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P62

Relationship between JAK2 V617F mutation status and Mpl protein expression in myeloproliferative disease

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Aim

The JAK2 V617F mutation is thought to play a role in the pathogenesis of different myeloproliferative disorders by inducing cytokine hypersensitivity in blood progenitor cells. The aim of this study was to detect the incidence of the JAK2 V617F mutation in a cohort of myeloproliferative patients and determine its correlation with abnormalities in platelet Mpl expression.

Methods

We studied 59 patients (26 PV, 33 ET) and 20 control subjects aged between 31 and 94 years of age. DNA was extracted from granulocytes and the JAK2 V617F abnormality detected by mutation specific PCR. Mpl expression was measured on platelet lysates by immunoblotting and compared with a control value by densitometry. Laboratory results were then correlated with clinical data.

Results

Control subjects with a normal haematological profiles were all JAK2 V617F negative and expressed a variable amount of platelet Mpl protein (mean 80%±17, n=20). Patients with a myeloproliferative disease were variably positive for the mutation with JAK2 V617F detected in 73% of PV and 63% of ET patients. In these myeloproliferative patients, Mpl was reduced compared to the control group (PV 46%±34, $p<0.01$ and ET 38%±30, $p<0.01$), and within each diagnostic classification the JAK2 V617F positive cohort had reduced Mpl expression compared to the patients with JAK2 V617F negative myeloproliferative disease (JAK2 V617F positive PV 35%±28, n=20 compared to JAK2 V617F negative PV 86%±18, n=6 and JAK2 V617F positive ET 31%±21, n=21 compared to JAK2 V617F negative ET 50%±39, n=12).

Conclusion

Decreased Mpl expression has been recognised to occur variably in myeloproliferative disease where its significance is uncertain. This study cohort suggests that myeloproliferative patients with decreased Mpl expression on platelets are more frequently positive for the JAK2 V617F mutation.

P63

Isolated duodenal granulocytic sarcoma presenting as bleeding polyp in an elderly patient with chronic myelomonocytic leukaemia – outcome following radiotherapy

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Aim

Granulocytic sarcoma (GS) is an extramedullary tumour composed of myeloid precursor cells, and may present in patients with myelodysplastic syndrome (MDS). It can affect any site but the gastrointestinal tract(GIT), especially the duodenum, is rarely affected. We describe and discuss the difficulties encountered in treating an elderly chronic myelomonocytic leukaemia (CMML) patient with bleeding duodenal GS.

Methods

A 70-years-old Chinese lady with CMML presented with melaena, which was complicated by acute myocardial infarction, in October 2004. Oesophagogastroduodenuoscopy (OGD) showed a bleeding duodenal polyp which was found to be GS on biopsy. Due to severe thrombocytopenia and high risks for surgery and chemotherapy, she was treated with localised RT. Endoscopic argon plasma coagulation (APC) was required to secure haemostasis of the duodenum GS. The duodenal GS resolved (as proven on OGD and CT scan) after a total of 3000cGy of radiation over 15 fractions. However, she continued to have persistent bleeding from radiation-induced telangiectasia of the stomach inspite of aggressive platelets and blood transfusions. Multiple sessions of endoscopic APC and adrenaline injection were done for haemostasis but to no avail. She transformed into AML in December 2004 and had worsening thrombocytopenia. She had prolonged hospital admissions, and continued to require massive blood products support until her demise in July 2005.

Results

There were only 2 previous duodenal GS reported and our patient is the first CMML patient reported to have this. She illustrated the difficulties in managing bleeding GIT GS in elderly MDS patients. Treatment of GIT GS may involve RT, chemotherapy or surgery.

Literature had shown superiority in outcome of chemotherapy over surgery or RT alone in the treatment of GS. However, elderly patients with GS like ours, may not be fit enough for chemotherapy. Hence, RT was chosen as the safest alternative for our patient.

Conclusion

In view of our experience with this patient, we recommend avoiding RT in GIT GS, as there is potential risk of radiation-induced GIT mucositis or telangiectasia which when coupled with concurrent severe thrombocytopenia, aggravates bleeding. If patients are fit, chemotherapy should be considered. Surgery may be required in the presence of surgical complications such as bleeding, obstruction or perforation.

P64

Comparison in TNC counts and CD34+ cells reported in unrelated cord blood units between cord blood banks and transplant centre: A single institution report

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Background

Engraftment after unrelated cord blood (UCB) transplantation is highly dependent on total nucleated cell (TNC) count and CD34+ cell content. Transplant centres would like reassurance that a frozen CB unit contains the appropriate TNC and CD34 count for the CB unit they have chosen provided by the CB Bank for safe transplantation of their patient. To our knowledge, no reports exist to establish whether CB bank numbers provide close enough numbers as determined by the transplant centre at time of infusion. Usually, the results of TNC counts and CD34+ cells in CB unit represents CB before freezing provided by all CB Banks. We compared these numbers to thawed CB values at our transplant centre provided to our laboratory for infusion between 2000 and Feb 2006.

Methods

Single 39 CB units were received at our laboratory (18 from Australian CB Banks and 21 from International CB Banks) for UCB transplantation for 13 adults and 20 pediatric patients. Six patients were infused with double CB units. All CB units were thawed with no washing prior to infusion except for 3/39 units. The CB units in this study were stored for a mean duration in liquid nitrogen tanks for 3.5 years (range 1-7 yrs) in CB banks to time of infusion. The mean volume of CB was 66.8 ml (range 25-300) for infusion.

Results

14/39 or 36% of CB Banks provided thawed values on CB units to transplant centre prior to CB release. The mean recoveries for TNC count and viable CD34+ cells counts for 39 units analysed was 78% (range 54-108%), and 67% (range 10-160%) respectively. In some cases reductions resulted in infusion of cell numbers considered inadequate for routine transplantation.

Conclusions

Taking into account of inter-laboratory testing and cord blood bank reporting of CB quality, we provide a standard of recovery of cells in the product at transplant centres following infusion, that is expected cell loss for all CB units for TNC and viable CD34+cells to transplant centres. We recommend that all CB banks provide data on thaw pilot vials as part of their standard policy to the transplant centre prior to final release of the CB unit.

P65

Safe treatment of patient with CML using dasatinib after prior retinal edema due to imatinib

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Background

Imatinib has revolutionised the management of CML. However ~10% of patients must discontinue due to adverse effects. Dasatinib, a novel tyrosine kinase inhibitor, has shown promising anti-leukaemic activity in patients of CML resistant or intolerant to imatinib.

Case

A previously-well 38 year old man diagnosed with chronic phase CML began imatinib 400 mg/day. Ophthalmic review for persisting visual disturbance revealed significant deterioration in visual acuity and macular and optic disc edema in the left and right eyes, respectively. Haematologic remission was achieved at 3 months. The visual disturbance persisted, was considered to be due to imatinib, leading to its cessation. On review 6 weeks later, the retinal oedema had resolved and visual acuity normalised. Disease was well controlled on hydroxyurea for 5 months. The patient then provided informed consent to participate in a phase-II trial of dasatinib, (70 mg bd). Ophthalmic evaluation 4 weeks after commencement of Dasatinib showed preserved visual acuity and no evidence of recurrence of the optic nerve edema. The patient is continuing on Dasatinib at 12+ months without any significant side-effects and has achieved a major molecular response.

Discussion

Fluid retention and edema are among the most common adverse effects of imatinib. Retinal and macular edema are rare, but well described side effects. Inhibition of PDGF receptors has been postulated as the cause of fluid retention based on data from animal studies.

This may suggest that differences in PDGFR inhibition between the two drugs may explain the different toxicity profiles in our patient, however the available data suggest both drugs effectively inhibit the PDGF receptor. The spectrum of tyrosine kinase inhibition of dasatinib needs further exploration considering the potential use in patients with PDGFR related toxicities with imatinib.

P66
IgA nephropathy in cutaneous T cell lymphomas

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Background

IgA nephropathy has been described rarely in association with cutaneous T cell lymphomas (CTCL). Here we describe two additional cases.

Case 1

A 68 year old man was diagnosed as having Sezary syndrome based on skin biopsies and morphology and immunophenotype of circulating atypical lymphocytes. Treatment was initiated with extracorporeal photopheresis and steroids followed by weekly methotrexate on disease progression . He was then commenced on a histone deacetylase inhibitor as part of a clinical trial. He had two episodes of bacteremia with staphylococcus aureus and pseudomonas. A progressive rise was noted in his serum creatinine from a baseline value of 0.11mmol/L to 0.18 mmol/L over a period of 2 months. Urine analysis revealed over 550 million glomerular red cells, 24 hour protein excretion of 0.3gm/day consistent with glomerulonephritis. A drug related renal dysfunction was thought to be unlikely and a renal biopsy was performed which revealed IgA nephropathy. His creatinine has plateaued and he is on regular monitoring .

Case 2

A 38 year old man was diagnosed with Mycosis Fungoides treated initially with PUVA followed by combination chemotherapy and radiotherapy. He then underwent reduced intensity allogeneic stem cell transplant. He relapsed and repeatedly responded to multiple donor lymphocyte infusions for recurrent disease. He then received a histone deacetylase inhibitor for progressive disease. Urine microscopy prior to study enrolment revealed macroscopic haematuria, one million glomerular red cells and urinary protein excretion of 1.65 gm/day. His creatinine remained normal throughtout. Renal biopsy performed to ascertain cause of acute glomerulonephritis revealed IgA nephropathy.

Discussion

Glomerular disease has been well described in association with lymphoma and there are only about a dozen reports in the literature reported to date for CTCL. However, the mechansim of renal disease remains unclear. Associated IgA nephropathy should be considered in patients with skin lymphoma and renal impairment.

P67
Keratinocyte growth factor in allogeneic stem cell transplant

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Aim

Keratinocyte growth factor (KGF, palifermin) has been shown to reduce both the severity and duration of mucositis in the autologous setting, with limited data in allogeneic transplants. The aim of this study was to audit our experience with the use of KGF in 8 allogeneic recipients.

Methods

All patients scheduled for HSCT with conditioning associated with a high risk of mucositis from April to September 2005 were offered KGF under compassionate supply from Amgen, Australia. KGF was administered at 60 mcg/kg on 3 consecutive days finishing 24 hours before conditioning, followed by a further 1-3 doses from Day 0 to day 2 avoiding concomitant use with methotrexate. Mucositis grading, duration, ADR's, TPN and analgesia usage were recorded. Historical controls, matched for disease and conditioning regimen were accessed from the Royal Melbourne Hospital BMT patient database.

Results

8 patients (6 allogeneic, 2MUDs, 7 AML, 1MDS) were consented to KGF. Conditioning given was Cy/TBI (4), BuCy (2), FluMel-ATG (1) and Flu/Mel (1) with CSA and MTX on days 1,3,6 and 11 as GVHD prophylaxis. 15 historical control patients were identified. The incidence of grade 3-4 oral mucositis was 75% in the cases compared to 80% incidence in the historical controls. Duration of mucositis, TPN and opiate usage were also very similar.

Conclusion

Compared to a cohort of patients prior to April 2005, KGF was not effective at significantly reducing the severity or duration of mucositis in our allograft patients. This observation stands in contrast to the experience with autologous patients. Possible causes include scheduling with methotrexate, omission of D+1 KGF to avoid concurrent methotrexate or allogeneic patients may be more heavily pre-treated with a

predisposition to mucositis. Further studies in the allogeneic setting are required.

P68
HSCT as part of an upfront strategy for the treatment of high risk HIV-related lymphomas

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Introduction

Highly active antiretroviral therapy (HAART) has had a significant positive impact on the incidence and outcome of Aids-related lymphomas (ARL). Nevertheless, certain subtypes of ARL continue to have a poor prognosis with conventional therapies.

