Australian and New Zealand Society of Blood Transfusion Ltd

1st Edition, January 2011

GUIDELINES

FOR

PREVENTION OF TRANSFUSION-ASSOCIATED GRAFT-VERSUS-HOST DISEASE (TA-GVHD)
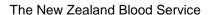
**(Update of the Guidelines for
Gamma Irradiation of Blood Components, May 2003)**

Australian & New Zealand
Society of Blood Transfusion Ltd

These guidelines are endorsed by:

The Australian Red Cross Blood Service

The New Zealand Blood Service

Disclaimer: Institutions using these guidelines should formulate their own policies according to their patient population and availability of irradiated components. By necessity, these policies may need to be broader than those in this document.

FOREWORD

ANZSBT Council is pleased to publish the first edition of the '*Guidelines For Prevention of Transfusion-Associated Graft-versus-Host Disease*' [formerly the '*Guidelines For Gamma Irradiation of Blood Components*'].

The revision of the Guideline title reflects its focus on the potential clinical consequences of transfusion-associated graft-versus-host disease, and underscores the importance of good communication between clinicians, laboratory staff and patients to identify at-risk patients and ensure they receive appropriate component therapy.

These guidelines are the considered opinion of the Council and Standing Committees of the Australian and New Zealand Society of Blood Transfusion. They have been endorsed by the national blood services of Australia and New Zealand. They are not intended as prescriptive statements but as guidance for best practice.

Particular thanks are due to Dr Zane Kaplan who undertook the literature review and drafted the revised document. Many others have provided valuable feedback during the review process, either as individuals, groups or on behalf of institutions. The final document reflects a consensus view based on the available (often quite limited) evidence in this area from the peer-reviewed literature and haemovigilance program reports.

Erica Wood
President ANZSBT
January 2011

SUMMARY OF AMENDMENTS TO THE 2003 GUIDELINES

Definite indications:

Aplastic anaemia: Has been removed from this section and placed in the possible indication section owing to a paucity of evidence in the literature regarding the association of TA-GvHD with aplastic anaemia. However, given that many of the treatments used are directed at T-cell function (especially anti-thymocyte globulin), the reviewers have suggested that serious consideration be given to the routine universal use of irradiated components in patients receiving treatment for aplastic anaemia.

Possible indications:

Chronic myeloid leukaemia (CML): Removed from the possible indication owing to a lack of evidence in the literature of increased risk of TA-GvHD.

Non-Hodgkin lymphoma (NHL): All cases including B- and T-cell NHL have been included as possible indications owing to several case reports and reporting to the UK Serious Hazards of Transfusion (SHOT) system, as discussed in the text.

All newborn infants: While there is no strong evidence, it is not always obvious whether a newborn has an immunodeficiency state. Therefore, to avoid the potential of missing a congenital cellular immunodeficiency, consideration could be given to offering irradiated components to all newborn infants.

Massive transfusion: Recent evidence suggests a degree of chimerism in patients after massive transfusion. The significance of this, however, remains uncertain. The consideration of provision of irradiated components in the setting of massive transfusion is an evolving area and, therefore, highlighted only for the sake of consideration. Note, however, that no firm evidence for deleterious effects of chimerism currently exists.

No Indication:

Thalassaemia and Haemophilia: These appeared in the previous Guideline but have been removed; there is no need to list all scenarios where irradiated blood components are not required.

Solid organ transplant: There is no evidence to support routine recommendation for irradiated components in patients undergoing solid organ transplantation unless other clinical circumstances warranting irradiated components exist.

Term infants: Moved to the possible indications section.

Tabulated Recommendations For Irradiated Cellular Components:

(Table reflects recommendations contained within the text of the Guidelines)

Definitions:
1. *Definite indications:* These are indications where there is strong evidence to support the requirement for use of irradiated blood components or where there is clear consensus on the requirement within published guidelines
2. *Possible indications:* This includes settings where case reports have been published but where no controlled studies are available
3. *No indication:* No cases have been reported or insufficient evidence to mandate routine irradiation

Definite indications	Clinical setting	Section(s)
I	Directed donations (from blood relatives)	2; 4.3.6
II	HLA-selected/matched platelet transfusions	2; 4.3.6
III	Granulocyte transfusions	3
IV	Intrauterine and all subsequent neonatal exchange transfusions	4.1
V	Congenital cellular immunodeficiency disorders	4.1
VI	Allogeneic and autologous haematopoietic stem cell transplantation	4.2.2; 4.3.2
VII	Hodgkin lymphoma	4.2.4
VIII	Patients receiving nucleoside analogues for malignant or non-malignant disorders	4.2.4; 4.3.1
IX	Patients receiving alemtuzumab for malignant or non-malignant disorders and transplantation	4.2.4; 4.3.2
Possible indications	**Clinical setting**	**Section(s)**
I	Premature infants and infants weighing less than 1300g	4.1
II	All newborn infants	4.1
III	Acute leukaemia	4.2
IV	Non-Hodgkin lymphoma	4.2.4
V	Patients with B cell malignancy who receive non-nucleoside analogue-containing chemotherapy and/or radiotherapy leading to lymphopenia $<0.5 \times 10^9$/L	4.2.4
VI	T cell malignancies	4.2.4
VII	Patients receiving high doses of chemotherapy and/or irradiation sufficient to cause lymphopenia $<0.5 \times 10^9$/L	4.2.4; 4.3.2
VIII	Patients receiving long term or high dose steroids as therapy for malignancies	4.2.4; 4.3.3
IX	Aplastic anaemia receiving immunosuppressive therapy	4.2.5
X	Massive transfusion for trauma	4.3.6
No indication	**Clinical setting**	**Section(s)**
I	HIV/AIDS (where none of the above apply)	4.3.5
II	Congenital humoral deficiency disorders	4.1
III	Solid organ transplantation (where none of the above apply)	4.3.4

INTRODUCTION

Transfusion-associated graft-versus-host disease (TA-GvHD) is a rare, but almost universally fatal, iatrogenic complication of transfusion. The inherent risk associated with an individual transfusion depends on the interplay of several factors, including the number and viability of contaminating lymphocytes in the transfused cellular component, the susceptibility of the patient's immune system to the engraftment of donor lymphocytes, and the degree of immunological (human leucocyte antigen, HLA) homology between the donor and the recipient (BCSH 2010). Gamma irradiation to inactivate viable T lymphocytes contained within the blood components remains the mainstay in prevention of TA-GvHD.

This document updates the Australian & New Zealand Society of Blood Transfusion (ANZSBT), Australian Red Cross Blood Service and New Zealand Blood Service *"Guidelines for Gamma Irradiation of Blood Components"* most recently published in 2003. The 2003 Guidelines drew heavily on the 1996 guidelines from the Blood Transfusion Task Force of the British Committee for Standards in Haematology (BCSH). In preparing these updated Guidelines, the recent literature and guidelines prepared by other professional and advisory bodies, including the BCSH, have been reviewed.

However, the evidence underpinning these and other guidelines remains scant pertaining to many of the clinical situations addressed here, generally being based on level III and IV evidence. Moreover, there are certain situations where irradiated components, if available, could or should be considered, but a definite recommendation cannot be made owing to a lack of evidence. Consideration has been given to changes in clinical transfusion practice, procedural aspects and compliance issues pertaining to gamma irradiation, and to defining patient cohorts who may benefit from receipt of irradiated blood components. The Guidelines also acknowledge and address potential future methods for lymphocyte inactivation. While no definite recommendations can be made regarding these evolving technologies, owing to a current lack of clear clinical evidence, reference to these strategies is included for completeness and to permit further consideration as they develop.

SCOPE

These Guidelines cover the pathogenesis and pathophysiology of TA-GvHD, procedural aspects, equipment dosimetry and maintenance, clinical indications for irradiated blood components, and future directions in preventing TA-GvHD.

SECTION 1

Pathogenesis & clinical features of TA-GvHD

TA-GvHD is a rare but serious complication of cellular blood component transfusion. The pathogenesis underpinning this condition is the transfusion of immunocompetent T lymphocytes which are not cleared (as normally occurs) by the recipient and subsequently engraft and proliferate in the recipient's bone marrow. Survival of these transfused lymphocytes may occur either as a result of shared human leucocyte antigen (HLA) epitopes and/or recipient immunosuppression. Engraftment and proliferation of CD4+ and CD8+ T cell lineages and subsequent immunologic responses culminate in GvHD. Recipient HLA class II antigens as well as minor histocompatibility antigens are presented to donor lymphocytes resulting in T lymphocyte activation, proliferation, cytolytic activity and cytokine liberation. The cytokine release and direct cytotoxic effect of the donor T cells produce the constellation of signs and symptoms associated with GvHD (Ruhl, et al 2009). Donor B cells have also been observed to produce cytotoxic antibodies (Vogelsang and Hess 1994).

