

Blood Matters

Quarterly information for hospitals served by the National Blood Service

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Editorial

This edition concentrates on services and therapies, which have more recently assumed a higher profile within the National Blood Service (NBS). These are the laboratories that process stem cell grafts from the blood and bone marrow producing increasingly sophisticated products for clinical transplantation and immunotherapy; those providing histocompatibility and immunogenetic services, the British Bone Marrow Registry (BBMR) and the NHS Cord Blood Bank (CBB). We also focus on the provision of HLA matched platelet concentrates, an essential resource for patients with haematological diseases including those undergoing blood and marrow transplant (BMT) procedures.

For some years it has been accepted that the processing of stem cells is a legitimate activity for the NBS, providing stem cell collection and processing activities for many large BMT programmes. In the 21st century the provision of stem cell processing services requires that facilities have good manufacturing practice (GMP) capability, advanced quality systems and good information technology. The NBS and other blood services have a good track record in this area. There are, of course, a number of excellent hospital facilities which also provide stem cell and immunotherapy services and, in fact, in England hospitals provide roughly 60% of these services with the NBS providing the other 40%.

Accreditation for BMT covers its clinical programme, bone marrow and peripheral blood stem cell collection facilities and stem cell processing laboratories. Accreditation presents an enormous challenge to these services. The EU Directive on Tissues and Cells described in this edition will become law in the UK from April 2006. It requires that stem cell collection and processing facilities comply with its requirements. Inspections will be carried out under the aegis of the newly formed Human Tissue Authority (HTA). Sections of the Directive apply also to stem cell registries. The other body involved in the accreditation of BMT services is the Joint Accreditation Committee of ISCT (International Society for Cell Therapy) and EBMT (European Group for Blood and Marrow Transplantation) – JACIE. Their guidelines are based on those originally established by the Foundation for Accreditation of Cell Therapy (FACT) in North America. Together FACT and JACIE will have a global profile in the establishment and maintenance of standards for high quality clinical, collection and laboratory services for stem cell transplantation. This means that currently there are two inspectorates with a remit to cover the collection and processing of stem cells, whilst JACIE alone inspects and accredits clinical BMT programmes. Presently it is unclear how the two will work together. There is a precedent in other European countries for JACIE to undertake all inspection and accreditation activities in the field of BMT and urgent discussions with the Department of Health and the HTA are required. In addition to the EU Directive and JACIE Guidelines there are a number of additional documents that stem cell and immunotherapy services will need to take note of. These include:

- European Commission Consultation on the Regulation of Human Tissue Engineering and Tissue Engineering Products
<http://pharmacos.eudra.org/F2/advtherapies/index.htm#pb>

- WHO Regulatory Requirements for Human Cells and Tissues for Transplantation
<http://www.whqlibdoc.who.int/publications/2005/9241593296.pdf>
- Council of Europe Guide to Safety and Quality Assurance for Organs, Tissues and Cells. Third edition: 2006 version.
http://www.bsbt.org/docs/EU_tissues_and_cells_directive_v3.pdf
- Council of Europe Research Group on Cellular Immune Therapies CDSP(2004)51.
http://www.coe.int/TIE/Social_Cohesion/Health/CIT%20report%20-%20final.doc
- World Marrow Donor Association (WMDA) Standards Bone Marrow Transplantation 2004(34), 103-110
<http://194.134.242.245/fileadmin/Publications/1704542a.pdf>

Further key developments for both the NBS and hospital based laboratories are:

- provision of products for the immunotherapy of patients with cancer and viral infections
- transplantation of non-haemopoietic cells e.g. to patients with cardiovascular and neurological disorders.

The observation in 1990 that transfusing lymphocytes, from their BMT donors, to three patients with chronic myeloid leukaemia (CML) who had relapsed after BMT caused them to enter a further remission was pivotal. Subsequent developments in this field are described in this edition.

The NBS operates the BBMR on behalf of three of the four UK Blood Transfusion Services (the Welsh Blood Service has its own registry). In the last three years it has expanded to its present size of more than 250,000 donors, the majority of whom are now fully typed by molecular biology techniques, which has involved an enormous amount of effort. Key objectives of the registry are:

- To maintain a registry size of 250,000 well-typed and readily accessible donors
- To obtain accreditation by the WMDA
- To obtain accurate follow-up data on patients who receive transplants from registry donors

The majority of donors on the BBMR are Caucasian and to increase the provision of donors for patients from a range of ethnic backgrounds, the NHS Cord Blood Bank (CBB) targets a significant proportion of its recruitment at donors from ethnic minority backgrounds, as described in this edition.

Patients with cancer who receive intensive chemotherapy and BMT procedures often develop severe thrombocytopenia and require intensive transfusion support. A proportion of these patients become refractory to platelet transfusions and may need matched products. Platelets matched for HLA and other antigens are an important but expensive and time-consuming resource and must be used appropriately. The NBS has made considerable efforts to refine and improve its services for these patients; these are summarised in this edition of Blood Matters.

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Realising the Potential of Stem Cell Therapies

Stem Cell Transplantation for Cancers of the Blood

There are around 24,500 newly-diagnosed cases of leukaemia, lymphoma, myeloma and related disorders in the UK every year. Put another way, around 1 in 25 people will develop a cancer of the blood during their lifetime. Taken together, these disorders are the 5th most common cause of cancer after lung, breast, bowel and prostate. They are the most common cause of cancer death in people aged 1 to 34.

Much progress has been made in treating leukaemia and related diseases. Today, almost 80% of children with the most common form of leukaemia will survive. Advances have also been made in the treatment of adults; the overall survival rate for adults with leukaemia is about 30%. These advances are a direct result of new medicines, more intensive combinations and the increased use of haemopoietic stem cell transplantation.

The NBS stem cell processing and testing laboratories currently support approximately 40% of stem cell transplants in England making the NBS the largest single supplier of stem cell services in the UK. Included in its portfolio of services are:

- The recruitment of bone marrow donors
- The HLA molecular typing of donors and maintenance of a register of the British bone marrow donors (the BBMR)
- The selection and counselling of stem cell donors
- The collection of stem cells by apheresis
- Stem cell testing, processing, cryopreservation and distribution
- Diagnostic testing of donors and stem cell recipients
- The NHS Cord Blood Bank (CBB)

Haemopoietic Stem Cell Graft Engineering

Despite huge advances in stem cell transplantation, the procedure continues to carry with it significant morbidity and mortality. These include the effects of graft versus host disease, graft rejection and immunosuppression. In contrast, the introduction of a donor immune system may be associated with beneficial effects such as the 'graft versus leukaemia' effect. In an effort to optimise the clinical efficacy of stem cell transplants, several strategies are now available to 'engineer' stem cell grafts to produce sub-populations of cells with desired characteristics. These procedures include:

- The use of magnetic cell selection devices to isolate large doses of CD34+ stem cells. This enables effective engraftment of haploidentical stem cells from a parent or sibling.
- The depletion of T lymphocytes to reduce the incidence and severity of graft versus host disease.
- The preparation and infusion of small doses of donor lymphocytes to exploit the 'graft versus leukaemia' effect.
- The immunomagnetic selection of relatively primitive stem cells expressing CD133.

- The use of assays to detect apoptotic cells as a means of predicting cell engraftment.

In this rapidly developing field, new technologies are being exploited to derive specialised immune cells (usually dendritic cells or T lymphocytes) capable of exerting very specific anti-cancer or anti-viral responses in patients. These new immunotherapies or 'cancer vaccines' are described elsewhere in this issue.

The Potential of Non-Haemopoietic Stem Cells

Recent studies have shown that adult stem cells from one type of tissue (bone marrow for example) seem capable of acquiring characteristics associated with stem cells of another tissue (for example muscle or liver). This important observation opens up the possibility of using stem cells to treat a range of disorders including Parkinson's disease, multiple sclerosis, cardiovascular disease and non-haemopoietic cancers. Some of the exciting possibilities are listed below.

Skin replacement: Keratinocytes (skin stem cells which normally reside in hair follicles) can be cultured to form an epidermal equivalent of the patient's skin. Such cultures might provide tissue for an autologous graft, bypassing the problems of rejection. This possibility may offer improved treatments for venous ulcers and burn victims.

