A phase I study of allogeneic Natural Killer Cell Therapy generated from Cord Blood Hematopoietic Stem and Progenitor Cells in Elderly Acute Myeloid Leukemia Patients

Harry Dolstra[1*], M Roeven[1,2,*], J Spanholtz[3], B Hangalapura[1], M Tordoir[3], F Maas[1], M Leenders[1], F Bohme[3], N Kok[3], C Trilsbeek[1], J Paardekooper[1], A van der Waart[1], P Westerweel[4], T Snijders[5], J Cornelissen[6], G Bos[7], H Pruijt[8], G Huls[2], A de Graaf[1], B van der Reijden[1], N Blijlevens[2], J Jansen[1], A van der Meer[1], J Cany[1], F Preijers[1], N Schaap[2]. [1,2]Radboud university medical center, [1]Department of Laboratory Medicine & [2]Department of Hematology, Nijmegen, the Netherlands; [3]Glycostem Therapeutics, Oss, the Netherlands; [4]Albert Schweitzer Hospital, Dordrecht, the Netherlands; [5]Medisch Spectrum Twente, Enschede, the Netherlands, [6]Erasmus MC Cancer Institute, Department of Hematology, Rotterdam, the Netherlands, [7]Maastricht UMC, Department of Internal Medicine, Maastricht, the Netherlands; [8]Jeroen Bosch Hospital, Department of Internal Medicine, 's Hertogenbosch, the Netherlands. *These authors contributed equally.

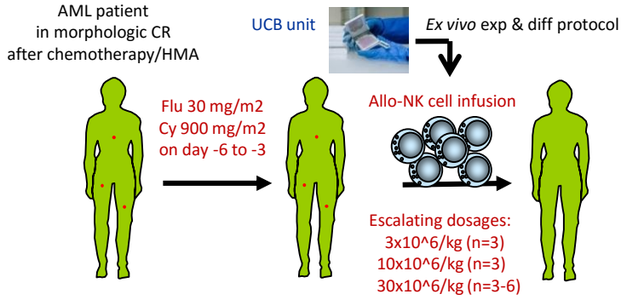
Introduction

Elderly AML patients have a poor prognosis due to high relapse rates following standard therapy. Natural Killer (NK) cell alloreactivity has found to significantly control relapse in AML in the HLA-mismatched haploidentical allo-SCT setting. Moreover, allogeneic NK cell infusions has shown to induce CR in patients with advanced AML. As a consequence, adoptive NK cell transfer may be a promising treatment for elderly AML patients, who are unfit or ineligible for allo-SCT. Most clinical studies exploited CD56+CD3- NK cell products enriched from leukapheresis of haploidentical donors containing low numbers of T cells that could have contributed to the observed therapeutic effects and potentially induced GVHD. Therefore, we have developed a GMP-compliant culture system for the generation of highly functional NK cells from umbilical cord blood (UCB)-derived CD34+ progenitor cells, without T cell contamination. Here, we report the feasibility, safety, toxicity and efficacy data of the first in man trial with this unique UCB-NK cell product.

Study design and intervention

A phase I dose escalation study was performed in elderly AML patients who achieved morphologic CR (<5% BM blasts) after standard treatment. Prior to NK cell infusion, patients received immunosuppressive conditioning consisting of cyclophosphamide (900 mg/m^2/day) and fludarabine (30 mg/m^2/day) for 4 consecutive days. On day 0, cohorts of 3 patients have received 3x10^6, 10x10^6 and 30x10^6 allogeneic NK cells per kg body weight generated *ex vivo* from CD34+ cells obtained from a partially HLA-matched allogeneic UCB unit. No systemic IL-2 was given to study if *in vivo* UCB-NK cell persistence and expansion could be obtained without cytokine support.