We consider the relapse rate in Burkitt's lymphoma treated with EPOCH to be unacceptably high and now routinely offer good performance status patients CODOX-M alternating with IVAC chemotherapy (6 cycles in total) with a view to autologous transplantation in CR-1.

Primary effusion lymphoma (PEL) is an HHV-8 associated ARL reported to have an exceptionally poor prognosis. We offered a good performance status patient in CR2 reduced intensity conditioning (RIC) allogeneic HSCT.

Results

8 cases of AIDS-related advanced stage Burkitt's lymphoma have been diagnosed at our institution since January 2005. 1/8 (73 years old) was offered only palliative therapy and died soon afterwards. Another was offered only CHOP/rituximab due to poor performance status and is in CR-1 at 63 days. 6/8 commenced CODOX-M/IVAC chemotherapy. One succumbed to progressive CNS disease. 5 have completed CODOX-M/IVAC to date and are in CR. So far, 2 of these 5 have successfully undergone autologous HSCT and have reached days 107 and 123. One remains platelet dependant.

The 34 year old with PEL had attained CR2 with F-GIV followed by autologous HSCT subsequent to early relapse post EPOCH chemotherapy. He underwent a sibling allogeneic RIC HSCT using melphalan/fludarabine conditioning with tacrolimus/sirolimus/methotrexate GVHD prophylaxis. Platelet and neutrophil engraftment was at day 13 and 17 respectively. He was discharged without significant infective complications and his CD4 count was 240 x 10^6 /L at day 47. He remains in CR2 at day 407 post transplantation and has returned to work.

Conclusion

Intensive chemotherapy may be given to ARL patients in the HAART era with acceptable toxicity. RIC allogeneic transplantation may also be safe and effective in well selected patients.

P69
Comparison of haematopoietic reconstitution after autologous transplantation using stem cells after short- (<12 months) and longer-term cryostorage

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Introduction

High-dose cytotoxic therapy (HDT) with autologous haematopoietic stem cell (HSC) rescue is an important treatment option for patients with non-Hodgkin's (NHL) and Hodgkin's Lymphoma (HL), particularly for those who have a suboptimal response to initial therapy, refractory and/or relapsed disease. Although it is customary to collect HSCs at the time the need is apparent, in theory harvesting stem cells during the early phases of treatment – preferably when the patient is in remission – may have advantages. In particular, the HSCs will have been less exposed to damaging cytotoxic therapy and moreover, early collections may be less contaminated with malignant cells. However, such "prophylactic" or "rainy day" harvests may need to be stored for extended periods before use, raising questions about their functional capacity.

Although laboratory studies of the effects of long-term cryostorage on the viability and functional capacity of HSCs have demonstrated that the cells remain functional, there are few studies of clinical outcomes after their reinfusion. Because we have a long-standing policy of carrying out rainy day harvests, we have had the opportunity to compare haematopoietic reconstitution after autologous HSC transplantation after short-term and longer-term cryostorage.

Aim

To evaluate the safety and efficacy of autologous HSC transplantation after short-term (<12 months) and longer-term storage of HSC; in terms of haematopoietic reconstitution and transplant-related complications.

Method

We performed a retrospective analysis of 114 patients with NHL and 19 with HL who had undergone HDT with autologous stem cell rescue at the Royal Hobart Hospital from June 1985 to June 2005. Patients who had HSC collected and used within a 12-month period were allocated to the short-term group (Range 6-52 weeks, median 12 weeks) and those who had HSC stored for greater than 12-months prior to use (Range 52-482 weeks, median 114 weeks), the longer-term group. We compared the rates of neutrophil and platelet engraftment,

and the 30-day transplant-related mortality (TRM).

Results

Of the 133 patients, 97 were in the short-term group and 36 in the longer-term group. TRM was 7/98 and 1/35 for the short- and longer-term groups respectively (P=0.7); no deaths occurred in the 77 patients transplanted after 1998.

Haematopoietic reconstitution was achieved in all 125 patients who survived to 30 days. Neutrophil recovery [absolute neutrophil count (ANC) >0.5 $x10^9$/L] was achieved by 12.2± 4.2 (SD) days and 14.2 ± 6.5 days post-reinfusion for the short- and the longer-term groups respectively (P=0.14). Platelet support was required for a mean 6.6 ± 9.5 and 7.5 ± 7.7 days respectively (P=0.7).

Conclusion

These clinical results, demonstrate that HSCs after longer-term cryostorage retain engraftment potential similar to that of HSCs stored for lesser periods, and that such cells are safe and efficacious for autologous transplantation.

P70
Does platelet size have diagnostic predictive value in patients with thrombocytopenia?

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Aim

To establish whether mean platelet volume (MPV) has diagnostic value in patients with thrombocytopenia by predicting the presence or absence of bone marrow disease.

To determine if results produced by a previous study (Bowles et al, 2005) can be reproduced using an Abbott Cell-Dyn 4000 automated analyser.

Methods

Data was retrospectively collected from full blood picture samples obtained from patients attending Fremantle Hospital. Samples were analysed on an Abbott Cell-Dyn 4000. MPV results were recorded for 179 patients with thrombocytopenia (platelet count <150 x 10^9/L). Based on clinical and laboratory information patients were divided into two groups; those with bone marrow disease (n=70) and those without evidence of bone marrow disease (n=109). Statistical analysis was performed on these groups.

Results

Bone marrow disease	mean platelet count	MPV mean	MPV media n	MPV SD	MPVrange
Present	82	9.21	9.00	1.93	5.77 – 16.80
Absent	101	9.11	8.81	1.79	6.00 – 16.60

Statistical analysis of data using the Student's t-test demonstrated a significant difference in platelet count between both groups of patients (p=0.003), but no significant difference in the mean MPV (p=0.699). These results are in contrast with the report by Bowles et al (2005) who demonstrated that patients with bone marrow disease had a lower MPV than those that did not. This difference may reflect different methods of automated platelet analysis (optical vs impedance), patient mix, and causes of thrombocytopenia. The effect of platelet transfusion and chemotherapy may also affect the MPV.

Conclusions

Our results do not support the use of the MPV (measured by the Abbott Cell-Dyn 4000) to predict the presence or absence of bone marrow disease in patients with thrombocytopenia. Further studies are required to determine if MPV can be used in the diagnosis of bone marrow disease

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P71
Chediak-Higashi syndrome–new insights on a rare morphological diagnosis

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Chediak-Higashi Syndrome (CHS) is a rare, autosomal recessive disorder characterised by hypopigmentation, immunodeficiency, neuropathy and platelet dysfunction. A defect of the CHS1 gene results in abnormal vesicular trafficking in many cells with the accumulation of storage granules, including lysosomes and melanosomes. Previously, this disease was usually fatal before 10 years age, with very few patients reaching adulthood.

A 7 year-old girl with fair hair and skin presented with a squint and cutaneous bruising. She was referred to an Ophthalmologist at a regional centre with a possible diagnosis of oculocutaneous albinism. Her mother was of Islander descent, and her father was Caucasian. At this review, a diagnosis of Hermansky-Pudlak syndrome (HPS) was queried, and a blood count requested. Full blood count parameters were essentially normal, however because of the history provided, the blood film was reviewed. The leucocytes contained large, abnormal granules. Platelet function analysis (PFA-100) confirmed an aspirin-like defect. A presumptive diagnosis of CHS syndrome was made, and the child was referred to a tertiary paediatric centre.

Her history revealed no major infective episodes, other than standard childhood illnesses and recurrent skin abscesses. She has mild learning difficulty, but no neuropathy. Electron microscopy and a bone marrow examination support the diagnosis of CHS. Tissue typing has confirmed a sibling donor match, providing the possibility of allogeneic stem cell transplantation, and long-term survival.

The case highlights the importance of accurate communication between clinician and the diagnostic laboratory. This rare, potentially treatable disorder may otherwise be overlooked, due to the current trend towards automated processing and reporting where blood count parameters are normal. The gene defects for CHS and the related HPS, and more recently a mutant zebra-fish model have been identified further elucidating their pathophysiology at a molecular level, and raising the hope for gene therapy in the future.

P72
Evans syndrome with durable response to Rituximab

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Introduction

Evans Syndrome is the association of autoimmune thrombocytopenia and autoimmune haemolytic anaemia without any underlying cause. The limited case reports in the literature on the use of rituximab in Evans syndrome indicate that responses to rituximab are not durable. We present a case report of a patient with Evans syndrome successfully treated with Rituximab with a durable response.

Method

Retrospective review of the medical records.

Results

A 33 year old man was diagnosed with Evans syndrome in November 1993 with a haemoglobin of 99g/L, platelets $41x10^9$/L and normal white cells. There was evidence of immune haemolysis with elevated reticulocytes, low haptoglobin, elevated lactate dehydrogenase, and a Coombs test strongly positive for IgG and C3d. A bone marrow biopsy was consistent with Evans syndrome. He responded initially to prednisone and splenectomy and remained in remission until relapse of the haemolytic anaemia in 1995. Again this was steroid sensitive. He then remained in remission until relapse in August 2000. Prednisone was restarted and a splenunculus removed. The response was adequate but the steroids were unable to be tapered. Dapsone was trialed but stopped due to an extensive rash. Azathioprine was introduced in May 2001 and allowed the prednisone to be stopped. His haemoglobin remained normal but his platelet count slowly decreased over one year to a level of approximately $50x10^9$/L. Because he was on warfarin for a very extensive DVT, more definitive treatment was sought. Rituximab (375mg/m^2 weekly for 4 doses) was given. Within 10 days of the first dose his platelets rose to $121x10^9$/L and subsequently normalised. The response has been durable and he remains in remission, off azathioprine, with normal blood counts 4 years post rituximab infusion.

Conclusion

Unlike previous cases of Evans Disease that have been described in the literature, rituximab has maintained a remission for over 4 years in this patient despite the failure of multiple previous regimens.

P73
Standardisation of real-time quantitative PCR methods for BCR-ABL mRNA analysis

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Aim

Serial analysis of BCR-ABL levels using real-time quantitative PCR (RQ-PCR) provides an appropriate monitoring strategy for patients with chronic myeloid leukaemia. However, the RQ-PCR methods used throughout the world are not standardised, which leads to variation of results. Also, there remains considerable uncertainty in terms of reproducibility and validity of data within and between laboratories. We present a study involving the distribution of BCR-ABL reference standards to 12 laboratories with the aim of determining laboratory specific conversion factors that allow conversion of data to an international reporting scale. This scale is fixed to a value with proven clinical efficacy for imatinib therapy.

Method

Reference standards were prepared with 4 BCR-ABL levels by diluting positive cells in normal cells. Standards were sent to 12 laboratories in Asia, USA, Europe and Australia, which use various RQ-PCR methods. Mean BCR-ABL values from each laboratory were correlated with those of the Adelaide laboratory. Conversion factors were calculated from the regression equations and validated by patient sample exchange.

Results

Prior to international scale conversion the range of BCR-ABL values for the reference standards varied from 7.5-fold to 48-fold. After conversion the values varied from 1.8-fold to 6.2-fold indicating an improved correlation between laboratories. To date validation of conversion factors has been undertaken in 5 laboratories for which there was a significant difference in the BCR-ABL values prior to conversion (P=0.012, Wilcoxin test) whereas after conversion there was no statistical difference.

Conclusion

Initial analysis indicates that alignment of data using various BCR-ABL methods is achievable by conversion to an international scale. By undertaking this study it is anticipated that an international scale for reporting BCR-ABL levels will be adopted which will have great impact on the accuracy of patient monitoring in an era of disease response that is beyond the measurement capacity of conventional cytogenetics.