Brain cell transplantation: The identification and localisation of neural stem cells in adults has been a major focus of current research. Potential targets of neural stem cell transplants include stroke, spinal cord injury, and neurodegenerative diseases such as Parkinson's disease.

Treatment for diabetes: It is now possible to create insulin-secreting cells from culture of stem cells. In addition, the cells self assemble to form structures which closely resemble normal pancreatic islets. The challenge now is to optimise conditions for insulin production with the aim of providing a stem cell-based therapy to treat diabetes.

Cardiac disease: Cardiac failure remains one of the major causes of mortality in the Western world. Recent studies have shown that the heart itself contains tissue resident stem cells although it is not yet clear whether these cells are bone marrow-derived, or represent a novel entity. The mobilisation and/or attraction of cardiac stem cells could be an extremely attractive option for therapy. In the clinical setting, bone marrow-derived or peripheral blood-derived stem cells have been administered to patients after myocardial infarction by intracoronary infusion. Such injections have been reported to improve several indices of heart function.

Future Challenges

To realise some of the potential therapies listed above, scientists must be able to easily and reproducibly manipulate stem cells so that they possess the necessary characteristics for successful differentiation, transplantation and engraftment. To be useful for transplant purposes, stem cells must be reproducibly made to:

- Proliferate extensively and generate sufficient quantities of tissue

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- Differentiate into the desired cell type(s)
- Survive in the recipient after transplant
- Integrate into the surrounding tissue after transplant
- Function appropriately for the duration of the recipient's life
- Avoid harming the recipient in any way
- Avoid immune rejection

NBS scientists are contributing to the world-wide effort to meet these technical hurdles both by undertaking basic research and by working collaboratively with academic units to explore the clinical efficacy of new treatments.

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The Impact of New EU Legislation on the Provision of Stem Cells for Transplantation

The EU Tissues and Cells Directive

In 1994, the Council of Europe organised a survey into the quality and safety of human tissues being used for transplantation in member states. A variety of practices were identified. In the UK, the Department of Health (DH) responded to the developing need for guidance in the area with the publication of its voluntary Code of Practice for Tissue Banks in 2001. By 2004, EU legislation entered the arena with Directive 2004/23/EC whose stated aims are to "set standards of quality and safety for donation, procurement, testing, processing, preservation, storage and distribution of human tissues". The Directive will become legally binding in the UK by April 2006.

The Directive calls *inter alia* for the establishment of technical criteria. These technical requirements will be set out in two Commission Directives (or 'technical annexes'). Unfortunately, these Commission Directives are unlikely to be finalised before Autumn 2005.

The Scope of the Directive

The Directive sets minimum standards for activities related to the procurement, processing, issue or use of human tissues and cells for clinical application. It does not prevent member states from using more stringent measures or from prohibiting the use or import of certain tissues or cells.

An indicative list of tissues and cells covered by the Directive is given in Table 1. The Directive excludes tissues already covered by existing legislation such as blood, blood components (which includes granulocytes) and vascularised organs. It also excludes tissues and cells used as an autologous graft within the same surgical procedure. Tissue engineered products are also outside the scope of the Directive except for aspects of donation, procurement and testing.

All stem cells destined for therapeutic use (haemopoietic and non-haemopoietic) from all sources (adult, fetal, peripheral blood, bone marrow, cord blood) fall within the remit of the Directive. Cellular therapies which use, for example, lymphocytes or dendritic cells are also covered by the

Table 1. Tissues and Cells Covered by the Directive

- **Haemopoietic stem cells from blood, bone marrow and cord blood including commercial cord blood banking activities**
- Cardiovascular tissues including heart valves and vessels
- Ocular tissues such as corneas and sclera
- Skin
- Gametes (sperm and eggs) and embryos
- Bone, cartilage, autologous chondrocyte implantation
- Ligaments, tendons, meniscus and other soft tissues
- Fetal tissues
- Donor leukocytes and other cellular therapies
- **Autologous cell systems**
- **Adult and embryonic stem cells**
- Endocrine tissues such as pancreatic islet cells
- Manufactured products derived from human tissues and cells (except 'tissue engineered products')

Directive. The Directive obliges NHS and independent transplant units to use only stem cells from licensed processing establishments who, in turn, must only process cells from licensed collection facilities.

Some Key Requirements of the Directive

The majority of the Directive's requirements will be familiar to establishments already accredited against the DH Code of Practice for Tissue Banks or by JACIE (Joint Accreditation Committee of the International Society for Cell Therapy Europe and the European Group for Blood and Marrow Transplantation) or Netcord. The main change from the requirements of the Code of Practice is the inclusion of stem cell collection. All establishments involved in the collection of stem cells from bone marrow, peripheral blood or cord blood must be accredited for that purpose by April 2006.

Licensing of Tissue Establishments. Member states must instigate a system of licensing to ensure that all relevant activities are compliant with the Directive. The licensing system will include a process of inspections.

Quality Management. The Directive requires establishments to operate a comprehensive quality system covering all aspects of tissue procurement, testing, processing, storage and distribution. In general, the requirements of the Directive are consistent with the principles of Good Manufacturing Practice (GMP):

- The basic elements of a quality system must be established so that procedures are documented and validated and all steps are traceable.
- Every activity that could affect the quality of the tissues or cells must be recorded and the data stored for a minimum of 30 years.
- There must be sufficient personnel who are adequately trained with clear job descriptions and documented responsibilities.
- Personnel must have access to ongoing training.
- Equipment must be designed, validated and maintained for the intended purpose

Donor Selection, Evaluation and Consent and Tissue Procurement. The Directive deals at length with donor-related issues. It prohibits the use of tissues which

have not been procured from (or imported by) a licensed institution. The Directive sets out requirements for donor selection and consent, testing, tissue procurement and traceability.

Import and Export of Human Tissues and Cells. The Directive delivers a framework of quality and safety standards intended to facilitate the exchange of tissues and cells destined for therapeutic use across the European community.

Reception, Processing, Storage and Distribution. For the most part, these criteria reflect the requirements of GMP. At the time of writing, environmental conditions for processing have not been finalised. However, it seems very likely that where tissues or cells are exposed to the environment during processing, an air quality of grade A as defined in the current European Guide to GMP, Annex 1, will be required. It follows that clean room facilities will be required for stem cell processing unless performed in "closed systems".

Relations Between Tissue Establishments and Third Parties. The Directive requires written agreements to be established between parties who provide any kind of activity capable of influencing the quality or safety of tissues or cells.

The NBS Response

In 2000, the NBS responded to the challenge of providing both existing and novel cellular therapies for patients by creating the "Stem Cell and Immunotherapy" function (SCI). SCI staff based at eight laboratories now support approximately 40% of stem cell transplants in England. Many of these Centres in collaboration with academic partners actively support clinical trials of new cell therapies. All of these activities have been developed against a background of increased regulation and impending legislation. To achieve full compliance with these requirements, SCI initiatives have included:

- The construction of laboratory and cleanroom facilities designed, operated and maintained to meet current and future regulatory requirements
- The development of a national quality manual
- The preparation of a training manual
- The development of a comprehensive library of documented procedures, policies and specifications designed to conform with relevant guidelines and standards.
- A system of internal audits
- The development of technical agreements with service users.

Additional Guidance

A DH Policy Collaborative, with input from the NBS, has developed guidance notes for managers seeking to comply with the EU Tissues and Cells Directive. This guidance is available at

<http://www.dh.gov.uk/PolicyAndGuidance/dfs/en>. The EU Directive is available at www.who.int/entity/ethics/en/ETH_EU_Directive_2004_23_EC.pdf.

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The JACIE Project: Accreditation of Blood and Marrow Transplant Programmes

What is JACIE?

JACIE is the Joint Accreditation Committee of the ISCT and the EBMT. It was established to provide a means by which Blood and Marrow Transplant (BMT) centres in Europe could demonstrate compliance with accepted best practice. The standards and inspection process were adopted from FACT (Foundation for the Accreditation of Cellular Therapy), the equivalent organisation in the US. Some pilot inspections were carried out in 2002 and the programme was fully implemented in 2004 with support from a 1-year EU Project Grant.

There is an Executive Committee, which meets monthly and a full Board, which meets 2-3 times a year. The JACIE office is at the EBMT secretariat in Barcelona, but most JACIE work is done over the Internet. JACIE has a website at www.jacie.org where you can find all relevant information, post questions and download the standards and other documentation.

