients survive disease-free 17 to 33 months post-transplant.

Transplantation using 2 partially-matched unrelated CB units did not appear to result in improvements either in engraftment or survival, as compared to a previous cohort of patients receiving single CB units. Further strategies appear to be needed to reduce the duration of severe neutropenia, and the high transplant-related mortality in these patients.

No conflicts of interest to disclose

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Can Otherwise Incurable Haematological Malignancies be Cured by KIR-ligand Mismatched Haploidentical Stem Cell Transplantation (haploHSCT)? – The Alfred Hospital Experience

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Introduction

Relapsed or refractory haematological malignancies are difficult to cure. Some patients with HLA-matched donors can be salvaged by allogeneic HSCT whereas those patients without donors will die. HaploHSCT, where donors and recipients are matched for one haplotype i.e., HLA-A, -B, -Cw and -DR mismatched, has been explored for more than a decade – with limited success. Ruggeri L, et al (*Science* (2002) 295:2097-00) have improved the results of haploHSCT with several modifications – Killer Immunoglobulin-like Receptor (KIR)-ligand mismatched donors, and highly T cell depleted megadose CD34+ stem cell infusions. KIR-ligand mismatch has been shown to generate a potent NK cell-driven graft-versus-leukaemia (GVL) effect in this setting – a phenomenon most prominent in myeloid malignancies.

Methods

We performed haploidentical transplantation in 12 patients with otherwise incurable haematological diseases (AML=7, CML-BC=1, MDS=1, VSAA=1, T-ALL=2) who did not have a suitable HLA-matched donor. All AML and ALL patients were refractory or in relapse. 3 had previously undergone autologous HSCT. Median age - 35.5 (22-58) yrs; 7 male, 5 female. Each patient/donor pair was haploidentical and KIR-ligand mismatched (GVL direction). Conditioning regimen - ATGAM, melphalan, fludarabine, thiotepe. G-CSF-mobilised PBSCs were CD34+ cell selected (CliniMACS or Isolex device). No post transplant immunosuppression was given. Caspofungin was used as fungal infection prophylaxis.

Results

Overall, 3 of 12 (25%) of patients are alive and disease-free (8, 4 and 3.5 yrs). Of the myeloid malignancies 3 of 9 (33%) are alive and disease-free. Both patients with T-ALL died of relapsed leukaemia, and the patient with VSAA died of transplant-related complications. The 3 surviving patients have no on-going transplant complications and Karnofsky scores of 100%. All patients engrafted with complete chimerism. Grade II-IV acute GVHD occurred in 3 of 11 evaluable patients. 3 developed chronic GVHD (1 limited and 2 extensive). 9 patients died – 3 of relapsed disease, 2 of multi-organ failure 1 each of chronic GVHD, interstitial pneumonitis, Scedosporium infection and VOD. No infections with Candida or Aspergillus occurred in the 11 patients who received caspofungin prophylaxis.

Conclusion

KIR-ligand mismatched haploidentical HSCT provided a significant proportion of patients, with otherwise incurable malignancies, long-term DFS. The 33% OS/DFS of the patients with myeloid malignancies compares favourably with traditional forms of alloHSCT in this refractory and heavily pretreated cohort. Caspofungin was effective antifungal prophylaxis. Future results may be improved if haploHSCT is considered earlier in the course of the disease – to decrease transplant-related mortality and perhaps relapse.

This research was supported by Merck Sharpe & Dohme who had no role in analysing the data or preparing the abstract.

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Delayed Relapse and Long-term Follow-up of Allogeneic Bone Marrow Transplantation for Chronic Myeloid Leukaemia

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Aim

To determine the factors affecting overall survival (OS), disease-free survival (DFS), and delayed relapse >3 years after BMT in CML patients treated with allogeneic BMT.

Methods

193 patients treated with allogeneic BMT for CML from 1981-2006 were identified. The data was analysed for OS, DFS and subgroups analysed for their effect on survival. Patients who were alive and disease free 3 years after BMT were studied for late relapse.

Results

The median age of recipients was 37 years (16-63). The disease stage at transplant was chronic phase (CP) 106, accelerated phase (AP) 44, and blast transformation (BT) 40. The conditioning was cyclophosphamide/TBI (111), busulfan/cyclophosphamide (73) or other (9). The donor was matched sibling (136) or matched unrelated/other related (57). Stem cell source was from marrow (157), blood (34) or double cord blood (2).

The median OS in all patients was 33 months and DFS 22 months. Preliminary univariate analysis showed that variables predicting better DFS included acute GVHD grade 0-1 but not source of stem cells or conditioning. Matched sibling donors showed a superior OS compared to other donors ($p=0.045$). This will be confirmed by multivariate analysis.

At 3 years overall DFS was 46%: 50% in matched sibling and 38% in alternate donors. Patients transplanted in CP had a better DFS > 3 years (median not reached) than AP or BT ($p=0.0$). There were 8 patients (5 CP, 3 AP) with delayed relapse. The median time to relapse >3 years was 4.6 years (3.1-20.8 years). 2/8 patients are alive after 6.4 and 6.8 months on imatinib therapy.

Conclusion

Survival following allogeneic transplant for CML is related to disease stage and severity of AGVHD but not donor type or conditioning. DFS beyond 3 years is not stable with small numbers of patients continuing to relapse especially in AP and BT.

No conflict of interest to disclose

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Plasma Exchange as Treatment of Transplantation-Associated Thrombocytopenic Microangiopathy (TA-TMA): Effect of Concomitant Acute GVHD on Efficacy and Outcome

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Aims

To review the outcome of patients treated at our institution with plasma exchange (PE) for transplantation-associated thrombocytopenic microangiopathy (TA-TMA) post-allogeneic stem cell transplantation (SCT).

Methods

Retrospective review of allogeneic SCT patients who developed TA-TMA. Patients were identified from a unit data-base, with data available from December 2001. Response to PE was defined as complete response (CR) if FBC, LDH and renal +/- CNS abnormalities returned to pre-TA-TMA values / function with PE, partial response (PR) if platelet counts improved to at least 50% of pre-TA-TTP values, and no response (NR) if no improvement in platelet counts occurred.

Results

In total, 15 patients with TA-TMA were identified, with 11/15 patients treated with PE. 4 patients did not undergo PE due to presence of active sepsis (n=1) and physician discretion (n=3). Overall, 3 patients responded to PE (CR), and 8 patients had NR. Of 7 patients treated with PE for TA-TMA developing in the setting of active acute GVHD, 0/7 responded. Of 4 patients treated with PE for TA-TMA in the absence of active acute GVHD (conditioning-related in 1, chronic GVHD in 2 and post resolution of acute GVHD in 2), 3/4 responded (p=0.024). In 2 of 7 patients with active acute GVHD and TA-TMA unresponsive to PE, TA-TMA eventually responded to increased immunosuppression / control of GVHD. For the whole group, only 2 patients remain alive, including 1/11 patients treated with PE. Median survival post onset of TA-TMA was 79 days (range 5-1845 days). Causes of death included infection-related (n=7), GVHD-related (n=4) and relapsed malignancy / PTLN (1 case each).

