Cellular Therapy for Leukemia

David L Porter, MD, Stephan Grupp, MD, Edus H Warren, MD
Cellular Therapy for Leukemia

• David L Porter, MD
  – Cellular therapy for treatment of relapse after allogeneic SCT
    • Donor T cells can induce a powerful GVL reaction for some patients with relapsed leukemia.
    • GVL induction with DLI is disappointing for all but patients with CML
    • New approaches to cellular therapy for leukemia are needed.

• Stephan Grupp, MD
  – Targeted cellular therapy for leukemia: right model, right CARs, right T cells.
    • Development of T cells expressing high affinity CARs for clinical use

• Edus H Warren, MD PhD
  – Therapy for leukemia with antigen-specific T cells.
    • T cells expressing conventional TCRs against potential leukemia-associated antigens (miHag, WT1, PR1, etc…)
Disease-Specific Treatment of Relapse after Allogeneic Transplantation

David L Porter, MD
University of Pennsylvania Medical Center
Abramson Cancer Center
Treatment of Relapse

- Cellular Immunotherapy
  - Withdrawal of immune suppression
  - DLI (± chemotherapy)
  - Second allogeneic SCT
    - Conventional or RIC?
  - Other cellular therapy
    - In-vivo and ex-vivo immune activation
    - Targeted cellular therapy
    - Combination cellular, antibody, drug therapies
Treatment of Relapse

- Non-Cellular therapies
  - Supportive and palliative care
    - May be appropriate, but not acceptable in many cases
  - Disease-specific chemotherapy and radiation.
    - Presumption is conventional therapies have failed.
    - Novel agents may hold significant but unproven promise.
  - Biological and targeted agents
    - Immunomodulators, cytokines, antibodies, DNA methyltransferase inhibitors, histone deacetylation, etc., etc,
Available data for treatment of relapse

- **CML-CP**: DLI is dramatically effective
  - Dose, schedule, toxicity well defined.
  - Role of TKI’s?
- **AML, ALL, NHL, HL, CLL, MM, CML-AP/BC**
  - DLI response rate (+),
  - Long term outcomes?
  - Second SCT?
  - Other therapies?

An embarrassing lack of data
Excuses (good ones)

- Patients are heterogeneous
  - Age, conditioning regimen intensity, GVHD prophylaxis, graft source (BM vs PBSC, sibling, URD, UCB, matched, mismatched…)
  - Clinical complications and co-morbidities after allogeneic SCT
    - May not tolerate therapies well
  - Active GVHD? Acute or chronic?
    - Use of immune suppression

- Disease-related issues are heterogeneous
  - Timing of relapse: early vs late relapse may be very different
  - Influence of prior therapies and likely drug resistance
  - Histology (particularly in NHL)
Excuses (good ones)

- Small numbers of patients studied with most diseases.
- Bias for:
  - Treatment selection
  - Patient selection
  - Reporting of outcomes
- Despite this, what do we know and where can we go?
Disease-Specific Treatment for Relapse
Relapsed CP CML

- DLI
  - CR 80%
  - DFS after CR >80%
  - Remissions are durable in >80% of patients
  - Dose and schedule
    - Strategies such as low dose DLI and dose escalation can limit GVHD while preserving GVL.
Relapsed CP CML

- Imatinib
  - CCyR in 73% (11/18) pts with cytogenetic relapse (Hess et al JCO 05)
  - CMR in 62% (23/37) of all patients
  - ?Durability
  - DLI given to 7 pts who did not achieve CMR or relapsed on IM
    - CMR in 4/7
    - MMR in 2/7 (1 was not assessable)
Newer considerations in treatment of relapsed CML after allogeneic SCT

- TKI plus DLI?
  - No prospective data
  - TKI could lead to rapid reduction in CML burden and disease control
  - DLI provides GVL in setting of minimal disease.
    - Use low T cell doses and minimize risk of GVHD.
  - TKI may not target leukemia stem cell and limit GVL reaction (Falkenburg)
- Identification of cells and/or antigens to be targeted
  - mHags or leukemia-associated antigens (PR1, WT1?) with antigen-specific T cells
- Vaccination of pt or donor with mHag, APC, TAA (PR1, WT1).
- Lymphocyte subsets as DLI (CD4 T cells)
- Is there a role for Interferon?
  - For DLI and TKI resistant pts or to potentiate onset and effect of DLI
Relapsed AML
Relapsed AML

• Relapse after allogeneic SCT: 20-60%
  – Depends on disease stage, cytogenetics, preparative regimen intensity.

• Treatment for relapsed AML
  – Supportive care
  – Withdraw immunosuppression (anecdotal)
  – Re-induction chemotherapy
  – DLI
  – Second BMT
  – Biological and targeted therapies (sorafenib for FLT3+ AML, 5-azacitididine, others)
<table>
<thead>
<tr>
<th>Ref</th>
<th>N=</th>
<th>CR</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collins, 97</td>
<td>46</td>
<td>6/39 (15%)</td>
<td>OS &lt;20%</td>
</tr>
<tr>
<td>Kolb, 95</td>
<td>19</td>
<td>5/17 (29%)</td>
<td>Median OS 248d all AML/MDS pts</td>
</tr>
<tr>
<td>Shiobara, BMT 00</td>
<td>21</td>
<td>8/21 (38%)</td>
<td>7% OS at 2 years</td>
</tr>
</tbody>
</table>
Limitations to DLI for Acute Leukemia

- Lack of response
- Toxicity
  - GVHD
  - Aplasia
  - Infections
- Rapid progression of leukemia before GVL response
Chemotherapy and DLI for AML

