IRRADIATION: The Past, the Present and the Future

Debra Lane, MD, FRCPC
Medical Officer, Canadian Blood Services
debra.lane@blood.ca
204 789-1079
WHY DO WE IRRADIATE BLOOD?

- Prevent Transfusion Associated Graft Versus Host Disease (TA-GVHD)

- Graft Versus Host Disease is an often fatal allo-immune disease after blood transfusion
TA-GVHD

- First described in 1960’s
  - Runting Disease
- Symptoms: 7-10 days post transfusion
  - Fever
  - Nausea
  - Vomiting
  - Diarrhea
  - Erythematous maculopapular eruption
  - May progress to erythroderma, bullae & frank desquamation
  - Hepatic dysfunction
  - Severe pancytopenia
DIAGNOSIS TA-GVHD

- Skin biopsy
- Dermal-epithelial layer separation
- Formulation of bullae
- Mononuclear cell migration into the epidermis
- Hyperkeratosis
- Dyskeratosis
DIAGNOSIS TA-GVHD
BONE MARROW

- Empty marrow
- Pancytopenia
- Fibrosis
- Lymphocyte infiltration
DIAGNOSIS TA-GVHD
LIVER BIOPSY

- Eosinophilic infiltration
- Degeneration of small bile ducts
- Peripheral inflammation
- Lymphocyte infiltration
POST MORTEM DIAGNOSIS

Donor lymphocyte infiltration in:

- Skin
- Lymph Nodes
- Liver
- GI Tract
DEFINITIVE DIAGNOSIS

- Serologic HLA typing
- DNA-based HLA Class II karyotyping
- Karyotype analysis
- Restriction fragment length polymorphism analysis using probes from HLA and non-HLA regions
- Genetic fingerprinting
- Fibroblasts & buccal cells are required since the patient’s lymphocytes are too reduced to perform standard HLA tests
- Parental samples may be needed to deduce HLA type
TA-GVHD INCIDENCE

- No adequate estimates of prevalence
- Japan; 1 case / 212 transfusions
- Serious Hazards Of Transfusions [SHOT] in UK documented 13 cases since 1996 [all fatal]
CLINICAL INDICATIONS FOR IRRADIATION

- Intrauterine transfusion
- Prematurity
- Congenital immunodeficiency
- Stem cell or marrow transplant
- Exchange transfusion for erythroblastosis
- Neuroblastoma
- Sarcoma
- Hodgkin's Disease with ablative chemo/or radiotherapy
- Fludarabine treatment
METHODS OF IRRADIATION

- Inactivates T-Cells by damaging nuclear DNA directly or indirectly
- Prevents donor T-Cells proliferation in the recipient
- Gamma rays or X-rays both inactivate T Cells
- Gamma rays come from $^{137}$ Cesium or $^{60}$ Cobalt
- X-rays are generated from the interaction of a beam of electrons with a metallic surface
Diagrammatic views of two common types of instrumentation used for blood irradiation.
A: Configurations of a freestanding irradiator using a cesium 137 source. B: Configuration of a linear accelerator.
CBS IRRADIATOR
RADIATION ROOM HANDLING SIGN

- **Cesium-137**: 55.4 TBq (1497 Curies)
- **Serial Number**: 012
- **Location**: Blood Product Management (Room 179A)
- **Form of Nuclear Substance**: Solid
- **Emergency Contact & Telephone Numbers**:
  - Tracey Pronyk-Ward (Production Mgr., RSO)
    - Bus: 204-789-1030
    - Cell: 204-801-5854
    - Home: 204-757-4483
  - Lynne Christie (Production Supervisor, RSO)
    - Bus: 204-789-1026
    - Cell: 204-806-5105
    - Home: 204-284-6798
CANNISTER IRRADIATORS (NORDION®)

- Blood components are placed in a metal canister
- Canister is on a rotating turntable
- Canister is rotated so gamma rays hit all parts of the component
- The irradiator is:
  - encased in lead
  - is kept under lock and key
  - and is very heavy: needs floor re-enforcement
WHAT COMPONENTS ARE IRRADIATED?

- Red cells (including frozen thawed red cells)
- Platelets – HLA matched
- Granulocytes
- # of viable lymphocytes determines whether the unit can cause TA-GVHD
RADIATION DOSE

- Selection of 1500 cGy was used in the 70’s
- Studies showed 500 cGy destroyed the MLC response of isolated lymphocytes
- Later studies showed 2500 cGy measured at the internal mid-plane is the best dose
- This was determined using a Limiting Dilution Assay (LDA) that measures the ability of CD4+ and CD8+ T cells in a functional assay after irradiation
FDA RECOMMENDATIONS

- Irradiation process must deliver 2500 cGy to the internal mid-plane of a free standing irradiation canister
- Minimum of 1500 cGy to any other point in the canister
DIRECTED DONATIONS

- Rarely occur
- May contain lymphocytes homozygous for and HLA haplotype
- May occur when close relatives or HLA matched platelets are provided
- CBS irradiates all Directed Donations
QUALITY CONTROL

📍 Like all instruments the irradiator must be quality controlled
📍 A process to ensure each unit is irradiated must be done
📍 Dose mapping is performed to ensure the delivery of irradiation is performed with a simulated blood component
QUALITY ASSURANCE GUIDELINE

- Dose 2500 cGy
- Turntable rotation checked daily
- Correction for Radioisotope Decay
  - $^{137}$Cs annually
  - $^{60}$Co quarterly
- Dose mapping
  - 1x/year for $^{137}$Cs
  - 2x/year for $^{60}$Co
  - After repairs
CONFIRMATION OF IRRADIATION