What are the aims of JACIE?

The primary aim of JACIE is to improve the quality of BMT in Europe, by

- providing a means whereby transplant centres, haemopoietic progenitor cells (HPC) collection facilities and processing facilities can demonstrate high quality practice
- running training courses in quality management for applicant centres
- running training courses for inspectors
- updating and revising the standards as necessary

An equally important and wider aim is to ensure harmonisation between JACIE standards and other national/international standards, including the EU "Tissue Directive" (Directive 2004/23/EC).

The JACIE Standards

The JACIE standards cover all aspects of clinical transplant programmes, collection facilities (BM collection and PBPC collection) and processing of HPC, as shown in the following table. Within each subsection are detailed lists of specific standards; for example the standard on donor evaluation and selection contains 33 specific items relating to clinical evaluation, laboratory testing, informed consent etc.

The standards also apply to therapeutic cells (TC) such as donor lymphocytes, but not to genetically engineered cells. The JACIE programme does not accredit Cord Blood collection and banking facilities as this is carried out by Netcord.

Centres may apply for accreditation as complete programmes comprising clinical unit, collection and processing or, for example, as collection and/or processing facilities serving a number of clinical programmes.

Clinical	Collection	Processing
Size and organisation		
Facilities	Facilities	Facilities
Staffing	Staffing	Staffing
Quality Management	Quality Management	Quality Management
Policies / procedures	Policies / procedures	Policies / procedures
Donor evaluation and selection	Donor evaluation and care	Donor evaluation and care
Administration of high dose therapy	Collection procedure (BM or PBPC)	Cryopreservation
Clinical Research	Labels	Labels
Data management	Records	Issue
Records		Transportation
		Storage and disposal
		Records

The Accreditation Process

Preparation by Centre The centre implements measures as described in the JACIE accreditation manual, and then applies for inspection by submitting some basic information about the programme/facility and a number of supporting documents including a self-assessment checklist.

Inspection An on-site visit is carried out by a team of trained inspectors, usually one per facility (clinical/collection/processing). The inspectors are doctors or scientists working in BMT. An inspection visit lasts 1 – 1.5 days and involves discussion with staff during their work, review of documents /records and completion of a detailed checklist relating to the standards. The inspectors write a report, noting any areas of non-compliance with the standards, which is reviewed by the Medical Director (MD). The MD writes a supplementary report to the centre noting the current level of compliance and making specific recommendations for corrections and improvements.

Centre Response The centre makes the agreed changes and submits evidence to confirm this. The documentation is reviewed by the inspectors and the MD. In some cases a limited revisit may be the best way to show that deficiencies have been remedied.

Accreditation The JACIE Executive Committee reviews all the reports and documentation and makes a recommendation to the JACIE Board. If approved, accreditation is awarded, valid for 3 years.

Experience So Far

25 centres in Western Europe have so far been inspected, including 5 in the UK. All were found to be functioning at a high level of excellence. Initial reports have been completed for 21 centres; 13 were found to be compliant at level 2 (minor deficiencies) and 8 at level 3 (significant deficiencies but not serious enough to require a reinspection). Examples of minor deficiencies are lack of references in SOPs or failure to include pregnancy assessment in donor evaluation. Significant deficiencies include lack of proper document control and

failure to monitor engraftment data. Eight centres have now completed corrections and have received full accreditation; the others are at various stages of the correction and review process.

Common Deficiencies

The most frequent deficiencies are in quality management (QM), particularly in the clinical programme. It is clear that there needs to be more education and support for the development of QM programmes in BMT facilities. The British Society for BMT has set up a quality forum which aims to share experience and expertise and welcomes new members (<http://www.bsbmt.org/quality.html>). Another common problem is with labelling. An International Labelling Group has recently been set up under the chairmanship of Paul Ashford, which plans to develop labels for HPC and TC that incorporate ISBT128 standards and are compatible with JACIE requirements.

Common Difficulties experienced by Centres

Afterwards, inspection centres are asked to complete a survey about their experience. The responses so far show that

- Over a year was needed for preparation in most cases; 30% took 18 months and 40% 2 years.
- Most centres had additional staff to manage project implementation, but these staff were only part-time in 52% centres and only 32% had any experience in QM.
- The area of greatest difficulty for most centres was in the clinical programme (clinical programme 67%; collection facility 38%; processing facility 5%).
- Most difficulty was found in implementing the QM system, adverse event reporting system and other documentation. Lack of a culture of QM in clinical facilities was cited as an important problem.

These results are consistent with the fact that the most common deficiencies noted at inspection are inadequacies in the QM system, particularly in the clinical programme. The survey also indicates that these arise from lack of trained staff and absence of QM culture in the clinical setting.

There is clearly an important need for training of clinical staff in QM. One of the major aims of JACIE over the next 1-2 years is to provide more educational material such as model documents and a guide to implementing QMS in a transplant centre. At a local and national level, resources are needed to enable centres to comply with the guidelines, in particular to employ a dedicated quality manager, at least on a part-time basis.

Despite the large amount of work needed to implement JACIE, all the centres reported that they felt it had been worthwhile and noted areas where they felt their programme had benefited, including, for example better documentation of adverse events.

JACIE and the EU Tissue Directive (Directive 2004/23/EC)

It is a fundamental aim of JACIE to ensure that as far as possible the JACIE standards are identical to other applicable national and international requirements,

including those of FACT, the EU and the WMDA. The current JACIE standards are entirely compatible with the requirements of the directive, though JACIE is more detailed in many areas. The technical annexes to the directive have not yet been finalised and JACIE is contributing to the consultation process on these annexes. Some minor modifications to the JACIE standards may be needed once these technical annexes have been finalised, to ensure there are no areas of conflict.

Other Aspects of JACIE

JACIE is currently working together with FACT on the 3rd edition of the Standards and Guidance, which is expected to be available for public consultation on the FACT website within the next 2-3 months. JACIE and FACT are also working with National and international Donor Registries and the WMDA to promote the use of accredited collection and transplant facilities for unrelated donor transplants wherever possible, without jeopardising the availability of HPC from unrelated donors.

Finally JACIE has applied to the EU for a 2-year project grant to facilitate outreach work in the less well-off countries of Europe. If successful this will involve reciprocal visits between centres in Western Europe that have already been inspected and those in Eastern Europe working towards accreditation.

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Immunotherapy Approaches in Patients with Solid Tumours

Immunotherapy can be looked upon as harnessing the body's own defence mechanism to combat cancer. About a hundred years ago William Coley first attempted to manipulate the immune system to fight cancer by injecting a mixed vaccine of streptococcal and staphylococcal bacteria with some success. Over the last century many groups tried this approach to treat solid cancers but it had not become a front line treatment until more recently. The tuberculosis vaccine, Baccillus Calmette-Guerin (BCG) is now routinely used in the treatment of bladder cancer whereby the tumour cells are destroyed following the induction of a localised inflammatory response.

The immunotherapy described above is non-specific whereas the real goal for cancer clinicians is a specific response by which the immune reaction is targeted to the tumour – the so called magic bullet. Specific immunotherapy can be achieved in one of two ways:

- vaccination where the immune system is stimulated to produce an anti-tumour immune response or
- adoptive transfer where active components of the immune system are administered.

A major aim of immunotherapy is to generate a cellular response directed at the tumour as, although antibodies

may have some tumouricidal effect, it is generally recognised that T cells are the main effectors in anti-tumour immune responses.

Vaccination

Success is dependent on the particular antigen used, the way the antigen is delivered, and the choice of adjuvant if any. A simple yet relatively crude vaccine uses tumour extracts which are irradiated to make them non viable before being re-injected into the patient together with an adjuvant such as BCG. This crude type of vaccine although previously used in colorectal cancer patients with limited success has now been superseded by peptide based vaccines targeting various types of solid tumour. These vaccine reagents are recognised when presented to the patients' T cells in association with major histocompatibility complex (MHC) molecules and the appropriate co-stimulatory molecules on antigen presenting cells (APC).