- Prospective trial of induction chemotherapy and G-CSF primed DLI.
- N=65 (AML and advanced CML, MDS)
- BMT to relapse 102 d (41-2515).
- Response:
  - CR 27/57 (47%)
    - Relapse after CR 11/27 (41%)
    - Non-relapse death 7/27 (26%)
    - Continued CR: 9/27 (33%)
    - 29 mo (4.7-43) 9/57 (16%)
  - No response: 30/57 (53%)
    - Death from PD 25/30

Levine, et al; JCO 20:405, 2002
DLI for AML

<table>
<thead>
<tr>
<th></th>
<th>1 yr OS</th>
<th>1 yr EFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR:</td>
<td>51%</td>
<td>34%</td>
</tr>
<tr>
<td>NR:</td>
<td>5%</td>
<td>0%</td>
</tr>
</tbody>
</table>

2 year survival 19%  
(95% CI 11-33%)

Overall Survival

Years After DLI

Relapse >6 mo from BMT

Relapse <6 mo from BMT

P < 0.001 at 1 year

Levine, et al;  JCO 20:405, 2002
DLI +/- Chemotherapy for Relapsed AML
Schmid et al (EBMT), JCO 07

• N=399 with 1\textsuperscript{st} relapse of AML
  – DLI (+/- chemotherapy), n=171
  – No DLI, n=228
  – Patients fairly similar, adjust for imbalances and differences in risk factors for relapse

• DLI recipients
  – Pre-DLI chemotherapy 75%
  – DLI in remission 12%
  – DLI at aplasia/nadir 21%
  – DLI with active leukemia 67%
DLI (± Chemotherapy) vs no DLI for Relapsed AML
Schmid et al (EBMT), JCO 07

- DLI 2 yr OS 21% (median 5 mo)
- Multivariate analysis for OS after relapse
  - age < 37 years ($P = .008$)
  - CR > 5 mos after HSCT; ($P < .0001$)
  - use of DLI ($P = .04$).
Factors Associated with Improved Survival After DLI for Relapsed AML: 3 Risk Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>P</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>% blasts at relapse &gt; 35%</td>
<td>.006</td>
<td>0.56</td>
<td>0.38 - 0.85</td>
</tr>
<tr>
<td>Female v male</td>
<td>.02</td>
<td>1.6</td>
<td>1.07 - 2.4</td>
</tr>
<tr>
<td>Cytogenetics (favorable v other)</td>
<td>.004</td>
<td>5.6</td>
<td>1.76 - 1.8</td>
</tr>
<tr>
<td>CR vs no CR at DLI</td>
<td>&lt;.0001</td>
<td>5.8</td>
<td>2.5 - 13.7</td>
</tr>
</tbody>
</table>

56±10% DLI in remission and/or favorable cytogenetics (n = 29)
21±8% no remission at DLI, but female and < 35% blasts at relapse (n = 24)
9±3% all other patients (n = 75)

• Pts treated with DLI appear to have better outcomes than patients who never receive DLI

• Inducing CR and MRD prior to DLI either:
  – improves GVL induction
  – selects patients most likely to benefit from DLI.
  – suggests pretreatment with chemotherapy may be useful to maximize response to DLI

• Confirms potent GVL effect from DLI for some patients with relapsed AML.
New Approaches for Relapsed AML

- Tumor-specific T cells
  - ? Target antigens
    - PR1, WT1, CD33, CD123, etc…
- NK cells (haploidentical, matched?)
- Vaccine therapies
- Activated T cells
- Novel and targeted agents ± DLI
**Novel Agents for Relapsed AML**

- **Sorafenib – FLT3 + patients** *(Metzelder Blood 09;113).*
  - After SCT, 3/3 pts responded; 1 CR ongoing 81 d.

- **5-Azacitididine**
  - 5-azacitididine f/b DLI *(Lubbert et al. BMT 2009)*
    - Response in 13/26 (50%)
    - 4 of 26 (16%) with sustained CR
  
  - **5-azacitididine for relapse** *(Jabbour et al. Cancer, 2009;115)*
    - Response in 5/9 (55%);
    - CR in 3/9 (33%)
    - Sustained CR in 2/9 (22%) for 4 and 17 months
Relapsed ALL
Relapsed ALL

- Conventional chemotherapy
  - Poor response, limited survival
- DLI
  - Response 0-20% with few long-term survivors.
  - May be more effective in children and with MRD.
    - Up to 30% of patients will respond.
- GVL is important to cure ALL with allogeneic SCT
  \((\text{Weiden NEJM 1979, Horowitz Blood 90, Goldstone 08})\)
- Speculation for lack of GV-ALL:
  - induction of T-cell anergy by ALL cells
  - inadequate expression of co-stimulatory or adhesion molecules.
  - Resistance to killing by NK cells (unlike AML)
## DLI for ALL: Selected Trials

<table>
<thead>
<tr>
<th>Ref</th>
<th>N=</th>
<th>CR</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collins, BMT 2000</td>
<td>44</td>
<td>7/40 (17%)</td>
<td>DLI +/- chemo OS 13% @ 3yr</td>
</tr>
<tr>
<td>Choi, BMT 2005</td>
<td>10</td>
<td>7/10 (70%)</td>
<td>2 alive:1 with leukemia,1 in CR 907 days</td>
</tr>
<tr>
<td>Kolb, Vox Sang 98</td>
<td>43</td>
<td>~0% at 2 years</td>
<td></td>
</tr>
</tbody>
</table>
Relapsed ALL

