BSBMT NEWS
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INSIDE...

Good evening and welcome to another pleasant summer's eve in the saloon bar of The Transplanter's Arms. Here, as the gloaming creeps across the river meadows outside and the gentle swish of the fly-baited rod drifts in through the opened window, I urge you to take a seat and listen to the murmur of conversation; for here you are amongst friends. As you tune in to the talk in the pub tonight you may overhear the following interesting snippets.

In pride of place, leaning on the bar, **Rachael Hough** and **Nigel Russell** are talking about cords. Cord blood transplantation appeared like a meteor in the haematological firmament during the noughties, but meteor-like again, now appears to be falling to earth, with a significant decrease in the number of cord blood transplants being done. They tell us why that is and why, perhaps, we should do something about it.

Sitting majestically on the large throne-like chair by the inglenook, where he is supping his beer from a richly engraved tankard, we welcome the new BSBMT President, **Charles Crawley**, who has brought with him his inaugural Presidential column. On the window seat **Cristina Navarrete** and **Colin Brown** from NHSBT are eager to tell us about how next generation sequencing is changing donor selection in their registry. **Simon Butler** is standing animatedly near the door wanting to let us know about The Anthony Nolan's 'Way Back' programme. Perched demurely on a stool in the corner, **Rui Zhao** is biding her time - waiting for a lull in conversation when she can tell us all a fascinating story about magnesium. I'm here too, of course, keeping a watchful eye on things from behind the beer engines. I'm ready to quickly scotch any of those stories that start 'you wouldn't believe the size of the stem cell harvest I got the other day...' And, as the evening draws to a close, I can report back to those hanging on until closing time what happened when I went to the annual **BSBMT Scientific Day**.

I hope you enjoy your evening in The Transplanter's Arms and I look forward to welcoming you back here soon. Cheers!

Patrick Medd
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HITTING THE RIGHT CORD

The advent of cord blood as an effective stem cell source for patients lacking a well matched sibling or adult unrelated donor has represented an important advance in the management of children and adults with high risk haematological malignancies and, increasingly in children, non-malignant diseases.

Over the last couple of months there have been a number of significant national developments that will consolidate the important role of cord blood transplantation within the U.K and inform its future use. These include:

- Accumulating evidence from UK trials of the clinical effectiveness of cord blood transplantation
- A national commissioning policy for double cord blood transplants
- Increased Government investment in growing the UK's cord inventory
- The development of a national cord blood selection algorithm in order to assist transplant centres in cord blood selection.

These improvements to the collection, selection, commissioning and most importantly to the evidence around outcomes is welcome given the news that cord blood use has recently stalled in the UK. The positive news comes amid warnings that funding and support will not continue if the resource is not used.

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HITTING THE RIGHT CORD

Investment in the National Cord Bank

Since the Stem Cell Strategic Forum report in 2010 the Government has provided around £9 million to NHS Blood and Transplant and Anthony Nolan to collect and bank over 9000 umbilical cord blood units. This year there will be an extra £1.75 million to continue as the 2014 follow-up report included evidence of how access to cord blood had increased availability to potentially curative allografts, particularly for patients from BME groups, as well as robust and novel evidence of cord blood's cost-effectiveness.

UK cord transplant outcomes are very good

Data showing improved outcomes after cord blood transplants in both adults and children continues to grow.

The BSBMT cord blood transplantation trials have shown very low day 100 transplant related mortality (the primary end-point) of less than 10% along with excellent survival rates. The overall survival in patients on the reduced intensity conditioning protocol (RIC CBT) is 63% and on the full intensity protocol (MAC CBT) is 73%.

Recent retrospective analyses of cord blood transplants in both adult patients (John Snowden *et al*) and paediatric patients (Paul Veys *et al*) also show that UK outcomes of cord blood transplants are excellent and comparable with those reported from around the globe. In 335 children and adolescents receiving a cord blood transplant in the UK between 1998-2012, the 4 year overall survival was 52% and 75% for malignant and non-malignant disorders respectively. In 154 adult CBT (≥18 years), the 4 year overall survival was 53% for early and 44% for intermediate stage of disease.

Possible new roles for cord blood transplantation

At the same time unpublished data from Anthony Nolan (Shaw *et al*, submitted) indicates that outcomes from 9/10 matched donors are less good than previously reported. In that study recipients matched for 10/10 HLA alleles had significantly better overall survival and reduced transplant related mortality compared to those matched at 9/10 or <9/10 (5yr OS: 43.1 vs 35.6 vs 28.4 respectively, p=0.001 and TRM at 1yr : 23.8% vs 32.0% vs 38.9% respectively, p=0.004). Mismatch at HLA-B and DQB1 appeared most adverse, while mismatching for HLA-A -C or -DRB1 resulted in no statistically significant difference in survival. These data are important, as previous UK studies have suggested a similar outcome for mismatched transplants and this no longer appears the case. Furthermore, this adverse outcome for an HLA mismatch was compounded if there was a CMV mismatch between donor and recipient, particularly R+/D- where the 5yr OS was 38.6%% but only 25.8% if there was a CMV mismatch. Although there are no studies which have directly compared cord blood with 9/10 matched donors the emerging UK data suggest that cord blood might have a role to play in transplants where previously a 9/10 matched adult would have been chosen.

Cord blood transplants are now routinely commissioned

So why have the number of cord blood transplants been stalling in the UK? The introduction of haploidentical donors has undoubtedly played a part.

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HITTING THE RIGHT CORD

And until recently there were substantial geographical variations regarding the commissioning of double cord blood transplants, with some centres being unable to secure funding. However, double cord transplants are now included in a national commissioning policy and there is no longer any requirement for individual funding requests (IFRs) or prior approval to be sought (although prior notification is still required).

Cost effectiveness of cord blood transplants

The 2014 report of the UK Strategic Oversight Committee addressed the important question of the health economics of cord blood transplantation. This detailed piece of work demonstrated that a cord blood inventory of 30,000 donations would achieve a cost per additional quality-adjusted life year (QALY) of £10,400. Importantly this compares favourably with the £15,000 threshold used by NHS England to evaluate the effectiveness of NHS spending decisions.

