Role of Natural Killer cells In RIC Allogeneic HSCT: Perspectives

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Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)
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• Classical understanding
  • Strong immunologic antileukemic action: GVL effect
  • High procedure-related mortality: GVHD reactions
  • Thus therapeutic limited to
    • young patients (GVHD increases with age)
    • Pts with HLA identical sibling (28% population) (toxicity increases otherwise)
    • Poor prognosis patients (too toxic for standard pts)
    • Limited to leukemia
Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

• Present developments
  • Transplant from unrelated donor equivalent to transplant from sibling (Higher possibility to perform transplant)
  • Transplant from alternative donors (Cord Blood; partially compatible): Promising developments
Increased Serum Levels of Tumor Necrosis Factor α Precede Major Complications of Bone Marrow Transplantation


Blood, Vol 75, No 4 (February 15), 1990: pp 1011-1016
Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

• **Present developments**
  • Transplant from unrelated donor equivalent to transplant from sibling (Higher possibility to perform transplant)
  • Transplant from alternative donors (Cord Blood; partially compatible): Promising developments

• **Reduced toxicity conditioning regimens**
  • Lower transplant related mortality (3 to 5 fold)
  • Allow for considering otherwise discarded populations
    • Patients with comorbidities (related to previous treatments…)
    • Alternative donors
    • Patients older than usual (Population suffering from cancer)
    • Other diagnoses: lymphoid malignancies, Solid tumors)
Impact of cGVHD on outcome in RIC context

0: No GVHD; 1: Mild; 2: Moderate; 3: Severe

Non Relapse Mortality

Relapse

p = 0.003

p = 0.005

41%
31%
17%
12%
## Thymoglobulin: One vs. Two days

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<th>day-5</th>
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AML patients and Matched Related SCT

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<tr>
<th>Variable</th>
<th>1-day ATG (N=53)</th>
<th>2-day ATG (N=22)</th>
<th>p-value</th>
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<tr>
<td>Age</td>
<td>49 [18-70]</td>
<td>60 [31-68]</td>
<td>0.032</td>
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<tr>
<td>Diagnosis: AML/MDS</td>
<td>47 (89%)/6 (11%)</td>
<td>18 (82%)/4 (18%)</td>
<td>0.325</td>
</tr>
<tr>
<td>High Risk Disease*</td>
<td>21 (40%)</td>
<td>10 (45%)</td>
<td>0.415</td>
</tr>
<tr>
<td>CsA/CsA+MMF</td>
<td>43 (81%)/10 (19%)</td>
<td>22(100%)/0</td>
<td>0.024</td>
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*High risk AML: secondary AML, Adverse caryotype, no CR at the time of transplantation
*High risk MDS: high risk IPSS, therapy related MDS

BMT, 2012
Medical Needs?

- Dramatic reduction of early mortality
- Better quality of life (control of GVHD)
- **Thus disease control has become the real remaining challenge**
BETTER LONG TERM DISEASE CONTROL?

• Patient selection
• Conditioning regimens modulation
• Post-graft approaches
  • Drug based immunomodulation
  • Tumor antigen vaccination
  • Donor based cellular immunotherapy
Natural Killer Cells

- Part of the Innate Lymphoid cells (ILC)
- Expression of Transcription Factor E4BP4
- Cytokine secretion: IFN gamma
- To distinguish stressed cells (infected, tumours) from healthy cells
- Ability to kill tumour cells

Recognition strategies

Missing self: inhibitory receptors
Stress-induced self: activating receptors

Modified from S Ugolini and E Vivier
Receptors expressed on NK cells

**Activating receptors**
- Nkp46
- CD16
- h Nkp30
- h Nkp44
- h Nkp80
- m NKR-P1C
- NK2D
- m NK2D-S
- h KIR-S
- m Act. Ly49
- CD94/NKG2C
- CRACC
- Ly9
- CD84
- NTBA
- 2B4

**Inhibitory receptors**
- h KIR-L
- h LILRB1
- CD94/NKG2A
- m Inh. Ly49
- m NKR-P1B
- m NKR-P1D
- KLRG-1
- TIGIT
- CEACAM-1
- LAIR-1

