Exploiting NK-cell alloreactivity in AML

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Clinical exploitation of alloreactive NK cells

Adoptive immunotherapy

HSCT

Handgretinger et al. Blood 2016
Factors influencing NK-cell based immunotherapy against cancer

Fang et al. Semin Immunol, 2017
Different manufacturing strategies to obtain NK cells are under investigation.

- PBMC
  - NK cell selection → Activation
  - Long-term expansion +/− Feeders
- hiPSC
- hESC
  - CD34+ cells → Differentiation
  - Xenogeneic feeders
- NK cell lines → Irradiation
- Activated NK cells
- Expanded NK cells
- Differentiated NK cells
- Clinical grade NK cell lines
Expansion of haploidentical NK cells after infusion into cancer patients

Five/19 poor-prognosis patients with AML achieved complete remission after infusion of partially purified haploidentical NK cells.

Miller et al. Blood 2005
Infused NK cells are alloreactive against AML

A

B

C

D

GL183+/NKG2A-  
EB6+/NKG2A-  
Z27+/NKG2A-

VNTR analysis

HLA-C1+ donor alloreactive NK clones in C1 missing patients

HLA-C2+ donor alloreactive NK clones in C2 missing patients

Curti et al. Blood 2011
Infused NK cells are capable of homing in recipient’s bone marrow

Long-lived NK cells proliferate homeostatically in the BM


Curti et al. Blood 2011
Larger NK alloreactivity is associated with reduced relapse

NK cells vs. control
HR 0.49 (95% 0.18-1.30)
P=0.138 Log Rank test

NK >8 vs. control
HR 0.15 (95% 0.03-0.70)
P=0.03 Log Rank test

Curti et al, Clin Cancer Res, 2016
A threshold of alloreactive NK cell clones is predictive for response

Curti et al, Clin Cancer Res, 2016
A threshold of alloreactive NK cell cells is predictive for response

Parisi et al, Front Immunol, 2017
The frequency of alloreactive NK cells may impact on the control of MRD in AML.
Response to NK infusion predicts durable remission after long-term follow up

Overall Survival

Disease-free Survival

p=0.0001

Parisi et al, EHA, 2018
The number of infused donor NK alloreactive cells correlates with prologed OS and DFS.

Overall Survival

- $\geq 2 \times 10^5$ NK/Kg
- $< 2 \times 10^5$ NK/Kg

Disease-free Survival

- $\geq 2 \times 10^5$ NK/Kg
- $< 2 \times 10^5$ NK/Kg

$p=0.030$

$p=0.029$

Parisi et al, EHA, 2018
Infused NK cells have immunoediting capacity of leukemia burden and reduce high-risk clones

Bjorklund et al, Clin Cancer Res, 2018
An algorithm for donor selection and cell processing based on NK functional dose

NKAML: A Pilot Study to Determine the Safety and Feasibility of Haploidentical Natural Killer Cell Transplantation in Childhood Acute Myeloid Leukemia

<table>
<thead>
<tr>
<th>NK Cell Graft</th>
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<tbody>
<tr>
<td>NK Cells (10^6/kg)</td>
<td>T Cells (10^5/kg)</td>
<td>B Cells (10^6/kg)</td>
</tr>
<tr>
<td>38.7</td>
<td>ND</td>
<td>0.106</td>
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<td>27.2</td>
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<td>13.4</td>
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AML DIAGNOSIS

INDUCTION CHEMOTHERAPY

IF CR

DONOR: SCREENING

PATIENT: CONSOLIDATION

PATIENT: SCREENING

DONOR: NK CELL PROCESSING AND COLLECTION

ALLOREACTIVE NK CELLS < 2 X 10^6 NK/Kg

DONOR: SECOND COLLECTION

PATIENT: NK CELLS INFUSION

ALLOREACTIVE NK CELLS ≥ 2 X 10^6 NK/Kg

Lemoli et al, Exp Hematol, 2017
**NK-based clinical program**

**NKAML:**
Infusion of alloreactive NK cells as consolidation strategy for adult acute myeloid leukemia patients: a multicenter clinical trial.

