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

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Aims

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

Methods

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

Results

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

Conclusion

The expression of chromatin remodelling enzymes, VDR, and survivin does not play a major role in augmented cytotoxic responses to combinations of DNA-damaging agents and HDACi's in HL-60 cells but may involve BCL6 upregulation via HDACi-induced p21 expression.

No conflict of interest to disclose

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Rapid Isolation of DNA Breakpoints in Leukaemia by Bottleneck PCR

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Aim

To isolate DNA breakpoints in chronic myeloid leukaemia (CML) and acute promyelocytic leukaemia (APML), as a first step for monitoring MRD using DNA

Methods

PCR primers were designed to cover the breakpoint site in CML and APML. For CML 6 forward primers covered a 3kb BCR and 282 reverse primers covered a 140kb ABL region, while for APML 6 forward primers covered a 3kb PML and 34 reverse primers covered 16kb RARa region. Primers were positioned approximately 500bp apart to minimise product size and ensure efficient amplification. Patient DNA from diagnosis was amplified in six PCRs, each containing one of the forward primers and all of the reverse primers. First round products were diluted and subjected to 2-4 rounds of a novel PCR technique we have termed bottleneck PCR. Due to the high number of individual primers in a reaction, the amplification of non-specific products, primarily reverse to reverse, is inevitable. The principle of bottleneck PCR is to use tagged reverse primers and adjust conditions of the PCR so as to ensure that the reverse primer or primers hybridises inefficiently. This modification results in selection against amplification of products resulting from reverse-reverse priming. Following several rounds of bottleneck, products were resolved by gel electrophoresis, with patient specific products sequenced to identify breaks.

Results

To date, breakpoints have been isolated and sequenced in 28/29 (97%) CML patients and in 2/2 APML patients. Further studies are ongoing.

Conclusion

The combination of multiplex and bottleneck PCR is a simple and a rapid strategy for sequencing the breakpoint in CML and APML, and may have application for other translocations. Sequence data from patients have been used to establish a DNA based quantitative PCR system to measure the level of MRD in CML patients.

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

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Dipeptidyl Peptidase Expression in Chronic Lymphocytic Leukaemia (B-CLL)

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Aim

The dipeptidyl peptidases (DPs) DP1V/CD26, DP8, DP9 and fibroblast activation protein (FAP) are a related group of serine proteases. Non-selective DP inhibition with ValboroPro/Talabostat is reported to cause apoptotic cell death in indolent CLL *in vitro*. Hence the aims were to:

1. Characterise mRNA and protein expression of known enzymes with DP activity in B-CLL lymphocytes
2. Compare DP expression to prognostic markers in B-CLL
3. Determine whether the DP1V/8/9 inhibitor p32/98 causes apoptosis in B-CLL and normal lymphocytes

Method

DP expression was assessed in sorted CD5⁺/CD19⁺ B-CLL cells by real-time qRT-PCR or by flow cytometry. Prognostic potential was assessed by comparing DP expression to IgVH mutational status, CD38, ZAP-70 expression and clinical features.

Results

DP8, DP9, DP11 and PEP (prolyl endopeptidase) were constitutively expressed in B-CLL. DP8 expression was significantly higher in B-CLL, than any other DP. Contrary to published data, CD26 expression was only detected in 4/40 patients. FAP mRNA and protein were not expressed. DP expression did not correlate with any prognostic or clinical indicators. Selective inhibition of DP activity by p32/98 at 100µM final did not cause apoptosis of B-CLL or normal lymphocytes.

Conclusion

Total rather than selective DP inhibition may be required to promote cell death. Due to the broad expression of DPs *in vivo*, this is unlikely to be tumour specific. CD26 expression is infrequent in CLL and not prognostically linked. The expression of DP8 in normal CD5⁺/CD19⁺ B-lymphocytes is currently being investigated for the significance of DP8 expression in B-CLL.

No conflicts of interest to disclose

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The BH3 Mimetic Compound ABT-737, Synergises with Standard Chemotherapy to Induce Apoptosis in Chronic Lymphocytic Leukaemia (CLL)

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Aim

Overexpression of Bcl-2 is universal in CLL and is associated with chemo-resistance. Targeting Bcl-2 with compounds that mimic its physiological antagonists (i.e., the BH3-only proteins) may have a role in treatment of CLL. ABT-737 is one such BH3 mimetic that shows potent *in vitro* cytotoxicity in CLL samples. We evaluated the relationship of established clinical prognostic factors and individual Bcl-2 family protein levels on *in vitro* sensitivity to ABT-737 in primary CLL cells. Additionally, synergistic combinations of ABT-737 with other anti-leukaemia agents were identified.

Methods

Circulating CLL cells from 30 patients were assessed *ex vivo* for sensitivity to ABT-737 alone or in combination with cytotoxic drugs. Cell lysates were analysed and quantified by Western blot for the level of expression of Bcl-2 family proteins. These data were correlated with the single agent and combination results.

Results

ABT-737 is efficacious ($LC_{50} < 100\text{nM}$) as a single agent in most (21/30) primary CLL samples, independent of response to prior therapy or prognostic markers (CD38, p53 status). Some CLL samples are sensitized by standard cytotoxic agents (dexamethasone, etoposide, fludarabine and mafosfamide) to killing by ABT-737. The synergistic response was not predicted by response to either ABT-737 or the cytotoxic drug as a single agent. Surprisingly, there was no direct correlation between the levels of Bcl-2 family proteins and cytotoxicity LC_{50} .

Conclusion

We have identified that a minority of CLL samples are relatively insensitive *in vitro* to Bcl-2 antagonism by ABT-737 as a single agent, but these are not readily predictable based on clinical features. Combination with a second anti-leukaemia agent may result in synergistic killing. Clinical trials are in progress with an orally bioavailable BH3 mimetic, ABT-263, that has a similar spectrum of activity and these results suggest a potential role for future combination therapy in this disease.

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Combining RAD001 (Everolimus) with Bortezomib Induces Synergistic Killing in Precursor-B Acute Lymphoblastic Leukemia

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Five year survival for patients with relapsed pre-B ALL is less than 10%, requiring novel approaches to therapy. Bortezomib has been shown to enhance chemosensitivity in pre-B ALL and demonstrates efficacy in relapsed disease. We hypothesized that RAD001 would enhance the sensitivity of the pre-B ALL cells to proteasome inhibition.

Combining RAD001 ($>2\mu\text{M}$) with Bortezomib (10nM) in vitro significantly ($p<0.05$) enhanced pre-B ALL cell kill, greater than the additive effect of individual therapies. Intracellular flow cytometry demonstrated up regulation of, bax, bim, puma and cleaved caspase 3, in the absence of a p53 response. This data indicates that enhanced killing is independent of p53.

We observed bortezomib to be a potent activator of caspase 8 and sought to determine if RAD001 induced synergy through up regulation of death receptor expression. We saw no increase in surface expression of DR4 or DR5 by RAD001, Bortezomib, or the combination of both agents.

Bortezomib is reported to induce apoptosis at G2/M via down regulation of NF κ B and BCL2. We sought to examine the cell cycle impact of combining RAD001 with Bortezomib. We observed Bortezomib alone and Bortezomib in combination with RAD001 ($>2\mu\text{M}$) resulted in an increased proportion of pre-B ALL cells in S phase and G2/M, relative to control. We hypothesize that combining RAD001 with Bortezomib enhances apoptosis signal induction at G2/M, providing a rationale for synergy.

Combining RAD001 with Bortezomib induces synergistic killing in pre-B ALL, independent of p53. Enhanced killing is associated with caspase activation and cell cycle accumulation in G2/M. We believe our data indicates combining RAD001 with Bortezomib has the potential to enhance responses for patients with pre-B ALL.

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Novartis supply of RAD001 for research use.
Novartis had no role in analyzing the data or preparing the abstract

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Dose Escalated RAD001 (Everolimus) Enhances Chemosensitivity in Precursor-B Acute Lymphoblastic Leukemia, through a JNK Dependent Impairment of Cell Cycle Arrest, in Response to DNA Damage or Microtubule Disruption

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Five year survival for patients with relapsed pre-B ALL remains dismal, requiring novel approaches to therapy. We evaluated the potential of mTOR inhibitor RAD001 to enhance chemosensitivity in pre-B ALL.

Combining 16 μ M RAD001 with DNA damage or vincristine in vitro, induced caspase-dependent synergistic killing ($p < 0.05$) of pre-B ALL cells. We observed 16 μ M RAD001 suppressed p53, markedly attenuating p21 responses. Lentiviral siRNA knock down of p53 in Nalm6 cells significantly increased ($p < 0.05$) cell death by vincristine relative to luciferase knockdown cells with an intact p53 response, indicating suppression of p53 enhances chemosensitivity.

Intracellular flow cytometry revealed combining 16 μ M RAD001 with DNA damage or vincristine activated the JNK pathway and c-Jun. c-Jun is reported to suppress the p53 and p21 promoters and prolong the half-life of p53 analogue, p73. Concordantly, we observed up regulation of p73, puma, bax, bim and cleaved caspase 3, indicating a p53 independent pathway to cell death.

We hypothesized that 16 μ M RAD001 enhances chemosensitivity through altered cell cycle regulation. 1.5 μ M RAD001 inhibited pRb, Ki67 and PCNA expression, increasing G0/1 cell cycle arrest in response to DNA damage or vincristine. In contrast, 16 μ M RAD001 increased pRb, cyclin D1, Ki67, CDC2 and PCNA expression. Enhanced DNA content, BrdU uptake and PCNA expression indicate cell cycle progression in response to DNA damage or vincristine when combined with 16 μ M RAD001. We observed JNK inhibition reduced PCNA expression at G0/1 and G2 in pre-B ALL cells exposed to DNA damage and G2/M with vincristine, indicating impairment of cell cycle arrest by 16 μ M RAD001 is JNK dependent.

RAD001 (16 μ M) enhances chemosensitivity independent of p53, associated with JNK dependent impairment of cell cycle arrest, in response to DNA damage or microtubule disruption. Our data indicates dose escalated RAD001 has the potential to enhance chemosensitivity for patients with pre-B ALL.

