

Ex vivo activated natural killer (NK) cells from myeloma patients kill autologous myeloma and killing is enhanced by elotuzumab

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Abstract

Immune-based therapies may improve outcome for multiple myeloma (MM) by eradicating chemo-resistant disease. Our recent trial utilizing IL2 activated, killer immunoglobulin-like receptor-ligand mismatched NK cell transfusions from haplo-identical donors yielded (n)CR in 50% of patients (Shi et al, BJH 2008;143:641-653). Unfortunately, after NK cell therapy, 2/10 patients had progressive disease, and the median duration of response for the other 8/10 patients was only 105 days (range 58-593). This may have been due to an insufficient dose of alloreactive NK cells and early rejection. Furthermore, appropriate donors were identified for only 30% of otherwise eligible patients. We therefore investigated whether NK cells from MM patients could be expanded and activated to kill autologous MM. We then examined whether pre-treatment of MM cell targets with elotuzumab, a humanized antibody to the MM tumor antigen CS1, could further enhance NK cell-mediated lysis.

PBMC from 8 MM patients were co-cultured for 14 days with irradiated K562 cells transfected with 4-1BBL and membrane bound IL15 in the presence of IL2 (300U/ml) as previously described (Imai et al, Blood 2005;106:376-383). The degree of NK cell expansion, NK immunophenotype, and ability to kill MM (4 hour ⁵¹Cr release assays) were assessed. To determine the ability of ex vivo expanded NK cells to traffic to bone marrow, activated NK cells were injected into the tail vein of NK cell depleted NOD-SCID mice, which were then sacrificed after 48 hours. Flow cytometry for human CD45, CD3, and CD56 was performed on cells from blood, marrow and spleen.

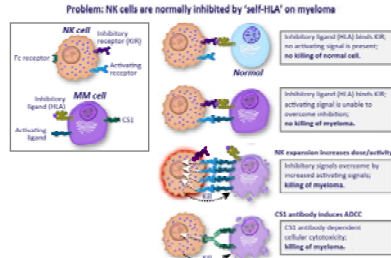
There was significant expansion of NK cells for 4 of 8 subjects (average 152 fold, range: 92-204) after 2 weeks of co-culture with K562 transfectants. Expansion of T cells was not observed. The NK cell activating receptor NKG2D, and natural cytotoxicity receptors NKP30, NKP44, and NKP46 were up-regulated following the expansion. Expanded NK cells were able to kill autologous MM (N=3 subjects, E:T ratio 10:1, average 30%, range 22-41%), whereas resting NK cells did not. Pretreatment of autologous MM cells with elotuzumab increased the activated NK cell-mediated killing by 1.7-fold over target cells pretreated with an isotype control antibody. This level of killing was similar to that of the highly NK kill-sensitive cell line K562. Autologous PHA blasts and CD34+ stem cells were not killed. Activated human NK cells were detectable in the bone marrow of NOD-SCID mice 48 hours after injection.

In conclusion, ex vivo activation of NK cells from MM patients with K562 transfectants can induce killing of autologous MM and produce large numbers of NK cells for potential therapy. The addition of elotuzumab to activated NK cell therapy enhances anti-MM effects by ADCC thus invoking an additional NK cell-mediated mechanism of MM killing. Importantly, ex vivo activated NK cells traffic to the bone marrow in mice. Autologous NK cell therapy eliminates the issues related to allo-donor availability and early NK cell rejection, and could provide an option for patients refractory to chemotherapy agents.

Background

Rationale for expanded autologous NK cell therapy incorporating elotuzumab

- No donor selection barrier
 - Only 30% of pts have an appropriately KIR-Ligand mismatched haplo-identical family donor and the level of alloreactivity is difficult to predict
- No risk of GvHD, no early rejection of transfused NK cells
- Expansion yields a large dose of highly active NK cells
- Elotuzumab is a humanized antibody to CS1 that induces NK cell mediated ADCC
- CS1 expression is uniformly high in myeloma but absent in most normal tissues



Methods

K562 transfectant stimulators:

- K562 is a human leukemia cell line
 - Lacks HLA Class I antigens, the major inhibitor of NK cell activity
- K562 cells are genetically modified* to express:
 - Membrane bound IL15 cytokine
 - essential NK cell growth and survival factor
 - induces proliferation of mature NK cells
 - enhances NK cell cytotoxicity
 - 4-1BB ligand
 - induces NK cell proliferation and cytokine secretion