Improvement in quality of the UK cord blood inventory

Unquestionably, the Government support and funding for cord blood collection and banking has resulted in the UK cord inventory being amongst the best in the world. Both UK cord blood banks are FACT accredited and UK cords are the most competitively priced of any CBB.

Bigger, better cords than ever before are being banked and high resolution typing of all new cords mean that there is less cost upfront. These changes and improvements to the search reports also mean that transplant centres can identify the units we need more quickly.

Support in selecting best cord units

BSBMT's Cord Blood Working Group received feedback that transplant centres needed more support in selecting cord blood units so we have developed national clinical consensus guidelines to help you choose the best cords and these will be published and circulated shortly. In the meantime if you need advice please do contact either of us and we will be happy to help.

The Cord Selection Committee has also been reformatted with a new chair, Dr Kay Poulton, to provide advice on specific cord blood unit selection for individual patients.

Summary

It is an exciting time for cord blood use in the UK. Studies continue to confirm the excellent outcomes for patients who receive either UK-sourced or international cord blood donations. The Government funding for cord blood collection and banking is resulting in bigger and better cords, typed to higher resolution and the commissioning environment is more conducive to double cord blood transplants.

Alongside this we have increased the support transplant centres can access to help them select the best units for individual patients and this will undoubtedly lead to more cord blood transplants happening and more patients having successful outcomes. There has never been a more optimistic time to be involved in cord blood transplantation.

Dr Rachael Hough and
Professor Nigel Russell

Co-chairs of the BSBMT Cord Blood Working Group

Images come from Wellcome Images, a website operated by Wellcome Trust, a global charitable foundation based in the United Kingdom.

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***Dissected newborn child showing connection to placenta**. Wellcome L0012880" by http://wellcomeimages.org/indexplus/obf_images/f8/ac/1428e9776c25e9dece1ff4f0dce9.jpgGallery: http://wellcomeimages.org/indexplus/image/L0012880.html. Licenced under CC BY 4.0 via Wikimedia Commons*

B ***The umbilical cord.** Wellcome L0012879" by http://wellcomeimages.org/indexplus/obf_images/d0/3b/16e0e4b5f654fa4c7590a67c*

69e6.jpgGallery: http://wellcomeimages.org/indexplus/image/L0012879.html. Licenced under CC BY 4.0 via Wikimedia Commons

Magnesium:

more than just another mineral?

XMEN disease: a new primary immunodeficiency affecting Mg^{2}+ regulation of immunity against Epstein-Barr virus Li F, Chaigne-Delalande B, Su H, Uzel G, Matthews H and Lenardo MJ ***Blood*** *2014* ***123****:2148-52*

As we take our first steps into the intimidating world of the transplant clinic we rapidly learn, after metaphorical clips around the ear from our elders and betters, to be vigilant about the ciclosporin levels, the viral PCRs, and perhaps, the magnesium. The reason for this last is that calcineurin inhibitors cause notorious renal wasting of magnesium and hypomagnesaemia can be dangerous, leading to the risk, at worst, of cardiac arrhythmias and seizures. Having checked the magnesium and, if necessary, supplemented it, we move on and turn our minds to other matters, like 'what's for lunch?'

Perhaps we dismiss it too rapidly though, as Li and co-workers review of their work on the newly described 'XMEN' syndrome tell us that magnesium may have a much more fundamental role in immune regulation than we previously had thought.

XMEN stands for X-linked immunodeficiency with magnesium defect, EBV infection, and neoplasia. The clinical features of the syndrome are listed in the table below. Li and colleagues describe their identification of a series of patients with persistently high EBV viraemia, an inverted CD4:CD8 ratio and a propensity to develop EBV positive lymphoproliferative disorders.

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Clinical Features of XMEN disease	
Major Features	Minor Features
EBV positive lymphoproliferative disorders	Sinusitis
Decreased CD4:CD8 ratio	Otitis media
Splenomegaly	Streptococcal pharyngitis
Dysglobulinaemia	Molluscum contagiosum
High EBV titres	Varicella and recurrent zoster
	Neutropenia and thrombocytopenia
	Haemolytic anaemia

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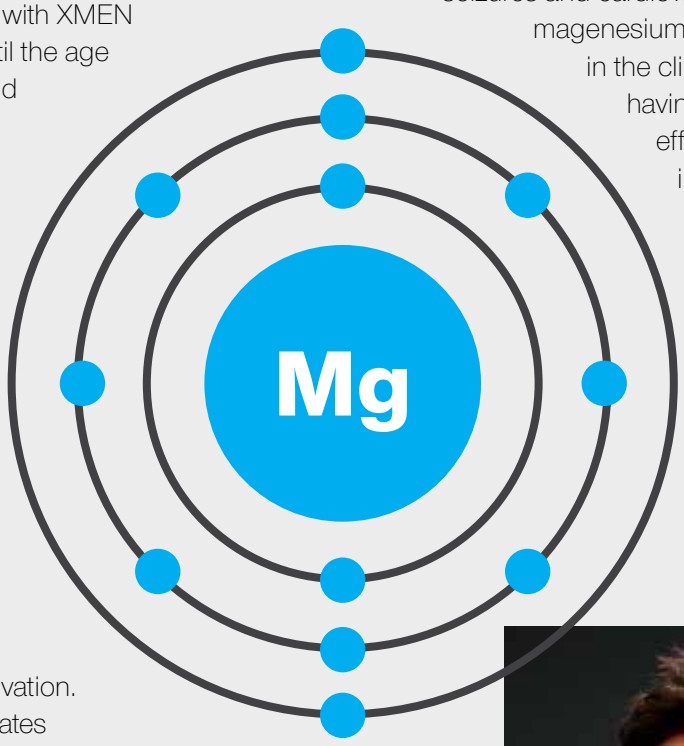
As the patients are all male it will come as no surprise that the mutated gene underlying the disorder finds itself on the X chromosome and has been identified as MAGT1. MAGT1 is a 70 kb gene with 10 exons encoding a 335 amino acid protein which functions as a transmembrane magnesium ion transporter. The gene is highly conserved and expressed in all mammalian cells, with particularly high expression in haematopoietic cells.