• DLI may not be standard for patients with ALL relapsing after SCT
  – Selection and expansion of leukemia-specific CTLs
  – Monoclonal antibodies (unconjugated and conjugated (calicheamicin, pseudomonas immunotoxin)
  – Bispecific antibodies (anti-CD19/anti-CD3ε; MT103, blinatumomab), anti-CD20/anti CD3)
  – NK cell therapies
  – Vaccine therapies (likely best with MRD)
  – T cells modified with chimeric antigen receptors to target tumor-specific antigens (i.e. CD19)
  – Activated DLI: ex-vivo activation through co-stimulation.
Activated DLI: Rationale

- Co-stimulation is needed for appropriate T cell activation without anergy.
- Immunotherapy may fail due to limited T cell activation.
- Many tumors have immune escape mechanisms:
  - Down-regulation of MHC
  - Low or absent co-stimulatory molecules
  - Secreted factors can inhibit T cells
- Activation through ex-vivo co-stimulation may bypass in-vivo suppression.
  - Reverse functional defects in patients with lymphoma*
  - Augment immune responsiveness after autologous SCT for myeloma**

**Ex-vivo T cell co-stimulation mimics physiologic T cell activation:** Super-paramagnetic microbeads approximate size and shape of APCs. Attached anti-CD3/anti-CD28 creates artificial APC to activate T cells. Super-paramagnetic nature of beads allows removal after activation.
aDLI: Patient Characteristics

- **N=18**
  - Diagnosis:
    - AML 4; ALL 7 (Ph+, 2); HD 1; NHL 3; CLL 1; CML 1; MM 1;
  - Age: 45 (12-57)
  - Sex mismatch donor: 5
  - Months BMT to relapse: 5 (2-90)

Activated DLI: Results

- Acute GVHD (17 evaluable patients)
  - Grade 0: 10
  - Grade I-II: 5 (skin only)
  - Grade III: 2
  - No patient died from complications related to GVHD.

- Response
  - CR: 8/17
    - CLL (1/1) 64+ mo
    - AML (2/4) 54+, 40 mo
    - NHL (1/3) 79+ mo
    - ALL (4/8) 8, 16, 22, 35, mo
  - PR: 1 (AML), 28 wk
  - NR/SD/NE: 9

Limitations to aDLI

- Lack of response (55%)  
- Late relapses (5/8) 8-40 mo after aDLI
- Late relapses
  - Tumor escape mechanisms?  
  - Loss of T cell responsiveness?  
  - Lack of maximal anti-leukemia activity
    - Target ALL cells more effectively  
      - i.e. use of CD19 CAR for relapsed ALL
2\textsuperscript{nd} Allogeneic SCT

- Extensive morbidity and mortality
- Long-term DFS historically $\sim$25%
  - Highly selected patients with excellent PS, longer duration SCT to relapse, minimal GVHD and organ toxicity.
  - Not often considered
- Factors associated with improved outcomes for 2\textsuperscript{nd} SCT?
  - Influence of timing of relapse?
  - Same or different donor?
  - Conditioning regimen intensity?
  - Influence of disease type and extent of relapse?
  - Manipulations to enhance GVT activity of second SCT?
- Available data is limited and should be reassessed in the “modern” era.
2nd transplant for acute and chronic leukemia relapsing after first HLA-identical sibling transplant (Eapen et al BMT 04)

• N=279
  – AML 125 (45)
  – ALL 72 (26)
  – CML 82 (29)

• Disease status at transplant
  – Remission 144 (52)
  – Relapse 135 (48)

• Conditioning regimen intensity
  – Myeloablative 84%
  – NMA/RIC 16%
Outcomes after 2\textsuperscript{nd} transplant for relapse after first HLA-identical sibling transplant (Eapen et al BMT 04)

- Relapse: 42 (36–48)\%
- TRM: 30 (24–36)\%,
- OS at 1 yr: 41\% (35–46)
- OS at 5 yrs: 28\% (23–34).
- Age >20 and relapse < 6 mo after 1\textsuperscript{st} transplant associated with:
  - TRM
  - Treatment failure
  - Worse survival
Second transplant for acute and chronic leukemia relapsing after first HLA-identical sibling transplant (Eapen et al BMT 04)

Age < 20 yrs, remission > 6 mo [51 (38–62)%]
Age > 20 yrs, remission > 6 mo
Age < 20 yrs, remission < 6 mo [3% (0.02–12)]
Age > 20 yrs, remission < 6 mo

Probability of overall survival after second transplantation
Second Allogeneic Transplant using RIC for Relapse (Shaw et al, BMT 08)

- Retrospective review from British Society for BMT registry
- N=71
- Same donor, 80%
- Conditioning for 1st SCT MA in 67%, RIC in 33%.
- 1 y OS 42%, 2 yr 27%.
  - 2 yr OS for early relapse (<11 mo) 23%
  - 2 yr OS for later relapse 31% (P=.014)
  - 2 yr OS for LPD 89%, AML 18%, ALL 22%, MDS 17%, CML and MPD disease 0%.
Second Allogeneic Transplant using RIC for Relapse (Shaw et al, BMT 08)

• Despite high risk disease features:
  – TRM reasonable
  – OS and probable cure for some patients.
  – Time from 1\textsuperscript{st} transplant to RIC transplant most significant predictor of outcome.

• Are outcomes better than DLI?

• Comparison of DLI to second allogeneic SCT with MA or RI conditioning?
Treatment of Relapse

• Not discussed:
  – Issues related to dose and schedule of DLI
  – Pre-emptive DLI (mixed chimerism, MRD, etc…)
  – Multiple new therapies

• For additional state of the art information:
Future Directions and Proposals

• Manipulation of DLI
  – Activation
  – T cell subset selection or depletion

• Tumor-specific targeting of T cells through selection, genetic modification, etc.
  – Isolating and expanding tumor reactive T cells
  – Chimeric antigen receptors or modification of TCR to redirect against specific antigens
    • miHAgS, novel tumor associated ags, over-expressed normal antigens, etc.

