Use of stem cells in haematology – the Singapore experience

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Singapore General Hospital
Standardization and Innovation

In haematopoietic stem cell transplant
Haematopoietic stem cell transplant (HSCT) in Singapore public hospitals

Started >30 years ago

Adult service:
– National University Hospital (NUH)
– Singapore General Hospital (SGH)
– National Cancer Center (NCC)

Paediatrics service:
– National University Hospital (NUH)
– KK Women’s and Children’s Hospital (KKH)
Number of transplants done in public hospitals in Singapore

Paediatrics: NUH, KKH (total = 408): auto=108, allo =300
Adults: NUH, SGH, NCC (total = 2204): auto =975, allo = 1229
Haematopoietic Stem Cell Transplant

• A highly specialized and complex modality of treatment for life-threatening diseases
• Associated with significant morbidity and mortality
• Multiple components involved:
  – Internal: collection, processing and clinical services
  – External: procurement of cell therapy products: unrelated donors and cord blood units from other centers within and outside the country

→ Standardization and harmonization needed
Efforts for standardization in HSCT

• FACT: Foundation for the Accreditation of Cell Therapy, in the US and Canada
• JACIE: Joint Accreditation Committee for ISCT Europe and EBMT, in Europe,
• AABB: American Association of Blood Banks

• Promote excellence and harmonization of hematopoietic SCT, achieved by measuring the compliance of candidate programs with a comprehensive set of international standards that cover the three major areas of the activity (clinical, collection and processing) as well as their interactions with external resources
FACT-JACIE International Standards

- Quality care can only be achieved if both clinical and laboratory issues are effectively addressed
- A set of requirements that include both clinical and laboratory practices.
- Apply to Hematopoietic Progenitor Cells (HPC) obtained from bone marrow, peripheral blood, and umbilical cord blood.
- Evidence-based requirements set by international teams of world-renowned experts vested in the improvement and progress of cellular therapy.
- Developed by consensus within committees consisting of knowledgeable clinicians, scientists, technologists, and quality experts
- Address every aspect of cell manufacturing and administration that impacts the quality of products and therapeutic care.
- Based upon published medical evidence whenever possible. When published data is not available, requirements are based upon accepted scientific theory.
A stepped process based on minimum standards to certify quality-assured BMT services
Standards focussed on driving pragmatic changes in working practices and implementing culture of quality improvement
AN UNMET NEED: QUALITY SYSTEMS FOR BMT IN LMIES

Centres that are currently accredited, inspected or have applied for JACIE accreditation are overwhelmingly in high-income economies in Europe. In addition, those economies usually have other regulatory frameworks within which transplant care is delivered. For many LMIEs, BMT is a prohibitively expensive and complex therapeutic strategy, and sometimes only delivered through private providers, with little or no access to care for the general population. Despite this, transplant activity is increasing outside the high-income economies in part due to initiatives by the local medical community to adapt established medical practice to their own context including economic constraints. It is therefore important that quality improvement and accreditation is not inhibitory to the development of BMT in LMIEs.

Through its expanding global network, the EBMT has increasing contact with transplant professionals in LMIEs who have expressed high levels of interest in implementing the FACT–JACIE standards in their units but see the process as organisationally and economically challenging. In response, JACIE has been developing a stepped process based on minimum standards to certify quality-assured BMT services particularly where they are provided to the broader population through public or not-for-profit healthcare providers.

The process is based not on inventing new standards, but instead on a selection of the existing FACT–JACIE standards, particularly those pertaining to quality management, policies and procedures (sections B/C/D 4 and 5 of the standards). In principle, most of these selected standards demand neither major financial investments in technology nor infrastructure, but are more focussed on driving pragmatic changes in working practices and implementing the culture of quality improvement. This model referred to as ‘First-Step’ aims to ensure that centres establish quality management systems for critical processes as a springboard to eventually meeting the more advanced requirements of the standards.