Tumour antigens are rarely specific to the tumour cell. Most often they are more highly expressed in or on the tumour cell compared to normal tissue. Numerous tumour associated antigens have been identified including melanocyte differentiation antigens (Melan A/Mart-1); testicular cancer antigens (MAGE-3, BAGE); antigens arising from point mutations (beta-catenin, MUM-1); over expressed "self" antigens (Her-2/Neu, p53 and MUC-1); and viral antigens (EBV, HCV and HPV). An alternative to the use of crude tumour lysate as the source of antigen are peptides, short protein sequences based upon the immunogenic regions of the tumour antigens. Peptide based vaccines are beneficial in that immune responses can easily be monitored, the peptide is readily processed before presentation to the T cells and the antigen is well defined unlike crude extracts. Furthermore, by using peptides the formulation of the vaccine such as dose, its combination with other immune stimulating factors, and the method of delivery should be more easily determined and controlled.

As indicated above, T cells only recognise antigen in the context of MHC presented to them on the surface of APC. The most important and efficient APC are the dendritic cells and their ability to stimulate T cells to great effect is being exploited in anti-cancer therapy. Dendritic cells can be isolated and grown ex vivo where they can be loaded or pulsed with antigen prior to transfer back into the patient. Alternatively, the dendritic cells can be treated with messenger RNA which is incorporated into their protein-making machinery, producing the 'tumour' antigen and presenting it to T cells. This approach has recently been shown to be successful in early prostate cancer trials.

Adoptive Transfer

This can involve:

- a monoclonal antibody either capable of inducing the destruction of a cancer cell on its own or by targeting a drug or radioactive substance to the cancer.
- a specific cell type most often a cytotoxic T cell that can mediate specific tumour cell killing following recognition of certain antigens on the cancer cell surface.

Monoclonal antibodies recognising so-called tumour specific antigens were demonstrated in the late 1970's to be capable of targeting tumour cells and human studies since then have resulted in tumour regression in some patients. However, a major limitation in the use of antibodies is that they cannot readily access large tumours. One antibody, Herceptin, targets the human epidermal growth factor receptor 2 (HER2) protein which is over expressed in 25-30% of primary breast cancers and has been shown to be effective in clinical trials.

Scientists have shown that T cells stimulated *ex vivo* and then transplanted back into a patient can destroy the remaining tumour cells. This technique has been used successfully in the U.S. by Rosenberg and colleagues who removed T cells infiltrating melanoma lesions, cultured them in the presence of IL-2 (a T cell growth factor) to generate large numbers of cells which were then transferred back to the patient. Studies have shown that if the cells are maintained within the patient, long term survival of up to seven years can be achieved. However, the T cells require that tumour cells express certain molecules on their surface for their recognition and killing, yet these are often down regulated or absent.

The Role of the NBS

It can be seen from this very short overview of immunotherapy in solid tumours that there is a clear need for the production of clinical cell preparations made under the auspices of GMP and which from April 2006 conform to the EU directive for Tissues. To this end, the NBS Stem Cell and Immunotherapy function has partnerships with cancer departments across the country. At these centres immune cells can be isolated, cultured, expanded and if required genetically manipulated prior to transfer back into the patient.

Projects ongoing include:

- NBS Birmingham who have adoptive immunotherapy projects with collaborators from the CRUK Centre for Cancer Studies and the University of Birmingham. These projects relate to the use of dendritic cells
 1. pulsed with hepatocellular carcinoma cell lysate or
 2. transfected with a plasmid-encoding melanoma antigens to induce anti-tumour responses upon transfer into patients.

Another project at Birmingham involves the selective reactivation and expansion of T cell clones targeting melanoma, EBV related and prostate tumours.

- NBS Leeds are also involved in projects to generate dendritic cells for adoptive immunotherapy. Here, in collaboration with the CRUK Clinical Centre Leeds, they are proposing to pulse dendritic cells with tumour lysates from renal tumours prior to re-infusion.
- NBS Manchester, where a collaboration has been set up with the Department of Clinical Oncology at the Christie Hospital to isolate, transfect and expand T cells from cancer patients. The transgene encodes for a single chain Fv (active part of an antibody) which recognises carcinoembryonic antigen (CEA) and this is linked to the zeta chain of the T cell receptor. This molecule allows the transfected T cells

to target CEA expressing tumour cells. Once the antibody has bound to the CEA the T cell will be activated and is able to kill the cancer cell.

The NBS is therefore actively involved in translational research in immunotherapy which may lead to significant advances in cancer therapy. Its quality infrastructure and GMP facilities has made it an ideal partner for these studies.

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Immunotherapy In Acute Lymphoblastic Leukaemia

Immunotherapy manipulates the activity of either the patients' own immune cells or, in the case of patients who have blood and marrow transplants (BMT), those of the donor, to attack and kill cancer cells. In BMT patients, particularly those with chronic myeloid leukaemia (CML), who relapse after the transplant, giving unmodified lymphocytes from the donor – donor leucocyte infusions (DLI) – can induce a further remission. It is usually the case, however, that lymphocytes have to be educated so that they recognise tumour cells as foreign before they are able to kill them. This can be done by culturing T-lymphocytes from either the patient, or their donor if they are post-allograft, in the laboratory with specialised antigen presenting cells called dendritic cells (DC) that may present tumour antigens to them. This can switch on their immune responsiveness. Alternatively the DC may be pulsed with relevant tumour antigens and injected into the patient to stimulate autologous lymphocyte proliferation. T cells when they become able to kill tumour cells are called cytotoxic T lymphocytes (CTL).

How Do T Cells Become Switched On?

One of the reasons that leukaemia and other cancers develop to life threatening proportions is that they appear to be unrecognised by the body's immune system. Cancer cells use a number of different mechanisms to avoid the radar of our immune defences. For example, to eliminate tumour cells, T and NK cells require that they display certain antigens involved in cell recognition such as CD80 and CD54. In acute lymphoblastic leukaemia (ALL) these antigens are downregulated which helps the tumour cells to avoid detection. There are many other immune escape mechanisms that operate in cancer cells and these are beyond the scope of this article. To become switched on, T cells may recognise small peptides derived from tumour cell digestion in DC following ingestion and processing and presented to them associated with HLA antigens on the DC surface. These HLA-peptide complexes interact with the T cell receptor (TcR). A second or co-stimulatory signal is given to T cells by the binding of CD80 and CD40 on DC to CD28 and CD40 ligand on T cells. This causes lymphoid growth factors such as interleukin (IL)-12 and IL-2 to be produced. T cells are then educated to recognise the tumour cells as foreign. If one of the co-stimulatory signals is missing then a state of tolerance develops. T cells may also interact with cancer cells directly.

Donor Leucocyte Infusions

Prior to 1990 attempts to stimulate patients' immune responses against tumour cells to prolong remission after chemotherapy were largely unsuccessful. That year a report was published of 3 patients with CML who relapsed after transplant but achieved a further remission when leucocytes from the transplant donor – DLI – were transfused to them. All 3 patients had survived 13 years from DLI therapy at the time of a recent report. Subsequently it was shown that DLI were effective in roughly 75% of CML patients. The response rate in acute leukaemia is much lower and in ALL less than 20% respond. ALL cells also evade attack by the bodies natural killer (NK) cells because they express little CD54 which is important for NK activity.

Immunotherapy Of ALL

70-80% of children and 20-25% of adults with ALL are cured using chemotherapy. Patients with poor initial prognostic features such as presence of the Philadelphia chromosome (Ph1) and failure to eliminate blast cells early in the course of treatment may require a more intensive approach. Many are offered an allogeneic BMT if they are less than 50 years and have a suitable donor; roughly 40-50% are successfully treated in this way. If patients are not cured by chemotherapy or transplant then immunotherapy may have a role. One way of producing CTL is to co-culture donor T cells with DC derived from the patient that also present ALL antigens. In fact it is presently unknown which antigens are important in triggering a CTL response against ALL blasts so current research uses cells which combine the features of both DC and ALL cells i.e. antigen presentation and possession of leukaemia antigens. This is done by:

- Culturing 'normal' DC from the patient while in remission and pulsing these with ALL cell lysates or apoptotic ALL cells
- Culturing normal DC from donor cells where the patient has had a BMT and pulsing these as above
- Fusing normal DC with ALL blasts
- Taking ALL blasts and culturing these to produce DC

In each case the aim is to generate cells which present ALL antigens. These can then be co-cultured with either the donor's T cells (allogeneic) or the patient's own T cells (autologous) aiming to switch on T cells with anti-ALL activity. To test whether or not this has been successful, the T cells can be further tested in the laboratory to check that they kill ALL blasts but that they show no lysis against normal cells from the patient e.g. peripheral blood lymphocytes or keratinocytes.