PBMC co-culture with K562 transfectant stimulators (in the presence of IL2)

Restimulation (day 7)

Harvest (day 14)

Fold Expansion
Cell counts

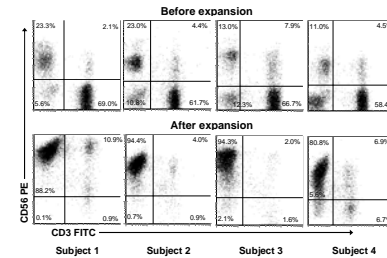
Phenotype
Flow Cytometry

Cytotoxicity
⁵¹Cr Release Assays

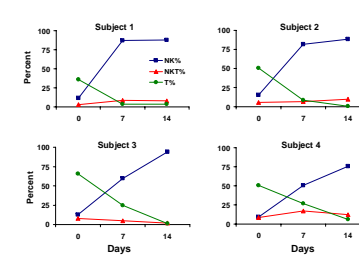
Trafficking
Murine Model

Results

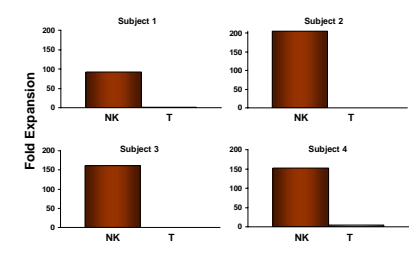
1. Myeloma patient derived NK cells expand after co-culture with modified K562 stimulators



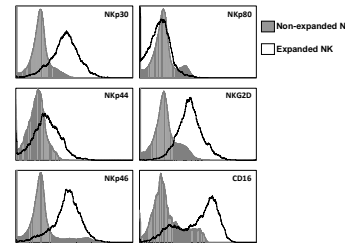
2. Kinetics of NK, T, and NKT cell subset growth and decline in co-cultures



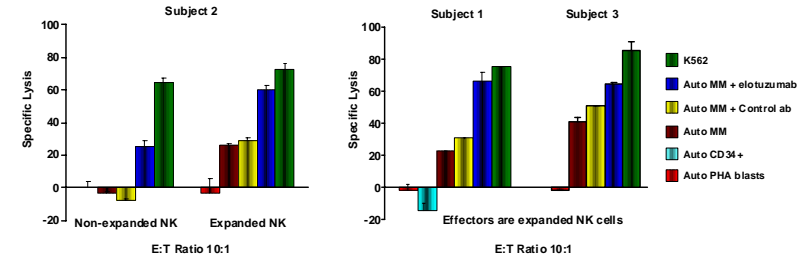
3. Myeloma patient derived NK cells expand but T cells do not after co-culture with modified K562 stimulators



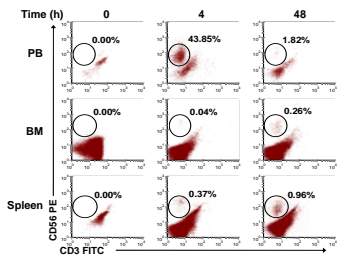
4. Expanded NK cells have an activated phenotype, with upregulation of NCRs, NKG2D, and the FC Receptor



5. Expanded myeloma patient derived NK cells kill primary myeloma and killing is enhanced by elotuzumab



6. Human activated NK cells are detectable in the PB, BM, and spleen of NOD-SCID mice 48 hours after injection



Conclusions

- Ex vivo expansion of NK cells from myeloma patients can produce large numbers of NK cells for therapy.
- Expanded NK cells have an activated phenotype.
- Expanded NK cells kill autologous myeloma cells but do not kill normal cells, including CD34+ progenitor cells.
- Elotuzumab enhances the anti-myeloma effect of activated, patient derived NK cells.
- Expanded NK cells can be detected in the bone marrow in mice even 48 hours post transfusion.
- Autologous NK cell therapy eliminates the issues related to allo-donor availability and early NK cell rejection, and could provide an option for patients refractory to chemotherapy agents.

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 - Employment: Daniel EH Afar is an employee of PDL BioPharma.
 - The remaining authors have nothing to disclose.