P74
Intrathecal Rituximab for primary CNS lymphoma

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Aim

Primary CNS lymphoma remains a therapeutic challenge, with relapses despite high-dose methotrexate and cranial irradiation. Rituximab added to CHOP chemotherapy has increased survival in systemic diffuse large B-cell lymphoma. If it could gain access to the CSF, it might improve the outcome of CNS lymphoma.

Rituximab given intravenously results in CSF levels only about 1% that of serum levels[1]. Direct injection into the spinal theca or intraventricular injection via an Ommaya reservoir results in much higher levels. Safety data on this mode of administration comes from animal studies and small series reported in humans[2-5].

Since the CNS is an “immunoprivileged” site, cytokine release reactions have not been regularly reported with this mode of administration. However, one report of a patient experiencing nausea and chills following ventricular administration of rituximab exists[2].

Methods

We have treated 2 patients with CNS lymphoma with rituximab given intrathecally by lumbar puncture(LP):

Upfront/Relapsed	**Other Rx**	**Dose(mg)**	**Schedule**
U	HD-MTX/idarubicin/systemic rituximab/dexamethasone/XRT	25	D1,4
R	HD-MTX/ XRT	25	D1,21

Results

Rituximab was given either as undiluted solution (25mg in 2.5ml) or diluted 1:1 in sterile saline.

Undiluted rituximab injection was further sterilised through a 0.22micron filter in the aseptic suite.

Patient 1 received prior systemic rituximab 375mg/sq.m. with the first 2 of 3 cycles of chemotherapy. The treatments were well-tolerated: patient 1 reported pain and tingling of the knee after the first LP; patient 2 reported no ill effects.

Conclusions

The efficacy of this treatment cannot yet be scientifically assessed, however, several case reports suggest favourable responses, including one of our patients with relapsed disease. We would recommend that the addition of intrathecal rituximab be studied in future randomised trials of treatment of CNS lymphoma.

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P75
Early experience with PET-CT in the assessment of treatment response in Hodgkin lymphoma (HL)

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Aim

To evaluate the utilisation of PET/CT in treatment response in HL.

Methods

From June 2003 to June 2006, 105 consecutive patients (53 female, 52 male; mean age=34 years) with HL were staged with PET/CT (LSO Biograph Duo). Histologies were: nodular sclerosing (n=53), mixed cellularity (17), lymphocyte rich (13), lymphocyte depleted (4) and NOS (18). Referrers staged patients according to the Ann Arbor classification. Maximum standard uptake value (SUV) was assessed in each case prior to treatment. SUVs were measured after treatment and a qualitative assessment was also made: complete response, residual disease and progressive disease. Isotope dose and scanning parameters were identical for all patients and scans.

Results

HL stage was: I (n=18), II (37), III (21), IV (29). Six patients were not included due to a short follow up interval and 24 had only a primary staging PET/CT. Thus there were 75 patients with 112 PET/CTs: 22 patients were scanned only at completion of treatment, 24 patients had scans (n=56) during and at completion and 29 had a second scan(s) (n=34) only during therapy. During treatment, scans were done after: 2 (n=24), 3 (13), 4 (13), 5 (2), 6 (3), 7 (1) cycles of chemotherapy and the number of cycles was not known (n=1). For the during-treatment scans, there was a complete response (CR) in 52, 5 had residual disease (RD) and 3 had progressive disease (PD). Over the last 12 mths there was a trend for scans to be requested mid-treatment rather than at the end of therapy.

Conclusions

Recent overseas data suggest early PET data have prognostic value. Our data indicate there is not a consistent local approach to the timing of assessment of response in HL with PET/CT.

P76
PET-CT in primary staging and management of Hodgkin lymphoma (HL) and Non-Hodgkin lymphoma (NHL)

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Aim
To evaluate PET-CT in staging and management of previously untreated HL and NHL.

Methods
From June 2003 to June 2006, 465 consecutive patients (223W, 242M; mean age 52 years) had PET-CT scans (LSO Biograph Duo). Isotope dose and scanning parameters were identical for all patients and scans. PET-CT scans were read without access to histology or other imaging. Histology was HL (n=105) and NHL (n=360); nodular sclerosing HL comprised 50% of HL and DLBCL accounted for 44% and follicular lymphoma 30% of NHL. Referrers were asked to provide the clinical stage, results of other investigations and the management plan prior to the scan; after PET-CT they were asked for a revised clinical stage and management plan based on the PET-CT. Pre- and post-staging and management plans were compared. The analysis was retrospective in 176 and prospective in 289 patients.

Results
PET-CTs were negative in 45 patients: 40 where the only site of disease was resected, and 5 where lesions were beyond the scanner resolution. All were included in the analysis. Pre- and post PET-CT staging was obtained in all 465 patients. PET-CT altered staging in 168 (36%) patients (38 HL, 130 NHL). Up-staging was seen in 149 (36 HL, 113 NHL). Pre and Post PET-CT management plans were obtained in 417. Patients without management plans (n=48) were excluded. Management was changed in 117 (28%) patients (20 HL, 97 NHL) comprising 55/149 (37%) patients upstaged, 56/297 (19%) where staging was unchanged and 6/19 (32%) patients downstaged.

Conclusion
In the largest PET-CT cohort yet reported our data indicate that in HL and NHL, PET-CT improves primary staging and referrers appear to alter management based on these data. The effect on management can be independent of stage where it relates to the volume and extent of disease.

P77
18F-FDG-Positron emission tomography scanning for staging, response assessment, and disease surveillance in patients with mantle cell lymphoma

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Positron emission tomography (PET) has become an important imaging modality in the staging and response assessment of various types of lymphoma. However data on its specific utility in mantle cell lymphoma are lacking.

Methods
The records of 28 patients with mantle cell lymphoma (MCL) who had one or more FDG-PET scans between March 1999 and November 2005 were retrospectively reviewed. One-hundred and one scans were performed, 80 using combined PET/CT. Nine patients had staging scans. The other scans were performed for response assessment or relapse surveillance.

Results
At baseline, FDG-PET had a site sensitivity of 96%. FDG-PET scans differed from the results of conventional imaging in 18 of 28 (64%) patients at some stage during their follow-up. Scans performed for assessment of response to therapy improved response from partial or unconfirmed complete response to complete metabolic response in 8 of 28 patients (35%), and showed more extensive disease without changing the stage or response quality in 2 of 28 patients (9%). Scans performed for relapse surveillance allowed earlier diagnosis in only 2 patients. PET compared to conventional means of assessing gastrointestinal involvement was directly assessable in only 3 patients, and was found to be of only modest utility.

Conclusions
MCL is an FDG-avid malignancy and FDG-PET is useful to accurately stage nodal disease. However, FDG-PET was not found to be highly sensitive in detecting subclinical bowel involvement. It is probable that FDG-PET has a role in assessing response of MCL to therapy, but this requires further investigation.

P78
SIADH and AML t(3;3): A case report

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Cytogenetic findings in Acute Myeloid leukaemia (AML) provide vital information with respect to prognosis and approach to therapy. It is recognised that chromosomal abnormalities involving chromosome 3 (in particular the 3p21:q26 region) have a distinctive constellation of clinical and pathological abnormalities. Clinically, this group of patients tend to have a high rate of treatment failure and subsequent early death. From a pathological viewpoint, patients in this group usually present with a normal or increased platelet count and bone marrow examination demonstrates abnormal megakaryopoiesis. A very small number of cases have also been reported to be associated with the syndrome of inappropriate antidiuretic hormone (SIADH.) This syndrome is characterised by partial impairment of water excretion leading to water retention mediated by the inappropriate excess production of antidiuretic hormone. We present the case of a 42 year old male presenting with neutropenia and a macrocytic anaemia who was subsequently diagnosed as AML involving t(3;3) and describe his subsequent progress and development of SIADH.

P79
The many faces of t(8;13): A case report

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Background
t(8;13) is a rare chromosomal translocation involving the short arm of chromosome 8. The abnormality fuses fibroblast growth factor receptor 1 (FGFR1) to ZNF108 gene on chromosome 13. The syndrome associated with t(8;13)(p11;q12) results in the constitutive activation of a receptor tyrosine kinase and subsequent cell proliferation.

It is well recognised as having an extremely poor prognosis as it frequently evolves to a refractory acute myeloid leukaemia within 1-2 years of diagnosis. We describe a case of a patient presenting with the t(8;13) translocation and show its progression from an initial diagnosis of a myeloproliferative disease to T-lymphoblastic lymphoma and finally to AML. A literature review of this distinctive clinicopathological syndrome will also be included.

P80
A rare classic: t(8;14)(q24;q11) in T-ALL

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Aim
Case report of a patient with T-cell Acute Lymphoblastic Leukaemia/ Lymphoblastic Lymphoma (WHO) and a rare cytogenetic abnormality.

Methods
Standard chromosome preparation and FISH techniques using bone marrow cells.

Results
A 22 year old man was referred for diagnostic cytogenetics with a provisional diagnosis of lymphoma with CNS, mediastinal and renal involvement. Cell marker analysis of bone marrow by Flow Cytometry showed no evidence of a clonal B-cell population. PCR demonstrated a monoclonal T-cell population and no evidence of B-cell disease. Cytogenetic analysis of mitogen stimulated cells revealed a small abnormal clone containing a translocation between chromosomes 8 and 14, t(8;14)(q24;q11). FISH was used to characterize the translocation.

Conclusions
This classical abnormality is rare with an incidence of 2% in T-ALL. It is associated with specific clinical features many of which were demonstrated in this patient.

P81
What a surprise: t(14;14)(q11;q32) in pre B-ALL

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Aim
Case report of a child with Trisomy 21 who was referred for cytogenetics with a diagnosis of acute leukaemia.

Method

Standard chromosome preparation and FISH techniques using bone marrow cells.

Results

Cell marker analysis showed the presence of a blast cell population with a phenotype consistent with precursor B-cell Acute Lymphoblastic Leukaemia (WHO). Karyotyping revealed a translocation involving both chromosomes 14, t(14;14)(q11;q32). This re-arrangement is usually associated with T-cell disorders and occurs only rarely with lymphoblastic leukaemia of B-lineage.

Conclusion

No other report of this abnormality in conjunction with Down Syndrome has been found. Reference is made to the few other relevant cases in the literature.

P82

Disseminated histoplasmosis presenting as pancytopenia in a patient with human immunodeficiency virus infection

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Introduction

Disseminated histoplasmosis, caused by the fungus Histoplasma capsulatum, is a life threatening condition in immunocompromised patients, for which prompt diagnosis and treatment is critically important due to the high mortality of untreated cases.

Case Report

A previously healthy 32-year-old female presented with a two-month history of lethargy, weight loss, night sweats and menorrhagia. She had immigrated from the Democratic Republic of Congo to New Zealand 7 years prior and then to Australia 3 months before presentation. Physical examination revealed pyrexia, bruising and hepatosplenomegaly. Full blood evaluation revealed severe pancytopenia with a leukoerythroblastic film and tear drop red cells. Serum lactate dehydrogenase was 594 U/L. Further investigation with bone marrow aspirate revealed extensive histiocyte engorgement with 3-4µm diameter oval-shaped uninucleate organisms on May-Grünwald-Giemsa (MGG) stain. The differential diagnosis was established as either Histoplasma capsulatum or Leishmania donovani infection. The patient was commenced on intravenous (IV) liposomal amphotericin B. Human immunodeficiency virus (HIV) testing was positive with an undetectable CD4 lymphocyte count. She commenced anti-retroviral therapy. Bone marrow trephine biopsy was strongly suggestive of disseminated histoplasmosis with the organisms being positive on both periodic-acid Schiff (PAS) and Grocott methenamine silver (GMS) stains. Cultures of bone marrow confirmed Histoplasma capsulatum variant capsulatum. The fevers resolved after 3 days and patient completed 14 days of IV amphotericin. She was discharged after 16 days from admission, on oral itraconazole.