Phase I dose escalation study in max. 12 AML patients ≥ 55 year and not eligible for allogeneic SCT



CCMO nr. NL31699 & Dutch Trial Register nr. 2818

ZonMw

Trial objectives

- Feasibility, Safety and Toxicity
- *In vivo* lifespan and expansion of transfused NK cells
- Biological activity of transfused NK cells
- Effect on residual disease determined by AML-MRD PCR and/or immunophenotyping

Results

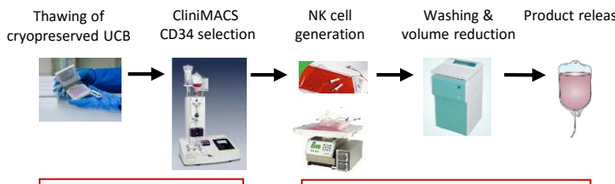
Patient & UCB donor characteristics

Seventeen elderly AML patients have been included of which 10 were treated with Flu/Cy and allogeneic UCB-NK cells (see table below). All patients were in morphologic CR1 (except UPN9 who was in CR3) after 2-3 standard chemotherapy courses (n=7), or 1 chemotherapy course followed by subsequent azacitidine or decitabine treatment (n=3).

UPN	Age /Sex	WHO type	FAB type	Cyto genetics	Molecular	Risk group	Missing ligand Recipient	Donor KIR type
1	72/M	AML with NPM1 mut.	M2	46 XY	NPM1	Good	C1 & Bw4	AA
2	68/M	AML	M7	46 XY	IDH1	Int	Bw4	AB
3	73/F	Therapy related AML	RAEB-t	Complex	TP53	Very poor	C1	AB
4	70/F	AML	M0	46 XX	IDH2, RUNX1	Int	C1	AB
5	72/F	AML with MDS-related features	M1	46 XX	IDH2, DNMT3A	Poor	-	AB
6	76/M	AML with MDS-related features	RAEB-t	46 XY	No known mutations	Poor	C1	AA
7	75/F	AML with MDS-related features	M0	46 XX +13	No known mutations	Poor	-	AB
8	71/M	AML	M0/M1	46XY Inv. 12	ASXL1, RUNX1	Very poor	C2 & Bw4	AA
9	71/M	AML	M5	46 XY	FLT3-ITD	Very poor	-	AA
10	73/M	AML	M5	47 XY+19(8) + 8 +19(7)	No known mutations	Poor	C2	AB

Results NK cell generation from CD34+ UCB cells

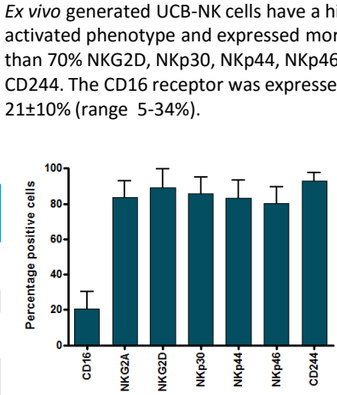
Six weeks prior to infusion (day -42), a partially HLA-matched UCB unit was thawed and CD34+ cells were enriched using CliniMACS. Enriched CD34+ UCB cells were subsequently used for *ex vivo* expansion and differentiation of CD56+CD3- NK cell products according to the validated prescription protocol and IMPD (see scheme & results below).

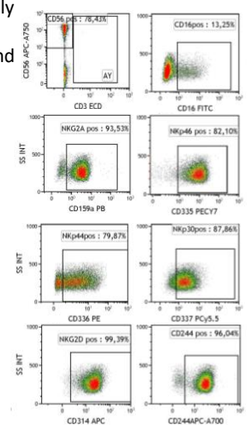


Results GMP manufacturing (n=10)	Median	Mean ± SD	Range
Purity CD56+CD3- cells (%)	74	70 ± 13	40-81
Viability CD56+CD3- cells (%)	95	95 ± 4	88-99
CD56+CD3- NK cells (x10^6/kg)	36	44 ± 32	6-113
CD3+ T cells (x10^4/kg)	0.00	0.16 ± 0.25	0.00-0.70
CD19+ B cells (x10^5/kg)	0.29	0.36 ± 0.39	0.00-1.10

Characteristics infused UCB-NK cell products

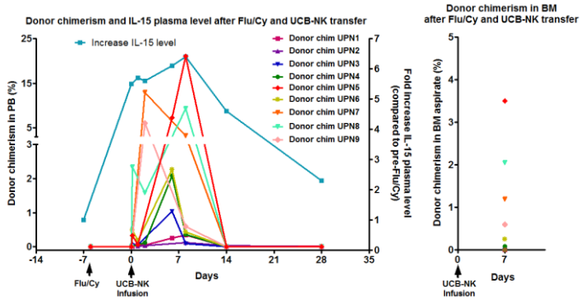
Ex vivo generated UCB-NK cells have a highly activated phenotype and expressed more than 70% NKG2D, NKp30, NKp44, NKp46 and CD244. The CD16 receptor was expressed 21±10% (range 5-34%).