Acknowledgements

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Novartis had no role in analyzing the data or preparing the abstract

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A New Generation IAP Inhibitor (IAPi) Induces Apoptosis of Human Myeloma Cells and Synergises with Conventional and Novel Anti-Myeloma Therapeutics

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Aim

To evaluate a novel IAP (Inhibitor of Apoptosis Protein) inhibitor (IAPi) as a potential therapeutic for multiple myeloma (MM)

Methods

Dose responsiveness to IAPi (5 to 50 μ M for 24 to 72 hours) was determined via MTS assays of 9 genetically heterogenous human myeloma cell lines (HMCL). Immunoblotting of caspases 3 and 7, PARP, ICAD, CAD and ROCK-1, with or without, specific caspase inhibition or siRNA knock-down of caspases 3 and 7 was performed to characterise the mechanism(s) of cell killing. Co-culture experiments with IL6, IGF-1 or the human stromal cell line HS5 (n = 3 for each) were used to quantify potential cytokine-mediated abrogation of IAPi anti-MM activity. Finally, the anti-MM activity of IAPi in combination with other therapeutics was investigated against both HMCL and primary MM samples.

Results

IAPi demonstrated IC_{50s} of 25 to 50 μ M against all 9 HMCL at 72 hours. Immunoblotting following IAPi treatment with and without caspase inhibitors or siRNA knock-down was consistent with activation of both the intrinsic and extrinsic apoptotic pathways. Rapid falls in ICAD and CAD plus cleavage of ROCK-1 and PARP confirmed IAPi-induced apoptosis via caspase 3 and 7. Surprisingly, HS-5 co-culture or contemporaneous addition of IL-6 or IGF-1 to IAPi treated cells showed enhanced MM cell apoptosis (median 1.2, 1.1 and 1.3 fold increase, respectively) compared to IAPi only treated controls. Combining IAPi with conventional cytotoxics, an anti-TRAIL agonistic antibody or a novel HSP90 inhibitor all demonstrated synergistic killing of MM cells with combination indices (CalcuSyn) of less than 1.

Conclusion

IAPi induces down-regulation of ICAD/CAD and ROCK-1/PARP cleavage via caspase 3 and 7 dependent processes. Furthermore, IAPi retains anti-MM activity in the context of relevant exogenous growth factor exposure and induces synergistic killing of MM cells when combined with conventional and novel anti-MM agents. IAPi represents a potentially novel therapeutic approach to MM.
This research was supported by Novartis Corporation. The company had no role in analysing the data or preparing the abstract

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Clinical and Immunohistochemical Features Associated with a Response to Bortezomib in Patients with Multiple Myeloma

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Aim

To identify clinical parameters and/or protein expression characteristics that are predictive of response to bortezomib therapy for relapsed or refractory multiple myeloma (MM).

Methods

We analysed the baseline clinical parameters and profiled the baseline MM cell expression of a range of immunohistochemical markers of cell cycle, apoptosis and angiogenesis (CD138, CyclinD1, Bcl2, Bcl-Xl, p53, p16^{INK4A}, p21^{CIP/WAF1}, relA, VEGF_{R1} and FGFR3) in pre-treatment trephines from a cohort (n=90) of relapsed MM patients recruited to one of two prospective multicentre trials of bortezomib salvage therapy. Univariate analyses were performed using the Student's t-test, Wilcoxon rank sum test or Fisher's exact test. Multivariate analysis was performed using multiple linear regression. Progression free survival (PFS) and overall survival (OS) were assessed using the Kaplan-Meier method.

Results

Response (CR or PR) to bortezomib was associated with a previous history of CR to alternative anti-MM treatment. Patients who expressed cyclin-D1 were more likely to respond (67% vs 47%; expression vs no expression, respectively; p = 0.08). In contrast, patients who expressed p16^{INK4A}, cytoplasmic p53 or high Bcl2 had poor responses (22% vs 57%, 42% vs 69% and 48% vs 74%; expression vs no expression, respectively; with p = 0.05, p = 0.01 and p = 0.01, respectively). A high likelihood of response (89%) was seen with p16^{INK4A} negative/Cyclin-D1 positive tumours. Conversely p53 positive/Cyclin-D1 negativity was associated with very low response rates (14%). Patients who did or did not express FGFR3 responded equally well to bortezomib. Patients who achieved a response to bortezomib and those patients who expressed cyclin-D1 demonstrated a significant OS advantage (p=0.0004 and p=0.05, respectively) whereas FGFR3 had no impact on survival.

Conclusion

Baseline clinical parameters and selective immunohistochemical markers can be used to identify patients that are most likely to achieve a meaningful clinical response to bortezomib salvage therapy.

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Targeting Lewis Y-positive Multiple Myeloma and Acute Myeloid Leukaemia with Gene-modified T cells demonstrating Memory Phenotype

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Aim/Background

Haematologic malignancies such as AML and MM are amenable to immunotherapy as evidenced by the immune mediated allogeneic graft versus leukaemia/myeloma effect. Studies of adoptive immunotherapy with gene modified T cells have shown clinical activity in solid tumours. Thus, our aim was to generate gene-modified clinical-grade T cells directed against malignancies expressing the carbohydrate antigen Lewis^Y (Le^Y). Moreover, we aimed to produce cells that possessed T cell memory, an attribute considered essential for *in vivo* T cell persistence and effective killing of tumour cell targets.

Methods/Results

We identified various cell lines that expressed Le^Y. Furthermore, 27% and 43% of primary MM and AML bone marrow samples were Le^Y-positive, respectively. We manufactured a novel retroviral vector construct resulting in efficient transduction of PBMC-derived T cells with resultant high expression of a single-chain anti-Le^Y chimeric T cell receptor. Using a GMP-conform protocol we achieved up to >100-fold expansion of T cells at the end of the culture (day 12). Anti-Le^Y T cells lysed Le^Y-positive tumour cells *in vitro* while sparing Le^Y-negative control tumour cells and Le^Y expressing neutrophils (low - moderate Le^Y expression). Detailed analysis of end-of-expansion T cells revealed similar transduction rates in CD4 and CD8 T cell subsets. Furthermore, T cells showed low expression levels of CD45RA and CCR7 and active proliferation in response to IL-2 and IL-15, suggesting an effector memory phenotype. Co-culture with Le^Y expressing tumour cells resulted in further proliferation and IFN- γ production of anti Le^Y T cells. We have developed a first in human phase I trial for patients with Le^Y-positive MM or AML.

Conclusion

Le^Y is a promising and immunologically relevant target for T cell immunotherapy and our product is likely to lead to *in vivo* persistence of anti-Le^Y T cells, an outcome which will be specifically addressed in our upcoming study.

No conflict of interest to disclose

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In Vitro Efficacy of Agonistic Antibody to TRAIL-R1 (Mapatumumab) and low dose Bortezomib in Multiple Myeloma

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Aim

The proteasome inhibitor Bortezomib (Bz) is a potent inducer of plasma cell apoptosis via its suppressive effects on NfKB. Bz is also associated with thrombocytopenia and inhibition of dendritic cell (DC) function, which may limit the induction of autologous T cell responses to myeloma antigens. In order to limit these effects of Bz we evaluated whether the novel agonistic antibody that targets TRAIL-R1 Mapatumumab (Mp) would allow significant downward titration of Bz dose whilst still inducing plasma cell death.

Method

Cultured human plasma cell lines (RPMI8226, U266, LP-1, NCI-H929, OPM-2 and JJN3) were treated with Mp and/or Bz for 24 or 48 hours. Cells were stained with annexin V-FITC and the viability dye 7-AAD and analyzed on the BD LSRII flow cytometer. In addition, the myeloma cell surface expression of Tumour Necrosis Factor Apoptosis-Inducing Ligand Receptors (TRAIL-R) 1 and 2 were assessed and correlated with Mp sensitivity.

Results

All cell lines were sensitive to 10nM Bz monotherapy. In contrast 4/6 cell lines were sensitive to Mp alone, RPMI8226 at 0.06ug/ml, U266 and OPM-2 at 1ug/ml each and LP-1 at 10ug/ml. When non-apoptosis inducing Bz doses were used in combination with titrated doses of Mp (from 0.01 to 50ug/ml) apoptosis of RPMI8226, U266 and OPM-2 was enhanced. In contrast, LP-1, JJN3 and NCI-H929 had no additional killing beyond that found by Mp alone. TRAIL-R 1/2 were expressed by RPMI8226 at a higher level than U266, correlating with enhanced sensitivity to combination Mp/Bz therapy.

Conclusion

Bortezomib is a potent anti-plasma cell therapy. The effective dose of Bz may be reduced significantly when used in combination with Mp suggesting that this combination therapy may be more efficacious in selected patients. These results emphasize the need for clinical studies to explore dose modification of Bz, which may in-turn reduce clinical side-effects and enhance endogenous immune responses.

This research was supported by Human Genome Sciences Inc. The company examined this abstract prior to submission

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Safety and Efficacy of Bortezomib Combined with the Deacetylase Inhibitor Romidepsin in Patients with Relapsed or Refractory Multiple Myeloma: Interim Results of a Phase I/II Trial

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Introduction

There are substantial pre-clinical data demonstrating synergistic activity of proteasome inhibitors and deacetylase inhibitors (DACi) in multiple myeloma (MM). This is the first clinical trial combining these two classes of drugs.

Methods

This is an ongoing open label single-centre single-arm phase I/II dose escalation trial of bortezomib, dexamethasone and romidepsin in patients with relapsed or refractory MM. All patients received bortezomib (1.3mg/m²; d1, 4, 8, 11) with dexamethasone (20mg d1, 2, 4, 5, 8, 9, 11, 12). The romidepsin dose escalation commenced at 8mg/m² IV d1, 8, 15 every 28d and involved an initial accelerated dose escalation phase, with intra-patient dose escalation of romidepsin to 10, 12 and 14mg/m². Response rates were assessed according to M-protein response criteria, CR documented to EMBT criteria.