So far, so puzzling – presumably there must be other proteins capable of transmembrane transport of magnesium, otherwise such a mutation would be lethal and one of the patients with XMEN syndrome did not present until the age of 45. Furthermore why should the loss of this magnesium transporter result in an immune deficit? The loss of this particular protein seems to result in a reduction in the intracellular concentration of free magnesium ions (which account for only about 5% of total intracellular magnesium). Loss of MAGT1 means loss of a rapid transient T cell receptor induced inward current of magnesium ions required for optimal T cell activation. The magnesium current activates phospholipase Cγ-1, which in turn controls the TCR-gated calcium flux from the endoplasmic reticulum necessary for T cell activation. It is this calcium pathway in T cells that is targeted by calcineurin inhibitors such as ciclosporin. Suddenly this starts to make sense.



Furthermore, the chronic reduction in free intracellular $Mg^{2}+$ appears to have an effect on cytotoxic T cell and NK cell function, as a certain basal level of intracellular free $Mg^{2}+$ is required to maintain expression of NKG2D. NKG2D is an activating receptor that signals cytotoxic killing in response to binding its ligand, which is expressed on virally infected cells and tumour cells. Thus low intracellular basal magnesium levels impair cytotoxic antiviral responses and tumour surveillance.

What comes next is perhaps the most interesting bit of all. Two of the patients in their series underwent allogeneic stem cell transplantation as treatment for the disorder. Unfortunately neither transplant was successful, with deaths from sepsis and from multiorgan failure with haemophagocytic syndrome. However, it appears that in patients with the condition, oral magnesium supplementation with magnesium threonate can increase intracellular $Mg^{2}+$ concentration, restore NKG2D expression and decrease EBV viral load *in vivo*. This raises the intriguing possibility that rather than just preventing seizures and cardiovascular toxicity, the magenesium supplements we dispense in the clinic each week may be having a much more profound effect on the reconstituting immune system of our patients.

Rui Zhao and Patrick Medd

What an X man looks like! *Hugh Jackman by Gage Skidmore" by Gage Skidmore. Licensed under CC BY-SA 3.0 via Wikimedia Commons - https://commons.wikimedia.org/wiki/File:Hugh_Jackman_by_Gage_Skidmore.jpg#/media/File:Hugh_Jackman_by_Gage_Skidmore.jpg*

BSBMT Scientific Day

7 May 2015

Thursday 7th May 2015 saw an event with an unexpected outcome that is likely to have profound effects on the direction the whole country takes for the next five years. I refer, of course, to the BSBMT Scientific Day; by strange co-incidence there was also a general election that day, but nobody remembers that now.

The day was divided into two halves: a morning scientific session chaired by Persis Amrolia and Karl Peggs and an afternoon devoted to the current state and future direction for clinical trials in SCT under the auspices of the clinical trials committee and chaired by Andy Peniket. Between these two halves, like the delicious filling in an artisan bread sandwich, came the BSBMT annual open meeting under the direction of its new President, Charles Crawley.

Gene Editing for Immune and Haematological Disorders

The morning opened with the first keynote speaker, **Professor Andrew Scharenberg** from Seattle Children's Hospital who spoke about his laboratory's work on translating gene editing therapies for hematologic diseases. Summarizing the present state of the gene-editing field, he noted that there are now many excellent platforms for generating nuclease reagents that specifically cleave at mutation sites in genes of patients affected by inherited hematologic diseases. The field is now focused on developing the processes to apply nuclease reagents to initiate homology-directed repair, and in refining methods for manufacturing personalized gene editing therapies. He showed data from his and his collaborator's work on utilization of mRNA-based nuclease expression and adeno-associated viral template delivery to achieve efficient homology directed repair in T-cells. These latest editing processes have now also been used to achieve efficient repair of the CD40L gene in T-cells from patients suffering from hyper-immunoglobulin M syndrome, and it is hoped that these methods will enter initial clinical application, most probably with collaborators in the UK, in 2016.

Professor Marina Cavazzana from Paris Descartes University then presented her work on the clinical application of gene therapy for inherited disorders. In the field of congenital immunodeficiencies gene therapy approaches may be able to provide an alternative to allo-SCT, through the use of gene-modified autologous SCT.

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Title	Presenter	Institution
RNAseq for identification of pathogens in cases of undiagnosed encephalitis in immunocomproised children	**Julianne Brown**	Great Ormond St Hospital
Pre-transplantation survival outcomes following allogeneic transplantation in Hodgkin lymphoma	**Yasmin Reyal**	University College London Hospitals
High rates of passive CMV antibody acquisition pre-allograft in patients receiving plasma-rich CMV unselected and leukodepleted blood components - a caution for donor selection	**Robert Lown**	University Hospital Southampton
Monocyte-derived macrophages in skin GVHD: a proinflammatory infiltrate and novel therapeutic target	**Laura Jardine**	Newcastle University
Genetic silencing of the glucocorticoid receptor in virus-specific T cells	**Laurie Menger**	UCL Cancer Institute
Generation of 252 HLA class I genomic sequences in a single sequencing reaction using DNA barcodes and SMRT sequencing technology	**Neema Major**	Anthony Nolan Trust

The requirements for this to be successful may include the need to select a disorder with a well-known pathophysiology, where the gene repair will provide a selective advantage to the cells, where the target cell is likely to have prolonged lifespan and where expression can be specifically targeted to haematopoietic stem cells. The various disorders where this approach has been tried meet these requirements to differing extents.
Thus for X-linked SCID the gene repair provides a significant selective advantage, there is no need for cytotoxic conditioning to eradicate the existing cell population, the target cell has a very long lifespan and there is no need for expression of the gene to be specifically limited to the target cell.
By contrast in attempting to use gene therapy to correct β-thalassaemia the gene repair provides at best a poor selective advantage for the cell, there is a need for myeloablative conditioning, the target cell lifespan is short and it's certainly required that the expression of the repaired gene (β–globin) is confined to the target cell population (erythrocytes).