Challenges remain. The feasibility of this approach has yet to be tested on the ground, and inevitably various assumptions will need revisiting after the first inspections against the graded
The Singapore Experience

HSCT programme of
• NUH and
• SGH
are AABB accredited
The Joint Accreditation Committee
ISCT-EBMT (JACIE)

hereby declares that

Haematopoietic Stem Cell Transplant Programme,
Singapore General Hospital
Singapore

has been found to meet the standards as set out in the FACT-JACIE International Standards for Cellular Therapy, edition 5 in the following area(s):

Autologous & Allogeneic Transplantation in Adult Patients
Collection of HPC, Marrow
Collection of HPC, Apheresis
Cell Processing - minimally manipulated

Programme Director: A/Prof Aloysius Ho

Certificate Number: 578
Date of Issue: 21/11/2016
Date of Expiry: 20/11/2020

NUH: FACT accredited
SGH: JACIE accredited
Standardization in haematopoietic stem cell transplant

• Clinical programme
  – Hospital: JCI
  – Transplant and cell therapy: FACT JACIE, AABB

• Supporting services
  – Donor registries: WMDA, NMDP
  – Tissue typing lab: ASHI
  – Enumeration of CD34 positive stem cells: ISHAGE
  – Blood bank: AABB
  – General and specialized haematology: CAP
  – Etc etc....
The WMDA Standards cover all aspects of HSC registry operations. In these standards, a registry is defined as an organisation that provides hematopoietic stem cells obtained from an individual recruited by that organisation as a volunteer donor to a patient in another country and facilitates exchanges on behalf of transplant centres in its country. Registries demonstrate their commitment to comply with WMDA Standards through the WMDA accreditation process. The WMDA Standards are the minimum guidelines for registries; registries are also required to conform to their governmental regulations and to the standards prevailing in the relevant community. The standards are focused on registry operations and do not cover aspects of unrelated donor transplantation that are included within the standards of other organisations, that focus on other aspects of HSC transplantation. The WMDA Standards require a registry to demonstrate the safety and quality of activities by associated
WMDA accreditation of entities involved in HSCT with unrelated donors and cord blood units

- General
- Organization of the registry
- Donor recruitment
- Donor characterization
- Information technology
- Facilitation of search requests
- Second and subsequent donations
- Collection, processing and transport of HSC
- Follow up of patient and donor
- Financial and legal liabilities
SPECIAL REPORT

Unrelated adult stem cell donor medical suitability: recommendations from the World Marrow Donor Association Clinical Working Group Committee

RN Lown1,2,11, J Philippe3,11, W Navarro4, SM van Walraven5, L Philips-Johnson4, M Fechter5, R Pawson6, M Bengtsson7, M Bekac8, S Field9, H Yang10 and BE Shaw2,3

The World Marrow Donor Association (WMDA) fosters collaboration between international registries to facilitate the exchange of hematopoietic stem cell products for unrelated stem cell donor transplantation. As indications for hematopoietic SCT grow, the movement of products across the world will increase. Although competent authorities may regulate products within their country, there is a need to protect the best interests of donors and recipients by identifying universal donor medical suitability criteria. Within this report the WMDA provides a background to unrelated adult donor and recipient safety, recommends a common framework for assessing the health of unrelated adult donors at each stage of the donation pathway and presents a novel mechanism for sharing international consensus criteria for individual medical and lifestyle conditions. Wherever possible, these criteria are evidence-based. By establishing a donor medical suitability working group, the WMDA has developed a process through which donor centers and registries may request a consensus opinion on conditions not already listed, as well as challenge existing criteria. Guidance from the WMDA is intended to complement, not supersede, guidance from national competent authorities and international regulatory bodies.
<table>
<thead>
<tr>
<th>Medical history</th>
<th>Specifically asked about</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>Ankylosing spondylitis; Crohn's disease; ulcerative colitis; myasthenia gravis; rheumatoid arthritis; sarcoidosis; systemic lupus erythematosus; multiple sclerosis; scleroderma/CREST. Any other autoimmune condition</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>HIV, hepatitis B, hepatitis C, HTLV, syphilis</td>
</tr>
<tr>
<td>Infectious diseases, including being a sexual partner of an infected individual</td>
<td>CJD (including familial and exposure risk, for example, neurosurgery, use of pituitary hormone), Chagas disease, tuberculosis, malaria</td>
</tr>
<tr>
<td>Infectious diseases, others</td>
<td>Sickle cell disease (or trait); thalassemia (including trait); inherited bleeding disorder; any other inherited disease</td>
</tr>
<tr>
<td>Inherited disease</td>
<td>Any acute or chronic back complaint, including cause, investigations, duration, medication and impact on activities of daily living</td>
</tr>
<tr>
<td>Back problems</td>
<td>Most recent blood pressure readings; medications; degree of control</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Coronary artery disease; evidence of valve disease, for example, murmur; arrhythmia</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>Degree of control; medications; use of oral steroids; hospital admissions; intensive care admissions/ventilation</td>
</tr>
<tr>
<td>Asthma</td>
<td>Medications; date of last seizure</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Number of pregnancies, including miscarriage; current/recent pregnancies; breastfeeding</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Receipt of a blood transfusion. Ask year and place of transfusion</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>The potential donor should be asked if they have any other past or current medical problems</td>
</tr>
<tr>
<td>Any other medical history</td>
<td>As defined by the registry’s national competent authority</td>
</tr>
<tr>
<td>Height and weight</td>
<td>When and where. Establish if at an establishment registered according to national regulations</td>
</tr>
<tr>
<td>High-risk sexual behavior</td>
<td>Non-prescription parenteral drug use</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>Alcohol use, consumption</td>
</tr>
<tr>
<td>Tattoo, acupuncture or body piercing</td>
<td>Current medications</td>
</tr>
<tr>
<td>Allergies</td>
<td>Medications</td>
</tr>
</tbody>
</table>
Unrelated adult stem cell donor medical suitability: recommendations from the World Marrow Donor Association Clinical Working Group Committee