Results

To date, 20 patients have entered the study with 16 evaluable for response and toxicity. Median number of prior therapies = 2 (2-5). Most patients had previously taken potential neurotoxic medications; vincristine (3), thalidomide (4), bortezomib (1). No DLTs were demonstrated at 8mg (n=1) or 10mg (n=3) of romidepsin. At 12mg, 3 episodes of Grade 4 thrombocytopenia and one episode of febrile neutropenia occurred. The maximum tolerated dose was declared as 10mg romidepsin. 10/15 patients have enrolled in phase II. Other toxicities include: Grade 3: fatigue (n=1), neutropaenia (n=1), sepsis (n=1); Grade 2: peripheral neuropathy (n=3), nausea (n=1), diarrhoea (n=1). Three patients required bortezomib dose reduction due to peripheral neuropathy. As of July 2008 the median number of treatment cycles delivered was 3 (1-8, N=20); Maintenance cycles was 7 (3-14, n=7). 5 patients have progressed. The overall response rate is 14/16 (87.5%), 4 CR, 6 PR and 4 MR.

Conclusion

These interim results demonstrate an extremely encouraging response rate, some durable responses and acceptable toxicity of a proteasome inhibitor-DACi combination in this patient group.

This research was supported by Gloucester Pharmaceuticals and Janssen-Cilag. The company had no role in analysing the data or preparing the abstract

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Bone Turnover, Bone Mineral Density and other Characteristics in Post-Transplant Myeloma Patients

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Aim

Given the frequency of bony complications in myeloma and controversies regarding optimal use of bisphosphonates, we investigate bone mineral density and biochemical markers of turnover in transplant patients. We investigated for associations with bisphosphonate use, presence of osteonecrosis of the jaw and myeloma disease characteristics.

Method

We identified all patients alive following previous autologous transplantation for myeloma at a single centre- Royal Perth Hospital, and reviewed clinical and laboratory data including cytogenetics. All patients received a survey questionnaire. Dental problems and use of bisphosphonates were recorded. Bone density, urine and blood metabolic markers were performed. Clinical records were reviewed including laboratory data and cytogenetics.

Results

54 patients were identified and contacted. Age range was 41-71 years, (46% males) and time since initial transplant 24-3621days. Two patients had received second autografts and one a later sibling allograft. The cytogenetics and laboratory characteristics were recorded. Bone turnover was found to be variable in this heterogeneous group of patients. While all had received autologous transplantation, there were significant time differences from the time of diagnosis, variations in steroid therapy and the use of bisphosphonates.

While a previous case of osteonecrosis of the jaw (ONJ) occurred in a patient having marked elevation in bone density and also a long bone fracture after receiving prolonged intravenous bisphosphonate, another patient with ONJ was found to have reduced bone density. Statistical and final full data will be available at the meeting.

Conclusion

Bone turnover and mineral density studies may assist in the management of patients with myeloma, including optimisation of bisphosphonate use. Varying results were identified in the studied post-transplant group of patients, who had received different duration and type of bisphosphonate and other therapies. Prospective studies from the time of myeloma diagnosis are planned in attempts to optimise future clinical care.

No conflict of interest to disclose

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No Bones About It: Determining the Optimal Aspiration Volume During Bone Marrow Harvest

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Background

The use of bone marrow (BM) as a stem cell source is increasing once again due to the lower rate of chronic GVHD compared with peripheral blood stem cells. There is no generally accepted technique for harvesting BM.

Method

BM was collected from the posterior iliac crests (PIC) in 2 separate bags: 10ml aspirates from the left and 20 ml aspirates from the right. Samples taken at the start and after 100, 150, 200, 250, & 500 ml were analyzed for TNC, CD34⁺ and CD3⁺ cell counts.

Results

Total numbers of harvests included in the study were four. The CD34 and CD3 cell number (mean \pm SEM $\times 10^6$) at the start of harvest were 5.8 ± 0.05 , 65.8 ± 12.0 respectively for 10 ml aspirates and 9.1 ± 0.5 , 106.9 ± 22.1 respectively for the 20ml aspirates. There is a rapid fall in the yield of CD34⁺ cells obtained with increasing harvest volume (19 and 25% of the initial number after 250 ml for 10 and 20 ml aspirates respectively; 14 and 11% respectively after 500 ml). In contrast the CD3⁺ cell numbers fall more slowly (43 and 50% after 250 ml, 45 and 38% after 500 ml). By the time 500 ml has been aspirated, there is no difference in the total number of CD34⁺ cells obtained from a 10 ml versus a 20 ml aspirate of bone marrow. The ratio of CD3 to CD34 was increasing both in 10 and 20 ml aspirates, indicating an increasing amount of PB contamination in the aspirates as the harvest volume increases

Conclusion

CD34⁺ cell yields fall rapidly when BM is harvested along the PIC. Using additional areas such as the anterior iliac crests or sternum or a second harvest may be preferable to a large volume PIC harvest for optimizing CD34⁺ stem cell collection. After 500 ml of BM has been harvested, 20 ml BM aspirates do not increase CD34⁺ cell numbers and 10 ml aspirates should be taken to minimize unnecessary blood loss and reduce T cell contamination.

No conflict of interest to declare

A193

Meeting Room 7
HSANZ Free Communications 11
O99

0830-1000
0845

Higher Incidence of CMV Reactivation Following Fludarabine Based Reduced Intensity Conditioning Transplants – A Retrospective Comparison with Myeloablative Transplants

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Limited data exist on the incidence of Cytomegalovirus (CMV) reactivation following fludarabine based reduced intensity conditioning (RIC) transplants. A retrospective cohort study of RIC and myeloablative transplants (MAT), performed between January 2002 and May 2008, was done to compare rates and risk factors for CMV reactivation. Pre-transplant ganciclovir was given to sero-positive recipients while all patients received Valaciclovir as herpes prophylaxis. Weekly monitoring for CMV was done using a qualitative PCR; if positive, quantification was done using either pp65 antigenemia or quantitative PCR (COBAS R). One hundred RIC transplants (27 unrelated) and 155 MAT (60 unrelated) were performed during the study period. The median age of RIC transplants (65 Males; 35 Females) was 52 years (range: 18 - 65). By CMV serostatus, 71 were high risk (D+/R+ or D-/R+), 15 intermediate (D+/R-) and 14 low risk (D-/R-). Acute graft versus host disease (GVHD) was observed in 44% of RIC and 64% of MAT. CMV reactivation was seen in 43 patients at a median of 48 days (range: 24 days – 64 months). In high risk patients, reactivation was significantly higher with RIC (61.4%) than MAT (41.6%; $p = 0.01$) and remained so even after adjusting for acute GVHD (adjusted OR 2.90, 95% CI: 1.49-5.68, $p=0.001$). Type of donor (sibling vs MUD) or use of ATG during conditioning did not influence reactivation. Of the 15 RIC transplants that received Campath, 10 had CMV reactivation. Analysis of patients with high risk serostatus who did not receive Campath ($n=166$) showed reactivation in 57% RIC compared to 42% MAT ($p=0.06$). Overall CMV disease was seen in 9% following RIC and 2.5% following MAT. The higher incidence of CMV reactivation in fludarabine based RIC transplants highlights the need for improved prophylactic and preemptive strategies including use of CMV specific CTL's in this vulnerable patient population.

No conflict of interest to disclose

A194

Meeting Room 7
HSANZ Free Communications 11
O100

0830-1000
0900

A Retrospective Comparison of the Outcome of Single Versus Double Unrelated Umbilical Cord Blood Units for Allogeneic Transplantation In Adults with Advanced Hematological Malignancies

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Unrelated umbilical cord blood (CB) has emerged as an alternative stem cell source for allogeneic transplantation for patients with hematologic malignancies, but in adults is limited by the low number of stem cells present in banked CB units. One strategy to overcome this problem is to transplant multiple CB units into each recipient, thereby increasing the stem cell dose, and potentially increasing the rate of engraftment and thereby reducing transplant mortality. We have compared the outcomes of transplants using 2 CB units in adults with poor prognosis hematologic diseases with those in a similar patient cohort who received transplants with single CB units. Eleven patients, median age 27 years and median weight 69 kg, received transplants of 2 partially-matched unrelated CB units after myeloablative conditioning therapy at Westmead Hospital, and the results were compared with an historical cohort of 9 patients undergoing single unit CB transplant at the same centre. Neutrophil recovery to 0.5×10^9 /L was seen by median day 32 (18-53), and platelet recovery to 50×10^9 /L by day 91 (56-381). These results were not significantly different from those reported in patients receiving single CB transplants. Acute graft versus host disease grades II-IV was seen in 4 patients, but no chronic graft versus host disease occurred. Transplant-related complications were responsible for the deaths of 5 patients in the first 3 months post-transplant, while 2 patients died of relapse of their hematologic malignancy. Four patients survive disease-free 17 to 33 months post-transplant.

Transplantation using 2 partially-matched unrelated CB units did not appear to result in improvements either in engraftment or survival, as compared to a previous cohort of patients receiving single CB units. Further strategies appear to be needed to reduce the duration of severe neutropenia, and the high transplant-related mortality in these patients.

No conflicts of interest to disclose

A195

Meeting Room 7
HSANZ Free Communications 11
O101

0830-1000
0915

Can Otherwise Incurable Haematological Malignancies be Cured by KIR-ligand Mismatched Haploidentical Stem Cell Transplantation (haploHSCT)? – The Alfred Hospital Experience

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Introduction

Relapsed or refractory haematological malignancies are difficult to cure. Some patients with HLA-matched donors can be salvaged by allogeneic HSCT whereas those patients without donors will die. HaploHSCT, where donors and recipients are matched for one haplotype i.e., HLA-A, -B, -Cw and -DR mismatched, has been explored for more than a decade – with limited success. Ruggeri L, et al (*Science (2002) 295:2097-00*) have improved the results of haploHSCT with several modifications – Killer Immunoglobulin-like Receptor (KIR)-ligand mismatched donors, and highly T cell depleted megadose CD34+ stem cell infusions. KIR-ligand mismatch has been shown to generate a potent NK cell-driven graft-versus-leukaemia (GVL) effect in this setting - a phenomenon most prominent in myeloid malignancies.

Methods

We performed haploidentical transplantation in 12 patients with otherwise incurable haematological diseases (AML=7, CML-BC=1, MDS=1, VSAA=1, T-ALL=2) who did not have a suitable HLA-matched donor. All AML and ALL patients were refractory or in relapse. 3 had previously undergone autologous HSCT. Median age - 35.5 (22-58) yrs; 7 male, 5 female. Each patient/donor pair was haploidentical and KIR-ligand mismatched (GVL direction). Conditioning regimen - ATGAM, melphalan, fludarabine, thiotepea. G-CSF-mobilised PBSCs were CD34+ cell selected (CliniMACS or Isolex device). No post transplant immunosuppression was given. Caspofungin was used as fungal infection prophylaxis.