Conclusions

Depending on the clinical circumstance in which TA-TMA develops, PE may be of therapeutic benefit. Responses to PE were seen in a majority of TA-TMA occurring in the absence of active acute GVHD. In contrast, TA-TMA occurring in association with active acute GVHD was unresponsive to PE, and as such, in this circumstance, we recommend therapy primarily be directed at controlling underlying GVHD.

No conflict of interest to disclose

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A Prospective Randomised Trial of Intravenous Iron Therapy versus Oral Iron for Iron Deficiency Anaemia in Pregnant Women

Alhossain Khalafallah^{1,2,3}, Amanda Dennis^{1,2}, Gerald Bates^{1,3}, Joanne Bates¹, Susie Shaw¹, Lauren Smith¹, John Bertram¹, David Seaton¹, Terry Brain¹, Michael Beamish¹ and Kim Rooney^{1,2}

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Introduction

To date, limited data are available regarding the prevalence of iron deficiency anaemia (IDA) during pregnancy in Australia, or the comparative efficacy of IV iron versus oral iron therapy in pregnant women.

Patients and Method

At the Launceston General Hospital we prospectively investigated 400 pregnant women between January and December 2007 with full blood count (FBC) and iron studies at the first or second antenatal visit. Among those, 100 women (25%) had iron deficiency anaemia, and were recruited to a prospective randomised trial to determine whether intravenous iron therapy (iron polymaltose) is superior to oral iron (ferrous sulphate 250 mg) for the management of IDA associated with pregnancy.

Results

At recruitment, median haemoglobin (Hb) was 108 g/L (range: 90-115, normal range: 120-160 g/L), while median serum ferritin was 11 µg/L and mean ferritin was 19 µg/L (normal range: 30-460 µg/L). After four weeks of treatment, the Hb level increased by a mean of 5.5 g/L on oral iron and by 9.6 g/L after IV iron. Mean/Median serum ferritin did not increase significantly in women on oral iron (15.3 and 14 respectively), but showed significant increase to a median of 228 µg/L and a mean of 241 µg/L in those given IV iron (P-value <0.001). Hb taken pre-delivery showed Hb-increase by a mean of 11.6 g/L on oral iron, and by 22.9 g/L for IV iron (P-value <0.001).

Conclusion

Our data indicate that iron deficiency is a common finding during pregnancy in the northern Tasmanian population, and intravenous iron therapy appears a safe and effective treatment in this cohort of patients.

The authors confirm that there is no conflict of interest in relation to this research.

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Compliance and Tolerability of Iron Therapy during Pregnancy

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Aim

To follow up pregnant women randomised either to oral iron or intravenous iron in order to assess the compliance and tolerability of iron therapy.

Methods

144 pregnant women with iron deficiency anaemia were enrolled in the trial, of which 72 were assigned to a single IV iron Polymaltose (Category A) infusion with a dose calculated according to body weight and Hb level, while 72 received daily oral iron with FGF (ferrous sulphate 250 mg, folic acid 300 mcg). Prior to iron infusion, antihistamine therapy with oral Polaramine 2 mg (Category A) was commenced. Compliance and tolerability were assessed via questionnaires at 2 and 4 weeks after administration had commenced.

Results

Compliance: Non-compliance in the oral arm was found in 12.5% of patients, and varied from missing a few tablets to neglecting to pick up repeat prescriptions. In the IV arm, one patient failed to present for her infusion. **Tolerability:** Approximately 22% of women in the oral arm experienced mild gastrointestinal symptoms, but were able to continue taking FGF without any ongoing problems. Five women were withdrawn from the oral iron due to GIT-upset. For those receiving an iron infusion, 28% reported mild symptoms such as tiredness during or after the infusion, although 80% of these were unlikely to be due to the infusion itself. Two women had a possible minor allergic reaction, and 2 had possible local reactions. 2 infusions were ceased before they were completed, but again, the symptoms related were unlikely to have been caused by the infusion but most likely due to antihistamine therapy with a drop of their blood pressure. None of these women required medical intervention.

Conclusion

Both oral iron and IV iron Polymaltose have been generally well tolerated in pregnancy. Compliance has been well within an acceptable limit.

The authors confirm that there is no conflict of interest in relation to this research

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Current Epidemiology of Thalassaemia and Haemoglobinopathy in New South Wales

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Background

During the 1990s, there has been increasing diversity of migration to Australia (and especially Sydney) with people originating from areas with a high incidence of thalassaemia / haemoglobinopathies, such as the Mediterranean, Middle East, South East Asia and Africa.

Method

Our laboratory receives HbEPG/thalassaemia screen requests from general and specialist medical, surgical and obstetric practices including antenatal screening, across New South Wales. We use High Performance Liquid Chromatography (BioRad VII) to quantitate different haemoglobins and run alkaline and acid gel electrophoresis to differentiate major haemoglobinopathies.

Results

Results for the last 5 years (2003-2007 inclusive) are as follows for thalassaemia: α - 687, β - 1631, $\delta\beta$ - 22. There were 150 patients with elevated HbF. Patient numbers with haemoglobinopathy were HbS - 117, HbE - 300, HbD - 24, HbC - 22, Hb Lepore - 30, Hb Constant Spring - 8, Hb Kempsey - 6, HbQ - 4. Figures are number of individual patients and include both heterozygous and homozygous inheritances.

Conclusion

β -thalassaemia is the most common form of thalassaemia seen. The incidence of β -thalassaemia in the post war years was largely due to Greek and Italian migration. The incidence is now increased due to migration from SE Asia. Migration from SE Asia is also responsible for the high incidence of Hb E, Hb Lepore, $\delta\beta$ -thalassaemia and Hb Constant Spring. Some other haemoglobinopathies likely reflect migration from Africa. A variety of rare haemoglobinopathies is seen and will be presented. The results reflect the changing ethnic diversity of the Australian population and patterns of migration.

No conflict of interest to disclose

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The Incidence of Haemoglobin disorders in Refugees migrating to Western Australia – a 5 year Retrospective Study, 2003 – 2007

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Aim

This was a retrospective study of the results of a screening programme for migrants undergoing health checks with the Refugee Migrant Health Clinic in Perth, WA.