It is worth pointing out that some of these approaches e.g. fusing DC and tumour cells have already been tested in the clinic. In 13 patients with renal carcinoma, 4 rejected all metastatic tumour lesions after vaccination with such hybrids and a further 3 showed a significant response.

Another strategy is to exploit the over-expression of certain antigens by leukaemia cells. These antigens include:

- Wilms Tumour (WT)-1; a transcription factor which is normally present in very few tissues but appears at very high levels in some leukaemias

- PR-3; a serine protease that is present in high levels in myeloid leukaemias
- Her2/neu; a transmembrane tyrosine kinase protein normally found in epithelial cells and over-expressed in 20-30% of breast cancers as well as some ALL's
- HB-1; a B cell associated antigen.

Investigators have succeeded in making autologous and/or allogeneic T cells that recognise these antigens and kill leukaemic cells. It remains to be seen whether or not these will be useful targets in clinical studies.

Another approach of great interest in allograft recipients is to exploit differences in the expression of minor histocompatibility antigens (mHA) between the donor and patient. For example the mHA HA-1 may be present in the patient but not the donor. Donor T cells can be cultured with donor DC that have been pulsed with HA-1 peptide. The result is to generate T cells with anti HA-1 specificity; in the laboratory it can be shown that these kill HA-1 positive ALL blasts taken from the patient. HA-1 and some other mHA are present only on haemopoietic cells which means that CTL with reactivity against them will not cause any collateral damage when injected.

What Are The Next Steps?

There is a lot of interest in immunotherapy in leukaemias where patients have not responded to chemotherapy and BMT. A small number of patients have been treated with CTL and shown responses. Current research is focused on defining which of the various strategies available will be most suited to particular types of leukaemia. CTL require more detailed study to elucidate their precise mode of action. In ALL they may need to be given after more chemotherapy aimed at eliminating the bulk of disease which will allow them to generate a robust anti-leukaemic effect. The patients to whom they are given will require careful monitoring e.g. using molecular techniques to measure the levels of residual leukaemia and also to ascertain that there are no unexpected side effects.

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The Current Status of The British Bone Marrow Registry

The British Bone Marrow Registry (BBMR) of unrelated bone marrow donor volunteers was established in 1987 and it is administered by the NBS on behalf of the Blood Transfusion Services of Scotland and Northern Ireland.

Currently the BBMR contains 246,040 bone marrow donors and nearly 7,000 cord blood units. All BBMR donors within the NBS are recruited from the blood donating population throughout England but the registration, typing and selection of donors is performed at 3 centres, Newcastle, Bristol and Colindale. Cord blood units are collected and registered by the NHS Cord Blood Bank at Edgware. Approximately 3% of the bone marrow donors and 40% of the cord blood units are from ethnic minority groups

The administration of all these activities is performed by the BBMR administrative HUB at the Bristol NBS centre who register these donors with the Bone Marrow Donor Worldwide database (BMDW) which contains over 9 million donors and is responsible for receiving all the national and international searches for donors.

The BBMR is now the 6th largest registry and all new donors (40,000/year) are currently typed for the HLA-A, -B, -Cw and -DRB loci using the latest DNA methodology for tissue typing. The result of this is that the NBS now has an extremely well typed Registry with 90% of these donors being HLA-A, -B and -DR-typed (61% are also HLA-C typed). The BBMR was the first registry in the world to introduce typing for alleles of the HLA-Cw loci. These alleles are now known to be important in the interaction with a subset of cells, NK cells, which contribute to the eradication of leukaemic cells.

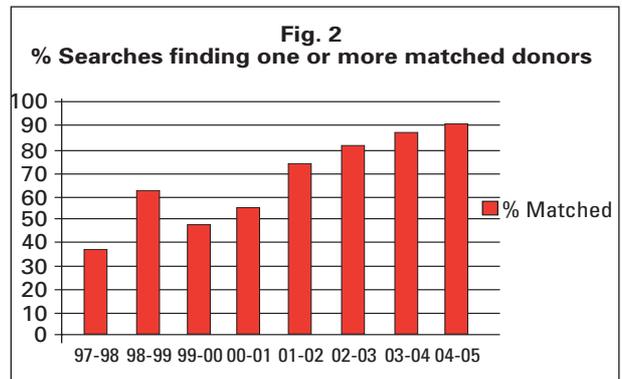
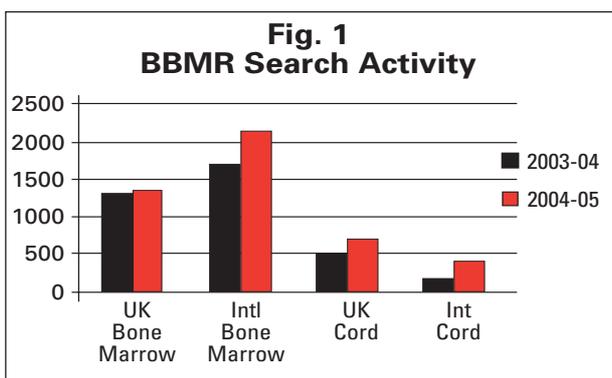
What is the effect of adding large numbers of well-typed donors to our registry?

We have seen a surge in the activity of the Registry over the last year with an increased number of donor searches carried out for patients in need of a transplant and this has led to an increase in the number of donors provided for transplantation. Figure 1 shows the search activity of the BBMR. For bone marrow and peripheral blood stem cells (PBSC) this increased from 3019 (1316 national and 1703 international) searches in 2003-04 to 3453 searches (1334 for national and 2119 for international) in 2004-05. Cord blood searches increased from 716 to 1094 in the same period. Also there has been a substantial increase in the percentage of searches that yield one or more HLA matched donors (Figure 2).

The number of final donors provided by the registry has increased from 78 in 2003-2004 to 126 in 2004-2005. In the latter, 61 donors were supplied to UK transplant centres and 65 to international transplant units.

Any other changes in the activity of the BBMR?

At present there are 3 sources of haematopoietic stem cells used for patients in need of haematological or immunological reconstitution, bone marrow, cord blood or peripheral blood. In the latter, the HSC have to be mobilised into the peripheral blood using granulocyte colony stimulating factor (G-CSF) and these are known as p mobilised peripheral blood stem cells (PBSC). Last year, for the first time, the number of PBSC supplied (n=67) was greater than bone marrow (n=59). This trend is likely to continue with the development of immunotherapy using PBSC.



Interestingly the number of cancelled transplants in the UK in the period described, for either patient or donor reasons, has fallen from 19 to 8.

The BBMR, with the support of Mr Peter Heard, is now steering towards obtaining World Marrow Donor Association accreditation and also to become an associated member of the NMDP registry of the USA. To this end, the BBMR has just issued its first comprehensive Operations Manual Document (MPD) to cover all its activities from recruitment to final donor work up. This quality-controlled set of detailed procedures is intended to ensure that the standards of excellence are maintained across all BBMR donor centres.

We have also appointed a new Data Manager and IT expert, Mr John Ord. His first achievement has been the design of our new format search report. These will be clearer with the ability to select any category of donor required e.g. male, young, CMV negative etc. We will also be providing additional scientific advice on the selection of the unrelated donors for each patient. In January, the BBMR data set will for the first time become integral with the rest of the NBS via the new IT system, Hematos.

Cord Blood Units

The BBMR contributed 6026 cord blood units to the total number of cord units registered within the BMDW and the Netcord databases. Transplantation using this new source of haematopoietic stem cells has substantially increased due to clinical outcome results indicating that cord blood transplantation is associated with a reduced risk of developing severe graft versus host disease (GVHD) even in the presence of some degree of HLA incompatibility. So far the NHS Cord Blood Bank has issued 95 units for transplantation through the BBMR or Netcord. This activity is likely to increase even more as a result of some new clinical protocols intending to use 2 cord blood units (double cord transplant) in adult patients. This protocol has been used in a big transplant centre in Minneapolis with great success.