Recently cases of long-lived lymphocyte survival and "engraftment" have been demonstrated in the setting of massive transfusion following trauma as well as in post-partum women. Such lymphocyte engraftment results in microchimerism without causing TA-GvHD. Why microchimerism can persist without evolving into overt disease remains to be elucidated, however, in some studies, the presence of such microchimerism has been linked to development of autoimmune disease.

TA-GvHD presents clinically like GvHD associated with allogeneic haematopoietic stem cell transplantation (HSCT), with multisystem and cutaneous involvement. However, when compared with transplant-associated GvHD, the transfusion-associated syndrome typically manifests earlier. Fever is most commonly the first presenting symptom, occurring as early as day 4 post-transfusion, with a median onset of 10 days, with most patients developing signs and symptoms 2-30 days after transfusion (Dwyre and Holland 2008). An erythematous maculopapular rash indistinguishable from that complicating HSCT subsequently develops. Gastrointestinal complications may range from abdominal pain to profuse bloody diarrhoea. Liver dysfunction manifests predominantly as a cholestatic hepatitis due to lymphoplasmacytic infiltration of portal tracts and damage to bile duct epithelium with consequent destruction of bile ducts. Bone marrow failure, presenting as progressive pancytopenia with neutropenic infections, occurs at a median of day 16. The bone marrow aplasia is the most significant contributor to the dismal prognosis in TA-GvHD. TA-GvHD is fulminant and rapidly fatal in the vast majority of cases. Death occurs on average 51 days following the transfusion (Parshuram, et al 2002). Immunosuppressive regimens including corticosteroids, antithymocyte globulin and OKT3 have yielded poor results with only a few documented survivors (Dwyre and Holland 2008).

The clinical presentation in infants and neonates is similar to that seen in adults, however, the interval between transfusion and symptom evolution may be delayed compared with that seen in adults (Ohto and Anderson 1996)

Incidence

The incidence of TA-GvHD is uncertain and may be underestimated in the world literature due to a lack of recognition and under-reporting. In susceptible recipients the frequency may be 0.1-1% without appropriate preventative strategies.

Diagnosis

TA-GvHD is a clinicopathologic diagnosis requiring a high index of suspicion. As the clinical signs and symptoms are non-specific and may mimic drug reactions, viral infections and autoimmune conditions, the diagnosis is often delayed and may be missed. Therefore, TA-GvHD should be suspected in any patient presenting with rash, fever, liver dysfunction and gastrointestinal symptoms with a recent history of transfusion (Agbaht, et al 2007). Histological diagnosis of TA-GvHD can be made from any affected tissue, although skin biopsies are usually the most easily accessible. While the histological findings are characteristic, they are not pathognomonic. Bone marrow biopsy frequently reveals a variably hypocellular marrow with a lymphocytic infiltrate. Haemophagocytosis may be prominent.

HLA typing plays a central role in the diagnosis. DNA-based analysis demonstrating foreign cells or DNA in the recipient's circulation or in tissue infiltrates is more sensitive than serological methods.

Blood components associated with TA-GvHD

All blood components containing viable lymphocytes potentially can cause TA-GVHD. Whole blood, red cells, platelets and granulocytes have been implicated as causes of TA-GVHD. Frozen components and fractionated components have not been implicated in TA-GvHD.

Risk factors for TA-GvHD

Recipient-related factors:
Transfusion recipients most vulnerable to TA-GvHD are those with congenital or acquired immunodeficiencies. These render the recipient at risk of engraftment by donor lymphocytes, due to an inability to recognise these cells as foreign and consequent failure to destroy them. Foetuses, low birth weight and premature babies are at particular risk, and some authors suggest all newborns should also be considered as at risk. Acquired cellular immunodeficiency may result either from disease states (such as haematologic malignancy) or be therapy-related (such as purine-based chemotherapeutic agents and in organ transplantation). Older age has also been implicated as a putative risk factor, with over 80% of patients with TA-GvHD being older than 65. However, it is not clear whether these reports simply reflect the typical age of transfusion recipients rather than any inherent susceptibility. Case series from the UK Serious Hazards of Transfusion (SHOT) data and other reports have highlighted coronary artery bypass grafting as a potential predisposing factor (Thaler, *et al* 1989; Williamson, *et al* 2007). Cardiopulmonary bypass and extracorporeal circuits have been shown to have an immunomodulatory effect, causing a transient reduction in lymphocyte function in immunocompetent patients (Hauser, *et al* 1991). However, it is unclear whether cardiac surgery produces the immunosuppression required for TA-GvHD or whether the increased incidence merely reflects the high number of donations used in this setting (Williamson, *et al* 2007).

Donor-related factors:
Presence of a one-way HLA match between donor and recipient (the donor is homozygous for an HLA haplotype for which the recipient is heterozygous) is associated with a significantly increased risk of TA-GvHD. Owing to HLA similarities between donor and recipient, donor lymphocytes are not rejected as foreign. This is the likely mechanism of underlying TA-GvHD in immunocompetent transfusion recipients. In a heterogeneous Caucasian population, the calculated risk of a patient with a particular HLA haplotype receiving a transfusion from a donor homozygous for the same HLA haplotype was reported as 1 in 7174, which translates to an actuarial risk of TA-GvHD due to homozygous HLA-haplotypes at 1 in 17,700-39,000. A significantly higher risk in populations such as the Japanese, who have higher degrees of HLA-homology, translates into the Japanese population risk being estimated at 1 in 1160 to 7900. The highest proportion of cases in the literature have been reported from Japan (Wagner and Flegel 1995).

Blood component-related factors:
The principal risk factor here is the number of viable lymphocytes in the component. The minimum number of viable T cells required for TA-GvHD is unknown. As few as 10^4 per kg may be lethal in immunocompromised hosts (Klein 2006). Further, reports have suggested that TA-GvHD may occur with transfusion of as few as 8×10^4 lymphocytes. The viability of lymphocytes is not constant throughout storage life and decreases as a function of component age, with the greatest risk being during the first 3 days, after which lymphocyte viability decreases exponentially. Further studies using flow-based assays detected essentially no viable T cells surviving in cold storage in red cells beyond 3 weeks (Vogelsang 1990).

Thus, three significant factors appear to directly relate to increased risk of TA-GvHD including:
1. Susceptibility of the recipient's immune system to engraftment by donor lymphocytes
2. HLA homology between recipient and donor
3. Number of viable lymphocytes transfused

Recommendations on diagnosis and notification:

There should be a high clinical index of suspicion of TA-GvHD in patients presenting with the constellation of symptoms, including fever, rash, liver dysfunction and gastrointestinal symptoms, and with a clear temporal relationship to a transfusion.

Diagnosis should be confirmed by DNA analysis.

Suspected cases should be notified to the pathology service provider, the blood service and relevant haemovigilance programmes.

SECTION 2

Principles of prevention of TA-GvHD

As TA-GvHD is almost invariably fatal, the prime objective is prevention. The principles central to prevention of TA-GvHD include the following:

Appropriate clinical use of blood transfusions:
Transfusion of blood components should be in accordance with relevant guidelines to ensure clinical appropriateness of indication, component and dose; to avoid unnecessary transfusions; and to thereby minimise transfusion-associated risks.

Irradiation of blood components:
Irradiation of cellular blood components is the mainstay of TA-GvHD prevention. Ionising radiation doses are employed that inhibit the proliferative ability of lymphocytes, while preserving the integrity of the component to be transfused.

Directed transfusions and donations from HLA-selected/matched donors:
Directed transfusions from family members significantly increase a recipient's susceptibility to TA-GvHD, as blood relatives share HLA haplotypes, thus increasing the risk of a one-way matched transfusion. Therefore, directed transfusion from relatives should not be encouraged outside specific medical indications. These are rare, and include patients who have rare blood groups or antibodies to high incidence antigens. Directed family donations and HLA-matched or otherwise selected donations should always be irradiated before being transfused.

Avoidance of transfusion of fresh blood?
There is some evidence that the fresher the blood the higher the risk of TA-GvHD. Case series have identified use of "fresh blood", evidenced by the fact that in about 90% of cases of TA-GvHD in the United States transfused blood was less than 4 days old (Ohto and Anderson 1996 and Petz*, et al* 1993). Further studies examining the influence of storage on T-cell function and survival have demonstrated that, after 2 weeks of storage, leucocytes progressively undergo apoptosis and lose their *in vitro* proliferative ability (Mincheff 1998).

Consideration could be given to using red cells that have been stored for greater than 1 week in at risk patients and cardiac surgery patients, as fresh blood (<3 days) has been demonstrated to cause TA-GvHD more commonly compared with blood stored for longer periods (>7 days) (Dwyre and Holland 2008). Outside of large volume transfusions to neonates, there are no other established specific medical indications for blood less than 5 days old (Williamson*, et al* 2007). However, there is little evidence to guide practice in this area and the potential adverse effects related to duration of storage, including potassium leakage and cytokine generation, must also be considered. Other potential adverse effects of the blood storage lesion are the subject of active research but are as yet unresolved.

