What the present and future holds

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History of Immunotherapy

- 1891. Spontaneous regression of tumour noticed by Coley, surgeon at M. Sloan Kettering
- Concomitant bacterial infection. Coley’s toxin
- Proof of principle of cancer immunotherapy
- Coley died penniless with the stock market crash in 1936
- Barnes and Loutit (1957); allogeneic graft prevented relapse of leukaemia while syngeneic did not
Red cell depletion in marrow transplants.

T cell depletion: sheep rosetting; Campath M/G/H; ATG

CD34 selection with immunomagnetic beads (CliniMACs): haplos; autoimmune

T cells essential for disease control, graft versus host disease and viral immunity

T cell depletion results in increased disease relapse but reduced GvHD.
ABO mismatched Tx and red cells in graft

- Prolonged red cell aplasia
- Effect on other progenitors increasingly recognised
- Increases morbidity and mortality (Worel 03)
- Plasma exchange; infusion of FFP

- Recent WMDA warning: 13 cases of which 12 received RBC replete units
- 2 developed severe ATN and needed ITU
- RBC replete needs to be processed
UK data: RIC Alemtuzumab for NHL with DLIs
Non Myeloablative with DLIs

- Altered paradigm of transplantation
- Basis for non myeloablative transplants: immunotherapy as cure
- Host T cell depletion to promote donor chimerism
- Component of escalating DLIs

- CD8 depleted DLIs
- CMV specific T cells
The Ideal Graft Engineered Transplant

- Minimal Conditioning (cells to facilitate engraftment)
- Graft enriched for haematopoietic progenitor cells
- Addback of Immune effectors to maximise anti tumour activity promote broad immune reconstitution enhance anti viral immunity abrogate clinical GvHD
Basis of Cellular Immunotherapy

- Autologous vs allogeneic
- T cells: Tregs; virus specific, tumour specific
- NK-T, CIK
- NK cells;
- T regulatory cells
- Mesenchymal stem cells
- Dendritic Cells
Selective/Intelligent T cell depletion

- Work of Cavazzana, M Koh and J Barrett
- CD25, CD69 and other activation antigens
- Ex vivo detection of alloreactive cells and selective removal
- Murine GvHD using the NOD/SCID mouse model (Koh et al., BJHaem2002)
- Anti CMV and anti-EBV activity preserved
Selective depletion of alloreactive donor lymphocytes: a novel method to reduce the severity of graft-versus-host disease in older patients undergoing matched sibling donor stem cell transplantation


We have selectively depleted host-reactive donor T cells from peripheral blood stem cell (PBSC) transplant allografts ex vivo using an anti-CD25 immunotoxin. We report a clinical trial to decrease graft-versus-host disease (GVHD) in elderly patients receiving selectively depleted PBSC transplants from HLA-identical sibling donors. Sixteen patients (median age, 65 years [range, 51-73 years]), with advanced hematologic malignancies underwent transplantation following reduced-intensity conditioning with fludarabine and either cyclophosphamide (n = 5), melphalan (n = 5), or busulfan (n = 6). Cyclosporine was used as sole GVHD prophylaxis. The allograft contained a median of 4.5 × 10^6 CD34 cells/kg (range, 3.4-7.3 × 10^6 CD34 cells/kg) and 1.0 × 10^9/kg (range, 0.2-1.5 × 10^9/kg) selectively depleted T cells. Fifteen patients achieved sustained engraftment. The helper T-lymphocyte precursor (HTLp) frequency assay demonstrated successful (mean, 5-fold) depletion of host-reactive donor T cells, with conservation of third-party response in 9 of 11 cases tested. Actuarial rates of acute GVHD were 46% ± 13% for grades II to IV and 12% ± 8% for grades III to IV. These results suggest that allodepletion of donor cells ex vivo is clinically feasible in older patients and may reduce the rate of severe acute GVHD. Further studies with selectively depleted transplants to evaluate graft-versus-leukemia (GVL) and survival are warranted. (Blood. 2005;106:1123-1129) © 2005 by The American Society of Hematology
Management

- Mismatched transplants
- 16 paediatric patients
- Patient APCs with donor lymphocytes in an MLR
- Riacin based immunotoxin
- 2/16 Grade II GvHD
- Improved immune reconstitution
- V-beta; TREC; functional EBV, CMV, adenovirus responses
- 9/16 relapsed: HR refractory population
Protective Conditioning for Acute Graft-versus-Host Disease

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ABSTRACT

BACKGROUND
 Conditioning with total lymphoid irradiation plus antithymocyte serum protects mice against acute graft-versus-host disease (GVHD) after hematopoietic-cell transplantation. We tested this strategy in humans.