Methods

All laboratory tests were performed according to standard diagnostic protocols, and included a FBP, Iron studies, HPLC, multiplex PCR for common alpha thalassaemia deletions and additional confirmatory tests as indicated by the results of initial screening.

Results

A total of 5523 samples were received over the review period. The majority of migrants were of African origin (n=4637), with 516 and 370 from Asia and the Middle East respectively.

Within the African population, alpha thalassaemia trait (heterozygous or homozygous $\alpha^{-3.7}$ deletion) was the most common abnormality, found in 12% of this population group. HbS was present in 8.2% of this population, most common in individuals of Central and West African origin (13.7% and 12% respectively). Beta thalassaemia trait was present in 6% of those from West Africa. It was rare in North Africa and was not seen in individuals from Central Africa. Other less common variants included HbC, Hb Stanleyville II and delta globin variants.

There has been a change in the pattern of migration from the Middle East and Asia over the study period. Whereas 370 individuals of Middle Eastern origin were screened in 2003 and 2004, this has been superseded by migration from South East Asia, with a total of 513 individuals since 2005.

The prevalence of alpha thalassaemia in the Middle Eastern group was 3.8%, and beta thalassaemia trait was seen in 1.4%.

The Asian migrants are mainly from Burma and Thailand. Alpha thalassaemia trait was seen in 6.2% of this population. Beta thalassaemia trait and HbE trait were seen in 6.4% and 4.3% respectively.

Conclusion

The pattern of Haemoglobin disorders reflects the changing demographics of the refugee migrant groups over time. Within these populations, clinically significant haemoglobin gene disorders are relatively prevalent, and clinicians should be alerted to this, particularly in the antenatal setting or in the context of pregnancy planning.

No conflict of interest to disclose

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The Role of Flow Cytometry in Myelodysplastic Syndromes (MDS)

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MDS is a heterogeneous group of myeloid neoplasms with abnormal maturation and differentiation of ≥ 1 cell lineage, resulting in bone marrow (BM) failure and a predisposition to leukaemia. Diagnosis is complex and standard assessment involves evaluation of peripheral blood (PB), BM and cytogenetics. Criteria for diagnosis and classification have recently been refined in a consensus publication, in which immunophenotyping (IP) was recommended as a co-criterion.

Although IP has been used extensively for diagnosis, classification, prognostication and detection of minimal residual disease for haematological malignancies, there is limited data in MDS. With an increased understanding of normal antigen expression, the detection of aberrations in blast and maturing myelomonocytic populations may provide a more objective assessment for sequential review. MDS flow-cytometric scoring systems have been developed to assist in the standardisation of this process (Wells et al. Blood 2003). The role of IP for discriminating early MDS and idiopathic cytopenias, as well as treatment response and remission status, in established MDS has not been defined.

We prospectively assessed the utility of IP, at diagnosis and after therapeutic intervention, by correlating standard assessment criteria, in particular morphology, with a modified MDS scoring system. Four colour-flow cytometric analysis was performed to assess blast and myelomonocytic populations. To establish "normal" antibody expression, we assessed marrow samples from 5 healthy volunteers and 16 patients with a normal PB and BM evaluation. Sequential assessment was then performed on 54 patients with possible and/or definitive diagnosis of MDS. This included sequential review in 10 patients treated with lenalidomide/stem cell factor in a phase II study.

We present our early data which demonstrates the potential utility IP as a diagnostic tool and in post treatment response assessment. However, larger numbers of patients are required in a prospective setting for confirmation of these findings.

No conflict of interest to be disclosed

Meeting Room 1
HSANZ Free Communications 12
O109

0830-1000
0945

Flow Cytometry as a Diagnostic Tool for Hereditary Spherocytosis- Westmead Hospital Experience

Nalini Pati, Mary Sartor, Sue Wong, Adrienne Nemet, Kenneth Bradstock
Department of Haematology, ICPMR, Westmead Hospital, Westmead, Sydney, Australia

Objectives

Flow cytometric analysis of eosin-5'-maleimide-labeled red blood cells has been proposed as a method of identifying hereditary spherocytosis (HS). The flow cytometric test measures the fluorescence intensity of intact red cells labelled with the dye eosin-5-maleimide (EMA), which reacts covalently with Lys-430 on the first extra cellular loop of band 3 protein. Patients with HS have reduced fluorescence compared to other patient groups and normal controls.

Aim

The aim of the present study was to assess the utility of flow cytometry in the diagnosis of hereditary spherocytosis by determining the sensitivity and specificity of this method within our laboratory.

Methods

Fresh peripheral blood was collected in Lithium Heparin, stained with the dye EMA and analysed by flow cytometry. A mean fluorescence intensity (MFI) range of 40.1 +/- 4.51 and Peak channel fluorescence (PCF) of 39.17 +/- 5.2 was considered positive for HS (these results were determined by analysing 7 known HS patients). Equivocal results are defined when the MFI remain in the range of 46-54 units, as opposed to normal healthy controls the MFI range between 55-74 units.

Results

A total of 96 samples were analysed with a female to male ratio of 1.06:1. Samples were investigated for HS for the reasons such as coombes's negative spherocytosis, positive family history (FH), neonatal hyperbilirubinaemia (NNH) and other haemolytic anaemia (HA). Within this cohort the group with positive FH of HS has highest positive and least equivocal results. The group with suspected HS (peripheral blood spherocytosis with negative coomb's test) resulted positive in 38% and equivocal in 26% cases. The group with NNH had positive results in 25%, and the group with HA in none. A further analysis of our data had shown the sensitivity and specificity of the test for HS were 80% and 100% respectively.

Conclusions

The highest number of equivocal results was obtained from the patient group with suspected HS possibly due to the fact that the specificity of this test method is for defects in band 3 protein and is less specific for other mutations or deletions in red cell membrane proteins. The EMA dye method by flow cytometry in the evaluation for HS with positive FH is specific, while in the group of patients with HA it is non contributory.

No conflict of interest to declare

Meeting Room 4
ANZSBT Free Communications 3
O110

0830-1000
0830

Massive Transfusion in Trauma

Romi Sinha, David Roxby, Ram Seshadri, Andrew Bersten
Flinders Medical Centre, Adelaide, South Australia, Australia

Aim

To determine blood products transfused, prevalence of coagulopathy on admission, clinical outcomes and the factors associated with massive transfusion in trauma patients.

Methods

A linked electronic database was developed using trauma, clinical and epidemiological and red cell transfusion databases. All trauma patients in the period 1998 to 2006 identified from the trauma database were linked by hospital medical number and reviewed for transfusion in the first 24 hours of injury.