Universal leucocyte depletion of cellular blood components?
Since the publication of the previous Guidelines, routine leucodepletion of all red cells and platelets is now in place in both New Zealand and Australia.

With the implementation of universal pre-storage leucodepletion for cellular components such as red cells and platelets in many centres around the world, there are emerging data to suggest that leucodepletion may reduce the incidence of TA-GvHD. For example, according to the UK SHOT data (SHOT report 2007), thirteen cases of TA-GvHD were reported between 1996 and 2001. Only two of these thirteen cases occurred in patients who received leucodepleted units. Of these 2 cases, one was a patient with myeloma and the other a child with relapsed acute leukaemia. No cases have been reported in immunocompetent recipients since the implementation of routine leucodepletion in the UK in 2001. While this may reduce the incidence of TA-GvHD, especially in immunocompetent recipients, by reducing the number of viable lymphocytes in a transfused component, it does not obviate the need for irradiation of blood components in at risk patients.

Institutional protocols to ensure recipients requiring irradiated components are identified:
Hospital, laboratory and blood service protocols should be in place to identify patients who should or must receive irradiated components. Over the first 10-year period of SHOT, there were 405 reports of patients for whom provision of irradiated components had been omitted in error on at least one occasion (SHOT reports; Williamson*, et al* 2007). Haemovigilance reports from both Australia and New Zealand have also highlighted instances where

recipients have received blood components with modifications not meeting their clinical needs (New Zealand Blood Service 2005 – 2009; Blood Matters, 2007).

Therefore, to attempt to minimise such events, mechanisms must be in place for appropriate and timely communication of information regarding special transfusion requirements (Blest and Howell 2007). Institutions must ensure communication regarding special transfusion requirements between clinicians, ward staff and laboratory blood bank staff. If patient care is transferred or shared between centres, procedures should be in place to ensure adequate and timely communication of special transfusion requirements. Efforts should also be made to educate patients about whether they require blood components with any specific modifications. In order to successfully implement the recommendations contained within these Guidelines, institutional education programmes and protocols should be devised for treating clinicians and emergency department staff, nursing staff, pathology and blood bank staff to ensure adherence both to these Guidelines and local policies.

Recommendations on prevention of TA-GvHD:

Decisions on indications for transfusion of blood components should be made in accordance with relevant clinical practice guidelines.

Institutions should have policies and procedures in place to ensure that all transfusion recipients who require irradiated components are identified and receive appropriate components.

Directed donations from blood relatives and HLA-selected/matched components must always be irradiated prior to transfusion.

Leucodepletion is not equivalent to irradiation and must not be used as an alternative to irradiation.

SECTION 3

Procedural aspects of irradiation

Manufacturing aspects:
The dose of radiation should be selected which inactivates T cells while maintaining the integrity of the blood components to be transfused.

Dose of irradiation:
The general consensus in the literature holds that doses in the range of 15 to 50 Gy are required to abrogate lymphocyte function while maintaining cellular function of the elements intended for transfusion. These doses have been derived using various assays which assess lymphocyte proliferative potential, such as assessment of response of lymphocytes to mitogens, limiting dilution assays (LDA) and responses of the lymphocytes to allogeneic cells in mixed lymphocyte cultures (Schroeder 2002). Based on experimental data from HSCT research, LDA are the most sensitive method for determining functional T cell numbers (Goes, *et al* 2006). A decrease in T cells of >2 log is needed to prevent GVHD (Schroeder 2002). Using LDA, Pelszynski et al demonstrated an incremental log reduction of T cells capable of proliferation with increasing dosages of γ irradiation. At a γ radiation dose of 25 Gy, there was a >5 $\log_{10}$ depletion of T cells to a level undetectable by LDA (Pelszynski, *et al* 1994). Further, Góes and colleagues, using LDA and flow cytometric based assays, revealed that at 25 Gy no T cell growth was detected in any of the experiments and a greater than 5 $\log_{10}$ reduction in functional T cells was noted (Goes, *et al* 2006). A recent study examining the biologic effect of ionising radiation on human blood *ex vivo* revealed that mitogenic capacity was reduced by greater than 90% by doses of 10-20 Gy, but residual proliferative capacity was demonstrable up to 50 Gy. The authors concluded that doses of 30-50 Gy abrogate lymphocyte proliferation almost completely (Weinmann, *et al* 2000). The above findings were also confirmed for platelets, with 25 Gy inactivating T cells as measured by LDA (Luban, *et al* 2000).

While γ irradiation has been traditionally used, X-ray irradiation has been investigated as an alternative as the machines are less expensive and do not have a radioactive source. X-ray irradiation has been shown to provide an equivalent effect on lymphocyte proliferation compared to γ irradiation (Janatpour, *et al* 2005).

In accordance with the above findings, international regulatory authorities and blood services have stipulated requirements for irradiation of blood components. The AABB *Standards* require a minimum dose of 25 Gy delivered to the central portion of the container with a minimum dose at any point in the component being at least 15 Gy (AABB 2009). Japanese guidelines also recommend a dose between 15 and 50 Gy (Asai, *et al* 2000). The Council of Europe and BCSH Blood Transfusion Task Force mandate a minimum dose in the irradiation field of 25 Gy, with no part receiving greater than 50 Gy (BCSH 2010 and Council of Europe Publishing 2008).

Recommendations on procedural aspects of irradiation:

Manufacturing practices must comply with regulatory authority requirements and these Guidelines.

The minimum dose achieved in the irradiation field should be 25 Gy, with no part receiving greater than 50 Gy.

γ-irradiation using dedicated instruments containing a long half-life, gamma-emitting source, or X-ray irradiation using linear accelerators or similar equipment, may be used providing dosages are calibrated and validated in accordance with the dose standards above.

If dedicated γ-irradiation blood irradiators are employed, such instruments will contain a long half-life, gamma-emitting source. The source must be double encapsulated. All specifications should be available. Adequate shielding must be provided to ensure that all dose rates are as low as is reasonably achievable at all accessible points. Radiation safety, dosimetry and personnel safety issues should be under the responsibility of a suitability qualified person in accordance with legislation.

The irradiator manufacturer, or their agent, should commission the irradiator and, on completion of commissioning, provide a calibration certificate for a dose rate at a specified point in the canister calibrated to traceable national Standards.

Following calibration/recalibration, a table must be produced which gives irradiation times for specified doses for a set period. Both the dose rate and the dose distribution must be checked upon installation, at least annually, and after any source change or mechanical alterations, particular to the rotating turntable.

Quality control procedures must be implemented and all operators must have been adequately trained in the use of the equipment.

Preventative maintenance procedures must be in place and wipe tests must be carried out at regular intervals, preferably six monthly, to check for leakage of radioactive contamination.

All facilities performing irradiation of blood components must ensure compliance with local legislation.

Institutions performing on site irradiation may either use γ-irradiation or X-ray irradiation providing their equipment and practices comply with the requirements stipulated above, are validated, comply with radiation regulatory and legislative requirements and the dosimetry must be calibrated for the dose rate at a specified point in the canister. Maintenance, preventative maintenance and quality control procedures must be in place.

Effect of irradiation on blood component efficacy and storage life:

The effect of irradiation on efficacy and safety of different components has been extensively evaluated. At radiation doses up to 50 Gy, the overall clinical efficacy does not appear to be adversely affected as there is no demonstrable compromise in function of blood cells other than lymphocytes, whose mitogenic stimulation is reduced by 98.5% (Schroeder 2002, Button, *et al* 1981 and Anderson, *et al* 1991). However, while not definitely clinically significant, physiological changes have been demonstrated in cellular components.

Effects on Red Cells:
Irradiation may affect the 24 hour recovery of transfused red cells, however, this effect is most pronounced only after prolonged storage (FDA 1993). Published data demonstrate that red cells irradiated within 24 hours of collection maintain satisfactory viability up to 28 days, while units stored for 42 days had unsatisfactory viability (Davey, *et al* 1992). Other studies have suggested that gamma irradiation did not significantly affect the 24-hour post-transfusion recovery of red cells stored for 35 days; further, red cell viability was not affected by whether the blood was irradiated at one or 14 days after collection (Mintz and Anderson 1993).

Gamma irradiation increases the supernatant potassium level. Extracellular potassium levels increase more rapidly during storage in irradiated compared with non-irradiated red cells (Dinning, *et al* 1991). Further, the increase correlates with the initial radiation dose. Rapid infusion of potassium can have deleterious cardiac effects. Generally, with "top-up" transfusions infused at usual rates, the potassium load is of little clinical significance. However, it is of concern in infants and in large volume transfusions such as exchange transfusion,

intrauterine transfusion and rapid massive transfusion in resuscitation settings. Therefore, in considering the clinical significance, both the speed and volume of the transfusion, as well as the age of the blood, must be taken into account.