• Study novel agents
  – azacitidine, decitabine, lenalidomide, bortezomib, sorafenib, etc.
Future Directions and Proposals

- Combination drug, antibody and/or cellular therapies.
- Vaccination strategies of patient/donor
  - mHag, tumor-specific antigens, APCs
- Readdress role of second SCT, particularly using RIC SCT.
- International multicenter collaboration to rapidly and definitively test and disseminate new treatment approaches for relapse.
PROUD

GLOOMY
Mice infused with 19-zeta lentiviral transduced T cells had no detectable luminescent after only 72 hours.

NSG mice infused with Nalm-6 ALL transduced with luciferase via lentiviral transfer. Fluorescense shows established marrow disease at Day 7.

These studies demonstrate that CARs against CD19 are effective against an aggressive pre-B ALL cell line.

Clinical trial with allogeneic CAR-19 for relapsed CD19+ ALL beginning 3/2010

Carl June, MD; Stephan Grupp, MD
Relapsed Hodgkin’s Disease, NHL, CLL, Myeloma

• Many overlapping issues
  – Limited data
  – Response rates?
  – Outcomes vary between
    • Early vs late relapse
    • Indolent vs aggressive disease
  – Nature of target antigens?
  – Small numbers of patients transplanted and treated for relapse
## Relapsed CLL, Myeloma, Hodgkin’s Disease, NHL

<table>
<thead>
<tr>
<th>Disease</th>
<th>N=</th>
<th>RR</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL</td>
<td>4-14</td>
<td>14-86%</td>
<td>Overall CR ~45% (33/73) Most relapse</td>
</tr>
<tr>
<td>MM</td>
<td>4-27</td>
<td>9-67%</td>
<td>Sustained response 0-18%, med OS ~23mo</td>
</tr>
<tr>
<td>HD</td>
<td>6-41</td>
<td>35-55%</td>
<td>Minority with durable responses (~30%)</td>
</tr>
<tr>
<td>NHL</td>
<td>3-17</td>
<td>0-76%</td>
<td>Heterogeneous diseases, long-term outcome best in indolent lymphoma</td>
</tr>
</tbody>
</table>
End presentation
Extra slides attached
Do not show
Disease-Specific Treatment of Relapse after Allogeneic Transplantation at the NCI sponsored 1st International Workshop on the Biology, Prevention and Treatment of Relapse after Allogeneic SCT:
Committee Members

<table>
<thead>
<tr>
<th>Fred Falkenburg</th>
<th>Karl Peggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joseph Antin</td>
<td>David Porter</td>
</tr>
<tr>
<td>Marcos de Lima</td>
<td>Jose Leis</td>
</tr>
<tr>
<td>Eli Estey</td>
<td>Nancy Hardy</td>
</tr>
<tr>
<td>John Levine</td>
<td>Nicolaus Kröger</td>
</tr>
<tr>
<td>Jacob Rowe</td>
<td>Edwin Alyea</td>
</tr>
<tr>
<td>Alan Wayne</td>
<td>Mike Bishop</td>
</tr>
<tr>
<td>David Maloney</td>
<td>Sergio Giralt</td>
</tr>
<tr>
<td>Koen van Besien</td>
<td></td>
</tr>
</tbody>
</table>
## Treatment options for relapsed Myeloma

<table>
<thead>
<tr>
<th></th>
<th><strong>ORR</strong></th>
<th><strong>CR</strong></th>
<th><strong>Overall survival</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DLI</strong></td>
<td>9 – 67 %</td>
<td>19 -30 %</td>
<td>med. ~23 mo</td>
</tr>
<tr>
<td><strong>CD8-depleted DLI</strong></td>
<td>71 %</td>
<td>43 %</td>
<td>2year: 55%</td>
</tr>
<tr>
<td><strong>Thalidomide</strong></td>
<td>29 – 83 %</td>
<td>0 – 22 %</td>
<td>3year: 25%</td>
</tr>
<tr>
<td><strong>Lenalidomide</strong></td>
<td>66 %</td>
<td>8 – 23 %</td>
<td>med. 19.9mo</td>
</tr>
<tr>
<td><strong>Bortezomib</strong></td>
<td>80 – 100 %</td>
<td>29 – 30 %</td>
<td>3year: 50%</td>
</tr>
<tr>
<td><strong>Thalidomide plus DLI</strong></td>
<td>67 %</td>
<td>22 %</td>
<td>2year: 100%</td>
</tr>
</tbody>
</table>

N Kroger, E Alyea
Low dose thalidomide (100mg) and escalating DLI

<table>
<thead>
<tr>
<th>Response</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR:</td>
<td>6/18</td>
<td>33%</td>
</tr>
<tr>
<td>PR:</td>
<td>4/18</td>
<td>22%</td>
</tr>
<tr>
<td>MR:</td>
<td>2/18</td>
<td>12%</td>
</tr>
<tr>
<td>SD/NC:</td>
<td>5/18</td>
<td>28%</td>
</tr>
<tr>
<td>PD:</td>
<td>1/18</td>
<td>5%</td>
</tr>
<tr>
<td>aGVHD II-IV</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

Med. time to response: 108 d (36 – 266)

Kroeger et al Blood 2004
## Second Transplant vs DLI (OS)

