Allogeneic cytokine-induced killer cells demonstrate anti-tumour activity in patients who relapse after allogeneic haemopoietic stem cell transplant for haematological malignancies

Yeh-Ching LINN
Singapore General Hospital

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Background

• Relapse of haematological malignancies after alloHSCT → poor prognosis

• Salvage treatment: high treatment related mortality and low response rate

• Additional modalities: Donor lymphocyte infusion (DLI) for its Graft vs Tumour (GVT) effect:
  • modest response
  • associated with Graft vs Host disease (GVHD)
Background: Cytokine-induced killer (CIK) cells

• Polyclonal T cells, cytokine expanded, non-MHC restricted
  – In vitro: kill tumour cells eg
    – Lymphoma cell lines,
    – primary acute (AML) and chronic myeloid leukemia (CML)
  – Mice:
    • Across MHC barrier: controls lymphoma with no or minimal GVHD as compared to unmanipulated splenocytes.
    • Persists at tumour sites but little infiltration of GVHD target tissue

• Clinical trial: Feasible to expand CIK cells in clinical scale from leukapheresis product of
  • healthy donors
  • patients undergoing chemotherapy
Materials and Methods: CIK expansion

- Phase I/II clinical study (clinicaltrial.gov NCT00460694)
- AlloCIK as “activated DLI” for patients who relapse post alloHSCT:
  - failed to respond to donor lymphocyte infusion (DLI), +/- chemotherapy, or
  - do not have access to further donor lymphocytes
- Leukapheresis product (fresh or frozen) from donors or patients (whose donors were unavailable)
- Expanded in bags in GMP facility
  - 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
  - Frozen in aliquots while awaiting microbiological clearance
Materials and Methods: clinical protocol

- **Infusion:**
  - Flexibility of salvage regimen based on individual case
  - If chemotherapy given: alloCIK infusion at lymphopenia
  - Pre-medication: iv Diphenhydramine, po Paracetamol and po Celecoxib
  - Dose: 10 million CD3/kg if no prior DLI, or 2-3x the dose of last DLI given
  - Intervals: at least 4 weeks

- **Dose escalation**
  - If no GVHD: dose doubled or tripled
  - If GVHD develops: withheld till controlled

- **Number of infusion**
  - Continued as long as cells available and no clinical contraindications
• Total of 34 CIK cultures (from 20 donors and 5 patients) for 24 patients
• 5 patients had cells harvested from themselves (UCBT=1, MUD=3, sibling = 1)
• Total of 55 infusions for 16 patients
• 8 patients did not receive CIK infusion: 7 deceased, 1 achieved remission before CIK infusion
<table>
<thead>
<tr>
<th></th>
<th>median</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD3+ T cell proportion (%)</strong></td>
<td>96.7%</td>
<td>58.4 – 99.7%</td>
</tr>
<tr>
<td><strong>CD3+T cell expansion (fold)</strong></td>
<td>9.33 fold</td>
<td>1.3 - 39.0 fold</td>
</tr>
<tr>
<td><strong>CD3+CD56+ cell proportion (%) (NK-like T cell)</strong></td>
<td>24.8%</td>
<td>3.8 – 73.0%</td>
</tr>
<tr>
<td><strong>CD3+CD56+ cell expansion (fold)</strong></td>
<td>27.77 fold</td>
<td>2.6 - 438.9 fold</td>
</tr>
<tr>
<td><strong>Cytotoxicity (40:1) vs allo AML target</strong></td>
<td>37%</td>
<td>0 - 69%</td>
</tr>
</tbody>
</table>