Irradiated red cells have been shown to contain more cell-free haemoglobin (approximately 50% increase) than control cells after equivalent periods of storage. There is no demonstrable, clinically significant effect of irradiation on red cell pH, glucose consumption, ATP or 2,3 DPG levels.

Effects on Platelets:
At doses of irradiation up to 50 Gy, there are no demonstrable significant changes in platelet function. Platelets irradiated at up to 5 days after collection show no alteration in post-irradiation function. Further, recent evidence suggests the timing of irradiation has no adverse effect of platelet function. Irradiation any time from day 1 to day 5 had no effect on platelet quality during a 7 day storage period (van der Meer and Pietersz 2005; Tynngard*, et al* 2008).

Effects on Granulocytes:
There is conflicting evidence for irradiation damage to granulocyte function. After 50 Gy, granulocytes have normal bacterial killing capacity, chemotactic mobility, and normal superoxide production after high-dose stimulation. Nitroblue tetrazolium reduction and ingestion stimulated by complement opsonized oil droplets were not diminished by 50-100 Gy (Button*, et al* 1981).

Recommendations on timing, storage and labelling of irradiated components:

Red cells may be irradiated at any time up to 14 days after collection and, thereafter, stored for a further 14 days from irradiation. Where the patient is at particular risk from hyperkalaemia, it is recommended that red cells be transfused within 24 hours of irradiation.
[Red cells can re-enter the inventory as long as the altered shelf-life is observed].

Platelets can be irradiated at any stage in their five-day storage and can, thereafter, be stored up to their normal shelf life of five days after collection.

Granulocytes for all recipients must be irradiated as soon as possible after production and, thereafter, transfused with minimal delay.

Labelling and documentation:

All irradiated components must be identified [and labelled immediately] with an approved label. The label should be permanent and include the date and dose of irradiation and any reduction in shelf life.

Each blood component pack being irradiated should be monitored using a radiation-sensitive label or device to indicate adequate exposure to gamma rays. All packs should be individually labelled.

A label indicating that irradiation has occurred should be affixed to each irradiated component to provide the end user with an assurance that irradiation has occurred.

There must be a permanent record of all components irradiated.

Blood components associated with TA-GvHD which require irradiation:

As described above, all non-frozen blood components containing viable lymphocytes have been implicated in TA-GvHD, including whole blood, red cells, platelets and granulocytes. Frozen blood components, such as fresh frozen plasma and cryoprecipitate, and fractionated products, such as albumin, factor concentrates and intravenous immunoglobulin, have not been implicated in TA-GvHD (Bernvil*, et al* 1994; Wieding*, et al* 1994). Fresh frozen plasma (FFP) has only been potentially implicated in one case of an infant with thymic hypoplasia who received only irradiated red cells and non-irradiated plasma (Wintergerst*, et al* 1989). This case has several confounding factors and overwhelming consensus from the literature is that FFP does not require irradiation (O'Shaughnessy*, et al* 2004).

There is a lack of consensus between the British (2010) and Japanese Guidelines (Asai*, et al* 2000) pertaining to the need to irradiate cryopreserved red blood cells. To date, there have been no documented cases of TA-GvHD

following transfusion of cryopreserved red blood cells despite the fact that greater than 1×10^7 lymphocytes may be present in this component (Dwyre and Holland 2008). The risk associated with this component is further reduced by the thorough washing during the deglycerolisation process. However, owing to infrequent use of cryopreserved red cells and the paucity of available evidence, no firm recommendation can be made regarding the necessity to irradiate this component prior to transfusion.

Granulocyte preparations contain high numbers of lymphocytes (5-10 $\times 10^9$ lymphocytes per unit) (Sanders and Graeber 1990). TA-GvHD has occurred following transfusion of non-irradiated granulocytes from random (non-related, non-HLA matched) donors, directed (blood relative) donors and HLA-matched donors (Ruhl, *et al* 2009). Thus, irrespective of the recipient's risk profile, all granulocyte components must be irradiated prior to transfusion.

These *Guidelines* will require review and updating should alternative fresh blood components become available.

Recommendation on blood components requiring irradiation:

Recipients in at risk groups (see section 4) should only receive red cells, platelets and granulocytes which have been irradiated.

Consideration should be given to irradiation of frozen red cells following the thawing and deglycerolisation process and prior to transfusion.

Frozen components (FFP, cryoprecipitate) and fractionated plasma products do not require irradiation.

Blood stem cells for the purposes of transplantation must not be irradiated.

SECTION 4

Clinical indications for gamma irradiated blood components:

The transfusion recipients most vulnerable to TA-GvHD are those with either congenital or acquired cellular immunodeficiencies. Acquired cellular immunodeficiency may result from disease state or therapy and render the patient incapable of recognising and/or destroying foreign lymphocytes.

4.1 Neonatal and paediatric practice:

Neonatal patients represent a cohort of relatively immunocompromised patients. The newborn may be at particular risk of TA-GvHD because of either physiological immune incompetence or, in some cases, because of an underlying congenital T-cell immunodeficiency. Although neonates, especially those who are extremely premature, are considered to be at increased risk for TA-GvHD, the degree to which they are at increased risk is controversial. This immunomodulatory effect is further compounded by the myriad of clinical conditions affecting such neonates, including sepsis, poor oral intake and nutritional status, as well as the need for surgery and multiple transfusions. Thus, the individual risk of a neonate requiring transfusion is difficult to calculate precisely (Strauss 2000). Neonates at particularly high risk are those who are very small (weighing less than 900g) or less than 28 weeks gestation, compared with infants weighing more than 1300g or greater than 28 weeks gestation, where a one-way HLA match appears to be required to confer increased risk (Ohto and Anderson 1996). Further, it is generally accepted that all premature infants are immunocompromised to some degree, and it has been suggested that they should receive irradiated blood components up to 7 months of age (Dwyre and Holland 2008).

Ohto and Anderson hypothesised that several mechanisms inherent in neonatal physiology may actually protect against TA-GvHD (Ohto and Anderson 1996). These mechanisms include the ability of the fetal thymus to abrogate donor cytotoxic T cell precursors which have the potential to attack recipient tissue. Further, as the antigen presenting cells in the foetus are immature and cannot provide co-stimulatory signals required for the immune response, the donor T cells may become inactivated/semi-tolerant of host tissues (Ohto and Anderson 1997).

The majority of cases of TA-GvHD reported in apparently immunocompetent infants have occurred in the setting of intrauterine transfusion (IUT) and/or following exchange transfusion. Indirect evidence seems to indicate that in large volumes, transfusion may result in either immunologic tolerance or further immune suppression increasing the risk of TA-GvHD. Although reports are scarce, the available evidence supports irradiation of cellular blood components for all neonates receiving exchange transfusions (ET) and IUT. Further, infants who received an IUT or ET should continue to receive irradiated blood components for subsequent "top-up" transfusions (BCSH 2010).

There is no consensus in the literature pertaining to use of irradiated components in neonatal practice, likely due to the lack of data. The majority of the recommendations are, therefore, based on level III evidence and grade B recommendations (Gibson*, et al* 2004). Some authors (Ohto and Anderson 1997) recommend that all cellular components transfused to newborns should be irradiated, whereas others have adopted a less stringent policy advocating irradiation of blood components only in the setting of IUT and ET (BCSH 2010 and Ruhl *et al*, 2009).

Transfusions to patients with congenital cellular immunodeficiency states account for the majority of paediatric cases of TA-GvHD. These congenital cellular immune deficiencies include severe combined immunodeficiency, DiGeorge syndrome, Wiskott-Aldrich syndrome and ataxia telangiectasia. Use of irradiated blood components in such patient groups is mandatory. Humoral immunodeficiency states, however, do not convey the same risk of TA-GvHD and routine irradiation of cellular blood components is not recommended. No cases of TA-GvHD have been reported in patients with isolated disorders of humoral immunity (BCSH 2010).

Clinical suspicion of an underlying immunodeficiency syndrome should be aroused by the presence of dysmorphic features, craniofacial abnormalities, cardiac anomalies, hypocalcaemia and lymphopenia. These features may be associated with partial deletion of chromosome 22 (del 22q11.2). T-cell function is reduced in up to 80% of patients with del 22q11.2 (Sullivan*, et al* 1998)

The issue that arises, however, is that it may be difficult to identify neonates with severe primary immunodeficiency who are at high risk of TA-GvHD and should receive irradiated cellular blood components (Strauss 2000). An underlying primary immunodeficiency disorder may not be overtly apparent after birth and, in

most instances of TA-GvHD, the immunodeficiency was undetected prior to the transfusion (Webb 1995). Therefore, some centres have advocated using irradiated blood components for all infants.