<table>
<thead>
<tr>
<th>Stage</th>
<th>Infectious disease</th>
<th>Recommended validated assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>CT/VT stage</td>
<td>HIV</td>
<td>HIV-1,2 antibody</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B</td>
<td>Hepatitis B surface antigen</td>
</tr>
<tr>
<td></td>
<td>Hepatitis C</td>
<td>Hepatitis C antibody</td>
</tr>
<tr>
<td>Work-up</td>
<td>HIV</td>
<td>HIV-1,2 antibody, p24 antigen, HIV RNA</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B</td>
<td>Hepatitis B surface antigen and antibody</td>
</tr>
<tr>
<td></td>
<td>Hepatitis C</td>
<td>Hepatitis C antibody, hepatitis C RNA</td>
</tr>
<tr>
<td></td>
<td>HTLV I+II</td>
<td>HTLV I+II antibody</td>
</tr>
<tr>
<td></td>
<td>Syphilis</td>
<td>Validated serological testing algorithm</td>
</tr>
</tbody>
</table>

Abbreviations: CT/VT = confirmatory/verification typing; HTLV = human T-lymphotropic virus.
World Marrow Donor Association framework for the implementation of HLA matching programs in hematopoietic stem cell donor registries and cord blood banks

The algorithm used to select a subset of potentially matched hematopoietic stem cell donors and then to order those donors for presentation on a search report is the cornerstone of any registry. These recommendations will assist registries in developing a matching program aimed at identifying all potentially suitable donors for any searching patient. The recommendations also guide the presentation of these donors in suitably sorted lists to aid transplant centers in rapidly identifying the best match for their patients.
• Started in 1972: for HLA typing for kidney transplant

• An international society of professionals dedicated to advancing the science, education and application of immunogenetics and transplant immunology
Enumeration of CD34 by flow cytometry

• Standardized method: International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines

• CD34pos cells: defined as CD45dim/low SSC/ CD34+/7AAD− population
American Association of Blood Bank

1958: 1st ed. of Standards for Blood Banks and Transfusion Services is published
1984: AABB develops standards and starts accrediting laboratories in the parentage testing field
1991: Requirements for HPCs and bone marrow are introduced in the 14th ed. of BBTS Standards
1996: 1st ed. of Standards for Hematopoietic Progenitor Cells is published
1999: 1st ed. of Standards for Immunohematology Reference Laboratories is published
2000: 1st ed. of Standards for Cord Blood Services is published
2001: New content covering clinical activities is added to the 6th ed. of Standards for Cellular Therapy Services
2004: Standards for HPCs and CB are merged, and the 1st ed. of Standards for Cellular Therapy Product Services is published
2008: 1st ed. of Standards for Molecular Testing for Red Cell, Platelet, and Neutrophil Antigens is published
2013: 1st ed. of Standards for Cellular Therapy Services is published
2014: 1st ed. of Standards for A Patient Blood Management Program is published
Standardization does not stifle Innovation
Henry Ford - Standards

“To standardize a method is to choose out of the many methods the best one, and use it. Standardization means nothing unless it means standardizing upward.