Results

From the trauma database 6519 patients were identified between 1998 and 2006. Four hundred and thirty eight patients (7%) were transfused in the first 24 hours of injury and 802 (12%) received at least one unit of red cell during their hospital stay. Three hundred and fifty nine patients received 1 to 9 units of red cells (RC) and 79 patients received ten or more red cells during first 24 hours of injury using a total of 1601 units. The median time from injury to admission for the massive transfusion group was 112 minutes with a median Injury Severity Score (ISS) of 38. The median RC: FFP (red cell to FFP) ratio was 2.5:1 in the massively transfused group of patients. 46% of the patients in this group presented with coagulopathy on admission. The overall mortality was 32% with 68 % of that mortality in the first 24-48 hours. The factors associated with massive transfusion were systolic blood pressure <90mm, ($p= 0.002$ [OR 3.6]), Hb <120g/L ($p= 0.01$ [OR 3.7]) and pH < 7.3 ($p= <0.001$ [OR 25.5]).

Conclusions

Trauma patients receiving more than 10 units of red cells are patients with severe injury and are associated with high risk of mortality and receive most of the blood components for the treatment and prevention of coagulopathy.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 3
O111

0830-1000
0845

Extent and Timing of Dilutional Coagulopathy and Thrombocytopaenia in Massively Transfused Obstetric Patients

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²Department of Haematology, Princess Margaret Hospital, Perth, WA, Australia

Aim

To determine the extent and timing of dilutional coagulopathy and thrombocytopaenia in obstetrics patients who receive massive transfusion of blood products, and based on these results, draft a specific protocol on the management of massive transfusion in obstetric patients.

Method

Retrospective case note and data base review of all obstetric patients who received ≥ 8 units of packed red blood cells (PRBC) between 2002-2008.

Result

58 patients received ≥ 8 units of PRBC between January 2002 and July 2008. The average number of blood products transfused were as follows: 13.7 units of packed red cells (95% CI 11.5 – 15.9), 7.4 units of fresh frozen plasma (95% CI 5.8 – 9.0), 6.9 units of cryoprecipitate (95% CI 5.0 – 8.8) and 1.1 units of platelets (95% CI 0.6 – 1.6). On average, this group of patients developed a significant coagulopathy when compared to baseline results using paired t-tests: peak INR 2.1 (95% CI 1.8 – 2.4, $p < 0.001$), peak APTT 77.1 seconds (95% CI 60.0 – 94.2, $p = 0.023$), nadir fibrinogen 1.1 g/L (95% CI 0.9 – 1.3, $p = 0.001$, nadir platelet count $76 \times 10^9/L$ (95% CI 65 – 86, $p < 0.001$). The median time to peak INR was 3.0 hrs (95% CI 2.4 – 3.6), peak APTT was 3.0 hrs (95% CI 2.5 – 3.5 hrs), nadir fibrinogen 3.7 hrs (95% CI 2.8 – 4.7). The median time to nadir platelet count was 7.5 hrs (95% CI 6.7 – 8.3 hrs) and this was significantly longer than the median time to development of coagulopathy (Kaplan Meier analysis, $p = 0.002$). A protocol for the management of massive transfusion in obstetric patients was developed and will be presented.

Conclusion

Obstetric patients who are transfused ≥ 8 units of PRBC develop a dilutional coagulopathy and this develops significantly earlier than dilutional thrombocytopaenia. These data show that adequate replacement of fresh frozen plasma and cryoprecipitate should be considered earlier than platelet transfusion in this patient group.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 3
O112

0830-1000
0900

Adherence to Transfusion Protocols and the Use of Recombinant Activated Factor VII

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¹ Monash University, Department of Epidemiology and Preventive Medicine, Melbourne, Victoria, Australia. ² Royal Prince Alfred Hospital, Sydney, New South Wales, Australia. ³ Royal North Shore Hospital, Sydney, New South Wales, Australia. ⁴ The Alfred Hospital, Melbourne, Victoria, Australia

Background

Most hospitals have clinical guidelines for the off-label use of recombinant activated Factor VII (rFVIIa, Novoseven), primarily as part of a massive transfusion protocol. Over the past years rFVIIa has increasingly been used outside the approved indications in haemophilia with inhibitors and Glanzmann's Thrombasthenia, particularly in trauma, cardiac surgery and other critical bleeding episodes. Use in these areas remains controversial.

Methods

Monash University established the Haemostasis Registry in 2005 (with an educational grant from NovoNordisk Pharmaceuticals) to monitor the use of rFVIIa throughout Australia and New Zealand. More than 80 hospitals are contributing data to the Registry including all major users of rFVIIa in Australia and New Zealand. As part of the process of joining the Registry project, participating hospitals are asked to supply copies of their protocols for use of rFVIIa.

Results

Over 2000 cases of rFVIIa use have been reported to the Register. Major areas of use are cardiac surgery (~ 43%), other surgery (~17%) and trauma (~15%). The majority of hospitals have documented protocols for rFVIIa use. Many of these are similar and are centred around situations of massive transfusion. However, most cases of rFVIIa use submitted to the Haemostasis Registry do not conform with these guidelines.

Conclusions

This is the largest case dataset of rFVIIa cases published to date and can now provide greater insight into the actual rather than theoretical use of rFVIIa in Australia and New Zealand. Lack of compliance with hospital protocols for rFVIIa use indicates either that the protocols do not reflect actual and appropriate use or that clinicians need to be further educated regarding what is currently considered appropriate use. In the absence of sound clinical trial evidence, consensus regarding appropriate use has not been achieved. In these circumstances, data from the Haemostasis Registry continues to be important in elucidating the safety and efficacy of rFVIIa and providing important feedback to doctors and hospitals. *This research was supported by NovoNordisk Pharmaceuticals Pty Ltd. The company had no role in analysing the data or preparing the abstract*

Meeting Room 4
ANZSBT Free Communications 3
O113

0830-1000
0915

Monitoring the Temperature of Red Cells Stored Between 2 to 6°C and the Time Taken to Reach 10°C at Room Temperature

Mary Zmijarevic, Lakshmi Shriram Nath

Department of Diagnostic Haematology, The Royal Melbourne Hospital, Victoria, Australia

Aim

To determine the time required for a unit of red cell concentrate stored between 2-6°C to reach 10°C once left a room temperature. Is the 30 minute rule still valid?

Method

Sixty units of expired red cell units (250mL) in additive solution (Adsol) were stored between 2 to 6°C. The probe of a calibrated digital thermometer was inserted into the port of the bag and left to sit on the wooden bench at room temperature (22°C). The temperature of the RCC was recorded in 5 minute intervals to 30 minutes. Seven units of whole blood stored between 2 to 6°C were also monitored with the calibrated digital thermometer and the temperature recorded in 5 minute intervals to 30 minutes.

