Natural killer cell therapy in children with refractory acute myeloid leukemia

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Objective

The use of natural killer (NK) cells as a cellular immunotherapy has increased over the past decade, especially for patients with hematologic malignancies. The advantages of NK cells for adoptive therapy are the high innate anticancer activity and low incidence of toxicity reactions. Today, there are different technologies and sources to obtain NK cells: immunomagnetic isolation, differentiation with cytokines or expansion with stimulatory/feeder cells such. We have previously reported the creation of K-562-based feeder cell line transduced to express 4-1BBL and membrane bound Interleukin-21 (mbIL-21). Here we describe four cases of NK immunotherapy for children with refractory acute myeloid leukemia (AML). Registered at www.clinicaltrials.gov as NCT04327037 and NCT05272293.

Methods

A total of 4 children with a median age of 16 (range, 11-17) with refractory AML were enrolled in the study. Peripheral blood samples of 4 haploidentical donors (3 mothers and 1 father) with a median age of 42.5 years (range, 40.6–50.4) were the source of PBMCs for NK cells expansion. NK expansion was induced by co-culturing of donor PBMCs with feeder line K562-mbIL-21-41BBL obtained in our laboratory. Course of immunotherapy consisted of a block of FLAG-based chemotherapy followed by NK cells infusions (from 1 to 3) during the period of cytopenia. NK cells were administered intravenously on day 0. Two patients received six dose of IL-2 ($1x10^6$ IU/m^2, Roncoleukin, LLC NPK BIOTECH, Russia) every second day starting on day -1, one patients received one dose of IL-2, another one was treated without IL-2.

Results

Totally 4 patients received 9 infusions of NK cells. The purity of NK cells was 95.5 % (77.3-98.3), median dose of infused NK cells was 41 (12-88)*10^6/ kg, CD3+ cells – 0.9 (0.8-11.5)*10^6 / kg. The majority of obtained NK cells had the phenotype of immature activated cells (NKG2A+, double bright CD56++CD16++, CD57-) expressing NKp30, NKp44, NKp46, NKG2D, CD69, HLA-DR and CD96.

Patient #1 received two course of immunotherapy with one NK cells infusion per course. This patient achieved complete morphological remission (CRm) and received matched unrelated HSCT, is alive.

Patient #2 received one course of immunotherapy with two NK cell infusions. After immunotherapy the level of blast cells in bone marrow decreased, however, the patient did not get the second course of immunotherapy because of infection status. The patient died from progression.

Patient #3 received one course of immunotherapy with two NK cell infusions, achieved CRm and received matched related HSCT, is alive.

Patient #4 received one course of immunotherapy with three NK cell infusions, achieved CRm. Further treatment was delayed because of infection complications. The patient is alive.

Table 1 Characteristics of patients and donors

Patients	Age	Sex	Diagnosis	Genetic aberrations	Disease status	Cycles of prior therapy	Chemotherapy before NK	Donor	Donor age	Missing KIR ligand	Donor NK alloreactivity	Donor KIR haplotype	Donor KIR haplotype score
P1	14	m	AML, M2	CBFb/MYH11	Refractory	3	FLAG-Ida	Mother	40,6	Yes / C2	No	telAB/cenAA	Neutral
P2	17	f	AML, M2	FLT3-ITD	Refractory	2	FLAI	Mother	50,4	No	No	telAA/cenAA	Neutral
P3	11	m	AML, M4	FLT3-ITD	Refractory	3	FLAG, Decitabine, Vorinostat, Sorafenib	Father	44,3	Yes /Bw4	Yes	telAA/cenBB	Best
P4	17	f	AML, M0	complex karyotype	Refractory	2	FLAG-Ida	Mother	40,6	Yes /Bw4	Yes	telAA/cenAA	Neutral

Table 2 NK cell product characteristics

Patients	Course	NK doses	Doses of infused cells					
			NK, %	NK, *10^6/ kg	CD3+56-, %	CD3+CD56-, *10^6/ kg	CD3+CD56+, %	CD3+CD56+, *10^6/ kg
P1	1	1	98,3	74	1,3	0,4	0,4	0,3
P1	2	1	77,3	41	1,3	0,7	20,3	10,8
P2	1	1	94,0	55	0,7	0,4	3,8	2,2
P2	1	2 cryo	92,9	12	1,8	0,2	5,1	0,7
P3	1	1	98,8	74	0,4	0,3	0,8	0,6
P3	1	2 cryo	99,1	88	0,5	0,4	0,4	0,4
P4	1	1	95,5	31	1,6	0,5	2,8	0,9
P4	1	2	96,4	24	1,7	0,4	1,6	0,4
P4	1	3 cryo	92,6	26	1,6	0,4	2,2	0,6

Table 3 Clinical response after NK immunotherapy

Patients	Blast cells (%) in BM		Remission	Treatment after NK	Outcome, follow up, days
	before	after			
P1	43	1,75	Yes, CRm	HSCT	Alive, +667
P2	47,7	9,5	No	Salvage therapy (5-azacitidine)	Death, +260, progression
P3	64,3	1,75	Yes, CRm	HSCT	Alive, +464
P4	63,5	1,5	Yes, CRm	Without treatment	Alive, +87

Conclusions

Infusions of haploidentical ex vivo expanded NK cells were safe and well tolerated, provided clinical response in 3 out of 4 poor prognosis patients with refractory AML. Encouraging results stimulate us to continue the investigation of NK immunotherapy for patients with AML.

Key words: NK cell immunotherapy, AML