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The Current Status of Cord Blood Banking

In most NHS Trust Maternity Units the placenta is considered a waste product, but in a small number of hospitals in and around North London the placenta and its blood are considered very special. Those babies

born at Barnet General, Northwick Park and Luton & Dunstable Hospitals all have the potential of saving the life of another. The NHS Cord Blood Bank (CBB), as part of the NBS, collects and stores cord blood for use in the treatment of unrelated patients. The bank currently has 7,500 units stored and has issued 95 donations for transplant.

Placental umbilical cord blood remaining in the placenta, following the birth of the baby, is a rich source of stem cells. These cord blood stem cells can be used in transplantation as an alternative to bone marrow and peripheral blood stem cells for the treatment of patients suffering from malignancies, bone marrow failure disorders and inherited metabolic and immunological disorders.

Patients requiring bone marrow transplantation cover a broad range of ethnically diverse groups, however ethnic minority groups are markedly under-represented in unrelated bone marrow registries - over 90% of our volunteer donors have North European ancestry. We have selected maternity units with which to work on the basis of high rates of normal deliveries and high ethnic diversity. The resulting collections improve the probability of patients from ethnic minority groups achieving a match from the bank. Approximately 40% of units in the NHS CBB have non-white Caucasian HLA types.

Cord blood is collected, tested for cell content, screened for infectious diseases, tissue-typed, frozen and placed in liquid nitrogen for long-term storage to provide an 'off the shelf' product. All procedures are designed to produce a safe and efficacious product. Once selected for transplantation a unit can be issued within 2 weeks; in urgent cases cord blood can be supplied within 24 hours.

The NHS CBB operates within a highly regulated environment, within the framework of good manufacturing practice (GMP) and good laboratory practice (GLP). Nationally the Bank is accredited by the Medicines and Healthcare products Regulatory Agency (MHRA) and internationally is one of only 7 CBB accredited by FACT-NETCORD. (FACT is the Foundation for Accreditation of Cell Therapy and the North American counterpart of JACIE; Netcord is an international organisation of CBBs and their inventories).

Donor Recruitment

Recruitment, consent and donor selection are performed by trained CBB staff in collaboration with the midwives. The CBB has a number of strategies to inform potential donors about the cord blood programme. At the initial donor mother interview the formal aspects of cord blood donation are discussed and fully informed consent is obtained for the donation, testing of both mother and cord blood samples and for both clinical use and research and development, if unsuitable for transplant.

Donor selection, to ensure the safety and appropriateness of the donation, is assessed through a standard questionnaire covering behavioural risks, travel and medical history. Two to three months post delivery the mother is contacted to check on her health and that of her baby.

Collection

The aim of the collection procedure is to harvest as much blood as possible from the placenta while

minimising the risks of contamination. Cord blood is collected from the placenta after it has been delivered in a dedicated area by trained staff. The placenta is suspended in a frame, the umbilical cord is robustly cleaned, a needle is inserted into a cord vessel and the blood drains out under gravity into a collection bag. Donation volumes range from 12 - 250ml with an average of 87ml units; less than 40ml are discarded as too small for use in the clinical setting, or are used for R&D with maternal consent.

Processing

Cord blood banking requires facilities for long-term storage of a large number of units. To reduce the stored volume, all cord blood donations are depleted of red cells and plasma, leaving the stem cells in a standard buffy coat volume of 20ml, whilst maintaining their cell quality and quantity. A cryopreservative is added to prevent cells from bursting during controlled-rate freezing, and the units are then stored in liquid nitrogen at temperatures of -196°C .

At various stages throughout the process samples are removed from the cord blood for testing, quality monitoring, and archiving for future testing at the time of issue for transplant.

Testing

- **Microbiology** Both the donor mother and the cord blood unit are tested to ensure safety for the transplant patient. Testing of the mother and cord blood for infectious diseases is the same as that for blood donors. This includes screening for HIV, hepatitis B and C, syphilis and HTLV. Further and more sensitive tests are performed when a cord blood unit is selected for transplant. A sample of cord blood is screened for bacterial and fungal contamination at the time of freezing.
- **Haematology** A full blood count is performed on a sample to assess the normality of the baby and to calculate the total nucleated cell content of the donation as an indicator of its engraftment potential. Only units that contain sufficient numbers of cells for transplantation - 40×10^7 nucleated cells - are accepted into the bank.
- **Tissue Typing** All cord blood units are tissue typed, to permit selection of a suitable donation for the patient.
- **Stem Cells** The number of CD34+ cells are enumerated as an estimate of the stem cell content of the donation.

Search and Release of Units for Transplant

On release from quarantine, following medical and quality review of the donor and donation data, the cord blood units are made available for search through the BBMR and the International Netcord Registry. Transplant Centres initiate a search for cord blood units in the same way as for bone marrow donors. The Transplant Centre searches for a cord blood unit that is the best possible match for their patient, although HLA matching for cord blood may be less stringent than that required for bone marrow donors - recommendations are that no more than two mismatches are acceptable. Selection also requires consideration of the nucleated

cell dose – recommendations are for at least 2.5×10^7 nucleated cells per kg patient body weight.

Once a unit has been selected for transplant the bank will provide anonymously all the information relating to the donation to the transplant centre, and initiate a number of additional and confirmatory tests to ensure the safety and identity of the unit.

Transplant Outcome

Transplant outcome data has been accrued and analysed by a number of groups, with the resulting data being used to evolve guidelines for the selection of suitable donations. The number of cells in the unit and the degree of HLA matching are the major factors associated with a successful outcome in both the paediatric and adult settings.

The cumulative experience of cord blood transplantation indicates that the outcome in overall survival is comparable with the outcomes using bone marrow or peripheral blood stem cells.

Summary

Cord blood, generally discarded as a waste product, can be collected without risk to the mother or donor, providing a life saving product for patients requiring a stem cell transplant who are unable to find a matched bone marrow donor.

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The Current Status Of HLA Selected Platelet Provision

Platelet refractoriness is defined by the failure to gain an adequate increment in platelet count, normally less than $10 \times 10^9/l$ when measured one hour post transfusion, following at least two platelet transfusions from random donors. Approximately 50% of alloimmunised, transfusion dependent patients experience platelet refractoriness which can be a life threatening complication of transfusion if poorly managed.

Refractoriness may be caused by both immune and non-immune factors and detailed knowledge of these factors are necessary for the effective management of such patients.

Immunological Platelet Refractoriness

HLA class I specific antibodies are the principal cause of immune destruction of transfused platelets but HPA specific antibodies, usually in conjunction with HLA antibodies, as well as high titre ABO antibodies may also be involved. Leucocytes present in transfused platelets are responsible for inducing the formation of HLA antibodies. Although universal leucodepletion was introduced in the UK during 1999, there can still be up to 5×10^6 leucocytes in an adult dose of platelets which are capable of initiating antibody production in previously sensitised patients, such as women who have been sensitised by pregnancy and patients who have previously received non-leucodepleted products.

Non-Immune Platelet Refractoriness

Transfused platelets can also be destroyed or removed from circulation through non-immune factors which include conditions such as splenomegaly, hepatomegaly, disseminated intravascular coagulation (DIC), septicemia, fever, infections, (e.g. CMV) and malignancies. In addition, drugs used as part of the patients' treatment including antibiotics, e.g. amphotericin B, vancomycin and ciprofloxacin, can also contribute to refractoriness.

In the majority of patients both immune and non-immune factors may be present and this necessitates a detailed discussion between the clinician treating the patient and the clinical scientists in the NBS Histocompatibility and Immunogenetics (H&I) laboratory receiving the initial request.

The management of platelet refractory patients involves a number of steps, which will be outlined below:

Clinical History

All initial requests for HLA matched platelets must come from the clinician responsible for the treatment of the patient. HLA selected platelets are a specialised and expensive product which must be used appropriately. The request must be made directly to the clinical scientists in the H&I department who will not only ensure that the patient fulfils the criteria for refractoriness but also record information relevant to the management of the patient, for example:

- Has the patient received blood group matched apheresis platelets?
- What were the pre and post transfusion platelet counts with the last two transfusions?
- Diagnosis
- Current treatment
- Drugs currently being used
- The patient ABO and Rh D type (D prophylaxis is advised for D negative women of child bearing age)
- Patient CMV status, will platelets from CMV positive donors be acceptable
- The number and frequency of doses required

Staff in the laboratory will need to perform investigations to determine whether the patient has produced HLA specific antibodies and to determine the HLA class I type of the patient.