Results

On average the time taken for a red cell unit to reach 10°C was 20 minutes. After 30 minutes, the average temperature of a red cell unit was 11.4°C. The time taken for a unit of blood to reach 10°C depended on the whether the starting temperature was closer to either 2 or 6°C. None of the 7 units of whole blood reached 10°C in 30 minutes.

Conclusion

The time taken for a unit of blood to reach 10°C depends on a number of factors including the volume, starting temperature, surface and surface temperature where the bag is placed and temperature of the room where the blood is infused. This study has led us to review our process of red cell return to the laboratory.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 3
O114

0830-1000
0930

Teaching Transfusion and Transplantation Science in an Information Communication Technology (ICT) Enabled Wet Laboratory Environment

Ralph Green¹, Elaine Batchelder¹, Stephen Shanahan¹ and Rebecca Crockett²
RMIT University, Melbourne, Victoria, Australia¹; St Vincent's Hospital, Melbourne, Victoria, Australia²

In 2006 we incorporated networked student workstations in our haematology laboratory which has provided us with the opportunity to create a blended learning environment. This ICT enabled learning space provides students with the opportunity to integrate both theory and practical learning during the one laboratory session.

While lecture sessions are still conducted in lecture theatres due to class size, all lectures are recorded as screencasts and made available as streaming videos, downloadable executable files and MP3 audio files. To assist students in their learning Multiple Choice Question (MCQ) self assessment tests and crosswords on course topics are included in the online learning resources.

All students are provided with a practical manual of techniques, however, the availability of the workstations also allows us to provide an on-screen step by step visual guide to the techniques, that include short video sequences where appropriate. Students are able to review the techniques as often as they wish and go back over parts of the lectures if they are unclear on any of the principles of the techniques or procedures they are undertaking.

Advanced practical classes are conducted as projects. Students are able to research the literature during class and record the results of their experiments in electronic workbooks. Advanced classes in Transplantation and Clinical Immunology employ student directed learning strategies based on the creation of a wiki that forms the student's collective knowledge base on the course topics. Instructor and peer assessment is used in assessing student contributions to the wiki.

Student feedback on this learning environment gained through questionnaires and focus groups has been very positive.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 3
O115

0830-1000
0945

Transfusing a Unit of Red Cells – What Does It Really Cost in Australia?

Erica Wood^{1,2}, Linley Bielby^{1,2}, Russell Hunt³, David Peterson¹, David Roxby³, David Westerman¹

¹ Australian Red Cross Blood Service, ² Peter MacCallum Cancer Centre, ³ Flinders Medical Centre, Adelaide and Melbourne, Australia

Background and Aims

Transfusion involves many important steps and multidisciplinary laboratory and clinical teams. Comprehensive process mapping and costings for the overall transfusion process have not been available previously in Australia.

Method

Process maps for transfusing one unit of red cells were constructed and validated. Clinical and laboratory databases provided deidentified, aggregate transfusion episode data for adult non-trauma patients from Jan-Dec 2006 at two university teaching hospitals. Hospital administrations provided personnel/financial data.

Result

26 major processes have been mapped in detail for inpatient and outpatient red cell transfusions, including: pretransfusion examination/clerical routines & informed consent; phlebotomy; transfusion-related testing/results management; component prescribing and ordering; ordering and shipping from blood centre to hospital; inventory management and distribution of units to clinical areas; administration, clean up and waste disposal; and transfusion reaction management.

These processes are complex. For example phlebotomy involves between 33 and 40 actions. Transfusion administration includes between 89 and 105 major steps. An acute severe transfusion reaction can trigger 40 or more major processes, involving hundreds of steps. Direct and indirect costs are being assigned, reflecting process steps and timings, including salaries, on costs and other outlays (e.g. training, assessment); clinical and laboratory equipment, reagents and consumables; quality programmes (e.g. audit, transfusion committee activities) and process-related generic overhead costs (e.g. cleaning, IT).

Conclusion

The process of transfusing a unit of red cells is complex, time-consuming and involves multiple staff and other resources. Preliminary financial data indicate very substantial costs. Understanding the real costs of the whole process should encourage better transfusion practice and use of alternatives where appropriate, to optimise red cell use, improve patient outcomes, and reduce exposures, risks and possibly costs. This model may be adaptable to determine costs associated with transfusion of other blood components.

This research was supported by a grant from Amgen Australia. The company had no role in analysing the data or preparing the abstract.

Meeting Room 2/3
ASTH Free Communications 2
O116

0830-1000
0830

Oral Surgery with Minimum Factor Support in Haemophilia and vWD Patients

Ian Hewson and Alison Street
Alfred Hospital, Melbourne, Australia

After several years using just **local measures** in warfarinised patients for post oral surgery haemostasis with excellent results, the Dental and Haemophilia Units at The Alfred Hospital decided to use these measures for our haemophilia and von Willebrand patients (who would normally require factor replacement) to see if as good post operative haemostasis could be achieved, without the need for additional factor infusion.

Local measures

Flood socket with 5% tranexamic acid, Place gelfoam or surgical

Close with 4-0 Monocryl.

No additional factors administered unless post-op haemorrhage a problem

Previous treatment

Factor infusions to increase levels to 70% - 100% before surgery and post operative dosage depending on complexity of procedure (for up to five days post op with removal of four wisdom teeth)

Protocol would vary with degree of oral surgery and patient's oral health

Previous Costs

For four wisdom teeth:

Daily 45-50U/kg for five days

Less extensive oral surgery:

Initial 30U/kg then 20U/kg for two days

At a cost @ AU\$ 1.00 per unit

Wisdom teeth = AU\$17,000.00 - \$19,000.00

Simple extractions = AU\$5,300.00

Study patients

No factors were infused either before or after oral surgery unless patient returned with a post operative bleed. Patients on regular prophylaxis maintained their usual regime.

Results

To date (May 08) thirty three patients on study, seven (21%) severe (<1% factor activity,) three (10%) moderate (1% - 5%) and twenty three (69%) mild (> 5%).

Of a total of 65 extractions, twenty five (39%) have been surgical. Inferior dental nerve block was used in 50% of cases. One patient required treatment for post operative bleeding.