Conclusion

Our case illustrates that differentiating histoplasmosis from other fungal or protozoan infections (including visceral leishmaniasis) can be difficult on morphology alone and special stains along with cultures are required for confirmation. Disseminated histoplasmosis is uncommon in immunocompetent patients and causes for immunocompromise (such as HIV co-infection) should be sought.

P83

Translocation (11;20): A novel rearrangement in a patient with chronic idiopathic myelofibrosis

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Introduction

Chronic idiopathic myelofibrosis (CIMF) has been associated with several cytogenetic abnormalities in a limited number of patient series. Trisomy 8 and deletion 12p have been associated with poorer prognosis, in addition to anaemia, leucocytosis and advanced age. Translocations between chromosomes 11 and 20 have not been previously recognised in myeloid disease including CIMF.

Case

A 66-year-old male was referred with leucocytosis and anaemia on routine testing. Physical examination revealed hepatosplenomegaly. Peripheral blood evaluation showed haemoglobin 106g/L, platelets 244 x 10^9/L and white blood cells 16.0 x10^9/L, with a leukoerythroblastic film and tear drop poikilocytes. Initial bone marrow aspirate was aparticulate, with unsuccessful cytogenetic analysis. The trephine biopsy showed moderately increased fibrosis on a variably hypocellular background providing a provisional diagnosis of CIMF (early fibrotic stage). The patient was observed periodically for 2 years until symptomatic anaemia and falling platelet count prompted repeat bone marrow biopsy. This revealed extensive marked increase in fibrosis consistent with CIMF. Cytogenetic analysis showed

46,XY,t(11;20)(p13;q13.3),del(12)(q24.1)[7]/46,XY,add(12)(q24.1)[3]/46,XY. Fifty-five per cent of metaphases examined were pseudodiploid. Two abnormal lines were identified. Seventy percent of the abnormal cells contained a translocation between chromosomes 11 and 20 and a partial deletion of 12q, while 30% contained additional material on 12q. Subsequent allele-specific polymerase chain reaction assay for JAK2 V617F mutation on peripheral blood was negative. The patient commenced hydroxyurea and transfusions of packed red blood cells for symptomatic anaemia. Hydroxyurea was ceased after 3 weeks due to worsening cytopenias. The patient has remained supported on fortnightly red blood cell transfusions for 12 months. His current survival is 36 months since diagnosis, consistent with low risk stratification predicted by Lille Scoring System for CIMF.

Conclusion

The possible role and prognostic significance of t(11;20) in CIMF remains to be determined.

P84
South Australia myelodysplastic syndromes (MDS) database

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Aim

Through the establishment of an SA MDS database project, we aim to collect information of (i) the local epidemiology data of MDS patients including their subtypes, cytogenetics subsets and (ii) the outcome data.

Methods

All patients that had been evaluated with bone marrow biopsies in the 76-month period (from Jan 2001 to April 2006) were included via active case findings and retrospective search through the marrow SNOPMED codes. Their diagnoses were verified, subtyped and an IPSS score given. Demographic information, blood and marrow findings, treatment, transfusion, and survival data were collected via an MDS database (MS Access).

Results

One hundred and nine patients were included; with a male to female ratio of 3.4. Median age of diagnosis was 67 years old (range: 24-87), with the majority in the age bracket 61-80 yrs. Overall, the WHO subtypes of RA, RARS, RCMD, RAEB, 5q- syndrome, and CMML made up 13, 7, 28, 31, 2 and 17% respectively. Younger patients had skewing of bad risk MDS subtypes. Favourable karyotypes were seen in 72%, intermediate karyotypes in 12% and poor risk cytogenetics in 26%, when complex karyotypes often involve chromosomes 5 and 7. The IPSS score gave a fairly good stratification (low risk 28%; intermed-1 38%; intermed-2 17%, high 4%). Only <10% patients received AML type of therapy and allografts. Survival results were generally consistent with what was described in the literature.

Conclusions

An MDS database project provides local Australian data of the epidemiological profile and outcome of our MDS patients.

P85
A case of imatinib-resistant, nilotinib-resistant chronic myeloid leukaemia (CML) responsive to dasatinib

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Background

A proportion of CML patients develop resistance to imatinib, many due to BCR-ABL kinase domain mutations which inhibit imatinib binding. Recently, the newly developed tyrosine kinase inhibitors dasatinib and nilotinib have entered clinical trials and each has shown considerable clinical and cytogenetic activity in patients unresponsive to or intolerant of imatinib. There are few data yet available on the possible cross-resistance between these “second generation” kinase inhibitors. We therefore describe a patient with CML previously resistant to both imatinib and nilotinib, with an early response to dasatinib.

Case report

65 yo man diagnosed with chronic-phase CML in 2003. Early treatment with imatinib 400mg/d attained a major cytogenetic response by interphase FISH (4% positive) with no further gains despite dose escalation to 800mg/day. Response was transient and after 17 months disease progressed and mutational analysis revealed a F359V non-P loop mutation. Treatment with nilotinib 400mg bd achieved a transient complete haematologic but minimal cytogenetic response with no improvement on 600mg bd. Treatment was complicated by exacerbation of congestive cardiac failure on background of ischaemic and valvular disease, and liver function abnormalities. At time of commencement of dasatinib 50mg bd in June 2006, disease had progressed to accelerated phase with BM blasts 10%, rapid increase peripheral leukocytosis (44.8 x 10^9/L) and platelets (1388 x 10^9/L) with BCR-ABL transcripts over 170%. After one month on stable dose dasatinib, a complete haematologic response was achieved; WBC 6.5 x 10^9/L and platelets 110 x 10^9/L with transient mild renal

impairment, fluid overload and stable cardiac status. Ongoing follow up of cytogenetic and molecular response will be presented.

Discussion

This case illustrates imatinib resistant CML due to a non P loop mutation not overcome by nilotinib, but sensitive to dasatinib consistent with recent reported cases with this mutation. This response may be due to the different binding mechanics, greater potency or broader kinase activity of dasatinib. It is likely that assessment of ABL mutational status will become essential in predicting response and possibly selecting the preferred second generation kinase inhibitor. In addition, the activity observed with these newer agents may stimulate further investigation into combinations and earlier use of alternate kinase inhibitors.

P86
Biphenotypic B Cell lymphoma analysed by a multidisciplinary approach: Integrated lymphoma diagnostics

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Aim

To utilize a combined histological, molecular and flow cytometric approach to investigate the clonality of biphenotypic tumours.

Methods and Results

A sixty-eight year old male with a 16 year past history of Non-Hodgkin's lymphoma, re-presented with widespread lymphadenopathy. A sample of right cervical posterior triangle lymph node was submitted for histological and flow cytometric analysis. H&E sections revealed partial involvement of the lymph node by follicular lymphoma, Grade 3a, with a proliferative index of 30% estimated by stains for MIB1. Immuno-histochemical stains showed positivity for CD10 within this follicular component but both immunostains and in-situ hybridisation for light chains did not reveal any staining. The interfollicular zone showed focal involvement by a population of small CD20-positive B-cells, staining positively for a cocktail employing both CD21 and CD35 in a single stain. This population showed a proliferative index of less than 5%.

Flow cytometry revealed two populations of B lymphocytes. One population was CD10 positive and kappa light chain restricted, they expressed IgG heavy chain and were negative for CD21. The other was CD10 negative, lambda light chain restricted, expressed IgM and IgD heavy chains, positive for CD21. The two populations were separated by cell sorting and submitted for molecular analysis. Flow cytometric analysis of a bone marrow sample demonstrated the presence of the lambda light chain restricted population only. The nodal cells were then flow sorted into Kappa only and Lambda only cell aliquots. Molecular analysis of flow sorted cells revealed a common immunoglobulin heavy chain gene rearrangement PCR band (FR3A-LJH amplicon) of 58bp. Further analysis revealed common usage of the leader VH6 and reverse J4 sequences, giving a 270bp amplicon for both the Lambda and Kappa clones. A minor band was also evident in the Kappa clone. All bands were sequenced for commonality and for somatic hypermutation.

Conclusion

This study demonstrates the effectiveness of an integrated multidisciplinary approach to diagnosis and biological understanding of NHL. New developments in multiparametric flow cytometry display the complexity of NHL not previously anticipated. The impact of this type of analysis on management is not yet evident. However, with greater understanding comes the potential to realise the biological differences in tumours that may explain variances in disease response to therapy.

P87
Acute leukaemia of ambiguous lineage with triphenotypic features based on the EGIL classification: A case report

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Aim

To describe the clinical and pathological features of a case of acute leukaemia of ambiguous lineage with triphenotypic features demonstrating myeloid, T and B lineages.

Method

Case report.

Results

A 25 year old man was diagnosed with Acute Leukaemia in May 2005 after presenting with a chest infection. Blood examination demonstrated leukoerythroblastic changes with numerous pleomorphic blasts. Bone marrow examination confirmed the extensive

pleomorphic blast infiltrate. Immunophenotyping studies of the blast population demonstrated expression of myeloid antigens: CD33, CD34, CD117 and cytoplasmic myeloperoxidase (MPO), as well as T lymphoid antigens: cytoplasmic CD3 and B lymphoid antigens: cytoplasmic IgM and CD10. Based on the European Group for the Immunologic Classification of Leukaemia (EGIL), he was confirmed to have a triphenotypic acute leukaemia, best classified in the WHO system as Acute Leukaemia of Ambiguous Lineage. Cytogenetic studies revealed an abnormal complex karyotype with 14 of 23 metaphases containing a derivative chromosome 3, deletion of the long arm of chromosome 5, trisomies 3, 6 and 8 and a t(9:11)(p22;q23) translocation. He underwent pre-induction with vincristine and prednisolone therapy followed by standard ICE (Idarubicin, Cytarabine, Etoposide) chemotherapy but had no response. He achieved remission with re-induction FLAG (Fludarabine, Cytarabine and G-CSF) and Idarubicin, which was also given as consolidation therapy. Tissue typing studies revealed no related or matched unrelated donors on all registries. He underwent double cord Allogeneic Bone Marrow Transplantation (BMT) in October 2005 followed by standard maintenance immunosuppression. He remains in ongoing remission.

Conclusions

Our patient with Acute Leukaemia of ambiguous lineage expressing triphenotypic features achieved remission following re-induction chemotherapy and allogeneic BMT. Ongoing monitoring continues.

P88
Extramedullary relapse of acute myeloid leukaemia post allogeneic stem cell transplant

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Aim

We report 3 cases of extramedullary relapse of acute myeloid leukaemia post allogeneic stem cell transplant

Background

Relapse remains the main cause of treatment failure in AML therapy, occurring in 50% of patients who achieve CR. Most of these involve the marrow, and most are early (60% in 1st year).

Isolated extramedullary relapses are infrequently seen, but are more likely to occur post transplant (allogeneic rather than autologous).

We report 3 cases which occurred over the last 12 months at Royal Perth Hospital.

The first case (MM) is a 40yo with M2 AML who underwent sibling allograft, with various sites of extramedullary relapse at different time points (testicular, humeral, tibia). These occur despite presence of chronic cutaneous and GIT GVHD. MM presented with a large intrathecal mass/CNS relapse and underwent intensive chemotherapy, which unfortunately was complicated by neutropaenic sepsis and multiorgan failure.