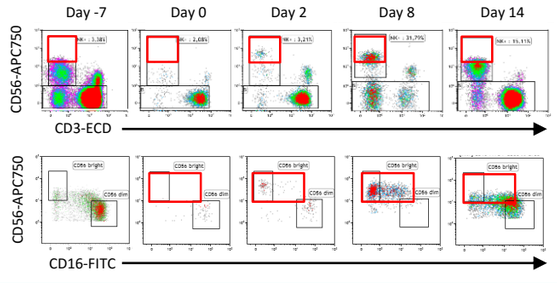
Donor chimerism after UCB-NK cell transfer

Donor chimerism was measured by Q-PCR for discriminating DNA polymorphisms. A temporary repopulation and persistence of UCB-NK cells could be detected in PB between days 1 and 8 post-infusion, which was associated with increased IL-15 plasma levels. Donor chimerism increased with higher doses of infused UCB-NK cells, and donor chimerism up to 3.5% was found in BM at day 7/8.



In vivo maturation of UCB-NK cells after adoptive transfer

Further UCB-NK cell maturation *in vivo* was observed by acquisition of CD16 (see data of UPN5 below) and KIR expression (data not shown), while expression of activating receptors was sustained.

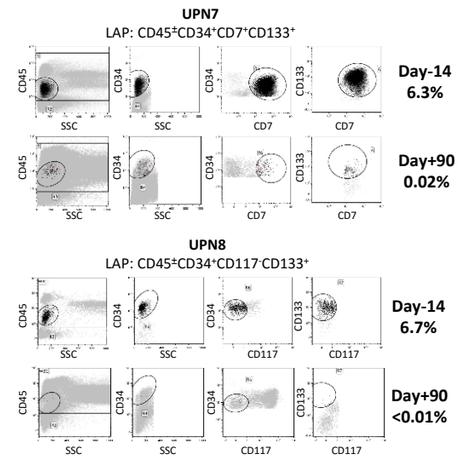


No UCB-NK cell related toxicity was observed

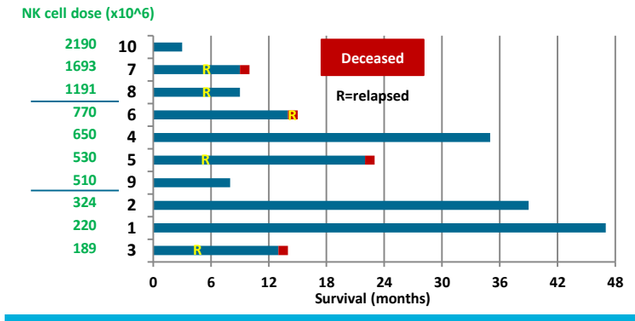
- Hematological toxicities as expected due to Flu/Cy regimen
- No dose-limiting UCB-NK cell related toxicities were observed
- No GVHD was observed after UCB-NK cell infusion

MRD and survival after UCB-NK cell treatment

Despite morphologic CR during azacitidine treatment, residual disease of 6-7% with a leukemia-associated phenotype could be detected by flow cytometry before NK cell infusion in BM of two patients (UPN7 and 8). In both patients MRD was reduced to less than 0.05% at 90 days after UCB-NK cell therapy.



Survival of UCB-NK cell treated patients from adoptive transfer



Conclusion

GMP-compliant UCB-NK cell products containing up to 30x10^6 NK cells/kg body weight can be safely infused in non-transplant eligible AML patients following immunosuppressive chemotherapy, showing no NK cell related toxicity. Infused UCB-NK cells repopulate, mature and migrate to BM without IL-2 infusion.

Address for correspondence:
Dr. H. Dolstra; harry.dolstra@radboudumc.nl; Dr. J. Spanholtz; jan.spanholtz@glycostem.com & Dr. N. Schaap; michel.schaap@radboudumc.nl

Radboudumc university medical center