Today’s standardization, instead of being a barricade against improvement, is the necessary foundation on which tomorrow’s improvement will be based.

If you think of “standardization” as the best that you know today, but which is to be improved tomorrow - you get somewhere. But if you think of standards as confining, then progress stops.”

Henry Ford, 1926
Today & Tomorrow
Innovations in HSCT

• Allogeneic transplant:
  – Conditioning: reduced intensity
  – Donor matching: high resolution HLA typing, improved algorithm
  – Donor source: only 30% of patients will have a HLA-matched sibling
    • Unrelated: Caucasians: 75% chance, Africans: 16-19%
    • (Local: Malays and Indians are less likely to find a full matched unrelated donor)
  • Cord blood
  • Haplo-identical
  – Cell manipulation: depletion and positive selection
  – Supportive care: anti-microbial therapy
  – A platform for adoptive immunotherapy
**The Singapore Experience: Improvement over time**

Overall survival of allogeneic transplant done for malignant diseases (adult)

Combined NUH + SGH data (n=705)

P=0.071
Alternative donor: Umbilical cord blood transplant (UCBT)

- Despite large adult volunteer registry, patients of minority ethnic background may not find a matched donor
- Cord blood as a rich source of haematopoietic stem cell: 1982
  - Studies on biology and cryopreservation of UCB cells
  - Established that UCB cells is a transplantable source

- 1988: First UCBT from sibling UCB through international collaboration
- UCB banks established to cryopreserve UCB for related and unrelated use
- 1993: First unrelated UCBT (Duke University Medical Center) for 25 children, with antigen mismatch
Biological & Immunological Superiority of UCB Grafts

- Readily available for immediate transplant
  - human leukocyte antigen (HLA) typed
  - infectious disease screening done
  - available from bank anytime

- Mismatched UCB
  - similar outcomes with fully matched BM/PBSC
  - reduced graft-versus-host-disease (GVHD)

- Greater tolerance for mismatches
  - mismatched transplants possible
  - much easier to find a match for transplant
Innovations in cord blood transplant

- To date: more than 30,000 UCBT worldwide
- Unrelated mismatched UCBT as compared to matched unrelated donor:
  delayed engraftment, less GVHD $\rightarrow$ similar overall survival and leukemia free survival

![Graphs showing A) Leukemia-free survival and B) Relapse](image_url)

*Blood* 2010;116(22):4693-4699
To overcome delayed engraftment: double cord blood transplant

Engraftment correlates with:
1. Degree of HLA match
2. Total nucleated cell dose
   • Efforts to increase cell dose

Days to neutrophil engraftment

Double UCBT (n=23): 100% median d23

Single UCBT (n=37): 65% median d32

p<0.01
To overcome delayed engraftment / immune reconstitution: cord blood expansion

Clinical Ex Vivo Expansion of Human Umbilical Cord Blood Stem and Progenitor Cells

ClinicalTrials.gov

Sponsor:
Singapore General Hospital

Collaborators:
Whitehead Institute for Biomedical Research
Blood Services Group, Health Sciences Authority of Singapore

Information provided by (Responsible Party):
Singapore General Hospital

ClinicalTrials.gov Identifier:
NCT01624701

First received: March 20, 2012
Last updated: November 26, 2015
Last verified: November 2015

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intervention</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Leukemia</td>
<td>Other: Ex vivo expanded cord blood cells</td>
<td>Phase 1</td>
</tr>
<tr>
<td>Chronic Leukemia</td>
<td></td>
<td>Phase 2</td>
</tr>
<tr>
<td>Myelodysplastic Syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myeloma</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
To overcome delayed engraftment / immune reconstitution: cord blood expansion
To overcome delayed engraftment: cord blood expansion

Controls: similar patients that meet NiCord® study eligibility criteria (age, disease, conditioning, CBU dose), transplanted with unmanipulated cord blood during the years 2010-2013, CIBMTR data
The Singapore Cord Blood Bank

Cumulative SCBB Cord Blood Inventory & Transplants

^Includes only clinical cord blood units
As of 30 April 2017
Cord blood transplant: the Singapore Experience

Outcome of UCBT for adults (combined NUH and SGH data)

- Our first case in 1998

- 90 adults with haem malignancies
- between 2005-2016
- single (N=12) or Double (N=78) unit UCB
- Long term overall survival = 42%