The next case (RS) is a 52yo with AML with antecedent MDS, treated with MUD and developed testicular relapse 5 years later. Investigations with CT/PET showed multiple nodal sites of relapse. Reinduction therapy was given. RS has since developed marrow relapse and is undergoing further chemotherapy and DLIs.

DP is a 33yo with AML t(8;21) who developed chloroma 3 years post MUD. PET scan was performed which revealed extensive extramedullary disease. Induction chemotherapy was administered following DLIs.

These cases illustrate the different pattern of relapse seen after conventional chemotherapy in comparison to allograft. Causes of relapse remain poorly defined. Postulated mechanisms include multidrug resistance secondary to expression of P glycoprotein, low levels of topoisomerase II and inhibition of apoptosis due to increased bcl-2 expression.

Our cases illustrated the potential role of PET and molecular monitoring if applicable in surveillance of extramedullary relapse.

Outcome however remained uncertain and there is a paucity of published data, with anedoctal reports only and a limited number of reviews. Guidelines for management are thus not possible at present.

Conclusion

Isolated extramedullary relapses remain a difficult management problem. Use of PET scans, molecular monitoring may improve detection rate and possibly treatment outcome. More studies are required to ascertain optimal strategies.

P89
Chronic myeloid leukaemia (CML) on imatinib: Morphological, cytogenetic and molecular monitoring

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Aim

Only a relatively small number of articles have been published regarding the bone marrow morphological changes seen in patients with

CML on imatinib, particularly beyond six months of therapy. The aim was to specifically assess these morphological changes in conjunction with cytogenetic and polymerase chain reaction (PCR) results.

Methods

We reviewed peripheral blood, bone marrow, cytogenetic and PCR results on 14 patients in chronic phase CML at imatinib initiation. The median age at diagnosis was 54. The follow-up period varied from 3 to 24 months.

Results

All patients showed a complete haematological response within 3-4 months, but the bone marrow morphological response was slower. The bone marrow trephine cellularity was increased in 9 of 14 patients prior to imatinib initiation, and in all patients except one the cellularity normalised over the analysis period. The quality of cytogenetic responses after 6 months (12/12 major cytogenetic response and 5/12 complete cytogenetic response) was higher than those seen in some reports, but our numbers are small. On PCR 3/12 patients had a major molecular response after 6 months, but none a complete molecular response.

One interesting finding, previously briefly mentioned in some publications, was the significant numbers of patients developing lymphoid collections while on imatinib. Most of the collections appeared morphologically benign, but small numbers were in a paratrabecular location or contained larger, more atypical lymphocytes.

Conclusions

Results are consistent with published data showing a rapid peripheral blood and subsequently bone marrow morphological response, but more delayed cytogenetic and molecular response to imatinib therapy. The significance of the increased number of lymphoid collections is still unclear.

P90

Syngeneic bone marrow transplant for paroxysmal nocturnal haemoglobinuria

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Method

A case presentation and review of the literature

Results

A previously well nineteen year old female was admitted with a ten day history of lower abdominal pain and diarrhoea. She had also recently received antibiotics for a suspected urinary tract infection after presenting to her GP with haematuria. Investigations revealed Hb 83, Plts 39, WBC 7.2, neut 4.3, CRP 148, ALP 163, GGT 43, LDL 311, haptoglobin 0.33, reticulocyte count 51. CT scan of the abdomen showed sub acute Budd-Chiari Syndrome with a pelvic abscess as the cause of her abdominal pain. Further investigations showed normal physiological coagulation inhibitors, tissue auto antibodies were not detected and no lupus or antiphospholipid antibodies were found also. Immunophenotyping of peripheral blood for GPI linked antigens CD14, CD16, CD55 and CD59 revealed 99% of neutrophils weakly expressing CD16 and were negative for CD55 and CD59 (type III PNH cells). 97% of RBC showed weak expression of CD59 and were negative for CD55 (type II). The moncytes were negative for CD14. These results confirmed Paroxysmal Nocturnal Haemoglobinuria. A bone marrow trephine was moderately hypocellular with some normal areas of haematopoiesis.

Treatment initially consisted of anticoagulation and blood product support. She then proceeded to a syngeneic bone marrow transplant from her identical twin sister (microsatellite studies confirmed that they were identical and her twin had no PNH clone identified).The patient received Cyclophosphamide 50mg/kg daily for 4 days prior to receiving peripheral stem cells from her donor. The patient was anticoagulated on admission with LMWH (previously had been on warfarin) until platelets <50 and this was recommenced on engraftment. The post-transplant course was fairly unremarkable. There was donor engraftment on day +12 (neut >0.5 and platelets >20).

Peripheral blood immunophenotyping on day +27 showed mixed chimeric status with a PNH clone of types I-III being identified in the red cell series and normal neutrophils. At day +56, there was no PNH clone in the neutrophil series and a decreasing clone in the red cell series and by day +189, no evidence of PNH was detected. Anticoagulation was stopped after the PNH clone was shown to be resolving post transplant. The patient has remained well with no further thrombotic episodes and immunophenotyping remains normal.

Conclusion

PNH is a disorder characterised by a defect in the GPI anchor due to an abnormality in the PIG-A gene which leads to partial or complete lack of expression of certain GPI-linked proteins, especially CD59 and CD55. The clinical manifestations are primarily related to abnormalities in haematopoietic function including haemolytic anaemia/aplastic anaemia and a hypercoaguable state leading to venous thrombosis particularly of the abdominal or cerebral veins. Syngeneic and HLA identical sibling haematopoietic cell transplantation (HCT) have been used successfully in selected patients with PNH. The largest reported series, from the International Bone Marrow Transplant Registry, described the outcome of HCT in 57 patients with PNH, of whom 2 received syngeneic stem cells. One received prior conditioning and one did not. The one who received no prior conditioning had graft failure and required a further transplant but both were still alive at 8 years and 12 years respectively with no evidence of PNH. In another syngeneic transplant, the absence of conditioning was associated with gradual loss of the transplanted cells and symptom recurrence at 17 months, suggesting a survival advantage for the PNH clone. Long term complete remission as assessed by flow cytometry in a recipient of a syngeneic transplant at 12 years has also been

reported.

For our patient and her twin, stem cells and T-cell subsets have been taken and co-cultured to assess reasons for the apparent survival advantage of the PNH clone. In summary, transplantation with prior conditioning, is proving to be the treatment of choice for young patients with PNH especially for those with a syngeneic donor, offering the possibility of long term cure.

P91
Audit of patients' experiences of bone marrow aspirate and/or trephine biopsies in adult haematology patients at Christchurch Hospital

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As part of our quality assurance and service improvement processes, an audit was performed to determine patients' experiences of bone marrow sampling procedures in our hospital. The aim was to determine the adequacy of explanations given regarding the procedure, determine the degree of pain felt by patients as our standard procedures were without sedation and whether patients were having ongoing pain after the procedure. Patients were asked to complete a questionnaire at their next visit after their bone marrow procedure.

51 patients completed questionnaires. 33% had received written information prior to the procedure. All patients gave consent for the procedure. In 82% this was immediately prior to the procedure. 88% felt less concerned regarding the procedure after the explanation given. The median degree of pain felt was rated at 4 out of 10. 18% had pain for more than 2 days after the procedure (range 0-17 days).

Overall we felt that our patients having bone marrow procedures being reassured by the explanations of the procedure provided. There is room for improvement in the usage of written information and in giving information earlier rather than just at the time of the procedure. Since this audit the use of Entonox has been instituted as analgesia for the procedure. The audit is to be repeated to assess the patients' experiences with this change. Some patients continued to have pain more than 48 hours after the procedure. This needs to be addressed in the consent process for the bone marrow procedure.

P92
Two cases of granulocytic sarcoma

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Aim

Granulocytic sarcoma (chloroma) may be encountered in de novo or relapsed acute myeloid leukaemia and may also be seen as an isolated event, perhaps before AML is evident. Two patients with granulocytic sarcoma are described and this unusual manifestation is reviewed.

Methods

Two cases studies are presented, followed by a brief review of the literature discussing the epidemiology, diagnosis, implications and treatment of granulocytic sarcomas.

Results/Conclusions

Patient 1 – A 47 year old woman presented with a 7 week history of back pain radiating down her left leg. She had been treated for AML 16 months previously and had attained a complete remission. When symptoms were first evident, her blood count was normal, although subsequent blood counts showed relapsed AML. Neurological examination was remarkable for generalised increased tendon reflexes. MRI of the vertebral column demonstrated an extramedullary tumour mass involving the T7-T11 paraspinal region causing significant canal stenosis and slight cord compression. There was also tumour mass filling the sacral canal and crowding the cauda equina. The patient was treated with idarubicin and cytosine arabinoside, along with dexamethasone and oral morphine, with good disease response thus far.

Patient 2 – A 77 year old man presented with a rapidly expanding mass in the left axilla which on FNA was shown to be a granulocytic sarcoma. The full blood count and bone marrow biopsy were normal. Aggressive treatment was not pursued due to the patient's age and comorbidities. The patient received low dose subcutaneous cytosine arabinoside followed by radiotherapy to the left axilla. Eleven months later he developed a right axillary mass and progressed to acute myeloid leukaemia despite recommencement of subcutaneous cytosine arabinoside.

Granulocytic sarcomas are seen more frequently in AML with a high presenting white count, t(8;21), or CD56 positivity. The correct diagnosis relies on cytochemistry and immunophenotyping. The ability of the blast cells to invade tissues has been attributed to overexpression of cell adhesion molecules. Treatment consists of radiotherapy combined with systemic chemotherapy. In some cases haemopoietic stem cell transplant may be indicated.

P93

Two cases of lymphoma containing both t(14;18) and rearrangement of IGH-cMYC, one with a cryptic insertion of IGH into MYC

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Case 1

A 73 year old male presented with a one week history of back and abdominal pain and a rapidly growing left axillary mass with widespread lymphadenopathy. In the peripheral blood, there was a mild lymphocytosis of 6.55 x109/L with an abnormal population of large lymphoid cells with irregular nuclei, prominent nucleoli and moderate amounts of basophilic cytoplasm.

Immunophenotyping of the lymphoid cells showed a lambda restricted clonal population of B cells that expressed CD19 (weak), CD20, CD79b (weak), and CD10. In the bone marrow a similar population of abnormal lymphoid cells was identified. Analysis of the cerebrospinal fluid excluded leptomeningeal involvement. Node biopsy was reported as diffuse large B cell lymphoma.

Case 2

A 63 year old female presented with abdominal pain, haematemesis, fevers and sweats. A CT scan showed a very large intra abdominal mass, and The LDH was markedly elevated at 7570 u/L. A biopsy of the gastric mass showed DLBCL. The bone marrow revealed involvement with Burkitt's type cells and the CSF showed no malignant cells.

Both cases have responded to intensive combination chemotherapy and rituximab

We report two patients with aggressive large cell lymphoma at presentation with both the t(14;18) and IGH-cMYC rearrangements. The IGH-cMYC rearrangement was cryptic in one patient and only detected with FISH analysis. This emphasises the importance of cytogenetics for the accurate diagnosis of lymphoma and of augmenting conventional cytogenetics with FISH analysis. Few studies of these two rearrangements in the same malignant cells have been reported in B-cell leukaemia/lymphoma. Most cases reported had a mature B cell phenotype with immature blastic morphology. Prognosis was very poor for this group of patients despite, in many cases, aggressive therapy. One study also found a high incidence of CNS relapse.

The recognition of this dual translocation in aggressive B cell leukaemia/lymphoma is important and may require consideration of novel treatment strategies.