Conclusions

It is safe to proceed with complex dental procedures with extensive local haemostatic measures without additional factor support.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 2
O117

0830-1000
0845

Retrospective Analysis of Desmopressin Responses in Patients With Type 1 and Type 2 Von Willebrand Disorder and Haemophilia A in South Australia

Ketan Bavishi, Elizabeth Duncan, Sue Rodgers, Simon McRae, Lay Tay, John Lloyd

Division of Haematology, Institute of Medical and Veterinary Science, South Australia, Australia

Aims

1. Review desmopressin responses in patients with VWD and mild/moderate haemophilia A, and to compare these against two international criteria. R
2. Develop our own criteria based on experience with desmopressin treatment in mild bleeding disorder. D

Methods

We reviewed forty patients with VWD and 16 haemophilia A. Blood samples were collected at 0, 0.5, 1, and 2 hours after desmopressin (0.3µg/kg) infusion over 30 minutes. For VWD, a complete response (CR) was defined as peak VWF:RCo and FVIII:C both 0.80 IU/ml or higher, AND > 0.6 IU/ml at 2 hours. For partial response (PR), peak of VWF:RCo or FVIII between 0.50-0.80 IU/ml and either > 0.40 IU/ml at 2 hours. No response, neither criteria were met. Only FVIII was used to assess response in haemophilia A.

Results

Using our own criteria in type 1 VWD, with baseline VWF:RCo > 0.20 IU/ml (n=25), 92% had a CR compared to 25% with VWF:RCo < 0.20 IU/ml (n=8, p=0.001). this response rate is similar to a recent literature report², but higher than earlier criteria.¹ There were seven type 2 VWD patients, 2 type 2A and 2 type 2N had CR whereas 2 unclassified patients and 1 type 2M did not respond. Of 11 haemophilia A patients without an inhibitor, 11 responded to desmopressin. Four patients with an inhibitor responded, one did not respond when the inhibitor was 640BU/ml, but responded when it was 8.0 BU/ml, and another with 16.5 BU/ml inhibitor (FVIII<0.01IU/ml) did not respond.

Conclusion

There was considerable variations in desmopressin responses between patients with type 1 and 2 VWD, and mild/moderate haemophilia A with or without inhibitor. It remains useful to determine the desmopressin responses in whom it might be effective treatment.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 2
O118

0830-1000
0900

A Report on Acquired Haemophilia A in South Australia from 1998 to 2008

Deepak Singhal, Lay Tay, Elizabeth Duncan, Susan Rodgers, Simon McRae, John Lloyd
Division of Haematology, Institute of Molecular and Veterinary Science, Adelaide, SA, Australia

Aim

To review Acquired Haemophilia A (AH) cases in SA between 1998-2008.

Method

IMVS laboratory and clinical records where available were used to assess laboratory results and management. The time to partial remission (PR) (inhibitor titre 0.5-1.0 BU/ml) and complete remission (CR) (inhibitor titre <0.5 BU/ml) were calculated.

Results

Eighteen cases of AH were identified (11 males/7 females). The median age at diagnosis was 74 years (range 27-89). The presentation median FVIII and FVIII inhibitor were 0.02 IU/ml (range 0.01-0.09) and 7.5 BU/ml (range 1.4-460) respectively. Only 10 patients had treatment details available (Table 1).

No	Age	Sex	Type of Bleed	FVIII (IU/ml)	FVIII Inh (BU/ml)	Bleed Rx	AH Rx
1	64	M	none	0.04	7.5	none	CVP, ivIg
2	68	M	skin, muscle	0.02	2.4	rFVIIa	Prednisolone
3	47	F	skin	0.01	460.0	pdFVIII, rFVIIa	Prednisolone, Azathioprine, ivIg, Cyclophosphamide, Rituximab
4	82	M	muscle	0.07	3.4	FFP	Prednisolone
5	27	F	PPH	0.04	28.0	pdFVIII	ivIg, Azathioprine, Prednisolone
6	82	F	joint	0.02	6.3	rFVIIa	Azathioprine
7	61	M	muscle	0.02	11.0	rFVIIa	Prednisolone, Azathioprine
8	70	F	skin	0.04	5.0	none	Prednisolone
9	47	F	skin	0.09	1.4	DDAVP	None
10	80	M	skin, joint	0.02	3.0	rFVIIa	Prednisolone, Azathioprine

Patient 6 died of intra-cranial haemorrhage, 2 patients (2,7) have not achieved remission yet and another two (5,10) relapsed after CR1. A total of 6 PRs and 8 CRs were assessed. Median time to PR and CR was 80.5 days (range 21-450) and 162.5 days (range 30-542) respectively. The FVIII at PR and CR were 0.35 IU/ml (range 0.22-0.51) and 1.09 IU/ml (range 0.41- 1.98). Seven achieved long term remission.

Conclusion

AH is a rare acquired bleeding disorder with FVIII autoantibody formation. Our cohort reflected the heterogeneity in behaviour and disease course, their outcomes correlate with published literature.

No conflict of interest to disclose

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Meeting Room 2/3
ASTH Free Communications 2
O119

0830-1000
0915

A Single Tertiary Centre Experience with Inhibitors in Paediatric Patients with Severe Haemophilia

Huy Tran¹, Simon Brown^{1,2}, Salena Griffin², Wendy Poulsen², Kelly Brady², John Rowell¹, Liane Lockwood²

¹ Clinical and Statewide Service, Pathology Queensland, Royal Brisbane & Women's Hospital, ² Haemophilia Centre, Royal Children's Hospital, Brisbane

Background

Inhibitor development to replacement therapy remains the most severe complication for individuals with severe haemophilia A and B (SHA/SHB), occurring in up to 30% and 5% of patients respectively. Immune tolerisation therapy (ITT) is successful in eliminating inhibitors in a majority of patients.

Aim

A retrospective study of all paediatric patients undergoing treatment for SHA and SHB from 1998-2008 at the Royal Children's Hospital was performed to observe factors relating to their incidence and response to ITT.

Results

50 patients with severe haemophilia A and B were identified from the database; 13 developed inhibitors (12/47 in SHA and 1/3 in SHB). 69% (9/13) of inhibitor patients were using recombinant factor replacement therapy prior to the time of inhibitor identification. Median age of detection was 2 years with a range from 0.5 to 9.5 years. 46% (6/13) had high titre inhibitors (defined as >5 Bethesda units/ml (BU)). The median peak titre level was 6.4 BU (range 0.6 to 3328 BU). The inhibitor titre is now <0.6 BU in 85% (11/13) of this cohort. Of these 11 patients, the median duration to a negative inhibitor was 9.6 months (range 0 months to 42 months). Of the two with unsuccessful tolerisation both had high peak titre levels (1792 and 3328 BU). One of these patients is receiving prophylaxis with FEIBA and has recently undergone a radiosynovectomy for synovial hypertrophy of a knee joint. Genetic mutation analysis was performed on 10 patients.