Recommendations for neonatal and paediatric practice:

Type of transfusion:
Intrauterine and exchange transfusion:
- *All red cells and platelets for intrauterine transfusion must be irradiated.*
- *It is essential to irradiate red cells for exchange transfusion if there has been a previous intrauterine transfusion, or if the donation comes from a blood relative. For other exchange transfusions, irradiation is recommended provided this does not unduly delay transfusion.*
- *Red cells less than 5 days of age should be used for intrauterine and exchange transfusion, and must be transfused within 24 hours of irradiation.*

Top-up transfusion: *It is not necessary to irradiate cellular components for routine 'top-up' transfusions unless there has either been a previous intrauterine transfusion or the blood has come from a blood relative, in which case the cellular component must be irradiated.*

Platelet transfusion:
- *Irradiation must be performed on platelets for transfusion in utero to treat alloimmune thrombocytopenia, and on platelet transfusions given after birth to infants who have received either red cells or platelets in utero.*
- *There is no requirement to irradiate platelets for pre-term infants >28 weeks and weighing >1300g, or term infants, unless these components are derived from blood relatives or are HLA-matched.*

Granulocyte transfusion: *All granulocyte transfusions must be irradiated for babies of any age and transfused as soon as possible after irradiation.*

Clinical considerations:

Premature neonates: *Particular consideration should be given to providing irradiated cellular components for premature infants <28 weeks or <900g, and consideration should also be given to using irradiated cellular blood components in premature infants weighing less than 1300g.*

All neonates: *Consideration should be given when possible to use of irradiated cellular components transfused to newborns.*

Congenital immunodeficiencies in infants and children:
- *There should be a high index of suspicion concerning coexisting cardiac defects and immunodeficiency. Dysmorphic features, craniofacial abnormalities, hypocalcaemia and lymphopenia are suggestive of an immunodeficiency syndrome – irradiated cellular components should be used if any suspicion of an underlying immunodeficiency state exists.*
- *Irradiation of cellular components is recommended for all infants/children with suspected or diagnosed T-cell immune deficiency states.*

Acquired immunodeficiency states in childhood: *There is no indication for the irradiation of cellular components for children who are HIV antibody positive, or who have AIDS.*

4.2 Haematologic disorders:

4.2.1 Acute leukaemia:

Previously acute leukaemia in both adults and children was not considered to be an indication for use of irradiated cellular blood components. However, since the publication of the previous Guidelines, a case has been reported of a 14-year-old girl being treated for relapsed acute lymphoblastic leukaemia who developed TA-GvHD (SHOT Report 2000-2001). She was treated with a regimen which did not contain nucleoside analogues and all cellular blood components received by this patient were leucodepleted.

> **Recommendations for acute leukaemia:**
>
> *While no definite recommendation can be made in light of the above case report, provision of irradiated cellular blood components should be considered for transfusion to all patients with acute leukaemia.*
>
> *HLA-selected/matched platelets must be irradiated.*
>
> *All granulocyte transfusions must be irradiated.*

4.2.2 Allogeneic haematopoietic stem cell transplantation (HSCT):

Patients undergoing allogeneic HSCT routinely receive irradiated cellular components. There is a paucity of literature regarding when it is safe to discontinue giving irradiated blood components post-allogeneic HSCT. In adult patients, CD4+ T cell counts plateau at about 50% of normal by six to twelve months (Williams and Gress 2008). However, this is significantly impacted by concurrent immunotherapy, presence of transplant-related GvHD, infections or relapse.

> **Recommendations for allogeneic HSCT:**
>
> *All recipients of allogeneic HSCT must receive irradiated cellular blood components from the time of initiation of conditioning chemo/radiotherapy.*
>
> *Transplant-associated GVHD may also be significantly immunosuppressive; therefore, in the setting of transplant-associated GVHD, patients should receive irradiated cellular blood components. Transfusion of only irradiated cellular blood components should be continued while the patient remains on post-transplant GVHD prophylaxis, usually for a minimum of twelve months or until lymphocytes are >1x10^9/L.*
>
> *Patients with active chronic transplant-related GvHD should continue to receive irradiated cellular blood components.*

4.2.3 Autologous haematopoietic stem cell transplantation:

There is a theoretical risk that viable T cells of blood donor origin may be collected along with harvested stem cells in patients undergoing bone marrow or peripheral blood stem cell harvesting, which have the potential for engraftment after re-infusion of autologous cells. Therefore, it is recommended that patients who are undergoing or are planned for stem cell or bone marrow harvest should be transfused only with irradiated blood components. Patients should continue to receive irradiated blood components until there is evidence of marrow engraftment and T cell recovery.

> **Recommendations for autologous HSCT:**
>
> *Patients undergoing bone marrow or peripheral blood stem cell harvesting for future autologous reinfusion should only receive irradiated allogeneic cellular blood components during, and for seven days before, the bone marrow/stem cell harvest to prevent the collection of viable allogeneic T lymphocytes.*
>
> *All patients undergoing autologous HSCT must then receive gamma irradiated cellular blood components from the initiation of conditioning chemo/radiotherapy until at least three months post-autograft. [or six months if Total Body Irradiation used].*

4.2.4 Lymphomas and related disorders:

Patients diagnosed with Hodgkin and non-Hodgkin lymphomas (NHL) may be considered immunodeficient. While the incidence of Hodgkin lymphoma is lower than that of NHL, the former has more frequently been implicated in case reports of TA-GvHD. Of 15 TA-GvHD cases reported to SHOT, 6 have occurred in patients with B cell malignancies (3 non-Hodgkin lymphoma, 1 Waldenstrom's macroglobulinaemia, 1 myeloma, and 1 case of acute lymphoblastic leukaemia). Cases of TA-GvHD have been documented to occur up to 2 years following treatment for NHL. Gelly *et al* reported a case of HLA-typing-proven TA-GvHD two years following treatment of high-grade B-cell NHL with CHOP (cyclophosphamide, hydroxydoxorubicin, vincristine, prednisolone) chemotherapy and involved field radiotherapy. The patient subsequently presented with angina associated with iron deficiency, for which he received four non-irradiated red cells. The patient died of TA-GvHD

and, at the time of death, was thought to be in complete remission from his lymphoma (Gelly, *et al* 2000). TA-GvHD has been documented in the setting of T cell NHL (Baglin, *et al* 1992).

Patients with lymphoma are frequently treated with nucleoside analogues including fludarabine, deoxycoformycin (pentostatin), chlorodeoxyadenosine (cladribine), clofarabine and bendamustine. These drugs significantly impair immunity and increase the risk of TA-GvHD through their inherent ability to cause a severe lymphocytopenia with prolonged reductions in CD4+ T cells. TA-GvHD has been documented to occur 11 months following the last dose of fludarabine therapy, underscoring the long term immunosuppressive effect of such therapy (Nollet and Holland 2003).

The optimal duration for which such patients should receive irradiated cellular blood components is not well defined. In view of the fact TA-GvHD has occurred up to two years following successful treatment in patients with lymphoma, prolonged utilisation of irradiated cellular blood components is recommended. However, owing to the lack of definitive scientific evidence an optimal duration is yet to be defined.

Alemtuzumab (Campath® anti-CD52 antibody) has recently emerged as a treatment for chronic lymphocytic leukaemia. The US Food and Drug Administration (FDA) has recently revised its recommendation pertaining to alemtuzumab use and stipulated that only irradiated blood components should be administered to such patients to avoid TA-GvHD, unless emergent circumstances dictate immediate transfusion (FDA, 2009). The duration for which patients require irradiated components is not well defined.

Recommendations for lymphoma and related disorders:

Patients with Hodgkin disease should receive irradiated cellular blood components at all stages of disease and therapy.

All patients treated with nucleoside analogues must receive irradiated cellular blood components; there are however, currently no data to support a stated period of time to use irradiated red cells and platelets for patients following treatment with nucleoside analogues; however, continued use for at least 1 year is recommended, and indefinite use could be considered.

All patients receiving alemtuzumab should be administered only irradiated blood components, unless emergent circumstances dictate immediate transfusion.

Consideration should be give to transfusing irradiated cellular blood components to patients with B cell malignancies who receive chemotherapy and/or radiotherapy leading to lymphopenia $<0.5 \times 10^9$/L.

Consideration should be given to transfusing irradiated cellular blood components to patients with B and T cell NHL.

4.2.5 Aplastic anaemia:

Most cases of acquired aplastic anaemia arise from immune destruction of haematopoietic precursors resulting in pancytopenia and a hypocellular bone marrow. One of the pathologic mechanisms is believed to be due to a deficiency of CD4+CD25+FOXP3+ regulatory T cells, as occurs in other autoimmune conditions (Bacigalupo 2007). This results in an oligoclonal expansion of cytotoxic T cells leading to immune destruction of the patient's haematopoietic precursors (Young, *et al* 2008). Thus, owing to the pathological basis of the condition and the fact that T cell immunity is generally not impaired, the patient is not likely to be inherently at increased risk of TA-GvHD. Only one case of TA-GvHD has been documented in a patient with severe aplastic anaemia who received a non-irradiated granulocyte transfusion (Lowenthal, *et al* 1975).