P94
Systemic mastocytosis – a cautionary tale with new diagnostic considerations

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A 55 year old female was referred for haematology assessment with a history of recurrent allergic manifestions during the last 20 years. These symptoms had escalated in severity during the last 12 months, to include anaphylaxis. Peripheral blood counts were normal. Bone marrow aspiration revealed scattered atypical mast cells (2% of nucleated cells), best demonstrated by Toluidine Blue stain. Trephine biopsy showed patchy cellularity with a fibrocellular interstitial lymphoid infiltrate. Clusters of mast cells, with both typical and atypical morphology were demonstrated in association with the lymphoid aggregates (Toluidine blue, Giemsa, CD 117 stains). Serum tryptase was significantly elevated. Molecular genetic analysis demonstrated the c-Kit D816V mutation, and was negative for IgH and TCR gene rearrangements. Despite precautions taken, marrow biopsy was followed by severe, atypical pain at the aspiration site requiring narcotic analgesia, but no systemic allergic phenomena.

Systemic mastocytosis is a rare and clinically elusive disorder that requires a careful approach to diagnosis with respect to procurement of the marrow biopsy, morphological assessment with appropriate cytochemical stains and grading of mast cell atypia, and clinical staging. Molecular diagnosis for the c-Kit D816V mutation may provide additional prognostic information, including the likelihood of response to newer therapies including Imatinib (Glivec). The accurate diagnosis and staging of the disease may in future allow a better evaluation of novel therapeutic interventions.

P95
Autologous haemopoietic stem cell transplantation for severe systemic sclerosis

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Aim

Haemopoietic stem cell transplantation (HSCT) has in recent years been suggested as a therapeutic modality in patients with systemic scleroderma (SSc) who have failed other therapies. We aim to assess the response to autologous HSCT in patients with refractory SSc.

Methods

We have conducted a pilot study of HSCT in SSc pts since 2002. The patients have failed monthly pulse cyclophophosphamide and at least 3 other DMARD's. Mobilisation was with cyclophosphamide 2g/m2, Methylprednisolone 1gm and G-CSF 10 micrograms/kg from day 2 until stem cell collection. Conditioning was with Cyclophosphamide 200mg and ATG 40mg/kg.

Results

Six patients (1M:5F), med age 37 years (range 28-47) have been entered into the program. Median stem cell yield was 20.37 x 10^6 CD34 cells/kg (2.14-38.6 x 10^6/kg). Follow up is now at a median of 27 months (8-47 months) with all patients demonstrating significant improvement including return to work, reductions in skin score, pain visual analogue score (VAS), and HAQ. Two patients have now relapsed, with one patient dying from disease progression (myocarditis) 15 months after transplant. The other patient has had minor worsening of clinical symptoms and autoimmune markers 11 months after transplant. The remaining four have had a sustained clinical response.

Conclusions

HSCT for severe SSc is a safe and promising treatment option in these patients. We plan to enter all future patients into the EBMT trial ASTIS to fully determine the role of HSCT in this disease.

P96
Splenic artery embolization and rituximab for Evans syndrome

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To describe the utilization of splenic artery embolization and Rituximab therapy in an adult Jehovah's Witness patient with Evans syndrome.

Method

A 24 year old female hepatitis B carrier was diagnosed with Evan's syndrome. Investigations confirmed autoimmune hemolytic anaemia with spherocytes, reticulocytosis, increased LDH and positive DAT. Platelet antibodies were positive and bone marrow showed hyperplasia. The anaemia and thrombocytopenia initially responded to high dose steroids. She was later diagnosed with the antiphospholipid antibody syndrome after a pulmonary embolism. This was followed by membranous glomerulonephritis secondary to hepatitis B requiring lamivudine therapy. Investigations for systemic lupus erythematosis were negative. 10 months after the initial diagnosis she relapsed with wet purpura and severe hemolytic anaemia unresponsive to high dose steroids, IVIG (3 doses) and Azathioprine. The patient was unfit for anaesthetic so splenic artery embolization was performed utilizing embospheres followed by embolization coils.

Results

The platelet count responded rapidly normalizing by day 5. The procedure was complicated by infarction of the tail of the pancreas and pneumonia. Methylprednisolone and Rituximab was administered after the Hb continued to fall reaching a nadir of 28g/l with severe dyspnoea and syncopal episodes. The Hb normalized within 2 weeks. Ongoing complications included subphrenic abscess and infected pancreatic pseudocyst (now resolved) and the patient remains in remission with a Hb of 127g/l and platelets of 446 at 12 months.

Conclusion

We describe a case treated successfully with splenic artery embolization and Rituximab therapy for severe symptomatic Evan's syndrome with limited treatment options. Rituximab therapy has been described to be successful in up to 67% of cases with Evan's syndrome and should be considered as an option in patients resistant to first line therapy.

P97
Blood cell morphology in Niemann Pick Type C: A case report

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Background

Niemann Pick Disease Type C (NPC) is an autosomal recessive lysosomal storage disorder due to a defect in cholesterol esterification which leads to the intracellular accumulation of lipids in a variety of tissues. NPC has a wide spectrum of clinical phenotypes, including the rapidly fatal neonatal cholestatic form described in this case. Definitive diagnosis of NPC requires studies on cultured fibroblasts.

Case Report

A 10 week old female infant of Aboriginal descent was transferred to the Royal Children's Hospital in Brisbane for further investigation of cholestatic hepatitis, having initially presented at 6 weeks of age with failure to thrive and jaundice. Examination revealed marked hepatosplenomegaly, however there were no dysmorphic features. Early problems included recurrent hypoglycaemia, coagulopathy, hypersplenism, and hyponatremia secondary to renal salt wasting. Initial investigations were for a primary liver disease however review of a blood film revealed unusual leukocyte vacuolation consistent with a storage disorder. Initial metabolic investigations were normal, including white cell and plasma lysosomal enzymes. A bone marrow aspirate demonstrated significant numbers of foam cells, and electron microscopy of skin showed concentric laminated inclusions. The diagnosis of NPC was ultimately confirmed by cholesterol esterification studies performed on cultured fibroblasts. The infant was managed palliatively, and died at 5 months as a result of progressive organ failure.

Conclusions

Review of a blood film by an experienced morphologist is a useful adjunct to the investigation of a possible storage disorder. The haematologic abnormalities may fluctuate during the clinical course, necessitating serial review where the index of suspicion is high. Bone marrow features are non-specific, but may provide additional supportive evidence.

P98
Cyclophosphamide/dexamethasone as initial treatment in newly diagnosed symptomatic multiple myeloma – a pilot study

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Aim

Historically VAD has been the most common induction regime used in myeloma but the contribution of the Vincristine and Doxorubicin in tumour burden reduction has not been clear. It is a relatively complicated therapy to administer and requires either an indwelling catheter for the continuous infusion or daily visits for four days to administer it with the shorter IV infusion regime. We wanted to have a simple, effective regimen that reduced toxicity and resulted in adequate harvests for high dose therapy and stem cell transplantation.

Methods

From 14 patients (8 males, 6 females) under the age of 69, median age 55 with newly diagnosed symptomatic myeloma received three cycles of Cy/Dex (Cyclophosphamide 100mg/m^2 day 1 and Dexamethasone 40mg days 1 – 4 and 9 – 12 as initial treatment. A bone marrow specimen was taken prior to therapy and also after the completion of three cycles of Cyclo/Dex. The patients then received Cyclophosphamide 1gm/m^2 IV plus Filgrastim SC as mobilisation therapy followed by stem cell harvest and subsequent high dose Melphalan 200mg/m^2 followed by autologous stem cell infusion (ASCT).

Results

All patients had bone marrow specimens submitted for cytogenetic testing by FISH using an extensive cocktail of probes.

All 14 patients had responsive disease with post therapy plasma cell samples decreasing to under 10%. Two of the patients (both over the age of 65, and one with prior back DXT) failed to mobilise. The rest mobilised very well with average CD34 dose of 8.2 x 10^6/kg so that enough stem cells were collected for a potential tandem transplant. Cytogenetic abnormalities were detected in 50% of the patients including two patients with t4;14 translocations. Toxicity was acceptable apart from the most elderly man, aged 69, who had several periods of neutropenia and an episode of herpetic oesophagitis. All transplants were relatively trouble free and mortality was 0%.

Conclusions

This pilot study did not identify any difference in the proportion of patients compared to conventional VAD who actually went on to receive ASCT. The response rate in terms of plasma cell reduction was excellent. We conclude that Cy/Dex is an effective alternative to VAD and does not require any need for hospitalisation and central venous access for continuous infusion. It also has the potential of being able to be combined with Thalidomide to produce even better results in induction therapy.

P99
Use of quantitative real-time PCR to monitor AML with t(8;21)(q22;q22): A case report

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Aim

Acute myeloid leukaemia (AML) with t(8;21) is generally associated with good prognosis but with a high risk of relapse. It is characterised by the AML1-ETO fusion gene, an ideal molecular target for monitoring disease. The role of residual disease detection has been controversial, with some long-term remission patients demonstrating persistent PCR positivity. Quantitative PCR (Q-PCR) now potentially

offers superior disease monitoring, as serial results can be more easily compared. Increasing Q-PCR results are predictive of emerging relapse and can be used as the basis for therapeutic intervention. However there is still contention about the ideal time-point and sample type for the most predictive monitoring. We report the successful application of Q-PCR to monitor AML1-ETO levels in an AML-M2 patient resulting in improved clinical management, and suggest a strategy for the molecular monitoring of this disease.

Method

Q-PCR using Taqman technology on the Applied Biosystem 7700 Sequence Detection System and qualitative reverse transcription nested PCR were used to monitor AML1-ETO levels.

Results

Qualitative and quantitative PCR results were concordant for the detection of residual disease, however Q-PCR provided the most informative results for prediction of relapse and precise modulation of therapy. Interestingly Q-PCR at molecular relapse showed higher disease levels in blood than marrow. PET scan revealed chest wall chloroma relapse with no evidence of bone marrow disease.

Conclusion

Q-PCR to monitor AML1-ETO levels in t(8;21) positive AML provides superior disease monitoring as a progressive increase in transcript numbers is more easily interpreted than non quantitative techniques. Peripheral blood offers a less invasive alternative for more regular monitoring with improved prediction of relapse, including extramedullary relapse.

P100
Ringed sideroblasts and thrombocytosis – a new provisional entity of a myelodysplastic/myeloproliferative overlap disorder

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Ringed sideroblasts with thrombocytosis (RST) is a provisional entity in the 2001 World Health Organisation classification scheme (MDS/MPD, unclassifiable). It is an uncommon overlap syndrome that shows features of both essential thrombocythemia and refractory anaemia with ringed sideroblasts.

Aim

To present a case study of a patient who fulfilled the criteria for RST and, for educational purposes, discuss the clinical features and prognosis of this new diagnostic category.

P101
Accelerated administration of rituximab – a pilot study

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Project Aim

Implement a pilot programme of accelerated Rituximab Administration to reduce treatment time, drug wastage and improve patient satisfaction

Background

Ritiximab (Mabthera) is a monoclonal antibody indicated in the treatment of a number of types of Non-Hodgkins lymphomas. It is being used increasingly and is traditionally administered slowly and incrementally due to reported reactions particularly related to cytokine response. The cytokine response, however, is most problematical during the initial exposure to the drug and in the presence of high tumour burden. Patients undergoing their second or subsequent infusion who have no history of cardiac or pulmonary co-morbidities and who did not react to their initial dose appear to be able to tolerate a faster infusion time. There have been a number of presentations at oncology and haematology conferences where rituximab has been administered at an accelerated rate with no adverse effects. In addition the BCCA protocol utilises the accelerated rate of administration.