Results

Overall, 3 of 12 (25%) of patients are alive and disease-free (8, 4 and 3.5 yrs). Of the myeloid malignancies 3 of 9 (33%) are alive and disease-free. Both patients with T-ALL died of relapsed leukaemia, and the patient with VSAA died of transplant-related complications. The 3 surviving patients have no on-going transplant complications and Karnofsky scores of 100%. All patients engrafted with complete chimerism. Grade II-IV acute GVHD occurred in 3 of 11 evaluable patients. 3 developed chronic GVHD (1 limited and 2 extensive). 9 patients died – 3 of relapsed disease, 2 of multi-organ failure 1 each of chronic GVHD, interstitial pneumonitis, *Scedosporium* infection and VOD. No infections with *Candida* or *Aspergillus* occurred in the 11 patients who received caspofungin prophylaxis.

Conclusion

KIR-ligand mismatched haploidentical HSCT provided a significant proportion of patients, with otherwise incurable malignancies, long-term DFS. The 33% OS/DFS of the patients with myeloid malignancies compares favourably with traditional forms of alloHSCT in this refractory and heavily pretreated cohort. Caspofungin was effective antifungal prophylaxis. Future results may be improved if haploHSCT is considered earlier in the course of the disease – to decrease transplant-related mortality and perhaps relapse.

This research was supported by Merck Sharpe & Dohme who had no role in analysing the data or preparing the abstract.

A196

Meeting Room 7
HSANZ Free Communications 11
O102

0830-1000
0930

Delayed Relapse and Long-term Follow-up of Allogeneic Bone Marrow Transplantation for Chronic Myeloid Leukaemia

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Aim

To determine the factors affecting overall survival (OS), disease-free survival (DFS), and delayed relapse >3 years after BMT in CML patients treated with allogeneic BMT.

Methods

193 patients treated with allogeneic BMT for CML from 1981-2006 were identified. The data was analysed for OS, DFS and subgroups analysed for their effect on survival. Patients who were alive and disease free 3 years after BMT were studied for late relapse.

Results

The median age of recipients was 37 years (16-63). The disease stage at transplant was chronic phase (CP) 106, accelerated phase (AP) 44, and blast transformation (BT) 40. The conditioning was cyclophosphamide/TBI (111), busulfan/cyclophosphamide (73) or other (9). The donor was matched sibling (136) or matched unrelated/other related (57). Stem cell source was from marrow (157), blood (34) or double cord blood (2).

The median OS in all patients was 33 months and DFS 22 months. Preliminary univariate analysis showed that variables predicting better DFS included acute GVHD grade 0-1 but not source of stem cells or conditioning. Matched sibling donors showed a superior OS compared to other donors ($p=0.045$). This will be confirmed by multivariate analysis.

At 3 years overall DFS was 46%: 50% in matched sibling and 38% in alternate donors. Patients transplanted in CP had a better DFS > 3 years (median not reached) than AP or BT ($p=0.0$). There were 8 patients (5 CP, 3 AP) with delayed relapse. The median time to relapse >3 years was 4.6 years (3.1-20.8 years). 2/8 patients are alive after 6.4 and 6.8 months on imatinib therapy.

Conclusion

Survival following allogeneic transplant for CML is related to disease stage and severity of AGVHD but not donor type or conditioning. DFS beyond 3 years is not stable with small numbers of patients continuing to relapse especially in AP and BT.

No conflict of interest to disclose

A197

Meeting Room 7
HSANZ Free Communications 11
O103

0830-1000
0945

Plasma Exchange as Treatment of Transplantation-Associated Thrombocytopenic Microangiopathy (TA-TMA): Effect of Concomitant Acute GVHD on Efficacy and Outcome

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Aims

To review the outcome of patients treated at our institution with plasma exchange (PE) for transplantation-associated thrombocytopenic microangiopathy (TA-TMA) post-allogeneic stem cell transplantation (SCT).

Methods

Retrospective review of allogeneic SCT patients who developed TA-TMA. Patients were identified from a unit data-base, with data available from December 2001. Response to PE was defined as complete response (CR) if FBC, LDH and renal +/- CNS abnormalities returned to pre-TA-TMA values / function with PE, partial response (PR) if platelet counts improved to at least 50% of pre-TA-TMA values, and no response (NR) if no improvement in platelet counts occurred.

Results

In total, 15 patients with TA-TMA were identified, with 11/15 patients treated with PE. 4 patients did not undergo PE due to presence of active sepsis (n=1) and physician discretion (n=3). Overall, 3 patients responded to PE (CR), and 8 patients had NR. Of 7 patients treated with PE for TA-TMA developing in the setting of active acute GVHD, 0/7 responded. Of 4 patients treated with PE for TA-TMA in the absence of active acute GVHD (conditioning-related in 1, chronic GVHD in 2 and post resolution of acute GVHD in 2), 3/4 responded (p=0.024). In 2 of 7 patients with active acute GVHD and TA-TMA unresponsive to PE, TA-TMA eventually responded to increased immunosuppression / control of GVHD. For the whole group, only 2 patients remain alive, including 1/11 patients treated with PE. Median survival post onset of TA-TMA was 79 days (range 5-1845 days). Causes of death included infection-related (n=7), GVHD-related (n=4) and relapsed malignancy / PTLD (1 case each).

Conclusions

Depending on the clinical circumstance in which TA-TMA develops, PE may be of therapeutic benefit. Responses to PE were seen in a majority of TA-TMA occurring in the absence of active acute GVHD. In contrast, TA-TMA occurring in association with active acute GVHD was unresponsive to PE, and as such, in this circumstance, we recommend therapy primarily be directed at controlling underlying GVHD.

No conflict of interest to disclose

A198

Meeting Room 1
HSANZ Free Communications 12
O104

0830-1000
0830

A Prospective Randomised Trial of Intravenous Iron Therapy versus Oral Iron for Iron Deficiency Anaemia in Pregnant Women

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Introduction

To date, limited data are available regarding the prevalence of iron deficiency anaemia (IDA) during pregnancy in Australia, or the comparative efficacy of IV iron versus oral iron therapy in pregnant women.

Patients and Method

At the Launceston General Hospital we prospectively investigated 400 pregnant women between January and December 2007 with full blood count (FBC) and iron studies at the first or second antenatal visit. Among those, 100 women (25%) had iron deficiency anaemia, and were recruited to a prospective randomised trial to determine whether intravenous iron therapy (iron polymaltose) is superior to oral iron (ferrous sulphate 250 mg) for the management of IDA associated with pregnancy.

Results

At recruitment, median haemoglobin (Hb) was 108 g/L (range: 90-115, normal range: 120-160 g/L), while median serum ferritin was 11 µg/L and mean ferritin was 19 µg/L (normal range: 30-460 µg/L). After four weeks of treatment, the Hb level increased by a mean of 5.5 g/L on oral iron and by 9.6 g/L after IV iron. Mean/Median serum ferritin did not increase significantly in women on oral iron (15.3 and 14 respectively), but showed significant increase to a median of 228 µg/L and a mean of 241 µg/L in those given IV iron (P-value <0.001). Hb taken pre-delivery showed Hb-increase by a mean of 11.6 g/L on oral iron, and by 22.9 g/L for IV iron (P-value <0.001).

Conclusion

Our data indicate that iron deficiency is a common finding during pregnancy in the northern Tasmanian population, and intravenous iron therapy appears a safe and effective treatment in this cohort of patients.

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

Meeting Room 1
HSANZ Free Communications 12
O105

0830-1000
0845

Compliance and Tolerability of Iron Therapy during Pregnancy

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Aim

To follow up pregnant women randomised either to oral iron or intravenous iron in order to assess the compliance and tolerability of iron therapy.

Methods

144 pregnant women with iron deficiency anaemia were enrolled in the trial, of which 72 were assigned to a single IV iron Polymaltose (Category A) infusion with a dose calculated according to body weight and Hb level, while 72 received daily oral iron with FGF (ferrous sulphate 250 mg, folic acid 300 mcg). Prior to iron infusion, antihistamine therapy with oral Polaramine 2 mg (Category A) was commenced. Compliance and tolerability were assessed via questionnaires at 2 and 4 weeks after administration had commenced.

Results

Compliance: Non-compliance in the oral arm was found in 12.5% of patients, and varied from missing a few tablets to neglecting to pick up repeat prescriptions. In the IV arm, one patient failed to present for her infusion. *Tolerability:* Approximately 22% of women in the oral arm experienced mild gastrointestinal symptoms, but were able to continue taking FGF without any ongoing problems. Five women were withdrawn from the oral iron due to GIT-upset. For those receiving an iron infusion, 28% reported mild symptoms such as tiredness during or after the infusion, although 80% of these were unlikely to be due to the infusion itself. Two women had a possible minor allergic reaction, and 2 had possible local reactions. 2 infusions were ceased before they were completed, but again, the symptoms related were unlikely to have been caused by the infusion but most likely due to antihistamine therapy with a drop of their blood pressure. None of these women required medical intervention.

Conclusion

Both oral iron and IV iron Polymaltose have been generally well tolerated in pregnancy. Compliance has been well within an acceptable limit.

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

A200

Meeting Room 1
HSANZ Free Communications 12
O106

0830-1000
0900

Current Epidemiology of Thalassaemia and Haemoglobinopathy in New South Wales

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Background

During the 1990s, there has been increasing diversity of migration to Australia (and especially Sydney) with people originating from areas with a high incidence of thalassaemia / haemoglobinopathies, such as the Mediterranean, Middle East, South East Asia and Africa.

Method

Our laboratory receives HbEPG/thalassaemia screen requests from general and specialist medical, surgical and obstetric practices including antenatal screening, across New South Wales. We use High Performance Liquid Chromatography (BioRad VII) to quantitate different haemoglobins and run alkaline and acid gel electrophoresis to differentiate major haemoglobinopathies.