Courtesy of Dr Koh Liang Piu, NUH HSCT
Cord blood transplant: the Singapore experience (paediatrics @ KKH)

Overall Survival
Malignant Disease (MSD vs Unrelated CBT)

UCB, malignant, 5 yr OS: 63.2%, n=20
Matched sibling, 5 y OS: 58.0%, n = 22

Overall Survival

Non-malignant: 5y OS: 78.8%, n = 12
Malignant: 5y OS: 63.2%, n = 20

Courtesy of Dr Tan Ah Moy, KKH Paeds HSCT
Alternative donor: haplo-identical donor

Almost everyone (>95%) has a haplo-identical donor

Problem: overcoming HLA barrier
- Graft failure
- Graft vs host disease

Solution
“super”-high dose ablative conditioning
Megadose CD34 cells
Stringent T cell depletion
TREATMENT OF HIGH-RISK ACUTE LEUKEMIA WITH T-CELL–DEPLETED STEM CELLS FROM RELATED DONORS WITH ONE FULLY MISMATCHED HLA HAPLOTYPE

ABSTRACT

Background  In this study we tried to achieve successful transplantation in patients with acute leukemia with the use of hematopoietic stem cells from donors who shared only one HLA haplotype with the recipient (a “full-haplotype mismatch”). To prevent graft failure, large doses of T-cell–depleted hematopoietic stem cells were transplanted after a conditioning regimen of enhanced myeloablation and immunosuppression was administered to the recipient.

leukemia. Transplantation-related mortality was 40 percent. After a median follow-up of 18 months (range, 8 to 30), 12 of the 43 patients were alive and free of disease. All surviving patients had a good quality of life.

Conclusions  The main limitations of transplantation of bone marrow from donors who are matched with the recipient for only one HLA haplotype — GVHD and graft failure — can be overcome. Since most patients have a relative with one haplotype mismatch, advances in this method will increase the availability of hematopoietic-cell transplantation as curative therapy for acute leukemia. (N Engl J Med 1998;339:1186-93.)
Earliest experience in haploidentical transplant

- Poor immune reconstitution
- High infection risk
- High treatment related mortality

**Figure 2.** Probability of Relapse.

**Figure 3.** Probability of Disease-free Survival in Patients with ALL or AML.
• Post transplant cyclophosphamide → prevents rejection and GVHD

• Non-ablative conditioning → lower toxicity

• Does not need mega-dose stem cells → more easily achievable

• No ex vivo depletion → widely used

HLA-Haploidentical Bone Marrow Transplantation for Hematologic Malignancies Using Nonmyeloablative Conditioning and High-Dose, Posttransplantation Cyclophosphamide

ABSTRACT
We evaluated the safety and efficacy of high-dose, posttransplantation cyclophosphamide (Cy) to prevent graft rejection and graft-versus-host disease (GVHD) after outpatient nonmyeloablative conditioning and T cell-replete bone marrow transplantation from partially HLA-mismatched (haploidentical) related donors. Patients with advanced hematologic malignancies (n = 67) or paroxysmal nocturnal hemoglobinuria (n = 1) received Cy 50 mg/kg i.v. on day 3 (n = 28) or on days 3 and 4 (n = 40) after transplantation. The median times to neutrophil (>500/μL) and platelet recovery (>20,000/μL) were 15 and 24 days, respectively. Graft failure occurred in 9 of 66 (13%) evaluable patients, and was fatal in 1. The cumulative incidences of grades II-IV and grades III-IV acute (aGVHD) by day 200 were 34% and 6%, respectively. There was a trend toward a lower risk of extensive chronic GVHD (cGVHD) among recipients of 2 versus 1 dose of posttransplantation Cy (P = .05), the only difference between these groups. The cumulative incidences of nonrelapse mortality (NRM) and relapse at 1 year were 15% and 51%, respectively. Actuarial overall survival (OS) and event-free survival (EFS) at 2 years after transplantation were 36% and 26%, respectively. Patients with lymphoid malignancies had an improved EFS compared to those with myelogenous malignancies (P = .02). Nonmyeloablative HLA-haploidentical BMT with posttransplantation Cy is associated with acceptable rates of fatal graft failure and severe aGVHD or cGVHD.
Haploidentical transplant with post transplant cyclophosphamide without ex vivo depletion