This increased rate of administration may reduce the patient's stay in the day care facility by up to half. In addition, all rituximab infusions have been bought in reconstituted from a pharmaceutical company. There were two issues with this;

a)

The order is made the day prior to treatment and the order may be cancelled due to an error in booking or a change in the patient's condition thus wasting the reconstituted drug ($2,710 per 700mg dose)

b)

The reconstituted rituximab is supplied in 1,000mls of Sodium Chloride for all patients regardless of fluid requirements/restrictions

Figures from the financial year ending June 2005 demonstrated that $20,000 were due to waste from cancelled Rituximab infusions. This is from inpatient records, outpatient records with regard to wastage are not obtainable.

It was decided that as part of the project, nurses would reconstitute the rituximab in 500mls of Sodium Chloride after administering the premedication, thus potentially eradicating waste of the drug. rituximab is a monoclonal antibody and not a chemotherapy drug and therefore was not a hazard in this regard for nurse reconstitution. The project nurses were given update training in aseptic technique and drug reconstitution and given an undisturbed area in a clean room to do this.

Methodology
This was a prospective project in which 20 patients were administered rituximab at an accelerated rate in keeping with the BCCA protocol and the pilot protocol undertaken at Brisbane and presented at HSANZ 05.

Patients undergoing their second or subsequent infusion who had no history of cardiac or pulmonary co-morbidities, who did not react to their initial dose and whose consultant was in agreement were prescribed at the accelerated rate. A total of 36 infusions (range 1-3, mean 1.6, median 2.5) were given to 20 patients. All patients were given a premedication (BCCA protocol) of Panadol 1 Gm, Phenergan 12.5mg and Hydrocortisone 100mg 1 hour prior to administration.

Patients were assessed for a cardiac or pulmonary history and any reaction to their initial rituximab infusion. Vital signs of Temperature, pulse, respirations, Blood Pressure and Oxygen saturations were taken prior to the accelerated infusion (baseline) and at +15, +30, +60, +90 minutes or at completion of the infusion.

A protocol and observation chart were drawn up .The 500mls infusion was administered as follows:

200mls/hour for 50mls, 300mls/hr for 170mls and 600mls/hr for 300mls.

Results
A total of 36 infusions were administered at the accelerated rate to 20 patients. 32 infusions were of 700mgs in 500mls of Sodium Chloride 0.9%, 2 of 600mg in 500mls, all of which were reconstituted immediately prior to infusion by the nursing staff taking 5-10 minutes each. 2 infusions were of 700mg in 1,000mls Sodium Chloride which were bought in reconstituted from an outside supplier.

Of these, 34 infusions, were administered with no reaction, one patient had a fever of 38 degrees 15 minutes after commencing but was neutropenic and subsequently found to have a positive blood culture result. One patient became tachypnoeic and febrile 15 minutes into the infusion and the rituximab was continued at the same administration rate with no further reaction.

Patients were asked to comment with regard to satisfaction.

19 surveys were returned. 12 patients reported that the shorter treatment time made the treatment ‘easier’ and 7 reported ‘the same’.

16 patients preferred receiving their Rituximab over a shorter time period and 3 didn’t mind.

18 patients reported that their visit to Liverpool CTC overall was shorter than for their first visit for Rituximab treatment and 1 reported ‘don’t know’

16 patients expressed a desire for a morning appointment and 3 preferred the option of treatment at midday.

Outcome
This appears to be a viable option for increased patient satisfaction, better patient flow and waste reduction.

The rituximab policy for administration has been revised and Haematologists, nursing and Pharmacy are in agreement that this is a valuable change in practice.

Next Steps
Simplify the administration rate in keeping with other protocols to 200mls per hour for 30 minutes and then 400mls per hour for 60 minutes (for a 500mls infusion)

Assess the requirement for hydrocortisone in the premedication

P102
Analysis of culture positive stem cell product

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Aim
To identify the incidence of culture positive stem cell product (CPSCP) in patients undergoing autologous peripheral blood stem cell transplant (APBSCT) and to correlate it with transplant outcome.

Methods

A review of 194 APBSCT performed at our institution from January 2000 to June 2006 was conducted. Samples from each product were subjected to aerobic and anaerobic microbiological culture before cryopreservation. An attempt was made to look for any impact CPSCP had on transplant outcome. Patient age, harvested CD34 yield, infused CD34 cell dose and the time to neutrophil engraftment were assessed. Groups who received the CPSCP (Group A, n =7) and the control group who received sterile product (Group B, n= 189) were compared using the student 't' test.

Results

We had 10 patients who had evidence of CPSCP. One of these was identified as false positive, giving an incidence of 4.6%. The most common organism identified was coagulase negative staphylococcus (6). The others included Bacillus (1), Staphylococcus aureus (1), and Diptheroids (1).

Seven out of these 9 patients received their CPSCP back after conditioning chemotherapy was administered. The study parameters were compared between groups (See table). There were no significant differences identified.

	Age(Median)	**Harvested CD34 count (x 10^6/kg body weight)**	**Infused CD34 count (x 10^6/kg body weight)**	**Time to Neutrophil engraftment (days)**
Group A(N= 7)	53.8	9.74	6.42	10.0
Group B(n=189)	53.6	10.34	5.95	10.8
P value	0.9	0.999	0.655	0.44

Two patients had evidence of infection with the same organism before the stem cell harvest was commenced. One of them had the same organism cultured after the harvest was infused. Four patients received prophylactic vancomycin during infusion of CPSCP. There was no transplant-related mortality in any of the patients who were infused with CPSCP.

Conclusion

Identification of CPSCP does not negatively impact on the immediate transplant course in-patients undergoing APBSCT. The precise implication of reinfusing CPSCP needs to be validated in larger studies.

P103

Detection of c-KIT exon 8 mutations in core-binding factor-AML patients using high resolution melting analysis

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Aim

Exon 8 c-KIT gain-of-function mutations have been detected in approximately 20%-30% of patients with "core-binding factor" (CBF) AML, perhaps more frequently in those with inv(16)(p13q22) than t(8;21)(q22;q22). Patients with c-kit mutations appear to have an inferior prognosis, and in-vitro and in-vivo studies of cells harbouring exon 8 deletions have shown response to imatinib mesylate. To facilitate identification of patients potentially suitable for clinical studies, a high-resolution melting assay (HRM) was developed for exon 8 mutations in CBF-AML patients.

Methodology

DNA was prepared from archival bone marrow slides of AML patients with inv(16)(p13q22) proven on cytogenetics. HRM was performed on the Corbett Rotorgene 6000 using SYTO 9 as the intercalating dye. Sequencing on an ABI3100 was also performed on all samples.

Results

Using HRM, 1 of the 6 patients studied to date showed a variant melting profile. Direct sequencing demonstrated a novel exon 8 deletion. The other 5 patients showed a normal exon 8 sequence. The mutation positive patient presented with skin and breast chloromas, with

bone marrow biopsy diagnosing AML M4Eo. Remission was attained with ICE induction; and maintained for 13 months. The patient underwent an HLA-identical allograft at relapse with remission for a further 6 months until progression with extramedullary skin and breast disease.

Conclusion

High resolution melting is an emerging technology which promises to revolutionise the assessment of clinical samples for both recurrent and novel mutations without the requirement for knowledge of the precise sequence or location of the mutation prior to testing. HRM is capable of detecting mutations which are present in as few as 5% of cells making this methodology very versatile for handling diverse clinical samples. Our preliminary c-kit mutation testing is concordant with CBF-AML literature. Further testing for D816V mutations will be undertaken given negative prognostic implications of these mutations in patients with CBF AML.

P104
The MAXIMA study

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Five randomized trials (four Phase III and one phase II) have confirmed that rituximab maintenance therapy provides clinically meaningful improvements in terms of Progression Free Survival, Event Free Survival and response duration for patients. Two studies have also found an overall survival advantage for rituximab maintenance therapy (Hoechster *et al.* 2005, van Oers *et al.* 2005) and a third study could demonstrate a strong trend towards overall survival advantage (Dreyling *et al.* 2006). A Cochrane meta-analysis of several randomised Phase III trials (Schulz *et al.* 2005) demonstrated that rituximab plus-chemotherapy for first-line treatment of Follicular Lymphoma is superior to chemotherapy alone and significantly prolongs overall survival.

To further broaden the available basis for maintenance treatment in the first-line and relapsed setting, the MAXIMA (MAintenance rituXImab in Follicular LymphoMA) trial has been initiated in August 2006 and will last 5 years. Patients with first line or relapsed/refractory advanced Follicular Lymphoma are included in this trial. In total 500 patients are planned for this international trial running in 23 countries.

Patients who achieve a Complete Remission, Complete Remission unconfirmed or Partial Remission after rituximab containing induction therapy (rituximab with or without chemotherapy) are eligible to enter the study to receive rituximab maintenance therapy administered at the standard dose of 375 mg/m^2 every 2 months for 2 years. This regimen is also investigated in the ongoing PRIMA study, and also in an ongoing SAKK study which investigates the benefit of rituximab maintenance therapy for up to five years.

The previous five randomized trials did not detect significant safety issues for rituximab maintenance therapy. The main objective of the MAXIMA trial is to confirm this safety data in a wider patient population.

Secondary objectives of the study include standard time dependent parameters (PFS, EF, OS). In addition, the effect of rituximab maintenance therapy on improving response quality (PR =>CR) after induction therapy will be evaluated.

P105
Overwhelming post-splenectomy infection: How well are we managing?

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Aim

Overwhelming Post-Splenectomy Infection (OPSI) is a potentially disastrous complication following splenectomy. This audit assessed the adequacy of current management and education in this group of patients.

Methods

Patients who underwent splenectomy between 1990 and 2001 in Christchurch were identified. A questionnaire was sent to the GPs whose patients remained alive in 2002. A separate questionnaire was sent out to contactable patients in 2006. Cause of admission and mortality were assessed from the National Inpatient Coding system and the National Mortality Dataset. Hospital notes were reviewed.

Results

All patients received vaccinations. 117 of the 247 patients identified had deceased by 2006. Of those who were not already at an end stage of their disease, only 1 patient was identified as having a bacterial infection leading to death. 75 out of 104 contactable patients participated in this study (response = 72.8%). Of these 75 patients, 5 were admitted with a bacterial infection, and only one required an admission to ICU. All were promptly seen and given antibiotics on arrival at hospital (median 1 and 1.5 hr, respectively). 72% of the 72 GPs who replied stated they had received no information about the patients' spleen status at discharge from hospital, while more than 50% felt they had not enough information for the management for such patients. 53.5% GPs stated that they had given follow-up education about OPSI to their patients, but only 39% of the patients believed that they had received ongoing education.

Conclusion

There was a vaccination rate of 100% and the incidence of OPSI is similar to that of other published studies. However there was poor communication between the hospital and GPs. The rate of ongoing education for patients was low during the study period. To address these issues a prospective splenectomy database is currently being developed.

P106
Acute spontaneous tumour lysis syndrome in a patient with Burkitt's lymphoma

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Aim

We describe the management and outcome of a young woman with a pelvic mass subsequently found to be Burkitt's lymphoma who presented with acute spontaneous tumour lysis syndrome, focusing primarily on outcomes of acute renal failure.

Case Report

A 32-year old woman admitted to our institution with abdominal pain and a pelvic mass underwent surgical debulking of what was subsequently found to be Burkitt's lymphoma. Spontaneous tumour lysis syndrome occurred intra-operatively with consequent hyperuricaemia, hyperkalaemia, hyperphosphataemia, metabolic acidosis and anuric acute renal failure. Therapy consisting of intravenous hydration, alkalinization, haemodialysis and rasburicase was commenced and renal function returned to normal within three weeks of presentation. A peak serum uric acid of 1.46 mmol/L was measured immediately after surgery with a reading of <0.03 mmol/L on the third post-operative day, after the second dose of rasburicase was administered. The use of these treatments permitted the early administration of appropriate full dose chemotherapy (CODOX-M/IVAC) and the patient remains in remission some nine months after diagnosis.