The results of early clinical work in these disorders are encouraging.
The Paris-London collaboration for treatment of X-linked SCID, where the defect is a mutation in the common γ–chain of the IL2/4/7/9/15 and 21 receptors, reports a 2 year EFS of 85% in the gene therapy group, with one report of insertional mutagnesis. In comparison with a haplo-identical SCT 'control' group, the gene therapy approach yields higher CD3 counts and thymic output. In patients with Wiscott-Aldrich syndrome (WAS) with no suitable 10/10, 9/10 or cord donor available, a phase I/II trial has demonstrated sustained expression of WAS protein is possible in B, T and NK cells to 36 months of follow-up.
In successful treatment the clinical syndrome resolves, and platelet function (although not number) improves.

The John Goldman Abstract Prizes

Karl Peggs (UCL Cancer Institute) then chaired the John Goldman Abstract Prize Competition. The titles, presenters and institutions of the six short-listed abstracts (three scientific and three clinical) are shown in the table above. The clinical abstract prize was won by **Julianne Brown** of GOSH for her presentation entitled 'RNAseq for identification of pathogens in cases of undiagnosed encephalitis in immunocomproised children'. In 63% of cases of encephalitis no causative organism is identified, partly because an organism will only be found if it is specifically looked for. To overcome this problem, she obtained post-mortem brain biopsy tissue from three paediatric cases where no pathogen had been identified by PCR. Deep-sequencing total RNA on the Illumina platform and discarding reads that matched human genome sequences, identified a viral genome in each of the cases.

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Dr Karl Peggs and Mrs Julianne Brown, winner of the clinical abstract prize


Dr Karl Peggs and Dr Laurie Menger, winner of the scientific abstract prize


BSBMT President Dr Charles Crawley presents the BSBMT long service award to Professor Charlie Craddock

Case one proved to be an astrovirus, despite astrovirus PCR being negative at the time of original investigation. Further work demonstrated this to be a novel virus whose sequence clustered with astroviruses found in animal hosts and which had not previously been described as a human pathogen. The second case identified a coronavirus of a relatively novel genotype (genotype E) only described for the first time in 2014. Case three was a mumps virus, which was sequenced and found to be 99.6% identical to the vaccine strain given to the patient pre-diagnosis. This elegant work already has clinical applications in the field and may have more with improved bio-informatics.

Laurie Menger of the UCL Cancer Institute won the scientific abstract prize for her work on the generation of glucocorticoid-resistant cytotoxic T cells. CMV infection remains one of the most common potentially lethal complications following allogeneic HSCT and the patients at greatest risk of CMV infection are those receiving enhanced immune suppression to treat GvHD. Since first-line treatment for GvHD is glucocorticoid therapy, which induces lympholysis, these patients cannot be treated with classic CMV-specific adoptive cellular therapies. In order to restore virus-specific T-cell immunity resistant to glucocorticoid treatment, she generated TALENs (Transcription Activator-Like Effector Nucleases)-induced Glucocorticoid Receptor gene inactivation in highly purified CMV-specific T cells. TALEN-modified CD8+ T cells retained specific killing activity against target cells pulsed with the CMV peptide pp65 in the presence of dexamethasone (DEX). Furthermore, when transferred into NOD-SCID Gamma (NSG) mouse recipients, TALEN-modified CD8+ T cells were efficacious in generating xeno-GvHD in the presence of DEX, hence providing evidence for their functional activity and resistance in vivo. Dr Menger pointed out that beyond this direct and immediate translational application in the co-treatment of CMV viraemia and GvHD, induction of glucocorticoid-resistance has the potential for therapeutic exploitation in T-cell based anti-cancer therapies - particularly in the therapeutic use of genetically redirected CAR (Chimeric Antigen Receptor)-modified T-cell therapies.

Following the BSBMT open meeting, the afternoon session opened with the presentation of the abstract prizes. This was followed by the presentation of a special award to **Professor Charlie Craddock** (University of Birmingham) in recognition of his numerous contributions to the field of SCT and his long and distinguished record of contributions to the work of the society.

The CTC session

Andy Peniket (Oxford University Hospitals) led the afternoon session discussing the work of the BSBMT clinical trials committee (CTC).

Dr Peniket opened with an overview of what the CTC might hope to achieve. Despite the success of SCT as a treatment in many areas, fundamental questions remain unanswered; transplant-related mortality and relapse remain problems and we lack an understanding of much of the basic biology underlying these processes. For instance, we don't really understand why some AML relapses after transplant and some doesn't, nor do we have a robust biological basis for the vast variations in the incidence and extent of GvHD.

He saw the role of the CTC as threefold: to contribute to understanding the biology of SCT, to help improve outcomes in SCT and to facilitate the training of the next generation of transplant physicians. The challenge will be to try and deliver these aims, which will require expensive prospective clinical trials, in a climate where the NHS specialist commissioning budget was overspent by £1 billion last year.

Prospective Studies

Charlie Craddock then re-took the stage to consider the state of prospective trials in SCT. Whilst the CTC is very successful in producing important retrospective studies, fewer than 5% of UK SCT are performed within a prospective clinical trial. The UK benefits from good clinical SCT teams, the work of the BSBMT and world-class science.

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However, the process of set up and opening of prospective clinical trials remains painfully slow. He identified the lack of a hub for SCT trials, the lack of an SCT trial network and the lack of resources to support trials within centres as key barriers to progress.

George Freeman, the Minister for Life Sciences, has spoken of his desire for the NHS to be a test bed for developing therapies and dramatically reducing the time taken for novel therapies to be delivered in routine clinical practice. Thus there is political will to improve the current situation. The Leukaemia and Lymphoma research fund (LLR) has sought to address this through the development of the Trials Acceleration Programme (TAP), which is modelled on the US Clinical Trials Network. TAP aims to reduce the time to trial set up by more than 50% and facilitate rapid recruitment to trials and its initial efforts have been promising, with trials such as MAJIC recruiting rapidly. A further aim is to embed good quality basic science within these studies.