HLA specific antibodies

A combination of different techniques are currently used to detect and define HLA specific antibodies. These include the classic complement dependent lymphocytotoxicity assay, and methods using purified HLA antigens, such as ELISA where the antigens are immobilised on a microtitre plate, or flow cytometry and Luminex based assays where the antigens are immobilised on microspheres. The latter techniques are extremely sensitive and are used throughout the NBS H&I laboratories allowing tests to be performed in the presence of therapeutic antibodies such as anti-thymocyte globulin (ATG) or anti-CD3. Where HLA antibodies have been detected the provision of HLA selected platelets is advised but it is important to HLA type the patient.

HLA typing

HLA typing is performed using DNA based techniques to define patients' HLA class I alleles. A significant proportion of patients under investigation for platelet refractoriness are awaiting or have received a haemopoietic stem cell transplant and the HLA type will be available in their notes and this can be faxed to the H&I laboratory to facilitate the search for compatible donors.

Although patients are typed for HLA-A, B and Cw, only the HLA-A and B loci are used for matching because the clinical significance of HLA-Cw antibodies in immunological refractoriness has not been established. This has been partly influenced by the poor serological detection of HLA-Cw antigens but with the availability of more sensitive DNA based techniques, the precise contribution of both the HLA-Cw antigens and -Cw antibodies to immunological platelet refractoriness may be elucidated. DNA technology has also allowed a more precise definition of the HLA specificities and contributed to definition of epitopes involved in antibody binding, which assists when matching donors with patients.

Matching

HLA selected platelets are collected by apheresis from HLA typed donors and are provided for refractory patients in whom HLA specific antibodies have been detected and where non-immune causes have been excluded. This approach can be used by the NBS for two main reasons; firstly, the NBS has a large panel of HLA typed platelet donors regularly attending apheresis clinics; secondly, the avoidance of HLA mismatches reduces the probability of future alloimmunisation which will decrease the compatible donor pool.

Selection of HLA compatible is based on a system two match grades:

- 'A grade' match, where there is no mismatch between the HLA-A and B type of the patient and donor
- 'B grade' match, where there is a mismatch between the HLA-A and/or B type of the patient and the donor. The mismatch may be described as B1, B2, B3, or B4 dependent on the whether there are 1, 2, 3 or 4 mismatches respectively.

An alternative approach to managing refractoriness is used in some countries that do not have access to large donor panels or the appropriate infrastructure and this is the provision of crossmatch negative platelets. Crossmatching may be useful where long term platelet therapy is not anticipated but this remains an area of controversy.

Follow up

All HLA selected platelets are issued for a named patient and clinicians are requested to ensure that a pre-transfusion platelet count and a one hour post-transfusion platelet count are performed. Patients that do not achieve an adequate increment with 'A grade' platelets will be investigated for HPA specific, ABO antibodies or non-immune causes of refractoriness. A small but significant group of patients requires both HLA and HPA selected or HLA and blood group matched platelets. HPA matched platelets are provided in conjunction with the NBS Platelet Immunology laboratories. Many patients, particularly those with uncommon HLA types, receive B grade matches and here

the feedback from hospitals also assists in the identification of unacceptable mismatches. This feedback is essential to guide both the clinician and the H&I staff on how to provide the most effective continued support with these specialised products, which requires ongoing discussion between NBS staff and the referring consultant.

New Developments

a) Epitope Matching

DNA typing and improved definition of epitopes recognised by HLA specific antibodies using recombinant HLA molecule technology is now standard practice in NBS laboratories. Studies are underway that will define patients in terms of the epitopes on their HLA antigens rather than traditional HLA specificities. Epitope matching between donor and patient may allow more scope for finding compatible platelets particularly for ethnic minority patients.

b) Out of Hours Service

HLA selected platelets by their very nature are not an 'off-the-shelf' product. However, there are some instances where suitably compatible products are available for patients, in emergencies. The NBS now offers an out of hours service for the support of patients already receiving selected platelets but requiring additional transfusions due to bleeding, the need to undergo an emergency procedure or a failure in the NBS system to provide platelets that have been ordered. Patients must fulfil either of these criteria to receive this out of hours service.

In conclusion the NBS H&I laboratories provide a comprehensive service to aid management of patients who are immunologically refractory to random platelet transfusion. The latest technologies for HLA antibody detection and definition and HLA typing are used in the selection of HLA compatible platelets and by working with hospital clinicians, platelet refractory patients can be effectively managed.

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The Demand for Blood

Reduction in demand

The demand for blood from hospitals has been falling consistently since the year 2000 and the reduction in demand is accelerating. Between 2002-03 and 2003-04 the fall in use was 2.3% but from 2003-04 to 2004-05 the reduction was 5.9%.

The reasons for the reduction in demand

It is thought that the main driver for the reduction in demand was the Health Service Circular HSC 2002/009 that encouraged hospitals to introduce appropriate use programmes. These included using a lower Hb trigger

and the use of cell salvage. It also encouraged active hospital transfusion committees and the introduction of hospital transfusion teams as well as the appointment of hospital transfusion practitioners. The Blood Stocks Management Scheme (BSMS) carried out a survey in late 2004 to try to understand the actual reasons behind the reduction. As well as the initiatives outlined previously other reasons given included increased awareness of transfusion protocols, the introduction of transfusion protocols, and participation in the BSMS. Over 50% of hospitals indicated at least five reasons for their reduction in use. It seems that HSC2002/009 has been the impetus for a cultural change in hospitals in relation to blood usage.

The NBS Demand Planning Group

The Demand Planning Group (DPG) was established to make projections on both the medium and long-term demand for blood and blood components, primarily red cells. It monitors demand on a regular basis and suggests changes to the demand target and reports to the NBS Executive on a quarterly basis.

Membership of the group extends to senior NBS managers and has recently been extended to ensure that it is well informed from a clinical perspective and for the provision of expert data analysis support. It is chaired by the BSMS manager.

Demand Forecasting

It is important for the NBS that demand forecasting is as accurate as possible. Predicted demand is used by the National Commissioning Group to agree the price of blood and by the NBS to prepare appropriate marketing and collection plans. The DPG has principally used environmental monitoring (assessing external influences on the demand for blood e.g. changes in surgical techniques) and trend analysis to forecast demand from year to year, although some modelling has also been used. The difficulty with using these techniques is that the factors that influence red cell demand are complex and poorly understood and therefore some factors that influence demand may be excluded leading to inaccuracies in the demand prediction. The DPG has commissioned a project to investigate the underlying reasons for varying red cell demand in hospitals, the output will be a set of key variables that influence demand. The project group is also examining demand projections for various different forecast time intervals. It is anticipated that more sophisticated automated mathematical modelling will be used.

How can hospitals help with demand planning?

The DPG assesses external influences on the demand for transfusion, e.g. changes in surgical techniques, to inform their discussion on demand forecasting. However they do not necessarily capture all the changes that may occur. Hospital blood transfusion managers can encourage hospital management to inform them of any planned changes to activity. They can help the DPG by providing this information to the BSMS and/or their hospital liaison manager.

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Year	No. of red cell units issued (Millions)	% change from previous year
2001-02	2.206	
2002-03	2.186	0.9%
2003-04	2.157	1.3%
2004-05	2.030	5.9%
2005-06 projected	1.93	

Improving the Evidence Base for Transfusion Medicine

Four years ago there was no supporting infrastructure for the development of the evidence-base for transfusion in England to, for example, support information provided in the Handbook of Transfusion Medicine or in guidelines produced by the British Committee for Standards in Haematology. The need for an evidence-base had been highlighted in the two 'Better Blood Transfusion' Health Service Circulars. In the second of these, 'systematic review and research into the clinical and cost-effectiveness of transfusion practice including alternatives to donor transfusion' was included as a recommendation that required specific work.

Three years ago, the NBA Trust Funds 'pump-primed' a new development to address this and established the Systematic Review Initiative (SRI) in Oxford. The main objective is to improve the evidence base for the practice of transfusion medicine. This objective is being achieved in a variety of ways, primarily the undertaking of systematic reviews. The findings of these are used to inform clinical practice and to identify gaps in the research literature and areas for further research.