<table>
<thead>
<tr>
<th></th>
<th>UK (Shaw 08)</th>
<th>IBMTR (Eapen 04)</th>
<th>EBMT (Bosi 01)</th>
<th>DLI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AML</strong></td>
<td>18%</td>
<td>27%</td>
<td>38%</td>
<td>9-56% (CR vs no?)</td>
</tr>
<tr>
<td><strong>ALL</strong></td>
<td>22%</td>
<td>30%</td>
<td>25%</td>
<td>0-15% (age)</td>
</tr>
<tr>
<td>Comments</td>
<td>All RIC</td>
<td>Most MA</td>
<td></td>
<td>(depends on risk factors)</td>
</tr>
</tbody>
</table>
Target Tumors with Bi-specific Antibodies

- Bi20 (FBTA05) [Buhmann et al, BMT 43; 09]
  - Anti CD20, anti CD3
  - Given with DLI to 6 pts with relapse after allo
  - 3/3 pts with CLL and 1/3 with NHL transient responses
- New antibodies with other targets
  - Anti-CD19 Bi-Specific T-Cell Engager (BiTE)
    - MEDI-538, Blinatumomab
      - 55 kD recombinant single chain variable fragments (scFv)
      - Anti-CD19 Fv (HD37)
      - Anti-CD3ε Fv (L2K-07)
      - Gly/Ser linker

Fv: variable fragment; V_H: variable heavy-chain; V_L: variable light-chain; sc: single chain
### Donor Lymphocyte Infusions for Relapsed CLL

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Preceding chemotherapy</th>
<th>CR</th>
<th>CD3 Dose</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Russell 2005</td>
<td>4</td>
<td>None</td>
<td>3 (75%)</td>
<td>2 x 10^7/kg</td>
<td>DLI-induced aplasia 2nd SCT in CR @ 14m</td>
</tr>
<tr>
<td>Ritgen 2004</td>
<td>9</td>
<td>None</td>
<td>7 (78%)</td>
<td>ND</td>
<td>CCR &gt; 2 years</td>
</tr>
<tr>
<td>Gribben 2005</td>
<td>7</td>
<td>None</td>
<td>6 (86%)</td>
<td>1 x 10^7 or 3 x 10^7/kg</td>
<td>50% gr II-IV aGVHD or extensive cGVHD</td>
</tr>
<tr>
<td>Marks 2002</td>
<td>7</td>
<td>None</td>
<td>1 (14%)</td>
<td>ND</td>
<td>0% CCR</td>
</tr>
<tr>
<td>Sorror 2005</td>
<td>8</td>
<td>5</td>
<td>1 (20%)</td>
<td>1 x 10^7 to 1.5 x 10^8/kg</td>
<td>1 PR, no durable response to chemotherapy</td>
</tr>
<tr>
<td>Khouri 2004</td>
<td>10</td>
<td>rituximab</td>
<td>7 (70%)</td>
<td>1 x 10^7 to 1 x 10^8/kg</td>
<td>2 PR Planned rituximab</td>
</tr>
<tr>
<td>Sorror 2008</td>
<td>3</td>
<td>“Antibody”</td>
<td>ND</td>
<td>ND</td>
<td>Antibody + DLI</td>
</tr>
<tr>
<td>Delgado 2006</td>
<td>14</td>
<td>none</td>
<td>3 (21%)</td>
<td>1 x 10^6 to 1 x 10^8/kg</td>
<td>1 PR, 2 died GVHD</td>
</tr>
<tr>
<td>Hoogendoorn 2007</td>
<td>11</td>
<td>none</td>
<td>5 (45%)</td>
<td>unknown</td>
<td>OS 67%, EFS 33% at 2 years</td>
</tr>
</tbody>
</table>

- Overall CR rate is 45% (33/73)
- Minority have durable responses
### DLI for Relapsed Hodgkin’s Disease

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Preceding chemotherapy</th>
<th>CR/PR</th>
<th>Response rate</th>
<th>Response rate (DLI only)</th>
<th>Response at latest follow-up: time from last DLI - median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. American survey (1999)</td>
<td>6</td>
<td>unknown</td>
<td>0/2</td>
<td>29%</td>
<td>unknown</td>
<td>2 PR 6+ and 18+ months</td>
</tr>
<tr>
<td>UK</td>
<td>16</td>
<td>3</td>
<td>8/1</td>
<td>56%</td>
<td>54%</td>
<td>5 CR 2223 days (1851-2388)</td>
</tr>
<tr>
<td>Spain</td>
<td>11</td>
<td>3</td>
<td>3/3</td>
<td>55%</td>
<td>N/A</td>
<td>None ongoing</td>
</tr>
<tr>
<td>UMN</td>
<td>2</td>
<td>unknown</td>
<td>0/2</td>
<td>100%</td>
<td>unknown</td>
<td>None ongoing</td>
</tr>
<tr>
<td>GITMO</td>
<td>9</td>
<td>0</td>
<td>3</td>
<td>33%</td>
<td>33%</td>
<td>unknown</td>
</tr>
<tr>
<td>MDACC</td>
<td>14</td>
<td>11</td>
<td>3/3</td>
<td>43%</td>
<td>33%</td>
<td>1 PR 264 days</td>
</tr>
<tr>
<td>DFCI</td>
<td>13</td>
<td>unknown</td>
<td>2/0</td>
<td>15%</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>EBMT Registry</td>
<td>41</td>
<td>23</td>
<td>13</td>
<td>32%</td>
<td>44%</td>
<td>unknown</td>
</tr>
<tr>
<td>EBMT Registry (adolescent)</td>
<td>6</td>
<td>unknown</td>
<td>1/0</td>
<td>17%</td>
<td>unknown</td>
<td>1 CR 1 year</td>
</tr>
</tbody>
</table>