Prof Craddock proposed that the UK should aim to develop a Transplant Trials Network (UKTTN) to try and deliver these aims specifically in the field of SCT. He suggested that work of such a network would be subject to peer review and oversight by the CTC. A key part of the network would be the development of a UK hub to coordinate its work. He estimated that the cost of running such a hub would amount to £3M for 4 years.

Following this excellent overview of the current landscape and its challenges were updates from the CIs of current prospective studies. Ronjon Chakraverty (Royal Free Hospital) reported on the ProT4 trial, which aims to explore in a randomised fashion the potential of CD4-enriched DLI to speed immune reconstitution, decrease GvHD incidence and improve SCT outcomes. Patients undergoing a 10/10 HLA-matched fludarabine/melphalan/campath conditioned sibling allo-SCT for haematological malignancy are eligible. If the patient is GvHD-free at day100 following a ciclosporin taper then they are randomised in a 2:1 fashion to receive a CD4-enriched DLI of 1 x 10^6 CD4+ cells/Kg or not. The primary endpoint is PFS at 1 year with secondary outcomes examining GvHD incidence, T cell reconstitution, chimerism, infection rates and TRM. Prof Chakreverty reported that, with 12 sites open, recruitment is above target, but that only 50% of patients are making it to randomisation due to more GvHD than anticipated.

Charlie Craddock then updated the audience on the FIGARO study that is examining whether the FLAMSA-Bu conditioning regimen has a role in improving outcomes of allo-SCT in high risk AML and MDS. To date 77 patients of a target of 122 have been recruited. The data monitoring committee have approved the continuation of the trial in November 2014 and the question of what a follow-on study might be is to be discussed at the trial meeting in July.

Kim Orchard (University Hospital Southampton) discussed a proposal for a study of targeted radiotherapy in ASCT conditioning for AL amyloidosis. ASCT is effective in this condition, but is hampered by a significantly higher TRM than is seen in myeloma ASCT. The proposed TRALA study would explore the role of an ^{90}Y-anti CD66 radio-immunoconjugate in delivering targeted radiotherapy to the marrow as SCT conditioning. This approach can potentially deliver 40Gy of radiotherapy to the marrow whilst limited the whole body dose to a much more tolerable 2Gy.

Lastly in this session Ram Malladi (University of Birmimgham) updated us on the progress of the AZTEC study that plans to assess the use of azacitidine as treatment for steroid-refractory GvHD in a single-arm open-label phase II design.

A lively and interesting general discussion of the state of prospective studies in SCT followed. There was a general feeling of collegiate optimism in the room and a feeling that a Transplant Trials Network was required to speed recruitment. Karl Peggs cautioned that there were threats to be considered as well. These include that fact that disease-specific rather than therapy-specific trials are often in competition for these patient groups and that there may exist a perception that the SCT community does not deliver prospective studies as effectively as it might.

Retrospective studies

Unlike prospective trials, retrospective studies in SCT suffer from far fewer obstacles and the CTC has a well-proven track record in delivering these. However, Dr Peniket emphasised that there was much that could be done to add value to these studies. This includes the delivery of better biological studies within the retrospective format and the incorporation of genomics information that should be so readily accessible in SCT patients and donors. In addition protocol harmonisation across the UK SCT community would increase the value of data from future retrospective studies by decreasing variability within the patient cohorts.

Rob Danby (Oxford University Hospitals) presented the results of his retrospective study on UK cord blood SCT. He examined all unmanipulated UK cord blood transplants performed between 1998 and 2012 collecting data via Eurocord.

The number of cord blood transplants dramatically increased in 2004-5 and, since 2011 has been decreasing while

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there is a corresponding increase in haplo-identical SCT. In paediatric malignant diseases overall survival at 5 years was 52% and improved outcomes were associated with higher cell doses and 6/6 HLA-matched cords. For non-malignant paediatric diagnoses 5y OS was 74%. In adults 154 transplants were examined which comprised 3% of UK unrelated donor allo-SCT. OS at 5 years was 35% with transplant in first CR associated with improved outcomes and T cell depletion significantly associated with impaired outcomes.

Chloe Anthias and Richard Szydlo (Anthony Nolan) presented data from the patient-donor project. Examining the outcome of campath-conditioned adult unrelated donor SCT they reported that 10/10 HLA-matched transplants were associated with significantly better outcomes that 9/10 matches. However, not all mismatches are equal, with HLA B and DQB1 mismatches being associated with poorer outcomes whilst HLA A, C and DR mismatched showed no such association. Once again their data demonstrate the importance of CMV matching, with the best outcomes being found for 10/10 HLA-matched and CMV-matched transplants. Dr Szydlo commented that the analysis was hampered by very significant amounts of missing data from the registries.

Matt Collin (Newcastle University) presented his work on protocol harmonisation. The number of variations in 'standard' SCT conditioning protocols is bewilderingly large and variations between them impede the interpretation of retrospective studies, where the criticism of a lack of uniformity in treatment can be made. Prof Collin felt that there was a general will to move towards harmonisation and that, due to requirements of the CRG and JACIE, we will probably have to do this in the end. He presented his work collating protocols gleaned from around the country. These could be plotted in a cluster dendrogram indicating how closely different versions of the FMC protocol, for instance, are related to each other. The next step follows an anonymous audit of the varied protocols and work to identify the most logical, most popular, most rational and shortest protocols. The ultimate aim would be to identify a single national version of each protocol for distribution.

Thus the day drew to a close and the delegates scurried away to cast their votes in the general election – probably an easier decision to make than agreeing on a national standard for SCT conditioning.

Successful implementation of Next Generation Sequencing (NGS) by NHSBT to improve the provision of high resolution HLA typed unrelated haematopoietic stem cell (HSC) donors

Cristina Navarrete and Colin Brown

HLA compatibility is an important risk factor in the outcome of haematopoietic stem cell transplantation.