However, while the underlying condition may not increase the risk of TA-GvHD, these patients are frequently treated with antithymocyte globulin (ATG) and cyclosporine which deplete T cells and inhibit function respectively. While these drugs pose a theoretically increased risk of TA-GvHD owing to their T cell immunosuppressive effect, there have only been two case reports of TA-GvHD in patients with aplastic anaemia, one in a patient 20 years post-ATG treatment for aplastic anaemia and in a second patient 10 years post-ATG treatment who also was a liver transplant recipient. These case reports and practice survey results from the European Group for Blood and Marrow Transplantation have prompted the BCSH, in the most recent revision of their irradiation guidelines, to recommend the use of irradiated blood components for aplastic anaemia receiving immunosuppressive therapy with ATG (and/or alemtuzumab) (BCSH 2010). Therefore, while no definite recommendations can be made owing to the paucity of evidence, given the theoretical risk, serious consideration should be given to, where

possible, irradiating all red cells and platelets transfused to aplastic anaemia patients receiving immunosuppressive therapy and especially those receiving ATG.

Recommendations for aplastic anaemia:

- *While no definite recommendations can be made, serious consideration should be given to transfusing irradiated red cells and platelets to patients with aplastic anaemia who are receiving immunosuppressive therapy.*

- *Patients not receiving immunosuppressive therapy do not definitively need to receive irradiated red cells and platelets.*

General recommendations regarding chemo- and immunotherapeutic agents and TA-GvHD:

New chemo- or immunotherapeutic regimens must be evaluated for their potential to predispose to TA-GvHD. Regular update of these Guidelines is required to include current recommendations relating to drugs and protocols with potent immunosuppressive effects.

4.3 Other patient groups:

4.3.1 Patients treated for non-malignant conditions with nucleoside analogues:

Case reports have emerged regarding patients with connective tissue disorders treated with fludarabine, who have developed TA-GvHD.

Leitman *et al* reported a case of TA-GvHD in a 42-year-old female with refractory lupus nephritis treated with three monthly cycles of fludarabine and cyclophosphamide. Three months following the final dose of fludarabine, she developed TA-GvHD, proven on HLA typing. This followed receipt of two units of red cells and 6 units of pooled random donor platelets, none of which was irradiated (Leitman*, et al* 2003). In patients treated for rheumatoid arthritis with fludarabine, studies have demonstrated profound T-cell immunosuppression with up to 70% reduction in CD4+ T cells. Following cessation of treatment, there is a prolonged recovery of CD4+ T cells, with less than 50% recovery at one year (Davis*, et al* 1998). Therefore, all patients who have been treated with fludarabine and other nucleoside analogues, such as deoxycoformycin (pentostatin) and chlorodeoxyadenosine (cladribine), should receive irradiated red cells and platelets irrespective of their underlying diagnosis. Only irradiated cellular blood components should be transfused for at least one year, and consideration should be given to life long utilisation.

Recommendations for patients receiving nucleoside analogues:

All patients who are treated with nucleoside analogues must receive irradiated cellular components. There are currently no data to support a specific period of time over which irradiated components should be used for patients following treatment with nucleoside analogues, however, continued use for at least one year is recommended and indefinite use could be considered.

4.3.2 Patients receiving alemtuzumab for non-malignant conditions:

Recently, alemtuzumab (Campath® anti-CD52 antibody) has been used in clinical trials of treatment of multiple sclerosis and in the renal transplant setting to reduce steroid dependence and graft rejection. For consistency with recommendations for use of irradiated blood components in patients receiving alemtuzumab for other conditions, and in line with the recently revised US FDA recommendation pertaining to alemtuzumab, only irradiated blood components should be administered to such patients to avoid TA-GvHD, unless emergent circumstances dictate immediate transfusion (FDA, 2009). The duration for which patients require irradiated components is not well defined.

Recommendations for patients receiving alemtuzumab for non-malignant conditions:

All patients receiving alemtuzumab should be administered only irradiated blood components, unless emergent circumstances dictate immediate transfusion.

4.3.3 Solid organ malignancies:

There is no clear evidence that solid organ malignancies increase the risk of TA-GvHD. A small number of cases of TA-GvHD have been reported, however, the majority of these occurred in the setting of additional risk factors, including autologous HSCT and the use of nucleoside analogues (Ruhl, *et al* 2009). Therefore, routine irradiation of red cells and platelets for patients with solid organ malignancies is not indicated. However, with the advent of newer and more aggressive chemotherapy regimens this recommendation may require revision. This is particularly relevant for patients receiving therapies that may cause marked lymphopenia ($<0.5 \times 10^9$/L) or those receiving long term, high dose steroids.

> **Recommendation for solid organ malignancies:**
>
> *Routine use of irradiated cellular components for patients with solid organ malignancies is not indicated unless other risk factors are present.*

4.3.4 Solid organ transplantation:

In comparison with patients who have received haematopoietic stem cell transplants, solid organ transplant recipients are generally less immunocompromised. Such patients are, therefore, more capable of abrogating the effect of donor lymphocytes and preventing engraftment than haemopoietic stem cell transplant recipients (Triulzi and Nalesnik 2001). There is a single report of TA-GvHD in the liver transplant setting, in a 14-month-old male with fulminant hepatic failure of unknown aetiology and pancytopenia with marrow hypoplasia. While this case provides credible evidence for TA-GVHD in liver transplant, it is debatable whether the risk can be extrapolated to all liver transplant recipients because of the patient's age and a pre-existing hematologic abnormality that may have placed the patient at increased risk for GVHD (Wisecarver, *et al* 1994). Rare cases of TA-GvHD have also been described in kidney (2 cases) and heart (1 case) transplant patients. These case reports are inconclusive, lacking definitive laboratory HLA or DNA typing.

> **Recommendations for solid organ transplantation:**
>
> *Routine irradiation of cellular blood components for solid organ transplant recipients is not required.*
>
> *The effect of new immunosuppressive treatments and regimens requires continuous observation, and these Guidelines must be updated in accordance with emerging data.*

4.3.5 HIV/AIDS:

Although patients with HIV may be significantly immunocompromised, TA-GvHD has not been recognised in patients with HIV/AIDS. A randomised study comparing leucodepleted versus unmodified blood components in HIV-positive patients demonstrated that sustained donor leucocyte microchimerism did not develop in any of these recipients. These data provide confirmatory laboratory evidence in support of the clinical impression that HIV is not a risk factor for TA-GvHD (Kruskall, *et al* 2001).

> **Recommendation for HIV:**
>
> *It is not necessary to irradiate cellular blood components for patients with HIV/AIDS.*

4.3.6 Massive transfusion:

Microchimerism, the persistence of a small population of allogeneic cells, has been recognised in transfusion recipients. Studies using PCR-based assays have demonstrated that the frequency of microchimerism is greatest among patients with severe traumatic injuries who receive relatively fresh blood components shortly after an episode of massive haemorrhage (Utter, *et al* 2007). Transfusion-associated microchimerism is demonstrable in approximately 10% of transfused trauma patients, with these cells enduring for years to decades at numbers approaching up to 5% of circulating leucocytes, with multiple immunophenotypic lineages suggestive of haematopoietic engraftment (Utter, *et al* 2007). At this stage, however, the clinical relevance of microchimerism either with regard to TA-GvHD or other potential adverse immunomodulatory effects remains to be fully elucidated.

> **Recommendations for massive transfusion:**
>
> *In the setting of massive transfusion, consideration could be given to transfusing irradiated cellular blood components. However, until further evidence becomes available, no definite recommendation can be made pertaining to irradiation of blood components capable of causing TA-GvHD in patients following massive transfusion. As this issue evolves, regular review of these Guidelines will be required to include up to date recommendations.*

4.3.7 Donations from family members (directed donations) and HLA-selected/matched donors:

Owing to the significant probability of shared HLA haplotypes within families, donations from family (blood relative) members pose a particular risk of TA-GvHD. Similarly, platelets obtained from non-related HLA-matched and or otherwise HLA-compatible donors pose a significant risk.

> **Recommendations for directed and HLA-selected/matched donations:**
>
> *Directed transfusions should be discouraged.*
>
> *All transfusions from blood relatives must be irradiated, even if the recipient is immunocompetent.*
>
> *All HLA-selected/matched platelets must be irradiated, even if the recipient is immunocompetent.*

4.4 Other issues regarding transfusion practice in patients at risk of TA-GvHD:

Haemovigilance reports continue to demonstrate that patients requiring irradiated blood components fail to receive them. This most often arises from errors in the transfusion process, including prescription and clerical errors.