Problems
- High relapse rate
- Delayed immune reconstitution
Haploidentical transplant: the Singapore experience

• Post transplant cyclophosphamide: first described in 2008
• Our first case in 2011

- Combined NUH and SGH
- 28 adults with haem malignancies
- between 2005-2016
- Haploidentical transplant with post transplant cyclophosphamide
Refinement of graft manipulation

Selective depletion of undesirable lymphocytes while preserving useful subsets

- TCR $\alpha \beta$ T cells: mediator of GVHD
- TCR $\gamma \delta$ T cells: innate immune system, anti-leukemic, does not cause GVHD

**CD3+T cells:**
- CD45RA+: naïve T cells, high potential for GVHD
- CD45RO+: memory T cells, anti-pathogen and anti-tumour

**NK alloreactivity**
- confers graft vs leukemia effect
- Facilitates engraftment

**Regulatory T cells:** reduces GVHD
Rapid memory T-cell reconstitution recapitulating CD45RA-depleted haploidentical transplant graft content in patients with hematologic malignancies

HPCs were obtained via G-CSF mobilization of the haploidentical donor and collection by leukapheresis on day 5 and 6 of G-CSF. The first HPC product collected on day 5 was T-cell depleted using the CliniMACS device and CD34 Microbead (Miltenyi Biotec, Auburn, CA, USA). Minimum cell dose required for the CD34+ enriched progenitor cell graft was $2 \times 10^6$ CD34+ cells/kg. Maximum CD3+ dose allowed for the CD34+ enriched HPC was $0.1 \times 10^6$ CD3+ cells/kg.

The HPC product collected on day 6 was processed for CD45RA+ cell depletion using the CliniMACS device and its ‘Depletion 3.1’ software. There was no target CD34+ dose or CD3+ dose on the CD45RA+ depleted product; however, release criteria of the product includes a $\geq 2 \log_{10}$ depletion of CD45RA+ cells. Two of the 17 patients did not meet the minimum CD45RA depletion after a single depletion step. Per standard operating procedure, a second run under the same conditions was performed and the requisite level of depletion was achieved in both products.

The NK cell product was collected by leukapheresis 5 days after the second HPC collection. It was a non-mobilized product and was processed on the CliniMACS device as previously described. All three cell products were infused fresh.

Refinement of graft manipulation

Precise prescription of cell therapy component

- haematopoietic stem cell
- CD45RA depleted T cells
- NK cells
Rapid memory T-cell reconstitution recapitulating CD45RA-depleted haploidentical transplant graft content in patients with hematologic malignancies
TCR αβ depleted, CD45RA depleted Haploidentical HSCT

Haplo 17 protocol

Combined KKH (n=1)
NUH (n=3)
SGH (n=2)

Day of HCT
-11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 +1

Harvesting DAY 0
- Day 1 apheresis product: split into 9: 1
  - 90%: TCR αβ depletion
  - 10%: CD45 RA depletion
- Infuse all on Day 0

Additional harvesting DAY +1
- if CD34 dose on day 1 is suboptimal (<4 x10^6/kg)
- Infused on Day +1
Cell therapy to prevent / treat relapse and infection

Graft-Versus-Leukemia Effect of Donor Lymphocyte Transfusions in Marrow Grafted Patients

European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia

Table 2. Response of Chronic and Acute Leukemia to the Treatment With Donor Lymphocyte Transfusions

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Studied</td>
<td>Evaluable*</td>
</tr>
<tr>
<td>CML</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytogen relapse</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Hematologic relapse</td>
<td>53</td>
<td>50</td>
</tr>
<tr>
<td>Transformed phase</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AML</td>
<td>23</td>
<td>17</td>
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<tr>
<td>MDS</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>ALL</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td>109</td>
</tr>
</tbody>
</table>
Activated donor lymphocyte: cytokine induced killer cells

Adoptive Immunotherapy with Cytokine-Induced Killer Cells for Patients with Relapsed Hematologic Malignancies after Allogeneic Hematopoietic Cell Transplantation