Conclusion

Incidence of inhibitor formation in SHA/SHB patients is 26% and 33% respectively. Factors pertaining to successful outcome using ITT reported in the literature were also evident in our study: peak level of inhibitor titre, duration of inhibitor and genotype. Although inhibitor development remains a serious complication for individuals with haemophilia, the eradication of inhibitors can be achieved in the majority of patients.

There is no conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 2
O120

0830-1000
0930

Low Incidence of High Titre FVIII Inhibitors in Children with Severe Haemophilia A While Only Treated with an Intermediate Purity FVIII Concentrate (BPL 8Y). Experience from Five Centres in the UK

Simon Brown

Haemophilia Centre, Royal Children's Hospital, Brisbane; Pathology Queensland, Royal Brisbane and Women's Hospital, Brisbane, Queensland

Aim

High titre FVIII inhibitors remain a significant complication of replacement therapy in severe haemophilia A (SHA). It remains controversial whether the type of FVIII concentrate is a risk factor for inhibitor development. Previous reports have demonstrated a low incidence of FVIII inhibitors in children with SHA who only received an intermediate purity FVIII concentrate (BPL 8Y). The aim of the current study was to obtain data on BPL 8Y use in relation to the incidence of high titre (>5 BU/mL) inhibitor development and the underlying FVIII gene mutations.

Method

Seventy-four children with SHA treated solely with BPL 8Y were identified. A retrospective analysis of the clinical and laboratory data identified patients who had developed high titre inhibitors. The length of time from first exposure to 8Y to either inhibitor development or switch to an alternative FVIII concentrate was used to analyse inhibitor development in this cohort.

Results

The median age at first exposure to BPL 8Y was 12 months (range 0.5-70) and the median duration on BPL 8Y was 78 months (range 15-149). The cumulative incidence of high titre inhibitors in this cohort was 3.2% over 72 months; in comparison the cumulative incidence of high titre inhibitors was 13% over 72 months for a recombinant FVIII concentrate. Although molecular analysis is incomplete, 26 of 54 individuals tested possess the intron 22 inversion. In a further 20 individuals, an underlying mutation in the FVIII gene has been detected. The Hamsters FVIII database revealed information on inhibitor development for 12 of these mutations (6 have been associated with FVIII inhibitor development). The two children who developed high titre FVIII inhibitors have failed to achieve long term tolerisation with ITI.

Conclusion

The use of an intermediate purity FVIII concentrate (BPL 8Y) was associated with a low incidence of high titre FVIII inhibitors.

No conflict of interest to disclose

Meeting Room 2/3
ASTH Free Communications 2
O121

0830-1000
0945

A Multi-Centre Retrospective Study to Assess the Safety and Efficacy of Biostate[®] in Children with von Willebrand's Disease (vWD)

Rebecca Howman^{1,2}, Chris Barnes³, Ram Suppiah⁴, Jamie Price¹, Julie Curtin⁵, Sue Russell⁶, Michael Seldon⁷, Lochie Teague⁸

1. Princess Margaret Hospital, Perth, WA,
2. King Edward Memorial Hospital, Perth, WA
3. Royal Children's Hospital, Melbourne, VIC
4. Adelaide Women's and Children's Hospital, Adelaide, SA
5. Children's Hospital Westmead, Sydney, NSW
6. Sydney Children's Hospital, Sydney, NSW
7. Mater Hospital, Newcastle, NSW
8. Starship Children's Hospital, Auckland, NZ

Aim

To evaluate the safety and efficacy of a high purity, double virus inactivated factor VIII/Von Willebrand factor concentrate (BIOSTATE[®]) in children with VWD.

Factor replacement therapy with Biostate[®] forms the mainstay of treatment in children with VWD in Australia and New Zealand although there is limited paediatric data on the clinical safety and efficacy of Biostate[®] when used in VWD.

Methods

A retrospective analysis of Biostate[®] use in children with VWD at 7 hospitals across Australia and New Zealand between April 2003 and November 2007 was conducted. Data was collected on patient demographics, treatment indication, dosage, adverse reactions, and haemostatic efficacy for each use of Biostate[®].

Results

A total of 41 VWD patients (21 VWD type 1, 13 VWD type 2, 6 VWD type 3, 1 unknown; 24 male/ 17 female, age range 5months-15 years) were treated identified. Seven dental procedures, 31 surgical procedures, 49 non surgical bleeding episode and 2 patients on prophylaxis were recorded. Efficacy was recorded as excellent / good for all dental procedures, 26/31 for surgical procedures (moderate 3 /31, poor 1/31) and 43 / 49 for non surgical bleeds (5/49 moderate). Efficacy for prophylaxis was regarded as excellent / good for 1 patient and moderate for the remaining patient. The only adverse event recorded was the development of an inhibitor in the patient on prophylaxis who recorded moderate effect.

Conclusions

Biostate[®] is both safe and effective in VWD paediatric patients who require a FVIII/VWF concentrate for the management and prophylaxis of bleeding.

This research was supported by CSL. The company had no role in analysing the data or preparing the abstract.

Meeting Room 4
HSANZ: ALLG Symposium

1100-1230
1100

Novel Therapeutic Targets in Acute Myeloid Leukemia

Martin S Tallman

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Acute myeloid leukemia (AML) represents an heterogeneous group of diseases which vary in genetics, clinical manifestations, and outcome. While overall survival (OS) of younger patients has improved during the last 3 decades with increasingly intensive post-remission strategies and transplantation to generate graft-versus-leukemia effect, the outcome for older adults remains poor and it is not clear that any post-remission chemotherapy is beneficial. Well-recognized prognostic factors include age, intensity of post-remission therapy (younger adults only) and cytogenetics which distinguish favorable, intermediate, and unfavorable groups with OS of 5 years of approximately 55%, 40%, and 10%, respectively. Among the large group of patients with normal karyotypes, recently described mutations in or overexpression of specific genes facilitate classification, contribute to prognosis and serve as targets for drug development. Patients with a mutation in NPM1, but not FLT3 appear to have a relatively favorable prognosis and may not benefit from allogeneic hematopoietic stem cell transplantation (HSCT). Alternatively, patients with core binding factor leukemias with mutations in c-KIT have an outcome similar to that of patients with unfavorable-risk cytogenetics and allogeneic HSCT appears justified. Gemtuzumab ozogamicin, an immunoconjugate directed at the antigen CD33, has modest single agent activity, but may be more effective when combined with chemotherapy. Similarly, while FLT3 inhibitors alone demonstrate some biologic activity with reduction in peripheral blood blasts and occasionally marrow blasts, but no remissions, their true benefit may emerge when combined with chemotherapy. Histone deacetylase inhibitors such as valproic acid, SAHA, and depsipeptide; hypomethylating agents such as 5-azacytadine and decitabine; antiangiogenesis agents such as Bevacizumab; and farnesyltransferase inhibitors are all promising. The novel nucleoside analog clofarabine appears particularly active in older adults including those with unfavorable cytogenetics. The discovery that the plant-derived sesquiterpene lactone parthenolide and its dimethyl analog target the leukemic stem cell is among the most exciting new developments.