Results

Results for the last 5 years (2003-2007 inclusive) are as follows for thalassaemia: α - 687, β - 1631, $\delta\beta$ - 22. There were 150 patients with elevated HbF. Patient numbers with haemoglobinopathy were HbS - 117, HbE - 300, HbD - 24, HbC - 22, Hb Lepore - 30, Hb Constant Spring - 8, Hb Kempsey - 6, HbQ - 4. Figures are number of individual patients and include both heterozygous and homozygous inheritances.

Conclusion

β -thalassaemia is the most common form of thalassaemia seen. The incidence of β -thalassaemia in the post war years was largely due to Greek and Italian migration. The incidence is now increased due to migration from SE Asia. Migration from SE Asia is also responsible for the high incidence of Hb E, Hb Lepore, $\delta\beta$ -thalassaemia and Hb Constant Spring. Some other haemoglobinopathies likely reflect migration from Africa. A variety of rare haemoglobinopathies is seen and will be presented. The results reflect the changing ethnic diversity of the Australian population and patterns of migration.

No conflict of interest to disclose

A201

Meeting Room 1
HSANZ Free Communications 12
O107

0830-1000
0915

The Incidence of Haemoglobin disorders in Refugees migrating to Western Australia – a 5 year Retrospective Study, 2003 – 2007

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Aim

This was a retrospective study of the results of a screening programme for migrants undergoing health checks with the Refugee Migrant Health Clinic in Perth, WA.

Methods

All laboratory tests were performed according to standard diagnostic protocols, and included a FBP, Iron studies, HPLC, multiplex PCR for common alpha thalassaemia deletions and additional confirmatory tests as indicated by the results of initial screening.

Results

A total of 5523 samples were received over the review period. The majority of migrants were of African origin (n=4637), with 516 and 370 from Asia and the Middle East respectively.

Within the African population, alpha thalassaemia trait (heterozygous or homozygous $\alpha^{-3.7}$ deletion) was the most common abnormality, found in 12% of this population group. HbS was present in 8.2% of this population, most common in individuals of Central and West African origin (13.7% and 12% respectively). Beta thalassaemia trait was present in 6% of those from West Africa. It was rare in North Africa and was not seen in individuals from Central Africa. Other less common variants included HbC, Hb Stanleyville II and delta globin variants.

There has been a change in the pattern of migration from the Middle East and Asia over the study period. Whereas 370 individuals of Middle Eastern origin were screened in 2003 and 2004, this has been superseded by migration from South East Asia, with a total of 513 individuals since 2005.

The prevalence of alpha thalassaemia in the Middle Eastern group was 3.8%, and beta thalassaemia trait was seen in 1.4%.

The Asian migrants are mainly from Burma and Thailand. Alpha thalassaemia trait was seen in 6.2% of this population. Beta thalassaemia trait and HbE trait were seen in 6.4% and 4.3% respectively.

Conclusion

The pattern of Haemoglobin disorders reflects the changing demographics of the refugee migrant groups over time. Within these populations, clinically significant haemoglobin gene disorders are relatively prevalent, and clinicians should be alerted to this, particularly in the antenatal setting or in the context of pregnancy planning.

No conflict of interest to disclose

A202

Meeting Room 1
HSANZ Free Communications 12
O108

0830-1000
0930

The Role of Flow Cytometry in Myelodysplastic Syndromes (MDS)

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MDS is a heterogeneous group of myeloid neoplasms with abnormal maturation and differentiation of ≥ 1 cell lineage, resulting in bone marrow (BM) failure and a predisposition to leukaemia. Diagnosis is complex and standard assessment involves evaluation of peripheral blood (PB), BM and cytogenetics. Criteria for diagnosis and classification have recently been refined in a consensus publication, in which immunophenotyping (IP) was recommended as a co-criterion.

Although IP has been used extensively for diagnosis, classification, prognostication and detection of minimal residual disease for haematological malignancies, there is limited data in MDS. With an increased understanding of normal antigen expression, the detection of aberrations in blast and maturing myelomonocytic populations may provide a more objective assessment for sequential review. MDS flow-cytometric scoring systems have been developed to assist in the standardisation of this process (Wells et al. Blood 2003). The role of IP for discriminating early MDS and idiopathic cytopenias, as well as treatment response and remission status, in established MDS has not been defined.

We prospectively assessed the utility of IP, at diagnosis and after therapeutic intervention, by correlating standard assessment criteria, in particular morphology, with a modified MDS scoring system. Four colour-flow cytometric analysis was performed to assess blast and myelomonocytic populations. To establish "normal" antibody expression, we assessed marrow samples from 5 healthy volunteers and 16 patients with a normal PB and BM evaluation. Sequential assessment was then performed on 54 patients with possible and/or definitive diagnosis of MDS. This included sequential review in 10 patients treated with lenalidomide/stem cell factor in a phase II study.

We present our early data which demonstrates the potential utility IP as a diagnostic tool and in post treatment response assessment. However, larger numbers of patients are required in a prospective setting for confirmation of these findings.

No conflict of interest to be disclosed

A203

Meeting Room 1
HSANZ Free Communications 12
O109

0830-1000
0945

Flow Cytometry as a Diagnostic Tool for Hereditary Spherocytosis- Westmead Hospital Experience

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Objectives

Flow cytometric analysis of eosin-5'-maleimide-labeled red blood cells has been proposed as a method of identifying hereditary spherocytosis (HS). The flow cytometric test measures the fluorescence intensity of intact red cells labelled with the dye eosin-5-maleimide (EMA), which reacts covalently with Lys-430 on the first extra cellular loop of band 3 protein. Patients with HS have reduced fluorescence compared to other patient groups and normal controls.

Aim

The aim of the present study was to assess the utility of flow cytometry in the diagnosis of hereditary spherocytosis by determining the sensitivity and specificity of this method within our laboratory.

Methods

Fresh peripheral blood was collected in Lithium Heparin, stained with the dye EMA and analysed by flow cytometry. A mean fluorescence intensity (MFI) range of 40.1 +/- 4.51 and Peak channel fluorescence (PCF) of 39.17 +/- 5.2 was considered positive for HS (these results were determined by analysing 7 known HS patients). Equivocal results are defined when the MFI remain in the range of 46-54 units, as opposed to normal healthy controls the MFI range between 55-74 units.

Results

A total of 96 samples were analysed with a female to male ratio of 1.06:1. Samples were investigated for HS for the reasons such as coombes's negative spherocytosis, positive family history (FH), neonatal hyperbilirubinaemia (NNH) and other haemolytic anaemia (HA). Within this cohort the group with positive FH of HS has highest positive and least equivocal results. The group with suspected HS (peripheral blood spherocytosis with negative coomb's test) resulted positive in 38% and equivocal in 26% cases. The group with NNH had positive results in 25%, and the group with HA in none. A further analysis of our data had shown the sensitivity and specificity of the test for HS were 80% and 100% respectively.

Conclusions

The highest number of equivocal results was obtained from the patient group with suspected HS possibly due to the fact that the specificity of this test method is for defects in band 3 protein and is less specific for other mutations or deletions in red cell membrane proteins. The EMA dye method by flow cytometry in the evaluation for HS with positive FH is specific, while in the group of patients with HA it is non contributory.

No conflict of interest to declare

A204

Meeting Room 4
ANZSBT Free Communications 3
O110

0830-1000
0830

Massive Transfusion in Trauma

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Aim

To determine blood products transfused, prevalence of coagulopathy on admission, clinical outcomes and the factors associated with massive transfusion in trauma patients.

Methods

A linked electronic database was developed using trauma, clinical and epidemiological and red cell transfusion databases. All trauma patients in the period 1998 to 2006 identified from the trauma database were linked by hospital medical number and reviewed for transfusion in the first 24 hours of injury.

Results

From the trauma database 6519 patients were identified between 1998 and 2006. Four hundred and thirty eight patients (7%) were transfused in the first 24 hours of injury and 802 (12%) received at least one unit of red cell during their hospital stay. Three hundred and fifty nine patients received 1 to 9 units of red cells (RC) and 79 patients received ten or more red cells during first 24 hours of injury using a total of 1601 units. The median time from injury to admission for the massive transfusion group was 112 minutes with a median Injury Severity Score (ISS) of 38. The median RC: FFP (red cell to FFP) ratio was 2.5:1 in the massively transfused group of patients. 46% of the patients in this group presented with coagulopathy on admission. The overall mortality was 32% with 68 % of that mortality in the first 24-48 hours. The factors associated with massive transfusion were systolic blood pressure <90mm, (p= 0.002 [OR 3.6]), Hb <120g/L (p= 0.01 [OR 3.7]) and pH < 7.3 (p =<0.001 [OR 25.5]).

Conclusions

Trauma patients receiving more than 10 units of red cells are patients with severe injury and are associated with high risk of mortality and receive most of the blood components for the treatment and prevention of coagulopathy.

No conflict of interest to disclose

Meeting Room 4
ANZSBT Free Communications 3
O111

0830-1000
0845

Extent and Timing of Dilutional Coagulopathy and Thrombocytopenia in Massively Transfused Obstetric Patients

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Aim

To determine the extent and timing of dilutional coagulopathy and thrombocytopenia in obstetrics patients who receive massive transfusion of blood products, and based on these results, draft a specific protocol on the management of massive transfusion in obstetric patients.

Method

Retrospective case note and data base review of all obstetric patients who received ≥ 8 units of packed red blood cells (PRBC) between 2002-2008.

Result

58 patients received ≥ 8 units of PRBC between January 2002 and July 2008. The average number of blood products transfused were as follows: 13.7 units of packed red cells (95% CI 11.5 – 15.9), 7.4 units of fresh frozen plasma (95% CI 5.8 – 9.0), 6.9 units of cryoprecipitate (95% CI 5.0 – 8.8) and 1.1 units of platelets (95% CI 0.6 – 1.6). On average, this group of patients developed a significant coagulopathy when compared to baseline results using paired t-tests: peak INR 2.1 (95% CI 1.8 – 2.4, $p < 0.001$), peak APTT 77.1 seconds (95% CI 60.0 – 94.2, $p = 0.023$), nadir fibrinogen 1.1 g/L (95% CI 0.9 – 1.3, $p = 0.001$, nadir platelet count $76 \times 10^9/L$ (95% CI 65 – 86, $p < 0.001$). The median time to peak INR was 3.0 hrs (95% CI 2.4 – 3.6), peak APTT was 3.0 hrs (95% CI 2.5 – 3.5 hrs), nadir fibrinogen 3.7 hrs (95% CI 2.8 – 4.7). The median time to nadir platelet count was 7.5 hrs (95% CI 6.7 – 8.3 hrs) and this was significantly longer than the median time to development of coagulopathy (Kaplan Meier analysis, $p = 0.002$). A protocol for the management of massive transfusion in obstetric patients was developed and will be presented.