- Overall response rates range from 35-55% (30/71 = 42%)
- A minority have durable responses (7/24 = 29%)
- Durable responses more frequent following T cell depleted HSCT?
DLI for Relapsed NHL

- Usually reported in context of larger transplant trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Cond for HCT</th>
<th>n</th>
<th>Histology</th>
<th>Chem/ XRT</th>
<th>CR/PR</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Russell 2005</td>
<td>T-dep (15)</td>
<td>17</td>
<td>DL (5), MCL (4)</td>
<td>9</td>
<td>11</td>
<td>PFS 3 y 52% OS 3 y 58%</td>
</tr>
<tr>
<td></td>
<td>T-replete (2)</td>
<td></td>
<td>FL (4), CLL (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bloor 2008</td>
<td>T-dep (16)</td>
<td>17</td>
<td>CLL (3), MCL (3)</td>
<td>8</td>
<td>13</td>
<td>10 in remission f/u 26 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FL (6), DL (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bishop 2008</td>
<td>RIC</td>
<td>5</td>
<td>DL (5)</td>
<td>4</td>
<td>3</td>
<td>3 CR 74+–83+mo</td>
</tr>
<tr>
<td>van Besien</td>
<td>ablative</td>
<td>3</td>
<td>DL (2), PL (1)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marks 2002</td>
<td>T-dep</td>
<td>15</td>
<td>FL (15)</td>
<td>8</td>
<td>7</td>
<td>7 CR 16+–40+ mo</td>
</tr>
<tr>
<td>Mandigers 2003</td>
<td>T-dep</td>
<td>7</td>
<td>FL (5), SL (2)</td>
<td>4</td>
<td>6</td>
<td>4 CR 43+–89+ mo</td>
</tr>
</tbody>
</table>
NHL Relapse Following Allogeneic HCT:

- Heterogenous diseases with different histologies
  - indolent to aggressive behavior
- Allogeneic SCT often used for advanced refractory disease.
  - after failed autologous HCT
  - with chemotherapy refractory disease
- Transplanted with a variety of conditioning intensities
  - myeloablative, reduced intensity, nonmyeloablative
  - T depleted or T replete grafts
  - HLA matched or mismatched, related, unrelated, cord blood or haploidentical stem cell sources
Figure 2. Kaplan-Meier estimates of overall and disease-free survival after aDLI


Copyright ©2006 American Society of Hematology. Copyright restrictions may apply.
Impact of Early vs Late NHL Relapse Post Allogeneic HCT

Progression-Free Survival after relapse
relapse < 6 mo after allo (n=12) vs > 6mo after allo (n=11)

P=0.03

8/23 durable remissions
4 LGL, 1 MCL, 1 HL, 2 DLB
6 Chemo, 2 DLI

Kenkre et al, ASH 09, abst 2247 (U Chicago)
Chemotherapy and DLI for AML

- Prospective trial of induction chemotherapy and G-CSF primed DLI.
- N=65
  - AML 50
  - CML AP/BC 11
  - MDS 4
- BMT to relapse 102 d (41-2515).
- Relapse to DLI 19 d (7-111)
- CD3 dose x 10^8 1.0 (0.8-4.2)

Levine, et al; JCO 20:405, 2002
Chemotherapy and DLI for AML

• Response:
  - CR 27/57 (47%)  
    - Relapse after CR 11/27 (41%)  
    - Non-relapse death 7/27 (26%)  
    - Continued CR: 9/27 (33%)  
      29 mo (4.7-43) 9/57 (16%)  
  - No response: 30/57 (53%)  
    - Death from PD 25/30  
    - CR to additional DLI 3/10

Levine, et al; JCO 20:405, 2002
Relapsed ALL

- DLI may not be standard for patients with ALL relapsing after SCT
- Novel approaches for relapsed ALL are needed
  - DLI as “pre-emptive” therapy.
    - When given for increasing chimerism, EFS 37% vs 0% for no DLI
      \( (Bader, JCO 04) \)
  - Selection and expansion of leukemia-specific CTLs
  - T cells modified with chimeric antigen receptors to target tumor-specific antigens (i.e. CD19)
  - Monoclonal antibodies (unconjugated and conjugated (calicheamicin, pseudomonas immunotoxin)
  - Bispecific antibodies (anti-CD19/anti-CD3ε; MT103, blinatumomab)
  - NK cell therapies
  - Vaccine therapies (likely best with MRD)
  - Activated DLI: ex-vivo activation through co-stimulation.
**Activated DLI: Hypothesis**

- DLI may not be effective if donor T cells are not appropriately activated *in-vivo*.

- *Ex-vivo* activation and co-stimulation may reverse functional T cell tolerance and/or augment GVT activity.

- *Ex-vivo* co-stimulated, expanded donor T cells may induce GVT in patients who do not respond well to standard DLI.
Treatment of Relapse After Allogeneic SCT

- In the absence of GHVD
  - Withdrawal of immunosuppression (IS)
  - Donor Lymphocyte Infusions (DLI)
- Monoclonal antibody therapy
- Chemotherapy +/- DLI
- Radiotherapy +/- DLI
- Immune stimulants (IL-2 etc)
- Targeted T cells (CARs, BITEs)
- Second allogeneic HCT
- Novel immunotherapies
Treatment of Relapse

- Something old
- Something new
- Something borrowed
- Something “blue” (or not)
Relapsed CP CML

- DLI
  - CR 80%
  - DFS after CR >80%
  - Remissions are durable in >80% of patients
  - Strategies such as low dose DLI and dose escalation can limit GVHD while preserving GVL.
Relapsed CP CML