The degree of HLA matching between recipient and donor strongly correlates with the risk of acute graft versus host disease (aGVHD) that, together with infection and disease relapse, is one of the three main causes of transplant related mortality (TRM). Although the best outcomes, in terms of aGVHD, are obtained using an HLA-identical donor, only 30% of patients have such a donor available. In the unrelated bone marrow or PBSC transplant setting, high-resolution (HR) or second field (i.e. >4 digit) HLA class I (HLA-A, -B, -C) and class II (-DRB1, -DQB1) matching between the recipient and the donor significantly improves clinical outcomes[1]. More recently the benefits of high-resolution HLA allele matching in the outcome of umbilical cord blood (UCB) transplants have also been documented, although in this setting other parameters, such as total nucleated cell (TNC) and CD34+ cell dose, are also important factors[2].

Unfortunately the majority of the over 25 million unrelated adult and over 600,000 cord blood donors registered and available through the national and international HSC

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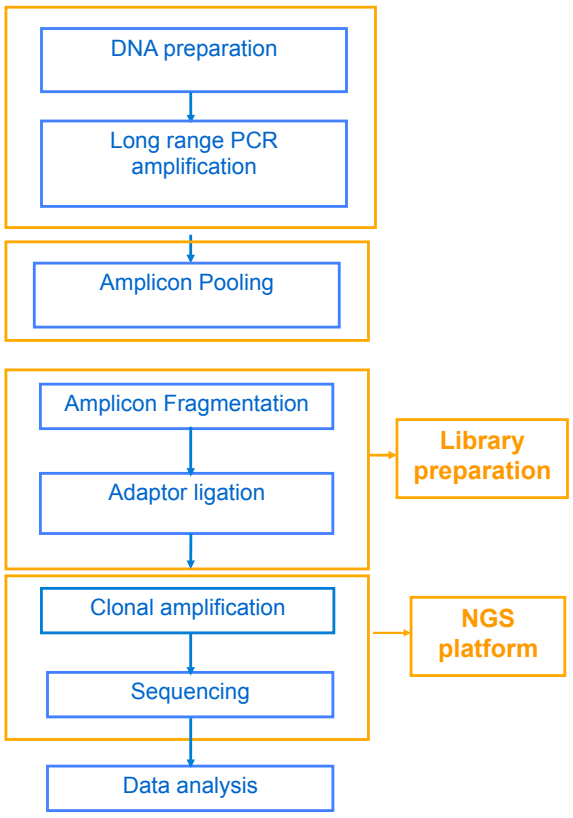
donor registries (such as BMDW - www.bmdw.org), have been typed at a lower or medium resolution level, delaying the availability of a HR HLA-matched donor with associated risks to the outcome of the transplant. Thus, the ability to register donors upfront with HR HLA types would certainly not only speed up the process of donor search and selection, but would also remove the need for additional HLA typing, and its associated costs, at the point of final donor selection.

HR allele HLA typing can be performed using conventional sequence based typing (SBT) using Sanger sequencing. However, this technique - which is not easily transferable to a high throughput setting - can sometimes yield ambiguous results, due to the co-amplification of the two chromosomes containing the HLA genes. This leads to difficulties in determining whether polymorphisms are on the same (*cis*) or different (*trans*) chromosomes. The *cis* and *trans* ambiguities can be overcome by using a new sequencing approach involving solid phase cloning and massive parallel sequencing[3]. This new technique, also known as Next Generation Sequencing (NGS), has the capacity to produce large amounts of data relatively quickly and cheaply. On single samples, NGS can generate a high proportion of an individual's genetic sequence in one experiment. However, when combined with novel bar-coding technology, it can also be used to target specific regions of the genome from many different individual samples in one pool e.g. the genes of the HLA region.

Figure 1: **Next Generation Sequencing Work Flow**



The NGS process is complex and involves many steps (see Figure 1), some of which are labour intensive. The first step includes DNA preparation with PCR amplification of the required region, gene or exon and pooling of the resulting amplicons. This is followed by the fragmentation of these amplicons and the ligation of adapters and indexes or identifiers, a step also known as library preparation. The fragmented and ligated products are then clonally amplified and sequenced and the sequence data are analysed using in-house or commercial software. Some of these steps can be automated, but require the use of sample tracking systems to transfer data between instruments and to generate control files for the liquid handling robots to ensure a high level of quality control. Although the laboratory skills and technology needed for NGS are similar to those used in current routine assays, the software needed to analyse the results is several levels more complex than that used for other HLA typing techniques. Because NGS generates hundreds of thousands of sequences, sorting and aligning of these sequences to the reference genome can be extremely challenging and, in order to achieve this, complex analysis algorithms have been developed. The success of any NGS implementation relies heavily on strong bioinformatics support in the laboratory.

Two main approaches have been used in the application of NGS to the HR definition of HLA alleles.

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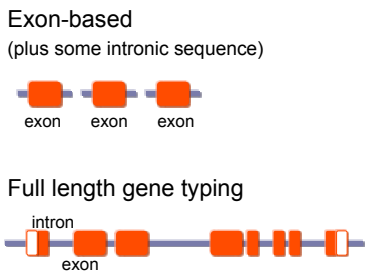
One involves the amplification and sequencing of the exons containing the polymorphic positions of the required genes (exons 2 and 3 for HLA –class I, A, B and C and exon 2 for HLA class II DR, DQ and DP)[4]. The second approach involves the amplification and sequencing of the full length of the gene of interest[5] (see Figure 2).

Figure 2:
Strategies for HLA typing using NGS



The main advantage of this latter approach, is that it allows the detection of polymorphisms of all exons and introns of the relevant gene, thus reducing the number of ambiguities obtained with the exon-based approach. An example is given in Table 1.

Following a period of extensive evaluation and validation the NHSBT H&I laboratory at Colindale we have now successfully implemented the use of Next Generation DNA Sequencing (NGS) for HR HLA- A, B, C, DR and DQ typing of adult donors and cord blood units registered with the British Bone Marrow Registry (BBMR), using the full length gene PCR approach.