METHODS
 Thirty-seven patients with lymphoid malignant diseases or acute leukemia underwent an experimental conditioning regimen with 10 doses of total lymphoid irradiation (80 cGy each) plus antithymocyte globulin, followed by an infusion of HLA-matched peripheral-blood mononuclear cells from related or unrelated donors who received granulocyte colony-stimulating factor.

RESULTS
 Of the 37 transplant recipients, only 2 had acute GVHD after hematopoietic-cell transplantation. Potent antitumor effects in patients with lymphoid malignant diseases were shown by the change from partial to complete remission. In the transplant recipients who underwent conditioning with total lymphoid irradiation and antithymocyte globulin, the fraction of donor CD4+ T cells that produced interleukin-4 after in vitro stimulation increased by a factor of five, and the proliferative response to alloantigens in vitro was reduced, as compared with normal control subjects and control subjects who underwent conditioning with a single dose of total-body irradiation (200 cGy).

CONCLUSIONS
 A regimen of total lymphoid irradiation plus antithymocyte globulin decreases the incidence of acute GVHD and allows graft antitumor activity in patients with lymphoid malignant diseases or acute leukemia treated with hematopoietic-cell transplantation.
Figure 1. Nonmyeloablative Conditioning Regimen of Total Lymphoid Irradiation and Antithymocyte Globulin. Panel A shows the experimental conditioning regimen, which consisted of 10 doses of total lymphoid irradiation (TLI) (at 80 cGy each) given over a period of 11 days (as indicated by arrows). Antithymocyte globulin (ATG) (at a dose of 1.5 mg per kilogram of body weight per day) was administered intravenously on days −11 through −7 (as indicated by arrows). An intravenous infusion of mobilized HLA-matched peripheral-blood mononuclear cells from a donor was administered on day 0. Panel B shows the
TLI and ATG conditioning with low risk of graft-versus-host disease retains antitumor reactions after allogeneic hematopoietic cell transplantation from related and unrelated donors


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A hematopoietic cell transplantation regimen was adapted from a preclinical model that used reduced-intensity conditioning (RIC) and protected against graft-versus-host disease (GVHD) by skewing residual host T-cell subsets to favor regulatory natural killer T cells. One hundred eleven patients with lymphoid (64) and myeloid (47) malignancies received RIC using total lymphoid irradiation (TLI) and antithymocyte globulin (ATG) followed by the infusion of granulocyte colony-stimulating factor-mobilized grafts. Included were 34 patients at least 60 years of age, 32 patients at high risk of lymphoma relapse after disease recurrence following prior autologous transplantation, and 51 patients at high risk of developing GVHD due to lack of a fully human leukocyte antigen (HLA)-matched related donor. Durable chimerism was achieved in 97% of patients. Cumulative probabilities of acute GVHD (grades II-IV) were 2 and 10% of patients receiving related and unrelated donor grafts. Nonrelapse mortality (NRM) at 1 year was less than 4%. Cumulative incidence of chronic GVHD was 27%. The 36-month probability of overall and event-free survival was 60% and 40%, respectively. Disease status at start of conditioning and the level of chimerism achieved after transplantation significantly impacted clinical outcome. The high incidence of sustained remission among patients with active disease at time of transplantation suggests retained graft-versus-tumor reactions. Active trials registration currently at clinicaltrials.gov under IDs of NCT00186640 and NCT00186615. (Blood. 2009;114: 1099-1109)
T regulatory cells

- Effect on GvHD and GvL: appears to be protective for GvHD and not affect GvL
- Rapamycin preserving Tregs
- Tregs in cord blood: potential for expansion
Antigen specific T cells

- Tumour specific
  non polymorphic: proteinase, bcr-abl, WT1
  polymorphic: mHag HA1, HA2 (Goulmy)

- Virus specific (CMV, EBV)

- Brenner’s work well established for CMV, EBV, Hodgkins

- Stauss: Allo-restricted CTLs. ?higher affinity
Anti-tumour and anti-viral

- CTL lines from peripheral blood (PB) or CB units that recognize multiple common viruses and provide antileukemic activity by transgenic expression of a chimeric antigen receptor (CAR) targeting CD19 expressed on B-ALL

- Previously published data on multi-specific generation of multi-virus specific CTLs