Conclusion

Obstetric patients who are transfused ≥ 8 units of PRBC develop a dilutional coagulopathy and this develops significantly earlier than dilutional thrombocytopenia. These data show that adequate replacement of fresh frozen plasma and cryoprecipitate should be considered earlier than platelet transfusion in this patient group.

No conflict of interest to disclose

A206

Meeting Room 4
ANZSBT Free Communications 3
O112

0830-1000
0900

Adherence to Transfusion Protocols and the Use of Recombinant Activated Factor VII

Louise Phillips¹, Vina Nguyen¹, Scott Dunkley², James Isbister³, Peter Cameron^{1,4}

¹ Monash University, Department of Epidemiology and Preventive Medicine, Melbourne, Victoria, Australia. ² Royal Prince Alfred Hospital, Sydney, New South Wales, Australia. ³ Royal North Shore Hospital, Sydney, New South Wales, Australia. ⁴ The Alfred Hospital, Melbourne, Victoria, Australia

Background

Most hospitals have clinical guidelines for the off-label use of recombinant activated Factor VII (rFVIIa, Novoseven), primarily as part of a massive transfusion protocol. Over the past years rFVIIa has increasingly been used outside the approved indications in haemophilia with inhibitors and Glanzmann's Thrombasthenia, particularly in trauma, cardiac surgery and other critical bleeding episodes. Use in these areas remains controversial.

Methods

Monash University established the Haemostasis Registry in 2005 (with an educational grant from NovoNordisk Pharmaceuticals) to monitor the use of rFVIIa throughout Australia and New Zealand. More than 80 hospitals are contributing data to the Registry including all major users of rFVIIa in Australia and New Zealand. As part of the process of joining the Registry project, participating hospitals are asked to supply copies of their protocols for use of rFVIIa.

Results

Over 2000 cases of rFVIIa use have been reported to the Register. Major areas of use are cardiac surgery (~ 43%), other surgery (~17%) and trauma (~15%). The majority of hospitals have documented protocols for rFVIIa use. Many of these are similar and are centred around situations of massive transfusion. However, most cases of rFVIIa use submitted to the Haemostasis Registry do not conform with these guidelines.

Conclusions

This is the largest case dataset of rFVIIa cases published to date and can now provide greater insight into the actual rather than theoretical use of rFVIIa in Australia and New Zealand. Lack of compliance with hospital protocols for rFVIIa use indicates either that the protocols do not reflect actual and appropriate use or that clinicians need to be further educated regarding what is currently considered appropriate use. In the absence of sound clinical trial evidence, consensus regarding appropriate use has not been achieved. In these circumstances, data from the Haemostasis Registry continues to be important in elucidating the safety and efficacy of rFVIIa and providing important feedback to doctors and hospitals.

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

A207

Meeting Room 4
ANZSBT Free Communications 3
O113

0830-1000
0915

Monitoring the Temperature of Red Cells Stored Between 2 to 6°C and the Time Taken to Reach 10°C at Room Temperature

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Department of Diagnostic Haematology, The Royal Melbourne Hospital, Victoria, Australia

Aim

To determine the time required for a unit of red cell concentrate stored between 2-6°C to reach 10°C once left a room temperature. Is the 30 minute rule still valid?

Method

Sixty units of expired red cell units (250mL) in additive solution (Adsol) were stored between 2 to 6°C. The probe of a calibrated digital thermometer was inserted into the port of the bag and left to sit on the wooden bench at room temperature (22°C). The temperature of the RCC was recorded in 5 minute intervals to 30 minutes. Seven units of whole blood stored between 2 to 6°C were also monitored with the calibrated digital thermometer and the temperature recorded in 5 minute intervals to 30 minutes.

Results

On average the time taken for a red cell unit to reach 10°C was 20 minutes. After 30 minutes, the average temperature of a red cell unit was 11.4°C. The time taken for a unit of blood to reach 10°C depended on the whether the starting temperature was closer to either 2 or 6°C. None of the 7 units of whole blood reached 10°C in 30 minutes.

Conclusion

The time taken for a unit of blood to reach 10°C depends on a number of factors including the volume, starting temperature, surface and surface temperature where the bag is placed and temperature of the room where the blood is infused. This study has led us to review our process of red cell return to the laboratory.

No conflict of interest to disclose

A208

Meeting Room 4
ANZSBT Free Communications 3
O114

0830-1000
0930

Teaching Transfusion and Transplantation Science in an Information Communication Technology (ICT) Enabled Wet Laboratory Environment

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*RMIT University, Melbourne, Victoria, Australia*¹; *St Vincent's Hospital, Melbourne, Victoria, Australia*²

In 2006 we incorporated networked student workstations in our haematology laboratory which has provided us with the opportunity to create a blended learning environment. This ICT enabled learning space provides students with the opportunity to integrate both theory and practical learning during the one laboratory session.

While lecture sessions are still conducted in lecture theatres due to class size, all lectures are recorded as screencasts and made available as streaming videos, downloadable executable files and MP3 audio files. To assist students in their learning Multiple Choice Question (MCQ) self assessment tests and crosswords on course topics are included in the online learning resources.

All students are provided with a practical manual of techniques, however, the availability of the workstations also allows us to provide an on-screen step by step visual guide to the techniques, that include short video sequences where appropriate. Students are able to review the techniques as often as they wish and go back over parts of the lectures if they are unclear on any of the principles of the techniques or procedures they are undertaking.

Advanced practical classes are conducted as projects. Students are able to research the literature during class and record the results of their experiments in electronic workbooks. Advanced classes in Transplantation and Clinical Immunology employ student directed learning strategies based on the creation of a wiki that forms the student's collective knowledge base on the course topics. Instructor and peer assessment is used in assessing student contributions to the wiki.

Student feedback on this learning environment gained through questionnaires and focus groups has been very positive.

No conflict of interest to disclose

A209

Meeting Room 4
ANZSBT Free Communications 3
O115

0830-1000
0945

Transfusing a Unit of Red Cells – What Does It Really Cost in Australia?

Erica Wood^{1,2}, Linley Bielby^{1,2}, Russell Hunt³, David Peterson¹, David Roxby³, David Westerman¹

¹ Australian Red Cross Blood Service, ² Peter MacCallum Cancer Centre, ³ Flinders Medical Centre, Adelaide and Melbourne, Australia

Background and Aims

Transfusion involves many important steps and multidisciplinary laboratory and clinical teams. Comprehensive process mapping and costings for the overall transfusion process have not been available previously in Australia.

Method

Process maps for transfusing one unit of red cells were constructed and validated. Clinical and laboratory databases provided deidentified, aggregate transfusion episode data for adult non-trauma patients from Jan-Dec 2006 at two university teaching hospitals. Hospital administrations provided personnel/financial data.

Result

26 major processes have been mapped in detail for inpatient and outpatient red cell transfusions, including: pretransfusion examination/clerical routines & informed consent; phlebotomy; transfusion-related testing/results management; component prescribing and ordering; ordering and shipping from blood centre to hospital; inventory management and distribution of units to clinical areas; administration, clean up and waste disposal; and transfusion reaction management.

These processes are complex. For example phlebotomy involves between 33 and 40 actions. Transfusion administration includes between 89 and 105 major steps. An acute severe transfusion reaction can trigger 40 or more major processes, involving hundreds of steps. Direct and indirect costs are being assigned, reflecting process steps and timings, including salaries, on costs and other outlays (e.g. training, assessment); clinical and laboratory equipment, reagents and consumables; quality programmes (e.g. audit, transfusion committee activities) and process-related generic overhead costs (e.g. cleaning, IT).

Conclusion

The process of transfusing a unit of red cells is complex, time-consuming and involves multiple staff and other resources. Preliminary financial data indicate very substantial costs. Understanding the real costs of the whole process should encourage better transfusion practice and use of alternatives where appropriate, to optimise red cell use, improve patient outcomes, and reduce exposures, risks and possibly costs. This model may be adaptable to determine costs associated with transfusion of other blood components.

This research was supported by a grant from Amgen Australia. The company had no role in analysing the data or preparing the abstract.

A210

Meeting Room 2/3
ASTH Free Communications 2
O116

0830-1000
0830

Oral Surgery with Minimum Factor Support in Haemophilia and vWD Patients

Ian Hewson and Alison Street
Alfred Hospital, Melbourne, Australia

After several years using just **local measures** in warfarinised patients for post oral surgery haemostasis with excellent results, the Dental and Haemophilia Units at The Alfred Hospital decided to use these measures for our haemophilia and von Willebrand patients (who would normally require factor replacement) to see if as good post operative haemostasis could be achieved, without the need for additional factor infusion.

Local measures

Flood socket with 5% tranexamic acid, Place gelfoam or surgical

Close with 4-0 Monocryl.

No additional factors administered unless post-op haemorrhage a problem

Previous treatment

Factor infusions to increase levels to 70% - 100% before surgery and post operative dosage depending on complexity of procedure (for up to five days post op with removal of four wisdom teeth)

Protocol would vary with degree of oral surgery and patient's oral health

Previous Costs

For four wisdom teeth:

Daily 45-50U/kg for five days

Less extensive oral surgery:

Initial 30U/kg then 20U/kg for two days

At a cost @ AU\$ 1.00 per unit

Wisdom teeth = AU\$17,000.00 - \$19,000.00

Simple extractions = AU\$5,300.00

Study patients

No factors were infused either before or after oral surgery unless patient returned with a post operative bleed. Patients on regular prophylaxis maintained their usual regime.

Results

To date (May 08) thirty three patients on study, seven (21%) severe (<1% factor activity,) three (10%) moderate (1% - 5%) and twenty three (69%) mild (> 5%).