**ENROLLING**

**MRDNK:**
Infusion of alloreactive NK cells for acute myeloid leukemia patients, eligible for allogeneic stem cell transplantation, with persistent minimal residual disease after conventional chemotherapy.

**UNDER APPROVAL**

*Financial Support by Italian Ministry of Health*
INFUSION OF ALLOREACTIVE NK CELLS AS CONSOLIDATION STRATEGY FOR ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS: A MULTICENTER CLINICAL STUDY

“NKAML”
Strategies to overcome the KIR-KIRL-mediated inhibition of NK cells

- Blocking KIR-KIRL interaction
- Activation of CD16 (Fc-receptor) on NK cells with an antibody directed against leukemic cells
- Bispecific and Trispecific killer engagers activate NK cells via the Fc-receptor against leukemia cells
- CAR-NK cells directed against leukemia antigens

Handgretinger et al. Blood 2016
NK cells “naturally” kill cell targets without prior sensitization

Handgretinger et al. Blood 2016
Combinatorial strategies with MICA-MICB mAb

The MICA-MICB mAb stabilizes these NKG2D ligands on tumor cells, inducing tumor cell killing by NK and CD8⁺ T cells. Combinations with immune checkpoint inhibitors (anti–PD-1 or anti–PD-L1), engineered immune cells, or antibodies blocking NKG2A or KIR could amplify antitumor activity. HLA-E, human leukocyte antigen E.

### Diagram

**NK cell**
- NKG2A
- KIR
- NKG2D
- CD16
- ADCC

**CD8⁺ T cell**
- PD-1
- Engineered T cell receptors
- Tumor antigen
- MHC I + antigen
- MMP, ADAM

**Tumor cell**
- MICA or MICB mRNA up-regulated by chemotherapy, radiotherapy, and HDAC inhibitors

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Adelheid Cerwenka, and Lewis L. Lanier Science
2018;359:1460-1461
How components of BM microenvironment may inhibit NK activity against AML

Parisi et al, Front Immunol, 2017
Patient-derived factors on alloreactive NK immunotherapy: the role of Tregs

Bachanova et al, Blood, 2014
ALT-803 is a novel IL-15 superagonist complex consisting of an IL-15 mutant (IL-15N72D) bound to an IL-15 receptor α/IgG1 Fc fusion protein. **ALT-803** has improved pharmacokinetic properties, longer persistence in lymphoid tissues and enhanced anti-tumor activity compared to native, non-complexed IL-15 in vivo.
Conclusions

- The demonstration of the significant clinical activity of alloreactive purified NK cells outside the transplantation setting is the rationale for exploiting this strategy as adoptive immunotherapy.

- The results from early safety studies have clearly paved the way for designing a new generation of efficacy clinical studies.

- Biological issues need full elucidation and clinical correlation.

- NK alloreactivity may represent the platform for expanding the field to innovative NK cell-based approaches.
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Time-line summary of alloreactive immunotherapy for AML

Krakow et al, Blood Reviews, 2014
The percentage of donor alloreactive NK cells correlates with relapse rate.
Infusion of alloreactive NK cells into AML patients in CR

- **Induction/Consolidation Chemotherapy**
- **Morphological or Better CR**
- **Immunosuppressive Chemotherapy Plus NK Cell Infusion**
- **Induction/Consolidation Chemotherapy**
- **Haploidentical Donor Selection**
- **Leukapheresis and Haploidentical NK Cell Purification**
- **Additional NK Cell Infusion (optional)**
- **Follow Up**

54 high risk AML patients were screened for the availability of one haploidentical KIR ligand mismatched donor.

26 patients (48%) had one suitable donor.

21 patients (38%) infused.

17 patients infused in CR.

16 evaluable patients for clinical response.

9 CR patients are disease-free after a median follow-up of 27 months.
KIR-KIRL mismatch in haploidentical SCT: the missing-self

Farag S et al, Blood 2004
Clinical impact of KIR-L mismatch on relapse rate after haploSCT

Defining the optimal donor: KIR-L mismatch plus activating KIRs

Handgretinger et al. Blood 2016
KIR-L mismatch and activating KIRS: improved clinical outcome after haploSCT

Mancusi et al. Blood 2015