Meeting Room 4
HSANZ: ALLG Symposium

1100-1230
1145

How Do We Manage Mantle Cell Lymphoma in 2008?

Simon Rule
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Mantle Cell Lymphoma (MCL) is a rare and aggressive form of non-Hodgkin's lymphoma (NHL) accounting for 6-8% of all cases. Patients with MCL have the shortest median time to progression and the shortest median survival of all lymphoma sub-types after first line treatment. Unlike some other lymphoma sub-types MCL is very rarely localised. Most patients present with widespread lymphadenopathy often with constitutional symptoms and an unusual feature of this disease is the predilection to involve the GI tract, which is virtually universal if it is actively looked for. There are many chemotherapy regimens used in MCL but no consensus as to the treatment of choice. It affects predominantly elderly patients, which means that many of the published treatment regimens are not applicable. CHOP based chemotherapy has been the commonest regimen adopted because of the aggressive nature of the condition. The addition of Rituximab to this and other regimens has been widespread despite compelling evidence that this improves outcome. In younger patients the use of high dose Ara-C as the cornerstone of the initial therapy looks to be well established but which precise regimen used is the subject of on-going studies. The consolidation of responses post such therapy with an autologous transplant has recently been shown to be highly effective and the precise role of an allograft is being defined. There is a small cohort of patients who exhibit an indolent clinical course, some of which present with a lymphocytosis in whom a watch and wait approach is legitimate. A number of single agent drugs have been shown to have activity in MCL. Perhaps the most exciting of these at the moment is Bortezomib (Velcade). Bortezomib demonstrates synergistic activity with a number of agents and these are being explored in the clinic. There are a number of other agents that have been used which demonstrate activity against MCL such as Temsirolimus, Lenalidomide, Flavopiridol, Bendamustine, Enzastaurin as well as histone deacetylase inhibitors, bcl-2 inhibitors and a range of other early agents. Where these and other agents will fit in the treatment schema of MCL will be defined as trials progress.

Riverside Theatre
Combined ANZSBT/ASTH: Thrombocytopenia
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1100-1230
1100

Thrombopoietic Agents in Patients with ITP

James Bussel

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ITP is a relatively common disorder of thrombocytopenia caused primarily by antibodies to platelets. These auto-antibodies cause peripheral platelet destruction and also apparently impaired platelet production. Bleeding is variable although generally related to the platelet count and serious bleeding occurs (intracranial hemorrhage, ICH) but is fortunately distinctly uncommon. Initial therapy consists of IVIG, IV anti-D, and steroids and in general intends to increase the platelet count by inhibiting platelet destruction. Second line treatments include rituximab, danazol and splenectomy. Their use depends upon individual physician and patient preference and the features of a given case not least of which may be the extent of the response to the treatment(s) that was tried initially. Third line treatments are primarily immunosuppressive including cyclophosphamide, azathioprin, interferon, mycophenolate mofetil, colchicine and other agents. Starting in 2006, several studies have been completed of the use of novel thrombopoietic agents in ITP. Thrombopoietin was initially cloned in 1994 and soon thereafter two forms of it went into clinical trial. Proof of principle was achieved early on and there was efficacy in the non-myeloablative setting but antibodies to these agents (primarily described for MGDF) lead to their discontinuation from therapy. Second generation agents have been primarily tried in patients with chronic ITP. A series of studies with 2 agents, AMG531 (romiplostim or Nplate) and Eltrombopag (promacta or revolade) have demonstrated substantial efficacy, little toxicity (thus far), and good tolerability. In addition, one of these agents has been used with success in allowing the treatment of hepatitis C infected patients with interferon and ribavirin. Finally two additional thrombopoietic agents have entered trial. The findings with these agents as of fall 2008 will be reviewed in detail and the newer areas of treatment considered.

Riverside Theatre
Combined ANZSBT/ASTH: Thrombocytopenia
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1100-1230
1140

The Molecular Control of Platelet Life Span

Emma Josefsson¹, Simone Schoenwaelder², Matthew Goschnick¹, Michael White¹, Kylie Mason¹, Andrew Roberts¹, Shaun Jackson², David Huang¹, **Benjamin Kile¹**

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We have recently demonstrated that the circulating life span of platelets is regulated by members of the Bcl-2 family of proteins, which control the intrinsic apoptosis pathway. Pro-survival Bcl-x_L is the critical regulator of platelet life span, functioning to keep pro-death Bak and Bax in check, thereby maintaining platelet viability. After 5-10 days in the circulation, platelets not consumed in hemostatic processes initiate a Bak and Bax-dependent cell death program and clearance from the bloodstream. Studies with the BH3 mimetic compound ABT-737, which inhibits Bcl-x_L, have shown that platelets induced to undergo cell death *in vitro* exhibit many of the hallmarks of apoptosis in nucleated cells, including mitochondrial damage, caspase activation and externalization of membrane phosphatidylserine (PS). PS exposure is also a feature of activated platelets, which employ it to drive pro-coagulant activity. We now have evidence to suggest that two distinct pathways lead to PS exposure in platelets, one regulated by Bcl-2 family proteins and caspases, the other dependent on calcium and calpains. We are currently examining the possibility of cross-talk between these pathways, and whether there is a role for Bak and Bax in mediating aspects of platelet function in addition to the control of life span.

Riverside Theatre
Combined ANZSBT/ASTH: Thrombocytopenia
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1100-1230
1205

Heparin Induced Thrombocytopenia - Current Controversies in Diagnosis and Management

Simon McRae

Affiliation: South Australia Pathology, The Queen Elizabeth and Royal Adelaide Hospitals, Adelaide, South Australia, Australia.

Heparin induced thrombocytopenia (HIT) is a immunological complication of heparin therapy associated with the potential for severe morbidity and even mortality. Prompt diagnosis and initiation of alternative anticoagulant therapy is required to avoid adverse clinical outcomes. Access to gold standard testing for HIT is limited in the majority of clinical centres, and alternative diagnostic strategies are therefore often used to confirm or exclude this diagnosis. The clinical utility of various approaches including pre-test probability scoring systems, enzyme-linked immunosorbent assays, and newer rapid laboratory assays, alone or in combination, will be discussed. The advantages and disadvantages of various treatment strategies will also be discussed, including management of patients with renal failure, and the potential role of fondaparinux. Finally, opportunities for future research will also be outlined.

ABSTRACTS - Wednesday 22



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