> **Recommendations regarding communications and hospital systems:**
>
> *Institutions must have systems in place to ensure that patients at increased risk of TA-GvHD are identified and receive irradiated components.*
>
> *Institutional guidelines must be in place to ensure appropriate communication regarding special transfusion requirements between treating clinicians and other ward staff and laboratory staff.*
>
> *Transfusion request forms should contain a field for irradiated cellular blood component requirements, to encourage the requestor to identify patients at risk.*
>
> *All patients who require irradiated blood components must have an alert on their electronic transfusion laboratory records.*
>
> *Laboratory systems must be in place to prevent cellular blood components that are not irradiated being ordered or released for transfusion to patients requiring such components.*
>
> *Institutional education programmes and policies should be devised for treating clinicians, emergency department staff, nursing staff and pathology and blood bank staff to ensure adherence to these Guidelines.*
>
> *Procedures must be in place to ensure adequate and timely communication of special transfusion requirements if patient care is transferred or shared between centres.*
>
> *Patients should receive education about whether they require cellular blood components with any specific modifications.*

SECTION 5

Future directions and emerging issues:

Pathogen reduction technologies:
Pathogen reduction methods were initially developed to reduce the risk of microbial contamination of blood components through disruption of DNA. These technologies may also, however, have a potential role in preventing TA-GvHD. Several methods exist or are in development for platelets, including amotosalen combined with ultraviolet A (UVA) light and riboflavin plus light, and UV C light alone (Schlenke 2004; Lockerbie, *et al* 2003). These systems appear to be robust, achieving up to a 5 $\log_{10}$ reduction of T lymphocytes in platelet concentrates (Grass, *et al* 1998), and therefore may have the potential to replace gamma irradiation of platelets without compromising platelet function (Pineda, *et al* 2006). Systems which do not require photoactivation to target nucleic acids have also been developed (Rios, *et al* 2006) and in murine models these have demonstrated efficacy in reducing or eliminating lymphocyte proliferation (Fast, *et al* 2002). Systems for pathogen inactivation of red cells are less well established.

> **Recommendations regarding pathogen reduction technologies:**
>
> *Pathogen inactivation or reduction technologies cannot yet be advocated as an alternative or equivalent to irradiation.*
>
> *Regular update of these Guidelines will be required to include recommendations pertaining to these technologies as they evolve.*

REFERENCES

Agbaht, K., Altintas, N.D., Topeli, A., Gokoz, O. & Ozcebe, O. (2007) Transfusion-associated graft-versus-host disease in immunocompetent patients: case series and review of the literature. *Transfusion,* **47,** 1405-1411.

American Association of Blood Banks. Committee on Standards. *Standards for blood banks and transfusion services,* 26th edition, 2009. AABB, Washington, DC.

Anderson, K.C., Goodnough, L.T., Sayers, M., Pisciotto, P.T., Kurtz, S.R., Lane, T.A., Anderson, C.S. & Silberstein, L.E. (1991) Variation in blood component irradiation practice: implications for prevention of transfusion-associated graft-versus-host disease. *Blood,* **77,** 2096-2102.

Asai, T., Inaba, S., Ohto, H., Osada, K., Suzuki, G., Takahashi, K., Tadokoro, K. & Minami, M. (2000) Guidelines for irradiation of blood and blood components to prevent post-transfusion graft-vs.-host disease in Japan. *Transfusion Medicine,* **10,** 315-320.

Bacigalupo, A. (2007) Aplastic anemia: pathogenesis and treatment. *Hematology Am Soc Hematol Educ Program,* **2007,** 23-28.

Baglin, T.P., Joysey, V.C., Horsford, J., Johnson, R.T., Broadbent, V. & Marcus, R.E. (1992) Transfusion-associated graft-versus-host disease in patients with Hodgkin's disease and T cell lymphoma. *Transfusion Medicine,* **2,** 195-199.

BCSH Blood Transfusion Task Force (1996). Guidelines on gamma irradiation of blood components for the prevention of transfusion-associated graft-versus-host disease. *Transfusion Medicine, 6, 261-271.*

BCSH Blood Transfusion Task Force (2010) Guidelines on the use of irradiated blood components. Available at http://www.bcshguidelines.com/pdf/draft_irrad_0710.pdf

Bernvil, S.S., Abdulatiff, M., al-Sedairy, S., Sasich, F. & Sheth, K. (1994) Fresh frozen plasma contains viable progenitor cells--should we irradiate? *Vox Sanguinis,* **67,** 405.

Blest, A. & Howell, C. (2007) Gamma Irradiation of Blood Components for the Prevention of TA-GvHD - Right Component to Right Patient Survey. *Transfusion Medicine,* **17S1,** 33-64.

Blood Matters, Department of Human Services, Victoria. Blood Matters Serious Transfusion Incident Reporting (STIR) program report for 2006-07. Available at: http://www.health.vic.gov.au/blood matters

Button, L.N., DeWolf, W.C., Newburger, P.E., Jacobson, M.S. & Kevy, S.V. (1981) The effects of irradiation on blood components. *Transfusion,* **21,** 419-426.

Council of Europe (2008) *Guide to the preparation, use and quality assurance of blood components, 14th edition. Council of Europe Publishing, Strasbourg*

Davey, R.J., McCoy, N.C., Yu, M., Sullivan, J.A., Spiegel, D.M. & Leitman, S.F. (1992) The effect of prestorage irradiation on posttransfusion red cell survival. *Transfusion,* **32,** 525-528.

Davis, J.C., Jr., Fessler, B.J., Tassiulas, I.O., McInnes, I.B., Yarboro, C.H., Pillemer, S., Wilder, R., Fleisher, T.A., Klippel, J.H. & Boumpas, D.T. (1998) High dose versus low dose fludarabine in the treatment of patients with severe refractory rheumatoid arthritis. *J Rheumatol,* **25,** 1694-1704.

Dinning, G., Doughty, R.W., Reid, M.M. & Lloyd, H.L. (1991) Potassium concentrations in irradiated blood. *BMJ,* **303,** 1110.

Dwyre, D.M. & Holland, P.V. (2008) Transfusion-associated graft-versus-host disease. *Vox Sanguinis,* **95,** 85-93.

Fast, L.D., DiLeone, G., Edson, C.M. & Purmal, A. (2002) Inhibition of murine GVHD by PEN110 treatment. *Transfusion,* **42,** 1326-1332.

FDA (1993) Recommendations Regarding License Amendments and Procedures for Gamma Irradiation of Blood Products. Food and Drug Administration.

FDA U.S. Food and Drug Administration (2009). Campath (alemtuzumab) Injection for intravenous use. Detailed View: Safety Labeling Changes Approved By FDA Center for Drug Evaluation and Research (CDER). Available at http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm163944.htm

Gelly, K.J., Kerr, R., Rawlinson, S., Norris, A. & Bowen, D.T. (2000) Transfusion-associated graft vs. host disease in a patient with high-grade B-cell lymphoma. Should cellular products for patients with non-Hodgkin's lymphoma be irradiated? *Br J Haematol,* **110,** 228-229.

Gibson, B.E., Todd, A., Roberts, I., Pamphilon, D., Rodeck, C., Bolton-Maggs, P., Burbin, G., Duguid, J., Boulton, F., Cohen, H., Smith, N., McClelland, D.B., Rowley, M. & Turner, G. (2004) Transfusion guidelines for neonates and older children. *Br J Haematol,* **124,** 433-453.

Goes, E.G., Borges, J.C., Covas, D.T., Orellana, M.D., Palma, P.V., Morais, F.R. & Pela, C.A. (2006) Quality control of blood irradiation: determination T cells radiosensitivity to cobalt-60 gamma rays. *Transfusion,* **46,** 34-40.

Grass, J.A., Hei, D.J., Metchette, K., Cimino, G.D., Wiesehahn, G.P., Corash, L. & Lin, L. (1998) Inactivation of leukocytes in platelet concentrates by photochemical treatment with psoralen plus UVA. *Blood,* **91,** 2180-2188.

Hauser, G.J., Chan, M.M., Casey, W.F., Midgley, F.M. & Holbrook, P.R. (1991) Immune dysfunction in children after corrective surgery for congenital heart disease. *Crit Care Med,* **19,** 874-881.

Janatpour, K., Denning, L., Nelson, K., Betlach, B., Mackenzie, M. & Holland, P. (2005) Comparison of X-ray vs. gamma irradiation of CPDA-1 red cells. *Vox Sanguinis,* **89,** 215-219.

Klein, H.G. (2006) Transfusion-associated graft-versus-host disease: less fresh blood and more gray (Gy) for an aging population. *Transfusion,* **46,** 878-880.

Kruskall, M.S., Lee, T.H., Assmann, S.F., Laycock, M., Kalish, L.A., Lederman, M.M. & Busch, M.P. (2001) Survival of transfused donor white blood cells in HIV-infected recipients. *Blood,* **98,** 272-279.