Of a total of 65 extractions, twenty five (39%) have been surgical. Inferior dental nerve block was used in 50% of cases. One patient required treatment for post operative bleeding.

Conclusions

It is safe to proceed with complex dental procedures with extensive local haemostatic measures without additional factor support.

No conflict of interest to disclose

A211

Meeting Room 2/3
ASTH Free Communications 2
O117

0830-1000
0845

Retrospective Analysis of Desmopressin Responses in Patients With Type 1 and Type 2 Von Willebrand Disorder and Haemophilia A in South Australia

Ketan Bavishi, Elizabeth Duncan, Sue Rodgers, Simon McRae, Lay Tay, John Lloyd
Division of Haematology, Institute of Medical and Veterinary Science, South Australia, Australia

Aims

1. Review desmopressin responses in patients with VWD and mild/moderate haemophilia A, and to compare these against two international criteria.
2. Develop our own criteria based on experience with desmopressin treatment in mild bleeding disorder.

Methods

We reviewed forty patients with VWD and 16 haemophilia A. Blood samples were collected at 0, 0.5, 1, and 2 hours after desmopressin (0.3µg/kg) infusion over 30 minutes. For VWD, a complete response (CR) was defined as peak VWF:RCo and FVIII:C both 0.80 IU/ml or higher, AND > 0.6 IU/ml at 2 hours. For partial response (PR), peak of VWF:RCo or FVIII between 0.50-0.80 IU/ml and either > 0.40 IU/ml at 2 hours. No response, neither criteria were met. Only FVIII was used to assess response in haemophilia A.

Results

Using our own criteria in type 1 VWD, with baseline VWF:RCo > 0.20 IU/ml (n=25), 92% had a CR compared to 25% with VWF:RCo < 0.20 IU/ml (n=8, p=0.001). this response rate is similar to a recent literature report², but higher than earlier criteria.¹ There were seven type 2 VWD patients, 2 type 2A and 2 type 2N had CR whereas 2 unclassified patients and 1 type 2M did not respond. Of 11 haemophilia A patients without an inhibitor, 11 responded to desmopressin. Four patients with an inhibitor responded, one did not respond when the inhibitor was 640BU/ml, but responded when it was 8.0 BU/ml, and another with 16.5 BU/ml inhibitor (FVIII<0.01IU/ml) did not respond.

Conclusion

There was considerable variations in desmopressin responses between patients with type 1 and 2 VWD, and mild/moderate haemophilia A with or without inhibitor. It remains useful to determine the desmopressin responses in whom it might be effective treatment.

No conflict of interest to disclose

A212

Meeting Room 2/3
ASTH Free Communications 2
O118

0830-1000
0900

A Report on Acquired Haemophilia A in South Australia from 1998 to 2008

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Division of Haematology, Institute of Molecular and Veterinary Science, Adelaide, SA, Australia

Aim

To review Acquired Haemophilia A (AH) cases in SA between 1998-2008.

Method

IMVS laboratory and clinical records where available were used to assess laboratory results and management. The time to partial remission (PR) (inhibitor titre 0.5-1.0 BU/ml) and complete remission (CR) (inhibitor titre <0.5 BU/ml) were calculated.

Results

Eighteen cases of AH were identified (11 males/7 females). The median age at diagnosis was 74 years (range 27-89). The presentation median FVIII and FVIII inhibitor were 0.02 IU/ml (range 0.01-0.09) and 7.5 BU/ml (range 1.4-460) respectively. Only 10 patients had treatment details available (Table 1).

No	Age	Sex	Type of Bleed	FVIII (IU/ml)	FVIII Inh (BU/ml)	Bleed Rx	AH Rx
1	64	M	none	0.04	7.5	none	CVP, ivIg
2	68	M	skin, muscle	0.02	2.4	rFVIIa	Prednisolone
3	47	F	skin	0.01	460.0	pdFVIII, rFVIIa	Prednisolone, Azathioprine, ivIg, Cyclophosphamide, Rituximab
4	82	M	muscle	0.07	3.4	FFP	Prednisolone
5	27	F	PPH	0.04	28.0	pdFVIII	ivIg, Azathioprine, Prednisolone
6	82	F	joint	0.02	6.3	rFVIIa	Azathioprine
7	61	M	muscle	0.02	11.0	rFVIIa	Prednisolone, Azathioprine
8	70	F	skin	0.04	5.0	none	Prednisolone
9	47	F	skin	0.09	1.4	DDAVP	None
10	80	M	skin, joint	0.02	3.0	rFVIIa	Prednisolone, Azathioprine

Patient 6 died of intra-cranial haemorrhage, 2 patients (2,7) have not achieved remission yet and another two (5,10) relapsed after CR1. A total of 6 PRs and 8 CRs were assessed. Median time to PR and CR was 80.5 days (range 21-450) and 162.5 days (range 30-542) respectively. The FVIII at PR and CR were 0.35 IU/ml (range 0.22-0.51) and 1.09 IU/ml (range 0.41- 1.98). Seven achieved long term remission.

Conclusion

AH is a rare acquired bleeding disorder with FVIII autoantibody formation . Our cohort reflected the heterogeneity in behaviour and disease course, their outcomes correlate with published literature.

No conflict of interest to disclose

A213

Meeting Room 2/3
ASTH Free Communications 2
O119

0830-1000
0915

A Single Tertiary Centre Experience with Inhibitors in Paediatric Patients with Severe Haemophilia

Huy Tran¹, Simon Brown^{1,2}, Salena Griffin², Wendy Poulsen², Kelly Brady², John Rowell¹, Liane Lockwood²

¹ *Clinical and Statewide Service, Pathology Queensland, Royal Brisbane & Women's Hospital,* ² *Haemophilia Centre, Royal Children's Hospital, Brisbane*

Background

Inhibitor development to replacement therapy remains the most severe complication for individuals with severe haemophilia A and B (SHA/SHB), occurring in up to 30% and 5% of patients respectively. Immune tolerisation therapy (ITT) is successful in eliminating inhibitors in a majority of patients.

Aim

A retrospective study of all paediatric patients undergoing treatment for SHA and SHB from 1998-2008 at the Royal Children's Hospital was performed to observe factors relating to their incidence and response to ITT.

Results

50 patients with severe haemophilia A and B were identified from the database; 13 developed inhibitors (12/47 in SHA and 1/3 in SHB). 69% (9/13) of inhibitor patients were using recombinant factor replacement therapy prior to the time of inhibitor identification. Median age of detection was 2 years with a range from 0.5 to 9.5 years. 46% (6/13) had high titre inhibitors (defined as >5 Bethesda units/ml (BU)). The median peak titre level was 6.4 BU (range 0.6 to 3328 BU). The inhibitor titre is now <0.6 BU in 85% (11/13) of this cohort. Of these 11 patients, the median duration to a negative inhibitor was 9.6 months (range 0 months to 42 months). Of the two with unsuccessful tolerisation both had high peak titre levels (1792 and 3328 BU). One of these patients is receiving prophylaxis with FEIBA and has recently undergone a radiosynovectomy for synovial hypertrophy of a knee joint. Genetic mutation analysis was performed on 10 patients.

Conclusion

Incidence of inhibitor formation in SHA/SHB patients is 26% and 33% respectively. Factors pertaining to successful outcome using ITT reported in the literature were also evident in our study: peak level of inhibitor titre, duration of inhibitor and genotype. Although inhibitor development remains a serious complication for individuals with haemophilia, the eradication of inhibitors can be achieved in the majority of patients.

There is no conflict of interest to disclose

A214

Meeting Room 2/3
ASTH Free Communications 2
O120

0830-1000
0930

Low Incidence of High Titre FVIII Inhibitors in Children with Severe Haemophilia A While Only Treated with an Intermediate Purity FVIII Concentrate (BPL 8Y). Experience from Five Centres in the UK

Simon Brown

Haemophilia Centre, Royal Children's Hospital, Brisbane; Pathology Queensland, Royal Brisbane and Women's Hospital, Brisbane, Queensland

Aim

High titre FVIII inhibitors remain a significant complication of replacement therapy in severe haemophilia A (SHA). It remains controversial whether the type of FVIII concentrate is a risk factor for inhibitor development. Previous reports have demonstrated a low incidence of FVIII inhibitors in children with SHA who only received an intermediate purity FVIII concentrate (BPL 8Y). The aim of the current study was to obtain data on BPL 8Y use in relation to the incidence of high titre (>5 BU/mL) inhibitor development and the underlying FVIII gene mutations.

Method

Seventy-four children with SHA treated solely with BPL 8Y were identified. A retrospective analysis of the clinical and laboratory data identified patients who had developed high titre inhibitors. The length of time from first exposure to 8Y to either inhibitor development or switch to an alternative FVIII concentrate was used to analyse inhibitor development in this cohort.

Results

The median age at first exposure to BPL 8Y was 12 months (range 0.5-70) and the median duration on BPL 8Y was 78 months (range 15-149). The cumulative incidence of high titre inhibitors in this cohort was 3.2% over 72 months; in comparison the cumulative incidence of high titre inhibitors was 13% over 72 months for a recombinant FVIII concentrate. Although molecular analysis is incomplete, 26 of 54 individuals tested possess the intron 22 inversion. In a further 20 individuals, an underlying mutation in the FVIII gene has been detected. The Hamsters FVIII database revealed information on inhibitor development for 12 of these mutations (6 have been associated with FVIII inhibitor development). The two children who developed high titre FVIII inhibitors have failed to achieve long term tolerisation with ITI.

Conclusion

The use of an intermediate purity FVIII concentrate (BPL 8Y) was associated with a low incidence of high titre FVIII inhibitors.

No conflict of interest to disclose

A215

Meeting Room 2/3
ASTH Free Communications 2
O121

0830-1000
0945

A Multi-Centre Retrospective Study to Assess the Safety and Efficacy of Biostate® in Children with von Willebrand's Disease (vWD)

Rebecca Howman^{1,2}, Chris Barnes³, Ram Suppiah⁴, Jamie Price¹, Julie Curtin⁵, Sue Russell⁶, Michael Seldon⁷, Lochie Teague⁸

1. Princess Margaret Hospital, Perth, WA,
2. King Edward Memorial Hospital, Perth, WA
3. Royal Children's Hospital, Melbourne, VIC
4. Adelaide Women's and Children's Hospital, Adelaide, SA
5. Children's Hospital Westmead, Sydney, NSW
6. Sydney Children's Hospital, Sydney, NSW
7. Mater Hospital, Newcastle, NSW
8. Starship Children's Hospital, Auckland, NZ

Aim

To evaluate the safety and efficacy of a high purity, double virus inactivated factor VIII/Von Willebrand factor concentrate (BIOSTATE®) in children with VWD.