**Chemotactic receptors**
- CCR2
- CCR5
- CCR7
- CXCR1
- CXCR3
- CXCR4
- CXCR6
- CX3CR1
- h Chem23R
- S1P5

**Cytokine receptors**
- IL-1R
- IL-2R
- IL-12R
- IL-15R
- IL-18R
- IL-21R
- IFNAR

**Adhesion receptors**
- CD2
- DNAM-1
- β1 integrins
- β2 integrins

Effector and regulatory functions of NK cells

- DNA damage
- Tumor transformation
- Intracytoplasmic microbial infection
- Others...

NK cells can eliminate stressed target cells through:
- Target cell lysis
- Cytokine secretion (IFN-γ, IL-10)

NK cells can induce:
- Antigen presentation
- Elimination of stressed target cells

NK cells are protected by:
- Healthy cells

NK cells can interact with:
- Antibodies
- T cells
- Mφ

Modified from S Ugolini and E Vivier
Targeting natural killer cells and natural killer T cells in cancer

Eric Vivier1,2,3,4, Sophie Ugolini1,2,3,5, Didier Blaise6,7, Christian Chabannon6,7 and Laurent Brossay5
Targeting natural killer cells and natural killer T cells in cancer

Eric Vivier\textsuperscript{1,2,3,4}, Sophie Ugolini\textsuperscript{1,2,3,5}, Didier Blaise\textsuperscript{6,7}, Christian Chabannon\textsuperscript{6,7} and Laurent Brossay\textsuperscript{6}
Anti-tumor effects of NK-cells

• Direct (preclinical) and indirect (clinical) evidence for a role of NK-cells against cancers and leukemias
  – In vitro: cytotoxic activity against various cell lines (K562)
  – Correlation between tumor infiltrating NK-cells and survival of lung, gastric or colo-rectal cancers (Coca, Cancer, 1997)

• Potent NK-cell versus
  – Leukemia effect
    • Correlation between NK-cell deficiency and prognosis of AML (Fauriat, Blood 2007)
    • NK-cells are effectors of the GVL effect (Ruggieri, Science 2002)
  – Myeloma effect
    • Correlation NK-cells and outcome (Garcia-Sanz R, Brit J Haem 1996).
    • NK-cells are effectors of the GVM effect (Kroger N, Brit J Haem 2005).
**Rationale for targeting NK-cells**

- **NK cells are frequent cytotoxic effectors**
  - Both in peripheral blood and tumor microenvironment
    - 13% (7-31%) in peripheral blood of healthy adults (Connans-Bitter J Pediatrics 1997)
  - Compares favorably with frequency of CTL directed against TAA

- **NK-cells should remain active on chemo-resistant cells**
  - Minimal residual disease after an intensive chemotherapy
  - Tumor which are intrinsically chemo resistant
    - e.g. p53- plasma cells of 17p- Multiple Myeloma
  - Quiescent cancer stem cells
Rational for a NK cell program in leukemia after allo-HSCT

- **NK cells: anti leukemic activity**
- **NK cells after allogeneic transplant**
  - Haplo mismatch Allo HSCT: Role of Kir mismatch (Ruggeri, 2006)
  - NK cells and allo Transplant
    - Not implicated in GVHD genesis (Dendritic cells) (Farag, 2002)
    - Activity not impaired by CSA (Wang, 2007)
  - HLA identical Allo after RIC: Role of post Transplant circulating NK cells (Kim, 2006, Dunbar, 2008)
- **Available Devices to obtain pure NK cell population if needed**
Ciclosporin

Donor NK cells do not recognize allogenic MHC molecules

Tolerance owing to lack of activating signal

Enhancement of alloreactivity and residual tumour cell elimination

Cytokines and cytotoxic mediators
Infusion of activated NK cells in tumor patients

Donor NK cells

In vitro NK cell activation

Leukopheresis

NK cell isolation

NK cell infusion

Cancer patient

Donor

White Blood Cell

Activating ligands

Activating receptors

Anti-cancer function

MHC class I

+ Tolerance

Patient cancer cell

Patient normal cell

Miller et al. Blood 2005

Courtesy from S Ugolini and E Vivier
NK Cells Allogeneic Immunotherapy
A two axes program