- **Imatinib**
  - CCyR in 73% patients with cytogenetic relapse (11/18) (Hess et al JCO 05)
  - CMR in 62% of all patients (23/37)
    - 78% if molecular relapse (14/18)
    - 47% if cytogenetic relapse (9/19)
  - ?Durability
    - DLI given to 7 pts who did not achieve CMR or relapsed on IM
      - CMR in 4/7
      - MMR in 2/7 (1 was not assessable)
Newer considerations in treatment of relapsed CML after allogeneic SCT

- **TKI plus DLI?**
  - No prospective data
  - TKI could lead to rapid reduction in leukemia burden and disease control
  - DLI provides GVL in setting of minimal disease.
    - Use low T cell doses and minimize risk of GVHD.
  - TKI may not target leukemia stem cell and limit GVL reaction (Falkenburg, et al)

- **Identification of cells and/or antigens to be targeted**
  - CML stem cell
  - mHags or leukemia-associated antigens (PR1, WT1?) with antigen-specific T cells

- **Vaccination of patient or donor with mHag, APC, tumor-associated Ags (PR1, WT1).**

- **Lymphocyte subsets as DLI (CD4 T cells)**

- **Is there a role for Interferon?**
  - For DLI and TKI resistant patients?
  - To potentiate onset and effect of DLI
Other than for CP CML, low response rates and late relapses limit successful DLI for relapsed disease after allogeneic SCT.
Activated DLI: Toxicity

- Mild infusional toxicity at higher dose levels
- Acute GVHD (17 evaluable patients)
  - Grade 0: 10
  - Grade I-II: 5 (skin only)
  - Grade III: 2
  - No patient died from complications related to GVHD.
- Chronic GVHD
  - Limited/extensive 2/2
    - eyes, oral mucosa, well controlled without systemic therapy
- Other
  - 1 ITP 6 months after aDLI successfully treated with prednisone.
  - No other unusual toxicities have been noted.

Relapsed Hodgkin’s Disease, NHL, CLL, Myeloma

• Many overlapping issues
  – Limited data
  – Response rates?
  – Outcomes vary between
    • Early vs late relapse
    • Indolent vs aggressive disease
  – Nature of target antigens?
  – Small numbers of patients transplanted and treated for relapse
## DLI for AML

### Survival Rates

<table>
<thead>
<tr>
<th>Time</th>
<th>Survival Rate</th>
<th>EFS Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 yr</td>
<td>51%</td>
<td>34%</td>
</tr>
<tr>
<td>No response</td>
<td>5%</td>
<td>0%</td>
</tr>
</tbody>
</table>

- **2 year survival**: 19% (95% CI 11-33%)

---

*Levine, et al;  JCO 20:405, 2002*
DLI for AML

Years After DLI

Overall Survival

P < 0.001 at 1 year

Relapse >6 mo from BMT

Relapse <6 mo from BMT

Levine, et al; JCO 20:405, 2002
New Approaches to test in relapsed CLL

- **Flavopiridol**
  - 45% response rate, 42% in p53 deleted, 72% in 11q deleted
- **Bendamustine**
  - ORR > 50%
- **Alemtuzumab**
  - ORR 33%, equivalent for p53 deleted
- **High-dose methylprednisolone**
  - 1 gram/m$^2$/day x 5 days + rituximab
  - ORR 78% including 5/9 p53-deleted with 1 cycle
  - Effect on GVL?
- **Ofatumumab** (Humanized anti-CD20 antibody)
  - Impressive activity in relapsed/refractory CLL (ORR 50%)
- **Lenalidomide**
  - 30% RR in 11q or 17p deletion CLL
- **Bi20** (trifunctional anti CD3/CD20) and DLI (Buhmann, BMT 09)

Byrd, Blood, 2007; Bergmann, Haematologica, 2005
Available data for treatment of relapse

- **CML-CP**: DLI restores durable CR in 80% of pts with CP relapse.
  - Dose, schedule, toxicity well defined.
  - Role of TKI’s?

- **AML, ALL, NHL, HL, CLL, MM, CML-AP/BC**
  - DLI response rate (±),
  - Long term outcomes?
  - Second SCT?
  - Other therapies?

An embarrassing lack of data
Newer considerations in treatment of relapsed CML after allogeneic SCT

- TKI plus DLI?
  - No prospective data
  - TKI could lead to rapid reduction in leukemia burden and disease control
  - DLI provides GVL in setting of minimal disease.
    - Use low T cell doses and minimize risk of GVHD.
  - TKI may not target leukemia stem cell and limit GVL reaction (Falkenburg)
  - Identification of cells and/or antigens to be targeted
  - CML stem cell
  - mHags or leukemia-associated antigens (PR1, WT1?) with antigen-specific T cells

- Vaccination of patient or donor with mHag, APC, TAA (PR1, WT1).
- Lymphocyte subsets as DLI (CD4 T cells)
- Is there a role for Interferon?
  - For DLI and TKI resistant patients or to potentiate onset and effect of DLI
## DLI for Relapse (non-CML)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>15-36%</td>
</tr>
<tr>
<td>ALL</td>
<td>0-18%</td>
</tr>
<tr>
<td>MDS</td>
<td>25-40%</td>
</tr>
<tr>
<td>Myeloma</td>
<td>9-50%</td>
</tr>
<tr>
<td>NHL</td>
<td>20-60%</td>
</tr>
</tbody>
</table>
• Pts treated with DLI appear to have better outcomes than patients who never receive DLI
  – OS 21% vs 9% at 2 years.
• Inducing CR and MRD prior to DLI either improves GVL induction, or at the least selects patients most likely to benefit from DLI.
  – Suggests pretreatment with chemotherapy may be useful to maximize response to DLI
• Confirms potent GVL effect from DLI for some patients with relapsed AML.
Activated DLI: Toxicity

- Mild infusional toxicity at higher dose levels
- Acute GVHD (17 evaluable patients)
  - Grade 0: 10
  - Grade I-II: 5 (skin only)
  - Grade III: 2
  - No patient died from complications related to GVHD.
- Chronic GVHD
  - Limited/extensive 2/2
    - eyes, oral mucosa, well controlled without systemic therapy
- 1 ITP 6 months after aDLI successfully treated with prednisone.
- No other unusual toxicities have been noted.