Comparable between donor-derived and patient-derived cultures
<table>
<thead>
<tr>
<th>ID</th>
<th>Disease</th>
<th>Treatment prior to first CIK infusion</th>
<th>Disease status at 1st CIK infusion</th>
<th>CIK dose (m=million CD3/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML#2 41 / F</td>
<td>AML M2 post salvage chemotherapy</td>
<td>Consolidation with 2nd FLAG followed by #1 CIK.</td>
<td>Hypocellular marrow</td>
<td>#1 = 10m</td>
</tr>
<tr>
<td>AML#3 60 / M</td>
<td>Flt3+ AML M1, In relapse</td>
<td>Po VP-16</td>
<td>Circulating blasts 5,740/uL</td>
<td>#1 = 10m, #2 = 20m, #3 = 25m</td>
</tr>
<tr>
<td>AML#4 59 / F</td>
<td>AML in relapse</td>
<td>None.</td>
<td>Circulating blasts 70/uL</td>
<td>#1 = 20m</td>
</tr>
<tr>
<td>AML#5 55 / M</td>
<td>Primary refractory AML</td>
<td>Po VP-16, oral hydroxyurea with CIK at each nadir.</td>
<td>Circulating blasts 9,990/uL</td>
<td>#1 = 15m, #2 = 30m</td>
</tr>
<tr>
<td>AML#6 28 / F</td>
<td>Primary refractory AML</td>
<td>Ida/clofarabine/Cytarabine followed by #1 CIK.</td>
<td>Hypocellular marrow post chemotherapy</td>
<td>#1 = 10m, #2 = 100m</td>
</tr>
<tr>
<td>HD#1 20 / M</td>
<td>Nodular sclerosing HD in relapse</td>
<td>Po VP-16/ pulse dexta 1 cycle before #1 CIK.</td>
<td>Progressive generalized lymphadenopathy</td>
<td>#1 = 10m, #2 = 20m</td>
</tr>
</tbody>
</table>

Results: No response in 6 patients
## Results: Unable to assess in 5 patients

<table>
<thead>
<tr>
<th>ID</th>
<th>Disease</th>
<th>Treatment prior to 1st CIK infusion</th>
<th>Disease status at 1st CIK infusion</th>
<th>CIK dose (m=million CD3/kg)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL#2 F/36</td>
<td>Ph+ ALL Second Haem relapse</td>
<td>Dasatinib with concomitant CIK.</td>
<td>Circulating blasts 510/uL</td>
<td>#1 = 20m #2 = 40m</td>
<td>CMR</td>
</tr>
<tr>
<td>AML#1 F/54</td>
<td>AML M5a in 3rd relapse</td>
<td>Ida/FLAG with alloPBSC rescue</td>
<td>Hypocellular marrow</td>
<td>#1 to #7 30m to 100m</td>
<td>Remission, relapsed 10mo later</td>
</tr>
<tr>
<td>AML#8 M/28</td>
<td>AML M1 at 1st relapse</td>
<td>Azacitidine/valproic</td>
<td>In partial remission At nadir of azacitidine</td>
<td>#1 = 30m #2 = 30m #3 = 20m</td>
<td>CR after 5 cycles</td>
</tr>
<tr>
<td>CML#1 F/46</td>
<td>CML in 2nd blast crisis</td>
<td>Dasatinib with concomitant CIK.</td>
<td>In complete haematological remission</td>
<td>#1 = 30m #2 = 30m #3 = 37m #4 = 40m</td>
<td>MMR</td>
</tr>
<tr>
<td>NHL#1 F/54</td>
<td>DLBCL in 3rd relapse</td>
<td>V-R-ESHAP 4 cycles with CIK at nadir of last 2 cycles.</td>
<td>In remission</td>
<td>#1 = 20m #2 = 30m #3 = 50m</td>
<td>Remission, relapsed 6 mo later</td>
</tr>
</tbody>
</table>
Results: Evidence of efficacy: Patient #1

- 30 y man with poor risk T-ALL, alloPBSCT in CR1
- Relapsed 13 months later: BM 61% blast, complex cytogenetics, extramedullary (EM) disease
Patient #1: T-ALL