- Radiation sensitive indicator labels are placed on the unit before irradiation
- Irradiation causes a visible change on the label
- Can come in 1500, or 2500 cGy levels
PROBLEMS WITH IRRADIATION

- Produces reactive oxygen species that cause lipid peroxidation and membrane damage
- Enhanced loss of potassium from inside the cells
- Large volume exchanges should have the supernatant plasma removed:
  - Washing
  - Supernatant removal
- Controversy on whether frozen plasma should be irradiated
  - May be progenitor cells
  - CBS does not irradiate plasma
Gamma Irradiation

The Good

• Inactivation of residual WBC
• Risk reduction of graft-versus-host disease

The Bad

• $\gamma$-irradiation induces hematological and biochemical changes in stored RBC
  • Accelerates potassium leakage
  • Accelerates loss of ATP
  • Increases rate of metabolism
  • Increases release of microparticles

• Resulting in a decrease in post-transfusion in vivo viability
Management of Quality Impact

- **AABB**
  - Max storage: 28d post-irradiation or 42d, whichever comes 1\textsuperscript{st}

- **Council of Europe**

- **British Society for Haematology**
Should We Be Concerned About the Quality of Irradiated RBCs?

- Canada uses the AABB standard.
- Products irradiated either at CBS or the hospital.
- Irradiated products are not part of the routine QC program.
- Unknown whether current North American practice is adequately accounting for the negative product quality effects of irradiation.
The Effect of Timing of Gamma-Irradiation on Hemolysis and Potassium

Katherine Serrano, Deborah Chen, Elena Levin, Jason Acker, Adele Hansen, Tracey Turner, Jayme Tchir, and Dana V. Devine
Canadian Blood Services, Vancouver and Edmonton, Canada
When Life Hands you Lemons…

October, 2012: Recognition of a breach of SOP in Donor Screening lead to a precautionary withdrawal of 12 months of inventory collected in one CBS Centre – including over 900 units of in-date RBCs.
When Life Hands you Lemons…

Make Lemonade!

Units used for a two part study:
(1) 84 RBC sent to the Acker lab for irradiation and weekly testing.
(2) The Devine lab irradiated the remaining ~900 units to develop a matrix of collection age and post-irradiation storage age impact on hemolysis.
Study Design

• n = 896 RCC units

• Matrix designed to spread units out to cover AABB regulation time period
  • 8-40d pre-irradiation, 1-28d post-irradiation
    » Youngest available unit was 8 days old at the beginning of the study
  • 42d max storage

Number of units in each block of the matrix

<table>
<thead>
<tr>
<th>Weeks pre-irradiation</th>
<th>Weeks post-irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>896</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

- Sample size calculation (80% power, 5% error, one-sided) n = 79

• Within each block, units spread out to maximize coverage
Method

- $\gamma$-irradiation at CBS according to SOP
- Storage at 2-6°C
- Units allowed to warm at RT for a minimum of 15 minutes prior to sampling with standardized 2 minute mixing of each unit prior to sampling
- Non-sterile sampling through cut tubing
- Measurements: (according to CBS QC SOP)
  - Total Hb (Advia-120 hematology analyzer)
  - Hematocrit
  - Supernatant Hb (Low Hb HemoCue)
Percent hemolysis of $\gamma$-irradiated RCC by study design matrix (grouped by weeks): a ‘mountain’ of data
Percent Hemolysis of $\gamma$-irradiated RCC at the end of allowable storage

How do the peaks of the mountains map against the regulatory standards?

N = 896 units

Council of Europe

British Council on Standards in Hematology

AABB

How do the peaks of the mountains map against the regulatory standards?
Units Failing RCC QC Hemolysis Standard (hemolysis > 0.8%), n = 40 (4.46%)
### Supernatant Potassium After Irradiation (n=84)

<table>
<thead>
<tr>
<th>Unit Age at Irradiation (days)</th>
<th>2</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>K+ (μM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>45.1 ± 4.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>43.4 ± 2.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>46.3 ± 3.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>52.2 ± 3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>54.8 ± 3.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Days of Storage Post-Irradiation

- 2 days: 53.3 ± 2.9, 60.7 ± 2.8, 64.2 ± 2.9, 67.7 ± 3.0
- 7 days: 56.0 ± 4.5, 62.4 ± 4.3, 66.0 ± 3.8, 68.9 ± 3.5
- 14 days: 54.2 ± 2.6, 60.4 ± 2.7, 65.0 ± 2.6
- 21 days: 55.3 ± 3.6, 61.2 ± 3.2
- 28 days: 59.4 ± 2.9
- 35 days: -
- 40 days: -
Summary of the Research

• Both time pre-irradiation and time post-irradiation affect % hemolysis.
• Most damaging combination seems to be ~24 days at irradiation followed by post-irradiation of ~18 days
SUMMARY OF RESEARCH-2

• Council of Europe irradiation guidelines would exclude most hemolysis failures. BCSH guidelines would exclude all hemolysis failures in this large data set.
• Data will be shared with the Canadian Standards Association for consideration of the appropriateness of the current Canadian standard.
• Increased potassium release occurs quickly after irradiation and levels are already 2/3rcds of maximum by 2 days post-irradiation.
FUTURE- PATHOGEN INACTIVATION

- Pathogen Inactivation methods using psoralens and long wave UV irradiation can bind nucleic acids by intercalation.
- UV light promotes covalent crosslinks between RNA and DNA.
- This prevents replication of DNA.
- This may be helpful for plasma and platelets but the density of red cells may inhibit the use of Pathogen Inactivation.
KEY MESSAGES

- Irradiation is important for select groups
- Irradiation has risks - should not be ordered if not indicated
- Standards should be revised in Canada/US to reflect the new data