- Virus specific CTLs followed by retro-viral gene transfer

- Disease specific (Micklethwaite et al; Blood 2010)
NK cells

- Previous clinical trials: uneven success. cytokine use, NK receptors not well characterised, not only Class I but combination of activatory and inhibitory receptors
- Ruggeri and Velardi: Perugia group (Science 2002)-haploidentical and mismatched transplants
- Alloreactive NK cells involved in GvL and suppression of GvH. Facilitates engraftment
Haplo m/m HSCT n=92
Extreme TCD by CD34 selection
High risk acute leukaemias (AML 57; ALL 35)

Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants

Loredana Ruggeri,1 Marusca Capanni,1 Elena Urbani,1 Katia Perruccio,1 Warren D. Shlomchik,2 Antonella Tosti,1 Sabrina Posati,1 Daniela Rogaia,1 Francesco Frassoni,3 Franco Aversa,1 Massimo F. Martelli,1 Andrea Velardi1*

<table>
<thead>
<tr>
<th>KIR ligand incompatibility in GVH direction</th>
<th>No</th>
<th>Yes</th>
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<tr>
<td>Number of transplants</td>
<td>58</td>
<td>34</td>
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| Donors displaying antirecipient NK clones | 1/58 | 34/34* |
| Disease                                   |      |       |
| ALL                                       | 21   | 14    |
| AML                                       | 37   | 20    |

| Transplantation outcomes                  |          |       |
| Rejection                                 | 15.5%    | 0%*   |
| Acute GVHD, ≥ grade II                    | 13.7%    | 0%*   |
| Probability of relapse at 5 years         |          |       |
| ALL                                       | 90%      | 85%   |
| AML                                       | 75%      | 0%**  |

$p \leq 0.01; \quad **p < 0.0008\ (22)$. 
Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer

- Jeffrey S. Miller, Yvette Soignier, Blood 2005

- Patients with renal cancer, melanomas, AML; HD
- Haplo-identical NK cells.

- Lympho-depletion with endogenous IL15 rise
- Remissions seen. IL2 given

- Ex vivo vs in vivo expansion
- Type of transplants

- Type of NK cell
- KIR mismatching
KIRs in the Asian setting

Effect of missing killer-immunoglobulin-like receptor ligand in recipients undergoing HLA full matched, non-T-depleted sibling donor transplantation: a single institution experience of 151 Asian patients

YC Linn¹, CY Phang², TJ Lim², SF Chong³, KK Heng¹, JJ Lee¹, Y Loh¹, W Hwang¹, YT Goh¹ and M Koh¹,²

No difference on multi-variate Analysis
—is KIR different in the Asian Context
-Is T cell depletion required?
-Other NK approaches.
Cytokine-induced killer cells

Non-MHC restricted T cells (CD3⁺CD56⁺ subset within LAK cell culture): Lanier 1986


Mechanism of cytotoxicity

* Granzyme - perforin
* +/- Fas mediated

Culture condition:
PBL: under specific cytokine stimulation

- IFN-γ: 1000 u/ml D1
- OKT3: 50ng/ml D2
- IL-2: 300u/ml D2
- Weekly feeding with IL-2 and fresh medium
- Mature by D21 - D28
CIK in clinical studies: Autologous CIK cells

1. *Post BMT relapse* (Phase I study, Stanford):

* 9 patients with relapsed HD/NHL given autologous CIK generated by large scale culture:
  * no toxicity
  * 2/9 PR & 2/9 stable disease

[The efficacy of chemotherapy in combination with auto-cytokine-induced killer cells in acute leukemia]

Jiang H, Liu KY, Tong CR, Jiang B, Lu DP.


图 1 两组患者 CCR 的生存率
CIK in clinical studies: Allogeneic CIK cells

Phase I trial (Stanford)

- Post allogeneic transplant relapses, n=10
- AML = 4, NHL = 2, Myeloma = 3, HD = 1
- 3 dose levels of CD3+ /kg: at 1x10^7, (n=3), 5x10^7 (n=6) and 1x10^8 (n=1)
- Chemotherapy prior to CIK for tumour debulking
- Infusional toxicity: ventricular arrhythmia in 2, transaminase elevation in 1
- Late toxicity: Grade I skin GVHD in 1, limited chronic GVHD in 2
- 1 year EFS = 20%, OS = 76%

ASH 2006, vol 108 (11), abstract #412
Mesenchymal stem cells

- Zhou H et al Nov 2009; BBMT
- 4 patients
- Sclerodermatous GvHD
- Th1 and Th2 responses
- No relapse seen
- Le Blanc: GvHD EBMT
- 3rd party MSCs
- Tissue repair post SCT: Hurlers. Haemorrhagic cystitis
- Cord blood