A systematic review starts with a clearly formulated question and uses systematic and explicit methods to identify, select and critically appraise relevant research and thereafter to collect and analyse data from the studies that are included in the review. Leading proponents of systematic reviews are the Cochrane Collaboration: part of our work is undertaken in association with the Cochrane Collaboration. To date we have completed systematic reviews on (publication details are given in brackets):

- Is fresh frozen plasma clinically effective: a systematic review of randomised controlled trials? (British Journal of Haematology 2004 (126) 139-152.)
- Prophylactic platelet transfusion for hemorrhage after chemotherapy and stem cell transplantation in hematological malignancies. (The Cochrane Library)¹.
- Antenatal interventions for fetomaternal alloimmune thrombocytopenia in the prevention of fetal and neonatal haemorrhage and death. (The Cochrane Library)¹.
- Desferrioxamine mesylate for managing transfusional iron overload in people with thalassaemia. (The Cochrane Library).

- Granulocyte transfusions for treating infections in patients with neutropenia or neutrophil dysfunction. (The Cochrane Library)

We have contributed to two systematic reviews undertaken by other research groups:

- The role of prophylactic fresh frozen plasma in decreasing blood loss and correcting coagulopathy in cardiac surgery. A systematic review. (Anaesthesia. 2004 Jun; 59(6): 550-8).
- The effectiveness and cost effectiveness of epoetin alfa, epoetin beta and darbepoetin alfa in anaemia associated with cancer, especially that attributable to cancer treatment. (www.nice.org.uk)

On-going systematic reviews include examining the efficacy of prophylactic granulocyte transfusions, the use of plasma exchange as treatment for TTP, the use of blood substitutes, recombinant factor VIIa for the management of severe bleeding in non-haemophilic patients, the use of red cell transfusion triggers in haematological malignancies and intravenous immunoglobulin for immune deficiency. In the planning stages are reviews of adverse events associated with red blood cell transfusion, the practice of the administration of blood and the management of acute gastro-intestinal haemorrhage. All completed systematic reviews will be updated biannually.

Two other activities we are undertaking to improve the evidence base for transfusion medicine are:

- the identification, review and critical appraisal of existing systematic reviews relevant to the field of transfusion medicine. This project is entitled 'Review of Reviews'.
- the hand searching of transfusion medicine and haematology journals and major conference abstracts to develop a database of randomized control trials in transfusion medicine.

The findings from both projects will provide an excellent resource for people interested in the practice of transfusion medicine by being used to inform practice and policy development and to define gaps in the evidence base where new systematic reviews and/or clinical trials are required. The findings of these projects will shortly be disseminated through the SRI section on the JPAC website (www.transfusionguidelines.org).

The ongoing existence of the SRI has now been maintained through core funding established through the blood price mechanism. Trust funding was invaluable to develop this programme in the first instance. The continued success of the SRI has been due to the mix of the persons working within the group. Led by Professors Mike Murphy and David Roberts of the NBS and Dr Brian McClelland from the Scottish National Blood Transfusion Service, the SRI includes a haematologist, an information scientist, a statistician and researchers with a specific interest in systematic reviews. The continued collaboration with a mix of health professionals from within the UK and internationally on all our systematic reviews will ensure the clinical relevance and appropriateness of the conclusions of the reviews.

The supporting infrastructure of the SRI is available to staff both within and outside the NBS. Hospital-based

investigators are encouraged to use the output of the SRI through information on the hospital section of the NBS web, the JPAC website (www.transfusionguidelines.org) and direct contact with the team via Susan Brunskill at susan.brunskill@nbs.nhs.uk

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¹ The Cochrane Library can be accessed freely through the website www.nelh.nhs.uk. These papers are within the 'Cochrane Reviews' section. To find the paper, search by the first word of the title, or by key title words in the 'search terms' box.

Frequently Asked Questions (FAQs)

If agitation of platelets during storage is interrupted, e.g. by equipment failure, do the platelets remain functional?

The purpose of agitation of platelet concentrates is to ensure that oxygen entering through the permeable plastic of the pack can reach the platelets and maintain their aerobic metabolism. The Standing Advisory Committee on Blood Components for the UK Transfusion Services have recently reviewed evidence in the literature with regard to platelet function following interruption of agitation.

Even in older types of storage packs i.e. those not suitable for extended platelet storage, studies showed that for up to 12 hours of no agitation, platelet function was not compromised. Mitchell showed that no agitation did affect parameters such as pH, hypotonic stress response and aggregation over a 4 day period, but this was abolished if platelets were manually mixed daily. Other reports have confirmed that up to 24 hours of no agitation does not affect platelet function either in vitro or in vivo. There is some evidence in addition that in vitro properties are maintained for longer periods e.g. 48 hours, though this has not yet been supported by in vivo studies.

The conclusion of the review therefore is as follows:- "It continues to be recommended that platelets be stored at 22°C with continuous gentle agitation. However if agitation is interrupted as a result of laboratory equipment breakdown or extended transportation outwith the blood bank, this is not an absolute reason to reject them for clinical use. For platelets stored in current types of platelet storage packs at ambient temperature, interruption of agitation for up to 24 hours has no adverse effects on in vitro or in vivo properties. As a check, if there is any doubt, maintenance of platelet swirling is good evidence that they remain viable."

This statement will be included in the "storage section" of platelet component descriptions in the 7th Edition of the Guidelines for the Blood Transfusion Services in the United Kingdom.

References are available on request from Dr Sheila MacLennan, Consultant

email: sheila.maclennan@nbs.nhs.uk

CPD Questionnaire

Q1. Cancers of the blood

- A. Are rare
- B. Are the 3rd most common cancer
- C. Are the 5th most common cancer
- D. Are the 7th most common cancer

Q2. Haemopoietic stem cell graft engineering

Which of the following are NOT strategies available to 'engineer' stem cell grafts?

- A. Use of magnetic cell selection devices
- B. Depletion of T cells
- C. Preparation and infusion of small doses of donor lymphocytes
- D. Immunomagnetic selection of cells expressing CD 130

Q3. Non-haemopoietic stem cells

- A. Can cure neurodegenerative disease
- B. Can be cultured to secrete insulin
- C. Can cure baldness
- D. Can cure heart failure

Q4. EU Directive 2004/23/EC

- A. May become legally binding in the UK
- B. Is, already, legally binding in the UK
- C. Will become legally binding in the UK by April 2006
- D. The NHS is exempt

Q5. EU Directive 2004/23/EC

- A. Covers blood components
- B. Covers donated kidneys
- C. Covers use of tissue engineered products
- D. Covers stored bone

Q6. JACIE

- A. Is a new teenage magazine
- B. Is the Joint Accreditation Committee of ISCT and EBMT
- C. Only accredits clinical bone marrow transplant units
- D. Accreditation lasts 5 years

Q7. JACIE

- A. Standards and inspection process have been adopted from Foundation for Accreditation of Cellular Therapy
- B. Only applicable to Spain
- C. Does not include collection facilities
- D. Quality management is not a major problem within most clinical facilities

Q8. Immunotherapy: Herceptin

- A. Targets human ductal growth factor receptor 2 protein
- B. Is ineffective in clinical trials of primary breast cancer
- C. Targets human epidermal growth factor receptor 2 protein
- D. Human epidermal growth factor receptor 2 protein is over expressed in 50% of primary breast cancers

Q9. Immunotherapy

- A. Donor leucocyte infusions are effective in over 60% of CML
- B. Donor leucocyte infusions are effective in over 60% of ALL
- C. Donor leucocyte infusions involve using modified donor lymphocytes
- D. ALL cells express excess CD54

Q10. BBMR

- A. Has less than 240,000 donors registered
- B. Does not include cord blood donations
- C. Is the smallest registry
- D. Is the 6th largest registry in the world

Q11. Cord blood banking

- A. Is not available in the UK
- B. Is only available in London
- C. Has increased the availability of non-caucasian stem cells
- D. Transplants with cord blood as a source of stem cells have a universally poor outcome

Q12. HLA selected platelets

- A. Will always improve platelet increments in the refractory patients
- B. Are always an exact HLA match to the patient
- C. Cannot be provided
- D. Require follow-up of platelet increments for best effect

*Blood Matters is prepared and issued by the National Blood Service, Reeds Crescent,
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