Enhance specificity of aDLI?

- Identify tumor-specific T cells and expand through ex-vivo co-stimulation.
  - After BMT for myeloid leukemia, T cells specific for potential leukemia-associated antigens identified through tetramer analysis (Beatty, Vonderheide; CCR 15, August 09 4944).
  - Cells do not expand.
    - Donor T cells are functionally unresponsive and have senescent
Second Allogeneic Transplant using RIC for Relapse (Shaw et al, BMT 08)
Activated DLI: Toxicity

- Mild infusional toxicity at higher dose levels
- Acute GVHD (17 evaluable patients)
  - Grade 0: 10
  - Grade I-II: 5 (skin only)
  - Grade III: 2
  - No patient died from complications related to GVHD.
- Chronic GVHD
  - Limited/extensive 2/2
    - eyes, oral mucosa, well controlled without systemic therapy)
- No other unusual toxicities have been noted.

## Activated DLI: Results

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CR:</strong></td>
<td><strong>8/17</strong></td>
<td></td>
</tr>
<tr>
<td>- CLL (1/1)</td>
<td><strong>64+</strong> mo</td>
<td></td>
</tr>
<tr>
<td>- AML (2/4)</td>
<td><strong>54+, 40 mo</strong></td>
<td></td>
</tr>
<tr>
<td>- NHL (1/3)</td>
<td><strong>79+</strong> mo</td>
<td></td>
</tr>
<tr>
<td>- ALL (4/8)</td>
<td>8, 16, 22, 35, mo</td>
<td></td>
</tr>
<tr>
<td><strong>PR:</strong></td>
<td>1 (AML), 28 wk</td>
<td></td>
</tr>
<tr>
<td><strong>NE:</strong></td>
<td>1 early death (ALL)</td>
<td></td>
</tr>
<tr>
<td><strong>NR/SD:</strong></td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Activated DLI: Toxicity

- Mild infusional toxicity at higher dose levels
- Acute GVHD (17 evaluable patients)
  - Grade 0: 10
  - Grade I-II: 5 (skin only)
  - Grade III: 2
  - No patient died from complications related to GVHD.
- Chronic GVHD
  - Limited/extensive 2/2
    - eyes, oral mucosa, well controlled without systemic therapy
- No other unusual toxicities have been noted.

Relapsed CP CML

• **Imatinib**
  – CCyR in 73% (11/18) pts with cytogenetic relapse (Hess et al JCO 05)
  – CMR in 62% (23/37) of all patients
    • 78% (14/18) if molecular relapse
    • 47% (9/19) if cytogenetic relapse, ?Durability
  – DLI given to 7 pts who did not achieve CMR or relapsed on IM
    • CMR in 4/7
    • MMR in 2/7 (1 was not assessable)
Treatment of Relapse

- **Cellular Immunotherapy**
  - Withdrawal of immune suppression
  - DLI (+ chemotherapy)
  - Second allogeneic SCT
    - Limited application for conventional second SCT
    - RIC second transplant as immunotherapy?
  - Other cellular therapy
    - In-vivo and ex-vivo immune activation
    - Targeted cellular therapy
    - Combination cellular, antibody, drug therapies
Other than for CP CML, low response rates and late recurrence limit successful DLI for relapse after allogeneic SCT.
Novel Agents for Relapsed AML

- Novel targeted agents for relapse:
  - Sorafenib – FLT3 + patients (Metzelder Blood 09;113).
    - After SCT, 3/3 pts responded; 1 CR ongoing 81 d.
  - 5-Azacitididine
    - 5-azacitididine f/b DLI (Lubbert et al. BMT 2009)
      - Response in 13/26 (50%)
      - 4 of 26 (16%) with sustained CR
    - 5-azacitididine for relapse (Jabbour et al. Cancer, 2009;115)
      - Response in 5/9 (55%);
      - CR in 3/9 (33%)
      - Sustained CR in 2/9 (22%) for 4 and 17 months
    - 5/8 pts remained in CR (median 17 mos, 14 - 26 mos).
    - 3/8 recurred
Excuses (good ones)

- Small numbers of patients studied with most diseases.
- Bias for:
  - Treatment selection
    - Depends on patient, disease activity, donor availability, prior therapies…
  - Patient selection
    - Age, co-morbidity, past and present transplant-related complications.
  - Reporting of outcomes
- Despite this, what do we know and where can we go?
Limitations to aDLI

- Lack of response (55%)
- Late relapses (5/8) 8-40 mo after aDLI
  - Time CR to relapse:
    - AML 40 mo
    - ALL 8, 16, 22, 35 mo
- Late relapses
  - Tumor escape mechanisms?
  - Loss of T cell responsiveness?
  - Lack of maximal anti-leukemia activity
    - Target ALL cells more effectively
Treatment of Relapse

• Cellular Immunotherapy
  – Withdrawal of immune suppression
  – DLI (± chemotherapy)
  – Second allogeneic SCT
  – Other cellular therapies

• Non-Cellular therapies
  – Supportive and palliative care
  – Conventional chemotherapy or radiation
  – Novel cytotoxic agents
  – Biological and targeted agents