Table 3. Patient Outcomes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose Level</th>
<th>Diagnosis</th>
<th>Best Response</th>
<th>Time from CIK to Relapse</th>
<th>Alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>AML</td>
<td>CR</td>
<td>19 months</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>NHL</td>
<td>PR/SD</td>
<td>13 months</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>AML</td>
<td>CR</td>
<td>4 months</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>NHL</td>
<td>CR/SD</td>
<td>Remission</td>
<td>Yes</td>
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<tr>
<td>5</td>
<td>2</td>
<td>MM</td>
<td>PD</td>
<td>9 days</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>MM</td>
<td>CR</td>
<td>6 months</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>HD</td>
<td>CR</td>
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<td>CR</td>
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<td>ALL</td>
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<td>CR</td>
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Activated donor lymphocyte: cytokine induced killer cells ------- the Singapore Experience

ORIGINAL ARTICLE

The anti-tumour activity of allogeneic cytokine-induced killer cells in patients who relapse after allogeneic transplant for haematological malignancies

Y-C Linn¹, M Niam², S Chu², A Choong², H-X Yong¹, K-K Heng¹, W Hwang¹, Y Loh¹, Y-T Goh¹, G Suck², M Chan² and M Koh²,³

• 55 infusions for 16 patients who had either failed or progressed after initial response to various individualized chemotherapy regimens and donor lymphocyte infusion (DLI),

• Response attributable to CIK cell infusion was observed in 5 patients: ALL (n=2), AML (n=1), Hodgkin’s disease (n=2), 2 of whom had a response sustained for more than 2 years.

• Acute GVHD occurred in 3 and was steroid responsive.
Donor lymphocytes for viral reactivation post allogeneic transplant

• CMV reactivation is a serious complication in post allo-HSCT patients
  – May cause recurrent reactivation and CMV disease
  – Anti-viral drugs not without toxicity
  – may sometimes be resistant to anti-viral drug therapy.

• Other opportunistic virus reactivation: EBV, AdV, HHV6, BKV

• Virus-specific T cells have been studied in clinical trials and shown success in preventing and eradicating various virus-related diseases post allo-HSCT
RECONSTITUTION OF CELLULAR IMMUNITY AGAINST CYTOMEGALOVIRUS IN RECIPIENTS OF ALLOGENEIC BONE MARROW BY TRANSFER OF T-CELL CLONES FROM THE DONOR

ELIZABETH A. WALTER, M.D., PHILIP D. GREENBERG, M.D., MARK J. GILBERT, M.D., ROSALYNDE J. FINCH, M.SC., KÄTHE S. WATANABE, M.SC., E. DONNALL THOMAS, M.D., AND STANLEY R. RIDELL, M.D.

Abstract  Background. Cytomegalovirus (CMV) disease in immunocompromised patients correlates with a deficiency of CD8+ cytotoxic T lymphocytes specific for CMV. We evaluated the safety and immunologic effects of immunotherapy with clones of these lymphocytes in recipients of allogeneic bone marrow transplants.

Methods. Clones of CD8+ cytotoxic T cells specific for CMV proteins were isolated from the blood of bone marrow donors. Fourteen patients each received four intravenous infusions of these clones from their donors beginning 30 to 40 days after marrow transplantation. The reconstitution of cellular immunity against CMV was monitored before and during the period of infusions and for up to 12 weeks after the final infusion. The rearranged genes encoding the T-cell receptor served as markers in evaluating the persistence of the transferred T cells.

Results. No toxic effects related to the infusions were observed. Cytotoxic T cells specific for CMV were reconstituted in all patients. In vitro measurements showed that cytotoxic activity against CMV was significantly increased (P<0.001) after the infusions in 11 patients who were deficient in such activity before therapy. The level of activity achieved after the infusions was similar to that measured in the donors. Analysis of rearranged T-cell-receptor genes in T cells obtained from two recipients indicated that the transferred clones persisted for at least 12 weeks. Cytotoxic-T-cell activity declined in patients deficient in CD4+ T-helper cells specific for CMV, suggesting that helper-T-cell function is needed for the persistence of transferred CD8+ T cells. Neither CMV viremia nor CMV disease developed in any of the 14 patients.