Factor replacement therapy with Biostate® forms the mainstay of treatment in children with VWD in Australia and New Zealand although there is limited paediatric data on the clinical safety and efficacy of Biostate® when used in VWD.

Methods

A retrospective analysis of Biostate® use in children with VWD at 7 hospitals across Australia and New Zealand between April 2003 and November 2007 was conducted. Data was collected on patient demographics, treatment indication, dosage, adverse reactions, and haemostatic efficacy for each use of Biostate®.

Results

A total of 41 VWD patients (21 VWD type 1, 13 VWD type 2, 6 VWD type 3, 1 unknown; 24 male/ 17 female, age range 5months-15 years) were treated identified. Seven dental procedures, 31 surgical procedures, 49 non surgical bleeding episode and 2 patients on prophylaxis were recorded. Efficacy was recorded as excellent / good for all dental procedures, 26/31 for surgical procedures (moderate 3 /31, poor 1/31) and 43 / 49 for non surgical bleeds (5/49 moderate). Efficacy for prophylaxis was regarded as excellent / good for 1 patient and moderate for the remaining patient. The only adverse event recorded was the development of an inhibitor in the patient on prophylaxis who recorded moderate effect.

Conclusions

Biostate® is both safe and effective in VWD paediatric patients who require a FVIII/VWF concentrate for the management and prophylaxis of bleeding.

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

A216

Meeting Room 4
HSANZ: ALLG Symposium

1100-1230
1100

Novel Therapeutic Targets in Acute Myeloid Leukemia

Martin S Tallman

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Acute myeloid leukemia (AML) represents an heterogeneous group of diseases which vary in genetics, clinical manifestations, and outcome. While overall survival (OS) of younger patients has improved during the last 3 decades with increasingly intensive post-remission strategies and transplantation to generate graft-versus-leukemia effect, the outcome for older adults remains poor and it is not clear that any post-remission chemotherapy is beneficial. Well-recognized prognostic factors include age, intensity of post-remission therapy (younger adults only) and cytogenetics which distinguish favorable, intermediate, and unfavorable groups with OS of 5 years of approximately 55%, 40%, and 10%, respectively. Among the large group of patients with normal karyotypes, recently described mutations in or overexpression of specific genes facilitate classification, contribute to prognosis and serve as targets for drug development. Patients with a mutation in NPM1, but not FLT3 appear to have a relatively favorable prognosis and may not benefit from allogeneic hematopoietic stem cell transplantation (HSCT). Alternatively, patients with core binding factor leukemias with mutations in c-KIT have an outcome similar to that of patients with unfavorable-risk cytogenetics and allogeneic HSCT appears justified. Gemtuzumab ozogamicin, an immunoconjugate directed at the antigen CD33, has modest single agent activity, but may be more effective when combined with chemotherapy. Similarly, while FLT3 inhibitors alone demonstrate some biologic activity with reduction in peripheral blood blasts and occasionally marrow blasts, but no remissions, their true benefit may emerge when combined with chemotherapy. Histone deacetylase inhibitors such as valproic acid, SAHA, and depsipeptide; hypomethylating agents such as 5-azacytidine and decitabine; antiangiogenesis agents such as Bevacizumab; and farnesyltransferase inhibitors are all promising. The novel nucleoside analog clofarabine appears particularly active in older adults including those with unfavorable cytogenetics. The discovery that the plant-derived sesquiterpene lactone parthenolide and its dimethyl analog target the leukemic stem cell is among the most exciting new developments.

A217

Meeting Room 4
HSANZ: ALLG Symposium

1100-1230
1145

How Do We Manage Mantle Cell Lymphoma in 2008?

Simon Rule

Portsmouth, UK

Mantle Cell Lymphoma (MCL) is a rare and aggressive form of non-Hodgkin's lymphoma (NHL) accounting for 6-8% of all cases. Patients with MCL have the shortest median time to progression and the shortest median survival of all lymphoma sub-types after first line treatment. Unlike some other lymphoma sub-types MCL is very rarely localised. Most patients present with widespread lymphadenopathy often with constitutional symptoms and an unusual feature of this disease is the predilection to involve the GI tract, which is virtually universal if it is actively looked for. There are many chemotherapy regimens used in MCL but no consensus as to the treatment of choice. It affects predominantly elderly patients, which means that many of the published treatment regimens are not applicable. CHOP based chemotherapy has been the commonest regimen adopted because of the aggressive nature of the condition. The addition of Rituximab to this and other regimens has been widespread despite compelling evidence that this improves outcome. In younger patients the use of high dose Ara-C as the cornerstone of the initial therapy looks to be well established but which precise regimen used is the subject of on-going studies. The consolidation of responses post such therapy with an autologous transplant has recently been shown to be highly effective and the precise role of an allograft is being defined. There is a small cohort of patients who exhibit an indolent clinical course, some of which present with a lymphocytosis in whom a watch and wait approach is legitimate. A number of single agent drugs have been shown to have activity in MCL. Perhaps the most exciting of these at the moment is Bortezomib (Velcade). Bortezomib demonstrates synergistic activity with a number of agents and these are being explored in the clinic. There are a number of other agents that have been used which demonstrate activity against MCL such as Temsirolimus, Lenalidomide, Flavopiridol, Bendamustine, Enzastaurin as well as histone deacetylase inhibitors, bcl-2 inhibitors and a range of other early agents. Where these and other agents will fit in the treatment schema of MCL will be defined as trials progress.

A218

Riverside Theatre
Combined ANZSBT/ASTH: Thrombocytopenia
Sponsored by Amgen

1100-1230
1100

Thrombopoietic Agents in Patients with ITP

James Bussel

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

ITP is a relatively common disorder of thrombocytopenia caused primarily by antibodies to platelets. These auto-antibodies cause peripheral platelet destruction and also apparently impaired platelet production. Bleeding is variable although generally related to the platelet count and serious bleeding occurs (intracranial hemorrhage, ICH) but is fortunately distinctly uncommon. Initial therapy consists of IVIG, IV anti-D, and steroids and in general intends to increase the platelet count by inhibiting platelet destruction. Second line treatments include rituximab, danazol and splenectomy. Their use depends upon individual physician and patient preference and the features of a given case not least of which may be the extent of the response to the treatment(s) that was tried initially. Third line treatments are primarily immunosuppressive including cyclophosphamide, azathioprin, interferon, mycophenolate mofetil, colchicine and other agents. Starting in 2006, several studies have been completed of the use of novel thrombopoietic agents in ITP. Thrombopoietin was initially cloned in 1994 and soon thereafter two forms of it went into clinical trial. Proof of principle was achieved early on and there was efficacy in the non-meloablative setting but antibodies to these agents (primarily described for MGDF) lead to their discontinuation from therapy. Second generation agents have been primarily tried in patients with chronic ITP. A series of studies with 2 agents, AMG531 (romiplostim or Nplate) and Eltrombopag (promacta or revolade) have demonstrated substantial efficacy, little toxicity (thus far), and good tolerability. In addition, one of these agents has been used with success in allowing the treatment of hepatitis C infected patients with interferon and ribavirin. Finally two additional thrombopoietic agents have entered trial. The findings with these agents as of fall 2008 will be reviewed in detail and the newer areas of treatment considered.

A219

Riverside Theatre
Combined ANZSBT/ASTH: Thrombocytopenia
Sponsored by Amgen

1100-1230
1140

The Molecular Control of Platelet Life Span

Emma Josefsson¹, Simone Schoenwaelder², Matthew Goschnick¹, Michael White¹, Kylie Mason¹, Andrew Roberts¹, Shaun Jackson², David Huang¹, **Benjamin Kile¹**

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²*The Australian Centre for Blood Diseases, Melbourne, Victoria, Australia*

We have recently demonstrated that the circulating life span of platelets is regulated by members of the Bcl-2 family of proteins, which control the intrinsic apoptosis pathway. Pro-survival Bcl-x_L is the critical regulator of platelet life span, functioning to keep pro-death Bak and Bax in check, thereby maintaining platelet viability. After 5-10 days in the circulation, platelets not consumed in hemostatic processes initiate a Bak and Bax-dependent cell death program and clearance from the bloodstream. Studies with the BH3 mimetic compound ABT-737, which inhibits Bcl-x_L, have shown that platelets induced to undergo cell death *in vitro* exhibit many of the hallmarks of apoptosis in nucleated cells, including mitochondrial damage, caspase activation and externalization of membrane phosphatidylserine (PS). PS exposure is also a feature of activated platelets, which employ it to drive pro-coagulant activity. We now have evidence to suggest that two distinct pathways lead to PS exposure in platelets, one regulated by Bcl-2 family proteins and caspases, the other dependent on calcium and calpains. We are currently examining the possibility of cross-talk between these pathways, and whether there is a role for Bak and Bax in mediating aspects of platelet function in addition to the control of life span.

A220

Riverside Theatre
Combined ANZSBT/ASTH: Thrombocytopenia
Sponsored by Amgen

1100-1230
1205

Heparin Induced Thrombocytopenia - Current Controversies in Diagnosis and Management

Simon McRae

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Heparin induced thrombocytopenia (HIT) is a immunological complication of heparin therapy associated with the potential for severe morbidity and even mortality. Prompt diagnosis and initiation of alternative anticoagulant therapy is required to avoid adverse clinical outcomes. Access to gold standard testing for HIT is limited in the majority of clinical centres, and alternative diagnostic strategies are therefore often used to confirm or exclude this diagnosis. The clinical utility of various approaches including pre-test probability scoring systems, enzyme-linked immunosorbent assays, and newer rapid laboratory assays, alone or in combination, will be discussed. The advantages and disadvantages of various treatment strategies will also be discussed, including management of patients with renal failure, and the potential role of fondaparinux. Finally, opportunities for future research will also be outlined.

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