Our approach has satisfied stringent quality requirements at each stage of the process and attained high levels of concordance with historical HLA types obtained in over 1000 samples. The first NGS HR HLA typing results were added to the BBMR in February 2015 and in future all adults and cord blood donors recruited to the BBMR will be HLA typed to the highest resolution possible.

This significant achievement has enabled the BBMR to become the first registry in the UK and one of the first registries in Europe listing large numbers of unrelated donors, in both national and international registries, with a detailed analysis of their HLA types at the DNA level. This information will allow a reduction in the time and costs involved in the selection of the best HLA-matched donor for individual patients, since it will avoid the need to perform further additional testing. Thus in accordance with the UK Stem cell Strategy, the implementation of NGS for the HR HLA typing of volunteer registry donors will improve the speed of identification of the most suitable HLA-compatible donor. The aim of this strategy is to improve the outcomes in patients transplanted from matched unrelated donors.

Currently, the most cost effective application of NGS is in a high throughput setting, where a rapid turnaround time is not essential. Thus, one of the remaining challenges of this technology for its successful application in a diagnostic setting, is the adaptation to a low throughput or single sample testing environment with a fast turn around time for reporting HLA typing results.

Table 1: **Advantage of NGS HLA full length gene sequencing: 'One step' high-resolution HLA typing**

Data Source	Results	Ambiguity
Sanger SBT (exons 2 and 3)	HLA-A *02:01:01G	*02:01:01:01, *02:01:01:02L, *02:01:01:03, *02:01:08, 02:01:11, *02:01:14Q, *02:01:15, *02:01:21, *02:01:48, *02:01:50, *02:09, *02:66, *02:75, *02:89, *02:97:01, *02:97:02, *02:132, *02:134, *02:140, *02:241, *02:252, *02:256, *02:266, *02:291, *02:294, *02:305N, *02:327, *02:329, *02:356N, *02:357
Sanger SBT (exons 2, 3, 4 plus GSSP)	HLA-A *02:01:01G	*02:01:01:01, *02:01:01:02L, *02:01:01:03
NGS using long range PCR	**HLA-A *02:01:01:01**	

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1. *Lee SJ , Klein J Haagenson M. et al (2007) High-resolution donor recipient HLA matching contributes to the succes of unrelated donor marrow transplantation. Blood.110:4576-4583.*
2. *Eapen M, Klein JP, Ruggeri A. et al (2014) Impact of allele-level HLA matching on outcomes after myeloablative single unit umbilical cord blood transplantation for hematological malignancy. Blood, 123;133-140*
3. *Metzker, M.L. (2010) Sequencing technologies - the next generation. Nature Reviews Genetics. 11, pp. 31-46.*
4. *Danzer M, Niklas N, Stabentheiner S, et al (2013) Rapid, scalable and highly automated HLA genotyping using next-generation seuencing: a transition from research to diagnostics, BMC Genomics, 14:221*
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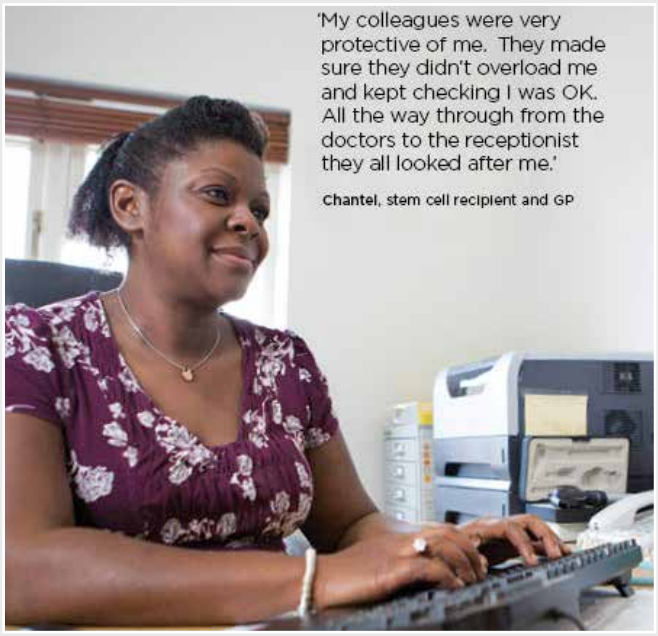
THE WAY BACK TO WORK

Simon Butler *is Policy and Public Affairs Manager at Anthony Nolan. Before joining the charity in June 2014 he worked in Parliament and as a lobbyist in the sport and recreation sector. @simonbutler86*

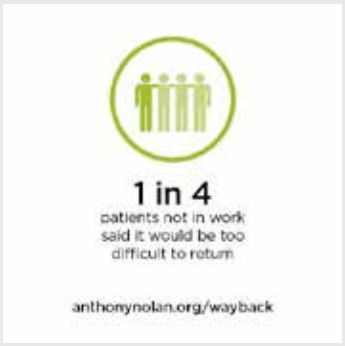


Road to recovery

Before we started work on our recent policy report, I admit I hadn't given a lot of thought to the challenges that patients face when it comes to going back to work after a stem cell transplant. Much of what we do at Anthony Nolan focuses on searching for donors and supporting people through their transplant, and so it becomes all too easy to think of a transplant as the end of the story. Of course the reality is that a successful stem cell transplant is actually the start of a long road to recovery.

Milestone

Returning to employment can be a very important step on that journey. When we did the research for *The Way Back To Work*, patients told us that they saw it as a chance to get back to the routine of every day life, and a welcome distraction from the recovery process.



Chantel, a stem cell recipient and GP, put it well: "Getting back to work was a big milestone. It gave me a sense of normality. I wasn't feeling like a patient – I was somebody contributing to society again." Crispin, also a recipient, told us that returning to his job as a postal worker "meant returning to the camaraderie of people I've worked alongside for many years."