Conclusion

Effective management of tumour lysis syndrome, including rasburicase can achieve excellent outcomes in terms of normalization of renal function. Patients presenting with a bulky tumour require metabolic screening for tumour lysis syndrome and appropriate preparatory measures prior to surgery and interventions.

P107
Acute bilineal leukaemia: Review of the literature and presentation of a case

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Aim

Acute bilineal leukaemia is a subtype of "acute leukaemia of ambiguous lineage" with co-existing separate myeloid and lymphoid blast populations. We report a case of acute bilineal leukaemia with an unusual presentation highlighting the diagnostic and therapeutic difficulties in this uncommon disorder.

Method

The clinical course and pathological findings of the patient were reviewed retrospectively. Flow cytometric data was acquired on a BD FACSCalibur using 3- and 4-colour monoclonal antibody combinations. Cytogenetics was performed using standard culture and metaphase analysis techniques.

Results

A 24-year old male presented for a punch biopsy of right cheek lesion with histology demonstrating a basal cell carcinoma. 20% of cells were P53 positive on immunohistochemistry. He developed generalised facial purpura post-biopsy. Subsequent investigation revealed pancytopenia, 90% blasts on bone marrow aspirate. These were P53 negative on immunostaining. Flow cytometry demonstrated a precursor T acute lymphoblastic leukaemia population situated in the typical blast region on CD45 versus side scatter analysis and a second blast population in the intermediate myeloid cell region. Cytogenetics revealed one major clone with complex karyotypic abnormalities including monosomy 17. These features were not lineage specific. The patient received ICE induction chemotherapy plus vincristine. Residual T-ALL was demonstrated at reassessment with clearance of the myeloid blasts. The T-ALL clone remained resistant to re-induction with Hyper-CVAD (B cycle) with clonal evolution demonstrated on cytogenetics. Remission was never achieved and the patient passed away 5 months following diagnosis.

Conclusion

This case highlights an unusual presentation of skin cancer in a young adult, with concomitant acute bilineal leukaemia. The karyotypic abnormalities raise the possibility of P53 in the pathogenesis in this case. Response to treatment was poor confirming previous literature reports of this aggressive neoplasm. New therapeutic approaches are indicated, ideally under a collaborative setting.

P108
Acute leukaemia of ambiguous lineage: The PeterMac experience

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Background

Acute leukaemia of ambiguous lineage is characterised by either absence or co-expression of lymphoid and myeloid markers. Biphenotypic and bilineal sub-groups are defined by the co-expression of different lineage markers on single or separate blast populations respectively. These rare leukaemias are associated with a poor prognosis with optimal therapy yet to be defined.

Aim

To audit the laboratory and clinical features of acute biphenotypic and bilineal leukaemia.

Methods

Cases of acute biphenotypic and bilineal leukaemia presenting to PeterMac between January 1998 and June 2006 were identified and assessed in terms of clinical characteristics, immunophenotype, karyotype, and remission-duration.

Results

One case of bilineal and eight cases of biphenotypic acute leukaemia presented during the audit period. This represented 3.4% of 261 patients presenting with acute leukaemia (200 myeloid and 52 lymphoid). The age of patients ranged from 25 to 83 (median 49) with 7 males and 2 females. All cases expressed a myeloid phenotype along with either B- (five) or T- (four) lineage markers. Five presented de novo, two were therapy related including one biphenotypic case diagnosed at relapse after initially achieving CR for pre-B-ALL, one transformed from CMML and one transformed from CML following a major molecular response (>3 log reduction in bcr-abl) to imatinib mesylate. Six of these patients demonstrated complex karyotype. Four patients achieved complete remission with a duration ranging from 3-13 months (median 11 months). Five patients died within six months following 'hybrid' therapy, two within thirteen months, one at 30 months while one patient remains alive at 25 months post HLA-mismatched allograft.

Discussion/Conclusion

These cases of acute biphenotypic and bilineal leukaemia support the published literature indicating that such leukaemias are rare and associated with a complex karyotype and poor prognosis. The low incidence of this disease represents a major impediment to research into the basic biology and optimal management.

P109
Raised Hb F investigation in a haemoglobinopathy screening program

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Aim

We carried out a retrospective study of patients with a raised Hb F to gain an understanding into its incidence and the common causes in which it is seen.

Method

The Haematology Department of Monash Medical Centre in Victoria performs Hb F estimation as part of its screening program for haemoglobinopathy testing. This is done on the Bio-Rad Variant HPLC system. The population tested included a broad ethnic and age group with various clinical conditions with a major component of antenatal patients. Raised Hb F is defined as >1% after the age of 6 months. From Jan 2004 to May 2006, 3800 patients were tested of which 439 patients had raised Hb F.

Results

Raised Hb F was seen in 165 adults (>12 years of age) with beta thalassaemia trait and a median Hb F of 1.7 % (range 1.1 to 26.8). Two of these patients had unusually high Hb F of 19.4% and 26.5%, they are currently undergoing extensive DNA testing and no defect has yet been identified in either the beta or the gamma globin genes. 75 adults were heterozygous for haemoglobin E, homozygous for Hb E and other structural Hb variants and had a median Hb F of 1.5% (range 1.1-7.8), 3.8% (range 1.7-9.0) and 2.9% (range 1.1-5.0) respectively. 39 female adults had a median Hb F of 1.3% (range 1.1-4.3) that could be attributed to pregnancy. 8 adults with probable delta beta thalassaemia trait/ HPFH had a median Hb F of 10.9% (range 6.4-30.6) while the remaining 60 adults had a median Hb F of 1.5% (range 1.1-5.4) for no obvious reason. The 102 non adults (<12 years of age) were divided into 2 groups depending on whether there was a beta globin gene abnormality or not. 25 patients with a beta globin chain abnormality aged 6-12 months and 1-2 years had a much higher median Hb F values (21.7% and 7.5% respectively) than the 46 patients that had normal red cell indices (median Hb F of 2.0% range 1.1-10.3) who may have been considered normal. Repeat testing of this young group would be of interest to verify the Hb F levels.

Conclusion

This study has enabled us to gain a better understanding of raised Hb F in the population studied, and in some cases we have not been able to identify a cause of the raised Hb F.

P110
Clinical experience with palifermin in peripheral blood stem cell transplantations

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Introduction

Oral mucositis, a debilitating side effect of high dose chemotherapy, is a significant clinical problem involving pain, the need for parenteral narcotics, total parenteral nutrition (TPN), an increased risk of infection and prolonged hospitalisation. From March 2005 until Kepivance™ (palifermin) gained marketing approval, all patients scheduled for a peripheral blood stem cell transplantation (PBSCT) at The Wesley Hospital were offered palifermin under the Special Access Scheme.

Aim

A clinical audit was conducted to compare the clinical events in patients undergoing PBSCT in those who received palifermin under the SAS scheme with historical controls who received standard medical therapy.

Methods

We considered 6 events in the analysis: days of neutropenia <1.5 (x109/l); temperature >38ºC; antibiotic use; TPN; morphine use and mucositis.

Results

A total of 112 transplants (89% autologous, 11% allogeneic) were undertaken in 107 patients in the period studied. Palifermin was given in 50% of the transplants.

The analyses indicated that the palifermin treated patients had significantly fewer days of morphine use or mucositis than those patients who did not receive palifermin. These differences persisted after adjustment for age, gender and disease. Of the patients with lymphoma, 25% of those treated with palifermin had some days of mucositis vs 50% who did not receive palifermin. The corresponding figures for patients with multiple myeloma were 46% vs 78%.

Conclusions

These results show that a significantly smaller proportion of patients required morphine and reported mucositis during treatment with palifermin. Analysis of the 112 consecutive transplants in a busy autograft unit without selection bias suggests that high risk groups can be identified that are likely to benefit from palifermin in terms of mucositis and pain. If therapy moves to a day-care based programme, significant bed day reductions could be made.

P111
Changes in transcription factor expression in malignant plasma cells following therapy

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Introduction

The differentiation of B-lymphocytes to plasma cells is regulated by the interaction of transcription factors in a lineage- and stage-specific process. As de-regulation of these transcription factors (e.g. Pax 5, Blimp-1 Oct-2) has been implicated in the pathogenesis of multiple myeloma, we hypothesized that the changes in the expression of these transcription factors may provide some insights into the patients' course and outcome.

Aim

To determine if the changes in the expression of the transcription factors in MM patients at diagnosis and following therapy (i) correlate with the response to treatment, and (ii) can predict patients' prognosis.

Method

Bone marrow (BM) aspirates were collected with informed consent from MM patients: at diagnosis (n=12), post-chemotherapy follow-up (8/12), at relapse (2/12), post-transplant follow-up (1/12), and transformation to plasma cell leukaemia (PCL) (1/12). CD138+ plasma cells were isolated from BM mononuclear cells followed by RNA extraction and reverse-transcription (RT)-PCR or RT-real-time PCR.

Result

RT-PCR results from 6/8 patients post-chemotherapy with good response to treatment showed re-expression &/or stronger expression of the full-length Pax 5 gene compared to that at diagnosis. Re-expression of full-length Pax 5 was also observed in the patient who received autologous stem cell transplant although it was undetectable in the post-chemotherapy sample. The presence of Pax 5 isoform with exon 9 deletion was observed in the two patients who had refractory disease and in the one whose disease had transformed to PCL. Both Blimp-1 and Oct-2 expressions were reduced in all patients following therapy (8/8 post-chemotherapy and post-transplant) but increased as disease progressed (2/2 refractory and transformation to PCL).

Conclusion

Our preliminary results suggest that sequential study of the expression of transcription factors can be used to monitor disease progression and to assess prognosis in MM patients.

P112
CNS relapse of myeloma following autologous stem cell transplant: Case presentation and review of the literature

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A 55 year old man was diagnosed with IgD lambda myeloma after presentation in November 2004 with spinal cord compression. Staging revealed multiple paravertebral masses and a sphenoid bone plasmacytoma (but no lytic bone lesions), mild anaemia, renal impairment (Cr 123umol/L) albumin 47g/L and B2M 2.7mg/L. Marrow analysis revealed 30% plasma cells with plasmablastic features, and no karyotypic abnormality. Initial treatment comprised spinal irradiation and systemic chemotherapy with oral cyclophosphamide,idarubicin and dexamethasone (CID regimen). Peripheral blood stem cells (8.2x10^9CD34/kg) were collected with cyclophosphamide mobilisation and infused after melphalan conditioning in June 2005. Eight months after transplantation the patient presented with symptoms of raised intracranial pressure and MRI revealed two intracerebral plasmacytomas. Biopsy of one of the lesions was consistent with plasmacytoma. Treatment with cranial irradiation, and systemic therapy with thalidomide and dexamethasone lead to clinical and radiological resolution, however the patient suffered leptomeningeal relapse four months later requiring intrathecal chemotherapy.

While spinal cord and nerve root compression are common complications of myeloma, CNS manifestations are rare, particularly post autologous SCT. Risk factors for CNS disease appear to be extension from contiguous cranial lesions, IgA and IgD subtypes, plasma cell leukaemia, and deletion of chromosome 13. CNS disease carries an extremely poor prognosis. Therapy with local radiotherapy and intrathecal chemotherapy has limited efficacy. Thalidomide has been used only in isolated cases of CNS disease and newer agents have not been studied in this context. The only two longer term survivors underwent allogeneic transplantation following CNS relapse.