Conclusions. The transfer of CMV-specific clones of CD8+ T cells derived from the bone marrow donor is a safe and effective way to reconstitute cellular immunity against CMV after allogeneic marrow transplantation. (N Engl J Med 1995;333:1038-44.)
Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA–peptide tetramers

Stem cell transplantation is used widely in the management of a range of diseases of the hematopoietic system. Patients are immunosuppressed profoundly in the early post-transplant period, and reactivation of cytomegalovirus (CMV) remains a significant cause of morbidity and mortality. Adoptive transfer of donor-derived CMV-specific CD8+ T cell clones has been shown to reduce the rate of viral reactivation; however, the complexity of this approach severely limits its clinical application. We have purified CMV-specific CD8+ T cells from the blood of stem cell transplant donors using staining with HLA–peptide tetramers followed by selection with magnetic beads. CMV-specific CD8+ cells were infused directly into nine patients within 4 h of selection. Median cell dosage was \(8.6 \times 10^7\)/kg with a purity of 98% of all T cells. CMV-specific CD8+ T cells became detectable in all patients within 10 d of infusion, and TCR clonotype analysis showed persistence of infused cells in two patients studied. CMV viremia was reduced in every case and eight patients cleared the infection, including one patient who had a prolonged history of CMV infection that was refractory to antiviral therapy. This novel approach to adoptive transfer has considerable potential for antigen-specific T cell therapy.
Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation

Ann M. Leen,1 Catherine M. Bollard,1 Adam M. Mendizabal,2 Elizabeth J. Shpall,3 Paul Szabolcs,4 Joseph H. Antin,5 Neena Kapoor,6 Sung-Yun Pai,5,7 Scott D. Rowley,8 Partow Kebriaei,2 Bimalangshu R. Dey,9 Bambi J. Grilley,1 Adrian P. Gee,1,10 Malcolm K. Brenner,1 Cliona M. Rooney,1,10 and Helen E. Heslop1

1Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children’s Hospital, The Methodist Hospital, Houston, TX; 2The EMMES Corporation, Rockville, MD; 3MD Anderson Cancer Center, Houston, TX; 4Duke University Medical Center, Durham, NC; 5Dana-Farber Cancer Institute, Boston, MA; 6Children’s Hospital of Los Angeles, Keck School of Medicine, University of Southern California, Los Angeles, CA; 7Boston Children’s Hospital, Boston, MA; 8John Theuer Cancer Center at Hackensack University Medical Center, Hackensack, NJ; 9Massachusetts General Hospital, Boston, MA; and 10Production Assistance for Cell Therapy Center at Baylor College of Medicine, Houston, TX

Key Points

- Banked third-party virus-specific T cells can safely and rapidly treat severe or intractable viral infections after HSCT.

Virus-specific T cell (VST) lines could provide useful antiviral prophylaxis and treatment of immune-deficient patients if it were possible to avoid the necessity of generating a separate line for each patient, often on an emergency basis. We prepared a bank of 32 virus-specific lines from individuals with common HLA polymorphisms who were immune to Epstein-Barr virus (EBV), cytomegalovirus, or adenovirus. A total of 18 lines were administered to 50 patients with severe, refractory illness because of infection with one of these viruses after hematopoietic stem cell transplant. The cumulative rates of complete or partial responses at 6 weeks postinfusion were 74.0% (95% CI, 58.5%-89.5%) for the entire group (n = 50), 73.9% (95% CI, 51.2%-96.6%) for cytomegalovirus (n = 23), 77.8% for adenovirus (n = 18), and 66.7% (95% CI, 36.9%-96.5%) for EBV (n = 9). Only 4 responders had a recurrence or progression. There were no immediate infusion-related adverse events, and de novo graft-versus-host disease developed in only 2 patients. Despite the disparity between the lines and their recipients, the mean frequency of VSTs increased significantly postinfusion, coincident with striking decreases in viral DNA and resolution of clinical symptoms. The use of banked third-party VSTs is a feasible and safe approach to rapidly treat severe or intractable viral infections after stem cell transplantation. This study is registered at www.clinicaltrials.gov as NCT00711035.

(Blood, 2013;121(26):5113-5123)
The Singapore experience: CMV-CIK

CIK rendered CMV-specific by pooled peptide stimulation and CD137 co-stimulation

- CMV-CIK exhibits CMV-specificity: kills CMV peptide-loaded autologous PHA blasts, but not control PHA blasts
- % cytotoxicity at E:T ratio of 1:1 ranges between 14 - 51%, at 10: 1 ranges between 49 – 82%
- Translatable to clinical trial
Conclusion

Haematology: “Bench to Bedside”
Standardization $\rightarrow$ ensures quality
$\rightarrow$ provides solid foundation for innovation
$\rightarrow$ results in continuous improvement

HSCT: a good example
THANK YOU!