These positive experiences are reflected in the data generated by our research. Our partners for the project, Justice Studio, surveyed over 120 patients to determine the impact of returning to work. They found that respondents who were in paid work at the time of the survey demonstrated improved wellbeing and better general health.

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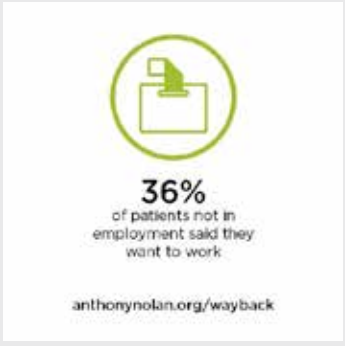
A difficult transition

What our survey also showed, however, is that going back to work is rarely easy for anyone recovering from the long-term impact of a stem cell transplant. In *The Way Back To Work* we report that only 54% of patients return to work within 3 years following their transplant – a figure that suggests there are significant barriers to overcome. Worryingly, 36% of stem cell recipients who weren't in employment told us they wanted to be in paid work, while another 25% told us it would be too difficult to go back.



For patients who are able to resume their career, the transition is often more difficult than they, their families or employers expect. During patient interviews we heard that going back often involves changes to responsibilities and working patterns, and were told that the challenge of going back to work full time can be particularly tough.

ANTHONY NOLAN
saving the lives of people with blood cancer

Support in the workplace

When it comes to supporting people to make the transition, employers have a key role to play. Support in the workplace – such as a phased return, flexible hours and extra sick leave – can make a huge difference to those returning after a transplant.

We heard a lot of praise for employers who had offered this kind of support. Paul, a Quality Assurance Programme Manager at a food retailer, told us: "Infection control was an issue, as my immune system was very weak. But I was able to work from home for a few months which worked really well."

Harun, a paramedic who last year featured in Anthony Nolan's 'After' exhibition, said: "My colleagues were fantastic; they kept my job open and were so supportive."

Making the case

However, its not just employers who can make a difference. Since the publication of *Road Map to Recovery* in 2013, Anthony Nolan has been making the case for all transplant patients to have access to high quality care and support throughout their recovery.

If NHS services adequately address the full range of long-term impacts of a stem cell transplant – psychological as well as physiological – more patients would receive the support they need to make a successful transition back to work.

The Way Back to Work helps to meet this challenge in two ways. Not only does it provide further evidence of the need for high quality services – which helps us make the case with politicians – but it also helps shape the information, guidance and support offered by our Patient Experience Team.

For more information about *The Way Back To Work* report, visit www.anthonynolan.org/wayback, or contact Simon Butler at simon.butler@anthonynolan.org.

THE PRESIDENT'S COLUMN

This is my first column as president of BSBMT having taken over the reins from Prof Gordon Cook who has so ably led to the Society over the last two years. Gordon has been intricately involved with BSBMT for many years, holding posts as secretary and subsequently chair of the clinical trials committee (CTC), President-elect, President and now past President. I'm sure he will continue to be actively involved in Society and I for one will greatly appreciate his on-going advice and help.

Indeed it is a little daunting taking over from such an illustrious series of past Presidents who have all made substantial contributions to the Society and also to the BMT and haematology communities as a whole. It was a great privilege at the Scientific Day in May to have the opportunity recognise the work of one of the past Presidents, Professor Charles Craddock, with the BSBMT long service award in recognition of his outstanding contribution to the society and to his commitment to developing stem cell transplant services. Professor Craddock was President of BSBMT between 2005-6 and is currently medical director of the Anthony Nolan Trust and chair of the Stem Cell Oversight Committee, which he established and which has done enormous work in improving access, particularly to cord blood transplantation in the UK. He is also one of the driving forces in the development of clinical trials in stem cell transplantation. The Figaro trial, of which he is CI, is one of the best recruiting trials in the field and his vision of a BMT clinical trials network has the potential to transform the clinical research base in BMT in the UK.

This year has seen other changes to the executive committee. Jenny Byrne has finally stepped down from the role of Secretary for the Society, a post that she has held for more years than is good for anyone, and I am delighted that she has accepted the role of President-elect. In her place as secretary we have a John Snowdon who has moved up from ordinary executive committee member has a launched himself into the role with huge enthusiasm. Emma Morris and Grant McQuaker have stepped down and I am very grateful to them for their contribution over the last 3 years. In their stead we have had the election of Steve Robinson, Maria Gilleece and Kavita Raj as ordinary committee members and it is a great pleasure to welcome them to the exec committee.

The Society ran two very successful meeting this in the last year; the educational meeting in October was extremely well attended and included Prof Per Ljungman and Prof Paul Carpenter as international speakers. The scientific day in May was organised by Karl Peggs and Persis Amrolia. It included an abstract competition at an exceptional standard and the CTC afternoon provided a forum for a wide discussion on clinical trials in BMT.

Keiren and her team at the registry continue to do sterling work in maintaining the database and providing a resource for clinical studies for the whole of the UK BMT community and they are currently working on the 6th commissioners report. One of the recurrent issues to come up again recently, is that the quality of registry studies is significantly weakened because of the divergences between transplant protocols at different centres. Some of the discrepancies have been helped by the work of Mike Potter and the guidelines subcommittee, which have produced five BCSH guidelines in the last three years. Nevertheless, there remain differences between transplant centres that are not explicable on the available evidence. We are currently embarking on a protocol harmonisation project, which is being led by Matt Collin from Newcastle. We have already gathered the details of some of the commonly used protocols together and we will be working to develop a standard consensus protocol for the more frequently used conditioning regimens which will be available on the BSBMT website. The hope is that a more uniform and consistent approach transparent conditioning regimens will both strengthen the CTC registry trials, and our position with NHSE commissioning.

Lastly my huge thanks to Patrick Medd who has put together the 17th newsletter, which I hope you found informative. The challenge laid down by Gordon was to be more efficient in returning my copy to Patrick than he had been - I'm not sure that I have achieved this on this occasion, but I hope not to disappoint Patrick in December.

Dr Charles Crawley