- **To amplify residual leukemic cell elimination/control**
  - Early strategy (prior day 100)
    - Lower MRD
    - Prior relapse occurrence (median = 6 months)
  - Retaining low toxicity profile: No GVHD

- **Two approaches to improve leukemic control**
  - Cellular therapy
    - NK selected donor lymphocyte infusion
  - NK response amplification by Kir blockade
Phase I/II of infusion of selected donor NK cells after HLA identical allogeneic stem cell transplantation prepared with reduced intensity conditioning

• **Primary Objective**
  – To establish the feasibility of donor NK cells infusion after HLA matched allogeneic transplant prepared by RIC

• **Secondary Objectives**
  – Ex vivo NK cell selection reproducibility
  – Clinical safety of NK cells infusion
  – Immunomonitoring:
    – Characterization of NK cells phenotypes, functions and survival
    – Other subpopulation
  – GVHD and relapse occurrence
  – Survival

Grant from INCa: PHRC 2010
protocole DLI-NK
plan expérimental / runs à blanc (n=3)

préparation de thérapipe cellulaire
CQ CTC = cyto (NFS / TNC / CD45 / CD3 / CD16 / CD56 / 7AAD) + hémoc + stabilité + DMSO
CQ NK = labo Vivier CIML

aphérèse 2 masses
stockage ON +4°C
déplaquetisation
incubation Ig non spécifiques
incubation anti-CD3+
déplétion CD3+
fraction CD3+
fraction CD3-
incubation anti-CD56+
sélection CD56+
fraction CD3-CD56+
fraction CD3-CD56-
Monoclonal antibody specific for NK cell inhibitory receptor

Patient NK cell

Healthy cell

Tolerance owing to lack of activating signal

Blockade of inhibitory signal

Patient NK cell

Tumour cell

Enhancement of NK cell activity and residual tumour cell elimination

Cytokines and cytotoxic mediators
**IPH2101 Anti-KIR monoclonal antibody**

- Fully human IgG4 monoclonal antibody (Innate Pharma, Marseille, France)
- Binds to KIR2DL1-2-3, and S1-2
- Blocks binding of KIR to HLA-C
- Induces NK cell-mediated lysis of HLA-C expressing AML blasts,
- Does not induce killing of normal PBMC

*Romagne, Blood, 2009*
Pharmacology of anti-KIR IPH2101 (1-7F9) drug candidate: *in vivo* efficacy model (NOD SCID mice)

- Injection of AML cells lines, and autologous bulk NK, with or without IPH2101

NK phenotype

![Graph showing survival rates](image)

Romagne et al, 2009, Blood, 24;114(13):2667-77
Phase I/II of IPH2102 MoAB after HLA identical allogeneic stem cell transplantation prepared with reduced intensity conditioning

- **Primary Objective**
  - To establish the feasibility of IPH2102 after HLA matched allogeneic transplant prepared by RIC

- **Secondary Objectives**
  - Clinical safety of IPH2102 administration
  - Pharmacokinetics and immunogenicity
  - Immunomonitoring:
    - Characterization of NK cells phenotypes, functions and survival
    - Other subpopulations
  - GVHD and relapse occurrence
  - Survival

Grant from ARC: Programme ARC 2011
Cellular therapy

**Hematopoietic Stem Cells**

- **Cancer patient**
  - **Day 0**: Transplantation
  - **Day 60**: NK cell infusion
  - **In vitro NK cell activation**

- **NK cell isolation**

- **Leukopheresis**
  - **White Blood Cell**

**Inhibitory receptor blockade**

- **Anti-KIR mAb**
  - **Day 60**

**Coutesy from S Ugolini and E Vivier**
Thanks!

• **Innate Pharma, Marseille**
  – F. Romagné
  – P. André

• **CIML, Marseille**
  – E. Vivier & S. Ugolini

• **Institut Paoli Calmettes Marseille**
  – D. Olive
  – C Chabannon
  – N Vey