BM hypocellular

9/06

10/06

11/06

12/06

1/07

2/07

EM disease in shoulder

RT to shoulder

HD MTX, ifosfamide, PBSCT

HD MTX, ifosfamide, VP-16

L-aspa, vcr, dexe
6-MP, MTX

BM no blast

BM no blast

BM no blast

Increase in size of EM disease in face

HD MTX, ifosfamide, VP-16

EM disease in orbit, zygoma, pterygoide fossae, maxillary and frontal sinus

HD MTX, ifosfamide

EM disease in shoulder

RT to shoulder

HD MTX, ifosfamide, PBSCT

HD MTX, ifosfamide, VP-16

L-aspa, vcr, dexe
6-MP, MTX

BM no blast

BM no blast

BM no blast
Patient #1: T-ALL

- Leukemia with aggressive biology manifesting as relentless extramedullary invasion despite chemotherapy,
- marrow was maintained in remission by CIK cells despite EM progression: Graft vs Leukemia (GVL) effect is known to be active for marrow leukemia while ineffective for EM disease

Multiple further chemotherapy, 4 CIK infusions and another transplant
Refractory and succumbed
Results: patient #2: B precursor-ALL

- 24 y man with B precursor ALL, UCBT done and relapsed 7 mo later
- Salvage chemotherapy × 2 cycles → CR2
- Generated CIK from himself: preferential expansion of NK cells instead

- Marrow maintained in remission for 5 months after CIK infusion despite CNS relapse which occurred one month: GVL effect of CIK on marrow
Results: patient #3: Hodgkin’s disease

- 23 y/F nodular sclerosing HD Nov 2005: ablative BMT in PR2
  - May 2006: PET showed positive chest and abdominal nodes
  - Initial response with DLI but progressed
  - Reduction in size and number of lung nodules after 2 CIK infusions but not durable

Timeline:

- **8/06**: DLI#1
- **9/06**: DLI#2
- **10/06**: DLI#3
- **11/06**: DLI#4
- **12/06**: CIK#1, 25m
- **1/07**: CIK#2, 50m
- **2/07**: CT: reduction in lymph nodes and lung nodules
- **3/07**: CT: increase in lymph nodes and lung nodules
- **4/07**: Bx: Hodgkin’s
- **5/07**: GVHD liver, CT: reduction in lung nodules
- **6/07**
- **7/07**
- **8/07**
Results: patient #4: Hodgkin’s disease

- 27 y/ M/ stage IV HD, autoPBSCT in CR1
- 7/2008: allo RICT in chemosensitive relapse after failing autologous PBSCT
- 11/2008: falling donor chimerism
- 11/08-1/09: DLI#1 - #3 at 4,8 and 14 million CD3/kg
- 5/2009 PET scan: new lymph nodes
- 7/2009 DLI#4: 20 million CD3/kg
- 8/2009 CIK #1: 30 million CD3/kg
- 9/2009 CT: nodes smaller
- 10/2009 liver GVHD;
- CT: no more enlarged nodes
- Remains in remission now 21 mo post CIK
Results: patient #5: AML

- 36 y/F/ AML in 1995, alloBMT in 1996
- First relapse: 12/2006

1st relapse
Ida/ara-c

2nd relapse
FLAG

CR2 = 23 months
CR2

CR3 = 30 months
CR3

CIK#1-#6

DLI#1

Still in CR

- Remains in remission with CR3 longer than CR2 which is unusual in the natural history of relapsed leukemia
- Suggestive of the GVL effect of additional CIK infusions in CR3
Results: adverse reaction

- Transient fever within 24h, controlled with symptomatic measures
- No hypersensitivity reactions seen

- Acute GVHD in 3 patients (2 showed evidence of response): predominantly raised alkaline phosphatase and transaminases, easily controlled with prednisolone
- Chronic GVHD pre-existing in 2 patients: unable to assess
Discussion

- AlloCIK is effective, in some may even be superior to DLI
  - Without higher incidence of GVHD
  - But some are not sustainable
- Efficacy far from satisfactory, ineffective against
  - Extramedullary leukemia
  - Lymphoma / leukemia in relapse without concomitant chemotherapy

→ There is still much room for improvement
- As a platform for further manipulations, eg
  - Expression of tumour associated antigen – specific receptors eg TCR or other receptor (eg antiCD19 for B-precursor ALL)
  - Concomitant administration with bispecific antibodies (eg CD3xCD19 bispecific molecule)

→ Enhanced specificity and potency of alloCIK
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Thank you 😊