Leitman, S.F., Tisdale, J.F., Bolan, C.D., Popovsky, M.A., Klippel, J.H., Balow, J.E., Boumpas, D.T. & Illei, G.G. (2003) Transfusion-associated GVHD after fludarabine therapy in a patient with systemic lupus erythematosus. *Transfusion,* **43,** 1667-1671.

Lockerbie, O., Rice, J., Janssens, M. & Goodrich, R. (2003) Prevention of Graft-Versus-Host Disease Using a Pathogen Reduction Process for Blood Products Using Riboflavin and UV Illumination. *Transfusion Medicine Hemotherapy*, 289–306.

Lowenthal, R.M., Grossman, L., Goldman, J.M., Storring, R.A., Buskard, N.A., Park, D.S., Murphy, B.C., Spiers, A.S. & Galton, D.A. (1975) Granulocyte transfusions in treatment of infections in patients with acute leukaemia and aplastic anaemia. *Lancet,* **1,** 353-358.

Luban, N.L., Drothler, D., Moroff, G. & Quinones, R. (2000) Irradiation of platelet components: inhibition of lymphocyte proliferation assessed by limiting-dilution analysis. *Transfusion,* **40,** 348-352.

Mincheff, M. (1998) Changes in donor leukocytes during blood storage. Implications on post-transfusion immunomodulation and transfusion-associated GVHD. *Vox Sanguinis,* **74 Suppl 2,** 189-200.

Mintz, P.D. & Anderson, G. (1993) Effect of gamma irradiation on the in vivo recovery of stored red blood cells. *Ann Clin Lab Sci,* **23,** 216-220.

New Zealand Blood Service (2005 – 2009) National Haemovigilance Programme Annual Reports 2005 to 2009. Available at: http://www.nzblood.co.nz.

Nollet, K.E. & Holland, P.V. (2003) Toward a coalition against transfusion-associated GVHD. *Transfusion,* **43,** 1655-1657.

O'Shaughnessy, D.F., Atterbury, C., Bolton Maggs, P., Murphy, M., Thomas, D., Yates, S. & Williamson, L.M. (2004) Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant. *Br J Haematol,* **126,** 11-28.

Ohto, H. & Anderson, K.C. (1996) Posttransfusion graft-versus-host disease in Japanese newborns. *Transfusion,* **36,** 117-123.

Ohto, H. & Anderson, K.C. (1997) Are guidelines for use of gamma irradiation for the prevention of transfusion-associated graft-versus-host disease adequate for newborns? *Transfusion Medicine,* **7,** 172-173.

Parshuram, C., Doyle, J., Lau, W. & Shemie, S.D. (2002) Transfusion-associated graft versus host disease. *Pediatr Crit Care Med,* **3,** 57-62.

Pelszynski, M.M., Moroff, G., Luban, N.L., Taylor, B.J. & Quinones, R.R. (1994) Effect of gamma irradiation of red blood cell units on T-cell inactivation as assessed by limiting dilution analysis: implications for preventing transfusion-associated graft-versus-host disease. *Blood,* **83,** 1683-1689.

Petz, L.D., Calhoun, L., Yam, P., Cecka, M., Schiller, G., Faitlowicz, A.R., Herron, R., Sayah, D., Wallace, R.B. & Belldegrun, A. (1993) Transfusion-associated graft-versus-host disease in immunocompetent patients: report of a fatal case associated with transfusion of blood from a second-degree relative, and a survey of predisposing factors. *Transfusion,* **33,** 742-750.

Pineda, A., McCullough, J., Benjamin, R.J., Cable, R., Strauss, R.G., Burgstaler, E., Porter, S., Lin, L., Metzel, P. & Conlan, M.G. (2006) Pathogen inactivation of platelets with a photochemical treatment with amotosalen HCl and ultraviolet light: process used in the SPRINT trial. *Transfusion,* **46,** 562-571.

Rios, J.A., Hambleton, J., Viele, M., Rugg, N., Sindermann, G., Greenwalt, T., Wages, D., Cook, D. & Corash, L. (2006) Viability of red cells prepared with S-303 pathogen inactivation treatment. *Transfusion,* **46,** 1778-1786.

Ruhl, H., Bein, G. & Sachs, U.J. (2009) Transfusion-associated graft-versus-host disease. *Transfus Med Rev,* **23,** 62-71.

Sanders, M.R. & Graeber, J.E. (1990) Posttransfusion graft-versus-host disease in infancy. *J Pediatr,* **117,** 159-163.

Schlenke, P. (2004) Protection against Transfusion-Associated Graft-versus-Host Disease in Blood Transfusion: Is Gamma-Irradiation the Only Answer? *Transfus Med Hemother*, 24-31.

Schroeder, M.L. (2002) Transfusion-associated graft-versus-host disease. *Br J Haematol,* **117,** 275-287.

Serious Hazards of Transfusion (SHOT) Annual reports 2001 – 2009. Available at: http://www.shotuk.org

Strauss, R.G. (2000) Data-driven blood banking practices for neonatal RBC transfusions. *Transfusion,* **40,** 1528-1540.

Sullivan, K.E., Jawad, A.F., Randall, P., Driscoll, D.A., Emanuel, B.S., McDonald-McGinn, D.M. & Zackai, E.H. (1998) Lack of correlation between impaired T cell production, immunodeficiency, and other phenotypic features in chromosome 22q11.2 deletion syndromes. *Clin Immunol Immunopathol,* **86,** 141-146.

Thaler, M., Shamiss, A., Orgad, S., Huszar, M., Nussinovitch, N., Meisel, S., Gazit, E., Lavee, J. & Smolinsky, A. (1989) The role of blood from HLA-homozygous donors in fatal transfusion-associated graft-versus-host disease after open-heart surgery. *N Engl J Med,* **321,** 25-28.

Triulzi, D.J. & Nalesnik, M.A. (2001) Microchimerism, GVHD, and tolerance in solid organ transplantation. *Transfusion,* **41,** 419-426.

Tynngard, N., Studer, M., Lindahl, T.L., Trinks, M. & Berlin, G. (2008) The effect of gamma irradiation on the quality of apheresis platelets during storage for 7 days. *Transfusion,* **48,** 1669-1675.

Utter, G.H., Reed, W.F., Lee, T.H. & Busch, M.P. (2007) Transfusion-associated microchimerism. *Vox Sanguinis,* **93,** 188-195.

van der Meer, P.F. & Pietersz, R.N. (2005) Gamma irradiation does not affect 7-day storage of platelet concentrates. *Vox Sanguinis,* **89,** 97-99.

Vogelsang, G.B. (1990) Transfusion-associated graft-versus-host disease in nonimmunocompromised hosts. *Transfusion,* **30,** 101-103.

Vogelsang, G.B. & Hess, A.D. (1994) Graft-versus-host disease: new directions for a persistent problem. *Blood,* **84,** 2061-2067.

Wagner, F.F. & Flegel, W.A. (1995) Transfusion-associated graft-versus-host disease: risk due to homozygous HLA haplotypes. *Transfusion,* **35,** 284-291.

Webb, D.K. (1995) Irradiation in the prevention of transfusion associated graft-versus-host disease. *Archives of Disease in Childhood,* **73,** 388-389.

Weinmann, M., Hoffmann, W., Rodegerdts, E. & Bamberg, M. (2000) Biological effects of ionizing radiation on human blood compounds ex vivo. *J Cancer Res Clin Oncol,* **126,** 584-588.

Wieding, J.U., Vehmeyer, K., Dittman, J., Hiddemann, W., Kohler, M. & Lanzer, G. (1994) Contamination of fresh-frozen plasma with viable white cells and proliferable stem cells. *Transfusion,* **34,** 185-186.

Williams, K.M. & Gress, R.E. (2008) Immune reconstitution and implications for immunotherapy following haematopoietic stem cell transplantation. *Best Pract Res Clin Haematol,* **21,** 579-596.

Williamson, L.M., Stainsby, D., Jones, H., Love, E., Chapman, C.E., Navarrete, C., Lucas, G., Beatty, C., Casbard, A. & Cohen, H. (2007) The impact of universal leukodepletion of the blood supply on hemovigilance reports of posttransfusion purpura and transfusion-associated graft-versus-host disease. *Transfusion,* **47,** 1455-1467.

Wintergerst, U., Meyer, U., Remberger, K. & Belohradsky, B.H. (1989) [Graft versus host reaction in an infant with DiGeorge syndrome]. *Monatsschr Kinderheilkd,* **137,** 345-347.

Wisecarver, J.L., Cattral, M.S., Langnas, A.N., Shaw, B.W., Jr., Fox, I.J., Heffron, T.G. & Rubocki, R.J. (1994) Transfusion-induced graft-versus-host disease after liver transplantation. Documentation using polymerase chain reaction with HLA-DR sequence-specific primers. *Transplantation,* **58,** 269-271.

Young, N.S., Scheinberg, P. & Calado, R.T. (2008) Aplastic anemia. *Curr Opin Hematol,* **15,** 162-168.